

DEVELOPING CRITERIA TO PRIORITIZE RAPID
REMOVAL OF AMERICAN ELM TREES INFECTED WITH
DUTCH ELM DISEASE

by

Matthew Russell

The University of Winnipeg
Department of Biological Sciences
Winnipeg, Manitoba, Canada

A thesis submitted to the Faculty of Graduate Studies
in partial fulfillment of the requirements for a
Master of Science in Bioscience, Technology, and Public Policy.

October 2021

ABSTRACT

During late summer and early fall in Manitoba, adult native elm bark beetles (NEBB) that carry Dutch Elm Disease (DED) emerge from brood galleries in the canopy and upper trunk of infected elm trees and move to the base and root flares of healthy trees to overwinter. In the spring, DED-carrying beetles disperse from these overwintering sites back to the canopy of healthy elm trees where they feed and construct new brood galleries, thus introducing new DED infections. The current practice after initial DED diagnosis is to remove diseased American and Siberian elm trees prior to emergence of overwintering adult NEBB vectors before the spring. In Manitoba and Saskatchewan, the preferred date for infected tree removal is before the end of March. In Winnipeg, the majority of trees are removed during late fall and winter although infected trees may remain standing into early summer.

Infected tree removal remains a vital and primary component of the integrated DED program in the City of Winnipeg, even though other DED management methods are practiced to augment infected tree removal, including insecticidal control of beetles, injection of fungicides for tree protection, sanitation pruning, etc. A significant constraint to this approach is that most infected trees are removed after NEBB adults have emerged in the fall and moved to overwintering sites on healthy trees. Delayed removals due to weather conditions, site accessibility and limitations in resources needed to remove trees have also resulted in infected elm trees remaining in place until the spring. All these issues diminish the success of the elm sanitation program. Removal of all diseased trees before mid-September could potentially reduce NEBB populations and thus, DED incidence, and spread. Logistical limitations are encountered when large numbers of infected trees require immediate removal, and it is impractical to remove that number between July and September. Preliminary research by Holliday (2016) suggested that a small percentage of diseased elm trees may support the majority of maturing NEBB brood. Confirmation of this trend and targeted removal of this small percentage of DED-infected trees carried out prior to the NEBB migration in the fall would greatly reduce DED incidence by decreasing the number of overwintering NEBB.

The current project, in collaboration with the University of Winnipeg (UW) and the City of Winnipeg (Forestry Branch), analyzed the correlation between NEBB densities in infected

elm trees and the expression of DED symptoms during the summers of 2017, 2018, and 2019. Trunk bark removal and bark removal of upper canopy branches were examined to predict the relationship between canopy NEBB densities and the expression of disease symptoms in the tree crown. A key question was whether specific trees within a larger group of infected trees could be visually confirmed to support large numbers of breeding NEBB during the summer.

Surveys were initiated in study neighbourhoods by Forestry Branch DED surveillance staff to confirm the presence of DED in mid-June each year. After DED-infected trees were identified, UW staff assessed a series of external disease symptoms in infected trees. Trees were first assessed in late June, continuing weekly for a minimum of four weeks until the end of August. Once the survey was completed, Forestry Branch sanitation crews removed infected study trees, and branch samples from these trees were taken to determine the number of NEBB brood galleries and percentage of DED staining was present in the canopy. In addition, bark was removed from the lower trunks of infected trees in 2017 to determine whether NEBB colonized this part of the tree during the summer and to examine the level of fungal staining in the lower trunk. During 2018 and 2019, sticky traps on DED-infected study trees were used to capture emerging NEBB and adults searching for overwintering sites. These collected NEBB were then tested for the presence of *Ophiostoma novo-ulmi* (DED) spores. The relationship between canopy variables recorded during the disease progression survey and NEBB brood gallery density were compared to determine which best predicted high density NEBB trees and could be used to implement a rapid tree removal program.

My results indicated that the percentage of dead canopy leaves, dead canopy branches, and DED infection sites were positively correlated with NEBB brood gallery density, whereas overall canopy cover and percentage green canopy leaves were negatively correlated with NEBB brood gallery density. Differences between trees were pronounced when infected trees were placed into two categories (no NEBB brood galleries detected versus NEBB brood galleries detected). Generalized linear models were employed to compare the external canopy variables with NEBB gallery density. Two models predicted which trees had high numbers of NEBB galleries; the first used percentage fungal staining (*i.e.*, proxy for NEBB density) as the response variable while the second model used trees grouped either into detectable or not detectable NEBB density as the response variable. The first model suggested that the percentage of dead leaves in the canopy was a useful predictor of NEBB density, while the second model found the

number of initial DED initial infection sites was the most significant predictor of NEBB densities.

These findings show that canopy die-back, the percentage of dead leaves in the canopy, and the number of infection sites assessed are the best indicators of NEBB densities. This suggests that if external DED symptoms are tracked during the first month of infection, then they can be used to identify trees and prioritize which need to be removed and disposed of first during July and August in order to prevent NEBB from emerging and dispersing to new trees in the fall.

ACKNOWLEDGMENTS

Thanks to my committee, Dr. Westwood, Dr. Park, and Martha Barwinsky for their continued guidance and feedback.

The Westwood Lab, Maureen Hanlon, Justis Henault, and Martine Balcaen for support over the three years of completing this thesis. Stephanie Sheard, Mia Kirbyson, and Lisa Jones whose help with field work and data collection was invaluable.

Everyone who we collaborated with at the City of Winnipeg, Karen Asmundson, Jeff Dempster, Shaugnessy Dagdick, Craig Martin, and Keri LaFrance, for their time and help with gathering the data for the project and forming it into a workable protocol.

Finally, I would like to dedicate this thesis to my parents, who without their support and love this project would not be possible.

Table of Contents

ABSTRACT.....	2
ACKNOWLEDGMENTS	5
LIST OF TABLES	8
LIST OF FIGURES	9
LIST OF FIGURES CONTINUED	10
LIST OF APPENDICES.....	11
LIST OF ABBREVIATED TERMS	12
CHAPTER 1 – INTRODUCTION.....	13
CHAPTER 2 – LITERATURE REVIEW	16
<i>Ophiostoma</i> and Dutch elm disease	16
Bark beetles associated with <i>Ophiostoma</i>	17
Attraction of vectors by <i>Ophiostoma</i>	19
Elm tree resistance.....	19
Historical spread of Dutch elm disease.....	20
Integrated pest management of Dutch elm disease.....	23
Integrated pest management in Manitoba	25
Importance of managing Dutch elm disease	25
Prior research by Holliday (2016).....	27
CHAPTER 3 – METHODS	29
General Methods.....	29
Disease progression survey	32
Trunk debarking.....	34
NEBB capture and fungal staining assessment.....	37
Mid-crown branch sampling	39

Disease symptoms calculation	41
Age estimation	44
Analysis of data groupings	44
NMDS ordination and ANOSIM	45
Generalized Linear modelling	47
CHAPTER 4 – RESULTS	50
Summary statistics for disease progression survey	50
Data groupings results	56
NMDS ordination and ANOSIM	65
Modelling – Regressions and GLM	68
Beetle capture	70
Debarking	70
Proportion of elm trees containing NEBB	72
CHAPTER 5 – DISCUSSION	74
Comparison to Holliday’s (2016) study	74
Assessment of canopy variables	75
Variable validity for modelling	77
Assessment of non-canopy variables	80
GLM Modelling discussion	82
Next steps with City of Winnipeg	84
NEBB beetle capture	85
Limitations	86
CHAPTER 9 - CONCLUSION	88
REFERENCES	90
APPENDIX	104

LIST OF TABLES

Table 1. Survey criteria for each component of the study over three years, showing the year each variable was recorded.	31
Table 2. Sample size of DED infected trees for each portion of the detectable/undetectable NEBB density groupings from 2017-2019.	45
Table 3. Generalized linear models developed for assessment of canopy branch brood gallery data and fungal staining and disease progression survey variables.....	48
Table 4. Disease progression survey and canopy sampling summary statistics collected between 2017-2019 in Winnipeg, MB	50
Table 5. Welch’s ANOVA results for neighbourhood groupings, indicating if variable tested showed significant difference between study neighbourhoods between 2017-2019.....	63
Table 6. Games-Howell post-hoc test for Welch’s ANOVA, indicating significance or not between each neighbour, for each variable tested.....	64
Table 7. Beta regression model results. Models compared to weighted fungal staining.....	69
Table 8. Generalized linear model results.....	70

LIST OF FIGURES

- Figure 1.** Study neighbourhoods in Winnipeg, MB, included in study (2017-2019). A: Minto, B: Wolsley, C: Crecentwood (2017), and D: Riverview and Lord Roberts.....28
- Figure 2.** Cambium plug (2017) taken to measure the moisture content after trunk debarking is completed. Cambium sample taken above trunk debarking ring. Tree sampled is DED-infected American elm tree in Winnipeg.....35
- Figure 3.** Trunk debarking completed in Winnipeg on DED-infected American elm trees. 2017 (left) and 2018 (right).....37
- Figure 4.** NEBB trapping program, traps on American elm tree in Minto (2018); 4 traps on each tree for each cardinal direction (left) and traps on American elm tree in Lord Roberts (2019); aligned parallel forming a ring around the tree trunk.....38
- Figure 5.** Mid-crown branch sampling completed in summer 2018. City of Winnipeg Forestry department crews while removing DED infected American elm trees would obtain mid-crown samples from each cardinal direction.....40
- Figure 6.** Histograms for diameter at breast height (A), tree height (B), and estimated age (C) for all American elm trees sampled between 2017 to 2019 in the study.....52
- Figure 7.** Boxplot of tree trunk diameter at breast height (DBH) (A), tree height (B), and estimated tree age (C) grouped by study year.....53
- Figure 8.** Boxplots of average percent canopy cover, 2017-2019. A: Average canopy cover for each study week over 3 years, B: Average canopy cover for each year.....54
- Figure 9.** Summary of infection sites recorded upon initial study tree assessment weekly (A), and boxplot showing NEBB brood gallery density per each level of infection site (B) over 2018 and 2019.....55
- Figure 10.** Boxplot of average diameter at breast height in each study year (meters), grouped by trees that during mid-crown sampling had either a detectable or undetectable density of NEBB brood galleries.....57

LIST OF FIGURES CONTINUED

- Figure 11.** Boxplot of average tree height (meters) in each study year, grouped by trees that during mid-crown sampling had either a detectable or undetectable density of NEBB brood galleries.....58
- Figure 12.** Boxplot of average estimated age in each study year, grouped by trees that during mid-crown sampling had detectable or undetectable density of NEBB brood galleries.....59
- Figure 13.** Grouping of study trees with detectable or undetectable levels of NEBB brood galleries compared to average canopy cover (A), percentage canopy green leaves (B), percent canopy dead leaves (C), and percent canopy dead branches (D), grouped by study year.....61
- Figure 14.** Grouping of study trees with detectable or undetectable levels of NEBB brood galleries, compared to percentage canopy fungal staining from tree canopy samples (A), infection sites recorded on initial tree assessment (B), and NEBB brood gallery density (Galleries per meter squatted) (C), divided by study year.....62
- Figure 15.** NMDS ordination of DED infected study trees, recoded in Winnipeg, MB. A. Ordination 2018 and 2019, with 7 variables. B. Ordination 2017, 2018, and 2019, with 9 variable vectors.....66
- Figure 16** NMDS ordination of DED infected study trees, recoded in Winnipeg, MB. A. Ordination 2017, 2018, and 2019. B. Ordination 2018 and 2019. Points in ordination are study trees included in ordination analysis.....67
- Figure 17.** NMDS ordination of DED infected study trees, recoded in Winnipeg, MB., grouped by Neighbourhood. A. Ordination 2017, 2018, and 2019. B. Ordination 2018 and 2019.....67
- Figure 18** NMDS ordination of DED infected study trees, recoded in Winnipeg, MB., grouped by trees with detectable or undetectable NEBB galleries by density. A. Ordination using data inclusive of 2017, 2018, and 2019. B. Ordination using data inclusive of 2018 and 2019.....68
- Figure 19.** Linear regression of 2017 trunk and canopy NEBB brood galleries.....72

LIST OF APPENDICES

- Appendix I.** Study trees and variables evaluated on initial assessment (2017-2019).
- Appendix II.** 2019 disease progression survey data.
- Appendix III.** 2018 disease progression survey data.
- Appendix IV.** 2017 disease progression survey data.
- Appendix V.** Mid-crown canopy branch sampling data (2017-2019).
- Appendix VI.** 2017 light intensity data.
- Appendix VII.** Trunk debarking data (2017 and 2018).
- Appendix VIII.** 2017 cambium and bark moisture percentages.
- Appendix IX.** Summary figures of percent canopy green leaves (2017 to 2019).
- Appendix X.** Summary figures of percent canopy dead leaves (2017 to 2019).
- Appendix XI.** Summary figures of percent canopy dead branches (2017 to 2019).
- Appendix XII.** Summary figures of percent fungal staining from pole pruning (2018 to 2019).
- Appendix XIII.** Summary of high canopy sampling variables (2017 to 2019).

LIST OF ABBREVIATED TERMS

1. Dutch elm disease - DED
2. Native elm bark beetle – NEBB
3. Integrated pest management - IPM
4. Riverview and Lord Roberts Neighbourhood - RVLNR
5. Diameter at breast height - DBH
6. Percentage canopy green leaves (in analysis) - G
7. Percentage canopy dead leaves (“...”) - DL
8. Percentage canopy dead branches (“...”) - DB
9. Fungal staining from pole-pruning samples (“...”) - PP
10. Non-metric multidimensional scaling - NMDS
11. Analysis of similarities - ANOSIM
12. Generalized linear model - GLM

CHAPTER 1 – INTRODUCTION

Since its introduction into North America in 1928, Dutch Elm Disease (DED) has spread throughout the continent within the range of the American Elm (*Ulmus americana*, L.) (Brasier 1991). DED in the central and northern range of *U. americana* in North America is caused by the fungus *Ophiostoma novo-ulmi* Brasier and transmitted by the smaller European bark beetle *Scolytus multistriatus* (Marsham, 1802) and the Native elm bark beetle (NEBB) *Hylurgopinus rufipes* (Eichoff) (Pines and Westwood 2008). North American elms have little natural resistance to *O. novo-ulmi*, which results in high mortality rates in trees after inoculation (Gibbs 1978; Hubbes 1999). *Ulmus americana* comprises a significant percentage of planted trees in Winnipeg and has a considerably narrow genetic base making breeding resistant varieties difficult. This has resulted in substantial DED mortality in urban elm populations across North America (Lester and Smalley 1969; Santamour 1973; Townsend et al 1991). DED mortality is further exacerbated where disease management programs are poor or not implemented, resulting in up to 90% elm mortality within ten years of DED introduction (Ackerberg 1977; Westwood 1991).

If properly implemented a successful integrated pest management (IPM) program can maintain annual elm tree losses below 5% per annum and maintain tree replacement rates to minimize the impact of DED on the structure of the urban forest (Campana and Stipes 1981; Dreistadt et al. 1990; Westwood 1991). Following the introduction of DED to Manitoba in 1975, the disease spread throughout the province, reaching Saskatchewan in 1990. After introduction, Manitoba implemented aggressive IPM strategies along with provincial DED legislation to provide a comprehensive approach for protection of the province's elm populations in cities and towns. Currently, the province and City of Winnipeg attempt to maintain yearly losses of elm trees to below 2.5% per year through IPM, including a focus on removal of diseased trees and destruction of potential brood material (Westwood 1991; Rioux 2003). While IPM is necessary to control the spread of the disease, removal of DED-infected *U. americana* is one of the primary management methods in the province. Preliminary research indicates that a significant amount of NEBB-vectored spores of *O. novo-ulmi* could be contained within relatively few trees in city neighbourhoods. Prioritized removal of infected elm trees with high NEBB densities could

remove a large portion of the vector population before it spreads to healthy trees thus resulting in a potential decrease in DED spread (Holliday 2016).

The goal of the present study is to develop criteria to identify American elm trees with high densities of NEBB in the first summer of infection to facilitate their rapid removal prior to beetle dispersal in the fall. I accomplish this through a disease symptom progression survey and mid-crown canopy sampling for NEBB galleries that allows for the modelling of internal NEBB brood gallery densities with external tree variables. Study objectives are to:

- 1) Determine effective (and intuitive) sampling method(s) for identifying American elm trees with DED that harbour high NEBB brood gallery densities and translate results into an operational approach and survey for use by the City of Winnipeg Forestry department;
- 2) Investigate and model the relationship between various external, measurable DED symptoms in infected American elm trees and NEBB brood gallery density; and
- 3) Establish whether the capture of NEBB beetles dispersing to overwintering sites in late fall can act as a predictor of NEBB brood gallery density in DED-infected American elm trees.

The following hypotheses were tested in this study:

- 1) A combination of external disease symptoms will correlate with the internal NEBB brood gallery densities in DED-infected American elm trees;
- 2) DED-infected American elm trees will show more accelerated progression of disease symptoms in canopy assessments of trees with larger NEBB brood gallery density; and
- 3) DED-infected American elm trees with large NEBB brood galleries will show larger than average captures of NEBB adults dispersing to overwintering sites.

My Study is comprised of three parts:

- 1) Survey of diseased American elm trees throughout the summer after DED infection symptoms are detected;
- 2) Capture of NEBB adults dispersing to overwintering sites (after part 1); and
- 3) Assessment of the percentage of fungal staining and NEBB brood gallery density in mid-crown samples from trees surveyed in part 1 and 2.

I predict DED-infected elm trees with increased signs of canopy die-back and disease symptoms will harbour higher densities of NEBB brood galleries and that these high-density NEBB trees will show rapid canopy dieback when compared to low-density NEBB brood gallery trees. Also, it is expected that high-density NEBB brood gallery containing elm trees will have higher rates of NEBB adults captured nearby when compared to infected trees with few brood galleries.

Once the canopy variables with the greatest potential to predict NEBB brood gallery densities are identified, a protocol will be developed to incorporate high NEBB-density trees into the City of Winnipeg's summer survey protocol. These trees will be given priority for removal before NEBB adults disperse to overwintering sites in late fall.

CHAPTER 2 – LITERATURE REVIEW

***Ophiostoma* and Dutch elm disease**

Dutch elm disease (DED) is a fungal wilt disease of elm trees (*Ulmus* spp.), caused by three fungal species in the Genus *Ophiostoma*. In Canada the primary fungal strain is *Ophiostoma novo-ulmi* Brasier which is spread mostly by the Native elm bark beetle (*Hylurgopinus rufipes* Eichoff, 1868.) (NEBB) (Pines and Westwood 2008). In Manitoba disease symptoms in American elm normally occur in early to mid-summer inducing sudden leaf wilting on one or more branches. Leaves turn yellow and eventually turn brown and fall prematurely. In late summer infections, leaves shrivel and turn brown but may persist through winter (Hildahl 1977). Internally the disease presents as long, dark, discontinuous streaks of mycelia and staining on the outer sapwood (Hildahl 1977).

Once the fungus is introduced into the tree, it spreads through the cells of the phloem and xylem, surrounding them and inhibiting water and nutrient transport (Hiratsuka 1987). *O. novo-ulmi* spreads in pupal chambers in the phloem tissue (Webber 2004). The speed of fungal spread throughout the tree is influenced by tree health, the virulence of the fungal strain, and the time of year of infection. Elms succumb to the fungus readily when under environmental stress (Hubbes and Jeng 1981; Hubbes 1988), but healthy, vigorous trees may be able to withstand infection for several years (Hildahl and Jeffrey 1980; Stipes and Campana 1981). Disease symptoms in infected elm trees are the product of water deprivation and decreased water transport (Hall and MacHardy 1981; van Alfen 1989). Toxins produced by *O. novo-ulmi* (chiefly cerato-ulmin) have also been proposed to cause DED disease symptoms in infected elm trees (Takai 1974; Sticklen 1991).

Ophiostoma ulmi (Buisman) caused the first Dutch elm disease pandemic, originating in Europe which quickly spread to North America, while *Ophiostoma novo-ulmi* is responsible for the current pandemic sweeping through North America (Brasier 1991). *Ophiostoma himal-ulmi* Brasier and Mehrotra 1995 is endemic to the western Himalaya region and occurs in elm trees with natural resistance to the disease (Brasier and Mehrotra 1995). These three fungal species make up the suite of DED causing fungi (Harrington et al. 2001).

Ophiostoma novo-ulmi is distinguished from *O. ulmi* through several definitive factors. Firstly, *O. novo-ulmi* has increased aggressiveness and pathogenicity traits in comparison to *O. ulmi*. They also differ from one another through colony morphology, growth rate, optimal growth temperature, mating frequency and both mitochondrial and nuclear DNA characteristics. Strong unidirectional fertility barriers are present between the two species (Brasier et al. 1981; Brasier 1991). Two different biotypes of *O. novo-ulmi* have been described, informally referred to as the Eurasian (EAN) and North America (NAN) races (Brasier 1979; Brasier 1991). Both races appear to be associated with and originated from different locations: Romania-Moldova-Ukraine for EAN and the southern Great Lakes in North America for NAN.

Colonies of EAN and NAN differ in their growth rates and morphology (Brasier et al. 1981). EAN further alternates between two colony morphologies with the dimorphism being absent in NAN (Brasier 1991). EAN exhibits a partial reproductive barrier against NAN, when EAN is the recipient and NAN the donor, growth is reduced by 90% (Brasier and Kirk 2010). The outbreak of *O. novo-ulmi* and its high virulence in North America demonstrates the rapid evolution of a pathogen after its introduction outside of its natural distribution (Brasier 2001). Recent research on the two races indicates the need to separate them into distinct subspecies (Brasier and Kirk 2010). *Ophiostoma novo-ulmi*'s hyper-virulence in comparison to *O. ulmi* is associated with a double-stranded RNA isolate Sh12B (Deng et al. 2003).

Bark beetles associated with *Ophiostoma*

Ophiostoma has a demonstrated ability to be carried by many beetles across many genera (Webber 2000). Many bark beetles are able to transmit *Ophiostoma* spores to elm, with the NEBB being the primary vector in the northern areas of DED incidence in North America (Swedenborg et al. 1988). Species of *Scolytus*, including *Scolytus scolytus*, the larger European bark beetle, (Fabricius 1775), *Scolytus multistriatus*, European elm bark beetle, (Marshall, 1812), and *Scolytus kirschi* (Skalitzky, 1876) are consistently associated with DED and are considered effective vectors of *Ophiostoma*. Of these *Scolytus* species, *S. multistriatus* and *S. scolytus* are the primary vector for *O. novo-ulmi* (Webber 1990) in Europe, with *S. multistriatus* being introduced into North America. *S. multistriatus* is less cold tolerant than NEBB and less common northward in North America. All three of the aforementioned species are present

throughout Europe with *S. multistriatus* also found in parts of North America (Faccoli and Battisti 1997). Many more bark beetle species are known to carry *Ophiostoma*. Thirteen other species of bark beetle endemic to Spain (Romón et al. 2007) and two species in Chile (Zhou et al. 2004) have been recorded carrying *Ophiostoma*.

Adult NEBB mate and feed in twig crotches in the canopy during the spring. After mating, female beetles construct brood galleries within the cambium, laying eggs in the galleries. Often brood galleries are formed in larger diameter branches of dying, diseased trees (Katson 1939; Whitten 1964; Thompson and Matthyse 1972; Lanier 1982; Pines and Westwood 1996; Swedenborg et al. 1998;). Brood galleries can be constructed in both the canopy and trunk. The larvae then feed and further develop in the galleries, eventually pupating within individual chambers (Hiratsuka 1987). Later in the summer newly emerged adults move to new trees to feed. If the host tree were infected with *O. novo-ulmi* NEBB will carry fungal spores upon emergence from brood galleries (Kondo et al. 1981). Adults will enter the tree canopy after emergence, and in the late fall move to the base of a new healthy tree or in some cases a still living infected tree to overwinter (Strobel and Lanier 1981; Anderson and Holliday 2003). After emerging from overwintering at the tree base adults will carry *Ophiostoma* spores, and during feeding the inoculum can be introduced into the xylem of healthy elms (Gardiner 1981). Inoculation by pathogenic fungi after exiting from bore holes is consistent for many beetle-fungi complexes (Molnar 1964). Increased pathogenicity of *O. novo-ulmi* may be associated with two phoretic mite species on NEBB that carry hyperphoretic fungal spores (Moser et al 2010).

The proportion of beetles in a vector population that bear spores on exit from a DED-infected elm tree varies between species. Fifty-eight percent of *S. multistriatus* and *S. pygmaeus* adults emerging from logs artificially loaded with *O. ulmi* spores carried DED spores, and there were no differences between species or sexes in those proportions (Faccoli and Battisti 1997). Webber (1990) found 6% of *Scolytus kirschii* Skalitzky carry *O. ulmi* spores, 64% of *S. multistriatus* carry *O. ulmi* spores, and 98% of *S. scolytus* carry *O. ulmi* spores. Beetles collected in summer had a significantly lower percentage (9.8%) of spores, thought to be due to higher temperature (Faccoli and Battisti 1997). Temperature is often a significant factor in fungal transmission (Six and Bentz 2007). *O. novo-ulmi* has an optimal growth temperature of 20-22 °C and upper limit at 33°C (Brasier et al. 1981). Oghiakhe and Holliday (2011) reported between 37.6% and 47.4% of *H. rufipes* captured carried *O. novo-ulmi* spores.

Attraction of vectors by *Ophiostoma*

Once an elm is infected with *O. novo-ulmi* the tree is metabolically manipulated by the fungus to increase production of semiochemicals, which enhance the trees' chemical attractiveness to NEBB aiding in inoculum dispersal (Pines and Westwood 2008). Mechanisms by which NEBBs are attracted to elms are not fully understood. Both pheromones and host volatiles (Gardiner 1979; Peacock 1979; Miller et al 1986) have been suggested as attractant mechanisms. Terpenes released from American elms have been shown to attract NEBB (Miller et al. 1986). Further study has indicated that four semiochemicals (one monoterpene and three sesquiterpenes) synergistically attract NEBB. Sesquiterpenes are up-regulated by *O. novo-ulmi* to attract NEBB (McLeod et al. 2005).

In response to beetle attack, elms may form necrotic lesions around infections and increase concentrations of allelochemicals with fungicidal properties. By modifying the chemicals released by these lesions *O. novo-ulmi* can increase vector attraction and spore dispersal by upregulating semiochemicals attractive to NEBB (Raffa 1988). Artificial compound mixtures of sesquiterpenes based on solvent extracts from elm trees have shown to be attractive to NEBB. However, no field-tested bait has proven as attractive as diseased elm tree controls (Miller et al. 1986).

Male adult NEBB make “chirps” similar to *Dendroctonus* species (Ryker and Rudinsky 1976). Since NEBB do not aggregate to overwhelm living trees, chemo-acoustic behaviour is used by males to locate suitable females, and the males will respond to host volatiles and short-range pheromones to locate gallery entrances. The stridulation of male NEBBs is also used in competition between males (Swedenborg et al. 1989).

Elm tree resistance

There are approximately 45 species of elm trees (*Ulmus*), six of which are found in North America (Brasier 2001). While the *Ulmus* genus is well defined, species delineations are a subject of controversy (Weigrefe 1994). The American elm (*Ulmus americana*) is the most susceptible to *Ophiostoma* infection, though all other elms endemic to North America (*Ulmus*

rubra Muhl., *Ulmus thomasii* Sarg., *Ulmus alata* Michx., *Ulmus serotina* Sarg., and *Ulmus crassifolia* Nutt. show various degrees of susceptibility (Hubbes 1999). Native North American species also consistently demonstrate less resistance than Asiatic elms (Gibbs 1978).

Left untreated, almost all *U. americana* that come into contact with *Ophiostoma* die within a few weeks to a few years. The susceptibility of elm stems from a lack of genetic resistance to a foreign pathogen. Elms native to the traditional distribution of *Ophiostoma* (i.e. Asiatic species) have significant genetic resistance due to co-evolution (Newhouse et al. 2007). Many resistant elm cultivars have been described (Townsend et al 1991). Research into elm resistance involves breeding from trees with measurable levels of resistance (Lester and Smalley 1969), introducing resistance genes into *U. americana* (Santamour 1973), and identifying genes involved in susceptibility and resistance (Redenbaugh et al 1981). Chinese elm (*Ulmus parvifolia* Jacq.) exhibits high DED resistance and has been hybridized with *U. americana*, creating progeny with comparably higher levels of resistance (Smalley and Guries 1993). Siberian elm (*Ulmus pumila*) is tolerant to DED outbreaks and was planted in Europe and the United States to replace native elms (Goidanich 1936; Smalley and Guries 1993; Santini et al. 2005; Zalapa et al 2010). Induced resistance has been explored by inoculating trees with strains of *O. ulmi* (or glycoprotein isolated from pathogen) (Hubbes and Jeng 1981; Hubbes 1993; Hubbes 2004). Induction of resistance showed promise against *O. ulmi*, but not *O. novo-ulmi*, and susceptible elm trees could not be sufficiently protected (Scheffer et al. 2008).

Verticillium isolates proved to effectively suppress DED in susceptible trees but would require pre-emptive inoculation (Scheffer 1990). Sola and Gol (2003) showed significant decrease in wilting following treatment with *Verticillium dahliae* Kleb (1913). Protection was only successful when *V. dahliae* was inoculated 15-30 days prior to tree infection with *O. novo-ulmi*. No protection was provided if inoculated with *V. dahliae* 45 days after DED infection. Sola and Gol (2003) showed *verticillium* is not an effective method of DED control in practice from either a economic or labour perspective.

Historical spread of Dutch elm disease

The historical spread of the DED epidemic was described by Gibbs (1978). Dutch elm disease first appeared in northwest Europe towards the end of World War 1. The disease was

recorded in France, Holland, Belgium, and Germany between 1918 and 1921, and later recorded in Britain in 1927. The first epidemic of Dutch elm disease is attributed to the spread of *O. ulmi*. Staining resembling *O. ulmi* inoculation was recorded as early as 1912 (Peace 1957), with unreliable reports citing fungal staining as early as 1900 (Liese 1940). Review of early reports by Spierenburg (1972) and Heybroek (1931) refer to the large elm bark beetle (*S. scolytus*) attacking stressed elms in Holland. The disease is thought to have originated in Asia, then spread through infected wood by ship to Europe (Holland) and eventually North America. Dutch researchers initially attempted control of DED in 1930 by removing 421,000 infected trees over 13 years until 1943 and implementing active disease management efforts to slow disease incidence rate (Went 1978). Coastal elms in the Netherlands were seemingly protected from the disease due to harsh coastal winds reducing tree growth (influencing vessel size) or affecting flight of vector beetle populations.

There is no evidence that the UK initiated coordinated efforts to control DED after it entered England. During the initial introduction Peace (1957) documented the disease progression from 1928 to 1955 in England, where the disease incidence was less severe than the rest of continental Europe. This was at the time explained by a cooler and humid climate along with an apparent tolerance of the fungus by elms planted in Britain (Peace 1957).

Dutch elm disease spread further eastward throughout Europe, reaching Italy and Czechoslovakia around 1930. The disease reached Poland and Ukraine by the 1940's causing severe losses in elm populations (Manka 1941). By 1960 the disease had spread through most of Europe except where cold climates limited disease spread. DED reached North America in the late 1920's and despite comprehensive sanitation efforts in New York City and the surrounding areas upon detection, the disease soon impacted 8850 square kilometres with localized incidences throughout eastern America after initial introduction (Hubbes 1999).

Spread in large urban centers like New York City was attributed to large breeding populations of the *S. multistriatus* and *H. rufipes*. (May 1934). *Scolytus* vectors of DED were reported in America as early as 1909 (Chapman 1910) with large number of *S. multistriatus* and *S. scolytus* discovered in shipments of elm logs from France in 1933 (Blackman 1934). The spread of the disease takes longer in colder climates in comparison to more southern areas within the geographical distribution of DED. In North America the disease reached the west coast

(Oregon) by 1973. In the 50 years after its introduction into North America the disease is estimated to have killed 50 to 100 million elms (Ackerberg 1977).

While initial spread and infection rates appeared lower than in Europe in the 1960's there was an increase in DED and widespread death of elms in England (Gibbs and Redfern 1977). This new increased incidence of DED was attributed to an “aggressive” strain of *Ophiostoma*, *O. novo-ulmi*. By 1976, the disease was wide spread throughout the British Isles (Gibbs 1978). The increase in disease rates in the UK directly coincided with the occurrence of the new fungal strain throughout Europe at this time. All cases of *O. novo-ulmi* are associated with significantly higher mortality than is the case with *O. ulmi* infections. European elm varieties that previously showed some resistance to *O. ulmi* had very little resistance to *O. novo-ulmi* (Martín et al. 2009). The new epidemic of *O. novo-ulmi* caused massive elm losses throughout unmanaged populations throughout North America (Sinclair 1978).

Modelling of the local spread of DED has been recently completed by Bajoux et al. (2020). When modelling Dutch elm disease spread it was found that significant differences occur between boulevard and park trees in regard to the spread rates, where focus on root spread of DED becomes much more important due to increased root connections in a park. It was found that topography of individual neighbourhoods had no role in DED spread. Neighbourhoods with increased root connections due to either the presence of parks or large densities of elm trees are at increased risk of DED spread. Harwood et al. (2011) also developed a DED spread model in England, however they did not account for infection spread through roots and did not take into account beetle dynamics and movements.

In managed urban forests the disease costs millions per year to keep disease incidence manageable. For example, the state of Minnesota spent \$105 million US over 6 years (1961-1967), losing 80, 000 trees, and the city of Minneapolis spent \$30 million US over a four-year period after disease discovery in 1965. When DED reached Manitoba in 1975, the first evidence of the disease was found in the towns of Selkirk and Brandon and the City of Winnipeg. From 1975 to 1980 the disease steadily spread throughout the south of the province, and throughout the 1980's the disease spread northward. By 1990, DED had reached southeastern Saskatchewan (Westwood 1991). The current distribution of DED has remained relatively stable in western Canada with no new infections reported further west of Saskatchewan (Rioux 2003).

Integrated pest management of Dutch elm disease

Control of DED is an expensive process due to the complex relationship between pathogen, host, vector, and the environment. Successful integrated pest management (IPM) of DED is based on reducing the probability of occurrence of new disease infections (Dreistadt et al. 1990). Integrated pest management has been used to manage DED (with varying success) in North America and Europe (Scheffer et al. 2008; Hintz et al. 2013). Due to the lethal nature of *O. novo-ulmi* infections, IPM efforts to control DED must focus primarily on reducing the probability of infection (Gibbs 1978). There are many facets to a successful IPM program for DED management. Sanitation involves the elimination of vector populations, through poisoning, trapping with attractants, enhancing biological enemies, and sanitation cutting of trees that could provide potential habitat (Westwood 1991). Successful sanitation requires the destruction of all potential brood habitat but is not usually possible due to the cost and sheer volume of infected wood that may have to be destroyed (Campana and Stipes 1981). Rather, Dutch elm disease must be controlled, instead of eradicated (Ganley and Bulman 2016).

Control of the vector population is an effective way to manage DED spread. However, population eradication is not feasible due to the large numbers of NEBB occupying areas with high numbers of elm (Lanier and Epstein 1978; Ganley and Bulman 2016). Insecticide spraying can prevent beetle feeding and breeding, while injection of insecticide into healthy tree conductive tissue provides partial control of NEBB populations (Campana and Stipes 1981; Doccola and Wild 2012), albeit with limited success (Scheffer et al. 1988).

Other control methods include severing root grafts between adjacent trees to prevent fungal spread, chemical injections of fungicides to increase tree resistance, and increased propagation of resistant elm species. Fungicides are an expensive control method that is usually only targeted at high value elm trees, especially since fungicide treatments do not protect against infections through root grafts (Scheffer et al. 2008). Attempts to control bark beetle populations through capture programs have not been successful after their implementation, even in cases of large capture of *S. multistriatus*, DED incidence was not significantly affected (Peacock 1984). Limited physical, human, and economic resources often make successful IPM of Dutch elm disease a challenging proposition. One of the advantages of a successful IPM program is that it

allows for preservation of the elm population over a longer time period thus spreading the cost of the IPM activities over multiple years (Westwood 1991). Prior to the disease arriving in an urban center implementation of tree maintenance and sanitation is important to ensure elms are as healthy as possible, and to decrease potential beetle breeding sites by removing dying or decadent trees and keeping the presence of dead branches in living trees to a minimum. DED management can be supplemented with bans on the movement of elm firewood from infected to non-infected areas (Hildahl 1977).

Integrated pest management is vital to controlling the spread and damage of DED, and shortfalls in management, such as slow response to infected trees and the lack of national scale management strategies, especially in Europe, have contributed to DED pandemic severity (Tomlinson and Potter 2010). Relaxation of DED surveillance and control resulted in DED-killed elm trees increasing from 6% to 62% over a five-year period of the total elm population (Gibbs 1978). IPM in the City of Hamburg, Germany has kept reported elm losses to less than 1% (Scheffer et al 2008).

Auckland, New Zealand provides an important case-study in DED management that can be compared to DED management in Winnipeg. Auckland has a population of 15,000 elm trees (Ganley and Bulman 2016). DED was initially discovered in 1989 (Bain 1991) due to a single introduction event from western Europe (Gadgil et al. 2000), and an eradication program was implemented. The program involved locating all elms in the infection area during beetle flight season (Ganley and Bulman 2016). Pheromone traps were used to monitor *S. multistriatus*, the only known DED vector present in New Zealand (Gadgil et al 2000). The cost of the program totalled NZD 4 million, and it resulted in a steady decline of DED incidence. However, an evaluation in 2004 showed that DED could only effectively be contained (not eradicated) and effective management required with more funding (Ganley and Bulman 2016). The program was stopped in 2008 and responsibility transferred to local municipalities rather than national or provincial agencies (Ministry for Primary Industries, 2008) however the case study proves management and control of DED using IPM can be effective at slowing the rate of tree loss for as long as appropriate resources are provided. Management of DED in New Zealand reportedly saved \$129 million in management costs and tree values compared to no management program (Ganley and Bulman 2016).

Integrated pest management in Manitoba

Before the arrival of DED in 1975, the objectives of elm management in Winnipeg were to maintain healthy elms and plant alternative tree species (Westwood 1991). After DED's arrival, a province wide program was implemented to aggressively control the disease (Hildahl 1977). The primary component to manage DED in its initial stages is the development of a site-specific inventory of elm trees in control areas, implementation of an elm sanitation program to remove infected and hazard elms (elm with significant deadwood or in an unhealthy condition), and the prompt removal and disposal of infected trees to prevent further disease spread.

In Manitoba, basal trunk insecticide spraying of elms to kill overwintering NEBB is used to control beetle populations. Fungicide tree injections are used as a prophylactic treatment to protect high value elms before they are infected. In 1981 the Province of Manitoba passed the Dutch Elm Disease Act regulating disease management and making implementation more effective (Westwood 1991). Cost sharing agreements between the province and communities with elm populations assisted in the management of DED, helped spread the economic cost of disease progression more evenly, and resulted in commitment from all government levels to manage DED in the province.

A cost analysis by Westwood (1991) showed that annual expenditures of \$2.06 million CDN throughout Manitoba kept elm losses to approximately 2.4% per year. Westwood (1991) estimated that even a marginal increase in the annual loss rate could increase the costs of elm removal dramatically, increase tree-replacement costs, result in declining ecological services and real-estate values in the affected areas. Overall, Westwood (1991) noted that an expenditure of \$10 million CDN by the province of Manitoba between 1981 and 1991 (plus the equivalent expenditures by over 50 towns and cities within the cost shared program) conserved CDN 276 million worth of elm trees. Furthermore, implementation of provincial buffer zones around selected communities markedly reduced rates of DED spread.

Importance of managing Dutch elm disease

When discussing management of a disease in the urban forest it is important to discuss the benefits and importance of managing and maintaining urban forests. Comprehensive assessments of the value of the urban forest are provided by Novak and Dwyer (2010). The value

of urban forests increases with the growth of urban landscapes. Urban forests, when properly managed, reduce negative environmental consequences of urban development by moderating climate, reducing energy use, absorbing and sequestering atmospheric carbon dioxide, reducing run-off and flooding, decreasing noise pollution, and increasing air quality (Schroeder 1989; Schroeder 2004).

Urban forests benefit citizens by providing social and economic benefits. Urban forests increase aesthetic quality (Schroeder 1989), improve mood, and provide opportunity for community engagement (, Dwyer et al. 1991; Westphal 1993; Schroeder 2004). Increases in urban forest cover save annual heating and cooling costs due to increased shade, lower ambient temperatures, and reduced wind speed (McPherson et al. 1997). Various studies have further reported the positive benefits on urban forests on mood, stress, general psychological benefits (Kaplan and Kaplan 1989; Sullivan 2001; Hammitt 2002) and decrease in health problems from ultraviolet radiation (Heisler et al. 1995). Lastly real estate values typically reflect positively in the presence of an urban forest, with increased sale prices due to landscaping and trees (Anderson and Cordell 1988; Novak and Dwyer 2010).

Urban forests are a valuable structural asset that often requires significant economic investment to maintain ecosystem services and societal benefits (Nowak et al. 2002, McPherson 2000, McPherson et al. 2005, Berland and Elliott 2014). While managing for DED it is important to manage other tree pests to help maintain over all urban forest resiliency as the removal of infected elm trees and replacement with other species as well as some elm can increase urban forest diversity (Miller 1997; Berland and Elliott 2014). Investing in urban forest protection further protects areas of high biodiversity in cities with little natural habitat (Alvey 2006).

Species diversification has been proposed as a method to provide long-term pest resistance to the urban forest (Barker 1975; Grey and Deneke 1986). Santamour (1990) proposed a 10/20/30 rule, which suggests that urban forest trees should be composed of no more than 10% of a given species, 20% of a single genus, and 30% from a specific family. Diversification efforts are sometimes challenging if there are few alternate tree species suited to an urban environment in some cities, especially in northern cities and if local tree nurseries carry a limited range of species (Richards 1983; Sydnor et al. 2010). New residential areas planted post-DED have emphasized diversification of the urban forest, but cities with low urban tree diversity are at risk

of further declines in species diversity due to pest species like the Emerald Ash Borer (*Agilus planipennis* (Fairmaire)) (Berland and Elliot 2014).

Prior research by Holliday (2016)

Recent research by Holliday (2016) has shown in several trials that although many symptomatic trees contain relatively low numbers of NEBB, a relatively small subset of infected elm trees may harbour much larger numbers of beetles. This small subset of DED-infected trees may produce most of the disease-spreading beetles, and if these trees were to be removed in a timely manner before the adult beetles emerge, the spread of DED could be mitigated. This finding prompted the current study to examine a large set of elm trees of greater DBH and located in a large urban neighbourhood setting.

Holliday (2016) suggested that external disease symptoms could be directly correlated with beetle density. Along with staff from the Provincial Forestry Branch, he examined the number of NEBB brood galleries in trunks and branches of DED-infected trees and related these to tree condition. The sample size where actual breeding beetle numbers were estimated was insufficient to strongly predict which trees had the potential to produce high beetle numbers although Holliday (2016) suggested that if trees carrying high NEBB densities could be identified in summer and promptly removed prior to beetles leaving the trees in the fall, there was potential to reduce disease spread.

Holliday (2016) further reported density of NEBB galleries in the branch samples were not correlated with branch DBH but that sampling at least two major branches could be representative of the overall NEBB gallery density throughout the canopy. It was concluded that results from branch samples are a reliable indicator of the density of NEBB galleries within the entire canopy. Of the 60 trees sampled, Holliday (2016) showed that 13% contained the majority of the NEBB galleries. He concluded that gallery densities of 24 m^2 (0.24 cm^2) or more could be used as a target level to identify trees for rapid removal.

Holliday (2016) found that trees with high densities of galleries in the canopy generally (but not always) had evidence of galleries in the trunk but a number of trees with moderate numbers of galleries in the crown showed no evidence of trunk galleries. Holliday (2016) suggested that more samples were required to establish a stronger link between number of

beetles in the trunk and overall beetle population in the tree, given that a limited number of trees in the study had trunk galleries while the majority had branch galleries. It is worth noting that the experiments were carried out using generally smaller diameter trees within stands of elms in park settings and on residential streets, thus some of the trees sampled were less representative of many older Winnipeg neighbourhoods which contain only large-diameter street elms.

The research by Holliday (2016) provided a starting point for my project and the objectives of my study build off the findings presented by Holliday (2016). In the project I will assess and contrast my findings with those reported by Holliday (2016), including the efficacy of trunk debarking as a NEBB gallery density predictor and the skew of NEBB galleries in a small subset of DED-infected trees.

CHAPTER 3 – METHODS

General Methods

I investigated the link between the appearance and progression of DED symptoms with NEBB brood gallery density in urban elm trees in the City of Winnipeg, Manitoba over a three-year period (2017 to 2019). Field work took place between May and September in each year. Four Winnipeg neighbourhoods were selected for the study (Figure 1). The Wolsey neighbourhood was selected to implement a pilot study in 2017. Some trees were sampled in Crescentwood in 2017 however they were not fully assessed. The study was then expanded to the Lord Roberts, Riverview, and Minto neighbourhoods in 2018 with the Sergeant Park neighbourhood added in 2019. Each neighbourhood was selected based on having a large number of accessible American elm trees located on city boulevards, ease of access to each tree for City of Winnipeg and University staff, and high proportions of the elm population being infected with DED. Study neighbourhoods were restricted to the above four due to the scope and labour available for the project.

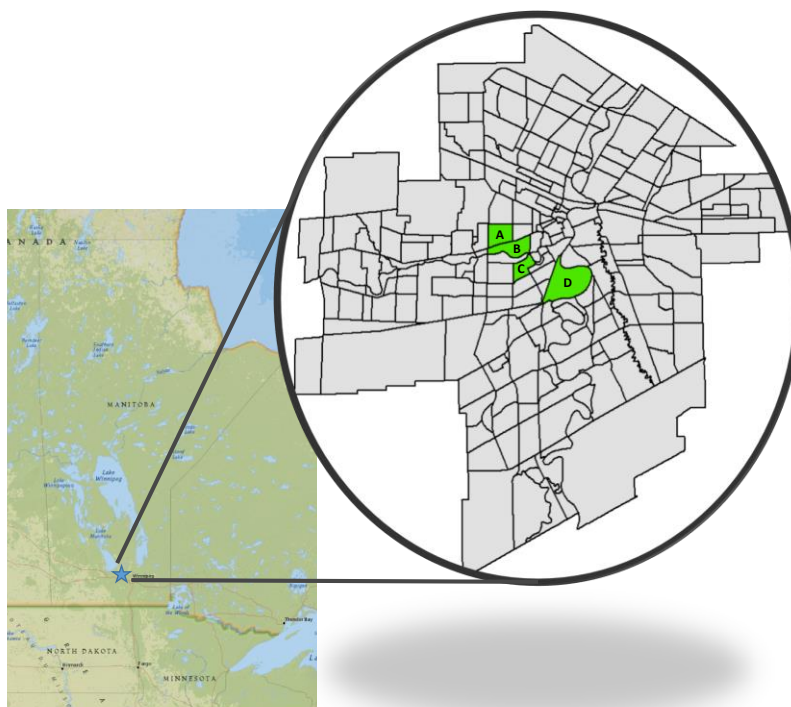


Figure 1. Study neighbourhoods in Winnipeg, MB, included in study (2017-2019). A: Minto, B: Wolsey, C: Crescentwood (2017), and D: Riverview and Lord Roberts.

Each year, DED-infected elm trees were examined for the appearance and progression of disease symptoms beginning in early summer. Following the survey for disease appearance and progression, DED-infected trees were removed by City of Winnipeg Forestry staff or tree removal contractors. For a portion of the trees monitored and removed in the disease progression survey, branches were removed from the mid-crown and examined to estimate the density of NEBB brood galleries. Infected trees in the survey were subsequently removed prior to the following field season and all study trees were identified with a unique identifier (supplied by the City of Winnipeg) including the year they were included in the study. Some survey methods in the study differed between years due to progression from a pilot study to a larger comprehensive survey. Survey methods varied slightly between years (2017 pilot study, and 2018-2019 comprehensive survey). Criteria and survey methods that were modified or improved are summarized in (Table 1).

Table 1. Survey criteria for each component of the of the disease progression survey over three years, showing the year each variable was recorded.

Experiment	Recorded Variable	Study Year		
		2017	2018	2019
Disease progression survey	Canopy Cover	Y	Y	Y
	Percent canopy green leaves	Y	Y	Y
	Percent canopy dead branches	Y	Y	Y
	Percent canopy dead leaves	N	Y	Y
	Pole pruning: % Fungal Staining	N	Y	Y
	Cambium moisture	Y	N	N
	Light intensity	Y	N	N
Trunk Debarking	Infection Sites	N	Y	Y
	Fungal Staining and NEBB brood gallery density	Y	2017 samples	N
	High Canopy Debarking	Y	Y	Y
Beetle capture survey	NEBB beetle capture	N	Y	Y

The methods are divided into four components. First, the disease progression survey was used to assess the appearance and progression of external symptoms in DED-infected elm trees in all three years of the study. Second, trunk debarking of infected elm trees was carried out to determine the presence of NEBB brood galleries and percentage fungal staining in 2017. Third, the mid-crown branches from infected trees were removed and sampled for the presence of NEBB brood galleries and percentage fungal staining in all three years of the study. The fourth

component involved placing sticky traps on DED-infected elm trees to determine whether capture rates of dispersing NEBB in the late fall could also be used as a suitable indicator of NEBB density in nearby elm trees. This was subsequently followed by determining the DED infection rate of dispersing beetles.

Disease progression survey

The disease survey began between early to mid-June and early July to late August in each year of the study when newly DED-infected elm trees become symptomatic and were identified and tagged by City of Winnipeg staff. Once City of Winnipeg DED surveillance staff located an infected elm tree it was given a unique code number and address. The code numbers of the infected elm trees examined in this study are shown in Appendix I, and the disease survey data sets are shown in Appendices II-IV. Once the identities and locations of the infected elm trees were received, I implemented the survey to assess the progression of various external variables exhibited by DED-infected trees throughout the remaining part of the summer. Trees were initially assessed in early to mid-June. Trees were surveyed until DED disease symptoms stabilized in late fall (Strobel and Lanier 1981; Swedenborg 1988). Physical features such as diameter at breast height (DBH) and tree height were recorded on initial visit.

The tree canopy survey of infected elms in 2017, 2018 and 2019 included an assessment of leaf condition (a critical component of disease detection surveys) including estimates of the percentage of green leaves, dead leaves, and dead branches in the tree canopy. Assessing the external canopy features allowed for comparison of external disease progression with the internal NEBB gallery densities. For the purpose of this study dead branches included both defoliated branches and dead wood. In study neighbourhoods, tree defoliation due to feeding caterpillars was minimal during the study period. The evaluations of canopy condition in each tree were made by two or three independent observers and then averaged. Canopy conditions were estimated by the surveyor while observing the canopy from various directions and angles to ensure the entire canopy was included. In 2017 and 2018 the percentage of yellow leaves in the canopy was recorded. Analysis of the 2017/2018 yellow leaf data set showed there was no significant change in the percentage of yellow leaves over the summer among the study trees, and for this reason, it was grouped together with percentage dead leaves in 2019. Diameter at

breast height (DBH, centimetres) and tree height (metres) were recorded on initial assessment of each study tree. DBH was recorded using a DBH tape, completed at breast height, and height was recorded with a clinometer (Suunto PM-5/360).

Each tree was surveyed three to six times (approximately 7 to 10 days apart) during the DED symptom assessment period through the summer to document the progression of external DED symptoms and to estimate the rate of tree decline. All canopy variables were measured each time the tree was assessed (excluding infection sites). Variability in survey weeks were due to variance of initial identification of DED, and tree access.

Canopy cover was estimated using a spherical densiometer (Forestry Suppliers – Concave Model A). A densiometer reading was taken at the four cardinal compass directions around the trunk at a distance of 1 m from the trunk. To determine canopy cover, the number of closed quadrant (presence of tree canopy detected) within each canopy square was counted using the densiometer. The densiometer had 24 canopy squares for a maximum recorded value of 96. The recorded value between 0 and 96 was converted to a percentage, thus a measurement of 96 would translate into 100% canopy cover at that cardinal direction. The four directions converted to canopy cover were then averaged to give the overall canopy cover. For example, an assessment value of 100% canopy cover would mean all the countable quadrants in each square on the densiometer were occupied by the canopy of the tree. An assessment of 0% canopy cover would indicate none of the countable quadrants being occupied by tree canopy. Densiometer measurements have been reported as relative to light intensity suggesting a good inexpensive method to assess canopy cover (Stickler 1959; Baudry et al. 2014; Werner 2019) and argued as equivalent to smartphone-based hemispherical photography and direct measurements of solar radiation (Russavage et al. 2021). Various studies have conversely deemed densiometers inconsistent and affected by user bias (Cook et al. 1995; Nuttle 1997, Vora 1998; Prasad et al. 2018). The use of densiometer measured canopy cover is further covered in Chapter 5.

In the 2017 pilot study, light intensity was recorded at two metres above the ground in the same locations that canopy cover was recorded (Appendix VI). Light intensity readings were recorded with a light meter (General Electric Company, USA) in lumens per meter squared (Lux). Preliminary analysis of the 2017 light intensity data showed no significant trends or correlations with DED symptoms between study trees and was discontinued in 2018. Light

intensity was recorded in the same manner as tree percentage canopy cover. Estimates of light intensity were recorded using a light meter in the four cardinal directions at 1 m from the tree trunk. Data for light intensity were recorded in Lux (lumens per square metre).

Beginning in 2018 and continuing into 2019, the number of initial DED infection sites in the tree crown were recorded on the first date of the DED symptom survey. DED spores are carried from infected elm trees by NEBB when the beetles emerge from overwintering sites (Kondo et al 1981). DED is then inoculated into healthy elm trees when NEBB feed and begin creating brood galleries after emerging from overwintering sites (Gardiner 1981). This leads to the first disease symptoms often being localized to the region where female NEBB enter under the bark. Often these first attack sites appear to be localized (if observed near the beginning of infection when first leaf symptoms occur) and a surveyor can determine the location and number of major limbs where the disease was first introduced into the tree (Solla and Gil 2002). Once the fungus starts growing through the tree and infection sites merge, then determining potential initial infection sites is not possible. Estimating the initial number of localized clusters of disease symptoms allows for an approximate estimate of the number of infection sites resulting from beetle attack at the beginning of the infection period (Strobel and Lanier 1981). Infection sites were only recorded once at the beginning of the summer survey.

In 2018 and 2019, the percentage of fungal staining in the sapwood was estimated from lower canopy branches via weekly pole pruning samples. Removed twigs had bark peeled back and the percentage of the sample containing DED fungal staining estimated. A minimum of four samples were taken at each tree and DED fungal staining was averaged across all samples.

Trunk debarking

To detect NEBB in elm trunks Holliday (2016) recommended that the entire circumference of the lower trunk could be sampled and that a bark removal sample with a vertical length of 50 cm should be sufficient to determine the presence of NEBB galleries. This would avoid sampling from the canopy of mature elm trees that requires the use of heavy equipment (aerial bucket truck and crew), thus is both expensive and time consuming. Holliday (2016) suggested that trunk galleries could be used as estimators of crown galleries present in the rest of the tree. This would allow surveyors to use less expensive trunk debarking to estimate

galleries in the crown of the tree where the majority of the NEBB brood galleries are present. During the pilot study in 2017, a section of trunk bark from infected trees was girdled to assess the density of NEBB brood galleries and percentage of fungus infection in August and early September following the protocol reported by Holliday (2016) and the Provincial Forestry Branch (Figure 2). Trunk debarking occurred after infected study trees had at least three DED symptom assessments since the date of first diagnosis. In spring 2018, a few elm trees infected with DED in 2017 that were left standing (not yet removed by sanitation crews) also had the trunk bark removed. The purpose of this was to see whether trees left standing in the fall and overwinter would have more evidence of NEBB brood galleries than trees that had the bark removed in the previous summer. These trees could potentially have increased numbers of brood galleries and the spring bark removal samples were intended to confirm whether brood galleries in the trunk were more evident in the spring one year after infection versus trunk bark removal in the previous August. These trees were recorded as having full canopy dieback or were tagged as dead/hazard trees in the 2017 DED progress survey assessment.



Figure 2. Cambium plug (2017) taken to measure the moisture content after trunk debarking is completed. Cambium sample taken above trunk debarking ring. Tree sampled is DED-infected American elm tree in Winnipeg.

A standard height of one meter from the base of the tree was chosen for the bottom edge of the trunk bark sample, and an area (~60 cm in vertical height from the bottom edge) was removed around the entire circumference of the tree. In trees sampled during the spring 2018,

two sections of trunk bark were removed per tree. Each section was approximately 45 cm in vertical height. The bottom section was approximately 25cm from the base of the tree with the top section 25cm above the top of the bottom section. In 2017 and the spring of 2018, NEBB brood galleries were counted within the total debarked area and the percentage of DED fungal staining in the cambium estimated.

Trunk debarking was discontinued in 2018 and 2019 because the method proved to be labour intensive, requiring a large time investment, with few beetles or brood galleries found either in 2017 or spring 2018. While NEBB were found to attack and colonize the crown branches during the summer (Strobel and Lanier 1981; Anderson 1996), Holliday (2016) reported that brood galleries in trunks may also have potential to assess levels of NEBB infestation within infected trees. See the Discussion section in Chapter 5 for further comments on the usefulness of trunk debarking. As there was little evidence of trunk NEBB brood galleries, the density of NEBB brood galleries was recorded only in the mid-crown of the tree for the remainder of the study (see Analysis, Trunk debarking data in Appendix VI).

In addition to determining NEBB brood gallery density and percentage fungus staining from the trunk bark samples in 2017 as described above, part of the removed bark samples with the cambium attached were returned to the laboratory to determine the cambial moisture content. The purpose of removing the bark/cambium samples was to determine whether removal of the initial trunk bark around the complete circumference of the tree caused an increase in the drying process and decreased NEBB habitat quality under the bark (as reported by Pines and Westwood 1996).

Bark samples were immediately frozen and processed in the laboratory at the University of Winnipeg between November 2017 and February 2018. Three to four weeks after the initial trunk bark samples were removed, a second bark/cambium sample was removed above the first bark/cambium removal location (Figure 3). The moisture content in the second bark removal samples was measured immediately after removal from the freezer and then bark samples were dried for four weeks and the moisture measured again. Each sample was frozen the day of collection. Samples were thawed and weighed, then dried at low heat in a drying oven and weighed again. Before and after difference is equal to weight of water removed from the sample was calculated and recorded as % total of original weight. If water movement was impacted in

the tree after the initial trunk bark removal, it would have been reflected in a lower moisture content of the second set of samples removed at the end of the season. Cambium data are included in Appendix VII, and similar to light intensity, were not included in further analysis due to non-significant preliminary results in 2017.



Figure 3. Trunk debarking completed in Winnipeg on DED-infected American elm trees. 2017 (left) and 2018 (right). Left: Trunk debarking completed in 2017, featuring a single large debarked section. Right: Trunk debarking completed in 2018, featuring two smaller debarked sections in comparison to 2017 sampling.

NEBB capture and fungal staining assessment

Subsequent to the disease progression surveys in 2018 and 2019 sticky, traps (BioQuip, 6x12”) were placed on DED-infected study trees during the fall of each year (Figure 4). Traps were placed in conjunction with the dispersal of NEBB adults in the late fall as they emerged from infected trees in search of overwintering sites at the base of nearby uninfected elm trees (Strobel and Lanier 1981; Anderson 1996). Sticky traps were used without the addition of bait semiochemicals or other attractants as studies have shown little effect of lures on increased NEBB capture (Landwehr et al. 1980; Swedenborg et al. 1988; Pines and Westwood 2008).



Figure 4. NEBB trapping program, traps on American elm tree in Minto (2018); 4 traps on each tree for each cardinal direction (left) and traps on American elm tree in Lord Roberts (2019); aligned parallel forming a ring around the tree trunk.

In 2018, yellow sticky traps were placed at an of height approximately of two metres above the ground, with one trap placed at each cardinal direction for a total of four traps per study tree. If other elm trees were directly adjacent to the study tree, traps were also placed on these elm trees facing the study tree. Modifications were made to the NEBB capture protocol in 2019 because the 2018 NEBB capture rate was low. Traps were placed in a band around the circumference of study trees forming a circle to maximize surface area covered. Traps were set up in early October and removed after the second hard frost in both years. After removal, traps were immediately frozen and stored at the University of Winnipeg. They were later removed, allowed to thaw to room temperature, and the number of NEBB captured were counted.

Once NEBB were identified on the traps (Strobel and Lanier 1981; Solomon 1995; Arnett et al. 2002), they were removed and transferred to petri dishes to determine which beetles carried spores of *O. novo-ulmi*. To prepare NEBB for DED cultures, NEBB were crushed and

subsequently plated on CSMA (cycloheximide-streptomycin malt agar) and incubated at room temperature, with no light exposure, for three weeks (Zhou et al 2004; Jacobi et al. 2006; Romon et al 2007). The CSMA medium is semi-selective for *Ophiostoma* (Harrington 1981; Jacobi et al. 2006). Other publications report PDA (potato dextrose agar) as another suitable growth medium (Tainter 1992; Oullette et al 1995; Oullette et al 1999).

Ophiostoma are separated from *Ceratocystis* through a lack of hyaline gelatinous sheath present on ascospores (Upadhyay 1981). Fungi grown from NEBB cultures were identified to genus through characteristic mycelial and synnematal stages (columns of erect bundles of hyphae) after being viewed under compound microscope (Porter et al. 1959; Hiratsuka and Takae 1978; Oullette et al. 1999; Stipes and Campana 1981). Culture growth and appearance are described and pictured in Liberato et al. (2006). After a negative or positive identification of *Ophiostoma* presence, NEBB that yielded or did not yield *Ophiostoma* were recorded.

Mid-crown branch sampling

Mid-crown branch samples were removed from a subset of infected elm trees that were part of the disease progression survey between 2017 and 2019. The samples were retrieved from study trees by City of Winnipeg tree removal crews or contract tree removal operators using an aerial bucket truck. In 2017, infected trees were removed after trunk debarking occurred. In 2018 and 2019, infected trees were removed as soon as possible after the conclusion of the disease progression survey and the crown branch removal (Figure 5).



Figure 5. Mid-crown branch sampling completed in summer 2018. City of Winnipeg Forestry department crews while removing DED infected American elm trees would obtain mid-crown samples from each cardinal direction.

When infected trees were removed, a sample branch was taken at each of the four cardinal directions. Initially one branch samples were taken per cardinal direction in 2017 but were increased to three per cardinal direction in 2018 and 2019. Sample branches were on average 60 cm in length and 12 cm in diameter. After samples were removed, they were either processed in the field or at the City of Winnipeg, Forestry Department by either R. Westwood, U of Winnipeg summer staff employed (2017 and 2018) or myself (2018 and 2019). Branches were debarked and NEBB galleries were counted. Each gallery was considered to be the initial gallery constructed by the female NEBB, not the subsequent galleries created by her offspring. DED fungal staining was recorded as the percentage of the sample exhibiting characteristic DED staining. See Appendix V for the mid-crown branch sampling data. Sampling of mid-crown branches is an effective method to estimate the number of NEBB brood galleries present as

Oghiakhe and Holliday (2011) reported the majority of galleries were found in the mid-crown during their study.

Disease symptoms calculation

Disease progression variables were modified so they could be used in the subsequent ordination and modelling analysis. Weekly canopy variables assessed during the disease progression survey were averaged for each tree across their respective study period, including percentage dead leaves, dead branches, green leaves, survey of fungal staining in twigs and small branches via pole pruning, and canopy cover. Values were averaged since single-week data would be unrepresentative of disease progression in each tree. To assess the weekly change in relation to beetle density and fungal staining, models were tested using the weekly rate of change for each variable.

For crown branch and trunk samples, gallery density and fungal staining were calculated as weighted averages of their respective measurements. Factoring in the sample dimensions allows for more accurate estimation of both variables. The surface area of each canopy sample was calculated as follows, where SA equaled the sample surface area, D was equal to the diameter (and in my case) width of mid-crown sample, and H equaled the height (and in my case) length of mid-crown sample.

$$\text{Equation 1: } SA = \pi DH$$

Weighted gallery density was calculated by taking the recorded staining percentage and enumerated NEBB brood galleries multiplied by the sample area, standardized to metres squared. SA equal to the sample surface area calculated in Equation 1. GD_{wt} was equal to the weighted gallery density and G was equal to the gallery count from mid-crown sample. Weighted fungal staining (St_{wt}) were calculated by taking the recorded fungal staining percentage (St) and multiplying it by the quotient of sample surface area (SA, calculated in Equation 1) and total surface area for all samples from a given tree (ΣSA).

$$\text{Equation 2: } St_{wt} = St \left(\frac{SA}{\Sigma SA} \right)$$

$$\text{Equation 3: } GD_{wt} = \left(\frac{G}{SA} \right) 10'000$$

Each tree's overall sum of sample area, fungal staining percentage, and gallery density (area total (m²), stain overall (weighted), and brood gallery density (weighted), respectively) were then used to calculate, average weighted staining, and average brood gallery density. Average weighted fungal staining ($AveSt_{wt}$) was obtained by summing the weighted fungal staining for each given tree (St_{wt}). Average weighted gallery density ($AveGD_{wt}$) was obtained by dividing weighted gallery density for the sample (GD_{wt}) by the total surface area for all samples from the given tree (ΣSA). Surface area was again calculated using Equation 1.

$$\text{Equation 4: } AveSt_{wt} = \sum St_{wt}$$

$$\text{Equation 5: } AveGD_{wt} = \frac{GD_{wt}}{\Sigma SA}$$

As noted above, models and ordinations feature an average of the disease progression survey variables over their respective study periods. I also assessed whether the change in these variables over the study season significantly related to the percentage of fungal staining or brood gallery density. Variable rate of change was calculated as the final variable value subtracted from the initial variable value, then divided by the study period. This provided the week-to-week change in each canopy variable, expressed as the rate of change per week.

$$\text{Equation 6: } \Delta V = \frac{V_F - V_I}{T}$$

Where ΔV was equal to the variable rate of change, V_F was equal to the final recording of the variable for the study period, V_I equal to the first recording of the variable for the study period, and T equal to the length of the study period (in weeks).

Lastly, each study tree was assigned to one of two groups based on whether NEBB beetle galleries were present or absent in the canopy branch sampling; referred to as either detectable NEBB gallery density or undetectable NEBB gallery density. This grouping showed particular importance in the exploratory analysis and the ordination/modelling (see later under Analysis). When calculating NEBB gallery density, the cardinal direction of the canopy branch sample was not included in the analysis as statistical models performed better (meaning had more sufficiently interpretable results) when samples were pooled. Cardinal direction was not used as both Holliday (2016) and Oghiakhe and Holliday (2011) found no effect of canopy direction on the location of NEBB brood galleries. Cardinal direction has been used in bark beetle modelling for

species colonizing tree trunks where there appears to be a preference for either the sunny or dark side (Shepherd 1966; Bleiker et al. 2013).

Trunk debarking (as described in Methods) had both weighted fungal staining and weighted NEBB gallery density calculated in the same way as the canopy branch samples. Canopy branch data are shown in Appendix V. Note, some of the canopy branch samples in Appendix V did not have an associated disease progression survey and were designated as such. These trees were also tagged in 2017 and had canopy branch samples taken in 2018. Trunk debarking variables can be found in Appendix VII.

Linear regressions were used to compare the relationship between trunk NEBB brood galleries and fungal staining, and canopy NEBB brood galleries and staining in 2017. As well, linear regression compared the relationship between canopy fungal staining and canopy NEBB brood galleries across all three study years. Three linear regressions were tested (similar to Holliday (2016)). NEBB brood galleries in canopy samples and weighted NEBB brood gallery density were compared to weighted percentage canopy fungal staining. Weighted NEBB brood gallery density was compared to weighted canopy percentage fungal staining. As recommended by Holliday (2016), all models were log-transformed to approximate a normal residual distribution and to more reliably model the relationship between NEBB galleries and fungal staining. Log-transformed regression lessened the impact of potential outlier trees with very high densities of NEBB brood galleries. Log transformations were done as $\log_{10}(x+1)$ to allow for the addition of zero counts in regression models. Linear regressions to correlate NEBB brood gallery density and DBH, and estimated tree age and height were both non-significant and are not further reported.

Beta regression was performed to compare percentage canopy fungal staining and NEBB brood gallery counts/weighted densities with a more suitable model than standard regression. Beta regression suits dependant variables (in my case percentage fungal staining) bound at 0 to 1. Since this variable is inherently proportional, beta regression is suitable whereas if each observation was 0 or 1, then a Poisson regression would be more suitable. Beta regressions were performed using the *betareg* function in *betarag* package in R (R Studio 1.4.1106, R v. 4.0.5, Cribari-Neto and Zeileis 2010).

Age estimation

Tree age was estimated for each study tree included in the disease progression survey and canopy sampling. The age of trees in each year of the study was assessed for potential correlation to internal fungal staining and NEBB gallery density. Age was estimated by using the City of Winnipeg's property value and land parcel database and locating the trees with their corresponding property addresses in the data base. Since the study elms used in Winnipeg's neighbourhood were planted on the boulevards in front of the homes when they were built, and neighbourhoods were built over a short period of time, we were able assign an estimated age to most of the study trees without having to take tree cores. We were able to estimate approximate planting dates for 21, 31 and 48 trees in 2017, 2018, and 2019, respectively.

To analyze whether different elm tree age demographics in our study were increased or decreased susceptibility to NEBB infection (showing higher NEBB gallery density), study trees in the canopy sampling component were assessed using linear regression. Linear regressions were used to compare NEBB brood gallery density and estimated age of study tree and also to compare NEBB brood gallery density and DBH and tree height. We were able to estimate approximate planting dates for 21, 31, and 48 trees in 2017, 2018, and 2019 respectively.

Analysis of data groupings

Data grouped by year, neighbourhood, and detectable/undetectable status of NEBB brood galleries were assessed for significant differences between study year for each canopy variable. Comparison of each variables response for each study year was performed with a Welch ANOVA and Games-Howell *post hoc* test as data did not meet the requirements of a typical ANOVA for variance and normal distribution. Welch ANOVA tests were performed in RStudio (1.4.1106, R v. 4.0.5) using the R package rstatix (Alboudkadel 2021 v0.7.0). The Welch ANOVA has lower type I error when applied to heterogenous data sets with large variances than other non-parametric equivalents such as Kruskal-Wallis (Moder 2010).

Pearson correlations were further analyzed using six symptom variables (variables covered all three years) or four variables (variables covered in 2018 and 2019). The variables were analyzed as groupings based on NEBB being detectable or undetectable in each year. A total of 100 study trees were included in this analysis, 59 with detectable NEBB galleries and 41

in which galleries were not detected. Further breakdown of sample sizes for detectable/undetectable NEBB density analysis are shown in Table 2.

Table 2. Sample size of DED infected trees for each portion of the detectable/undetectable NEBB density groupings from 2017-2019. Presence indicates DED infected trees that contained galleries in mid-crown sampling. Absence indicates a lack of galleries found when DED infected trees were sampled. Sum total for both presence/absence is included (“Study Total”), and yearly sum for total number of trees samples is included as “Year Totals”.

Study Year	2017	2018	2019	Study Total
Presence	10	14	16	40
Absence	11	17	32	60
Year Totals	21	31	48	100

NMDS ordination and ANOSIM

Non-metric multidimensional scaling (NMDS) was used to summarize and visualize the relationship of external canopy variables with the results of the canopy branch sampling. NMDS was performed using a Sorensen (Bray-Curtis) distance measure with a random starting configuration and Monte Carlo testing with 250 runs for both observed and randomized data. NMDS was selected over principal components analysis (PCA) for ordinating the data. PCA performs better when data show a linear response to environmental variables and there are few null data points in the data (Ramette 2012). Commonly the requirements for a PCA may not be met in some ecological studies and other methods are preferred (Ramette 2012) as is the case for my data set. NMDS is commonly used in ecological studies and is generally applied to identify patterns among multiple variables, especially when parametric tests are not ideal (Minchin 1987; Hirst and Jackson 2007). NMDS is an iterative method that generates a dissimilarity matrix, and then ordinated the data to visualize the similarities in data points to one another. It is important to mention it is not meaningful to assess correlations between ordination axis and study variables, as only the axis configuration is relevant in the NMDS, while axis directions are arbitrary. Further, NMDS arranges data points to minimize rank-order correlations between actual variable

distance, and their respective ordination space distances (Shafii et al. 2013). Study variables were represented in the ordination space as arrows with the direction of the arrow indicating the direction and strength of the correlation between study variables and ordinated points (Vare and Oksanen 1995).

NMDS ordination was used to assess the relationship of canopy variables to one another, and to various data groupings (NEBB gallery presence/absence, study year, and study neighbourhood). This allowed the patterns in disease progression and canopy sampling variables to be assessed and helped inform decisions in modeling (described later). Ordinations were run for all three study years (7 variables) and for only 2018 and 2019 study years (9 variables). Doing both sets of ordination ensures each variable can be included in the ordination analysis. To ensure that the NMDS results are valid for interpretation, stress levels for the ordination were assessed. The literature suggests that ordinations with stress below 20% are sufficient to show meaningful trends (Clark 1993) and Shepard's plots (stress plot) were generated to further assess stress values for the NMDS ordinations. The plot provides stress values for the tested number of dimensions and show decrease in ordination stress and ordination dimensions are increased. Large scatter around the regression line indicates that original dissimilarities are not well preserved in reduced numbers of ordination dimensions. Lastly, a non-parametric Monte Carlo permutation test was performed that randomly permutes the sampled data repeatedly (Gittins 1985) and generates a test statistic for each permutation cycle. If the generated test statistic is excessively different to the reference than study variables and sampling data can be inferred to have a relationship (Van Wijngaarden et al. 1995) Significant result of the Monte Carlo test would indicate canopy variables have a significant effect on the sample trees in the NMDS ordination (Brink et al. 2003).

Stress values, visualization through Sheperd's plot, and Monte Carlo test are sufficient evidence that an NMDS are satisfactory and suitable for evaluation (Dexter et al. 2018). NMDS ordination, along with Shepard's plot and Monte Carlo test were run using PC-Ord Version 6 (McCune and Grace 2002; McCune and Mefford 2011). The ordination was replicated in R using the vegan package to generate figures (Oksanen et al. 2020 v2.5-7).

Analysis of similarities (ANOSIM) is a nonparametric test for assessing differences between two or more groups based on the distance measures in an NMDS ordination (Clarke

1993; Rem 2012). The test compares the distance ranks between groups and the distance ranks within groups. These are compared and the resulting R statistics measures if groups are separated ($R=1$, distances within groups have more similarities than others) or whether no separation occurs ($R=0$, distances between groups have more similarities than within). ANOSIM were calculated using R package *vegan* (Oksanen et al. 2020 v2.5-7) and *ecodist* (Goslee and Urban 2007). Clarke and Gorley (2001) provide descriptions for assessing the R statistic provided by the ANOSIM test. R values exceeding 0.75 interpret groups as being well separated. R values above 0.5 indicate groupings that are separated but have overlap, lastly R values lower than 0.25 indicated grouping that are mostly undifferentiated. ANOSIM, in comparison with similar test methods (i.e. MANOVA) are preferable due to fewer assumptions of the data distribution required for the test to be valid because it is based on the ranks of distance (Ramette 2012).

Generalized Linear modelling

Generalized linear models (GLM) were used to assess the relationship of canopy symptoms from the disease progression survey to NEBB brood gallery densities recorded from canopy branch samples. Three models were created, results of which are reported in Table 3. The first and second model used a quasibinomial error structure with internal fungal staining percentage as a proxy for internal beetle galleries. Model 1 independent variables were percentage canopy dead leaves, dead branches, and infection sites. Model 2 independent variables were percentage canopy green leaves, average canopy cover (densiometer measured), and infection sites. The third model used a binomial distribution with a logit link function and beetle detectability as the response variable. In all three models, the independent variables were the percentage of dead leaves, percentage of dead branches, and number of infection sites. Modelling was completed in RStudio (RStudio Team 2021 v1.4.1106, R v. 4.0.5). Models 1 and 3 were tested with their coefficients replaced with their equivalent weekly rate change. Rather than variables being averaged over the study years, models were created with the variable of weekly rate of change.

Table 3. Generalized linear models developed for assessment of canopy branch brood gallery data and fungal staining and disease progression survey variables. For model 1-3 the distribution, link function, response variable, and effect variables are listed (DB = % canopy dead branches, DL = % canopy dead leaves, G = % canopy green leaves, and InfSites = Infection sites recorded on infected tree initial visit).

Model	Distribution	Link	Response variable	Effect variables
1	Quasibinomial	Logit	% Fungal staining	DB + DL + InfSites
2	Quasibinomial	Logit	% Fungal Staining	G + AveCC + InfSites
3	Binomial	Logit	NEBB detectability	DB + DL + InfSites

In initial exploratory analysis of canopy symptom correlation to internal beetle density, the variables chosen for model 1 to 3 showed correlation with beetle densities, supported by the NMDS ordination (see Analysis). Further, percentage canopy green leaves and average canopy cover were collinear to the aforementioned variables and their inclusion in the model would produce non-representative results. Collinearity between variables in a model leads to inflated apparent correlations (Brunsdon et al. 2012), hence running models were done for both groupings (percent canopy dead leaves/percent canopy dead branches, and percentage canopy green leaves/average canopy cover). The explanatory variables also must be easily observed to match the project objectives, which removed fungal staining from pole pruning, and any internal measurements from being included in the GLM.

Additional variance presence in the datasets used for the first two models required a GLM with a quasibinomial distribution. Quasibinomial distributions are suitable to correct for the over-dispersed data in this study (Zuur 2009; Sentis et al. 2016). Over-dispersed data is the presence of increased data variability than expected. A binomial distribution was chosen for the third model assessing beetle detectability and the response variable. Since the response variable was binary (either NEBB were present or absent in the branch samples), a GLM with a binomial distribution and log link function was considered suitable (Nicholls 1989; Lenihan 1993; Guisan 1998).

Fungal staining was used as a proxy for internal beetle density since fungal staining correlated significantly with internal beetle density (see Analysis), fit the requirements of the quasibinomial distribution, and featured a more normal distribution compared to the over-dispersed NEBB gallery density. Internal fungal staining showed more normal and less skewed data distribution and variance as a response variable in the generalized linear model compared to NEBB brood gallery density. While this model did not directly predict gallery density, it still provided a meaningful prediction about the internal condition of the tree. Fungal staining as a proxy in modelling inherently predicted the presence or absence of NEBB galleries in the canopy. Trees without evidence of galleries would be expected to have a decreased amount of fungal staining. For trees with extensive fungal staining at the time of initial inspection in the tree crown, I predicted that there would be multiple infection sites (*i.e.*, the presence of NEBB galleries and increased NEBB gallery densities). High fungal staining would indicate larger NEBB densities since not all NEBB carry *O. novo-ulmi* when attacking American elm trees.

I performed diagnostic tests to assess the model adherence to standard regression assumptions. Plots of residuals against values of each predictor in the model created basic diagnostic figures to test for non-linearity. Normality of residuals, absence of heteroscedacity, and absence of outliers among residuals indicated that GLM model assumptions had been met (Wang 1987; Zuur 2009). Examination of residual plots was added with a lack-of-fit test. Models were assessed for influential and outlier points through and influence index plot. Lastly, QQ plots were generated that approximated normality in data deviance, allowing me to evaluate whether the response distributions were suitable.

Linear models were used in a similar fashion as Holliday (2016) to assess the correlation between NEBB brood galleries in trunk debarking and mid-crown canopy samples. Regressions were used to assess the potential relationship between NEBB brood galleries and percent fungal staining (both weighted). For each regression, canopy NEBB brood galleries (independent) and trunk NEBB brood galleries were tested, with both variables log transformed to compensate for data distribution

CHAPTER 4 – RESULTS

Summary statistics for disease progression survey

There were 350 trees included in the disease progression survey portion of the study (91 in 2017, 150 in 2018, and 109 in 2019). Of these, 100 trees were included in the ordination and modelling analysis (trees containing disease progression survey and mid-crown canopy sampling). Certain DED-infected study trees were omitted in the ordination and modelling analysis as either unrepresentative (excessively small size) of a typical boulevard elm tree or had portions of required data missing. Table 4 shows the disease progression survey and canopy variables along with relevant summary statistics. Table 4 includes all study trees that were suitable for analysis; the full data set with all trees for each year appears in Appendices II-IV.

Table 4. Disease progression survey and canopy sampling summary statistics collected between 2017-2019 in Winnipeg, MB (N = number of trees sampled). Variables in disease progression survey: DBH = Diameter at breast height (recorded in cm), HT = Tree height recorded in meters, CC = Percentage canopy cover, G = Percent canopy green leaves, DL = Percentage canopy dead leaves, DB = Percentage canopy dead branches, PP = Percentage DED fungal staining recorded from twig samples obtained through pole pruning, InfSites = Number of infection sites recorded on initial visit to infected elm tree. Canopy survey variables are as follows: Stain = Percentage DED fungal staining from mid-crown canopy samples, Galleries = Gallery density recorded from mid-crown canopy samples (NEBB brood galleries per meter squared).

Study Component	Variable	N	Min	Max	Mean	Std Dev.	Year					
							2017		2018		2019	
							Mean	Std Dev.	Mean	Std Dev.	Mean	Std Dev.
	DBH (cm)	350	8	110	64.7	13.7	67.8	16.0	52.2	12.8	64.1	13.4
	HT (m)	350	6	24	14.5	2.3	14.2	3.1	11.2	4.9	12.4	1.9
	CC (%)	350	0	100	33.5	29.1	24.4	16.7	22.4	20.1	50.8	34.5
Disease Progression Survey	G (%)	259	0	100	49.9	31.0			50.6	29.9	49.2	32.3
	DL (%)	259	0	63	15.6	14.6			16.1	14.6	15.0	14.7
	DB (%)	350	0	100	34.6	28.2	40.9	35.2	31.3	27.6	34.3	31.1
	PP (%)	259	0	85	25.2	20.2			31.1	21.2	22.4	19.2
	InfSites	259	0	7	3.3	1.7			3.0	71.2	3.5	2.1
High	Stain	100	0	100	49.1	37.7	32.9	25.4	40.4	43.6	63.3	36.9
Canopy Survey	Galleries (Gals/m2)	100	0	5	0.2	1.0	0.0	0.2	0.1	0.2	0.5	1.0

The mean tree diameter at breast height (DBH) over the three study years was 64.7cm (SD=13.7) (Table 4). Figure 6 shows the distribution of the data for DBH, tree height, and age for all three years of the study. There was no significant difference in DBH between the three study years ($F_{2,195.49}=1.16$, $p=0.316$) (Figure 7a). Mean tree height over the three years was 14.5m (SD=2.3) and there was a significant difference in height of trees between study years ($F_{2,184.41}=55.6$, $p<0.001$) (Figure 7b), with the tallest trees sampled in 2017 and the smallest trees sampled in 2019. The mean estimated tree age was 92.4 years of age (SD=15.6). Age was significantly different between study years ($F_{2,40.992} = 3.90$, $p = 0.0282$), with the estimated oldest trees surveyed in 2017 and the youngest in 2019 (Figure 7c).

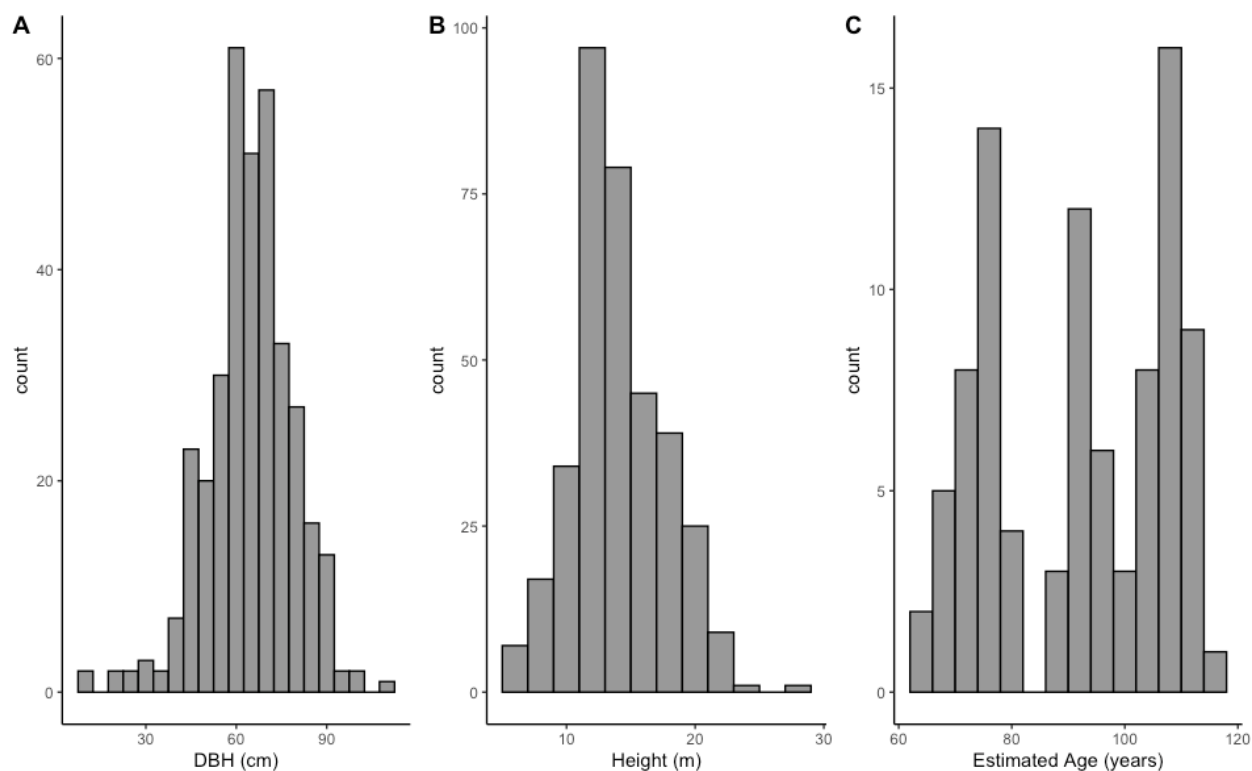


Figure 6. Histograms for diameter at breast height (A), tree height (B), and estimated age (C) for all American elm trees sampled between 2017 to 2019 in the study. Sample count is DED infected trees included in the study.

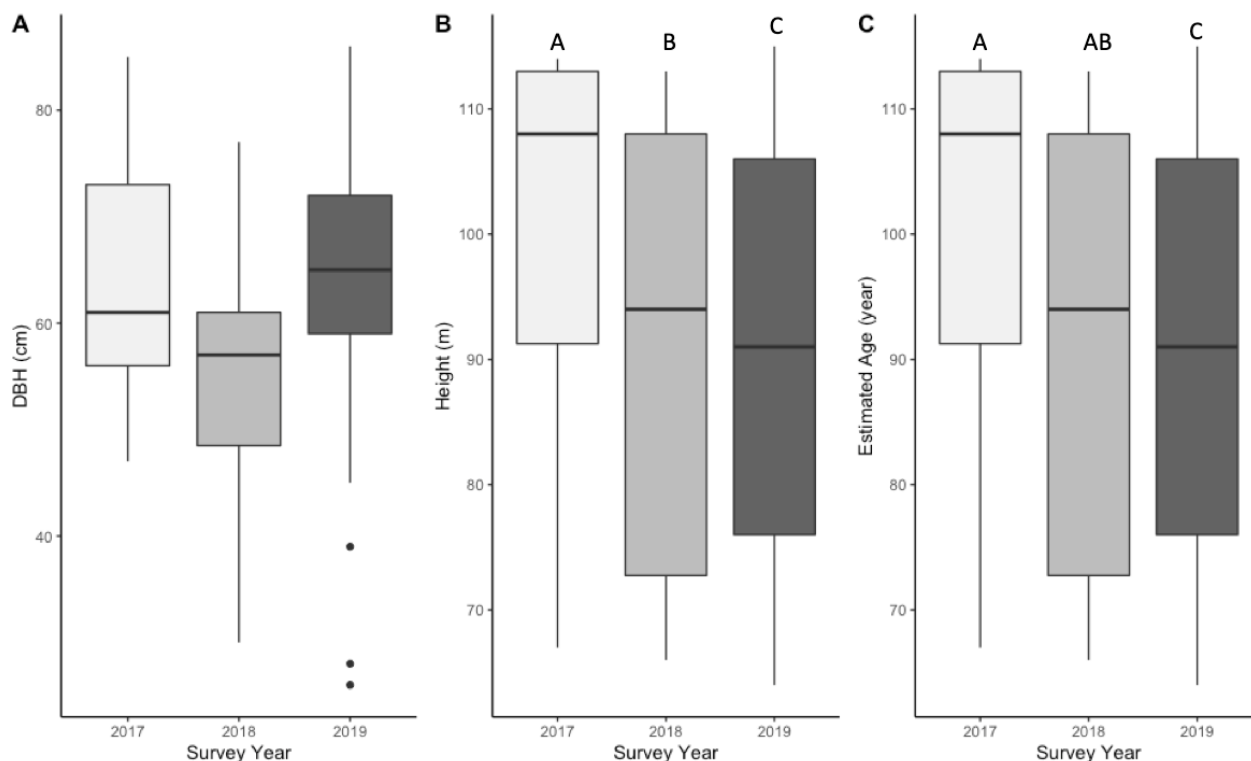


Figure 7. Boxplot of tree trunk diameter at breast height (DBH) (A), tree height (B), and estimated tree age (C) grouped by study year. Bars with different letters are significantly different (Games-Howell post hoc test) at $p < 0.05$.

Mean percentage canopy cover (CC) was significantly different between the three years ($F_{2,215.84} = 35.8$, $p < 0.001$), averaging 35% across all study years (Figure 8). Canopy cover was higher towards the last survey week in comparison to initial survey weeks throughout all years, with 2017 and 2018 having smaller canopy cover values than 2019, and 2019 showing larger variance than the two previous years. Canopy cover variability is addressed later in the discussion. The number of infection sites showed no significant difference across study years, however the percentage canopy fungal staining did increase as infection sites increased (Figure 9a). DED fungal staining was greater in trees with 5-7 infection sites, especially in comparison to trees with 1-2 infection sites. Trees with 3-4 infection sites had a large range of DED fungal staining. There were few NEBB galleries recorded in study trees with < 5 infection sites while

those trees with 5-7 infection sites had higher NEBB brood gallery densities (Figure 9b). Average infection sites over the three years were 3.3 (SD=1.7).

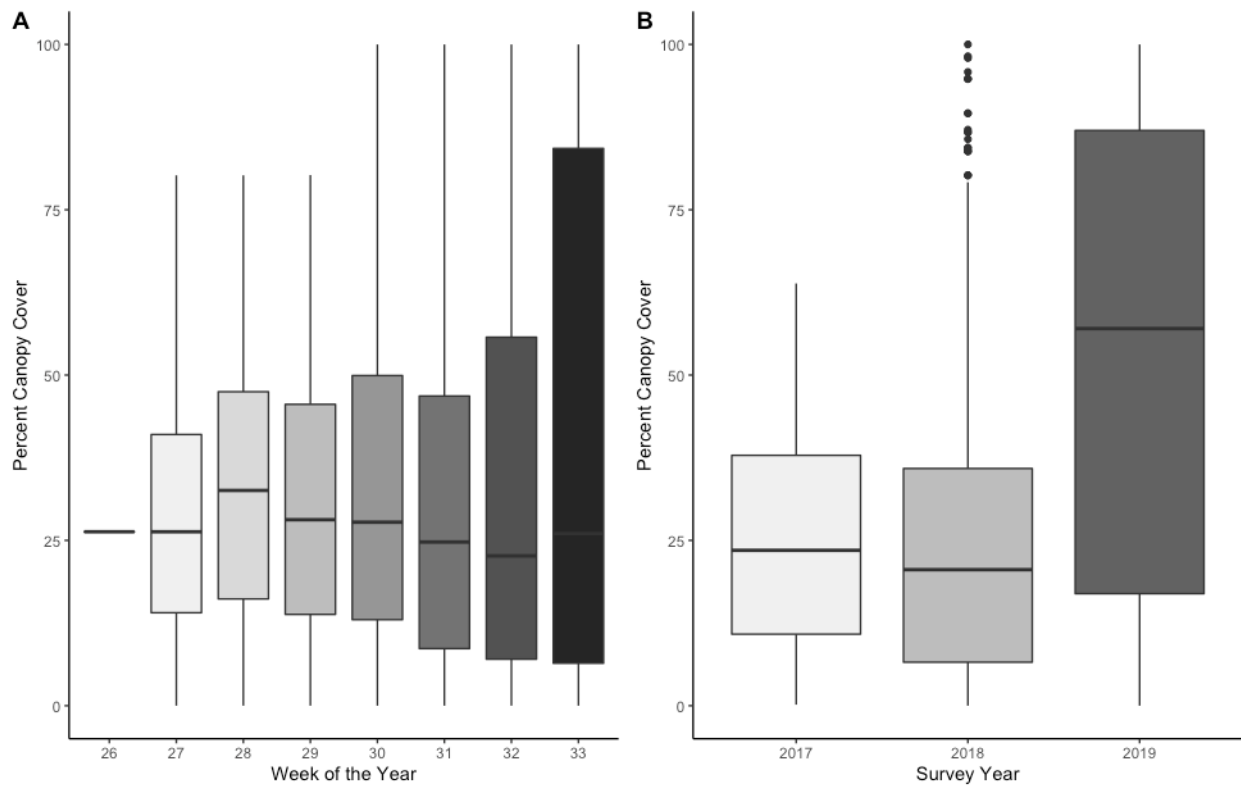


Figure 8. Boxplots of average percent canopy cover, 2017 to 2019 in Winnipeg, MB. A: Average canopy cover for each study week over 3 years, B: Average canopy cover for each year.

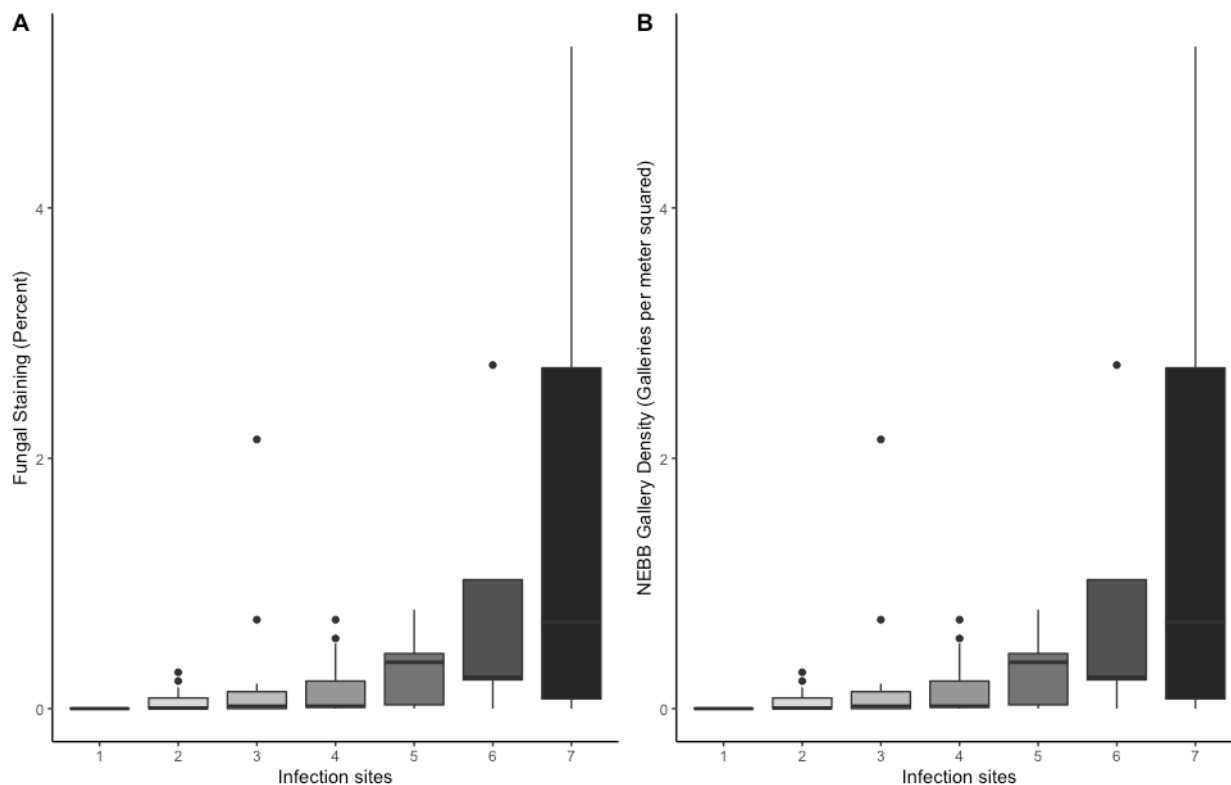


Figure 9. Summary of infection sites recorded upon initial study tree assessment weekly (A), and boxplot showing NEBB brood gallery density per each level of infection site (B) over 2018 and 2019. Infection sites recorded as major limbs showing evidence of significant canopy dieback localized to sites of beetle attack.

The remaining canopy variables showed no significant difference across study years (Figures showing year-to-year averages are in Appendix IX). Along with the non-significant canopy variables, percentage canopy fungal staining and NEBB brood gallery density from mid-crown sampling did not differ significantly between years. Averages across all three years for canopy variables were: Percentage canopy cover of green leaves ($47.32 \pm 29.64\%$), percentage canopy dead leaves ($22.96 \pm 15.39\%$), percentage canopy dead branches ($34.6 \pm 28.2\%$), and percentage fungal staining from pole pruning samples ($25.2 \pm 20.2\%$).

Data groupings results

Study trees were grouped into those with or without a detectable level of NEBB gallery density. These two groups in each study year (six categories) were assessed by each disease progression symptom and tree canopy variable. The percentage of fungal staining from pole pruning was not assessed due to low sample size in trees with canopy samples. Average trunk diameters between detectable and non-detectable NEBB gallery groups were significantly different ($F_{5,35.442} = 3.722$, $p=0.008$) over the study period; the differences occurred in the trees with detectable NEBB galleries, 2017 and 2019 showed greater DBH than 2018 (2017 and 2018 $p=0.015$; 2018 and 2019 $p=0.009$) (Figure 10).

Height between detectable and non-detectable galleries in trees was significantly different ($F_{5,33.335} = 5.1512$, $p=0.001$) over the study period with 2017 undetectable showing greater tree height than 2019 undetectable ($p=0.019$) and 2019 showing greater height than 2018 ($p=0.003$) in trees with detectable NEBB galleries; and (Figure 11). Estimated tree age was also significant ($F_{5,35.172} = 3.113$, $p=0.022$) but only between 2017 and 2019 NEBB gallery detectable trees ($p=0.015$) with 2017 have older trees (Figure 12).

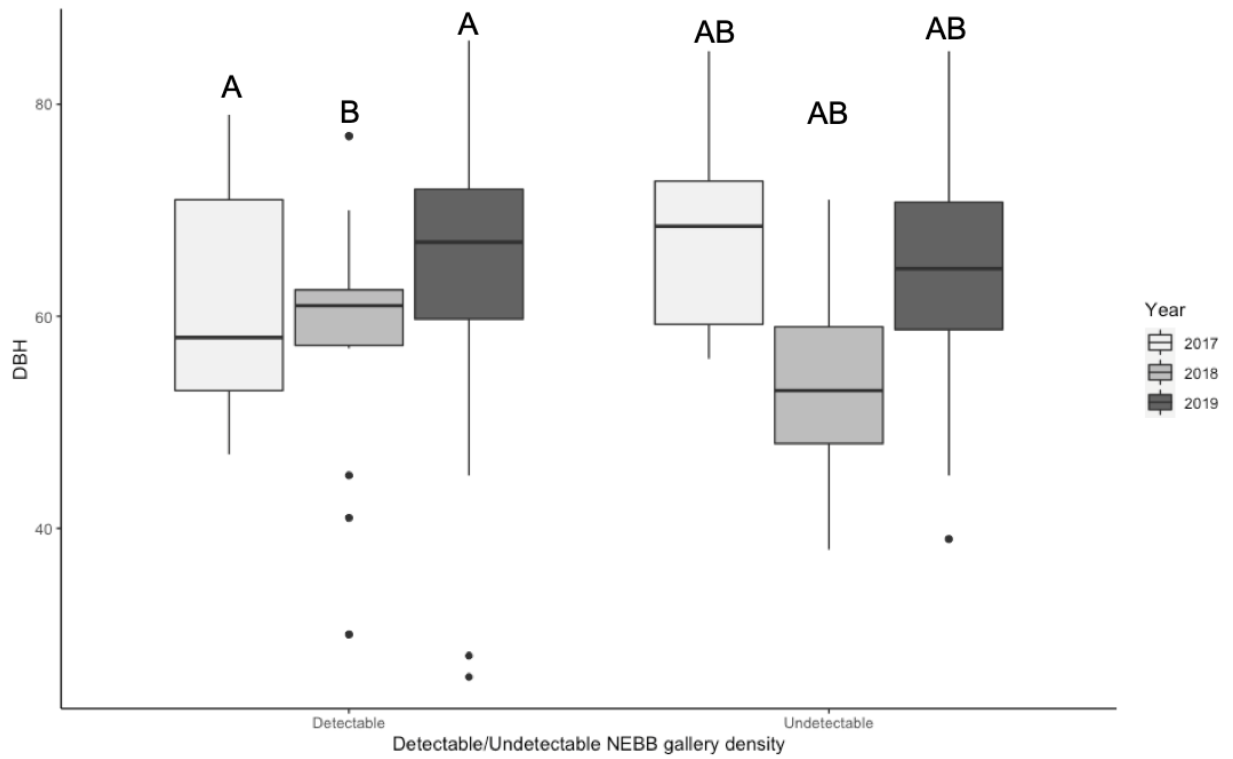


Figure 10. Boxplot of average diameter at breast height in each study year (meters), grouped by trees that during mid-crown sampling had either a detectable or undetectable density of NEBB brood galleries. Samples taken from Winnipeg, MB between 2017 and 2019.

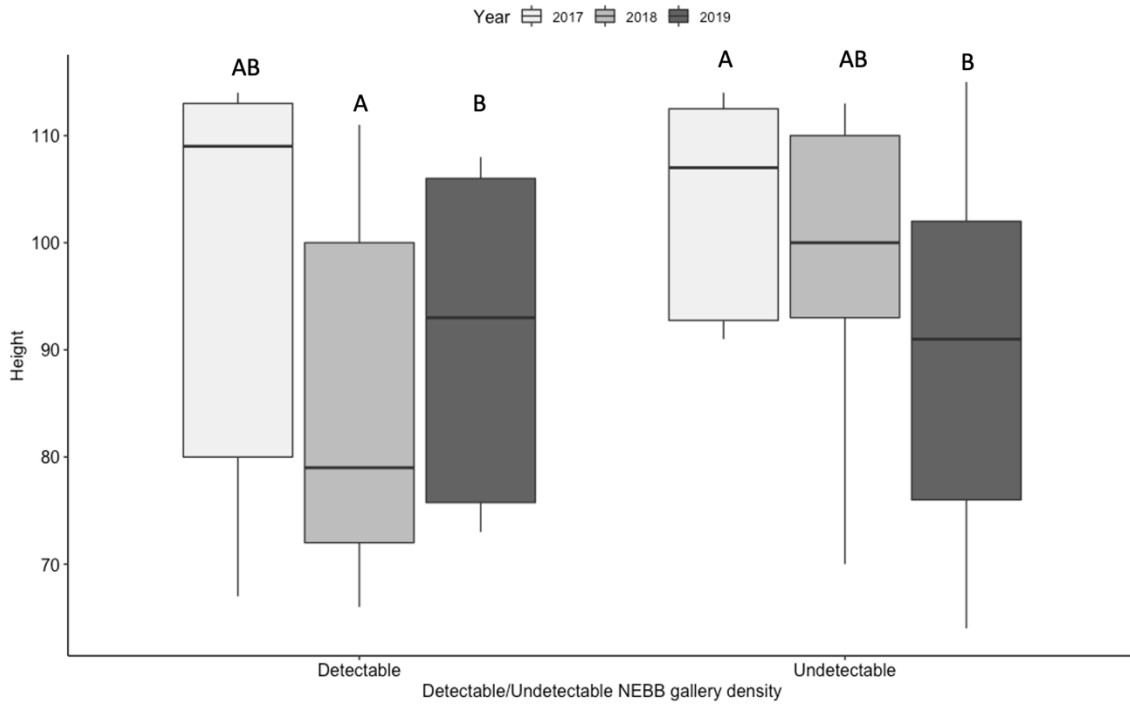


Figure 11. Boxplot of average tree height (meters) in each study year, grouped by trees that during mid-crown sampling had either a detectable or undetectable density of NEBB brood galleries. Samples taken from Winnipeg, MB between 2017 and 2019.

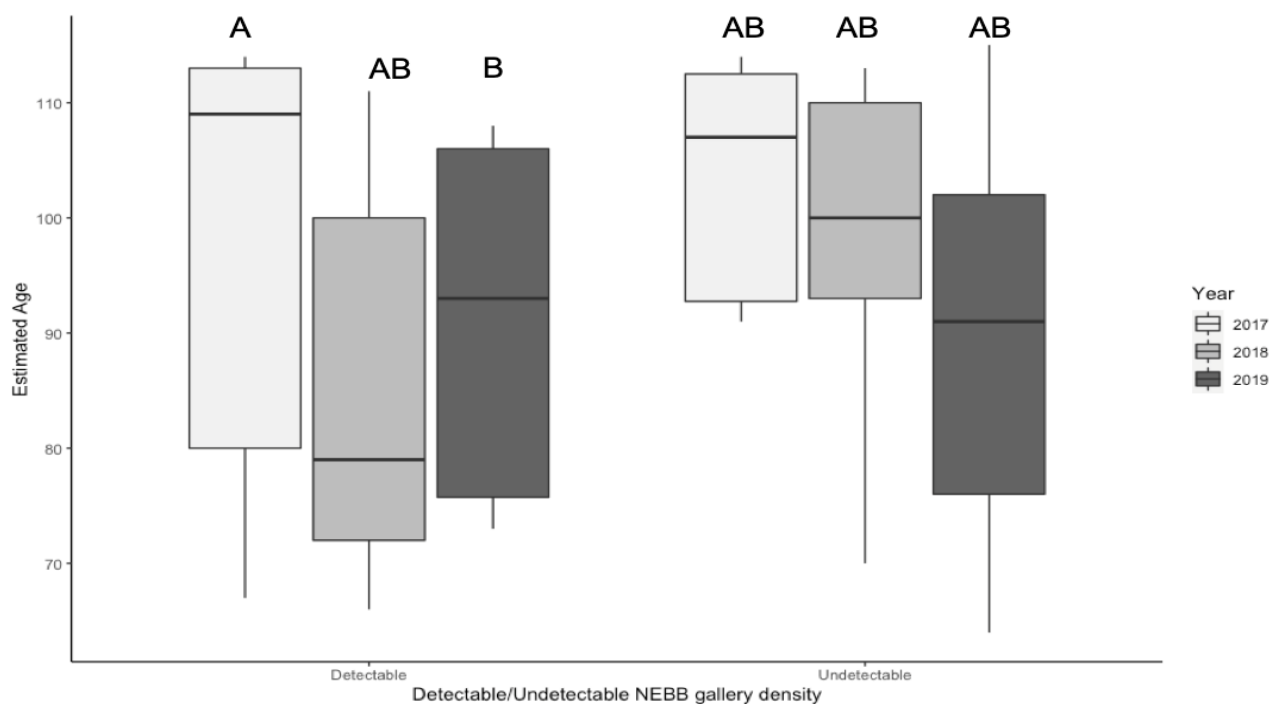


Figure 12. Boxplot of average estimated age in each study year, grouped by trees that during mid-crown sampling had either a detectable or undetectable density of NEBB brood galleries. Samples taken from Winnipeg, MB between 2017 and 2019.

Average canopy cover between trees with detectable NEBB galleries and those without galleries was significant ($F_{5,34.506} = 5.09$, $p = 0.001$), with 2017 undetectable being significantly less than and 2018 undetectable trees ($p = 0.004$), and 2017 detectable being significantly less than 2019 detectable trees ($p = 0.011$). Trees with undetectable NEBB gallery density showed higher average canopy cover in 2018 especially in comparison to 2017 trees with undetectable NEBB gallery density (with the smallest average canopy cover) (Figure 13).

Percentage canopy green leaves between trees with detectable NEBB galleries and those without galleries was significant ($F_{3,37.146} = 7.41$, $p = 0.005$) with the difference being 2018 trees with undetectable NEBB galleries being larger than 2019 detectable NEBB galleries ($p < 0.001$) (Figure 13).

Percentage canopy dead leaves between trees with detectable NEBB galleries and those without galleries was significant ($F_{3,35.951} = 3.14$, $p=0.037$) with 2018 trees with undetectable NEBB being smaller than 2019 detectable NEBB galleries ($p=0.044$). Percentage canopy dead branches between trees with detectable NEBB galleries and those without galleries were significant ($F_{5,35.154} = 4.24$, $p=0.004$) with significantly less percentage canopy dead branches in undetectable trees in 2017 and undetectable trees in 2018 ($p=0.010$). Trees with undetectable gallery densities in 2017 were also significantly greater than undetectable trees in 2019 ($p=0.025$) (Figure 13).

There was a significant difference in the number of infection sites ($F_{3, 37.213} = 8.262$, $p<0.001$) between 2018 trees with undetectable NEBB galleries having fewer infection sites than trees in 2019 with detectable galleries ($p<0.001$). Overall, there were a greater number of infection sites in trees with detectable NEBB gallery density than undetectable, and 2019 infection sites in comparison to 2018 for both treatments. There was also a significant difference in tree canopy weighted percentage fungal staining ($F_{5,35.172} = 8.262$, $p<0.001$) between trees with detectable and undetectable NEBB galleries. Trees with detectable NEBB galleries had a larger percentage of fungal staining than trees with NEBB galleries.

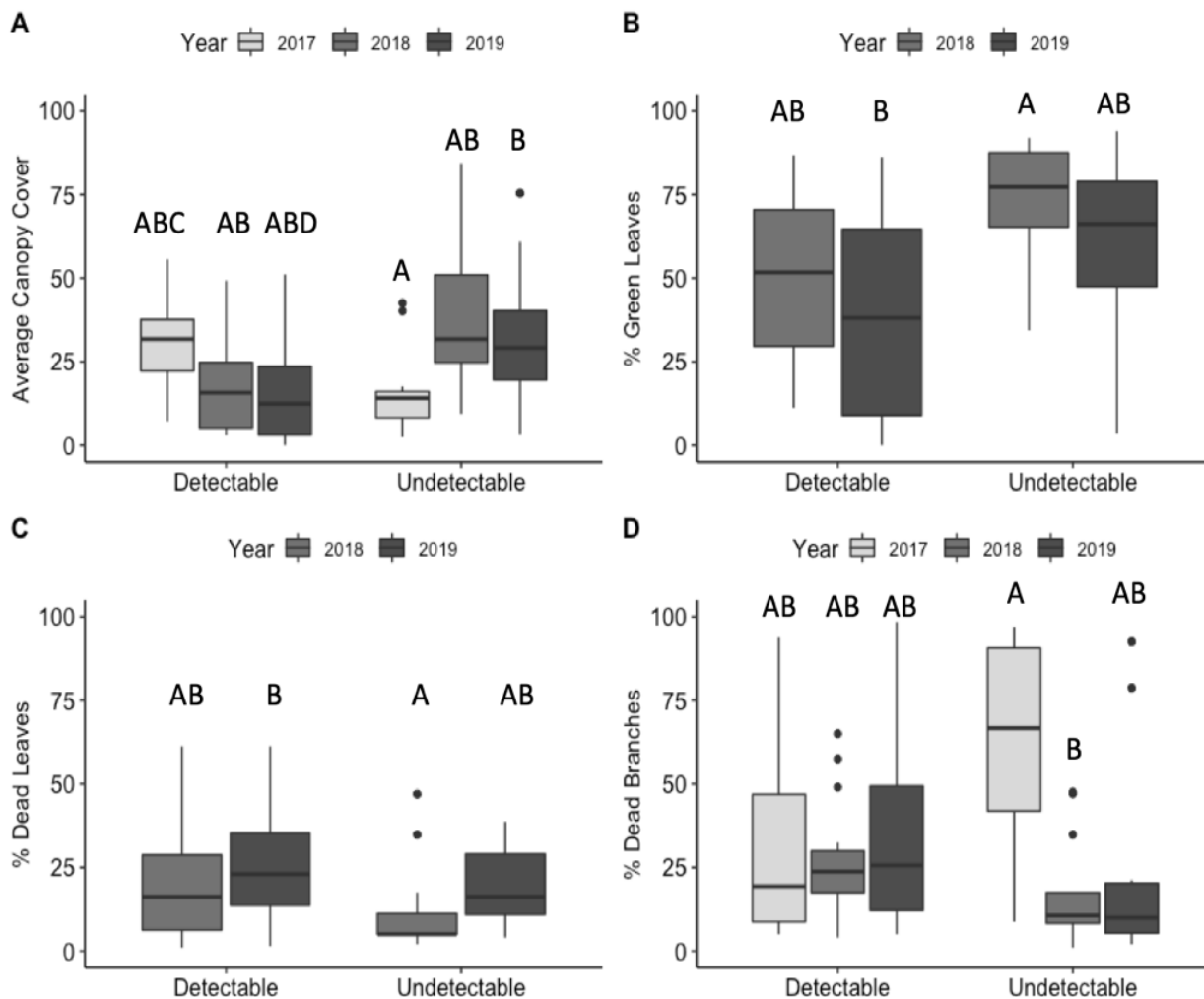


Figure 13. Grouping of study trees with detectable or undetectable levels of NEBB brood galleries compared to average canopy cover in Winnipeg, MB between 2017 and 2019 (A), percentage canopy green leaves (B), percentage canopy dead leaves (C), and percentage canopy dead branches (D), grouped by study year. Bars with different letters indicate are significantly different (Games-Howell post hoc test) at $p < 0.05$.

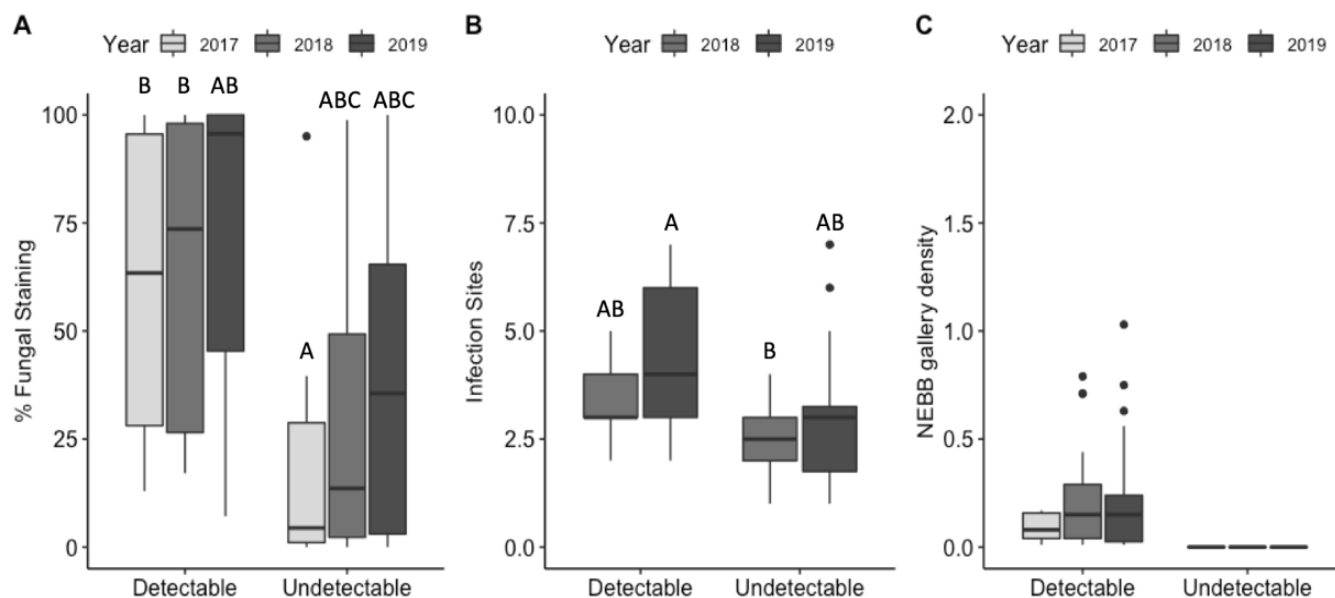


Figure 14. Grouping of study trees with detectable or undetectable levels of NEBB brood galleries, compared to percentage canopy fungal staining from tree canopy samples in Winnipeg, MB between 2017 and 2019 (A), infection sites recorded on initial tree assessment (B), and NEBB brood gallery density (galleries per meter squatted) (C), divided by study year. Letters denote significant differences between groups. Bars with different letters indicate significant difference (Games-Howell post hoc test) at $p < 0.05$.

Canopy branch data were examined between neighbourhoods to assess whether relative location had an impact on either disease progression survey or tree canopy variables. Only trees with canopy branch data were used ($n=100$) in the assessment, with five trees from Crescentwood, 48 from Minto, 30 for Lord Roberts/Riverview (RVL) and 17 from Wolsley. Given the unequal sample sizes between neighbourhoods and the small sample sizes from Crescentwood and Wolsley, summary data are provided only for statistical inference as these comparisons may be misleading. Welch's ANOVA and Games-Howell post-hoc tests are reported in Table 5 and Table 6, respectively.

Table 5. Welch's ANOVA results for neighbourhood groupings, indicating if variable tested showed significant difference between study neighbourhoods between 2017-2019. (DBH = Diameter at breast height (cm), HT = tree height (m), Stain = Percentage fungal staining recorded from mid-crown sampling, W. NEBB gallery density = Weighted NEBB brood gallery density recorded from mid-crown sampling (Galleries per meter squared).

Variable Tested	Groupings	Num. degrees of freedom	Den. Degrees of freedom	F Statistic	p-value
DBH	Neighbourhood	3	24.221	7.384	0.001
HT	Neighbourhood	3	15.877	6.558	0.004
Stain	Neighbourhood	3	16.691	2.03	0.149
W. NEBB gallery density	Neighbourhood	3	56.998	3.291	0.037

Table 6. Games-Howell post-hoc test for Welch's ANOVA, indicating significance or not between each neighbour, for each variable tested. (DBH = Diameter at breast height (cm), HT = tree height (m), Stain = Percentage fungal staining recorded from mid-crown sampling, W. NEBB gallery density = Weighted NEBB brood gallery density recorded from mid-crown sampling (Galleries per meter squared)).

Variable	Tested grouping	p-value
DBH	Crecentwood - Minto	0.034
	Crecentwood - RVLR	1
	Crecentwood - Wolsley	>0.999
	Minto - RVLR	0.015
	Minto - Wolsley	0.731
	RVLR - Wolsley	0.004
HT	Crecentwood - Minto	0.772
	Crecentwood - RVLR	0.317
	Crecentwood - Wolsley	0.239
	Minto - RVLR	0.013
	Minto - Wolsley	0.008
	RVLR - Wolsley	0.921
Stain	Crecentwood - Minto	0.832
	Crecentwood - RVLR	0.998
	Crecentwood - Wolsley	0.9988
	Minto - RVLR	0.329
	Minto - Wolsley	0.119
	RVLR - Wolsley	0.876
W. NEBB Gal. Density	Crecentwood - Minto	0.038
	Crecentwood - RVLR	0.272
	Crecentwood - Wolsley	0.838
	Minto - RVLR	0.158
	Minto - Wolsley	0.054
	RVLR - Wolsley	0.477

NMDS ordination and ANOSIM

NMDS was used to examine variables sampled in 2018 and 2019 only and also for all variables sampled in 2017, 2018, and 2019 to allow all variables to be expressed through ordination. Both NMDS ordinations had optimum stress values with a two-dimensional solution. Ordination for 2017 to 2019 had a stress value of 10.1% with 55 iterations. The ordination for 2018-2019 had a stress value of 13.2% with 54 iterations. Both had a significant Monte Carlo test ($p=0.004$) indicating significant interaction between study trees and test variables. The 2018-2019 ordination had a R^2 value of 0.714 for axis 1, and R^2 value of 0.204 for axis 2, with a cumulative R^2 value between both axes of 0.919. The 2017-2019 ordination had a R^2 value of 0.473 for axis 1 and R^2 of 0.375 for axis 2, with a cumulative R^2 of 0.848 for both axes.

For both the 2018-2019 and 2017-2019 ordinations, the Pearson correlation coefficients for the variables greater than $r^2=0.400$ are reported. Correlations greater than 40% in 2018-2019 ordination were with axis 1: infection sites ($r^2=0.539$), percentage canopy green leaves ($r^2=0.865$), percentage canopy dead branches ($r^2=0.649$), average canopy cover ($r^2=0.517$), and percentage fungal staining in mid-crown canopy samples ($r^2=0.536$). There were correlations greater than 40% in 2017-2019 ordination with both axes. Correlations greater than 40% on Axis 1 included average canopy cover ($r^2=0.414$) and percentage fungal staining in mid-crown canopy samples ($r^2=0.766$). On Axis 2, correlations greater than 40% were percentage canopy dead branches ($r^2=0.498$). DBH, height, and estimated age had no correlations greater than 40% in either ordination.

Average canopy cover and percentage canopy green leaves are found at opposite ends of the ordination space from percentage canopy dead branches and infection sites, both which appear to be colinear. DBH and height appear at the opposite side of the ordination to percentage canopy dead leaves and percentage fungal staining from tree canopy sampling. The grouping of percentage canopy dead branches, percentage canopy dead leaves, both percentage fungal staining and NEBB brood gallery density, and infection sites occupy the same area of the ordination. DBH and height appear closely related in the ordination in addition to average

canopy cover and percentage canopy green leaves, which also appear to be closely associated (Figure 15).

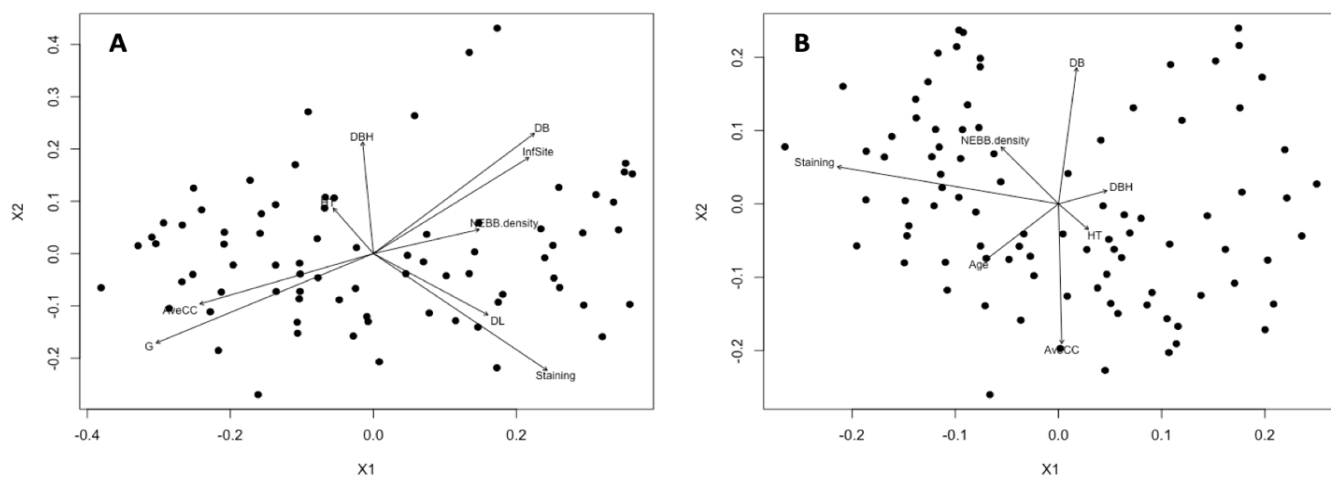


Figure 15. NMDS ordination of DED infected study trees, recoded in Winnipeg, MB. A. Ordination 2018 and 2019, with 7 variables. B. Ordination 2017, 2018, and 2019, with 9 variable vectors. Points in ordination are study trees included in the ordination analysis.

The ANOSIM test of both ordinations by year resulted in a non-significant result for the 2018-2019 ordination ($R=0.037$, $p=0.109$) and a significant result for 2017-2019 ($R=0.080$, $p=0.014$) (Figure 16). An ANOSIM test grouping both ordinations by neighbourhood produced a non-significant result for 2018-2019 ($R=0.039$, $p=0.111$) and a significant result for 2017-2019 ($R=0.085$, $p=0.018$) (Figure 17). An ANOSIM test for grouping by detectable or undetectable NEBB brood gallery densities produced a significant result for both 2018-2019 ($R=0.186$, $p=0.002$) and 2017-2019 ($R=0.203$, $p=0.001$) (Figure 18).

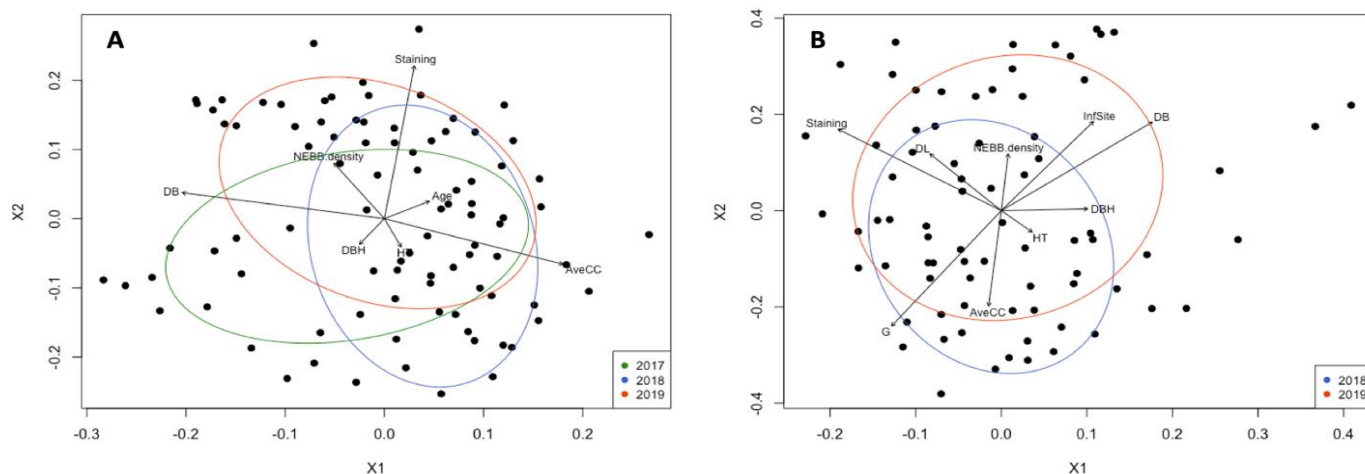


Figure 16. NMDS ordination of DED infected study trees, recoded in Winnipeg, MB. A. Ordination 2017, 2018, and 2019. B. Ordination 2018 and 2019. Points in ordination are study trees included in ordination analysis.

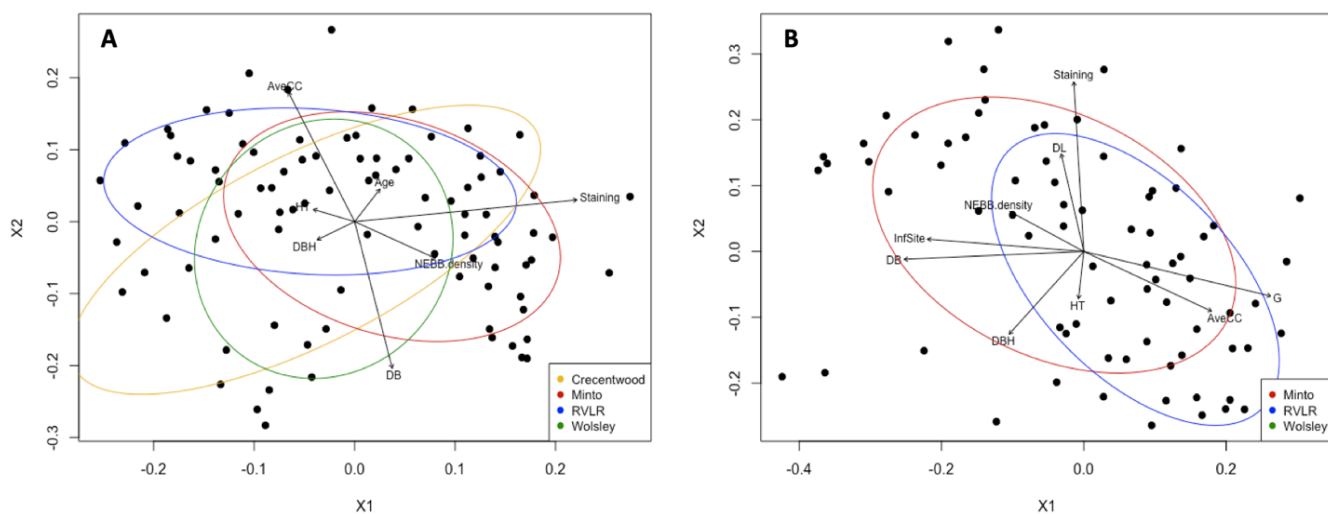


Figure 17. NMDS ordination of DED infected study trees, recoded in Winnipeg, MB., grouped by Neighbourhood. A. Ordination 2017, 2018, and 2019. B. Ordination 2018 and 2019, note that there are three neighbourhood groups and only two grouping circles due to insufficient data points in Wolsley across 2018-2019 to generate an envelope. Points in ordination are study trees included in ordination analysis.

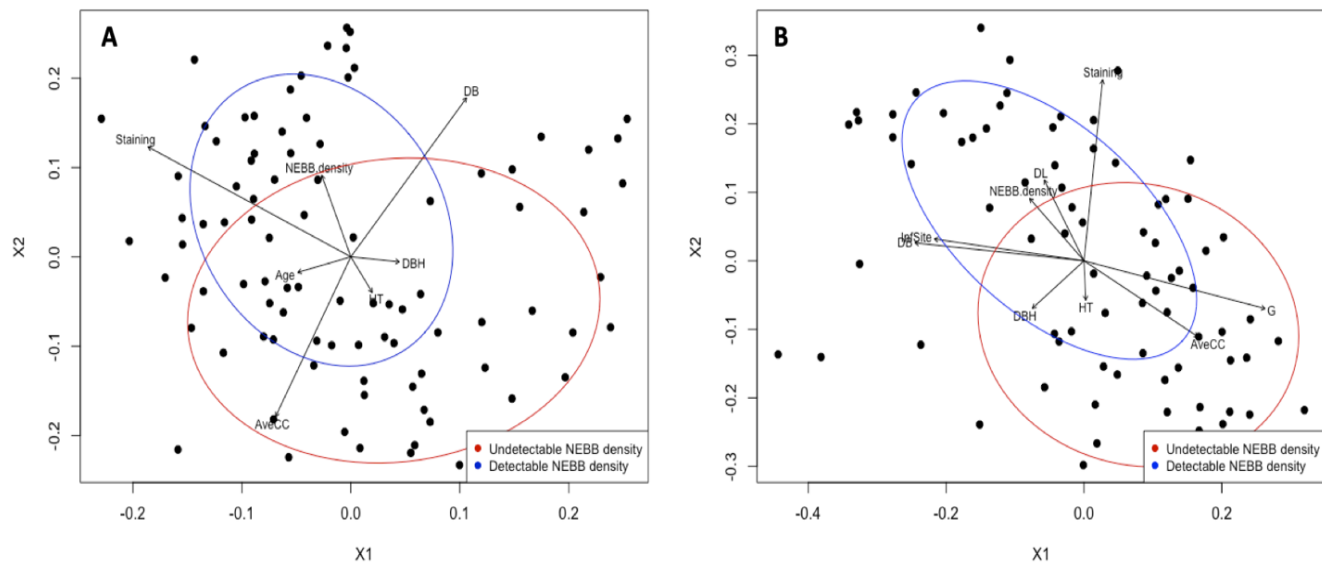


Figure 18. NMDS ordination of DED infected study trees, recoded in Winnipeg, MB., grouped by trees with detectable or undetectable NEBB galleries by density. A. Ordination using data inclusive of 2017, 2018, and 2019. B. Ordination using data inclusive of 2018 and 2019. Points in ordination are study trees included in ordination analysis.

Modelling – Regressions and GLM

Counts of NEBB brood galleries were positively but weakly related to log-transformed weighted fungal staining ($R^2=0.0497$, $p=0.004$) and log-transformed weighted NEBB brood gallery density and log-transformed weighted canopy percentage fungal staining were significant ($R^2=0.0393$, $p=0.0392$). Beta regression between counted NEBB brood galleries, weighted NEBB brood gallery density, and log-transformed NEBB brood gallery density were all significant with p-values <0.001 (Table 7).

Table 7. Beta regression model results. All models compared to weighted fungal staining. For model coefficients, Gallery count = count of NEBB brood galleries on mid-crown sample. Gallery density = weighted NEBB brood gallery density from mid-crown samples, Gallery density (log) = log-transformed weighted NEBB brood gallery density from mid-crown samples.

Coefficients	Estimate	Std. Error	Z-Value	P-value
Gallery Count	0.0051984	0.001	5.377	<0.001
Gal Density	0.42892	0.082	5.256	<0.001
Gal Density (log)	0.71316	0.065	5.282	<0.001

In Generalized linear models, model 1 (weighted percentage fungal staining as a proxy for NEBB brood gallery density as the response variable), the percentage of canopy dead leaves was significant ($p=0.005$). Percentage canopy dead branches was non-significant however it had a relatively low p-value (0.088). Infection site was non-significant in Model 1. In model 2 (weighted percentage fungal staining as proxy for NEBB brood gallery density as the response variable), there was a significant effect of average canopy cover ($p=0.039$). No other variables were significant. In model 3 (detectable/undetectable NEBB brood gallery density as the response variable), there was no significant effect of percentage canopy dead branches or dead leaves but a significant effect of infection sites on the response variable. To test the effect of the rate of change of the canopy variables over the survey season in the models 1 and 3, the same response variable was used with substituted coefficients for the rate equivalent (variable change per week, calculated as shown in the Methods) and showed no significant effect. Results of Models 1, 2, and 3, along with confidence intervals as well as relevant Wald test results, are shown in Table 8.

Table 8. Generalized linear model results. For model coefficients, see Methods: GLM for details. Model 1 and 2 uses weighted percentage fungal staining as a proxy for NEBB gallery density as response variable. Model 3 uses detectable/undetectable NEBB gallery density as response variable.

Coefficients		Estimate	Std. Error	t-value	p-value	Confidence intervals	
						2.50%	97.50%
Model 1	Intercept	-1.246	0.477	-2.613	0.011	-2.180	-0.311
	Canopy Dead Leaves	0.039	0.014	2.899	0.005	0.013	0.066
	Canopy Dead Branches	0.020	0.011	1.728	0.088	-0.003	0.042
	Infection Sites	0.075	0.178	0.423	0.673	-0.273	0.423
Model 2	Intercept	0.906	1.584	0.572	0.567	2.198	4.010
	Canopy Dead Leaves	-0.007	0.016	-0.436	0.663	0.039	0.024
	Canopy Dead Branches	-0.043	0.020	-2.158	0.031	0.083	0.004
	Infection Sites	0.310	0.266	1.166	0.244	0.211	0.832
Model 3	Intercept	-1.878	0.718	-2.615	0.009	-3.285	-0.470
	Canopy Dead Leaves	0.028	0.018	1.499	0.134	-0.008	0.640
	Canopy Dead Branches	0.002	0.017	0.139	0.889	-0.031	0.036
	Infection Sites	0.526	0.267	1.968	0.049	0.002	1.049

		Chi-sq.	df	p-value
Model 1		15.1	3	0.002
Model 2	Wald Test	83.5	3	<0.001
Model 3		7.9	3	0.048

Beetle capture

Trapping results for the 123 traps placed in 2018 captured 22 NEBB (14 of which showed fungal growth typical of *O. novo ulmi*). The most beetles captured on a single trap were five. Fourteen traps accounted for the 22 NEBB captures, and there was only one instance of multiple traps at a single tree capturing multiple NEBB. The overall capture number was insufficient to provide any meaningful further analysis. In 2019, the beetle traps captured only four beetles and were not plated for *O. novo-ulmi* testing or assessed further.

Debarking

Trunk debarking during the summer 2017 (N = 87) identified 10 trees with NEBB brood galleries. Of these 10 trees, four were tagged as being infected with DED in August 2016 or

approximately one year before the pilot study began. These four trees most likely had NEBB breeding for one year before assessments began in the summer of 2017. These 2016 tagged trees had minimal canopy cover in 2017, with 75 -100% missing leaves (dead or yellow) and greater than 75% deadwood. Three of the 2016 tagged trees contained galleries in both the trunk and canopy.

Ten DED-infected trees identified in 2017 (as part of the canopy symptom response survey) were left to overwinter and trunk debarked during the spring 2018. Seven of these trees contained NEBB galleries in the canopy and two contained galleries in the trunk. Another 36 trees tagged in both 2016 (N=3) and 2017 (N=33) were debarked in spring 2018 after overwintering without canopy symptoms recorded in the summer of 2017. The seven trees with canopy branch data showed no correlation between trunk gallery density, average galleries, and average staining with canopy closure.

Branch samples were removed from 48 trees by University of Winnipeg staff accompanying the City of Winnipeg crews in the summer 2017. Twenty-two of these trees contained galleries in the canopy (45.8%). In 2017, the trees with the most galleries included the four trees tagged for DED infection in 2016. There was no correlation between canopy closure and trunk bark moisture (N = 69) and trunk cambium moisture (N = 52). There was also no correlation between bark cambium moisture and percent deadwood in the canopy (N = 48 and N = 34, respectively). Similarly, there was no significant relationship between both the percentage dead and percentage yellow leaves in the canopy with either cambium branch or trunk DED staining. However, there was a positive significant relationship between trunk and branch galleries ($R^2 = 0.869$, $P = 0.005$, $N = 8$) although the sample size was small as few trees actually had trunk galleries. It does appear that when trunk galleries are present, canopy branches will also have NEBB beetle galleries.

Linear regressions comparing correlations between trunk and canopy NEBB brood galleries showed no significant difference for 3 of the 4 models. Only the untransformed and unweighted linear regression between NEBB brood galleries in the trunk and canopy showed significant differences ($R^2=0.081$, $p=0.036$). Linear regressions are displayed in Figure 19, with p-values and R^2 values in Table 6.3.

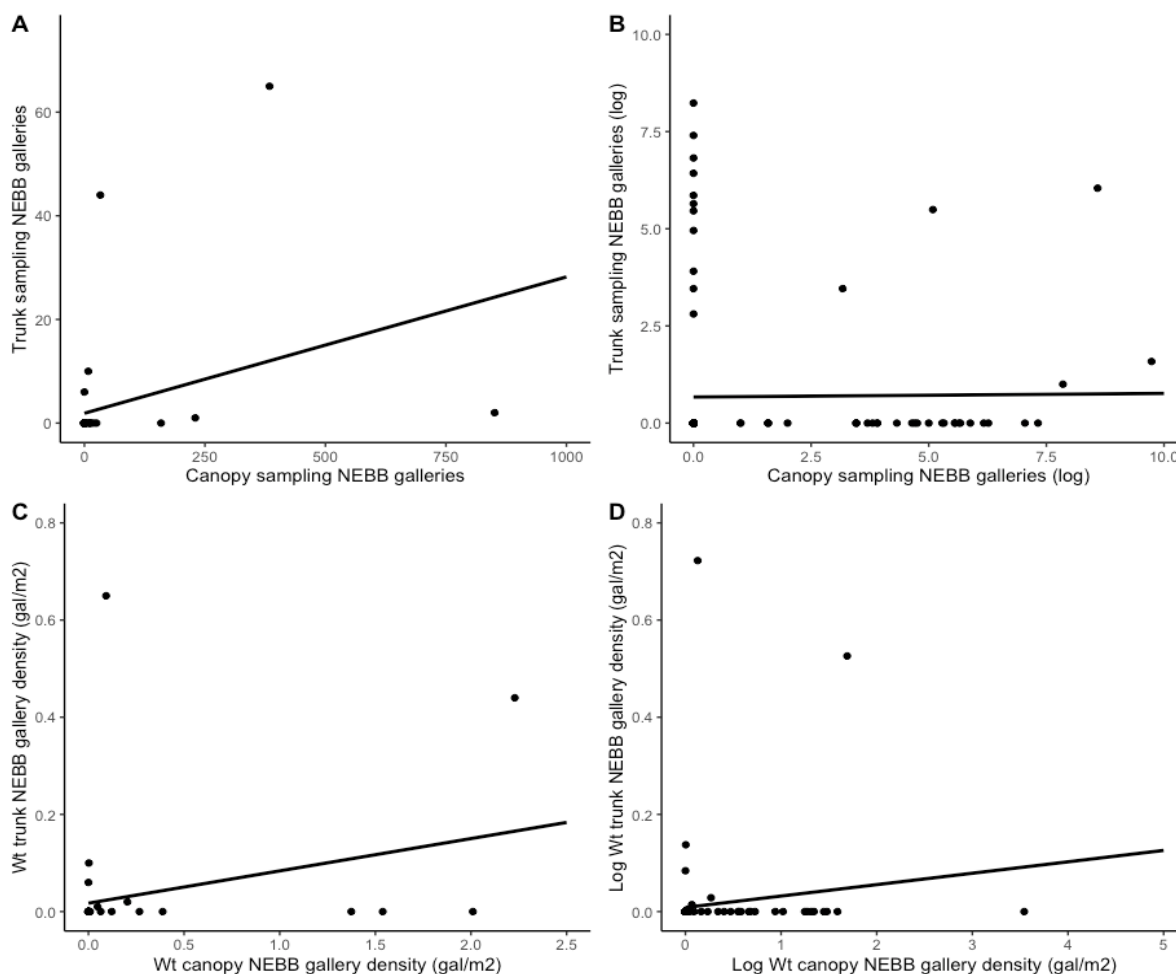


Figure 19. Linear regressions of 2017 trunk and canopy NEBB brood galleries. A: untransformed and unweight trunk and canopy NEBB brood galleries. B: Log transformed unweighted trunk and canopy NEBB brood galleries. C: Weighted trunk and canopy NEBB gallery densities, untransformed. D: Weighted trunk and canopy NEBB brood gallery density, log transformed.

Proportion of elm trees containing NEBB

Figure 20 is constructed to compare the galleries found in Holliday (2016) with our study. Each point represents a study tree in either mine or Holliday's study, with the y-axis showing the number of NEBB galleries found in that study tree. Both studies follow a very similar trend, and only the top 50 trees from both studies are shown. Holliday (2016) reported 90% of NEBB brood galleries found in mid-crown sampling from 13% of study trees, whereas we found 74% of NEBB brood galleries found in approximately 10% of study trees.

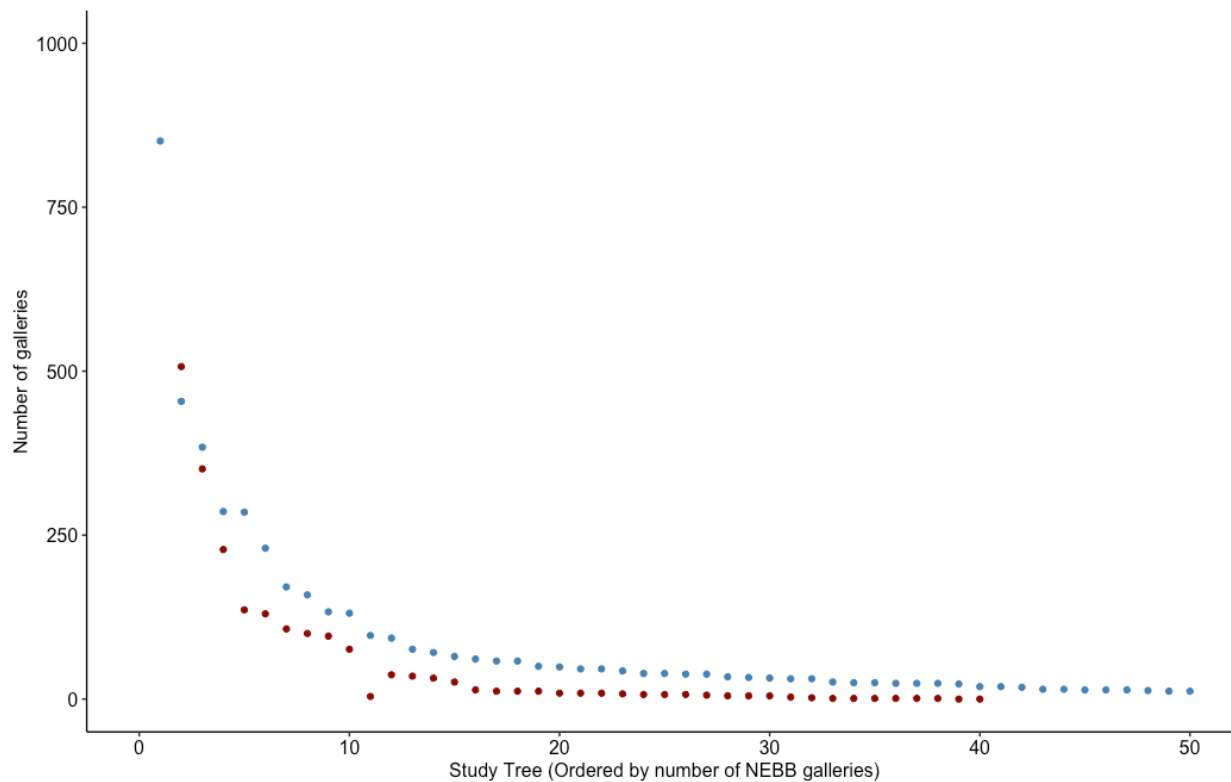


Figure 20. Scatterplot comparing Holliday (2016) and the current study. Each point represents a tree in current study (Blue points) or Holliday's (Red points) study, ordered from left to right by number of galleries found in mid-crown sampling.

CHAPTER 5 – DISCUSSION

Comparison to Holliday's (2016) study

The initial results reported by Holliday (2016) provided a base line to further investigate the question that drove this research: Does a subset of DED-infected elm trees harbour the majority of NEBB brood galleries and produce most of the adult beetles in the fall? Holliday reported that 90% of NEBB brood galleries were found in only 13% of DED-infected elm trees (n=60). Using NEBB galleries recorded from our canopy branch sampling, I found that 74% of NEBB brood galleries in 15% of the sampled trees.

Differences between the two studies as to the percentage of infected trees with brood galleries may be attributed to the differences in the elm tree populations. The average DBH over my three-year study was $64.7 \pm \text{SD}=13.7$ cm compared to $28.7\text{cm} \pm 11.1$ cm for Holliday (2016). The 60 trees included by Holliday (2016) (collected in 2013 and 2015) included 33 trees from public boulevards in Selkirk, Manitoba, with the rest being from Selkirk Park and the community of Headingly, Manitoba. There were 355 trees examined in my study. Both Holliday (2016) and my results indicate that DBH is not correlated with the number of NEBB brood galleries (trunk and canopy galleries). There were fewer trees in Holliday's study than this study, and a little over half of these were park/non-boulevard trees. Thus, Holliday's findings may not be directly comparable with my results for large DBH trees although the trends are similar. The differences in the number of trees with NEBB brood galleries may be due to differences in tree size, location, and sample size between Holliday (2016) and the current study. My study was designed to be more representative of mature elm trees in an urban environment, especially on boulevards. In my study the 100 trees with canopy samples are quite representative of Winnipeg's urban elm forest in many neighbourhoods and are representative of average tree DBH and height of a typical boulevard elm tree in most older neighbourhoods in the city.

Holliday (2016) did not report actual year of DED infection for study trees so it is unknown whether any trees had the disease for more than 2 to 3 months (perhaps some sample trees survived to the second year with the infection before succumbing), thus direct comparison to my study where the date of recorded infection was known may also reflect differences in brood gallery presence. I observed several infected elm trees identified the year before removal (*i.e.*, identified in summer 2016 and removed 2017) that produced large amounts of NEBB brood

galleries in the mid-crown canopy alongside evidence of galleries on the trunk. As such, overwintered trees that were not removed until many months after infection could significantly skew reported NEBB brood galleries and brood gallery density and thus were not included in my analysis.

Assessment of canopy variables

Assessment of potential indicators of infected elm trees harbouring large densities of NEBB brood galleries started with assessing the trends of each canopy and non-canopy variable on a weekly and monthly basis. Most canopy variables, including percentage canopy green leaves, percentage canopy dead leaves, percentage canopy dead branches, and pole pruning fungal percentage showed a progression of symptom development when comparing the start to the end of surveying. When examined individually over the sample period in each year, the degree of change for most of the variables was not significant after the first week or two. Average canopy cover was the only weekly variable that differed significantly in response to study year. Average canopy cover was considerably lower in 2019 compared to 2017 and 2018. The last four weeks of the study in 2019 had a wide range in canopy closure measurements between trees when compared with the lower variability noted in 2017 and 2018.

The inconsistency in average canopy cover could be attributed to several causes. Average canopy cover can be challenging to measure on boulevards for elm trees. Since measurements are recorded at the base of the tree, changes or lack thereof in the lower canopy of an elm tree will primarily influence the recorded canopy cover and can be less representative of the mid- and upper canopy than the lower canopy. Secondly, the increase in canopy cover variance in 2019 could be a result of multiple surveyors recording canopy cover. While estimating canopy cover appeared to be fairly consistent within years, there were several new surveyors between years and new trees each year, therefore the potential exists for user bias, especially between study years. It should be noted however, that average canopy cover is the only canopy variable to show a significant difference between recorded values between years.

Recent studies describe the spherical densiometer as an inexpensive and suitable alternative to more expensive and complicated methods to measure canopy density (Baudry and Charmetant 2013; Baudry et al. 2014; Russavage et al. 2021). However, some authors have

suggested that densiometers yield large between-observer biases and inconsistent canopy cover estimates between individual observers compared to other techniques and instruments (Vales and Bunnell 1988). Densiometers may have more merit in research studies focused on measuring canopy cover in natural forests compared to single trees in urban forests where inexpensive and rapid methods for large areas are required. The use of spherical densiometers for very thin canopies, as are seen in DED-infected elm trees with extensive dieback, has been suggested to result in a greater error than when measuring true canopy cover in natural forests (Vora 1988). Densiometers have also been reported to overestimate canopy cover (Cook et al 1995; Nuttle 1997; Prasad et al. 2018), especially when cover ranges between 35-70% (Ko et al. 2009), which can be further exacerbated in very open environments (such as boulevard measurements in an urban forest) (Jennings et al. 1999). Average canopy cover comparisons using a densiometer between years should be used with some caution given that user bias between years and different trees being measured may add additional variation. To help remedy the potential inconsistencies of densiometer measurements, I measured tree canopy cover in all four cardinal directions.

Percentage canopy dead leaves and yellow leaves (measured in 2017) was combined into one measurement for 2018 and 2019 to increase the accuracy of estimating this variable. While yellow leaves are an accepted indicator of DED infection, they can often be similar to dead leaves in the canopy. Estimation of the two variables together removes the uncertainty that comes from trying to distinguish between canopy dead leaves and canopy yellow leaves.

Preliminary regressions completed in a report to the City of Winnipeg prior to the 2018 study season (see 4.5 2017 preliminary results) showed no significant correlation between either cambium moisture or light intensity and the other surveyed canopy variables. Furthermore, there was no correlation between either cambium moisture or light intensity and NEBB brood galleries or percentage fungal staining in either trunk or canopy samples. Cambium samples were taken during the infection period of some study elm trees to determine whether removal of lower trunk bark and cambium impacted the progression of external DED symptoms, but no correlation with tree moisture content was found. Bark removal, while extensive and completely encompassing the trunk in this study, appears to provide less disruption to the movement of water than the use of chain saw plunge cuts or frill cuts around the circumference of the tree (Pines and Westwood 1996). Neither light intensity nor cambium moisture showed any potential as predictors of NEBB brood galleries, and similarly, cambium moisture showed no correlation with DED infection or

levels of NEBB brood infestation in this study. The method and amount of trunk debarking in this study appeared to have no measurable effect on trunk moisture content.

In comparison to differences in variables from year to year, assessing canopy variables in trees with and without detectable levels of NEBB brood galleries yielded several useful indicators of the level of beetle activity within trees. Differences in this grouping within observed canopy variables were small, however, it is important to note that the percentage of green leaves, average canopy cover, and percentage of dead branches were all higher in trees with detectable NEBB brood gallery densities than those without. It might be worth modelling canopy variables together to see whether there is an over-arching trend between canopy variables and detectable/undetectable NEBB brood galleries densities.

Tree DBH, height, and estimated age in comparison to canopy variables showed little significance in relation to NEBB brood galleries nor percentage fungal staining from canopy samples. This is most likely due to the consistent size of trees included in the present study. Most boulevard trees surveyed in a broad sense were similar, and trees that were excessively small (and not representative of a typical American Elm boulevard tree) were not sampled. As tree DBH, height, and estimated age were not significantly different for explaining staining or beetle gallery density, they were not included in the beetle infestation models.

We can assume that the study population represented a typical sampling of urban boulevard American elm trees (based on DBH, height, and estimated age) in many older Winnipeg neighbourhoods. Furthermore, the expression of DED symptoms in the neighbourhoods themselves was consistent, thus neighbourhood was not included as a variable in further modelling. It should be noted that the neighbourhoods were concentrated in south-central Winnipeg, and future surveys of a more diverse selection of neighbourhoods would help confirm or modify what we believe is typical tree DBH, height, and age for a Winnipeg neighbourhood (Neighbourhoods ranged from being directly adjacent to approximately 5km apart).

Variable validity for modelling

Results of both the 2017-2019 and 2018-2019 NMDS ordinations helped guide my choice of potential variables used in GLM modelling and as criteria for rapid tree removal. Both

ordinations had acceptable stress levels (10.1% and 13.2% respectively) with 2017-2019 results having 84.8% of ordination explained on both axes and 2018-2019 having 91.9% explained on both axes. Low stress, along with visual assessment of stress plots, indicated ordinations provided useful guidance on choosing potential variables to predict beetle gallery density. Both ordinations had significant Monte Carlo permutation tests indicating an overall significant relationship between canopy variables and DED-infected study trees.

In both ordinations, average canopy cover, percentage canopy green leaves, percentage canopy dead branches, and percentage fungal staining from mid-crown sampling were significant (Pearson correlation) with the ordination axis. Infection sites were significant in the 2018-2019 ordination indicating a positive relationship between infection sites and presence/absence of NEBB brood galleries in mid-crown sampling. These canopy variables all showed potential as criteria for predicting beetle presence or absence in the model building process. Ordination vectors in NMDS ordination space showed that average canopy cover and percentage canopy green leaves were co-linear with percentage canopy dead branches (and percentage canopy dead leaves, however this showed low correlation in ordination space). Percentage canopy green leaves and percentage canopy dead branches are intuitively co-linear since canopy dead branches increase with a decrease in canopy green leaves. As these two variables were co-linear in the GLM analysis, I used average canopy cover/percentage canopy green leaves in one model (model 2) and percentage canopy dead branches/dead leaves in another (Models 1 and 3).

Analysis of similarities (ANOSIM) served to compare whether groupings of the data showed significant differences in NMDS ordination. Detectable/undetectable NEBB brood gallery densities showed a significant value in ANOSIM indicating that study trees in one of these two groups have more in common within their group than between groups. The difference lends credence to exploring the detectable/undetectable tree categories in further modelling and exploring which canopy variables can potentially predict which trees will fall into detectable or undetectable NEBB brood gallery density categories. In comparison, ANOSIM showed no significance when assessing study trees grouped by year or neighbourhood, except for 2017-2019, which showed significance when assessing the neighbourhood. This is likely due to the inclusion of Crescentwood, which has a very small sample size and would affect the ANOSIM result. Non-significant results indicate that there is equal or more similarity between groups than within. Non-significance in ANOSIM for both ordination by year, and in 2018-2019 ordination

for neighbourhood, indicates that both these groupings do not need to be included as random effects in modelling, and that there were no major differences in study trees between years or between neighbourhoods.

For development of the GLM Models 1 to 3 weighted percentage canopy fungal staining was used as a proxy for weighted NEBB brood gallery density. While beetle gallery density was the main variable I tried to predict, fungal staining, as the response variable, produced more reliable results. The skewed nature of NEBB gallery data weighted or count data was greater than canopy percentage fungal staining. Furthermore, when testing correlations between fungal staining and NEBB brood gallery count and density, three of the four tested linear regressions were significant. The only non-significant regression (with a p-value of 0.07) was non-log transformed, and as described by Holliday (2016), regressions comparing fungal staining and NEBB brood galleries (count or density) should be log-transformed as the data distribution becomes less skewed and better represented in the linear regression.

As discussed, the co-linear relationships between canopy dead branches/dead leaves and canopy green leaves/average canopy cover dictated that only one of these groupings should be included in the generalized linear model. Both canopy percentage dead leaves and canopy percentage green leaves showed strong Pearson correlations in ordination space, and both trended towards groupings of detectable and undetectable NEBB brood gallery densities. Both showed equal probability of being successful predictors of levels of NEBB colonization. Average canopy cover showed strong Pearson correlation in ordination space in both run ordinations (2017-2019 and 2018-2019) and had higher r^2 values from the Pearson correlation in comparison to r^2 values from percentage canopy dead leaves. From this we can predict that percentage canopy dead leaves and percentage of green leaves reflect the presence of increased NEBB gallery densities in infected trees. While average canopy cover may be a stronger predictor the issues with densiometer measured canopy cover still remain.

Average canopy cover had greatest variance as described previously and is perhaps a less suitable candidate for predicting beetle gallery density when compared to the more consistent measurements of percentage canopy dead leaves. To cover both options for Models 1 and 2, each included one of these groups (Model 1, canopy percentage dead leaves/canopy percentage dead branches and Model 2, canopy percentage green leaves and average canopy cover). Results of

the Model (Table 5) indicated that the use of canopy dead leaves and canopy dead branches were the more suitable as both had low p-values whereas Model 2 coefficients only showed significance with average canopy cover.

When coefficients in Model 1 and 3 were replaced with their equivalent rate of change per week, there was no significance in the tested model suggesting that the speed of variable change after DED infection does not indicate the severity of infection. In regard to the disease progression survey and its implementation as a protocol to diagnose high density NEBB brood trees, variable assessment could be limited to one assessment.

Model 3 assessed how the coefficients deemed most suitable from Models 1 and 2 relate to grouping DED-infected elm trees into with or without a detectable levels of NEBB brood gallery categories. Model 3 only showed significance in infection sites recorded on the initial visit by a surveyor. While both ordinations showed a significant difference in detectable/undetectable NEBB brood galleries, both canopy variables (percentage canopy dead leaves and percentage canopy dead branches) did not show potential as predictors of trees with detectable NEBB beetle gallery densities. With only infection sites indicative of whether or not an infected tree had NEBB galleries at a detectable level, it should be included in criteria for rapid removal.

Assessment of non-canopy variables

In the present study, trunk debarking was not useful as a diagnostic criterium for predicting beetle density. The results from trunk debarking and subsequent mid-crown sampling were quite different. Firstly, trunk galleries are not readily found in infected elm trees (at least within several months after the initial infection), with few infected trees having any evidence of trunk galleries. Of a total of 45 trees with trunk debarking, only 13% (n=6) contained trunk galleries. Compared to the 59% (n=59) of 100 trees with mid-crown samples that yielded NEBB brood galleries. These results are consistent with Holliday (2016) that NEBB primarily colonize the upper canopy on initial infection.

In addition, trunk debarking represents a significant investment of time and financial cost when compared to observing the canopy as a potential criterion for rapid removal. Trunk

debarking, especially for large DBH elm trees (which make up the majority of boulevard trees in many neighbourhoods in Winnipeg) represent a significant time and labour commitment to debark and would not be feasible nor financially practical to implement on a city-wide basis. Removing a comparatively small proportion of bark off the infected elm did not reveal the presence of galleries. It should be noted the areas debarked on each tree in this study were on average greater than areas debarked in Holliday (2016) and did still not prove useful for finding galleries. Similar to Holliday (2016), I found no significant effect of cardinal direction on the number of trunk galleries, however with so few galleries found in trunks, this trend may just not have been evident.

Linear regression correlating trunk and canopy NEBB brood galleries (results reported in Table 6) were run similar to Holliday (2016) to assess whether trunk galleries were correlated with mid-crown canopy galleries. Log-transformed models were included since they reduced the effect of trees with excessively high NEBB brood galleries in either the trunk or canopy. While log-transformed data are preferable when comparing trunk and canopy NEBB brood galleries, they represent added complexity when the log-transformed results are translated into practical guidelines to identify elm trees for rapid removal in the field. All the regressions between canopy and trunk NEBB brood galleries, either log-transformed or weighted density for NEBB brood galleries, showed no significant difference except for the untransformed and unweighted NEBB brood galleries for both branch and trunk. As mentioned above, untransformed regression allowed study trees with excessively large NEBB brood galleries to adversely affect the outcome. Results of both log-transformed and weighted NEBB brood gallery densities were more reliable as study trees with excessively large NEBB brood galleries had reduced influence on the regression results. Since the only regression showing significant correlation between trunk and canopy NEBB brood galleries was non-log transformed and used unweighted NEBB brood gallery density, there is likely very little to no relationship between trunk and canopy galleries in the first year after DED infection exists. Reasons for this trend may be explained by NEBB colonizing the canopy during the initial infection disproportionately compared to the trunk and studies show the majority of NEBB brood galleries are found in the mid-crown (Pines and Westwood 1996; Swedenborg et al. 1998; Oghiake and Holliday 2011). My results suggest that trunk debarking is not a reliable criterion to predict mid-crown canopy galleries and is not satisfactory as a criterion to determine rapid tree removal.

GLM Modelling discussion

Model 1, with the percentage of canopy dead branches and percentage of canopy dead leaves, showed potential as diagnostic criteria. While percentage canopy dead branches showed a marginally non-significant p-value (0.088) in Model 1, it could still be considered as a potential diagnostic variable since canopy dead branches (or dieback) have been shown to relate to beetle infestation densities in other bark beetle species (Anulewicz et al. 2007; Ellison et al 2020). Further, DED-infected elm trees with significant portions of dead wood serve as suitable habitat for NEBB adults, and from my observations, DED-infected trees left standing until the next summer have canopies fully dead supporting a large number of beetles (2016 tagged trees removed in 2017). While not strictly significant here, it still warrants inclusion, especially since adherence to strict p-values in ecological modelling is debated. Moran (2003) suggests rejecting strict adherence to the Bonferroni rule in ecological studies due to imposing awkward constraints, reducing the freedom of the researcher to interpret data more freely and logically, and avoiding the loss of potentially meaningful results. Past debate on adherence to strict p-values has been a subject of controversy with various articles arguing against strict adherence (Perneger 1998; Perneger 1999; Cabin and Mitchell 2000; Feise 2002; Garcia 2004). Further arguments suggest strict adherence to ‘significant’ p-values results in science that is harder to reproduce and can result in publication bias (Amrhein et al. 2017). In the present case, removing such a relatively low p-value (0.088) from consideration, even with ecological explanation, could be deleterious at such an early stage of the research into rapid removal criteria.

Model 2 supports average canopy cover as a potentially diagnostic criterion. The unreliable nature of densiometer measured canopy cover makes the use of average canopy cover to predict NEBB brood galleries less reliable perhaps because of potential surveyor bias. Model 3 demonstrated that infection sites were a suitable predictor of whether a tree had NEBB brood galleries at a detectable level. Finally, the model rate of canopy variables used in Models 1 and 3 showed no significance in the rate of change in any variable in relation to internal NEBB brood galleries. From these results, I arrived at a suite of predictors that could be used in rapid-removal assessment, namely percentage canopy dead leaves, dead branches, and infection sites can all

contribute to a set of criteria for rapid removal of potential high NEBB brood gallery density infected elm trees.

Translating these three variables into exact cut-off points for the decision to target a tree for rapid removal would be challenging as I did not discover a variable that could provide a direct correlation between the measures that predict the actual density of beetle galleries required for a tree to be removed. Rather, I can strongly infer that if beetle galleries are found throughout the canopy (and there is coincidentally large percentages of fungal staining from canopy samples), then based on my results these trees are most likely candidates for rapid removal. The presence of beetle galleries in the canopy based on a relatively small sample of canopy branches indicated that beetle galleries were most likely well distributed in the canopy. I recommend using all three variables to assign a priority category to each infected elm tree in the City of Winnipeg. This will create groups of priority trees by neighbourhood so that trees can be removed as the resources of the City of Winnipeg Forestry department allows. It is not feasible to provide distinct cut-offs for the model variables that will predict large NEBB brood gallery densities. My recommendations include using three diagnostic criteria to predict NEBB brood gallery density and determine which trees can be assigned a priority for removal.

Since NEBB brood gallery density data had a left skewed data distribution, modelling was unable to provide meaningful results, suggesting that percentage fungal staining be used as a comparable variable. Holliday (2016) and my results both show a correlation between these two variables, however due to the data distributions of both, the comparison should be used cautiously. Beta regressions using the percentage fungal staining as the dependent variable were significant and further support the correlation of percentage fungal staining and NEBB brood galleries and gallery density in mid-crown sampling. These results should be interpreted with some caution since my model used the percentage fungal staining directly from canopy samples of infected trees predicted to have high fungal staining. Previous work (Webber 1990; Faccoli and Battisti 1997; Six and Bentz 2007) as well as the results here on the percentage of NEBB carrying *Ophiostoma* spores indicates that it may take a large amount of NEBB attacking a tree to result in high fungal staining in canopy samples, at least in the initial stages of infection. It is a fairly safe assumption that trees with large amounts of staining would also have a high density of NEBB brood galleries trees. Thus, when assigning trees to a priority will likely include trees with high NEBB brood gallery density.

Next steps with City of Winnipeg

Various components of this research were implemented into the DED detection survey over the summer of 2021 in collaboration with the City of Winnipeg to determine whether the results of the current study could be used to implement practical DED rapid removal protocols for infected elm trees. Each of the three best potential diagnostic variables were included in the assessment made by DED field inspection crews upon initial identification of an American elm tree infected with DED. Infection sites, percentage canopy dead leaves, and percentage dead branches in the canopy were estimated. However, to maintain consistency and maximize time efficiency, the measures of percentage canopy dead leaves and canopy dead branches were divided into six categories for recording. The first category included trees with no evidence of the three variables ranging to the sixth category with trees having extensive evidence of advanced conditions of all three diagnostic variables. The remaining categories were chosen as a range of percentages (1-25%, 26-50%, 51-75%, and 76-99%). It was thought that these groupings would reduce the impact of individual bias in estimating the canopy and help maintain consistency across surveyors. Going forward, the City will record infection sites as described initially here, with a maximum assignable infection site value of six to match the groupings of percentage canopy dead leaves and dead branches.

Using these six groupings, it is proposed that each tree receive a numerical value, equal to a sum of the infection sites, and the categories of both percentage canopy dead leaves and percentage canopy dead branches. The value of the categories across the three canopy variables assessed will be summed, and trees with the highest resulting value will have first priority for removal, and will be removed as soon as City of Winnipeg crews are able to do so. This will allow the City to remove trees first that are likely to contain a high density of NEBB brood galleries with available time and resources. An exception to this priority will come from the results here and Holliday (2016) where both studies identified trees as DED-infected with large numbers of NEBB brood galleries that were not removed until the following spring. Any trees that are not removed until the spring of the year following when they were tagged should be removed before trees found with new DED infections as they are likely to harbour large amounts

of NEBB brood, and in the case of Holliday (2016), were significant outliers to the rest of the data set.

The pilot test by the City of Winnipeg in 2021 will be used to determine the effectiveness of the criteria for rapid removal for permanent inclusion in the operational program of the City. After infected DED trees are identified over the summer, trees with the highest total scores (infections sites, percentage canopy dead branches, and percentage canopy dead leaves) will have samples removed from their canopy following the protocol described in this study. This will provide an opportunity to assess if the predicted high-density NEBB brood gallery trees do in fact contain large densities of NEBB brood galleries and corroborate the findings of my work. If the pilot test by the City of Winnipeg is successful, then this protocol for rapid removal of high density NEBB brood gallery containing infected DED trees will become an additional tool in the City's current IPM program for DED management.

NEBB beetle capture

The primary goal of using sticky traps to capture NEBB adults dispersing to overwintering sites was to determine whether traps in the vicinity of trees with larger NEBB brood gallery densities would result in larger beetle captures than those close to trees with lower NEBB brood gallery densities. Unfortunately, few adult NEBB were caught thus no meaningful analysis could be done to answer this question.

The trapping method used to capture NEBB adults dispersing to overwintering sites resulted in low capture rates. While both pheromones and host volatiles have been suggested as potential attraction mechanisms (Gardiner 1979; Peacock 1979; Miller et al. 1986), no chemical lures have proven to increase capture rates of NEBB adults compared to diseased elm wood (Miller et al. 1986). *Ophiostoma novo-ulmi* up-regulates semiochemical production to attract adult NEBB (Pines and Westwood 2008), however the use of attractants with traps has proven ineffective (Miller et al. 1986). In conjunction, necrotic lesions formed by American elm trees in response to *O. novo-ulmi* infection (Raffa 1988) may make diseased elm trees more attractive to NEBB adults than our traps. Lastly, weather during both trapping periods likely increased the likelihood of poor capture. Significant storms after traps were placed in both 2018 and 2019 resulted in many traps having to be replaced or lost before they could be collected.

While NEBB monitoring was unsuccessful in capturing large numbers of adults, the percentage of captured NEBB contaminated with *O.novo-ulmi* spores provided useful data. While past studies have not focused on the proportion of NEBB carrying *O. novo-ulmi* spores, the percentage of *Scolytus scolytus* carrying DED spores has been reported to be as few as 6%, and as high as 96% (Webber 1990). While not a focus of my project, 63% of the beetles captured here carried *O.novo-ulmi*, which is comparable to Faccoli and Battisti (1997) who found 58% of *S. multistriatus* and *S. pygmaeus* carried *Ophiostoma* spores. Compared to Oghiakhe and Holliday (2011), my findings for the percentage NEBB carrying *O. novo-ulmi* spores was higher as they found between 37.6% and 47.4% in captured NEBB in 2008. The higher rate of *O. novo-ulmi* fungal spores contamination on NEBB here is likely also due to the capture period, as beetles captured in the fall (in lower temperatures than summer) have been shown to have higher percentages of carrying *Ophiostoma* fungal spores (Faccoli and Battisti 1997).

Limitations

Various limitations were present in my study that may have influenced the data I collected including the total sample size of trees. The difficulty in collecting large numbers of mid-canopy branch samples proved to be a limitation in maximising the number of samples that could be assessed for brood galleries, thus the estimation of the presence and abundance of NEBB brood galleries could have been improved. While more than 300 trees were surveyed for external DED indicator variables over three study years, not all trees had mid-crown canopy samples removed due to the logistics of having the City of Winnipeg and contract bucket crews available. To increase the number of samples obtained in 2019, bark removal was done both in the field and also on branches that were stored indoors to be debarked and processed later. Previously, all debarking occurred in real time in the field at the time the tree was removed. However, it is recommended that processing of canopy branch samples be done in the field as fungal staining is easier to assess immediately after tree takedown when the wood is still “wet”.

Annual modifications to the sampling protocol over the three study years resulted in some data not being used in the analysis. The first year (2017) was a pilot study and certain variables were not assessed (example: no infection site data was collected in 2017) while trunk bark sampling was discontinued in subsequent years. The recording of certain predictor variable

as a single percentage (example: canopy closure = 50%) rather than placing the measure into a category may have been less useful in assessing trees when a variety of surveyors could be involved in a future operational survey. This issue was partially addressed in 2021 as the City of Winnipeg survey crews moved to a sliding range category to measure percentage canopy dead branches and dead leaves. Since the majority of variables included in the present study were estimated and not quantitative measures, there is a degree of error to be expected with multiple surveyors involved in collecting the data.

CHAPTER 9 - CONCLUSION

The results from my study will allow the City of Winnipeg Forestry department to continue field testing of the protocol and for confirmation of these recommendations. City-wide tree surveying using a consistent study protocol to identify and rapidly remove specific trees in the future should confirm the results of the present study. Surveying of all neighbourhoods with elms using the rapid removal diagnostic protocol in Winnipeg will provide a wider range of growth habits for elm. Removal of trees following subsequent application of our suggested protocol and assessing canopy brood gallery density can be used to validate the models.

With the shortcomings of many existing DED control methods, removal of DED trees that are predicted to harbour high NEBB levels could provide a major decrease in the overall intensity of a DED outbreak over time, thus reducing resources needed to control the disease. Since the survey of disease symptoms is straightforward and can be incorporated into existing surveys, economic costs to implement are likely to be minimal.

Identifying DED-infected elm trees harbouring large NEBB populations for rapid removal is a complex process that is simplified through the findings of my study. Models presented along with supporting analysis provide a method for improving DED management in Winnipeg's urban forest by removing large numbers of DED vectors before they can emerge to infect new trees.

Based on the canopy variables assessed, indicators of poor canopy health are correlated with larger beetle infestations that allow for the best designation of high NEBB population trees. Visual estimation of the percentage presence of several canopy condition indicators (Canopy dead leaves, dead branches and infection sites) can be deemed sufficient to determine which trees may be good candidates for priority removal before beetle emergence in the fall. The boulevard elm trees in the current study neighbourhoods were similar in age and size. I saw no evidence of NEBB preferring smaller or larger trees, which suggests that tree DBH and height are not practical indicators of NEBB brood gallery density.

I developed models using percentage canopy dead leaves and dead branches along with the number of DED infection sites to assess the distribution of fungal staining found in the tree canopy. Canopy fungal staining showed a significant correlation with the presence of NEBB

brood galleries suggesting that when canopy NEBB galleries are found, these trees have significant potential to harbour numerous NEBB. While a sliding scale of NEBB gallery density could not be developed that correlate with large numbers of beetles, being able to classify trees as either positive or negative for the presence of NEBB should be sufficient to identify those for rapid removal. The results of my study demonstrate that within an urban forest in Winnipeg neighbourhoods where elms are the dominant species, there exists a range of infected trees (10.649 gal/m² to approximately 1.000 gal/m²) that harbour a significant portion of the beetle population. Removal of all or some of these trees prior to beetle emergence in the fall should reduce the number of newly-infected trees in the year following removal.

At the time of completion for this thesis, further field studies by the City of Winnipeg Forestry Branch to test its findings in neighbourhoods with large elm populations will allow for the expansion of the models developed here to predict NEBB density. The simplification of my study protocol, in collaboration with the City of Winnipeg, will allow for a low-cost application of the survey methods proposed here. In this way, the efficacy of this protocol will be field tested to determine whether percentage canopy dead leaves, dead branches, and infection sites are accurate predictors of NEBB brood gallery density.

REFERENCES

- Alboukadel Kassambara 2021. rstatix: Pipe-Friendly Framework for Basic Statistical Tests. R package version 0.7.0. <https://CRAN.R-project.org/package=rstatix>
- Alvey, A.A. 2006. Promoting and preserving biodiversity in the urban forest. *Urban Forestry and Urban Greening*. 5(4): 195-201.
- Amrhein, V., F. Korner-Nievergelt, and T. Roth. 2017. The earth is flat ($p > 0.05$): Significance thresholds and the crisis of unreplicable research. *PeerJ*. DOI 10.7717/peerj.3544.
- Anderson, L.M., and H.K. Cordell. 1988. Influence of trees on residential property in Athens, Georgia (USA): A survey based on actual sales prices. *Landscape Urban Planning*. 15:153-164.
- Anderson, P.L. 1996. Overwintering Behaviour of the Native elm bark beetle, *Hylurgopinus rufipes* (Eichoff) (Coleoptera: Scolytidae), in Manitoba. MSc thesis. University of Manitoba.
- Arnett R.H., M.C. Thomas, P.E. Skelley, and J.H. Frank. 2002. *American Beetles*. Polyphaga: Scabaeoidea through Circulinoidea. CRC Press. 876p.
- Anderson, P. L. and Holliday, N. J. 2000. Overwintering of the native elm bark beetle, *Hylurgopinus rufipes* (Coleoptera: Scolytidae), in Siberian elm, *Ulmus pumila*. *Proceedings of the Entomological Society of Manitoba*, 55, (1999), 28–31.
- Anulewicz, A.C., D.G. McCullough, and D.L. Cappaert. 2007. Emerald ash borer (*Agrilus planipennis*) density and canopy dieback in three North American ash species. *Arboriculture and Urban Forestry*. 33(5): 338-349.
- Bark, P.A. (1975). Ordinance control of street trees. *Journal of Arboriculture*. 1:212-216.
- Barwinsky, M. and Domke, D. 2012. Comprehensive strategy to enhance Dutch elm disease management in the City of Winnipeg. Department of Public Works, City of Winnipeg, October 19, 2012. Retrieved on 20 December, 2013 from <http://www.scribd.com/doc/114141093/Dutch-Elm-Report>.

- Bain, J. 1991. Dutch elm disease – the New Zealand experience. In: Arthur T.E., and J.D. Hitchmough, eds. Does the Elm Have a Future in Australia? Burnley, AUS: Victorian College of Agriculture and Horticulture. 40-44.
- Bajeux, N., J. Arino, S. Portet, and R. Westwood. 2020. Spread of Dutch elm disease in an urban forest. *Ecological modelling*. 438. <https://doi/10.1016/j.ecolmodel.2020.109293>.
- Baudry, O., C. Charmetant, C. Collet, Q. Ponette. 2014. Estimating light climate in forest with the convex densiometer: operator effect, geometry and relation to diffuse light. *European Journal of Forest Research*, Springer Verlag. 133(1): 101-110.
- Brasier C.M., Lea J., Rawlings M.K. 1981. The aggressive and non-aggressive strains of *Ceratocystis ulmi* have different temperature optima for growth. *Transactions of the British Mycological Society* 76, 213-218.
- Berland, A., and P. Elliott. 2014. Unexpected connections between residential urban forest diversity and vulnerability to two invasive beetles. *Landscape Ecology*. 29: 141-152.
- Bleiker, K.P., M.R. O'Brien, G.D. Smith., and A.L. Carroll. 2013. Characterisation of attacks made by the mountain pine beetle (Coleoptera: Curculionidae) during its endemic population phase. *Canadian Entomology*. 00: 1-14.
- Brunsdon, C., M. Charlton, P. Harris. 2012. Living with Collinearity in Local Regression Models. In: Accuracy 2012 – 10th International Symposium on Spatial Accuracy Assessment in Natural Resources and Environmental Sciences, 10th-13th July, Florianopolis, SC, Brazil.
- Cabin, R.J., and R.J. Mitchell. 2000. To Bonferroni or not to Bonferroni: When and how are the question. *ESA Bulletin*. 81: 246-248.
- Campana, R. J. and Stipes, R. J. 1981. Dutch elm disease in North America with particular reference to Canada: success or failure of conventional control methods. *Canadian Journal of Plant Pathology*, 3, 252–259.
- Clarke, K.R. 1993. Non-parametric multivariate analysis of changes in community structure.

- Austral Ecology 18: 117-143
- Clarke, K.R., and R.N Gorley. 2001. PRIMER v5: User manual/tutorial, PRIMER-E. Plymouth UK, 91.
- Cook, J.G., T.W. Stutzman, C.W. Bowers, K.A. Brenner, and L.L. Irwin. 1995. Spherical densiometers produce biased estimates of forest canopy cover. Wildlife Society Bulletin. 23(4). 711-717.
- Cribari-Neto, F., and A. Zeileis. 2010. Extended beta regression in R: Shaken, stirred, mixed and partitioned. Journal of Statistical Software. 48(11): 1-25.
- Dexter, E., G. rollwagen-Bollens, S.M. Bollens. 2018. The trouble with stress: A flexible method for the evaluation of nonmetric multidimensional scaling. Limnology and Oceanography.: Methods. 16: 434-443.
- Doccola, J.J, and P.M. Wild. 2012. Tree injection as an alternative method of insecticide application. In: Soloneski, S., and M. Larramendy (eds) Insecticides – Basic and Other Applications. InTech. Rijeka, Croatia.
- Dodds, K.J., H.M. Hull-Sanders, N.W. Siegert, and M.J. Bohne. 2014. Colonization of three maple species by Asian longhorned beetle, *Anoplophora glabripennis*, in two mixed-hardwood forest stands. Insects. 5(1): 105-119
- Domke, D. A. 2005. 30 years of Dutch elm disease in Winnipeg. Urban Forester, Summer. Retrieved on 18 October, 2012 from <http://www.savetheelms.mb.ca/publications.htm>.
- Domke, D. 2012. Comprehensive strategy to enhance the protection of our urban forest from Dutch elm disease. Scenario 2: Full enhanced management. Minutes – Standing Policy Committee on Infrastructure Renewal and Public Works. City of Winnipeg.
- Dwyer, J.F., H.W. Scroeder, and P.H. Gobster. 1991. The significance of urban trees and forests: Towards a deeper understanding of values. Journal of Arboriculture. 17:276-284.
- Ellison, E.A., D.L. Peterson, and D. Cipollini. 2020. Fate of white fringetree through the

- invasion wave of emerald ash borer and its variation in resistance to attack. *Environmental Entomology*. 49(2): 489-495.
- Faccoli, M., and A. Battisti. 1997. Observation on the transmission of *Ophiostoma ulmi* by the smaller elm bark beetles (*Scolytus* spp.). Proceedings, Integrating Cultural Tactics into the Management of Bark Beetle and Reforestation Pests. USDA Forest Service General Technical Report NE_236.
- Feise, R.J. 2002. Do multiple measures require p-value adjustments?. *British Medical Bulletin* 2:8.
- Gadgil, P.D., L.S. Bulman, M.A. Dick, J. Bain, and C.P. Dunn. 2000. Dutch elm disease in New Zealand. In: C.P. Dunn, ed. *The Elms: Breeding, Conservation, and Disease Management*. Boston, MA, USA: Kluwer Academic Publishers. 189-199.
- Garcia, L.V. 2004. Escaping the Bonferroni iron claw in ecological studies. *Oikos*. 105(3): 657-663.
- Gardiner, L.M. 1981. Seasonal activity of the native elm bark beetle, *Hylurgopinus rufipes*, in central Ontario (Coleoptera: Scolytidae). *Can. Ent.* 113: 341-348.
- Gibbs, J.N. 1978. Development of the Dutch elm disease epidemic in southern England. 1971-6. *Annals of Applied Biology*. 88: 219-228.
- Gittins, R. 1985. *Canonical Analysis. A review with applications in ecology*. Berlin: Springer-Verlag.
- Goslee, S.C., D.L. Urban. 2007. The ecodist package for dissimilarity-based analysis of ecological data. *Journal of Statistical Software*. 22(7): 1-19.
- Grey, G.W., and F.J. Deneke. 1986. *Urban Forestry*. Krieger, Malabar.
- Guisan, A., S.B. Weiss, and A.D. Weiss. 1998. GLM versus CCA spatial modeling of plant species distribution. *Plant ecology* 143: 107-122.
- Harrington, T.C., D. McNew, J. Steimel, D. Hofstra, and R. Farrell. 2001. Phylogeny and

- Taxonomy of the *Ophiostoma piceae* complex and the Dutch elm disease fungi. *Mycologia*. 93(1): 111-136.
- Harwood, T.D., I. Tomlinson, C.A. Potter, and J.D. Knight. 2011. Dutch elm disease revisited: past, present and future management in Great Britain. *Plant Pathology*. 60: 545-555.
- Haugen, L. 2007. How to identify and manage Dutch elm disease. United States Department of Agriculture. Northeastern Area: State and Private Forestry. NA-PR-07-98.
- Heisler, G.M., R. Grant, H. Grimmond, and C. Souch. 1995. Urban forests' cooling our communities? in *Proceedings of the Seventh National Urban Forestry Conference* (C. Kollin and M. Barratt, eds.), American Forests, Washington, DC. Pp. 31-34.
- Heybroek, H.M. 1993. Why bother about the elm? In: Stricklen M.B., J.L. Sherald, eds. *Dutch elm disease research - Cellular and Molecular Approaches*. New York, USA. Springer, 1-8.
- Hildahl, V. 1977. Recognition and control of Dutch elm disease in the prairie provinces. 1977. *Blue Jay*. 35(2): 67-73.
- Hintz, W.E., J.S. Carneiro, I. Kassatenko, A. Varga, and D. James. 2013. Two novel mitoviruses from a Canadian isolate of the Dutch elm pathogen *Ophiostoma novo-ulmi* (93-1224). *Virology Journal*. 10: 252.
- Hirst, C.N., and D.A. Jackson. 2007. Reconstructing community relationships: the impact of sampling error, ordination approach, and gradient length. *Diversity and Distributions*. 13: 361-371.
- Holliday, N. J. 2016. Sampling to develop a rapid prioritization method for DED removals: Preliminary results from 2013 and 2015 City of Winnipeg Report: 12 pp.
- Hubbes, M. 1993. Mansomones, elicitors and virulence. Pages 1-8 in: *Dutch Elm Disease: Cellular and Molecular Approaches*. M.B. Sticklen and J.L. Sherald, eds. Springer-Verlag, New York.
- Hubbes, M. 2004. Induced resistance for the control of Dutch elm disease. *Invest Agrar-Sist R*:

- Sist. Recur. For. 13(1): 185-196.
- Hubbes, M., and R.S Jeng. 1981. Aggressiveness of *Ceratocystis ulmi* strains and induction of resistance in *Ulmus americana*. European Journal of Forest Pathology. 11: 257-264.
- Jacobi, W.R., R.D. Koski, T.C. Harrington, and J.J. Witcosky. 2007. Association of *Ophiostoma novo-ulmi* with *Scolytus schevyrewi* (Scolytidae) in Colorado. Plant Disease. 91(3): 245-247.
- Jennings, S.B., N.D. Brown, and D. Sheil. 1999. Assessing forest canopies and understorey illumination: Canopy closure, canopy and other measures. Forestry. 72(1): 59-74.
- Jin, H., Webster, G. R. B., Holliday, N. J., Pines, P. A. and Westwood, A. R. 1996. An elm bark beetle bioassay for residual efficacy of chlorpyrifos and cypermethrin used for the control of Dutch elm disease in Manitoba. Journal of Environmental Science and Health, B31, 751-761.
- Karnosky, D.F. 1979. Dutch elm disease – a review of the history, environmental implications, control, and research needs. Environmental Conservation, 6, 311-322.
- Kondo E.S, Y. Hiratsuka, W.B. Denyer, eds. Proceedings of the Dutch elm disease symposium Workshop, 1981. Winnipeg, Canada: Manitoba Department of Natural Resources. 406-426.
- Ladwig, L.M., S.J. Meiners. 2010. Liana host preference and implications for deciduous forest regeneration. Journal of the Torrey Botanical Society. 137(1): 103-112
- Landwehr, V.R., W.J. Phillipsen, M.E. Ascerno, and R. Hatch. 1981. Attraction of the native elm bark beetle to American elm after the pruning of branches. Journal of Economic Entomology. 74(5): 577-580.
- Landwehr, V., Phillipsen, W. and Ascerno, M. 1982. An integrated approach to managing native elm bark beetle populations in Minnesota. In Kondo, E. S., Hiratsuka, Y. and Denyer, W. B. G. (Eds.). Proceedings of the Dutch elm disease symposium and workshop, Winnipeg,

- MB, October 5–9, 1981 (pp. 454–465). Manitoba Department of Natural Resources, Winnipeg, MB.
- Lanier, G. N. 1989. Trap trees for control of Dutch elm disease. *Journal of Arboriculture*, 15, 105–111.
- Lenihan, J.M. 1993. Ecological responses surfaces for North American tree species and their use in forest classification. *Journal of Vegetative Science*. 2: 667-680.
- Liberato, J.R., R.S. Cameron, M.A. Dick, and C. Inglis (2006). Dutch elm disease (*Ophiostoma*). <http://www.padil.gov.au>.
- McCune, B., and J.B. Grace. 2002. *Analysis of Ecological Communities*. MiM Software Design, Glenden Beach, Oregon.
- Mcune, B., and M.J. Mefford. 2011. *PC-ORD: Multivariate analysis of Ecological Data*. Version 6. MiM Software Design, Gleneden Beach Oregon.
- McPherson, E.G. 2000. Expenditures associated with conflicts between street tree root growth and hardscape in California, United States. *J. Arboric.* 26:289-297.
- McPherson, E.G., J.R. Simpson, P.J. Peper, S.E. Maco, and Q. Xiao. 2005. Municipal forest benefits and costs inf five US cities. *J. For.* 103:411-416.
- McPherson, E.G., D. Nowak, G. Heisler, S. Grimmond, C. Souch, R. Grant, and R. Rowntree. 1997. Quantifying urban forest structure, function, and value: the Chicago urban forest climate project. 1: 49-61.
- Miller, R.W. 1997. *Urban Forestry: planning and managing urban greenspaces*. Waveland, Long Grove. Waveland Press, Inc.
- Minchin, P.R. 1987. An evaluation of the relative robustness of techniques for ecological ordination. *Vegetation*. 69: 89-107.
- Moder, K. 2010. Alternatives to F-Test in One Way ANOVA in case of heterogeneity of

- variances (a simulation study). *Psychological Test and Assessment Modeling*. 52(4): 343-353.
- Moran, M.D. 2003. Arguments for rejecting the sequential Bonferroni in ecological studies. Department of Biology. Hendrix College.
- Newbanks, D., A. Bosch, and M.H. Zimmermann. 1983. Evidence for xylem dysfunction by embolization in Dutch elm disease. *Phytopathology*: 73(7): 1060-1063.
- Nicholls, A. O. 1989. How to make biological surveys go further with generalized linear model. *Biological Conservation*. 50: 51-75.
- Novak, D.J., and D.E. Crane, J.F. Dwyer. 2002. Compensatory value of urban trees in the United States. *Journal of Arboriculture*. 28: 194-199.
- Novak, D.J., and J.F. Dwyer. 2010. Understanding the benefits and costs of urban forest ecosystems. *Urban and Community Forestry in the Northeast*, 2nd ed., eds. J.E. Kuser.
- Nuttle, T. 1997. Densimeter bias: Are we measuring the forest or the trees? *Wildlife Society Bulletin*. 25(3). 610-611.
- Oghiakhe, S and Holliday, N. J. 2011. Evaluation of insecticides for control of overwintering *Hylurgopinus rufipes* (Coleoptera: Curculionidae). *Journal of Economic Entomology*, 104, 899 – 894.
- Oksanen, J., F. Guillaume Blanchet, M. Friendly, R. Kindt P. Legendre, D. McGlenn, P.R. Minchin, R.B. O'Hara, G.L. Simpson, P. Solymos, M. Henry, H. Stevens, E. Szoecs, and H. Wagner. 2020. *Vegan: Community Ecology Package*. R package version 2.5-7. <https://CRAN.R-project.org/package=vegan>.
- Oullette, G.B., H. Chamberland, A. Goulet, M. and Lachapelle. 1999. Fine structure of the extracellular sheath and cell walls in *Ophiostoma novo-ulmi* growing on various substrates. *Canadian Journal of Microbiology*. 45: 582-597.
- Oullette, G.B., C. Cote, N. Methot, H. Chamberland, and J.G. Lafontaine. 1995. Cytology of

- irregular growth forms of *Ophiostoma ulmi* and *Ophiostoma novo-ulmi* growing through millipore filter membranes and sterilized elm wood sections. *Canadian Journal of Microbiology*. 41(12) 1095-1110.
- Peacock, J.W. 1981. Citywide mass trapping of *Scolytus multistriatus* with multilure. In: Kondo E.S, Y. Hiratsuka, W.B. Denyer, eds. Proceedings of the Dutch elm disease symposium Workshop, 1981. Winnipeg, Canada: Manitoba Department of Natural Resources. 406-426.
- Perneger, T.V. 1998. What's wrong with Bonferroni adjustments. *British Medical Journal*. 316: 1236-1238.
- Perneger, T.V. 1999. Multiple testing. *British Medical Journal*. 322: 226-231
- Pines, I.L., and A.R. Westwood. 1996. Evaluation of monosodium methane arsenate for the suppression of native elm bark beetles, *Hylurgopinus rufipes* (Eichhoff) (Coleoptera: Scolytidae). *Canadian Entomologist*. 128:435-441.
- Pines, I.L. and A.R. Westwood. 2008. A mark-recapture technique for Dutch elm disease vector the Native elm bark beetle, *Hylurgopinus rufipes* (Coleoptera: Scolytidae). *Arboriculture and Urban Forestry*. 34(2): 116-122.
- Porter, J.N., J.J. Wilhelm, and H.D. Tresner. 1959. Method for the preferential isolation of Actinomycetes from Soils. Biochemical Research Section, Lederle Laboratories, American Cyanide Company, New York. 9: 174-178.
- Prasad, P., J. Ram, and J. Bhatt. 2018. A comparison of canopy cover measured through transect and densiometer in oak forest of Central Himalaya, India. *ENVIS Bulletin Himalayan Ecology*. 26. 29-32.
- Richards, N.A. 1983. Diversity and stability in a street tree population. *Urban Ecology*. 7: 159-171.
- Romon, P., Z. XuDong, J.C. Iturrondobeita, M.J. Wingfield., and A. Goldarazena. 2007.

- Ophiostoma* species (Ascomycetes: Ophiostomatales) associated with bark beetles (Coleoptera: Scolytinae) colonizing *Pinus radiata* in northern Spain. *Canadian Journal of Microbiology*. 53(6): 756-767.
- Russavage, E., J. Thiele, J. Lumbsden-Pinto, K. Schwager, T. Green, M. Dovciak. 2021. Characterizing canopy openness in open forests: Spherical densiometer and canopy photography are equivalent but less sensitive than direct measurements of solar radiation. *Journal of Forestry*. 119(2): 130-140.
- Santamour, F.S. 1990. Trees for urban planting: diversity, uniformity, and common sense. In: *Proceedings of the 7th conference of the metropolitan tree improvement alliance*. Pp 57-65.
- Santini, A., N. La Porta, L. Ghelardini, L. Mittempergher. 2008. Breeding against Dutch elm disease adapted to the Mediterranean climate. *Euphytica*. 163:45-56.
- Scheffer, R.J. 1990. Mechanisms involved in biological control of Dutch elm disease. *Journal of Phytopathology*. 130: 265-276.
- Scheffer, R.J. J.G. Voeten, and R.P. Guries. 2008. Biological control of Dutch elm disease. *Plant Disease*. 92: 192-200.
- Schlyter, F., and J. Löfqvist. 1990. Colonization pattern in the pine shoot beetle, *Tomicus piniperda*: Effects of host declination, structure and presence of conspecifics. *Entomologia Experimentalis et Applicata*. 54(2)
- Schroede, H.W. 1989. Environment, behaviour, and design research on urban forests, in *Advances in Environment, Behaviour, and Design*. Eds. E.H. Zube and G.L. Moore. Plenum Press, New York. Pp. 87-107.
- Schroeder, H.W. 2004. *Special Places in the Lake Calumet Area*, USDA Forest Service North Central Research Station, General Technical Report 249, St Paul MN, 23pp.
- Sentis, A, C. Gemard, B. Jaugeon, S, and S. David. 2016. Predator diversity and environmental

- change modify the strengths of trophic and nontrophic interactions. *Global Change Biology*. Doi: 10.1111/gcb.13560.
- Shepherd, R.F. 1966. Factors influencing the orientation and rates of activity of *Dendroctonus penderosae* (Coleoptera, Scolytidae), on lodgepole pine. *Canadian Entomology*. 103: 1607-1625.
- Six, D.L., and B.J. Bentz. 2007. Temperature determines symbiotic abundance in a multipartite bark beetle-fungus ectosymbiosis. *Microbial Ecology*. 54: 112-118.
- Solla, A. and L. Gil. 2002. Influence of water stress on Dutch elm disease symptoms in *Ulmus minor*. *Canadian Journal of Botany*. 80(8): 810-817.
- Solomon, J.D. 1995. Guide to insect borers of North American broadleaf trees and shrubs. *Agriculture Handbook*. 706. Washington, D.C.: U.S. Department of Agriculture, Forest Service. 735 p.
- Smalley, E.B., and R.P. Guries. 1993. Breeding elms for tolerance to Dutch elm disease. *Annual Review of Phytopathology*. 31:325-352.
- Stipes, R. J. 2000. The management of Dutch elm disease. In Dunn, C. P. (Ed.). *The elms: breeding, conservation, and disease management* (pp. 157–172). Kluwer Academic Publishers, Boston, MA.
- Strickler, G.S. 1959. Use of the densiometer to estimate density of forest canopy on permanent sample plots. *Forest Science*. 2: 314-320.
- Strobel, G.A., and G.N. Lanier. 1981. Dutch elm disease. *Scientific American*. 13 pages.
- Swedenborg, P.D., R.L. Jones, M.E. Ascerno, and V.R. Landwehr. 1988. *Hylurgopinus rufipes* (Eichoff) (Coleoptera: Scolytidae): Attraction to broodwood: Host colonization behaviour, and seasonal activity in Central Minnesota. *The Canadian Entomologist*. 120(12): 1041-1050.
- Swedenborg, P. D., Jones, R. L., Ascerno, M. E. and Landwehr, V. R. 1988. *Hylurgopinus*

- rufipes* (Eichhoff) (Coleoptera: Scolytidae): Attraction to broodwood, host colonization behavior, and seasonal activity in central Minnesota. *Canadian Entomologist*, 120, 1041–1050.
- Sydnor, T.D., S. Subburayalu, M. Bumgardner. 2010. Contrasting Ohio nursery stock availability with community planting needs. *Arboriculture and Urban Forestry*. 36:47-54.
- Tainter, F.H. 1992. *Ceratocystis*. In: *Methods for research on soilborne phytopathogenic fungi*. Eds. L.L. Singleton, J.D. Higail., and C.M. Rush. American Phthopathological society press, St. Paul Minnesota.
- Tomlinson, I., and C. Porter. 2010. ‘Too little, too late’? Science, policy and Dutch elm disease in the UK. *Journal of Historical Geography*. 36: 121-131.
- Upadhyay, H.P., 1981. A monograph of *Ceratocystis* and *Ceratocystiopsis*. Univiersit of Georgia Press, Athens. 176 pp.
- Vales, D.J., and F.L. Bunnell. 1988. Comparison of methods for estimating forest overstory cover. I. Observer effects. *Canadian Journal of Forest Research*. 18(5): 606-609.
- Vare, H., J. Oksanen. 1995. Effects of reindeer grazing on vegetation in dry *Pinus sylvestris* forests. *Journal of Vegetation Science*. 6: 523-530.
- Van der Brink, P.J. N.W. Van der Brink, and C.J. Ter Braak. 2003. Multivariate analysis of ecotoxicological data using ordination: Demonstrations of utility on the basis of various examples. *Australasian Journal of Ecotoxicology*. 9:141-156.
- Van Wingngaarden, R.P., P.J. Van Den Brink, J.H. Oude, and P. Leeuwangh. 1995. Ordination techniques for analysing response of biological communities to toxic stress in experimental ecosystems. *Ecotoxicology*. 4:61-77.
- Veilleux, J., Leferink, J. and Holliday, N. J. 2012. Rapid removal of symptomatic trees reduces Dutch elm disease infection rates. *Arboriculture and Urban Forestry*, 38, 99–104.
- Vesely, E. 2007. Green for green: the perceived value of a quantitative change in the urban tree

- estate of New Zealand. *Ecological Economics*. 63: 605-615.
- Vora, R.S. 1988. A comparison of the spherical densiometer and ocular methods of estimating canopy cover. *Great Basin Naturalist*. 48(2): 10.
- Wang, P.C. 1987. Residual plots for detecting nonlinearity in generalized linear models. *Technometrics*. 29(4): 435-438.
- Webber J. F. 1990. The relative effectiveness of *Scolytus scolytus*, *S. multistriatus* and *S. kirschii* as vectors of Dutch elm disease. *European Journal of Forest Pathology*, 20,184–192.
- Webber, J.F. 2000. Insect vector behaviour and the evolution of Dutch elm disease. In: Dunn C.P. (eds) *The Elms*. Springer, Boston MA. https://doi.org/10.1007/978-1-4615-4507-1_3
- Wiegrefe, S.J., K.J. Sytsma, and R.P. Guries. 1994. Phylogeny of Elms (*Ulmus*, Ulmaceae); molecular evidence for a sectional classification. *Systematic Botany*. 19(4): 590-612
- Werner, L. 2019. Standard Operating Procedure EAP064, Version 1.1: Determining canopy closure using a concave spherical densiometer, Model C for the Type N Experimental buffer treatment study in incompetent lithologies. Publication No. 19-03-221. Washington State Department of Ecology, Olympia.
- Westphal, L.M. 1993. Why trees? Urban forestry volunteer values and motivations, in managing urban and high use recreation settings. (P.H. Gobster, ed.) Gen. Tech. Rep. NC-163, USDA Forest Service, North Central Forest Experiment Station, St. Paul, MN. Pp. 19-23.
- Westwood, A. R. 1991. A cost-benefit analysis of Manitoba's integrated Dutch elm disease management program 1975–1990. *Proceedings of the Entomological Society of Manitoba*, 47, 44–59.
- Zalapa, J.E., J. Brunet, R.P. Guries. 2010. The extent of hybridization and its impact on the genetic diversity and population structure of an invasive tree, *Ulmis pumila* (Ulmaceae). *Evololutionary Applications*. 3:157-168
- Zhou, X.D., Z.W. de Beer. R. Ahumada, and B.D. Wingfield. 2004. *Ophiostoma* and

Ceratocystiopsis spp. associated with two pine- infesting bark beetles in Chile. Fungal Diversity. 15: 261-274.

Zuur, A., EN Leno, N Walker, AA Saveliev, GM Smith. 2009. Mixed effect models and extensions in ecology with R. Springer, New York, NY, USA.

APPENDIX

Appendix I: Study tree data, with selected variables evaluated on initial tree assessment. Abbreviations for the neighbourhoods are as follows: RVL: River view and Lord Roberts, CW: Crecentwood.

	Study tree tag ID	Year Assessed	Street Address	Study Area	DBH (cm)	Tree Height (m)	Infection Sites
1	UnknownA	2019	506 Newman	Wolsley	39	9	7
2	19-0239	2019	909 Palmerston Ave	Wolsley	61	12	3
3	19-0240	2019	869 Palmerston Ave	Wolsley	65	12	6
4	19-0244	2019	489 Basswood Pl	Wolsley	61	12	3
5	19-0245	2019	513 Basswood Pl	Wolsley	43	6	3
6	19-0246	2019	520 Basswood Pl	Wolsley	68	15	2
7	19-0247	2019	525 Basswood Pl	Wolsley	73	14	3
8	19-0249a	2019	528 Basswood Pl	Wolsley	69	14	2
9	19-0249b	2019	532 Basswood Pl	Wolsley	64	12	5
10	19-0480	2019	465 Craig	Wolsley	45	12	1
11	19-0506	2019	643 Strathcona	Minto	81	13	6
12	19-0507	2019	666 Strathcona	Minto	59	10	3
13	19-0509	2019	684 Strathcona	Minto	56	12	1
14	19-0510	2019	685 Strathcona	Minto	67	12	1
15	19-0511	2019	689 Strathcona	Minto	65	13	2
16	19-0512	2019	864 Strathcona	Minto	47	13	2
17	19-0513	2019	816 Strathcona	Minto	62	12	2
18	19-0514	2019	712 Strathcona	Minto	77	14	3
19	19-0515	2019	584 Ashburn	Minto	52	12	5
20	19-0517	2019	588 Ashburn	Minto	82	13	3
21	19-0519	2019	690 Ashburn	Minto	60	11	3
22	19-0520	2019	691 Ashburn	Minto	61	11	2
23	19-0521	2019	747 Ashburn	Minto	80	17	3
24	19-0522	2019	754 Ashburn	Minto	65	12	4
25	19-0524H	2019	804 Ashburn	Minto	57	10	1
26	19-0525	2019	849 Ashburn	Minto	61	11	1
27	19-0526	2019	869 Ashburn	Minto	66	12	4
28	19-0529	2019	674 Spruce	Minto	45	12	2
29	19-0532	2019	584 Spruce	Minto	65	13	6
30	19-0533	2019	580 Spruce	Minto	53	11	2

Appendix I: Continued from previous page.

	Study tree tag ID	Year Assessed	Street Address	Study Area	DBH (cm)	Tree Height (m)	Infection Sites
31	19-0535	2019	802 Clifton	Minto	71	13	6
32	19-0540	2019	585 Clifton	Minto	67	12	7
33	19-0543	2019	716 Ashburn	Minto	58	11	7
34	19-0550	2019	592 Goulding	Minto	77	14	6
35	19-0701	2019	490 Basswood Pl	Wolsley	55	12	3
36	19-0702	2019	495 Basswood Pl	Wolsley	71	12	5
37	19-0703	2019	509 Sprague	Wolsley	63	14	
38	19-0704	2019	1140 Portage Ave	Wolsley	45	12	3
39	19-0706	2019	499 Greenwood	Wolsley	65	12	4
40	19-0707	2019	533 Greenwood	Wolsley	80	14	7
41	19-0708	2019	1110 Portage Ave	Wolsley	61	13	0
42	19-0721	2019	255 Garfield	Wolsley	91	15	7
43	19-0723	2019	229 Garfield	Wolsley	32	9	3
44	19-0724	2019	114 Sherburn	Wolsley	44	12	0
45	19-0725	2019	223 Ruby St	Wolsley	45	13	4
46	19-0726	2019	219 Ruby St	Wolsley	62	13	3
47	19-0727	2019	160 Ruby St	Wolsley	82	14	7
48	19-0730	2019	279 Evanson St	Wolsley	64	13	2
49	19-0732	2019	210 Evanson St	Wolsley	85	13	6
50	19-0733	2019	203 Home St	Wolsley	72	12	5
51	19-0734	2019	199 Home St	Wolsley	81	15	0
52	19-0737	2019	213 Canora St	Wolsley	44	11	1
53	19-0749	2019	209 Walnut	Wolsley	66	14	6
54	19-0778	2019	312 Baltimore	RVLR	54	18	1
55	19-0787	2019	352 Bartlet	RVLR	65	16	5
56	19-0792	2019	187 Morley	RVLR	75	17	2
57	19-0793	2019	117 Morley	RVLR	74	18	2
58	19-0795	2019	125 Morley	RVLR	60	13	2
59	19-0796	2019	134 Morley	RVLR	59	12	1
60	19-0798	2019	345 Balfour	RVLR	65	15	3

Appendix I: Continued from previous page.

	Study tree tag ID	Year Assessed	Street Address	Study Area	DBH (cm)	Tree Height (m)	Infection Sites
61	19-0858	2019	550 Jubilee Ave	RVLR	49	11	2
62	19-0859	2019	524 Jubilee Ave	RVLR	41	9	1
63	19-0860	2019	553 Jubilee Ave	RVLR	52	12	0
64	19-0861	2019	515 Jubilee Ave	RVLR	62	13	7
65	19-0863	2019	685 Jubilee Ave	RVLR	71	13	3
66	19-0880	2019	666 Rosedale Ave	RVLR	87	17	2
67	19-0883	2019	588 Rathgar Ave	RVLR	71	15	2
68	19-0900a	2019	511 Clifton	Wolsley	62	11	5
69	19-0900b	2019	511 Clifton	Wolsley	50	12	5
70	19-0902	2019	631 Goulding	Minto	83	14	4
71	19-0903	2019	633 Goulding	Minto	60	14	2
72	19-0904	2019	635 Goulding	Minto	73	13	4
73	19-0905	2019	636 Goulding	Minto	86	15	3
74	19-0906	2019	640 Goulding	Minto	58	14	4
75	19-0907	2019	642 Goulding	Minto	65	13	3
76	19-0908	2019	649 Goulding	Minto	85	13	4
77	19-0912	2019	677 Goulding	Minto	80	13	5
78	19-0913	2019	685 Goulding	Minto	68	12	6
79	19-0914H	2019	691 Goulding	Minto	67	12	7
80	19-0915H	2019	699 Goulding	Minto	56	12	7
81	19-0916H	2019	706 Goulding	Minto	70	12	7
82	19-0917	2019	708 Goulding	Minto	61	12	3
83	19-0918H	2019	715 Goulding	Minto	81	13	7
84	19-0919	2019	720 Goulding	Minto	54	12	7
85	19-0947	2019	1033 Strathcona	Minto	69	12	4
86	19-0948	2019	1026 Strathcona	Minto	57	8	3
87	19-0949	2019	1003 Strathcona	Minto	90	16	1
88	19-1022	2019	1033 Strathcona	Minto	91	14	1
89	19-1023	2019	965 Strathcona	Minto	69	12	3
90	19-1024	2019	639 Ashburn	Minto	28	9	1

Appendix I: Continued from previous page.

	Study tree tag ID	Year Assessed	Street Address	Study Area	DBH (cm)	Tree Height (m)	Infection Sites
91	19-1025	2019	633 Spruce	Minto	74	13	1
92	19-1066	2019	462 Rosedale Ave	RVLR	72	13	1
93	19-1068	2019	665 Beresford Ave	RVLR	60	10	2
94	19-1069	2019	573 Beresford Ave	RVLR	72	13	7
95	19-1070	2019	566 Beresford Ave	RVLR	50	11	3
96	19-1071	2019	509 Beresford Ave	RVLR	75	14	4
97	19-1073	2019	657 Rathgar Ave	RVLR	59	11	4
98	19-1108	2019	128 Oakwood	RVLR	56	12	1
99	19-1109	2019	118 Morley	RVLR	46	15	5
100	19-1161	2019	43 Alloway	Wolsley	57	12	4
101	19-1163	2019	13 Purcell	Wolsley	72	13	1
102	19-1164	2019	25 Purcell	Wolsley	64	11	5
103	19-1171	2019	55 Sherburn	Wolsley	55	11	7
104	19-1173	2019	57 Lipton St	Wolsley	92	15	1
105	19-1179	2019	43 Arlington St	Wolsley	78	13	1
106	19-1187	2019	133 Chestnut St	Wolsley	66	12	2
107	19-1351	2019	907 Ashburn	Minto	39	9	3
108	19-1352	2019	890 Ashburn	Minto	45	7	3
109	19-1353	2019	949 Spruce	Minto	66	14	4
110	19-1354	2019	947 Spruce	Minto	61	13	2
111	19-1355	2019	911 Spruce	Minto	84	15	5
112	19-1356	2019	903 Spruce	Minto	52	12	3
113	19-1357(1)	2019	895 Spruce	Minto	66	13	7
114	19-1357(2)	2019	895 Spruce	Minto	59	14	3
115	19-1358	2019	891 Spruce	Minto	72	14	5
116	19-1359	2019	877 Spruce	Minto	69	15	1
117	19-1360	2019	915 Clifton	Minto	69	12	4
118	19-1361	2019	908 Clifton	Minto	70	15	4
119	19-1362H	2019	834 Clifton	Minto	72	12	7
120	19-1363	2019	828 Clifton	Minto	59	12	3

Appendix I: Continued from previous page.

	Study tree tag ID	Year Assessed	Street Address	Study Area	DBH (cm)	Tree Height (m)	Infection Sites	
121	19-1364	2019	802	Goulding	Minto	26	9	3
122	19-1365	2019	807	Goulding	Minto	65	12	4
123	19-1366	2019	814	Goulding	Minto	63	12	4
124	19-1367	2019	820	Goulding	Minto	71	14	5
125	19-1368	2019	825	Goulding	Minto	64	10	7
126	19-1553	2019	118	Garfield	Wolsley	82	13	1
127	19-1554	2019	112	Garfield	Wolsley	60	11	6
128	19-1555a	2019	60	Sherburn	Wolsley	81	19	1
129	19-1555b	2019	60	Sherburn	Wolsley	60	15	1
130	19-1657	2019	711	Erin	Minto	59	8	4
131	19-1920	2019	728	Goulding	Minto	64	12	7
132	19-9794	2019	120	Morley	RVLR	51	12	5
133	29-0774	2019	196	Baltimore	RVLR	68	20	1
134	UnknownB	2019	729	Clifton	Minto	71	15	5
135	17-0743	2018				79	19	
136	18-0113	2018	771	Walker	RVLR	8	6	4
137	18-0114	2018		Argue	RVLR	8	7	5
138	18-0129	2018	312	Baltimore	RVLR	46	19	5
139	18-0251	2018	1146	Portage Ave	Wolsley	51	9	5
140	18-0252	2018	742	Ingersol	Minto	81	22	2
141	18-0253	2018	706	Ingersol	Minto	78	16	5
142	18-0258	2018	681	Strathcona	Minto	61	17	2
143	18-0260	2018	677	Ashburn	Minto	68	19	2
144	18-0261	2018	766	Spruce	Minto	67	16	5
145	18-0262	2018	732	Clifton	Minto	58	16	4
146	18-0263	2018	633	Clifton	Minto	68	16	5
147	18-0264	2018	661	Wall	Minto	52	11	2
148	18-0265	2018	642	Minto	Minto	51	13	4
149	18-0266	2018	601	Goulding	Minto	72	14	2
150	18-0267	2018	591	Minto	Minto	83	15	1

Appendix I: Continued from previous page.

	Study tree tag ID	Year Assessed	Street Address	Study Area	DBH (cm)	Tree Height (m)	Infection Sites
151	18-0268	2018	1111 Portage	Minto	62	12	3
152	18-0269	2018	651 Greenwood	Minto	46	13	4
153	18-0270	2018	591 Garfield	Minto	61	13	1
154	18-0271	2018	1091 Portage	Minto	43	9	1
155	18-0272	2018	526 Sherburn	Minto	77	21	3
156	18-0274	2018	496 Ingersol	Minto	91	19	5
157	18-0275	2018	987 Strathcona	Minto	59	18	3
158	18-0276	2018	996 Strathcona	Minto	66	20	2
159	18-0277	2018	972 Ashburn	Minto	69	19	3
160	18-0278	2018	954 Ashburn	Minto	56	12	4
161	18-0279	2018	945 Spruce	Minto	64	17	5
162	18-0280	2018	915 Spruce	Minto	91	28	1
163	18-0281	2018	725 Garfield	Minto	60	17	3
164	18-0755	2018	190 Lipton St	Wolsley	71	22	5
165	18-0756	2018	888 Portage Ave	Wolsley	35	10	5
166	18-0757	2018	3 Borrowman Ave	Wolsley	64	13	4
167	18-0762	2018	960 Wolseley Ave	Wolsley	56	12	4
168	18-0770	2018	740 Ingersol	Minto	68	18	2
169	18-0771	2018	779 Ingersol	Minto	76	20	3
170	18-0772	2018	738 Ingersol	Minto	79	21	4
171	18-0774	2018	753 Ingersol	Minto	78	18	2
172	18-0775	2018	747 Ingersol	Minto	62	19	2
173	18-0776	2018	741 Ingersol	Minto	64	21	5
174	18-0777	2018	702 Ingersol	Minto	74	19	5
175	18-0778	2018	721 Ingersol	Minto	68	20	3
176	18-0779	2018	715 Ingersol	Minto	76	18	2
177	18-0780	2018	678 Ingersol	Minto	69	18	4
178	18-0787	2018	506 Basswood Pl	Wolsley	69	22	5
179	18-0788	2018	545 Basswood Pl	Wolsley	82	20	3
180	18-0789	2018	526 Basswood Pl	Wolsley	63	22	4

Appendix I: Continued from previous page.

	Study tree tag ID	Year Assessed	Street Address	Study Area	DBH (cm)	Tree Height (m)	Infection Sites
181	18-0790	2018	446 Greenwood Pl	Wolsley	67	20	1
182	18-0791	2018	114 Garfield St S	Wolsley	72	18	2
183	18-0792	2018	91 Sherburn St	Wolsley	66	18	2
184	18-0793	2018	204 Lipton St	Wolsley	85	17	3
185	18-0794	2018	186 Lipton St	Wolsley	54	12	1
186	18-0795	2018	179 Lipton St	Wolsley	67	22	3
187	18-0796	2018	218 Lenor St	Wolsley	67	20	2
188	18-0797	2018	230 Arlington St	Wolsley	77	18	3
189	18-0798	2018	203 Home St	Wolsley	67	20	3
190	18-0800	2018	191 Canora St	Wolsley	75	20	4
191	18-0852	2018	335 Balfour	RVLR	53	20	3
192	18-0854	2018	231 Bartlet	RVLR	59	17	4
193	18-0855	2018	571 Walker	RVLR	66	16	4
194	18-0856	2018	598 Walker	RVLR	60	19	4
195	18-0857	2018	767 Walker	RVLR	62	16	4
196	18-0858	2018	539 Osborne	RVLR	47	12	3
197	18-0859	2018	539 Osborne	RVLR	61	14	3
198	18-0861	2018	326 Morley	RVLR	66	14	4
199	18-0862	2018	722 Nassau	RVLR	52	16	4
200	18-0863	2018	432 Arnold	RVLR	73	15	4
201	18-0864	2018	329 Arnold	RVLR	62	14	2
202	18-0865	2018	287 Arnold	RVLR	52	15	3
203	18-0866	2018	492 Carlaw	RVLR	56	15	4
204	18-0867	2018	655 Nassau	RVLR	57	7	3
205	18-0868	2018	10 Eccles	RVLR	53	12	4
206	18-0869	2018	136 Balfour	RVLR	18	19	2
207	18-0870	2018	126 Ashland	RVLR	61	15	2
208	18-0871	2018	122 Ashland	RVLR	70	15	1
209	18-0872	2018	103 Baltimore	RVLR	53	14	2
210	18-0873	2018	111 Baltimore	RVLR	61	17	2

Appendix I: Continued from previous page.

	Study tree tag ID	Year Assessed	Street Address	Study Area	DBH (cm)	Tree Height (m)	Infection Sites	
211	18-0874	2018	115	Baltimore	RVLR	38	15	3
212	18-0876	2018	123	Baltimore	RVLR	63	13	2
213	18-0877	2018	122	Baltimore	RVLR	77	16	3
214	18-0878	2018	126	Baltimore	RVLR	74	19	3
215	18-0879	2018	136	Baltimore	RVLR	60	19	3
216	18-0881	2018	345	Baltimore	RVLR	61	13	2
217	18-0883	2018	183	Baltimore	RVLR	49	18	2
218	18-0884	2018	163	Baltimore	RVLR	49	15	2
219	18-0885	2018	138	Oakwood	RVLR	85	21	1
220	18-0886	2018	294	Oakwood	RVLR	72	16	3
221	18-0887	2018	125	Maplewood	RVLR	53	9	3
222	18-0888	2018	253	Maplewood	RVLR	61	14	2
223	18-0890	2018	48	Morley	RVLR	73	17	2
224	18-0891	2018	57	Morley	RVLR	48	14	5
225	18-0892	2018	58	Morley	RVLR	96	22	3
226	18-0893	2018	254	Morley	RVLR	71	16	4
227	18-0894	2018	247	Morley	RVLR	69	18	4
228	18-0895	2018	242	Morley Ave	RVLR	61	15	3
229	18-0896	2018	114	Morley Ave	RVLR	77	19	2
230	18-1172	2018	668	Jubilee Ave	RVLR	58	12	1
231	18-1173	2018	556	Jubilee Ave	RVLR	44	12	2
232	18-1174	2018	635	Jubilee Ave	RVLR	65	15	3
233	18-1175	2018	649	Jubilee Ave	RVLR	75	18	3
234	18-1176	2018	665	Jubilee Ave	RVLR	41	11	2
235	18-1180	2018	616	Rosedale Ave	RVLR	74	20	3
236	18-1181	2018	578	Rosedale Ave	RVLR	59	15	4
237	18-1183	2018	465	Rosedale Ave	RVLR	45	16	3
238	18-1184	2018	515	Rosedale Ave	RVLR	45	16	3
239	18-1185	2018	454	Rosedale Ave	RVLR	71	16	4
240	18-1186	2018	351	Rosedale Ave	RVLR	30	8	4

Appendix I: Continued from previous page.

	Study tree tag ID	Year Assessed	Street Address	Study Area	DBH (cm)	Tree Height (m)	Infection Sites
241	18-1189	2018	665 Rathgar Ave	RVLR	77	18	4
242	18-1190	2018	665 Beresford Ave	RVLR	44	10	3
243	18-1199	2018	182 Morley Ave	RVLR	67	17	5
244	18-1302	2018	54 Picardy Pl	Wolsley	71	17	2
245	18-1307	2018	70 Maryland St	Wolsley	58	14	5
246	18-1308	2018	78 Ruby St	Wolsley	70	21	2
247	18-1309	2018	108 Lenore St	Wolsley	79	20	3
248	18-1310	2018	81 Lenore St	Wolsley	71	17	2
249	18-1311	2018	80 Lenore St	Wolsley	65	20	1
250	18-1312	2018	72 Lenore St	Wolsley	61	13	2
251	18-1313	2018	141 Arlington St	Wolsley	77	18	3
252	18-1314	2018	849 Wolseley Ave	Wolsley	83	17	4
253	18-1315	2018	97 Chestnut St	Wolsley	72	18	5
254	18-1316	2018	39 Home St	Wolsley	89	20	1
255	18-1317	2018	17 Evanson St	Wolsley	74	21	3
256	18-1318	2018	26 Evanson St	Wolsley	71	16	4
257	18-1319	2018	960 Wolseley Ave	Wolsley	70	17	3
258	18-1319	2018	960 Wolseley Ave	Wolsley	70	20	4
259	18-1320	2018	26 Ruby St	Wolsley	80	17	2
260	18-1321	2018	1000 Palmerston Ave	Wolsley	48	16	2
261	18-1322	2018	31 Lipton St	Wolsley	73	16	2
262	18-1323	2018	52 Lipton St	Wolsley	76	21	4
263	18-1324	2018	1046 Wolseley Ave	Wolsley	56	13	4
264	18-1325	2018	1120 Palmerston Ave	Wolsley	20	11	2
265	18-1327	2018	856 Westminster Ave	Wolsley	71	14	4
266	18-1328	2018	848 Westminster Ave	Wolsley	59	16	4
267	18-1329	2018	852 Westminster Ave	Wolsley	70	18	2
268	18-1330	2018	758 Westminster Ave	Wolsley	70	15	2
269	18-1331	2018	750 Westminster Ave	Wolsley	83	22	3
270	18-1332	2018	73 Chestnut St	Wolsley	54	18	4

Appendix I: Continued from previous page.

	Study tree tag ID	Year Assessed		Street Address	Study Area	DBH (cm)	Tree Height (m)	Infection Sites
271	18-1334	2018	1197	Wolseley Ave	Wolsley	59	18	3
272	18-1831	2018	152	Garfield St S	Wolsley	71	17	4
273	19-0259	2018	686	Ashburn	Minto	85	21	2
274	UnknownA	2018	644	Gourding St	Minto	88	19	3
275	UnknownB	2018	147	Garfield St S	Wolsley	62	19	4
276	16-0168	2017	926	Wolseley Ave	Wolsley	97	16	
277	16-0234	2017	900	Westminster Ave	Wolsley	59	11	
278	16-0605	2017	806	Wolseley Ave	Wolsley	89	12	
279	16-0921	2017	67	Maryland St	Wolsley	102	14	
280	17-0714	2017	39	Fawcett	Wolsley	54	14	
281	17-0716H	2017	19	Alloway	Wolsley	88	24	
282	17-0717	2017	13	Purcell	Wolsley	48	13	
283	17-0719	2017	104	Walnut	Wolsley	90	22	
284	17-0720	2017	100	Walnut	Wolsley	65	17	
285	17-0721	2017	81	Walnut	Wolsley	79	17	
286	17-0722	2017	28	Woodrow	Wolsley	68	12	
287	17-0723	2017	750	Wolseley Ave	Wolsley	23	9	
288	17-0724	2017	145	Walnut	Wolsley	72	16	
289	17-0726	2017	163	Walnut	Wolsley	65	15	
290	17-0727	2017	188	Walnut	Wolsley	74	17	
291	17-0728	2017	210	Walnut	Wolsley	79	22	
292	17-0735	2017	154	Maryland St	Wolsley	43	11	
293	17-0736	2017	192	Chestnut St	Wolsley	59	16	
294	17-0737	2017	153	Chestnut St	Wolsley	85	15	
295	17-0739	2017	145	Canora St	Wolsley	70	11	
296	17-0740	2017	165	Canora St	Wolsley	58	15	
297	17-0741	2017	170	Canora St	Wolsley	38	14	
298	17-0742	2017	176	Canora St	Wolsley	57	12	
299	17-0743	2017	802	Preston	Wolsley	79	20	
300	17-0744B	2017	821	Preston	Wolsley	60	14	

Appendix I: Continued from previous page.

	Study tree tag ID	Year Assessed	Street Address	Study Area	DBH	Tree Height	Infection Sites
301	17-0744C	2017	821 Preston	Wolsley	72	14	
302	17-0744E	2017	821 Preston	Wolsley	61	10	
303	17-0747	2017	871 Wolsley Ave	Wolsley	79	20	
304	17-0748	2017	130 Ethelbert	Wolsley	74	16	
305	17-0749	2017	133 Ethelbert	Wolsley	80	18	
306	17-0750	2017	160 Ethelbert	Wolsley	62	15	
307	17-1019	2017	456 Greenwood	Wolsley	52	18	
308	17-1020	2017	462 Greenwood	Wolsley	69	18	
309	17-1023	2017	139 Garfield	Wolsley	37	7	
310	17-1024	2017	128 Garfield	Wolsley	88	16	
311	17-1025	2017	112 Garfield	Wolsley	77	15	
312	17-1026	2017	233 Garfield	Wolsley	75	18	
313	17-1027	2017	121 Sherburn	Wolsley	56	15	
314	17-1038	2017	174 Aubrey	Wolsley	53	13	
315	17-1039	2017	24 Lipton St	Wolsley	67	13	
316	17-1040	2017	72 Ruby St	Wolsley	47	9	
317	17-1042	2017	137 Ruby St	Wolsley	68	14	
318	17-1043	2017	129 Ruby St	Wolsley	83	14	
319	17-1230	2017	453 Greenwood	Wolsley	62	11	
320	17-1401	2017	195 Ethelbert	Wolsley	45	12	
321	17-1405	2017	822 Preston	Wolsley	64	14	
322	17-1406	2017	175 Home St	Wolsley	69	14	
323	17-1407	2017	242 Arlington St	Wolsley	88	15	
324	17-1410	2017	19 Arlington St	Wolsley	82	15	
325	17-1411	2017	20 Arlington St	Wolsley	110	18	
326	17-1413	2017	188 Evanson St	Wolsley	62	13	
327	17-1414	2017	53 Evanson St	Wolsley	63	14	
328	17-1415	2017	10 Evanson St	Wolsley	47	8	
329	17-1416	2017	55 Lenore	Wolsley	40	12	
330	17-1418	2017	193 Lenore St	Wolsley	66	12	

Appendix I: Continued from previous page.

	Study tree tag ID	Year Assessed		Street Address	Study Area	DBH	Tree Height	Infection Sites
331	17-1419	2017	114	Lenore St	Wolsley	73	14	
332	17-1420	2017	7	Lenore St	Wolsley	83	15	
333	17-1421	2017	1071	Palmerston Ave	Wolsley	73	14	
334	17-1423	2017	1065	Palmerston Ave	Wolsley	66	15	
335	17-1433	2017	812	Wolseley Ave	Wolsley	100	15	
336	17-1434	2017	66	Home St	Wolsley	61	14	
337	17-1435	2017	157	Aubrey	Wolsley	73	15	
338	17-1436	2017	747	Westminster Ave	Wolsley	63	14	
339	17-1437	2017	756	Westminster Ave	Wolsley	69	17	
340	17-1440	2017	22	Alloway	Wolsley	63	12	
341	17-1441	2017	31	Alloway	Wolsley	80	15	
342	17-1466	2017	186	Dromore	CW	84	11	
343	17-1701	2017	960	Westminster Ave	Wolsley	80	13	
344	17-1702	2017	154	Ruby St	Wolsley	72	13	
345	17-2027	2017	183	Waverly	CW	50	10	
346	17-2036	2017	194	Montrose	North River	66	15	
347	17-2356	2017	550	Wellington	CW	45	8	
348	17-2357	2017	514	Wellington	CW	50	11	
349	17-2357	2017	514	Wellington	CW	53	6	
350	17-2368	2017	73	Harvard	CW	54	9	
351	17-2378	2017	53	Harvard	CW	61	10	
352	17-2379	2017	2	Avonherst	CW	54	11	
353	17-2381	2017	34	Avonherst	CW	57	11	
354	17-2383	2017	561	Wellington	CW	70	11	
355	17-2452	2017	175	Oxford St	CW	59	17	

Appendix II: 2019 Disease progression survey data. Abbreviations are as follows, CC - average canopy cover, G - percent canopy green leaves, DB - percent canopy dead branches, DL - percent canopy dead leaves and PP - pole pruning result as percent fungal staining.

Study tree tag ID	Variable and week of year assessed																								
	CC					G					DL					DB					PP				
	29	30	31	32	33	29	30	31	32	33	29	30	31	32	33	29	30	31	32	33	29	30	31	32	33
1 19-0506	70.6	67.2	76	94	45	45	30	30	45	45	55	55	10	10	15	15	80	25	56.3	83.8					
2 19-0507	75	62.5	77.6	94.4	60	55	40	40	20	25	35	30	20	20	25	30	0	0	5	10					
3 19-0509	80.5	39.3	78.9	94.7	95	92	90	90	2	5	5	5	3	3	5	5	7.5	5	7.5	2.5					
4 19-0510	81.3	83.9	83.9	96	95	93	85	70	2	4	10	20	3	3	5	10	21.3	33.8	15	11.3					
5 19-0511	58.9	57	57	89.3	95	90	90	75	2	5	5	15	3	5	5	10	20	38.8	21.3	25					
6 19-0512	66.9	60.9	65.9	91.5	85	85	75	75	2	2	5	5	13	13	20	20	5	5	1.25	5					
7 19-0513	45.1	49.7	48.2	87	90	80	85	83	3	5	5	7	7	10	10	10	0	3.75	2.5	2.5					
8 19-0514	60.7	44.8	67.7	91.9	80	75	75	75	5	5	5	5	15	25	25	25	16.3	0	0	0					
9 19-0515	95.6	93.5	96.1	99	30	25	15	10	45	45	55	55	25	30	30	35	62.5	77.5	66.3	80					
10 19-0517	84.9	84.4	88.5	97.1	60	60	60	60	32	27	20	20	8	13	20	20	3.75	2.5	13.8	10					
11 19-0519	62	63.5	64.8	91.2	80	75	75	60	10	15	15	25	10	10	10	15	31.3	36.3	32.5	25					
12 19-0520	58.3	59.4	60.7	90.2	70	70	70	70	15	15	10	10	15	15	20	20	42.5	43.8	47.5	48.8					
13 19-0521	59.4	59.9	62.2	90.6	65	65	60	60	15	15	20	20	20	20	20	20	21.3	20	11.3	20					
14 19-0522	77.9	78.4	79.9	95	55	55	55	50	35	35	30	30	10	10	15	20	70	61.3	83.8	67.5					
15 19-0524H	31.5	34.6	36.2	84	60	60	55	55	2	0	3	3	38	40	42	42	0	0	0	0					
16 19-0525	24	26.3	25	81.3	75	75	75	75	15	15	15	15	10	10	10	10	1.25	0	0	0					
17 19-0526	54.9	55.7	63	90.8	65	65	65	60	25	25	25	30	10	10	10	10	45	36.3	43.8	36.3					
18 19-0529	34.9	37.2	37	84.2	80	80	80	80	15	15	15	15	5	5	5	5	17.5	0	5	11.3					
19 19-0532	94.3	95.3	97.9	99.5	25	20	15	5	65	60	70	50	10	20	15	45	57.5	42.5	0	23.8					
20 19-0533	61.2	63.5	64.3	91.1	85	85	85	80	10	10	10	15	5	5	5	5	18.8	17.5	32.5	35					
21 19-0535	94.5	95.6	96.9	99.2	15	15	10	5	15	10	10	5	70	75	80	90	11.3	7.5	17.5	16.3					
22 19-0540	97.9	98.7	99.2	99.8	2	1	1	1	15	15	15	15	83	84	84	84	0	0	0	0					
23 19-0543	99.5	100	100	100	5	5	3	2	60	60	62	58	35	35	35	40	66.3	65	62.5	100					
24 19-0550	77.1	78.6	81.3	95.3	65	65	55	45	20	15	25	35	15	20	20	20	47.5	45	41.3	31.3					
25 19-0902	66.4	68.2	70.6	92.6	90	85	85	85	5	5	5	5	5	10	10	10	40	10	27.5	30					
26 19-0903	56.3	58.1	60.4	90.1	80	80	80	75	10	10	10	15	10	10	10	10	35	18.8	21.3	18.8					
27 19-0904	78.4	84.9	83.1	95.8	65	65	60	65	20	15	20	20	15	20	20	15	37.5	25	40	28.8					

Appendix II: Continued from previous page.

Study tree tag ID	Variable and week of year assessed																								
	CC					G					DL					DB					PP				
	29	30	31	32	33	29	30	31	32	33	29	30	31	32	33	29	30	31	32	33	29	30	31	32	33
28	19-0905	52.9	53.9	58.6	89.6	75	75	70	60	20	20	25	35	5	5	5	5	8.75	27.5	41.3	35				
29	19-0906	89.6	90.6	91.7	97.9	20	20	15	15	35	35	40	40	45	45	45	45	37.5	37.5	48.8	47.5				
30	19-0907	70.8	71.9	74.2	93.6	65	65	55	55	25	25	35	35	10	10	10	10	30	27.5	43.8	35				
31	19-0908	51	53.4	57	89.3	55	55	55	55	25	20	20	20	20	30	30	25	37.5	28.8	36.3	26.3				
32	19-0912	81.8	81.5	88	97	45	45	35	30	45	25	35	40	10	30	30	30	45	25	45	40				
33	19-0913	83.9	82.6	88.8	97.2	15	20	5	3	40	35	45	47	45	45	50	50	43.8	16.8	22.5	18.8				
34	19-0914H	100	100	100	100	0	0	0	0	2	2	2	2	98	98	98	98	0	0	0	0				
35	19-0915H	100	100	100	100	0	0	0	0	0	0	0	0	100	100	100	100	0	0	0	0				
36	19-0916H	100	100	100	100	1	0	0	0	5	4	2	2	94	96	98	98	0	0	0	0				
37	19-0917	83.9	84.6	89.3	97.3	55	55	55	50	35	35	35	35	10	10	10	15	0	0	0	0				
38	19-0918H	100	100	100	100	0	0	0	0	2	2	1	1	98	98	99	99	0	0	0	0				
39	19-0919	100	100	100	100	0	0	0	0	30	30	30	30	70	70	70	70	0	0	0	0				
40	19-0947	82	91.4	93	98.2	40	35	15	10	20	20	20	20	40	45	65	70	22.5	27.5	23.8	20				
41	19-0948	86.5	89.1	95.6	98.9	25	20	15	5	10	10	15	10	65	70	70	85	28.8	8.75	10	5				
42	19-0949	35.9	33.6	37	84.2	90	90	90	90	2	2	2	2	8	8	8	8	18.8	15	21.3	8.75				
43	19-1022	37	38.5	42.2	85.5	94	94	92	91	1	1	3	4	5	5	5	5	2.5	6.25	10	12.5				
44	19-1023	81	84.4	84.6	96.2	65	60	50	40	25	25	30	35	10	15	20	25	13.8	11.3	11.3	10				
45	19-1024	4.17	6.77	9.9	77.5	75	75	50	45	20	20	35	40	5	5	15	15	20	23.8	26.3	43.8				
46	19-1025	75	72.7	78.4	94.6	95	95	93	93	3	3	5	5	2	2	2	2	23.8	20	7.5	17.5				
47	19-1351	82	98.7	100	100	5	3	0	0	55	57	60	60	40	40	40	40	15	0	0	0				
48	19-1352	91.1	91.1	91.9	98	45	45	40	40	20	20	25	30	35	35	35	30	26.3	0	3.75	5				
49	19-1353	59.1	70.1	76	94	75	70	50	45	20	25	40	40	5	5	10	15	18.8	7.5	23.8	27.5				
50	19-1354	75	76.6	77.1	94.3	80	75	70	60	15	15	20	25	5	10	10	15	22.5	17.5	37.5	27.5				
51	19-1355	96.6	96.4	97.4	99.3	15	15	10	7	20	10	10	13	45	65	80	80	30	5	10	5				
52	19-1356	89.3	93.2	95.8	99	30	25	10	5	40	25	40	65	30	30	50	30	36.3	15	42.5	10				
53	19-1357(1)	100	100	100	100	1	0	0	0	24	25	20	20	75	75	80	80	0	0	0	0				
54	19-1357(2)	72.9	72.9	75.5	93.9	85	85	80	75	10	10	15	20	5	5	5	5	40	36.3	52.5	28.8				
55	19-1358	69.8	71.1	72.4	93.1	85	80	80	73	10	15	15	20	5	5	5	7	41.3	35	35	51.3				
56	19-1359	49.5	51	53.1	88.3	80	75	70	60	15	20	25	35	5	5	5	5	5	0	10	11.3				
57	19-1360	47.4	53.9	54.2	88.5	75	75	70	70	20	20	25	25	5	5	5	5	38.8	46.3	53.8	42.5				
58	19-1361	60.2	64.1	76.3	94.1	60	55	45	30	30	30	30	35	10	15	25	35	33.8	7.5	5	8.75				
59	19-1362H	93.2	95.3	97.1	99.3	5	5	2	2	5	5	2	4	90	90	96	94	17.5	15	21.3	16.3				

Appendix II: Continued from previous page.

Study tree tag ID	Variable and week of year assessed																									
	CC					G					DL					DB					PP					
	29	30	31	32	33	29	30	31	32	33	29	30	31	32	33	29	30	31	32	33	29	30	31	32	33	
60	19-1363		72.7	80.2	77.6	94.4		50	50	40	35		30	30	40	40		20	20	20	25		13.8	5	7.5	18.8
61	19-1364		95.3	96.1	96.9	99.2		65	55	45	25		25	30	40	60		10	15	15	15		70	27.5	32.5	33.8
62	19-1365		68.8	69.3	70.8	92.7		60	70	70	50		35	20	20	40		5	10	10	10		47.5	33.8	37.5	42.5
63	19-1366		32.8	35.2	41.9	85.5		75	75	60	60		20	20	35	35		5	5	5	5		66.3	46.3	72.5	32.5
64	19-1367		33.3	34.9	39.6	84.9		85	85	80	80		10	10	15	15		5	5	5	5		32.5	15	16.3	17.5
65	19-1368		100	100	100	100		0	0	0	0		20	20	20	20		80	80	80	80		0	0	0	0
66	19-1657		77.1	82.6	88	97		45	40	35	30		25	30	35	30		30	30	30	40		22.5	21.3	21.3	22.5
67	19-1920		87	86.5	89.8	97.5		45	30	20	15		45	55	75	75		10	15	5	10		70	35	72.5	38.8
68	unknownA		84.1	85.4	86.5	96.6		40	40	40	5		15	15	30	25		45	45	30	70		2.5	5	10	26.3
69	19-1071	24.5	24.5	15.8	11.3		63	63	57	22		1	1	1	5		35	41	41	72						
70	19-1070	23.5	17.3	15.3	15.3		68	63	63	62		1	1	2	2		28	33	33	33						
71	19-1069	0	0	0	0		0	0	0	0		0	0	0	0		100	100	100	100						
72	19-1068	18.3	12	6.5	4		82	67	41	15		2	3	7	10		15	18	53	75						
73	19-0859	32	32.5	32.5	32.5		98	97	97	97		0	1	1	1		1	1	1	1						
74	19-0858	51.5	25.3	8.75	8.5		88	78	54	53		7	7	7	7		3	13	37	38						
75	19-0860	65.8	65.8	65.8	65.8		98	98	98	98		1	1	1	1		1	1	1	1						
76	19-0863	45.3	45.3	41.5	19		66	61	61	55		2	2	2	3		27	27	32	33						
77	19-0861	11	3.75	2	0		30	5	2	0		27	27	20	20		42	67	78	78						
78	19-0883	21.8	18.8	12.8	10.3		82	43	35	35		2	11	9	9		15	45	55	55						
79	19-1073	14	4.5	3.75	3.75		61	29	5	5		3	5	5	5		35	66	90	90						
80	19-1066	60.3	59.8	59.8	59.8		90	89	89	89		0	0	0	2		5	6	6	6						
81	19-0880	63.5	63.5	23.3	23.3		79	79	72	62		15	15	15	25		5	5	13	13						
82	19-0798	15.5	15.5	15.5	15.5		79	79	63	63		5	5	5	5		15	15	31	31						
83	29-0774	25.5	25.5	25.5	25.5		97	97	97	97		1	1	1	1		2	2	2	2						
84	19-0778	33.3	19.5	19.5	19.5		92	91	91	91		5	5	5	5		2	4	4	4						
85	19-0787	15.3	12	8.5	8.5		25	20	5	5		31	30	30	30		54	50	65	65						
86	19-0793	21.8	13	11.5	11.5		84	75	75	52		1	10	5	25		15	15	20	27						
87	19-9794	11	11	11	11		30	30	30	30		10	10	10	10		59	59	59	59						
88	19-0795	29.3	36.3	36.3	16.8		69	70	68	30		5	5	5	10		25	25	27	59						
89	19-0796	22	24.5	24.5	35		91	91	91	58		2	2	2	5		6	6	6	27						
90	19-0792	28.3	28.3	25.3	25.3		82	79	74	74		10	10	10	10		7	10	15	15						
91	19-1109	5.25	5.25	5.25	5.25		20	20	15	15		10	10	10	10		69	69	74	74						

Appendix II: Continued from previous page.

Study tree tag ID	Variable and week of year assessed																								
	CC					G					DL					DB					PP				
	29	30	31	32	33	29	30	31	32	33	29	30	31	32	33	29	30	31	32	33	29	30	31	32	33
92	19-1108	57.5	57.5	55.8	55.8	89	89	89	89		3	3	3	3		8	8	8	8						
93	19-1161		13.3	12.8	12.8		22	19	19			5	5	5			71	72	74						
94	19-1179		70.3	66.8	30		72	68	68			10	12	10			7	9	31						
95	19-0244	80.3	80.3	80	79.5	83	80	75	75		5	5	5	5		16	19	19	21						
96	19-0701	21.3				45					35					20									
97	19-0702	11				37					1					62									
98	19-0245	28.3	18.8	3	1.75	77	57	41	7		7	10	10	10		16	30	44	73						
99	19-0246	18.8	8	3	0	75	55	13	0		7	15	20	15		18	30	54	75						
100	19-0247	20	17.8	13	11.5	68	66	45	43		28	30	42	44		3	4	7	12						
101	19-0249a	39.5	39.5	39	39	95	89	89	89		2	3	3	3		3	8	8	8						
102	19-0249b	6.5	4	3	1	35	15	15	4		5	10	7	3		60	75	73	93						
103	19-0737		38.5	33	33		88	86	87			5	5	4			6	8	8						
104	19-1187		15.5	16.5	8.75		81	77	73			5	5	5			13	17	21						
105	19-0900a	0.5	0.5	0	0	5	5	0	0		5	5	10	10		90	90	90	90						
106	19-0900b	5.5	5.5	1	1	35	25	5	2		25	25	25	20		40	50	70	78						
107	19-0480	7.75	6.75	3.75	1.5	55	40	15	1		2	5	7	7		43	55	78	92						
108	19-0730		53.3	49.3	46.3		76	63	37			1	1	1			23	33	59						
109	19-0732		0.75	0	0		3	0	0			1	1	1			96	99	99						
110	19-1553		62.3	54.5	20.3		93	85	72			1	3	5			6	12	23						
111	19-0721		0	0	0		13	0	0			2	2	2			85	98	98						
112	19-1554		17.3	9.75	8		49	27	21			5	7	3			45	69	76						
113	19-0706	4.5	3	1.75	1.25	25	20	11	3		1	1	1	1		74	79	88	96						
114	19-0707	7.25	4.25	2.75	0	15	10	6	2		1	6	6	6		84	84	87	92						
115	19-0734		39.3	33.5	27		92	88	85			5	7	7			2	4	7						
116	19-0733		17	17	2		33	33	1			15	15	35			52	52	66						
117	19-0723		27.8	11	2		55	40	0			20	30	72			25	25	28						
118	19-1173		22.3	21.3			81	81				5	5				3	3							
119	unknownB	0	0	0	0	9	0	0	0		1	1	1	1		82	89	99	99						
120	19-0240	2.5	2.5	1.25	0	8	8	4	0		4	4	4	4		88	88	92	96						
121	19-0239	46.5	46.5	44.8	35.8	90	90	85	84		3	3	3	3		7	7	12	13						
122	19-1163		27.8	35.8	35.8		95	95	95			3	3	3			2	2	2						
123	19-1164		17	9.75	3		46	41	5			15	15	30			38	43	64						

Appendix II: Continued from previous page.

Study tree tag ID	Variable and week of year assessed																								
	CC					G					DL					DB					PP				
	29	30	31	32	33	29	30	31	32	33	29	30	31	32	33	29	30	31	32	33	29	30	31	32	33
124	19-0727	4.75	4.75	4.75		5	5	5			0	0	0			95	95	96							
125	19-0725	10.3	8.25	7.25		61	47	23			1	1	1			38	52	76							
126	19-0726	10	9.25	9.25		70	58	54			10	10	10			20	32	36							
127	19-1171	0	0	0		0	0	0			10	10	10			90	90	90							
128	19-1555a	19.3	14.8	19.5		95	91	70			0	1	1			5	8	29							
129	19-1555b	23	23	12.3		88	88	91			1	1	1			11	11	8							
130	19-0724	82	82	81		98	93	92			1	3	3			0	2	4							
131	19-0703	49.3	49.3	49.3	64	95	92	92	92		0	3	3	3		5	5	5	5						
132	19-0749	13	6.25	5		70	31	25			5	15	10			25	54	65							
133	19-0708	49.3	49.3	49.3	49.3	98	98	98	98		1	0	0	0		2	2	2	2						
134	19-0704	13	11.8	11.8	11.8	55	10	1	1		5	5	5	5		40	45	45	44						

Appendix IV: 2017 Disease progression survey data. Abbreviations are as follows, CC – average canopy cover, and DB - percent canopy dead branches.

Study tree tag ID	Variable and week of year assessed													
	CC							DB						
	27	28	29	30	31	32	33	27	28	29	30	31	32	33
1	16-0168			0.2			0.2				100			100
2	16-0234			0.2			0.2				100			100
3	16-0605					0.2	0.2					100		100
4	16-0921					0.2	0.2					100		100
5	17-0714	11	8.7			6.9	0.4	15	60			85		95
6	17-0716H	0.2	0.2	0.2			0.2	99	100	100				100
7	17-0717	49	40	36				5	10	10				
8	17-0719	20	27		23			5	15		30			
9	17-0720	32	21		35			15	20		20			
10	17-0721	28	33		37			5	5		10			
11	17-0722	19	21	14			7.4	60	80	80				80
12	17-0723	0.2	0.2	0.2				75	95	95				
13	17-0724	34	42	30				15	40	40				
14	17-0726	43	38		40			15	15		15			
15	17-0727	36	48		44			5	15		15			
16	17-0728	17	23		27			10	15		20			
17	17-0735		23	22	26		20		35	35	35			35
18	17-0737		59	47	42		49		5	5	5			5
19	17-0736		41	27	24				5	10	10			
20	17-0739	50	31		26		25	10	25		30			30
21	17-0740		15	17	9.8				60	85	90			
22	17-0741	52	25		24			5	10		10			
23	17-0742	43	25	24			27	5	5	5				5
24	17-0743	23	33				12	2	30	50			80	95
25	17-0747	24	22		22		30	5	5		5			5
26	17-0748	47	27		42		44	5	10		10			10
27	17-0749	44	36		39		37	10	10		10			10
28	17-0750	57	41		45		50	10	15		15			20
29	17-1019		25	18			24		15	15				15
30	17-1020		63	45			53	54	5	5			5	5

Appendix IV: Continued from previous page.

Study tree tag ID	Variable and week of year assessed													
	CC						DB							
	27	28	29	30	31	32	33	27	28	29	30	31	32	33
31	17-1023		13	13	14				50	60	60			
32	17-1024		35	27	34				30	30	30			
33	17-1025		43	32	41		40		5	20	20			30
34	17-1026		46	43	46				30	35	45			
35	17-1027		21	16	13		14		10	65	70			75
36	17-1038		16	16	14		12		90	90	90			95
37	17-1039			12	11	5.6	0.2			85	95	95		100
38	17-1040			17	13		12			55	65			80
39	17-1042				11	5.9	6.7	3.3			95	95	99	99
40	17-1043				34	32	21	13			25	25	25	50
41	17-1230				24	27	26	20			30	50	50	50
42	17-1401	38	31	33				36	10	15	15			15
43	17-1405			33		43	47	47			5		5	5
44	17-1406			0.7	0.2	0.2				99	100	100		
45	17-1407			38	34			19			25	50		80
46	17-1410		30		35			33		2		2		28
47	17-1411		52		48			42		5		5		15
48	17-1414		46	50				42		5	5			5
49	17-1413		10	8.7	6.7					95	100	100		
50	17-1415		23	18			16	15		45	45		45	50
51	17-1418			19	23		20	19			50	50	50	55
52	17-1419		38		39			37		5		5		5
53	17-1420		2.5	1.7	2			0.2		99	99	99		99
54	17-1421		24	20			7.4	3		50	55		60	80
55	17-1423			44	46		41	40			5	5	10	10
56	17-1433					0.2		0.2					100	100
57	17-1434			20	22		23	24			20	25	25	30
58	17-1435			34	36		33	35			15	15	15	15
59	17-1436			29	34			39			2	10		10
60	17-1437			46	58			48			5	5		5

Appendix IV: Continued from previous page.

Study tree tag ID	Variable and week of year assessed															
	CC						DB									
	27	28	29	30	31	32	33	27	28	29	30	31	32	33		
61	17-1440			21	21		20	18			15	15		15	15	
62	17-1441			29	33		32	33			10	10		15	15	
63	17-1466		29		14			6.1		40		50			60	
64	17-1701			5.9	4.3		2.2	1.2			90	90		90	90	
65	17-1702			64	61			61			2	5			5	
66	17-2027				1.7	0.2	0.2	0.2				95	95	95	99	
67	17-2036				49		54	42				5		10	10	
68	17-2356				16	18	17	18				30	30	30	30	
69	17-2357					1.7	3	2.8					90	90	90	
70	17-2357					5.6	5.6	2.2					95	95	95	
71	17-2368					16	13	14					20	20	20	
72	17-2378					9.8	11	9					65	65	70	
73	17-2379						40	41						5	5	
74	17-2381						8.7	11						0	15	
75	17-2383						10	7.2						80	80	
76	17-2452					59		52						5	5	
77	17-0744B						26	23	14					30	60	60
78	17-0744C				40	25	17	19				30	40	40	40	
79	17-0744E					62	50	48					2	10	10	
80	17-1416				7.7	7.7	5.1	1.2					20	20	30	40

Appendix V: Mid-crown canopy branch sampling data for all study years. Trees with an ID in orange highlight are 2017 trees removed in 2018 (with no disease progression survey data attached). Weighted NEBB gallery density are recorded as galleries/m².

	Study tree tag ID	Count of NEBB galleries	Weighted Fungal Staining %	Weighted NEBB gallery density	NEBB galleries detectable / undetectable
1	17-0587	38	12.3	0.656	Detectable
2	17-0714	8	10.8	0.002	Detectable
3	17-0716H	851	75.0	0.204	Detectable
4	17-0717	0	25.0	0.000	Undetectable
5	17-0719	0	36.2	0.000	Undetectable
6	17-0720	0	16.0	0.000	Undetectable
7	17-0721	0	6.0	0.000	Undetectable
8	17-0722	230	56.8	0.048	Detectable
9	17-0724	0	4.4	0.000	Undetectable
10	17-0726	0	8.7	0.000	Undetectable
11	17-0727	0	18.1	0.000	Undetectable
12	17-0728	0	2.5	0.000	Undetectable
13	17-0735	19	56.2	0.008	Detectable
14	17-0736	0	9.7	0.000	Undetectable
15	17-0739	0	31.2	0.000	Undetectable
16	17-0740	10	46.2	0.004	Detectable
17	17-0741	0	30.0	0.000	Undetectable
18	17-0744	10	24.6	0.388	Detectable
19	17-0744A	0	47.5	0.000	Undetectable
20	17-0744C	0	0.0	0.000	Undetectable
21	17-0744E	0	0.0	0.000	Undetectable
22	17-0746	0	48.1	0.000	Undetectable
23	17-0747	0	12.9	0.000	Undetectable
24	17-0748	0	15.5	0.000	Undetectable
25	17-0750	0	18.1	0.000	Undetectable
26	17-1023	0	20.0	0.000	Undetectable
27	17-1024	0	26.9	0.000	Undetectable
28	17-1026	0	46.2	0.000	Undetectable
29	17-1038	14	13.5	0.003	Detectable
30	17-1039	10	46.7	1.539	Detectable

Appendix V: Continued from previous page.

	Study tree tag ID	Count of NEBB galleries	Weighted Fungal Staining %	Weighted NEBB gallery density	NEBB galleries detectable / undetectable
31	17-1042	1	35.1	0.064	Detectable
32	17-1043	0	30.2	0.000	Undetectable
33	17-1054	25	25.2	2.010	Detectable
34	17-1122	131	10.2	1.788	Detectable
35	17-1125	46	15.3	1.028	Detectable
36	17-1126	46	9.9	0.603	Detectable
37	17-1131	0	7.0	0.000	Undetectable
38	17-1134	2	6.3	0.033	Detectable
39	17-1136	0	0.4	0.000	Undetectable
40	17-1137	71	15.7	1.726	Detectable
41	17-1138	13	15.9	0.323	Detectable
42	17-1139	58	19.8	1.486	Detectable
43	17-1140	31	13.0	0.582	Detectable
44	17-1141	39	8.3	0.497	Detectable
45	17-1142	26	9.7	0.463	Detectable
46	17-1148	49	8.1	1.419	Detectable
47	17-1348	1	6.7	0.007	Detectable
48	17-1405	0	19.3	0.000	Undetectable
49	17-1406	384	72.1	0.092	Detectable
50	17-1412	0	17.8	0.000	Undetectable
51	17-1413	0	53.7	0.000	Undetectable
52	17-1415	2	45.4	0.267	Detectable
53	17-1416	0	30.3	0.000	Undetectable
54	17-1427A	12	27.3	1.374	Detectable
55	17-1427B	0	24.7	0.000	Undetectable
56	17-1433	76	40.0	0.000	Undetectable
57	17-1434	3	20.0	0.000	Undetectable
58	17-1435	0	26.2	0.000	Undetectable
59	17-1441	0	9.4	0.000	Undetectable

Appendix V: Continued from previous page.

	Study tree tag ID	Count of NEBB galleries	Weighted Fungal Staining %	Weighted NEBB gallery density	NEBB galleries detectable / undetectable
60	17-1701	2	30.6	0.122	Detectable
61	17-1907	0	2.5	0.000	Undetectable
62	17-2027	159	30.3	10.649	Detectable
63	17-2078	33	19.4	2.229	Detectable
64	17-2357	0	33.1	0.000	Undetectable
65	17-2942	14	9.4	0.175	Detectable
66	17-3444	24	15.2	0.476	Detectable
67	17-4101	50	8.6	0.917	Detectable
68	17-4104	0	9.7	0.000	Undetectable
69	18-0129	18	100.0	0.440	Detectable
70	18-0858	65	100.0	0.710	Detectable
71	18-0859	0	2.9	0.000	Undetectable
72	18-0862	1	100.0	0.010	Detectable
73	18-0866	15	66.0	0.160	Detectable
74	18-0867	0	81.0	0.000	Undetectable
75	18-0868	24	98.0	0.150	Detectable
76	18-0870	1	25.0	0.010	Detectable
77	18-0871	0	4.4	0.000	Undetectable
78	18-0872	8	88.9	0.100	Detectable
79	18-0873	0	0.0	0.000	Undetectable
80	18-0874	9	17.1	0.040	Detectable
81	18-0876	0	29.3	0.000	Undetectable
82	18-0877	0	2.1	0.000	Undetectable
83	18-0879	0	0.0	0.000	Undetectable
84	18-0881	0	2.7	0.000	Undetectable
85	18-0883	7	52.2	0.080	Detectable
86	18-0884	39	100.0	0.290	Detectable
87	18-0887	3	26.0	0.030	Detectable
88	18-0888	0	56.0	0.000	Undetectable
89	18-0891	43	76.2	0.790	Detectable
90	18-1172	0	22.8	0.000	Undetectable

Appendix V: Continued from previous page.

	Study tree tag ID	Count of NEBB galleries	Weighted Fungal Staining %	Weighted NEBB gallery density	NEBB galleries detectable / undetectable
91	18-1174	10	26.5	0.150	Detectable
92	18-1176	0	0.0	0.000	Undetectable
93	18-1181	58	73.6	0.710	Detectable
94	18-1183	0	98.8	0.000	Undetectable
95	18-1184	9	59.5	0.090	Detectable
96	18-1185	2	21.3	0.020	Detectable
97	18-1186	0	92.2	0.000	Undetectable
98	18-1189	0	29.1	0.000	Undetectable
99	18-1831	31	78.8	0.240	Detectable
100	19-0506	32	100.0	2.745	Detectable
101	19-0512	4	27.5	0.020	Detectable
102	19-0513	0	0.5	0.000	Undetectable
103	19-0519	4	12.9	0.030	Detectable
104	19-0520	14	60.8	0.170	Detectable
105	19-0521	0	20.3	0.000	Undetectable
106	19-0525	0	2.1	0.000	Undetectable
107	19-0529	0	31.5	0.000	Undetectable
108	19-0532	24	100.0	0.230	Detectable
109	19-0533	25	98.6	0.220	Detectable
110	19-0535	0	0.0	0.000	Undetectable
111	19-0543	6	37.5	0.040	Detectable
112	19-0550	133	100.0	1.030	Detectable
113	19-0902	1	33.8	0.010	Detectable
114	19-0903	0	64.0	0.000	Undetectable
115	19-0904	3	60.9	0.010	Detectable
116	19-0905	0	83.0	0.000	Undetectable
117	19-0906	93	100.0	0.560	Detectable
118	19-0907	0	3.3	0.000	Undetectable
119	19-0908	2	35.9	0.020	Detectable
120	19-0912	23	100.0	0.370	Detectable

Appendix V: Continued from previous page.

	Study tree tag ID	Count of NEBB galleries	Weighted Fungal Staining %	Weighted NEBB gallery density	NEBB galleries detectable / undetectable
121	19-0913	34	100.0	0.250	Detectable
122	19-0914H	454	100.0	5.290	Detectable
123	19-0916H	285	100.0	3.390	Detectable
124	19-0917	19	76.6	0.150	Detectable
125	19-0918H	38	100.0	0.630	Detectable
126	19-0919	97	99.7	0.750	Detectable
127	19-0947	12	13.0	0.100	Detectable
128	19-1023	2	7.1	0.010	Detectable
129	19-1024	0	39.6	0.000	Undetectable
130	19-1025	0	69.7	0.000	Undetectable
131	19-1351	4	100.0	0.160	Detectable
132	19-1352	6	97.8	0.060	Detectable
133	19-1353	1	41.6	0.010	Detectable
134	19-1354	2	46.6	0.010	Detectable
135	19-1355	3	93.5	0.030	Detectable
136	19-1356	12	100.0	0.200	Detectable
137	19-1357(1)	6	92.3	0.090	Detectable
138	19-1357(2)	171	100.0	2.150	Detectable
139	19-1358	0	44.4	0.000	Undetectable
140	19-1359	0	55.7	0.000	Undetectable
141	19-1361	2	28.2	0.000	Undetectable
142	19-1362H	0	0.0	0.000	Undetectable
143	19-1363	0	95.1	0.000	Undetectable
144	19-1364	0	100.0	0.000	Undetectable
145	19-1366	61	73.6	0.520	Detectable
146	19-1368	286	100.0	2.500	Detectable
147	19-1657	15	93.2	0.220	Detectable

Appendix VI: Continued from previous page.

Study tree tag ID	Days since initial light intensity assessment.																												Ave. Light intensity (Lux)						
	I.	1	5	10	12	13	14	17	18	19	29	21	24	25	26	27	28	31	32	33	34	35	38	39	40	41	47	48		49	50	51	52		
31	17-1024			10		13								41																				21.3	
32	17-1025			13		13						44																		7			18.9		
33	17-1026			13		13						37																						20.8	
34	17-1027			25		45				36																					12			29.4	
35	17-1038						2					55	51																		14			30.3	
36	17-1039						12					51		10																	9			20.6	
37	17-1040						14					62																		11				28.9	
38	17-1042						13					26		14																	15			17.0	
39	17-1043						14					55		15																	36			30.0	
40	17-1230																22						20		36					7				21.3	
41	17-1401		4			17					14																			16				12.6	
42	17-1405						16						30														3		7					14.0	
43	17-1406						15					40									9													21.1	
44	17-1407						1									18															10			9.7	
45	17-1410						6						26																		10			14.0	
46	17-1411						4						22																		11			12.2	
47	17-1413						1					40	14																					18.4	
48	17-1414						0									44															12			18.8	
49	17-1415						15					20					23														14			17.7	
50	17-1418						19						12								11							11						13.3	
51	17-1419						29						13																		15			18.8	
52	17-1420						15					49								14								16						23.3	
53	17-1421						21					51										7							44					30.8	
54	17-1423						17									13						6							12					11.9	
55	17-1433																			10								34						21.7	
56	17-1434											24				19						9										11			15.6
57	17-1435											24				25												8		46					25.8
58	17-1436											36				49															20				34.8
59	17-1437											23				39															22				27.8
60	17-1440											25				34												11		8					19.4

Appendix VI: Continued from previous page.

Study tree tag ID	Days since initial light intensity assessment.																												Ave. Light intensity (Lux)					
	I.	1	5	10	12	13	14	17	18	19	29	21	24	25	26	27	28	31	32	33	34	35	38	39	40	41	47	48		49	50	51	52	
61	17-1441											59				64										44	21							46.8
62	17-1466						8										13															2		7.5
63	17-1701											40				23							18					6					21.5	
64	17-1702											19				14														12			14.9	
65	17-2027															31				18	30						7					21.3		
66	17-2036															49				39											2		30.0	
67	17-2356															24				52		17					33						31.5	
68	17-2357																			78	96						74						82.8	
69	17-2368																			21					45					17			27.6	
70	17-2378																			14					51					21			28.3	
71	17-2379																							11						10			10.6	
72	17-2381																							11									10.8	
73	17-2382																													16			16.3	
74	17-2383																									55					12			33.6
75	17-2452																					10										1		5.6

Appendix VII: Trunk debarking data from 2017 and 2018. Weighted fungal staining (%) and weighted NEBB gallery density (galleries per square meter) calculated as high canopy sampling values were calculated (see analysis). Trees in 2017 (only “Bottom” sample) had a single bark strip taken.

ID	Debark Date	DBH (cm)	Bottom		Top		Weighted fungal staining (%)	Weighted NEBB gallery density	
			Fungal Staining (%)	NEBB Gallery count	Fungal Staining (%)	NEBB Gallery count			
1	16-0719	6-11-18	69	100.0	31	100.0	12.0	100.0	0.2
2	16-1002	6-11-18	57	98.8	76	98.8	92.0	98.8	0.8
3	17-0359	6-04-18	75	0.0	0	0.0	0.0	0.0	0.0
4	17-0361	6-04-18	45	0.0	0	0.0	0.0	0.0	0.0
5	17-0366	6-06-18	56	100.0	124	100.0	176.0	100.0	1.5
6	17-0374	6-06-18	53	55.0	38	68.8	11.0	61.9	0.2
7	17-0753	6-06-18	40	78.8	0	78.8	0.0	78.8	0.0
8	17-0765	6-11-18	48	80.0	0	80.0	0.0	80.0	0.0
9	17-0765	6-11-18	62	91.3	0	91.3	0.0	91.3	0.0
10	17-1578	6-12-18	69	18.8	0	20.0	0.0	19.4	0.0
11	17-1596	6-13-18	57	0.0	0	0.0	0.0	0.0	0.0
12	17-1597	6-13-18	65	7.8	0	7.8	0.0	7.8	0.0
13	17-1598	6-13-18	72	80.0	0	80.0	0.0	80.0	0.0
14	17-1599	6-13-18	65	99.3	24	99.3	33.0	99.3	0.3
15	17-1950	5-31-18	59	0.0	0	0.0	0.0	0.0	0.0
16	17-2014	6-12-18	69	97.5	0	97.5	0.0	97.5	0.0
17	17-2051	5-31-18	61	23.8	0	24.0	0.0	23.9	0.0
18	17-2052	6-01-18	69	22.5	0	27.5	0.0	25.0	0.0
19	17-2055	6-01-18	65	52.5	4	56.3	6.0	54.4	0.1
20	17-2091	6-01-18	55	0.0	0	0.0	0.0	0.0	0.0
21	17-2460	6-12-18	58	35.0	0	35.0	0.0	35.0	0.0
22	17-3477	6-04-18	64	18.8	0	18.8	0.0	18.8	0.0
23	17-3479	6-04-18	48	15.0	0	15.0	0.0	15.0	0.0
24	17-3647	6-13-18	60	56.3	50	56.3	62.0	56.3	0.6
25	17-3852	6-04-18	66	21.3	0	21.3	0.0	21.3	0.0
26	17-4095	6-06-18	63	87.5	0	87.5	0.0	87.5	0.0
27	16-0168	8-03-17	97	57.5	30			57.5	0.3
28	16-0234	8-01-17	59	16.3	0			16.3	0.0
29	16-0605	8-08-17	89	95.0	85			95.0	0.9
30	16-0921	8-07-17	102	67.5	14			67.5	0.1

Appendix VII: Continued from previous page.

ID	Debark Date	DBH (cm)	Bottom		Top		Weighted fungal staining (%)	Weighted NEBB gallery density
			Fungal Staining (%)	NEBB Gallery count	Fungal Staining (%)	NEBB Gallery count		
31	17-0714	8-11-17	51	51.7	10		51.7	0.1
32	17-0716	7-24-17	89	67.5	2		67.5	0.0
33	17-0717	7-27-17	48	3.8	0		3.8	0.0
34	17-0719	8-03-17	90	55.0	0		55.0	0.0
35	17-0720	8-02-17	66	30.0	0		30.0	0.0
36	17-0721	8-02-17	78	58.8	0		58.8	0.0
37	17-0722	7-24-17	68	12.5	1		12.5	0.0
38	17-0723	7-24-17	23	48.8	0		48.8	0.0
39	17-0724	7-26-17	72	2.5	0		2.5	0.0
40	17-0726	8-04-17	64	95.0	0		95.0	0.0
41	17-0727	8-03-17	74	1.3	0		1.3	0.0
42	17-0728	8-03-17	79	16.3	0		16.3	0.0
43	17-0735	7-25-17	43	35.0	0		35.0	0.0
44	17-0736	8-03-17	59	47.5	0		47.5	0.0
45	17-0739	8-15-17	70	20.0	0		20.0	0.0
46	17-0740	7-25-17	58	31.3	0		31.3	0.0
47	17-0741	7-27-17	38	10.0	0		10.0	0.0
48	17-0743	7-27-17	79	37.5	0		37.5	0.0
49	17-0744	7-27-17	50	0.0	0		0.0	0.0
50	17-0744	8-16-17	52	50.0	0		50.0	0.0
51	17-0746	8-17-17	46	15.0	0		15.0	0.0
52	17-0747	8-07-17	79	0.0	0		0.0	0.0
53	17-0748	8-08-17	74	0.0	0		0.0	0.0
54	17-0749	8-08-17	80	1.3	0		1.3	0.0
55	17-0750	8-08-17	62	13.8	0		13.8	0.0
56	17-1020	8-23-17	69	10.0	0		10.0	0.0
57	17-1023	7-25-17	37	12.5	0		12.5	0.0
58	17-1024	8-01-17	88	7.5	0		7.5	0.0
59	17-1025	7-28-17	77	13.8	0		13.8	0.0
60	17-1026	7-31-17	75	22.5	0		22.5	0.0

Appendix VII: Continued from previous page.

ID	Debark Date	DBH (cm)	Bottom		Top		Weighted fungal staining (%)	Weighted NEBB gallery density
			Fungal Staining (%)	NEBB Gallery count	Fungal Staining (%)	NEBB Gallery count		
61	17-1027	7-26-17	56	55.0	0		55.0	0.0
62	17-1038	7-31-17	53	32.5	0		32.5	0.0
63	17-1039	8-01-17	67	56.3	0		56.3	0.0
64	17-1042	8-01-17	68	57.5	0		57.5	0.0
65	17-1052	8-11-17	26	57.5	0		57.5	0.0
66	17-1054	8-15-17	50	52.3	0		52.3	0.0
67	17-1055	8-15-17	26	94.8	0		94.8	0.0
68	17-1230	8-16-17	62	80.0	0		80.0	0.0
69	17-1401	7-27-17	45	0.0	0		0.0	0.0
70	17-1405	8-23-17	64	0.0	0		0.0	0.0
71	17-1406H	8-09-17	69	88.8	65		88.8	0.7
72	17-1412	8-23-17	46	3.8	0		3.8	0.0
73	17-1413	7-31-17	62	43.8	0		43.8	0.0
74	17-1415	8-07-17	47	77.5	0		77.5	0.0
75	17-1416	8-16-17	40	89.5	0		89.5	0.0
76	17-1418	8-10-17	66	32.5	0		32.5	0.0
77	17-1420	8-08-17	83	98.5	0		98.5	0.0
78	17-1421	8-11-17	73	41.3	0		41.3	0.0
79	17-1423	8-17-17	66	10.0	0		10.0	0.0
80	17-1427	8-11-17	23	93.8	0		93.8	0.0
81	17-1434	8-11-17	61	51.3	0		51.3	0.0
82	17-1435	8-23-17	73	42.5	0		42.5	0.0
83	17-1440	8-18-17	63	60.0	0		60.0	0.0
84	17-1441	8-18-17	80	15.0	6		15.0	0.1
85	17-1701	8-14-17	80	48.8	0		48.8	0.0
86	17-2027	8-14-17	50	62.5	0		62.5	0.0
87	17-2078	8-23-17	57	88.8	44		88.8	0.4
88	17-2356	8-15-17	45	73.5	0		73.5	0.0
89	17-2357	8-14-17	53	63.8	0		63.8	0.0
90	17-2375	8-29-17	104	87.5	0		87.5	0.0

Appendix VII: Cambium and bark moisture percentages recorded in 2017. Samples taken in trunk were removed before trunk debarking occurred, samples in “Circle” were taken after trunk debarking and on the site of removed bark. Samples taken in “Upper” were removed through the bark above the removed area post trunk debarking.

Sample Tag ID	Trunk		Circle		Upper	
	Bark Moisture %	Cambium Moisture %	Bark Moisture %	Cambium Moisture %	Bark Moisture %	Cambium Moisture %
1 16-0168	24.9	80.4			17.9	
2 16-0234	4.8	77.2				
3 16-0605	17.4			34.0		
4 16-0618			7.1	15.1	7.5	
5 16-0921	21.6	36.7				
6 17-0714	16.9		13.0	48.6		
7 17-0716H	1.4	57.9	24.2	87.2		
8 17-0717	72.9					
9 17-0719	48.3	33.7				
10 17-0720	9.3	75.2				
11 17-0721	55.0	54.7				
12 17-0722	10.7	131.0			12.8	
13 17-0723	55.2					
14 17-0724	48.6	90.8			15.2	
15 17-0726	27.7	111.3				
16 17-0727	6.1	54.5	16.5	37.0		
17 17-0728	68.1	80.2				
18 17-0729			15.6	55.3		
19 17-0735	0.8	26.8	21.6	55.7	30.3	14.3
20 17-0736	11.5	57.7	8.7	58.5		
21 17-0739	10.5	53.4	10.4	33.9		
22 17-0740			10.6	64.6		
23 17-0741	27.9		10.1	40.5		
24 17-0743	5.2	55.0	9.6	40.2		
25 17-0744A	5.1	36.2	-0.9	30.9		
26 17-0744B	1.8	33.6	9.5	33.0		
27 17-0744C						
28 17-0744D	88.7		17.9	51.6		
29 17-0744E	26.8		30.0	20.8		
30 17-0745	1.4	79.2				

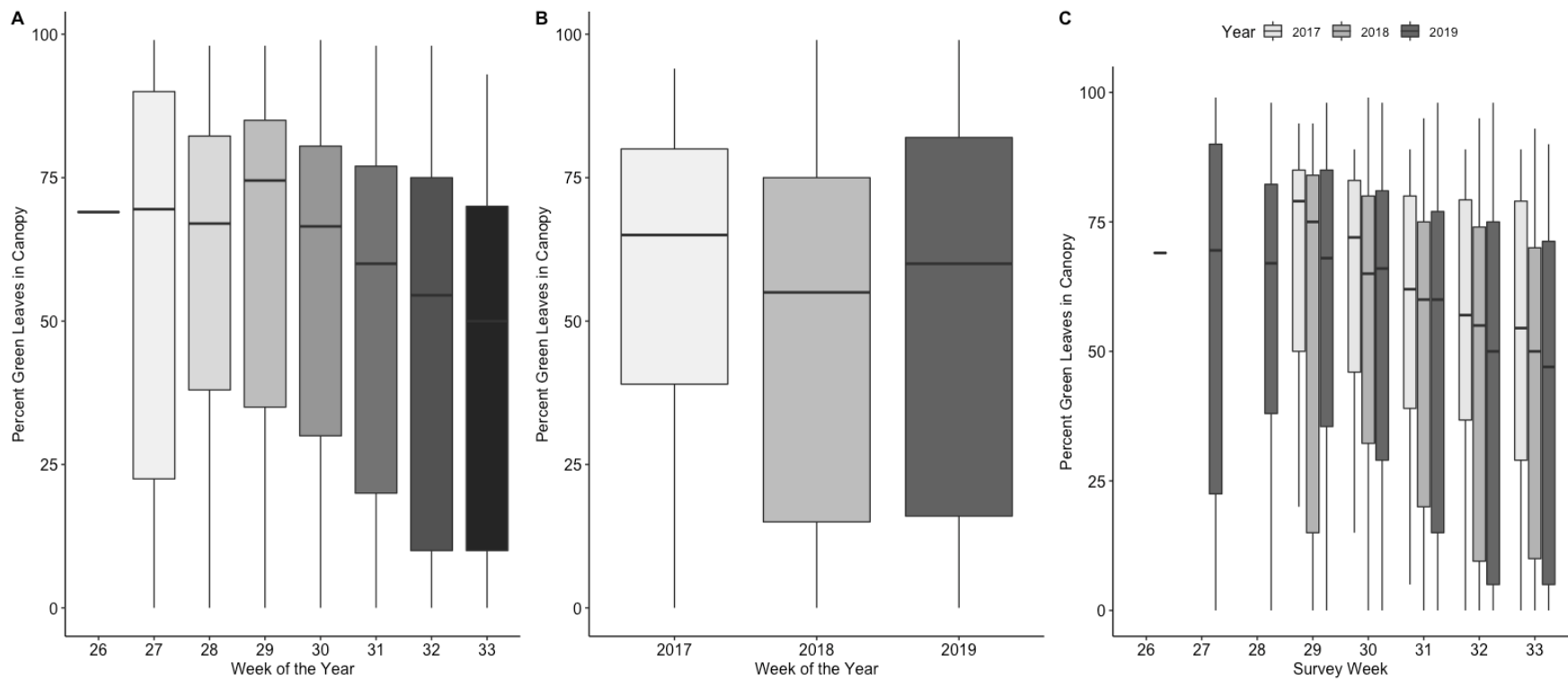
Appendix VIII: Continued from previous page.

Sample Tag ID	Trunk		Circle		Upper	
	Bark	Cambium	Bark	Cambium	Bark	Cambium
	Moisture %	Moisture %	Moisture %	Moisture %	Moisture %	Moisture %
31 17-0746	54.9		29.7			
32 17-0747	10.2	72.0	15.4	23.8		
33 17-0748	20.4	105.1	9.2	47.4		
34 17-0749	43.2	43.2	11.0	45.1		
35 17-074y			34.4	34.8		
36 17-0750	20.8	57.9	12.4	47.1		
37 17-1020	29.5		13.6	32.5		
38 17-1023	67.8	36.0	177.4	49.4	33.3	
39 17-1024	37.7	54.5	11.4	26.8		
40 17-1025	21.4	86.5	16.8	40.4		
41 17-1026	22.0	82.9	12.5	52.6	33.6	
42 17-1027	3.7	65.2	13.9	35.5		
43 17-1038	4.8	60.0				
44 17-1039	3.6	76.5				
45 17-1042	8.5	111.8	12.6	27.5		
46 17-1043	4.1	42.7	5.0	61.0		
47 17-1052	42.7		20.0	36.2		
48 17-1054	50.5	57.9	18.2	63.7		
49 17-1055	15.9	51.6	12.3	91.7		
50 17-1083			11.8			
51 17-1230	23.0		12.6	-8.2		
52 17-1401	58.2		46.7	75.0		
53 17-1405	15.2	60.0	13.4	50.0		
54 17-1406	23.2	62.7				
55 17-1406H	61.8	36.4				
56 17-1412	67.3	53.3	54.3			
57 17-1413	4.0	52.8	7.8	48.9	23.8	
58 17-1415	93.0		6.6	-8.4		
59 17-1416	23.4		16.7	53.3		

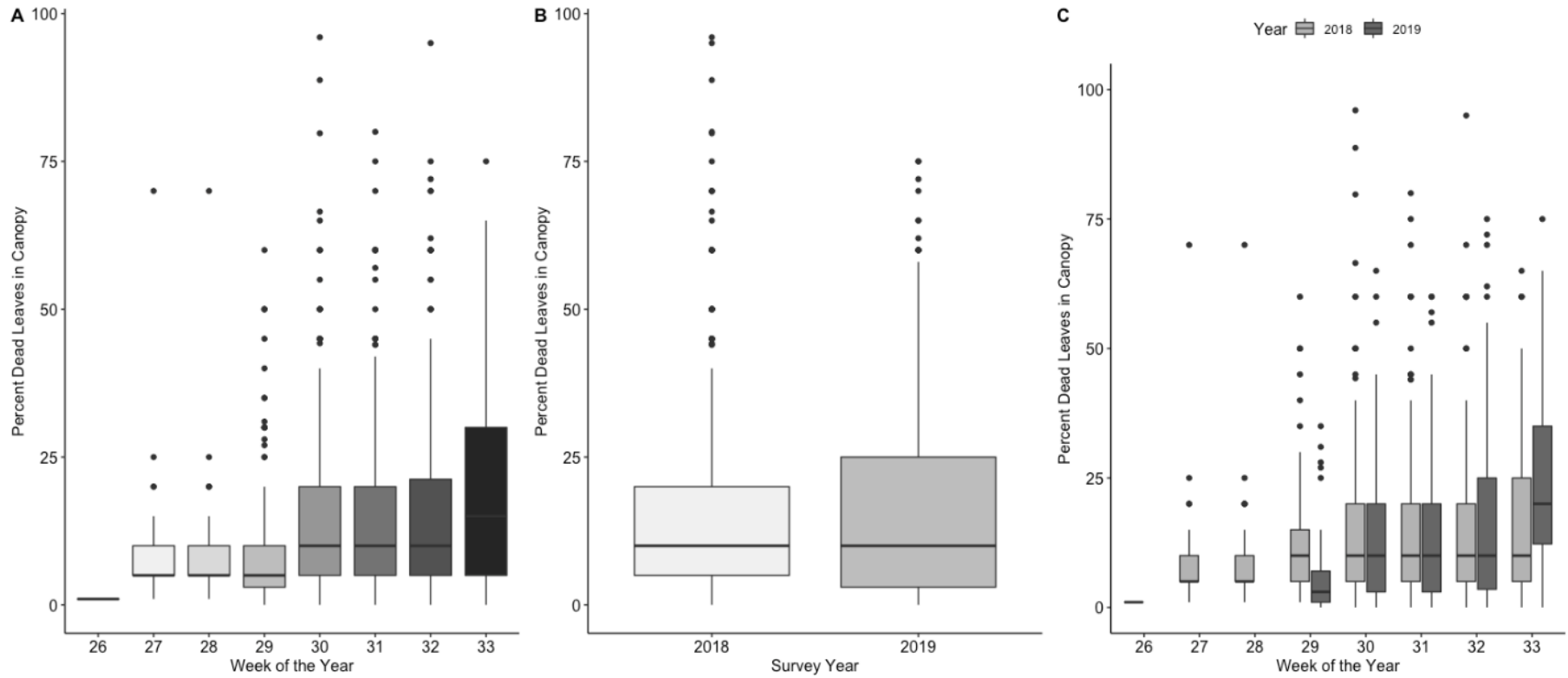
Appendix VIII: Continued from previous page.

Sample Tag ID	Trunk		Circle		Upper	
	Bark	Cambium	Bark	Cambium	Bark	Cambium
	Moisture %	Moisture %	Moisture %	Moisture %	Moisture %	Moisture %
60 17-1418	7.5	80.4	5.0	41.2		
61 17-1420	-32.0	30.5	8.0	55.2		
62 17-1421	24.0	38.9	13.8	39.7		
63 17-1423	25.2	35.3	13.8	32.4		
64 17-1427	115.5		131.3			
65 17-1433	38.7	111.7	9.5	31.9		
66 17-1434	17.2		8.7	50.0		
67 17-1435			14.8	41.8		
68 17-1437	45.2	75.9				
69 17-1440	36.5	15.7	7.6	64.2		
70 17-1441	2.7	55.0	13.8	58.5		
71 17-1701	127.4	127.4	14.2			
72 17-2027	65.8		12.9	55.6		
73 17-2078	35.2	102.7	11.9	78.0		
74 17-2356	56.4	15.3	11.8	57.5		
75 17-2357	40.7	61.8	18.7	64.2		
76 17-2375	8.5	56.3				

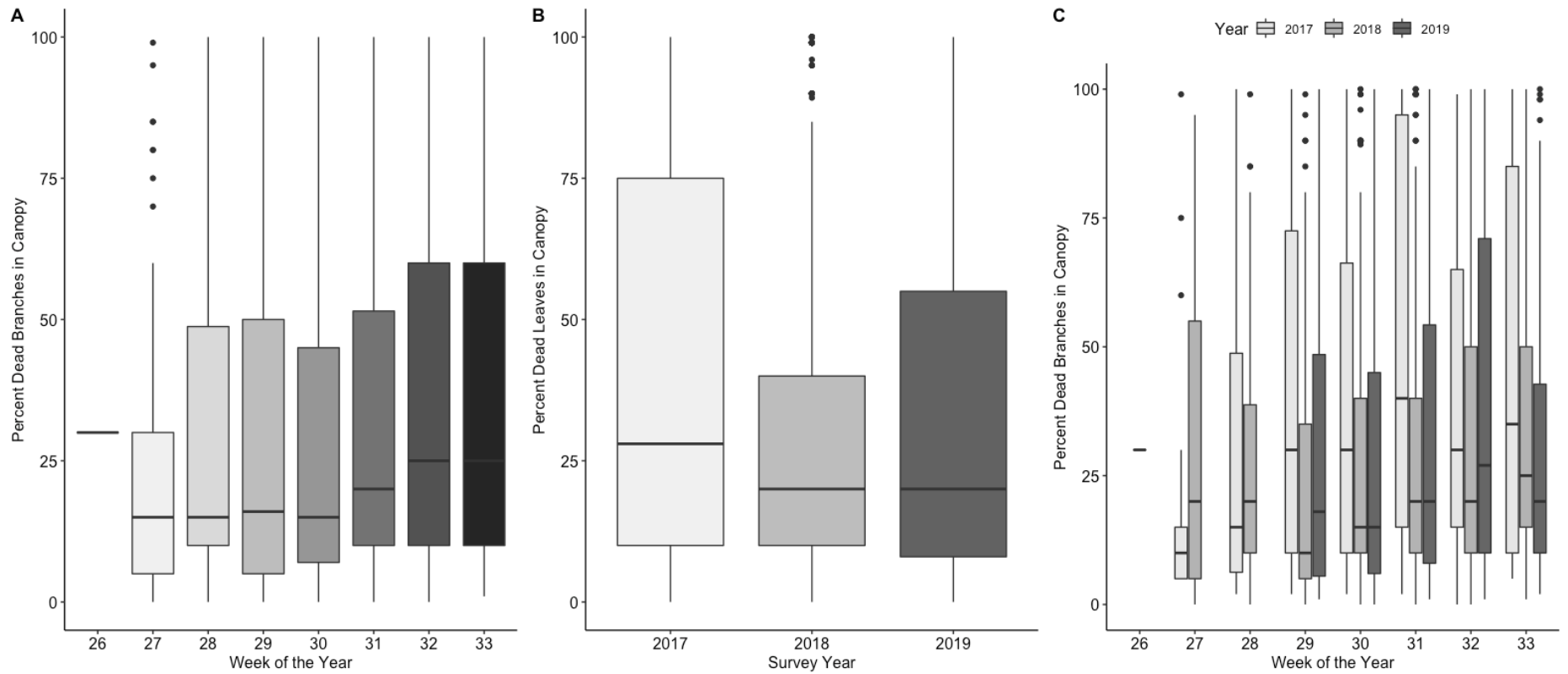
Appendix IX: Summary of percent canopy green leaves, 2017 to 2019. A. Boxplot of percent canopy green leaves for each study week over 3 years. B. Boxplot of percent canopy green leaves for each year. C. Boxplot of percent weekly canopy green leaves, for each year by study week.



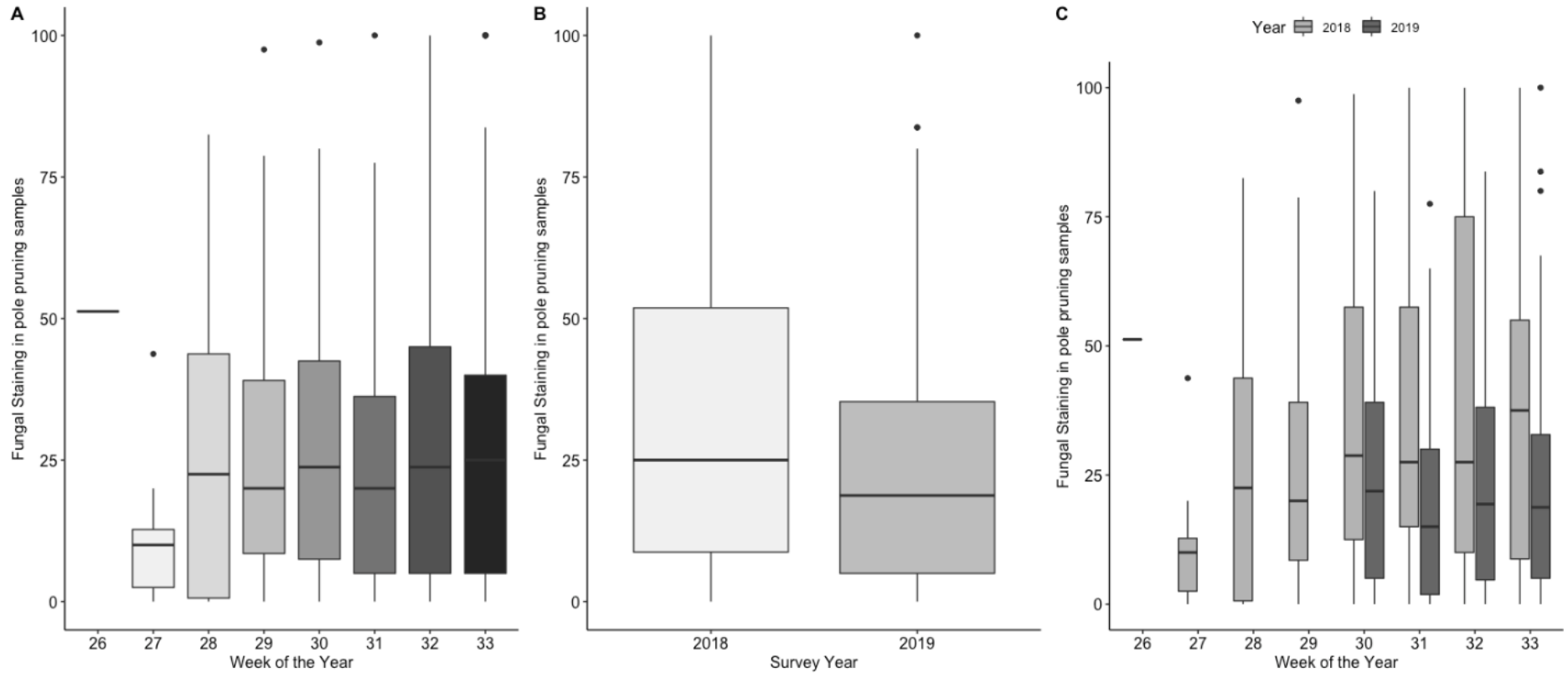
Appendix X: Summary of percent canopy dead leaves, 2017 to 2019. A. Boxplot of percent canopy dead leaves for each study week over three years. B. Boxplot of percent canopy dead leaves for each year. C. Boxplot of percent canopy dead leaves, for each year by study week.



Appendix XI: Summary of percent canopy dead branches, 2017 to 2019. A. Boxplot of percent canopy dead branches for each study week over three years. B. Boxplot of percent canopy dead branches for each year. C. Boxplot of percent canopy dead branches, for year by study week.



Appendix XII: Summary of percent fungal staining from pole pruning samples, 2018 and 2019. A. Boxplot of variable average each week over two years. B. Boxplot of variable average for each year. C. Boxplot variable average, for each year by study week.



Appendix XIII: Summary of mid-crown variables recorded during tree canopy sampling. A: Boxplot of percent fungal staining from tree canopy samples by study year. B: Boxplot of NEBB brood gallery density per each study year.

