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## Chapter 1: Introduction

Black spruce (Zesegaandag [Ojibwe]; *Picea mariana* [Mill] BSP) is a dominant and wide-ranging species of the North American boreal forest whose composition and structure are influenced by both stand-replacing, high-intensity fires and small-scale, frequent disturbances from wind and pathogens resulting in gap dynamics (Viereck and Johnston, 1990; Fenton et al., 2009). The boreal forest reaches its southern and western edge in Minnesota, USA, where black spruce comprises roughly 9% of the state's 17.4 million forested acres (Miles, 2017). The soils in these poorly drained, nutrient-poor systems are upwards of 4,000 years old. They are dominated by peatlands soils (75%) that can reach an excess of 15 feet in-depth, where black spruce, eastern larch (*Larix laricina* [(Du Roi) K. Koch]; mashkiigwaatig [Objibwe], and balsam fir (*Abies balsamea* [(L) Miller]; zhingob [Ojibwe]) is the primary cover type (Aaseng et al., 2003; Heinselman, 1963). The understory vegetation in peatland forests and swamps is dominated by ericaceous shrubs such as Labrador tea (*Rhododendron groenlandicum* Oeder), bog laurel (*Kalmia polifolia* Wangenh.), small cranberry (*Vaccinium oxycoccos* L.), and leatherleaf (*Chamaedaphne calyculata* (L.) Moench), that sits atop a moss layer composed of varying species of sphagnum.

Unsurprisingly, black spruce is the dominant tree species within the black spruce forest type. It is a long-lived species whose site index values at 50 years can range from nearly 15 feet on lower-quality sites to almost 45 feet on higher-quality places (Johnston, 1977). Black spruce is an important species both ecologically and economically. Mature black spruce stands provide essential habitat for wildlife such as the snowshoe hare (*Lepus americanus* [Erxleben]), spruce grouse (*Falcipennis canadensis* [Linnaeus]), American marten (*Martes americana* [Turton]), salamanders (Salamandridae family), cavity-nesting birds, as well as numerous small mammals (Cimon-Morin et al., 2010). Black spruce is also the second most harvested pulpwood species, with 226,129 cords of spruce harvested in 2017 in Minnesota (Minnesota Forest Resource Report, 2021); long fibers of black spruce (and regionally white spruce (*Picea glauca* (Moench) Voss)) are essential for the production of high-quality paper and pulpwood products (Viereck and Johnston, 1990; Sabourin and Hart, 2010).

Black spruce has relatively few native and invasive biotic disturbance agents, which differs from other forest ecosystems in the region and nationally. One of the most common small-scale disturbance agents which results in the mortality of black spruce is the eastern dwarf mistletoe (*Arceuthobium pusillum* [Peck]) (EDM) (Baker and French, 1991; Ostry and Nicholls, 1979). EDM is a native disturbance agent across the range of black spruce. Within Minnesota, EDM prevalence is variable, with estimates ranging from 10% to 50% of black spruce stands infected in northern Minnesota (Baker et al., 2012; Gray et al., 2021).

The wide range of variability is significant to note since once a black spruce tree is infected, EDM has been shown to cause mortality in roughly seventeen years, with a rate of infection of 2.42 feet per year (Baker and French, 1980). The high likelihood of mortality in a relatively short time period may be related to its relatively newer evolutionary history within the region (i.e., black spruce is not as well-equipped to fend off EDM compared to both white and red spruce (*Picea rubens* Sarg.) (Baker et al., 1980). EDM's relatively quick and certain mortality differs from many other mistletoes in North America. Other dwarf mistletoe species (*Arceuthobium*, Viscaceae) are often considered “nuisances" from a timber perspective because there are declines in growth, decreased timber quality, and some levels of mortality (Geils and Hawksworth, 2002; Singh, 1982; Villier et al., 2017). However, these mistletoes often are noted to provide important nesting sites for different bird species (Queijeiro-Bolaños et al., 2018).

The sometimes-cryptic infections of EDM, coupled with the commercial importance of black spruce, make management practices more complex, especially if *Arceuthobium* infections potentially have synergistic impacts with other disturbance agents. One such disease agent is *Armillaria* (Agaricales, Basidiomycota), a prolific fungal species that causes *Armillaria* root rot in both hardwoods and conifers; *Armillaria* has been noted to infect upwards of five hundred woody trees and shrub host species across various systems (Devkota and Hammerschmidt, 2020; Kubiak et al., 2017). *Armillaria* is a facultative necrotroph; it can actively attack living cambial tissue when able. Once the pathogen has succeeded in colonizing its host, it will switch modes and become a saprobe, causing a white-rot type of decay. It will also colonize any dead woody material the mycelium may encounter (Devkota and Hammerschmidt, 2020). The virulence of *Armillaria* infection varies from species to species, with *A. mellea* and *A. ostoyae* ([Vahl] P. Kumm) being one of the most virulent and common species found in forested systems (Devkota and Hammerschmidt, 2020; Shaw and Kile, 1991).

The environments observed within forested systems are highly variable, and virulence and proliferation of *Armillaria* are impacted by climatic variables such as temperature and soil oxygen levels (Kubiak et al., 2017). Rhizomorph production in peat soils increased, compared to lower nutrient sand soils, possibly due to the increased oxygen gradient found in the organic horizons of peatlands. Furthermore, in peat soils, the rhizomorphs tend to be concentrated in the humus layer due to the oxygen gradient, and it is thought that the location of the rhizomorphs in the humus is related to an increased level of infection via the root collar of the host trees (Kubiak et al., 2017; Thormann et al. 2001). Thormann and colleagues (2001) also noted that rhizomorphs were absent in organic soil layers in Finland unless shallow peat deposits and flowing groundwater were found. Rhizomorphs have been documented to be missing from soils that are highly saturated with water, especially for a majority of the year, such as in fens; *Armillaria* rhizomorphs require soil moisture to grow and proliferate, but an excess of moisture in the rhizosphere will inhibit growth (Thormann et al., 2001).

Given the limited understanding of the broader stand development processes within the black spruce forest, my study has two main goals. The purpose of Chapter 2 is to better understand the effect that residual overstory trees and saplings, grouped in clumps, have on stand regeneration and structure, the influence stand boundaries have on EDM disease ecology, and to examine the implementation and treatment effectiveness of the Minnesota Department of Natural Resource's 5-foot cutting rule. Our objective was to investigate the above questions by resampling recently harvested lowland black spruce stands with documented pre-harvest stand conditions.  The purpose of Chapter 3 is better to understand the diversity and abundance of fungi, with an emphasis on pathogens, across four different study treatments in managed black spruce bogs: harvested black spruce bogs with an EDM infection; uninfected, harvested black spruce bogs; unharvested black spruce bogs with an EDM infection; uninfected, unharvested black spruce bogs. Our objective was to investigate whether the above treatments affected fungal diversity and abundance. Together these studies will help increase our understanding of the black spruce forest type and potential options for management related to EDM.

## Chapter 2: Influence of Eastern Dwarf Spruce Mistletoe (*Arceuthobium pusillum*) on Regeneration Health and Composition in Lowland Black Spruce (*Picea mariana)* Post-Harvest

### Overview

Black spruce (*Picea mariana* [Mill] Britton, Sterns and Poggenb) is a dominant and wide-ranging North American boreal forest species. It reaches its southwestern range limit in Minnesota, USA. Forest managers in Minnesota have become increasingly aware of how eastern dwarf mistletoe (*Arceuthobium pusillum* [Peck]) (EDM) may be influencing the ability of black spruce to regenerate after harvest naturally. This study aimed to explore regeneration dynamics in black spruce stands, quantify residual tree density and impact from EDM, and quantify EDM infestation in neighboring stands. Ten stands were surveyed over three years in northern Minnesota pre-and post-harvest. Eastern larch comprised almost 70% of the regeneration in EDM infected stands and over 50% of the regeneration in the uninfected stands, suggesting that the light intensive environment is favorable for eastern larch regeneration. A significant difference was observed between the total percentage of black spruce regeneration recorded, with uninfected stands having a higher proportion of black spruce ($p = 0.06$). The diameter at breast height in inches and the square feet of basal area per acre were greater in stands with EDM infections due to residual trees left in clumps. While clearcuts are a viable option for mitigating EDM, the disease will continue to occur if the 5-foot cutting rule is not followed. Likewise, neighboring stands can act as a source for future EMD infections even if the 5-foot cutting rule is efficiently applied within a stand. Management decisions for EDM should consider infection both within the stand and at the broader landscape level (neighboring stands) when developing management recommendations.

## Introduction

In the North American boreal forest, black spruce (Zesegaandag (Ojibwe) *Picea mariana* [Mill] BSP) is a dominant and widespread species. In northern Minnesota, USA, black spruce accounts for about 1.5 million (9%) of the state's nearly 17.4 million forested acres (Miles, 2017). In Minnesota, and throughout the region, black spruce is an important species. From an economic standpoint, black spruce is the second most harvested pulpwood species; in 2017, the state of Minnesota alone had well over 200,000 cords of black spruce pulpwood harvested (Minnesota Department of Natural Resources, 2021). The importance of black spruce as a pulp species can be found in the long fibers contained in its wood structure that produce high-quality paper (Sabourin and Hart 2010). Wildlife makes excellent use of black spruce ecosystems, as well, with essential habitat found for the American marten (*Martes americana* [Turton]) and the spruce grouse (*Falcipennis canadensis* [Linnaeus]), to name a few (Cimon-Morin et al., 2010).

Historically, the structure and composition of black spruce were influenced by frequent localized pest, pathogen, and wind events and infrequent, high-intensity fires that would cause complete stand mortality events (Viereck and Johnston, 1990; Fenton et al., 2009; Heinselman, 1984). While black spruce has few pests and pathogens that are cause for concern, the eastern dwarf mistletoe (*Arceuthobium pusillum* [Peck]) (EDM), a native parasitic plant, results in mortality of black spruce (Baker and French, 1991; Ostry and Nicholls, 1979). The cause for the concern among forest managers can be highlighted by work done by Baker and French (1991) that shows how after initial infection, a single tree will succumb to EDM mortality within 20 years, and up to 75% of a stand may also experience the same mortality event in a ten to twenty-year period as the infection radially spreads from the initial point of entry at a rate of almost two and a half feet per year (Baker and French, 1980). Furthermore, as black spruce stands become more infected with EDM, there is mounting evidence that black spruce will lose dominance in the stand and give way to a lowland conifer and hardwood mixed forest (Skay et al., 2021; Gray et al., 2021; Baker and Knowles, 2004).

EDM's current forest management practices within Minnesota rely on a clearcut regeneration harvest with an aerial seeding of black spruce on rotations between 60 and 120 years due to site quality (MN DNR, 2019). An essential part of the clearcut regeneration harvest, especially on lands managed by the Minnesota Department of Natural Resources (MN DNR), is the implementation of the 5-foot cutting rule in EDM infected stands. The 5-foot cutting rule removes all black spruce (infected or not) over five feet tall within the stand and all infected black spruce trees that are located up to 2 chains (132ft) beyond the stand's perimeter. These trees are commonly felled or trampled and left on site (MN DNR 2019). If other tree species are present in the overstory (e.g., tamarack (*Larix laricina* (Du Roi) K. Kochor) or northern white cedar (*Thuja occidentalis* L.)), those trees are recommended to be left as residual trees as part of best management practices (Minnesota Forest Resources Council, 2013). However, Roth and colleagues (2021) found that, according to surveys and focus groups with loggers and foresters, the 5-foot cutting rule is not consistently implemented. For example, flattening every stem over five feet tall across an entire stand may not be a financially viable option for some contractors.

According to the Minnesota DNR (2019) post-harvest regeneration surveys must be conducted within one year of the harvest of an EDM infected black spruce stand. At the time of the post-harvest infection survey, it is determined whether further treatments are needed to decrease future EDM infections and spread. Regardless of the EDM status of a stand, regeneration surveys must be conducted after a minimum of five years have elapsed to deem if the stand is well-stocked with a minimum of 600 trees per acre (TPA) of desirable growing stock (MN DNR, 2019).

Regeneration surveys can be a valuable tool in determining the trajectory of black spruce regeneration and volume. Residual seedling and sapling characteristics immediately post-harvest have been documented to influence future growth and yield of black spruce; for example, at the time of release, the larger the residuals, the higher the yield will be at the end of the rotation (Pothier et., al, 1994). However, Baker and Knowles (2004) reported that in black spruce stands with EDM infections, regeneration declined, resulting in high proportions of infected trees to uninfected trees and volume loss at the time of harvest; Skay and colleagues (2021) corroborated these findings by reporting that regeneration, no matter the species, was significantly lower in stands infected with EDM, compared to uninfected stands, and that sites that lacked EDM saw a significantly higher proportion of black spruce seedlings.

As previously reported by Roth and colleagues (2021), the DNR's 5-foot cutting rule is not always implemented for multiple reasons, which may result in infected residual black spruce of various sizes still being present in the overstory after a harvest. The resulting residual trees may result in similar conditions as a shelterwood with reserve harvest; Anderson and colleagues (2020) using 50 years of data, found successful regeneration in shelterwood harvests. Furthermore, depending on the size of the residual clumps, gaps may form within the residual overstory trees; in Quebec, St-Denis and colleagues (2009) observed successful black spruce regeneration in gaps, even in stands upwards of 200 years of age that reside on poor site classes. The successful regeneration of black spruce documented in gaps, after a shelterwood treatment, and the potential for increased growth, highlight the potential for alternative management practices in black spruce.

The goals of this study were to quantify stand structure and composition after harvest in EDM infected and non-infected stands. Specifically, the study quantified regeneration of desirable growing stock, residual tree clumps containing both overstory trees and saplings, and EDM presence in stand boundaries. We utilized these data to explore changes in structure and composition between EDM infected and EDM non-infected stands with the goal of better understanding how the Minnesota Department of Natural Resource's 5-foot cutting rule is implemented and how those choices may influence future stand development. Although Baker and Knowles (2004) and Skay and colleagues (2021) have documented declines in growth, yield, and regeneration of black spruce in stands infected with EDM, there has been little work examining the role that residual clumps have on black spruce regeneration and stand structure and therefore represents a new line of questioning.

## Methods

### *Study Region*

The boreal forest reaches its southern extent in Northern Minnesota, USA, and comprises diverse forested ecosystems, from upland conifer and hardwood forests to lowland conifer swamps and bogs. Precipitation consists of rain in the warmer months and snow in the colder months, adding up to about 53 to 68 inches per year, with nearly half of the precipitation events occurring during the growing season (Hanson and Hargrave, 1996). With yearly temperatures ranging from mild summers to cold winters, the growing season for black spruce in Northern Minnesota is relatively short, averaging about 105 days a year, with the normal annual temperature ranging from about 33 ◦F to roughly 40 ◦F (Aaseng et al., 2003).

### *Study Area*

Stands were located in the northeast corner of Minnesota on the Department of Natural Resources (DNR) administered lands in the following counties: Lake, St. Louis, Koochiching, Itasca, Beltrami, Lake of the Woods, and Roseau (Figure 2.1). Stands ranged in age from 88 to 160 years, with an average age of 115. The site index ranged from 30 to 40 feet in height at 50 years of age, with an average site index value of 35. The average stand size was 44 acres, ranging from 8 to 102 acres.

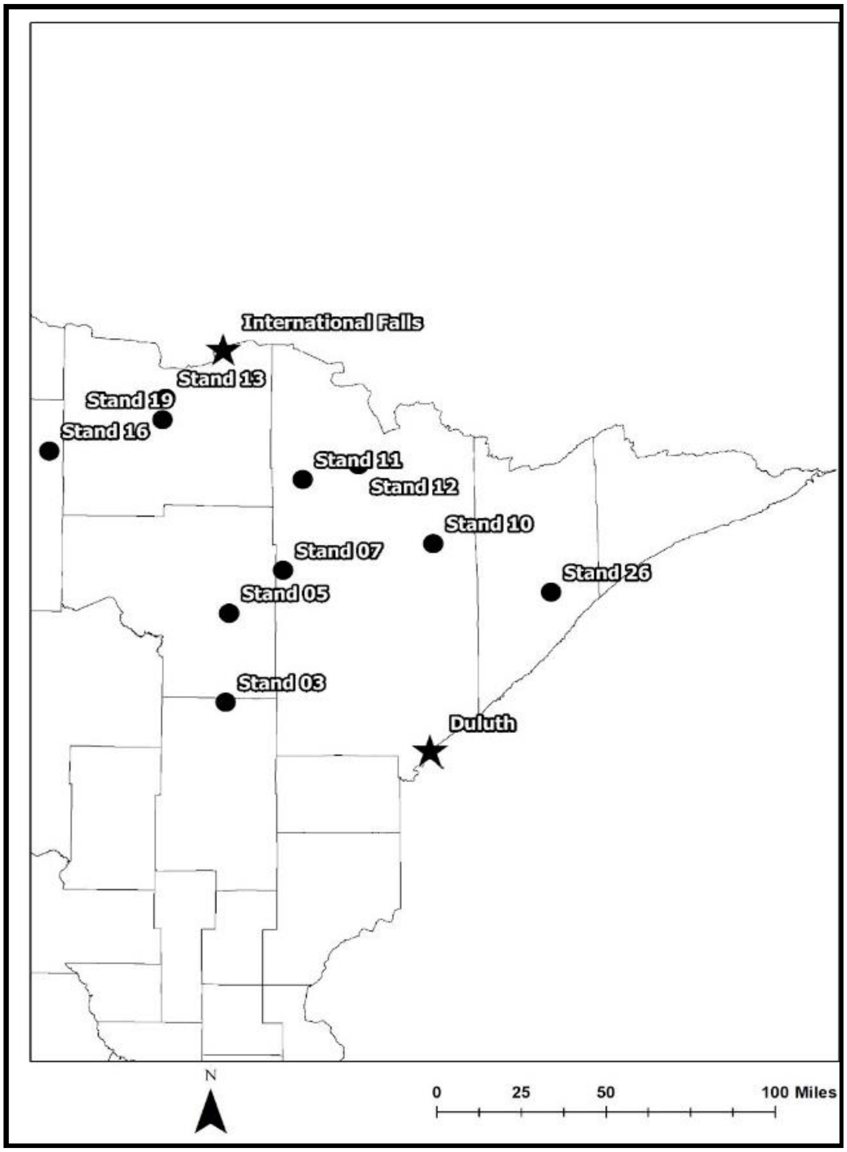


**Figure 2**.1 Site map of the ten study stands across Northern Minnesota and their relation to the municipalities of Duluth and International Falls, Minnesota, USA.

**Table 2.1** Locations and descriptions of the ten stands sampled in this study. After post-harvest inventory, five stands were found to have EDM, and five were found to lack EDM.

| Stand ID | Pre-Harvest EDM Status | Post-Harvest EDM Status | Stand Age | Site Index at 50 years (ft) | Stand Size (acres) | Dominate Soils | County | Stand Location (lat, long) |
|---|---|---|---|---|---|---|---|---|
| 3 | No | No | NA | NA | 8.3 | Sago muck, 0 to 1 percent slopes, frequently ponded | Aitkin | 47.00724, -93.37688 |
| 5 | Yes | Yes | NA | NA | 15 | Loxley peat | Itasca | 47.41066, -93.35782 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 7 | Yes | Yes | NA | NA | 10 | Cathro muck, occasionally ponded, 0 to 1 percent slopes | St. Louis | 47.6048, -93.01643 |
| 10 | No | Yes | 88 | 40 | 59 | Tacoosh mucky peat, 0 to 1 percent slopes | St. Louis | 47.72388, -92.06308 |
| 11 | Yes | Yes | 127 | 38 | 30 | Rifle-Rifle, ponded, complex, 0 to 1 percent slopes | St. Louis | 48.01686, -92.88955 |
| 12 | No | No | 108 | 39 | 27 | Rifle soils, spooner catena, 0 to 1 percent slopes | St. Louis | 48.08011, -92.5368 |
| 13 | No | No | 141 | 30 | 99.2 | Cathro and Tacoosh soils, suomi catena, 0 to 1 percent slopes | Koochiching | 48.37948, -93.77147 |
| 16 | Yes | Yes | 90 | 32 | 81.2 | Lupton-Lupton, ponded, complex, 0 to 1 percent slopes | Beltrami | 48.13014, -94.51935 |
| 19 | No | No | 160 | 30 | 101.5 | Greenwood-Greenwood, ponded, complex, 0 to 1 percent slopes | Koochiching | 48.28555, -93.78912 |
| 26 | Yes | Yes | 89 | 34 | 12.3 | Rifle-Rifle ponded complex, 0 to 1 percent slopes, dense substratum | Lake | 47.49357, -91.32469 |

*Pre-harvest sampling design*

Twenty-six stands were initially sampled between 2018 and 2019. These stands were selected because they had an active timber sale (e.g., the wood was sold to a logging business owner). Timber sale contracts can last between four and upwards of six years based on numerous factors, including weather conditions; the frozen ground is required for harvesting black spruce. The survey design for pre-harvest inventories (Figure 2.2) was a modified version of Skay and colleagues (2021).

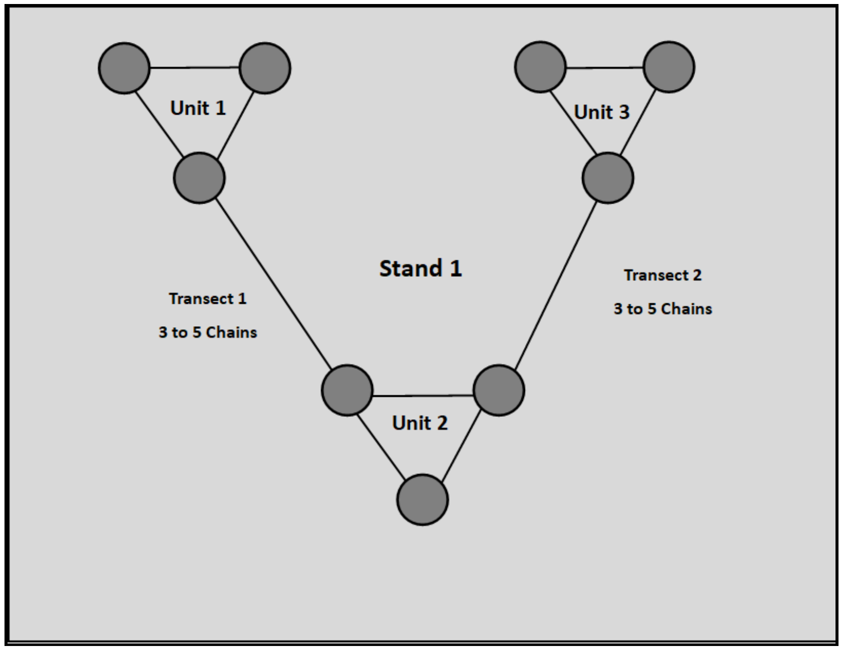


**Figure 2.2** Pre-harvest stand inventory layout.

Pre-harvest sampling included three sampling units per stand (figure 2.2). Each sampling unit consisted of three 1/50th acre overstory plots (Figure 2.3). Within each 1/50th acre plot, every tree with a diameter at breast height (DBH) greater than 3.5 inches was tallied. Each tree's species, mortality, EDM infection status, and health description were recorded. The first tree of every overstory plot was measured for its height and a core was extracted (figure 2.4).

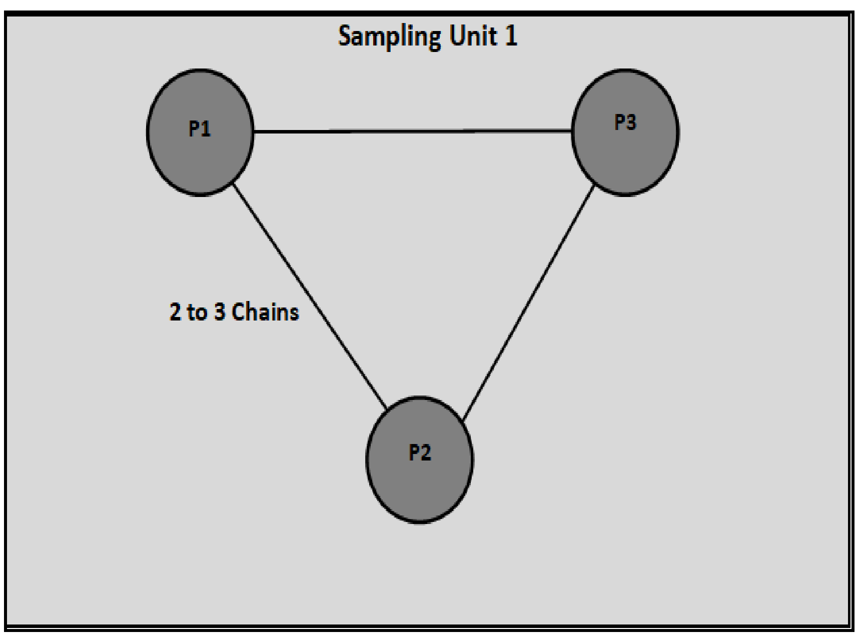


**Figure 2.3**. Pre-harvest stand inventory layout.

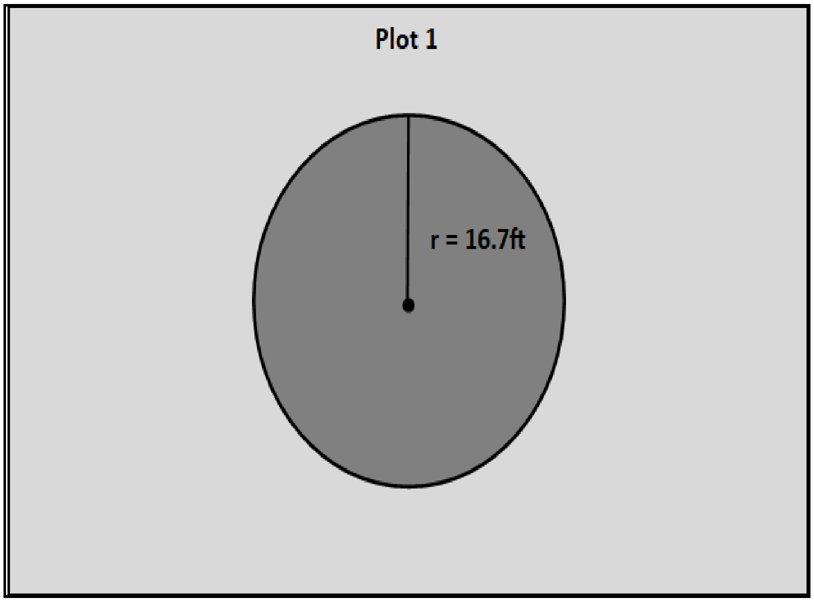


**Figure 2.4**. Pre-harvest overstory plot layout.

Within each overstory plot were two 1/500th acre regeneration subplots. Within each 1/500th acre subplot, all trees less than one-inch DBH were tallied by species. Each subplot was established 10 feet from the plot center of the 1/50th acre plot on a north (0°) south (180°) axis (figure 2.5).

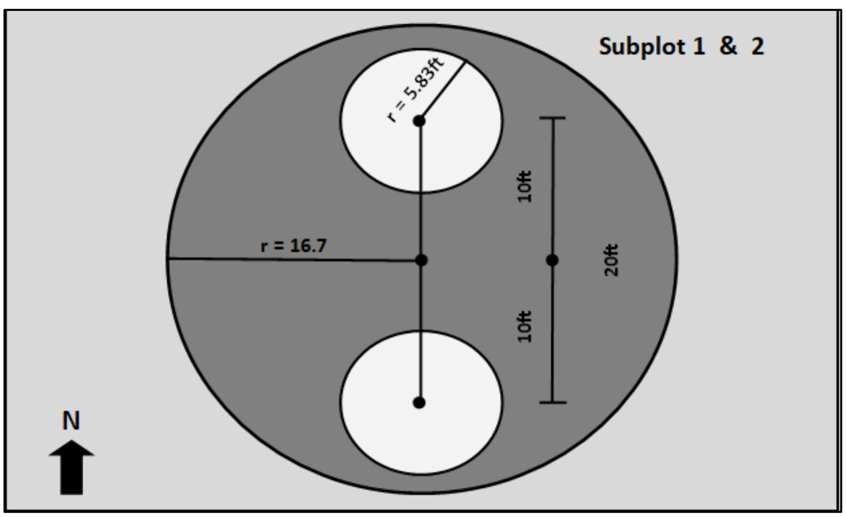


**Figure 2.5** Pre-harvest subplot layout.

Between sampling units, a three to five chain transect, with the length of each transect predetermined given the acreage and shape of the overall stand, measuring ten feet wide (five feet on either side of the transect), was laid out beginning at the last overstory plot of the previous sampling unit and ending at the first overstory plot of the next unit. The goal of the transect was to walk along a predetermined azimuth to inspect every overstory tree (>3.5” DBH) and record its EDM status. Once the end of the transect was reached, an offset of a half or complete chain along a random azimuth was used to establish the first overstory plot within the next sampling unit (figure 2.6).

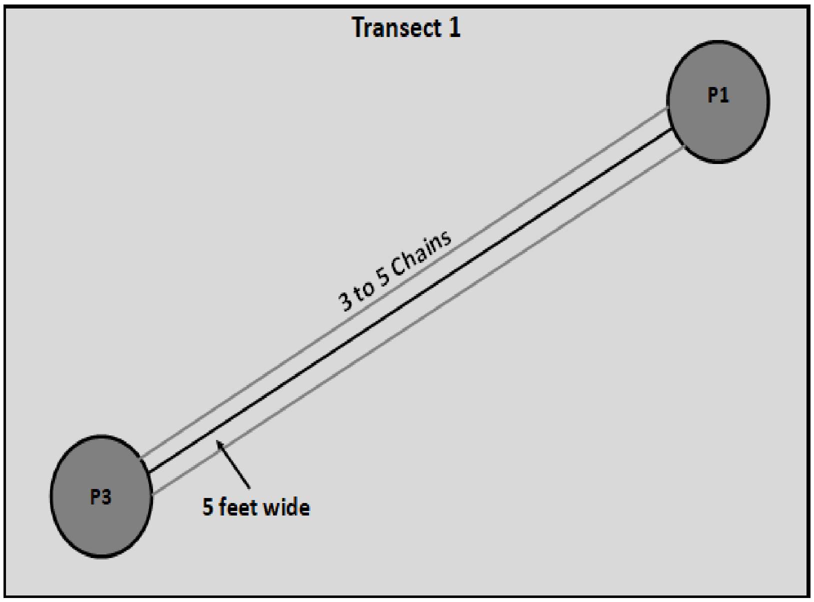


**Figure 2.6** Pre-harvest transect layout.

*Post-harvest sampling design*

Of the 26 stands sampled for pre-harvest data, only ten stands were harvested by the 2021 growing season. Only the ten stands harvested were included in the analysis. Of the ten harvest stands sampled, five contained EDM, and five were reported to lack EDM.

The ten stands sampled post-harvest followed a modified sampling procedure that included adding more units (figure 2.7) and plots (figure 2.8), expanding the data that were collected to better capture stand regeneration, such as height classes: 1 = < 6in, 2 = 6.1in to 3ft, 3 = >3.1ft (Table 2.3). A modified EDM rating (DMR) was used to better capture EDM infection; the modified DMR follows: 0 = no EDM, 1 = less than 50% EDM, 2 = ~50% EDM, and 3 = greater than 50% EDM. Since the stands were harvested, no overstory transects were conducted during post-harvest sampling.

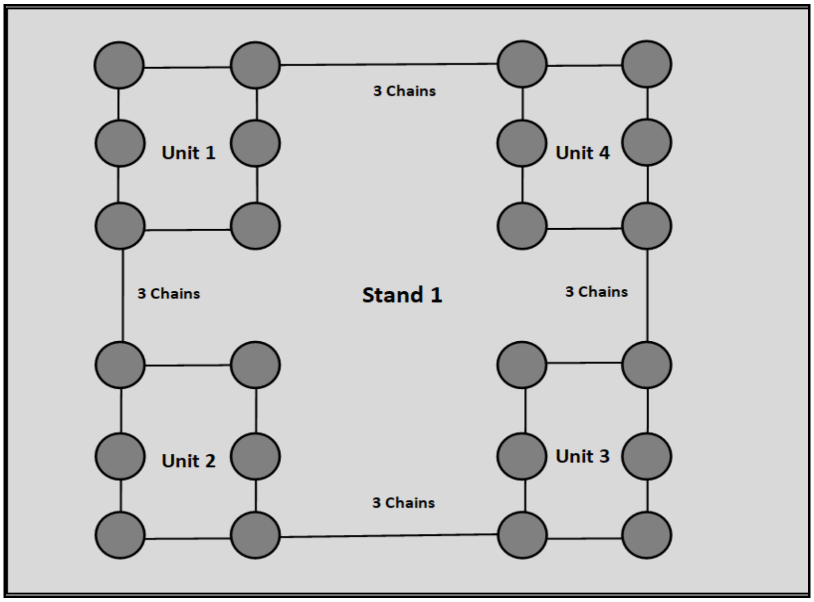

**Figure 2.7** Post-harvest stand inventory layout.

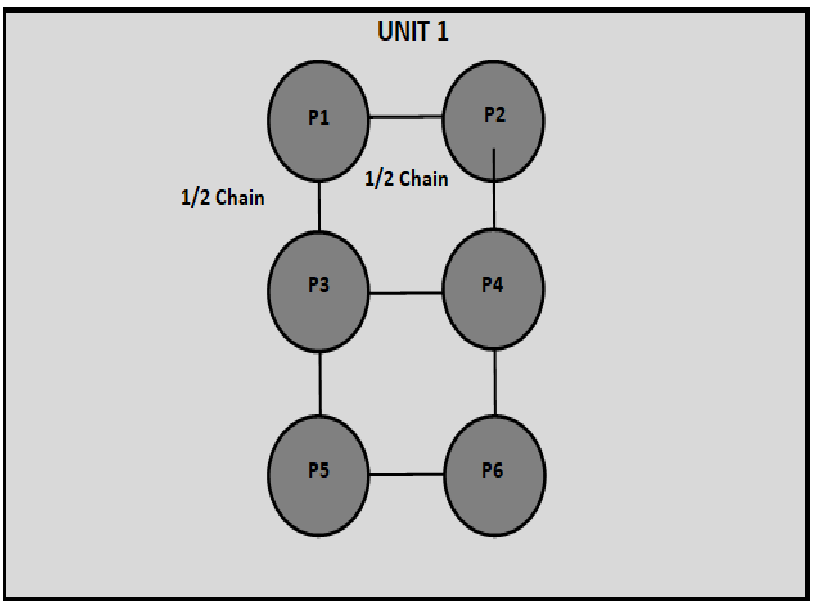

**Figure 2.8** Post-harvest sampling unit layout.

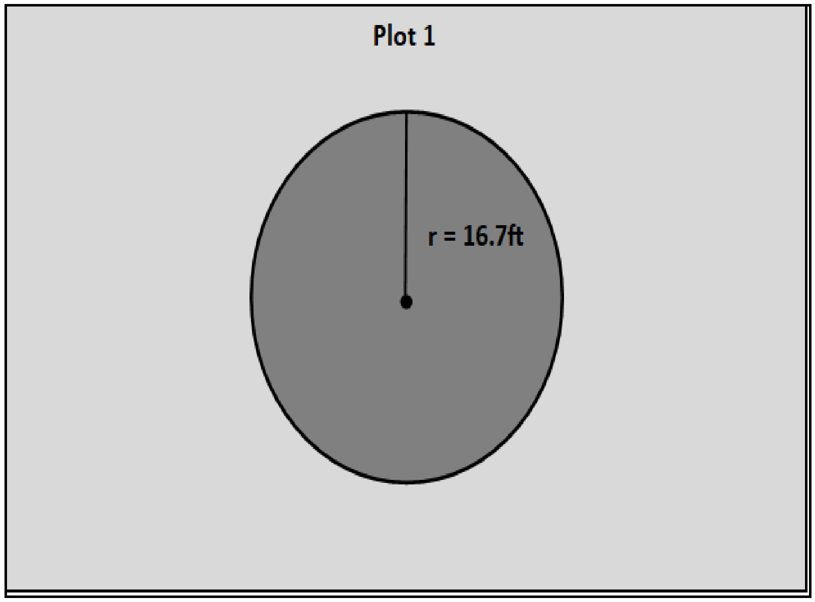


**Figure 2.9** Pre-harvest overstory plot layout.

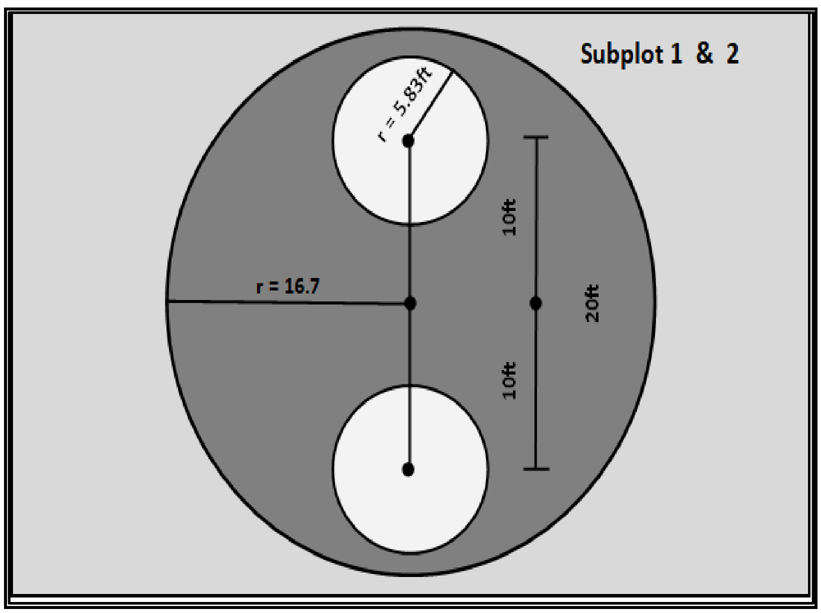


**Figure 2.10** Post-harvest subplot layout.

Data were collected on residual clumps of overstory trees to assess the species composition, stand structure, and EDM status in residual clumps. The boundaries of these residual clumps were mapped using the mobile software application, *Avensa Maps,* to gain a spatial understanding of their role in the stand. All residual trees over 3.5in DBH in diameter were classified as overstory trees and were sampled for species, height, DBH, mortality, EDM, and DMR. All saplings in the residual clumps that were 5 feet tall and greater than 3.5 inches DBH were tallied for species, EDM, DMR, and mortality.

Residual clumps were found and sampled in the five stands determined to have EDM based on post-harvest surveys. Residual clumps were further separated into two categories, EDM, and No EDM, regardless of the infection status of the stand in which they were found (Table 2.4; Table 2.5).

Stand boundaries were walked to assess whether the unharvested stands adjacent to the harvested stand could be acting as an infection source. Boundary points were taken along the stand boundary once every three chains (198ft). A qualitative sample was taken at each point to assess the level of EDM infection present at each point. The sampling protocol was as follows: 0 = No EDM, 1= Some EDM, 2 = A lot of EDM.

*Statistical analysis*

Data were summarized at the plot level and organized by EDM infection status at the stand level. There were five infected stands and five uninfected stands; a stand was categorized as having an EDM infection if any of the trees in the stand were determined to be infected. The infection status of a stand was determined after the pre-harvest inventories, and those designations were carried through to the post-harvest inventories and analyses.

Trees per acre (TPA) was calculated for the regeneration based on the 1/500th acre plots and for the residual overstory based on the 1/50th acre plots; basal area per acre ($ft^2$/ac) (BA) was calculated in the same manner as TPA. TPA and BA were calculated for each species, with black spruce separated by infection status within the stand and denoted by "Black Spruce_EDM" for infected and "Black Spruce" for uninfected. However, several species were found during sampling that did not have a high enough frequency to warrant separate analysis and were therefore lumped together based on whether they were conifer or hardwood species.

Pre- and post-harvest stand conditions were not directly compared and tested for significant differences. The prescriptions were all laid out as clearcuts, which produces a substantial difference in TPA, BA, and DBH pre-and post-harvest.

Each height class of trees (<3in; 3.1in-6ft; >6.1ft) was analyzed separately to avoid overestimating stand density metrics, such as TPA. Diameter distributions were created using the diameter at breast height (DBH) data collected and separated into two-inch DBH classes. Both the height classes and the diameter distributions were made and analyzed based on the EDM infection status of the stand in which data were collected.

A modified EDM rating (0 = no EDM, 1 = less than 50% EDM, 2 = ~50% EDM, and 3 = greater than 50% EDM) was assigned to every individual that had a discernable EDM infection, regardless of mortality. However, since EDM causes such definitive mortality, a binary variable of 0 (No EDM) or 1 (EDM) was also assigned to each recorded individual, regardless of mortality status.

The mobile software application, *Avensa Maps*, was used to collect point and polygon data for residual clumps and stand boundaries. The polygon data were uploaded to ArcMap 10.8; polygon data were analyzed for the area of clumps.

Using a Welch's Two Sample t-test, a level of significance of $\alpha < 0.1$ was used to determine significance due to the high variability present in forested systems. Microsoft Excel calculated summary statistics such as averages, standard deviations, and standard errors. Microsoft Excel was also used to create tables and figures. All other statistical analyses were performed in R and R Studio.

## Results

### *Pre- and Post-Harvest Overstory Stand Structure*

Overstory density and composition were variable in infected and uninfected pre-harvest stands (Figure 2.11). Significant differences between infected and uninfected stands were found in the pre-harvest trees per acre across all species ($p = 0.004$), infected black spruce ($p = 0.03$), and uninfected black spruce ($p = 0.0004$) (Table 2.2). Pre-harvest overstory (figure 2.13) stand density of infected and uninfected stands saw significant differences in the basal area ($ft^2/ac$) across all species ($p = 0.79$) and infected ($p = 0.03$) and uninfected

($p$ = $0.01$) black spruce, respectively (Table 2.2). Significant differences were found in the pre-harvest DBH (figure 2.15) between stands with and without EDM infections across all species ($p = 0.05$) and infected black spruce ($p = 0.00006$) (Table 2.2).

All uninfected stands, post-harvest, were free of any overstory residual trees. Overstory TPA comparison from the post-harvest surveys (figure 2.14) revealed no significant differences (Figure 2.12; Table 2.2). Post-harvest basal area ($ft^2/ac$) (figure 2.14) of infected and uninfected stands was significantly different ($p = 0.08$) for all species; there were no other significant differences (Table 2.2). The only significant difference ($p = 0.08$) observed between post-harvest DBH (figure 2.16) was across all species between infected and uninfected stands (Table 2.2).

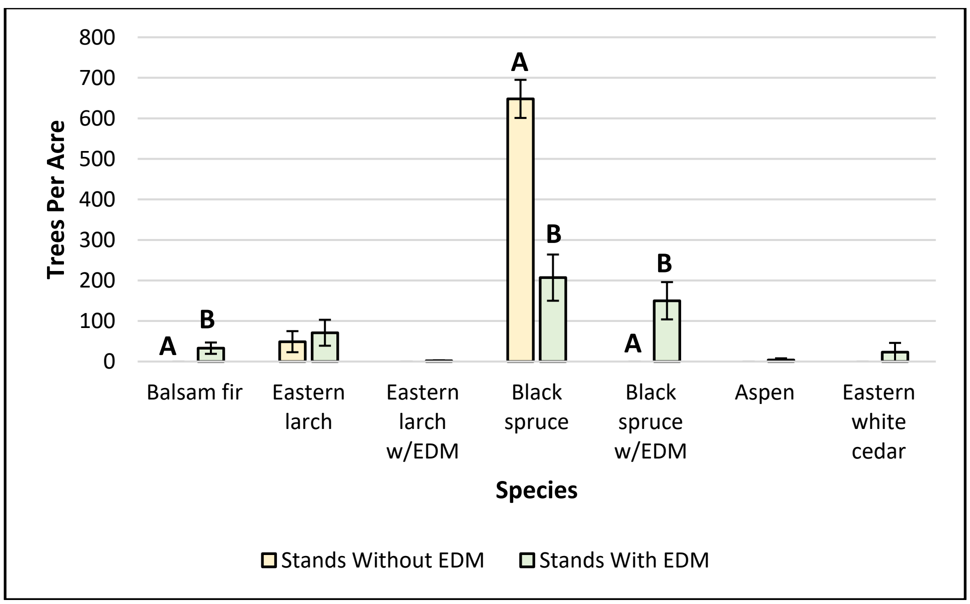


**Figure 2.11** Average trees per acre during pre-harvest conditions. Species with different letters are significantly different ($p < 0.1$) from one another.

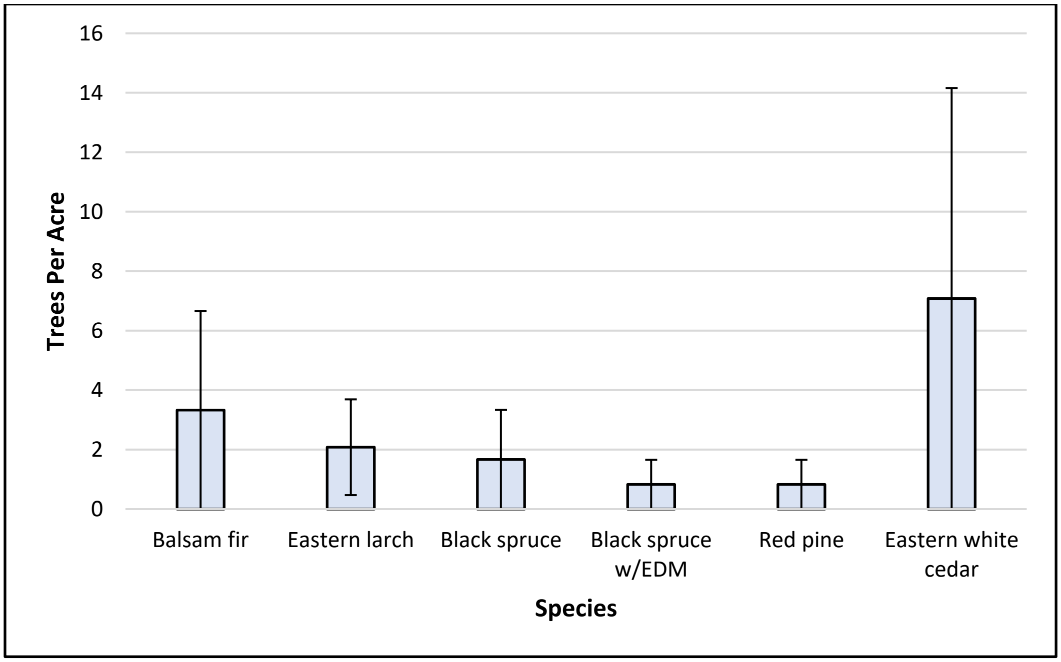


**Figure 2.12** Average trees per acre by species during post-harvest stand inventories. No residuals were left in uninfected stands.

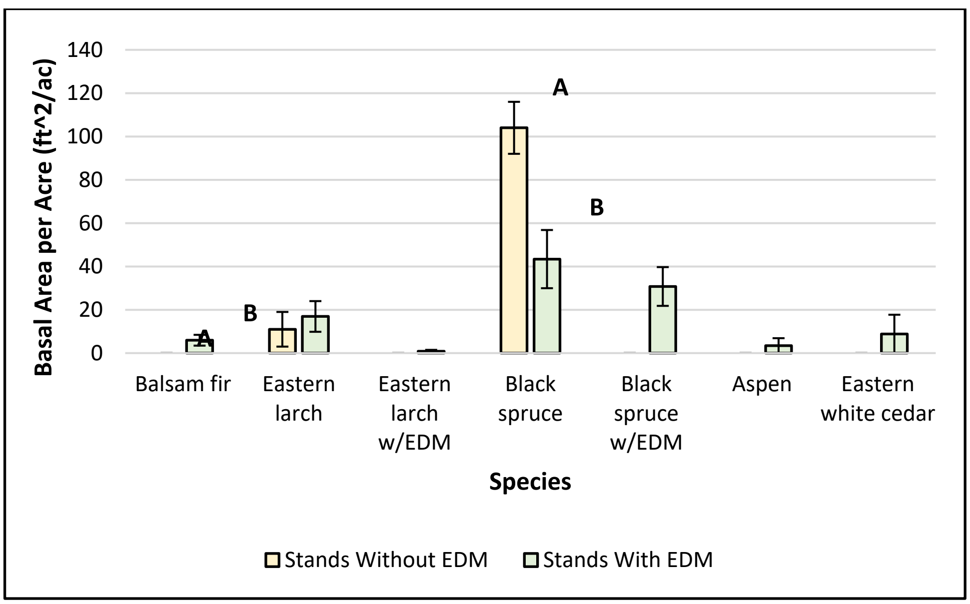


**Figure 2.13** Average square feet of BA/ac ($ft^2/ac$) during pre-harvest conditions. Species with different letters are significantly different ($p < 0.1$) from one another.

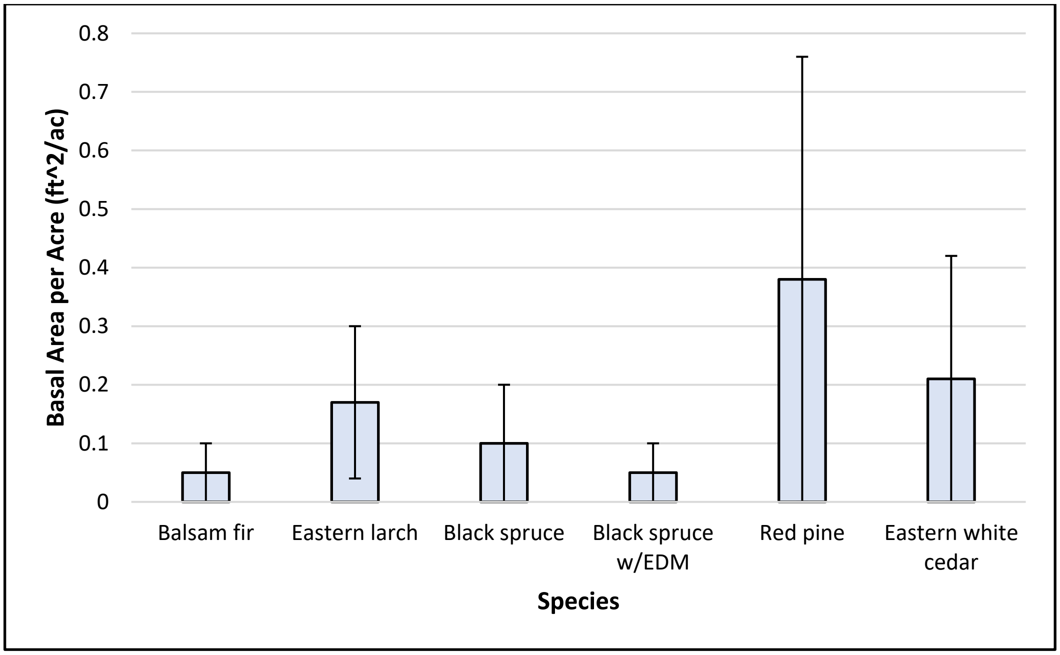


**Figure 2.14** Average square feet of BA/ac ($ft^2/ac$), per species, during post-harvest conditions. No residuals were left in uninfected stands.

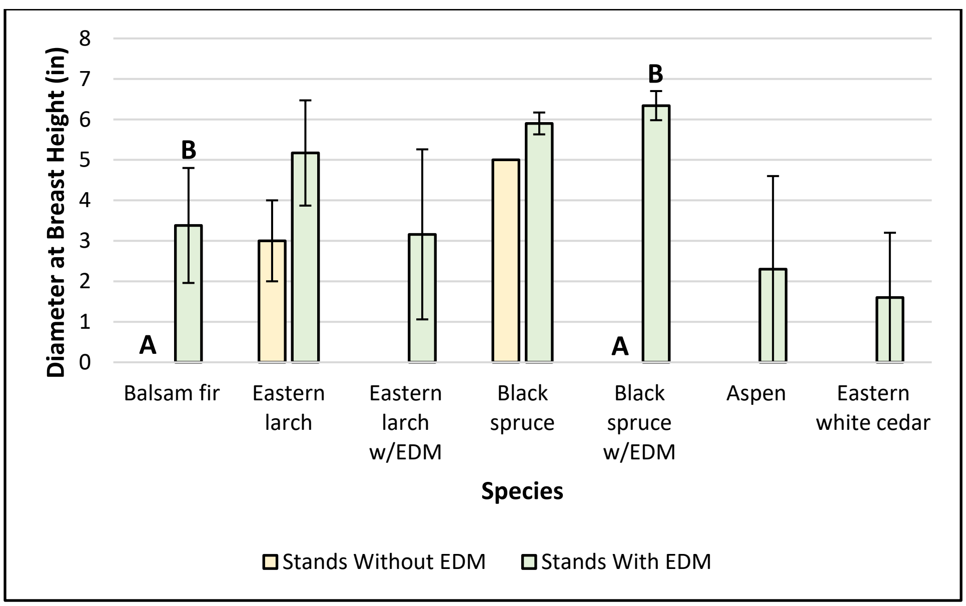


**Figure 2.15** Average diameter at breast height in inches during pre-harvest conditions. Species with different letters are significantly different ($p < 0.1$) from one another.

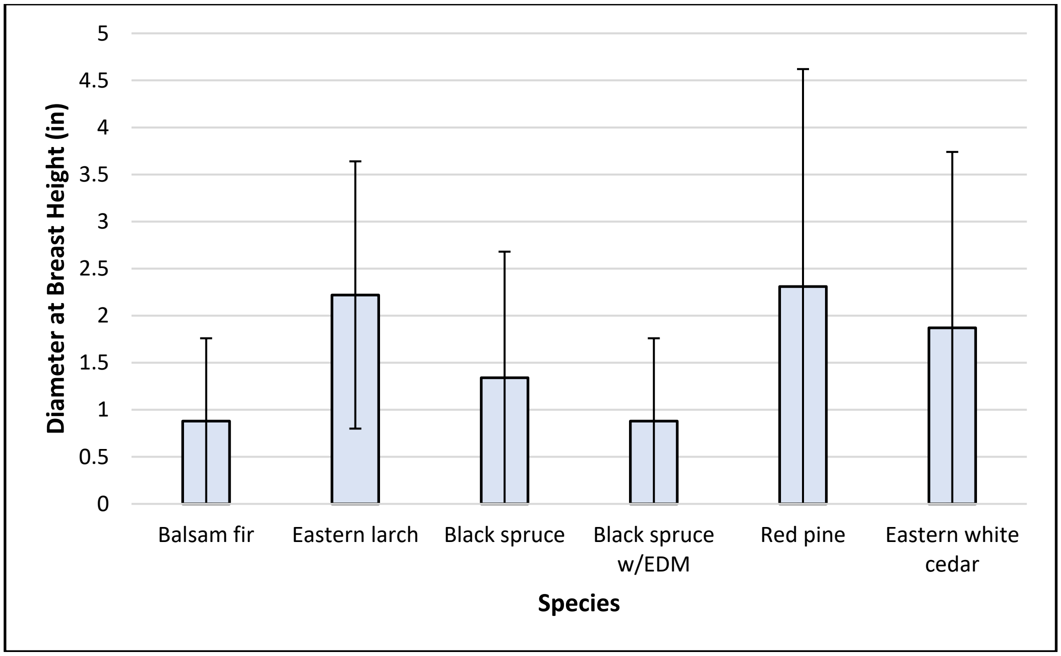

**Figure 2.16** Average diameter at breast height in inches, per species, during post-harvest conditions. No residuals were left in uninfected stands.

*Regeneration*

Regeneration was highly variable among species and height classes for EDM infected and uninfected stands. The greatest density of regenerating seedlings was observed in the smallest height class (<3in), with a total of 9,065 (± 1,245) TPA recorded in stands with an EDM infection and 5,042 (± 557.4) TPA in stands without an infection; most of those seedlings were eastern larch with an average TPA of 7,585 (±6,115) and 3,140 (±598) for EDM infected and uninfected stands, respectively (Table 2.3).

EDM had little impact on regenerating black spruce across all height classes, regardless of the infection status of the stand. However, a significant difference ($p = 0.06$) was observed in size class one between the average TPA of uninfected black spruce seedlings in infected stands (727 ± 296) and uninfected stands (1,754 ± 363); no other significant differences were observed between any species, regardless of height classes or stand infection status (Table 2.3).

**Table 2.2** Pre- and post-harvest overstory conditions are summarized by all species, Black Spruce (black spruce without EDM) and Black Spruce_EDM (black spruce with EDM). All p-values less than 0.1 represent a significant difference.

| Pre-Harvest | | | | | |
|---|---|---|---|---|---|
| | | Sand w/EDM | Stands w/o EDM | t-statistic | p-value |
| All Species | | | | | |
| | BA/ac | 110.1 | 114.7 | -0.28 | 0.79 |
| | TPA | 491 | 697 | -4.41 | 0.004 |
| | DBH | 6.19 | 5.32 | 2.3 | 0.05 |
| Black Spruce | | | | | |
| | BA/ac | 43.39 | 103.8 | -3.35 | 0.01 |
| | TPA | 207 | 658 | -5.99 | 0.0004 |
| | DBH | 5.9 | 5.3 | 1.58 | 0.15 |
| Black Spruce_EDM | | | | | |
| | BA/ac | 30.75 | 0 | 3.48 | 0.03 |
| | TPA | 150 | 0 | 3.24 | 0.03 |
| | DBH | 6.34 | 0 | 17.5 | 0.00006266 |
| **Post-Harvest** | | | | | |
| | | Sand w/EDM | Stands w/o EDM | t-statistic | p-value |
| All Species | | | | | |
| | BA/ac | 0.95 | 0 | 2.28 | 0.08 |
| | TPA | 15.83 | 0 | 1.79 | 0.15 |
| | DBH | 4.61 | 0 | 2.3 | 0.08 |
| Black Spruce | | | | | |
| | BA/ac | 0.1 | 0 | 1 | 0.37 |
| | TPA | 1.67 | 0 | 1 | 0.37 |
| | DBH | 1.34 | 0 | 1 | 0.37 |
| Black Spruce_EDM | | | | | |
| | BA/ac | 0.05 | 0 | 1 | 0.37 |
| | TPA | 0.83 | 0 | 1 | 0.37 |
| | DBH | 0.88 | 0 | 1 | 0.37 |

The average TPA of aspen in all three height classes, while not significantly different, was higher in infected stands compared to uninfected stands, with an average TPA in infected stands of 256 (± 120), 1,983 (± 1,210), and 213 (± 150) compared to an average of 133 (± 63), 1,575 (± 1,428), and 156 (± 156) in uninfected stands across height class one, two, and three, respectively. Furthermore, the average TPA of aspen in height class two was the species most frequently observed in infected stands, at 1,983 (± 1,210) stems per acre.

Balsam fir follows a similar trend as aspen across height classes; there were more trees per acre of balsam fir in infected stands compared to uninfected stands, with height classes two and three having no recorded individuals in uninfected stands. Height class one had an average of 179 (± 94) balsam fir TPA in infected stands compared to an average of 2 trees per acre of balsam fir in uninfected stands (Table 2.3).

**Table 2.3** Average trees per acre of regeneration across three height classes (less than 3 inches, 3.1 inches to 3 feet, greater than 3.1 feet) were broken down by species and the stand's infection status. P-values less than 0.1 are significantly different. Black spruce was categorized by EDN infections status: Black Spruce (black spruce without EDM) and Black Spruce_EDM (black spruce with EDM)

| | Species | EDM TPA | No EDM TPA | t-statistic | p-value | Standard Error EDM | Standard Error No EDM |
|---|---|---|---|---|---|---|---|
| Size Class 1 | | | | | | | |
| | Balsam fir | 179 | 2 | 1.8797 | 0.1333 | 94 | 2 |
| | Eastern Larch | 7585 | 3140 | 0.7236 | 0.5087 | 6115 | 598 |
| | Black Spruce | 727 | 1754 | -2.1943 | 0.0608 | 296 | 363 |
| | ack Spruce_ED | 0 | 0 | NA | NA | 0 | 0 |
| | Aspen | 256 | 133 | 0.9107 | 0.3972 | 120 | 63 |
| | Aisc. Hardwood | 310 | 10 | 1.1002 | 0.3329 | 273 | 8 |
| | Misc. Conifers | 6 | 2 | 0.6447 | 0.5485 | 6 | 2 |
| Size Class 2 | | | | | | | |
| | Balsam fir | 165 | 0 | 1.5542 | 0.1951 | 106 | 0 |
| | Eastern Larch | 1827 | 3527 | -0.5218 | 0.6253 | 949 | 3117 |
| | Black Spruce | 811 | 1075 | -0.2648 | 0.7979 | 534 | 578 |
| | ack Spruce_ED | 0 | 0 | NA | NA | 0 | 0 |
| | Aspen | 1983 | 1575 | 0.2181 | 0.8330 | 1210 | 1428 |
| | Aisc. Hardwood | 115 | 0 | 1.3909 | 0.2366 | 82 | 0 |
| | Misc. Conifers | 19 | 2 | 1.3495 | 0.2485 | 14 | 0 |
| Size Class 3 | | | | | | | |
| | Balsam fir | 54 | 0 | 1.3609 | 0.2452 | 40 | 0 |
| | Eastern Larch | 40 | 33 | 0.1446 | 0.8888 | 27 | 33 |
| | Black Spruce | 15 | 52 | -1.0579 | 0.3445 | 8 | 35 |
| | ack Spruce_ED | 2 | 0 | 1.0000 | 0.3739 | 2 | 0 |
| | Aspen | 213 | 156 | 0.2606 | 0.8010 | 150 | 156 |
| | Aisc. Hardwood | 23 | 0 | 1.7225 | 0.1601 | 13 | 0 |
| | Misc. Conifers | 10 | 2 | 0.7932 | 0.4692 | 10 | 2 |

*Residual Clumps*

A significant difference (*p = 0.06*) was found in the average percentage of area that clumps occupied in a stand infected with EDM compared with acreage in stands without EDM. The average acreage in clumps with and without recorded EDM infections was 2.17 and 1.66 acres, respectively (Table 2.4).

*Residual Clump Saplings*

Residual saplings, defined as trees being 5 feet or more in height with a diameter of three and a half inches or less, were only found in EDM-infected stands. Approximately 3.5% (± 2.5) of black spruce saplings in infected clumps were found to have active EDM infections but were living. Conversely, 2% (± 2.0) of black spruce saplings were found dead in infected clumps; no significant differences were recorded (Table 2.4).

**Table 2.4** Attributes and metrics of residual clumps found in the five stands determined to be infected with eastern EDM post-harvest.

| | EDM | No EDM | t-statistic | p-value |
|---|---|---|---|---|
| avg Clump AC | 2.14 (± 0.64) | 0 | 0.27 | 0.8 |
| avg % Clump of Admin Area | 10.79% (± 0.13) | 0% | 2.7 | 0.06 |
| % of black spruce living in clumps with EDM | 3.56% (± 2.45) | 0% | 2.02 | 0.11 |
| % of black spruce dead in clumps with EDM | 2.18% (± 2.03) | 0% | 1.67 | 0.17 |

*Residual Clump Overstory*

Overstory trees in residual clumps ranged from 3.5 inches to 17.2 inches in DBH. It is important to note that no clumps were found in uninfected stands, only stands infected with EDM had residual clumps present. Associated heights of residuals ranged from 5.1 to 76.7 feet. The DBH class most frequently measured was the 3.0-4.9 in. class, with a count of 354 trees averaging 26.4 feet in height (Table 2.5).

The majority (62%) of dead trees in clumps were black spruce, regardless of individual infection status; the vast majority (64%) of black spruce was dead and had a small diameter (3.0-4.9 in.) (Figure 2.17). Living residual overstory trees in clumps were composed of balsam fir (31%) and paper birch (*Betula papyrifera* [Marshal]; wiigwaasaatig [Ojibwe]) (23%); living black spruce accounted for 13% of the residual trees. Of those living black spruce, the majority (52%) were small-diameter trees in the 3.0 - 4.9 in. class (Figure 2.18).

Mortality and EDM status had little effect on overstory black spruce diameter in residual clumps. However, there was a significant difference found between the average TPA of live and dead uninfected black spruce in both the 5.0 - 6.9 in. ($p = 0.04$) and the 7.0 - 8.9 in. ($p = 0.02$) inch diameter class (Table 2.6).

**Table 2.5** Count and average height of the two-inch diameter class distributions of overstory trees found in residual clumps.

| Diameter Class (in) | Average Height (ft) | Count |
|---|---|---|
| 3.0-4.9 | 26.4 | 354 |
| 5.0-6.9 | 30.8 | 198 |
| 7.0-8.9 | 35.5 | 93 |
| 9.0-10.9 | 44.1 | 41 |
| 11.0-12.9 | 41.5 | 18 |
| 13.0-14.9 | 50.9 | 3 |
| 15.0-16.9 | 70.1 | 3 |
| 17.0-18.9 | 76.0 | 1 |

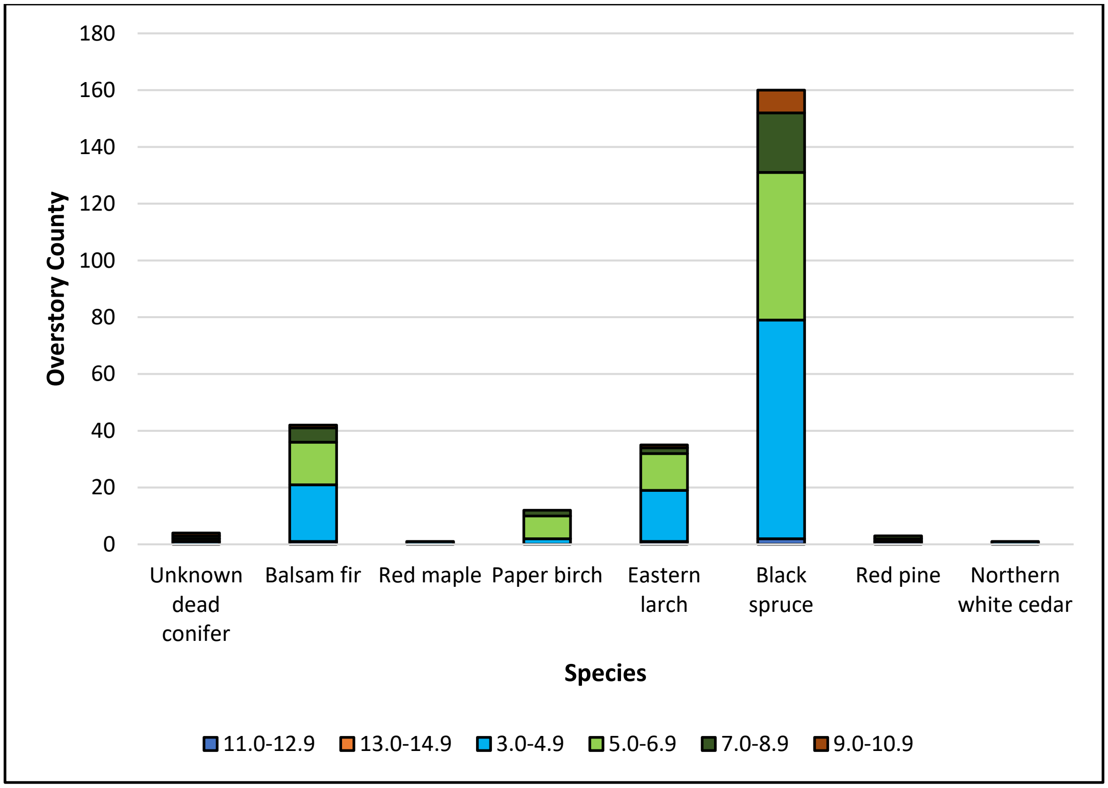


**Figure 2.17** Count of dead residual overstory trees, by species and diameter class, found in residual clumps.

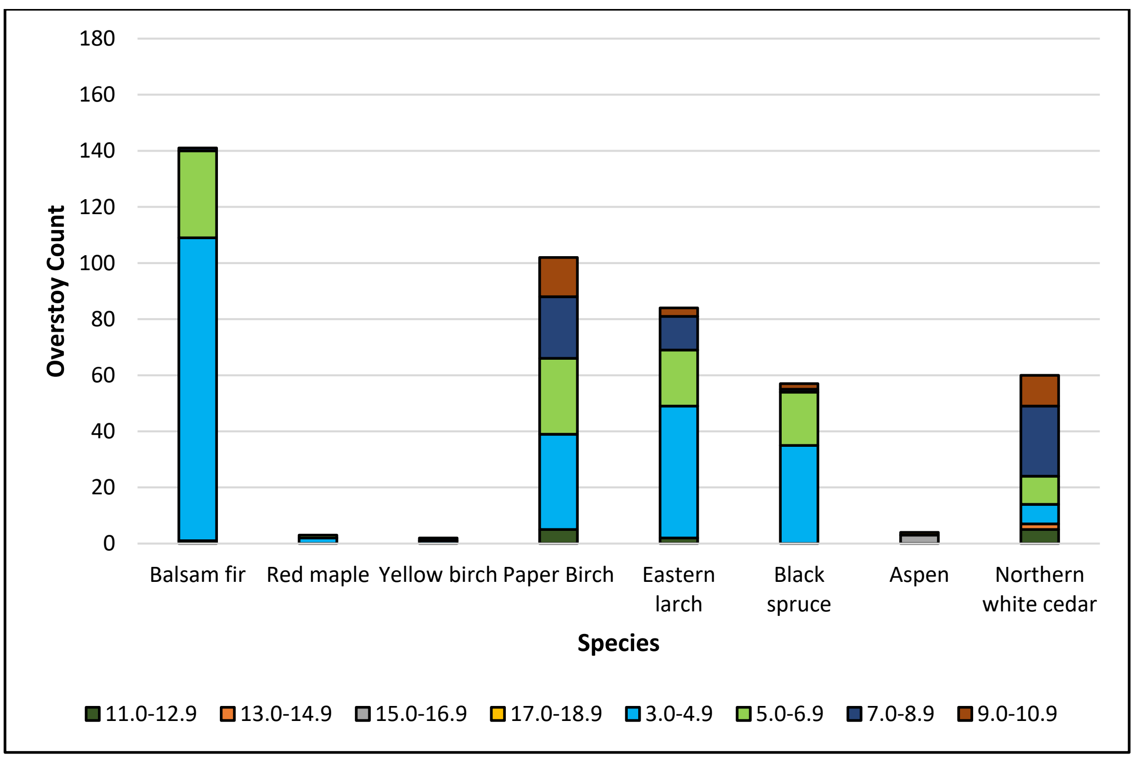


**Figure 2.18** Count of live residual overstory trees, by species and diameter class, found in residual clumps.

**Table 2.6** Average trees per acre by diameter class of infected (Black Spruce_EDM) or uninfected (Black Spruce), overstory black spruce, either living or dead, in residual clumps. A p-value of less than 0.1 denotes significant differences.

| | | PIMA Live | | PIMA Dead | | | |
|---|---|---|---|---|---|---|---|
| Diameter Class (in) | | Average | Standard Error | Average | Standard Error | t-statistic | p-value |
| 3.0-4.9 | | | | | | | |
| | Black Spruce | 0.35 | 0.14 | 1.14 | 0.27 | -0.58213 | 0.5623 |
| | Black Spruce_EDM | 0.47 | 0.16 | 0.65 | 0.2 | -1.572 | 0.1226 |
| 5.0-6.9 | | | | | | | |
| | Black Spruce | 0.13 | 0.12 | 0.56 | 0.16 | -2.0965 | 0.03986 |
| | Black Spruce_EDM | 0.29 | 0.15 | 0.6 | 0.19 | 1.0875 | 0.2805 |
| 7.0-8.9 | | | | | | | |
| | Black Spruce | 0 | 0 | 0.26 | 0.14 | -2.4968 | 0.01825 |
| | Black Spruce_EDM | 0.03 | 0.08 | 0.42 | 0.25 | -1.0844 | 0.2867 |
| 9.0-10.9 | | | | | | | |
| | Black Spruce | 0.07 | 0.13 | 0.2 | 0.17 | -1.0583 | 0.3007 |
| | Black Spruce_EDM | 0.07 | 0.13 | 0.33 | 0.29 | -0.78446 | 0.4449 |
| 11.0-12.9 | | | | | | | |
| | Black Spruce | 0 | 0 | 0 | 0 | NA | NA |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Black Spruce_EDM | 0 | 0 | 0.17 | 0.22 | -1 | 0.3388 |
| 13.0-14.9 | | | | | | | |
| | Black Spruce | 0 | 0 | 0 | 0 | NA | NA |
| | Black Spruce_EDM | 0 | 0 | 0 | 0 | NA | NA |
| 15.0-16.9 | | | | | | | |
| | Black Spruce | 0 | 0 | 0 | 0 | NA | NA |
| | Black Spruce_EDM | 0 | 0 | 0 | 0 | NA | NA |
| 17.0-18.9 | | | | | | | |
| | Black Spruce | 0 | 0 | 0 | 0 | NA | NA |
| | Black Spruce_EDM | 0 | 0 | 0 | 0 | NA | NA |

*EDM Presence in Stand Boundaries*

Stands with EDM had a significantly greater presence of EDM in the stand boundaries, 73.3% ∓ 20% of points, compared to uninfected harvested stands, 16.4% ∓ 27% of points ($p = 0.02$). Of the 115 boundary points, 55 were recorded in stands without a documented EDM infection, and 60 were recorded in infected stands. Of the boundary points recorded in uninfected stands, 3.64% were found to have no EDM, 12.73% were found to have low levels of EDM infection, and 3.64% were found to have high levels of EDM infection present. It is important to note that only one of five stands determined to be uninfected during pre-harvest surveys had EDM along the boundary. Of the boundary points collected in stands determined to have EDM infections, 15.79% were found to have no EDM, 34.36% were found to have low levels of EDM infection, and 38.94% were found to have high levels of EDM infection present (Table 2.7).

**Table 2.7** Average occurrence of EDM along stand boundaries in stands with and without an EDM infection. Different letters denote a significant difference of less than 0.1.

| | Stands Without EDM | Stands With EDM | t-statistic | p-value |
|---|---|---|---|---|
| Average Occurrence of EDM Along Stand Boundary | 16.36% | 73.30% | 3.02 | 0.02 |
| Standard Error | 0.27 | 0.20 | | |

## Discussion

*Pre- and Post-Harvest Overstory Stand Structure*

A lower abundance, density, and diameter of all recorded species, including a significant decrease in black spruce in historically black spruce-dominated stands, was seen in the presence of EDM before harvest. This shift corroborates the findings by both Gray and colleagues (2021) and Skay and colleagues (2021) that EDM, especially in highly infected stands, is causing a change in stand structure and species composition. At the same time, post-harvest EDM had no significant effects on the abundance, density, and diameter of black spruce in the overstory, regardless of the infection status of the stand. This lack of an influence post-harvest may be due to the low levels of overstory residuals that fell within the 1/50th acre overstory plots.

During sampling, an interesting observation was that uninfected stands saw no overstory trees standing. Hence, the only overstory trees recorded in the 1/50th acre plots were in infected stands. Furthermore, only 16% of the species recorded in the overstory plots were black spruce (Table 2.2). Regardless of the silvicultural treatment implemented (e.g., clearcut versus shelterwood), the abundance of non-black spruce species post-harvest may start to shift the seed source for stand regeneration away from black spruce (Skay et al., 2021), especially considering that 75% of the black spruce in a stand will succumb to EDM induced mortality within a 15 to 20-year timeframe (Baker and French, 1980).

The differences in overstory TPA post-harvest may be due to access, or lack thereof, to funds needed for operators to fulfill the silvicultural treatment in its entirety, or perhaps the reason for the absence of overstory trees in uninfected stands was the presumed increase in yield and profitability in stands lacking EDM (Roth et al., 2021).

*Regeneration*

Most regenerating seedlings were found in the smallest height class, which contained trees that were three inches or less and is what one would expect to happen after a stand-replacing disturbance. This is consistent with the findings of Anderson and colleagues (2018) that a high-intensity treatment, such as a clearcut, results in more growing space for regenerating and residual trees. While Skay and colleagues found EDM to significantly influence the levels of regeneration between unharvested stands with and without EDM, EDM's lack of influence on regeneration levels post-harvest might be explained by the sudden availability of growing space and access to a high-light environment. One can infer that this sudden increase in light availability is the reason for the high numbers of eastern larch regeneration (Table 2.3), as eastern larch is shade intolerant and needs high light environments to establish (Johnston, 1990). The high density of eastern larch in the smallest size class likely means these individuals regenerated after the harvest; it is hard to know if the eastern larch in size class 2 (Table

2.3) also regenerated after the harvest or may have been advanced regeneration. It is likely that eastern larch, as well as other species in size class 1, will suffer high levels of mortality due to variability in both annual weather patterns and competition.

Furthermore, it was unsurprising to see such high levels of aspen in recent cuts, mainly if an abundant seed and root sucker source was located nearby, as aspen regenerates well after disturbances and can quickly become the dominant species (Lieffers et al., 1996). Eastern larch and aspen, along with the species that represented the miscellaneous conifers and hardwoods in this study, such as paper birch and northern white-cedar were found in higher numbers in stands with EDM infections, corroborating the findings from Skay and colleagues (2021) that EDM infections can shift the species composition in black spruce bogs, resulting in greater species diversity.

EDM's most significant impact on stand regeneration post-harvest was the significantly fewer regenerating black spruce free of EDM (Table 2.3). This is consistent with the findings from Baker and Knowles (2004) that as the infected stand ages, more and more of the regeneration will become infected and never reach the overstory where they will then reseed a stand.

Black spruce stands with an absence of EDM will continue to function as black spruce-dominated systems that will likely not shift in terms of species or structure through successional dynamics (Anderson et al., 2019; Skay et al., 2021; Gray et al., 2021). However, there is evidence by Anderson and colleagues (2018; 2019) and Skay and colleagues (2021) that alternative silvicultural practices provide sufficient regeneration without the use of aerial seeding, along with increased complexity stand structure.

Harvest operations in the study stands took place over the last two to three years. French and colleagues (1968) conducted surveys for EDM after harvest in Northern Minnesota. They found that over 60% of their plots had become infected three years post-harvest due to residual infected trees. The work by French and colleagues (1968) is consistent with the data from this study that shows height class three, where trees are greater than 3.1 feet, to be the only height class containing infected black spruce, presumably because they established soon after harvest or because they were advanced regeneration prior to harvest and were already infected.

With the regeneration data from numerous studies (French et al., 1968; Baker and Knowles, 2004; Anderson et al., 2019; Skay et al., 2021; Gray et al., 2021) in mind, it can be predicted that there is more than ample regeneration (Table 2.3) to meet the goal of 600 or more tree per acre of desirable growing stock (MN DNR, 2019) in EDM infected stands. However, as the infected stands mature, more and more of the regeneration will become infected and succumb to EDM mortality, other lowland conifers and hardwoods will regenerate in the newly available space, and the stand will ultimately shift out of black spruce dominance due to a lack of regenerating stock.

*Residual Clumps*

This study did not seek to investigate the reasoning behind leaving clumps of residual saplings and overstory trees. EDM management in black spruce is a complicated task. While EDM is a natural disturbance agent and a native forest pathogen (Ostry and Nicholls, 1979), there are many people involved in the management, sale, procurement, and harvest of black spruce that personify the complexities of managing timber alongside EDM. According to the forester and logger's perceptions of EDM by Roth and colleagues 2021, there is a lack of communication and planning on the successful treatments and objectives for EDM management. For example, if eradication of EDM from a site is the goal, there is an expressed need for better training on identifying early-stage EDM infections and the need for more funding to implement the 5-foot cutting rule. However, forest managers need to assess if the 5-foot cutting rule is genuinely required. While both foresters and wildlife biologists were both concerned about forest health, Roth and colleagues (2021) reported that these concerns are at odds with one another. Biologists advocate for leaving trees to foster wildlife habitat and overall species diversity, with Queijeiro-Bolaños and colleagues (2018) reporting that when considering dwarf mistletoes on a global scale "at least 97 vertebrate families use mistletoe for food, and other 50 for nesting sites", while foresters advocate for the removal of every stem to help prevent further infection (MN DNR, 2019). To further the complexities of EDM management, residual clumps create heterogeneity in stand age and structure (St. Denis et al., 2010), but as Baker and Knowles (2004) found, at a cost to the health of the newly regenerating saplings and the advanced regeneration.

*Residual Clump Saplings*

Residual saplings were only found under a residual overstory canopy within clumps, and it should be noted that residual clumps were only found within stands that contained EDM. This study defined residual samplings as being advanced regeneration if stems were taller than 5 feet with a diameter at breast height of less than three and a half inches. Approximately 5% of the advanced regeneration in clumps were black spruce, presumably due to the lack of residual black spruce in the overstory, or because what black spruce was present in the overstory had succumbed to EDM induced mortality, which is consistent with the work by Baker and Knowles (2004) and Gray and colleagues (2021) that EDM infections have the ability to reduce a stand's seed source by inducing mortality in overstory black spruce.

Site index values for black spruce at 50 years range from 15 to 45 feet (Johnston, 1977), so the diameter and height of the residual samplings were not a surprise. Black spruce is likely to have its annual diameter and basal area growth suppressed for the first twelve years after harvest while under an established canopy, where the differences are negligible after those twelve years (Groot and Hokka, 2000). Furthermore, black spruce under an uneven-aged silvicultural system, with entries every 20 to 25 years, or a shelterwood cut where advanced regeneration is already present, offer a viable management strategy resulting in stocked stands and a commercial harvest while providing structural heterogeneity and increased yield to a stand. (Groot and Hokka, 2000; Anderson et al., 2019; Pothier et., al, 1994; Groot, 2014). However, in stands that have an EDM infection, a clearcut has the potential to offer better gains by way of EMD abatement, as opposed to an uneven-aged system.

*Residual Clump Overstory*

Residual clump overstories were primarily comprised of living species other than black spruce, such as balsam fir and paper birch (Figure 2.18). However, most of the standing dead trees in clumps were black spruce (Figure 2.17). Clumps were only found in infected stands; it can be presumed that the vertical dead black spruce had succumbed to EDM-induced mortality sometime before harvest based on the appearance of the recorded standing dead trees. The high level of black spruce mortality and low levels of residual black spruce seedlings helps to solidify further the points made by Skay and colleagues (2021), Gray and colleagues (2021), and Baker and Knowles (2004) that the structure and species composition in these stands are shifting due to EDM infections. A further concern for forest managers is that EDM can infect the trees in their immediate vicinity and that infections spread out radially as well (Baker and French, 1991). The radial enlargement of EDM-induced mortality centers stemming from infected residual clumps will only continue to act as a perpetual infection source, infecting regeneration efforts and potentially infecting neighboring uninfected stands.

*EDM Presence in Stand Boundaries*

With nearly half of the recorded boundary points across all stands containing some level of EDM infection, it is little wonder why managing EDM is such a long and complicated process. With nearly 75% of the boundary points collected in infected stands showing signs of EDM, it can be predicted, based on the work by Baker and French (1980) and Baker and Knowles (2004), that these stand adjacent infections will spread to the regenerating stand.

Surveying the post-harvest stand boundary may allow managers some insight into the future of the health of the stand. However, EDM infections can be cryptic and hard to detect, adding to management complexities (Gray et al., 2021). For example, of the ten stands surveyed for this study, five were determined pre-harvest to be free of EDM, and five were determined to be infected. It was not until the post-harvest inventory that it was revealed that one of the five stands classified as uninfected was found to contain EDM based on the survey of the boundary. Within this stand, EDM was also detected in residual clumps on advanced regeneration but was not readily detectable on these trees until observations were made up close when measurements were made. Depending on the management objectives, missing an early EDM infection could result in a severe loss of volume at the end of a 60 to 120-year rotation since 75% of the black spruce may succumb to EDM mortality (MN DNR, 2019; Baker and Knowles, 2004). Furthermore, a missed EDM infection along a stand boundary could cause what was once a black spruce stand at the beginning of a 120-year rotation to transition to a different cover type altogether at the end of the rotation (Gray et al., 2021; Skay et al., 2021; Baker and Knowles, 2004).

**Management implications**

EDM management is complicated and depends on the land manager's objectives. EDM is a native pathogen and disturbance agent in black spruce bogs (Ostry and Nicholls, 1979). Eradicating it is an all but impossible task; it has also proved very difficult to predict where infections are most likely to occur (Gray et al., 2021). Additionally, infections in stands with high tree densities can be hard to discern and require extensive sampling, and trees from neighboring stands or residual clumps may act as a continual infection source (Baker and Knowles, 2004).

Clearcutting black spruce stands infected with EDM seem to be the most efficient means of managing the adverse effects of infection (Anderson et al., 2019; Skay et al., 2021). However, if the 5-foot cutting rule is not followed, a clear-cut harvest as a means of pathogen abatement is rendered useless and acts as an EDM seed tree treatment. If a clearcut cannot be implemented for logistical or financial reasons, an effort should be made to avoid infected clumps of trees to try and keep EDM localized in the hope that EDM will not immediately spread to new and advanced regeneration.

However, a successful clearcut cannot always be implemented. A solution might stem from the shift in stand structure created by EDM-induced black spruce mortality. Gray and colleagues (2021) found that in stands where 50% or more of the black spruce are infected with EDM, species other than black spruce will begin to establish, with Skay and colleagues (2021) predicting that these highly infected stands will see young, infected black spruce not reach the overstory and that the stands will eventually transition to a lowland conifer mix or a lowland conifer-hardwood cover type.

About half of the stands sampled in this study contained or were adjacent to an aspen seed source regardless of a stand's infection status. In some cases, seed trees within the stand were upwards of 17 inches in diameter and over 70 feet in height. The combination of aspen and spruce is a common feature of the landscape in boreal forests, with aspen dominating quickly after disturbances, and is often referred to as a mixed-wood system and generally implies a mix of quaking aspen and white spruce (Kabzems et al., 2016; Lieffers et al., 1996). Several studies have shown that the mixed-wood system creates a higher level of biomass and yield at the end of the rotation, compared to that of a solely white spruce-dominated stand (Kabzems et al., 2016; Man and Lieffers, 1999; MacPherson et al., 2001; Wang et al., 1995).

One alternative approach to managing EDM in black spruce would be to implement a mixed wood system with black spruce and aspen. Aspen regenerates relatively quickly, often with a 60-year rotation (Man and Lieffers, 1999) compared to the 60 to 120-year rotation length of black spruce (MN DNR, 2019). Given black spruce's shade tolerance, black spruce may be able to "hold" as advanced regeneration until the aspen is harvested and then released. These differences in rotation length lend themselves to both a single rotation of black spruce and aspen, as well as a two-staged harvest.

In the boreal forests of Canada, Bokalo and colleagues (2007) found that while high aspen densities decreased the height to root collar ratio in white spruce, aspen self-thinned about a decade after establishment, effectively releasing the spruce. Furthermore, the initial high density of aspen may benefit from a mechanical thinning twenty years post-establishment, with a delayed thinning having the potential to increase spruce diameter, decrease knot size and increase wood quality (Comeau, 2021). If EDM is creating a shift in species that favors aspen, managers should consider the pros and cons of losing a black spruce-dominated stand but gaining a black spruce-aspen mixed-wood stand.

Stands, where less than 50% of black spruce were infected with EDM saw little overstory mortality, and only a small number of other species established in the EDM induced canopy gaps (Gray et al., 2021). It can then be presumed that stands with no or little EDM presence will maintain an ample black spruce seed source for some time, with the uninfected stands in this study averaging almost 2,000 (± 363) black spruce seedlings per acre in height class one (Table 2.3). However, black spruce regeneration in infected stands only averaged slightly more than 300 trees per acre across all height classes, well below the 600 TPA the MN DNR (2019) requires at 5 years. Still, when you incorporate eastern larch, the average TPA jumps up to 1500 (Table 2.3).

Compared to eastern larch and other species, the lack of regenerating black spruce in infected stands alludes to the difficulty of regenerating black spruce naturally that Henderson and colleagues (1992) documented in Ontario, Canada. While Henderson and colleagues (1992) reported that artificial regeneration via aerial seeding was a viable option for regenerating black spruce, Anderson and colleagues (2019) in northern Minnesota found enough natural regeneration of desired stock after harvest. However, focusing only on black spruce regeneration in infected stands, this study found that there may not be adequate levels of growing stock, and an argument could be made for aerial seeding; with over 7,500 (± 6,115) eastern larch seedlings regenerating in height class one, two to three years post-harvest, it may be appropriate to conduct an aerial seeding treatment of black spruce after the five-year post-harvest regeneration survey is complete. EDM presents another logistical challenge on a landscape level: how to determine which of Minnesota's 1.5 million acres of black spruce are infected with EDM, and if so, what is the intensity of the infection, and what silvicultural and regeneration practices are appropriate.

Across the landscape, EDM may very well be inadvertently increasing the resiliency of black spruce forests by adding complexities to stand structure. However, EDM can also limit seed sources (Baker and Knowles, 2004), eventually decreasing black spruce's presence on the landscape altogether. Thought should also be given to the findings by

Anderson and colleagues (2019) that show how alternative silvicultural practices still provide ample growing stock and combined with the findings by Hart and colleagues (2018), uneven-aged black spruce stands very well may be more resilient to fire and climate change than even-aged stands. EDM is both a native plant and disturbance agent, as well as a serious plant pathogen to Minnesota's 1.5 million acres of black spruce. It is important to consider the various management options needed to sustain the black spruce timber source, increase black spruce climate and fire resiliency, but also acknowledge the important ecological role that EDM as a forest disturbance plays in the southern boreal region.

## Appendix

**Table 2.8** List of species found across sampling classes and distributed by EDM status. Ojibwe names came from the Ojibwe People's Dictionary.

| | | | Stands with EDM | | | | Stands Without EDM | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Scientific | Common | Ojibwe | Regen | OVST | Clump Saplings | Clump OVST | Regen | OVST | Clump Saplings | Clump OVST |
| *Abies balsamea* (L.) Mill. | Balsam fir | Zhingob | x | x | x | x | x | | | |
| *Acer rubrum* L. | Red maple | Aninaatig | x | | x | x | x | | | |
| *Betula alleghaniensis* Britton | Yellow birch | Wiinizik | x | | x | x | | | | |
| *Betula papyrifera* Marshal | Paper birch | Wiigwaasaatig | x | | x | x | | | | |
| *Larix laricina* (Du Roi) K. Koch | Tamarack | Mashkiigwaatig | x | x | x | x | x | | | |
| *Picea mariana* (Mill.) Britton, Sterns and Poggenb. | Black spruce | Zesegaandag | x | x | x | x | x | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Pinus resinosa Aiton* | Red pine | Bapakwanagemag | | x | | x | | |
| *Pinus strobus* L. | Eastern white pine | biisaandago-zhingwaak | x | | x | | x | |
| *Populus grandidentata* Michx. | Bigtooth aspen | Azaadi | x | | | | | |
| *Populus tremuloides* Michx. | Quaking aspen | Azaadi | x | | x | x | x | |
| *Salix* spp. | Willow species | Oziisigobiminzh | | | x | | | |
| *Thuja occidentalis* L. | Northern white-cedar | Giizhik | x | x | x | x | | |
| *Toxicodendron vernix* (L.) Kuntze | Poison sumac | | | | x | | | |

**Table 2.9** List of species found across height classes and distributed by EDM status. Ojibwe names came from the Ojibwe People's Dictionary.

| | | | Stands with EDM | | | Stands Without EDM | | |
|---|---|---|---|---|---|---|---|---|
| Species Scientific Name | Species Common Name | Species Ojibwe Name | Size Class 1 | Size Class 2 | Size Class 3 | Size Class 1 | Size Class 2 | Size Class 3 |
| *Abies balsamea* (L.) Mill. | Balsam fir | Zhingob | x | x | x | x | | |
| *Acer rubrum* L. | Red maple | aninaatig | x | x | x | x | | |
| *Betula alleghaniensis* Britton | Yellow birch | Wiinizik | | x | x | | | |
| *Betula papyrifera* Marshal | Paper birch | wiigwaasaatig | x | x | x | x | | |
| *Larix laricina* (Du Roi) K. Koch | Tamarack | mashkiigwaatig | x | x | x | | x | x |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Picea mariana* (Mill.) Britton, Sterns and Poggenb. | Black spruce | zesegaandag | x | x | x | | x | x |
| *Pinus resinosa Aiton* | Red pine | bapakwanagemag | | | | | | |
| *Pinus strobus* L. | Eastern white pine | biisaandago-zhingwaak | | x | | x | | x |
| *Populus grandidentata* Michx. | Bigtooth aspen | Azaadi | x | x | | | | |
| *Populus tremuloides* Michx. | Quaking aspen | Azaadi | x | x | | x | x | x |
| *Salix* spp. | Willow species | oziisigobiminzh | | | | | | |
| *Thuja occidentalis* L. | Northern white-cedar | Giizhik | x | x | x | | | |
| *Toxicodendron vernix* (L.) Kuntze | Poison sumac | | | | | | | |

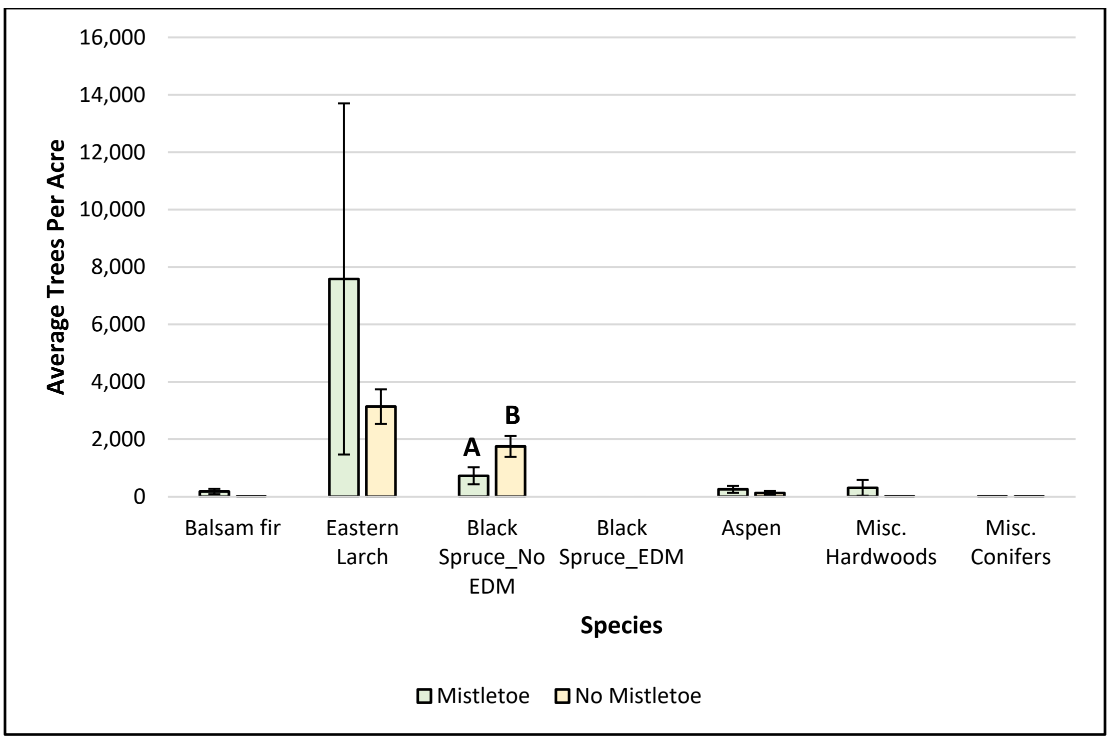


**Figure 2.19** Average trees per acre of regenerating species in size class one.

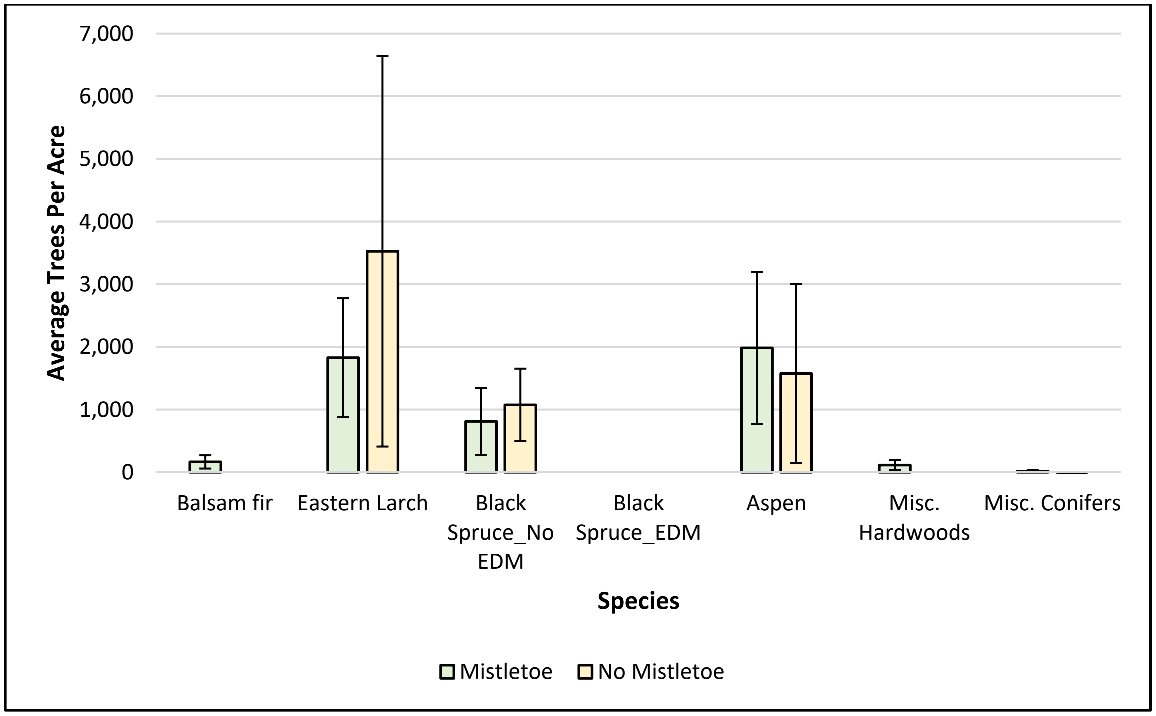


**Figure 2.20** Average trees per acre of regenerating species in size class two.

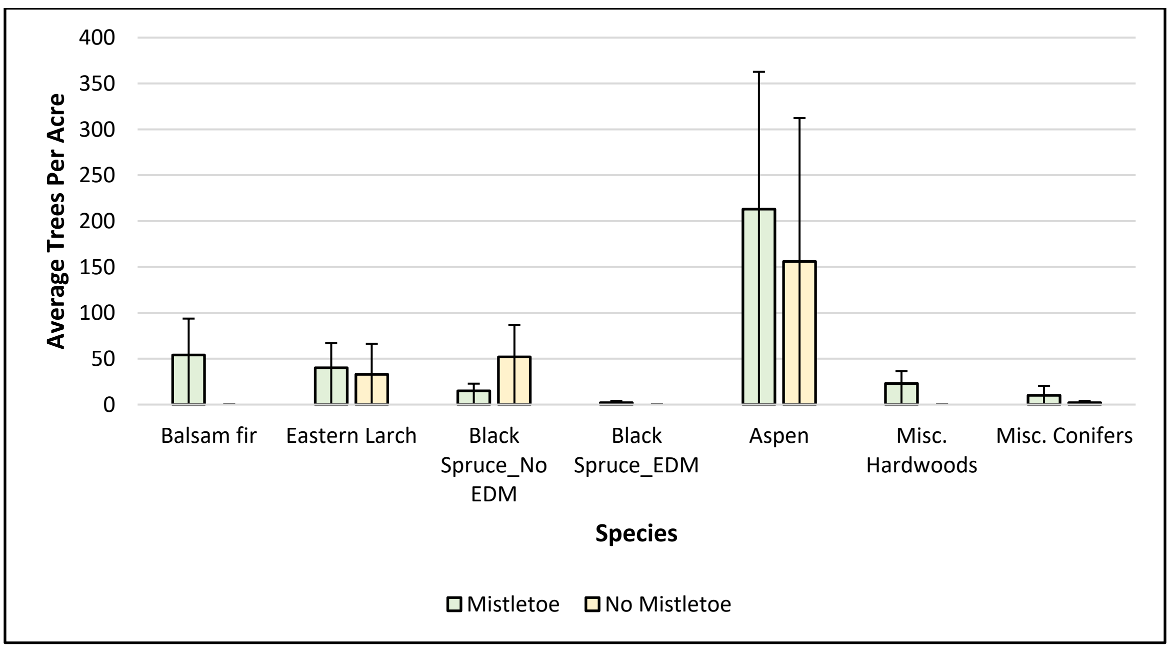


**Figure 2.21** Average trees per acre of regenerating species in size class three.

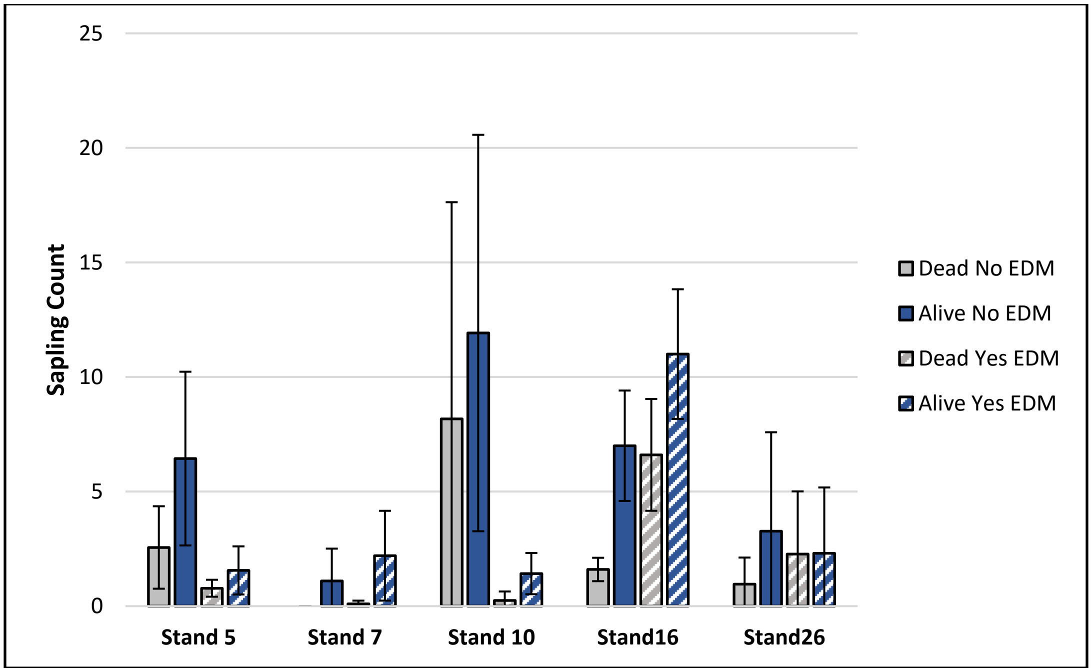


**Figure 2.22** The average count of living and dead black spruce with and without EDM infections tallied within residual clumps.

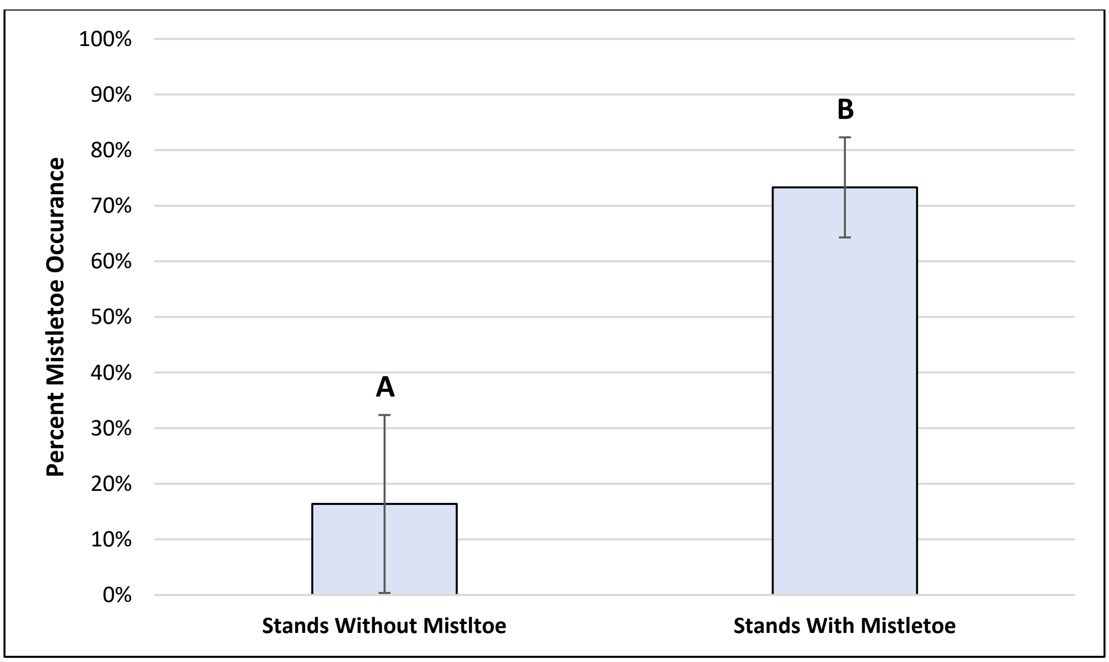


**Figure 2.23** Average occurrence of EDM along stand boundaries in stands with and without an EDM infection. Different letters denote a significant difference of less than 0.1.

## Chapter 3: Wood Decay Fungi Associated with Black Spruce Stands with Eastern Dwarf Mistletoe

### Overview

Twelve actively managed black spruce (*Picea mariana* [Mill] Britton, Sterns & Poggenb) stands in Northern Minnesota, USA, were sampled to better understand the diversity and abundance of fungi present in black spruce bogs affected by eastern dwarf mistletoe (*Arceuthobium pusillum* [Peck]). The twelve stands were divided evenly between recently harvested (six stands) and unharvested (six stands); additionally, half of the harvested and unharvested stands were infected with eastern dwarf mistletoe, and half were uninfected. Due to the potential for fungal infections to act as secondary disturbance agents that could expedite black spruce mortality, an emphasis was placed on investigating the presence of fungal pathogens in the Basidiomycota. Thirty-nine distinct fungal species were identified across all twelve study stands. Of all the species identified, less than half (43%) were determined to be fungal pathogens. No significant differences in fungal diversity were found between the study treatments. Additional work in black spruce systems should be considered to increase the understanding between potential above and below ground disturbance agents.

### Introduction

While there has been a recent re-investment in the exploration of southern boreal forest dynamics, these systems are understudied forest ecosystems, especially when considering disturbance dynamics such as the influence of eastern dwarf mistletoe (EDM) (*Arceuthobium pusillum* [Peck]; EDM) in black spruce forest ecosystems (*Picea mariana* [Mill] Britton, Sterns and Poggenb) (Skay et al., 2021; Anderson et al., 2019; Gray et al., 2021). For other dwarf mistletoe species, environmental factors, such as stand structure, drought, and simultaneous infections by fungi and insects, can influence the rate and severity of a mistletoe infection and subsequent management decisions (Queijeiro-Bolaños et al., 2017; Hanks et al.; Geils and Hawksworth, 2002).

Dwarf mistletoes acquire most of their photosynthate, carbon, water, and mineral nutrients from the host tree (Clark and Bonga, 1970). A 2017 study conducted by de Villier and colleagues (2017) observed that varying levels of symptom manifestation of EDM in white spruce (*Picea glauca* [Moench] Voss) depended on the level of infection (e.g., depressed photosynthate capacity in severely infected trees) and symptoms of the disease became visible once the vigor of the tree declined. Giels and Hawksworth (2002) reported that mistletoe infections across *Arceuthobium* species resulted in the potential weakening of the tree's defenses, allowing secondary impacts from both insects and fungi. It is important to note that more resinous conifers such as spruce *(Picea* spp.), pine (*Pinus* spp.), and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) may be more resistant to secondary infection than the less resinous species of fir (*Abies* spp.), larch (*Larix* spp.), and hemlock (*Tsuga* spp.) (Geils and Hawksworth, 2002).

While the impacts of EDM on black spruce have been documented in forest management and forest ecology (Baker and Knowles, 2004; Gray et al., 2021; Skay et al., 2021), there has been little documentation on the potential for secondary disturbance agents to influence EDM induced black spruce mortality. One of the most prolific and virulent disturbance agents in North America that has the potential to contribute to black spruce mortality is the fungus *Armillaria* (Agaricales, Basidiomycota) (Devkota and Hammerschmidt, 2020). *Armillaria* causes *Armillaria* root rot, a disease that actively decays the root tissues while a tree is still alive (Devkota and Hammerschmidt, 2020).

In heavily managed forests, regardless of type, older, more mature stands can be a source of *Armillaria* and cause infections in recently planted or seeded trees, with inoculum persisting in the soil for years (Kubiak et al., 2017; Labbé et al., 2015). In British Columbia, where *A. ostoyae* is the only *Armillaria* species documented to cause mortality in Douglas-fir plantations, there was an increased chance of infection after thinning treatments in young stands (Cruickshank et al. 1997). *Armillaria* infections, especially in stressed trees, have been found to predispose trees to secondary infestation by bark beetles (Kubiak et al., 2017). In one instance, James and Goheen (1981) found that 80% of the root diseased trees they surveyed also experienced a secondary infestation from bark beetles.

Several disturbances, such as dwarf mistletoes and fungal pathogens, play essential roles in forested ecosystems, including those in the southern boreal forest (Skay et al., 2021; Thormann et al., 2001). The Minnesota Department of Natural Resources (1994) reports that all black spruce stands over the age of 30 will have root rot. In the understudied black spruce ecosystems, it is unknown what fungi are present and if there is a facultative relationship between infections of fungal pathogens and eastern dwarf mistletoe that could lead to expedited mortality of black spruce. The relationship among multiple interacting disturbance agents has not been heavily explored in the actively managed black spruce peat bogs in the southern boreal forest and represents a new line of questioning. Furthermore, there is a lack of documentation in the literature regarding the presence of any fungi, regardless of their mode of nutrition or ecological role, in black spruce ecosystems.

This study aimed to better understand the diversity and abundance of fungi, emphasizing pathogens within the Basidiomycota, found within managed black spruce ecosystems, which included both recently harvested and unharvested stands and stands infected by EDM and those uninfected by EDM. Our objective was to explore differences in fungal communities across treatments and management activities. Increased understanding of potential below-ground pathogens could help natural resource managers in decisions related to forest management in black spruce ecosystems.

## Methods

### *Study Region*

Northern Minnesota, USA, represents the southern boundary of the boreal forest. The southern boreal forest region features mixed conifer, hardwood, conifer-hardwood upland forests, and lowland conifer bogs and swamps.

One of the dominant tree species in peatland ecosystems is black spruce. The growing season for black spruce in Northern Minnesota is relatively short compared to those closer to the equator, averaging roughly 105 days per year. Yearly precipitation ranges from 134 to 172 centimeters, with half of the precipitation falling during the growing season; the colder months see the precipitation as snow, while the warmer months see it as rain. The average annual temperature for the southern boreal forest is about 1◦C to roughly 4◦C (Aaseng et al., 2003; Hanson and Hargrave, 1996).

*Study Area*

Stands were located in Northern Minnesota, USA, on Department of Natural Resources (DNR) administered lands in the following counties: Aitkin, Lake, St. Louis, Koochiching, and Beltrami (Figure 3.1).

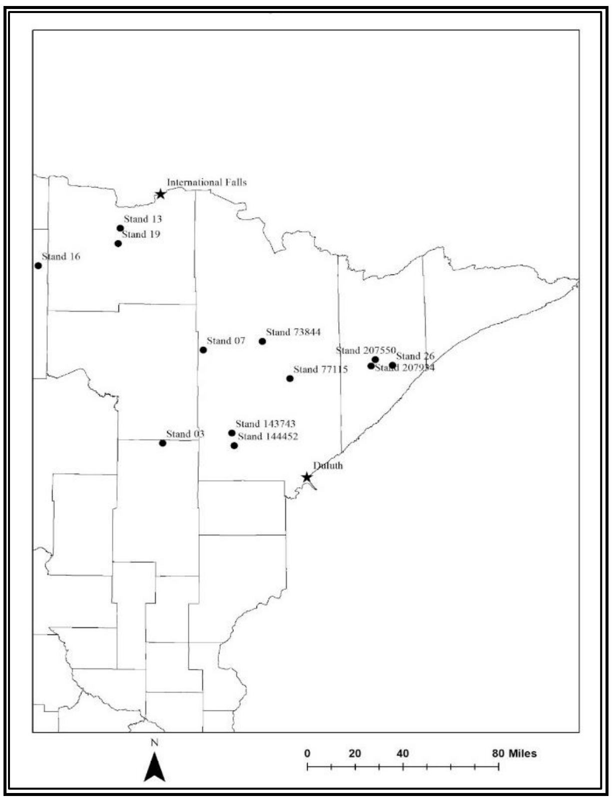


**Figure 3.1** Map of Northern Minnesota, USA, displaying the location of stands used in this study.

DNR stands were identified by their EDM infection status and harvest status and were broken down into the following classifications: 3 harvested + EDM positive; 3 harvested + EDM negative; 3 unharvested + EDM positive; 3 unharvested + EDM negative. Resulting in an even distribution in the number of stands between the two categories – management (harvested versus unharvested) and EDM level (present versus absent).

Study stand size ranged from 3.36 to 40.15 hectares, with an average of 17.48 hectares. Tree density went from 947 trees per hectare (TPH) to 3,883, with an average TPH of 2,002. The overstory basal area in the study stands spanned 19.6 $m^2$/ha to 34.1$m^2$/ha, with an average of 27.31 m2/ha. Study stands had a wide range of ages, with the youngest being 65 and the oldest being 160 years, averaging about 97 years. Soil types were categorized as being mucky, ponded, and peaty, with 0 to 1 percent slopes (Table 3.1).

**Table 3.1** Descriptions and locations for the twelve study stands. Of the twelve stands sampled, six were harvested, six were infected with eastern dwarf mistletoe (EDM), six were unharvested, and six were uninfected by EDM.

| Stand ID | Harvested | EDM | Stand Age | Site Index at 50 years (m) | Stand Size (hectares) | Overstory BA ($m^2$/ha) | TPH | Dominant Soils | County | Stand Location (lat, long) |
|---|---|---|---|---|---|---|---|---|---|---|
| 3 | Yes | No | NA | NA | 3.36 | 34.1 | 1606 | Sago muck, 0 to 1 percent slopes, frequently ponded | Aitkin | 47.00724, -93.37688 |
| 7 | Yes | Yes | NA | NA | 4.05 | 27.8 | 1016 | Cathro muck, occasionally ponded, 0 to 1 percent slopes | St. Louis | 47.6048, -93.01643 |
| 13 | Yes | No | 141 | 9 | 40.15 | 29.3 | 1771 | Cathro and Tacoosh soils, Suomi catena, 0 to 1 percent slopes | Koochiching | 48.37948, -93.77147 |
| 16 | Yes | Yes | 90 | 10 | 32.86 | 22.4 | 1290 | Lupton-Lupton, ponded, complex, 0 to 1 percent slopes | Beltrami | 48.13014, -94.51935 |
| 19 | Yes | No | 160 | 9 | 36.09 | 30.0 | 1881 | Greenwood-Greenwood, ponded, complex, 0 to 1 percent slopes | Koochiching | 48.28555, -93.78912 |
| 26 | Yes | Yes | 89 | 10 | 4.98 | 19.7 | 947 | Rifle-Rifle ponded complex, 0 to 1 percent slopes, dense substratum | Lake | 47.49357, -91.32469 |
| 207934 | No | Yes | 91 | 14 | 22.22 | 30.9 | 2506 | Cathro muck, occasionally ponded, 0 to 1 percent slopes | Lake | 47.4989, -91.5268 |
| 143743 | No | No | 82 | 13 | 15.99 | 25.0 | 2333 | Greenwood-Greenwood, ponded, complex, 0 to 1 percent slopes | St. Louis | 47.0711, -92.75762 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 207550 | No | Yes | 86 | 13 | 8.86 | 32.3 | 2078 | Toimi stony fine sandy loam, 3 to 8 percent slopes, very stony | Lake | 47.5158, -91.50379 |
| 144452 | No | Yes | 72 | 12 | 7.12 | 19.6 | 1344 | Rifle-Rifle ponded complex, 0 to 1 percent slopes, dense substratum | St. Louis | 46.9888, -92.73881 |
| 77115 | No | No | 65 | 11 | 14.12 | 29.3 | 3372 | Daisybay peat, 0 to 1 percent slopes | St. Louis | 47.418, -92.24121 |
| 73844 | No | No | 93 | 11 | 19.91 | 27.4 | 3883 | Greenwood soils, 0 to 1 percent slopes | St. Louis | 47.6561, -92.48443 |

*Field Data Collection*

EDM-induced mortality centers were targeted to collect biological samples of fungal pathogens. Mortality centers often exhibit a chronosequence of mortality, making live, dead, and dying black spruce readily available to sample for potential fungal pathogen infection. Standing dead and dying trees, coarse woody debris, and stumps were targeted and sampled using a Pulaski to liberate rot. A sample is defined as wood rot measuring no more than one cubic foot. Samples were deliberately taken from black spruce and tamarack stumps or snags or unidentifiable conifer stumps using a Pulaski or a pocketknife. If basidiocarps were found when sampling target species, they were collected (i.e., best chance of getting viable culture and DNA). Ten samples per stand were collected. Samples were placed in sterile bags and stored in a freezer or a refrigerator until processed in the lab.

*Lab Methods*

Wood samples were cut into small segments and placed in culture media for isolations. Four to five segments were placed onto each of three different media: 1.5% malt extract agar amended with lindane (M þ L) for mite control (15 g of Difco Bacto-agar, 15 g of Difco Bacto malt extract, 0.01 g of Aldrich lindane per L of deionized water with 0.1 g streptomycin sulfate added after autoclaving); modified Sabouraud Dextrose Agar (adapted from Harrington, 1981) amended with lindane (SDA þ L) (15 g of Difco Bacto-agar, 15 g of Difco Bacto malt extract, 0.01 g of Aldrich lindane per L of deionized water, with 0.1 g Aldrich cycloheximide, 0.1 g streptomycin sulfate added after autoclaving), and

Basidiomycota Selective Agar (adapted from Worrall, 1991) amended with lindane (BSA þ L) (15 g of Difco Bacto agar, 15 g of Difco Bacto malt extract, 2 g of Difco yeast extract, 0.06 g, Aldrich benomyl, 0.03 g of Aldrich lindane per L of deionized water, and 0.1 g streptomycin sulfate, 2 ml lactic acid added after autoclaving). Plates were sealed with Parafilm® and incubated at room temperature for one to two weeks. After initial inoculation and incubation, pure cultures were obtained by subculturing colonies to malt extract agar (MEA) (15 g Difco Bacto malt extract, 15 g Difco Bacto agar, and 1 L deionized water) plates. They were incubated at room temperature for one to two weeks. DNA extraction was performed using mycelia from pure cultures using a CTAB procedure according to Blanchette et al. (2016) or a NaOH protocol according to Osmundson et al., (2013). Polymerase chain reaction (PCR) was performed to amplify the rDNA internal transcribed spacer region (ITS) using ITS1F and ITS4 primers (Gardes and Bruns, 1993), according to Blanchette et al. (2016). Amplicons were visualized via 1% agarose gel electrophoresis with SYBR green I pre-stain and visualized with transillumination on a Dark Reader DR45. Sanger sequencing was done with PCR primers on an ABI 3730xl DNA sequencer (Applied Biosystems e Foster City, CA. Consensus sequences were assembled using Geneious 9.0 (Kearse et al., 2012) and were subjected to the BLASTn algorithm (Zhang et al., 2000) using the megablast option in NCBI GenBank. Identification of cultures was based on the highest BLAST score matching a species-level accession from a published taxonomic or type specimen study. Sequences representative of each taxon were deposited in GenBank and given genera or higher-level classification. Values of less than 97% best BLAST match were given the species name of closest identity (Koljalg et al., 2013; Schoch et al., 2012).

*Statistical Methods*

All descriptive and summary statistics, such as averages and standard deviations, were calculated using Microsoft Excel. A p-value of 0.1 was used to accommodate the variation found in forested systems. Using R and R Studio, Welch's Two Sample t-test was used to test for significant differences between stand treatments.

## Results

*ITS Sequence Results*

Of the 39 samples sequenced for species identification, 26 genetically distinct species were identified (Table 3.2). The red-banded polypore (*Fomitopsis mounceae* (Polyporales, Basidiomycota) Haight and Nakasone) was the most abundant species, representing 15% of all samples collected. Two other relatively abundant species were *Coniophora puteana* (Schumach.) P. Karst. and *Irpex lacteus* (Fr.) Fr.; both were about half as abundant as *F. mounceae*, with each species making up nearly 7.5% of the identified species (Table 3.2).

Samples were assigned a known species name if the BLAST returned a 97% or higher result. However, some samples returned a BLAST value of less than 97% but higher than 88%. These included: *Athelia decipiens* (Höhn. and Litsch.) J. Erikss., *Rhizochaete americana* (Nakasone, C.R. Bergman and Burds.) Gresl., Nakasone and Rajchenb., *Hyphoderma tenue* (Pat.) Donk, *Phlebia aurea* (Fr.) Nakasone and were labeled according to the most closely matched species (Table 3.3).

**Table 3.2** Phylogenetic organization of 26 distinct species identities was obtained through BLAST results from ITS1 sequences.

| Division | Class | Order | Family | Genus | Species | Count |
|---|---|---|---|---|---|---|
| **Basidomycota** | **Agaricomycetes** | **Atheliales** | **Atheliaceae** | ***Athelia*** | **decipiens** | **1** |
| | | **Boletales** | **Coniophoraceae** | ***Coniophora*** | ***Arida*** | 1 |
| | | | | | ***Puteana*** | 2 |
| | | | **Serpulaceae** | ***Serpula*** | ***himantioides*** | 2 |
| | | **Cantharellales** | **Hydnaceae** | ***Sistostrema*** | ***brinkmannii*** | 2 |
| | | | | | ***raduloides*** | 1 |
| | | | | | ***sp.*** | 1 |
| | | **Gloephyllales** | **Gloeophyllaceae** | ***Neolentinus*** | ***lepideus*** | 1 |
| | | **Hymenochaetales** | **Hymenochaetaceae** | ***Porodaedalea*** | ***Pini*** | 1 |
| | | | **Incertae sedis** | ***Trichaptum*** | ***abietinum*** | 1 |
| | | | **Rickenellaaceae** | ***Resinicium*** | ***furfuraceum*** | 1 |
| | | **Polyporales** | **Dacrybolaceae** | ***Postia*** | ***sericemollis*** | 2 |
| | | | **Fomitopsidaceae** | ***Fomitopsis*** | ***mounceae*** | 6 |
| | | | **Hyphodermataceae** | ***Hyphoderm*** | ***tenue*** | 1 |
| | | | **Incertae sedis** | ***Calcipostia*** | ***guttulata*** | 1 |
| | | | **Irpicaceae** | ***Irpex*** | ***lacteus*** | 3 |
| | | | **Meruliaceae** | ***Phlebia*** | ***aurea*** | 1 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | ***tremellosa*** | 1 |
| | | | **Phanerochaetaceae** | ***Phanerochaete*** | ***livescens*** | 1 |
| | | | | ***Rhizochaete*** | ***americana*** | 1 |
| | | | **Polyporaceae** | ***Perenniporia*** | ***valliculorum*** | 1 |
| | | | | ***Poriella*** | ***subacida*** | 4 |
| | | **Russulales** | **Stereaceae** | ***Xylobolus*** | ***frustulatus*** | 1 |
| | **Dacrymycetes** | **Dacrymycetales** | **Dacrymycetaceae** | ***Calocera*** | ***viscosa*** | 1 |
| **Ascomycota** | **Sordariomycetes** | **Hypocreales** | **Clavicipitaceae** | ***Pochonia*** | ***sp.*** | 1 |

**Table 3.3** ITS1 BLAST results from the 40 samples sequenced. Samples with 97% or greater similarity to a type species or a published sequence were given the species name. Samples with a likeness of less than 97% were acknowledged as potentially being a different or new species, but for the scope of this study, they were given the name of the species with the greatest similarity. Known host, known region, and known documentation in North American spruce bogs were reported as found in the literature.

| EDM | Harvested | Sample ID | % Ident | GenBank Accession # | Pathogen | Rot Type | Known Host | Documented in North American Spruce Bogs |
|---|---|---|---|---|---|---|---|---|
| Yes | No | *Athelia decipiens* | 87.96% | ON611810 | No | White | *Larix Occidentalis, Picea, Pinus Ponderosa, Abies concolor* | Yes |
| Yes | No | *Calcipostia guttulata* | 99.01% | ON611811 | Yes | Brown | *Picea* | NA |
| No | No | *Calocera viscosa* | 97.46% | ON611812 | Yes | Brown | *Pseudotsuga menziesii, Picea, Abies, Larix, Pinus, Picea abie* | No |
| Yes | Yes | *Coniophora arida* | 99.77% | ON611813 | Yes | Brown | *Populus* | Yes |
| Yes | Yes | *Coniophora puteana* | 100.00% | ON611814 | Yes | Brown | *Picea sitchensis, Pinus Ponderosa, Picea, Pinus conotorta var. murrayana, Quercus petraea* | No |
| No | No | *Coniophora puteana* | 99.86% | ON611815 | Yes | Brown | | |
| No | No | *Coniophora puteana* | 99.53% | ON611816 | Yes | Brown | | |
| No | Yes | *Fomitopsis mounceae* | 100.00% | ON611817 | No | Brown | Conifers and Hardwoods | Yes |
| Yes | Yes | *Fomitopsis mounceae* | 100.00% | ON611818 | No | Brown | | |
| Yes | No | *Fomitopsis mounceae* | 100.00% | ON611819 | No | Brown | | |
| Yes | No | *Fomitopsis mounceae* | 99.84% | ON611820 | No | Brown | | |
| Yes | No | *Fomitopsis mounceae* | 99.83% | ON611821 | No | Brown | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Yes | Yes | *Fomitopsis mounceae* | 99.65% | ON611822 | No | Brown | | |
| Yes | No | *Hyphoderma tenue* | 95.45% | ON611823 | No | Brown | *Quercus, Acer, Betula, Picea, Abies* | No |
| No | Yes | *Irpex lacteus* | 100.00% | ON611824 | No | White | | |
| No | Yes | *Irpex lacteus* | 100.00% | ON611825 | No | White | Hardwoods | No |
| Yes | No | *Irpex lacteus* | 100.00% | ON611826 | No | White | | |
| Yes | Yes | *Neolentinus lepideus* | 99.66% | ON611827 | No | Brown | *Larix, Pinus, Pinus contorta* var *murrayana, Pinus ponderosa* | No |
| No | No | *Perenniporia valliculorum* | 100.00% | ON611828 | Yes | White | Unknown | No |
| Yes | Yes | *Phanerochaete livescens* | 100.00% | ON611829 | No | White | *Pinus sylvestris* | No |
| No | Yes | *Phlebia aurea* | 96.68% | ON611830 | No | White | *Fagus, Acer, Fraxinus* | No |
| No | No | *Phlebia tremellosa* | 99.41% | ON611831 | No | White | *Betula, Larix, Picea abies, Fagus, Betula populifolia , Magnolia , Populus trichocarpa, Quercus garryana, Acer rubrum* | No |
| Yes | No | *Poriella subacida* | 100.00% | ON611832 | Yes | White | | |
| Yes | Yes | *Poriella subacida* | 99.83% | ON611833 | Yes | White | *Abies, Picea, Tsuga, Pinus, Betula, Abies balsamea,* | No |
| Yes | Yes | *Poriella subacida* | 99.83% | ON611834 | Yes | White | | |
| No | No | *Poriella subacida* | 99.66% | ON611835 | Yes | White | | |
| No | No | *Porodaedalea pini* | 99.69% | ON611836 | Yes | White | *Abies, Chamaecyparis, Larix, Libocedrus, Picea, Pinus, Pseudotsuga, Taxus, Thuja, and Tsuga* | No |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Yes | Yes | *Postia sericeomollis* | 100.00% | ON611837 | Yes | Brown | *Thuja plicata, Picea glauca, Picea abies, Abies concolor, Pinus koraiensis, Fagus, Larix, Cedrus, Taxus, Pinus sylvestris* | No |
| Yes | No | *Postia sericeomollis* | 99.84% | ON611838 | Yes | Brown | | No |
| Yes | Yes | *Resinicium furfuraceum* | 99.41% | ON611839 | No | White | *Thuja plicata, Tsuga heterophylla, Pinus monticola, Pinus koraiensis, Pinus sylvestris* | No |
| Yes | No | *Rhizochaete americana* | 88.15% | ON611840 | Yes | White | *Acer saccharum* | No |
| Yes | No | *Serpula himantioides* | 99.47% | ON611841 | Yes | Brown | *Salix, Picea, Fagus, Pseudotsuga menziesii, Alnus, Abies concolor, Abies magnifica* | No |
| Yes | No | *Serpula himantioides* | 99.34% | ON611842 | Yes | Brown | | |
| No | Yes | *Sistotrema brinkmannii* | 100.00% | ON611843 | No | Brown | *Salix, Pinus resinosa, Picea* | No |
| Yes | No | *Sistotrema brinkmannii* | 99.81% | ON611844 | No | Brown | | |
| No | Yes | *Sistotrema raduloides* | 99.27% | ON611845 | No | White | *Quercus, Tsuga heterophylla, Populus tremuloides, Betula* | No |
| No | No | *Sistotrema sp.* | 99.64% | ON611846 | No | White | | |
| Yes | No | *Trichaptum abietinum* | 99.22% | ON611847 | No | White | *Abies, Pseudotsuga menziesii, Picea, Larix, Pinus, Pinus sylvestris, Fagus* | Yes |
| Yes | Yes | *Xylobolus frustulatus* | 97.00% | ON611848 | No | White | *Quercus, Betula, Pseudotsuga menziesii, Liriodendron tulipifera* | No |

*Stand Treatment Results*

Species were separated into categories depending on whether they were pathogenic and their associated wood-rot type. Of the 39 species identified, most (5 or 13%) were non-pathogenic, brown-rot producing fungi found in uninfected, unharvested stands; the highest level (4 or 10%) of white-rot fungi occurred in stands that were uninfected and harvested and were species determined to be non-pathogenic (Table 3.4). No significant differences were found in the number of fungi present in the different stand treatments (Table 3.5).

**Table 3.4** The species identified were separated into categories describing the stand treatment in which they were collected and their mode of nutrition and associated rot type. The count of species in each category and their percentage of the total species count are reported.

| Mistletoe | Harvested | Pathogen | Rot Type | Count of Species | Percent of Total |
|---|---|---|---|---|---|
| Yes | No | No | Brown | 5 | 13% |
| No | No | Yes | Brown | 4 | 10% |
| No | Yes | No | White | 4 | 10% |
| Yes | No | Yes | Brown | 4 | 10% |
| Yes | No | No | White | 3 | 8% |
| Yes | Yes | No | Brown | 3 | 8% |
| Yes | Yes | No | White | 3 | 8% |
| Yes | Yes | Yes | Brown | 3 | 8% |
| No | No | No | White | 2 | 5% |
| No | No | Yes | White | 2 | 5% |
| No | Yes | No | Brown | 2 | 5% |
| Yes | No | Yes | White | 2 | 5% |
| Yes | Yes | Yes | White | 2 | 5% |

**Table 3.5** Treatment results of the Welch's two-tailed t-test of the count of species found between treatment types.

| | | t-statistic | p-value |
|---|---|---|---|
| Stands with Mistletoe | Stands Without Mistletoe | 3.16 | 0.16 |
| Harvested Stands | Unharvested Stands | -0.55 | 0.64 |
| Pathogen | Non-Pathogen | -0.06 | 0.95 |
| Brown-Rot | White-Rot | 1.78 | 0.11 |

## 4. Discussion

Fungal communities within black spruce ecosystems in the southern boreal forest are relatively unknown.  We observed 39 unique fungal species. All the Basidiomycota identified were of the Agaricomycotina, a subdivision containing about one-third of the fungal species known to Western science (Hibbett, 2006). Species within the Agaricomycotina are further divided into three classes: the Agaricomycetes, the Dacrymycetes, and the Tremellomycetes (Kües and Navarro-González, 2015). No species in the Tremellomycetes were found; however, 95% and 2.5% of the species in this study belonged to the Agaricomycetes and Dacrymycetes, respectively. Of the 25 species within the Agaricomycetes, the majority (52%) belonged to the Polyporales, an order containing almost 2,000 species. Many species within the Polyporales are significant wood-decay fungi of living and dead conifers (Hussein et al., 2018). The high presence of wood decay fungi suggests ample host substrate available for fungal colonization and to support an abundance of fungi, especially in both harvested and unharvested stands.

Several species (17%) were white-rot fungi that can colonize the heartwood of standing, live trees, such as *Porodaedalea pini* (Brot.) Murril. However, it was surprising that there was a lack of root-rot species identified from the samples collected. While there is little general information about what fungal species are commonly found in black spruce bogs, there is documentation of *Armillaria* (Kubiak et al., 2017; Thormann et al., 2001) and *Onnia tomentosa (*Fr.)  P. Karst. Furthermore, *O. tomentosa* is one of the most widespread root pathogens in North America, especially in the boreal and sub-boreal forests (Bernier and Lewis, 1998; Germain et al., 2009).

The absence of common root-rot species like *Armillaria* and *O. tomentosa* might be explained by the relatively low number of samples that produced viable cultures for sequencing, as this study was by no means meant to build an exhaustive list of the fungi found in the study system. While 120 samples were initially collected, only 40 isolates were obtained to sequence. Therefore, we cannot assume the fungi found in this study to be the only fungi associated with black spruce and EDM. Most samples were taken from dead and dying trees and stumps ranging in decomposition levels. The lack of root-rot fungi could also be due to: the samples collected had advanced decay status of the substrate and were no longer conducive to Armillaria growth, the presence of root-rot fungi in black spruce bogs is lower than was initially hypothesized, or bias could have skewed sample collection towards more decayed wood and wood that was more easily accessible.

Further surprising was the presence of species that have yet to be documented in North American black spruce bogs, due to the lack of research looking into fungal diversity in these systems, such as *Postia sericeomollis* (Romell) Jülich, *Xylobolus frustulatus* (Pers.) Boidin, *Irpex lacteus* (Fr.) Fr., and a species such as *Phlebia aurea* (Fr.) Nakasone that has no documentation in the literature of being on the North American Continent. *Postia. sericeomollis* is known to infect western red cedar (*Thuja plicata* [Donn ex D. Don]) and Alaskan yellow cedar (*Callitropsis nootkatensis* [(D. Don) Oerst. ex D.P. Little]). *I. lacteus* is commonly found on angiosperms (Novotný et al., 2000), however, has been recently found colonizing red pine stumps in Minnesota (Otto et al., 2021). At the same time, *X. frustulatus* is known to infect certain oak species in central and southern Minnesota (Otjen and Blanchette, 1984).

No significant differences were found between the fungal species characteristics (e.g., rot type) and the study stand treatment (e.g., unharvested). Again, this could, in part, be due to the number of samples taken or the substrate targeted when sampling. However, this is not to say that there is no link between fungi and a stand's harvest and EDM infection status. Pathogenic fungi might find entry to a black spruce tree due to its weakened defenses from battling an EDM infection; a commercial harvest could leave residual trees stressed and weakened due to harvesting damage or the sudden change in stand structure, allowing for fungi to more easily invade living tissue. Finally, the longevity of an unharvested stand may provide the downed woody debris and stand structure required for certain species of fungi to thrive; stands with a higher site class may give way to more fungal abundance or diversity.

There is limited literature on the management of black spruce infected with EDM, and information about how secondary disturbances may influence black spruce post EDM infection is scarce. Results from this study provide new information on the fungi associated with black spruce and EDM. While no significant differences were found between treatment types, the fungi found provides important new information about the fungal species that are potentially influencing black spruce stand dynamics and species composition. Future research should focus on how varying disturbance agents impact black spruce forest management, as well as continuing to build up the list of documented fungi present in black spruce bogs.

## 8. Appendix

**Table 3.6** The species identified were separated into categories describing the stand treatment in which they were collected. The count of species in each category and their percentage of the total species count are reported.

| | Species Count | Percent of Total | Average Per Stand | Standard Deviation | Standard Error |
|---|---|---|---|---|---|
| Harvested and Infected Stands | 11 | 28% | 2.75 | 0.5 | 0.22 |
| Unharvested and Infected Stands | 14 | 35% | 3.5 | 1.29 | 0.58 |
| Harvested and Uninfected Stands | 7 | 18% | 2.33 | 1.53 | 0.68 |
| Unharvested and Uninfected Stands | 8 | 20% | 2.67 | 1.15 | 0.52 |

**Table 3.7** The species identified were separated into categories describing the stand treatment in which they were collected and their mode of nutrition and associated rot type. The count of species in each category and their percentage of the total species count are reported.

| | Species Count | Percent of Total | Average Per Stand | Standard Deviation | Standard Error |
|---|---|---|---|---|---|
| Infected Stands | 25 | 63% | 3.13 | 0.99 | 0.44 |
| Uninfected Stands | 15 | 38% | 2.50 | 1.22 | 0.55 |
| Harvested | 18 | 45% | 2.57 | 0.98 | 0.44 |
| Unharvested | 22 | 55% | 3.14 | 1.21 | 0.54 |
| Pathogen | 17 | 43% | 2.83 | 0.98 | 0.44 |
| Non-Pathogen | 23 | 58% | 2.88 | 1.25 | 0.56 |

| | | | | | |
|---|---|---|---|---|---|
| Brown Rot Fungi | 21 | 53% | 3.50 | 1.05 | 0.47 |
| White Rot Fungi | 18 | 45% | 2.57 | 0.79 | 0.35 |
| Brown Rot Pathogen | 11 | 28% | 3.67 | 0.58 | 0.26 |
| Brown Rot Non-Pathogen | 10 | 25% | 3.33 | 1.53 | 0.68 |
| White Rot Pathogen | 6 | 15% | 2.00 | 0.00 | 0.00 |
| White Rot Non-Pathogen | 12 | 30% | 3.00 | 0.82 | 0.37 |

## Chapter 4: Conclusion

Black spruce is an essential species in Minnesota, comprising 9% of the forested lands in the state, but black spruce is also an important species in the broader Lakes States region of the US and Canada (Miles, 2017; Viereck and Johnston, 1990). There has been an increasing awareness of the lack of knowledge regarding black spruce management. While there has been more work on the biology of eastern dwarf mistletoe (*Arceuthobium pusillum* [Peck]) (EDM), there is much to learn regarding the interactions and influence of EDM on stand dynamics in black spruce forests.

EDM complicates management practices on a local and landscape level (Skay et al., 2021; Gray et al., 2021; Anderson et al., 2019; Baker and Knowles, 2004). EDM is difficult to detect as it has very small aerial plant bodies (Baker and French, 1980; Gray et al., 2021). Furthermore, the findings from this thesis show that it is sometimes not until after a harvest has been completed that EDM becomes visible along stand boundaries and in any residual overstory and advanced regeneration.

Historically, the main landscape disturbance agent in black spruce forests was fire (Heinselman, 1984). With the absence of fire in these systems, one can infer that EDM has now become the main disturbance agent, causing shifts in stand structure and species composition, creating gaps, and increasing stand heterogeneity. However, fire may soon return to black spruce systems as the boreal forest is predicted to become warmer and drier due to climate change, lowering the resilience of black spruce-dominated stands (Baltzar et al., 2021; Whitman et al., 2019; Hart et al., 2018). Furthermore, the warmer and drier climate is expected to decrease the growth rates of boreal black spruce, and if fires become too frequent, it has been predicted that there will be a lack of seed sources due to immature black spruce stands being less resistant to fire (Girardin et al., 2015; Baltzar et al., 2021; Whitman et al., 2019; Hart et., al 2018). Currently, work by Baltzar and colleagues (2021) shows that black spruce across boreal North America is moderately resistant to the adverse effects of climate change.

Increased resilience on a stand and landscape-level might be possible with the spruce-aspen mixed-wood system. In Saskatchewan, Canada, fire has become a more dominant feature on the landscape in recent decades, and Hart and colleagues (2018) have documented an increased resilience to fire in stands of mixed conifer and hardwoods as opposed to pure stands of spruce. Drobyshev and colleagues (2012) found that stands of aspen and black spruce mutually benefit one another and increase stand and landscape resiliency in the face of climate change, with black spruce favoring cooler and wetter periods and aspen favoring warmer and drier periods.

We may know relatively little regarding EDM compared to other forest pathogens in other systems. Still, we know even less about the fungi and fungal relationships that influence both black spruce and EDM. While root pathogens were not documented in this study, plenty of literature (Devkota and Hammerschmidt, 2020; Kubiak et al. 2017; Shaw and Kile, 1991; Thormann et al. 2001; Labbé et al., 2015) allows us to presume that fungal root pathogens are in black spruce bogs and, like EDM, cause mortality. There is much to be learned about how black spruce, EDM, and their associated fungi interact, and it is an exciting time for research regarding those organisms.

It is essential to understand and acknowledge that while EDM and certain fungi are forest pathogens, they are also native forest disturbances that have been shaping black spruce forests for millennia (Ostry and Nicholls, 1979). In the management of black spruce stands and landscapes, it is imperative now, more than ever, in the face of climate change, that managers consider their goals and objectives and how their implementation might alter the southern boreal landscape towards a more or less fire and climate-resistant future.

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