A field investigation of the environmental factors associated with explosive seed dispersal in the dwarf mistletoe *Arceuthobium americanum*

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Introduction

Dwarf mistletoes (DMs), genus *Arceuthobium*, are dioecious, flowering plants with a broad geographical distribution. More specifically, they are obligate aerial parasites that utilize members of *Pinaceae* and *Cupressaceae* for water, carbohydrates, and other nutrients, and are rooted to their host by an extensive endophytic system. On the host's surface, DM are characterized by reproductive shoots that are drastically reduced in size and, on the female plant, develop bicoloured fruits (Hawksworth and Wiens 1996). *Arceuthobium americanum*, a North American species of DM, is parasitic on lodgepole pine trees throughout British Columbia (Geils *et al*. 2002). Like almost all DMs, the seeds of *A. americanum* are dispersed through an explosive mechanism in which the rupture of mature fruit expels the seed from the parent plant. The sticky coating of viscin cells allows these seeds to adhere to the new host trees on which they land. This dispersal phenomenon occurs in the early fall, while germination of the new DM does not ensue until the following spring when light, temperature and moisture are suitable (Hawksworth and Wiens 1996, Geils *et al*. 2002). Several years of endophytic growth occur before reproductive shoots become apparent, and at least one to two more years of growth occur before flowering takes place. Following pollination via wind or insects, another year elapses before the fruit mature and explode in the early fall (Geils *et al*. 2002). Although their prolonged lifecycle does not allow for rapid forest infestations, DM's dependence on its host detrimentally affects forest productivity. Infections decrease the quality of host wood, weaken the immunity of the trees against other diseases, and ultimately shorten the lifespan of the trees (deBruyn *et al*. 2015). Furthermore, the branches of the trees infected by the DM become dry and brittle, which poses a major forest fire hazard. Forestry industries suffer extensive losses due to the decreased wood quality resulting from DM infections (Geils *et al*. 2002). Increasing our understanding of how the DM disperse could give foresters insights into controlling these independently dispersing parasites. The purpose of this study was to investigate whether there are any weather variables, such as temperature and humidity, associated with the seed dispersal mechanisms of lodgepole pine DM, and comprised three main objectives. The first objective was to determine the weather pattern of the field site by recording environmental variables using a weather station. The second objective involved tracking the pattern of fruit loss over a chosen set of sample DMs, determined by analysis of daily photographs of 11 samples and time lapse footage of one sample, from which light-intensity information was also obtained. Lastly, the data resulting from the first two objectives were combined to accomplish the last objective; namely, to determine if there are significant relationships between fluctuations and trends in the weather variables, and the occurrence of fruit explosions throughout the dispersal period.

Materials and methods

Field preparation

The field site for this study is located in the Stake Lake region of BC (50° 30′ latitude, 120° 29′ longitude), about 20 km southwest of Kamloops. It is a quiet, secluded area just off the Lac Le Jeune road that experiences no traffic disturbances and few pedestrians. On the first preparatory field day (August 22, 2016), I selected 12 DM female inflorescences bearing maturing fruit (i.e., "fruit clusters") on host trees all located in relatively close proximity to each other. The localized nature of the site is important, as it will ensure that the data logged from the weather station accurately represents the environmental conditions experienced by each fruit cluster. The host branches associated with each DM sample were marked with flagging tape and labelled with letters A through L. The GPS coordinates of each fruit cluster were recorded and averaged (n=3) using a *Garmin etrex10* GPS device. One of the 12 samples was chosen to be the subject of time lapse data collection. A *Brinno* time lapse camera in a waterproof case was positioned approximately 2 feet from this sample using an adjustable metal tripod fastened to the trunk of the host tree. The positioning of the camera was optimized for both light capture and ideal orientation of the sample fruit cluster. A *TP1080WC ProWeather* station was secured to a post that was located centrally to all samples. The associated wireless datalogger was stored nearby in an insulated bag where it was protected from the elements but still able to detect and log the variables recorded from the weather station. The weather station began recording immediately after set up and continued recording for the remainder of the dispersal period, providing the data necessary for the completion of objective one in this study.

Data collection

For 22 consecutive days, August 22 to September 12, a photo of each of the 12 sample plants was taken. It was ensured that the angle of each photo was as similar as possible to the previous photo to enable quick visual comparisons over the plant's dispersal period. The time lapse recording and weather data were also uploaded daily to a laptop for backup purposes, although recordings from both devices were cumulative throughout the duration of the study. The weather station logged recordings of the following variables in 5 minute intervals: temperature, wind chill, relative humidity, hourly/daily/weekly/monthly rainfall, dew point, wind speed and direction, gust levels and atmospheric pressure.

Data analysis

Analyzing weather patterns

Upon upload of the weather data from the datalogger, the information was displayed on the computer using the *EasyWeather* software program. Due to complications with the datalogger adjusting to the colder overnight temperatures, a few series of data were not retained during the collection period. The recordings from the Ministry of Transportation and Infrastructure's Walloper weather station (21091) were obtained and the temperature and humidity data from this station were compared to the weather data collected in this study over the same time period. Two-tailed T-tests found the two data sets were significantly different, so the data from this weather station was used to fill in the missing sections of data lost in the present study. The data sets with the five-minute interval recordings obtained from the weather station were reduced to hourly intervals to correlate with the substitute data and simplify the graphing procedure. The weather variables such as temperature, humidity, and rainfall were then graphed using Excel software to visualize the weather patterns over the collection period and outline any major trends or fluctuations. This analysis marked the completion of objective one.

Determining fruit loss per day

Daily photo "stills"

For each sample fruit cluster, the daily photos taken were lined up consecutively in a photo editing software. The initial number of mature fruit in the first photo were counted and marked off using a drawing tool. In the following photo, the corresponding fruit that were still present were counted. The difference between these two values was thus representative of the fruit lost during the time period between the two photos. This step-by-step counting process was repeated until the given sample showed zero fruit from that angle. This procedure was then repeated for the remaining 10 samples. (The plant that was photographed continuously by the time lapse camera was not photographed daily). This analysis represents partial completion of objective two, as it illustrated the time periods when the majority of seeds were dispersed, and thus gave a good starting point for examining the time lapse footage.

Time lapse and light intensity

In total, the time lapse footage comprised 22.5 MB of data. Initially the images were captured every 2 minutes from 9 am to 5 pm (Aug 22 – 24). The range was then increased from 8 am to 6 pm and the rate was increased to 30 second intervals (Aug 25 – Aug 30). As the fruit cluster began notably diminishing, the range and rate were increased to an 8 pm endpoint and 15 second intervals, respectively (Aug 31 – Sept 11). All time lapse footage was analyzed using ImageJ software. Using this program, each individual frame that comprised each video could be viewed separately and consecutively. Viewing the first and last frames in sequence allowed us to determine which exact fruit were lost at some point along the duration of the video. The remaining bulk of the video was then viewed repeatedly to pinpoint the exact frames where each fruit was last present and when it disappeared, marking the full completion of objective two. These times were then used in graphical analyses to determine if correlations were present with the weather variables recorded at 5 minute intervals. Furthermore, the light intensity of each frame of time lapse footage was measured using ImageJ software, providing another variable to test against explosion events.

Graphical and statistical analyses

Using excel software, the average daily explosions from the 11 samples were plotted against average weather variables such as temperature, change in temperature and humidity (Figures 1 and 2). Statistical analyses on this data are yet to be conducted. Excel software was also used to plot the time lapse explosions against the weather variables recorded at 5 minute intervals (Figure 3 and 4), as well as the light intensity recordings measured from each frame of the footage (Figure 5). This is the process through which objective three will be accomplished following the completion of pending statistical analyses.

Results

Daily photos

The main purpose of this work was to determine if the total number of explosions each day correlated with the average weather variables for that same time period. The cumulative fruit loss between the 11 samples (not including time lapse) were totalled for each time period between daily photos. This explosion number was then plotted against average temperature and max change in temperature for that time period (Figure 1). The explosion number was also plotted against average relative humidity for that time period (Figure 2).

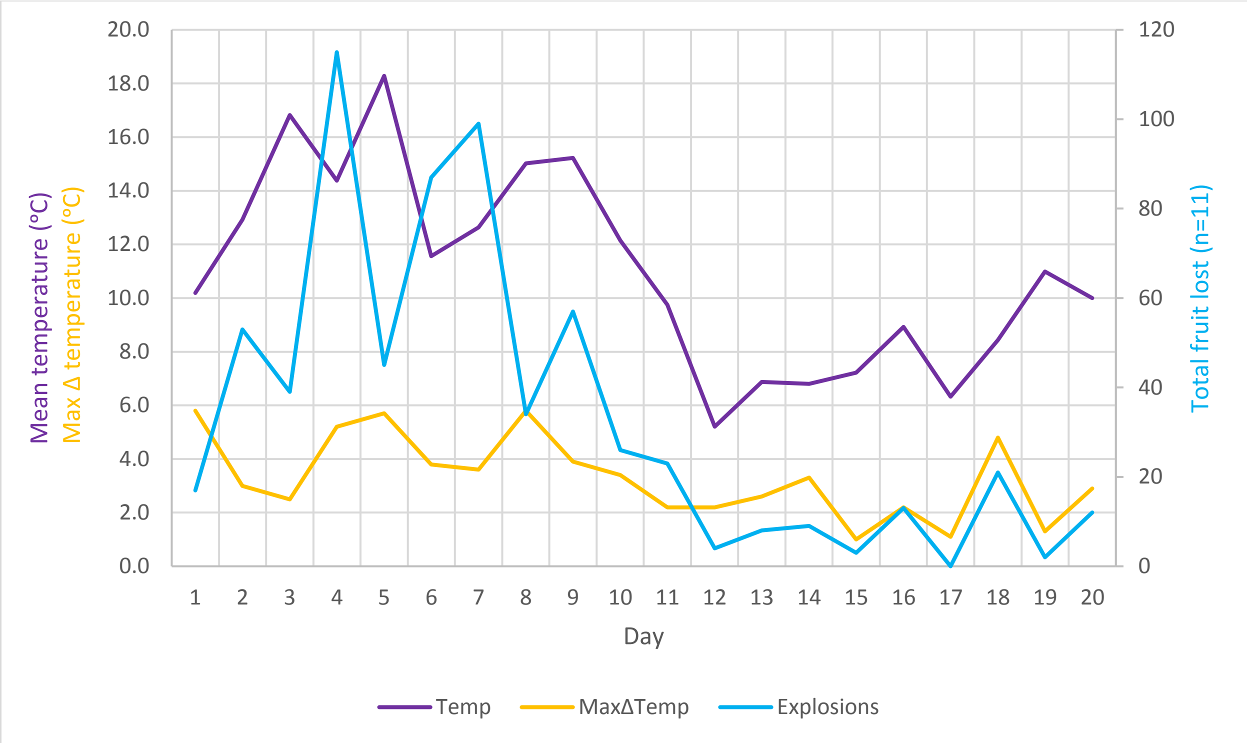

Figure 1. Total fruit loss (blue) plotted against average daily temperature (purple) and max change in temperature (orange) over the 20-day explosion period.

There appears to be a positive correlation between both mean temperature and total explosions. Majority of the seeds dispersed in days 1 through 10 when temperatures were highest. For the remaining days, mean temperature, max change in temperature, and total explosions are all much lower. Regression analysis of mean temperature and total explosions gave an R^2 value of 0.368. Further statistical analyses are yet to be conducted.

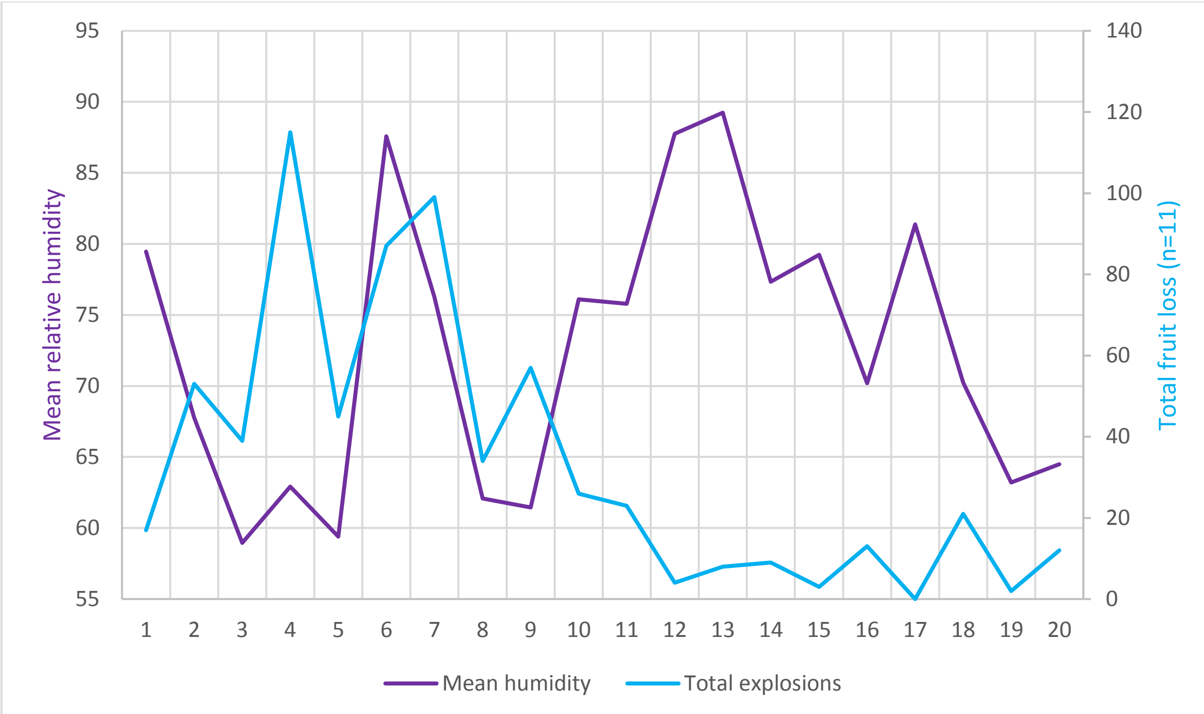

Figure 2. Total fruit loss (blue) plotted against average relative humidity (purple) over the 20-day explosion period.

There appears to be a negative correlation between total explosions and average relative humidity. Apart from one spike on day 6, relative humidity was quite low during the first 10 days when majority of fruit exploded. For the remaining days when fewer fruit were lost, relative humidity was quite high in comparison.

Time lapse and light intensity

The second goal of this work was to use the time lapse footage to pinpoint exact times when fruit were lost, and correlate these points with fluctuations in the weather patterns. Throughout the dispersal period, a total of 68 explosions were pinpointed within the videos. These explosions were plotted as single points (purple) along the temperature trend (Figure 3), with the explosion point being represented by the frame where the fruit was first missing. The same was done with the humidity recordings (Figure 4). Lastly, the explosions were plotted against changes in light intensity (Figure 5). Using ImageJ software, the light intensity of each frame in the video footage was determined. The light intensity throughout the videos were then assembled into a line graph, against which the explosions were plotted. In this case, each explosion is represented by two points: the frame when the fruit was last present, and the next frame where the fruit had disappeared. Four explosions occurring across increasing light intensities are included here as an example (Figure 5); however, all 68 explosions were plotted in the same format.

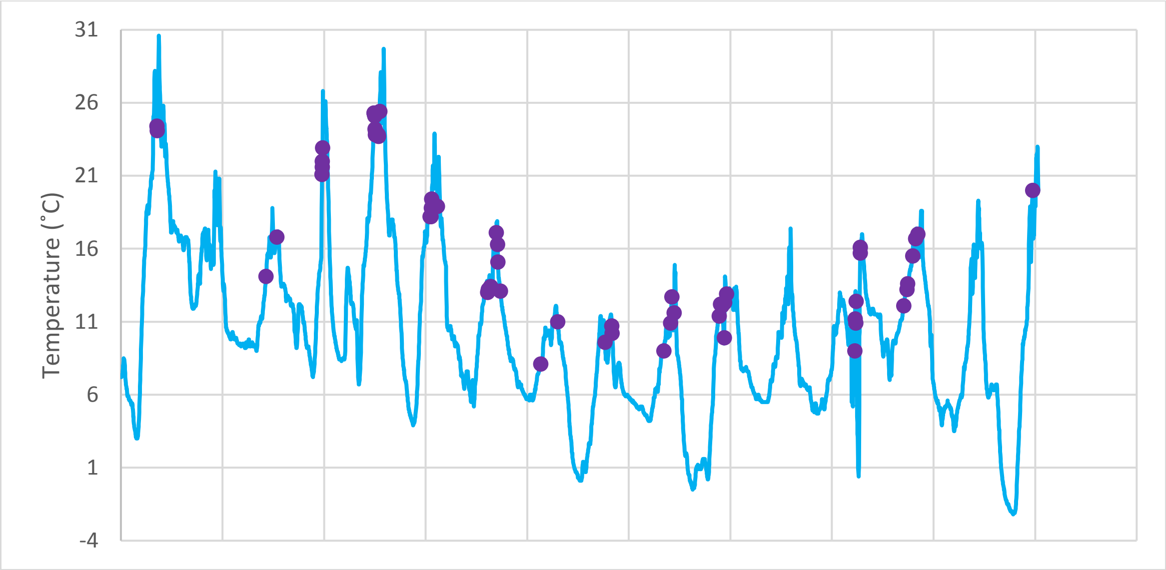

Figure 3. The 68 time lapse explosions (purple dots) plotted against temperature throughout the dispersal period. Each point represents the frame where a fruit first disappeared.

The majority of the explosions appear to be occurring when temperatures are highest. More specifically, most of the explosions seem to be occurring across increasing temperatures. No statistical analyses have been conducted on this data as of yet.

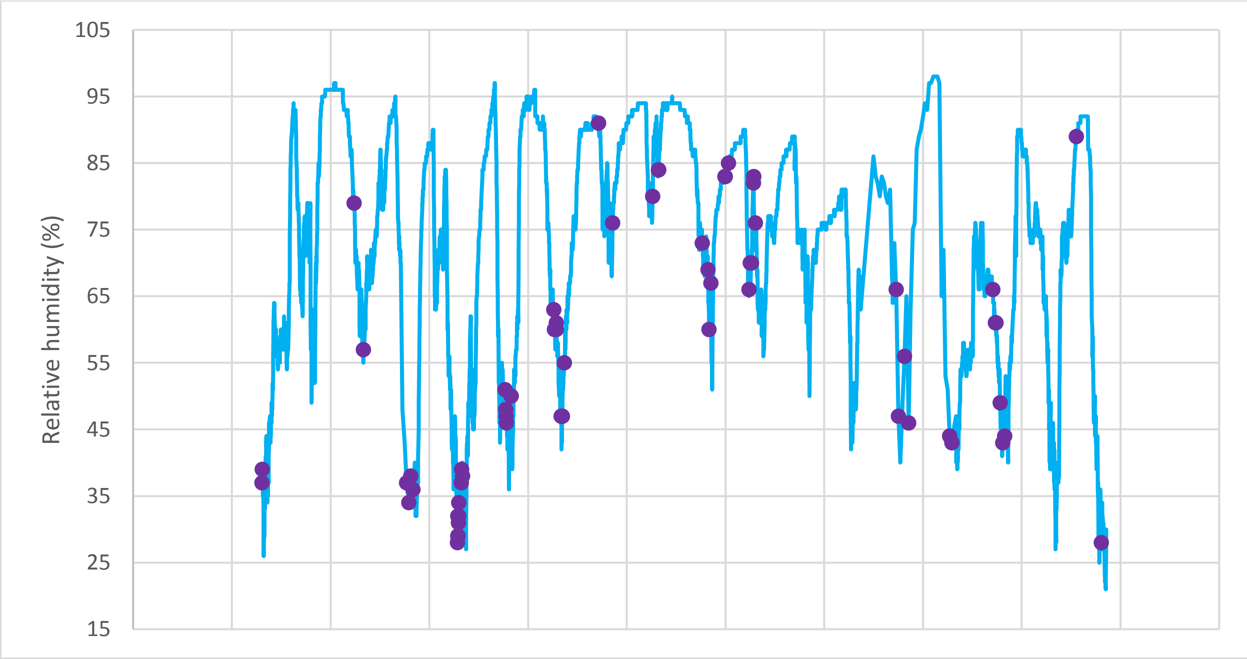

Figure 4. The 68 time lapse explosions (purple dots) plotted against humidity throughout the dispersal period. Each point represents the frame where a fruit first disappeared.

Explosions occur at varying levels of relative humidity. There does not appear to be a trend as to whether they occur across increasing or decreasing levels of humidity. Again, no statistical analyses have been conducted on this as of yet.

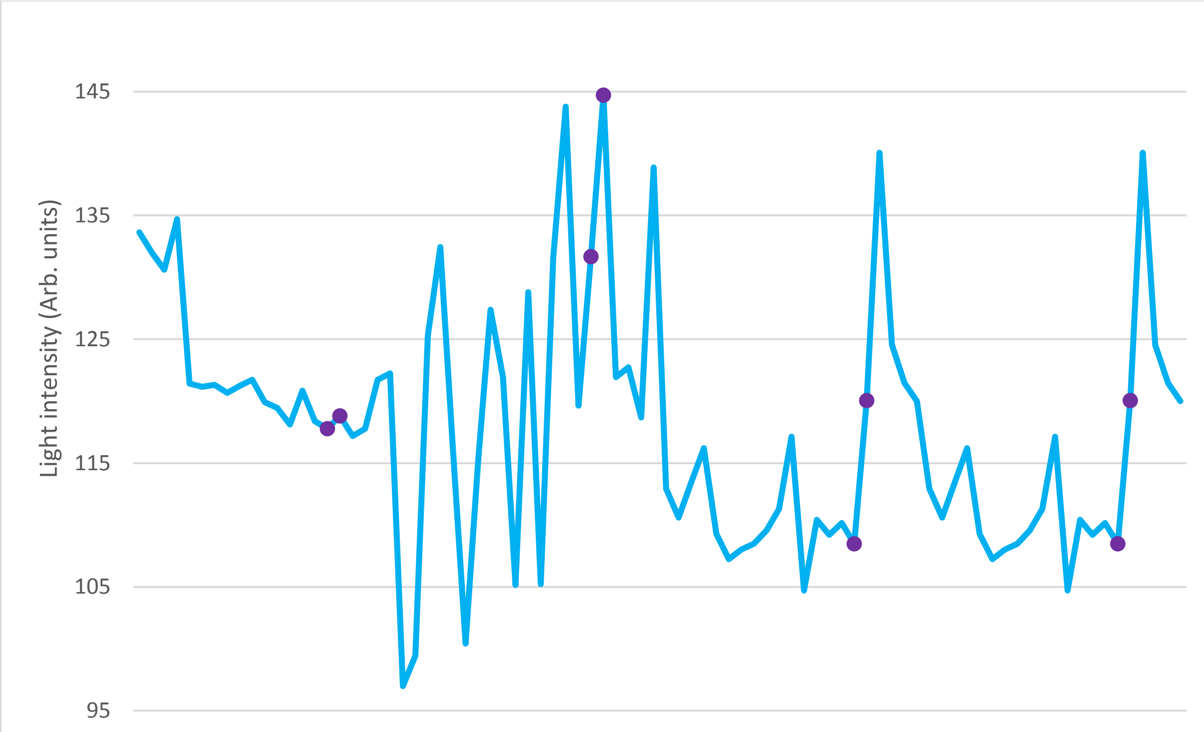

Figure 5. A sample graph showing four of the 68 explosions plotted across light intensity recorded every 20 seconds. Each explosion is represented by a pair of purple dots, one for the last frame where the fruit was present and one for the next frame where the fruit was missing.

These four sample explosions are showing dispersal events occurring across increasing light intensities. When all 68 explosions were analyzed together, 51.5% of the explosions occurred across increases in light intensity. Decreases in light intensity were evident in 38.2% of explosions, and the remaining 10.3% of explosions occurred across unchanged light intensities.

Discussion

The parasitic lodgepole pine DM is detrimental to forest productivity in BC (DeBruyn *et al*. 2015). The seeds of the DM are dispersed through an explosive mechanism in the late summer/early fall following the maturation of fruit on the female plant (Hawksworth and Wiens 1996, Geils *et al*. 2002). This study was conducted to determine if there are any weather variables associated with the dispersal events of this DM.

The three objectives included (1) determining the weather patterns of the field site under study; (2) pinpointing time periods when majority of explosions occur; and (3) seeing if these two variables demonstrate any correlation. From the period of August 22nd, 2016 to September 12th, 2016, weather variables such as temperature, humidity, wind, and rainfall were recorded every 5 minutes. Twelve sample plants were monitored for fruit loss via both daily photographs and time lapse footage.

Although statistical analyses are yet to be conducted, preliminary results suggest that temperature has the strongest influence on the DM seed dispersal events. Average daily temperatures and max daily change in temperature show a positive correlation with total fruit loss from 11 sample plants (Figure 1). When looking at specific explosions captures on the time lapse footage, it appears that all 68 explosions occurred during a time when temperatures were highest (Figure 3). Lastly, the majority of these explosions seem to be occurring across increases in temperature, supporting the finding that these dispersal events are likely to be correlated with a change in temperature.

A study conducted by deBruyn *et al*. in 2015 discovered that endogenous heat production, referred to as thermogenesis, is associated with seed dispersal in this species of DM. During their work in developing this theory, they found that ambient heating in the laboratory triggered the explosion of the fruit by initiating the thermogenic process that initiates these dispersal events (deBruyn *et al*. 2015). Furthermore, germination rates of DM are known to increase in the spring as temperatures rise (Hawksworth and Wiens 1996). Knowing that the DM are capable of responding to this variable later in their lifecycle further supports the findings of this study. The evidence presented here strongly suggests that temperature is involved in the DM seed dispersal process.

The relationship between seed dispersal and humidity is not confirmed at this point. Daily humidity averages suggest a negative correlation (Figure 2), but there are approximately four conflicting data points that cannot be explained by this trend. The 68 explosions captured on the time lapse occur across the entire range of humidity, and do not appear to favour increases or decreases – although statistical analyses of this remain to be conducted. However, when considering the mechanism through which DM disperse their seeds, it is not doubtful that humidity may be involved. Upon maturation of the fruit, the pedicel supporting the fruit elongates, and the fruit's internal hydrostatic pressure increases. Eventually, a contraction occurs, which causes the fruit to explode, launching the single seed into flight (Geils *et al.* 2002). The association of hydrostatic contractions to propel seeds from mature fruit implies that humidity of the air around the fruit may also play a role in triggering DM seed discharge (Hawksworth and Wiens 1996). Looking at another stage of the DM life cycle, response to water seems inconclusive. Some species of DM show enhanced embryonic development with increased water availability, whereas others seem to grow independently of humidity conditions (Hawksworth and Wiens 1996).

DM are exposed to a variety of light intensities throughout the day, and previous work has shown the plants to be receptive to these changes. These responses included increased germination rates in the spring when light intensity is higher than winter month, as well as poliferationof DM upon thinning of the overhead canopy (i.e., tree removal) that allows more light to reach the forest floor (Geils *et al.* 2002, Hawksworth and Wiens 1996). The analysis of light intensity in this work involved only the time lapse sample. Light intensity measurements in each frame of the time lapse footage were determined using computer software. For each of the 68 dispersal events captured, 5 minutes of this light intensity data was graphed prior to the point which represented the explosion. The necessary amount of lead-up time was inferred from the findings of deBruyn *et al*. (2015) who found the response time of the DM to be around 2-3 minutes. Upon analyzing these graphs, it was noted that over half of the explosions occurred across increasing light intensities. It is unknown at this point whether this is significant, or if the sunlight association is a reflection of the relationship between seed dispersal and temperature. Correlating these changes in light intensity with temperature fluctuations may not be possible with this data set – the location of the weather station and the heavy foliage around the time lapse sample may prevent this interpretation from being accurate, but will be attempted anyway. It has been noted previously that DM growth is suppressed in low light conditions and promoted in conditions where the amount of light is increased (Geils *et al.* 2002). The dynamic response of DM to changes in light intensity implies that they adaptively respond to sun exposure; with this in mind, it is not unreasonable to suggest that light intensity might be a particularly important factor in the discharge process.

Due to the many combination of environmental factors acting upon DM simultaneously, it will be challenging to isolate a single variable that is correlated with explosive seed discharge. Because this study is taking place in the natural environment of DM, many variables will be observed at once. However, it is important to point out that studying the DM in their natural habitat prevents the potential of artificial results that can be generated in a laboratory setting. Furthermore, it is very possible that the trigger or associated variable we are trying to discover is a not single event, but more likely a combination of a few weather variables.

Conclusion and future directions

Preliminary results of this study suggest that seed dispersal in the lodgepole pine DM is positively correlated with temperature, negatively correlated with humidity, and associated with increases in light intensity. The rest of this study will involve completion of statistical analyses to determine significance of these relationships. This area of data analysis will mainly be focused on generalized linear models and linear mixed models. Further work in this project could include repetition of the study for solidification and verification of the findings. Eventually, these results could be combined with a physiological study that could lead to the development of a herbicide or some other preventative measure that targets the DM specifically and does not harm the host trees.

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