THE EFFECT OF *BACILLUS THURINGIENSIS* SEROTYPE *KURSTAKI* (*BTK*) AS AN INSECTICIDE ON JACK PINE BUDWORM IN THE 2019 NORTHERN ONTARIO SPRAY PROGRAM IN COMPARISON TO HISTORICAL USE

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ABSTRACT

Holmlund, K.A. 2020. The effect of *Bacillus thuringiensis* serotype *kurstaki* (*Btk*) as an insecticide on jack pine budworm in the 2019 Northern Ontario spray program in comparison to historical Use. 58pp.

Bacillus thuringiensis (*Bt*) has been used historically as an insecticide by the Ontario Ministry of Natural Resources and Forestry (OMNRF) to suppress jack pine budworm populations in dense outbreaks. In some previous spray programs the methods used to determine spray initiation and calculate success of the spray program was not performed by OMNRF resource technicians, but rather had been contracted out to external workers. 2019 was the first year since the 1990's that OMNRF resource technicians based out of Thunder Bay Ontario performed on site observations to track spray initiation and success. A comparison of the 2019 operation with previous efforts showed the effectiveness of *Btk* as a jack pine budworm suppression, contrasting to previous efforts on historical outbreaks. The methodology followed was provided and carried out by the OMNRF forest health program. The results showed that the effectiveness of *Btk* as a biological insecticide has not decreased over the time it has been used in Ontario. The change of internally performing the development and assessment of the spray led to the 2019 spray program as a success alongside previous spray programs in the province.

Key Words: jack pine (*Pinus banksiana*), budworm, *Bacillus thuringiensis* seryotype *kurstaki*, *Bt*k, insecticide, Northwestern Ontario.

CONTENTS

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I would like to thank the Jack Pine Budworm Forest Health Terrestrial Technicians crew who conducted larval development surveys, pre and post spray observations, and laboratory procedures. Additionally, the project head Dan Rowlinson for organizing the methodologies and communicating with the crew, affected communities, and spray pilots. The Senior Terrestrial Technician Vance Boudreau for managing the crew, participating in field procedures, and providing guidance and revision of this thesis. The Forest Health Technicians that operated the core forest health operations contributing to the data of pheromone traps and aerial mapping. Dr. Don Henne, entomologist in the Faculty of Natural Resources and Forestry at Lakehead University for supervising the production of this thesis through the writing and editing process.

INTRODUCTION

This thesis focuses on one of many naturally occurring insect defoliators that are actively part of Canada's native forest disturbance cycles: in Eastern Canada the eastern hemlock looper, and locally in Northern Ontario, predominantly forest tent caterpillar, and spruce and jack pine budworms. These species have overlapping ranges and outbreak cycles spreading across extensive areas of the boreal forest. From 1984 to 1994 annual defoliation caused by these insects in Canadian forests was just shy of 1/3rd the volume of annual harvest (Hardy 1995).

Jack pine budworm (JPBW) (*Choristoneura pinus pinus* Freeman, Lepidoptera: Tortricidae) is one defoliator species that has had cyclic outbreaks in Ontario for many decades. The OMNR 2018 annual report once again identified JPBW as a current major forest insect causing disturbance to jack pine in the Northwest region of Ontario. JPBW relies on jack pine (*Pinus banksiana*) as a host species, and occasionally scots pine (*Pinus sylvestris*) as a secondary host. In 2018, 870 ha of Jack pine mortality was attributed to JPBW, occurring within 627,455 ha of moderate to severe defoliation (OMNRF 2018).

JPBW is similar to another common defoliator, eastern spruce budworm (*Choristoneura fumiferana)*. Until 1953 they were not recognized as separate species (Canada 2015). The two can be differentiated by differences in cyclic outbreak behaviour and their hosts; spruce budworm has a longer outbreak cycle and primarily defoliates spruce (*Picea glauca*) and balsam fir (*Abies balsamea*) (OMNRF 2018).

JPBW tends to occur in central Canada; Ontario, Manitoba and Saskatchewan. In the spring larvae emerge from the bark where they overwinter, and immediately feed and develop on new shoots and flowers. In mid summer full-grown larvae pupate in the crown and then emerge into adult moths by August. Eggs are laid on host trees as well, and through late summer to early fall, newly-emerged larvae retreat under bark to wait for spring (Canada 2015). The insect tends to outbreak into large areas, and naturally decline after a few years; outbreaks tend to occur on an 8-10 year cycle. The last large outbreak in Northwestern Ontario occurred in 2009 (OMNRF 2009).

Annual tracking of JPBW presence across forest health plot locations across all of northern Ontario have been observed since the mid 1990's. Health plots are recorded to address overall health and flower production. In 2018, 107 plots were observed, and 81 of those locations also contained pheromone traps to specifically attract and count adult male JPBW (OMNRF 2018).

Bacillus thuringiensis (*Bt*) has been used as a control insecticide for JPBW and is effective on other lepidoptera species. JPBW control spray programs have occurred in Ontario since 1968, however since 1985 *Bt* has been the only approved treatment used on crown land. The use of a single deterrent poses the risk of treatment being less successful over time and with repeated use (Meating 1994).

OBJECTIVE & HYPOTHESIS

As a result of JPBW damage incurred in 2018, a 2019 control spray program occurred in the Northwest region of Ontario through the Ministry of Natural Resources and Forestry. The goal of the project was to protect the most highly valued stands that fall under the procedural requirements of spraying *Bacillus thuringiensis* serotype *kurstaki*, and maintain those stands in relatively good health until the outbreak runs its course.

The 2019 spray program was performed through field and lab studies, and spray procedures were followed by the terrestrial technicians operating out of the Northwest Biodiversity and Monitoring Unit in Rosslyn, Ontario. The results of the program and its procedures were compared to the 2009 and other historical spray operational programs, observing the current effectiveness of *Btk* on the JPBW, as a contrast to previous efforts. Any changes in the effectiveness of the substance over time were quantified. Spray operations were previously contracted out to external workers. The 2019 spray program was the first operation since the 1990's where timing and assessment of the spray was conducted entirely by ministry employees.

Observations were collected from a plot network of 52 locations across the region of disturbance; from Dryden into Red Lake district, covering north towards Pikangikum First Nation. These included both areas that were aerially sprayed and control plots for comparative study.

The 2019 effort was expected to be, at minimum, equally successful as previous operations within the province. This is expected since *Btk* has been widely used, and the

province has treated similar outbreaks in the past with *Btk*; the 2009 outbreak occurred in essentially the identical area as the current outbreak (OMNRF 2009). Due to the JPBW's natural occurrence, the spray program's goal was not to eliminate the pest, but to slow its rate of spread and reduce the level of defoliation. Thus, success was measured by considering changes in defoliation, in contrast to simply causing a JPBW population decline. The results may also differ due to procedural changes inevitably caused by methods being carried out by terrestrial technicians of the OMNRF rather than external contracts.

LITERATURE REVIEW

JACK PINE BUDWORM BIOLOGY & POPULATION ECOLOGY

Jack Pine (*Pinus banksiana*)

JPBW is a member of the insect order Lepidoptera (butterflies and moths); it is native to North America and defoliates its host tree, jack pine (*Pinus banksiana*) (Nealis 1995). Although JPBW can depend on some other *Pinus* species, jack pine is the preferred and nearly exclusive host (Howse & and Meating 1995). Due to the relationship between JPBW and jack pine, to understand the biology and population ecology of JPBW, one must look at the ecology of the jack pine itself. Many studies have observed the relationship of jack pine and natural disturbances, such as armillaria root disease and fire, subsequently linking them to the population dynamics of both JPBW and jack pine (Nealis and Lomic 1994; Mallett 1995; McCullough 2000; Nealis et. al. 2003).

Jack pine is a heavily harvested tree in Ontario, accounting for 12.3% of growing stock in the province and 30% of annual harvest (Howse and Meating 1995; Meating et. al. 1995; Scarr 1995). Jack pine is found across the southern extent of the boreal forest extending throughout the rest of the boreal forest in North America (Mallet 1995; McCullough 2000). Jack pine has adapted to forest fire as a natural disturbance and relies on the cyclic nature of forest fires to promote regeneration with their serotinous cones that open in response to high heat (Mallett 1995; McCullough 2000). Severe JPBW outbreaks leave an extensive amount of fuel in the form of dry and dead standing trees that promote the ignition of forest fires (McCullough 2000). Thus, throughout this

thesis the initiation of natural disturbance cycles that promote natural forest ecology will be acknowledged.

Jack Pine Budworm (*Choristoneura pinus pinus* Freeman)

Until 1953, JPBW was not distinguished from eastern spruce budworm (*Choristoneura fumiferana*), another common insect defoliator in the boreal forest (Nealis 1995). The species of *Choristoneura* are mainly identifiable by their host-specific tendencies and therefore biogeographic isolations presented by host species range (Nealis 1995). The JPBW and spruce budworm are very similar in distribution, the separation of the species was proven and named by Freeman (1953); the main differences include outbreak population cycles and host species; spruce budworm mainly defoliates *Abies* and *Picea* species (Nealis 1995). Additionally, the two species are limited by differences in sex pheromones, preventing the hybridization of the species (Weatherston et. al. 1971).

JPBW begin as egg clusters from which emerge larvae that pass through seven larval stages (instars), and finally pupate and adult moths emerge that breed (Lejeune 1950; Nealis 1995; Nat. Resour. Can. 2015). Eggs are laid in July-August on needle clusters, they hatch after two weeks and the hatchlings seek overwintering shelter in their first instar larval stage (L1) (Nealis 1995; Nat. Resour. Can. 2015). In the early spring, the larvae emerge from overwintering in their second instar larval stage (L2) and begin to make their way into the fresh shoots and developing flowers (Nealis & Lomic 1994; Nealis 1995; Nat. Resour. Can. 2015). Once emerged as L2 and feeding, larvae develop through the remainder of larval instar stages (L2-L7) moving toward foliage (preferring current year foliage) defoliating it before pupation in mid-July; after which

the adults emerge and begin the cycle again (Lejeune 1950; Nealis 1995; Nat. Resour. Can. 2015).

Jack Pine Budworm Population Dynamics

JPBW outbreaks tend to occur every 6-12 years (Volney 1988; Nealis and Lomic 1994; Nat. Resour. Can. 2015). The population dynamics typically follow a low background (endemic) population creating minimal defoliation, followed by a sudden dramatic increase in population, which causes major defoliation for 2-4 years followed by a mirrored dramatic population collapse to the low background population (Volney 1988; Nealis and Lomic 1994; Nealis 1995; McCullough 2000; Nealis et. al. 2003; Nat. Resour. Can. 2015). The population dynamics of JPBW are not fully understood and are commonly related to studies that have been done on spruce budworm (Nealis and Lomic 1994, Nealis 1995).

The predominant research on the JPBW population collapse has been on the flower presence response of the jack pine trees. JPBW larvae typically consume the available pollen cones (flowers) in their early larval stages (up to L3), before moving to other foliage (Nealis and Lomic 1994; Nealis 1995; McCullough 2003; Nealis et. al. 2003; Nat. Resour. Can. 2015). The relationship between pollen cones and JPBW had been studied before it was officially identified as a species distinct from the spruce budworm (Lejeune 1950). In multiple published works on the JPBW's density dependence of jack pine flowers for early larvae survival, population collapse was linked to the severe stress response of jack pine to stop producing an abundance of

flowers following the beginning of an outbreak and high defoliation. The lack of flowers for young larvae to feed on is therefore thought to influence a population collapse (Nealis and Lomic 1994; Nealis 1995; Nealis et. al. 2003). Secondly, parasitoids occurring in larvae have also been linked to a population collapse in combination with a pollen cone dependency (Nealis 1995; McCullough 2000).

JPBW outbreaks have been associated with other biotic forest disturbances such as armillaria root disease (Mallet 1995). After JPBW (or other insect disturbances) begin to put stress on a tree, armillaria root disease can more easily infect a tree. The compiled disturbance of defoliation and root disease magnifies mortality during JPBW outbreak (Mallet 1995).

HISTORICAL JACK PINE BUDWORM OUTBREAKS & CONTROL METHODS IN ONTARIO

Historical Outbreaks of Jack Pine Budworm

This thesis will focus on JPBW in Ontario; however, it is important to note that defoliation by JPBW is widespread through the range of the boreal forest (Mallet 1995; McCullough 2000).

Within the province of Ontario, The Ontario Ministry of Natural Resources and Forestry recognises four different regions defined by locality (Howse and Meating 1995). The Southern and Central portions are named as such, and the Northern portion of the province is divided into the Northwest and Northeast (Howse and Meating 1995). This thesis has a specific focus on the Northwest region.

As presented in the 1995 symposium, with information collected by the Forest Insect and Disease Survey Unit in Ontario, after an outbreak that peaked in the late 1960's at around 2.2 million ha, during the early 1970's to early 1980's no severe outbreak of JPBW occurred (Howse and Meating 1995). The next outbreak peaked in 1985, with over 3.5 million ha of defoliation across the Northwest and Northeast collapsing by 1990 (Howse and Meating 1995). After this, the next outbreak began in 2008 (OMNRF 2009).

Aerial Control Operations

JPBW outbreaks threaten the optimal growth and survival of jack pine (Hopkin and Howse 1995). Since jack pine is considered a high value tree in Ontario, along with black spruce (*Picea mariana*), the protection of jack pine health and production is important to the OMNRF (Ontario Ministry of Natural Resources and Forestry) (Hopkin and Howse 1995; Howse and Meating 1995; Meating et. al. 1995; Scarr 1995). As a result, the OMNRF has performed control programs against JPBW since the outbreak in the 1960's to protect high value stands and prevent excessive forest fire (Meating et. al. 1995).

Outbreak and control operations were summarised and presented by Meating et. al. in the 1995 symposium on JPBW biology and management by Natural Resources Canada and the Canadian Forest Service. Control programs involve the aerial application of insecticides across selected infested jack pine stands (Meating et. al. 1995). During the 1960's outbreak, fenitrothion was applied to selected regions with infested jack pine (Meating et. al. 1995).

The next outbreak occurred in the mid to late 1980's. This outbreak was more severe, peaking at over 3.5 million ha. At this time aerial control operations took place over 840,597 ha of infested jack pine, at this time *Bacillus thuringiensis* (a biological insecticide) was introduced as the only approved product to treat JPBW outbreaks (Meating et. al. 1995).

A smaller-scale outbreak was detected in the mid-1990's and an aerial operational program took place at that time, effectively controlling the outbreak (Meating et. al. 1995). This was the first-time over-wintering surveys indicating future defoliation were considered to be a part of control programs, as opposed to only trees subjected to previous defoliation (Meating et. al 1995).

Preceding the 1995 symposium, suggestion of the development of more than one control method for the future was proposed. However, *Bt* (*Bacillus thuringiensis*) was, and still is, the method of choice, including use in the 2019 spray program to be studied in this thesis. *Bt* is the only method approved for this type of use (Meating et. al 1995; OMNRF 2009).

The last aerial control operation that occurred before the current one was in 2009 (OMNRF 2009; OMNRF 2018). The spray occurred in the Northwest region after 135,000 ha of defoliation was detected in 2008 and predicted to substantially increase (OMNRF 2009). *Btk* (*Bacillus thuringiensis* var. *kurstaki*) was applied to 58,146 ha, mostly in the Red Lake area of the Northwest region (BioForest 2010).

In collaboration with the OMNRF, BioForest Technologies was responsible for the timing and assessment of the spray program. The Canadian Forest Service

technology "BIOSIM" software used historical population data, and weather data to predict the emergence date of L2 larvae and assist in determining the aerial application date (Regniere et. al. 1995). New foliage and larval development were observed in preparation for proper application, and pre-spray and post-spray observations were collected across 57 spray plots and 18 control plots to determine success of the spray. The results demonstrated a successful spray program, as defoliation was significantly decreased across all areas of the aerial operation (BioForest 2010).

A LOOK AT *BTK* (*BACILLUS THURINGIENSIS* VAR. *KURSTAKI*)

Insecticidal application has been used across Canada as a successful suppression of forest defoliators (Shoesmith 1995; Meating et. al. 1995). *Bacillus thuringiensis* is a bacterial insecticide that commonly contains spores, and crystals within the spores that, when ingested by Lepidoptera larvae, bind to the midgut, breaking down cell membranes (cell lysis) and eventually causes death (Mommaerts et. al. 2009; Sanchis 2012). *Kurstaki* refers to the subspecies of *Bacillus thuringiensis* used in the 2009 spray operation (World Health Organization 1999; BioForest 2010). *Bt* is more widely accepted than chemical insecticides from an environmental perspective, due to its smaller impact on the environment and non-target species (Bellocq et. al. 1992; Meating et. al. 1995; Norton et. al. 2001; Sanchis 2012). Before *Bt*, Fenitrothion was the only registered insecticide for use (before 1985). However, after expression of concern from environmental groups, *Bt* was introduced as an alterative (Meating et. al. 1995). *Bt* is considered among the safest bacterial insecticides for the environment, and multiple

studies have labeled *Bt* as safe for non-lepidoptera species including mammals and birds (Bellocq et. al. 1992; Meating et. al. 1995; World Health Organization 1999; Norton et. al. 2001; Sanchis 2012). Areas in Canada for forest use are treated with a concentration of 20-30 BIU/ha (Meating et. al. 1995; BioForest 2010). A single application at this concentration has been proven effective at significantly reducing defoliation in JPBW (Meating et. al. 1995; BioForest 2010).

MATERIALS & METHODS

PREPARATION FOR THE 2019 SEASON

Increasing JPBW presence in pheromone traps and foliage damage was recorded by forest health technicians in the summers of 2016, 2017, and 2018 forest health reports (OMNRF 2018). The outbreak was beginning to dramatically increase, and the early larval (L2) observation surveys in the 2018 winter season indicated that the outbreak was going to increase even further. Below, **Figure 1** shows the mapped outbreak data from aerial observations in 2018.

Due to these reports coming in from the Red Lake District and southwards, internal funding was approved for a 2019 spray program, with timing and assessment run by OMNRF employees.

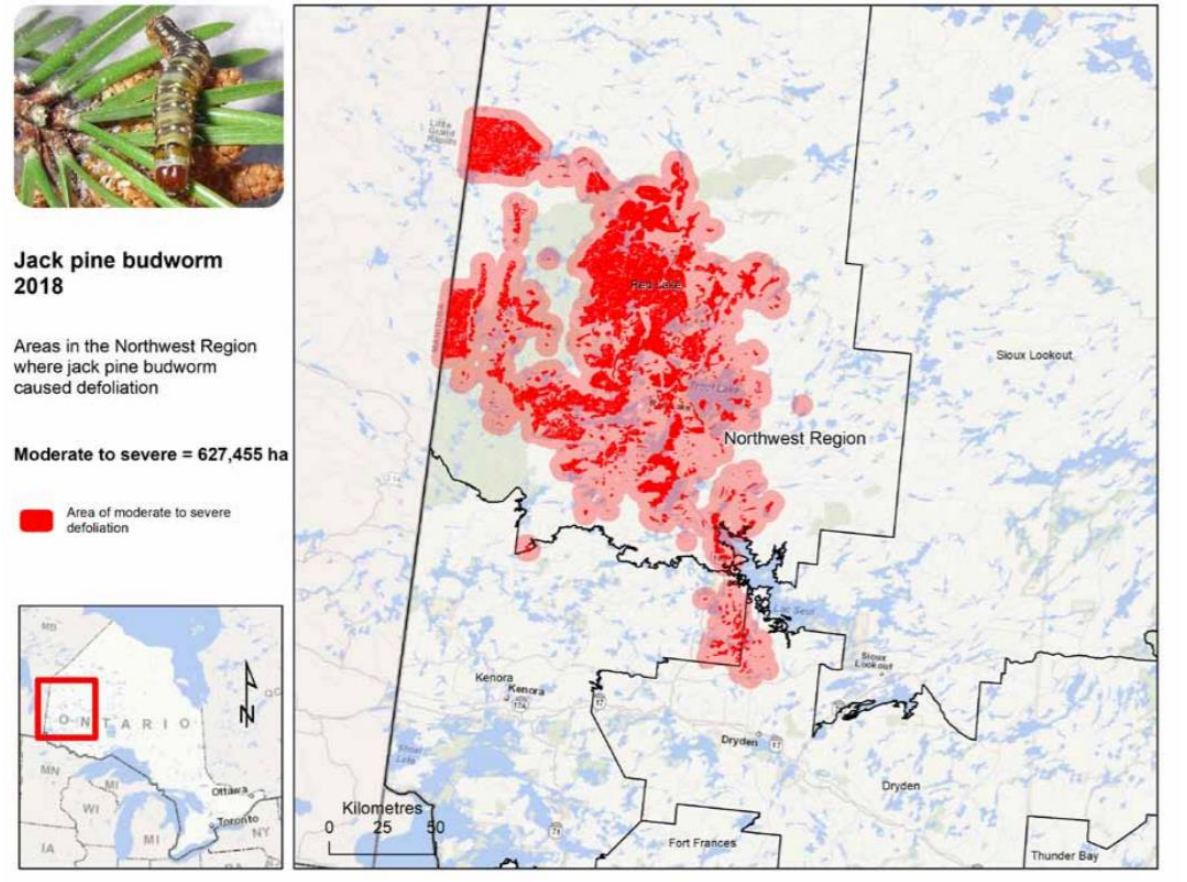


Figure 1. 2018 JPBW defoliation in the northwest region (Source: OMNRF 2018)

The 2019 season started with upper management collecting aerial reports, compiled with L2 numbers. A total of 627,455 ha of moderate to severe defoliation was reported in 2018 (OMNRF 2018). eFRI data were used to confirm the localities of jack pine stands that were composed of over 40% jack pine cover and were a minimum of 40 years of age to be included in the spray program. **Figure 2** shows the entire map of the Northwest region with the 76 timing and assessment plots; there are control plots (not sprayed) marked in green for comparison, and spray plots are shown in red. See **appendix I** for close maps of each project area and **appendix II** for increasing defoliation maps from 2016-2018.

Across thee project areas OMNRF staff based through Dryden and Red Lake put up spray warning signs at every road access point in English, French and Ojibwa. It stated the use of *Btk* in the area, and the general dates of the project (June 2019 – August 2019).

Outreach in local communities was conducted by Dan Rowlinson (OMNRF forest health lead in Ontario). As a result, most of the public was aware and supportive of the project. First nation land in block 5c (See **figure 2**) was removed before the procedures began as they decided they did not want *Btk* treatment in that area.

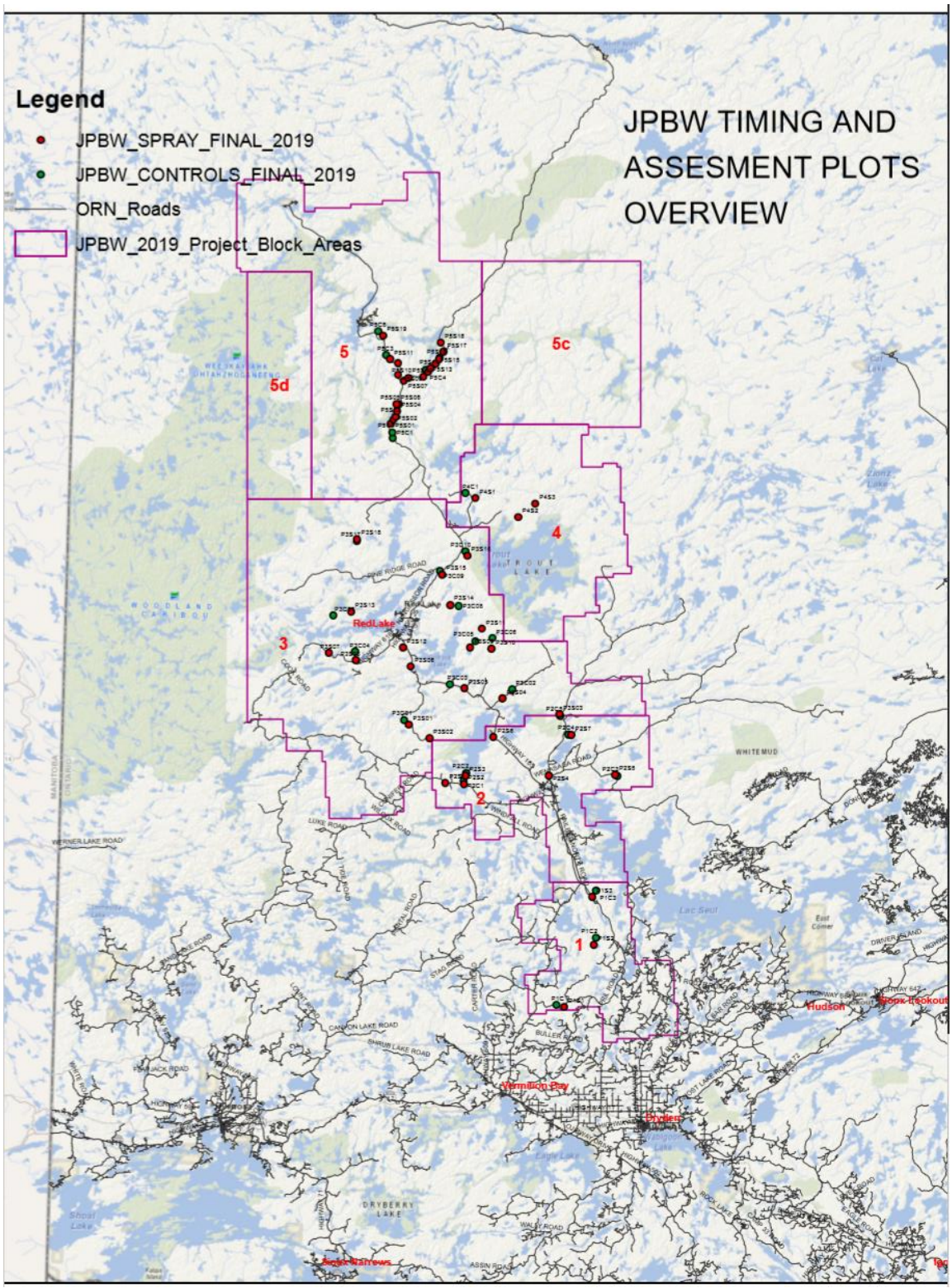


Figure 2. Overview of the original 76 control and spray plots used for timing and assessment

PLOT ESTABLISHMENT

The OMNRF JPBW crew began work in early May. They were based out of the Thunder Bay Biodiversity and Monitoring Unit OMNRF location on 25th Sideroad in Rosslyn, ON. During shifts in the field staff stayed in Ear Falls, ON. The first duration of field work was in the first week of June. Staff members from the Central and Northeast districts joined in Ear Falls to help train and cover more ground.

First, all plots were visited; at each plot, ten jack pine trees were marked and labeled (1-10) with orange flagging tape and black permanent marker. The first tree began 40 m straight in from the edge of the stand as measured with a reel measuring tape. All trees were approximately 5 m apart; trees one through six where selected in a line as the stand allowed, and trees four through ten hooked in any direction. Thus, the final plot was in a general 'J' shape (see **figure 3**). Trees that had an accessible crown and allowed maneuverability of pruning poles where favoured. When plots were inaccessible (i.e. poor road conditions and walking distance over 2 km), or when the eFRI data did not represent a true stand (expected 60% jack pine cover was not there) plots were moved up to 100 m when possible or removed from the project at staff's discretion. Original plot coordinates were in GPS units and copies of all paper maps were in every truck that staff were traveling in. GPS coordinates were modified once a plot was established; new coordinates were added at the first tree of every plot. Staff traveled by truck in pairs to different project areas to establish all the plots. Flagging tape was placed alongside the bush roads during establishment to help identify plot locations. During this time, a forest fire ignited in project area 5 and north towards Pikangikum First Nation, so no staff travelled to that area in the first field shift.

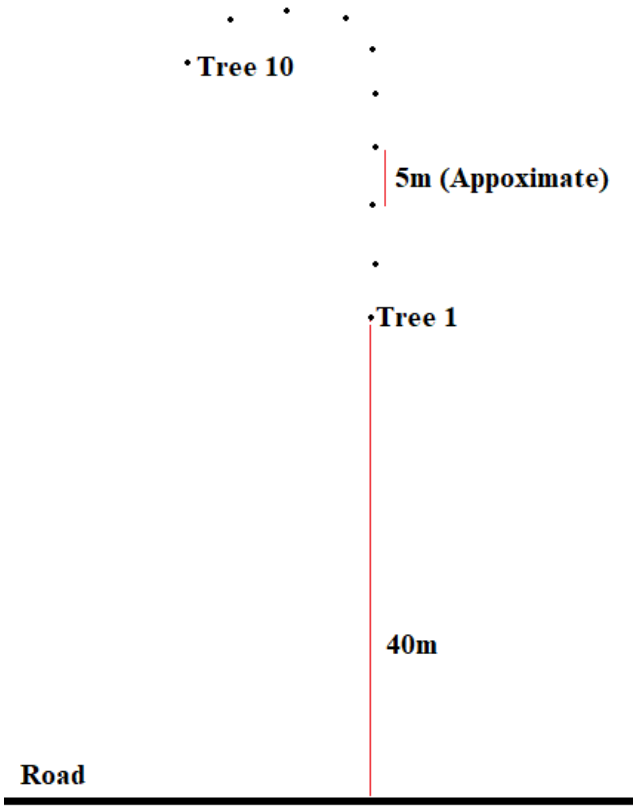


Figure 3. Timing and assessment plot set up, 40m into the stand, 10 marked trees 5m apart, hooking after tree 6.

DEVELOPMENT ASSESSMENT: TIMING

Applying the spray treatment of *Btk* needs to occur while the larvae are out and feeding on new flowers and shoots, this is indicated by the larval phase of the budworm and the "shoot class" from the OMNRF protocol for timing and assessment (not published). According to the protocol the spray will be most effective when the budworms are in their third larval stage (L3) and the shoots are in class 3; this is the time when the larvae are out and feeding but not yet doing significant damage, and the shoots are beginning to open (**see appendix VII**)

During the spring, the BIOSIM computer model was continuously updating and predicting the emergence date of the JPBW using past data and current weather. As according to the model, staff travelled back up for a second shift starting in the last week of June and some staff remained on the field until all spray blocks had opened on June 21st. Development assessments began at this time to observe the development of new shoots, and larvae. Development samples were collected at varying plot locations though all five project areas. Since the project was so widespread, the northmost plots in project area five were expected to develop later than plots in project area one. Using pruning poles, samples were taken from the mid to upper crown of jack pine trees that were not one of the ten trees flagged for assessment. Six branches (approximately 61cm in length) were collected from ten different random trees at the site.

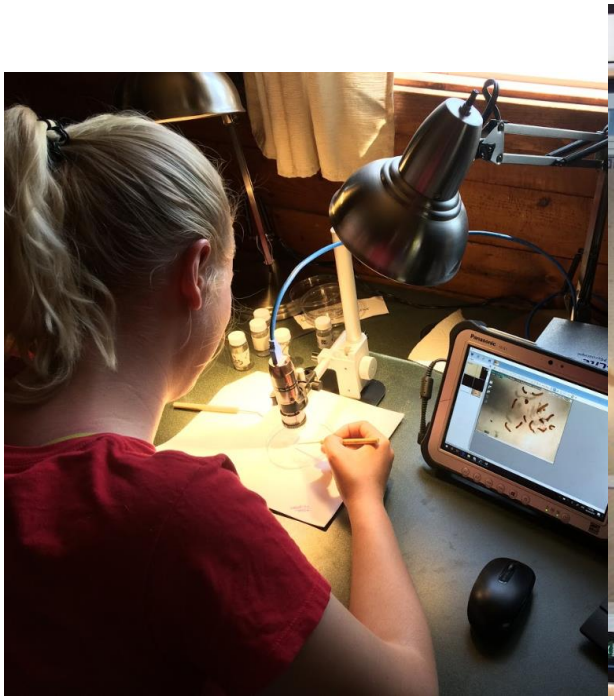
Development sample collections started less frequently (every 2-3 days from all five project areas) and increased over 14 days until daily samples where taken from all five project areas. In the field, for each of the six branches, 20 current-year shoots were observed and classified using the shoot class definitions (**see appendix VII**) and the host index was calculated based on the classes of all the shoots, this data was recorded on a host development sheet (see **appendix III**). When the host index and larval index reached at least 3, the spray could be initiated in the respective project area; for host and larval index calculation methods, see **appendix IV**.

Development kits that contained probes, tweezers, isopropyl alcohol, markers, vials, pencils, and masking tape were used to collect a total of 50 larvae off of the six branches (attempting the maximum representativeness of five larvae from each branch, and larvae off of both flowers and shoots). Tweezers and probes were used to pick

through shoots and flowers looking for larvae, and placing them into vials with isopropyl alcohol. Each vial was labeled each with masking tape and marker to show the plot lactation and data (see **figures 4 - 6**). Each larva was tallied by it's locality on the sample (i.e. on flower or on foliage) and was recorded on a larval development sheet (**see appendix III**). The vials were taken into the field-lab in Ear Falls, which was essentially a microscope that connected to a computer tablet screen, with a program to measure length on the microscope image via the computer screen after calibrating the computer with the magnification (see **figures 7 - 9**). Each of the 50 larvae were lined up on a petri dish under the microscope and the width (in mm) of their head cap was measured on the tablet computer to determine larval stage (L1-7). The measurements were pasted into an excel file which calculated the larval index. In the beginning phase of development, larvae were scarcer; sometimes less than 50 were found across the samples, but later in the development phase as more larvae developed and emerged, they were plentiful.

Figure 4 & 5. Development observations: searching and collecting JPBW larvae.

Figure 6 & 7. JPBW in development collection vial (left); and JPBW on a petri dish in the Ear Falls field lab for development observation.



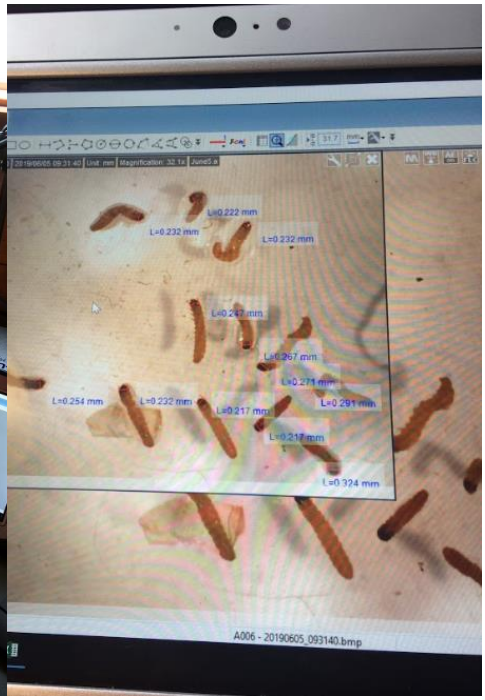


Figure 8 & 9. Ear Falls field lab set up for determining larval index, and screen display on tablet computer of measured head cap widths of JPBW for a development plot.

PRE-SPRAY ASSESSMENT

In the Field

Simultaneous to the development observations, other staff members were collecting pre-spray samples from all of the established plot locations, including what was left post-fire in project area five. Pre-spray sampling involved visiting every site and cutting a 61 cm branch off of the mid to upper crown off of each of the ten marked trees. Pruning poles were used for cutting, "tarps" (see **figures 10 - 11)** were used for measuring (utilized in the post spray differently). Each 61 cm branch was cut up and placed into a large brown paper bag, which was marked with the plot, tree, date of collection, and presence or absence of flowers. The ten staple-sealed paper bags from each plot were placed in a burlap sac and put into the back of the truck. These pre-spray samples were not immediately placed into a cooler on site in Ear Falls because the date of larva moving into L3 or L4 was later than expected. To allow more accurate total counts that would take place in the lab, bags were stored in room temperature to allow some further larval development/increase in size. All plot burlap sacs containing their ten paper bags were brought back to the Rosslyn OMNRF location and put into a cooler. The cooler malfunctioned and the temperature went below zero, thus the mortality data from the pre-spray was not accurate, however the count was not affected.

Figure 10 & 11. Length verification of branch cut from the jack pine crown (left) and jack pine crew using pruning poles to cut a sample from the mid to upper crown of the jack pine.

In the Lab

In the Rosslyn lab, every crew member had a station with a desk light, probes, magnifying glass, tweezers, and pre-spray tally recording sheet (see **appendix V**). Each brown bag was opened, and a total larvae count was made for each tree. Microscope checks where used to confirm species identification (see **figures 12 – 14**). The plot location, tree number, flower presence, collection date, count date, and crew members who collected and counted the sample were all recorded onto the pre-spray sheet and the petri-dish or vial used. Every bag was checked three times by three different crew members too ensure no larva were missed in the count. Once the total count was complete, plot data was entered into an excel spreadsheet to await the post-spray comparisons.

When all ten samples from a plot were complete, the count, and date data were recorded onto a foliage examination summary sheet (see **appendix VI**). The total budworm count from all ten tree branches was recorded and divided by ten to create an average budworm count per branch; this was done for every plot.

Figure 12. Rosslyn lab station set up with sample and data sheet.

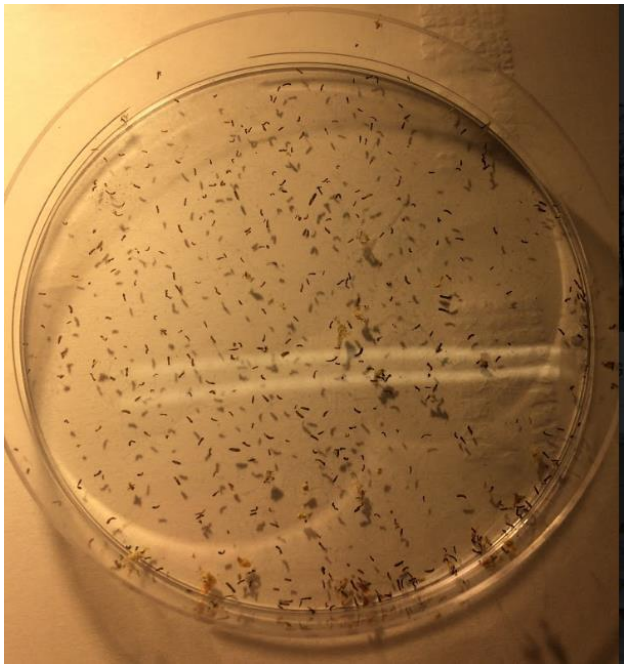


Figure 13. Pre-spray larvae from a sample branch counted in a petri-dish in the Rosslyn lab.

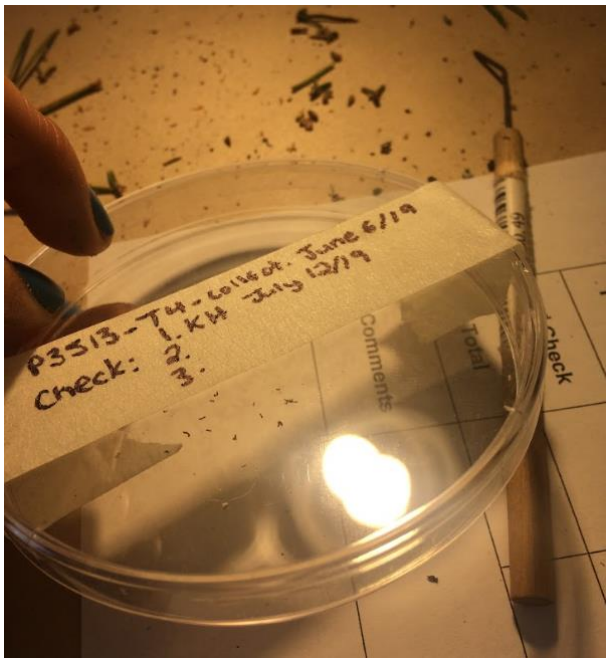


Figure 14. Pre-spray larvae petri dish, labeled with plot, tree, and observation information in the Rosslyn lab.

THE SPRAY

The spray began in project one, two, and three on June 17th, 2019, project four on June 20th, 2019, and project five on June 21st, 2019. A total of approximately 100,360 ha was sprayed at a concentration of 30 BIU/1.5L/ha of *Btk*.

POST-SPRAY ASSESSMENT

In the Field

Once the pre-spray counts and the aerial spray were complete the crew headed back to Ear Falls to begin the post spray observations. The same methodology was practiced as the pre-spray branch collection, with the addition of the following: each branch that was cut was caught in the tarp to avoid large larvae falling off and being missed in the count, and based on ocular estimates, staff recorded defoliation of new shoots on the paper bag by 5% increments.

At the end of each day burlap sacs were immediately placed into an on-site cooler to avoid further development of larvae. It is important to note that defoliation was recorded immediately after each branch was cut and before it was placed in the paper bag, so any defoliation that occurred within the bag was not recorded. Flower presence was also recorded. See **figure 15**.

A few more plots were lost due to fire in project area 4 and new inaccessibility issues (i.e. significant washouts). These plots were not counted in the post spray. See **figure 16**.

Figure 15. Observing sample branch and confirming length during post-spray.

Figure 16. Post-fire in project area five, burned stand.

In the Lab

All burlap sacs were taken from the Ear Falls cooler, brought back to Rosslyn, and placed into the cooler at the Rosslyn location. In the lab a similar methodology as the pre-spray was practiced. Differences in the post spray included the defoliation data being copied off of the sample bags onto the lab data sheet, and differentiation of larvae were added to the recording sheet as some JPBW had moved into the pupal stage or even emerged into adults (see **appendix V**). Since the budworms that were present had progressed in life stages and were larger as a result, only two counts by two different crew members was completed as detection was not as challenging (see figures **17 & 18**).

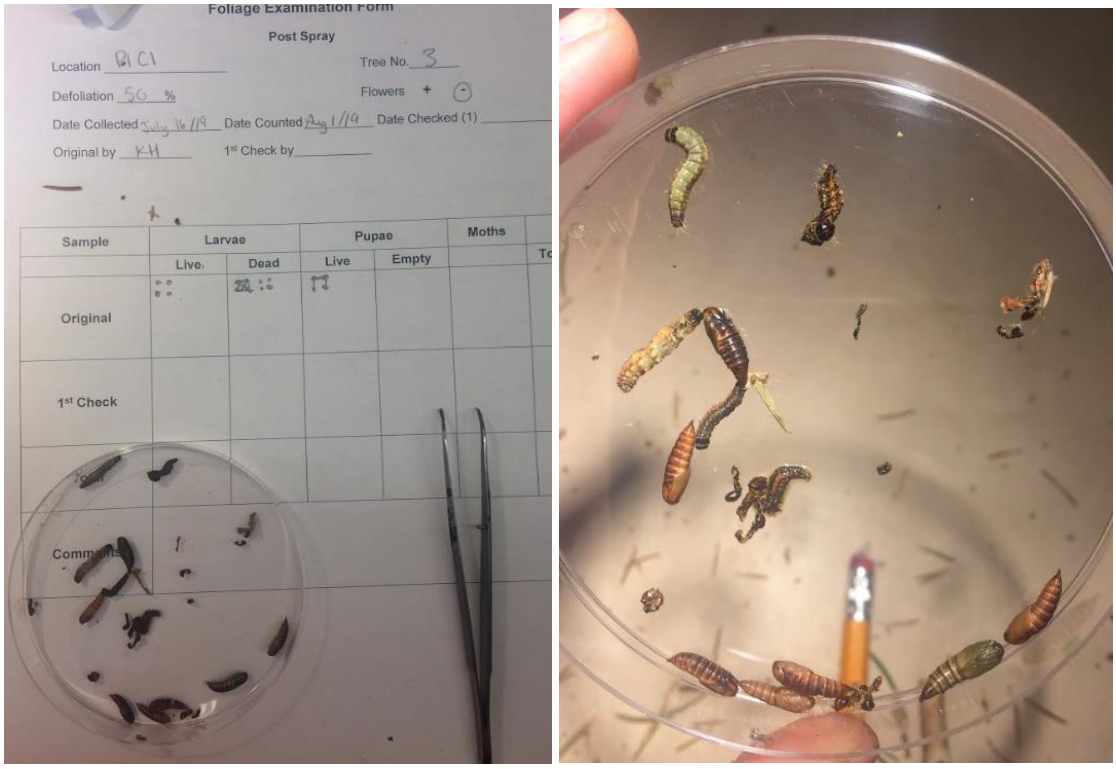


Figure 17 & 18. Post-spray counts of JPBW across many life stages in the Rosslyn lab.

When all ten samples from a plot were complete, the count, date and defoliation data were recorded onto a foliage examination summary sheet (see **appendix VI**). The total budworm count from all ten tree branches was recorded and divided by ten to create an average budworm count per branch; this was done for every plot.

RESULTS

2019 SPRAY PROGRAM

The data presented is collated from the pre and post spray assessments. At each plot location ten trees were sampled to represent the number of jack-pine budworm per 61cm branch on each tree. With unforeseen circumstances (such as fire, washouts, or any accessibility problems) the results contain 19 control plots and 37 sprayed plots. If flagging had fallen off a tree or it was poorly marked during the pre-spray sampling, a new tree may have been selected. Thus, at each of the 56 sampled plots 10 trees were successfully sampled and brought to the lab, totalling 560 sample counts. However, in the post-spray process trees that were not still marked or unclearly marked could not be replaced, as the method requires the same tree to be counted twice, resulting in any missing trees being removed from the data. Additionally, 3 out of 56 plots became inaccessible for post-spray evaluation. Three post-spray control plots contained less than ten samples; P3C2, P3C8, P3C10: with 9, 8, and 9 trees, respectively, and four post-spray treated plots contained less than ten samples; P2S5, P3S6, P3S12, P5S4 with 5, 9, 9 and 7 trees, respectively. With those missing trees and missing plots considered, 519 samples from 53 plots were collected in the post-spray process. Altogether 1079, 61cm branch samples from both pre and post spray assessment were collected.

The following tables present the plots lost from the original maps displayed in the appendix. Again, some plots were lost due to inaccessibility, EFRI error, or fire. The tables show which plots were lost during plot marking/pre-spray and which were lost before post spray. Due to fire in project area four, no data was collected for pre or post spray. **See fig. 19** for the summary map.

Table 1. Control plot loss over the duration of the project.

Project Area	Original Plot Set	Pre-Spray Plots	Post-Spray Plots
P1	C1	C1	C1
	C2	C3	C3
	C3		
P2	C1	C1	C1
	C2	C2	C2
	C3	C3	C3
	C4	C4	C4
	C5	C5	C5
P3	C1		
	C2	C2	C2
	C3	C3	C3
	C4	C4	C4
	C5	C5	
	C6	C6	C6
	C7	C7	C7
	C8	C8	C8
	C9	C9	C9
	C10	C10	C10
P4	C1	C1	
P5	C1	C1	C1
	C2	C2	C2
	C3		
	C4		
	C5		
	C6		

Table 2. Spray plot loss over the duration of the project. Continued next page.

Project Area	Original Plot Set	Pre-Spray Plots	Post-Spray Plots
P1	S1	S1	S1
	S2		
	S3	S3	S3
P2	S1	S1	S1
	S2	S2	S2
	S3	S3	S3
	S4	S4	S4
	S5	S5	S5
P2	S6	S6	S6
	S7	S7	S7

Project Area	Original Plot Set	Pre-Spray Plots	Post-Spray Plots
P3	S1	S1	S1
	S2	S2	S2
	S3	S3	S3
	S4	S4	S4
	S5	S5	S5
	S6	S6	S6
	S7		
	S8	S8	S8
	S9	S9	S9
	S10	S10	S10
	S11	S11	S11
	S12	S12	S12
	S13	S13	S13
	S14	S14	S14
	S15		
	S16		
	S17	S17	S17
	S18	S18	S18
P4	S1	S1	
	S2	S2	
	S3		
P5	S1		
	S2	S2	S2
	S3	S3	S3
	S4	S4	S4
	S5	S5	S5
	S6	S6	S6
	S7	S7	S7
	S8	S8	S8
	S9	S9	S9
	S10	S10	S10
	S11		
	S12	S12	S12
	S13	S13	S13
	S14		
	S15		
	S16		
	S17		
	S18		
	S19		

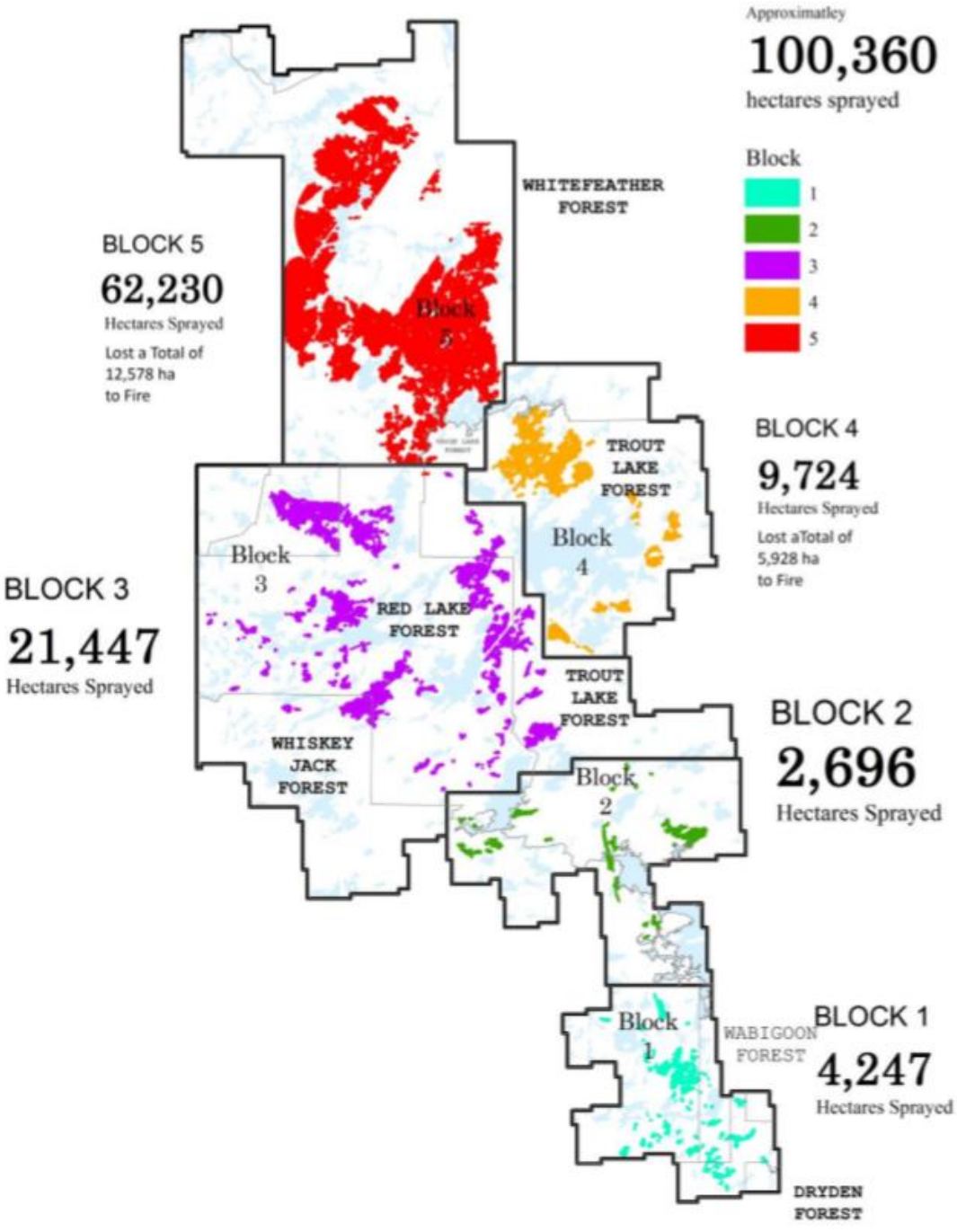


Figure 19. A summary map produced by OMNRF 2019 forest health staff, displaying area sprayed across all project areas (blocks) and significant loss due to fire.

Following**, figures 20 & 21** summarize average number of budworms per branch for each spray and control plot location, respectively. This was determined by taking the total budworm count across all ten trees within a plot, and then taking the mean of that value to represent the plot. In cases where less than 10 trees were sampled the calculation was adjusted based on the total mean value for the number of trees that were sampled. This was done so that comparisons between pre and post sprays could still be shown with the 53 plots that were sampled twice.

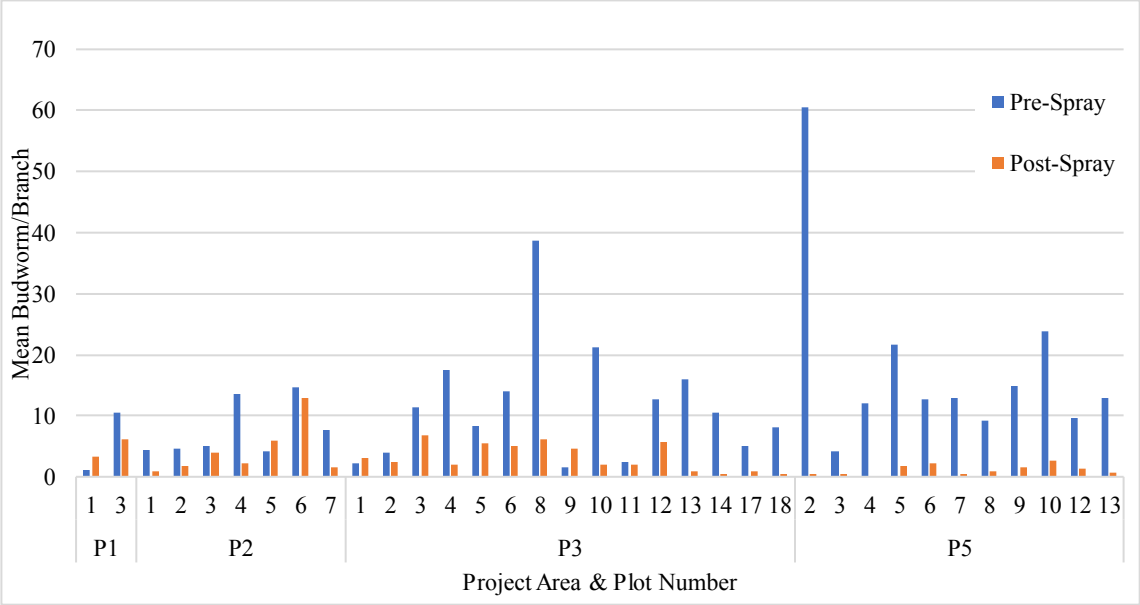


Figure 20. A comparison of jack-pine budworm population presence occurring on a 61 cm branch in the spray plots from the pre-spray and post-spray assessments.

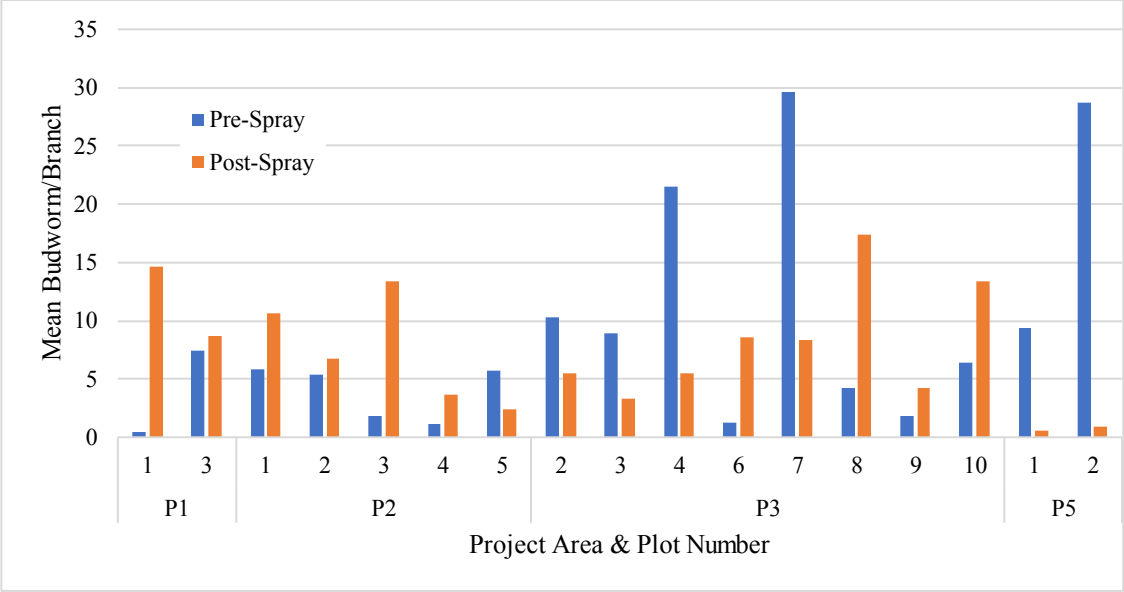


Figure 21. A comparison in jack-pine budworm population presence occurring on a 61 cm branch in the control plots from the pre-spray and post-spray assessment.

Below, **figure 22** compares control plots and spray plots by the percent defoliation of the sample. The defoliation data was collected after the spray.

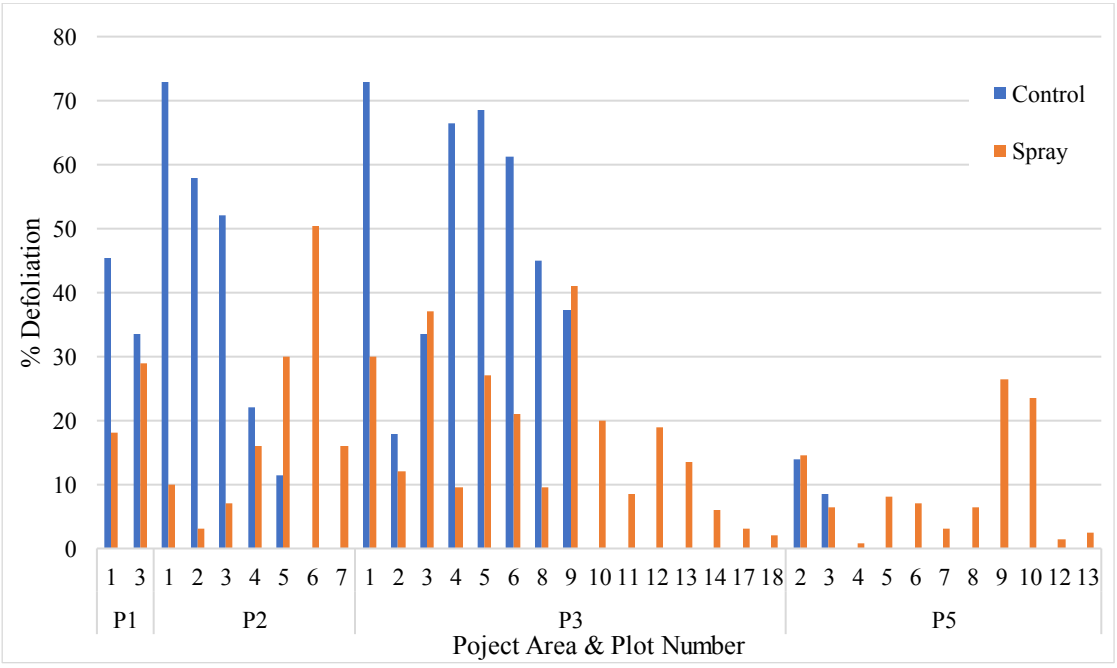


Figure 22. Defoliation of samples from each plot, comparing spray and control plots.

A summary table (Table 3) was created with the data from figures 20-22. A direct comparison of mean values for population counts and percent defoliation data is displayed for control and spray plots.

Table 3. Summary of mean values from data presented in figures 20-22.

	Population/Branch Pre-Spray	Population/Branch Post-Spray	Percent defoliation
Control Plots	8.8	7.5	42.4
Spray Plots	12.4	2.8	15.4

To best compare current with past sprays, the same statistical analysis was used according to the 2009 efficiency report (BioForest Technologies Inc. 2010). Before proceeding to analysis of post spray populations across control and spray plots, a standard single factor ANOVA conducted in Excel determined that pre-spray budworm population counts showed no significant difference between the control and spray plots ($p=0.26$). Single factor ANOVA was used to analyze all relations following. The JPBW population per branch had no significant difference between pre and post spray counts though the control plots ($p=0.6$). The jack pine population decline between pre and post spray was significant through spray plots, $p<00001$. There was additionally a significant difference between the post spray populations and defoliation when comparing the control and spray plots, $p<00001$ for both. All ANOVA results were comprised of complete data sets; if a population count occurred in the pre-spray but not the post, that plot was removed from the analysis. Standard deviations among all means (pre and post budworm populations and percent defoliation for both control and spray plots) was fairly high.

COMPARING 2019 TO HISTORICAL OPERATIONS

The fist JPBW control operation in Ontario using *Bt* was in 1985, when two formulations (dipel 132 and thuricide) were used, with no significant differences found in effectiveness recorded. In 1985, most blocks were sprayed at a rate of 20 BIU/1.6L/ha. Overall, there was an average of 15.6 larvae per 61cm branch in spray blocks and 13.8 larvae per branch in control plots. Percent defoliation in sprayed areas averaged 27% compared to 54% in unsprayed control areas (Meating et. al. 1995).

In 1994 the same formulation, concentration, and application rate (30 BIU/1.5L/ha of Foray 76B (*Btk*)) was later used in 2009 and 2019. No specific data from this operation was recovered. However, the defoliation rates were presented as significantly lower in spray plots (Meating et. al. 195).

The most recent spray operation in Ontario occurred in 2009 when 58,146.2 ha were sprayed (much less than the 100,360 ha sprayed in 2019), with the same target as 2019; to limit defoliation to 40% or less. Foray 76B (*Btk*) was used at a concentration and rate of 30 BIU/1.5L/ha. According to the efficiency report prepared by BioForest Technologies (the contracted staff for 2009) a significant difference was found between the spray and control plots, post-spray population counts, and between spray and control plots defoliation assessments. The 2009 report separated the northern and southern plot statistics due to the initial ANOVA run determining that a significant difference existed between the control and spray plots pre-spray budworm population counts, and this difference was attributed to variation across the stands from north to south. The separation of the north and south regions then allowed for reliable comparisons, and thus no significant differences were found between pre-spray control and spray budworm

population counts for plots occurring within their respective localities (BioForest Technologies Inc. 2010).

The following table presents the results from historical sprays. The 1994 spray program was not included due to the lack of data. For the 2009 spray program, northern and southern plots were weighted as to the number of plots present in the northern vs southern regions in order to present meaningful statistics to be used for comparison. In 2009 the northern plots were comprised of 41 spray and 10 control plots, while the southern plots were comprised of 16 spray and 8 control plots. For percent defoliation 21 spray and 5 control plots, and 8 spray and 4 control plots for the northern and southern plots, respectively, were used for post-spray population counts.

Table 4. A comparison of mean values from the 1985, 2009 and 2019 spray operations in Ontario. Spray and Control plots are designated with a "S" and "C", respectively.

	1985	**2009**	**2019**
Concentration	20 BIU/1.6L/ha	30 BIU/1.5L/ha	30 BIU/1.5L/ha
Defoliation (%)	S: 27 C: 54	S: 20.5 C: 39.2	S: 15.4 C: 42.4
Post-Spray Pop. (JBPW/Branch)	S: 15.6 C: 13.8	S: 4.0 C: 8.1	S: 2.8 C: 7.5

Attention can be drawn to the different concentration used in 1985 and the less dramatic results, although percent defoliation remained under 40% in the sprayed areas.

DISSCUSION

The single application of *Btk* at 30 BIU/1.5L/ha in the 2019 JPBW spray operation was successful at reducing the percent defoliation level and budworm population count (# budworms per branch) across the area sprayed. This was shown by the significant difference that was indicated by the ANOVA statistical test when comparing percent defoliation between control and spray plots, comparing population counts before and after the spray, and the differences in post spray populations between spray and control plots.

Based on the results found, the effectiveness of *Btk* has not decreased, despite repeated use in Ontario from 1985, 2009, and 2019. The 2019 spray program was successful by way of a target percent defoliation goal of under 40% across spray blocks, ANOVA data analysis, and by comparison to previous operations. This statement can conclude that the change of internally conducting the development and assessment of the JPBW by OMNRF staff did not affect the success of the program.

According to all of the results collected in the 2019 spray program and comparisons to previous spray programs over the last 35 years; *Btk* continues to be effective at decreasing JPBW larval populations and defoliation effects by the larvae in their late instar stages.

Special attention to the 2009 results that displayed similar yet slightly less dramatic outcomes to the 2019 results shows that, despite spray applications across the same area (as the 2009 outbreak covered almost the same geographic location as the 2019 outbreak), the increase in JPBW control success documented in the 2019 program

could be attributed to a change in the amount of data collected. In 2009 the same protocol for plot establishment, including the J shaped plot, was followed, but there were differences in the number of plots and amount of data collected from those plots. In 2009, 58,146.2 ha were sprayed compared to 100,360 sprayed in 2019 (BioForest Technologies Inc. 2010). Even though a larger area was sprayed in 2019, fewer plots were successfully used to collect complete datasets. In 2009, 57 spray plots and 26 control plots were assessed, whereas in 2019, 35 spray plots and 17 control plots were assessed (BioForest Technologies Inc. 2010). The fire that occurred though the project area significantly impacted the number of plots that had full datasets in the 2019 operation, as the original set was to include 50 spray and 25 control plots, still less than 2009. Another large difference in the data collection from 2009 to 2019 was the post-spray population counts. In 2009, all plots were assessed for defoliation, however only 29/57 spray plots and 9/26 control plots were assessed for post-spray population counts (BioForest Technologies Inc. 2010). In 2019, all plots that were assessible had post-spray population counts completed. These differences between 2009 and 2019 could be the reason for the change in recorded effectiveness, although the differences did not change the result that both operations were a success.

CONCLUSION

Based on results collected from the 2019 JPBW spray program and comparisons with previous aerial spray programs, *Btk* continues to be an effective biological insecticide for use on JPBW infestations in northwestern Ontario. The change from previous harmful chemical insecticides to *Btk* as a biological insecticide has proven to be a successful approach to maintaining tolerable levels of jack-pine budworm defoliation in Ontario's commercial forests while negating harmful effects of chemical alternatives. Through the review of numerous published works on the potential effects of *Btk*, it has been referenced as a safe option with no significant known effects on the natural forest flora or fauna other than lepidoptera species.

As repeatedly presented in the 1995 symposium, further research on different JPBW management tools may still be valuable due to a potential for decreasing effectiveness with continual use of a single management strategy, including *Btk*. Although this thorough review of the 2019 spray operation and comparison to previous spray programs has not indicated that *Btk* has become any less effective or more dangerous in its use to protect commercial jack-pine stands, the potential of exploring other options for controlling natural forest pests could yield positive improvements in the future. More research would be crucial if different options are to be explored; the importance of maintaining forest health, protection of biodiversity and the commercial forest upon which we rely can't be underestimated during that process.

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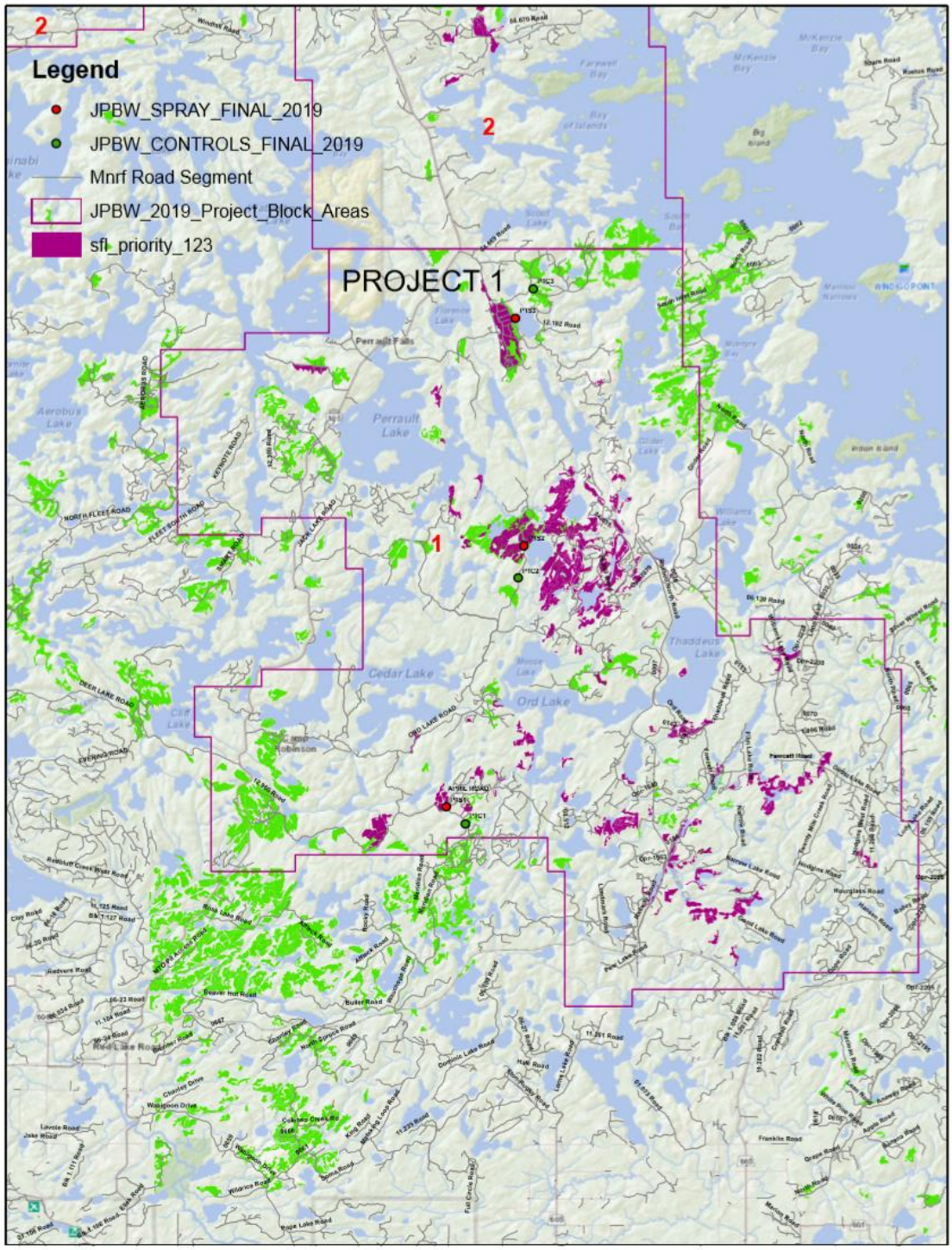
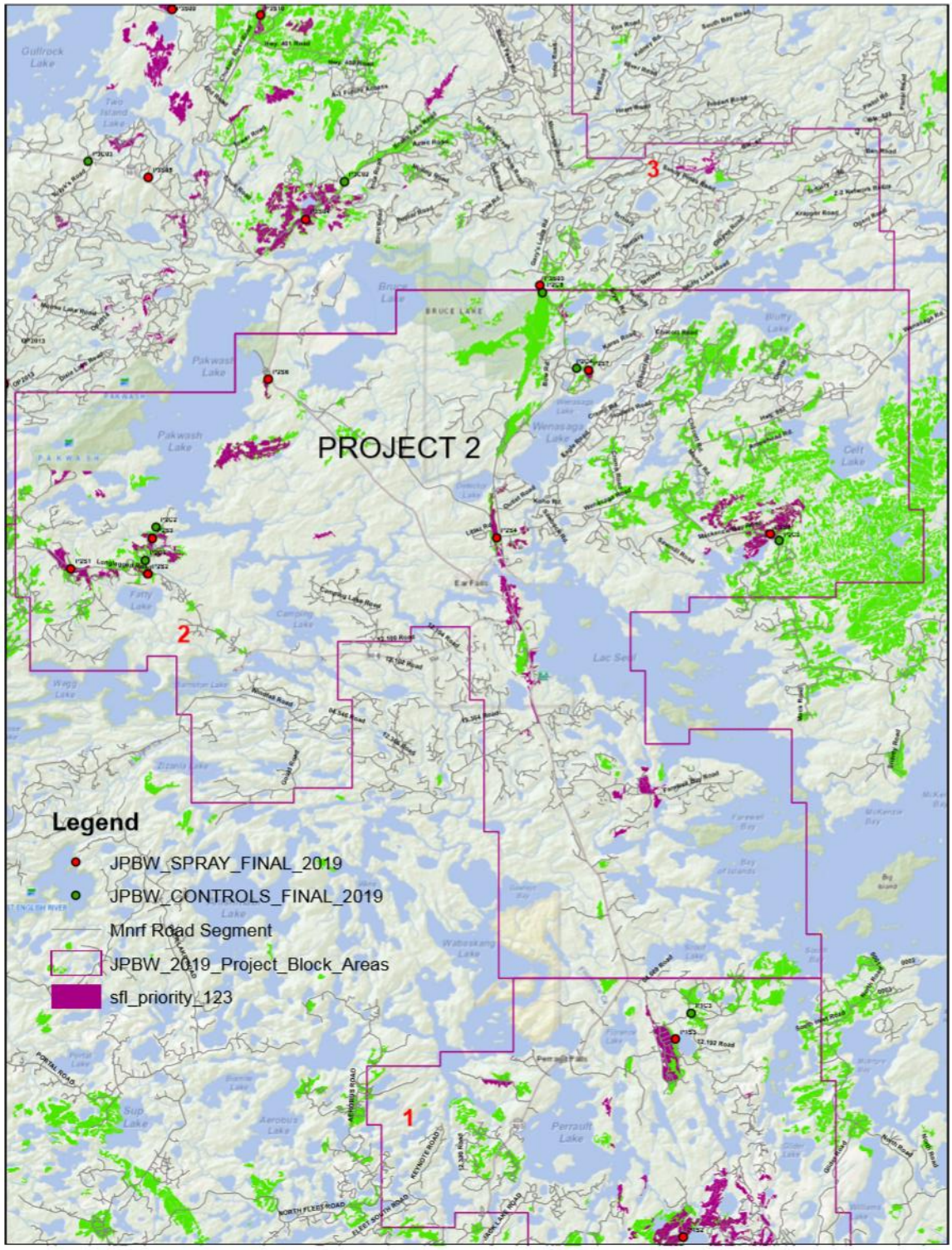
APPENDICES

Appendix I

Individual Project Area Maps Created by northern Ontario OMNRF staff:

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1
2
3
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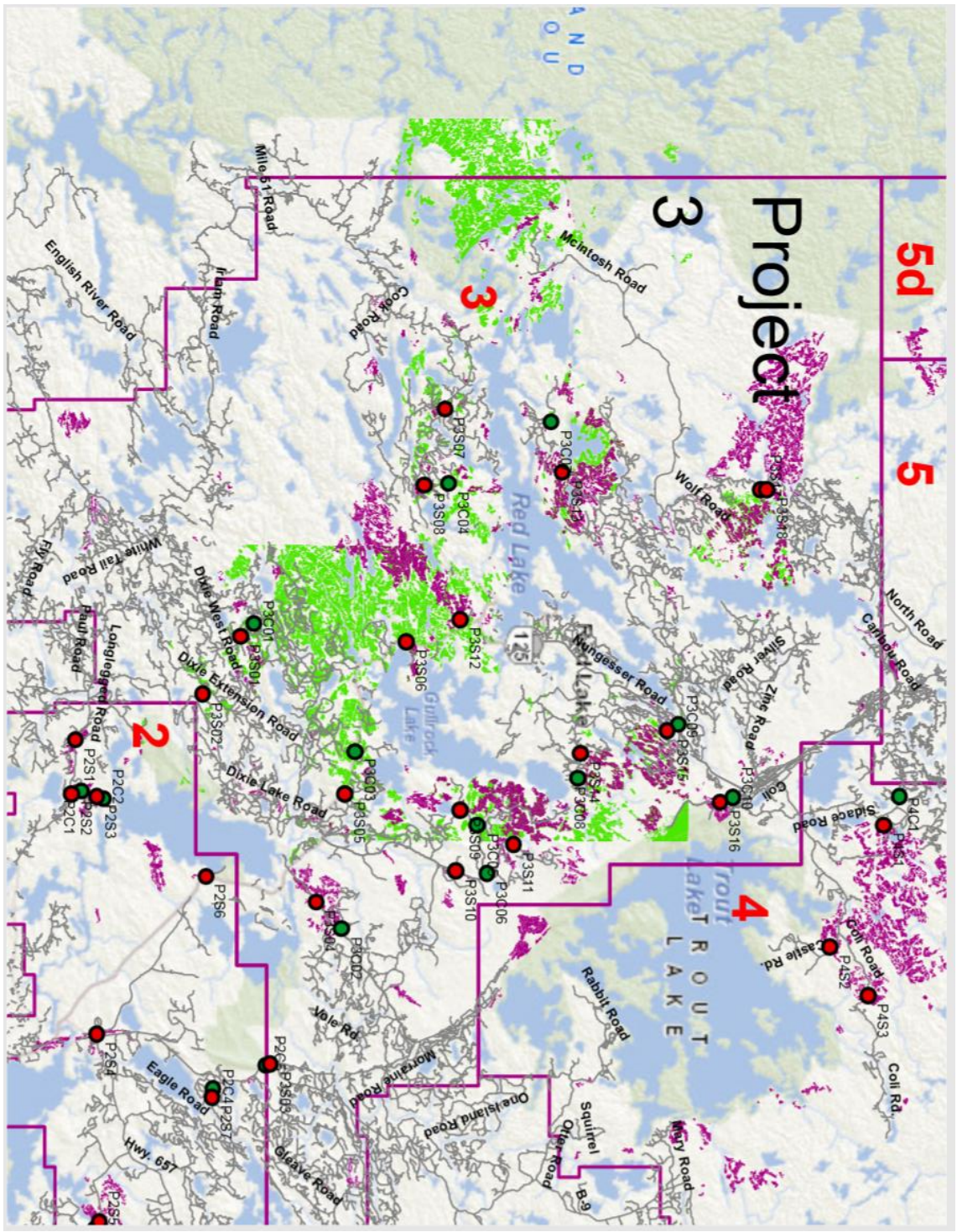
Project
5d
5
3
4
2
Red Lake
Trout Lake
Gullrock Lake
Mile 61 Road
English River Road
Irish Road
Cook Road
McIntosh Road
Wolf Road
North Road
Carlhoff Road
Silver Road
Zine Road
Coil
Slidge Road
Coil Road
Castle Rd.
Coil Rd.
Rabbit Road
Squirrel
Otter Road
B-9
Mary Road
Ryngesser Road
Dixie West Road
Dixie Extension Road
Dixie Lake Road
Longlegged Road
Paul Road
White Tail Road
Fly Road
Vole Rd.
Morraine Road
Eagle Road
Hwy. 657
Gleave Road
One Island Road
P3S07
P3C04
P3S08
P3C00
P3S13
P3S14
P3S12
P3S06
P3C01
P3S01
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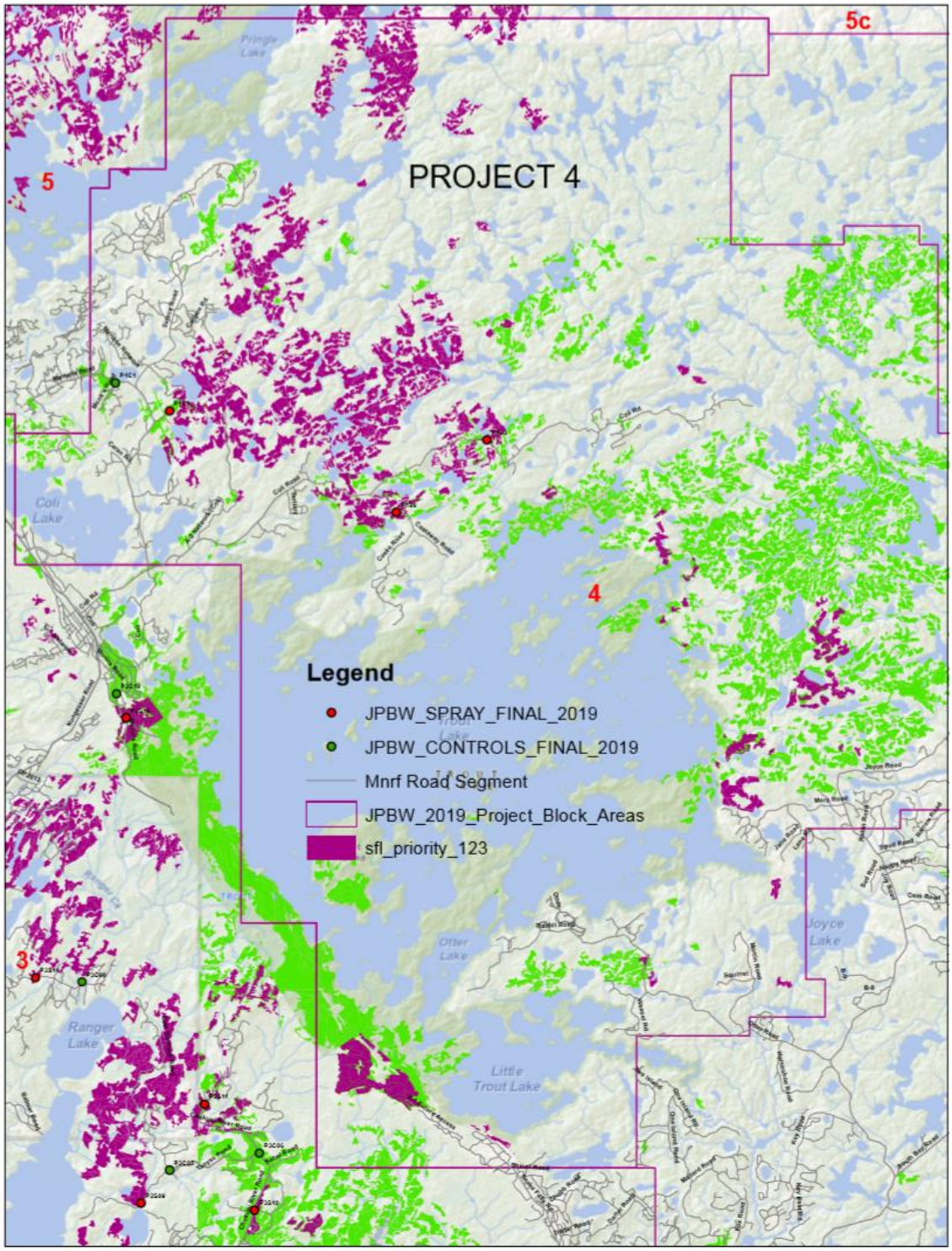
P3C03
P3S05
P3S09
P3C05
P3C06
P3S11
P3S10
P3S04
P3C02
P3S03
P3C08
P3S15
P3S16
P3C10
P3S17
P4C1
P4S1
P4S2
P4S3
P2S6
P2S4
P2C4
P2S7
P2C3
P2S5
P2C2
P2S3
P2S2
P2C1
P2S1


PROJECT 4
5
5c
4
3
Legend
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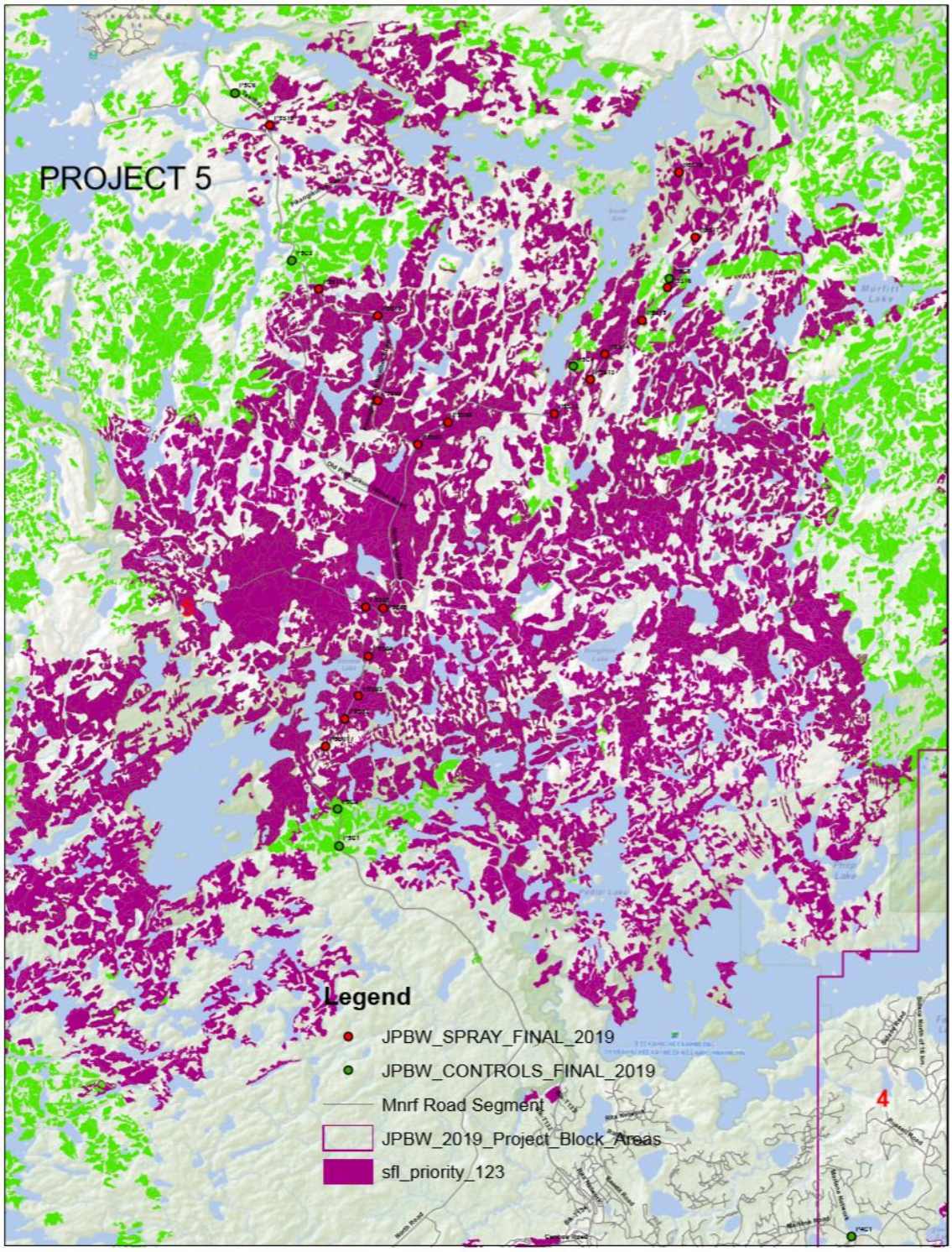
JPBW_CONTROLS_FINAL_2019
Mnrf Road Segment
JPBW_2019_Project_Block_Areas
sfl_priority_123
Pringle Lake
Coli Lake
Otter Lake
Ranger Lake
Little Trout Lake
Joyce Lake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Legend
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JPBW_CONTROLS_FINAL_2019
Mnrf Road Segment
JPBW_2019_Project_Block_Areas
sfl_priority_123
4

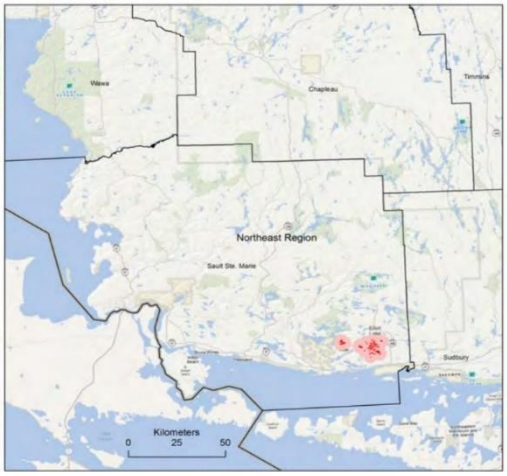
Appendix II

Increasing JPBW defoiation from 2016-2018. Source: Ontario Forest Health Conditions, OMNRF 2016-2018. (Source: OMNRF 2016, 2017, 2018).

Jack pine budworm 2016

Northeast Region
Areas-within-which jack pine budworm caused defoliation.
2,403 ha

Area of Moderate-to-Severe Defoliation

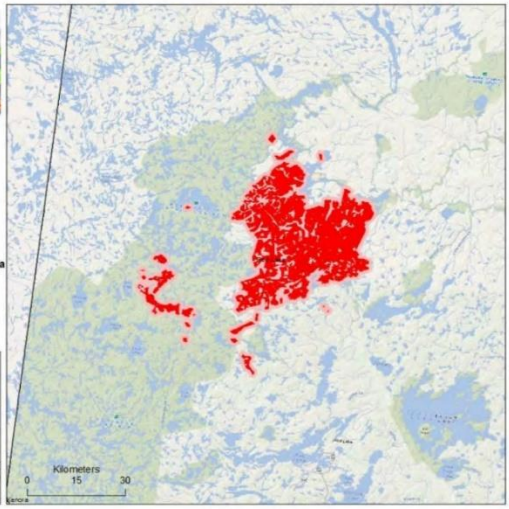


Jack Pine Budworm 2017

Northwest Region
Areas-within-which jack pine budworm caused defoliation

Moderate-to-severe = 100,187 ha

Area of moderate-to-severe defoliation

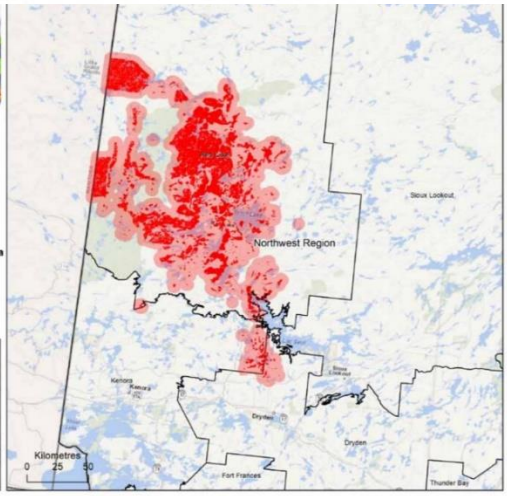


Jack pine budworm 2018

Areas in the Northwest Region where jack pine budworm caused defoliation

Moderate to severe = 627,455 ha

Area of moderate to severe defoliation



Appendix III

Development Data Sheets (blank and filled out) for Host and Larvae

Jack Pine Budworm Host and Larval Development Datasheet

Project Area: _____ Plot Number: _____ Crew: _____

UTM Zone: _____ Easting: _____ Northing: _____

Collection Date: _____ Processing Date: _____

Host Development

Branch	Class 1	Class 2	Class 3	Class 4	Class 5	Class 6
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						

Class	1	2	3	4	5	6	Total
# Shoots							
Product	x1	x2	x3	x4	x5	x6	

$$\textit{Host Index} = \frac{\textit{Sum of Products}}{\textit{Total Number of Shoots}} = \_\_\_\_\_ = \_\_\_\_\_$$

Larval Development

	Bud	Needle	Flower	Wandering	Total
Number of Larvae					

JACK PINE BUDWORM HOST DEVELOPMENT

Location PIC 1

UTM co-ordinates : Zone: 15 Easting: 0490162

Crew: Kristianna + Emerson Northing: 5548939

Tree species: Jackpine Date Collected: June 6th 2019

Branch	Class					
	1	2	3	4	5	6
1	⊠·	⊠				
2	⊠	⊠				
3	⊠	⊠·				
4	⊠··	⊠·				
5	⊠	⊠				
6	⊠	⊠·				
7						
8						
9						
10						

SUMMARY

CLASS	1	2	3	4	5	6	TOTAL
# OF SHOOTS	61	59					
PRODUCT	61	118					

HOST INDEX = $\frac{\text{SUM OF PRODUCTS}}{\text{TOTAL \# OF SHOOTS}} = \frac{179}{120} =$ 1.49

JACK PINE BUDWORM LARVAL DEVELOPMENT

Location PIC1

UTM co-ordinates : Zone: 15 Easting: 0490162

Crew: Kristianna + Emerson. Northing: 5548939

Tree species: Jackpine Date Collected: June 6/19

Minimum of 5 branches

LOCATION (WHERE LARVA FOUND)	BUD	NEEDLE	FLOWER	WANDERING	TOTAL
NUMBER OF LARVAE	⊠ ⊓	·	⊠ ⊠ ⊠ ⊠:·		

INSTAR	II	III	IV	V	VI	VII	PUPAE	TOTAL
NUMBER OF LARVAE								
PRODUCT	X2	X3	X4	X5	X6	X7	X8	

LARVAL INDEX = $\frac{\text{SUM OF PRODUCTS}}{\text{TOTAL \# OF LARVAE}} =$ 2

Appendix IV

Larval and Host Index Calculations

To calculate the Host Index and Larval Index:

The sum of tallies from each of the 20 shoots on each of the 6 respective branches is determined for each class: example from Appendix III shown in following table.

Branch Number	Shoots in Class 1	Shoots in Class 2	Total Shoots/Branch
1	11	9	20
2	10	10	20
3	9	11	20
4	12	8	20
5	10	10	20
6	9	11	20
SUM	**61**	**118**	**120**

Sum of Shoots in Class 1 = 61

Sum of Shoots in Class 2 = 59

Total Shoots Observes = 20 x 6 = 120

Host Index = sum [(Sum of shoots in class) x (class number)] / Total Number of Shoots

= [(61 x 1) + (59 x 2)] / 120

= [61 + 118] / 120

= 1.49

For the larval index, the same mathematical procedure is used, the sum of larva is used in replacement of sum of shoots. The larva is separated into larval stage (L1-7) instead of classes, thus, the sum of larvae in each stage is multiplied by the stage number. Branch numbers are irrelevant for larval index. The larval index was calculated on excel after the lab measurements determined the larval stage of 50 larvae. The larval index equation would look as follows:

Larval Index = sum [(sum of larvae in larval stage) x (larval stage number)] / Total Larvae

PRE-SPRAY Foliage Examination Datasheet

Project Area _____ Plot _____ Tree _____ Date Collected _____

	Original Count	First Check	Second Check
Date Counted			
Staff Initials			

	Foliage and Bag Examination		
	Live Larvae	Dead Larvae	Total Budworms
Original Count			
1st Check			
2nd Check			
Total			

Comments:

Foliage Examination Form

Pre-Spray ♀ Post-Spray ♀

Location PIC1 Plot No _____ Tree No. T1

Defoliation _____ %

Date Collected June 5/19 Date Counted June 22 2019 Date Checked (1) June 22

Date Checked (2) June 22 2019

Original by RD 1st Check by HJ 2nd Check by KH

Sample	Foliage from bag (larvae)		Petrie Check (microscope)		
	Live	Dead	Live	Dead	Total
Original	/	/	/	/	0
1st Check	\	\	\	\	Ø
2nd Check	/	..	/	..	2
Total	0	2	0	2	2
Comments					

POST-SPRAY Foliage Examination Datasheet

Project Area _____ Plot _____ Tree _____ Date Collected _____

Defoliation _____% Flowers + -

	Original Count	Check
Date Counted		
Staff Initials		

	Larvae		Pupae			Moths	Total
	Live	Dead	Live	Dead	Empty		
Original Count							
Check							
Total							

Comments:

Foliage Examination Form

Post Spray

Location P1C1 Tree No. 1

Defoliation 40 % Flowers + (-)

Date Collected July 16/19 Date Counted Aug 1/19 Date Checked (1) Aug 1/19

Original by HJ 1st Check by CL

Sample	Larvae		Pupae		Moths	
	Live	Dead	Live	Empty		Total
Original						15
1st Check						1
Total	1	4	11	0	0	16
Comments						

Appendix VI

Example Folliage Examination Summary for Plot P1C1 (Project 1; Control 1)

FOLIAGE EXAMINATION SUMMARY

Location Project 1 Plot C1

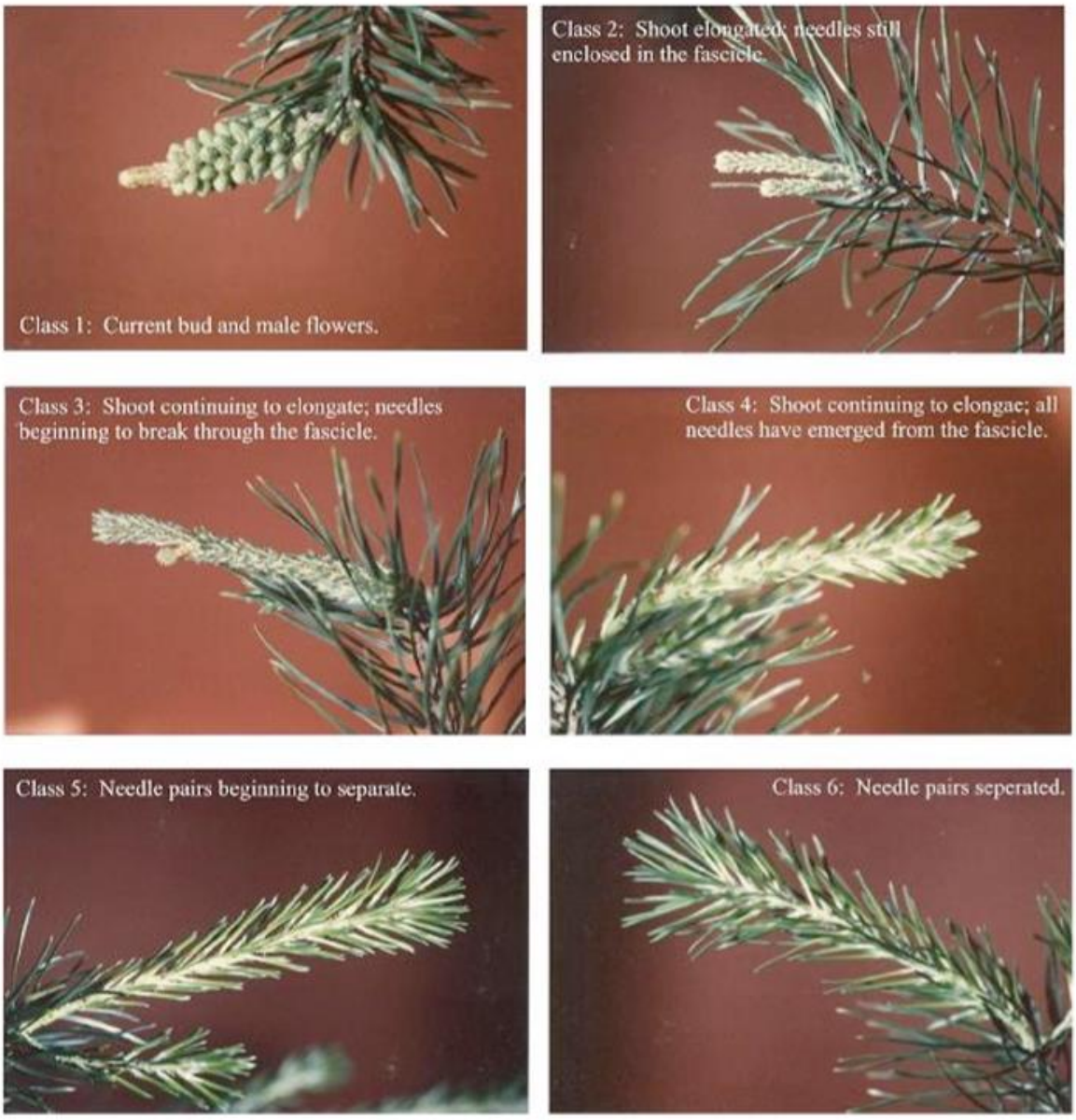
Date Collected Jun 5, 2019 (Pre) Jul 16, 2019 (Post)

	Pre-Spray			Post-Spray		
Tree No.	Total Budworm	Living Budworm	Defoliation (%)	Total Budworm	Living Budworm	Defoliation (%)
1	2	0		16	12	40
2	0	0		3	1	20
3	1	1		25	11	50
4	0	0		2	1	40
5	1	0		14	11	40
6	0	0		11	5	35
7	0	0		3	1	25
8	0	0		21	13	35
9	0	0		7	4	85
10	0	0		44	26	85
Total	4	1		146	85	
Avg.	0.4	0.1		14.6	8.5	45.5

Comments:

Appendix VII

Shoot Class Images



Source: BioForest Technologies 2010.

LIST OF TABLES

LIST OF FIGURES