A PROPOSAL TO IDENTIFY AND PRODUCE WEEVIL-RESISTANT WHITE PINE

Theme 3

Principal Investigators: Michael S. Greenwood (Mike_Greenwood@umit.maine.edu) and Robert S. Seymour (rseymour@maine.edu) ; School of Forest Resources, University of Maine, 5755 Nutting Hall, Orono, Maine, 4469-5755

Graduate Research Assistant: Jason Schatz

Cooperator: Greg Adams, Silviculture Manager, JD Irving Ltd. Sussex Tree Nursery

RR4, Sussex, NB EOE1PO

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A replicated clonal plantation of eastern white pine was established on the University of Maine's Demeritt Forest during June, 2008, for the purpose of studying resistance to attack from the white pine weevil (*Pissodes strobi* Peck). Procedures for capturing and rearing weevils, to ensure equal levels of attack among the planted clones, were also developed.

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http://www.nsrcforest.org

Project Summary

- The white pine weevil (*Pissodes strobi* Peck) is a serious pest of eastern white pine (*Pinus strobus* L.) throughout its range, and is especially damaging when pine saplings grow vigorously in open conditions. This pest effectively prevents white pine from being planted under conditions where it would otherwise thrive. If genetic resistance to weevil attack could be demonstrated, then the silviculture of this important northeastern tree species could change radically for the better, since artificial regeneration would thus be feasible.
- We acquired over 64 clonally propagated strains of eastern white pine from two different sources in New Brunswick, Canada, selected mainly for superior growth and quality, and in 2008, established a replicated field study in Old Town, Maine, to test the hypothesis that there is genetic variability, and ideally, resistance, to weevil attack. We also developed field and laboratory procedures to capture weevil pupae in the wild and rear them under controlled conditions to the adult stage where they can then be released on host trees to ensure that all individuals in the test population are subject to equal rates of attack.
- During the growing season (2008) after planting, a seedling debarking weevil (*Hylobius congener*) reached epidemic populations and caused some seedling mortality before being controlled by insecticide treatment. The following year (2009) grass competition on this fairly fertile site became severe, causing further mortality, mainly of the containerized seedlings which were fairly small when planted. During 2010, all trees were treated with the pre-emergent herbicide Simazine, which resulted in very effective grass control. Four of the 10 main 64-clone replicates were restored to full stocking by replacing dead trees with extras from a surplus population established on the same site in 2008. All trees in these surviving replicates were then protected with 4 x4 foot brush blankets, which effectively removed all competition from the nearby environment of the seedlings and resulted in excellent recovery and growth. All seedlings were measured for height, and the tallest reached slightly over one meter. At the beginning of the 2011 growing season, most trees were reaching the stage where they are potentially susceptible to weevil attack.

Background and Justification

- White pine has been a mainstay of the timber industry in New England for centuries, and nearly 10% of the sawtimber volume in New England forests is white pine, more than any other single species.
- About 700 million board feet are sawn in New England each year (almost all of it in Maine), with a value of about 300 million dollars.
- 48% of mill owners report difficulty in obtaining white pine logs, and many are concerned about their quality.
- Plantation-grown *radiata* pine from New Zealand and Chile is competing with white pine, since its wood is similar in appearance and cheaper to obtain.
- White pine is relatively fast growing, does well in mixed-wood stands and is easy to plant, but is heavily attacked by the white pine weevil during the sapling stage when growing in the open.

Background and Justification

- The white pine weevil (*Pissodes strobi* Peck), a native insect which attacks the terminal shoots of white pine, is ubiquitous through out the range of white pine. Adult weevils emerge from the duff in the spring, climb or fly to the top of the tree and lay eggs in feeding cavities at the base of the bud cluster of the terminal shoot. The larvae feed on the inner bark and later the cambium and developing xylem of the expanding shoot, and if numerous enough, kill the new shoots completely (Fig. 1A).

Background and Justification

- While weevil attacks are rarely lethal, but often result in multi-stemmed trees, that while healthy, have no value for lumber (Fig. 1B). When weevil attack occurs, height growth is reduced by 40-60% in that year (Hamid et al. 1997). Losses of about 40% of potential volume and board quality reductions of 1 to 3 grades have been reported.
- A white pine provenance test in western Maine experienced 40% of the trees being attacked each year, with attack frequency varying from 15 to 70%. Most of the trees were successfully attacked 2-7 times each over 11 years. Trees exhibiting more rapid growth were attacked with a significantly higher frequency than those growing less rapidly.

Background and Justification

- Genetic resistance to the white pine weevil has been demonstrated in interior spruce in British Columbia. Progeny of full-sib families created from resistant parents were 4-fold more resistant to the weevil than progeny from susceptible parents (20% of the resistant progeny showed top kill versus nearly 90% for susceptible progeny). Rapid evaluation of resistance was achieved by inoculating each tree with 3-4 weevils, which created uniform weevil pressure throughout the tests, and effective screening could be completed in 3-4 years. Weevil resistance is highly heritable in interior spruce.
- Selection of weevil resistant trees is difficult when working with material of unknown genetic background because the ratio of truly resistant trees to inherently susceptible escapes may be low even when attacks are frequent. At best, one must wait many years to detect resistant individuals within a test, and even then resistance shown by individual trees must be confirmed by testing their progeny.
- Deploying clones of individual trees, where individual genotypes can be replicated throughout a test, will permit the most precise identification possible of truly resistant individual genotypes. A clonal test, containing a number of clones can be grown under optimum conditions at close spacing, and then each tree can be inoculated with weevils harvested from field-grown trees. This could greatly increase the precision of testing for weevil resistance over a much shorter period of time than has been previously possible.

Methods – Part I:
Weevil Collection, Rearing, and Artificial Inoculation

Leader Collection

- During the last week of July, when most weevils are in either the late larval or pupal stage of their life cycle, weevil-infested leaders were collected from four local sources:
- Leaders were clipped from the main stem at least 3 inches below the lowest extent of weevil damage; weevil damage was marked by dead needles, dying lateral branches, resin exudation streaks, softness of the bark (indicating that the stem had been girdled), and senescence of the stem. Infested trees from which leaders were collected ranged in height from approximately 4 to 20 ft, with most being under 8 ft tall.
- Clipped terminals were trimmed of most lateral branches and placed in a bucket to be carried back to the vehicle. The bucket contained about 1 inch of water in order to keep cut leaders moist, but this was not necessary.

Methods – Part I:
Weevil Collection, Rearing, and Artificial Inoculation

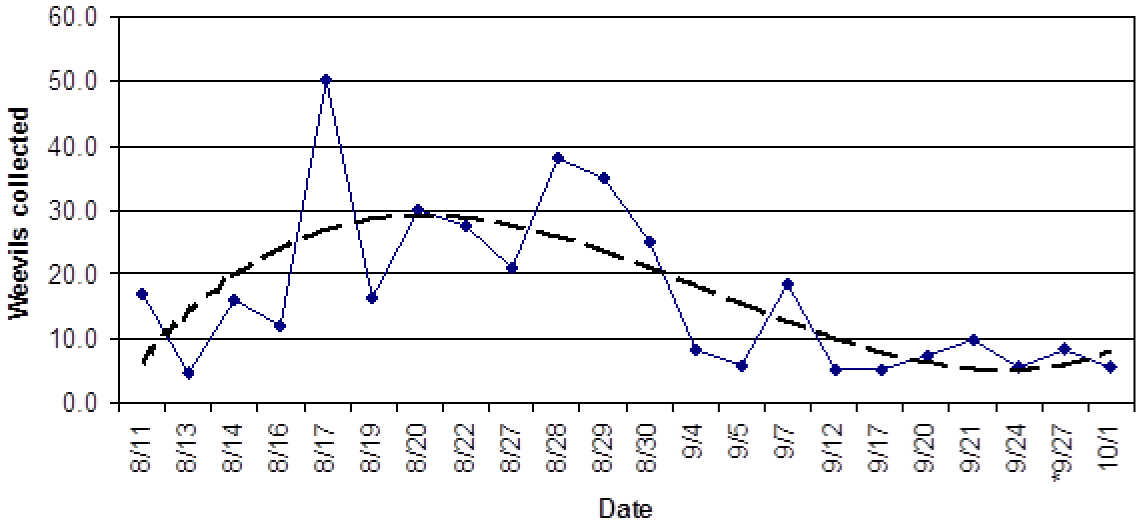
Weevil Collection and Rearing

- Leaders were distributed among nine 42-gallon plastic garbage bins (hereafter called emergence cages), with 15-23 leaders in each emergence cage. The cages were housed beneath plastic sheeting in order to prevent the bins from filling with water and drowning the weevils.
- The bottom of the emergence cages was covered with a two-inch layer of peat moss in which the cut end of leaders was buried. The peat was kept moist in order to prevent leaders from drying out. The emergence cages were covered by nylon screen, which was fastened tightly to the emergence cage with wire. Fresh pine branches were placed in each cage in order to attract and nourish emerging weevils. Pine branches were replaced weekly.

Methods – Part I:
Weevil Collection, Rearing, and Artificial Inoculation

Weevil Collection and Rearing (continued)

- During the second week of August, weevils began to emerge from leaders. Most weevils were found either on the screens or on the fresh pine branches. Weevils were collected every 1-3 days and placed in screen-covered 10-gallon buckets (hereafter called rearing cages). Weevils do fly, particularly on warm days, and can quickly crawl out of lidless buckets, so instead of transferring weevils one-at-a-time from collection cages to rearing cages, I recommend first collecting weevils in a small container with a tightly fitting lid, and then transferring those weevils to the rearing cages all at once.



Methods – Part I:
Weevil Collection, Rearing, and Artificial Inoculation

Weevil Collection and Rearing (continued)

- Rearing cages were protected from rain by overhead plastic sheeting. Fresh pine branches were placed in each rearing cage in order to nourish the weevils. The branches were placed in a plastic cup filled with moist peat moss in order to keep the branches from drying out, though the water and peat moss were probably not necessary and actually may have negatively impacted weevil survival/collection rates by drowning weevils and/or making them difficult to find. New pine branches were placed in the cage at least once per week. Old branches were typically left in the cages until they had senesced completely, because week-old branches often still hosted weevils that had to be picked off before the branches could be removed, and unnecessary handling of the weevils was avoided.
- About 50 weevils, on average, were placed in each rearing cage, though 105 weevils occupied one cage with no apparent problems. As long as weevils are provided with sufficient food, population density does not seem to be an issue.

Methods – Part I:
Weevil Collection, Rearing, and Artificial Inoculation

Weevil Emergence

- Approximately 96 spruce leaders (mainly black spruce, with a few Norway spruce) and 56 white pine leaders were collected.
- On average, 3.9 weevils were collected per spruce leader and 6.5 weevils were collected per white pine leader.
- In total, 742 weevils were collected from 152 leaders. This emergence rate is lower than that reported by most studies, but several observations suggest that more weevils emerged than were collected.
- Several weevils were observed escaping the cages. In fact, only 557 of the 742 weevils originally collected were recovered from rearing cages for the inoculation trials.
- The cut ends of the leaders were buried in a two-inch layer of moist peat moss in order to prevent the leaders from drying out. The peat provided the weevils with ample hiding places, and weevils buried in the peat were very difficult to find. Casual observations suggested that several dozen weevils, if not more, were hiding in the peat.

Methods – Part I:
Weevil Collection, Rearing, and Artificial Inoculation

Artificial Inoculation to augment natural populations

- During the second week of October, 2007, five weevils were placed on each of 100 white pine naturally regenerated saplings in the future plantation location in order to augment natural weevil populations. Trees varied in leader dimensions and ranged in height from 1-3m, which is the proposed height at which our resistance study trees will be inoculated.
- Because most weevils overwinter beneath the trees from which the emerge, our weevils probably went into hibernation beneath the target trees by the end of October or beginning of November. Assuming 40-60% overwintering survival (as has been reported in other studies), 2-3 weevils would emerge to inhabit each tree in the spring (in addition to weevils from the background population).
- In resistance trials, it is important to ensure both high numbers and an even distribution of weevils in order to expose all trees to a similar level of attack. If high infestation rates are achieved, then augmentation can be judged as successful. Also, we expected this pilot study to provide valuable information on the heights and leader dimensions that trees have to attain before weevil augmentation and resistance trials become practical in the planted clonal population.

Results – Part I: Weevil Collection, Rearing, and Artificial Inoculation

- In mid-July, 2008, inoculated trees were surveyed for weevil damage. Of the 100 inoculated trees, 13 were successfully weeviled (Table 1) and most trees showed evidence of at least some weevil feeding.

Table 1.

Height (m)	Leader length (m)	Top diam (mm)	Middle diam (mm)	Bottom diam (mm)
1.55	0.67	7.5	9	12
1.88	0.67	7.5	9.6	12.3
1.12	**0.33**	4.5	5.1	6
1.94	0.37	5.6	7.5	10.9
1.17	0.36	5.4	6.6	7.7
1.55	0.77	5.2	7	9.5
1.91	0.46	5.8	6.9	11
1.96	0.58	5.8	7.5	10.6
1.91	0.80	7.8	10.4	13.5
1.66	0.47	6.3	7.2	8.8
1.99	0.65	7.5	10	13
0.91	0.39	**4.2**	**4.4**	**5.6**
1.13	0.65	5.2	6.6	8.6

Results – Part I: Weevil Collection, Rearing, and Artificial Inoculation

- Height did not significantly affect weevil attack incidence, though weeviled trees were taller, on average, than unweeviled trees (Table 2).
- Weevil attack incidence was significantly affected by leader dimensions, with longer, thicker leaders having a greater likelihood of attack (Table 2). This suggests that as long as our study trees produce sufficiently large leaders, the height and age of our trees are not necessarily relevant.
- One successfully attacked tree (the 2nd to last tree in Table 1) was <1m in height with a relatively thin leader, but most attacked trees were larger and tended to be growing in relatively open areas (Jason Schatz, personal observation).

Table 2.

Parameter	Unweeviled(n=87)	Weeviled (n=13)	Overall Range	Effect on weeviling (p-value)
Height (m)	1.40 ± 0.05	1.59 ± 0.11	0.5 - 3.1	NS
Leader length (m)	0.43 ± 0.01	0.55 ± 0.05	0.15 - 0.80	< 0.01
Top diam (mm)	4.98 ± 0.13	6.02 ± 0.33	3.2 - 8.4	< 0.01
Middle diam (mm)	6.05 ± 0.17	7.52 ± 0.50	3.3 - 13.4	< 0.01
Bottom diam (mm)	7.80 ± 0.24	9.96 ± 0.70	3.4 - 16.2	< 0.01

Methods – Part II:
Establishment and Maintenance of a Clonal Test Plantation

Study Site

- The study site is located in the Dwight B. Demeritt forest on the College Avenue Extension in Old Town, ME, adjacent to the University Forest offices and bordered by the Lucy Thompson forest loop road.

Site Preparation

- In 2007, the overstory was dominated by red pine in the northern ¾ of the study site and Norway spruce on the southern ¼. The site was clearcut in fall 2007, creating an approximately two-acre open area for planting. Herbicide was applied in late fall 2007, and significant herbaceous competitor growth did not arise again until early summer 2008.

Methods – Part II:
Establishment and Maintenance of a Clonal Test Plantation

Plant Material

- Seedlings of 115 total clones were acquired in early May 2008 from J.D. Irving, Ltd. (contact: Greg Adams) and the Canadian Forest Service (contacts: Yil Sung Park, Ian MacEacheron). 67 clones had 10 or more replicates and became the focus of the resistance trials.
- The seedlings from J.D. Irving were three-year old potted seedlings (photo, lower L) and those from the Canadian Forest Service were either one-year-old containerized seedlings from which a second flush of growth was forced during winter 2007-2008 in greenhouses at the Canadian Wood Fibre Centre, or two-year-old seedlings that had been overwintered in a freezer during winter 2007-2008 (photo, lower R)

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- Seedlings from the Canadian Forest Service were kept in an outdoor shadeframe covered with 60% shadecloth between the date of seedling acquisition (May 2nd) and the dates of planting. Seedlings from J.D. Irving were kept in partial shade (approximately 50%) at the planting site in the Demerritt Forest between the date of seedling acquisition and the dates of planting.

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Methods – Part II:
Establishment and Maintenance of a Clonal Test Plantation

Planting (2008)

Main Blocks - 1-10:

- From May 12th-16th, 2008, 10 replicates of 64 clones were planted at 1.5 m spacing in 10 randomized 8x8 tree blocks with one of each clone planted in each block. Seedlings were watered on May 15th, 21st, 27th, and 30th during a period of warm, dry weather.

Main Blocks - 11-15:

- From June 16th-20th, 2008, 320 seedlings were planted at 1.5 m spacing in 5 randomized 8x8 tree blocks. These blocks were mainly comprised of additional replicates of clones planted in blocks 1-10 as well as other well-replicated clones.

Border row:

- On June 24th, 2008, 132 seedlings were planted at 1.5 m spacing in a randomized border row around the previously planted seedlings (blocks 1-15) in order to create more uniform light exposure conditions for seedlings in blocks 1-15. Generally, only clones with seven or fewer replicates were planted in the border row.

Methods – Part II: Establishment and Maintenance of a Clonal Test Plantation

Extras for Main Blocks

- On June 25th-26th, 2008, 276 seedlings were planted at 1 m spacing in a single randomized 12x24 tree randomized block at the north end of the planting site. These seedlings are extras from the 64 clones planted in blocks 1-10 and are intended for transplanting in spring 2009 when mortality has been assessed in blocks 1-10. Seedlings were watered on June 26th in order to help seedlings become established during a period of warm, dry weather.

Clonal Blocks

- On July 2nd, 135 seedlings were planted at 1.5 m spacing at the south end of the planting site. Each of 12 different clones was planted in an approximately 2x6 tree single-clone block, with blocks of each clone located adjacent to one another. The clonal blocks were intended to demonstrate phenotypic differences between different clones.
- All seedlings were labeled with sturdy aluminum tags with their clone number.

Methods – Part II:
Establishment and Maintenance of a Clonal Test Plantation

Seedling Mortality from *Hylobius congener*

- By mid-July 2008, approximately 5% of planted seedlings had died and another 5% exhibited damage similar to that observed on dead seedlings.
- Some minor browsing, apparently by deer or small mammals, was observed, and several seedlings appeared to have died due to desiccation, probably caused by insufficient root development in some smaller seedlings and a rainless period during the first three weeks of July, 2008.
- The majority of damage and mortality was caused by the seedling debarking weevil (*Hylobius congener*). The debarking weevil is common in eastern North America and is attracted to open areas of recently cut coniferous forests. For the first two years after cutting, adults breed in stumps, logs, and slash and feed on the bark of softwood seedlings. The bark is eaten down to the wood (Fig. 2A-C), which can girdle branches or entire trees if feeding is sufficiently heavy.

Methods – Part II: Establishment and Maintenance of a Clonal Test Plantation

Typical damage to a white pine seedling lateral branch attacked by the seedling debarking weevil (*Hylobius congener*).

Methods – Part II:
Establishment and Maintenance of a Clonal Test Plantation

- Seedling damage was surveyed on July 31st, 2008. Injuries evidently caused by the debarking weevil were recorded as 0 (dead), 1 (mortally injured), 2 (severely injured), 3 (moderately injured), 4 (slight injury), and 5 (no damage).
- As of July 31st, 2008, 9% of planted seedlings had died and an additional 7% were severely damaged (i.e. injury levels 1 and 2) (Table 4A).
- In all, 25% of seedlings were either dead or appeared to be in some danger of dying (i.e. injury levels 0-3).
- Of the original 67 clones with 10 or more ramets, 56 had 10 or more ramets at injury levels 4-5, while 59 had 10 or more ramets at injury levels 3-5.

All Seedlings **A**

Injury level	Count of seedlings	Percent of Seedlings	Cumulative %
0	136	9.1	9.1
1	35	2.3	11.4
2	71	4.7	16.1
3	133	8.9	25.0
4	165	11.0	36.0
5	962	64.0	100.0
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Methods – Part II:
Establishment and Maintenance of a Clonal Test Plantation

Debarking Weevil Control

- The simplest control method is to delay planting until two years after cutting in order to allow the weevils to complete their lifecycle. However, several insecticides are also effective in controlling the debarking weevil. Prior to planting, seedlings can be treated with a dip. During planting, seedlings can be treated with soil systemics, such as Imidacloprid. Systemics can be applied after planting but should ideally be administered during the active growth phase of the seedlings in order to facilitate uptake.
- During active adult phases of the weevil life cycle, sprays such as permethrin can be effective (chlorpyrifos and carbofuran are also recommended, though carbofuran is for nursery seedlings only).
- In our study, the site was treated with Bifenthrin on August 5th, 2008 by spraying each seedling and the immediate surrounding area.

Methods – Part II:
Establishment and Maintenance of a Clonal Test Plantation

Competition Control (Simazine treatment) – Spring, 2010

- Although the initial site preparation herbicide was quite effective, the plantation was overtaken by a dense mat of grass during the 2009 growing season, which was especially rainy.
- During early April, 2010, when the vegetation was still dormant, the plantation was sprayed with Simazine (an effective pre-emergent herbicide) at a rate of 5 pounds per acre, in spots about 3 feet in diameter around each visible surviving tree.

Methods – Part II:
Establishment and Maintenance of a Clonal Test Plantation

Competition Control (Simazine treatment) – Spring, 2010

- The Simazine treatment was highly effective, resulting in excellent grass control that extended well into the late summer.

Methods – Part II:
Establishment and Maintenance of a Clonal Test Plantation

Competition Control (Brush Blankets) and Mortality Replacement – May, 2010

- During May, 2010, we utilized teams of first-year forestry students enrolled in summer camp to refill dead trees and install brush blankets around living trees.
- Blankets are 4’ x 4’ with a central hole for the tree.
- 9 staples (each corner, the midpoints of each site, plus the center near the tree) hold the blankets tightly to the ground

Methods – Part II:
Establishment and Maintenance of a Clonal Test Plantation

Competition Control (Brush Blankets) and Mortality Replacement – May, 2010

- Workers positioning blankets around a JD Irving potted seedling.

Methods – Part II:
Establishment and Maintenance of a Clonal Test Plantation

Competition Control (Brush Blankets) and Mortality Replacement – May, 2010

- Worker digging a hole to replace a dead seedling from the same clone in the replacement block.

Methods – Part II:
Establishment and Maintenance of a Clonal Test Plantation

Competition Control (Brush Blankets) and Mortality Replacement – May, 2010

- Worker replanting a replacement seedling of the same clone that died. Note the effective grass control by the Simazine treatment about one month before.

Methods – Part II:
Establishment and Maintenance of a Clonal Test Plantation

Competition Control (Brush Blankets) and Mortality Replacement – May, 2010

- A small containerized seedling that had previously been completely covered by dense grass competition.

Methods – Part II:
Establishment and Maintenance of a Clonal Test Plantation

Competition Control (Brush Blankets) and Mortality Replacement – May, 2010

- Two views of the plantation after successful installation of the blankets and refilling mortality trees from the replacements.
- In all, 4 of the 10 original replicates were completely restored.

Methods – Part II: Establishment and Maintenance of a Clonal Test Plantation

Height Measurements – May, 2010

- All trees on the 4 restored replicates were measured for height at the end of the 2009 growing season. This was the tallest seedling, slightly over 1 m tall and entering the “window” where susceptibility to the white pine weevil begins.

Methods – Part II:
Establishment and Maintenance of a Clonal Test Plantation

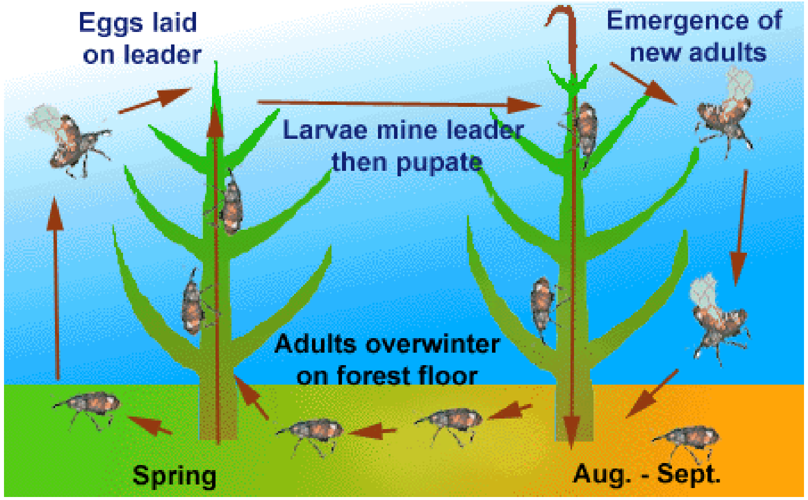
Current Status – April, 2011

- The photos below show the condition of the experiment after the 2010 growing season.
- On the left, we see a portion of the plantation without blankets; on the right, we see the restored replicates with blankets (mostly still under the snow).
- Casual inspection showed excellent survival of the replacement trees, as well as excellent leader growth and recovery of the surviving trees.

Methods – Part II:
Establishment and Maintenance of a Clonal Test Plantation

Future Plans

- After the 2011 growing season, the study trees should be susceptible to weevil attack.
- Although the establishment stage of the study is complete, we plan to continue monitoring and measurement after the 2011 growing season.
- Complete measurements will be made on the 4 restored blocks.
- Survival on the remaining blocks will also be assessed.
- If resources permit, we will collect and rear weevils over the upcoming winter, and challenge the trees during May of 2012.



Implications and applications in the Northern Forest region

- If any clones prove to be resistant, then artificial regeneration of white pine (via somatic embryogenesis) could revolutionize the white pine industry in the Northeast by allowing artificial regeneration in open environments.

Products

- This study focused on developing **rearing procedures** for the white pine weevil (to ensure equal probabilities of attack), and on establishing a **clonal test plantation** for future research on this important topic. Although the plantation experienced unexpected mortality from *Hylobius congener* and grass competition, both objectives were completed successfully.
- Jason Schatz, Graduate Research Assistant (who was funded for one year on this project) produced an exhaustive, 75-page review of the literature entitled *"The White Pine Weevil (Pissodes strobi Peck): Biology, Resistance, & Experimental Methods" (available from PI Seymour upon request)*
- This review includes 20 Tables, 13 Figures, and nearly 200 references on this topic.
- This document also fully describes the field study design and the sources of all clones planted.