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Enhancing Stand Structure through Snag Creation in Northeastern U.S. Forests: Using Ethanol Injections and Bark Beetle Pheromones to Artificially Stress Red Maple and White Pine

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Abstract: We investigated two methods to create white pine and red maple snags in a forested setting. The first involved injecting trees with ethanol at two times (single Ethanol (ETOH) and double ETOH injections) to increase attractiveness to insects and elicit attacks on trees. The second method was unique to white pines and involved both injection treatments in combination with baiting trees with *Ips*-specific pheromones. Three of five white pines from the double ETOH treatment died in the second year. Species including *Ips pini* (Say), *Ips grandicollis* Eichhoff, *Orthotomicus caelatus* Eichhoff, *Crypturgus borealis* Swaine and *Monochamus notatus* (Drury) responded more strongly to at least one of the treatments over control trees. However, there were no differences found in individual Scolytinae or Cerambycidae species response to treatments in red maple. Fitness (F_V/F_M) and vitality (PI_{abs}) were both significantly reduced in both ETOH treatments compared to controls in white pine. In red maple, fitness was reduced in the double ETOH treated trees but the final mean F_V/F_M values were within the approximate optimal of health. Ethanol injections, in combination with *Ips*-specific semiochemicals, show promise for creating standing coarse woody debris (CWD) in white pine. Injecting ethanol was not effective for stressing red maple.

Keywords: coarse woody debris; tree stress; tree colonization; tree fitness; tree vitality; arboreal beetles

1. Introduction

Forests of the northeastern United States have undergone dramatic changes since European settlement in the 1600s [1]. Landscape level reforestation occurring after the industrial revolution led to large areas of second growth forest that were structurally similar and more homogenous in the eastern U.S. [2–4]. Structural characteristics, such as downed wood and standing snags were not traditionally important considerations in managed forests. However, as a better understanding of CWD importance to healthy forests developed [5], natural resource managers began integrating these structural characteristics into management plans.

Coarse woody debris occurs in the form of standing dead trees (snags) and downed dead tree limbs, boles and roots in various stages of decay classes. Coarse woody debris forms structural features that serve key ecological functions in the form of habitat for organisms [6,7], and energy flow in the form of nutrient cycling [8]. Forests develop over time with a continual flux of CWD as stand dynamics transition from early-successional pioneer to late-successional old growth. This results

in a diverse array of vertical and horizontal CWD in various stages of decay providing habitats to diverse communities.

Snag management is considered important when developing stand structural features suitable for cavity nesting wildlife and other species dependent on this community component [7,9]. Snag habitat varies by seral stage or forest management history, but snags are generally more abundant and diverse in older and unmanaged forests [10–14]. A wide array of organisms depend on the presence of snags and downed wood, ranging from microorganisms to vertebrates [15–19]. Insects and microorganisms are particularly important for CWD decomposition and initiate decay in much of this material [20].

Trees experience various biotic and abiotic stressors over time and stand density and composition drive resource competition that ultimately generates snags throughout a stand. The physiological cost of metabolic defense against these stressors ultimately weakens trees, making them more susceptible to future stress events. Volatile synthesis, particularly ethanol, acetaldehyde, ethylene, and ethane [21] often results in response to severe stress. Ethanol has been found to accumulate in high enough concentrations, during severe stress episodes, that it acts as a primary attractant for ambrosia beetles that typically colonize recently dead and dying trees [22]. Techniques have been developed to artificially inject trees with ethanol to promote ambrosia beetle colonization [23,24]. These techniques have largely been developed to monitor flight and colonization to properly time insecticide applications in nursery stock [23,25–27].

Various methods have been attempted to increase snag abundance in forests. Artificial methods, including using mechanical wounding (*i.e.*, girdling, topping, or shooting), fungal inoculation, herbicide injection, and insect pheromone baiting, and a combination of these methods have been used with mixed results [28–31]. Some of these methods are not appropriate for use in eastern forests, or are problematic because of regulations or management desires (e.g., pesticides).

Ethanol accumulation has been documented in water-stressed trees where it diffuses into live cells in the stem and foliage [32,33]. As trees experience increased stress (drought, resource competition, extreme temperatures, nutrient deficits, insect and disease attack), photosynthesis is reduced and plants shift towards photoprotection mechanisms to safely dissipate excess energy [34]. Chlorophyll fluorescence is a measure of photosynthetic efficiency and illustrates the relationship between structure and function of Photosystem II (PSII), Reaction Center (RC) and core complexes [35]. Chlorophyll fluorescence has been used to measure the physiological status of plant performance (fitness and vitality) under a large range of conditions [36], including the effects of insect herbivory [37,38]. More specifically, chlorophyll *a* fluorescence provides direct information on the molecular structure and function of Photosystem II (PSII). Photochemical changes due to stress can be measured through a unique group of fluorescence parameters derived from chlorophyll *a* fluorescence kinetics. The maximum quantum yield of PSII photochemistry (F_V/F_M) can be used to measure solar energy conversion to fixed carbon and serve as a strong indicator of overall plant fitness [39]. Plant vitality is characterized by the Performance Index (PI_{abs}) [40] to best explain the functionality of both photosystems I and II while providing quantitative data on plant performance under stress [41].

We investigated the potential of two methods for creating white pine and red maple snags. The first method involved injecting trees with ETOH and was used on both tree species. Ethanol injections have been effective for artificially stressing hardwoods and attracting ambrosia beetles [23,27], but have not been tested for effectiveness in forested settings. The second method used was unique to white pine and involved baiting trees with *Ips*-specific pheromones with the hope of initiating mass attack by *Ips pini* and/or *Ips grandicollis*. These two methods have the potential to promote beetle activity and the necessary stress to create snags in a managed forest. Incoming arboreal beetles were captured and tree decline was monitored through chlorophyll fluorescence. We chose a recently harvested stand that is being managed to promote horizontal coarse woody debris recruitment (slash was left on site during silviculture treatments) but lacks standing dead trees. Our objective was to use ethanol injections and semiochemicals to test methods for recruiting standing dead and stressed trees in managed forests.

2. Materials and Methods

2.1. Site

This study was conducted on a ~67 ha woodlot owned and managed by the Mount Grace Land Conservation Trust located in the town of Ashburnham, MA, USA. The soils are classified as mostly dry, rocky tills and the overstory is dominated by white pine, *Pinus strobus* L., northern red oak, *Quercus rubra* L., and red spruce, *Picea rubens* Sarg., with a mix of red maple, *Acer rubrum* L., sugar maple, *Acer saccharum* L., black birch, *Betula lenta* L., and black cherry, *Prunus serotina* Ehrh., as minor components. Site elevation ranged between 327–345 m. A shelterwood harvest conducted in 2011 targeted the poorest quality and lowest vigor trees. A second entry to remove the final overstory will occur in 10–15 years.

2.2. Ethanol Injections

Fifteen red maple and fifteen white pine were randomly selected from residual overstory trees distributed throughout the harvested area. Trees from each species were paired based on crown class, diameter at breast height (dbh) similarities, and spatial location. All experimental trees were in the codominant/dominant canopy class. Average dbh of red maple was 23.8 $\pm$ 0.61 cm and white pine was 27.7 $\pm$ 0.66 cm. All trees appeared healthy at the initiation of the experiment and had no perceptible insect or pathogen damage on boles. Canopy condition of trees was also healthy with no significant damage to limbs or branches present.

Selected trees were grouped within species and randomly assigned to either a single ETOH injection, a double ETOH injection, or a control. Experimental trees were grouped as close to one another as possible but generally separated by at least 25 m. At least 50 m separated experimental blocks from one another. Single ETOH treatment trees were injected with 500 mL of 95% ETOH approximately 30 cm from the base of the trees on 26–27 March 2012, and double ETOH treatment trees were treated with an additional 500 mL of ETOH on 7 June 2012. All treatment trees were injected again on 7–9 May 2013 and double ETOH treatment trees were treated with an additional 500 mL of ethanol on 5 June 2013. An Arborjet Tree I.V. (Arborjet, Woburn, MA, USA) injection system along with #4 plugs was used to inject ethanol in both tree species. Four evenly spaced holes were drilled approximately equidistant from one another around the base of each tree. New holes were drilled equidistant from previous holes, and new plugs were used for double ETOH treated trees. Holes passed through the bark and phloem and into the sapwood. Injection plugs were set into holes with a soft mallet. Viper valve assemblies with needles were pushed into injection plugs at all four points and opened sequentially after liquid tanks were filled with 60 psi of pressure. No holes were drilled into control trees. Average dbh for the white pine control trees was 28.9 $\pm$ 1.63 cm, 26.9 $\pm$ 1.73 cm for the single ETOH treatments, and 25.9 $\pm$ 1.47 cm for the double ETOH treatments. Average dbh for red maple control trees was 23.1 $\pm$ 0.64 cm, 24.1 $\pm$ 1.27 cm for the single ETOH treatments, and 24.9 $\pm$ 1.85 cm for the double ETOH treatments.

In addition to the single or double ETOH injections, pheromones targeting *I. grandicollis* and *I. pini* were also attached to white pine treatment trees. Ipsenol, ipsdienol, and lanierone (Contech Inc., Victoria, BC, Canada) were attached at breast height and changed every six weeks. Release rates for ipsenol, ipsdienol, and lanierone were approximately 0.2 mg/d, 0.2 mg/d, and 0.02 mg/d, respectively. These compounds are also kairomones for other bark beetle and woodborer species [42–44].

2.3. Traps

One 12-unit multiple-funnel trap (Synergy Semiochemicals, Burnaby, BC, Canada) with a wet collection cup was affixed to every tree at approximately 3 m from the ground. A 0.4 m plant hanger mounted on a piece of brown aluminum roof flashing and affixed to trees with two compression straps was used to attach traps to trees. Compression straps were used instead of screws or nails to avoid damage to control trees that would result in volatile release and an additional attraction for

beetles. A line was attached to traps and fed through a carabiner attached to the plant hanger to allow for lowering and raising during collections. RV antifreeze (Prestone® RV antifreeze, FRAM Group, Lake Forest, IL, USA) was used as the collecting agent and traps were collected every two weeks from 28 March 2012 to 24 October 2012. Scolytinae and Cerambycidae were identified using Wood [45] and Lingafelter [46], respectively. Voucher specimens were deposited in the forest insect collection at the U.S. Forest Service, Durham Field Office, Durham, NH, USA.

Beetle collections were analyzed using ANOVA in JMP version 10 (SAS Institute Inc., Cary, NC, USA). Only species with greater than 50 specimens collected were statistically analyzed. Data were checked for normality and heteroscedacity and transformed to meet the assumptions of ANOVA when needed. Tukey's honest significant difference ($\alpha = 0.05$) test was used to compare differences in trap catches among controls and the two ETOH injection treatments. Paleontological Statistics (PAST) [47] was used for diversity measures and individual-based rarefaction estimates.

2.4. Tree Surveys

All control and treatment trees were examined for signs of bark and ambrosia beetle attacks in August 2012. Exposed bark from the base of the trees up to approximately 3 m were searched for entrance holes, resin exudation, and/or frass that would indicate bark or ambrosia beetle attacks. Characteristics of entrance holes (e.g., size, resin and frass characteristics, and height on the bole) were used to assign a genera or species to each hole. Beetles were not excavated from experimental trees.

2.5. Chlorophyll Fluorescence

Healthy and unhealthy vegetation demonstrate differences in pigment and moisture content. Healthy cells are more capable of quenching and utilizing light energy towards photosynthetic processes than dead or damaged cells. Chlorophyll fluorescence is the process of light being re-emitted by chlorophyll molecules and serves as an indicator of photosynthetic energy conversion in vegetation. A Handy PEA fluorimeter (Hansatech Instruments, Norfolk, UK) using a high light emitting device (LED) light source with rapid measurement capabilities was used to capture the polyphasic rise in chlorophyll fluorescence known as the Kautusky Induction [48]. Each control, single ETOH, and double ETOH tree was used for chlorophyll fluorescence measurements. All foliage was collected from sunlit branches, stored in plastic collection bags, and kept cool until they were brought back to the lab for analysis. All foliar samples were processed within 12 h of collection. All samples were placed into a dark adaptation clip for 30 min and then subjected to a saturating red actinic light intensity of 1500 $\mu Mol \cdot m^{-2} \cdot s^{-1}$ for a duration of 1 second. All samples were grouped by treatment (control, single ETOH treatment, double ETOH treatment). The maximum quantum efficiency of PSII photochemistry (fitness) and Performance Index (vitality) values were analyzed using PROC GLIMMIX in SAS 9.3 (SAS Institute Inc., Cary, NC, USA) and are defined below. Tukey's honest significant difference ($\alpha = 0.05$) was used for pairwise comparisons among fluorescence means within and between treatments.

Fv/Fm is defined as:

$$F_V/F_M = (F_M - F_0)/F_M, \quad (1)$$

PIabs is defined as:

$$PIabs = \frac{1 - (F_0/F_M)}{M_0/V_J} \times \frac{F_M - F_0}{F_0} \times \frac{1 - V_J}{V_J}, \quad (2)$$

where F_M is the maximal fluorescence and F_0 is the minimal fluorescence, V_J is the relative variable fluorescence at 2 ms (J-step), and M_0 represents the initial slope of fluorescence kinetics.

3. Results

3.1. Beetles on White Pine

A total of 60 species and 3537 individuals of Scolytinae and Cerambycidae were captured on white pine control and stress treatments during the experiment. There were 3297 Scolytinae from

33 species, and 240 Cerambycidae from 27 species captured. Fifty-four percent of the Scolytinae were *Ips* species. *Orthotomicus caelatus* (12.7%) and *Gnathotrichus materiarius* (Fitch) (8.2%) were relatively common species on trees. *Monochamus notatus* (21%), *M. scutellatus* (Say) (15%), and *Acmaeops proteus* (Kirby) (10%) were the most abundant Ceambycidae captured in traps on white pine.

Traps on treated trees captured significantly more total beetles ($F_{2, 12}$ = 20.8, P = 0.0001), Cerambycidae ($F_{2, 12}$ = 10.6, P = 0.002), and Scolytinae ($F_{2, 12}$ = 20.9, P = 0.0001) than traps on control trees (Table 1). Likewise, more *I. pini* ($F_{2, 12}$ = 15.6, P = 0.0005), and *I. grandicollis* ($F_{2, 12}$ = 8.2, P = 0.006) were captured in traps on the two pheromone-baited ETOH injected treatments compared to traps on control trees. *Crypturgus borealis* were captured in higher numbers in traps on the pheromone-baited double ETOH injection treatment compared to the control and pheromone-baited single ETOH injection treatment ($F_{2, 12}$ = 5.3, P = 0.02). *Orthotomicus caelatus* ($F_{2, 12}$ = 6.1, P = 0.01) and *M. notatus* ($F_{2, 12}$ = 10.4, P = 0.002) were captured at higher numbers in the pheromone-baited double ETOH injection treatment compared to the control, but pheromone-baited single ETOH injection treatment catches were intermediate. No response to treatments was found for *Xyloterinus politus* (Say) ($F_{2, 12}$ = 0.8, P = 0.47), *Pityogenes hopkinsi* Swaine ($F_{2, 12}$ = 0.12, P = 0.88), *I. calligraphus* (Germar) ($F_{2, 12}$ = 3.5, P = 0.06), *Hylastes opacus* Erichson ($F_{2, 12}$ = 0.15, P = 0.86), *Gnathotrichus materiarius* ($F_{2, 12}$ = 0.19, P = 0.83), or *Dendroctonus valens* LeConte ($F_{2, 12}$ = 2.1, P = 0.17).

Table 1. Mean (±SE) number of Scolytinae and Cerambycidae captured on white pine treatment and control trees. Both ethanol injection treatments were also baited with pheromones. Means followed by the same letter within a row are not significantly different (Tukey's honest significant difference $P > 0.05$).

	Control	Single ETOH Injection	Double ETOH Injections	*P*-Value
Total Scolytinae and Cerambycidae	66 ± 9.9 B	318 ± 45.6 A	322 ± 30.2 A	0.0001
Total Scolytinae	61.4 ± 9.4 B	297.2 ± 43.4 A	300.8 ± 27.1 A	0.0001
Crypturgus borealis	2.4 ± 0.4 B	3.8 ± 1.2 B	10.0 ± 2.7 A	0.022
Dendroctonus valens	2.0 ± 0.95	5.0 ± 2.6	7.2 ± 1.5	0.1706
Gnathotrichus materiarius	15.2 ± 3.1	13.6 ± 2.6	17.8 ± 6.9	0.8265
Hylastes opacus	5.0 ± 2.5	5.0 ± 2.2	3.6 ± 1.5	0.8613
Ips calligraphus	0.0 ± 0.0	3.2 ± 1.0	28.6 ± 14.4	0.0624
Ips grandicollis	0.8 ± 0.4 B	23.6 ± 3.8 A	27.8 ± 7.9 A	0.0056
Ips pini	0.4 ± 0.4 B	190.2 ± 33.1 A	114.8 ± 25.6 A	0.0005
Orthotomicus caelatus	9.8 ±2.7 B	21± 6.6 AB	54.2 ± 14.5 A	0.0147
Pityogenes. hopkinsi	8.4 ± 1.7	9.0 ± 2.8	7.6 ± 1.1	0.8844
Xyloterinus politus	2.6 ± 0.7	5.2 ± 1.8	3.8 ± 1.6	0.4661
Total Cerambycidae	5.4 ± 1.6 B	20.8 ± 2.6 A	21.8 ± 3.9 A	0.0022
Monochamus notatus	0.0 ± 0.0 B	4.0 ± 1.4 AB	8.0 ± 1.6 A	0.0024

More species were captured on pheromone-baited single ETOH (49) and pheromone-baited double ETOH (48) injected trees compared to control trees (43). The same pattern was seen for total abundance where 1590, 1613, and 334 beetles were captured on pheromone-baited single ETOH, pheromone-baited double ETOH, and control trees, respectively. Simpsons index was highest on control trees (0.89) compared to pheromone-baited single ETOH (0.63) and pheromone-baited double ETOH (0.82) treatments. Individual-based rarefaction curves demonstrated that the two pheromone-baited ETOH injection treatments had similar numbers of species per number of specimens collected (Figure 1). Control trees captured slightly fewer species, but with almost five times fewer specimens collected.

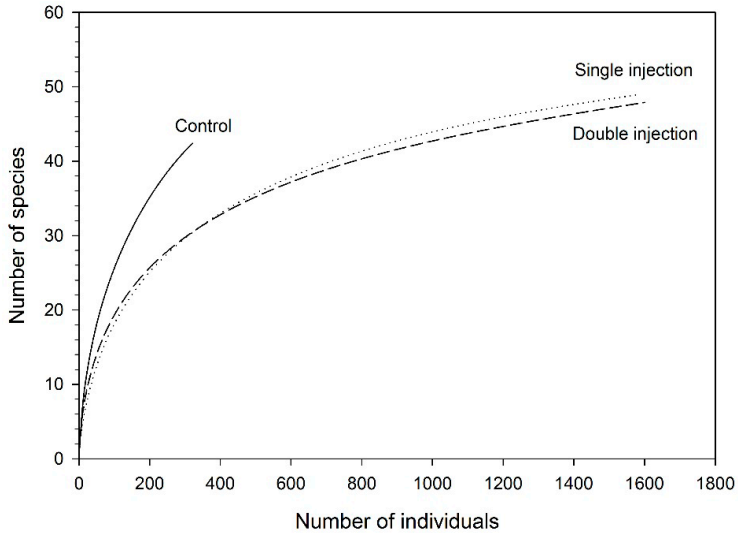

Figure 1. Individual based rarefaction curves for pheromone-baited ethanol injected treatments and control white pines.

3.2. Beetles on Red Maple

A total of 58 species and 925 individuals of Scolytinae and Cerambycidae were captured on red maple control and stress treatments during the experiment. There were 850 Scolytinae from 32 species and 75 Cerambycidae from 26 species captured. *Xyloterinus politus* (22%) and *Gnathotrichus materiarius* (17%) were the most abundant Scolytinae in traps on red maple. Other common Scolytinae included *Pityogenes hopkinsi* (10%), *Orthotomicus caelatus* (9.3%), and *Monarthrum mali* (Fitch) (7%). No Cerambycidae species represented more than 10% of total trap catches. *Trachysida mutabilis* (Newman) (9%), *Acmaeops proteus* (8%), *Urographis fasciatus* (DeGeer) (7%), *Tetropium cinnamopterum* Kirby (7%), and *Clytus ruricola* Olivier (7%) were the most common cerambycids.

There were no differences among average trap catches of total Scolytinae, Cerambycidae, or any of the individual species tested on red maple (Table 2). More species were captured on the single ETOH (32) and double ETOH (37) injected trees than control trees (29). The same pattern was seen for total abundance where 340, 378, and 207 beetles were captured on single ETOH, double ETOH, and control trees, respectively. Simpsons estimates were similar among the control (0.90), single ETOH (0.87) and double ETOH (0.90) treated trees. Individual-based rarefaction demonstrated that double ETOH injected trees captured more species per total numbers collected, while single ETOH and control trees caught fewer (Figure 2).

Table 2. Mean (±SE) number of Scolytinae and Cerambycidae captured on red maple treatment and control trees.

	Control	Single ETOH Injection	Double ETOH Injections	*P*-Value
Total Scolytinae and Cerambycidae	41.4 ± 4.5	68.0 ± 26.1	75.6 ± 14.3	0.4
Total Scolytinae	36.6 ± 3.5	64.6 ± 25.2	68.8 ± 12.6	0.35
Gnathotrichus materiarius	3.6 ± 1.2	8.8 ± 3.6	11.0 ± 3.6	0.25
Hylastes opacus	2.4 ± 0.5	4.2 ± 1.8	3.6 ± 0.9	0.58
Monarthrum mali	0.6 ± 0.4	8.0 ± 4.3	5.6 ± 3.5	0.29
Orthotomicus caelatus	5.0 ± 0.5	5.2 ± 2.4	7.2 ± 2.0	0.65
Pityogenes hopkinsi	6.2 ± 1.2	5.0 ± 2.5	6.0 ± 1.8	0.89
Xyloterinus politus	7.0 ± 1.1	18.6 ± 7.8	14.2 ± 2.4	0.26
Total Cerambycidae	4.8 ± 1.5	3.4 ± 1.2	6.8 ± 3.1	0.5

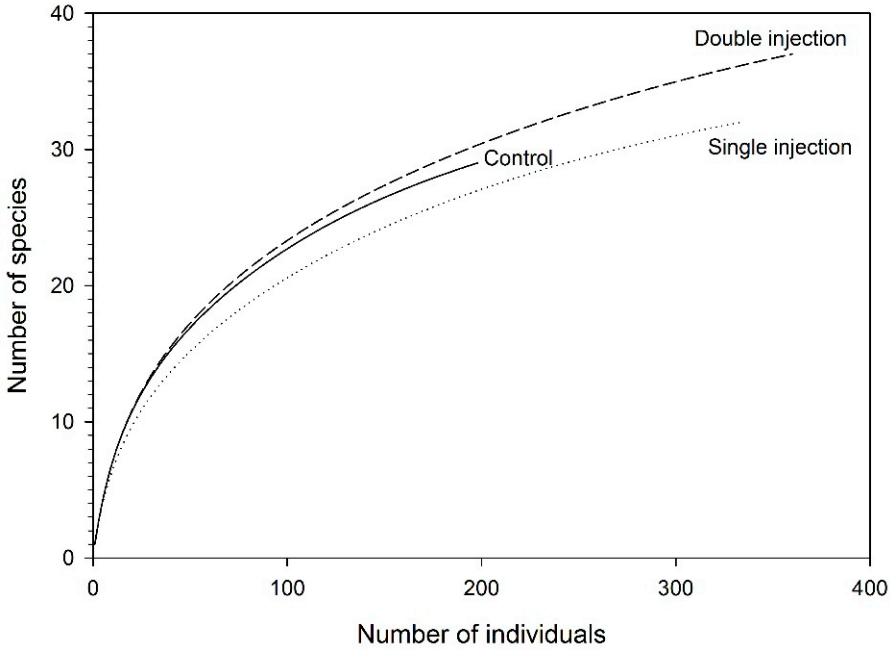

Figure 2. Individual based rarefaction curves for ethanol treated and control red maple.

3.3. Tree Surveys

Four of the five (80%) pheromone-baited single ETOH treated and all of the pheromone-baited double ETOH treated white pine had signs of bark beetle attack in the lower 2.5 m of tree bole. All of these attack sites were attributed to *Ips* species, and, based on trapping results, were most likely *I. pini.* No signs of bark or ambrosia beetles were found on control trees. Surveys of red maple control and treatment trees did not locate any bark or ambrosia beetle attacks.

3.4. Chlorophyll Fluorescence

Three (60%) of the pheromone-baited double ETOH treated white pine died during the second year of this study. There was no visible red maple mortality. In white pine, there was a significant interaction between year and treatment for mean F_V/F_M ($F_{2,2} = 39.48$, $P < 0.001$). Untreated white pine control trees exhibited similar F_V/F_M values within the approximate optimal range in both years, while mean F_V/F_M values for pheromone-baited single- and double-treated white pine significantly declined in 2013, indicating severe moisture stress and an overall reduction in fitness (Figure 3). There was no significant interaction between year and treatment for mean PI_{abs}. However, white pine vitality (PI_{abs}) in both pheromone-baited ETOH treatments was significantly lower than the control ($F_{2,2} = 35.99$, $P < 0.001$) (Figure 4). In red maple, there was a significant interaction between year and treatment for mean F_V/F_M ($F_{2,2} = 32.68$, $P < 0.001$). There was no significant difference between control and single-treated red maple in either 2012 or 2013. However, the double-treated red maple treatment demonstrated a significant drop in overall fitness with a mean that fell below the optimal range (Figure 5). There was a significant interaction between year and treatment for mean red maple PI_{abs} ($F_{2,2} = 15.66$, $P < 0.001$). Mean PI_{abs} for the control and single ETOH treatments improved from 2012 to 2013 and significantly decreased for the double ETOH treatment from 2012 to 2013 (Figure 6).

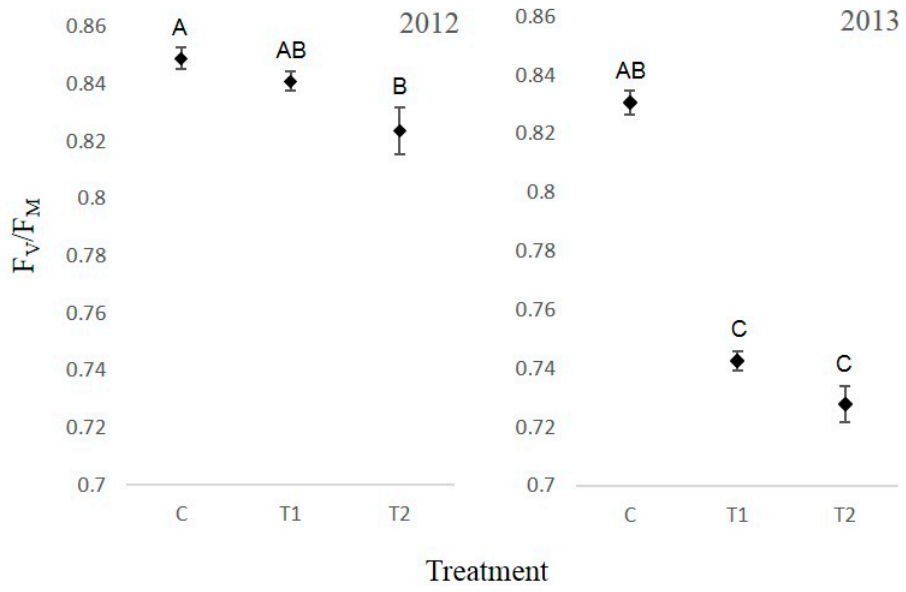

Figure 3. Mean maximum quantum efficiency of Photosystem II photochemistry (F_V/F_M) ± SEM for three levels of white pine treated with ethanol (Control = C, pheromone-baited single ethanol treatment = T1, and pheromone-baited double ethanol treatment = T2) in 2012 and 2013. Mean separation letters were obtained through the least squares means option in PROC GLIMMIX. Three of the five T2 trees died between 2012 and 2013.

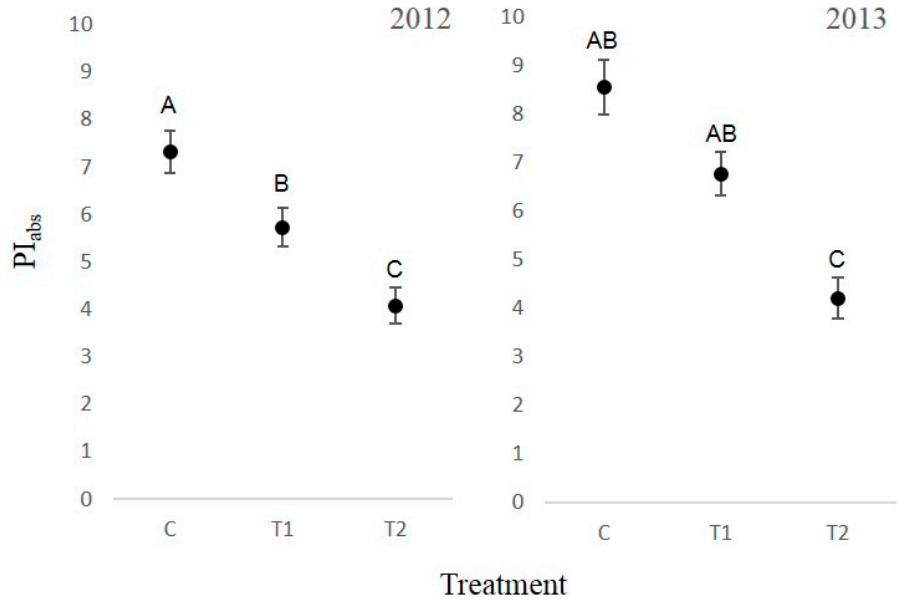

Figure 4. Mean Performance Index (PI_{abs}) ± SEM for three levels of white pine treated with ethanol (Control = C, pheromone-baited single ethanol treatment = T1, and pheromone-baited double ethanol treatment = T2) in 2012 and 2013. Mean separation letters were obtained through the least squares means option in PROC GLIMMIX. Three of the five T2 trees died between 2012 and 2013.

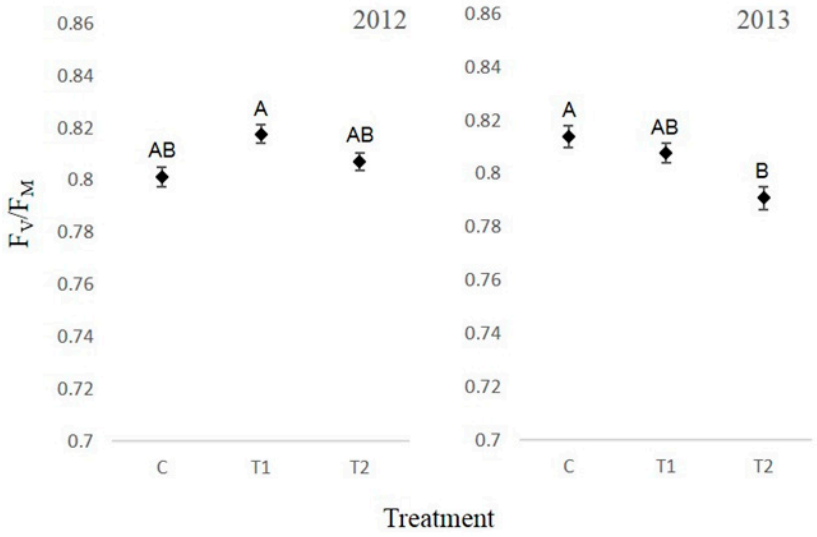

Figure 5. Mean maximum quantum efficiency of PS II photochemistry (F_V/F_M) ± SEM for three levels of red maple treated with ethanol (Control = C, Single ethanol treatment = T1, and double ethanol treatment = T2) in 2012 and 2013. Mean separation letters were obtained through the least squares means option in PROC GLIMMIX.

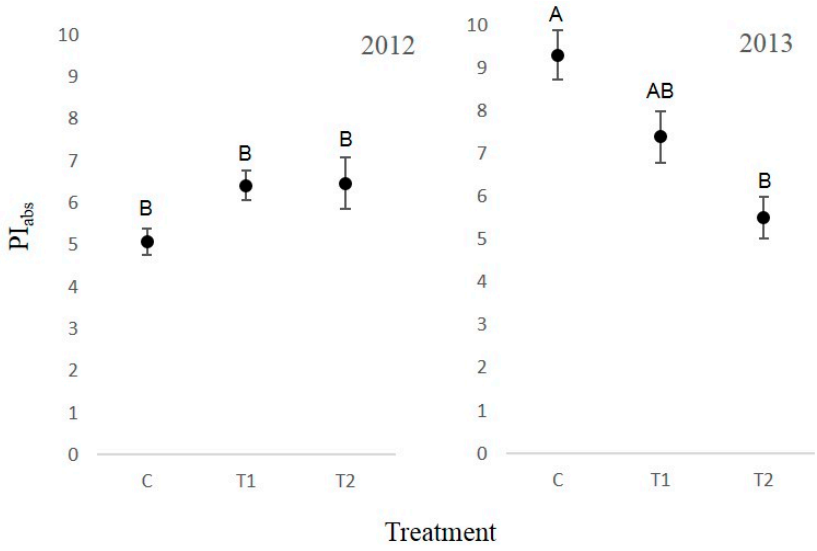

Figure 6. Mean Performance Index (PI_{abs}) ± SEM for three levels of red maple treated with ethanol (Control = C, Single ethanol treatment = T1, and double ethanol treatment = T2) in 2012 and 2013. Mean separation letters were obtained through the least squares means option in PROC GLIMMIX.

4. Discussion

Attempts were made to stress white pine and red maple in a mixed hardwood-pine stand that had undergone recent thinning operations. This stand was devoid of vertical structure and was being managed for multiple uses including timber, recreation, and wildlife. The combination of ethanol and aggregation pheromones targeting *Ips* species were successful at stressing white pine and ultimately led to the creation of CWD in the form of standing snags. However, the ethanol only treatment of red maple did not result in any significant effects on this tree species. The lack of a semi-aggressive insect that mass colonizes red maple was likely an important factor in our inability to push red maple into a stressed condition.

The stand where this experiment was established was recently heavily thinned, potentially inciting stress in residual trees, or providing conditions where it was not possible to determine the influence of our stem injections from background stress on control and treatment trees. Consequently, any actual influence our tree injections may have had, especially on red maple, could have been lost because trees were already responding to significant disturbance.

Insect communities respond to stressed trees in relatively predictable patterns based on tissue use and various abiotic host measures (e.g., moisture). These responding insects can facilitate tree mortality by their actions or the actions of microorganisms they inoculate into host trees. Healthy, vigorous trees are typically able to overcome insect attack through defense mechanisms. However, as stress increases, photosynthetic activity is reduced and plants shift toward photoprotection methods that tax limited reserves [34].

Fitness and vitality in treated white pine were generally lower than control trees, indicating that treated trees were physiologically more stressed than control trees. This difference in tree health was also evident by the abundance and richness of beetles arriving at trees. More species were captured on treated trees compared to control trees. Individual-based rarefaction curves for pheromone-baited single and double ETOH treated white pines were very similar to one another and close to asymptote, suggesting that most species present in the environment were sampled. Control trees, however, captured relatively high numbers of species per far fewer beetles. The most abundant Scolytinae was *Ips pini*, and this species was practically absent from control trees while abundant in traps and found attacking host trees. Trees were baited with ipsdienol and lanierone, both aggregation pheromones used by *I. pini* during host colonization [49]. This beetle has been reported to occasionally kill living trees [50–52] but generally behaves as a secondary species in eastern North American forests [53,54]. Mass attack by *Ips pini* and inoculation of its associated fungi into trees was likely responsible for ultimately killing trees.

The Scolytinae *O. caelatus*, *I. grandicollis*, and *C. borealis* were all captured at higher numbers on at least one of the treatments. *Orthotomicus caelatus* and *I. grandicollis* have previously been reported to be attracted to traps baited with pheromones used to bait trees in this experiment [43,49]. Low numbers of *I. calligraphus* were captured in the experiment, but a trend existed suggesting this species also responded more strongly to treated trees than control trees, likely due to the presence of ipsdienol on trees [49]. One Cerambycidae, *M. notatus*, was also captured at higher numbers on double ETOH treated trees. This species is also attracted to the pheromones used to bait trees [42,44] and likely host volatiles [55,56]. Interestingly, a suite of Scolytinae showed no significant response to treated trees over control trees. These Scolytinae, including *X. politus*, *P. hopkinsi*, *H. opacus*, *G. materiarius*, and *D. valens*, all inhabit stressed trees and some use host volatiles to locate hosts [57,58], while others may locate trees through random landing [59]. It is also possible that, for beetles using host volatiles to locate trees, there was not a perceptible difference among the composition or abundance of compounds emitted from treated and control trees. With the exception of *M. mali*, the most abundant bark beetles collected were conifer infesting species.

The double ETOH treated red maple exhibited subtle symptoms of decline in overall fitness while the control and single ETOH treatments showed signs of improvement in both fitness and vitality. No differences among the responses of Scolytinae or Cerambycidae to the ethanol injections in red maple were found, nor were any ambrosia beetle attack sites found. However, more species were captured in traps on the treated trees than control trees. None of the individual-based rarefaction curves for treated or control trees reached asymptote, suggesting that more species were present in the environment than were captured on treatment or control trees. The lack of a Scolytinae aggregation pheromone to compliment the ethanol injections likely limited our ability to further stress trees through the action of attacking beetles or their associated microorganisms.

While an aggregation pheromone was not implemented for red maple trees, various ambrosia beetles (Scolytinae) respond strongly to ETOH injected trees and baited traps [23,27,60]. These species are of particular interest because some are damaging and often carry pathogenic fungi [61]. We

captured eleven species of ambrosia beetle on red maple and white pine, but generally in very low abundance. The exotic ambrosia beetles *Xylosandrus germanus* Blandford, *Xyleborus seriatus* Blandford, *Xyleborinus saxeseni* (Ratzeburg), and *Xyleborinus alni* (Niisima) were all captured at numbers too low to statistically analyze but were found only on ETOH injected trees. Native ambrosia beetles, including *Anisandrus sayi* (Hopkins) and *Monarthrum fasciatum* (Say), were captured at low total numbers but also found more often on treated trees than control trees. *Monarthrum mali* was found at higher numbers on ETOH injected trees and was almost entirely absent from control trees, suggesting ETOH or related volatiles emanating from red maple served as an attractant for this species. Conversely, *Xyloterinus politus* showed no response to ETOH treatments.

The low numbers of ambrosia beetles collected was surprising given the large amounts of CWD remaining on the ground from the silvicultural treatment that provided plentiful habitat for beetle reproduction. It is possible that this material was more attractive, or more easily exploited, than experimentally manipulated trees. Low trap numbers could also be a result of asynchrony between ethanol injections (26–27 March, 7 June) and dispersal flights of ambrosia beetles. We injected trees in March because of an unseasonably warm spring and concern for an earlier than usual flight of ambrosia beetles in the northeast. Two weeks of milder temperatures with heavy rain followed tree injections, likely limiting beetle dispersal. It is also possible that our trap height resulted in lower catches of some ambrosia beetles. We chose to hang traps at 3 m with the hope of catching beetles that preferred basal portions of trees as well as species that attacked higher bole portions. Some species of ambrosia beetles, including *Xylosandrus germanus*, have been captured in higher numbers in traps lower than the trapping height used in our study [62]. However, these species have also been captured at relatively high numbers on forest trees at heights between 1.5 m and 6 m in previous studies [63,64]. No ambrosia beetle attacks were found at or below our trapping height during tree surveys, so it is unlikely that our trapping methods significantly underestimated these species.

Patterns suggest that there were some attractiveness differences among control and treated trees. White pine were successfully stressed with the combination of ETOH injections and aggregation pheromones. While the methods we used to assess tree health did not detect noticeable differences in red maple health, there is some indication that beetles perceived these trees differently than control trees. While only captured at low numbers, ambrosia beetles did respond to ETOH injected trees at higher numbers than controls. Had larger local beetle populations focused attacks on ETOH injected red maple, trees may have suffered more significant decline.

Unfortunately, we were unable to include a pheromone only and ethanol only treatment of white pine that would have helped elucidate what was driving the tree decline. Previous deployment of *Ips* pheromones in forested settings on traps suggested that these beetles would be unlikely to mass attack trees (*i.e.*, no spill-over attacks) with only pheromones. Consequently, we chose to use both methods with the hope of adequately stressing trees and providing information to natural resource managers interested in more naturally creating snags.

5. Conclusions

There are several situations where natural resource managers would want to create stressed trees [65,66]. Stressed trees can act as a detection tool, attracting insects that respond to host volatiles or stress volatiles [23,67]. Another reason is to supplement dead wood in managed forests to increase structure. Many managed forests are denude of dead trees, limiting habitat for dead-wood dependent species. Various attempts at creating snags have been made, but most of these involve mechanisms that do not allow for the natural decline optimal for community development. We tested two methods (ethanol injections, pheromone tree baiting) for creating white pine and red maple snags in a managed woodlot in Massachusetts, USA. The combination of ethanol injections and *Ips* aggregation pheromones successfully created snags from healthy mature overstory white pine in northeastern forests. Ethanol injections alone were unsuccessful at stressing or killing red maple. Other methods should be investigated for stressing and killing red maple in forested settings.

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