The Effects of Climate Change and Forest Disturbances on Terpene Production of White Pine (Pinus strobus) and Red Pine (Pinus resinosa) in Northern Michigan

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The effects of climate change and forest disturbances on terpene production of white pine (Pinus strobus) and red pine (Pinus resinosa) in Northern Michigan

Author: Troy deHagen

Chairs: Dr. Steve Bertman & Dr. David Karowe
Abstract

The secondary impacts that climate change may have on the interactions between forests and the atmosphere through forest disturbances is of increasing interest as the climate continues to change. Anthropogenic climate change, which is a direct result of coal and fossil fuel emissions, is likely to impact forest ecology through increased temperatures and extreme precipitation events, thus increasing the frequency and severity of climate-induced forest disturbances. Forest disturbances may have a secondary impact on the production and emission of biogenic volatile organic compounds (BVOC) within forests. In the presence of oxides of nitrogen ($NO_x$) produced from fossil fuel emissions, BVOC react to produce ozone ($O_3$), a greenhouse gas (GHG) and potent oxidant. Furthermore, in the presence of atmospheric particles, $O_3$ can interact with the particles to produce smog, thus impacting regional air quality. BVOC produced within forests are responsible for the vast majority (~90%) of VOC emissions in forested regions, with isoprene and terpenes being the most dominant forms. This study looked at the response of white and red pine terpene production to selective and non-selective forest disturbance types. It was found that terpene production in white pine increased with selective forest disturbance and declined over time after a non-selective disturbance. However, in red pine, neither disturbance type was correlated with terpene production. The species-specific terpene production and response to disturbance observed in red and white pine suggest the need for further investigation into the aspects of forest disturbances that elicit a change in terpene production and the underlying physiology within varying tree species.

Introduction

The impacts of forest disturbances on forest-atmosphere interactions requires a detailed understanding of how disturbances influence chemical production within forests. Forest ecosystems are predicted to experience increased disturbances from extreme weather events caused in part by climate change (Kharin et al., 2013; Wanders et al, 2015). Although healthy forest ecosystems regularly undergo disturbances under natural conditions, the impact that more frequent and severe disturbances will have on the production of volatile organic compounds (VOCs) and their emission from foliage is not well understood. The extent to which any specific forest will experience disturbances is largely dependent on its geographical location, composition, and resiliency to changes in temperature and precipitation (Stevens-Rumann, 2018). Several studies have suggested that forest-atmosphere interactions, such as carbon cycling (Curtis & Gough, 2018), are altered through forest disturbances. A study by Goldstein et al. (2004) suggested that monoterpene emissions in California ponderosa pine were enhanced by forest thinning, a common forest management practice. However, no study thus far has investigated the direct link between forest disturbances and VOC production by individual tree species.

Forest disturbances can be categorized as either selective or non-selective. A selective disturbance disproportionately impacts one or more tree species within a forest. Selective forest disturbances provide the opportunity for disturbance-resilient tree species to thrive, whereas less resilient species succumb to the environmental pressures that are generated. Drivers of selective forest disturbance include increased temperatures, irregular precipitation events, and pest and pathogen outbreaks, which often preferentially parasite tree species (Seidl, 2017). The impacts of varying degrees of selective disturbance within forests on VOC production in disturbance-resilient tree species can provide valuable information about forest-atmosphere chemistry. Additional to direct changes in VOC production, selective disturbance can also result in a forest compositional change, thus causing changes in the VOC composition of a forest. Non-selective forest disturbances are such that the severity of disturbance within a forest is shared among all tree species within the forest, examples of which include forest fires or clear-cutting. After a non-selective forest disturbance, species composition of a forest is often homogenous and dominated by early successional trees and the transition back to late successional trees can take dozens to hundreds of years depending on the severity of the disturbance and the forest type. The temporal impacts of non-selective disturbances on VOC production and composition has received little attention thus far.

Various biogenic volatile organic compounds (BVOCs) are produced by plants and a variety of purposes have been identified. For example, some serve as chemical defenses, taking part in thermostolerance (Loreto et al., 1998), oxidative tolerance (Loreto & Velikova, 2001), and herbivory defense ( Unsicker et al. 2009; Gershenzon & Dudareva, 2007; Schnee et al., 2006). In addition to their physiological role, BVOCs readily diffuse from plant leaf tissue and are emitted into the atmosphere, which impacts regional atmospheric chemistry (Atkinson, 1998; Kesselmeier & Staudt, 1999). Although BVOCs are present in trace amounts in the atmosphere, they are extremely reactive, with lifetimes ranging from minutes to hours ( Atkinson, 1998; Guenther, 1995). In the atmosphere, BVOCs react with oxides of nitrogen ($NO_x = NO + NO_2$) to produce ozone ($O_3$) (Atkinson, 1998; Holloway & Wayne, 2010), which influences the oxidizing potential of the troposphere (Arneth et al., 2011; Taraborrelli et al., 2012), acts as a greenhouse gas, and leads to photochemical smog events. Presence of BVOC in the atmosphere can also influence tropospheric carbon monoxide (CO), formaldehyde (HCHO), and peroxyacetyl nitrate (PAN) concentrations (Pfister et al. 2008), lead to the formation of secondary organic aerosols (SOAs) (Andreae, 2009; Laathawornkitkul et al., 2009; Ng et al, 2006; Hao et al., 2011), and result in atmospheric nanoparticle formation (Riipinen et al., 2012).
The terrestrial biosphere is the largest emitter of BVOCs into the atmosphere, responsible for over 90% of global VOC emissions (Guenther, 1995). The most abundant BVOC emitted from foliage are isoprene (~70%) and terpenes (~24%). Recent modelling of BVOC emissions estimates an annual contribution of $6.6 \times 10^{14}$ g C yr$^{-1}$ (Messina, 2016), compared to past estimates of $1.2 \times 10^{15}$ g C yr$^{-1}$ (Muller, 1992; Guenther et al., 1995), signifying the evolving knowledge of BVOC production, emissions, and modelling in recent years. The ability to accurately estimate and predict atmospheric BVOC concentrations is difficult because it depends on a thorough understanding of many factors that control BVOC production and emission. Temperature and light variables have been standard in BVOC modelling, but other emission factors and feedback mechanisms are continuously being implemented into newer models.

An analysis of terpene production in two pine species of Northern Michigan in response to selective and non-selective forest disturbances may shed light on the future of terpene emissions in the Midwest and global trends. *Pinus strobus* (white pine) and *Pinus resinosa* (red pine) were chosen for this study, two species prevalent in Michigan. In the temperate forests of North America, pine species are predicted to succeed in future climate scenarios due to their resilience to hotter and dryer conditions and the terpene BVOCs they produce are extremely diverse, providing valuable and novel insight into forest-atmosphere interactions. Needle concentrations are used as a proxy for gas-phase emission as described in (Toma & Bertman, 2012). Using constitutive needle concentrations provides the capability of many rapid measurements across a large area, thus providing the ability to scale measurements from individual stands to forest with more precision.

**Materials and Methods**

**Study site** Samples were obtained from the University of Michigan Biological Station (UMBS) (45°30’N, 84°42’W) in Pellston, Michigan during the summer of 2018. Four forest sites located within one kilometer of each other with similar soil conditions and tree cover types were sampled. The Ameriflux forest (A) and Riggsville road forest (R) were selected for control sampling of undisturbed white and red pine, respectively. The FASET (F) and experimental burn forests (B) served as sites for collection of selective disturbance samples and non-selective disturbance samples. The FASET forest is a result of the Forest Accelerated Succession Experiment (FASET), which involved the selective stem girdling of aspen and birch trees in the Spring of 2008. Basal area within several 20 m radius (0.08 ha) plots along seven transects from a central carbon flux tower was measured before and after girdling to obtain percent basal area senesced, hereafter referred to more simply as percent disturbance severity. The experimental burn forest has several plots that were artificially clear cut and burned on-site to mimic forest fires and subsequently allowed to regrow naturally. This study includes five plots in the burn forest that were burned in 1936, 1948, 1954, 1980, and 1998, respectively.

![Map of experimental forests at UMBS](image)

**Sample Acquisition** Samples were collected from Ameriflux (n=18 white pine), Riggsville road (n=3 red pine), FASET (n=84 white pine; n=23 red pine), and the burn plots (n=15 white pine; n=15 red pine). All samples were collected from understory trees around 2 m tall and fascicles were chosen from southern-most branches nearest to 1.5 m from the ground. Disposable nitrile gloves were used when sampling and no form of volatile personal products or bug spray were worn to avoid sample contamination. Needle samples were obtained by using flat-tipped steel forceps. White and red pine samples were taken using separate forceps to avoid contamination. Before and after sampling, forceps were rinsed in a vial of HPLC grade hexane to remove any contamination between sampling. For each sample, 10-15 fascicles (5 and 2 needles per fascicle in white and red pine, respectively) were placed inside a sampling bag (Thermo Scientific, PN: 6255-0406) and immediately placed in a cooler with dry ice. Sample bags were transferred to a -80 °C freezer until processing.

**Measured Variables and Light Availability Determination** The following variables were measured for each sampled tree: height, age, diameter at breast height (DBH), and surrounding temperature. Percent of light available to each tree was also measured using an ACCUPAR ceptometer model LP-80. Light measurements were conducted on sunny days with minimal cloud cover by taking a baseline, ‘maximum light’, measurement in an unshaded area with direct sunlight, followed by 3 readings at 1.5 m height around each tree. Each reading was calculated as a fraction of the
baseline measurement and the three measurements were averaged to obtain the percent light availability of each tree.

**Sample Processing** Needles were removed from the sampling bag, cut into approximately 5mm pieces. Fascicle ends were discarded. Approximately 0.2 g of fresh needle mass was weighed on weigh paper using an analytical balance. Needle trimmings were transferred to a 7 mL glass scintillation vial with PTFE-line screw-cap lids. 2.0 mL HPLC grade hexane with 100 μM tridecane internal standard was added to each vial for liquid extraction of terpenes from the needle samples. Vials were placed in an isotherm water bath, set at 23.0 °C for 24 hours. After incubation, samples were centrifuged at 3000 rpm for 5 minutes and supernatant was transferred to a gas chromatograph autosampler vial to be analyzed via GC-MS. The vials containing the needle samples were placed in a drying oven for 24 hours to obtain dry needle mass after extraction.

**Gas Chromatography-Mass Spectrometry Calibration and Analysis** Instrumentation for analysis included a Thermo Scientific Trace 1310 Gas Chromatograph with a Thermo TG5 MS 45 m column (ID: 0.25 mm, film: 0.25 μm) and a Thermo Scientific ISQ LT Single Quadrupole Mass Spectrometer. The GC oven settings used for white and red pine are tabulated below and a Thermo Scientific ISQ LT Single Quadrupole Mass Spectrometer. The GC oven settings used for white and red pine are tabulated below and a Thermo Scientific ISQ LT Single Quadrupole Mass Spectrometer. The GC oven settings used for white and red pine are tabulated below and a Thermo Scientific ISQ LT Single Quadrupole Mass Spectrometer. The GC oven settings used for white and red pine are tabulated below and a Thermo Scientific ISQ LT Single Quadrupole Mass Spectrometer.

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Table 1: GC oven settings for white pine FASET samples. Total run time 44.00 minutes.

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Table 2: GC oven settings used for red pine FASET samples and burn plot samples. Total run time of 30.00 minutes.

Calibration was conducted using authentic monoterpenes and sesquiterpene standards. (1R)-(+)-α-Pinene, Camphene, (1S)-(−)-β-Pinene, β-Mycene, α-Phellandrene, 3-Carene, Limonene, Terpinolene, β-Caryophyllene, α-Humulene were purchased from... A 500 μM stock solution containing the authentic standards was created and serially diluted to create 250 μM, 100 μM, 40 μM, and 5 μM solutions. HPLC grade hexane with a 100μM internal standard was used as the solvent for each dilution. Averaged calibrations of monoterpenes or sesquiterpenes were used for calculations of compounds for which there was no authentic standard available. For the calibration of Germecrene D-4-ol, elemental mass spectrometry was used... The calibrations for each instrument method were used for samples that were run with the same method. Linear regression provided an equation that was used to calculate the concentrations of each compounds in the pine needle samples. An excel LINEST array function was used to obtain slope, slope error, and standard deviations for each calibration.

**Statistical Analysis** SPSS software was used for statistical analysis. A normality test was run on all samples and least-squares linear regression was used for statistical significance. Linear regression analysis of terpene concentrations against other possibly confounding variables such as light availability was also conducted.

**Results**

**Species-Specific Terpene Production** An analysis of terpene composition of red and white pine shows that the two species produce different absolute terpene concentrations as well as unique proportions of individual terpenes (Fig. 2). Averaging the sums of the concentrations of terpenes found in the needles of white pine showed that they consist of 63.8% monoterpenes, 21.1% sesquiterpenes, and 15.0% sesquiterpenols, which are alcohol derivatives of sesquiterpenes. In comparison to the variety of terpene compounds that are produced by white pine, red pine displays a more homogeneous terpene pool. On average, monoterpenes in red pine samples constitute over 95% of total terpenes produced. Total sesquiterpenes and sesquiterpenols in red pine needle samples were 4.1% and 0.1% of the total pool, respectively. Of the individual terpene compounds produced, α-pinene was the most abundant in white pine, constituting 36.7% of the total terpenes, whereas β-pinene was the predominant terpene in red pine, accounting for over 50%. Furthermore, red and white pine produce distinctly different proportions of sesquiterpenols, alcohol derivatives of sesquiterpenes. Germecrene D-4-ol was observed to be the predominant component in white pine, making up 12.4% of the total terpenes, but only trace amounts of sesquiterpenols were observed in red pine samples.
Fig. 2: Percent composition of major terpenes in the needles of white pine (A) and red pine (B) as well as proportions of individual terpene in the needles of white pine (C) and red pine (D) averaged from all analyzed samples.

**Terpene Concentrations as a Function of Selective Disturbance** Total terpene concentrations increased linearly in response to selective forest disturbance in the white pine samples obtained from the FASET experimental forest (Fig. 3A). This response holds true for statistical analysis of monoterpenes (p = 0.004, R² = 0.08), sesquiterpenes (p = 0.001, R² = 0.10), and sesquiterpenols (p = 0.018, R² = 0.06). In contrast to the response of white pine to disturbance, there was not found to be a significant response of terpene production in red pine to selective disturbance (Fig. 3B). Similarly, there was no trend for monoterpenes (p = 0.55, R² = 0.01) or sesquiterpenes (p = 0.31, R² = 0.03).

Fig. 3: Total terpene concentrations (umol/DWg) of white pine (A) and red pine (B) in FASET and undisturbed forests as a function of disturbance severity, averaged by plot. R² and p-value were obtained using all obtained samples.
**Terpene Production in Response to Non-Selective Disturbance** In the burn plots, it was found that white pine total terpene production was greatest in plots that had been disturbed more recently and that concentrations decreased nearly significantly over time since the non-selective stand removal and burn-type disturbance occurred (Fig. 4). Further statistical analysis shows that white pine monoterpenes production decreased significantly over time since disturbance, whereas sesquiterpene production showed no significant trend. Interestingly, sesquiterpenols displayed an upward trend over time since burn rather than downward.

In the red pine samples, similarly to the response, or lack thereof, to selective forest disturbance, there was no significant response in terpene production as a function of time since burn (Fig. 5). This was consistent among monoterpenes \(p = 0.75, R^2 = 0.01\) and sesquiterpenes \(p = 0.71, R^2 = 0.01\).

**Discussion**

Although they are closely related species, white and red pine are observed to have vastly different correlations between terpene concentrations and disturbance severity. Changes in constitutive leaf concentrations may indicate a unique chemical production response to forest disturbance that begs further investigation. Furthermore, constitutive leaf levels are used as a proxy for gas-phase emissions, but a comparison of these data to direct emission measurements in response to forest disturbance would provide a valuable step toward a more complete model.

White pine terpene production is split between monoterpenes, sesquiterpenes, and sesquiterpenols, whereas red pine terpene products are dominated almost entirely by monoterpenes, which are more volatile than sesquiterpenes and are typically more reactive (Atkinson, 1998). Furthermore, the total concentration of terpenes was found to be, on average, more than 25% greater in red pine than in white pine. This species-specific terpene production may indicate a need for consideration in forest management practices, especially in pine plantation decisions, as greater production of monoterpenes by red pine may adversely impact regional...
ozone production and air quality in areas where anthropogenic sources of NOx are present.

The relatively high constitutive levels of sesquiterpenols, predominately germecrene D-4-ol, found in white pine but not red pine is also a mystery. Germecrene D-4-ol is especially curious due to its opposite trend of production in response to time since burn compared to all other terpene compounds. This may suggest that Germecrene D-4-ol serves a physiological role in pine species distinct from other terpene compounds.

The observed species-specific responses to forest disturbance further support the complexity involved in predicting and modeling BVOC within forests. In general, red pine displayed no significant response in terpene production in response to disturbance. Conversely, white pine were observed to respond to forest disturbances with increased terpene production in the case of more recent and more severe disturbances. The temporal nature of the terpene production response is visible in the burn plot chronosequence samples obtained from the experimental burn plots, but the long-term response of white pine to selective disturbance is not known. A longer study with annual analysis of the same trees over time after a selective disturbance would be necessary for an investigation of long-term effects.

Forest disturbance as a driver of changes in BVOC production is a relatively new concept. Rather than a single variable, forest disturbance is a mixture of changing variables, thus creating a desire to know what components of forest disturbance cause the change in forest chemical composition. In this study, several variables were measured to test if any were correlated with forest disturbance or terpene production. There was found to be no statistical correlation between terpene concentrations and tree age, height, temperature, or DBH. However, it was found that light availability was positively correlated with the severity of disturbance and terpene concentrations increased with light availability (Fig. 6). The relationship between light available and disturbance severity is likely a result of canopy gap formation following tree mortality. The relationship between light availability and terpene concentration is also plausible as a result of increase in photosynthetic activity. This, however, does not adequately explain the magnitude of increased terpene concentrations in highly disturbed tree samples.

In Fig. 3A, showing total terpene production by white pine as a function of forest disturbance, R² = 0.10, suggesting that forest disturbance explains about 10% of the variation in the total terpene production. When plotted as a function of light availability, this provides R² = 0.04, suggesting light availability explains about 4% of the variation in total terpene production. Therefore, there is an approximately 6% gap in explanatory power that is provided by disturbance severity that light availability does not account for. This then leads us to the conclusion that there is either an intrinsic property of forest disturbance that explains the variation in terpene production or that there is some other component of forest disturbance that is involved. Future studies of BVOC production in response to forest disturbance may look to explore this gap in explanatory power. Variables, such as nutrient availability and nutrient competition in disturbed forests could provide a basis for a deeper understanding.

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