Interactive comment on "The atmospheric potential of biogenic volatile organic compounds from needles of White Pine (Pinus strobus) in Northern Michigan" by S. Toma and S. Bertman

We would like to thank both reviewers for their time and insightful comments. Both reviewers were thorough and thoughtful and have made some excellent suggestions. Some of the suggestions are beyond the scope of this study. Most are very helpful and have been incorporated in a revised manuscript.

Our main changes are:

1. *Figure S1 was moved from the supplementary material and inserted in the main paper as Figure 1. Therefore, original Figures 1 and 2 are changed to Figures 2 and 3.*
2. *We modified Figure 2 (Figure 3 in the current version) Figure S3 (Figure S2 in the current version)*
3. *At least 9 new citations were added.*
4. *All suggested grammatical and editorial changes were made and the revised manuscript has been further proofread.*

Suggestions from Anonymous Referee #1

While the paper is generally well written, it lacks some rigor in developing the principle conclusions presented. First, the paper needs to build a stronger case to link leaf BVOC content to atmospheric emissions. Second, the increase in OH reactivity due to limonene should be considered for the sum of BVOCs rather than a binary system with α-pinene for the 'true' atmospheric potential to be realized.
Lines 17-19, page 26851. The crux of the paper is that leaf concentrations are directly related to atmospheric emissions. The authors propose that the "atmospheric potential" or OH reactivity can be inferred from the leaf BVOC composition without measuring any leaf emissions. This point deserves a more complete treatment especially since one of the two provided references (Lerdau et al., 1994) did not find this relationship for all terpene species (i.e., Δ-carene).

The reviewer is quite right that we don't have direct measurements of white pine emissions and, in fact, we had a longer version of this section that was shortened before submission. We replaced the original wording of Lines 17-21 with the following:

"Measuring BVOC concentration in leaves can be an alternative to the branch enclosure technique if there is a relationship between BVOC emission rate and their concentrations. Lerdau et al. (1995, 1994) studied the relationship of three monoterpenes (α-pinene and β-pinene, and Δ-carene) in ponderosa pine (1994) and Douglas-fir (1995). In ponderosa pine α-pinene and β-pinene concentration were positively related to emission rate, but Δ-carene was not. In Douglas-fir, all three terpenes showed a positive relationship. Lerdau (1995) suggested that terpenes concentration of leaves could be used as an important parameter to predict emission rates in models. While the present work does not explicitly explore the relationship between gas-phase emissions and needle concentrations, the relationship is plausible and needle concentrations provide a benchmark for potential BVOC emissions in a whole forest.

Relationships between chemical compounds and emission rates vary for tree species the ratio of compounds within a needle likely are related to gas-phase emissions scaled by volatility. Measuring BVOC concentration of leaves allows easy measurement of a large number of samples from a wide area in a short time and hence estimation with precision of the BVOC content of an area of forest."

The wording of the final sentence in Introduction is much too strong for what is 'reported' in the manuscript. It leaves the reader expecting a link between BVOC levels and genetic diversity as well as an analysis of the genetic diversity of pine populations. Support is not provided for either of those. Consider rewording to match the results in the paper.

We have thought a lot about the potential drivers for the variability we observed, including the possibility of environmental factors as well as genetic variations. We recorded many environmental parameters and analyzed the data for possible relationships. We found no statistically significant relationships between needle concentrations and environmental factors such as temperature and light environment. We left most of the analysis of environmental factors out of the original manuscript. In the revised version we have included a table in the supplementary material including the factors measured for each sample and the Mode it expressed. Given the numbers of samples and the proximity of samples in both Modes I and II, we are currently exploring the genetic basis in collaboration with molecular biology colleagues. The referee is correct that we cannot attribute our observations to genetics. But we feel confident saying that there is chemotypic diversity within the populations of trees studied. We do not want to suggest we understand the mechanism yet, but simply to report that there could be more variation is terpenoid emissions than commonly suggested.

We edited the last sentence of the Introduction to: "This work reports BVOC levels in white pine needles in northern Michigan measured over three growing seasons and attempts to estimate the atmospheric influence of chemotypic diversity within a population of plant sepcies."

Section 3.2. The explanation of why BVOC leaf concentrations were higher in the top of the canopy compared to bottom of the canopy should focus more on BVOC biosynthesis rather than what is known on emissions (temperature and light dependencies). Leaf-level emissions are not relevant to explain the leaf concentrations.

The reviewer is right that leaf concentrations should be related to BVOC biosynthesis. It is currently believed that MT and SQT are produced by different biosynthetic pathways in different parts of the cell (e.g. Martin et al., 2002), so they could be differently sensitive to environmental conditions. Gleizes et al. (1980) shows that synthesis of MT is more sensitive to light than SQT. We are not aware of citations that specifically look at temperature effects on partitioning of BVOC into MT and SQT. Environmental conditions appear not, though, to affect the ratio of limonene/pinene. That is, the same trees expressed Mode II in all years despite large variation in environmental conditions and there is no difference in this ratio between the top and bottom of canopy trees.

Section 3.5. The discussion of environmental factors to explain the chemotype variation observed is unclear. Noting that α-pinene/limonene ratios were invariant for individual trees over

a growing season does not fully eliminate differences in their environ- mental growing conditions. Alternative environmental factors should be fully elucidated and supported with additional data.
Was the age of tree or leaf type (across modes) considered (Loreto et al., 2000)?
Did the light environment differ between the two modes (sun vs. shade)?
Could one mode have been resource limited (nitrogen or water) or stressed (e.g., competition from a nearby canopy dominant)?

We looked at many environmental factors, temperature, light environment, tree age, and DBH. We were careful to sample only mature needles from each tree. There were no significant correlations with any of these factors, (including saplings versus canopy trees). We tracked ratios of compounds throughout growing seasons and over the course of several years. The trees in both modes were located in the same forest and in some cases trees of similar age and size were as close as 2 m apart. The distribution of trees of different modes is essentially random in the forest, as shown in Figure S1 (new Figure 1). We have listed the environmental factors measured in the text and have included a table in the supplementary material that includes measurements of environmental factors for each sample.

Several pieces of evidence strongly suggest that environmental factors are not major explainers of the two Modes observed and why we are pursuing genetically related factors:

1) *There is a lack of correlation with recorded environmental parameters.*
2) *Multi-year data show that the same trees express the same Mode each year.*
3) *Trees in both Modes are randomly distributed throughout the forest.*
4) *Trees in both Modes are found in very close proximity, too close to experience vastly different environmental factors.*
5) *The strongly bimodal nature of the observed ratio between limonene and a-pinene and the consistency of the ratios between trees and between years.*

We "measured" light environment in two ways. The first was qualitative by observation, the second employed the analysis of hemispheric photos taken next to each tree using Gap Light Analyzer software. Again, we found both Mode I and Mode II trees in all light environments and did not find any significant relationship between needles concentrations and calculated Leaf Area Index from the hemispheric photos.

Leaf-level biological exchange data (e.g., assimilation rates, stomatal conductance) would greatly help clarify leaf-level environmental differences between the two modes. Finally, it is not clear from the supplemental Figure, S1, that "tress of both modes were within 2 m of each other" if using figure S3 to identify trees of differing modes.

With Figure S1 (which we have now included as Figure 1) we show the proximity and random distribution of trees of different Modes, which suggests there is no pattern of resource allocation. With Figure S3 (now Figure S2) we attempt to show simply that the same trees expressed the same Mode every year. We feel this is supporting evidence that the bimodal behavior is not an induced effect. Martin et al. (2003) conducted an inductional experiment using Methyl Jasmonate in needles of Norway spruce and showed that the activity of monoterpene and sesquiterpene synthases declined 15 days after treatment. If the length of induction is on the *order of weeks, the consistency of behavior in white pines over the course of years makes it unlikely that induction is responsible.*

Section 3.5. The authors hypothesize that the chemotype variation is due to genetic variation (page 26857) after ruling out environmental variation but provide no support or citations for this assertion (e.g., could it be due to the high level of genetic variation among populations (Buchert, 1994)). Is there prior work on genetic variation and BVOC synthesis? Have gene studies on UMBS white pines been conducted? What could explain the small pockets of genetically different trees within the forest?

There are no reports of terpene synthase gene sequencing from white pine in the GenBank database. We are in attempting to sequence the terpene synthase genes in the different populations of white pine that we sampled, but extracting pure RNA from pine needles is very challenging. We determined by chiral GC that (-)-limonene is almost exclusively responsible for the increased ratio of limonene/pinene in trees exhibiting Mode II behavior in trees at UMBS. The levels of (+)-limonene do not change much from tree to tree or from year to year. We believe that this behavior further supports the hypothesis that the bimodal chemotype observations are related to biosynthesis differences due to genetics. We can include the data on chiral analysis if that would make our argument for a genetic basis stronger.

Section 3.5. Regarding the calculation of the impact of Mode II trees (elevated limonene) on OH reactivity. The 11% increase in OH reactivity is based on a binary system of only limonene and α-pinene (equations 1-3) yet those two compounds only account for ~30-50% of the terpenes measured. Since the title of the paper concerns the atmospheric potential of these BVOCs, it seems more appropriate to consider the relative increase in OH reactivity due to limonene based on the full terpene profile measured. While it is biologically interesting that two modes were observed, the atmospheric implication of such should not be overstated.

Yes, this is a good point, and we hope that we have not overstated the atmospheric importance of this specific observation. We think the data suggest that chemotypic variation within an ecosystem could affect emissions, especially when considering in-canopy chemistry. We have expanded the analysis to include total terpene reactivity. The analysis now includes an assessment that includes vapor pressure, for which we have used some estimated parameters, for compounds that are still inadequately studied. Assuming Raoult's Law applies to needles as solutions, we estimate partial pressure of each compound based on average needle concentrations in order to estimate reactivity.

We have modified the analysis and revised the wording of section 3.5 to clarify the atmospheric impact.

References to be added to manuscript:

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