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Biogeosciences Discussions

Interactive comment on "Short term changes in methanol emission and pectin methylesterase activity are not directly affected by light in *Lycopersicon esculentum*" *by* P. Y. Oikawa et al.

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We would first like to thank T. Sharkey, S.M. Noe, P. Harley, and editor G. Wolfahrt for their helpful critiques and suggestions. Most of the suggestions have been incorporated into the revised manuscript and we feel the manuscript is much improved. We were pleased that all the referees generally agreed that the manuscript is a valuable contribution to methanol (MeOH) emission modeling efforts. We herein address all issues raised in the referee comments.

T. Sharkey suggested that we could improve our study by feeding leaves abscisic acid (ABA) to induce stomatal closure. This experiment could show that under constant

light, and during stomatal closure, MeOH emissions significantly decrease. An additional experiment such as this could "strengthen the case that all apparent light responsiveness of MeOH emission is caused by biophysics of diffusion from the leaf". S.M. Noe and P. Harley agreed that the proposed experiment by Sharkey would strengthen our study. We agree that adding an ABA treatment to the paper might reinforce the results, and we appreciate this suggestion.

My co-authors and I discussed the possibility of using ABA to induce stomatal closure. We decided not to use this plant hormone, however, because of its effects on expansion and growth. Previous studies have shown that ABA is associated with reductions in cellular expansion (Zhang and Davies, 1990; Bacon et al., 1998). As we were testing whether light's direct influence on cell expansion influences MeOH production through the PME pathway, we did not feel ABA would be an appropriate tool for inducing stomatal closure. That is, while a positive result would support our conclusion, a negative result might stem from ABA's impact on expansion, rather than its impact on stomata. In addition, this experiment has already been done in a similar capacity by Nemecek-Marshall et al. (1995), in which they applied ABA to a young stem of Populus vulgaris under constant light and showed MeOH emissions decreasing with conductance.

T. Sharkey also suggested that other possible effects of light on PME may not have been picked up in our enzyme assay and that those undetected effects should be noted in the manuscript. Sharkey offered that rapidly reversible post-translational effects could be induced by the light treatment and not detected by our PME assay as they are transitory and easily missed. We agree with this point and have added to the revised manuscript a brief discussion of the processes that may have been missed in our assay measurements. In addition to reversible post-translational effects, we discuss the issue of individual PME activity rates responding to the light treatment and not being detected in our assay of total PME activity. We can therefore state that total PME activity was not significantly influenced by light, but the influence of light on individual PME activity rates remains unknown.

S.M. Noe suggested that another possible experiment would be to demonstrate, as done previously by Hüve et al. (2007), that after a period of stomatal closure, a MeOH emission burst should be seen with subsequent decay. Although this could provide further evidence for stomatal control over MeOH emission, as mentioned by S.M. Noe, this has been previously demonstrated and we do not feel it is a necessary addition to our study.

P. Harley requested an explanation for why the data were not collected in darkness. We agree that it would have been valuable to have a dark treatment. The reason we did not have a dark treatment was due to the difficulty involved with maintaining open stomata in the dark. It was a struggle to maintain high enough stomatal conductance at 50 μmol m2 s-1 and bringing the leaf into total darkness while maintaining open stomata would have proven difficult. However, it might be useful to investigate the significance of darkness for MeOH production rates. For example, there is a possibility that MeOH production rates significantly change between low light to dark conditions. MeOH production rates in total darkness have yet to be investigated.

P. Harley questioned our normalization procedure for the data presented in Figure 1. Harley suggested that "if light were to affect both MeOH production and conductance similarly, the effect on production could well be masked by normalizing the data." This is a very good suggestion, and we thank him for it. The point Harley raises is justified, and we agree that Figure 1 cannot determine whether or not light directly influences MeOH emission. We have, as a result, adjusted our interpretation of Figure 1. For example, on page 423, line 18 we have removed the sentence stating that Figure 1c suggested that "gs is responsible for apparent short-term MeOH emission responses to light." Instead we state in the discussion that "MeOH emissions normalized for gs suggested that changes in gs were capable of explaining changes in MeOH emission in response to light." We have paired this interpretation with the following disclaimer: "It is important to note that if light influenced gs and MeOH emission similarly, then the normalization of MeOH emission by gs would have resulted in the removal of the effect

of light on MeOH emission as well." In the revised text, we believe it is now clear that the data in Figure 1 were used to explore the relationships between MeOH emission, gs and light. Additional experimentation was clearly required in order to investigate the direct effects of light on MeOH production. Again, we thank the reviewer for bringing this to our attention.

P. Harley also suggested that the data in Figure 2 would be more compelling if non-normalized MeOH emissions rather than normalized emissions were shown. In order to remove all influence of gs on MeOH emission, we normalized the MeOH emission data by the small changes in gs that occurred across the five light treatment levels. Using the normalized data was preferable as the normalization removed any influence the small changes in stomatal conductance had on MeOH emission across the light treatments. We also graphed and analyzed the non-normalized MeOH emission data and found that they behaved similarly and were not related to light. Those data are not shown but are very similar to the figures presented in the manuscript. In the results section of the revised manuscript, we have added information concerning these unreported data along with our reasoning for using normalized emission data.

P. Harley requested clarification concerning the statistical analysis, specifically whether the analysis was conducted on normalized or non-normalized MeOH emission data. The analysis was conducted on normalized emission data. This has been clarified in the revised text. As mentioned above, non-normalized MeOH emission data were also analyzed and found to not be related to light. Those data are not reported in the manuscript.

As suggested by P. Harley, we revised the sentence on page 416, line 20 to read "and the remainder coming from" instead of "and the remainder's coming from".

Harley requested clarification concerning our definition of mature leaves. On page 419, lines17-19 we refer to mature leaves as leaves that are fully expanded. We therefore consider fully expanded leaves to be mature.

Finally, P. Harley clarified that the Niinemets-Reichstein model, referred to on page 418, line 15, assumes that MeOH production increases exponentially with temperature, but does not include a direct light effect. We appreciate this clarification and have re-worded the sentence to read as follows: “Harley et al. (2007) applied the Niinemets-Reichstein model to predict MeOH emissions from several species, assuming temperature-regulated MeOH production while accounting for changes in gs and gas- and liquid-phase MeOH pool sizes.”

Literature Cited

Bacon, M. A., Wilkinson, S., and Davies, W. J.: pH-Regulated Leaf Cell Expansion in Droughted Plants Is Abscisic Acid Dependent, Plant Physiology, 118, 1507-1515, 10.1104/pp.118.4.1507, 1998. Harley, P., Greenberg, J., Niinemets, U., and Guenther, A.: Environmental controls over methanol emission from leaves, Biogeosciences, 4, 1083-1099, 2007. Hüve, K., Christ, M. M., Kleist, E., Uerlings, R., Niinemets, U., Walter, A., and Wildt, J.: Simultaneous growth and emission measurements demonstrate an interactive control of methanol release by leaf expansion and stomata, Journal of Experimental Botany, 58, 1783-1793, 2007. Nemecek-Marshall, M., Macdonald, R. C., Franzen, F. J., Wojciechowski, C. L., and Fall, R.: Methanol emission from leaves - enzymatic detection of gas-phase methanol and relation of methanol fluxes to stomatal conductance and leaf development, Plant Physiology, 108, 1359-1368, 1995. Zhang, J., and Davies, W. J.: Does ABA in the Xylem Control the Rate of Leaf Growth in Soil-Dried Maize and Sunflower Plants?, Journal of Experimental Botany, 41, 1125-1132, 10.1093/jxb/41.9.1125, 1990.

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