Interactive comment on “Impact of heat stress on the emissions of monoterpenes, sesquiterpenes, phenolic BVOC and green leaf volatiles from several tree species” by E. Kleist et al.

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Interactive comment on “Impact of heat stress on the emissions of monoterpenes, sesquiterpenes, phenolic BVOC and green leaf volatiles from several tree species” Kleist et al.
We thank both reviewers and in particular the interested reader Peter Harley for their comments. All remarks were considered.
Response to remarks of referee #1 (RC C4094)

Remark Referee #1: General comments - The manuscript by Kleist et al. aims to investigate the impact of heat stress on various biogenic volatiles organic compounds (BVOCs) belonging to some plant species. Interestingly, the application of the elegant 13CO2 labeling technique during rising temperatures contributed to dissect BVOCs synthesized by using freshly carbon assimilated through photosynthesis from the release of BVOCs stored in preexisting pools. Despite the manuscript faces a very important issue regarding the effects of Climate Change on BVOCs emission from the vegetation, I have serious concerns about the physiological and ecological meaning of this study.

Our response: Referee #1 is correct claiming that the physiological meaning of BVOC emissions and their changes in response to stress is an interesting item. However, we here explore the changes of emission strengths as a response to heat stress from the point of view of atmospheric chemistry. Changing source strength in response to stress may have impacts on photochemical ozone formation, on the oxidation capacity of the troposphere and on the formation of secondary aerosols the latter being important for climate change. The impact of BVOC on tropospheric chemistry (and also on biogeochemical carbon cycles) is high and justifies research on changes of BVOC emissions with stress. We therefore submitted the manuscript to BG rather than to a journal focusing on plant physiological aspects of BVOC in this manuscript. To underline our viewpoint we changed the beginning of the abstract accordingly. This text passage now reads:

"Climate change will induce extended heat waves to parts of the vegetation more frequently. Heat may act as stress on plants changing emissions of biogenic volatile organic compound (BVOC). As BVOC impact the atmospheric oxidation cycle and aerosol formation it is important to explore possible changes of BVOC emissions under heat stress conditions. We measured heat stress induced changes in BVOC emissions from European beech, Palestine oak, Scots pine, and Norway spruce in a laboratory..."
setup. Considering only irreversible changes of BVOC emissions as stress impacts we found that heat decreased the de novo emissions of monoterpenes, sesquiterpenes and phenolic BVOC. Decreasing emission strength with heat stress was independent of...”

Remark Referee #1: Indeed kind of ‘heat stress’ has been applied in an arbitrary manner to some plant individuals without a clear ecological reason behind the species selection. Plants were forced to emit BVOCs without any assessment of the physiological performances thus making the scientific outcome difficult to apply to the real world. Then, an attempt to combine abiotic (heat) with biotic (insect) stress has been made without addressing neither the kind or the level of herbivores infestation found on the plant investigated.

Our response: With respect to severeness and duration of heat application please see also our response to the remark of Peter Harley. Parts of the response are repeated here:

We agree with Peter Harley, that in combined heat and water stress situations the temperatures chosen here are conceivable. Furthermore we would like to point out, that comparing visible responses of plants treated here with those observed in the environment show that the plants in our experiment were less affected. During heat waves such as the summer 2003 in mid Europe many deciduous trees (we observed this in particular for Silver birch) lost most of their leaves. This behavior is well known and certainly due to the connected impacts of heat and drought. However, none of the plants used during our measurements showed visible stress symptoms of comparable severeness. We therefore conclude that the stress application (with heat alone) was less severe than the stress appearing periodically in the real environment. We are aware that both, heat and drought were responsible for the plants’ responses in the environment. However to understand the impacts of both stresses requires to check the impacts separately in a first step and thereafter checking the combined effects. This was the reason to first determine impacts of heat for well watered plants although such situations will most probably be uncommon in the environment. More common will be combined effects. In combination both stresses will put much more stress on the plants than that we applied.

Concerning the combination of biotic stress and heat, the aim of our work was to check the impact of heat on both, constitutive and stress induced emissions. Heat stress decreased biotic stress induced emissions (except GLV) similar as was observed for other de novo emissions. The trend of heat stress to decrease de novo BVOC emissions is independent of the emission being constitutive or stress induced. It is therefore also independent on the level of herbivore attack. It is important to know that also biotic stress induced emissions are decreased when heat acts as stressor. We therefore leave the description of suppressed biotic stress induced emissions in the manuscript.

Remark Referee #1: As a consequence, the results shown in the manuscript risk to appear only a list of sterile measurements that do not add any novelty to the research field of BVOCs.

Our response: In the peer reviewed literature we found only few examples where suppression of monoterpene emissions with heat stress on deciduous tree species is described. We found no descriptions of suppression of sesquiterpene emissions with heat, no descriptions of suppressed MeSa emissions and we found no descriptions of the effects observed for conifers. We therefore are convinced that our results do add significant novelty to the research field of BVOC.

Remark Referee #1: Overall, the manuscript should be carefully re-worked and (re-thought) before being accepted for publication. Following my major and minor reviews. Done.

Major reviews:

Remark Referee #1: The whole manuscript focuses on the impact of heat stress on BVOCs without any discussion about the basic role of BVOCs (especially isoprenoids)
in plants protection by exerting a direct antioxidant action (Vickers et al. 2009), or by indirectly contribute to regulate stress-related internal signaling processes (Farmer EE, 2001; Baldwin IT et al. 2006).

Our response: To repeat, referee # 1 is correct claiming that the physiological meaning of BVOC emissions and their changes in response to stress are interesting items. But, we here describe the changes of emission strengths as an important finding with respect to atmospheric chemistry and climate. The importance of BVOC in this field of research justifies our research and we do not add discussion on possible plant protective properties of BVOC as they would be speculative in our experiments where plant internal parameters have not been measured.

Remark Referee #1: Although in Material&Methods section the author mentioned that concentrations of water vapor, CO2 were measured at the same time than other gases, no results have been plotted in the manuscript but only a quick list of data is reported (page 9541 lines 15-19). Indeed it is somehow surprisingly since the kinetics of such fundamental parameters will make a significant contribution to assess plant performances during heat stress, highlighting the physiological meaning of the results achieved.

Our response: We agree with this reviewer and added the new figure 7 including data on net photosynthesis (see also remarks of Peter Harley and our responses to his remarks)

Remark Referee #1: Moreover, there is no reason why the author have investigated only ‘impacts of heat stress on the emissions of BVOC’ without providing any information about the recovery period. In fact most often the recovery is a very much interesting period to evaluate plants ability to acclimate to previously applied stress events by activating diverse defense mechanisms.

Our response: As can be seen from figure 4 we studied the recovery period for constitutive emissions. In this case it took nearly 2 month until monoterpene emissions had reached values as before heat stress application. Using data from other experiments and fitting the temporal behavior in an exponential manner allowed estimating recovery times of constitutive MT emissions also for these experiments. However, similar to the amplitudes of BVOC emission increases, recovery times were highly variable. Using our data alone does not allow quantitative statements on recovery. To give some more information on the recovery of de novo emissions we added information on 1,8-cineole emissions for the experiment no. 6 in Table 3: “Five days after the heat stress 1,8-cineole had recovered but emissions of SQT and MeSa remained negligibly low.”

We probably produced confusion by writing the sentence (p. 9553 line 23): “However, so far we have no data on such a recovery.” We therefore changed this sentence to: “However, so far we have not much data on recovery of de novo emissions.”

Remark Referee #1: In my opinion ‘killing’ plants with heat does not give any useful information to investigate the effect of future global change in ‘living’ plants as the author expected to do.

Our response: As can easily be seen from the new figure 7 this plant recovered with respect to assimilation within less than one day. Although the behavior shown in figure 7 was not general and it took up to two months until plants recovered, we did not kill the plants.

Remark Referee #1: The explanation given in Material&Methods section on experimental plant material selection is very shallow and it appears to be a random choice without a real logic behind. Therefore, a clear ‘selection strategy’ should be addressed as it will also give more ecological relevance to this study.

Our response: Regarding possible changes in BVOC source strength with stress the most important species are those that exhibit strong BVOC emissions and that are widespread. European beech, Scots pine, Norway spruce and Palestine oak are strong and widespread monoterpene emitters (see also P. 9536 lines 6 ff.). Furthermore the conifers were used as proxy for monoterpene storing species and the broadleaf species

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were used as proxy for species showing de novo monoterpene emissions (P. 9537 lines 24 ff.). Our selection strategy was guided by this logic and it was clearly described in the manuscript. We therefore left the text on our ‘selection strategy’ as it was.

Remark Referee #1: Since in Table 2 the author shows fluxes of BVOCs in PICOmol m-2s-1, a calibration plot demonstrating the sensitivity of the analytical system employed is more than welcome because such tiny values are very much prone to artifacts.

Our response: There seems to be a misunderstanding. The signal intensity obtained by GC-MS analysis depends on the amount of sampled BVOC mass. The amount of sampled BVOC mass indeed depends on flux densities. But, the amount of sampled BVOC mass also depends on the amount of probed biomass and on the throughput of air through a chamber. With our set-up we are able to use quite large plants and the leaf area of the Palestine oak investigated in the respective experiment was \( \sim 0.27 \text{ m}^2 \). Investigating this plant with an air flow of \( \sim 15 \text{ l min}^{-1} \) (\( \sim 0.01 \text{ mol s}^{-1} \)) leads to mixing ratios of about 250 ppt at flux densities of 10 picomol m-2 s-1. Emission rates of most of the monoterpenes listed in Table 2 were well above this, resulting in mixing ratios in the ppb range. For a GC-MS system with detection limits below 10 ppt (Heiden et al., 1999) measurements of such concentrations are no problems at all. For the strongest emissions signal to noise ratios were well above 1000:1. As signal to noise ratios in this range are not very much prone to artifacts and furthermore our calibration method was often described in the literature before, we do not add further text on this but add some references including our calibration method: "(Schuh et al., 1997, Heiden et al., 1999, Komenda et al., 2001; Heiden et al., 2003)"

Remark Referee #1: Despite the very high temperature used to simulate heat waves (sometime reaching 51 °C) refers to air-temperature, the author stated that this is somehow realistic (page 9547 lines 10-18) because during severe stress event (like drought) leaves inevitably overheat and therefore can experience even higher temperature than air. This is true, but since the author increased the air temperature (and not the leaf temperature), the explanation results faulty. -

Our response: Indeed leaf temperature can be much higher than air temperature, in particular when plants suffer from drought. But, it should be considered that our plants were well watered. Differences between leaf temperatures and air temperatures therefore were \( \sim 2 \) to 3 °C. This was clearly mentioned in the Materials and Method section (BGD manuscript p. 9538 lines 27, 28). With respect to the absolute temperatures we here again refer to our response to the respective remarks of Peter Harley.

Remark Referee #1: I kindly ask the author to avoid too much broad speculations as the ones reported at the beginning of page 9556 (lines1-7). –

Our response: The text written at the beginning of page 9556 reads: "Induction of biotic stress induced emissions requires activation of the respective biosynthetic pathways. This activation will certainly depend on the plant species, on the kind of infestation and probably also on the degree of infestation. The degree of infestation will also depend on changes of the plants environment with on-going climate change. The development of interactions between plants and their biotic environment is uncertain (Arneth and Niinemets, 2010) and therefore no predictions are possible so far."

We believe that it is not too much broad speculation if we claim that activation of biosynthetic pathways depends on plant species and on the kind and degree of infestation; we furthermore suggest that such interactions may change with on-going climate change but note that it is uncertain how these interactions change and add a peer reviewed reference where this is described. In contrast we intended the whole paragraph including the text referred to by referee #1 as advice against too early generalization of our results. We nevertheless checked the whole text with respect to this comment. We found two statements / text passages that were probably misleading or interpreted as too early generalization. One of them at the end of the abstract, the other one on p.
9549 lines 5 ff. Both were abated by extending the respective sentence, deleting text passages and including another possibility.

Abstract: “Otherwise the overall effect of heat stress will be a lower increase in BVOC emissions than predicted by algorithms that neglect stress impacts.” P. 9549 line 5ff:
The whole paragraph was deleted and the text changed to: “We therefore propose that future heat waves will either increase GLV emissions or leave them unaffected. If heat waves act as stress GLV emissions are the only de novo emissions that may increase with the increasing stress.”

Remark Referee #1: The author should avoid to mix up information about Materials&Methods within the Result section: page 9541 lines 7-12;
Our response: Done. As the respective information can be obtained from table 1 as well as from figure 1, the respective part was exchanged by the sentence: “A three years old beech seedling was exposed to heat stress (details see Table 1) and emission rates measured at a temperature of 31 °C and a PPFD = 800 µmol m-2 s-1 before and after the heat stress, respectively were compared.” Table 1 was changed accordingly.

page 9541-9542 lines 23-28 and lines 1-5, respectively;
Our response: Done. The respective information was also included in table 1 and the respective part was deleted.

page 9543 lines 14-17;
Our response: Remark is correct; this is indeed a mix up of “Materials and Methods” with “Results”. However, test reading by scientists not directly involved in our measurements showed that they did not understand figures 1 and 2 referred to in this part of the text without information on the time schedule of 13CO2 application and heat near to the figures. We leave this short description for clarity.

page 9544 lines 14-19).
Our response: This part reads: “During a time period of about ten weeks (mid of April to end of June, Fig. 4) the plants were held without stress application. MT emissions were fairly stable during that time (e.g. emission rate of lA3-pinene = 4 nmol m-2 s-1, lA3 standard deviation of ± 0.7 nmol m-2 s-1, see Fig. 4). Then the plant chamber temperature increased to 45 °C for 48 h. As observed for the other pines this heat stress induced MT emissions.” Only the first sentence is “Materials and Methods”. The other sentences belong to “Results” and are left in this chapter. We see the first short sentence near to a figure as an aid for clarity and leave this sentence in chapter “Results” for easy reading.

Remark Referee #1: English language needs to be polished. In particular, please avoid the abuse of the article “the” as well as the adjective “this/these” in order to improve the fluency of the manuscript.
Our response: Done.

Minor reviews:
- page 9534 (line 25): all the vegetation?
Our response: To avoid misunderstanding or misinterpretation of this sentence it was changed to: “Vegetation is an important source of reactive biogenic volatile organic compounds (BVOC).”

page 9534 (line 26): “The source strength of these …… which ones? page 9534-9535 (lines 26-1): “The source strength of these …… which ones?
Our response: “Done, the word “these” is deleted.

page 9535 (line 11): For sure there are more ‘up-to-date’ references regarding BVOCs modeling than the one mentioned (see Guenther et al. 2012).
Our response: Thanks for this remark; the reference Guenther et al. (2012) is added.

page 9538 (line 12): it is written “chamber temperatures (between 12 and 31 C)”;
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it mean 12 C=min and 31=max? If so, why did the author set such a big range of temperature variation?

Our response: This was made to determine the temperature dependence of BVOC emissions from pine and used to control whether or not the plants showed the “normal” behavior before the heat stress was applied. Within the error limits temperature dependencies were the same as those reported in literature. To avoid unnecessary extension of the manuscript these numbers were not listed.

page 9539: the mathematical formula shown in his page most likely misses a “Æl” letter; please double check it.

Our response: We checked the formula. As written in BGD it is correct.

page 9541 (line 7): it is written “A three years old beech seedling”; does it mean only ONE tree? I remind the author that (as a rule of thumb) measurements should be always taken at least in 3 different plant individuals to take into account for natural occurring biodiversity.

Our response: In this manuscript we describe the response of de novo emissions with respect to elevated temperatures. Decreasing de novo emissions were observed when heat acted as stress and the effect was observed for de novo emissions from all plants: European beech, Palestine oak, Scots pine, and Norway spruce (in total 12 independent experiments with different individuals). We do not quantitatively compare results between different species which would indeed require more measurements; we distinguish between the two basic emission mechanisms. Plants of certain species are used as representatives for the respective mechanisms that furthermore were tested by labeling experiments. We therefore believe that the number of experiments is sufficient to draw the conclusions given in the manuscript.

The only comparison of data obtained for European beech to data on other species is that of the surprising heat tolerance. Our observation of heat tolerance is based on the results obtained from 4 individuals of European beech seedlings in independent experiments (P 9541, lines 3 - 6). It is furthermore consistent to remarks on the heat tolerance of European beech in peer reviewed literature. As the number of investigated beeches is higher than the rule of thumb and our finding with this respect just confirms existing knowledge, we believe that also our comparison of the heat tolerance of European beech to that of other species is justified from statistical reasons.

page 9544 (line 11): what does it mean “1h twilight”?

Our response: Our way to simulate twilight is now included in the respective sentence and reads: “...diurnal rhythm: 12 h illumination, 10 h darkness, and 1 h twilight simulated by switching on or off single lamps with a time delay of ∼5min. in the morning and in the evening, respectively.”

page 9544 (line 23-24): what is written in these two lines in not supported by any figures and/or tables in the manuscript.

Our response: To show more data on net photosynthesis we added the new figure 7.

page 9547 (line 13-14): please rephrase it.

Our response: Text was rephrased and now reads: “Heat and drought periods are often coupled. Thus the impact of heat may be enhanced by parallel drought because reduction of transpiration causes less cooling of leaves.”

page 9548 (line 11-13): what it is stated here is puzzling; please rephrase it.

Our response: The sentence: “This temporal dynamics certainly superimposes any diurnal variation ...” was deleted.

page 9548-9549 (line 25-28 and lines 1-4): what it is stated here is puzzling; please rephrase it.

Our response: Text is rephrased and now reads: “GLV emissions are related to the degree of membrane damage (Fall et al., 1999; Beauchamp et al., 2005; Behnke et al.,
2009). Assuming that heat stress does not repair damaged membranes consequently leads to the hypothesis that heat stress cannot cause decreasing GLV emissions. We therefore propose that future heat waves will either increase GLV emissions or leave them unaffected.”

Our response: The text was changed to: “The general response of such stress induced de novo emissions to heat stress was similar to that of constitutive de novo emissions. In all cases when the heat acted as stress de novo emissions decreased. An increase was not observed. The reason for the decrease of biotic stress induced de novo emissions is likely the same as that causing decreases of constitutive de novo MT emissions; a general decrease of the plants performance as a consequence of the heat. The decrease in performance may be due to the denaturation of enzymes which synthesize the respective VOC, the breakdown of plant internal signalling cascades, or reduction of carbon supply caused by decreased CO2 uptake.”

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