Interactive comment on “Monoterpene and sesquiterpene emissions from Quercus coccifera exhibit interacting responses to light and temperature” by M. Staudt and L. Lhoutellier

M. Staudt and L. Lhoutellier

michael.staudt@cefe.cnrs.fr

Received and published: 12 September 2011

We thank both referees, Dr. Tiffany Duhl and Dr. Guenther Seufert for the insightful and helpful comments on our manuscript. We are very pleased that both reviewers find our work useful and encourage its publication in Biogeosciences. We have endeavored to respond to all suggestions. We will first answer to general concerns addressed by the referees (i.e. the apparent missing data of leaf temperature and transpiration) and reply then point-by-point to single comments and explain our suggestions for improvements of the manuscript.

Leaf temperature: In our flat leaf chamber, temperature is measured by two thermo-
couples. One inserted through a small hole in the chamber bottom serves as pilot of the chamber temperature regulation (i.e. the water circulating in the chamber frame is heated/cooled until the temperature measured by this thermocouple achieves the programmed target temperature); the second one is clamped together with the leaf inside the chamber and is usually used to assess leaf temperature. In many years of study using different plant species, we could rarely observe clear leaf-to-air temperature differences with this system, and if so, only in experiments with very big-leaved species, probably because, as supposed by Dr. Seufert, the air exchange rates are fast and the fan is placed only a few mm below the plane where the leaves are normally placed. Quercus coccifera, the oak species used in the present study, had small leaves that were very quite tough and often undulated. Consequently, several leaves had to be clamped inside the chamber to get a sufficient biomass and it was impossible to have all of the enclosed lamina surfaces perpendicular to the light source. The leaf-to-air temperature differences indicated by the two thermocouple measurements were variable, overall small and not clearly related to PPFD levels or other measured variables. For example in the light response curves they rarely exceeded 0.3 °C and were on average smaller than 0.1 °C. In the temperature responses, maximum temperature differences approached values of only +0.5°C that were – unlike expected - recorded in the low PPFD series. Temperature differences between these two thermocouples were also observed in the light and temperature responses run without leaves (empty chamber runs) indicating that much of the observed differences could be due to a position effect of the second thermocouple. In light of this, we decided to use the air temperature data for the evaluation and processing of the emission data, i.e. the data of the thermocouple piloting temperature control, whose position never changed during the experiments. We feel that these data are more representative than the “leaf” temperature measured somewhere by chance on a single spot of a shoot with quite complex foliage. Nevertheless, some minor leaf-to-air temperature differences might have been existed and we agree with the referees that this uncertainty cannot completely be ignored in the discussion of our results, especially as we mention in
the DISCUSSION the possible occurrence of dramatic leaf-over temperatures during Mediterranean summer conditions. To remedy this problem we suggest:

- to include in the revised description of the chamber system 2.1 (see our comments to ref 2): ‘A second thermocouple was clamped inside to assess leaf temperature. These data were however not considered in the data evaluation, because the temperature difference between the two thermocouples was small (< 0.5 °C) and variable, obviously unrelated to true leaf-to-air temperature differences.’

- to add in 3.4 after the sentence ‘...gave only marginally different response curves for the 30°C and 37°C series (P<0.1)’: ‘Moreover, it cannot be ruled out that small unaccounted memory effects and leaf temperature variations have influenced the light responses.’

- to insert end of 4.2 after ‘In that case, the large excess of light energy...’ the sentence: ‘Furthermore, some leaf overheating, not detected by our temperature measurements, may have accentuated stress at high PPFD levels.’

- to reword in 4.3 the part ‘However, our simulation we ignored...’ to ‘However, our simulation assumed that leaf temperatures were equal to air temperatures measured above the canopy, because no consistent leaf-to-air temperature differences were detected in our enclosure system during light and temperature ramping. Nevertheless, leaf over-heating may frequently happen in natural conditions (e.g. Singsaas et al. 1999), and be particularly strong in open Mediterranean shrublands during the summer period when plant transpiration is reduced by drought...’

Additional remark: While we could never see strong leaf-to-air temperature differences in our leaf chamber studies, we could indeed observe (using both, thermocouples and infrared thermometers) them in the field on oak saplings during calm sunny summer days. On un-shaded leaves close to the ground we recorded during midday hours leaf temperatures exceeding 45°C. At the same time the surface temperature of the bare soil reached values between 50 and 60°C (!), while air temperature remained
below 35°C.

Missing transpiration data: Transpiration was indeed measured but data were not reported in our first version, because we considered them as irrelevant for the interpretation of the emission data. Perhaps also the humidity sensors and possible condensation problems during and after exposure to very high temperatures have limited the precision of these measurements. In the revised ms, we suggest to mention the H2O measurement in the section METHODS at the end of 2.2 (see below comments to ref 2) and to add a brief description of the transpiration data in the RESULTS under 3.1. and 3.2.: 3.1. Transpiration rate increased with increasing light from about 0.3 mmol m-2 s-1 in the dark to values around 2.0 mmol m-2 s-1, and tended to decrease at highest PPFD levels in the 37°C-series (data not shown). 3.2.: Transpiration data (not shown) were rather scattered over the whole range of temperatures, but tentatively increased with increasing temperature, especially at low assay PPFD.

We do not suggest incorporating H2O data in figures 1 and 2, because we feel that this would overcharge figures and make them less attractive to readers.

Other comments by the referees (point-by-point):

Referee 1 Additional minor comments and suggestions for typographical corrections:
1. Delete ‘already’ p 5692, line 23. Answer: deleted
Referee 1: 2. Insert a hyphen in the phrase ‘man made’ p 5693, line 16. Answer: done
Referee 1: 3. Change ‘level’ to ‘levels’, p 5693, line 21 Answer: changed
Referee 1: 4. Change ‘Early, light’ to ‘Early on, light’, p 5693, line 22 Answer: changed
Referee 1: 5. Change ‘have been’ to ‘were’, p 5693, line 22 Answer: changed
Referee 1: 6. Move ‘such as the isoprene synthase’ from the end of the sentence on p 5694, line 19 to after the word ‘enzymes’ (and insert a comma after ‘enzymes’), on line 18 (p 5694). Answer: corrected
Referee 1: 7. Change ‘more insight in the emission control’ to ‘more insight into the controls over emissions’, p 5694, line 22 Answer: changed

Referee 1: 8. Change ‘understanding on’ to ‘understanding of’, p 5694, line 24 Answer: changed

Referee 1: 9. Delete ‘however’, p 5694, line 26 Answer: deleted

Referee 1: 10. Change ‘overview’ to ‘overviews’, p 5695, line 4 Answer: changed

Referee 1: 11. Delete ‘been’, p 5695, line 10 Answer: deleted

Referee 1: 12. Insert ‘, even then,’ after ‘conditions and’ and before ‘over a rather limited’ to read ‘conditions and, even then, over a rather limited’ , p 5695, line 11 Answer: inserted

Referee 1: 13. Change ‘metabolisms’ to ‘metabolites’, p 5695, line 17 Answer: changed

Referee 1: 14. Change ‘endogenic’ to ‘endogenous’, p 5695, line 21 Answer: changed

Referee 1: 15. Insert a hyphen in the phrase ‘stress induced’ p 5697, lines 12-13 Answer: Hyphen inserted

Referee 1: 16. Change ‘rise’ to ‘increase’, p 5697, line 13 Answer: changed

Referee 1: 17. Could the variable humidity range of 30-60% affect emissions observed during the experiment? (p 5698, line 19) Answer: To our knowledge there is little evidence for direct effects of air humidity on isoprenoid emissions. The relatively large humidity range in our study comes from the difficulty to control humidity in our leaf chamber over a 30°-temperature range (20 to 50 °C). Also at temperatures above 40 °C the relative humidity was kept lower to avoid water condensation in cold spots and spots with pressure drops (air filters, IRGA, VOC sample cartridges...).

Referee 1: 18. Were the same leaves used to measure F(m), as described on p 5699
lines 9-10, which were exposed to intensely high white light levels (10,000 micromoles), then placed in the enclosure and used for BVOC emissions measurements? If so, could this large pulse of light have damaged any leaf tissue/photosynthetic apparatuses prior to emissions measurements and thereby affected results? Answer: Yes, the same leaves were used (mentioned in the beginning of section 2.2). The application of a strong light pulse of around 10000 micromoles is standard procedure in determining Fm and Fm’. The high light intensity is necessary to ensure that all PSII reactions centers become closed (reduced). PSII is not damaged, because the pulse is very brief (<1 sec). However, after a saturation pulse, complete relaxation of PSII (to make fluorescence reaching a new steady-state) usually takes several minutes, which has to be considered when making replicate measurements on a same leaf. Therefore, we spotted different parts of the leaf lamina during replicate measurements of the quantum yield (as mentioned in the second paragraph of 2.2).

Referee 1: 19. Suggest re-wording of ‘on overnight dark-adapted leaves’ on p 5699, line 5 because this is hard to understand. Do you mean leaves were simply left in the dark for 1 night prior to measurement? Answer: Please, see below under 20.

Referee 1: 20. Similarly to comment 19, reword ‘temperatures response and again in the morning afterwards’ to ‘temperature response and again the following morning’, p 5699, line 6 Answer: We suggest rewording this part as follows: Maximum photochemical efficiency of photosystem II (Fv/Fm) was determined in the morning prior running a light or temperatures response and again the following morning. Leaves were equipped with Walz leaf clips in the evening before and left in the dark overnight.

Referee 1: 21. Insert a hyphen in the phrase ‘dark adapted’ p 5699, line 10 Answer: corrected

Referee 1: 22. Change ‘data bases’ to ‘databases’ p 5700, line 28 Answer: changed

Referee 1: 23. I am confused by what you are trying to say the 2 sentences on p 5701 lines 19-22 that begin with ‘The decreases were faster’: :: is this just meant to let the
reader know that artifact effects were considered and minimized by observing how long it took for emissions to decline when leaves were removed from the enclosure? If this is what you meant, state it as such. Answer: Yes, these preliminary experiments were made to assess the maximum memory effect of the plant and enclosure system after a given stabilization time (here 1 hour). Such memory effects (if strong) may affect the apparent shape of emission responses to light and temperature. Following the system response (postillumination BVOC concentration decreases) with and without plant allowed us to discern between memory effects inside the leaves (depletion of BVOC precursor pools + non-specific BVOC storage) and outside the leaves (reversible adsorption of emitted BVOCs on chamber walls, sampling ports a.s.o.). We agree that these details are confusing and are not much relevant for the interpretation of the results. We suggest to simplify this part by mentioning in two sentences only the total memory effects: ‘The results showed that memory effects were small. After 1 hour, concentrations of non-oxygenated and oxygenated monoterpenes were respectively decreased to 1-2 % and 2-8 % of their initial values.’

Referee 1: 24. I think it’s important to emphasize that there were, in some (or most?) instances only a few hours allowed for emissions to stabilize after installation of the enclosures and prior to initiation of BVOC sample collection, and many studies have reported longer equilibration times of 12-24 h necessary for confidence that emissions are not stress-induced (see for example, Duhl et al., 2008 which is cited in the manuscript). Answer: As mentioned in section 2.4., the stabilization time we applied after leaf installation and between each light and temperature increase was about 1 hour. According to our results from kinetic studies, one hour was enough to stabilize CO2/H2O gas exchange and constitutive VOC emissions. We agree that longer equilibration times of 12-24h are absolutely necessary when studying VOC-storing plants, where even moderate mechanical stress can lead to long-lasting emission bursts. For non-VOC storing species, such as oaks, shorter equilibration times might be acceptable or even more reasonable, because here stress-induced changes in VOC emissions can behave in different ways depending on the regarded VOC class. For example GLV emissions
usually emerge almost instantaneously upon stress exposure and disappear also very rapidly. Instead, the induction of stress-induced SQTs, MTs and volatile phenolic compounds may take several hours to few days (see e.g. Staudt et al. 2010 (cited in the ms) for the induction kinetics of different VOC classes). Hence, longer equilibration times could rather favor than avoid the risk seeing stress-induced emissions of SQTs. This is also why we never used same trees/shoots for replicate measurements of light and temperature responses.

Referee 1: 25. In methods section 2.4, was there just one measurement made per tree? I think this is not very clear to the reader. Answer: We measured one response curve (either light or temperature) per tree and shoot in order to avoid the problems of “pseudo repetitions” and of the possible induction of “stress-VOCs” in assayed shoots/trees (see above), and to get a more representative figure of the studied oak species. We slightly changed the text in the new manuscript to be more explicit.

Referee 1: 26. Insert ‘and sampling time’ after ‘airflow’, and ‘the’ before ‘airflow’, to read ‘multiplied by the airflow and sampling time and divided by’, p 5702, line 14 Answer: We suggest to change the text as follows: The BVOC emission rate was calculated as the difference between the air concentration in the chamber enclosing a shoot and the concentration measured in the empty chamber multiplied by the airflow and divided by the projected leaf area (ng m−2 s−1) or leaf dry mass (µg g−1 h−1). The air concentration in a given VOC sample was calculated as the amount of VOC sampled on the cartridge divided by the sampling volume.

Referee 1: 27. Change ‘Eq. (1)’ to ‘Eq. (2)’ on p 5703, line 1, and also change ‘Eq. (2)’ to ‘Eq. (1)’ on p 5703, line 2 because these are apparently swapped in their descriptions. Answer: changed

Referee 1: 28. Eucalyptol is an oxygenated monoterpene even though it is apparently grouped by the authors with non- oxygenated monoterpenes on p 5704, line 6 and again on p 5713, line 24 Answer: Yes, Eucalyptol is indeed an oxygenated MT,
which behaved however like pinenes, limonene... and therefore we grouped it with non-oxygenated MTs. To clarify the point, we suggest adding at the end of 3.1.; “However, the oxygenated MT Eucalyptol behaved like MT-hc and therefore was grouped with these compounds. . . .

Referee 1: 29. Why didn’t the authors repeat the dark BVOC emissions measurements again after the light- and temperature-ramping experiments? This could have shed more light (no pun intended) on whether the GLV emissions were in fact caused by damage to leaf tissues during enclosure installation (and sampling soon after installation) or whether they could also be associated with low light levels? Answer: We did not, because - as it often happens - the detailed evaluation of the chromatograms (a long-lasting job) was finished later when experiments were already done. Also, our light-to-dark transition experiments with this and other oak species did not reveal substantial GLV emissions in response to dark exposures, and to our knowledge there is no literature reporting such a response. Nevertheless we fully agree that our present knowledge on GLV emissions is insufficient. Definitely, we need more studies on these ubiquitous VOCs.

Referee 1: 30. Could the observation of Germacrene-D (often associated with stress-induced emissions) being emitted at the higher temperature level of 37 deg C possibly be related to activation of the Shikimate pathway caused by thermal stress? Perhaps this should be discussed in more detail. Answer: We think that Germacrene D is mainly made in the mevalonate pathway as other sesquiterpenes. We do not know to which extend interactions between the mevalonate and shikimate pathways exist and could have affected Germacrene D synthesis/emissions in our study. However, we agree that Germacrene D and many other SQTs found in the emissions of kermes oak have been seen in stress-induced VOC bouquets of other pant species. In fact, stress-induced VOCs and constitutive VOCs are not always distinguishable; many of typical stress-induced VOCs can also be found in the emissions of apparently healthy plants, albeit at much lower amounts. In any case, the possibility that in our study SQT synthesis
has been somewhat activated by (oxidative) stress associated with temperature and light treatments and perhaps contributed to shape the SQT light and temperature responses cannot be completely ruled out. We do not think that such a stress activation of SQT biosynthesis affected the light response at $37\,^\circ\text{C}$ (Fig 1), because: the mean SQT Es of the $37\,^\circ\text{C}$ series was not higher than the mean SQT Es of the $30\,^\circ\text{C}$ series (24 versus 31 ng/m$^2$sec, t-test: $P = 0.56$), SQT emissions rather decreased than increased at final highest light levels and there is no evidence from fluorescence data or GLV emissions that leaves experienced much more stress in the $37\,^\circ\text{C}$ series than in the $30\,^\circ\text{C}$ series. It is more likely that stress-activated SQT synthesis affected the temperature response at high light (Fig 2), because: the mean SQT Es of the 1000 PPFD series was quite higher than that of the 150 PPFD series (35 versus 13 ng/m$^2$sec, t-test: $P = 0.07$), and because SQT emissions re-increased at final highest temperatures together with the occurrence of photooxidative stress as indicated by fluorescence and GLV emission data. We do not believe that this was the major mechanism that boosted SQT emissions under high light and high temperature, because, as mentioned above, stress-induced activation of SQT synthesis usually proceeds slowly (it requires gene activations). Therefore, other more direct and faster mechanisms have likely been involved, as discussed in section 4.2. Nevertheless, we suggest to add at the end of the discussion 4.2 a sentence mentioning this possibility: 'Finally, the oxidative stress and membrane damages that occurred during heat and high PPFD exposure could have also induced some up-regulation of the biosynthesis of SQTs (Loreto and Schnitzler, 2010) and contributed to increase SV emissions from Q. coccifera leaves.'

Referee 1: 31. Suggest using a different word than 'primordial' on p 5714, line 25; this word sounds strange here. Maybe try 'paramount' instead? Answer: 'Paramount' sounds great, we changed

Referee 1: 32. Insert ‘for non-terpene storing vegetation species’ in between ‘processes’ and ‘should’, Page 5716, line 1 Answer: inserted

Referee 1: 33. Change ‘of’ to ‘from’, p 5716, line 5 to read ‘far from being accomplished’
Referee 2: The plant enclosure and exposure system is homemade and requires some more detail description, in case the authors could not provide a published reference of their experimental setup. E.g., dimension of the chamber, how was the flushing done, Answer: We agree and completely revised the paragraph describing the plant exposure system as follows: ‘Response-curves of foliar BVOC emissions to light and temperature were determined by means of a dynamic, temperature and light controlled enclosure system consisting of a flat rectangular chamber of approx. 105 ml vol (10.5 x 5 x 2 cm). The chamber was made of a double walled water-jacketed stainless steel frame and a lid holding a 50 µm PTFE-film. Chamber and lid were equipped with silicon gaskets to ensure tightness and fine nylon nets to maintain leaves in horizontal position. Homogenous mixing of the chamber air was maintained by a small PTFE fan inserted through the chamber bottom. The chamber was continuously flushed with compressed air (Ingersoll Rand compressor Mod. 49810187) at a constant rate of 0.5 L min-1 (regulated by a Brooks 5815 mass flow controller), which was cleaned and dried in a clean air generator (AIRMOPURE, Chromatotec, France) and re-humidified to achieve relative humidity of 30 to 60 % in the chamber outlet by by-passing a variable portion of the air stream through a washing bottle. Chamber and plant were illuminated with a white light source (OSRAM 1000 W) filtered by a 5-cm water bath. Variation in chamber illumination was achieved by changing the distance between light source and chamber, and by covering the chamber with neutral density filters (Kodak Wratten Gelatin Filters). Chamber air temperature was regulated by a temperature controller (STATOP 4849, Chauvin Arnaux), whose output was connected to a modified heating unit of a laboratory water-bath, which circulated water through the chamber frame. Input temperature was measured by a thermocouple (Chrom-Constantan, OMEGA) inserted through a small hole in the chamber bottom. A second thermocou-
ple was clamped inside to assess leaf temperature. These data were however not considered in the data evaluation, because the temperature difference between the two thermocouples was small (< 0.5 °C) and variable, obviously unrelated to true leaf-to-air temperature differences. The whole system was installed in an air-conditioned laboratory adjacent to the greenhouse. During temperature response measurements, the air temperature of the laboratory was progressively increased by about 10 °C to avoid water condensation in sampling lines and instruments.’

Referee 2: did the chamber work in (what) overpressure? Answer: Yes, the chamber was run in overpressure, which could be seen by a slight swelling of the Teflon film when the chamber was closed. We have not measured this overpressure.

Referee 2: Does the PPFD exposure measured outside the chamber represent the factual leaf exposure to photon flux density and spectrum? Answer: Tests with our PAR probe indicated that the 50-µm-Teflon-film covering the chamber has little effect on photon flux density and spectrum. On the other hand, it is clear that the PPFD level measured by the probe outside the chamber can only be taken as an estimate of the mean PPFD received by all enclosed leaf laminas, especially because leaves were undulated and their laminas could not always exactly positioned perpendicular to the light source. However, we believe that the relative error was similar during all measurement series.

Referee 2: How was variation of temperature exposure done? Answer: Please, see our response above (chamber description) and below (P5702 L4).

Referee 2: Wording in general is fluent and understandable but sometimes the sentences are much too long, e.g. more than 12 lines in last para of p.5693 Answer: We are aware that our English is far of being perfect and are grateful to both referees for their efforts to improve wording and style.

Referee 2: Specific comments (I tried to list below only minor comments and typos in addition to those highlighted already by T. Duhl) P5699-Measurements of photosyn-
thesis: the CI-301 is not in stock anymore at CID - was it running in differential mode? Be more specific. A pity the authors did not measure H2O and transpiration/stomatal conductance - would be most informative in the context of T/light response curves. Answer: We ran the instrument in absolute mode and transpiration was measured and results are now mentioned in the revised manuscript (please, see also our general comments above). We suggest to change the description of photosynthesis and transpiration measurement end of 2.2 as follows: ‘Photosynthesis (net-CO2-assimilation, An) and transpiration were measured by drawing a constant portion of the inlet and outlet air through a CI-301 infrared CO2 gas analyzer run in absolute mode (CID Inc., Camas, WA, USA) via tubes enclosing two humidity sensors with integrated temperature probes (HIH-3602C, Honeywell Inc., IL, USA). CO2 and humidity data were recorded three times during the period of VOC sampling. An and transpiration were calculated according to von Caemmerer and Farquhar (1981).’

Referee 2: P5699-L16: replace “after of a pulse” by “after a pulse” Answer: Replaced

Referee 2: P5700-BVOC emission measurements: It is not clear which samples were analysed by GC-FID and GC-MS and why Answer: We agree that the combination of GC-FID and GC-MS measurements in our study needs to be better explained. In fact VOC sampling for GC-FID analysis was done during all measurements in all experiments and these data were exclusively used to quantify BVOC emissions (as mentioned at the end of 2.3). VOC sampling for GC-MS analysis never replaced sampling for GC-FID measurements. It was done in addition, usually twice during each series, once when emissions were expected to be low and once when emissions were expected to be high. GC-MS data served exclusively to identify compounds. This was possible because both analyses were run with the same set-up (column, temperature programs...) yielding chromatograms with a very similar fingerprint. We proceeded in this way, because FID is a less specific detector than MS. The extrapolation of response factors gained from BVOC standards to others BVOCs of the same classes is less critical with FID than with MS. We suggest to reword the sentence ‘In addi-
tion, BVOC samples were taken...’ as ‘To sustain peak identification of GC-FID measure-
ments, additional BVOC samples (two per response curve) were taken on Perkin
Elmer adsorption cartridges (300 mg Tenax TA, 20-35 mesh, Chrompack) for GC anal-
yses coupled with mass spectrometry (Varian CP3800/Saturn2000 MS equipped with
a Perkin-Elmer Turbomatrix thermo-desorber). Both GCs were run with the same ana-
lytical set-up and program.’

Referee 2: P5701-L24: terminal shoot of the upper tree crown is misleading: a 3 years
old sapling of Kermes Oak I would call a sapling and not a tree Answer: We agree and
changed accordingly

P5702-L3: The responses to temperature WERE measured Answer: please, see our
next answer.

P5702-L4: how was this done, the exposure temperature in 5degC increments between
20 and 50degC? Heating up the water jacket? The twig in the chamber was at 50_C
and the rest of sapling outside at 20_C? This would be a relevant experimental con-
dition and needs to be mentioned Answer: Yes, variation of the chamber temperature
was done by heating up the water circulating in the chamber steel frame. Hence only
the leaves inside were exposed to temperature ramps and not the rest of the saplings,
although we somewhat increased lab temperature during temperature ramps in order
to boost the air humidification system and to minimize water condensation problems
(now mentioned in the new description). We agree that from a physiological viewpoint
it would be better having the whole plant under the same conditions; to our knowledge
most studies on light temperature responses of VOC emissions have used small leaf or
shoot chambers as in our study. It would be interesting to see whether heating or not
the whole plant would alter VOC emission responses at leaf level. On the other hand,
running temperature responses in climate chambers may impose technical constraints
such as the use of VOC sampling lines - nasty for measurements of semivolatiles. To
be more explicit, we changed the description of chamber system, temperature control
and protocol in the sections 2.3 and 2.4 (please, see above).
Referee 2: P5702-L7 –“At the end”- of what? Of one day/one response curve? Answer: Usually at the end of a measurement series (mentioned in the new ms), i.e. after the second Fv/Fm determination in the morning afterwards.

Referee 2: P5705-L19- tended to saturate at lower light levels: I think it is relevant that this happened at 37°C at 3 time higher emission levels compared to 30°C. At 37°C and 1000PPFD one observes about 2300ng of MThc emissions in the light response curve of Fig. 1 and around 800 ng in the temperature response curve in Fig. 2 – such difference is striking and should be discussed Answer: Yes, in the light response at 37°C, emissions tended to level off at lower light levels than at 30°C with a 3fold difference in the absolute emission rates. Indeed this difference cannot totally be explained by the difference in assay temperature. When looking on Fig 2 or Fig 3, one can see that emissions increase about 2fold between 30 and 37°C. Oppositely, MT-hc emission levels were similar in the two temperature response series despite the difference in the assay PPFD. We can also observe huge differences within the series (error bars). Hence it is clear that additional unknown sources of variation existed that determined the overall emission capacities of kermes oak leaves, perhaps weather effects but above all a large tree-to-tree (and/or leaf-to-leaf) variability. This large between-tree/between-shoot variability is mentioned several times in the MS, in the section RESULTS (3.1, 3.2, beginning of 3.4:”Yet, individual replicates largely differed in their absolute emission rates...” and in the section DISCUSSION (4.1). Given that our data cannot really answer to this question, we feel that a more detailed discussion is beyond the scope of our study primarily focusing on light and temperature responses of emissions. We suggest to slightly change the text in 4.1: ‘There were relative large differences among individuals in both quantity and quality of MT emissions, probably associated with inherent differences in the trees capacity to produce MTs, as it has been observed in populations of other MT emitting oak species (Staudt et al., 2004).’

Referee 2: P5706-L11: I do not see this big difference in Fig. 2, until 35deg I see increase to about 800 vs 1000ng, obviously not significant Answer: By “consistently”
we meant always. Between 20 and 35 °C mean emission rates were always higher in the 1000 PPFD series than in the 150 PPFD series, but differences were indeed quite small and for sure not significant. To be more prudent we suggest replacing “consistently” by “somewhat”. As already mentioned above, we believe that between-tree/between-shoot variability perhaps also weather affects were important modulators of the absolute amounts of BVOCs released by kermes oak leaves. Because absolute emission rates are often found to be variable, response curves were statistically compared only on a relative scale (as we state in the INTRODUCTION and at the beginning of chapter 3.4).

Referee 2: P5706-15- equal emission rates at both temperatures: not clear, Fig. 2 shows something different: under 1000 PPFD I see ca 1000 ng at 35°C and lower emissions of ca 700ng at 40C Answer: This statement referred to the individual replicates within the high PPFD series: in two of three replicates the maximum rate was observed at 35 °C; in one replicate we observed about equal rates at 35 and 40 °C. We agree that these details are somewhat confusing and suggest replacing them by a more simplified description: “Under high assay PPFD, emissions of MT-hc peaked around 35 °C, dropped rapidly at higher temperatures and were very low at 50°C.”

Referee 2: P5724 Fig.1 caption: replace traingles with triangles I guess Answer: replaced

Referee 2: P5727-caption Fig. 3: the coefficients of T and light responses are important for eventual users of the results, maybe better to present in a separate table Answer : We first planed to show the coefficient in an extra table, but later abandoned this idea, because the absolute coefficient values should not be taken as granted. We think that much more studies on light and temperature responses are necessary on different plant species and under different conditions to generate some kind of mean responses (if these exist). Actually, our results demonstrate that light and temperature responses are quite variable and that coefficients gained from a given study cannot be easily extrapolated to others. Therefore, we would prefer not showing the coefficients
in an extra table (also in order to keep the paper in length). Nevertheless, we feel that some of our study outputs such as the light dependency of SQT emissions are useful and should be taken in consideration for the prediction of VOC emissions from non-VOC-storing vegetation.

Interactive comment on Biogeosciences Discuss., 8, 5691, 2011.