Emission of volatile organic compounds from plants shows a biphasic pattern within an hormetic context

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Abstract: Biogenic volatile organic compounds (BVOCs) are released to the atmosphere from vegetation. BVOCs aid in maintaining ecosystem sustainability via a series of functions, however, VOCs can alter tropospheric photochemistry and negatively affect biological organisms at high concentrations. Due to their critical role in ecosystem and environmental sustainability, BVOCs receive particular attention by global change biologists. To understand how plant VOC emissions affect stress responses within a dose-response context, dose responses should be evaluated. This commentary collectively documents hormetic-like responses of plant-emitted VOCs to external stimuli. Hormesis is a generalizable biphasic dose response phenomenon where the response to low doses acts in an opposite way at high doses. These collective findings suggest that ecological implications of low-level stress that may alter BVOC emissions should be considered in future studies. This commentary promotes new insights into the interface between biological systems and environmental change that influence several parts

of the globe, and provide a base for advancing hazard assessment testing strategies and protocols to provide decision makers with adequate data for generating environmental standards.

Keywords: dose-response; hormesis; risk assessment; U-shape curve; volatile organic compounds

Capsule: The emission of volatile organic compounds (VOCs) from plants displays a biphasic pattern within a hormetic context.

Introduction

Volatile organic compounds (VOCs) are abundant in the Earth's atmosphere as a result of natural (e.g. vegetation) and anthropogenic emissions. Recently, VOCs received particular attention due to their contribution in the formation of ground-level ozone (O_3). Such O_3 levels can be significantly elevated in the Northern Hemisphere, posing risks to animals and vegetation (Agathokleous et al., 2016; Kampa & Castanas, 2014; Li et al., 2017).

Despite their potential to affect the formation of O_3, VOCs help to maintain ecosystem sustainability (Peñuelas & Staudt, 2010; Tumlinson, 2014). For example, VOCs are part of an external communications network which transfers plant-to-plant signals (Peñuelas & Staudt, 2010; Tumlinson, 2014). A further crucial role is mediating interactions between plants and (a)biotic factors (Peñuelas & Staudt, 2010; Tumlinson, 2014). For instance, when a plant is attacked by herbivores or other organisms such as fungi, it releases a complex VOC blend which will be transferred to neighboring plants preconditioning against potential threats within a defined time window (Himanen et al., 2010; Piesik et al., 2017). Plant-emitted VOCs can be of profound significance to insects which rely on them for tracing their route to host plants (Piesik et al., 2014); plants may also rely on VOCs to attract insects as is the case of the Venus flytrap (*Dionaea muscipula*) which relies on food smell mimicry to attract prey. In addition to VOC utilization by animals to trace host/prey, animals utilize VOCs for sexual communication, i.e. pheromones (Tumlinson, 2014). VOCs, therefore, are relevant to a variety of scientific disciplines, including agriculture, behavioral science, biology, chemistry, earth and planetary sciences, ecology, engineering, environmental science, and physiology.

Within the context of global change, it is necessary to understand how plant VOC emissions affect stress responses in a dose-response framework. Three common dose-response models are the threshold, linear-non-threshold (LNT) and hormetic (Fig 1). The threshold and LNT models have dominated the literature throughout the 20^{th} century (Calabrese 2017a,b).

Hormesis appeared in the mainstream only after the 2000s, creating a dose-response revolution via extensive documentation and challenging the belief that the fundamental nature of dose-response is biphasic rather than a type of combined set of threshold and linear frameworks (Agathokleous, 2018; Calabrese, 2011; Calabrese & Blain, 2011; Calabrese & Mattson, 2017).

Assessment of more than ten thousand dose responses revealed that hormesis is a highly generalizable biological phenomenon, with hormetic dose responses being independent of biological model, endpoint, and stressor, and having maximum response in the low dose zone less than 2-fold the control and width of the low-dose, potentially beneficial, zone lower than 10-fold in dose range (Agathokleous, 2018; Agathokleous et al., 2018; Calabrese, 2011, 2013, 2014, 2017c; Calabrese & Blain, 2009; 2011; Calabrese & Mattson, 2011, 2017; Cedergreen et al., 2007; Hasmi et al., 2014; Kim et al., 2018; Poschenrieder et al., 2013, Vargas-Hernandez et al., 2017).

Analysis

An earlier hypothesis that VOCs emitted by plants may display a hormetic response to environmental factors (Calfapietra et al., 2009) along with recently-reported biphasic emission kinetics of the lipoxygenase (LOX) pathway in cucumber (*Cucumis sativus* L.) in response to time-dependent exposure to methyl jasmonate (MeJA) stimulated the need to assess whether VOCs emission by plants is biphasic by time and by dose in response to stress (Jiang et al., 2017). Emission kinetics of benzenoid methyl salicylate, 4,8-dimethyl-nona-1,3,7-triene (DMNT), sesquiterpene (*E*,*E*)-α-farnesene, LOX, or monoterpene (*E*)-β-ocimene tended to be biphasic in response to the time *Alnus glutinosa* plants were exposed to: feeding from moth *Cabera pusaria* larvae; feeding from sawfly *Monsoma pulveratum* larvae (a); drought (b); or combination of (a) and (b) (Copolovici et al., 2011, 2014). Data suggesting a biphasic emission of a variety of green leaf VOCs were also found in other plants in response to the time exposed to herbivory or

mechanical wounding (Harren & Cristescu, 2013; Piesik et al., 2013), drought (Jubany-Marí et al., 2010), or light (Rasulov et al., 2009, 2011).

In addition to the time-dependent biphasic VOCs responses, concentration-dependent biphasic VOCs responses to external stimuli were also reported (Carriero et al., 2016; Rasulov et al., 2009, 2011). For instance, the response of isoprene emission rate to CO_2 levels was biphasic in aspen (*Populus tremula* × *P. tremuloides*) leaves (Fig 2*A*); isoprene emission rate was closely related to the chloroplastic DMADP pool (Rasulov et al., 2009). In the same aspen hybrid, signs for a biphasic dose response of isoprene emission rate to CO_2 (Fig 2*B*), O_2 (Fig 2*C*), and leaf temperature (Fig 2*D*) were observed (Rasulov et al., 2011). Biphasic emission of isoprenoids is supported by further studies, particularly in response to O_3 (Calfapietra et al., 2009; Tani et al., 2017; Yuan et al., 2016). Emission rates of several compounds (α-pinene, β-pinene, limonene, ocimene, hexanal and DMNT) from birch (*Betula pendula* Roth) plants exposed to O_3 and 10, 30 or 70 kg N ha^{-1} yr^{-1} consistently indicate the occurrence of hormesis (Carriero et al., 2016), however, unlike isoprene, with reduced emission in the low-dose region (Fig 2*E*,*F*,*G*,*H*). Interestingly, the latter results (Carriero et al., 2016) raised the question that some biphasic dose responses may have two maxima, in agreement with results from other studies (Jiang *et al.*, 2017). Reduced emission potential of sesquiterpenes in the low-dose region has been also reported for Norway spruce (*Picea abies* (L.) H. Karst.) which displayed typical hormetic dose response of sesquiterpenes to O_3 (Bourstsoukidis et al., 2012). Similarly, evidence for O_3-induced hormesis in the emission rate of several VOCs was revealed in tobacco (*Nicotiana tabacum* L. 'Wisconsin') plants which were exposed to 0, 400, 600, 800, and 1000 ppb for 30 min and their VOCs emission rates were measured at 0.5, 3, 10, 24, and 48 h (Kanagendran et al., 2018).

Biphasic isoprene emissions, with maximum in the low-dose zone (Fig 2*A*,*B*,*C*,*D*), are particularly important to air quality and environmental health as isoprene is non-specifically

stored in the plants and has the largest emission rate among VOCs thus being the major biogenic VOC affecting tropospheric photochemistry. Hence, higher emissions under low-level stress would result in more isoprene in the atmosphere. However, biphasic emissions of VOCs specifically stored in plant tissues are also important to ecological health because reduced emission in the low-dose zone may have implications for plant tolerance against environmental stress and to the interactions among plants, herbivores, and other organisms, therefore with potential consequences to ecosystem sustainability (Ormeño et al., 2011).

The biphasic dose responses of plant-emitted VOCs as noted above reveal hormesis, a highly generalizable dose response phenomenon (Agathokleous, 2018; Calabrese & Blain, 2009; 2011; Calabrese & Mattson, 2011, 2017). The evolutionary basis of hormesis (Agathokleous, 2018; Calabrese & Mattson, 2017; Kim et al., 2018) suggests that plants/VOCs biology and accompanying VOC emissions have evolved within the context of enhancing communication, conditioning, protection, and defense functions.

Conclusions & Perspectives

This commentary promotes new insights into the interface between biological systems and environmental change that influences several parts of the globe, by collectively documenting hormetic-like responses of plant-emitted VOCs to stimuli. Hormetic acting VOCs challenge the environmental pollution research community to consider the ecological implications of low-level stress that may alter VOC emissions. The blend of VOC emissions also warrants further examination within the context of their evolutionary adaptations and how these may be mediated within a dose response framework.

The study of hormetic dose responses will be challenging, requiring stronger study designs with more doses, especially in the low dose zone, and probably with repeat measure-time components. However, the continued ignoring of the possibility of hormesis in ecological system

responses by inadequate hazard assessment testing strategies and protocols will provide decision makers with inadequate data upon which to base environment standards. With the use of more rigorous and demanding protocols that permit an evaluation of the entire dose response continuum, preferably over time, the scientific and policy rewards should be substantial.

Acknowledgements EA is an International Research Fellow (ID No: P17102) of the Japan Society for the Promotion of Science (JSPS). This research was supported by JSPS KAKENHI Grant Number JP17F17102. JSPS is a non-profit, independent administrative institution. EJC acknowledges longtime support from the US Air Force (AFOSR FA9550-13-1-0047) and ExxonMobil Foundation (S18200000000256). The U.S. Government is authorized to reproduce and distribute for governmental purposes notwithstanding any copyright notation thereon. The views and conclusions contained herein are those of the author and should not be interpreted as necessarily representing policies or endorsement, either expressed or implied. Sponsors had no involvement in study design, collection, analysis, interpretation, writing and decision to and where to submit for publication consideration.

References

Agathokleous, E., 2018. Environmental hormesis, a fundamental non-monotonic biological phenomenon with implications in ecotoxicology and environmental safety. Ecotox. Environ. Safe. 148, 1042–1053.

Agathokleous, E., Kitao, M., Calabrese, E.J., 2018. The rare earth element (REE) lanthanum (La) induces hormesis in plants. *Environmental Pollution*. doi:10.1016/j.envpol.2018.02.068

Agathokleous, E., Saitanis, C.J., Wang, X., Watanabe, M., Koike, T., 2016. A review study on past 40 years of research on effects of tropospheric O_3 on belowground structure,

functioning, and processes of trees: a linkage with potential ecological implications. Wat. Air Soil Poll. 227, 33.

Bourtsoukidis, E., Bonn, B., Dittmann, A., Hakola, H., Hellén, H., Jacobi, S., 2012. Ozone stress as a driving force of sesquiterpene emissions: a suggested parameterisation. Biogeosciences 9, 4337–4352.

Calabrese, E.J., 2011. Toxicology rewrites its history and rethinks its future: Giving equal focus to both harmful and beneficial effects. Environ. Toxicol. Chem. 30, 2658–2673.

Calabrese, E.J., 2013. Biphasic dose responses in biology, toxicology and medicine: Accounting for their generalizability and quantitative features. Environ. Pollut. 182, 452–460.

Calabrese, E.J., 2014. Hormesis: a fundamental concept in biology. Microb. Cell, 1, 145–149.

Calabrese, E.J., 2017a. The threshold vs LNT showdown: Dose rate findings exposed flaws in the LNT model part 1. The Russell-Muller debate. Environ. Res. 154, 435–451.

Calabrese, E.J., 2017b. The threshold vs LNT showdown: Dose rate findings exposed flaws in the LNT model part 2. How a mistake led BEIR I to adopt LNT. Environ. Res. 154, 452-458.

Calabrese, E.J., 2017c. Hormesis commonly observed in the assessment of aneuploidy in yeast. Environ. Pollut. 225, 713–728.

Calabrese, E.J., Blain, R.B., 2009. Hormesis and plant biology. Environ. Pollut. 157, 42–48.

Calabrese, E.J., Blain, R.B., 2011. The hormesis database: The occurrence of hormetic dose responses in the toxicological literature. Regul. Toxicol. Pharm. 61, 73–81.

Calabrese, E.J., Mattson, M.P, 2011. Hormesis provides a generalized quantitative estimate of biological plasticity. J. Cell Commun. Signal. 5, 25–38.

Calabrese, E.J., Mattson, M.P., 2017. How does hormesis impact biology, toxicology, and medicine? Aging Mech. Dis. 3, 13.

Calfapietra, C., Fares, S., Loreto, F., 2009. Volatile organic compounds from Italian vegetation

and their interaction with ozone. Environ. Pollut. 157, 1478–1486.
Carriero, G., Brunetti, C., Fares, S., Hayes, F., Hoshika, Y., Mills, G., Tattini, M., Paoletti, E. 2016. BVOC responses to realistic nitrogen fertilization and ozone exposure in silver birch. Environ. Pollut. 213, 988–995.
Cedergreen, N., Streibig, J.C., Kudsk, P., Mathiassen, S.K., Duke, S.O., 2007. The occurrence of hormesis in plants and algae. Dose-Response 5, 150–162.
Copolovici, L., Kännaste, A., Remmel, T., Vislap, V., Niinemets, Ü., 2011. Volatile emissions from Alnus glutionosa induced by herbivory are quantitatively related to the extent of damage. J. Chem. Ecol. 37, 18–28.
Copolovici, L., Kännaste, A., Remmel, T., Niinemets, Ü., 2014. Volatile organic compound emissions from Alnus glutinosa under interacting drought and herbivory stresses. Environ. Exp. Bot. 100, 55–63.
Harren, F.J.M., Cristescu, S.M., 2013. Online, real-time detection of volatile emissions from plant tissue. AoB Plants 5, plt003.
Hasmi, M.Z., Naveedullah, Shen, H., Zhu, S., Yu, C., Shen, C., 2014. Growth, bioluminescence and shoal behavior hormetic responses to inorganic and/or organic chemicals: A review. Environ. Inter. 64, 28–39.
Himanen, S.J., Blande, J.D., Klemola, T., Pulkkinen, J., Heijari, J., Holopainen, J.K. 2010. Birch (*Betula* spp.) leaves adsorb and re-release volatiles specific to neighbouring plants – a mechanism for associational herbivore resistance? New Phytol. 186, 722–732.
Jiang, Y., Ye, J., Li, S., Niinemets, Ü., 2017. Methyl jasmonate-induced emission of biogenic volatiles is biphasic in cucumber: a high-resolution analysis of dose dependence. J. Exp. Bot. 68, 4679–4694.
Jubany-Marí, T., Prinsen, E., Munné-Bosch, S., Alegre, L., 2010. The timing of methyl jasmonate, hydrogen peroxide and ascorbate accumulation during water deficit and

subsequent recovery in the Mediterranean shrub *Cistus albidus* L. Environ. Exp. Bot. 69, 47–55.

Kampa, M., Castanas, E., 2014. Human health effects of air pollution. Environ. Pollut. 151, 362-367.

Kanagendran, A., Pazouki, L., Shuai, L., Liu, B., Kännaste, A., Niinemets, Ü., 2018. Ozone-triggered surface uptake and stress volatile emissions in *Nicotiana tabacum* 'Wisconsin'. J. Exp. Bot. 69, 681–697.

Kim, Se.-A., Lee, Y.-M., Choi, J.-Y., Jacobs, D.R.Jr., Lee, D.-H., 2018. Evolutionarily adapted hormesis-inducing stressors can be a practical solution to mitigate harmful effects of chronic exposure to low dose chemical mixtures. Environ. Pollut. 233, 725–734.

Li, P., Feng, Z., Calatayud, V., Yuan, X., Xu, Y., Paoletti, E., 2017. A meta-analysis on growth, physiological, and biochemical responses of woody species to groundlevel ozone highlights the role of plant functional types. Plant Cell Environ. 40, 2369–2380.

Ormeño, E., Goldstein, A., Niinemets, Ü., 2011. Extracting and trapping biogenic volatile organic compounds stored in plant species. Trends Anal. Chem. 30, 978–989.

Peñuelas, J., Staudt, M., 2010. BVOCs and global change. Trends Plant Sci. 15, 1360-1385.

Piesik, D., Delaney, K.J., Wenda-Piesik, A., Sendel, S., Tabaka, P., Buszewski, B., 2013. *Meligethes aeneus* pollen-feeding suppresses, and oviposition induces, *Brassica napus* volatiles: beetle attraction/repellence to lilac aldehydes and veratrole. Chemoecology 23, 241–250.

Piesik, D., Kalka, I., Wenda-Piesik, A., Bocianowski, J., 2014. *Apion miniatum* Germ. herbivory on the mossy sorrel, *Rumex confertus* Willd.: induced plant volatiles and weevil orientation responses. Pol. J. Environ. Stud. 23, 2149–2156.

Piesik, D., Wenda-Piesik, A., Weaver, D.K., Morrill, E.L., 2007. Influence of *Fusarium* crown rot disease on semiochemical production by wheat plants. J. Phytopathol. 155, 488–496.

Poschenrieder, C., Cabot, C., Martos, S., Gallego, B., Barceló, J., 2013. Do toxic ions induce hormesis in plants? Plant Sci. 212, 15–25.

Rasulov, B., Huve, K., Valbe, M., Laisk, A., Niinemets, U., 2009. Evidence that light, carbon dioxide, and oxygen dependencies of leaf isoprene emission are driven by energy status in hybrid aspen. Plant Physiol. 151, 448–460.

Rasulov, B., Hüve, K., Laisk, A., Niinemets, Ü., 2011. Induction of a longer term component of isoprene release in darkened aspen leaves: origin and regulation under different environmental conditions. Plant Physiol. 156, 816–831.

Tani, A., Ohno, T., Saito, T., Sohei, I., Yonekura, T., Miwa, M., 2017. Effects of ozone on isoprene emission from two major *Querqus* species native to East Asia. J. Agric. Meteorol. 73, 195–202.

Tumlinson, J.H., 2014. The importance of volatile organic compounds in ecosystem functioning. J. Chem. Ecol. 40, 212-213.

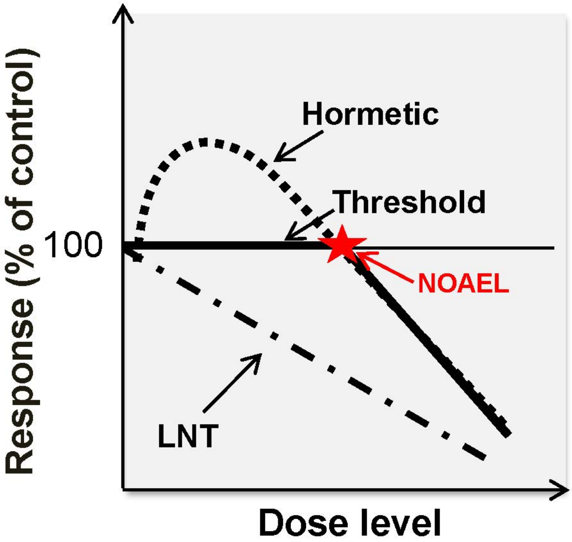
Vargas-Hernandez, M., Macias-Bobadilla, I., Guevara-Gonzalez, R.G., De Romero-Gomez, S.J., Rico-Garcia, E., Ocampo-Velazquez, R.V., De Alvarez-Arquieta, L.L., Torres-Pacheco, I. 2017. Plant hormesis management with biostimulants of biotic origin in agriculture. Front. Plant Sci. 8, 1762.

Yuan, X., Calatayud, V., Gao, F., Fares, S., Paoletti, E., Tian, Y., Feng, Z., 2016. Interaction of drought and ozone exposure on isoprene emission from extensively cultivated poplar. Plant, Cell Environ. 39, 2276–2287.

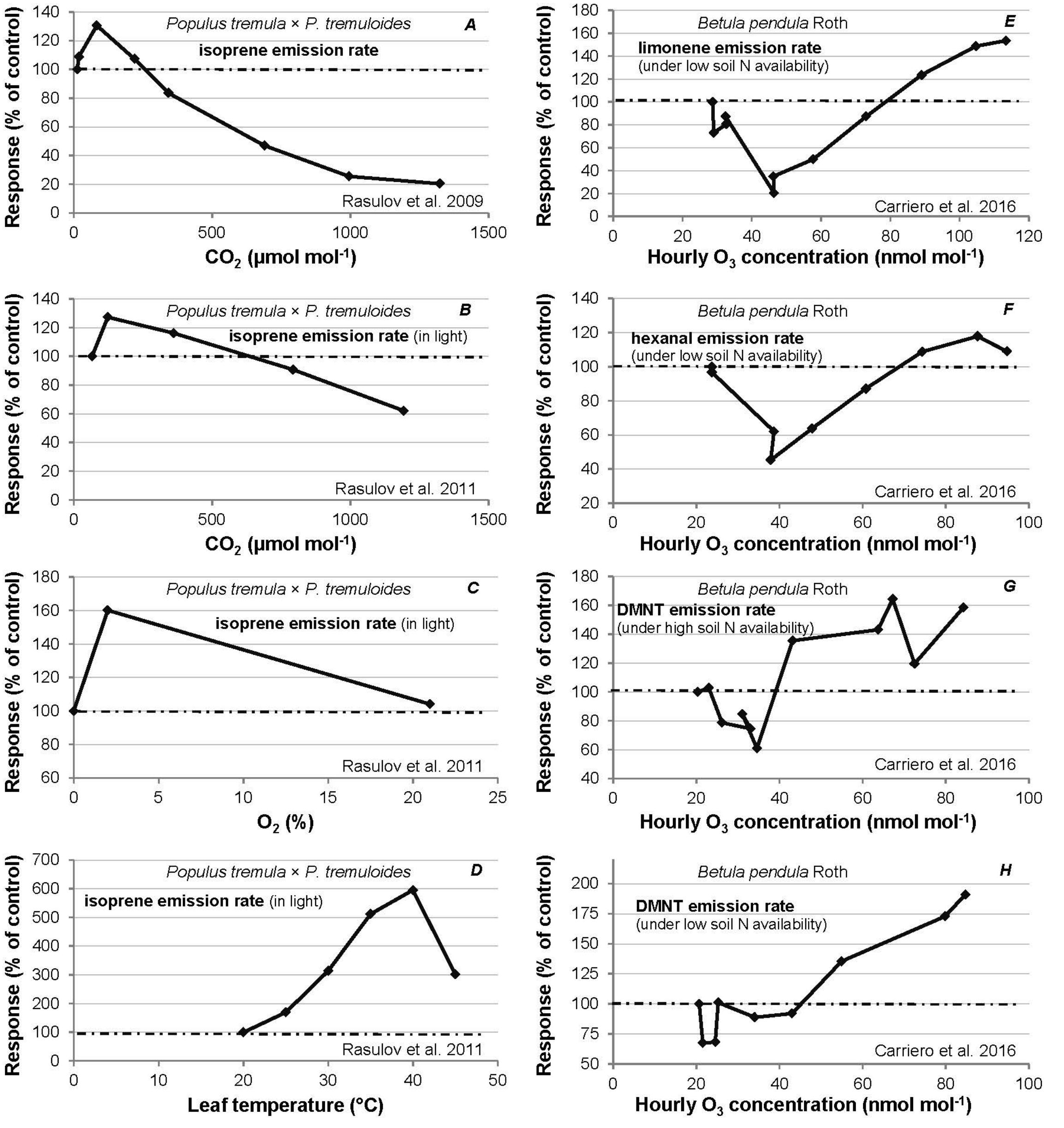
Captions

Fig 1. Hypothetical hormetic, threshold, and linear non-threshold (LNT) dose-response models. The five-point star indicates the no-observed-adverse-effect-level (NOAEL) for the threshold and hormesis models. The LNT model assumes that response increases linearly with increasing dose.

Fig 2. Typical preliminary examples of hormetic-like emissions of volatile organic compounds (VOCs) from plants. Dose and response data presented only in figures in the reviewed articles were estimated using image analysis software (Adobe Photoshop CS4 Extended v.11, Adobe Systems Incorporated, CA, USA).



Response (% of control)
100
Hormetic
Threshold
NOAEL
LNT
Dose level



Populus tremula × P. tremuloides
A
isoprene emission rate
Rasulov et al. 2009
Response (% of control)
CO2 (µmol mol-1)
Betula pendula Roth
E
limonene emission rate
(under low soil N availability)
Carriero et al. 2016
Hourly O3 concentration (nmol mol-1)
B
isoprene emission rate (in light)
Rasulov et al. 2011
F
hexanal emission rate
C
O2 (%)
G
DMNT emission rate
(under high soil N availability)
D
Leaf temperature (°C)
H