***New Phytologist* Supporting Information**

Article title: Green leaf volatile production by plants affect biotic interactions: a meta-analysis
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Article acceptance date: 02 May 2017

The following Supporting Information is available for this article:

Methods S1 Data acquisition and selection for the meta-analysis

Publications were collected using the online search tools: Web of Science ™ and Google Scholar. A combination of the following keywords was used in the online search: plant, volatile, green leaf volatile, hexen*, headspace analysis, insect, wounding, damage, fungus, pathogen. Only studies were withheld which met following criteria:

- ≥3 repetitions
- At least two GLVs are reported
- Control and treatment GLV measurements are reported
- Standard deviations or standard errors are reported
- Masses or molar concentrations are reported (no peak areas or peak intensities)

Studies regarding the effect of abiotic stress or bacterial, viral or oomycete infection on GLV production were not included in this meta-analysis as they did not meet all the requirements.

Notes S1 References used for the meta-analysis

Allmann S, Späthe A, Bisch-Knaden S, Kallenbach M, Reinecke A, Sachse S, Baldwin IT, Hansson BS. 2013. Feeding-induced rearrangement of green leaf volatiles reduces moth oviposition. *Elife* **2**: e00421.

Beck JJ, Porter N, Cook D, Gee WS, Griffith CM, Rands AD, Truong TV, Smith L, San Román I. 2015. In□field volatile analysis employing a hand□held portable GC□MS: emission profiles differentiate damaged and undamaged yellow starthistle flower heads. *Phytochemical Analysis* **26**: 395-403.

Blackmer J, Rodriguez-Saona C, Byers J, Shope K, Smith J. 2004. Behavioral response of Lygus hesperus to conspecifics and headspace volatiles of alfalfa in a Y-tube olfactometer. *Journal of Chemical Ecology* **30**: 1547-1564.

Blande JD, Korjus M, Holopainen JK. 2010. Foliar methyl salicylate emissions indicate prolonged aphid infestation on silver birch and black alder. *Tree Physiology* **30**: 404-416.

Chen Y, Whitehill JG, Bonello P, Poland TM. 2011. Feeding by emerald ash borer larvae induces systemic changes in black ash foliar chemistry. *Phytochemistry* **72**: 1990-1998.

Colazza S, McElfresh JS, Millar JG. 2004. Identification of volatile synomones, induced by Nezara viridula feeding and oviposition on bean spp., that attract the egg parasitoid Trissolcus basalis. *Journal of Chemical Ecology* **30**: 945-964.

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. *Journal of Chemical Ecology* **37**: 18-28.

D'Auria JC, Pichersky E, Schaub A, Hansel A, Gershenzon J. 2007. Characterization of a BAHD acyltransferase responsible for producing the green leaf volatile (Z)□3□hexen□1□yl acetate in *Arabidopsis thaliana*. *The Plant Journal* **49**: 194-207.

Danner H, Boeckler GA, Irmisch S, Yuan JS, Chen F, Gershenzon J, Unsicker SB, Köllner TG. 2011. Four terpene synthases produce major compounds of the gypsy moth feeding-induced volatile blend of Populus trichocarpa. *Phytochemistry* **72**: 897-908.

Degenhardt DC, Refi-Hind S, Stratmann JW, Lincoln DE. 2010. Systemin and jasmonic acid regulate constitutive and herbivore-induced systemic volatile emissions in tomato, *Solanum lycopersicum*. *Phytochemistry* **71**: 2024-2037.

Engelberth J, Alborn HT, Schmelz EA, Tumlinson JH. 2004. Airborne signals prime plants against insect herbivore attack. *Proceedings of the National Academy of Sciences of the United States of America* **101**: 1781-1785.

Erb M, Veyrat N, Robert CA, Xu H, Frey M, Ton J, Turlings TC. 2015. Indole is an essential herbivore-induced volatile priming signal in maize. *Nature communications* **6**: 6273.

Gossner MM, Weisser WW, Gershenzon J, Unsicker SB. 2014. Insect attraction to herbivore-induced beech volatiles under different forest management regimes. *Oecologia* **176**: 569-580.

Himanen S, Vuorinen T, Tuovinen T, Holopainen JK. 2005. Effects of cyclamen mite (*Phytonemus pallidus*) and leaf beetle (*Galerucella tenella*) damage on volatile emission from strawberry (*Fragaria× ananassa* Duch.) plants and orientation of predatory mites (*Neoseiulus cucumeris*, *N. californicus*, and *Euseius finlandicus*). *Journal of Agricultural and Food Chemistry* **53**: 8624-8630.

Huang CH, Yan FM, Byers JA, Wang RJ, Xu CR. 2009. Volatiles induced by the larvae of the Asian corn borer (*Ostrinia furnacalis*) in maize plants affect behavior of conspecific larvae and female adults. *Insect Science* **16**: 311-320.

Jiang Y, Ye J, Veromann L-L, Niinemets Ü. 2016. Scaling of photosynthesis and constitutive and induced volatile emissions with severity of leaf infection by rust fungus (*Melampsora larici-populina*) in *Populus balsamifera* var. suaveolens. *Tree Physiology* **36**: 856-872.

Kariyat RR, Mauck KE, De Moraes CM, Stephenson AG, Mescher MC. 2012. Inbreeding alters volatile signalling phenotypes and influences tri□trophic interactions in horsenettle (*Solanum carolinense* L.). *Ecology Letters* **15**: 301-309.

Kessler A, T Baldwin I. 2004. Herbivore□induced plant vaccination. Part I. The orchestration of plant defenses in nature and their fitness consequences in the wild tobacco Nicotiana attenuata. *The Plant Journal* **38**: 639-649.

Khaling E, Li T, Holopainen JK, Blande JD. 2016. Elevated Ozone Modulates Herbivore-Induced Volatile Emissions of *Brassica nigra* and Alters a Tritrophic Interaction. *Journal of Chemical Ecology* **42**: 368-381.

Li T, Blande JD. 2015. Associational susceptibility in broccoli: mediated by plant volatiles, impeded by ozone. *Global change biology* **21**: 1993-2004.

Matsui K, Sugimoto K, Mano Ji, Ozawa R, Takabayashi J. 2012. Differential Metabolisms of Green Leaf Volatiles in Injured and Intact Parts of a Wounded Leaf Meet Distinct Ecophysiological Requirements. *Plos One* **7**.

Mattiacci L, Rocca BA, Scascighini N, D'alessandro M, Hern A, Dorn S. 2001. Systemically induced plant volatiles emitted at the time of "danger". *Journal of Chemical Ecology* **27**: 2233-2252.

McCall PJ, Turlings TC, Loughrin J, Proveaux AT, Tumlinson JH. 1994. Herbivore-induced volatile emissions from cotton (*Gossypium hirsutum* L.) seedlings. *Journal of Chemical Ecology* **20**: 3039-3050.

Michereff MFF, Laumann RA, Borges M, Michereff-Filho M, Diniz IR, Neto ALF, Moraes MCB. 2011. Volatiles mediating a plant-herbivore-natural enemy interaction in resistant and susceptible soybean cultivars. *Journal of Chemical Ecology* **37**: 273-285.

Morrison III WR, Ingrao A, Ali J, Szendrei Z. 2016. Identification of plant semiochemicals and evaluation of their interactions with early spring insect pests of asparagus. *Journal of Plant Interactions* **11**: 11-19.

Ngumbi E, Chen L, Fadamiro HY. 2009. Comparative GC-EAD responses of a specialist (*Microplitis croceipes*) and a generalist (*Cotesia marginiventris*) parasitoid to cotton volatiles induced by two caterpillar species. *Journal of Chemical Ecology* **35**: 1009-1020.

Peñaflor MFGV, Erb M, Robert CAM, Miranda LA, Werneburg AG, Dossi FCA, Turlings TC, Bento JMS. 2011. Oviposition by a moth suppresses constitutive and herbivore-induced plant volatiles in maize. *Planta* **234**: 207-215.

Piesik D, Miler N, LemaÅ„czyk G, Bocianowski J, Buszewski Ba. 2015. *Botrytis cinerea* infection in three cultivars of chrysanthemum in 'Alchimist' and its mutants: Volatile induction of pathogen-infected plants. *Scientia Horticulturae* **193**: 127-135.

Piesik D, Panka D, Delaney KJ, Skoczek A, Lamparski R, Weaver DK. 2011a. Cereal crop volatile organic compound induction after mechanical injury, beetle herbivory (*Oulema* spp.), or fungal infection (*Fusarium* spp.). *Journal of Plant Physiology* **168**: 878-886.

Piesik D, Panka D, Jeske M, Wenda-Piesik A, Delaney KJ, Weaver DK. 2013. Volatile induction of infected and neighbouring uninfected plants potentially influence attraction/repellence of a cereal herbivore. *Journal of applied entomology* **137**: 296-309.

Piesik D, Wenda-Piesik A, Kotwica K, Łyszczarz A, Delaney KJ. 2011b. *Gastrophysa polygoni* herbivory on Rumex confertus: single leaf VOC induction and dose dependent herbivore attraction/repellence to individual compounds. *Journal of Plant Physiology* **168**: 2134-2138.

Piesik D, Wenda-Piesik A, Krasińska A, Wrzesińska D, Delaney K. 2015. Volatile organic compounds released by *Rumex confertus* following *Hypera rumicis* herbivory and weevil responses to volatiles. *Journal of applied entomology* **140**: 308-316

Pinto-Zevallos DM, Strapasson P, Zarbin PH. 2016. Herbivore-induced volatile organic compounds emitted by maize: Electrophysiological responses in *Spodoptera frugiperda* females. *Phytochemistry Letters* **16**: 70-74.

Pinto DM, Blande JD, Nykänen R, Dong W-X, Nerg A-M, Holopainen JK. 2007. Ozone degrades common herbivore-induced plant volatiles: does this affect herbivore prey location by predators and parasitoids? *Journal of Chemical Ecology* **33**: 683-694.

Rodriguez-Saona C, Crafts-Brander SJ, Pare PW, Henneberry TJ. 2001. Exogenous methyl jasmonate induces volatile emissions in cotton plants. *Journal of Chemical Ecology* **27**: 679-695.

Rodriguez-Saona C, Crafts-Brandner SJ, Cañas LA. 2003. Volatile emissions triggered by multiple herbivore damage: beet armyworm and whitefly feeding on cotton plants. *Journal of Chemical Ecology* **29**: 2539-2550.

Rodriguez-Saona C, Poland TM, Miller JR, Stelinski LL, Grant GG, De Groot P, Buchan L, MacDonald L. 2006. Behavioral and electrophysiological responses of the emerald ash borer, Agrilus planipennis, to induced volatiles of Manchurian ash, Fraxinus *mandshurica*. *Chemoecology* **16**: 75-86.

Rostás M, Ton J, Mauch-Mani B, Turlings TC. 2006. Fungal infection reduces herbivore-induced plant volatiles of maize but does not affect naive parasitoids. *Journal of Chemical Ecology* **32**: 1897-1909.

Rostás M, Turlings TCJ. 2008. Induction of systemic acquired resistance in *Zea mays* also enhances the plant's attractiveness to parasitoids. *Biological Control* **46**: 178-186.

Schwartzberg EG, Böröczky K, Tumlinson JH. 2011. Pea aphids, *Acyrthosiphon pisum*, suppress induced plant volatiles in broad bean, *Vicia faba*. *Journal of Chemical Ecology* **37**: 1055-1062.

Stanton MA, Preβler J, Paetz C, Boland W, Svatoš A, Baldwin IT. 2016. Plant-mediated pheromone emission by a hemipteran seed feeder increases the apparency of an unreliable but rewarding host. *New Phytologist* **211**: 113-125

Sugimoto K, Matsui K, Iijima Y, Akakabe Y, Muramoto S, Ozawa R, Uefune M, Sasaki R, Alamgir KM, Akitake S, Nobuke T, Galis I, Aoki K, Shibata D, Takabayashi J. 2014. Intake and transformation to a glycoside of (Z)-3-hexenol from infested neighbors reveals a mode of plant odor reception and defense. *Proceedings of the National Academy of Sciences of the United States of America* **111**: 7144-7149.

Thaler JS, Karban R, Ullman DE, Boege K, Bostock RM. 2002. Cross-talk between jasmonate and salicylate plant defense pathways: effects on several plant parasites. *Oecologia* **131**: 227-235.

Tooker JF, De Moraes CM. 2007. Feeding by Hessian fly [*Mayetiola destructor* (Say)] larvae does not induce plant indirect defences. *Ecological Entomology* **32**: 153-161.

Verheggen FJ, Haubruge E, De Moraes CM, Mescher MC. 2013. Aphid responses to volatile cues from turnip plants (*Brassica rapa*) infested with phloem-feeding and chewing herbivores. *Arthropod-Plant Interactions* **7**: 567-577.

Vuorinen T, Nerg A-M, Vapaavuori E, Holopainen JK. 2005. Emission of volatile organic compounds from two silver birch (*Betula pendula* Roth) clones grown under ambient and elevated CO_2 and different O_3 concentrations. *Atmospheric Environment* **39**: 1185-1197.

Wei J, Wang L, Zhu J, Zhang S, Nandi OI, Kang L. 2007. Plants Attract Parasitic Wasps to Defend Themselves against Insect Pests by Releasing Hexenol. *Plos One* **2**.

Wenda-Piesik A. 2011. Volatile Organic Compound Emissions by Winter Wheat Plants (*Triticum aestivum* L.) under *Fusarium* spp. Infestation and Various Abiotic Conditions. *Polish Journal of Environmental Studies* **20**: 1335-1342.

Williams L, Rodriguez□Saona C, Paré PW, Crafts□Brandner SJ. 2005. The piercing□sucking herbivores *Lygus hesperus* and *Nezara viridula* induce volatile emissions in plants. *Archives of insect biochemistry and physiology* **58**: 84-96.

Yan ZG, Wang CZ. 2006. Similar attractiveness of maize volatiles induced by *Helicoverpa armigera* and *Pseudaletia separata* to the generalist parasitoid *Campoletis chlorideae*. *Entomologia Experimentalis Et Applicata* **118**: 87-96.

Zebelo SA, Matsui K, Ozawa R, Maffei ME. 2012. Plasma membrane potential depolarization and cytosolic calcium flux are early events involved in tomato (*Solanum lycopersicon*) plant-to-plant communication. *Plant Science* **196**: 93-100.

Methods S2 Methodology used for the meta-analysis

Treatment effect

The meta-analysis is largely based on the methodology used in Rowen & Kaplan (2016) with minor modifications. In short, the treatment effect was calculated using Hedges' g, which represents the standardized mean difference between a treatment and control (Hedges, 1981). As input, the sum of all green leaf volatiles was used. Calculations and plots were made using the packages "meta" and "ggplot2", respectively in R (build 3.2.4).

Publication bias

Funnel plots were created to verify the presence of potential publication bias (i.e. studies that report higher treatment effects are more likely to be published than studies with lower treatment effect) (Sterne & Egger, 2001). In the absence of publication bias, the studies will be distributed symmetrically around the mean treatment effect in a funnel plot. Asymmetry in funnel plots may indicate publication bias. Funnel plots were created using the package "meta" in R and are displayed in Fig. S1. Interpreting the funnel plots, we can see that for the "Fungus" and "Wounding" treatment, the funnel plots are asymmetrical and that a publication bias may be present.

Robustness of the data

To ascertain whether the reported treatment effect is robust and cannot be attributed to the potential publication bias, we calculated the fail safe number (Table 1), using the Rosenberg method (Rosenberg, 2005). The fail-safe number represents the amount of cases with a nonsignificant treatment effect that should be added to the meta-analysis to obtain a non-significant treatment effect. The fail-safe number for each treatment is calculated using the package "meta" in R.

Table 1: Overview of the fail safe numbers (Rosenberg approach) for the different treatments and the number of studies which was used in this meta-analysis.

Treatment	Fail-safe number (Rosenberg approach)	Number of studies included in the analysis
Fungus	1856	26
Insect	3847	88
Wounding	6099	48

Hedges LV. 1981. Distribution theory for Glass's estimator of effect size and related estimators. *Journal of Educational and Behavioral Statistics* **6**: 107-128.

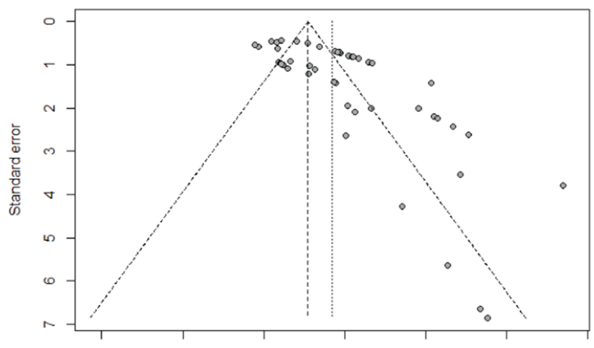
Rosenberg MS. 2005. The file-drawer problem revisited: a general weighted method for calculating fail-safe numbers in meta-analysis. *Evolution* **59**: 464-468.

Rowen E, Kaplan I. 2016. Eco-evolutionary factors drive induced plant volatiles: a meta-analysis. *New Phytologist* **210**: 284-294.

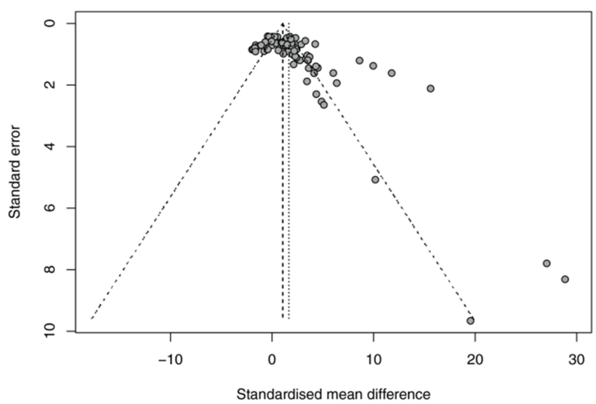
Sterne JA, Egger M. 2001. Funnel plots for detecting bias in meta-analysis: guidelines on choice of axis. *Journal of clinical epidemiology* **54**: 1046-1055.

Fig. S1 Funnel plots for total GLV production after treatment by a fungus, insect or wounding. Funnel plots depict the standardized mean difference (Treatment effect) on the x-axis and the standard error of the treatment effect on the y-axis. Each point represents an experiment of the studies included in the meta-analysis. If no publication bias is present, studies will cluster symmetrically around the mean treatment effect.

Fungi



Insects



Wounding

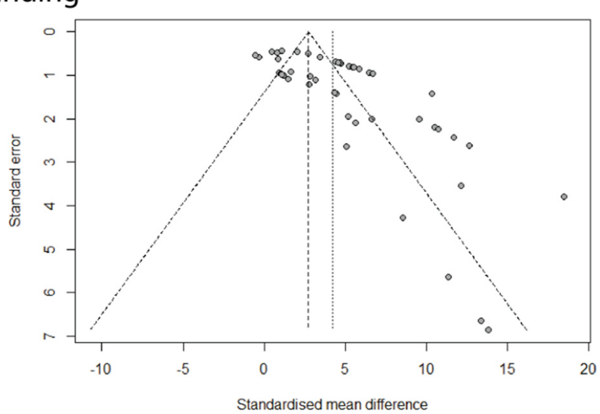


Table S1 Treatment effect and one sample, two sided t-test (H0: Treatment effect=0) for the different treatments

Treatment	Treatment effect (mean)	Treatment effect (median)	n	t-value	P-value
Fungus	12.19	9.67	26	5.744	<0.001
Insect	2.88	1.86	88	5.207	<0.001
Wounding	5.47	4.69	48	8.676	<0.001
Chewing	3.60	2.12	64	5.000	<0.001
Piercing	0.83	0.36	23	2.722	0.012

Table S2 Two-way ANOVA on the treatment effect with Treatment (Fungus, Insect, Wounding) and Taxa (monocots, eudicots) as fixed factors.

Source	Type III sum of squares	df	Mean Square	F	P-value
Treatment	2611.276	2	1282.488	45.917	<0.001
Taxa	754.924	1	779.856	27.921	<0.001
Treatment*Taxa	1712.171	2	836.906	29.964	<0.001
Error	4711.753	156	27.931		
Corrected Total	8382.494	161			

Fig. S2 Treatment effects are not significantly different (P>0.05) between studies which use wounding + oral secretions or real herbivory. The number of studies used to calculate the effect is shown inside the boxplots. Significance of differences was calculated using student T-test