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Goelen, T., Sobhy, I. S., Vanderaa, C., Wäckers, F., Rediers, H., Wenseleers, T., Jacquemyn, H. and Lievens, B. 2020. Bacterial phylogeny predicts volatile organic compound composition and olfactory response of an aphid parasitoid. – Oikos doi: 10.1111/oik.07301

Appendix 1

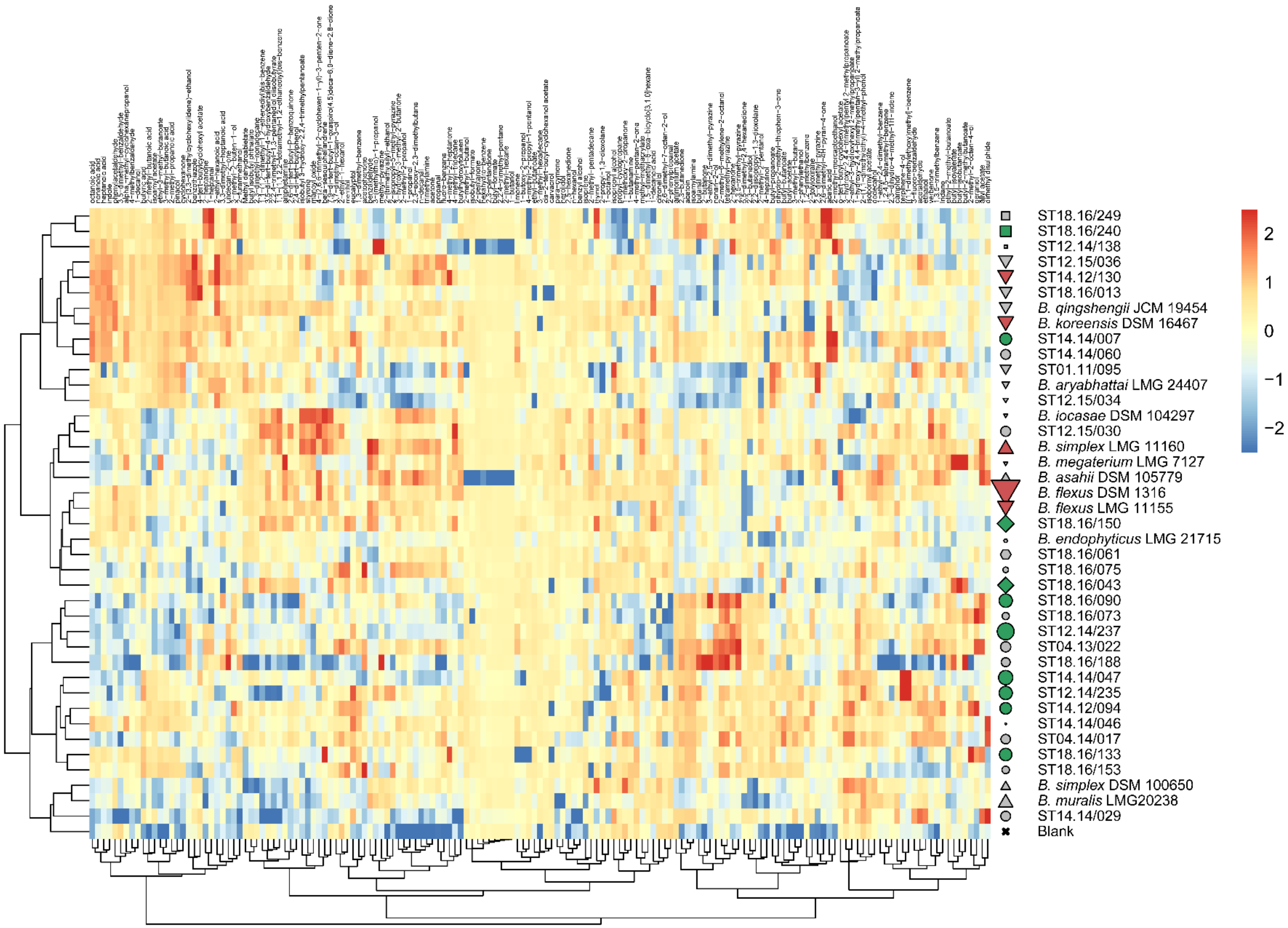

Figure A1. Heat map of the mVOC composition for the 40 *Bacillus* strains investigated in this study. Data are presented in the form of mean centred, log transformed average peak areas of compounds of three biological replicates (*n* = 3). mVOCs and *Bacillus* strain treatments were clustered by using Manhattan distances and a Ward.D2 clustering algorithm. Symbol shapes indicate the clade to which each of the strains belongs (see also Fig. 2): circle = clade A, square = clade B, hexagon = clade C, diamond = clade D, upward facing triangle = clade E, and downward facing triangle = clade F. Symbol colours indicate the effect of the mVOCs on the olfactory response of *Aphidius colemani*, i.e. green = significantly attractive, grey = neutral, and red = significantly repellent. Symbol sizes are proportional to the absolute values of the preference index (PI) as determined in the olfactometer bioassay.

Table A1. Primers used for PCR amplification and sequencing.

Target	**Primer**[a]		**Sequence**	**Spectrum (Clade)**[b]	**Reference**
16S rRNA gene	27F	Forward	5′-AGAGTTTGATCMTGGCTCAG-3′	A,B,C,D,E,F	Lane (1991)[c]
	1492R	Reverse	5′-GGTTACCTTGTTACGACTT-3′	A,B,C,D,E,F	Lane (1991)[c]
rpoB	*rpoB*_r1f	Forward	5’-AGGTCAACTAGTTCAGTATGG-3’	A,C,D,E,F	Cai et al. (2017)[d]
	*rpoB*_r1fB	Forward	5'-AGGTCAACTAGTTCAATACGG-3'	B	This study
	*rpoB*_r1r	Reverse	5’-TAATTCAGCAAGCGGGTTCG-3’	A	Cai et al. (2017)[d]
	*rpoB*_r1rB	Reverse	5'-GTTAACTCTGCTAATGGGTTTG-3'	B,C	This study
	*rpoB*_r1rD	Reverse	5'-GAGTCAATTCAGCTAATGGATTTG-3'	D	This study
	*rpoB*_r1rEF	Reverse	5'-CAATTCGCCTAATGGATTCG-3'	E,F	This study

[a]Primers were combined to obtain amplicons for all strains studied.
[b]For the different clades, see Fig. 2.
[c]Lane D.J. 1991. 16S and 23S rRNA sequencing. – In: Stackebrandt, E. and Goodfellow, M. (eds), Nucleic acids techniques in bacterial systematics. Wiley, pp. 115–175.
[d]Cai, X., Xi, H., Liang, L., Liu, J., Xue, Y. and Yu, X. 2017. Rifampicin-resistance mutations in the *rpoB* gene in *Bacillus velezensis* CC09 have pleiotropic effects. – Front. Microbiol. 8: 178.

Table A2. Results of the olfactometer bioassay.

Isolate identifier	**Preference Index (PI)**[a]	p-**value**
ST14.14/060	0.22	0.107
ST12.14/235	0.32	**0.027**
ST14.14/007	0.28	**0.043**
ST14.14/047	0.35	**0.027**
ST14.14/029	0.22	0.106
ST14.14/046	0.00	0.995
ST04.14/017	0.21	0.112
B. aryabhattai LMG 24407	-0.04	0.794
B. asahii DSM 105779	-0.19	0.176
ST18.16/150	0.33	**0.011**
B. endophyticus LMG 21715	0.07	0.601
ST18.16/061	0.25	0.078
B. flexus DSM 1316	-0.55	**<0.001**
B. flexus LMG 11155	-0.29	**0.030**
ST12.15/030	0.23	0.099
B. iocasae DSM 104297	-0.03	0.796
B. koreensis DSM 16467	-0.28	**0.038**
ST18.16/073	0.15	0.244
ST12.15/036	-0.25	0.078
ST18.16/013	-0.22	0.079
ST12.15/034	-0.06	0.681
ST01.11/095	-0.18	0.212
ST14.12/130	-0.27	**0.046**
B. megaterium LMG 07127	-0.04	0.791
B. muralis LMG 20238	0.25	0.064
ST12.14/138	-0.05	0.699
ST18.16/133	0.30	**0.027**
ST14.12/094	0.27	**0.046**
B. qingshengii JCM 19454	-0.21	0.119
B. simplex DSM 100650	-0.15	0.280
B. simplex LMG 11160	-0.26	**0.049**
ST18.16/153	0.16	0.229
ST18.16/188	0.20	0.112
ST18.16/075	0.11	0.423
ST04.13/022	0.23	0.113
ST12.14/237	0.42	**0.005**
ST18.16/043	0.31	**0.025**
ST18.16/090	0.32	**0.018**
ST18.16/240	0.29	**0.030**
ST18.16/249	0.21	0.152

[a]Results are presented by calculating the preference index (PI) by dividing the difference between the number of parasitoids choosing the bacterial odours and the parasitoids choosing the control by the total number of responding insects. p-values in bold indicate statistically significant differences.

Table A3. Summary of phylogenetic signal indices and Mantel test used to analyse the mVOC profiles for the presence of a phylogenetic signal in individual compounds.

Compound	Pagel's λ	Moran's I	Abouheif's Cmean	Mantel test
butanal	**0.21**	**0,25**	**0.28**	**24.2**
2,3-butanedione	**0.70**	**0.65**	**0.66**	**28.4**
ammonium acetate	**0.57**	**0.44**	**0.46**	**25.5**
acetoin	**0.95**	**0.85**	**0.86**	**28.8**
isoamylamine	**0.88**	**0.83**	**0.83**	**29.0**
methyl-methacrylate	**0.35**	**0.31**	**0.32**	**24.6**
3-methyl-1-butanol	**0.52**	**0.32**	**0.35**	**22.4**
2-methyl-propanoic acid	**0.71**	**0.49**	**0.51**	**24.3**
2,3-butanediol	**0.75**	**0.44**	**0.47**	**21.9**
2-hexanone	**0.56**	**0.53**	**0.54**	**25.2**
ethyl-3-methyl-butanoate	**0.83**	**0.58**	**0.59**	**24.7**
cyclohexanone	**0.60**	**0.42**	**0.44**	**25.3**
benzaldehyde	**0.51**	**0.42**	**0.44**	**24.4**
2,3,5-trimethyl-pyrazine	**0.35**	**0.34**	**0.35**	**23.3**
eucalyptol	**0.29**	**0.28**	**0.29**	**25.4**
phenylacetaldehyde	**0.29**	**0.32**	**0.33**	**25.5**
2-methyl-6-methylene-2-octanol	**0.39**	**0.42**	**0.44**	**26.3**
tetramethyl-pyrazine	**0.42**	**0.45**	**0.46**	**25.6**
nonan-2-ol	**0.70**	**0.64**	**0.64**	**25.1**
2-ethyl-hexanoic acid	**0.58**	**0.51**	**0.52**	**25.3**
nonanoic acid	**0.36**	**0.32**	**0.33**	**24.7**
4-tert-butylcyclohexyl acetate	**0.37**	**0.26**	**0.27**	**24.2**
1-hydroxy-2,4,4-trimethylpentan-3-yl) 2-methylpropanoate	**0.62**	**0.38**	**0.40**	**25.2**
3-hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	**0.34**	**0.41**	**0.42**	**25.6**
2-ethyl-3-hydroxyhexyl 2-methylpropanoate	**0.40**	**0.26**	**0.27**	**24.4**
butyric acid	**0.62**	**0.38**	**0.40**	23.6
3-methyl-butanoic acid	**0.75**	**0.52**	**0.53**	24.6
2-methyl-butanoic acid	**0.97**	**0.25**	**0.27**	20.9
2-heptanone	**0.90**	**0.36**	**0.38**	22.5
butyl-propanoate	**0.70**	**0.37**	**0.39**	24.1
2,6-dimethyl-4H-pyran-4-one	**0.76**	**0.36**	**0.38**	17.9
5,5-dimethyl-2,4-hexanedione	**0.87**	**0.38**	**0.39**	19.0
4-methyl-2-propyl-1-pentanol	**0.49**	**0.36**	**0.38**	10.8
2-phenylpropanal	**0.42**	**0.28**	**0.30**	21.3
3,5-dimethyl-benzaldehyde	**0.24**	**0.26**	0.27	**23.2**
2,2-diisopropyl-1,3-dioxolane	**0.67**	**0.33**	0.34	15.4
butyl isobutyl phthalate	**0.31**	0.21	0.22	**22.6**
Methyl dehydroabietate	**0.27**	0.18	0.19	**23.3**
2-methyl-hexane	**1.00**	-0.05	0.03	6.2

isobutyl-formate	**1.00**	-0.01	0.03	9.7
butyl-formate	**1.00**	-0.05	-0.01	5.5
1-butanol	**1.00**	-0.05	0.03	6.2
2,2,4-trimethyl-pentane	**1.00**	-0.06	0.02	6.5
2-pentanone	**0.99**	-0.05	0.03	8.5
2,3-pentandione	**1.00**	-0.07	-0.03	6.7
3,7-dimethyl-octan-3-ol	**0.49**	0.19	0.22	21.5
2-phenylethanol	**0.72**	0.15	0.18	19.3
3-methyl-hexadecane	**0.99**	0.02	0.05	8.2
ethyl-butanoate	0.13	**0.52**	**0.52**	**14.4**
pinacol	0.21	**0.27**	**0.28**	**23.0**
dimethyl disulphide	0.00	**0.25**	**0.26**	21.3
butyl-acetate	0.51	**0.36**	**0.38**	23.7
ethyl-2-methyl-butanoate	0.44	**0.26**	**0.29**	21.9
2-methyl-2-octen-4-ol	0.00	**0.21**	**0.23**	20.0
geraniol	0.00	**0.29**	**0.31**	23.2
isobornyl acetate	0.09	**0.32**	**0.33**	20.5
2,3-epoxy-2,3-dimethylbutane	0.26	0.18	0.19	**24.8**
1,5-dimethyl-6-oxa-bicyclo[3,1,0]hexane	0.15	0.12	0.14	**17.0**
2-methyl-2-pentanol	0.23	0.12	0.15	**23.8**
?-methylstyrene	0.19	-0.03	-0.01	**24.4**
4-methylbenzaldehyde	0.13	0.06	0.07	**17.4**
2,3-dihydro-4-methyl-1H-indene	0.23	0.18	0.19	**20.7**
alpha,-methyl-cyclohexanepropanol	0.15	0.13	0.14	**22.7**
1-decanol	0.09	0.07	0.08	**14.2**
indole	0.18	0.13	0.14	**25.3**
2-(1,1-dimethylethyl)-4-methyl-phenol	0.25	0.14	0.16	**24.9**
1,1-(1,1,2,2-tetramethyl-1,2-ethanediyl)bis-benzene	0.12	-0.03	-0.02	**23.2**
PC1	**0.61*****	**0.72*****	**0.72*****	**137*****
PC39	0.00	-0.28	-0.30	91.2
Mantel test		***Z* = 6.29x10^{10}**	***P* < 0.001**	

Pagel's λ, Moran's I and Abouheif's C_{mean} were calculated and a Mantel test was performed using normalized peak area data of every mVOC produced by each of the *Bacillus* strains and a phylogenetic tree based on a concatemer of the 16S rRNA gene and *rpoB* sequences. The same tests were performed on the eigenvectors (PC1 and PC39) resulting from the pPCA. Only the compounds showing a significant phylogenetic signal in one or more indices, or test are shown. A Mantel test was used to analyse the complete dataset of all mVOC produced by the *Bacillus* strains. Values in bold indicate a significant phylogenetic signal (*, p < 0.05; **, p < 0.01; ***, p < 0.001).