Investigating the effects of diesel exhaust and flower color on flower visitation by free-flying honey bees

Title:

Investigating the effects of diesel exhaust and flower color on flower visitation by free-flying honey bees

Authors:

Lusebrink I. [1, 2, *]

* Corresponding author

[1] Centre for Biological Sciences, University of Southampton, Life Sciences Building 85, Southampton SO17 1BJ, UK

Current affiliation:

[2] Georg August University of Göttingen, Büsgen-Institute, Department of Forest Zoology and Forest Conservation, Büsgenweg 3, 37077 Göttingen, Germany

E-Mail corresponding author: inka.lusebrink@uni-goettingen.de

https://orcid.org/0000-0003-2937-202X

Girling R. D. [1, 3]

[3] Department of Sustainable Land Management, School of Agriculture, Policy and Development, University of Reading, Reading RG6 6EU, UK

https://orcid.org/0000-0001-8816-8075

Dobrindt L. [4]

[4] Göttingen, Germany

Jackson C. W. [1]

[1] Centre for Biological Sciences, University of Southampton, Life Sciences Building 85, Southampton SO17 1BJ, UK

Newman T. A. [5]

[5] CES, Faculty of Medicine, Institute for Life Sciences, University of Southampton, Southampton SO17 1BJ, UK

https://orcid.org/0000-0002-3727-9258

Poppy G. M. [1]

[1] Centre for Biological Sciences, University of Southampton, Life Sciences Building 85, Southampton SO17 1BJ, UK

Abstract:

Previous laboratory studies have shown that diesel exhaust can differentially degrade the volatile organic compounds (VOCs) that constitute floral odors. Furthermore, in proboscis extension response studies honey bees have been shown to have reduced recognition to these degraded floral odors. In this study, we investigated whether flower odors exposed to diesel exhaust reduce foraging in free-flying bees and if flower color influences bees' behavior. Therefore, we conducted a field study in which honey bees were trained to visit the locations of two arrays of artificial flowers. From the artificial flowers, honey bees were presented with floral VOCs combined with either fresh air or diesel exhaust, through different colored flowers (black, blue, red, and yellow). Honey bee visitation rate did not differ between volatiles delivered with fresh air or with diesel exhaust, suggesting that revisitation of previously rewarding flower patches may be unaffected by air pollution. We also observed a significant interaction between treatment and color: blue flowers were more attractive when volatiles were delivered with diesel exhaust, which was the other way around for red and black and played no role in yellow flowers. Generally, honey bee foraging behavior seemed to be influenced by their previous experiences.

Key words: diesel exhaust, foraging, honey bee, volatile organic compounds, air pollution

Statements and Declarations

Funding

Funding for this research has been provided through the Leverhulme Trust Research Project Grant RPG-089.

Conflict of interest

The authors declare no conflicts of interest.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Contributions

RDG, TAN and GMP conceived the study. IL designed the artificial flower setup and conducted the experiment. IL and LD performed the analysis and wrote the manuscript. CWJ took the UV-VIS measurements and UV pictures. All authors discussed the results and commented on the manuscript.

Acknowledgements

Special thanks go to Martin Fischlechner for the introduction to laser cutting and Przemyslaw Ociepa for loaning me his Hitachi camcorder.

Introduction

Managed European honey bees (*Apis mellifera*) provide critical pollination services for the production of a wide variety of flowering agricultural crops worldwide, the yields of which can be limited by insufficient insect pollination (Reilly et al. 2020). Whilst there is evidence that wild pollinators can be more efficient (Breeze et al. 2011; Garibaldi et al. 2013), a large proportion of current agricultural systems rely on honey bees for pollination service provision (Calderone 2012; Garibaldi et al. 2013). Furthermore, evidence suggests that honey bees are also the most important pollinators in natural habitats worldwide (Hung et al. 2017).

Flower location and recognition is a critical step in the provision of pollination services. During this process, honey bees integrate multisensory stimuli such as odor, color, size and shape (Chittka & Raine 2006), but floral color and odor are considered to be the most important cues (Burger et al. 2010). Visual cues such as floral color are mainly used for short-distance orientation (Dafni et al. 1997) but also contribute to flower constancy, during which honey bees visit the same species of flower, even though other flowers are available. For example, Chittka et al. (2001) demonstrated a relationship between bee-subjective color differences and flower constancy. Honey bees have three receptors for color vision: UV, blue and green (Peitsch et al. 1992). Flower-naïve honey bees prefer bee UV-blue (around 410 nm), bee blue and bee green (around 430 nm and 530 nm, respectively) (Giurfa et al. 1995), but bees can be trained to associate rewards with different floral colors (Menzel 1985).

Floral odors act as long-distance attractants and as both landing and feeding cues (Raguso 2008); these odors are comprised of mixtures of volatile organic compounds (VOCs), which can be plant-species specific (Knudsen et al. 2006). They are easily learned and remembered by a range of insect pollinators (Wright & Schiestl 2009), which contributes to foraging success, increased foraging efficiency (Dötterl & Vereecken 2010) and flower constancy in honey bees (Menzel 1999; Giurfa 2007). However, pollinators forage in a range of environments, both urban and rural, and there is growing evidence to suggest that air pollutants common to both rural (e.g. ozone) and urban (e.g. diesel exhaust) areas can react with the VOCs that constitute floral odors. For example, laboratory studies have shown that ozone can interrupt VOCs that are important for plant-plant signaling (Blande et al. 2010) and that are used as floral odors (Saunier and Blande 2019). Diesel exhaust can affect the ability of honey bees to detect a floral odor indirectly by differentially degrading individual components of that floral odor (Girling et al. 2013; Lusebrink et al. 2015). Atmospheric chemistry modelling of the reaction kinetics of floral odor VOCs with common air pollutants suggests that VOC degradation could dramatically reduce the distances over which floral odors are detectable (McFrederick et al. 2008). Furthermore, recent field studies have suggested that air pollution can reduce flower visitation by pollinating insects, including honey bees, and therefore the pollination services they provide (Ryalls et al. 2022). In addition, diesel exhaust exposure can have negative effects on the learning behavior of honey bees and reduce their tolerance to additional stress factors (Reitmayer et al. 2019).

Due to changing traffic volumes throughout the day (e.g. rush hours vs. times with less traffic) air pollution from diesel exhaust will vary accordingly. Therefore, if diesel exhaust interferes with the attractiveness of a flower, this effect may vary over time, which in return might influence foraging success, foraging efficiency and flower constancy. Therefore, we investigated the effects of exposing floral VOCs to diesel exhaust on honey bee foraging, using a free-flight field-based assay. Using an artificial flower setup our objective was to evaluate whether floral odor delivered alongside diesel exhaust, emitted by a diesel generator, reduces foraging in free flying honey bees and whether this foraging behavior is influenced by flower color.

Material and Methods

Study site

The experiments took place in a walled garden in Chilworth, Hampshire, United Kingdom (50° 57′ 49.7″N, 1° 25′ 23.2″W), which housed three British Standard National beehives and eight nucleus hives. Bees were trained by luring them to a small petri dish (diameter 6 cm) with 30% sucrose solution at the hive entrance, which was slowly moved to an intermediate spot approximately 20 m away from the hives and 30 m from the nucleus hives between two specific locations (~10 m apart) within the garden (also see supplementary file 1 for more detail).

Experimental setup

An artificial flower set up capable of emitting synthetic flower volatiles was designed (Fig. 1; for more details see supplementary file 1). Two setups were built each containing four flowers. Each flower was made of a 7 cm long custom-made glassware adapter with a 14/23 insert joint (flower opening) and a straight 6 mm tubing connection (flower stipe). A Perspex® disk was cut, using a laser cutter, to fit into each glass adapter insert. The disk had a center hole to hold a 0.2 mL PCR tube for offering a sugar reward, and eight holes evenly spaced around the center to allow floral volatile emission. Flower petals were made of 2 mm foam sheets in the colors black, blue, red and yellow that were attached around the Perspex® disk. These colors were chosen with the aim of eliciting a range of behavioral preferences; honey bees are attracted to and can distinguish between yellow and blue (Hill et al 1997), red flowers are less preferred but can be perceived by honey bees (Reisenman and Giurfa 2008) and black flowers are rare in nature. The flowers were inserted into a custom-made Perspex® box, which was covered with camouflage netting and housed the volatile delivery system, which consisted of a battery driven pump, a gas washing bottle, an airflow control valve, and tubing. The volatiles were delivered with a flow of 0.5 L/min through each flower.

For the experiment we used a VOC blend, which contained eight common floral compounds that occur in more than half of all the families of seed plants (Knudsen et al. 2006). All compounds (purity) were purchased through Sigma Aldrich (St. Louis, Missouri, USA) and added to the blend in equal amounts: β-pinene (98%), myrcene (90%), limonene (98%), β-ocimene (≥ 90%), benzaldehyde (99%), β-caryophyllene (≥ 80%), methyl salicylate (≥ 99%), and benzyl alcohol (99%).

Diesel exhaust was pumped from the exhaust pipe of a diesel generator (Suntom SDE 6500 E, Fuzhou Suntom Power Machinery Co., Ltd. Fuzhou, China) through Teflon tubing into 2 bags (~20 L each), which were made by sealing off a polyethylene terephthalate (PET) tube (22 cm diameter, 50 cm length, 25 µm thickness; Kalle UK Ltd, Witham, UK) at both ends. A valve was attached to the bags with Parafilm® (Bemis Company, Inc, Oshkosh, Wisconsin, USA) which allowed the exhaust bags to be filled and stored prior to each experimental run and then be attached to the gas sampling pump of the volatile delivery system of the artificial flower setup.

Experimental design

The two identical artificial flower setups were randomly assigned each day, on four different days, to either location and to one of two treatments: fresh air or diesel exhaust. All four flower colors(black, blue, red, and yellow) were used each day and the order they were presented was randomized. All flowers were of the same color between treatments within one experimental run.

Before each experimental run, 1 µL of the above mentioned synthetic floral volatile blend was added to a filter paper and placed into the gas washing bottle of the artificial flower setup. To each flower a PCR tube filled with a 30% sucrose solution was added as a reward. One experimental run lasted 30 min (Fig. 2). For the first 15 min, volatiles were delivered at a flow rate of 0.5 L/min with fresh air for both treatments. For the second 15 min, the flow rate remained the same but the volatile delivery in the diesel exhaust treatment was switched to diesel exhaust. The diesel exhaust bags were swapped after 7.5 min. Throughout the experimental period of 30 min the sucrose solution was refilled every 10 min. All four colors were run on one day and the experiment was repeated on four days and filmed with a Veho Muvi HD and Hitachi Full HD DZHV 593E camcorder mounted on tripods.

Data analysis

For analysis, videos were played back using the VLC media player and data for one flower at a time was recorded. A flower visit was noted when a worker honey bee landed on the flower and extended her proboscis into the PCR tube holding the sucrose solution. For each flower, the visitation rate per minute was recorded. In most instances the visitation rate (bees per flower per minute) was calculated by dividing the total number of bees which visited a flower during the experimental periods of 15 min by fifteen. However, on a few occasions individual bees accidentally removed the PCR tube with the sugar solution from a flower and hence the reward was not offered for the total experimental period. In these instances, the total number of bees, which have visited the flowers, were divided by the total time that the sugar reward was actually offered.

Bee activity at the artificial flower set up was statistically analyzed using the R software environment (version 3.4.3.; R Development Core Team 2018). A linear mixed-effects model from the *nlme* package (Pinheiro et al. 2015) was fitted. The response variable was the number of bee visits per minute in the second experimental period. Treatment, color, and their interaction were modelled as fixed effects while the visitation rate of the first experimental period was included as a covariate. The date of the visitation was modelled as a random effect. Visual inspection of the residual plots showed no deviations from homoscedasticity and normality. Pairwise comparisons with a Tukey p-value adjustment were conducted using the *pairs* method from the *lsmeans* package (Lenth 2016).

Results

Red flowers were visited significantly more than flowers of any other color ($F_{(3,116)} = 33.759$, $P < 0.001$; Fig. 3A). Bee visits per minute in the first experimental period predicted bee visits of the second period ($F_{(1,116)} = 52.879$, $P < 0.001$). Whether the flower volatiles were delivered by diesel or fresh air did not influence the visitation rate ($F_{(1,116)} = 0.129$, $P = 0.719$; Fig. 3B). However, a significant interaction of color and treatment influenced how many bees visited per minute ($F_{(3,116)} = 9.339$, $P < 0.001$; Fig. 3C). Red and black flowers were visited more in the fresh air treatment (8.28 ± 0.66 SE, 6.32 ± 0.39 SE) compared to the diesel treatment (7.77 ± 0.59 SE, 5.45 ± 0.49 SE). For yellow flowers both treatments were visited at an almost equal rate (clean air: 5.49 ± 0.58 SE, diesel: 5.46 ± 0.76 SE), but blue flowers were more attractive when the volatiles were delivered with diesel exhaust (6.19 ± 0.75 SE) compared to delivery with fresh air (5.14 ± 0.59 SE).

Discussion

In a previous laboratory study, we found that when we exposed the same common flower volatiles used in this study to diesel exhaust, the blend altered significantly. The amount of myrcene decreased, β-ocimene became undetectable, and β-caryophyllene was transformed into its geometric isomer isocaryophyllene. In a behavioral assay (Proboscis Extension Response) we demonstrated that these alterations reduce the ability of honey bees to recognize the floral blend (Lusebrink et al. 2015). However, these previous results were not supported by those of the current field assay, which suggested that whether this common flower volatile blend was delivered using fresh air or diesel exhaust had no influence on the overall visitation rate to the artificial flowers by free-flying honey bees. Whilst there were interactions with flower color, there was no consistent effect of diesel treatment. Honey bee behavior may have been influenced by experience gained beforehand; the bees having previously been trained through positive association to visit the artificial flowers' locations. Floral odors are commonly used in long-range attraction (Raguso 2008) and are likely to be more important for new flower location discovery than for revisitation of an established foraging site. Our result suggests the possibility that the ability to learn the location of successful foraging sites during periods of low air pollution could be sufficient to negate the predicted disruption to foraging that may occur during high air pollution events. We encourage further studies that specifically address this new hypothesis.

Generally, bees preferred the red flowers, which is an unexpected result since the trichromatic color vision of honey bees peaks at a wavelength of 544 nm (green) outside of the red spectrum of human vision (Daumer 1956; Peitsch et al. 1992). Chittka & Waser (1997) reported that the L-receptor of honey bees, which is their long wavelength type photoreceptor, has an extended tail towards longer wavelengths reaching zero at 650 nm and hence bees can perceive the color red. However, bees usually prefer short wavelength stimuli and only exhibit a weak preference for some longer wavelengths (Menzel 1967). Visual discrimination by free-flying honey bees does not seem to be an absolute phenomenon (Avarguès-Weber et al. 2010) but is at least partly dependent on experience (Reser et al. 2012), therefore the preference for the red flowers in our field assay could be related to experiences the honey bees gained before the start of our experiment.

Additionally, the bees' foraging behavior was influenced by treatment when blue flowers were on display. Bees visited more often when the volatiles were delivered by diesel exhaust. This might be due to the fact that toluene, which is part of diesel exhaust (see Lusebrink et al. 2015 supplementary material) is chemically similar to benzaldehyde, a flower volatile which occurs in 64% of seed plant families (Knudsen et al. 2006). Riffel et al. (2014) showed that in *Maduca sexta* moths toluene elicited a strong antennal response and activated the same olfactory sensory neurons that respond to benzaldehyde. It is possible that the honey bees in our study could have recently foraged on blue flowers which emitted benzaldehyde as part of their floral odor, like some *Petunia* species (Stuurman et al. 2004), and hence learned to associate the color blue with benzaldehyde.

The interaction between treatment and color could also be explained by the innate color preference of bees, since we only observed a lower visitation rate in the diesel exhaust treatment, when the flower color on display was not favored by bees (black and red), but not when the other colors were presented. Naïve honey bees favor blue flowers (ranging from UV-blue at around 410 nm to bee blue at 430 nm; Giurfa et al. 1995), which corresponds with our blue foam petals and might explain why, even when the odor signal was altered, the blue flowers were visited more frequently. The color preference for blue is followed by a preference for "bee green" (around 530 nm; Giurfa et al. 1995), which corresponds with our yellow flowers that were visited equally in both treatments. Similarly, Gumbert (2000) showed that free-flying bumblebees, which have similar color preferences to honey bees, revert to their innate preferences under changing circumstances.

The results of this study demonstrate that, in order to understand the potential ecological impacts of air pollution on insect foraging behavior and success, it is crucial to study such interactions under field conditions.

References

Avarguès-Weber A, Giurfa M (2014) Cognitive components of color vision in honey bees: how conditioning variables modulate color learning and discrimination. J Comp Physiol A:1-13. https://doi.org/10.1007/s00359-014-0909-z

Blande JD, Holopainen JK, Li T (2010) Air pollution impedes plant-to-plant communication by volatiles. Ecol Lett 13:1172–81. https://doi.org/10.1111/j.1461-0248.2010.01510.x

Breeze TD, Bailey AP, Balcombe KG, Potts SG (2011) Pollination services in the UK: How important are honeybees? Agric, Ecosyst Environ 142:137-143. https://doi.org/10.1016/j.agee.2011.03.020

Calderone NW (2012) Insect Pollinated Crops, Insect Pollinators and US Agriculture: Trend Analysis of Aggregate Data for the Period 1992–2009 PLOS ONE 7:e37235. https://doi.org/10.1371/journal.pone.0037235

Chittka L, Raine NE (2006) Recognition of flowers by pollinators. Current Opinion in Plant Biology 9:428–435. https://doi.org/10.1016/j.pbi.2006.05.002

Chittka L, Spaethe J, Schmidt A, Hickelsberger A (2001) Adaptation, constraint, and chance in the evolution of flower color and pollinator color vision. In: Thomson JD, Chittka L (eds) Cognitive Ecology of Pollination: Animal Behaviour and Floral Evolution. Cambridge University Press, Cambridge, pp 106–126. https://doi.org/10.1017/CBO9780511542268.007

Chittka L, Waser NM (1997) Why red flowers are not invisible to bees. Israel Journal of Plant Sciences 45:169–183. https://doi.org/10.1080/07929978.1997.10676682

Dafni A, Lehrer M, Keyan P (1997) Spatial flower parameters and insect spatial vision. Biological Reviews 72:239–282. https://doi.org/10.1111/j.1469-185X.1997.tb00014.x

Daumer K (1956) Reizmetrische Untersuchung des Farbensehens der Bienen. Zeitschrift für vergleichende Physiologie 38:413–478. https://doi.org/10.1007/BF00340456

Dötterl S, Vereecken NJ (2010) The chemical ecology and evolution of bee-flower interactions: a review and perspectives. Canadian Journal of Zoology 88:668–697. https://doi.org/10.1139/Z10-031

Garibaldi LA et al. (2013) Wild Pollinators Enhance Fruit Set of Crops Regardless of Honey Bee Abundance. Science 339:1608-1611. https://doi.org/10.1126/science.1230200

Girling RD, Lusebrink I, Farthing E, Newman TA, Poppy GM (2013) Diesel exhaust rapidly degrades floral odours used by honeybees. Scientific Reports 3 Artn 2779. https://doi.org/10.1038/Srep02779

Giurfa M (2007) Behavioral and neural analysis of associative learning in the honeybee: a taste from the magic well. Journal of Comparative Physiology A 193:801–824. https://doi.org/10.1007/s00359-007-0235-9

Giurfa M, Núñez J, Chittka L, Menzel R (1995) Colour preferences of flower-naive honeybees. Journal of Comparative Physiology A 177:247–259. https://doi.org/10.1007/BF00192415

Gumbert A (2000) Color choices by bumble bees (*Bombus terrestris*): innate preferences and generalization after learning. Behav Ecol Sociobiol 48:36-43 doi:10.1007/s002650000213

Hill PSM, Wells PH, Wells H (1997) Spontaneous flower constancy and learning in honey bees as a function of colour. Animal Behaviour 54: 615-627. https://doi.org/10.1006/anbe.1996.0467

Hung K-LJ, Kingston JM, Albrecht M, Holway DA, Kohn JR (2018) The worldwide importance of honey bees as pollinators in natural habitats. Proceedings of the Royal Society B: Biological Sciences 285:20172140. https://doi.org/10.1098/rspb.2017.2140

Knudsen J, Eriksson R, Gershenzon J, Ståhl B (2006) Diversity and distribution of floral scent. The Botanical Review 72:1–120. https://doi.org/10.1663/0006-8101(2006)72[1:DADOFS]2.0.CO;2

Lenth RV (2016) Least-Squares Means: The R Package lsmeans. Journal of Statistical Software, 69(1), 1-33. https://doi.org/10.18637/jss.v069.i01

Lusebrink I, Girling RD, Farthing E, Newman TA, Jackson CW, Poppy GM (2015) The effects of diesel exhaust pollution on floral volatiles and the consequences for honey bee olfaction. Journal of Chemical Ecology 41:904–912. https://doi.org/10.1007/s10886-015-0624-4

McFrederick QS, Kathilankal JC, Fuentes JD (2008) Air pollution modifies floral scent trails. Atmospheric Environment 42:2336–2348. https://doi.org/10.1016/j.atmosenv.2007.12.033

Menzel R (1967) Untersuchungen zum Erlernen von Spektralfarben durch die Honigbiene (*Apis mellifica*). Zeitschrift für vergleichende Physiologie 56:22–62. https://doi.org/10.1007/BF00333562

Menzel R (1985) Learning in honey bees in an ecological and behavioral context. Fortschritte der Zoologie (Stuttgart) 31:55–74.

Menzel R (1999) Memory dynamics in the honeybee. Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology 185:323–340. https://doi.org/10.1007/s003590050392

Peitsch D, Fietz A, Hertel H, de Souza J, Ventura DF, Menzel R (1992) The spectral input systems of hymenopteran insects and their receptor-based colour vision. Journal of Comparative Physiology A 170:23–40. https://doi.org/10.1007/BF00190398

Pinheiro J, Bates D, DebRoy S, Sarkar D and R Core Team (2018) nlme: Linear and Nonlinear Mixed Effects Models. https://cran.r-project.org/web/packages/nlme/nlme.pdf.

Raguso RA (2008) Wake up and smell the roses: The ecology and evolution of floral scent. Annual Review of Ecology, Evolution, and Systematics 39:549–569. https://doi.org/10.1146/annurev.ecolsys.38.091206.095601

Reilly J, Artz D, Biddinger D, Bobiwash K, Boyle N, Brittain C, Brokaw J, Campbell J, Daniels J, Elle E (2020) Crop production in the USA is frequently limited by a lack of pollinators. Proceedings of the Royal Society B 287:20200922. https://doi.org/10.1098/rspb.2020.0922

Reisenman CE, Giurfa M (2008) Chromatic and achromatic stimulus discrimination of long wavelength (red) visual stimuli by the honeybee *Apis mellifera*. Arthropod-Plant Interactions 2: 137-147. https://doi.org/10.1007/s11829-008-9041-8

Reitmayer CM, Ryalls JMW, Farthing E, Jackson CW, Girling RD, Newman TA (2019) Acute exposure to diesel exhaust induces central nervous system stress and altered learning and memory in honey bees. Scientific Reports 9:5793. https://doi.org/10.1038/s41598-019-41876-w

Reser DH, Wijesekara Witharanage R, Rosa MGP, Dyer AG (2012) Honeybees (*Apis mellifera*) learn color discriminations via differential conditioning independent of long wavelength (green) photoreceptor modulation. PLOS ONE 7:e48577. https://doi.org/10.1371/journal.pone.0048577

Riffell JA, Shlizerman E, Sanders E, Abrell L, Medina B, Hinterwirth AJ, Kutz JN (2014) Flower discrimination by pollinators in a dynamic chemical environment. Science 344:1515–1518. https://doi.org/10.1126/science.1251041

Ryalls JMW, Langford B, Mullinger NJ, Bromfield LM, Nemitz E, Pfrang CP, Girling RD (2022) Anthropogenic air pollutants reduce insect-mediated pollination services Environmental Pollution., 297: 118847. https://doi.org/10.1016/j.envpol.2022.118847

Saunier A, Blande JD (2019) The effect of elevated ozone on floral chemistry of Brassicaceae species Environ Pollut 255:113257. https://doi.org/10.1016/j.envpol.2019.113257

Stuurman J, Hoballah ME, Broger L, Moore J, Basten C, Kuhlemeier C (2004) Dissection of floral pollination syndromes in Petunia. Genetics 168:1585–1599. https://doi.org/10.1534/genetics.104.031138

Wright GA, Schiestl FP (2009) The evolution of floral scent: the influence of olfactory learning by insect pollinators on the honest signalling of floral rewards. Functional Ecology 23:841–851. https://doi.org/ 10.1111/j.1365-2435.2009.01627.x

Figures legends:

Figure 1: Artificial flower setup: A) general design, custom-made Perspex® box housing the volatile delivery system, B) artificial flower, made of a glassware adapter with a Perspex® disk in the middle and flower petals made of foam sheets and C) while in use in the field, covered with camouflage netting and attached bag filled with diesel exhaust.

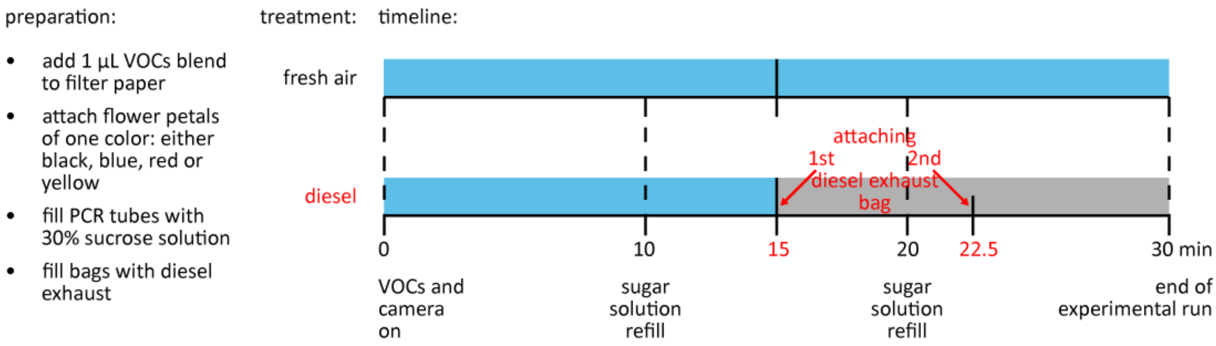

Figure 2: Preparation for and timeline of one experimental run: as preparation for each experimental run, 1 µL of VOCs blend was added to a filter paper and placed into the gas washing bottle of the VOCs delivery system. Flower petals of the same color were attached to the Perspex® disks of each flower (eight in total) and a PCR tube filled with a 30% sucrose solution was added to each flower. The diesel exhaust bags were filled with the exhaust from a diesel generator. At the start of each experimental run the camcorders and the gas sampling pump of the VOCs delivery system were turned on. For the first 15 min, VOCs were delivered with fresh air for both treatments. For the second 15 min the VOCs delivery in the diesel exhaust treatment was switched to diesel exhaust. During the experiment the sucrose solution was refilled every 10 min and the diesel exhaust bag was swapped after 7.5 min.

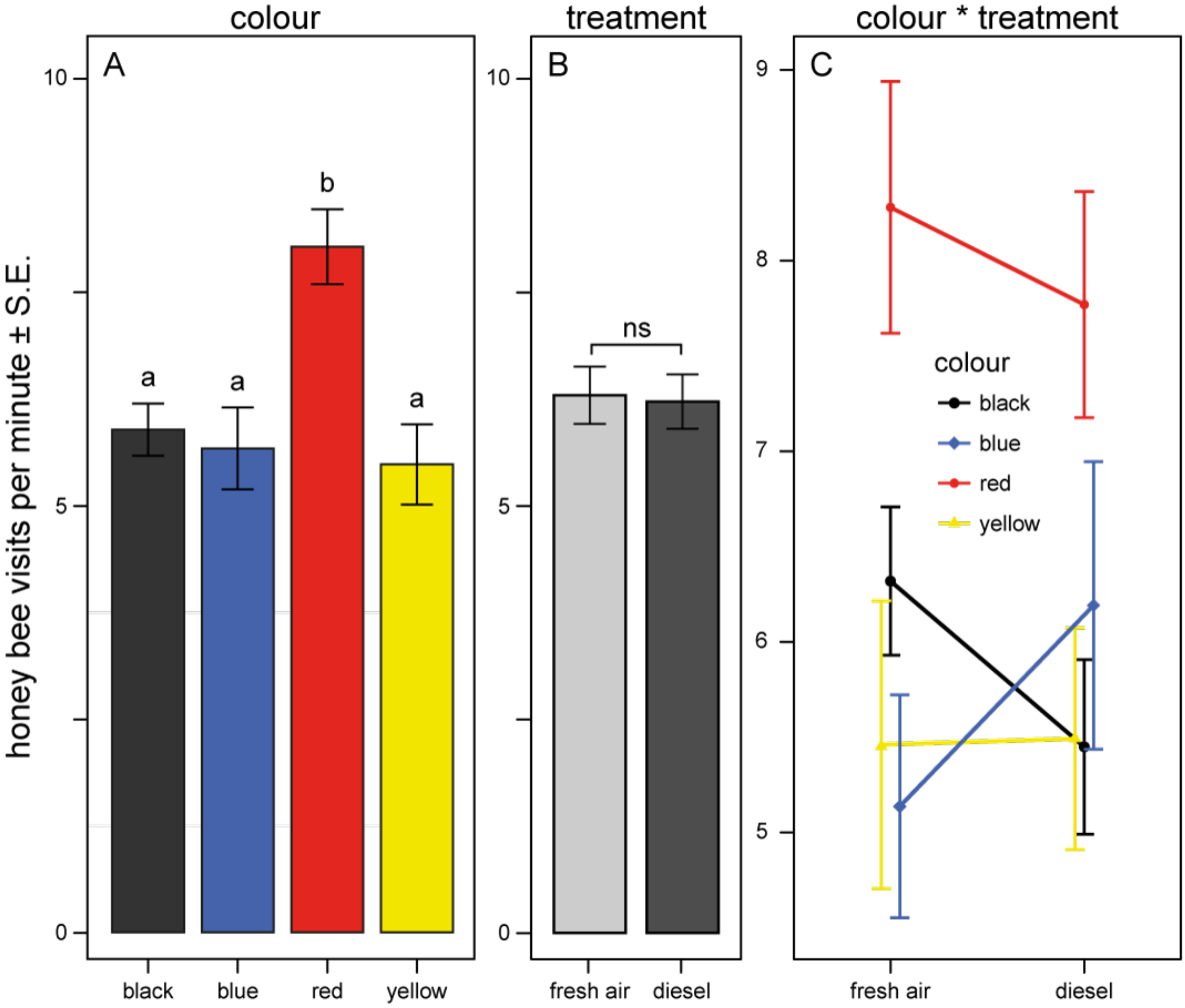

Figure 3: Bar graphs of main effects of A) color, different letters above the bars indicate statistically significant difference (P < 0.05) B) treatment, abbreviation ns: non-significant, and line graph of C) interactions of color and treatment on honey bee visits per minute at the artificial flower set up. The error bars represent standard errors (S.E.).