GEORG-AUGUST-UNIVERSITÄT GÖTTINGEN IN PUBLICA COMMODA SEIT 1737

The effect of global change on multitrophic interactions of sugar beet

Dissertation

To obtain the degree Doctor of Philosophy (Ph.D.)

International Ph.D. Program for Agricultural Sciences in Göttingen (IPAG)

Division of Agricultural Entomology

Faculty of Agricultural Sciences

Georg-August-Universität Göttingen-Germany

Submitted by

Md Shahinoor Rahman

Born in Pabna, Bangladesh

Göttingen, July 2023

Name of referee: Prof. Dr. Michael Rostás
Name of co-referee: Prof. Dr. Mark Varrelmann

Date of examination: 29 August 2023

Dedicated to
my beloved parents and wife

“Success is not the key to happiness. Happiness is the key to success. If you love what you are doing, you will be successful”

---*Albert Schweitzer*

Table of contents

The effect of global change on multitrophic interactions of sugar beet

General introduction

Global climate change is an alarming issue involving significant long-term environmental changes, notably warming temperatures and precipitation patterns (Duchenne-Moutien & Neetoo, 2021). Warmer temperatures enhance evaporation, leading to a reduction in surface water and the desiccation of soil and vegetation, ultimately resulting in drought (Dai et al., 2018). Due to climate change, many countries are now facing more frequent, severe, and long-lasting droughts. Climate change models also predicted increased extreme drought events in central Europe, which have been observed in reality in recent years (Boergens et al., 2020; *IPCC*, 2014), that significantly impact agricultural outputs by impairing plant growth, development, and physiology (Fahad et al., 2017).

Soil salinity is an additional consequence of climate change that adversely affects coastal agricultural land. The continuous rise in sea levels over a period of 25 years has resulted in a significant escalation of salinity levels, leading to an increase in soil salinity from 1% to 33% (Rahman et al., 2018). It affects over 160 countries, covering around 20% of the world's land area (Shahid et al., 2018). Fifty percent of irrigated land of the Asian continent is affected by soil salinity (Pitman & Läuchli, 2002). It negatively affects soil biology and stability, consequently reducing crop yields (Hill & Koenig, 1999). Till now, salinity is not a big problem for Europe; however, the data related to the salinity-affected area in Europe is controversial (Daliakopoulos et al., 2016). According to the current European Union map, salinity in Europe may affect many coastal areas with inland seawater intrusion (Costantini, 2020). The rise in mean temperature increased in Europe in the last few years and the consequent increase in evapotranspiration may have boosted the soil salinity in many parts of Europe (*EIP-AGRI*, 2020). So, salinity is a potential future risk for European agriculture. Therefore, it is of utmost importance to address the forthcoming risks associated with salinity in agricultural crops due to changing climatic conditions.

Sugar beet (*Beta vulgaris*), a member of the Amaranthaceae family, is an economically important industrial crop (Nikan & Manayi, 2019). It is typically grown for its high sugar content, which is extracted from roots. Sugar beet is

utilized to produce granulated sugar, molasses, and other sweeteners, with additional byproducts including pulp for livestock feed, betaine for various applications, vinasse as a fertilizer, pectin in food production, and energy from residual biomass. In 2021 the global sugarbeet production was 270.16 million metric tons (*FAO*, 2023). The European Union (EU) is the world's leading sugar beet producer, with around half of the global production; and in the year 2020-21, Germany is the top producer of sugar from sugar beet among all EU countries (Shahbandeh, 2023). Drought stress affects the yield of sugarbeet. Sugar beet plants need sufficient soil moisture to achieve the maximum potential sugar yield (Rajabi & Taleghani, 2022). On the other hand, the early stages of sugarbeet seedlings are sensitive to salinity, and in the late maturity stages, it is considered tolerant to salinity (Liu et al., 2014). However, the salinity tolerance threshold of sugar beet plants depends on the cultivars, soil water regime, and climatic conditions (Yolcu et al., 2021).

Black bean aphid, *Aphis fabae* (Order: Hemiptera, Family: Aphididae) is an economically important pest for sugar beet (Golizadeh et al., 2016). It is a dark-bodied aphid, a major pest of many agricultural crops causing yield loss through sap-sucking but also through virus transmission (Wamonje et al., 2020), however, its impact is constrained due to limited mobility. Infestations of this pest typically occur in scattered hot spots or along the edges of fields, rather than uniformly affecting the entire field (Nguyen & Nansen, 2018). When natural enemies are lacking, insecticidal application is performed to control this aphid (Roubos et al., 2014). The effect of drought stress on plant-aphid interactions is well documented (Leybourne et al., 2021). Nevertheless, there is limited documentation on the impact of soil salinity on plant-aphid interactions. Furthermore, our understanding of drought and salinity effects on the sugar beet-aphid interactions remains inadequate.

Aphidius colemani (Hymenoptera: Braconidae) is a solitary, koinobiont endoparasitoid of aphids, exhibiting a host range that encompasses over 41 aphid species (Stary, 1975). The larvae of koinobiont parasitoids maintain an intimate association with their host (Strand & Obrycki, 1996). Consequently, the distribution, abundance, and performance of *A. colemani* are contingent upon the quality of its host aphid, and the performance of *A. colemani* might depend on the

stressor from the first trophic level (Prado et al., 2015). However, the indirect effects of drought and soil salinity on parasitoids, are still poorly understood (Han et al., 2019; Kansman et al., 2021).

Beet leaf miner (*Pegomya cunicularia*) is another economically important sugar beet pest. Its larvae tunnel inside the sugar beet leaves, creating large irregular blotch-shaped mines in the leaves and occasionally causing serious damage to the beet (Michelsen, 1980). Global climate change, such as drought, might have the potential to alter the ecological adaptations of beet leaf miners that may create conditions more conducive to their reproduction. Moreover, the ban on neonicotinoids in several European countries has left sugar beet production vulnerable to insect pests, resulting in escalating pest pressure, as evidenced by recent studies (Viric Gasparic et al., 2021). After the ban of neonicotinoid insecticides, the increase of leaf miners was recently observed and threatening sugar beet production. To date, no comprehensive study has confirmed the global climate change effect on sugar beet leaf miners.

The interactions among plants, insect herbivores, and their natural enemies like parasitoids, play a crucial role in shaping multitrophic food webs and significantly influence community dynamics (Tariq et al., 2013). Plants experience simultaneous pressures from above and below-ground insect herbivores, potentially affecting them through plant-mediated interactions (Bezemer et al., 2003). Above-ground herbivores can increase stress in plants and modulate plant physiology. Similarly, drought and soil salinity stresses also alter the plant's physiology. As a result, these stressors may strongly affect the quality and quantity of plant nutrients and central metabolites available to herbivores (Ahuja et al., 2010; Masters et al., 1993). Physiological and chemical changes induce various responses within plants and directly impact foliar insects and their parasitoids (Kaplan et al., 2008). The performance of herbivores can demonstrate variability, encompassing negative, positive, or neutral effects, contingent upon the specific interaction mechanisms, feeding behavior, or the sequence of arrival on the host plant (Erb et al., 2011; Johnson et al., 2012). Drought and salinity may affect the diversity of herbivorous insects, their abundance, and physiology by changing plant physiology (Tariq et al., 2012). Frequency, duration, and/or severity of drought and salinity can alter the structure and composition of the

ecosystem. As a result, global changes empirically addressed the rapid and faster alternations of the multitrophic interactions. It has been suggested that drought and salinity alter the concentrations of plant defense compounds. However, some studies have revealed that the impacts of drought and salinity on plant herbivores can vary, exhibiting both positive and negative effects depending on the type and intensity of the stress and the feeding guild of the herbivores. During multitrophic interactions, plants exhibit complex defense strategies (Van der Putten et al., 2001) that involve the emission of volatile organic compounds (VOCs) and the alternation of their central metabolites in response to herbivore attacks (Zhou et al., 2015). These adaptive responses enhance the efficiency of parasitoids, thereby increasing their effectiveness in controlling herbivorous insects. Plants release VOCs in response to insect herbivory, which serves as chemical cues for natural enemies to locate and target the insect herbivores as hosts (Tumlinson, 2023). The plant VOC emission and changes of primary metabolome induced by foliar herbivores (aphids, leaf miners) can be influenced by drought and soil salinity. So, plant VOC emission and central metabolites changes are influenced by both biotic (aphid, leaf miner) and abiotic (drought and soil salinity) stresses, thus, may directly interfere with herbivore-parasitoid interactions. The behavior and performance of parasitoids can be influenced by their host, host diet, and environmental conditions (Benrey, 2023). The development of parasitoids has also been linked with plant internal metabolites conditions (Yuan et al., 2023).

No comprehensive study has confirmed the effect of global climate change on sugar beet. After the ban of neonicotinoid insecticides, there has been an increase in aphid and leaf miner infestations in Europe, posing a threat to sugar beet production. Therefore, it is essential to understand the interactions between sugar beet, aphids, leaf miners, and their parasitoids under changing climate, particularly in drought and salinity conditions.

Goals of the dissertation

Based on the knowledge gap and relevant areas of study, the goals of this dissertation are as follows:

(i) In order to gain insights into the effects of drought on the interactions between sugar beet, its herbivore (*A. fabae*), and its parasitoid (*A. colemani*), this study aims to address the lack of

information regarding the impact of water-limiting conditions. Specifically, the study focuses on examining the influence of drought on sugar beet growth and morphology and how the drought-induced stress in sugar beet affects the performance of aphids and their parasitoids. Additionally, the study investigates the changes in the profile of sugar beet VOCs due to drought and aphid infestation and explores the consequences of these alterations on the responses of parasitoids.

(ii) Secondly, salinity poses a potential threat to Europe in the future, and the increasing population pressure of aphids could be alarming for sugar beet production. The ultimate goal of this part of the research is to enhance our understanding of how soil salinity and aphid infestation alter sugar beet VOCs emission, primary metabolome, phytohormones, and their impact on the performance of aphid (*A. fabae*), and its parasitoid (*A. colemani*).

(iii) Very limited information is available regarding the beet leaf miner, and currently we have no knowledge about sugar beet and leaf miner interactions under drought conditions. Therefore, the objectives of this dissertation are to investigate how drought affects the central metabolites and VOCs of sugar beet, as well as its impact on the performance and preferences of beet leaf miners. The aim is to fill the knowledge gap and gain a comprehensive understanding of the effects of drought on the interactions between sugar beet and the beet leaf miner, thereby contributing to the management and protection of sugar beet crops.

References

Ahuja, I., de Vos, R. C. H., Bones, A. M., & Hall, R. D. (2010). Plant molecular stress responses face climate change. Trends in Plant Science, 15(12), 664–674. https://doi.org/10.1016/j.tplants.2010.08.002

Benrey, B. (2023). The effects of plant domestication on the foraging and performance of parasitoids. Current Opinion in Insect Science, 57, 101031. https://doi.org/10.1016/j.cois.2023.101031

Bezemer, T. M., Wagenaar, R., Van Dam, N. M., & Wäckers, F. L. (2003). Interactions between above- and belowground insect herbivores as mediated by the plant defense system. Oikos, 101(3), 555–562. https://doi.org/10.1034/j.1600-0706.2003.12424.x

Boergens, E., Güntner, A., Dobslaw, H., & Dahle, C. (2020). Quantifying the Central European Droughts in 2018 and 2019 With GRACE Follow-On. Geophysical Research Letters, 47(14), 1–9. https://doi.org/10.1029/2020GL087285

Costantini, E. A. C. (2020). EIP-AGRI- Focus Group-Soil salinization, pp. 1–16. https://ec.europa.eu/eip/agriculture/sites/default/files/eip-agri_fg36_soil_salinisation_starting_paper_2019_en.pdf

Dai, A., Zhao, T., & Chen, J. (2018). Climate Change and Drought: A Precipitation and Evaporation Perspective. Current Climate Change Reports, 4(3), 301–312. https://doi.org/10.1007/s40641-018-0101-6

Daliakopoulos, I. N., Tsanis, I. K., Koutroulis, A., Kourgialas, N. N., Varouchakis, A. E., Karatzas, G. P., & Ritsema, C. J. (2016). The threat of soil salinity: A European scale review. The Science of the Total Environment, 573, 727–739. https://doi.org/10.1016/j.scitotenv.2016.08.177

Duchenne-Moutien, R. A., & Neetoo, H. (2021). Climate Change and Emerging Food Safety Issues: A Review. Journal of Food Protection, 84(11), 1884–1897. https://doi.org/10.4315/JFP-21-141

EIP-AGRI (2020). EIP-AGRI- Focus Group-Soil salinization Final Report, pp. 1-37. https://ec.europa.eu/eip/agriculture/sites/default/files/eip-agri_fg_soil_salinisation_final_report_2020_en.pdf

Erb, M., Robert, C. A. M., Hibbard, B. E., & Turlings, T. C. J. (2011). Sequence of arrival determines plant-mediated interactions between herbivores. Journal of Ecology, 99(1), 7–15. https://doi.org/10.1111/j.1365-2745.2010.01757.x

Fahad, S., Bajwa, A. A., Nazir, U., Anjum, S. A., Farooq, A., Zohaib, A., Sadia, S., Nasim, W., Adkins, S., Saud, S., Ihsan, M. Z., Alharby, H., Wu, C., Wang, D., & Huang, J. (2017). Crop Production under Drought and Heat Stress: Plant Responses and Management Options. Frontiers in Plant Science, 8. https://www.frontiersin.org/articles/10.3389/fpls.2017.01147

FAO. (2023). Sugar beet production worldwide from 1965 to 2021 (in million tons)*. https://www.statista.com/statistics/249609/sugar-beet-production-worldwide/

Golizadeh, A., Abedi, Z., Borzoui, E., Golikhajeh, N., & Jafary, M. (2016). Susceptibility of Five Sugar Beet Cultivars to the Black Bean Aphid, Aphis fabae Scopoli (Hemiptera: Aphididae). Neotropical Entomology, 45(4), 427–432. https://doi.org/10.1007/s13744-016-0383-0

Han, P., Becker, C., Sentis, A., Rostás, M., Desneux, N., & Lavoir, A.-V. (2019). Global change-driven modulation of bottom–up forces and cascading effects on biocontrol services. Current Opinion in Insect Science, 35, 27–33. https://doi.org/10.1016/j.cois.2019.05.005

Hill, R., & Koenig, R. (1999). Water Salinity and Crop Yield. All Archived Publications, 425(3), 1–6. https://digitalcommons.usu.edu/extension_histall/18/

IPCC (2014). "Climate change 2014 – impacts, adaptation and vulnerability. Part A: global and sectoral aspects," in Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Field, C.B., V.R. Barros, D.J. Dokken, K.J. Mach, M.D. Mastrandrea, T.E. Bilir, M. Chatterjee, K.L. Ebi, Y.O. Estrada, R.C. Genova, B. Girma, E.S. Kissel, A.N. Levy, S. MacCracken, P.R. Mastrandrea, and L.L. White (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, p. 1132.

Johnson, S. N., Clark, K. E., Hartley, S. E., Jones, T. H., McKenzie, S. W., & Koricheva, J. (2012). Aboveground-belowground herbivore interactions: A meta-analysis. Ecology, 93(10), 2208–2215. https://doi.org/10.1890/11-2272.1

Kansman, J. T., Ward, M., LaFond, H., & Finke, D. L. (2021). Effects of Plant Stress on Aphid–Parasitoid Interactions: Drought Improves Aphid Suppression. Environmental Entomology, 50(3), 713–718. https://doi.org/10.1093/ee/nvab005

Kaplan, I., Halitschke, R., Kessler, A., Rehill, B. J., Sardanelli, S., & Denno, R. F. (2008). Physiological integration of roots and shoots in plant defense strategies links above- and

belowground herbivory. Ecology Letters, 11(8), 841–851. https://doi.org/10.1111/j.1461-0248.2008.01200.x

Leybourne, D. J., Preedy, K. F., Valentine, T. A., Bos, J. I. B., & Karley, A. J. (2021). Drought has negative consequences on aphid fitness and plant vigor: Insights from a meta-analysis. Ecology and Evolution, 11(17), 11915–11929. https://doi.org/10.1002/ece3.7957

Liu, Y., Fu, T. T., Sui, N., Ding, T. L., Du, X. H., Song, J., & Wang, B. S. (2014). Response of Sugar Beet to Salinity during the Stages of Seedling Emergence and Plant Growth. Applied Mechanics and Materials, 522–524, 1102–1108. https://doi.org/10.4028/www.scientific.net/AMM.522-524.1102

Masters, G. J., Brown, V. K., & Gange, A. C. (1993). Plant Mediated Interactions between Above- and Below-Ground Insect Herbivores. Oikos, 66(1), 148–151. https://doi.org/10.2307/3545209

Michelsen, V. (1980). A revision of the beet leaf-miner complex, Pegomya hyoscyami s.lat. (Diptera: Anthomyiidae). Insect Systematics & Evolution, 11(3), 297–309. https://doi.org/10.1163/187631280794824631

Nguyen, H. D. D., & Nansen, C. (2018). Edge-biased distributions of insects. A review. Agronomy for Sustainable Development, 38(1), 11. https://doi.org/10.1007/s13593-018-0488-4

Nikan, M., & Manayi, A. (2019). Chapter 3.5—Beta vulgaris L. In S. M. Nabavi & A. S. Silva (Eds.), Nonvitamin and Nonmineral Nutritional Supplements (pp. 153–158). Academic Press. https://doi.org/10.1016/B978-0-12-812491-8.00021-7

Pitman, M. G., & Läuchli, A. (2002). Global Impact of Salinity and Agricultural Ecosystems. In A. Läuchli & U. Lüttge (Eds.), Salinity: Environment—Plants—Molecules (pp. 3–20). Springer Netherlands. https://doi.org/10.1007/0-306-48155-3_1

Prado, S. G., Jandricic, S. E., & Frank, S. D. (2015). Ecological Interactions Affecting the Efficacy of Aphidius colemani in Greenhouse Crops. Insects, 6(2), 538–575. https://doi.org/10.3390/insects6020538

Rahman, A. K. M. M., Ahmed, K. M., Butler, A. P., & Hoque, M. A. (2018). Influence of surface geology and micro-scale land use on the shallow subsurface salinity in deltaic coastal areas: A case from southwest Bangladesh. Environmental Earth Sciences, 77(12), 423. https://doi.org/10.1007/s12665-018-7594-0

Rajabi, A., & Taleghani, D. (2022). Drought Stress Management in Sugar Beet (Beta vulgaris L.) Cultivation. In V. Misra, S. Srivastava, & A. K. Mall (Eds.), Sugar Beet Cultivation, Management and Processing (pp. 429–440). Springer Nature. https://doi.org/10.1007/978-981-19-2730-0_21

Roubos, C. R., Rodriguez-Saona, C., & Isaacs, R. (2014). Mitigating the effects of insecticides on arthropod biological control at field and landscape scales. Biological Control, 75, 28–38. https://doi.org/10.1016/j.biocontrol.2014.01.006

Shahbandeh. (2023). Sugar beet: Sugar production in European countries 2021. Statista. https://www.statista.com/statistics/1126827/sugar-beet-sugar-production-in-european-countries/

Shahid, S. A., Zaman, M., & Heng, L. (2018). Soil Salinity: Historical Perspectives and a World Overview of the Problem. In M. Zaman, S. A. Shahid, & L. Heng (Eds.), Guideline for Salinity Assessment, Mitigation and Adaptation Using Nuclear and Related Techniques (pp. 43–53). Springer International Publishing. https://doi.org/10.1007/978-3-319-96190-3_2

Stary, P. (1975). Aphidius colemani Viereck: Its taxonomy, distribution and host range (Hymenoptera, Aphidiidae). Acta Entomologica Bohemoslovaca, 72(3), 156–163. https://www.cabdirect.org/cabdirect/abstract/19750527699

Strand, M. R., & Obrycki, J. J. (1996). Host Specificity of Insect Parasitoids and Predators. BioScience, 46(6), 422–429. https://doi.org/10.2307/1312876

Tariq, M., Wright, D. J., Bruce, T. J. A., & Staley, J. T. (2013). Drought and Root Herbivory Interact to Alter the Response of Above-Ground Parasitoids to Aphid Infested Plants and

Associated Plant Volatile Signals. PLOS ONE, 8(7), e69013. https://doi.org/10.1371/journal.pone.0069013

Tariq, M., Wright, D. J., Rossiter, J. T., & Staley, J. T. (2012). Aphids in a changing world: Testing the plant stress, plant vigour and pulsed stress hypotheses. Agricultural and Forest Entomology, 14(2), 177–185. https://doi.org/10.1111/j.1461-9563.2011.00557.x

Tumlinson, J. H. (2023). Complex and Beautiful: Unraveling the Intricate Communication Systems Among Plants and Insects. Annual Review of Entomology, 68(1), 1–12. https://doi.org/10.1146/annurev-ento-021622-111028

Van der Putten, W. H., Vet, L. E. M., Harvey, J. A., & Wäckers, F. L. (2001). Linking above- and belowground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. Trends in Ecology & Evolution, 16(10), 547–554. https://doi.org/10.1016/S0169-5347(01)02265-0

Viric Gasparic, H., Lemic, D., Drmic, Z., Cacija, M., & Bazok, R. (2021). The Efficacy of Seed Treatments on Major Sugar Beet Pests: Possible Consequences of the Recent Neonicotinoid Ban. Agronomy, 11(7), 1277. https://doi.org/10.3390/agronomy11071277

Wamonje, F. O., Tungadi, T. D., Murphy, A. M., Pate, A. E., Woodcock, C., Caulfield, J. C., Mutuku, J. M., Cunniffe, N. J., Bruce, T. J. A., Gilligan, C. A., Pickett, J. A., & Carr, J. P. (2020). Three Aphid-Transmitted Viruses Encourage Vector Migration From Infected Common Bean (Phaseolus vulgaris) Plants Through a Combination of Volatile and Surface Cues. Frontiers in Plant Science, 11. https://doi.org/10.3389/fpls.2020.613772

Yolcu, S., Alavilli, H., Ganesh, P., Panigrahy, M., & Song, K. (2021). Salt and Drought Stress Responses in Cultivated Beets (Beta vulgaris L.) and Wild Beet (Beta maritima L.). Plants, 10(9), 1843. https://doi.org/10.3390/plants10091843

Yuan, J., Wen, T., Yang, S., Zhang, C., Zhao, M., Niu, G., Xie, P., Liu, X., Zhao, X., Shen, Q., & Bezemer, T. M. (2023). Growth substrates alter aboveground plant microbial and metabolic properties thereby influencing insect herbivore performance. Science China Life Sciences. https://doi.org/10.1007/s11427-022-2279-5

Zhou, S., Lou, Y.-R., Tzin, V., & Jander, G. (2015). Alteration of Plant Primary Metabolism in Response to Insect Herbivory. Plant Physiology, 169(3), 1488–1498. https://doi.org/10.1104/pp.15.01405

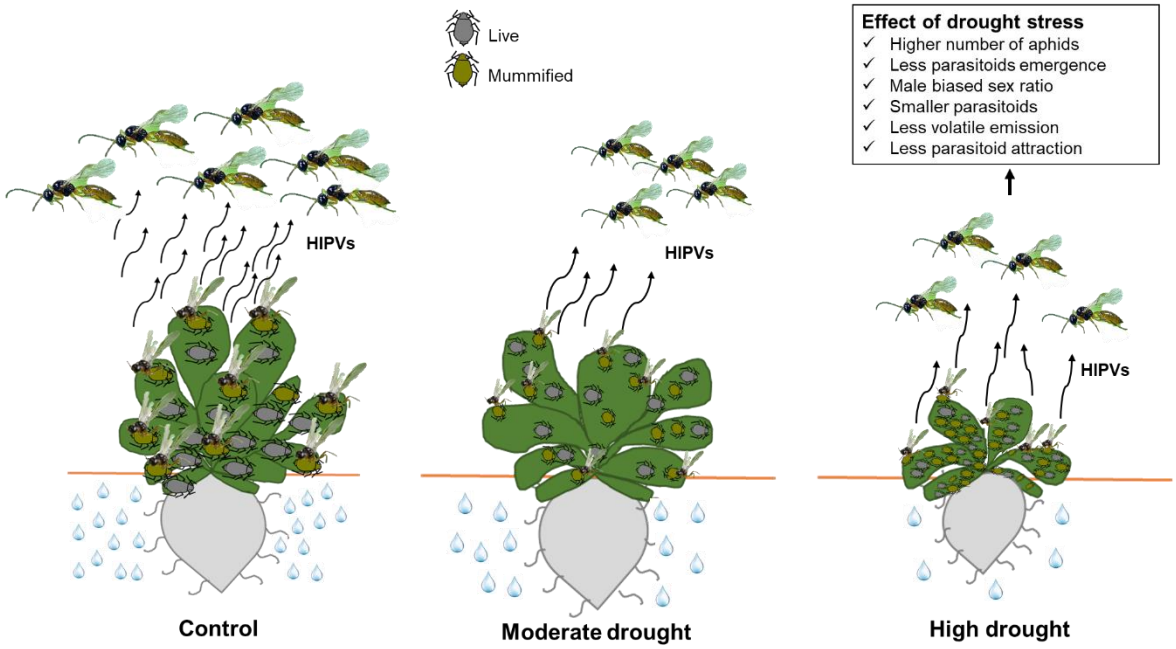
Drought aggravates plant stress by favouring aphids and weakening indirect defense in a sugar beet tritrophic system

Shahinoor Rahman*, Michael Rostás, and Ilka Vosteen*

Department of Crop Sciences, Division of Agricultural Entomology, Georg-August-University of Goettingen, Germany

*Correspondence: shahinoor_ent@yahoo.com; ilka.vosteen@uni-goettingen.de

Graphical abstract



Abstract

Climate change leads to more frequent droughts that may alter multitrophic networks in agroecosystems by changing bottom-up and top-down effects on herbivorous insects. Yet, how bottom-up effects of drought alter tritrophic interactions remains poorly understood. This study investigated two intensities of drought stress in the tritrophic system consisting of sugar beet (*Beta vulgaris*), an aphid (*Aphis fabae*), and its parasitoid (*Aphidius colemani*). We thoroughly investigated each trophic level, examining the performance of plants, insects, and parasitoids, as well as the attraction of parasitoids to herbivore-induced plant volatiles (HIPVs). Drought stress negatively affected plant growth but benefited *A. fabae,* leading to faster development and a higher reproduction rate. Drought-stressed plants also emitted less plant volatiles, which resulted in reduced attraction of *A. colemani* to aphid-infested plants. Drought indirectly affected parasitoid performance, as evidenced by lower emergence rates and production of fewer females, although mummification rates were higher on drought-stressed plants. Reduced parasitoid attraction and performance on drought-stressed plants may exert lower top-down pressure on aphid populations. Combined with increased aphid performance, this may facilitate aphid outbreaks, which could further weaken drought-stressed plants. Our findings highlight the need to study multiple trophic levels and emphasize the importance of incorporating HIPVs and parasitoid attraction when assessing combined abiotic and biotic stresses in crops.

Keywords: *Aphis fabae*, *Aphidius colemani*, *Beta vulgaris*, Biocontrol services, Bottom-up effects, Multitrophic interactions, HIPVs

Introduction

Climate change models predict altered rainfall patterns and an increased number of extreme drought events, as experienced recently in Central Europe (Boergens et al., 2020; IPCC, 2014). Prolonged water deficit can have a broad range of impacts on arable cropping systems and their arthropod communities by altering the interactions among species and thus changing multitrophic networks (Jamieson et al., 2012; Walter, 2018). Drought negatively affects plant growth and morphology (Grzesiak et al., 2019; Yang et al., 2021; Zhang et al., 2018), and the prime symptoms are decreased plant height (Anjum et al., 2017; Li et al., 2020; Misra et al., 2020; Patmi et al., 2020), shoot and root biomass (Benjamin & Nielsen, 2006), number and area of leaves (Khaleghi et al., 2019; Mishra et al., 2018), as well as wilting and rolling of leaves (Willick et al., 2018).

Drought also alters phytohormonal signaling (Gupta et al., 2020; Jogawat et al., 2021; Mubarik et al., 2021) and the biochemical composition of plant tissues (Bettaieb Rebey et al., 2012) which may improve (Khan et al., 2010) or decrease herbivore performance (Xie et al., 2020). The effects of drought on herbivore performance can depend on the magnitude of the experienced stress (Mody et al., 2009; Tariq et al., 2012). Aphids, for example, respond differently to moderate and severe levels of drought which can alter aphid-parasitoid interactions (Kansman et al., 2021). Drought stress further has bottom-up effects on the third trophic level by altering the behavior and performance of parasitoids (Shehzad et al., 2020; Tariq et al., 2013).

However, reported bottom-up effects of drought stress on aphid performance are inconsistent and may depend on the level of drought stress as well as the plant and aphid species studied (Cui et al., 2021; Luo & Gilbert, 2022; Mewis et al., 2012; Shehzad et al., 2020; Xie et al., 2020). Studies on aphid parasitoids, representing the third trophic level, are scarce and mainly focus on parasitization rates and mummification success (e.g. Ahmed et al., 2017; Kansman et al., 2021). Parasitoid wasps specialized on aphids develop within the body of their hosts. Thus, their performance is significantly influenced by changes in the physiology or behavior of aphids (Brodeur & Boivin, 2004; Kaplan et al., 2016). Several studies showed indirect effects of plant drought stress on parasitoid performance, but the responses range from positive (Romo &

Tylianakis, 2013) to negative (Ahmed et al., 2017; Johnson et al., 2011; Nguyen et al., 2018).

Another essential aspect is the ability of parasitoids to find host-infested plants under drought-stress conditions. Parasitoids use herbivore-induced plant volatiles (HIPVs) to detect their hosts (e.g. Turlings & Erb, 2018); because HIPVs provide information to parasitoids that help suppressing the herbivore attack, HIPVs can be considered as a plant's information-mediated indirect defense (Kessler & Heil, 2011). To date only few studies have assessed the effects of drought on HIPV emission, parasitoid attraction and the underlying mechanisms (Martini & Stelinski, 2017; Salerno et al., 2017; Weldegergis et al., 2015) and only one other has focused on aphids (Tariq et al., 2013). Hence, more research is needed before a comprehensive understanding can be obtained on how drought stress modulates the plant's indirect defense.

Sugar beet (*Beta vulgaris* spp. *vulgaris*) is an important crop and a significant source of sugar in temperate regions, contributing to a third of the world's annual sugar production (Dohm et al., 2014). Since neonicotinoid seed treatment in sugar beet has been phased out in many European countries in 2018, increasing pest pressure has been observed (Viric Gasparic et al., 2020). Naturally occurring parasitoids and their conservation are therefore likely to play a more prominent role as biocontrol agents in the future by reducing aphid populations in sugar beet fields. How the tritrophic system, consisting of sugar beet, the important herbivore *Aphis fabae,* and its parasitoid *Aphidius colemani,* is affected by water limitation is entirely unknown. In this comprehensive study, we specifically addressed the effects of different levels of drought on (i) sugar beet plant growth and morphology (ii) aphid performance on drought-stressed sugar beets (iii), parasitoid performance on aphids reared on drought-stressed plants, (iv) volatile emission from aphid-infested and uninfested sugar beets and (v) parasitoid attraction towards aphid-infested and uninfested plants. Our results show that *A. fabae* benefits from drought stress as they develop faster and are harder to detect for parasitoids due to the lower HIPV emission from drought-stressed plants. These findings should be considered when developing novel biological pest control strategies in the context of global climate change.

Materials and methods

Plant and insects

Seeds of *B. vulgaris* subspec. *vulgaris* cultivar 'Vasco' (SESVanderHave, Belgium) were sown in plastic trays (54 holes, each 3.5 cm diameter) filled with quartz sand (0.2-0.8 mm). Trays were placed in a controlled climate room with high-pressure sodium vapour lamps (16L:8D photoperiod, light intensity: 130 ± 10 µmol/(s m^2), relative humidity: 65 ± 5 %, temperature: 20 ± 1 °C) and were supplied with tap water. Twelve days after sowing (DAS), seedlings were supplied with half-strength modified Hoagland solution (HS) (Hoagland & Arnon, 1938) for up to 23 DAS to provide nutrients. At 24 DAS, seedlings were transferred to the drought system with full strength HS. For preparing HS, $Na_2MoO_4.2H_2O$ was used instead of $H_2MoO_4.H_2O$, and $C_{10}H_{12}FeN_2NaO_8.3H_2O$ replaced $C_{12}H_{12}Fe_2O_{18}$ with concentrations of 0.12 mg/l and 22.5 mg/l, respectively.

Colonies of *Aphis fabae* (Order: Hemiptera, Family: Aphididae) were reared on the same genotype of sugar beet as used in the experiments and grown in separate insect rearing room. *A. colemani* was reared on *A. fabae,* and sugar syrup (10% sucrose solution) was supplied as food source for adult parasitoids.

Drought system and experimental setup

As described by Marchin *et al.*, 2020, a modified capillary action-based drought system was used for all drought stress experiments. PVC-U (polyvinyl chloride without plasticizers) cylinders (diameter: 6.5 cm; height: 12.5 cm) with their bottom part fitted with fine nylon mesh with four fine holes were filled with quartz sand (0.2-0.8 mm) and used as a plant holder. Porous floral foam (length: 23 cm, width: 11 cm, height: 8 cm) (BIG-mosy, Mosy GmbH, Germany) was cut into three equal pieces and placed in black plastic box boxes (length: 46 cm, width: 30 cm, height: 16 cm) (Iris Ohyama, Germany). Six plants were placed in each box on top of the floral foam, and HS was added to the box with an adjusted water level (Figure S1). By maintaining the depth of the water level, three treatments were imposed as- i) control: 40% volumetric water content (VWC), ii) moderate drought: 16% VWC, and iii) high drought: 10% VWC. Prior to the experiment, the maximum water holding capacity of the specified volume of quartz sand (40% VWC) and the permanent wilting point (below 9%) of sugar beet plants were determined in a preliminary experiment. Each morning at 10

a.m., the VWC was checked with a digital soil moisture meter (ThetaProbe type ML3, Delta-T, Cambridge, UK), and water levels were adjusted by applying HS as needed. From 24 DAS to 26 DAS, all plants were kept at 40% VWC with full strength HS for acclimation. Subsequently, plants were exposed to the drought treatments described above.

To answer the first three questions regarding plant, aphid, and parasitoid performance, three separate experiments were conducted in the same manner, where three drought boxes (each box contained 6 plants and was considered a block) were used for each treatment (control, moderate drought, high drought). This resulted in a total of n = 18 plants per treatment.

A fourth experiment was conducted to assess parasitoid olfactory behavior to plant volatiles; in this case, six boxes were used to prepare the six different odor sources: i) control, ii) control + aphid, iii) moderate drought, iv) moderate drought + aphid, v) high drought, and vi) high drought + aphid. A total of n = 6 plants per treatment were tested.

Monitoring plant performance

To test the effect of drought stress on sugar beet, plant height was measured every seven days, starting at the two true leaf stage (26 DAS), and prior to drought application, until 54 DAS. Plants were harvested at 73 DAS, and total biomass, root and shoot weight, root and shoot length, and the number of leaves were recorded.

Aphid performance

To explore the influence of drought stress on the reproductive performance of *A. fabae,* three wingless adults were placed on the second leaf of each sugar beet plant at 30 DAS (day 4 of drought treatment). A perforated polypropylene bag (15 × 25 cm) (Nette GmbH, Germany) was carefully placed over each aphid-infested leaf. After 24 h, all aphids except two neonate nymphs were removed from the leaf. These nymphs (F0 generation) were left undisturbed for 8-9 days. Before reproduction began, one aphid was selected as the F0 mother and the other was removed. The F0 mother was allowed to produce its first progeny (F1) before being transferred to the third leaf of the same plant. Two first F1 offspring were kept undisturbed on leaf two until either one started to reproduce. In the

meantime, the number of offspring of the F0 mother (now on leaf three) was recorded and nymphs were removed daily until the death of the F0 mother. The number of offspring until their own first offspring (F1) started to reproduce (*Nd*), the total number of offspring, the duration of the reproductive period, and the average number of offspring per day were calculated for the F0 mother. After the F1 aphids had started to reproduce, one was randomly selected as the F1 mother, while the other was removed (see also Figure S2). The pre-reproductive period and longevity of the F1 mother were recorded. The following equation was used to calculate the intrinsic rate of increase (Rm): $Rm = 0.738 (log_e Nd)/d$ (*d* = reproductive periods; *Nd* = number of offspring of the F0 mother until her own first offspring reproduces) (Wyatt & White, 1977).

Parasitoid performance

Five wingless adult aphids were placed on the second leaf of a sugar beet plant (30 DAS), and the entire plant was covered with a perforated polypropylene bag (15 × 25 cm, Nette GmbH, Germany). After 24 h, the adults and all offspring except five neonate nymphs were removed. The nymphs were left undisturbed for nine days (pre-reproductive stage). At 40 DAS, one naïve female *A. colemani* was released into each polypropylene bag for five hours to parasitize the aphids. After removal of the parasitoids, aphids were checked daily to record the following parameters: proportion of 1) mummified, surviving, and dead aphids, 2) time from parasitization to mummification, 3) time from mummification to adult emergence, 4) total parasitoid development time, 5) emergence rate, 6) sex ratio and 7) hind tibia length of the emerged parasitoids. *A. colemani* adults were preserved in 96% (v/v) ethanol and hind tibia length of both legs was measured using a stereomicroscope (Leica WILD M3Z) equipped with an ocular micrometer.

Parasitoid host finding behavior and plant volatile analysis

A six-arm olfactometer (Turlings et al., 2004) was used to trap plant volatiles and to simultaneously measure the attraction of female *A. colemani* to six odor sources. Aphid-infested and non-infested plants subjected to the three drought treatments (control, moderate drought, high drought) were used as the six odor sources. All plants were tested at the same age (40 DAS) and had been

exposed to 13 days of drought stress (start at 27 DAS). In the case of aphid treatments, 40 *A. fabae* (mixture of all ages) were released on each plant at 30 DAS and were allowed to feed and reproduce for 10 days. A single plant from each treatment was randomly assigned to each arm of the olfactometer. Instead of placing each sugar beet plant into an odor source glass vessel, the plant was bagged using an inert oven bag (Bratschlauch, Toppits, Germany).

Collections of volatiles were carried out for 24 h (from 09:00 to 09:00 next day), and within this time, parasitoid behavioral assays were conducted (from 10:00 to 16:00; see supporting information for details of the collection procedure). Every day, a total of 30 naïve females of *A. colemani* (2-5 days old) were tested in five experimental rounds. In each experimental round, six parasitoids were released simultaneously. After 60 minutes, the choices made by the parasitoids were recorded, and all parasitoids were removed before the next group was released. After every experimental day, the glass and Teflon parts of the olfactometer were first cleaned with demineralized water and then rinsed with 99.5% acetone. After evaporating the solvents, all cleaned glassware was placed in an oven for two hours at 180°C. This procedure was repeated on six experimental days, with a fresh set of plants used each day. Each day after the volatile collection, the above-ground biomass was measured with a balance (KERN PEJ 4200-2M, Kern & Sohn GmbH, Germany), leaf areas were measured by an automatic leaf area meter (LI-3100C, LI-COR® Biosciences GmbH, Germany) to calculate volatile emission as ng/h/g FW and ng/cm^2 leaf area.

After headspace collection, the trapped volatiles were eluted with 150 µl dichloromethane (DCM) and analyzed by gas chromatography – mass spectrometry (5977B HES MSD, Agilent Technologies; see supporting information for details of the GC-MS analysis and compound identification).

Statistical analysis

All data were analyzed using the statistics package R-version 4.1.2 integrated with R-Studio Desktop-version 2021.09.1+372. Various models were considered for analyzing different types of data, and the most appropriate model was selected based on its assumptions. Detailed information about the statistical analysis can be found in the supporting information.

Results

Effect of drought stress on the performance of sugar beet plant

Drought stress had a negative effect on sugar beet height over time (GLMM: Drought: $\chi^2 = 57.76$, $p < 0.001$; Time: $\chi^2 = 510.26$, $p < 0.001$; Fig. 1; Table S1). Before applying drought at 26 DAS, plant height was not significantly different between the treatments (Figure 1). After one week of drought treatments (33 DAS), the height of plants exposed to medium or severe drought was strongly reduced ($p < 0.001$) compared to control plants. Plants exposed to severe drought were the smallest and height differences remained constant throughout sampling times. After four weeks of drought (54 DAS), moderately and highly stressed sugar beet plants were 15.2% and 30.9% shorter than controls.

Similarly, moderate and high drought significantly reduced total plant biomass (g FW) (LMM: $F_{2,15} = 224.16$, $p < 0.001$), root weight (g FW) (LMM: $F_{2,15} = 16.574$, $p < 0.001$), and shoot weight (g FW) (LMM: $F_{2,15} = 139.71$, $p < 0.001$) (Table 1) compared to control plants. Plants exposed to severe drought showed significantly reduced weights compared to plants from the moderate drought treatment. Moderately and highly stressed plants had 29.1% and 58.4% reduction in total plant biomass; 22.6% and 36.2% reductions in root weight; and 31.7% and 67.52% reduction in shoot weight, respectively. Shoot length (cm) was also higher in control plants compared to stressed plants (LMM: $F_{2,15} = 70.287$, $p < 0.001$); however, root length (cm) was lowest in control plants (GLMM: $\chi^2_{(df\ 2)} = 40.41$, $p < 0.001$) (Table 1). Root to shoot ratio by weight and by length was lowest in control plants and highest in highly stressed plants ($p < 0.001$) (Table 1). Number of leaves on plants grown in moderate and high drought conditions were significantly reduced compared to control plants ($\chi^2_{(df\ 2)} = 39.54$, $p < 0.001$) (Table 1).

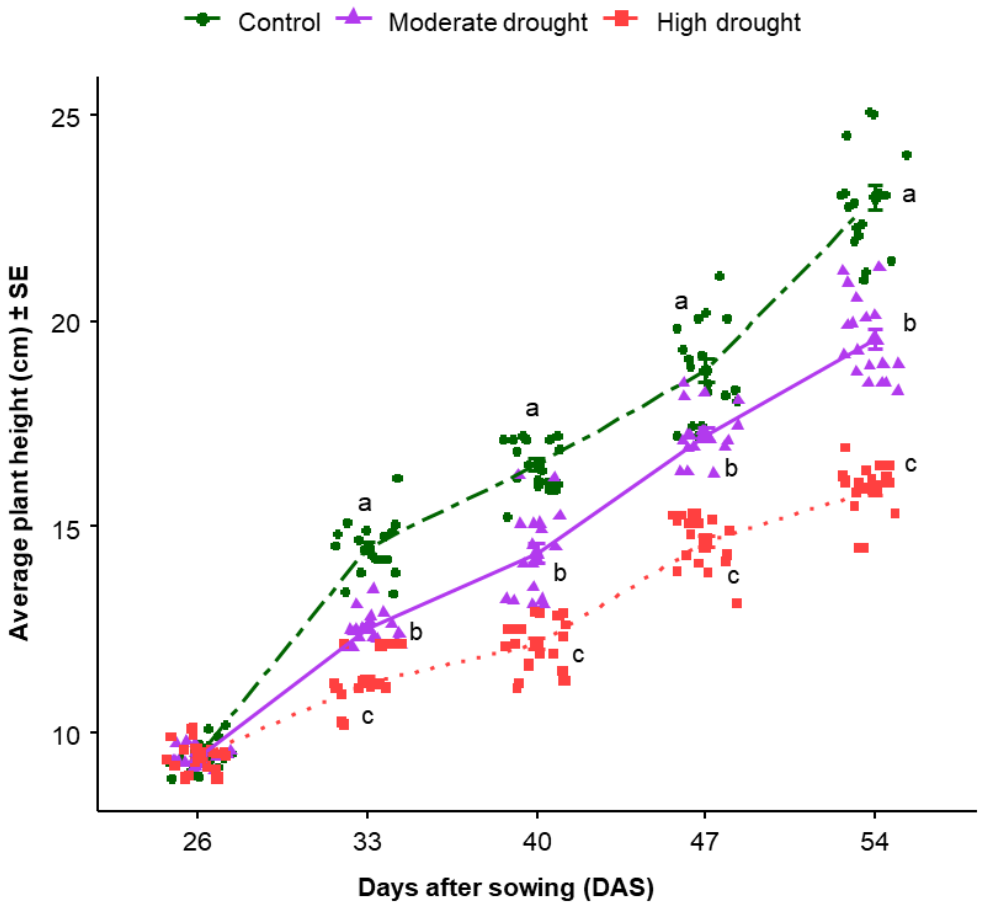


Figure 1: Line graphs depicting the effects of drought (Control = ~ 40% volumetric water content (VWC), Moderate drought = ~ 16% VWC, High drought = ~ 10% VWC) on plant height of sugar beet. Parameters were measured starting at 26 days after sowing (DAS) (before application of drought stress) in seven day intervals. Data points represent individual replicates and different letters ($p \leq 0.05$) indicate significance among treatments, n = 18

Table 1: Effect of different magnitude of drought stress on sugar beet plant performance (mean ± SE, n=18) at 73 days after sowing. Different letters indicate significant differences among treatments.

Parameter	Control	Moderate drought	High drought	Statistics
Total Biomass (g FW)	39.20±0.65 a	27.80±0.56 b	16.3±0.57 c	$F_{2,15} = 224.16$, $p < 0.001$
Root weight (g FW)	11.24±0.43 a	8.690±0.27 b	7.17±0.25 c	$F_{2,15} = 16.574$, $p < 0.001$
Shoot weight (g FW)	27.96±0.54 a	19.07±0.68 b	9.08±0.54 c	$F_{2,15} = 139.71$, $p < 0.001$
Root shoot ratio by weight (g FW)	0.412±0.01 b	0.470±0.03 b	0.86±0.07 a	$\chi^2_{(df\ 2)} = 21.29$, $p< 0.001$
Root length (cm)	17.10±0.32 c	22.10±0.62 b	23.9±0.45 a	$\chi^2_{(df\ 2)} = 40.41$, $p < 0.001$
Shoot length (cm)	28.00±0.44 a	23.00±0.51 b	19.8±0.34 c	$F_{2,15} = 70.287$, $p < 0.001$
Root shoot ratio by length (cm)	0.620±0.01 c	0.970±0.03 b	1.21±0.02 a	$\chi^2_{(df\ 2)} = 52.46$, $p < 0.001$
Total number of leaves	12.61±0.26 a	10.94±0.24 b	7.44±0.37 c	$\chi^2_{(df\ 2)} = 39.54$, $p < 0.001$

Effect of drought stress on the performance of Aphis fabae

There were significant differences in the pre-reproductive period (GLMM: $\chi^2_{(df\ 2)}$ =18.69, p < 0.001) and reproductive period (GLMM: $\chi^2_{(df\ 2)}$ = 12.47, p = 0.0019) of *A. fabae* feeding on drought-affected plants, (Figure 2A). Aphids developing on highly drought-stressed plants matured faster by shortening their pre-reproductive period (control vs high: p < 0.001; control vs moderate: p = 0.975; moderate vs high: p < 0.001) and extended their reproductive phase (control vs high: p < 0.001; control vs moderate: p = 0.14; moderate vs high: p = 0.061) without changing their total life span (GLMM: $\chi^2_{(df\ 2)}$ = 0.079, p = 0.961).

The fecundity of *A. fabae* differed significantly depending on drought treatments (GLMM: $\chi^2_{(df\ 2)}$ = 26.02, p < 0.001). Aphids on moderately and highly drought-stressed plants produced more offspring than aphids on control plants (Figure 2B). Similar patterns were observed in the number of offspring that were produced by the F1 generation until their own first progeny reproduced (*Nd*) (GLMM: $\chi^2_{(df\ 2)}$ = 23.45, p < 0.001), the average number of offspring produced per day (GLMM: $\chi^2_{(df\ 2)}$ = 7.20, p = 0.027) and the intrinsic rate of increase (*Rm*) (GLMM: $\chi^2_{(df\ 2)}$ = 30.58, p < 0.001) (Figure S3).

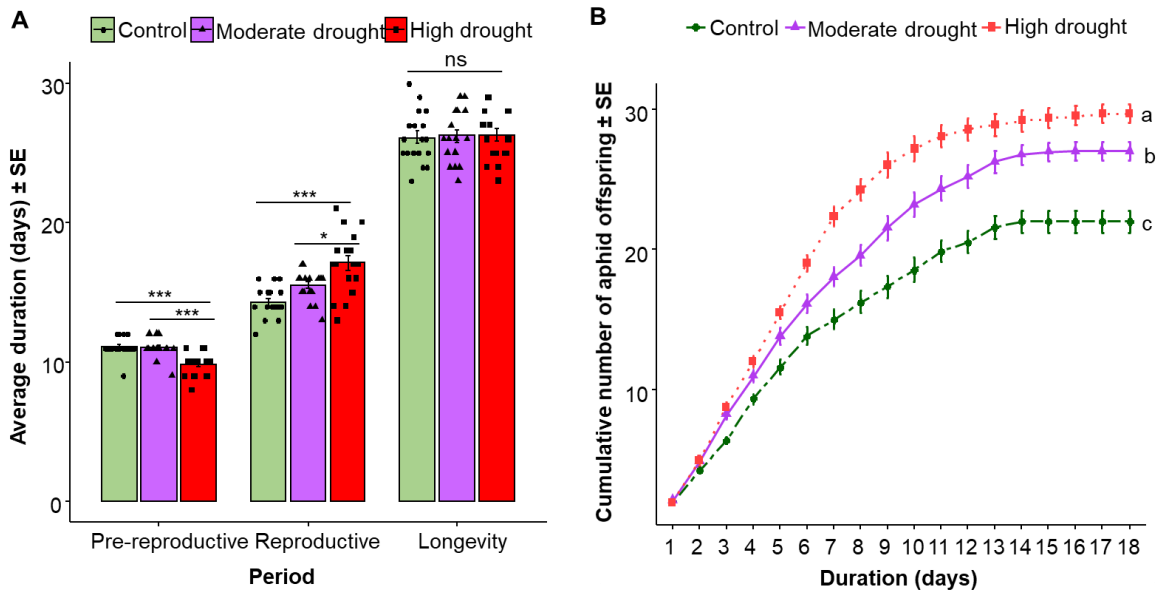


Figure 2: Effect of drought stress (~ 40% volumetric water content (VWC), ~ 16% VWC, ~ 10% VWC) on the performance of *Aphis fabae*. Bar graphs represent the average duration of pre-reproductive and reproductive period as well as longevity of *A. fabae* (A) and line graphs represent the cumulative offspring number of *A. fabae* (B). Data points in bar graphs represent individual replicates. Asterisks (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$) and different letters ($p \leq 0.05$) indicate significance among treatments, n = 18.

Effect of drought stress on the performance of Aphidius colemani

Significantly more aphid mummies were formed on highly drought-stressed plants than on control plants (GLMM: $\chi^2_{(df\ 2)} = 10.40$, P = 0.005, control vs moderate: $p = 0.388$, control vs high: $p = 0.0017$, moderate to high: $p = 0.056$) and significantly less aphids survived (Kruskal-Wallis: $\chi^2_{(df\ 2)} = 7.03$, $p = 0.029$), even though the percentage of aphids that died a few days after parasitization was the same in all treatments (Kruskal-Wallis: $\chi^2_{(df\ 2)} = 2.46$, $p = 0.29$) (Fig. 3A). The highest mummification rate (57.77%) was found in drought-stressed plants, while control plants showed the lowest mummification rate (42.22%) (Figure 3A).

Total developmental time (oviposition to adult emergence) of *A. colemani* was shorter on drought-stressed than on control plants (GLMM: $\chi^2_{(df\ 2)} = 25.57$, $p < 0.001$; control vs high: $p < 0.001$; control vs moderate: $p = 0.0007$; moderate vs high: $p = 0.39$) (Figure 3B). Specifically, within the developmental period, drought boosted the oviposition to mummification time (GLMM: $\chi^2_{(df\ 2)} = 60.51$, $p < 0.001$) but not the time from mummification to adult emergence (GLMM: $\chi^2_{(df\ 2)} = 4.25$, $p = 0.119$) (Figure 3B).

Drought had negative effects on the adult emergence rate of *A. colemani*. Adults emerged successfully from 84.30% of the mummies on control plants, from 53.70% of mummies on moderately drought-stressed plants and from 40.27% of mummies plants on highly drought stressed plants (Kruskal-Wallis: $\chi^2_{(df\ 2)} = 22.82$, $p < 0.001$, control vs moderate: $p = 0.005$, control vs high: $p < 0.001$, moderate to high: $p = 0.091$) (Figure 3C). Sex ratio was also significantly altered by drought stress (Kruskal-Wallis: male: $\chi^2_{(df\ 2)} = 6.77$, $p = 0.033$, and female: $\chi^2_{(df\ 2)} = 10.31$, $p = 0.005$). The proportion of mummies from which males emerged was not strongly affected by drought stress, but the proportion of mummies from which females emerged was significantly lower in moderately and highly drought-stressed plants compared to controls (Figure 3C).

Hind tibia length of male (LM: $F_{2,36} = 19.91$, $p < 0.001$) and female (LM: $F_{2,31} = 8.66$, $p < 0.001$) *A. colemani* showed significant differences depending on the intensity of drought stress. Parasitoids that emerged from aphids on drought-stressed plants had shorter hind tibia lengths compared to aphids on control plants. This was the case for both male (control vs high: $p < 0.001$; control vs

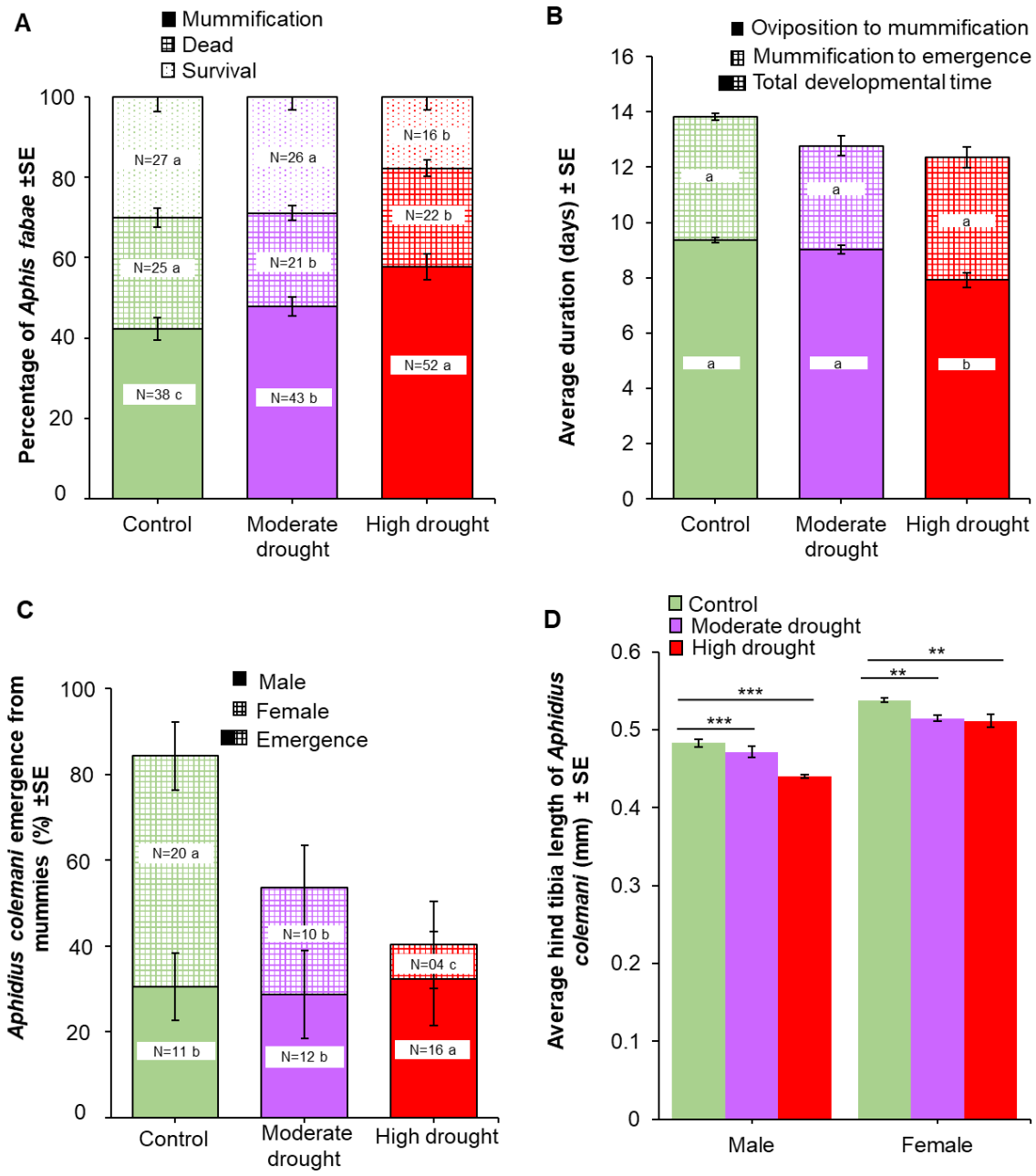


Figure 3: Effect of drought stress (~ 40% volumetric water content (VWC), ~ 16% VWC, ~ 10% VWC) on the performance of the parasitoid *Aphidius colemani*. Stacked bar graph represents the percentage of aphids that formed mummies, died or survived (A); time from oviposition to mummification and mummification to emergence (B); and percentage of male and female parasitoids (C). Bars show the hind tibia lengths of the emerged male and female *A. colemani* (D). Asterisks and different letters indicate significance among treatments ($p \leq 0.05$). * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, n =18, N = exact number of sample evaluated

moderate: $p = 0.27$; moderate vs high: $p < 0.001$) and female parasitoids (control vs high: $p = 0.013$; control vs moderate: $p = 0.004$; moderate vs high: $p = 0.87$) (Figure 3D).

Six-arm olfactometer bioassay and analysis of VOCs

Overall, 80% of the released *Aphidius colemani* females were attracted by sugar beet VOCs and were found in one of the arms of the six-arm olfactometer at the end of the trial. Drought stress and aphid presence significantly influenced the attraction of parasitoids to VOCs emitted by the sugar beet plant (binomial GLM:$\chi^2_{(df\ 5)} = 220.68$, $p < 0.001$) (Figure 4). Aphid-infested control plants attracted the highest number of parasitoids (46.11%), while non-infested controls attracted the second highest number of parasitoids (18.88%), but significantly less than the aphid-infested controls. Plants subjected to moderate drought stress in the absence of aphids attracted similar numbers of parasitoids as the non-infested control plants. However, aphid-infested plants exposed to moderate drought stress attracted significantly fewer parasitoids (0.55%) than the non-infested plants exposed to moderate drought stress (10.55%). Only few parasitoids were attracted to highly drought-stressed plants with and without aphids (1.11% and 3.33%, respectively). These numbers did not differ significantly from the number of parasitoids attracted to the aphid-infested plants exposed to moderate drought stress.

Strong differences in volatile emission were observed between the different treatments. While well-watered plants without aphids emitted 21 VOCs, aphid-induced well-watered plants emitted 29 different VOCs. Seven of these compounds were not detected in any other treatment (Figure 5). Aphid-induced highly drought-stressed plants emitted 14 VOCs, and highly drought-stressed plants without aphids emitted only six different compounds. However, non-infested, moderately drought-stressed plants released more (13 VOCs) compounds than aphid-induced moderately drought-stressed plants (8 VOCs) (Figure 5).

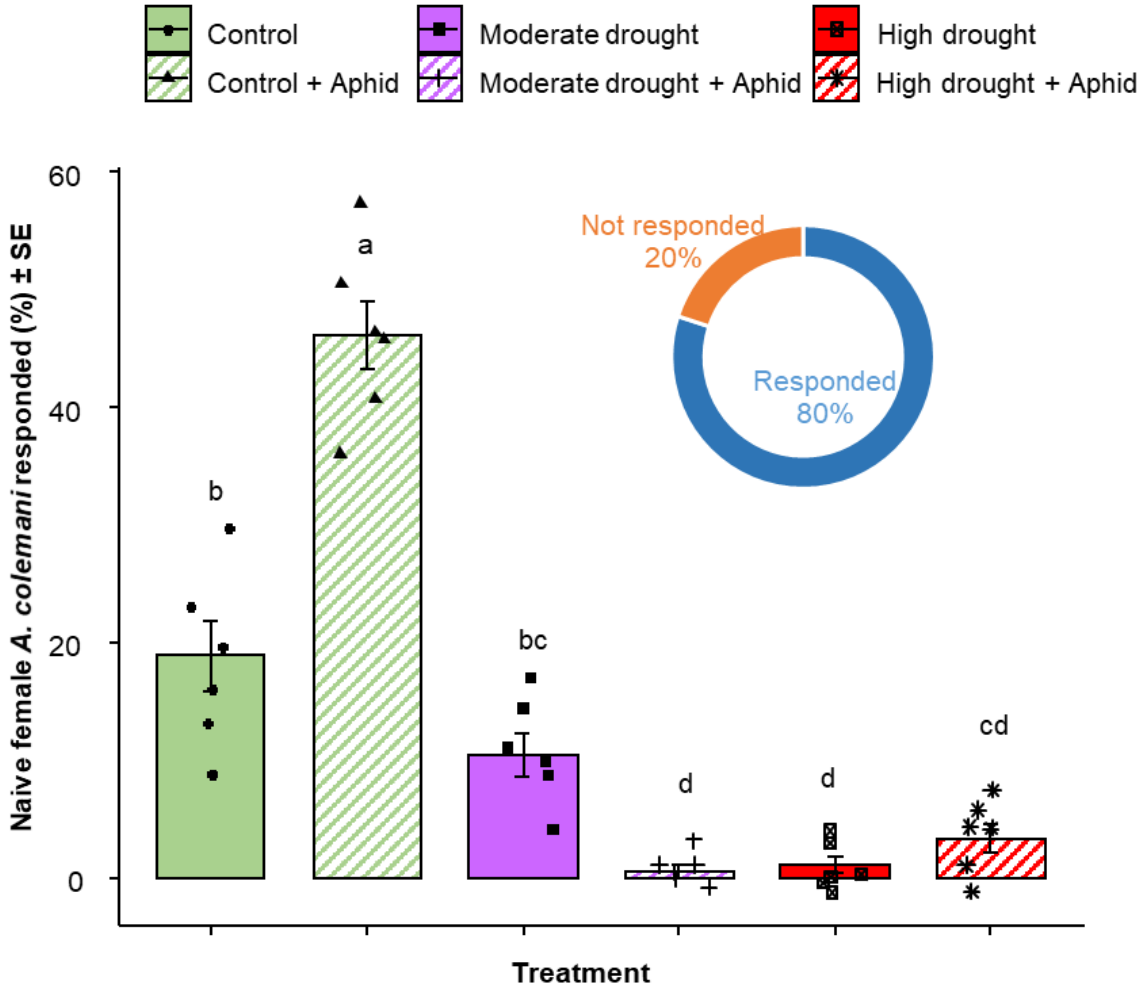


Figure 4: Effect of aphid infestation and watering regime on volatile preference of female *A. colemani.* Bar graph depicting the proportion of active females that chose the respective odour source. Pie chart represents the total percentage of female *A. colemani*, which made a choice among the treatments. Different letter indicate significance among treatments ($p < 0.05$). In total 6 different groups of plants were tested on 6 days. Each day, 5 groups of 6 parasitoid each were released in the six arm olfactometer to choose among the six different odour sources.

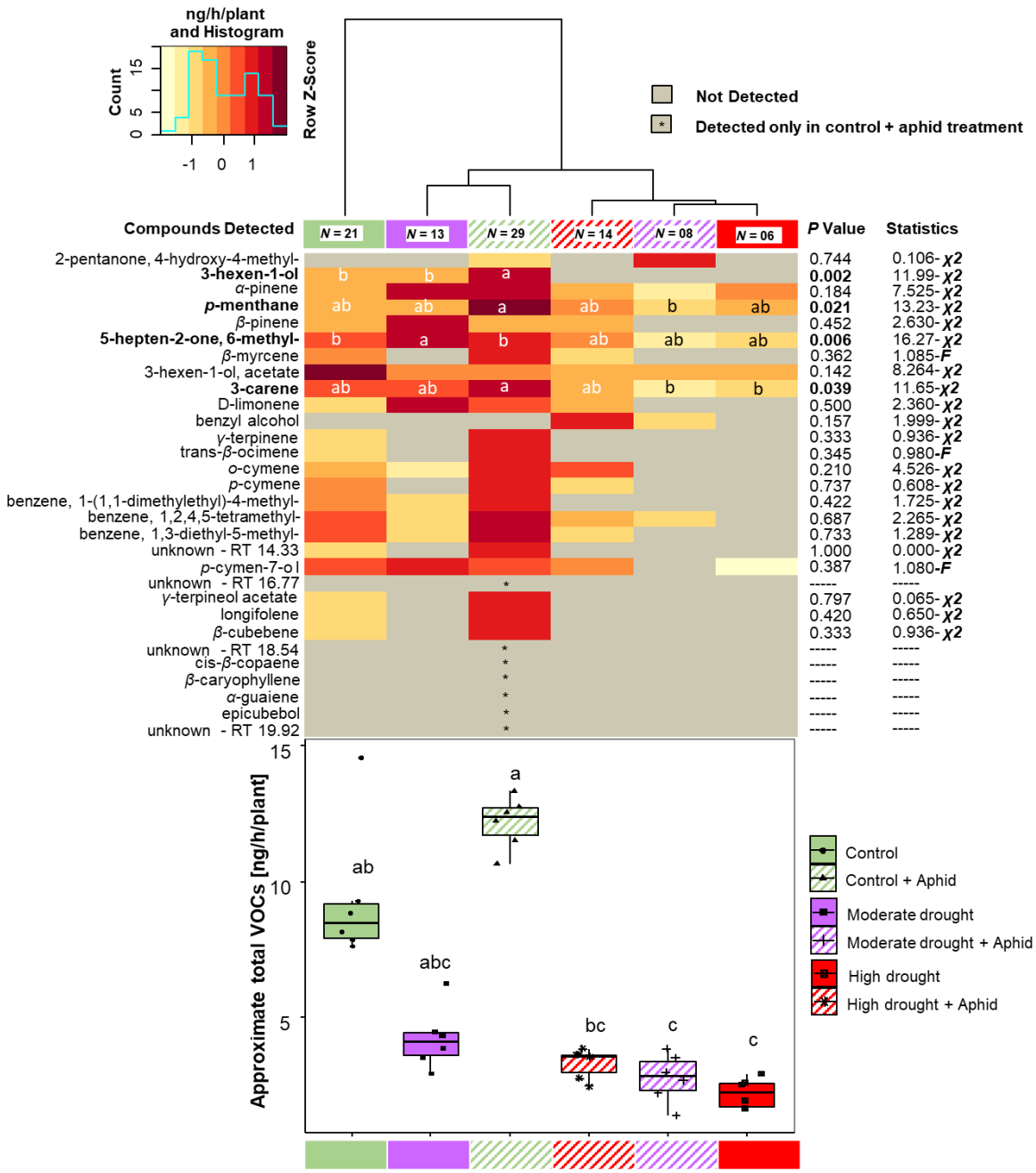


Figure 5: Effect of aphid infestation and drought stress on volatile emission. Heat map represents emission rates of specific volatile organic compounds (VOCs) and the boxplot represents the total amount of VOCs emitted from differently treated sugar beet plants. Compound names and *P* values in bold indicate significant differences among treatments. Different letters show statistically significant differences in total VOCs between treatments ($p \leq 0.05$, n = 6). *N* = total number of different compounds detected in the respective treatment.

Hierarchical clustering of the emitted plant volatile blends revealed that the blend from non-infested well-watered controls was distinct from all other treatments (Figure 5). Among the different VOCs, emission of 3-hexen-1-ol; *p*-menthane; 5-hepten-2-one, 6-methyl; and 3-carene were significantly different among treatments when emission rates were analyzed per plant (Figure 5, Table S3).

When VOCs emission rates were standardized by leaf biomass (ng/h/g FW), 5-hepten-2-one, 6- methyl; *p*-menthane; *o*-cymene; and 3-hexen-1-ol were significantly different among different treatments (Figure S4, Table S4). After standardized by leaf area (ng/cm^2), emissions rates of 5-hepten-2-one, 6- methyl; *p*-menthane; *p*-cymen-7-ol; *o*-cymene; and 3-hexen-1-ol were significantly different (Figure S4, Table S5). For all emitted compound, compound classes, retention time, and indices from experiments and literature are summarized in Table S2.

Drought and aphid infestation had a significant effect on total VOC emission from the whole plant (LM: Drought: $F_{2,30} = 136.0$, $p < 0.001$; Aphid: $F_{1,30} = 3.39$, $p = 0.075$; Drought × Aphid: $F_{2,30} = 7.86$, $p = 0.0017$) (Figure 5), per gram fresh weight (LM: Drought: $F_{2,30} = 36.45$, $p < 0.001$; Aphid: $F_{1,30} = 6.97$, $p = 0.013$; Drought × Aphid: $F_{2,30} = 11.10$, df = 2, $p < 0.001$) and also when standardized by leaf area (cm^2) (LM Drought: $F_{2,30} = 35.46$, df = 2, $p < 0.001$; Aphid: $F_{1,30} = 2.42$, $p = 0.13$; Drought × Aphid: $F_{2,30} = 8.26$, $p = 0.0013$) (Figure S4, Table S4, Table S5). Aphid-induced control plants emitted the highest amounts of VOCs per plant, followed by control plants without aphids. The emission was significantly lower in moderately and highly drought-stressed plants regardless of aphid infestation (Figure 5). After standardization of VOC emission per gram leaf biomass or leaf area (cm^2), this pattern changed due to drought-stressed plants having significantly lower biomass and smaller leaf area than well-watered plants (Table S6). When corrected for plant biomass or leaf area, no differences were found in emission rates from highly drought-stressed plants with aphids and well-watered plants (with and without aphids) (Figure S4). A positive correlation ($R = 0.9$, $p < 0.001$) was found between total VOC emission per plant and the number of attracted parasitoids (Figure 6).

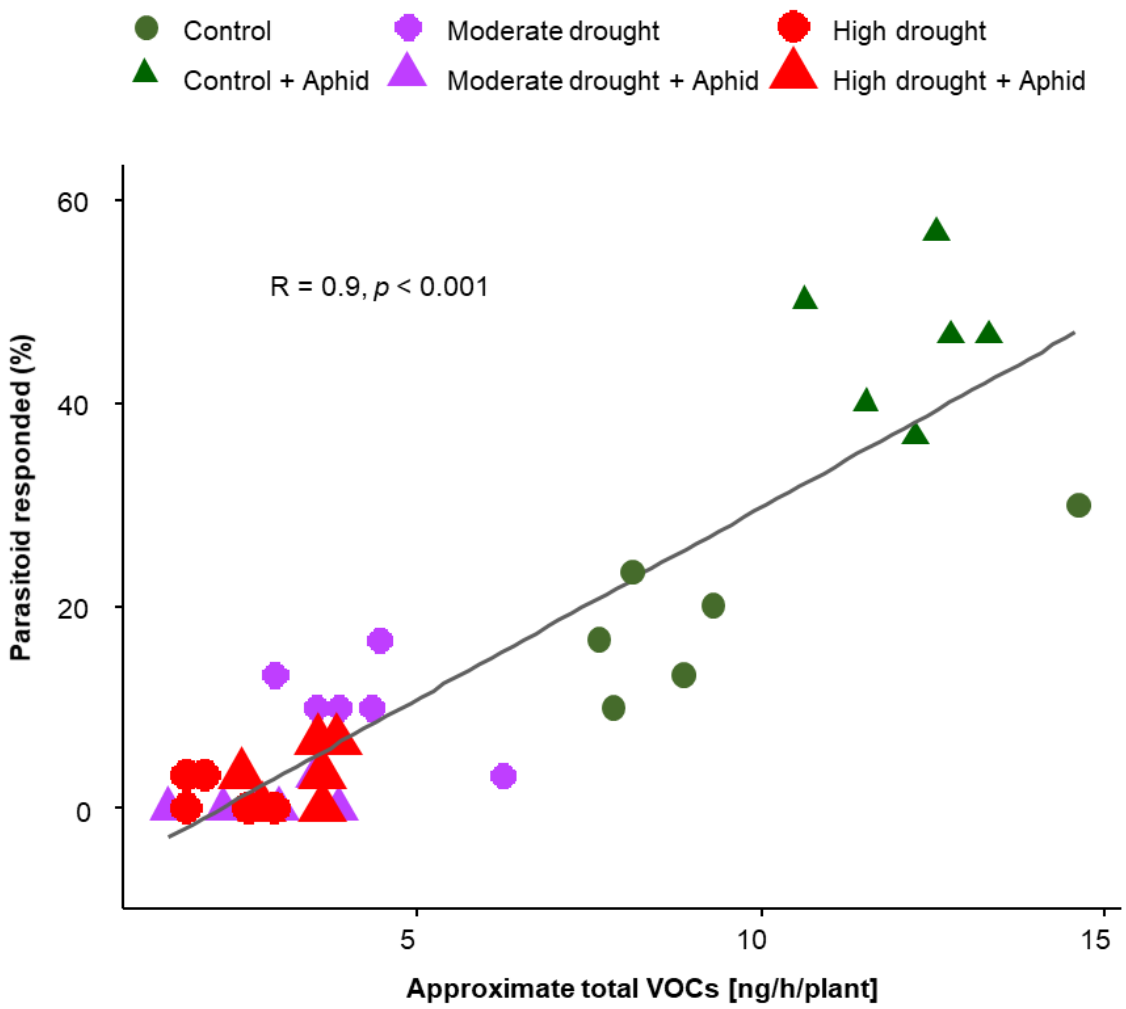


Figure 6: Correlation between parasitoid preferences and total VOCs emission per plant.

Discussion

Our study documents that drought stress differentially affects the three trophic levels associated with sugar beet. While drought stress had direct negative effects on sugar beet plants by reducing their size and biomass, it benefited *Aphis fabae*, as these aphids developed faster and produced more offspring on drought-stressed plants. Even though more parasitoid mummies were formed on drought-stressed plants, fewer adult parasitoids emerged from the mummies on drought-stressed plants. In addition, HIPV emission was drastically reduced by drought stress and parasitoids were less attracted to drought-stressed plants with and without aphids when compared to well-watered plants. This suggests that biological pest control by parasitoids might be severely impaired by drought stress in this system.

Drought stress severely alters plant growth and morphology and leads to many physiological and biochemical responses. Water limitation triggers a phytohormonal signaling cascade, involving abscisic acid (ABA) and induces stomatal closure, resulting in reduced gas exchange and ultimately in reduced photosynthesis (Ding et al., 2018; Farooq et al., 2009). Drought also interferes with mitosis and the loss of turgor inhibits cell elongation (Fahad et al., 2017; Farooq et al., 2009). In addition, it disturbs the water balance, membrane permeability, mineral nutrition, and enzyme activities in the plant (Dubey et al., 2021). Taken together, the effects of drought stress on plant physiology typically result in reduced growth and lower biomass production, as was observed in this study and in other crops, such as maize (Anjum et al., 2017), sugarcane (Misra et al., 2020), wheat and rice (Patmi et al., 2020; Zhang et al., 2018). The increase in root:shoot ratio reported here is also a typical plant response that enhances water uptake and thus mitigates the effects of drought (Fang & Xiong, 2015; Kurepa & Smalle, 2022). In our study, drought stress symptoms were already visible at moderate drought levels and were even more pronounced in the high drought treatment. Plant stress caused by water limitation can be further exacerbated if it makes plants more susceptible to herbivores, either directly or indirectly by reducing their ability to attract natural enemies for defense.

In this study we found that the performance and fecundity of individual *Aphis fabae* were highest on plants receiving high drought treatment, intermediate on plants experiencing moderate drought and lowest on well-watered plants. Effects of drought stress on aphid performance are variable (Leybourne et al., 2021), indicating that there is no general response of aphids towards drought-stressed host plants. The effects of drought stress on aphids can further depend on the timing and magnitude of the water limitation experienced (Luo & Gilbert, 2022; Tariq et al., 2013) and on specific interactions between aphid species and host plant species (Leybourne et al., 2021; Mewis et al., 2012). Reasons for enhanced aphid performance on drought-stressed plants could be an increase in the nutritional quality of the drought-stressed plant (Smith et al., 2019), which includes sugar and or/ amino acid concentrations in the phloem sap (Fàbregas & Fernie, 2019; Hale et al., 2003). Phytohormonal crosstalk between different stress-related phytohormones such as jasmonic acid,

salicylic acid and abscisic acid, further affects inducible defenses against aphids (Guo et al., 2016; Kansman et al., 2022) and may benefit the aphids on drought-stressed plants.

The emission of VOCs can vary both in quantity and quality, depending on the biotic and abiotic stress factors involved, and these changes can influence the attraction of natural enemies to herbivore-infested plants (Dicke & Baldwin, 2010; Kugimiya et al., 2010). Drought has been shown to affect the foraging success of a parasitoid by altering plant volatile emissions. This resulted in less attractive (Tariq et al., 2013) or even unrecognizable signals (Martini & Stelinski, 2017), but positive (Salerno et al., 2017) or neutral effects of drought stress on parasitoid attraction have also been reported (Weldegergis et al., 2015).

In our study, aphid infestation on well-watered plants resulted in a strong increase in total VOC emission and in the release of eight VOCs that were not detected in the well-watered controls without aphids. The well-watered aphid-infested plants were the most attractive to the parasitoid *A. colemani*. In the drought-stressed plants, on the other hand, the number of compounds detected and the total emission of volatile compounds per plant were greatly reduced. Not all plant VOCs are perceived by parasitoids (Goelen et al., 2021; Li et al., 2022), but in our study we found a strong correlation between the total amount of emitted VOCs and the attraction of *A. colemani*. Aphid-infested plants from the well-watered treatment and from the high drought treatments emitted significantly more volatiles and attracted more parasitoids than the uninfested plants subjected to the same watering regime. This shows that, despite the lower overall attraction of *A. colemani* to drought-stressed plants, the parasitoids were still able to discriminate between aphid-infested and non-infested plants under severe drought stress. Interestingly, in the moderate drought treatment, we found a suppression of volatile emission during aphid infestation and parasitoids were significantly less attracted to the aphid-infested plants compared to the non-infested plants under moderate drought stress, suggesting that parasitoids may be unable to find aphids on moderately drought-stressed plants.

All studies on HIPV emission in relation to drought stress report changes in the emission of some VOC compounds, but the effects of altered HIPV bouquets on parasitoid attraction are variable and the mechanisms leading to

altered behavior are not well understood (Catola et al., 2018; Pagadala Damodaram et al., 2021; Salerno et al., 2017; Tariq et al., 2013; Weldegergis et al., 2015). Similar to our results, *A. colemani* and *Diaeretiella rapae* preferred aphid-infested well-watered Brussels sprouts to aphid-infested drought-stressed plants. Emission rates of allyl isothiocyanate, limonene and *β*-phellandrene from drought-stressed aphid-infested plants were more similar to the undamaged well-watered controls than to aphid-infested well-watered plants (Tariq et al., 2013), suggesting that reduced differences in emission rates of behaviorally active compounds from infested and undamaged plants under drought stress may reduce parasitoid attraction. Similarly, drought-stressed plants might become more attractive to parasitoids if drought stress increases the differences between VOC blends from infested and non-infested plants.

The physiological mechanisms that lead to changes in VOC emission upon drought stress are also not fully understood and may depend on the exact drought stress treatment. Acute drought usually leads to reduced stomatal conductance (Daszkowska-Golec & Szarejko, 2013), while plants that have recovered from previous drought stress still show the results of altered phytohormonal signaling and physiological adaptations to drought stress (Weldegergis et al., 2015). Different magnitudes and durations of drought stress and pulsed or continuous stress treatments lead to different physiological and metabolic changes in the plant, resulting in different changes in VOC emission (e.g. (Salerno et al., 2017). The reduced VOC emission of drought-stressed plants in our study can partially be explained by the reduced biomass of drought-stressed plants. After standardizing VOC emission by plant biomass or leaf area, severely drought-stressed plants with aphids emitted similar amounts of VOCs as well-watered plants with aphids, indicating that VOC emission was not affected by severe drought stress. However, moderately drought-stressed plants with aphids emitted significantly less VOCs than the well-watered plants with aphids, and reduced emission during drought stress was found in uninfested plants (Figure S4). VOCs are mainly released through the stomata and the reduced emission of VOCs from drought-stressed plants may be partly the result of low stomatal conductance (Harley, 2013; Lin et al., 2022; Niinemets et al., 2004; Seidl-Adams et al., 2015). Stomatal closure should have strong effects on the emission rates of compounds

with low Henry's law volatility constant (H^{pc}, for definition see Sander, 2015) such as alcohols, carbonyls, aldehydes and oxygenated monoterpenes. Emission rates of compounds with a high H^{pc} such as non-oxygenated monoterpenes should not be affected by stomatal conductance (Lin et al., 2022). Most non-oxygenated monoterpenes emitted by sugar beet (e.g. *β*-pinene, *p*-menthane, *β*-myrcene, 3-carene, D-limonene, *o*-cymene etc.) were released in higher quantities from aphid-infested plants than from undamaged plants belonging to the well-watered and the high drought treatments. Thus, these compounds may be reliable cues for parasitoids to indicate host presence even under drought stress conditions. Notably, most of these compounds were absent or emitted only in low amounts from the aphid-infested plants in the moderate drought treatment, coinciding with low parasitoid attraction. Emission rates of *p*-menthane, *β*-myrcene, 3-carene, D-limonene and *o*-cymene standardized by plant biomass were highest from severely drought-stressed aphid-infested plants, suggesting that the production of these potentially important compounds might be upregulated in highly drought-stressed plants upon aphid feeding to compensate for the decrease in plant biomass. Moreover, *γ*-terpinene and trans-*β*-ocimene, two monoterpenes with a high H^{pc} that should not be effected by stomata conductance, were absent from the blends emitted by drought-stressed plants. This may indicate that other regulatory processes such as phytohormonal signaling are involved in the production and release of VOCs, and are altered by drought stress.

Aphidius colemani may actually benefit from a reduced attraction to aphids on drought-stressed sugar beet plants, because emergence rate and adult size were strongly reduced on drought-stressed plants. This may be due to the smaller body size of aphid hosts on drought-stressed sugar beets (Rahman *et al.* unpublished). Aphid body size can be affected by drought stress (Ahmed et al., 2017; Kansman et al., 2021). Aphid body size and the size of emerging parasitoids are usually positively correlated, and parasitoid emergence rate can be related to host size and quality (e.g. Garratt et al., 2010; Tariq et al., 2013; Yasir Ali et al., 2022).In our study, emerging adults of *A. colemani* were indeed smaller when developing in aphids on drought-stressed plants. Interestingly, only the emergence of females was negatively affected by drought stress in our

experiment, with just four females emerging from aphids on highly drought-stressed plants compared to 16 males. Under moderate drought, the sex ratio was almost equal, while on well-watered plants, 2/3 of the emerging parasitoids were females. A lower emergence rate and a shift from female-biased to a male-biased sex ratio of *A. colemani* and *D. rapae* under severe drought stress was also observed by Tariq et al., 2013 and Shehzad et al., 2020. Aphid parasitoids can actively control sex allocation by laying more unfertilized eggs, resulting in more male offspring on poor quality hosts (Cloutier et al., 1991; Pandey & Singh, 1999). Alternatively, female parasitoids, with their larger body size, may require larger hosts to develop successfully, and female larvae may not have been able to develop successfully in small hosts on drought-stressed plants. Indeed, smaller males emerged from aphids on highly drought-stressed plants than from aphids on moderately drought-stressed plants, while the body sizes of female parasitoids did not differ. This suggests that female *A. colemani* have already reached the lower limit of their body size on moderately drought-stressed plants. Tariq et al. (2013) also found no effect of drought stress on the size of female *A. colemani*, but female *D. rapae* were smaller on drought-stressed plants. In contrast, Shehzad et al. 2020 reported negative effects of severe drought and positive effects of mild drought on the body size of both parasitoid species. Reduced attraction to drought-stressed, aphid-infested sugar beet plants as a result of reduced VOC emissions may thus help *A. colemani* to avoid oviposition on low quality hosts, especially when plants without water deficit are available in close proximity.

In our study, the duration of parasitoid larval development was shorter on highly drought-stressed plants compared to well-watered controls, which coincides with faster aphid development in this treatment. In contrast, parasitoid development time increased with increasing drought stress in the study by Ahmed et al. (2017). Changes in the duration of development might alter the predation risk of parasitoid larvae, which often become intraguild prey of aphid predators (Mottaghinia et al., 2018) and thus affecting the size of the parasitoid population. However, these effects might be minor compared to the reduced female emergence on drought-stressed plants observed in our study.

More mummies were formed on highly drought-stressed plants when compared to the well-watered controls. The higher mummification rate could have been the result of higher attack rates on highly drought-stressed plants, because smaller aphids require less handling time by the parasitoid (Wu et al., 2011). Alternatively, immune responses that kill some of the parasitoid eggs or larvae may have been compromised in aphids that developed on highly drought-stressed plants. Immunity to parasitoids is encoded in the aphid genome (Martinez et al., 2014) and provided by defensive endosymbionts such as *Hamiltonella defensa* (Rouchet & Vorburger, 2014). Heat stress negatively affects the immunity provided by *H. defense*, but has no negative effect on another defensive endosymbiont (Benjamin & Nielsen, 2006; Guay et al., 2009). It remains to be tested whether plant-mediated effects of abiotic stress, such as drought, also influence aphid immune responses against parasitoids.

Due to the intimate relationship between aphid parasitoids and their hosts, there are multiple ways in which drought stress can affect parasitoid performance and foraging behavior, and it is difficult to predict how this would affect pest control. Suppression of aphid populations by *A. colemani* in a cage experiment was strongest under mild drought stress and moderate under severe drought stress (Kansman et al., 2021). In our study, the effects of drought stress on parasitoids were mainly negative, due to reduced numbers of emergent females and a reduced attractiveness of VOCs emitted by drought-stressed plants. A reduced ability of parasitoids to find aphid-infested plants combined with a low female emergence rate can have devastating effects on pest control. Moreover, drought stress often coincides with high temperatures and low atmospheric humidity and these additional factors may exacerbate the effects of drought stress. For example, low atmospheric humidity negatively affects parasitoid activity and host-finding success in the field (Vosteen et al., 2020). Drought stress combined with high temperatures negatively affected the ability of parasitoids to control aphid populations, despite the positive effects of these factors when tested alone (Romo & Tylianakis, 2013).

Overall, our findings indicate that drought stress exerts positive plant-mediated, bottom-up effects on aphids infesting drought-stressed sugar beets, resulting in a positive impact on the *A. faba* population while negatively affecting

its parasitoid, *A. colemani*. Consequently, we anticipate that with an increased frequency of drought events projected under future climate change conditions, aphid outbreaks could be facilitated in this system. This can be attributed to improved food resources and reduced top-down pressure, thereby creating a conducive environment for aphid population growth.

Supporting information

Additional supporting information can be found in the supplementary files section at the end of this article.

Author contributions

Shahinoor Rahman: conceptualization, conduction of experiments, data collection, data analysis, and writing the first draft. Michael Rostás: conceptualization, review, editing, and supervision. Ilka Vosteen: conceptualization, checking data analysis, manuscript editing and supervision. The authors declare no conflicts of interest.

References

Ahmed, S. S., Liu, D., & Simon, J.-C. (2017). Impact of water-deficit stress on tritrophic interactions in a wheat-aphid-parasitoid system. *PLOS ONE*, *12*(10), e0186599. https://doi.org/10.1371/journal.pone.0186599

Anjum, S. A., Ashraf, U., Tanveer, M., Khan, I., Hussain, S., Shahzad, B., Zohaib, A., Abbas, F., Saleem, M. F., Ali, I., & Wang, L. C. (2017). Drought Induced Changes in Growth, Osmolyte Accumulation and Antioxidant Metabolism of Three Maize Hybrids. *Frontiers in Plant Science*, *8*, 69. https://doi.org/10.3389/fpls.2017.00069

Benjamin, J. G., & Nielsen, D. C. (2006). Water deficit effects on root distribution of soybean, field pea and chickpea. *Field Crops Research*, *97*(2), 248–253. https://doi.org/10.1016/j.fcr.2005.10.005

Bettaieb Rebey, I., Jabri-Karoui, I., Hamrouni-Sellami, I., Bourgou, S., Limam, F., & Marzouk, B. (2012). Effect of drought on the biochemical composition and antioxidant activities of cumin (Cuminum cyminum L.) seeds. *Industrial Crops and Products*, *36*(1), 238–245. https://doi.org/10.1016/j.indcrop.2011.09.013

Boergens, E., Güntner, A., Dobslaw, H., & Dahle, C. (2020). Quantifying the Central European Droughts in 2018 and 2019 With GRACE Follow-On. *Geophysical Research Letters*, *47*(14), 1–9. https://doi.org/10.1029/2020GL087285

Brodeur, J., & Boivin, G. (2004). Functional Ecology of Immature Parasitoids. *Annual Review of Entomology*, *49*(1), 27–49. https://doi.org/10.1146/annurev.ento.49.061703.153618

Catola, S., Centritto, M., Cascone, P., Ranieri, A., Loreto, F., Calamai, L., Balestrini, R., & Guerrieri, E. (2018). Effects of single or combined water deficit and aphid attack on tomato volatile organic compound (VOC) emission and plant-plant communication. *Environmental and Experimental Botany*, *153*, 54–62. https://doi.org/10.1016/j.envexpbot.2018.05.001

Cloutier, C., Lévesque, C. A., Eaves, D. M., & Mackauer, M. (1991). Maternal adjustment of sex ratio in response to host size in the aphid parasitoid Ephedrus californicus. *Canadian Journal of Zoology*, *69*(6), 1489–1495. https://doi.org/10.1139/z91-208

Cui, H., Wang, L., Reddy, G. V. P., & Zhao, Z. (2021). Mild Drought Facilitates the Increase in Wheat Aphid Abundance by Changing Host Metabolism. *Annals of the Entomological Society of America*, *114*(1), 79–83. https://doi.org/10.1093/aesa/saaa038

Daszkowska-Golec, A., & Szarejko, I. (2013). Open or Close the Gate – Stomata Action Under the Control of Phytohormones in Drought Stress Conditions. *Frontiers in Plant Science*, *4*. https://www.frontiersin.org/articles/10.3389/fpls.2013.00138

Dicke, M., & Baldwin, I. T. (2010). The evolutionary context for herbivore-induced plant volatiles: Beyond the 'cry for help.' *Trends in Plant Science*, *15*(3), 167–175. https://doi.org/10.1016/j.tplants.2009.12.002

Ding, L., Lu, Z., Gao, L., Guo, S., & Shen, Q. (2018). Is Nitrogen a Key Determinant of Water Transport and Photosynthesis in Higher Plants Upon Drought Stress? *Frontiers in Plant Science*, *9*. https://www.frontiersin.org/articles/10.3389/fpls.2018.01143

Dohm, J. C., Minoche, A. E., Holtgräwe, D., Capella-Gutiérrez, S., Zakrzewski, F., Tafer, H., Rupp, O., Sörensen, T. R., Stracke, R., Reinhardt, R., Goesmann, A., Kraft, T., Schulz, B., Stadler, P. F., Schmidt, T., Gabaldón, T., Lehrach, H., Weisshaar, B., & Himmelbauer, H. (2014). The genome of the recently domesticated crop plant sugar beet (Beta vulgaris). *Nature*, *505*(7484), 546–549. https://doi.org/10.1038/nature12817

Dubey, A., Kumar, A., Malla, M. A., Chowdhary, K., Singh, G., Ravikanth, G., Harish, null, Sharma, S., Saati-Santamaria, Z., Menéndez, E., & Dames, J. F. (2021). Approaches for the amelioration of adverse effects of drought stress on crop plants. *Frontiers in Bioscience (Landmark Edition)*, *26*(10), 928–947. https://doi.org/10.52586/4998

Fàbregas, N., & Fernie, A. R. (2019). The metabolic response to drought. *Journal of Experimental Botany*, *70*(4), 1077–1085. https://doi.org/10.1093/jxb/ery437

Fahad, S., Bajwa, A. A., Nazir, U., Anjum, S. A., Farooq, A., Zohaib, A., Sadia, S., Nasim, W., Adkins, S., Saud, S., Ihsan, M. Z., Alharby, H., Wu, C., Wang, D., & Huang, J. (2017). Crop Production under Drought and Heat Stress: Plant Responses and Management Options. *Frontiers in Plant Science*, *8*. https://www.frontiersin.org/articles/10.3389/fpls.2017.01147

Fang, Y., & Xiong, L. (2015). General mechanisms of drought response and their application in drought resistance improvement in plants. *Cellular and Molecular Life Sciences: CMLS*, *72*(4), 673–689. https://doi.org/10.1007/s00018-014-1767-0

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., & Basra, S. M. A. (2009). Plant drought stress: Effects, mechanisms and management. *Agronomy for Sustainable Development*, *29*(1), 185–212. https://doi.org/10.1051/agro:2008021

Garratt, M. P. D., Leather, S. R., & Wright, D. J. (2010). Tritrophic effects of organic and conventional fertilisers on a cereal-aphid-parasitoid system. *Entomologia Experimentalis et Applicata*, *134*(3), 211–219. https://doi.org/10.1111/j.1570-7458.2009.00957.x

Goelen, T., Vuts, J., Sobhy, I. S., Wäckers, F., Caulfield, J. C., Birkett, M. A., Rediers, H., Jacquemyn, H., & Lievens, B. (2021). Identification and application of bacterial volatiles to attract a generalist aphid parasitoid: From laboratory to greenhouse assays. *Pest Management Science*, *77*(2), 930–938. https://doi.org/10.1002/ps.6102

Grzesiak, S., Hordyńska, N., Szczyrek, P., Grzesiak, M. T., Noga, A., & Szechyńska-Hebda, M. (2019). Variation among wheat (Triticum easativum L.) genotypes in response to the drought stress: I – selection approaches. *Journal of Plant Interactions*, *14*(1), 30–44. https://doi.org/10.1080/17429145.2018.1550817

Guay, J.-F., Boudreault, S., Michaud, D., & Cloutier, C. (2009). Impact of environmental stress on aphid clonal resistance to parasitoids: Role of Hamiltonella defensa bacterial symbiosis in association with a new facultative symbiont of the pea aphid. *Journal of Insect Physiology*, *55*(10), 919–926. https://doi.org/10.1016/j.jinsphys.2009.06.006

Guo, H., Sun, Y., Peng, X., Wang, Q., Harris, M., & Ge, F. (2016). Up-regulation of abscisic acid signaling pathway facilitates aphid xylem absorption and osmoregulation under drought stress. *Journal of Experimental Botany*, *67*(3), 681–693. https://doi.org/10.1093/jxb/erv481

Gupta, A., Rico-Medina, A., & Caño-Delgado, A. I. (2020). The physiology of plant responses to drought. *Science*, *368*(6488), 266–269. https://doi.org/10.1126/science.aaz7614

Hale, B. K., Bale, J. S., Pritchard, J., Masters, G. J., & Brown, V. K. (2003). Effects of host plant drought stress on the performance of the bird cherry-oat aphid, Rhopalosiphum padi (L.): A mechanistic analysis. *Ecological Entomology*, *28*(6), 666–677. https://doi.org/10.1111/j.1365-2311.2003.00563.x

Harley, P. C. (2013). The Roles of Stomatal Conductance and Compound Volatility in Controlling the Emission of Volatile Organic Compounds from Leaves. In Ü. Niinemets & R. K. Monson (Eds.), *Biology, Controls and Models of Tree Volatile Organic Compound Emissions* (pp. 181–208). Springer Netherlands. https://doi.org/10.1007/978-94-007-6606-8_7

Hoagland, D. R., & Arnon, D. I. (1938). *The water-culture method for growing plants without soil*. University of California, College of Agriculture, Agricultural Experiment Station.

IPCC (p. 1132). (2014). "Climate change 2014 – impacts, adaptation and vulnerability. Part A: global and sectoral aspects," in Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Field, C.B., V.R. Barros, D.J. Dokken, K.J. Mach, M.D. Mastrandrea, T.E. Bilir, M. Chatterjee, K.L. Ebi, Y.O. Estrada, R.C. Genova, B. Girma, E.S. Kissel, A.N. Levy, S. MacCracken, P.R. Mastrandrea, and L.L. White (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1132.

Jamieson, M., Trowbridge, A., Raffa, K., & Lindroth, R. (2012). Consequences of Climate Warming and Altered Precipitation Patterns for Plant-Insect and Multitrophic Interactions. *Plant Physiology*, *160*, 1719–1727. https://doi.org/10.1104/pp.112.206524

Jogawat, A., Yadav, B., Chhaya, Lakra, N., Singh, A. K., & Narayan, O. P. (2021). Crosstalk between phytohormones and secondary metabolites in the drought stress tolerance of crop plants: A review. *Physiologia Plantarum*, *172*(2), 1106–1132. https://doi.org/10.1111/ppl.13328

Johnson, S. N., Staley, J. T., McLeod, F. A. L., & Hartley, S. E. (2011). Plant-mediated effects of soil invertebrates and summer drought on above-ground multitrophic interactions. *Journal of Ecology*, *99*(1), 57–65. https://doi.org/10.1111/j.1365-2745.2010.01748.x

Kansman, J. T., Basu, S., Casteel, C. L., Crowder, D. W., Lee, B. W., Nihranz, C. T., & Finke, D. L. (2022). Plant Water Stress Reduces Aphid Performance: Exploring Mechanisms Driven by Water Stress Intensity. *Frontiers in Ecology and Evolution*, *10*. https://www.frontiersin.org/articles/10.3389/fevo.2022.846908

Kansman, J. T., Ward, M., LaFond, H., & Finke, D. L. (2021). Effects of Plant Stress on Aphid–Parasitoid Interactions: Drought Improves Aphid Suppression. *Environmental Entomology*, *50*(3), 713–718. https://doi.org/10.1093/ee/nvab005

Kaplan, I., Carrillo, J., Garvey, M., & Ode, P. J. (2016). Indirect plant–parasitoid interactions mediated by changes in herbivore physiology. *Current Opinion in Insect Science*, *14*, 112–119. https://doi.org/10.1016/j.cois.2016.03.004

Kessler, A., & Heil, M. (2011). The multiple faces of indirect defences and their agents of natural selection. *Functional Ecology*, *25*(2), 348–357. https://doi.org/10.1111/j.1365-2435.2010.01818.x

Khaleghi, A., Naderi, R., Brunetti, C., Maserti, B. E., Salami, S. A., & Babalar, M. (2019). Morphological, physiochemical and antioxidant responses of Maclura pomifera to drought stress. *Scientific Reports*, *9*(1), 19250. https://doi.org/10.1038/s41598-019-55889-y

Khan, M. a. m., Ulrichs, C., & Mewis, I. (2010). Influence of water stress on the glucosinolate profile of Brassica oleracea var. Italica and the performance of Brevicoryne brassicae and Myzus persicae. *Entomologia Experimentalis et Applicata*, *137*(3), 229–236. https://doi.org/10.1111/j.1570-7458.2010.01059.x

Kugimiya, S., Shimoda, T., Tabata, J., & Takabayashi, J. (2010). Present or Past Herbivory: A Screening of Volatiles Released from Brassica rapa Under Caterpillar Attacks as Attractants for the Solitary Parasitoid, Cotesia vestalis. *Journal of Chemical Ecology*, *36*(6), 620–628. https://doi.org/10.1007/s10886-010-9802-6

Kurepa, J., & Smalle, J. A. (2022). Auxin/Cytokinin Antagonistic Control of the Shoot/Root Growth Ratio and Its Relevance for Adaptation to Drought and Nutrient Deficiency Stresses. *International Journal of Molecular Sciences*, *23*(4), 1933. https://doi.org/10.3390/ijms23041933

Leybourne, D. J., Preedy, K. F., Valentine, T. A., Bos, J. I. B., & Karley, A. J. (2021). Drought has negative consequences on aphid fitness and plant vigor: Insights from a meta-analysis. *Ecology and Evolution*, *11*(17), 11915–11929. https://doi.org/10.1002/ece3.7957

Li, M., Xia, S., Zhang, T., Williams, L., Xiao, H., & Lu, Y. (2022). Volatiles from Cotton Plants Infested by Agrotis segetum (Lep.: Noctuidae) Attract the Larval Parasitoid Microplitis mediator (Hym.: Braconidae). *Plants*, *11*(7), Article 7. https://doi.org/10.3390/plants11070863

Li, W., Wang, Y., Zhang, Y., Wang, R., Guo, Z., & Xie, Z. (2020). Impacts of drought stress on the morphology, physiology, and sugar content of Lanzhou lily (Lilium davidii var. Unicolor). *Acta Physiologiae Plantarum*, *42*(8), 127. https://doi.org/10.1007/s11738-020-03115-y

Lin, P.-A., Chen, Y., Ponce, G., Acevedo, F. E., Lynch, J. P., Anderson, C. T., Ali, J. G., & Felton, G. W. (2022). Stomata-mediated interactions between plants, herbivores, and the environment. *Trends in Plant Science*, *27*(3), 287–300. https://doi.org/10.1016/j.tplants.2021.08.017

Luo, R., & Gilbert, B. (2022). Timing of short-term drought structures plant–herbivore dynamics. *2022*(1). https://doi.org/10.1111/oik.08860

Marchin, R. M., Ossola, A., Leishman, M. R., & Ellsworth, D. S. (2020). A Simple Method for Simulating Drought Effects on Plants. *Frontiers in Plant Science*, *10*, 1715. https://doi.org/10.3389/fpls.2019.01715

Martinez, A. J., Ritter, S. G., Doremus, M. R., Russell, J. A., & Oliver, K. M. (2014). Aphid-encoded variability in susceptibility to a parasitoid. *BMC Evolutionary Biology*, *14*(1), 127. https://doi.org/10.1186/1471-2148-14-127

Martini, X., & STELinski, L. L. (2017). Drought stress affects response of phytopathogen vectors and their parasitoids to infection- and damage-induced plant volatile cues. *Ecological Entomology*, *42*(6), 721–730. https://doi.org/10.1111/een.12439

Mewis, I., Khan, M. A. M., Glawischnig, E., Schreiner, M., & Ulrichs, C. (2012). Water Stress and Aphid Feeding Differentially Influence Metabolite Composition in Arabidopsis thaliana (L.). *PLOS ONE*, *7*(11), e48661. https://doi.org/10.1371/journal.pone.0048661

Mishra, B. K., Srivastava, J. P., & Lal, J. P. (2018). Drought Resistance in Lentil (Lens culinaris Medik.) in Relation to Morphological, Physiological Parameters and Phenological Developments. *International Journal of Current Microbiology and Applied Sciences*, *7*(1), 2288–2304. https://doi.org/10.20546/ijcmas.2018.701.277

Misra, V., Solomon, S., Mall, A. K., Prajapati, C. P., Hashem, A., Abd_Allah, E. F., & Ansari, M. I. (2020). Morphological assessment of water stressed sugarcane: A comparison of waterlogged and drought affected crop. *Saudi Journal of Biological Sciences*, *27*(5), 1228–1236. https://doi.org/10.1016/j.sjbs.2020.02.007

Mody, K., Eichenberger, D., & Dorn, S. (2009). Stress magnitude matters: Different intensities of pulsed water stress produce non-monotonic resistance responses of host plants to insect herbivores. *Ecological Entomology*, *34*(1), 133–143. https://doi.org/10.1111/j.1365-2311.2008.01053.x

Mottaghinia, L., Hassanpour, M., Razmjou, J., Chamani, E., & Hosseini, M. (2018). Intraguild Predation on the Parasitoid Wasp Aphidius colemani by the Predator Aphidoletes aphidimyza: Effect of Host Plant Cultivars. *Journal of Agricultural Science and Technology*, *20*(3), 533–542.

Mubarik, M. S., Khan, S. H., Sajjad, M., Raza, A., Hafeez, M. B., Yasmeen, T., Rizwan, M., Ali, S., & Arif, M. S. (2021). A manipulative interplay between positive and negative regulators of phytohormones: A way forward for improving drought tolerance in plants. *Physiologia Plantarum*, *172*(2), 1269–1290. https://doi.org/10.1111/ppl.13325

Nguyen, L.-T.-H., Monticelli, L. S., Desneux, N., Metay-Merrien, C., Amiens-Desneux, E., & Lavoir, A.-V. (2018). Bottom-up effect of water stress on the aphid parasitoid Aphidius ervi. *Entomologia Generalis*, *38*(1), 15–27. https://doi.org/10.1127/entomologia/2018/0575

Niinemets, Ü., Loreto, F., & Reichstein, M. (2004). Physiological and physicochemical controls on foliar volatile organic compound emissions. *Trends in Plant Science*, *9*(4), 180–186. https://doi.org/10.1016/j.tplants.2004.02.006

Pagadala Damodaram, K. J., Gadad, H. S., Parepally, S. K., Vaddi, S., Ramanna Hunashikatti, L., & Bhat, R. M. (2021). Low moisture stress influences plant volatile emissions affecting herbivore interactions in tomato, Solanum lycopersicum. *Ecological Entomology*, *46*(3), 637–650. https://doi.org/10.1111/een.13012

Pandey, S., & Singh, R. (1999). Host size induced variation in progeny sex ratio of an aphid parasitoid Lysiphlebia mirzai. *Entomologia Experimentalis et Applicata*, *90*(1), 61–67. https://doi.org/10.1046/j.1570-7458.1999.00423.x

Patmi, Y. S., Pitoyo, A., Solichatun, & Sutarno. (2020). Effect of drought stress on morphological, anatomical, and physiological characteristics of Cempo Ireng Cultivar Mutant Rice (Oryza sativa l.) strain 51 irradiated by gamma-ray. *Journal of Physics: Conference Series*, *1436*, 012015. https://doi.org/10.1088/1742-6596/1436/1/012015

Romo, C. M., & Tylianakis, J. M. (2013). Elevated Temperature and Drought Interact to Reduce Parasitoid Effectiveness in Suppressing Hosts. *PLOS ONE*, *8*(3), e58136. https://doi.org/10.1371/journal.pone.0058136

Rouchet, R., & Vorburger, C. (2014). Experimental evolution of parasitoid infectivity on symbiont-protected hosts leads to the emergence of genotype specificity. *Evolution; International Journal of Organic Evolution*, *68*(6), 1607–1616. https://doi.org/10.1111/evo.12377

Salerno, G., Frati, F., Marino, G., Ederli, L., Pasqualini, S., Loreto, F., Colazza, S., & Centritto, M. (2017). Effects of water stress on emission of volatile organic compounds by Vicia faba, and consequences for attraction of the egg parasitoid Trissolcus basalis. *Journal of Pest Science*, *90*(2), 635–647. https://doi.org/10.1007/s10340-016-0830-z

Sander, R. (2015). Compilation of Henry's law constants (version 4.0) for water as solvent. *Atmospheric Chemistry and Physics*, *15*(8), 4399–4981. https://doi.org/10.5194/acp-15-4399-2015

Seidl-Adams, I., Richter, A., Boomer, K. B., Yoshinaga, N., Degenhardt, J., & Tumlinson, J. H. (2015). Emission of herbivore elicitor-induced sesquiterpenes is regulated by stomatal aperture in maize (Zea mays) seedlings. *Plant, Cell & Environment*, *38*(1), 23–34. https://doi.org/10.1111/pce.12347

Shehzad, M., Gulzar, A., Staley, J. T., & Tariq, M. (2020). The effects of drought stress and type of fertiliser on generalist and specialist herbivores and their natural enemies. *Annals of Applied Biology*, *178*(2), 377–386. https://doi.org/10.1111/aab.12654

Smith, M. R., Veneklaas, E., Polania, J., Rao, I. M., Beebe, S. E., & Merchant, A. (2019). Field drought conditions impact yield but not nutritional quality of the seed in common bean (Phaseolus vulgaris L.). *PLOS ONE*, *14*(6), e0217099. https://doi.org/10.1371/journal.pone.0217099

Tariq, M., Wright, D. J., Bruce, T. J. A., & Staley, J. T. (2013). Drought and Root Herbivory Interact to Alter the Response of Above-Ground Parasitoids to Aphid Infested Plants and Associated Plant Volatile Signals. *PLOS ONE*, *8*(7), e69013. https://doi.org/10.1371/journal.pone.0069013

Tariq, M., Wright, D. J., Rossiter, J. T., & Staley, J. T. (2012). Aphids in a changing world: Testing the plant stress, plant vigour and pulsed stress hypotheses. *Agricultural and Forest Entomology*, *14*(2), 177–185. https://doi.org/10.1111/j.1461-9563.2011.00557.x

Turlings, T. C. J., Davison, A. C., & TamÒ, C. (2004). A six-arm olfactometer permitting simultaneous observation of insect attraction and odour trapping. *Physiological Entomology*, *29*(1), 45–55. https://doi.org/10.1111/j.1365-3032.2004.0362.x

Turlings, T. C. J., & Erb, M. (2018). Tritrophic Interactions Mediated by Herbivore-Induced Plant Volatiles: Mechanisms, Ecological Relevance, and Application Potential. *Annual Review of Entomology*, *63*(1), 433–452. https://doi.org/10.1146/annurev-ento-020117-043507

Viric Gasparic, H., Grubelic, M., Dragovic Uzelac, V., Bazok, R., Cacija, M., Drmic, Z., & Lemic, D. (2020). Neonicotinoid Residues in Sugar Beet Plants and Soil under Different Agro-Climatic Conditions. *Agriculture*, *10*(10), Article 10. https://doi.org/10.3390/agriculture10100484

Vosteen, I., van den Meiracker, N., & Poelman, E. H. (2020). Gone with the wind: Low availability of volatile information limits foraging efficiency in downwind-flying parasitoids. *Animal Behaviour*, *165*, 59–70. https://doi.org/10.1016/j.anbehav.2020.04.025

Walter, J. (2018). Effects of changes in soil moisture and precipitation patterns on plant-mediated biotic interactions in terrestrial ecosystems. *Plant Ecology*, *219*, 1449–1462. https://doi.org/10.1007/s11258-018-0893-4

Weldegergis, B. T., Zhu, F., Poelman, E. H., & Dicke, M. (2015). Drought stress affects plant metabolites and herbivore preference but not host location by its parasitoids. *Oecologia*, *177*(3), 701–713. https://doi.org/10.1007/s00442-014-3129-x

Willick, I. R., Lahlali, R., Vijayan, P., Muir, D., Karunakaran, C., & Tanino, K. K. (2018). Wheat flag leaf epicuticular wax morphology and composition in response to moderate drought stress are revealed by SEM, FTIR-ATR and synchrotron X-ray spectroscopy. *Physiologia Plantarum*, *162*(3), 316–332. https://doi.org/10.1111/ppl.12637

Wu, G.-M., Barrette, M., Boivin, G., Brodeur, J., Giraldeau, L.-A., & Hance, T. (2011). Temperature Influences the Handling Efficiency of an Aphid Parasitoid Through Body Size-Mediated Effects. *Environmental Entomology*, *40*(3), 737–742. https://doi.org/10.1603/EN11018

Wyatt, I. J., & White, P. F. (1977). Simple Estimation of Intrinsic Increase Rates for Aphids and Tetranychid Mites. *Journal of Applied Ecology*, *14*(3), 757–766. https://doi.org/10.2307/2402807

Xie, H., Shi, J., Shi, F., Xu, H., He, K., & Wang, Z. (2020). Aphid fecundity and defenses in wheat exposed to a combination of heat and drought stress. *Journal of Experimental Botany*, *71*(9), 2713–2722. https://doi.org/10.1093/jxb/eraa017

Yang, X., Lu, M., Wang, Y., Wang, Y., Liu, Z., & Chen, S. (2021). Response Mechanism of Plants to Drought Stress. *Horticulturae*, *7*(3), Article 3. https://doi.org/10.3390/horticulturae7030050

Yasir Ali, M., Lu, Z., Ali, A., Bilal Amir, M., Afaq Ahmed, M., Shahid, S., Liu, T.-X., & Pan, M. (2022). Effects of Plant-Mediated Differences in Aphid Size on Suitability of Its Parasitoid, Aphelinus varipes (Hymenoptera: Aphelinidae). *Journal of Economic Entomology*, *115*(1), 74–80. https://doi.org/10.1093/jee/toab245

Zhang, J., Zhang, S., Cheng, M., Jiang, H., Zhang, X., Peng, C., Lu, X., Zhang, M., & Jin, J. (2018). Effect of Drought on Agronomic Traits of Rice and Wheat: A Meta-Analysis. *International Journal of Environmental Research and Public Health*, *15*(5), 839. https://doi.org/10.3390/ijerph15050839

Drought aggravates plant stress by favoring aphids and weakening indirect defense in a sugar beet tritrophic system

Shahinoor Rahman*, Michael Rostás, and Ilka Vosteen*

Department of Crop Sciences, Division of Agricultural Entomology, Georg-August-University of Goettingen, Germany

*Correspondence: shahinoor_ent@yahoo.com; ilka.vosteen@uni-goettingen.de

Supporting information

Volatile collection

Ten fluorescent lamps (PAR inside odor source vessels: 130 µmol/(s m^{-2}) at 3 cm distance from lamps) illuminated the plants during VOC collection for 16 hours during the day and were switched off for 8 hours during night conditions. Volatile collection traps (7 cm glass tube) containing 30 mg of 80-100 mesh Porapak Q (Volatile Collection Trap LLC, FL, USA) were kept in place by two fine mesh metal screens and attached to each oven bag. Two activated charcoal filters (400 ccs, Alltech, Deerfield, IL, USA) were used to filter the air; then filtered and humidified air was pushed into each vessel at a rate of 1.0 l/min originating from a central in-house compressor. With a vacuum pump (N816.3KN.18, Laboport®, Germany), 0.5 l/min of air was pulled through the trapping filter.

Volatile analysis

After headspace collection, the trapped volatiles were eluted with 150 µl dichloromethane (DCM) into a 1 ml glass vial and stored at -80 °C for further analysis. Before the analysis, 200 ng of tetralin (1,2,3,4 tetrahydronaphthalene, Sigma-Aldrich, Taufkirchen, Germany) was added to each sample as an internal standard. Each sample's qualitative and quantitative VOC composition was analyzed by gas chromatography – mass spectrometry (5977B HES MSD, Agilent Technologies). A 2 µl sample was injected in pulsed splitless mode with an automated injection system. The oven temperature was held at 40 °C for 3 min and then increased gradually to a final temperature of 220 °C, which was held for 10 min. Helium (1.5 ml/min) was used as the carrier gas. The software MSD ChemStation with the NIST17 and Wiley11 mass spectral libraries was used to tentatively identify compounds by their mass spectra and retention indices.

Compound quantification was achieved by comparing peak areas to the peak area of the internal standard.

Statistical analysis

Prior to the analysis of each dataset, different models were tested with different family distributions were tested, depending on the type of dataset. Models were simplified when necessary, model assumptions were checked, performance of mixed effects models was simulated and compared using the Performance (Lüdecke et al., 2021) and DHARMa (Florian Hartig, 2021) packages to find the best model for each data set.

Mixed effects models were used to account for the fact that three or six plants were placed together in one plastic box to manipulate water availability. Each plastic box was considered as one block and the effect of a block was included as a random factor in the mixed effects models.

The effect of drought on plant height at different times (days) was analyzed by a generalized linear mixed effects model (GLMM) using the glmmTMB function (Brooks et al., 2017) with a log link to Gaussian to account for repeated measurements. Drought stress and time were used as fixed factors, but the interaction between drought stress and time was not included. Plant identity, nested in blocks, was used as a random factor in this analysis. Linear mixed effects models (LMMs) were used to analyze total biomass, root weight, shoot weight, and shoot length. Root-shoot ratio by weight was analyzed using GLMM with log link to Gaussian. Root length, root-shoot ratio by length, reproductive rate, intrinsic growth rate of aphids were analyzed by GLMM with Gaussian family. Male and female hind tibia length of parasitoids were analyzed by linear mixed effect models (LMM) with block as a random factor. Pre-reproductive period, reproductive period, longevity, number of offspring until own first offspring reproduce, cumulative aphid count data from the last day of the experiment, mummification to oviposition time of parasitoid, oviposition to emergence time of parasitoid, total development time of parasitoid, and number of leaves were analyzed by GLMM with Conway-Maxwell Poisson distribution (family = compois) (Huang, 2017), considering block as a random factor. Parasitoid responses to the six different odor sources were analyzed using general linear model (GLM) with binomial family distribution, considering number of successes (= all parasitoids

that went to each odor source) and number of failures (= all parasitoids that went to the other five odor sources) as two-vector response variables. Percent mummification of parasitoid data was analyzed by GLMM with beta binomial family distribution. When the data set violated the assumptions for conducting an ANOVA, an alternative approach was used. A non-parametric Kruskal-Wallis test followed by Dunn's test with Holm's method for post hoc analysis was considered. The data included variables such as survival, dead and parasitized aphids by parasitoids, parasitoid emergence rate and proportion of male and female parasitoids. Total volatile emissions were analyzed by two-way ANOVA with drought stress and aphid presence as explanatory variables, followed by Tukey HSD post-hoc test for multiple comparisons. Hierarchical clustering was calculated for VOCs based on the length of the straight line drawn, followed by Euclidean distance with complete linkage. The Pearson correlation coefficient was calculated to determine the relationship between VOCs and parasitoid response. One-way ANOVA was performed on the emission rates of individual VOC compounds, but for those individual VOCs that did not meet the model assumptions, we used the Kruskal-Wallis test followed by the Dunn test with the Holm method for post hoc analyses.

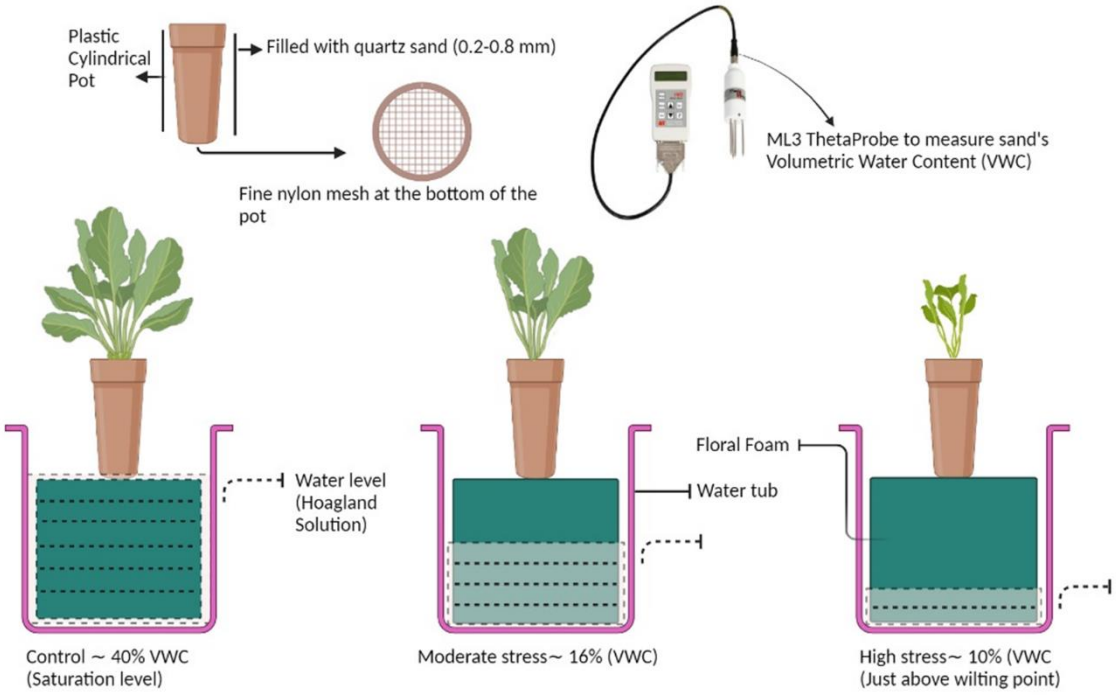


Figure S1: Schematic diagram of the capillary action drought system.

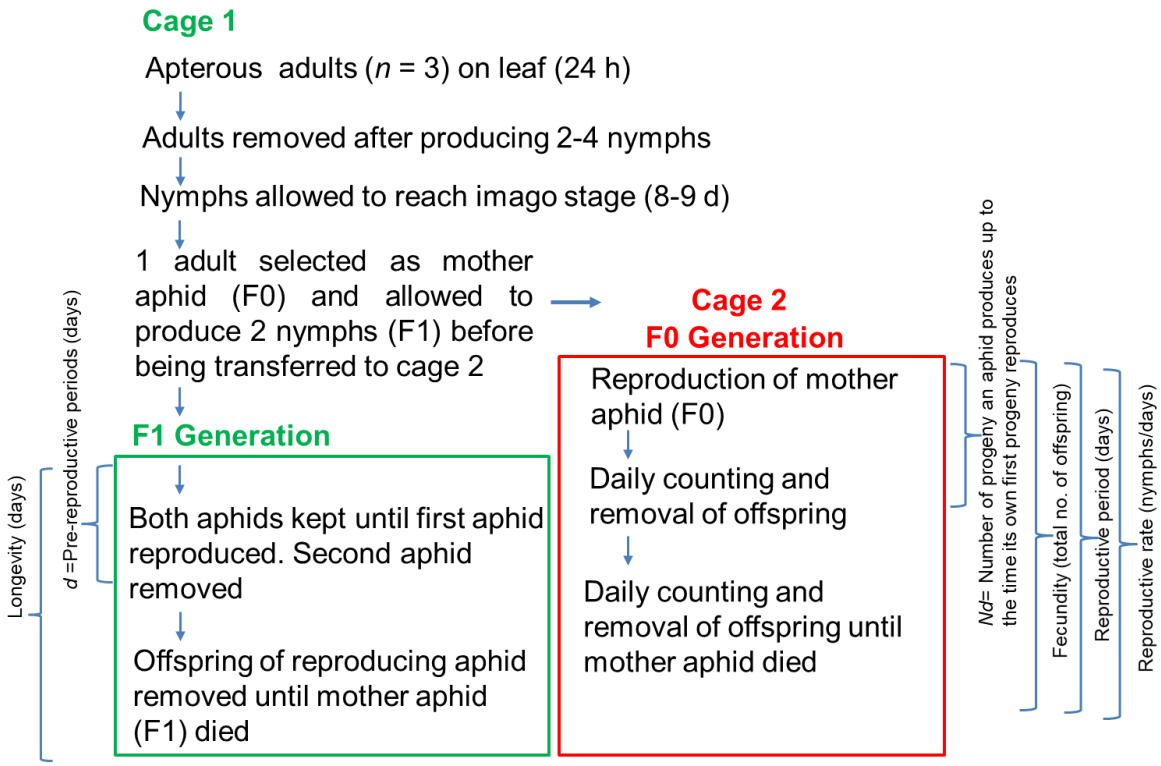


Figure S2: Protocol for testing aphid performance.

Table S1: Summary of statistical results of drought stress on sugar beet. The experiment evaluated plant height over time.

	Plant Height		
Predictors	**Estimates**	**Statistic (z value)**	***p***
(Intercept)	2.68 ***	214.82	**<0.001**
	(0.01)		
Treatment [Moderate drought]	-0.14 ***	-9.28	**<0.001**
	(0.01)		
Treatment [High drought]	-0.31 ***	-20.36	**<0.001**
	(0.02)		
Time [40DAS]	0.12 ***	11.33	**<0.001**
	(0.01)		
Time [47DAS]	0.28 ***	28.39	**<0.001**
	(0.01)		
Time [54DAS]	0.43 ***	45.54	**<0.001**
	(0.01)		
	Random Effects		
σ^2	0.56		
$\tau_{00\ Identity:Block}$	0.00		
$\tau_{00\ Block}$	0.00		
ICC	0.00		
$N_{Identity}$	54		
N_{Block}	18		
Observations	216		
Marginal R^2 / Conditional R^2	0.071 / 0.072		

* p<0.05 ** p<0.01 *** p<0.001

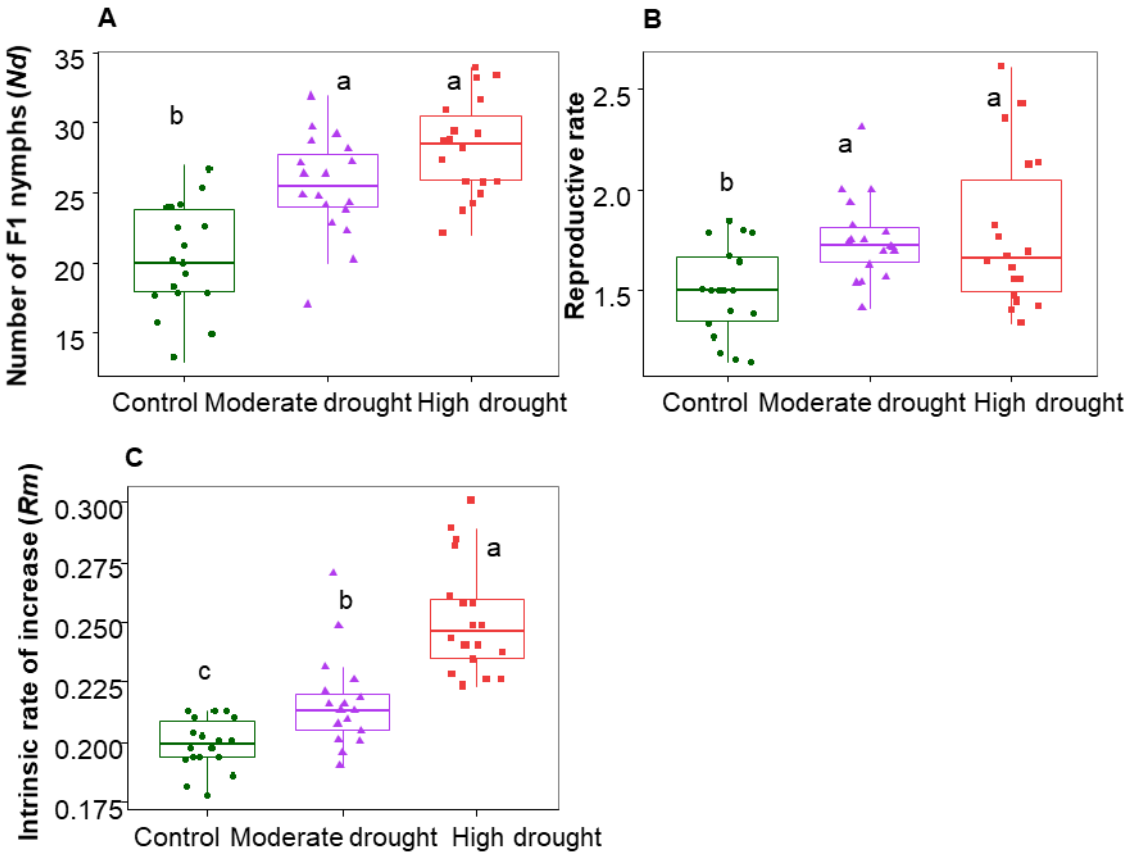


Figure S3: Effect of drought stress (~ 40% volumetric water content (VWC), ~ 16% VWC, ~ 10% VWC) on the performance of *Aphis fabae*. Figure shows the number of nymphs (F1) produced by a single adult aphid (F0) until the first F2 aphid emerges (A), the reproductive rate (average number of offspring per day) (B), and the intrinsic rate of increase (*Rm*) (C). Bottom, middle, and top lines in the box plots represent the first quartile, median, and third quartile, respectively. Data points represent individual replicates and different letters indicate significance among treatments ($p < 0.001$), n= 18.

Table S2: Volatile compounds detected in the headspace of sugar beet plants. Literature retention indices were extracted from the NIST chemistry webbook (https://webbook.nist.gov/chemistry/), based on best matching GC-MS method (Van Den Dool and Kratz RI, non-polar column, temperature ramp).

Compound Group	Compound name	Retention time	Retention Index Experimental	Retention Index Literature
Monoterpenoids	α-pinene	9.39	937.0	937
	p-menthane	9.69	950.7	968
	β-pinene	10.34	980.3	980
	β-myrcene	10.59	991.8	992
	3-carene	11.05	1013.4	1013
	D-limonene	11.43	1032.0	1033
	γ-terpinene	11.60	1049.6	1047
	trans-β-ocimene	11.8	1050.0	1050
	o-cymene	12.05	1059.1	1027.7
	p-cymene	12.44	1080.7	1033
	p-cymen-7-ol	15.83	1263.2	1287
	γ-terpineol acetate	11.60	1319.6	1341
Benzenoids	benzyl alcohol	11.53	1036.4	1033
	benzene, 1-(1,1-dimethylethyl)-4-methyl-	12.73	1095.6	1101
	benzene, 1,2,4,5-tetramethyl-	13.32	1124.6	1115.8
	benzene, 1,3-diethyl-5-methyl-	13.75	1147.5	1143
Fatty acid derivatives	2-pentanone, 4-hydroxy-4-methyl-	7.38	842.2	841.3
	3-hexen-1-ol	7.65	858.1	858
	5-hepten-2-one, 6-methyl-	10.52	988.7	988
	3-hexen-1-ol, acetate	10.92	1007.7	1005
Sesquiterpenoids	longifolene	17.81	1374.8	1390
	β-cubebene	18.03	1387.8	1388
	unknown -RT 18.54	18.54	1419.5	-----
	cis-β-copaene	18.67	1427.5	1428
	β-caryophyllene	17.81	1376.4	1390
	α-guaiene	18.88	1440.8	1439
	epicubebol	19.69	1494.3	1494
Unidentified	unknown –RT 19.92	19.92	1507.1	-----
	unknown –RT 14.33	14.33	1177.8	-----
	unknown -RT 16.77	16.77	1313.2	-----

Table S3: Estimated emission rate (ng/h/plant) of above-ground VOCs from differently treated sugar beet plants. CA- = Control without aphid, CA+ = control with aphid, MDA- = moderate drought without aphid, MDA+ = moderate drought with aphid, HDA- = high drought without aphid, HDA+ = high drought with aphid.

Compound	**VOC emission per plant ng/h/plant ±SE**					
	CA-	**CA+**	**MDA-**	**MDA+**	**HDA-**	**HDA+**
3-hexen-1-ol, acetate	5.59±1.80	2.28±0.55	2.08±0.67	1.34±0.55	1.51±0.28	1.53±0.54
3-carene	0.16±0.04 ab	0.23±0.04 a	0.16±0.07 ab	0.06±0.02 b	0.07±0.02 b	0.11±0.02 ab
5-hepten-2-one, 6-methyl-	0.13±0.05 b	0.17±0.07 b	0.20±0.08 ab	0.01±0.004 a	0.06±0.03 ab	0.12±0.04 ab
p-menthane	0.032±0.06ab	0.094±0.05 a	0.036±0.01ab	0.026±0.008 b	0.029±0.005 ab	0.042±0.006 ab
α-pinene	0.15±0.05	0.28±0.07	0.27±0.19	0.08±0.04	0.18±0.16	0.14±0.05
p-cymen-7-ol	0.72±0.19	0.72±0.19	0.83±0.13	------	0.37±0.15	0.67±0.15
β-pinene	0.07±0.02	0.13±0.04	0.83±0.24	------	------	0.04±0.01
benzene, 1,2,4,5-tetramethyl-	0.24±0.16	0.39±0.24	0.03±0.07	0.02±0.01	------	0.04±0.02
benzene, 1,3-diethyl-5-methyl-	0.20±0.13	0.36±0.21	0.06±0.01	------	------	0.03±0.01
o-cymene	0.09±0.03	0.16±0.05	0.04±0.02	------	------	0.13±0.05
D-limonene	0.11±0.05	0.16±0.04	0.19±0.15	------	------	0.13±0.03
benzene, 1-(1,1-dimethylethyl)-4-methyl-	0.16±0.09	0.28±0.16	0.05±0.02	------	------	------
β-myrcene	0.14±0.06	0.18±0.06		------	------	0.07±0.03
3-hexen-1-ol	0.02±0.006 b	0.26±0.10 a	0.01±0.005 b	------	------	------
p-cymene	0.11±0.07	0.19±0.09	------	------	------	0.07±0.03
trans-β-ocimene	0.06±0.02	0.11±0.04	------	------	------	------
γ-terpinene	0.06±0.02	0.10±0.03	------	------	------	------
longifolene	0.47±0.32	0.80±0.56	------	------	------	------
β-cubebene	0.38±0.25	0.67±0.49	------	------	------	------
γ-terpineol acetate	0.28±0.2	0.51±0.36	------	------	------	------
2-pentanone, 4-hydroxy-4-methyl-	------	1.08±0.61	------	1.23±0.67	------	------
α-guaiene	------	0.27±0.20	------	------	------	------
epicubebol	------	0.52±0.38	------	------	------	------
benzyl alcohol	------		------	0.03±0.01	------	0.21±0.12
β-caryophyllene	------	0.31±0.22	------	------	------	------
cis-β-copaene	------	0.51±0.38	------	------	------	------
unknown 14.33 RT	0.20±0.11	0.32±0.49	------	------	------	------
unknown 16.77 RT	------	0.16±0.10	------	------	------	------
unknown 18.54 RT	------	0.55±0.40	------	------	------	------
unknown 19.92 RT	------	0.40±0.31	------	------	------	------

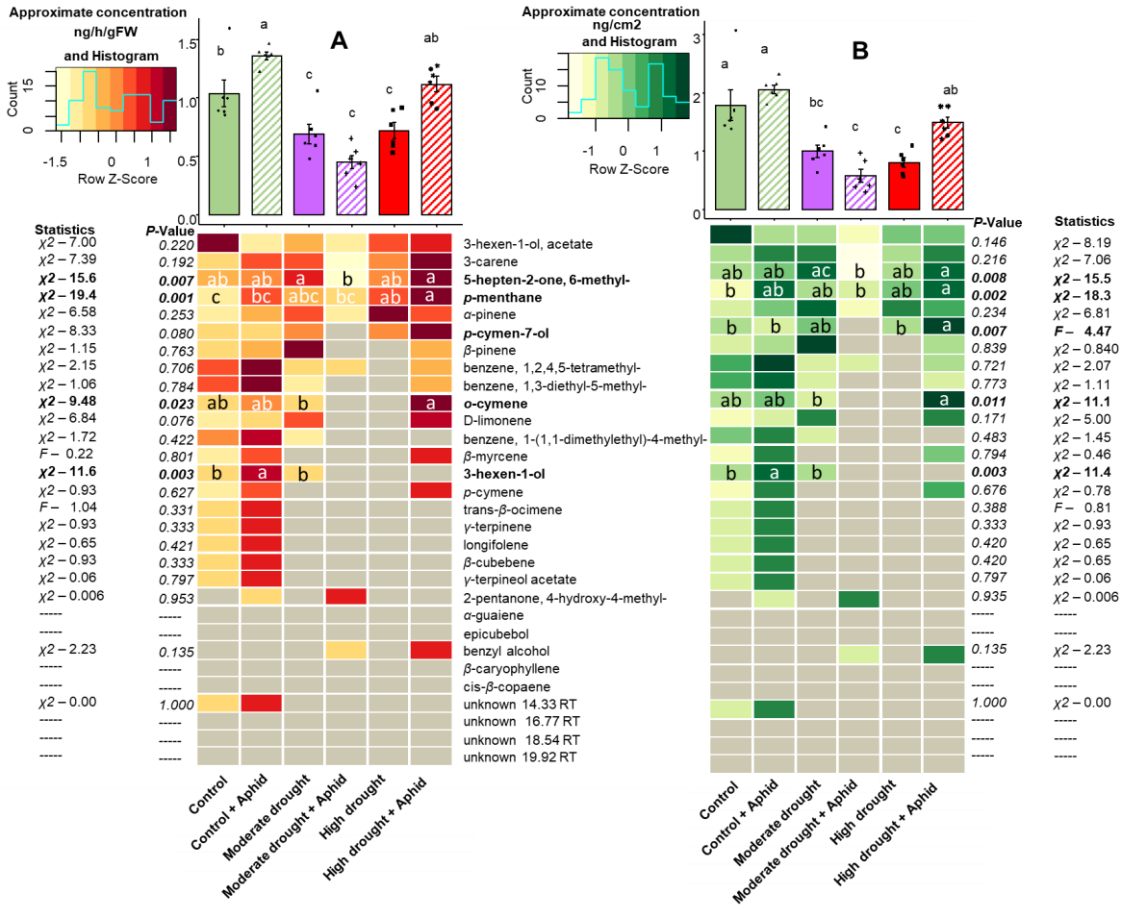


Figure S4: Effects of drought and aphid infestation on volatile emission. Heat map represents emission rates of volatile organic compounds (VOCs) from treated sugar beet plants. Volatile emission was standardized by plant biomass and is expressed as ng/h/gFW (A); volatile emission was standardized by leaf area and is expressed as ng/cm^2 (B), n = 6.

Table S4: Estimated emission rate (ng/h/g FW) of above-ground VOCs from differently treated sugar beet plants. CA- = control without aphid, CA+ = control with aphid, MDA- = moderate drought without aphid, MDA+ = moderate drought with aphid, HDA- = high drought without aphid, HDA+ = high drought with aphid.

Compound	**VOC emission standardized by plant biomass ng/h/g FW ±SE**					
	CA-	**CA+**	**MDA-**	**MDA+**	**HDA-**	**HDA+**
3-hexen-1-ol, acetate	0.62±0.19	0.26±0.06	0.34±0.11	0.22±0.09	0.49±0.08	0.49±0.16
3-carene	0.02±0.005	0.03±0.004	0.03±0.01	0.01±0.003	0.02±0.008	0.03±0.008
5-hepten-2-one, 6-methyl-	0.01±0.006 ab	0.02±0.08ab	0.03±0.01 a	0.002±0.003 b	0.02±0.008 ab	0.04±0.008 a
p-menthane	0.004±0.0007 c	0.01±0.05 bc	0.01±0.0003 abc	0.004±0.001 bc	0.01±0.001 ab	0.02±0.001 a
α-pinene	0.02±0.005	0.03±0.008	0.04±0.03	0.01±0.007	0.06±0.05	0.05±0.01
p-cymen-7-ol	0.08±0.02	0.08±0.02	0.14±0.02	------	0.12±0.04	0.25±0.05
β-pinene	0.01±0.003	0.01±0.005	0.05±0.03	------	------	0.01±0.005
benzene, 1,2,4,5-tetramethyl-	0.03±0.01	0.04±0.02	0.005±0.002	0.003±0.001	------	0.01±0.007
benzene, 1,3-diethyl-5-methyl-	0.02±0.01	0.04±0.02	0.007±0.002	------	------	0.01±0.006
o-cymene	0.01±0.004 ab	0.02±0.06ab	0.01±0.004 b	------	------	0.05±0.01 a
D-limonene	0.01±0.005	0.02±0.004	0.03±0.02	------	------	0.04±0.01
benzene, 1-(1,1-dimethylethyl)-4-methyl-	0.02±0.01	0.03±0.01	0.01±0.004	------	------	------
β-myrcene	0.02±0.006	0.02±0.007	------	------	------	0.02±0.009
3-hexen-1-ol	0.003±0.007 b	0.03±0.01 a	0.002±0.0009 b	------	------	------
p-cymene	0.01±0.008	0.02±0.01	------	------	------	0.02±0.009
trans-β-ocimene	0.01±0.002	0.01±0.005	------	------	------	------
γ-terpinene	0.01±0.003	0.01±0.003	------	------	------	------
longifolene	0.05±0.03	0.09±0.06	------	------	------	------
β-cubebene	0.04±0.02	0.07±0.05	------	------	------	------
γ-terpineol acetate	0.03±0.02	0.06±0.04	------	------	------	------
2-pentanone, 4-hydroxy-4-methyl-	------	0.12±0.06	------	0.20±0.11	------	------
α-guaiene	------	0.03±0.02	------	------	------	------
epicubebol	------	0.06±0.04	------	------	------	------
benzyl alcohol	------		------	0.01±0.002	------	0.07±0.03
β-caryophyllene	------	0.03±0.02	------	------	------	------
cis-β-copaene	------	0.06±0.04	------	------	------	------
unknown 14.33 RT	0.02±0.01	0.04±0.02	------	------	------	------
unknown 16.77 RT	------	0.02±0.01	------	------	------	------
unknown 18.54 RT	------	0.06±0.04	------	------	------	------
unknown 19.92 RT	------	0.04±0.03	------	------	------	------

Table S5: Estimated emission rate (ng/cm^2 leaf area) of above-ground VOCs from differently treated sugar beet plants. CA- = control without aphid, CA+ = control with aphid, MDA- = moderate drought without aphid, MDA+ = moderate drought with aphid, HDA- = high drought without aphid, HDA+ = high drought with aphid.

Compound	**VOC emission standardized by leaf area ng/cm^2 ± SE**					
	CA-	**CA+**	**MDA-**	**MDA+**	**HDA-**	**HDA+**
3-hexen-1-ol, acetate	1.13±0.38	0.42±0.11	0.48±0.15	0.22±0.09	0.56±0.11	0.60±0.16
3-carene	0.03±0.007	0.04±0.008	0.04±0.01	0.01±0.004	0.03±0.008	0.04±0.01
5-hepten-2-one, 6-methyl-	0.02±0.01 ab	0.03±0.01ab	0.05±0.02 a	0.00±0.001 b	0.02±0.01 ab	0.05±0.01 a
p-menthane	0.01±0.001 b	0.02±0.08ab	0.01±0.0004 ab	0.01±0.001 b	0.01±0.002 ab	0.02±0.0008 a
α-pinene	0.03±0.009	0.05±0.01	0.06±0.04	0.02±0.009	0.06±0.05	0.05±0.02
p-cymen-7-ol	0.13±0.03 b	0.12±0.03 b	0.20±0.03 ab	------	0.13±0.05 b	0.40±0.09 a
β-pinene	0.01±0.005	0.02±0.007	0.07±0.06	------	------	0.01±0.006
benzene, 1,2,4,5-tetramethyl-	0.04±0.02	0.07±0.04	0.01±0.004	0.004±0.002	------	0.02±0.008
benzene, 1,3-diethyl-5-methyl-	0.04±0.02	0.06±0.03	0.01±0.003	------	------	0.01±0.008
o-cymene	0.01±0.005 ab	0.03±0.09ab	0.01±0.003 b	------	------	0.09±0.03 a
D-limonene	0.02±0.009	0.03±0.008	0.05±0.03	------	------	0.05±0.01
benzene, 1-(1,1-dimethylethyl)-4-methyl-	0.03±0.01	0.05±0.02	0.01±0.007	------	------	------
β-myrcene	0.02±0.01	0.03±0.01	------	------	------	0.02±0.01
3-hexen-1-ol	0.004±±0.00 b	0.04±0.0 a	0.003±0.001b	------	------	------
p-cymene	0.02±0.01	0.03±0.01	------	------	------	0.02±0.01
trans-β-ocimene	0.01±0.003	0.02±0.007	------	------	------	------
γ-terpinene	0.01±0.005	0.02±0.004	------	------	------	------
longifolene	0.08±0.05	0.13±0.09	------	------	------	------
β-cubebene	0.07±0.04	0.11±0.07	------	------	------	------
γ-terpineol acetate	0.05±0.03	0.08±0.06	------	------	------	------
2-pentanone, 4-hydroxy-4-methyl-	------	0.19±0.11	------	0.30±0.16	------	------
α-guaiene	------	0.05±0.03	------	------	------	------
epicubebol	------	0.09±0.07	------	------	------	------
benzyl alcohol	------	------	------	0.01±0.003	------	0.09±0.04
β-caryophyllene	------	0.05±0.3	------	------	------	------
cis-β-copaene	------	0.08±0.06	------	------	------	------
unknown 14.33 RT	0.03±0.02	0.05±0.03	------	------	------	------
unknown 16.77 RT	------	0.02±0.01	------	------	------	------
unknown 18.54 RT	------	0.09±0.06	------	------	------	------
unknown 19.92 RT	------	0.06±0.04	------	------	------	------

Table S6: Summary of fresh leaves weight (g) and leaf area (cm^2). Different letters indicate significant differences among treatments. CA- = control without aphid, CA+ = control with aphid, MDA- = moderate drought without aphid, MDA+ = moderate drought with aphid, HDA- = high drought without aphid, HDA+ = high drought with aphid.

Treatment	Leaf Fresh Weight (g) ±SE		Leaf Area (cm^2) ±SE	
CA-	9.016±0.074	a	141.20±4.413	a
CA+	8.933±0.128	a	146.42±4.012	a
MDA-	6.133±0.111	b	101.49±2.35	b
MDA+	6.116±0.101	b	103.03±2.70	b
HDA-	3.066±0.076	c	66.31±1.78	c
HDA+	3.083±0.079	c	64.69±3.27	c

References

Brooks, M. E., Kristensen, K., Benthem, K. J. van, Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Mächler, M., & Bolker, B. M. (2017). GlmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. The R Journal, 9(2), 378–400.

Florian Hartig. (2021). DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. R package version 0.4.3. Https://CRAN.R-project.org/package=DHARMa.

Huang, A. (2017). Mean-parametrized Conway-Maxwell-Poisson regression models for dispersed counts. ArXiv:1606.03214 [Stat]. http://arxiv.org/abs/1606.03214

Lüdecke, D., Ben-Shachar, M., Patil, I., Waggoner, P., & Makowski, D. (2021). performance: An R Package for Assessment, Comparison and Testing of Statistical Models. Journal of Open Source Software, 6(60), 3139. https://doi.org/10.21105/joss.03139

Salinity stress and aphid infestation modulate sugar beet phytochemistry: impacts on aphid-parasitoid trophic interactions

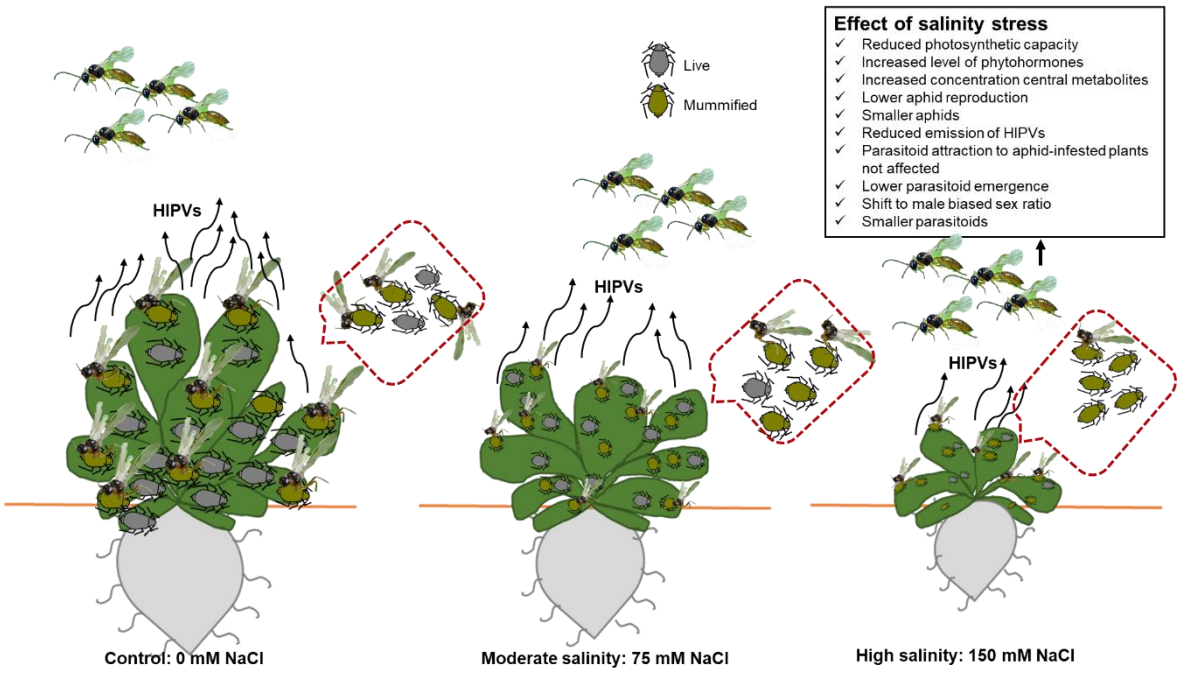
Shahinoor Rahman*[1], Musrat Zahan Surovy[2], Mohammad Alhussein[1], Michael Rostás[1], and Ilka Vosteen*[1],

[1]Department of Crop Sciences, Division of Agricultural Entomology, Georg-August-University of Goettingen, Germany

[2]Department of Crop Sciences, Division of Plant Pathology and Crop Protection, Georg-August-University of Goettingen, Germany

*Correspondence: shahinoor_ent@yahoo.com; ilka.vosteen@uni-goettingen.de

Graphical abstract



Abstract

Climate change can greatly impact sugar beet production by causing soil salinization, which can change plant chemical composition. But, little is known about how this affects the interactions between plants, herbivorous insects, and their natural enemies. Our study comprehensively investigated how herbivory by aphids (*Aphis fabae*) and two magnitudes of salinity alter the morphology, physiology, and phytochemistry (central metabolites, phytohormones) of sugar beet (*Beta vulgaris*). In addition, how salinity and aphid herbivory affects the emission of volatile organic compounds (VOCs), and preferences and performances of parasitoids (*Aphidius colemani*). Rising salinity caused a decrease in plant growth, biomass, and leaf size in sugar beets, but no changes were observed due to aphid infestation. As salinity levels increase, sugar beet plants exhibited a decline in their ability to photosynthesize, as indicated by lower chlorophyll fluorescence that was further exacerbated by aphid infestation. Both salinity and aphid herbivory significantly changed levels of several plant hormones, such as abscisic acid (ABA), 12-oxophytodienoic acid (OPDA), salicylic acid (SA), and jasmonates (JAs), which in turn affected the plant's metabolism. Central metabolome analysis showed that increasing salinity stress increased concentrations of amino acids, organic acids, fatty acids, and sugar metabolites. Salinity stress further resulted in reduced reproduction and smaller size of aphids and decreased parasitoids fitness as evidenced by lower emergence rate, altered sex ratio, and reduced body size. The alteration of central metabolites due to salinity stress resulted in decreased overall quantity and diversity of volatile organic compounds (VOCs), leading to a diminished attraction of parasitoids toward salinity-stressed plants. However, salinity-stressed plants with aphid herbivory emitted VOC that aided in preserving parasitoid attraction. Our findings indicate that salinity stress reduces the aphid population and impairs the performance and preference of parasitoids by altering phytohormonal signals and central metabolites, consequently affecting the emission of VOC. These alterations disrupt multitrophic interactions in our system.

Keywords: Aphid, Central metabolites, Multitrophic interactions, Parasitoid, Photosynthesis, Phytohormone, Salinity stress, Sugar beet, Volatiles

Introduction

Soil salinity is a major problem in many parts of the world, particularly in arid and semi-arid regions with high evaporation rates and limited freshwater resources (Farahani et al., 2020). Soil salinity is a problem in over 160 countries, affecting an estimated 20% of the global land area, particularly prevalent in Asia, where it is estimated to affect around 50% of the irrigated land (Pitman & Läuchli, 2002; Shahid et al., 2018). In terms of the impact of soil salinity on agricultural productivity, it is estimated that soil salinity reduces crop yields by an average of 10-20% worldwide. Soil salinization is a globally intimidating issue of crop productivity, and by 2050, it is anticipated that salinity will worsen on 50% of the world's arable land (Butcher et al., 2016).

Soil salinity interferes with plant water equilibrium, reduces turgor pressure, and interferes with mineral absorption, causing ion imbalance; oxidative stress through increased production of reactive oxygen species (ROS). It leads to Na+ and Cl- ions accumulation while inhibiting K+ and Ca2+ uptake and causing ion toxicity (Arif et al., 2020; Zörb et al., 2019). Plants use various mechanisms to adapt to salinity conditions. These mechanisms include sophisticated molecular, physiological, and biochemical changes (Neil Willy, 2016; Shahid et al., 2020), like regulating toxic ion accumulation and nutrient status (Kamran et al., 2019), modulating phytohormones (Cao et al., 2017; Fahad et al., 2015), regulating K^+/Na^+ relationship for stomata functioning and photosynthetic activity (Assaha et al., 2017), and modulating plant primary (Dias et al., 2015) and secondary metabolism (Jan et al., 2021). Still, salinity negatively impacts plant's morphological features by inducing oxidative and toxic stress, resulting in stunted growth, reduced cell elongation, decreased stomatal aperture, decreased leaf chlorophyll fluorescence (Fv/Fm), metabolic adaptations, etc. (Chele et al., 2021; Munns & Tester, 2008; Shin et al., 2021; van Zelm et al., 2020).

Salinity stress was shown to alter the internal chemistry of sugar beets by altering central metabolic processes (Cui et al., 2022; Liu et al., 2020). Salinity also changes the levels and ratios of various central metabolites, such as sugars, amino acids, and secondary metabolites (Akula & Ravishankar, 2011; Teklić et al., 2021). These changes can have a range of impacts on the growth and

development of the plant, as well as plant responses to insect herbivory (Kerchev et al., 2012; Stallmann et al., 2022; Zhou et al., 2015). Through bottom-up effects, plant traits like nutritional value, chemical and mechanical defenses, distribution, and abundance may affect aphids' fitness and performances either positively (Eichele-Nelson et al., 2018) or negatively (Ghodoum Parizipour et al., 2021; Laney et al., 2018), might depending on plant, insect species, and feeding guild (Dong et al., 2020; Quais et al., 2020; Schile & Mopper, 2006).

Phytohormones play a vital role in enabling plants to adapt to various biotic and abiotic stresses by influencing growth, differentiation, nutrient allocation, and the balance between source and sink tissues (Fahad et al., 2015). Hormone signal transduction cascades, known as cross-talk, play a vital role in plant development and responses to stresses. Hormone synthesis, transduction, perception, and cross-talk form a complex network influencing hormone action, developmental processes, and gene expression (Fujita et al., 2006; Khan et al., 2020). Phytohormones such as salicylic acid (SA), jasmonic acid (JA), ethylene (ET), and abscisic acid (ABA) primarily regulate the protective responses of plants against both biotic and abiotic stresses via synergistic and antagonistic actions (Bostock, 2005; Fujita et al., 2006). Phytohormonal cross-talk facilitates the coordination of numerous genes and their regulators involved in stress mitigation and remediation (Pieterse et al., 2012).

High soil salinity levels can significantly affect multitrophic interactions in agricultural crops. These interactions refer to the complex relationships between different species at multiple levels of the food web, including plants, herbivores, and natural enemies. In agricultural systems, soil salinity can affect the growth and development of crops, which can, in turn, impact the availability and nutritional quality of the plants as a food source for herbivores (Harmon & Daigh, 2017). The nutritional quality of host plants also changes herbivore physiology which alters parasitoid performances (Kaplan et al., 2016; Sarfraz et al., 2009). Moreover, plant chemical cues play a critical role in attracting herbivore enemies (Clavijo McCormick et al., 2012). High levels of soil salinity affect plant growth and development, potentially leading to changes in the production and release of plant volatile organic compounds (VOCs) (Forieri et al., 2016; Landi et al., 2020). Parasitoids use herbivore-induced plant volatiles (HIPVs) to locate their hosts;

because HIPVs provide information to parasitoids that help suppress the herbivore attack, HIPVs can be considered as a plant's information-mediated indirect defense (Kessler and Heil, 2011). However, there is limited research on how soil salinity affects HIPV emissions, preferences, and performance of parasitoids, which occupy the third trophic level in the food web (Forieri et al., 2016).

Sugar beet (*Beta vulgaris* spp. *vulgaris*) is an important crop and a significant source of sugar in temperate regions, providing a significant global sugar supply, accounting for approximately one-third of the world's annual sugar production (Dohm et al., 2014). With the discontinuation of neonicotinoid seed treatment in sugar beet across several European countries in 2018, there has been a noticeable increase in pest pressure, particularly aphids (Viric Gasparic et al., 2020). As a result, the conservation and utilization of naturally occurring parasitoids are expected to assume a more prominent role as biocontrol agents in sugar beet fields, contributing to the reduction of aphid populations in the future. How the tritrophic system, consisting of sugar beet, herbivore *Aphis fabae,* and its parasitoid *Aphidius colemani,* is affected by salinity in relation to sugar beet phytochemistry is entirely unknown. In this comprehensive study, we specifically addressed the effects of different levels of salinity stress and aphid infestation on (i) sugar beet plant morphology, photosynthesis efficiencies, phytohormones, central metabolites, and emission of VOC (ii) parasitoid performance on aphids reared on salinity-stressed plants and (iii) parasitoid attraction towards aphid-infested and uninfested plants.

Materials and Methods

Insects, plants, hydroponic system, and experimental setup

Colonies of *Aphis fabae* (Order: Hemiptera, Family: Aphididae) was reared on *Beta vulgaris* subspec. *vulgaris* cultivar 'Vasco' (SESVanderHave, Belgium) in a controlled insect raring room (16L:8D photoperiod, light intensity 130 ± 10 µmol/(s m^2), relative humidity: 65 ± 5 %; temperature: 20 ± 2 °C). *Aphidius colemani* was reared on *A. fabae,* and sugar syrup (10% sucrose solution) was supplied as food source for adult parasitoids.

From seedlings to whole experiments were conducted in a separate experimental room followed by above mentioned climatic conditions. Seeds of the sugar beet genotype (mentioned above) were shown in plastic trays (54 holes, each 3.5 cm diameter) filled with quartz sand (0.2-0.8 mm) and let grow by supplying tap water for nine days. From 10 DAS to 19 DAS (days after sowing) plants were supplied with half-strength modified Hoagland solution (HS) (Hoagland and Arnon, 1938) to supply appropriate nutrients. For preparing HS, Na2MoO4.2H2O was used instead of H2MoO4.H2O, and C10H12FeN2NaO8.3H2O replaced C12H12Fe2O18 with concentrations of 0.12 mg/l and 22.5 mg/l, respectively. At 20 DAS, seedlings were transferred to the hydroponic system with full-strength HS.

For all the experiments, a hydroponic system was constructed (Figure S1), where black plastic boxes (46 × 30 × 16 cm) served as a reservoir of 10 L. HS was aerated continuously (24/7) by setup the two super silent power air pumps (AQUA FORTE V-20, Germany) connected with bubbling release air stones. Six holes were made at the lid of the boxes, each 3 cm in diameter, for holding the plants. For all experiments, 23 DAS sugar beet plants were treated with three different magnitudes of salt stress as i) Control: (Hoagland solution with 0 mM NaCl, EC= ~ 2.2 dS/m), ii) Moderate salinity (Hoagland solution with 75 mM NaCl, EC= ~ 10.2 dS/m), and iii) High salinity (Hoagland solution with 150 mM NaCl, EC= ~ 17.4 dS/m) and from 26 DAS aphid stress were imposed. Electrical conductivity (EC) was checked daily, and additional HS was provided to keep the reservoir full when required. PH was also checked daily and maintained at 5.9-6.2 by adding 1N KOH & 1N HCL when required, and HS was changed every seven days.

Experiment 1: Aphid performances

To explore the influence of salinity stress on the life cycle performance of *A. fabae,* three wingless adults were placed on the second leaf of each sugar beet plant at 26 DAS (day 4 of salinity treatment). Aphid performances like pre-reproductive period, reproductive period, longevity, number of offspring of the mother aphid until her own first offspring reproduces (*Nd*), reproductive rate (RR), and intrinsic rate of increase (*Rm*) were measured according to the protocol described in Chapter 1.

Experiment 2: Monitoring of plant morphology, aphid population dynamics, aphid body size, and leaf chlorophyll fluorescence

Plant height was measured in three-time points starting at two true leaf stages (22 DAS before salt stress), 37 DAS, and 52 DAS. At 54 DAS plants were harvested, and total biomass, root weight, shoot weight, root length, shoot length, root shoot ratio by weight and length, number of total leaves, and the average leaf area (LI-3100C Area Meter, LI-COR Biosciences, Germany) were recorded.

In order to study the effects of different levels of salinity stress on aphid population dynamics, one adult aphid per plant (9 days aged, ready to go reproductive phase, based on experiment 1) was released at 26 DAS and let them reproduce. Every seven days number of aphids were counted (33 DAS, 40 DAS, 47 DAS, and 54 DAS). During harvest at 54 DAS, the five largest aphids on each of the 12 plants (12*5 =60) were visually observed. They were then placed on adhesive tape, photographed, and analyzed using Image-J software to measure their body length and coverage area.

The leaf chlorophyll fluorescence parameters (photosynthetic efficiencies) were measured at 22 DAS (before salt stress), 37 DAS, 42 DAS, and 47 DAS. The plants were kept in the dark for 30 minutes before measurement. The photosynthesis yield analyzer (MINI-PAM-II, Heinz Walz GmbH, Germany) was used to determine the maximum photochemical quantum yield of Photosystem II (Fv/Fm), the effective photochemical quantum yield of PS II (Φ_{PSII}), and the electron transport rate (ETR). Since *A. fabae* mostly feeds on the lower side of sugar beet leaves, care was taken to only measure from the upper side of the leaf to avoid interference with honeydew.

Experiment 3: Parasitoid performances

Five wingless adult aphids were placed on the second leaf of a sugar beet plant (26 DAS), and parasitoid performance was evaluated according to the protocol described in Chapter 1.

Experiment 4: Parasitoid host finding behavior and plant volatile analysis

A six-arm olfactometer (Turlings et al., 2004) was used to trap plant volatiles and to simultaneously measure the attraction of female *A. colemani* to six odor sources (n= 6) (for detail, see chapter 1). However, in brief, treated plants

were exactly 36 DAS aged, having 10 days of Aphid treatment (40 aphids per plant) and 13 days of salinity stress treatment and a total output of six different treatments, namely control, control + aphid, moderate salinity, moderate salinity + aphis, high salinity, high salinity + aphid. Collections of volatiles were carried out for 24 h (from 09:00 to 09:00 next day), and within this time, parasitoid behavioral assays were conducted (from 10:00 to 16:00). Every day, a total of 30 naïve females of *A. colemani* (2-5 days old) were tested in five experimental rounds. In each experimental round, six parasitoids were released as they did not interfere with each other's choices. After 60 minutes, the choices made by the parasitoids were recorded, and a new one replaced the group.

After headspace collection, the trapped volatiles were eluted with 150 µl dichloromethane (DCM) and analyzed by gas chromatography – mass spectrometry (5977B HES MSD, Agilent Technologies; see chapter 1 for details). The retention indices of the compounds were determined in the experiment and compared with the values reported in the literature. The summarized results can be found in supporting information (Table S1).

Experiment 5: Analysis of phytohormones

Plants were grown as the methodology described earlier. However, in this case, salt stress treatments were implemented at 33 days of the plants. At day 36, 30 *A. fabae* (mixture of all ages) were placed on the second leaf of the plant and covered with a perforated polypropylene bag (15 × 25 cm, Nette GmbH, Germany), and leaf samples were collected at three different time points (from day 37, 38, and 39). So, collected leaves (n = 6) were subjected to 4, 5, and 6 days of salt stress and 24, 48, and 72h of aphid infestation. Before collecting leaf samples, all aphids were removed quickly from the leaves with the help of a paint-brush, cut at the leaf's base, placed into the falcon tube (50 ml), and immediately dipped in liquid nitrogen. Then the samples were lyophilized, powdered, and stored at -80°C.

About 40 mg powdered samples were weighed (KC BA 100, Sartorius Micro, Germany) into 1.5 ml Eppendorf tubes, then added 1ml of methanol (99.95 % v/v LC-MS Grade) + 0.2% formic acid (v/v), vortexed, and ultra-sonicated (VWR-600THD, Malaysia) for 1 min (VWR-600THD, Malaysia) finally, Eppendorf tubes were kept in the ice bag and placed on a shaker in dark condition for 1h.

After that, samples were centrifuged at 15800 g for 10 min at 4°C (Eppendorf-5425, Germany), and supernatant (900 µl) was transferred into another 1.5 ml Eppendorf tubes and again centrifuged for 5 min. After the second centrifugation, the supernatant (800µl) was transferred into 5 ml Eppendorf tubes, and 2.4 ml Millipore water was added to make a working solution. C_{18} columns were prepared by washing with 2 ml methanol (99.95 % v/v LC-MS Grade) and 2 ml Millipore water with the help of a solid phase extraction vacuum machine (SPE Vacuum Manifold CHROMABOND®, Germany). The working solution was filtrated by C_{18} column and collected on falcon tubes, and then C_{18} column was rewashed with 1 ml Millipore water. Ethyl acetate (2.4 ml) with 0.1% formic acid (v/v) was added to the falcon tubes, vortexed, and centrifuged for 5 min. The upper part of the solution (~ 2 ml) was eluted and transferred 1 ml at once to 1.5 ml Eppendorf tubes for drying in a speed vac (RVC 2-25 CD plus, Germany) at 30°C. Finally, phytohormone extraction was collected in the above Eppendorf tubes from C_{18} column with 400 µl of methanol (99.95 % v/v LC-MS Grade), vortexed for 1 min, and kept at -20°C. Before analysis with Ultra high-performance liquid chromatography (UHPLC), samples were vortexed, ultrasonicated for 2 min, and centrifuged for 5 min. Then, 300 µl supernatant was transferred into HPLC amber glass vials and analyzed instantly.

UHPLC-MS-MS was used for the quantitative analysis of phytohormones according to Posada-Vergara et al., (2022), and the following chemicals were used as authenticating standards: abscisic acid (AA), 12-oxo- Phytodienoic acid (OPDA), salicylic acid (SA), jasmonic acid (JA) and jasmonic acid isoleucine (JAI).

Experiment 6: Analysis of sugar beet plant central metabolites

The EDTA-facilitated phloem extraction procedure was followed to analyze the central metabolites. Plants were grown in the same fashion as described in experiment 5. In brief, on day 36, 30 *A. fabae* (a mixture of all ages) were placed on the second leaf of the plant and covered with a perforated polypropylene bag (15 × 25 cm, Nette GmbH, Germany) to prevent escape. To minimize the error, leaves from control plants (without aphids) were also covered with a perforated polypropylene bag. Phloem exudates were collected (n= 6) at

one point, i.e. 3 days after the aphid infestation and 6 days after the salinity stress (39 DAS).

Phloem was collected according to the protocol described by Tetyuk et al., (2013) with necessary modifications. In brief, for the collection of phloem sap, second leaf was cut at the base of the petiole and immediately submerged the petiole in 1.5 ml Eppendorf tubes containing 1 ml of 15 mM K_2-EDTA solution and placed in a dark chamber having a relative humidity of ~98%. After 1 h, the leaves were gently removed and washed thoroughly with Millipore water to remove all EDTA, and placed another Eppendorf tube containing 1 ml of double distilled autoclaved water and back again the dark chamber for the collection of phloem exudates. After collection of phloem exudates for 5 h, Eppendorf tubes were dipped into the liquid nitrogen and placed at -80°C. Blank samples were also prepared without phloem exudates. To prepare samples for GC-MS, samples were lyophilized, suspended with 120µl methanol (99.95% v/v GC-MS Grade), vortexed, shaken for 1 h, and transferred to a glass insert with a GC-MS glass vial. To each sample, 20 µl adonitol (prepared as 20 ng/µl of 99.95 % LC-MS Grade methanol) was added as an internal standard and then dried with a speed vac at 30°C for 180 min (RVC 2-25 CD plus, Germany). Argon gas was added to each sample to prevent oxidation, and samples were stored overnight at -80°C. The next day, 80 µl methoxyamination reagent (prepared by 20 mg/ml of methoxyamine hydrochloride in pure pyridine) was added to each sample, including blanks. The mixtures were vortexed, short centrifuged, and placed in a shaker for 90 min. After that, 20 µl samples were transferred to a glass insert with a GC-MS glass vial, and 20 µl N-Methyl-N-(trimethylsilyl) trifluoracetamide (MSTFA) was also added to each sample and vortexed for 10 sec and the samples were ready for GC-MS.

Samples were run with Agilent Technologies (GC: 7890B, MSD: 5977B, USA) fitted with Restek Rtx-5 w/Integra-Guard column (30 m × 0.25 mmID × 0.25 µm df, USA). PAL autosampler (PAL RSI 85, Switzerland) was used to inject 1 µl of samples. Each sample was run two times with two split ratios, one was 0.1:1, and another was 10:1. Split ratio 10:1 was only used to detect highly concentrated metabolites (eg. sucrose, galactose etc). We utilized 0.1:1 ratio to detect less concentrated metabolites and blacked out the specific peak location (retention

time) from 10:1 ratio to avoid unnecessary damage to GC-MS. Other GC-MS setups and the analysis of peaks were done according to the protocol described in chapter 3.

Statistical analysis

All data were analyzed using the statistics package R-version 4.2.1 integrated with R-Studio Desktop-version 2022.07.02+576 and the MetaboAnalyst 5.0 software. Various models were considered for analyzing different types of data, and the most appropriate model was selected based on its assumptions. Detailed information about the statistical analysis can be found in the supporting information.

Results

Effect of salt stress on the life cycle of an individual aphid Aphis fabae

There were significant differences in the pre-reproductive period (Kruskal-Wallis: $\chi^2_{(df\ 2)} = 37.34$, $p < 0.001$), reproductive period (GLMM: $\chi^2_{(df\ 2)} = 26.14$, $p < 0.001$), and life span of aphid (Kruskal-Wallis: $\chi^2_{(df\ 2)} = 50.17$, $p < 0.001$). Aphids developing on high salinity-stressed plants matured slower by shortening their pre-reproductive period (control vs moderate: $p < 0.001$; control vs high: $p < 0.001$; moderate vs high: $p = 0.04$) and shortening their reproductive phase (control vs moderate: $p < 0.001$; control vs high: $p < 0.001$; moderate vs high: $p < 0.001$) that reduced their total life span (control vs moderate: $p < 0.001$; control vs high: $p < 0.001$; moderate vs high: $p < 0.001$) (Figure S2 A). Aphids on salinity-stressed plants reproduce significantly less number of nymphs (upto its own first progeny, *Nd*) than in control plants (GLMM: $\chi^2_{(df\ 2)} = 28.45$, $p < 0.001$) (Figure S2 B). A similar significant pattern was also observed on the reproduction rate of aphids (GLMM: $\chi^2_{(df\ 2)} = 13.79$, $p < 0.001$) (Figure S2 C), however, their intrinsic rate of increase (*Rm*) was non-significant (GLMM: $\chi^2_{(df\ 2)} = 5.88$, $p = 0.053$) (Figure S2 D).

Effect of salinity-aphid interactions on plant morphology, leaf chlorophyll fluorescence, and aphid population dynamics & aphid size

Salinity stress has a significant negative impact on plant height over time (LMM: Salinity: $F_{2,15} = 371.04$, $p < 0.001$; Time: $F_{1,67} = 5135.13$, $p < 0.001$; Salinity × Time: $F_{2,67} = 122.34$, $p < 0.001$). However, the population of aphids did not

significantly impact the height of sugar beet plants (Figure 1A). Furthermore, only salinity affects the total biomass (LMM: $F_{2,15}$ = 232.36, p < 0.001) and average individual leaf area (LMM: $F_{2,15}$ =176.39, p < 0.001) (Figure 1B, C).

Salinity stress and aphid infestation significantly affected the photosynthetic efficiencies in sugar beet leaves over time. The presence of salinity stress resulted in a notable decrease in the maximum quantum yield of PS II (Fv/Fm) (LM: $F_{2,264}$ = 231.64, p < 0.001). This reduction was particularly pronounced in plants exposed to high salinity levels over an extended period (LM: $F_{3,264}$ = 608.42, p < 0.001). Additionally, aphid infestation further exacerbated the decrease in photosynthetic efficiencies, with a more pronounced effect observed in high salinity-stressed plants ($F_{1,264}$ = 55.38, p < 0.001) (Figure 1D). A similar trend was noticed in the effective photochemical quantum yield (Φ_{PSII}) of PS II and electron transport rate (ETR) (Figure S3 A, B). The specific statistical interactions can be found in Table S2.

High salinity significantly reduces the overall aphid population over time (GLMM: $\chi^2_{(df\ 2)}$ = 44.47 , p < 0.001; control vs moderate: p = 0.167; control vs high: p < 0.001; moderate vs high: p < 0.001) (Figure 1E). Moreover, salinity also reduces the aphid body length (LM: $F_{2,33}$ = 70.44 , p < 0.001; control vs moderate: p = 0.198; control vs high: p < 0.001; moderate vs high: p < 0.001) (Figure 1F), and aphid body coverage area (LM: $F_{2,33}$ = 171.62, p < 0.001; control vs moderate: p = 0.47; control vs high: p < 0.001; moderate vs high: p < 0.001) (Figure S3 C).

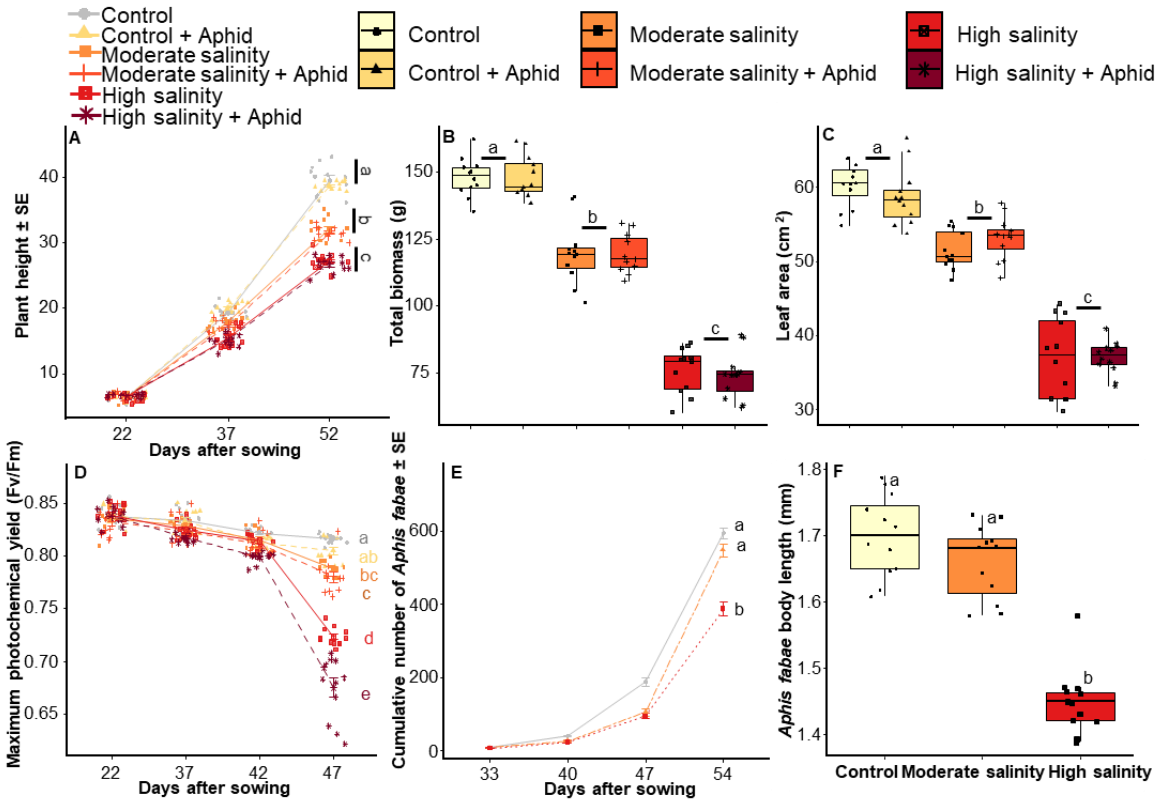


Figure 1: Effect of salinity stress (0 mM NaCl, 75 mM NaCl, 150 mM NaCl) and aphid infestation on sugar beet plant height (A) (Test: LMM); total biomass (B) (Test: LMM); leaf area (C) (Test: LMM), photosynthetic efficiency represented as the maximum quantum yield of PSII (Fv/Fm) (D) (Test: LM). Effect of salinity stress on aphid population (E) (Test: GLMM) and aphid body length (F) (Test: LM, n= 60). Data points represent individual replicates, and different letters ($p \leq 0.05$) indicate significance among treatments, n= 12 plants.

Effect of salt stress on the performance of parasitoid Aphidius colemani

More mummies were formed on high salinity-stressed plants (73.33%) than on moderate salinity (61.11%) and control plants (55.55%) (Binomial GLM: $\chi^2_{(df\ 2)} = 6.54$, $p = 0.03$). Significantly fewer aphids survived on high salinity-stressed plants (Binomial GLM: $\chi^2_{(df\ 2)} = 8.83$, $p = 0.01$), even though the percentage of aphids that died a few days after parasitization was the same in all treatments (Binomial GLM: $\chi^2_{(df\ 2)} = 0.18$, $p = 0.91$) (Figure 2A).

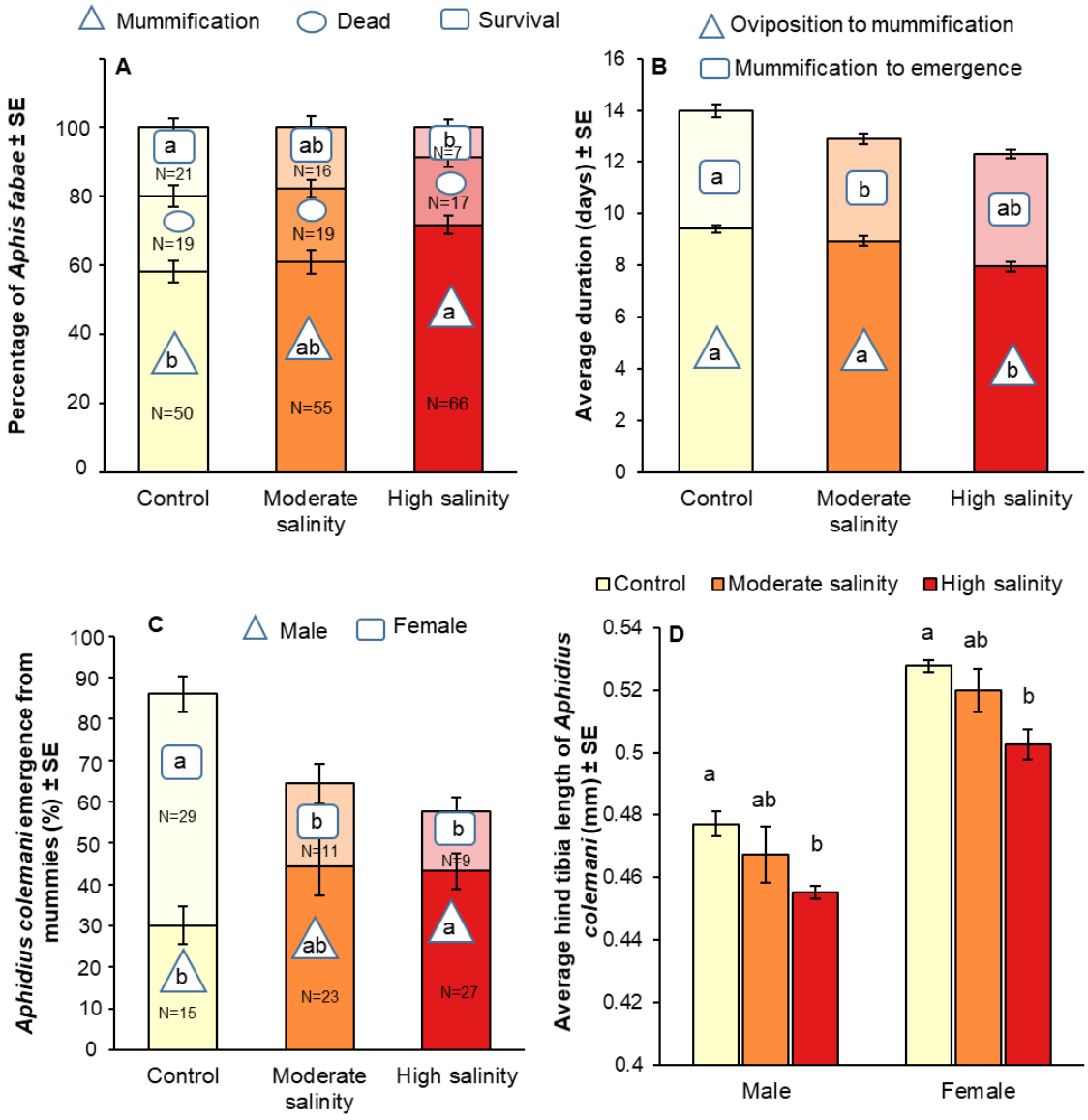


Figure 2: Effect of salinity stress (0 mM NaCl, 75 mM NaCl, 150 mM NaCl) on the performance of parasitoid *Aphidius colemani*. Stacked bar graph represents the percentage of aphids that formed mummies, died or survived (A) (Test: Binomial GLM); time from oviposition to mummification and mummification to emergence (B) (Test: GLMM); and percentage of male and female parasitoid (C) (Test: Kruskal-Wallis). Bars show the hind tibia lengths of the emerged male and female *A. colemani* (D) (Test: LM). Asterisks and different letters indicate significant differences among treatments ($p \leq 0.05$). * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, n =18, N = exact number of sample evaluated

Parasitoids complete their developmental phase very quickly in high salinity stressed plants than control plants (GLMM: $\chi^2_{(df\ 2)} = 47.93$, $p < 0.001$; control vs moderate: $p < 0.001$; control vs high $p < 0.001$; moderate vs high: $p < 0.001$) (Figure 2B). Moreover, within the developmental phase, salinity fastest the oviposition to mummification time (GLMM: $\chi^2_{(df\ 2)} = 75.76$, $p < 0.001$) and mummification to emergence time (GLMM: $\chi^2_{(df\ 2)} = 9.17$, $p = 0.01$) (Figure 2B).

However, interestingly, significantly more parasitoids emerged from control plants (~86%) compared to moderate (~64%) and highly stressed (~57%) plants (Binomial GLM: $\chi^2_{(df\ 2)} = 16.90$, $p < 0.001$), and more interestingly sex ratio was also altered by salinity stress (Kruskal-Wallis: male: $\chi^2_{(df\ 2)} = 8.59$, $p = 0.013$), (Kruskal-Wallis: female: $\chi^2_{(df\ 2)} = 21.48$, $p < 0.001$) (Figure 2C).

Hind tibia length of male (LM: $F_{2,43} = 3.91$, $p = 0.027$) and female (LM: $F_{2,35} = 6.44$, $p = 0.004$) *A. colemani* showed significant differences depending on the intensity of salinity stress. Parasitoids that emerged from aphids on salinity-stressed plants had shorter hind tibia lengths compared to aphids on control plants. This was the case for both male (control vs moderate: $p = 0.49$; control vs high: $p = 0.02$; moderate vs high: $p = 0.27$) and female parasitoids (control vs moderate: $p = 0.46$; control vs high: $p = 0.002$; moderate vs high: $p = 0.07$) (Figure 2D).

Six-arm olfactometer bioassay and analysis of VOCs

Hierarchical clustering analysis of the volatile blends emitted by the differently treated plants revealed two main groups - uninfested controls and plants at moderate salinity with and without aphids in one group and aphid-infested controls and plants at high salinity stress with and without aphids in the second group (Figure 3 A). Interestingly, blends emitted by plants that experience the same level of salinity stress emit the most similar blends irrespective of aphid presence. Among 21 different compounds detected in the blends, emission rates of 3-hexen-1ol, 1-octen-3-ol, 3-octanone, trans -*β*-ocemine, and *β*-caryophyllen differed significantly between the treatments (Figure 3A, Table S3) and showed highly distinct emission patterns. 3-hexen-1ol was absent from the blend emitted by aphid-infested plants at high salinity stress, the lowest emission was detected from uninfested plants at high salinity stress, and the highest amounts were emitted by aphid-infested control plants. Emission rate of 3-octanone was not influenced by aphid presence and declined with increasing severity of salinity stress. After standardization of emission rates per gram fresh weight, most significant differences between the different treatments disappeared, and only the emission rates of trans -*β*-ocemine, and *p*-cymen-7-ol were significantly different (Figure S4 A, Table S4). In the case of VOCs emission per cm^2 of the leaf, only the emission of *p*-cymen-7-ol was significantly different between the treatments

(Figure S4 B, Table S5). Total volatile emission per plant was significantly higher in unstressed control plants compared to plants grown in salinity conditions (LM: $F_{2, 31} = 6.98$, $p = 0.003$). The highest emission rates were measured from aphid-infested control plants, but these emission rates did not differ from the uninfested controls (Figure 3B). More interestingly, the parasitoid *A. colemani* preferred the blends emitted by the aphid-infested plants over those emitted by the uninfested plants (Binomial GLM: $\chi 2(df\ 5) = 31.13$, $p < 0.001$), irrespective of the salinity treatment (Figure 3C).

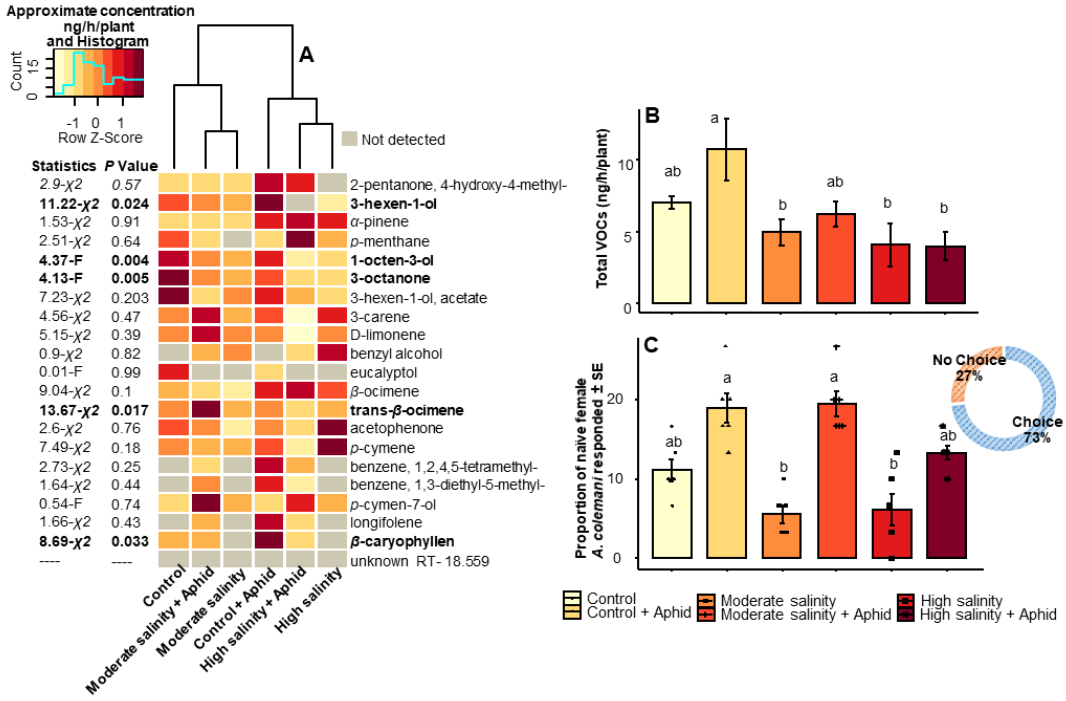


Figure 3: Effect of salinity stress (0 mM NaCl, 75 mM NaCl, 150 mM NaCl) and aphid infestation on Volatile emission. Heat map represents the hierarchical clustering and the emission rates of specific volatile organic compounds (VOCs) (A), compound names, and P values in bold indicate significant differences among treatments; emission of approximate total volatile organic compound (Test: LM) (B); and the proportion of active females that chose the respective odor source (Test: Binomial GLM) (C). Pie chart represents the total percentage of female A. colemani, which made a choice among the treatments. Different letter indicate significance among treatments ($p < 0.05$). In total 6 different groups of plants were tested on 6 days. Each day, 5 groups of 6 parasitoid each were released in the six arm olfactometer to choose among the six different odour sources.

Effect of salinity-aphid interaction on sugar beet leaf phytohormones

Salinity and aphid stress significantly affected the profile of important phytohormones in sugar beet leaves over time. Abscisic acid (ABA) concentration was mainly influenced by salinity stress (LM: $F_{2,84} = 728.06$, $p < 0.001$, Figure 4A) and increased with increasing salinity levels. ABA concentration of the salinity-

stressed plants decreased over time (LM: $F_{2,84}$= 23.68, p < 0.001), and aphid presence resulted in slightly lower ABA levels (LM: $F_{1,84}$ = 34.52, p < 0.001), but only after 72 h, ABA levels of salinity-stressed plants were significantly lower when aphids were present (see table S6 for significant interactions). 12-oxo phytodienoic acid (OPDA) concentration varied over time depending on salinity level and aphid presence (Figure 4B,Table S6). After 4 days of salinity stress (24 h of aphid infestation), ODPA levels were significantly increased in salinity-stressed plants without aphids, while no differences to the low salinity treatment were observed in aphid-infested plants. After 5 days of salinity stress (48 h of aphid infestation), ODPA concentration was highest in unstressed plants without aphids and lowest in the unstressed plants with aphids, while no significant differences were observed in the other treatments. After 6 days of salinity stress (72 h of aphid infestation), ODPA levels were highest in the unstressed plants, medium in the highly salt-stressed plants and lowest in the medium stressed plants, while aphid presence had no effect. Jasmonic acid (JA) concentration is mainly influenced by aphid infestation ($F_{1,84}$ = 349.77, p < 0.001), as a similar pattern observed in Jasmonic acid isoleucine (JAI) ($F_{1,84}$ = 359.04, p < 0.001) (Figure 4C, D, Table S6). JA and JAI levels were lower at 72 h aphid infestation than earlier, and no difference was observed at moderate salinity between infested and uninfested plants. Salicylic acid (SA) concentration remains more or less constant due to salinity stress over time (Figure 4E, Table S6). However, due to aphid stress, SA increased significantly, more visible after 48 h of aphid infestation.

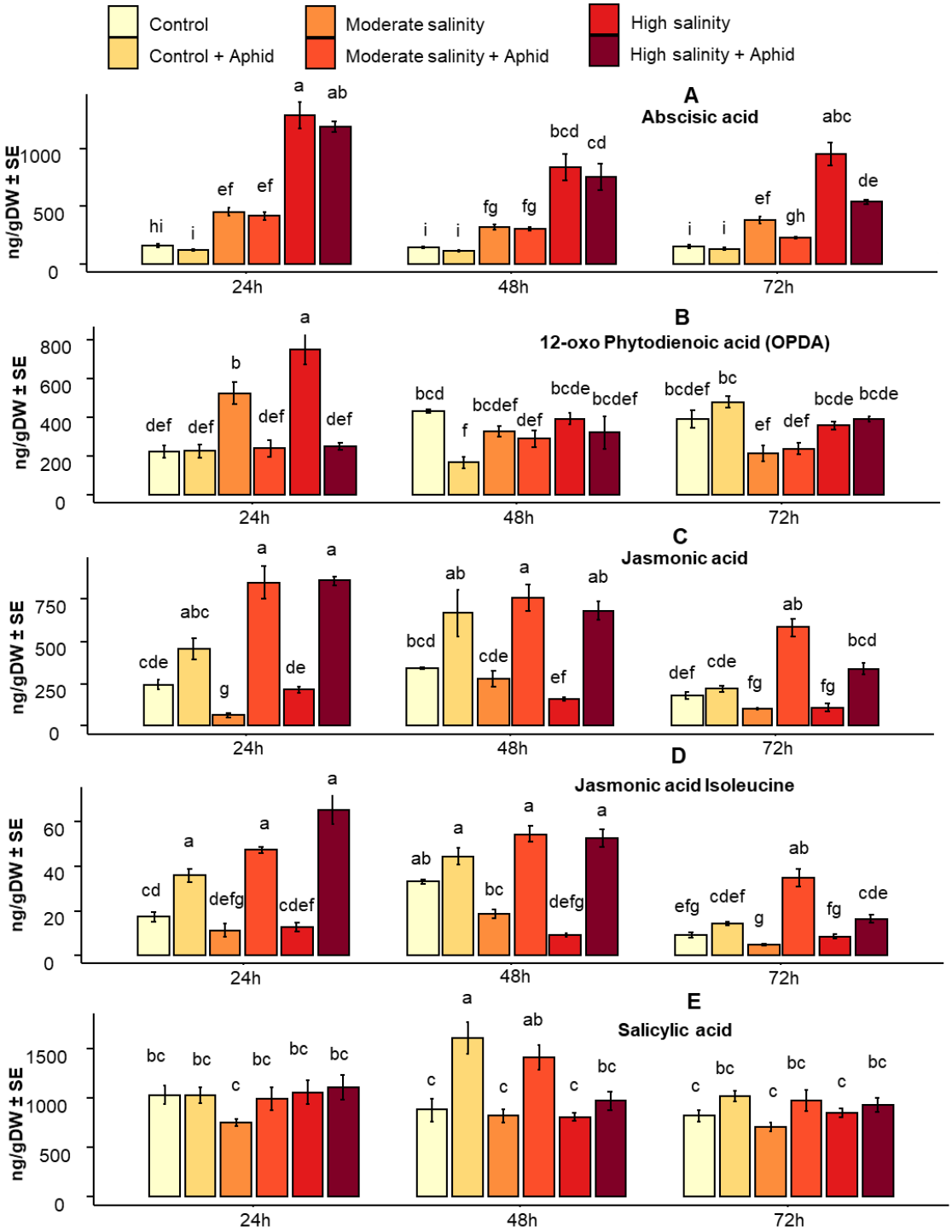


Figure 4: Effect of salinity stress (0 mM NaCl, 75 mM NaCl, 150 mM NaCl) and aphid infestation on the profile of phytohormones of sugarbeet. Abscisic acid (Test: LM $_{log10\ transformed}$) (A); 12-oxo phytodienoic acid (Test: LM) (B); Jasmonic acid (Test: LM $_{log10\ transformed}$) (C); Jasmonic acid isoleucine (Test: LM $_{log10\ transformed}$) (D); and Salicylic acid (Test: LM) (E). Different letters indicate significance among treatments ($p \leq 0.05$), n = 6.

Effect of salinity-aphid interaction on sugarbeet plant central metabolites, collected from phloem exudates

A total of 119 metabolites were identified from the phloem samples and classified as five major metabolites (Figure 5A). Hierarchical clustering analysis of qualitative and quantitative differences among the different metabolites reveals that the metabolites from high-salinity + aphid plants are distinct from all other treatments (Figure S5). Amino acid concentration mainly increased by salinity stress (LM: salinity: $F_{2,30} = 93.46$, $p < 0.001$; aphid: $F_{1,30} = 0.35$, $p = 0.55$; salinity × aphid: $F_{2,30} = 5.93$, $p = 0.006$), however, due to aphid infestation in control plants, slightly reduced concentration of amino acid was observed (Figure 5B). Concentration of total sugar metabolite was increased only due to salinity stress (LM: $F_{2,33} = 25.33$, $p < 0.001$) (Figure 5C). Moreover, salinity is the predominant factor responsible for the elevated ratio between sugar and amino acid concentrations (LM: salinity: $F_{2,30} = 3.69$, $p < 0.001$; aphid: $F_{1,30} = 1.94$, $p = 0.17$; salinity × aphid: $F_{2,30} = 3.49$, $p = 0.043$) (Figure 5D). The elevation of organic acid concentration was influenced by both salinity and aphid infestation (LM: salinity: $F_{2,30} = 114.74$, $p < 0.001$; aphid: $F_{1,30} = 41.05$, $p < 0.001$; salinity × aphid: $F_{2,30} = 3.95$, $p = 0.029$). A similar trend is observed in fatty acid concentration (LM: salinity: $F_{2,30} = 84.70$, $p < 0.001$; aphid: $F_{1,30} = 37.48$, $p < 0.001$; salinity × aphid: $F_{2,30} = 10.34$, $p < 0.001$). The concentration of organic and fatty acids is predominantly influenced by aphid infestation when plants experience salinity stress (Figure 5E, F).

The PCA (principal component analysis) was performed to reduce the dimensionality and visualize the relationship among different treatments. 2D-score plot and synchronized 3D plot of PCA showed a clear separation in different treatments. PC-1 explained 70.7% of the total variation, PC-2 explained 5.7%, and PC-3 explained 3.3% of the variation (Figure 6 A, B). A similar separation was also observed when performing the partial least square-discrimination analysis (PLS-DA), where the overview of PLS-DA plot (Figure S6 A) showed the variation among the five components, 2D-scores plot, and 3D-synchronized plot (Figure S6 B, C) represents the separation of treatment group. Based on VIP (variable importance of projection) score >1, the top thirty-five compounds were identified and summarized along with their relative concentration in Figure 6 C,

and D, which represents stress-responsive metabolites were different based on salinity (Figure 6 C) and aphid infestation (Figure 6 D).

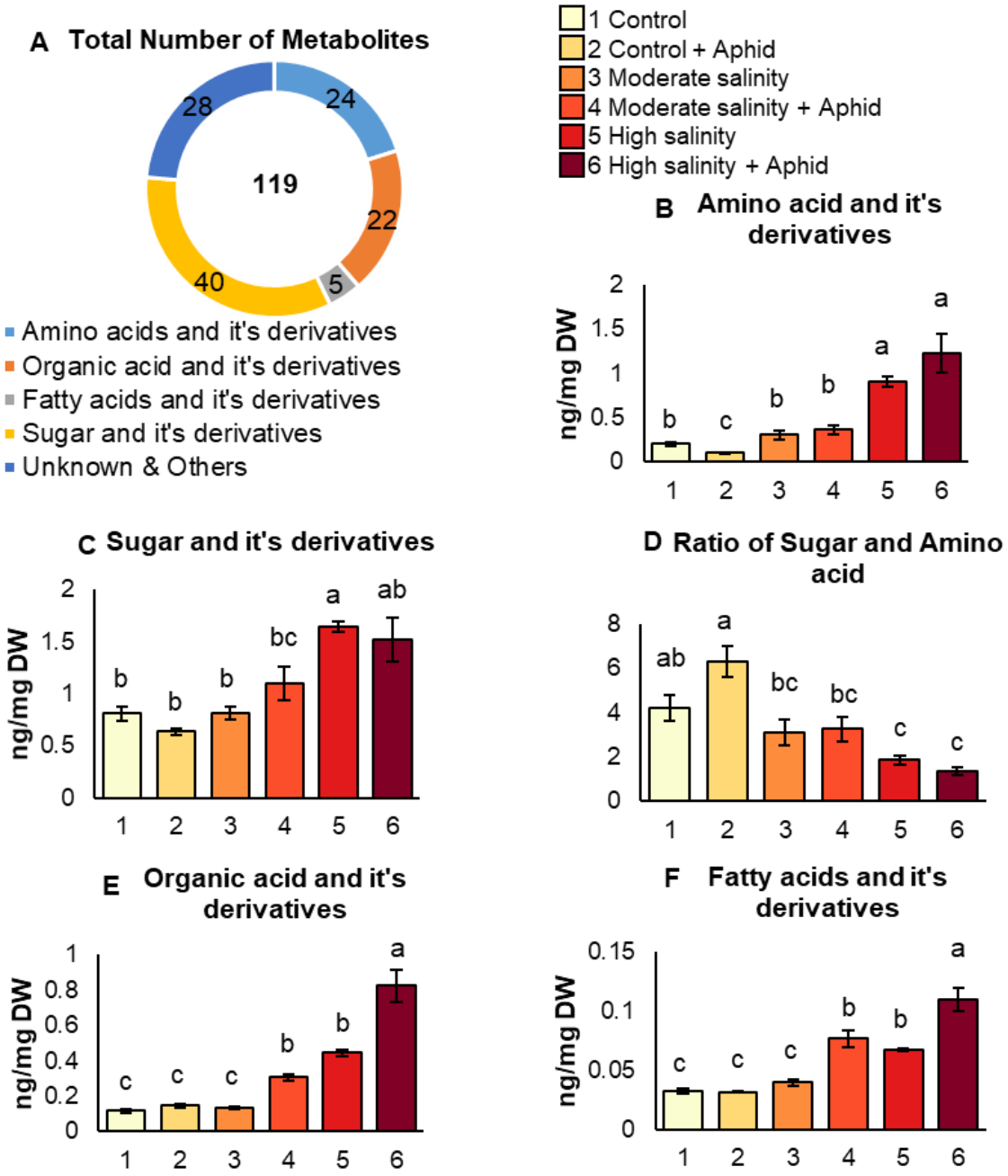


Figure 5: Effect of salinity stress (0 mM NaCl, 75 mM NaCl, 150 mM NaCl) and aphid infestation on sugar beet plant central metabolites. Total number of metabolites and their derivatives of major classes (A); Approximate concentration of amino acids (Test: LM $_{log10\ transformed}$) (B); sugars (Test: LM); ratio of sugars and amino acids (Test: LM) (D); organic acids (Test: LM $_{log10\ transformed}$) (E); and fatty acids (Test: LM $_{log10\ transformed}$) (F). Different letters indicate significance among treatments ($p \leq 0.05$), n = 6.

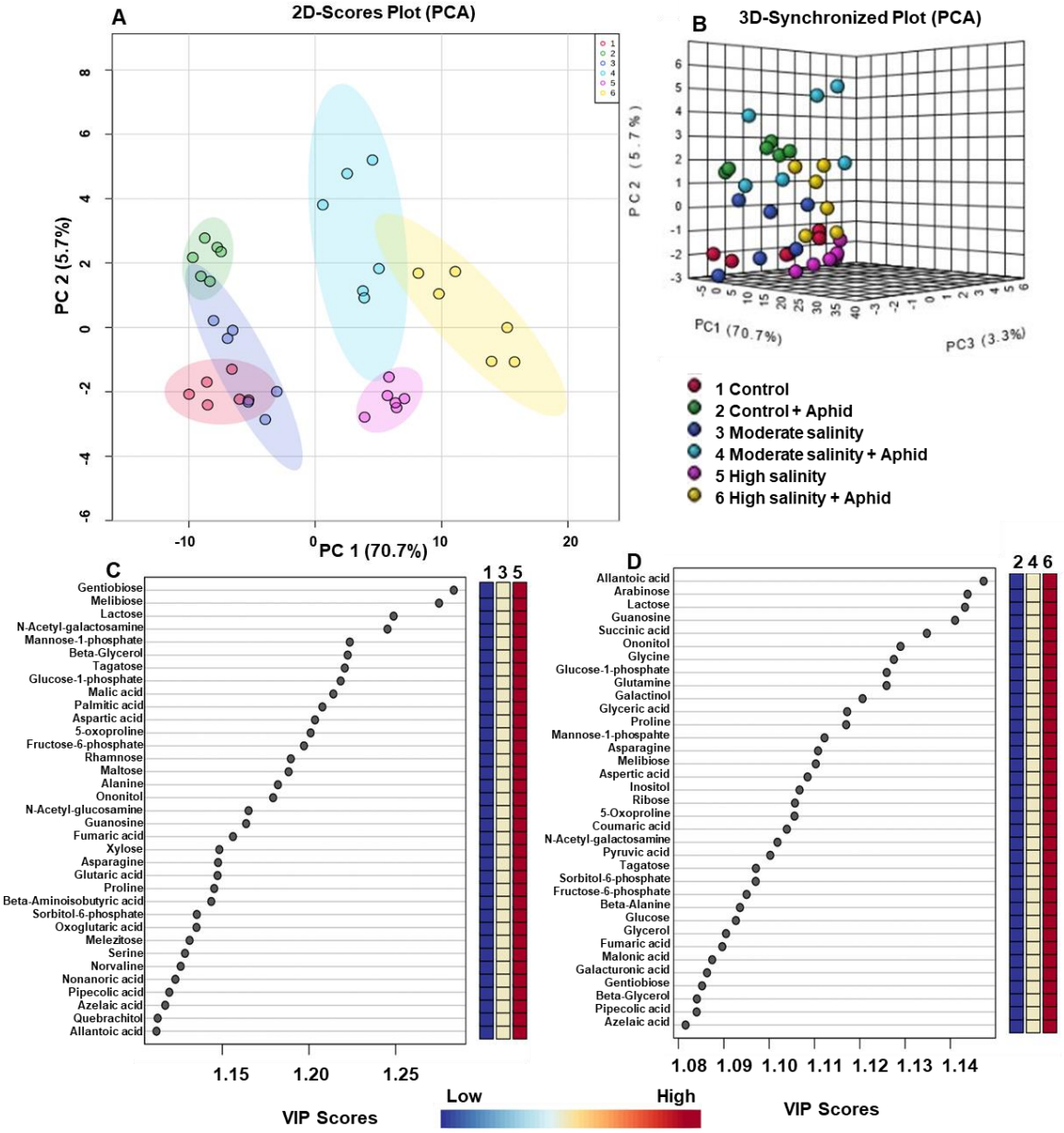


Figure 6: Principal component analysis (PCA) of metabolites profile among different treatments are illustrated as 95% confidence intervals for each group as 2D-scores plot (A); and 3D-synchronized plot (B). In response to different treatments, important metabolites are identified by partial least square-discriminate analysis (PLS-DA). Thirty-five top metabolites are shown according to the variable importance of projection (VIP) score due to salinity stress (C); and aphid infestation (D). Colored boxes indicate the relative concentrations of the corresponding metabolites in each group. n= 6.

Discussion

Our study reports that an increase in salinity had direct negative effects on plant morphological features by reducing plant height, biomass, leaf area etc. and these negative effects cascaded through the trophic system. Aphids were smaller and had fewer offspring on salinity-stressed plants, resulting in slower population growth, while parasitoid emergence rate and size were decreased on salinity-stressed plants. Parasitoid sex ratio further shifted from female based on control plants to male based on salinity-stressed plants. Soil salinity and the interaction with aphids negatively impacted sugarbeet leaf photosynthetic efficiency and altered the plants' phytohormonal signaling and metabolic profiles. Total volatile emission was reduced in salinity-stressed plants, and a significant increase in total VOC emission upon aphid infestation was observed in control and at moderate salinity plants. Still, parasitoids preferred the aphid-infested over the uninfested plants, indicating that ability of sugar beet plants to attract parasitoids as an indirect defense against aphids was not compromised at high salinity levels. In the following paragraphs, we discuss in detail how salinity stress and aphid infestation alter plant photosynthetic efficiencies, phytohormonal signaling, and phloem metabolome and how this, in turn, may affect aphid performance. Afterward, we explore possible links to how these bottom-up changes cascade through the trophic system and affect parasitoid performance and host-finding abilities.

Salinity stress creates toxicity and an ionic imbalance in plants, which negatively impacts photosynthesis, disrupts homeostasis, alters phytohormones and metabolites, and thus manifests symptoms such as slow growth, reduced plant height, decreased germination rate, and withered leaves (Hao et al., 2021). Phytohormones are signaling molecules that regulate various cellular activities in plants. These hormones play a significant role in organizing and coordinating various signal transduction pathways during the response of plants to abiotic and biotic stresses (Pieterse et al., 2009). Abscisic acid (ABA) (Gurmani et al., 2013), salicylic acid (SA) (Khan et al., 2013), jasmonic acid (JA) (R Khan & A Khan, 2013), Jasmonic acid isoleucine (JAI) (de Ollas et al., 2015), ethylene (ET) (Iqbal et al., 2012), 12-oxophytodienoic acid (OPDA) (Maynard et al., 2018), are known for their strong role in imparting stress tolerance in plants. In our study, ABA levels

were significantly increased upon salinity stress. ABA modulates stomata conductance, and rapid increases in ABA levels are typical for plants subjected to salinity or drought stress to reduce water loss by transpiration (Chen et al., 2020; Ding et al., 2018; Farooq et al., 2009; Huang et al., 2021; Min et al., 2015; Niu et al., 2018; Yu et al., 2020). Stomata closure further results in reduced gas exchange and ultimately in reduced photosynthesis (Ding et al., 2018; Farooq et al., 2009). Similarly, ODPA has a regulatory effect on stomata conductance under drought- and salinity stress and regulates ion transport and osmotic adjustment in salinity-stressed plants and also JA has been reported to induce stomata closure under drought stress (Müller & Munné-Bosch, 2021). As a precursor for JA, OPDA further plays in a key role in defense induction against herbivores, including aphids, and increased levels of JA have been reported in sugar beet plants upon aphid infestation. Interestingly, ODPA levels were significantly increased after 4 days of salinity stress in plants without aphids, indicating a regulatory role in salinity-stress responses in sugar beet. This increase of ODPA in response to salinity stress was not observed in the aphid-infested plants, which showed a strong increase in JA levels 24 and 48 h after aphid infestation. JA levels after 24 h of aphid infestation were considerably higher in salinity-stressed plants, even though this difference was not significant, suggesting that production of JA qickly depleted the excess of ODPA that had accummulated in response to salinity stress. Similar to our results, Forieri et al. 2016 detected higher JA levels upon caterpillar herbivory in salinity-stressed plants. Levels of JAI, which is the main signaling molecule in defense induction by the JA signaling pathway, closely resemble JA levels in our study. 72 h after aphid infestation, JA and JAI levels of aphid-infested plants were considerably lower than at the earlier time points, and no differences were detected between aphid-infested and uninfested plants in the low salinity treatment. JA signaling may further play a direct role in plants' responses to salinity stress, and an increase in JA levels upon salt stress was found in tobacco, Arabidopsis, and wheat (Chen et al., 2016; Valenzuela et al., 2016; Zhao et al., 2014). JA signaling was reported to be involved in salt-inhibited root growth (Valenzuela et al., 2016, Zhao et al., 2014), salt stress-induced leaf senescence (Kurotani et al., 2015) and in maintaining ROS homeostasis upon salt stress (Abouelsaad & Renault, 2018).

Crosstalk between between ABA signaling and defense signaling pathways has been reported (Guo et al., 2016; Quais et al., 2020) and some aphid species seem to activate ABA signaling to suppress JA and SA related defenses (Hillwig et al., 2016; Studham & MacIntosh, 2013). Similarly, JA and SA signaling pathways often exhibit antagonistic interactions, meaning that they can negatively regulate each other's pathways. Different plant species may exhibit varying degrees of SA-JA crosstalk and prioritize different defense pathways in response to aphid feeding. In some plant species, an increase in JA levels can suppress SA-mediated defense responses (Morkunas et al. 2011). However, the relationship between JA and SA in sugar beet defense against aphids is not yet well-characterized. We found increased SA levels in aphid-infested sugar beet plants at low and moderate salinity levels after 48 h of aphid feeding, while no differences were observed for the other time points and treatments, suggesting that SA signaling does not play an important role in sugar beet reaction to salinity stress and aphid-infestation. Taken together, our results suggest that salinity stress enhances JA signaling in aphid-infested sugar beets due to increased levels of the JA-precursor OPDA in salinity-stressed plants which gets converted to JA upon aphid feeding.

High levels of ABA, ODPA, and JA result in stomata closure, which is an important mechanism to reduce water loss upon drought stress (Müller & Munné-Bosch, 2021). Plant responses to drought and salinity are very similar as both conditions lead to water stress, causing the closure of stomata, which ultimately reduces carbon assimilation through photosynthesis (Ma et al., 2020). Within the first 19 days after the onset of salinity stress, we did not see any strong effect on photosynthetic capacity, suggesting that salinity-induced stomata closure did not affect photosynthesis in our system. However, after 24 days, we observed a strong decline in photosynthetic efficiency. This decline was strongest in aphid-infested plants under severe salinity stress. Contrary to our results, Forieri et al. 2016 already observed a decline in photosynthetic efficiency two days after the onset of salinity stress in maize.

Aphids are phloem feeders and phloem composition thus has a strong impact on aphid performance. Six days after the onset of salinity stress and three days after the start of aphid infestation, phloem exudates from stressed and non-

stressed plants were collected. We identified 119 metabolites from the phloem that were classified as central metabolites (Figure 5A). Total amounts of amino acids, sugars, organic acids, fatty acids, and their respective derivatives increased with increasing levels of salinity, while aphid infestation only had small effects (Figure 5 B, C, E, F). This increase in metabolite concentration in the phloem might partially be the result of reduced water availability caused by osmotic stress. Moreover, the sugar-to-amino acid ratio in the phloem decreased from 4.20 to 3.08 in plants under moderate salinity and further reduced to 1.86 under high salinity (Figure 5 D), which might be the result of the reduced photosynthetic capacity found in our experiment. Hierarchical clustering based on the amounts of central metabolite revealed two major groups among the different treatments: the first consisting of control plants with and without aphids and plants without aphids under moderate salinity stress, while the second group consisted of the highly-stressed plants with and without aphids and the moderately-stressed plants with aphids (Figure S5). This suggests that changes in central metabolites increase with the intensity of the combined stressors rather than a specific metabolic response to each type of stress.

Plants have a number of mechanisms that allow them to resist or alleviate the damage caused by salt stress, including the accumulation of small-molecule osmotic adjustment substances. When plants are subjected to high levels of salt in the soil, they can respond by synthesizing and accumulating small molecules such as proline, betaine, and trehalose, which help to balance the concentration of solutes inside and outside of the cells and maintain the proper osmotic potential (Abobatta, 2020; Hassan et al., 2015; Koyro et al., 2012). Indeed, we identified top 35 small molecules based on high variable importance of projection (VIP) score (Figure 6 C) that increased in concentrations with increasing salinity stress, such as gentiobiose, melibiose, lactose, malic acid, alanine, proline, norvaline etc. among the phloem exudates of sugar beet. These small molecules help reduce the negative effects of salt stress on plant growth and development by acting as osmoprotectants, which help protect the plant cells from damage caused by high salinity levels (Li et al., 2017). However, metabolic changes due to aphid infestation over different salinity levels (Figure 6 D), we observe that metabolites showed different VIP scores and the order of the most-important

metabolites differed, suggesting that combined stresses might alter osmoprotectants to maintain cell turgor pressure via osmoregulation, and protection of cellular components via reduction of ionic toxicity (Singh et al., 2022).

Changes in the levels and ratios of specific metabolite pools, including sugars, amino acids, and organic acids, often characterize the response of plants to salinity stress. In response to insect herbivory, plants undergo a variety of modifications in their main metabolism. Herbivore-infested plants are thought to increase their production of amino acids for the synthesis of defensive metabolites while attempting to limit the herbivores' access to free amino acids (Zhou et al., 2015). Our study found that the concentrations of major metabolite groups, including amino acids, sugars, organic acids, and fatty acids, showed a significant increase irrespective of salinity levels and aphid infestation as determined by phloem analysis. Li et al. (2017) found that the roots of wild soybean seedlings can respond to stressors such as salt by increasing the synthesis of amino acids, fatty acids, sugars, and organic acids, as well as the secondary metabolism of antioxidants. An increased amount of amino acids, organic acid, and sucrose metabolites was found in other plants (Hartzendorf & Rolletschek, 2001; Khan et al., 2020), including sugarbeet roots (Liu et al., 2020). However, when compared with specific metabolite responses in plants, it might be increased or decreased depending on treatments (Sanchez et al., 2008; Widodo et al., 2009). Particularly in the case of some aphids, there is good evidence of increased free amino acid levels being the direct result of insect feeding (Koyama et al., 2004; Sandström et al., 2000).

Aphids are expected to benefit from the elevated nutrient concentration in the phloem, as well as the reduced sugar-to-amino acid ratio (Abisgold et al., 1994; Jakobs & Müller, 2018; Nowak & Komor, 2010; Ryan et al., 2015). Furthermore, previous studies have also reported positive effects of high soil salinity on aphids (Eichele-Nelson et al., 2018; Polack et al., 2011). Contrary to this, Araya et al., 1991 demonstrated that salinity negatively impacted cereal aphids, and we also observed negative effects of high salinity levels on the performance and reproduction of individual aphids as well as aphid population growth. One factor might impact aphid performance is phloem viscosity. Due to

the higher concentration of sugars, phloem viscosity might be increased, which might have a negative effect on phloem uptake. Another factor that might affect aphid feeding and performance is the accumulation of toxic ions, such as Na+, Cl−, Mg2+, SO42-, or HCO3, in plants growing under saline conditions (Zörb et al., 2019). These toxic ions may negatively influence aphid performance when they are ingested with the phloem or xylem sap during drinking, and aphids may reduce their feeding to avoid the uptake of these toxic ions.

In the 3rd trophic level, we investigated aphid parasitoid *Aphidius colemani*, and we hypothesized that there might be an effect on parasitoid performance due to the negative effect on aphids. More parasitoid mummies developed on the highly stressed plants, and parasitoid development time was decreased on salinity-stressed plants (Figure 2 A, B). However, salinity negatively impacted the parasitoid emergence rate and adult size. Salinity altered the male-biased sex ratio of emerged parasitoids (Figure 2 C, D). In our study, aphid body size was smaller on salinity-stressed plants, which might explain the lower body size of adult parasitoids because parasitoid emergence rate can be related to host size and quality (Garratt et al., 2010; Yasir Ali et al., 2022). The potential reason for a higher proportion of males among parasitoids under high salinity stress could be due to the females actively controlling the sex ratio, with both male and female parasitoid larvae having similar survival rates under such conditions. Aphid parasitoids tend to produce more female offspring (by laying fertilized eggs) when infesting large hosts, and more male offspring (by laying non-fertilized eggs) when infesting small hosts (Cloutier et al., 1991; Pandey & Singh, 1999).

Another important aspect that we investigated here is to address the question of how salinity and aphid stress alter the emission of sugarbeet plant volatiles along with the preferences of parasitoid *Aphidius colemani* on 3rd trophic level. We observed that more parasitoids were attracted to the volatiles emitted by aphid-infested plants compared to undamaged plants, regardless of the salinity treatment (Figure 3C). This suggests that the signal encoded in the volatile blend remained intact despite the reduced total emission from aphid-infested plants under severe salinity stress (Figure 3 B). 1,2,4,5-tetramethyl benzene, 1,3-dimethyl-5-methyl benzene, longifolene, and *β*-caryophyllene were only emitted by aphid-infested plants or in much higher amounts than from the

undamaged plants (Figure 3A) and may thus play an important role as the key components in attracting parasitoids in our system.

In summary, our research revealed that high salinity has detrimental effects on all components of our tri-trophic system: sugar beet plants, aphids, and parasitoids. The salinity and aphid stress increased the concentration of central metabolites in the phloem and altered phytohormone signaling. Plant growth was negatively influenced by salinity stress, but not by aphid feeding, while both factors had negative effects on photosynthetic efficiency. Even though salinity reduced the VOC emission from aphid-infested plants when compared to aphid-infested control plants grown at low salinity levels, attraction of parasitoids to VOCs emitted from the aphid-infested plants remained unaffected by salinity stress. Our findings highlight the necessity of studying multiple trophic levels and the importance of phytochemistry, including HIPVs and parasitoid attraction, when assessing crop combined with both abiotic and biotic stresses.

Supporting information

Additional supporting information can be found in the supporting information section at the end of this article.

Author contributions

Shahinoor Rahman: conceptualization, conduction of experiments, data collection, data analysis, and writing the first draft. Musrat Zahan Surovy: helps during the conduction of experiments, analysis of metabolome data, and construction of graphical abstract. Ilka Vosteen: conceptualization, manuscript editing, and supervision. Mohammad Alhussein: helps with phytohormone analysis in UHPLC-MS-MS platform. Michael Rostás: conceptualization, review, editing, and supervision. The authors declare no conflicts of interest.

References

Abisgold, J. D., Simpson, S. J., & Douglas, A. E. (1994). Nutrient regulation in the pea aphid Acyrthosiphon pisum: Application of a novel geometric framework to sugar and amino acid consumption. Physiological Entomology, 19(2), 95–102. https://doi.org/10.1111/j.1365-3032.1994.tb01081.x

Abobatta, W. F. (2020). Plant Responses and Tolerance to Combined Salt and Drought Stress. In M. Hasanuzzaman & M. Tanveer (Eds.), Salt and Drought Stress Tolerance in Plants: Signaling Networks and Adaptive Mechanisms (pp. 17–52). Springer International Publishing. https://doi.org/10.1007/978-3-030-40277-8_2

Abouelsaad, I., & Renault, S. (2018). Enhanced oxidative stress in the jasmonic acid-deficient tomato mutant def-1 exposed to NaCl stress. Journal of Plant Physiology, 226, 136–144. https://doi.org/10.1016/j.jplph.2018.04.009

Akula, R., & Ravishankar, G. A. (2011). Influence of abiotic stress signals on secondary metabolites in plants. Plant Signaling & Behavior, 6(11), 1720–1731. https://doi.org/10.4161/psb.6.11.17613

Araya, F., Abarca, O., Zúñiga, G. E., & J. Corcuera, L. (1991). Effects of NaCl on glycine-betaine and on aphids in cereal seedlings. Phytochemistry, 30(6), 1793–1795. https://doi.org/10.1016/0031-9422(91)85014-Q

Arif, Y., Singh, P., Siddiqui, H., Bajguz, A., & Hayat, S. (2020). Salinity induced physiological and biochemical changes in plants: An omic approach towards salt stress tolerance. Plant Physiology and Biochemistry, 156, 64–77. https://doi.org/10.1016/j.plaphy.2020.08.042

Assaha, D. V. M., Ueda, A., Saneoka, H., Al-Yahyai, R., & Yaish, M. W. (2017). The Role of Na+ and K+ Transporters in Salt Stress Adaptation in Glycophytes. Frontiers in Physiology, 8, 509. https://doi.org/10.3389/fphys.2017.00509

Bostock, R. M. (2005). Signal Crosstalk and Induced Resistance: Straddling the Line Between Cost and Benefit. Annual Review of Phytopathology, 43(1), 545–580. https://doi.org/10.1146/annurev.phyto.41.052002.095505

Butcher, K., Wick, A. F., DeSutter, T., Chatterjee, A., & Harmon, J. (2016). Soil Salinity: A Threat to Global Food Security. Agronomy Journal, 108(6), 2189–2200. https://doi.org/10.2134/agronj2016.06.0368

Cao, D., Lutz, A., Hill, C. B., Callahan, D. L., & Roessner, U. (2017). A Quantitative Profiling Method of Phytohormones and Other Metabolites Applied to Barley Roots Subjected to Salinity Stress. Frontiers in Plant Science, 7. https://www.frontiersin.org/articles/10.3389/fpls.2016.02070

Chele, K. H., Tinte, M. M., Piater, L. A., Dubery, I. A., & Tugizimana, F. (2021). Soil Salinity, a Serious Environmental Issue and Plant Responses: A Metabolomics Perspective. Metabolites, 11(11), Article 11. https://doi.org/10.3390/metabo11110724

Chen, K., Li, G.-J., Bressan, R. A., Song, C.-P., Zhu, J.-K., & Zhao, Y. (2020). Abscisic acid dynamics, signaling, and functions in plants. Journal of Integrative Plant Biology, 62(1), 25–54. https://doi.org/10.1111/jipb.12899

Chen, X., Zhang, X., Jia, A., Xu, G., Hu, H., Hu, X., & Hu, L. (2016). Jasmonate mediates salt-induced nicotine biosynthesis in tobacco (Nicotiana tabacum L.). Plant Diversity, 38(2), 118–123. https://doi.org/10.1016/j.pld.2016.06.001

Clavijo McCormick, A., Unsicker, S. B., & Gershenzon, J. (2012). The specificity of herbivore-induced plant volatiles in attracting herbivore enemies. Trends in Plant Science, 17(5), 303–310. https://doi.org/10.1016/j.tplants.2012.03.012

Cloutier, C., Lévesque, C. A., Eaves, D. M., & Mackauer, M. (1991). Maternal adjustment of sex ratio in response to host size in the aphid parasitoid Ephedrus californicus. Canadian Journal of Zoology, 69(6), 1489–1495. https://doi.org/10.1139/z91-208

Cui, J., Li, J., Dai, C., & Li, L. (2022). Transcriptome and Metabolome Analyses Revealed the Response Mechanism of Sugar Beet to Salt Stress of Different Durations. International Journal of Molecular Sciences, 23(17), Article 17. https://doi.org/10.3390/ijms23179599

de Ollas, C., Arbona, V., & GóMez-Cadenas, A. (2015). Jasmonoyl isoleucine accumulation is needed for abscisic acid build-up in roots of Arabidopsis under water stress conditions. Plant, Cell & Environment, 38(10), 2157–2170. https://doi.org/10.1111/pce.12536

Dias, D. A., Hill, C. B., Jayasinghe, N. S., Atieno, J., Sutton, T., & Roessner, U. (2015). Quantitative profiling of polar primary metabolites of two chickpea cultivars with contrasting responses to salinity. Journal of Chromatography B, 1000, 1–13. https://doi.org/10.1016/j.jchromb.2015.07.002

Ding, L., Lu, Z., Gao, L., Guo, S., & Shen, Q. (2018). Is Nitrogen a Key Determinant of Water Transport and Photosynthesis in Higher Plants Upon Drought Stress? Frontiers in Plant Science, 9. https://www.frontiersin.org/articles/10.3389/fpls.2018.01143

Dohm, J. C., Minoche, A. E., Holtgräwe, D., Capella-Gutiérrez, S., Zakrzewski, F., Tafer, H., Rupp, O., Sörensen, T. R., Stracke, R., Reinhardt, R., Goesmann, A., Kraft, T., Schulz, B., Stadler, P. F., Schmidt, T., Gabaldón, T., Lehrach, H., Weisshaar, B., & Himmelbauer, H. (2014). The genome of the recently domesticated crop plant sugar beet (Beta vulgaris). Nature, 505(7484), 546–549. https://doi.org/10.1038/nature12817

Dong, Y.-C., Wang, Z.-J., Bu, R.-Y., Dai, H.-J., Zhou, L.-J., Han, P., Amiens-Desneux, E., Bearez, P., & Desneux, N. (2020). Water and salt stresses do not trigger bottom-up effects on plant-mediated indirect interactions between a leaf chewer and a sap-feeder. Journal of Pest Science, 93(4), 1267–1280. https://doi.org/10.1007/s10340-020-01258-y

Eichele-Nelson, J., DeSutter, T., Wick, A. F., Harmon, E. L., & Harmon, J. P. (2018). Salinity Improves Performance and Alters Distribution of Soybean Aphids. Environmental Entomology, 47(4), 875–880. https://doi.org/10.1093/ee/nvy072

Fahad, S., Hussain, S., Matloob, A., Khan, F. A., Khaliq, A., Saud, S., Hassan, S., Shan, D., Khan, F., Ullah, N., Faiq, M., Khan, M. R., Tareen, A. K., Khan, A., Ullah, A., Ullah, N., & Huang, J. (2015). Phytohormones and plant responses to salinity stress: A review. Plant Growth Regulation, 75(2), 391–404. https://doi.org/10.1007/s10725-014-0013-y

Farahani, M. H. D. A., Vatanpour, V., & Taheri, A. (2020). Desalination: Challenges and Opportunities. BoD – Books on Demand.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., & Basra, S. M. A. (2009). Plant drought stress: Effects, mechanisms and management. Agronomy for Sustainable Development, 29(1), 185–212. https://doi.org/10.1051/agro:2008021

Forieri, I., Hildebrandt, U., & Rostás, M. (2016). Salinity stress effects on direct and indirect defence metabolites in maize. Environmental and Experimental Botany, 122, 68–77. https://doi.org/10.1016/j.envexpbot.2015.09.007

Fujita, M., Fujita, Y., Noutoshi, Y., Takahashi, F., Narusaka, Y., Yamaguchi-Shinozaki, K., & Shinozaki, K. (2006). Crosstalk between abiotic and biotic stress responses: A current view from the points of convergence in the stress signaling networks. Current Opinion in Plant Biology, 9(4), 436–442. https://doi.org/10.1016/j.pbi.2006.05.014

Garratt, M. P. D., Leather, S. R., & Wright, D. J. (2010). Tritrophic effects of organic and conventional fertilisers on a cereal-aphid-parasitoid system. Entomologia Experimentalis et Applicata, 134(3), 211–219. https://doi.org/10.1111/j.1570-7458.2009.00957.x

Ghodoum Parizipour, M. H., Rajabpour, A., Jafari, S., & Tahmasebi, A. (2021). Host-targeted salt stress affects fitness and vector performance of bird cherry-oat aphid (Rhopalosiphum padi L.) on wheat. Arthropod-Plant Interactions, 15(1), 47–58. https://doi.org/10.1007/s11829-020-09795-0

Guo, H., Sun, Y., Peng, X., Wang, Q., Harris, M., & Ge, F. (2016). Up-regulation of abscisic acid signaling pathway facilitates aphid xylem absorption and osmoregulation under drought stress. Journal of Experimental Botany, 67(3), 681–693. https://doi.org/10.1093/jxb/erv481

Gurmani, A. R., Bano, A., Ullah, N., Khan, H., Jahangir, M., & Flowers, T. J. (2013). Exogenous abscisic acid(ABA) and silicon (Si) promote salinity tolerance by reducing sodium (Na+) transport and bypass flow in rice (“Oryza sativa” indica). Australian Journal of Crop Science, 7(9), 1219–1226. https://doi.org/10.3316/informit.619232089838636

Hao, S., Wang, Y., Yan, Y., Liu, Y., Wang, J., & Chen, S. (2021). A Review on Plant Responses to Salt Stress and Their Mechanisms of Salt Resistance. Horticulturae, 7(6), Article 6. https://doi.org/10.3390/horticulturae7060132

Harmon, J. P., & Daigh, A. L. M. (2017). Attempting to predict the plant-mediated trophic effects of soil salinity: A mechanistic approach to supplementing insufficient information. Food Webs, 13, 67–79. https://doi.org/10.1016/j.fooweb.2017.02.002

Hartzendorf, T., & Rolletschek, H. (2001). Effects of NaCl-salinity on amino acid and carbohydrate contents of Phragmites australis. Aquatic Botany, 69(2), 195–208. https://doi.org/10.1016/S0304-3770(01)00138-3

Hassan, M. A., Fuertes, M. M., Sánchez, F. J. R., Vicente, O., & Boscaiu, M. (2015). Effects of Salt and Water Stress on Plant Growth and on Accumulation of Osmolytes and Antioxidant Compounds in Cherry Tomato. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 43(1), Article 1. https://doi.org/10.15835/nbha4319793

Hillwig, M. S., Chiozza, M., Casteel, C. L., Lau, S. T., Hohenstein, J., Hernández, E., Jander, G., & MacIntosh, G. C. (2016). Abscisic acid deficiency increases defence responses against

Myzus persicae in Arabidopsis. Molecular Plant Pathology, 17(2), 225–235. https://doi.org/10.1111/mpp.12274

Hoagland, D. R., Arnon, D. I., & Arnon, D. J. (1938). The Water-culture Method for Growing Plants Without Soil. University of California, College of Agriculture.

Huang, Y., Zhou, J., Li, Y., Quan, R., Wang, J., Huang, R., & Qin, H. (2021). Salt Stress Promotes Abscisic Acid Accumulation to Affect Cell Proliferation and Expansion of Primary Roots in Rice. International Journal of Molecular Sciences, 22(19), 10892. https://doi.org/10.3390/ijms221910892

Iqbal, N., Masood, A., & Khan, N. A. (2012). Phytohormones in Salinity Tolerance: Ethylene and Gibberellins Cross Talk. In N. A. Khan, R. Nazar, N. Iqbal, & N. A. Anjum (Eds.), Phytohormones and Abiotic Stress Tolerance in Plants (pp. 77–98). Springer. https://doi.org/10.1007/978-3-642-25829-9_3

Jakobs, R., & Müller, C. (2018). Effects of intraspecific and intra-individual differences in plant quality on preference and performance of monophagous aphid species. Oecologia, 186(1), 173–184. https://doi.org/10.1007/s00442-017-3998-x

Jan, R., Asaf, S., Numan, M., Lubna, & Kim, K.-M. (2021). Plant Secondary Metabolite Biosynthesis and Transcriptional Regulation in Response to Biotic and Abiotic Stress Conditions. Agronomy, 11(5), Article 5. https://doi.org/10.3390/agronomy11050968

Kamran, M., Parveen, A., Ahmar, S., Malik, Z., Hussain, S., Chattha, M. S., Saleem, M. H., Adil, M., Heidari, P., & Chen, J.-T. (2019). An Overview of Hazardous Impacts of Soil Salinity in Crops, Tolerance Mechanisms, and Amelioration through Selenium Supplementation. International Journal of Molecular Sciences, 21(1), 148. https://doi.org/10.3390/ijms21010148

Kaplan, I., Carrillo, J., Garvey, M., & Ode, P. J. (2016). Indirect plant–parasitoid interactions mediated by changes in herbivore physiology. Current Opinion in Insect Science, 14, 112–119. https://doi.org/10.1016/j.cois.2016.03.004

Kerchev, P. I., Fenton, B., Foyer, C. H., & Hancock, R. D. (2012). Plant responses to insect herbivory: Interactions between photosynthesis, reactive oxygen species and hormonal signalling pathways. Plant, Cell & Environment, 35(2), 441–453. https://doi.org/10.1111/j.1365-3040.2011.02399.x

Kessler, A., & Heil, M. (2011). The multiple faces of indirect defences and their agents of natural selection. Functional Ecology, 25(2), 348–357. https://doi.org/10.1111/j.1365-2435.2010.01818.x

Khan, M. I. R., Iqbal, N., Masood, A., Per, T. S., & Khan, N. A. (2013). Salicylic acid alleviates adverse effects of heat stress on photosynthesis through changes in proline production and ethylene formation. Plant Signaling & Behavior, 8(11), e26374. https://doi.org/10.4161/psb.26374

Khan, N., Ali, S., Zandi, P., Mehmood, A., Ullah, S., Ikram, M., Ismail, I., Shahid, M. A., & Babar, M. A. (2020). Role of sugars, amino acids and organic acids in improving plant abiotic stress tolerance. Pakistan Journal of Botany, 52(2). https://doi.org/10.30848/PJB2020-2(24)

Koyama, Y., Yao, I., & Akimoto, S.-I. (2004). Aphid galls accumulate high concentrations of amino acids: A support for the nutrition hypothesis for gall formation. Entomologia Experimentalis et Applicata, 113(1), 35–44. https://doi.org/10.1111/j.0013-8703.2004.00207.x

Koyro, H.-W., Ahmad, P., & Geissler, N. (2012). Abiotic Stress Responses in Plants: An Overview. In P. Ahmad & M. N. V. Prasad (Eds.), Environmental Adaptations and Stress Tolerance of Plants in the Era of Climate Change (pp. 1–28). Springer. https://doi.org/10.1007/978-1-4614-0815-4_1

Kurotani, K., Hayashi, K., Hatanaka, S., Toda, Y., Ogawa, D., Ichikawa, H., Ishimaru, Y., Tashita, R., Suzuki, T., Ueda, M., Hattori, T., & Takeda, S. (2015). Elevated Levels of CYP94 Family Gene Expression Alleviate the Jasmonate Response and Enhance Salt Tolerance in Rice. Plant and Cell Physiology, 56(4), 779–789. https://doi.org/10.1093/pcp/pcv006

Landi, M., Araniti, F., Flamini, G., Piccolo, E. L., Trivellini, A., Abenavoli, M. R., & Guidi, L. (2020). "Help is in the air": Volatiles from salt-stressed plants increase the reproductive success of receivers under salinity. Planta, 251(2), 48. https://doi.org/10.1007/s00425-020-03344-y

Laney, A. G., Chen, P., & Korth, K. L. (2018). Interactive effects of aphid feeding and virus infection on host gene expression and volatile compounds in salt-stressed soybean, Glycine max (L.) Merr. Arthropod-Plant Interactions, 12(3), 401–413. https://doi.org/10.1007/s11829-017-9590-9

Li, M., Guo, R., Jiao, Y., Jin, X., Zhang, H., & Shi, L. (2017). Comparison of Salt Tolerance in Soja Based on Metabolomics of Seedling Roots. Frontiers in Plant Science, 8. https://www.frontiersin.org/articles/10.3389/fpls.2017.01101

Liu, L., Wang, B., Liu, D., Zou, C., Wu, P., Wang, Z., Wang, Y., & Li, C. (2020). Transcriptomic and metabolomic analyses reveal mechanisms of adaptation to salinity in which carbon and nitrogen metabolism is altered in sugar beet roots. BMC Plant Biology, 20(1), 138. https://doi.org/10.1186/s12870-020-02349-9

Ma, Y., Dias, M. C., & Freitas, H. (2020). Drought and Salinity Stress Responses and Microbe-Induced Tolerance in Plants. Frontiers in Plant Science, 11. https://www.frontiersin.org/articles/10.3389/fpls.2020.591911

Maynard, D., Gröger, H., Dierks, T., & Dietz, K.-J. (2018). The function of the oxylipin 12-oxophytodienoic acid in cell signaling, stress acclimation, and development. Journal of Experimental Botany, 69(22), 5341–5354. https://doi.org/10.1093/jxb/ery316

Min, J.-H., Chung, J.-S., Lee, K.-H., & Kim, C. S. (2015). The CONSTANS-like 4 transcription factor, AtCOL4, positively regulates abiotic stress tolerance through an abscisic acid-dependent manner in Arabidopsis. Journal of Integrative Plant Biology, 57(3), 313–324. https://doi.org/10.1111/jipb.12246

Morkunas, I., Mai, V. C., & Gabryś, B. (2011). Phytohormonal signaling in plant responses to aphid feeding. Acta Physiologiae Plantarum, 33(6), 2057–2073. https://doi.org/10.1007/s11738-011-0751-7

Müller, M., & Munné-Bosch, S. (2021). Hormonal impact on photosynthesis and photoprotection in plants. Plant Physiology, 185(4), 1500–1522. https://doi.org/10.1093/plphys/kiaa119

Munns, R., & Tester, M. (2008). Mechanisms of Salinity Tolerance. Annual Review of Plant Biology, 59(1), 651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911

Neil Willy. (2016). Environmental Plant Physiology (1st ed.). Garland Science. https://doi.org/10.1201/9781317206231

Niu, M., Xie, J., Chen, C., Cao, H., Sun, J., Kong, Q., Shabala, S., Shabala, L., Huang, Y., & Bie, Z. (2018). An early ABA-induced stomatal closure, Na+ sequestration in leaf vein and K+ retention in mesophyll confer salt tissue tolerance in Cucurbita species. Journal of Experimental Botany, 69(20), 4945–4960. https://doi.org/10.1093/jxb/ery251

Nowak, H., & Komor, E. (2010). How aphids decide what is good for them: Experiments to test aphid feeding behaviour on Tanacetum vulgare (L.) using different nitrogen regimes. Oecologia, 163(4), 973–984. https://doi.org/10.1007/s00442-010-1652-y

Pandey, S., & Singh, R. (1999). Host size induced variation in progeny sex ratio of an aphid parasitoid Lysiphlebia mirzai. Entomologia Experimentalis et Applicata, 90(1), 61–67. https://doi.org/10.1046/j.1570-7458.1999.00423.x

Pieterse, C. M. J., Leon-Reyes, A., Van der Ent, S., & Van Wees, S. C. M. (2009). Networking by small-molecule hormones in plant immunity. Nature Chemical Biology, 5(5), Article 5. https://doi.org/10.1038/nchembio.164

Pieterse, C. M. J., Van der Does, D., Zamioudis, C., Leon-Reyes, A., & Van Wees, S. C. M. (2012). Hormonal Modulation of Plant Immunity. Annual Review of Cell and Developmental Biology, 28(1), 489–521. https://doi.org/10.1146/annurev-cellbio-092910-154055

Pitman, M. G., & Läuchli, A. (2002). Global Impact of Salinity and Agricultural Ecosystems. In A. Läuchli & U. Lüttge (Eds.), Salinity: Environment—Plants—Molecules (pp. 3–20). Springer Netherlands. https://doi.org/10.1007/0-306-48155-3_1

Polack, L. A., Pereyra, P. C., & Sarandón, S. J. (2011). Effects of Plant Stress and Habitat Manipulation on Aphid Control in Greenhouse Sweet Peppers. Journal of Sustainable Agriculture, 35(7), 699–725. https://doi.org/10.1080/10440046.2011.606489

Posada-Vergara, C., Lohaus, K., Alhussein, M., Vidal, S., & Rostás, M. (2022). Root Colonization by Fungal Entomopathogen Systemically Primes Belowground Plant Defense against Cabbage Root Fly. Journal of Fungi, 8(9), Article 9. https://doi.org/10.3390/jof8090969

Quais, M. K., Munawar, A., Ansari, N. A., Zhou, W.-W., & Zhu, Z.-R. (2020). Interactions between brown planthopper (Nilaparvata lugens) and salinity stressed rice (Oryza sativa) plant are cultivar-specific. Scientific Reports, 10(1), Article 1. https://doi.org/10.1038/s41598-020-64925-1

R Khan, M. I., & A Khan, N. (2013). Salicylic Acid and Jasmonates: Approaches in Abiotic Stress Tolerance. Journal of Plant Biochemistry & Physiology, 1(4). https://doi.org/10.4172/2329-9029.1000e113

Ryan, G. D., Sylvester, E. V. A., Shelp, B. J., & Newman, J. A. (2015). Towards an understanding of how phloem amino acid composition shapes elevated CO2-induced changes in aphid population dynamics. Ecological Entomology, 40(3), 247–257. https://doi.org/10.1111/een.12181

Sanchez, D. H., Siahpoosh, M. R., Roessner, U., Udvardi, M., & Kopka, J. (2008). Plant metabolomics reveals conserved and divergent metabolic responses to salinity. Physiologia Plantarum, 132(2), 209–219. https://doi.org/10.1111/j.1399-3054.2007.00993.x

Sandström, J., Telang, A., & Moran, N. A. (2000). Nutritional enhancement of host plants by aphids—A comparison of three aphid species on grasses. Journal of Insect Physiology, 46(1), 33–40. https://doi.org/10.1016/S0022-1910(99)00098-0

Sarfraz, M., Dosdall, L. M., & Keddie, B. A. (2009). Host plant nutritional quality affects the performance of the parasitoid Diadegma insulare. Biological Control, 51(1), 34–41. https://doi.org/10.1016/j.biocontrol.2009.07.004

Schile, L., & Mopper, S. (2006). The deleterious effects of salinity stress on leafminers and their freshwater host. Ecological Entomology, 31(4), 345–351. https://doi.org/10.1111/j.1365-2311.2006.00799.x

Shahid, M. A., Sarkhosh, A., Khan, N., Balal, R. M., Ali, S., Rossi, L., Gómez, C., Mattson, N., Nasim, W., & Garcia-Sanchez, F. (2020). Insights into the Physiological and Biochemical Impacts of Salt Stress on Plant Growth and Development. Agronomy, 10(7), Article 7. https://doi.org/10.3390/agronomy10070938

Shahid, S. A., Zaman, M., & Heng, L. (2018). Soil Salinity: Historical Perspectives and a World Overview of the Problem. In M. Zaman, S. A. Shahid, & L. Heng (Eds.), Guideline for Salinity Assessment, Mitigation and Adaptation Using Nuclear and Related Techniques (pp. 43–53). Springer International Publishing. https://doi.org/10.1007/978-3-319-96190-3_2

Shin, Y. K., Bhandari, S. R., & Lee, J. G. (2021). Monitoring of Salinity, Temperature, and Drought Stress in Grafted Watermelon Seedlings Using Chlorophyll Fluorescence. Frontiers in Plant Science, 12. https://www.frontiersin.org/articles/10.3389/fpls.2021.786309

Singh, P., Choudhary, K. K., Chaudhary, N., Gupta, S., Sahu, M., Tejaswini, B., & Sarkar, S. (2022). Salt stress resilience in plants mediated through osmolyte accumulation and its crosstalk mechanism with phytohormones. Frontiers in Plant Science, 13, 1006617. https://doi.org/10.3389/fpls.2022.1006617

Stallmann, J., Pons, C. A. A., Schweiger, R., & Müller, C. (2022). Time point- and plant part-specific changes in phloem exudate metabolites of leaves and ears of wheat in response to drought and effects on aphids. PLOS ONE, 17(1), e0262671. https://doi.org/10.1371/journal.pone.0262671

Studham, M. E., & MacIntosh, G. C. (2013). Multiple Phytohormone Signals Control the Transcriptional Response to Soybean Aphid Infestation in Susceptible and Resistant Soybean Plants. Molecular Plant-Microbe Interactions®, 26(1), 116–129. https://doi.org/10.1094/MPMI-05-12-0124-FI

Teklić, T., Parađiković, N., Špoljarević, M., Zeljković, S., Lončarić, Z., & Lisjak, M. (2021). Linking abiotic stress, plant metabolites, biostimulants and functional food. Annals of Applied Biology, 178(2), 169–191. https://doi.org/10.1111/aab.12651

Tetyuk, O., Benning, U. F., & Hoffmann-Benning, S. (2013). Collection and Analysis of Arabidopsis Phloem Exudates Using the EDTA-facilitated Method. Journal of Visualized Experiments : JoVE, 80, 51111. https://doi.org/10.3791/51111

Valenzuela, C. E., Acevedo-Acevedo, O., Miranda, G. S., Vergara-Barros, P., Holuigue, L., Figueroa, C. R., & Figueroa, P. M. (2016). Salt stress response triggers activation of the jasmonate signaling pathway leading to inhibition of cell elongation in Arabidopsis primary root. Journal of Experimental Botany, 67(14), 4209–4220. https://doi.org/10.1093/jxb/erw202

van Zelm, E., Zhang, Y., & Testerink, C. (2020). Salt Tolerance Mechanisms of Plants. Annual Review of Plant Biology, 71(1), 403–433. https://doi.org/10.1146/annurev-arplant-050718-100005

Viric Gasparic, H., Grubelic, M., Dragovic Uzelac, V., Bazok, R., Cacija, M., Drmic, Z., & Lemic, D. (2020). Neonicotinoid Residues in Sugar Beet Plants and Soil under Different Agro-Climatic Conditions. Agriculture, 10(10), Article 10. https://doi.org/10.3390/agriculture10100484

Widodo, Patterson, J. H., Newbigin, E., Tester, M., Bacic, A., & Roessner, U. (2009). Metabolic responses to salt stress of barley (Hordeum vulgare L.) cultivars, Sahara and Clipper, which differ in salinity tolerance. Journal of Experimental Botany, 60(14), 4089–4103. https://doi.org/10.1093/jxb/erp243

Yasir Ali, M., Lu, Z., Ali, A., Bilal Amir, M., Afaq Ahmed, M., Shahid, S., Liu, T., & Pan, M. (2022). Effects of Plant-Mediated Differences in Aphid Size on Suitability of Its Parasitoid, Aphelinus varipes (Hymenoptera: Aphelinidae). Journal of Economic Entomology, 115(1), 74–80. https://doi.org/10.1093/jee/toab245

Yu, Z., Duan, X., Luo, L., Dai, S., Ding, Z., & Xia, G. (2020). How Plant Hormones Mediate Salt Stress Responses. Trends in Plant Science, 25(11), 1117–1130. https://doi.org/10.1016/j.tplants.2020.06.008

Zhao, Y., Dong, W., Zhang, N., Ai, X., Wang, M., Huang, Z., Xiao, L., & Xia, G. (2014). A Wheat Allene Oxide Cyclase Gene Enhances Salinity Tolerance via Jasmonate Signaling. Plant Physiology, 164(2), 1068–1076. https://doi.org/10.1104/pp.113.227595

Zhou, S., Lou, Y.-R., Tzin, V., & Jander, G. (2015). Alteration of Plant Primary Metabolism in Response to Insect Herbivory. Plant Physiology, 169(3), 1488–1498. https://doi.org/10.1104/pp.15.01405

Zörb, C., Geilfus, C.-M., & Dietz, K.-J. (2019). Salinity and crop yield. Plant Biology, 21(S1), 31–38. https://doi.org/10.1111/plb.12884

Salinity stress and aphid infestation modulate sugarbeet phytochemistry: impacts on aphid-parasitoid trophic interactions

Shahinoor Rahman*[1], Musrat Zahan Surovy[2], Mohammad Alhussein[1], Michael Rostás[1], and Ilka Vosteen*[1],

[1]Department of Crop Sciences, Division of Agricultural Entomology, Georg-August-University of Goettingen, Germany

[2]Department of Crop Sciences, Division of Plant Pathology and Crop Protection, Georg-August-University of Goettingen, Germany

*Correspondence: shahinoor_ent@yahoo.com; ilka.vosteen@uni-goettingen.de

Supporting information

Statistical analysis

Experiment 1

Three boxes (six plants of each box considered a block) were used for each treatment (control, moderate salinity, high salinity), resulting in a total of n = 18 plants per treatment. Pre-reproductive period and the longevity of aphids were analyzed by Kruskal-Wallis non-parametric test followed by the Dunn test for posthoc analysis. Reproductive period, Reproductive rate (*RR*), and Intrinsic rate of increase (*Rm*) were calculated by generalized linear mixed model (GLMM) with glmmTMB function with the gaussian family distribution, followed by Tukey HSD posthoc test for multiple comparisons. Number of the progeny of an aphid produced upto its' own first progeny reproduce (*Nd*) was calculated by generalized linear mixed model (GLMM) with glmmTMB function with Conway-maxwell-Poisson (compois) family distribution, followed by Tukey HSD posthoc test for multiple comparisons.

Experiment 2

Three boxes (four plants of each box considered a randomized block) were used for each treatment (control, control + aphid, moderate salinity, moderate salinity + aphid, high salinity, high salinity + aphid), resulting in a total of n = 12 plants per treatment. Repeated measure 3-way ANOVA was performed by linear

mixed effect model (LMM) for analyzing sugar beet plant height, followed by Tukey HSD posthoc test for multiple comparisons. 2-way ANOVA was performed by linear mixed effect model (LMM) for analyzing sugar beet plant biomass and leaf area, followed by Tukey HSD posthoc test for multiple comparisons. Repeated measure 3-way ANOVA was performed by the linear model (LM) for analyzing sugar beet leaf chlorophyll fluorescence (photosynthetic efficiencies) parameters like maximum quantum yield of PS II (Fv/Fm), quantum yield (YII) of PS II, and the electron transport rate (ETR), followed by Tukey HSD posthoc test for multiple comparisons. Aphid population dynamics was calculated by generalized linear mixed model (GLMM) with glmmTMB function with Conway-maxwell-Poisson (compois) family distribution, followed by Tukey HSD posthoc test for multiple comparisons. Aphid body length and body coverage were performed by the linear model (LM), followed by Tukey HSD posthoc test for multiple comparisons.

Experiment 3

To know how salt stress influences the life cycle of an individual parasitoid, three boxes (six plants of each box considered a block) for each treatment (control, moderate salinity, high salinity), resulting in a total of n = 18 plants per treatment. Parasitoid performances to three different magnitudes of salinity stress, mummified, dead, survival, and emerged, were analyzed by the generalized linear model (GLM) with binomial family distribution, followed by Tukey HSD posthoc test for multiple comparisons. The number of successes (= mummified/ dead/ survived/ emerged) and the number of failures (= not mummified/ not dead/ not survived/ not emerged) were considered a two-vector response variable. Oviposition to mummification period, mummification to oviposition period, and total development period of parasitoid were analyzed by generalized linear mixed model (GLMM) with glmmTMB function with Conway-maxwell-Poisson (compois) family distribution, followed by Tukey HSD posthoc test for multiple comparisons. Percent male and female parasitoids were analyzed by Kruskal-Wallis non-parametric test followed by the Dunn test. The hind tibia length of male and female parasitoids was evaluated by the linear model (LM), followed by Tukey HSD posthoc test for multiple comparisons.

Experiment 4

Hierarchical clustering was computed for VOCs based on the length of the straight line drawn followed by Euclidean distance with complete linkage. Statistics were performed for individual VOCs, where the F-value comes from the linear model (LM), and χ^2 for the Kruskal-Wallis non-parametric analysis (n= 6). 2-way ANOVA was performed to analyze the total volatile organic compound from whole plant, followed by Tukey HSD posthoc test for multiple comparisons. The total volatile emissions were standardized by dividing the values by the fresh weight (FW) and leaf area (cm^2). Subsequently, a Kruskal-Wallis non-parametric test was performed to analyze the data. Parasitoid responses to the six different odor sources were analyzed by the generalized linear model (GLM) with binomial family distribution, followed by Tukey HSD posthoc test for multiple comparisons. The response variable was considered as a two-vector, with the number of successes representing all parasitoids that went to the respective odor source and the number of failures representing all parasitoids that went to the other five odor sources.

Experiment 5

Leaf phytohormones (n= 6) were analyzed with 3-way ANOVA by the linear model (LM), followed by Tukey HSD posthoc test for multiple comparisons. Salicylic acid (SA), and 12-oxo phytodienoic acid (OPDA) were computed from the original data set; however, Abscisic acid (ABA), Jasmonic acid (JA), and Jasmonic acid isoleucine (JAI) were computed after $\log_{10}$ transformation.

Experiment 6

Metabolomics data sets were obtained from phloem exudate samples (n= 6). A linear model was employed (LM) to analyze total amino acid, organic acid, fatty acid, and sugar metabolites and the sugar-to-amino acid ratio. Subsequently, a two-way ANOVA was conducted, and multiple comparisons were performed using the Tukey HSD posthoc test. The calculations for sugar content and the sugar-to-amino acid ratio were derived directly from the original dataset, while amino acid, organic acid, and fatty acid values were computed after applying a $\log_{10}$ transformation to the data. Other metabolites data were analyzed with MetaboAnalyst 5.0 software packages. Before the analysis, data were

normalized by log_{10} and auto-scaling (mean-centered and divided by the standard deviation of each variable). Both supervised partial least square-discrimination analysis (PLS-DA), and unsupervised principal component analysis (PCA) method was performed to reduce the dimensionality and visualize the relationship among different treatments. The top thirty-five compounds were identified based on the variable importance of projection (VIP) score.

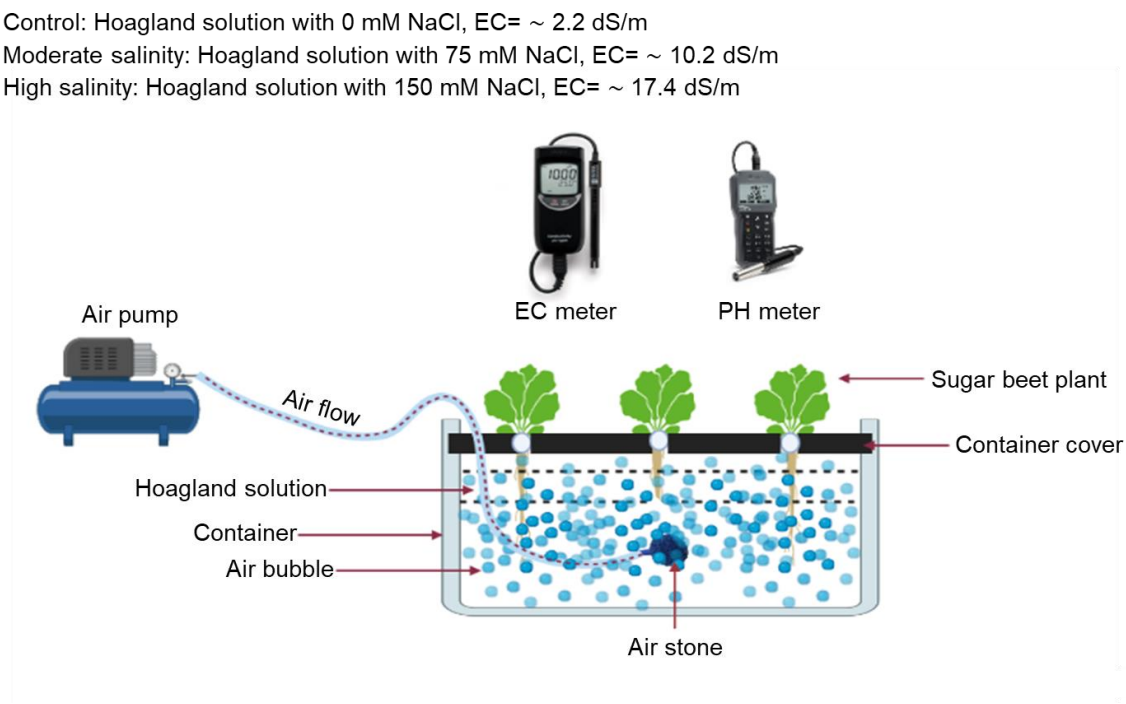


Figure S1: Schematic diagram of the hydroponic system to grow sugar beet plants for the conduction of salinity experiments.

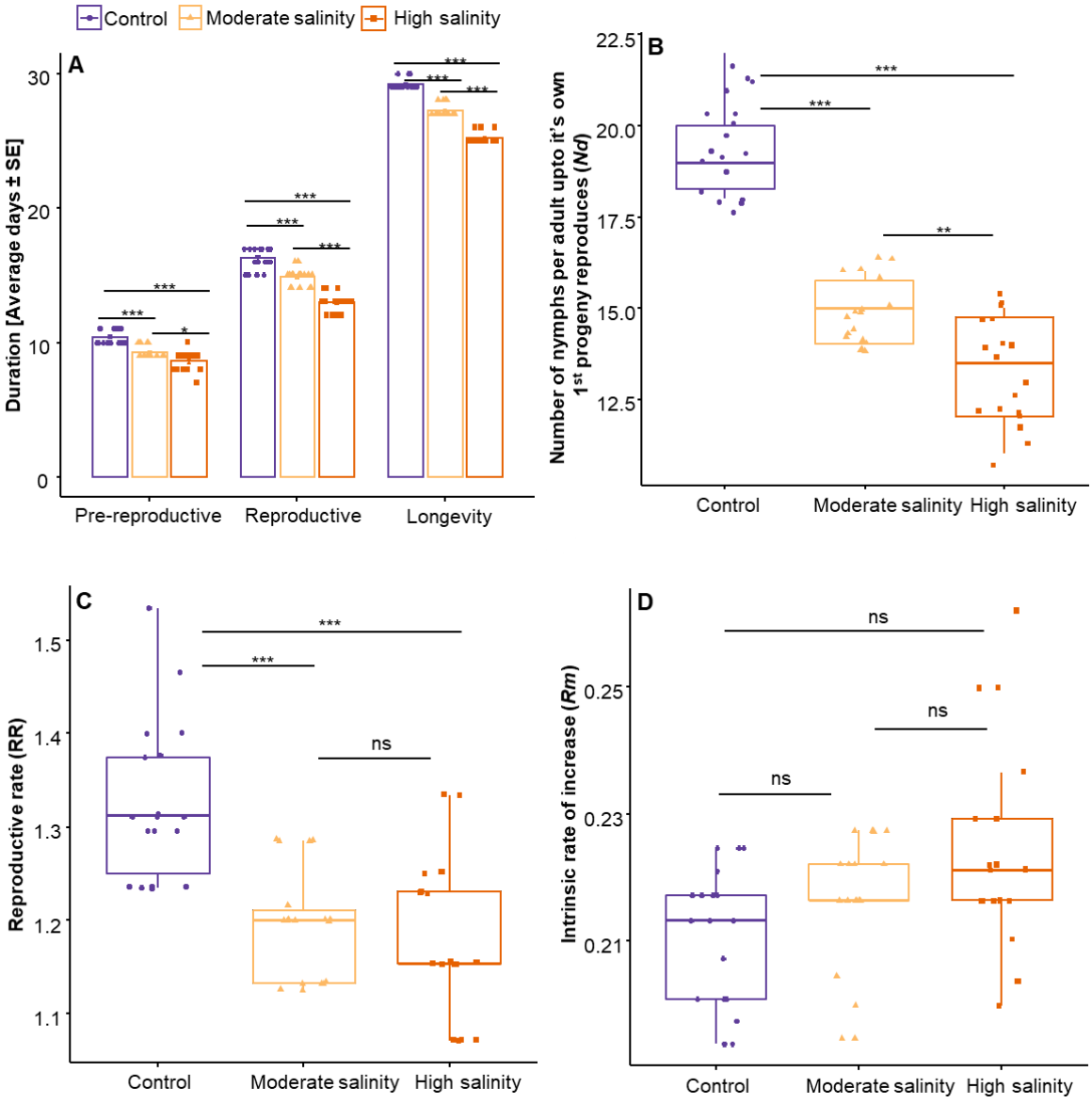


Figure S2: Effect of salinity stress (0 mM NaCl, 75 mM NaCl, 150 mM NaCl) of an individual life cycle of *Aphis fabae* on pre-reproductive period (Test: Kruskal-Wallis), reproductive period (Test: GLMM) and longevity (Test: Kruskal-Wallis) (A); number of aphid offspring (Test: GLMM) (B); average number of aphid per day (Test: GLMM) (C); and intrinsic rate of increase (Test: GLMM) (D). Data points represent individual replicates, and asterisks (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$) indicate significance among treatments, ns= non-significant, n= 18.

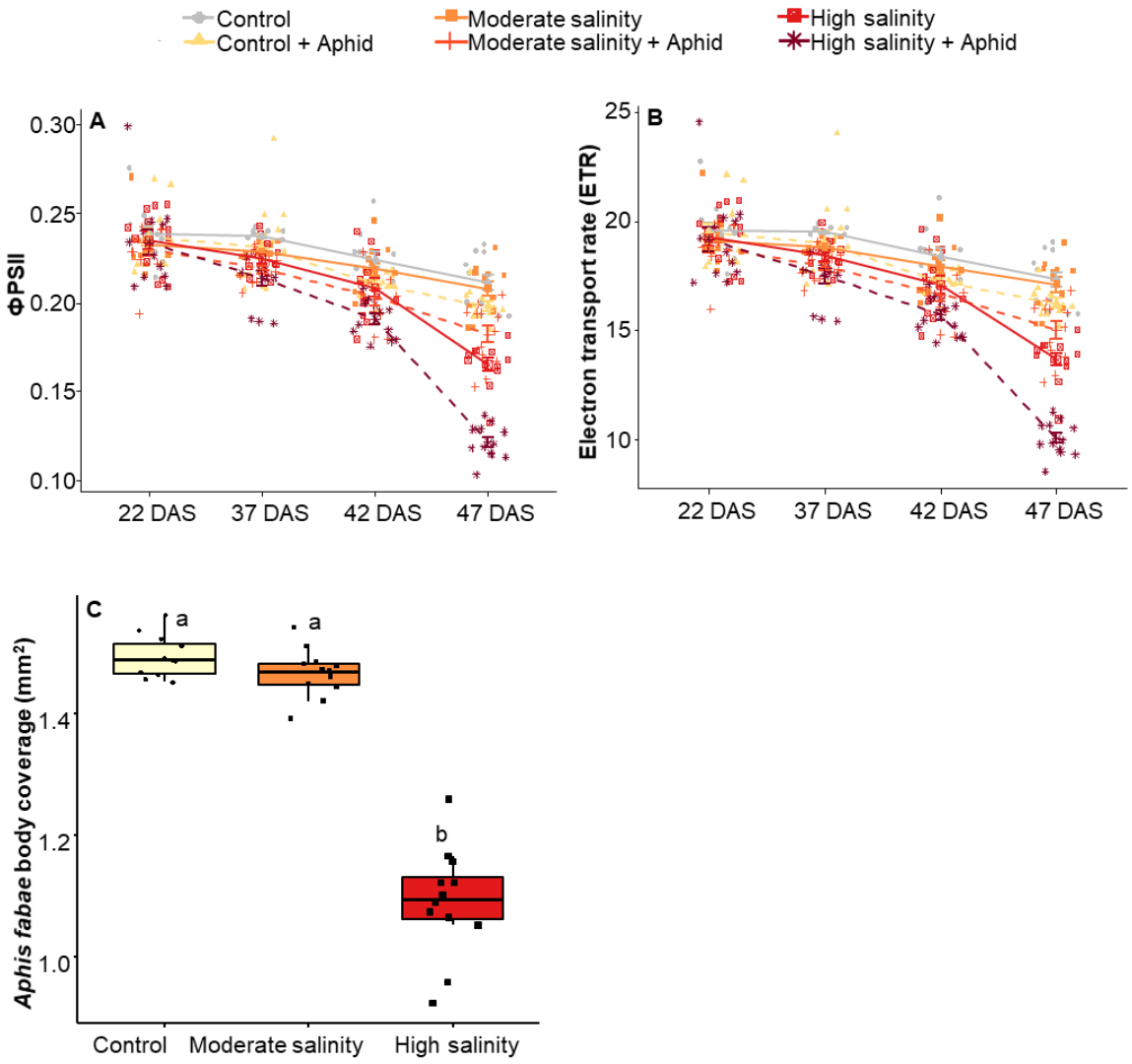


Figure S3: Effect of salinity stress (0 mM NaCl, 75 mM NaCl, 150 mM NaCl) and aphid infestation on sugar beet photosynthetic efficiencies measured as effective photochemical quantum yield (Φ_{PSII}) of PS II (Test: LM) (A); electron transport rate (ETR) (Test: LM) (B). Effect of salinity stress on aphid body coverage (Test: LM, n= 60 aphid) (C). Data points represent individual replicates, and different letters ($p \leq 0.05$) indicate significance among treatments, n= 12 plants.

Table S1: Volatile compounds detected in the headspace of sugar beet plants. Literature retention indices were extracted from the NIST chemistry webbook (https://webbook.nist.gov/chemistry/), based on best matching GC-MS method (Van Den Dool and Kratz RI, non-polar column, temperature ramp).

Compound name	Retention Index Experimental	Retention Index Literature
2-pentanone, 4-hydroxy-4-methyl-	845.25	841.3
3-hexen-1-ol	858.53	858
α-pinene	936.63	937
p-menthane	950.75	968
1-octen-3-ol	980.12	981
3-octanone	987.98	998
3-hexen-1-ol, acetate	1007.46	1005
3-carene	1013.47	1013
D-limonene	1032.22	1033
benzyl alcohol	1036.38	1037
eucalyptol	1036	1035
β-ocimene	1039.63	1044
trans-*β*-ocimene	1050.29	1050
acetophenone	1071.46	1073
p-cymene	1080.91	1033
benzene, 1,2,4,5-tetramethyl-	1125.25	1115.8
benzene, 1,3-diethyl-5-methyl-	1147.84	1143
p-cymen-7-ol	1259.96	1287
longifolene	1375.09	1387
β-caryophyllen	1402.77	1406
unknown RT- 18.559	1420.3	-----

Table S2: Statistical interactions of leaf photosynthetic efficiencies parameters

Photosynthetic efficiencies	Factors	Statistics
Maximum quantum yield of PS II (Fv/Fm)	Salinity	$F_{2,264} = 231.64$, $p < 0.001$
	Aphid	$F_{1,264} = 55.38$, $p < 0.001$
	Time	$F_{3,264} = 608.42$, $p < 0.001$
	Salinity × Aphid	$F_{2,264} = 10.42$, $p < 0.001$
	Salinity × Time	$F_{6,264} = 150.07$, $p < 0.001$
	Aphid × Time	$F_{3,264} = 12.45$, $p < 0.001$
	Salinity × Aphid × Time	$F_{6,264} = 5.27$, $p < 0.001$
Quantum yield (YII) of PS II	Salinity	$F_{2,264} = 70.38$, $p < 0.001$
	Aphid	$F_{1,264} = 63.12$, $p < 0.001$
	Time	$F_{3,264} = 191.40$, $p < 0.001$
	Salinity × Aphid	$F_{2,264} = 2.69$, $p = 0.06$
	Salinity × Time	$F_{6,264} = 23.89$, $p < 0.001$
	Aphid × Time	$F_{3,264} = 10.39$, $p < 0.001$
	Salinity × Aphid × Time	$F_{6,264} = 1.41$, $p = 0.20$
Electron transport rate (ETR)	Salinity	$F_{2,264} = 69.82$, $p < 0.001$
	Aphid	$F_{1,264} = 62.61$, $p < 0.001$
	Time	$F_{3,264} = 188.33$, $p < 0.001$
	Salinity × Aphid	$F_{2,264} = 2.68$, $p = 0.06$
	Salinity × Time	$F_{6,264} = 22.87$, $p < 0.001$
	Aphid × Time	$F_{3,264} = 10.21$, $p < 0.001$
	Salinity × Aphid × Time	$F_{6,264} = 1.55$, $p = 0.20$

Table S3: Estimated emission rate (ng/h/plant) of above-ground VOCs from differently treated sugar beet plants. CA- = Control without aphid, CA+ = Control with aphid, MSA- = Moderate salinity without aphid, MSA+ = Moderate salinity with aphid, HSA- = High salinity without aphid, HSA+ = High salinity with aphid.

Compound	**VOC emission per plant ng/h/plant ±SE**					
	CA-	**CA+**	**MSA-**	**MSA+**	**HSA-**	**HSA+**
2-pentanone, 4-hydroxy-4-methyl-	0.02±0.006	0.83±0.80	0.02±0.01	0.07±0.04	-----	0.73±0.70
3-hexen-1-ol	0.05±0.004 ab	0.08±0.02 a	0.03±0.01 ab	0.04±0.009 ab	0.01±0.004 b	-----
α-pinene	0.34±0.03	1.77±1.35	0.35±0.09	0.43±0.13	1.85±1.51	2.36±2.08
p-menthane	0.04±0.0009	0.04±0.009	-----	0.04±0.007	0.04±0.01	0.05±0.01
1-octen-3-ol	0.70±0.15 a	0.58±0.14 ab	0.35±0.06abc	0.40±0.09 abc	0.23±0.05 bc	0.12±0.05 c
3-octanone	0.62±0.13 a	0.42±0.09 ab	0.27±0.05 ab	0.32±0.06 ab	0.18±0.05 b	0.17±0.06 b
3-hexen-1-ol, acetate	2.10±0.60	1.82±0.64	1.14±0.45	0.66±0.19	0.78±0.30	0.96±0.44
3-carene	0.23±0.03	0.25±0.06	0.21±0.06	0.29±0.07	0.28±0.11	0.15±0.02
D-limonene	0.52±0.03	0.50±0.14	0.52±0.18	0.68±0.17	0.52±0.18	0.24±0.14
benzyl alcohol	-----	-----	0.51±0.17	0.43±0.15	0.67±0.30	0.41±0.19
eucalyptol	0.47±0.04	0.47±0.10	-----	-----	-----	-----
β-ocimene	0.33±0.05	0.59±0.24	0.16±0.07	0.24±0.03	0.51±0.39	0.62±0.54
trans-*β*-ocimene	0.18±0.03 ab	0.21±0.06 ab	0.12±0.02 ab	0.46±0.17 a	0.11±0.04 ab	0.04±0.01 b
acetophenone	0.43±0.02	0.42±0.09	0.34±0.12	0.41±0.06	0.52±0.19	0.36±0.06
p-cymene	0.16±0.01	0.19±0.07	0.14±0.06	0.13±0.06	0.31±0.14	0.05±0.02
benzene, 1,2,4,5-tetramethyl-	-----	0.32±0.18	-----	0.10±0.08	-----	0.12±0.07
benzene, 1,3-diethyl-5-methyl-	-----	0.21±0.11	-----	0.17±0.08	-----	0.09±0.06
p-cymen-7-ol	0.80±0.09	0.79±0.12	0.82±0.05	1.04±0.18	0.82±0.13	0.94±0.16
longifolene	-----	0.49±0.34	-----	0.19±0.11	-----	0.12±0.09
***β*-caryophyllen**	0.06±0.01 ab	0.42±0.26 a	-----	0.11±0.06 ab	-----	0.03±0.02 b
unknown RT- 18.559	-----	0.32±0.18	-----	-----	-----	-----

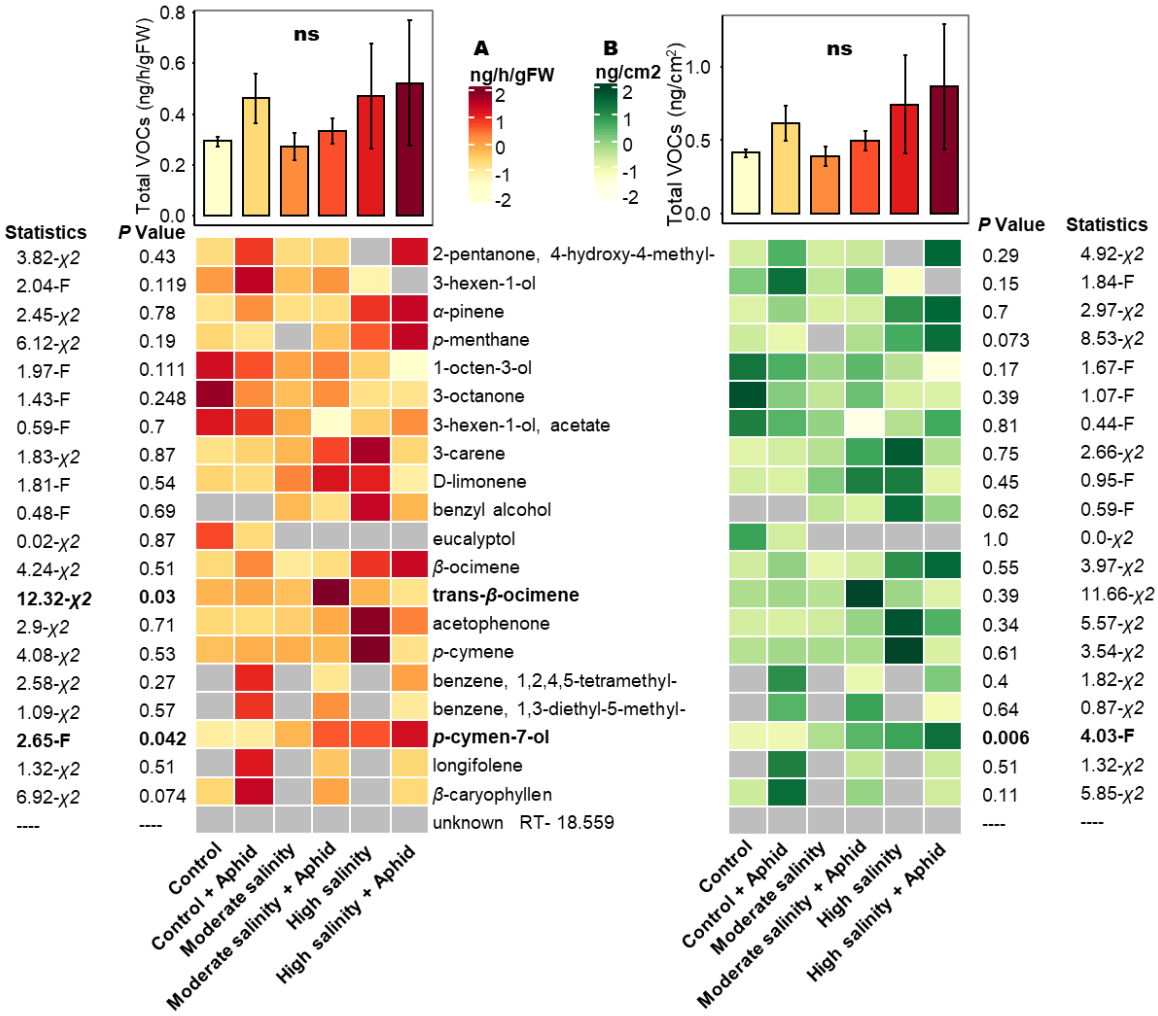


Figure S4: Effect of salinity stress (0 mM NaCl, 75 mM NaCl, 150 mM NaCl) and aphid infestation on volatile emission. Heat map represents emission rates of volatile organic compounds (VOCs) from treated sugar beet plants. Compound names and *P* values in bold indicate significant differences among treatments ($p \leq 0.05$). Volatile emission was standardized by plant biomass and is expressed as ng/h/gFW (A); volatile emission was standardized by leaf area and is expressed as ng/cm^2 (B), n= 6.

Table S4: Estimated emission rate (ng/h/g FW) of above-ground VOCs from differently treated sugar beet plants. CA- = Control without aphid, CA+ = Control with aphid, MSA- = Moderate salinity without aphid, MSA+ = Moderate salinity with aphid, HSA- = High salinity without aphid, HSA+ = High salinity with aphid.

Compound	**VOCs emission standardized by plant biomass ng/h/g FW ±SE**					
	CA-	**CA+**	**MSA-**	**MSA+**	**HSA-**	**HSA+**
2-pentanone, 4-hydroxy-4-methyl-	0.001±0.0002	0.039±0.03	0.001±0.0006	0.004±0.002	-----	0.049±0.04
3-hexen-1-ol	0.002±0.0001	0.004±0.001	0.002±0.0007	0.002±0.0005	0.001±0.0003	-----
α-pinene	0.014±0.001	0.081±0.06	0.019±0.005	0.023±0.007	0.127±0.10	0.160±0.14
p-menthane	0.002±0.0001	0.002±0.0004	-----	0.002±0.0004	0.003±0.001	0.003±0.0008
1-octen-3-ol	0.029±0.006	0.024±0.005	0.019±0.003	0.022±0.005	0.016±0.003	0.009±0.004
3-octanone	0.026±0.005	0.018±0.003	0.015±0.003	0.018±0.004	0.012±0.003	0.012±0.004
3-hexen-1-ol, acetate	0.087±0.02	0.081±0.02	0.061±0.02	0.035±0.01	0.054±0.02	0.067±0.03
3-carene	0.010±0.001	0.010±0.002	0.012±0.003	0.016±0.003	0.019±0.007	0.010±0.001
D-limonene	0.021±0.001	0.020±0.005	0.029±0.01	0.037±0.009	0.035±0.01	0.017±0.01
benzyl alcohol	-----	-----	0.028±0.01	0.023±0.008	0.046±0.02	0.029±0.01
eucalyptol	0.019±0.009	0.019±0.003	-----	-----	-----	-----
β-ocimene	0.014±0.002	0.026±0.01	0.009±0.004	0.013±0.001	0.035±0.02	0.042±0.03
trans-*β*-ocimene	0.007±0.001 ab	0.009±0.002 ab	0.007±0.001ab	0.024±0.009 a	0.008±0.003ab	0.003±0.001 b
acetophenone	0.018±0.002	0.018±0.003	0.019±0.007	0.022±0.003	0.036±0.01	0.025±0.005
p-cymene	0.007±0.0006	0.008±0.002	0.008±0.003	0.007±0.003	0.021±0.009	0.003±0.001
benzene, 1,2,4,5-tetramethyl-	-----	0.013±0.006	-----	0.005±0.004	-----	0.009±0.005
benzene, 1,3-diethyl-5-methyl-	-----	0.214±0.004	-----	0.165±0.004	-----	0.085±0.004
***p*-cymen-7-ol**	0.033±0.004 b	0.034±0.004 b	0.045±0.003 b	0.056±0.01 b	0.057±0.008 b	0.066±0.01 a
longifolene		0.019±0.01	-----	0.010±0.006	-----	0.009±0.007
β-caryophyllen	0.002±0.0007	0.017±0.01		0.006±0.003	-----	0.002±0.001
unknown RT- 18.559	-----	0.013±006	-----	-----	-----	-----

Table S5: Estimated emission rate (ng/cm^2 leaf area) of above-ground VOCs from differently treated sugarbeet plants. CA- = Control without aphid, CA+ = Control with aphid, MSA- = Moderate salinity without aphid, MSA+ = Moderate salinity with aphid, HSA- = High salinity without aphid, HSA+ = High salinity with aphid.

Compound	**VOC emission standardized by leaf area ng/cm^2 ± SE**					
	CA-	**CA+**	**MSA-**	**MSA+**	**HSA-**	**HSA+**
2-pentanone, 4-hydroxy-4-methyl-	0.001±0.0003	0.046±0.04	0.001±0.001	0.006±0.003	-----	0.083±0.08
3-hexen-1-ol	0.003±0.0002	0.004±0.001	0.002±0.0009	0.003±0.0008	0.001±0.0005	-----
α-pinene	0.020±0.001	0.098±0.07	0.027±0.007	0.035±0.01	0.205±0.16	0.272±0.24
p-menthane	0.003±0.0001	0.002±0.0005	-----	0.003±0.0006	0.004±0.001	0.006±0.001
1-octen-3-ol	0.041±0.008	0.034±0.008	0.027±0.004	0.032±0.007	0.025±0.006	0.014±0.006
3-octanone	0.036±0.007	0.025±0.005	0.021±0.004	0.026±0.005	0.020±0.006	0.019±0.006
3-hexen-1-ol, acetate	0.123±0.035	0.106±0.038	0.092±0.037	0.052±0.01	0.085±0.03	0.110±0.05
3-carene	0.013±0.001	0.015±0.003	0.017±0.005	0.023±0.005	0.030±0.01	0.017±0.002
D-limonene	0.030±0.002	0.029±0.008	0.040±0.01	0.055±0.01	0.055±0.01	0.027±0.01
benzyl alcohol	-----	-----	0.040±0.01	0.035±0.01	0.071±0.03	0.046±0.03
eucalyptol	0.027±0.002	0.027±0.006	-----	-----	-----	-----
β-ocimene	0.019±0.002	0.034±0.01	0.012±0.005	0.019±0.002	0.057±0.04	0.071±0.06
trans-*β*-ocimene	0.011±0.002	0.012±0.003	0.009±0.001	0.037±0.01	0.012±0.005	0.005±0.002
acetophenone	0.025±0.001	0.025±0.005	0.026±0.009	0.033±0.004	0.056±0.01	0.041±0.008
p-cymene	0.009±0.0008	0.011±0.004	0.011±0.004	0.010±0.005	0.033±0.01	0.006±0.002
benzene, 1,2,4,5-tetramethyl-	-----	0.019±0.01	-----	0.008±0.006	-----	0.014±0.008
benzene, 1,3-diethyl-5-methyl-	-----	0.013±0.006	-----	0.013±0.006	-----	0.010±0.007
***p*-cymen-7-ol**	0.047±0.006 b	0.046±0.007 b	0.065±0.003ab	0.083±0.01 ab	0.090±0.01 ab	0.106±0.01 a
longifolene	-----	0.028±0.02	-----	0.015±0.008	-----	0.014±0.01
β-caryophyllen	0.004±0.001	0.025±0.01	-----	0.009±0.005	-----	0.003±0.002
unknown RT- 18.559	-----	0.019±0.01	-----	-----	-----	-----

Table S6: Statistical interactions of the phytohormonal data set

Phytohormones	Factors	Statistics
Abscisic acid (ABA) log10 transformed	Salinity	$F_{2,84} = 728.06$, $p < 0.001$
	Aphid	$F_{1,84} = 34.52$, $p < 0.001$
	Time	$F_{2,84} = 23.68$, $P < 0.001$
	Salinity × Aphid	$F_{2,84} = 0.25$, $p = 0.77$
	Salinity × Time	$F_{4,84} = 6.16$, $p < 0.001$
	Aphid × Time	$F_{2,84} = 5.20$, $p = 0.007$
	Salinity × Aphid × Time	$F_{4,84} = 2.63$, $p = 0.039$
12-oxo Phytodienoic acid (OPDA)	Salinity	$F_{2,84} = 13.74$, $p < 0.001$
	Aphid	$F_{1,84} = 33.61$, $p < 0.001$
	Time	$F_{2,84} = 2.45$, $p = 0.09$
	Salinity × Aphid	$F_{2,84} = 3.04$, $p = 0.052$
	Salinity × Time	$F_{4,84} = 13.19$, $p < 0.001$
	Aphid × Time	$F_{2,84} = 20.39$, $p < 0.001$
	Salinity × Aphid × Time	$F_{4,84} = 9.75$, $p < 0.001$
Jasmonic acid (JA) log10 transformed	Salinity	$F2,84 = 0.90$, $p = 0.409$
	Aphid	$F_{1,84} = 349.77$, $p < 0.001$
	Time	$F_{2,84} = 34.99$, $p < 0.001$
	Salinity × Aphid	$F_{2,84} = 35.08$, $p < 0.001$
	Salinity × Time	$F_{4,84} = 9.02$, $p < 0.001$
	Aphid × Time	$F_{2,84} = 7.47$, $p = 0.001$
	Salinity × Aphid × Time	$F_{4,84} = 5.75$, $p < 0.001$
Jasmonic acid isoleucine (JAI) log10 transformed	Salinity	$F_{2,84} = 2.88$, $p = 0.061$
	Aphid	$F_{1,84} = 359.04$, $p < 0.001$
	Time	$F_{2,84} = 81.50$, $p < 0.001$
	Salinity × Aphid	$F_{2,84} = 26.48$, $p < 0.001$
	Salinity × Time	$F_{4,84} = 5.07$, $p = 0.001$
	Aphid × Time	$F_{2,84} = 3.45$, $p = 0.036$
	Salinity × Aphid × Time	$F_{4,84} = 7.75$, $p < 0.001$
Salicylic acid (SA)	Salinity	$F_{2,77} = 3.19$, $p = 0.046$
	Aphid	$F_{1,77} = 31.57$, $p < 0.001$
	Time	$F_{2,77} = 7.09$, $p = 0.001$
	Salinity × Aphid	$F_{2,77} = 3.88$, $p = 0.024$
	Salinity × Time	$F_{4,77} = 3.38$, $p = 0.013$
	Aphid × Time	$F_{2,77} = 7.25$, $p = 0.001$
	Salinity × Aphid × Time	$F_{4,77} = 1.33$, $p = 0.26$

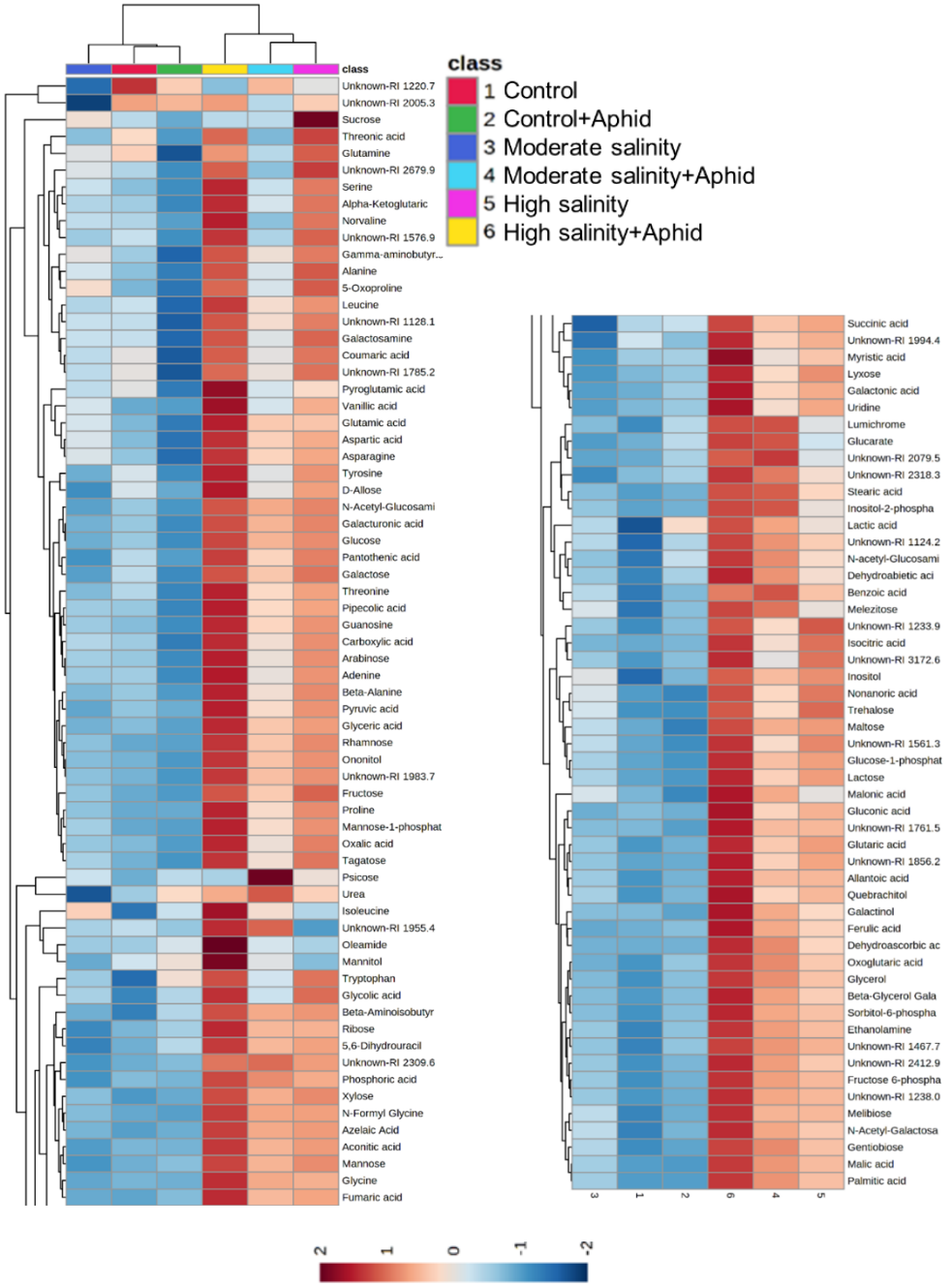
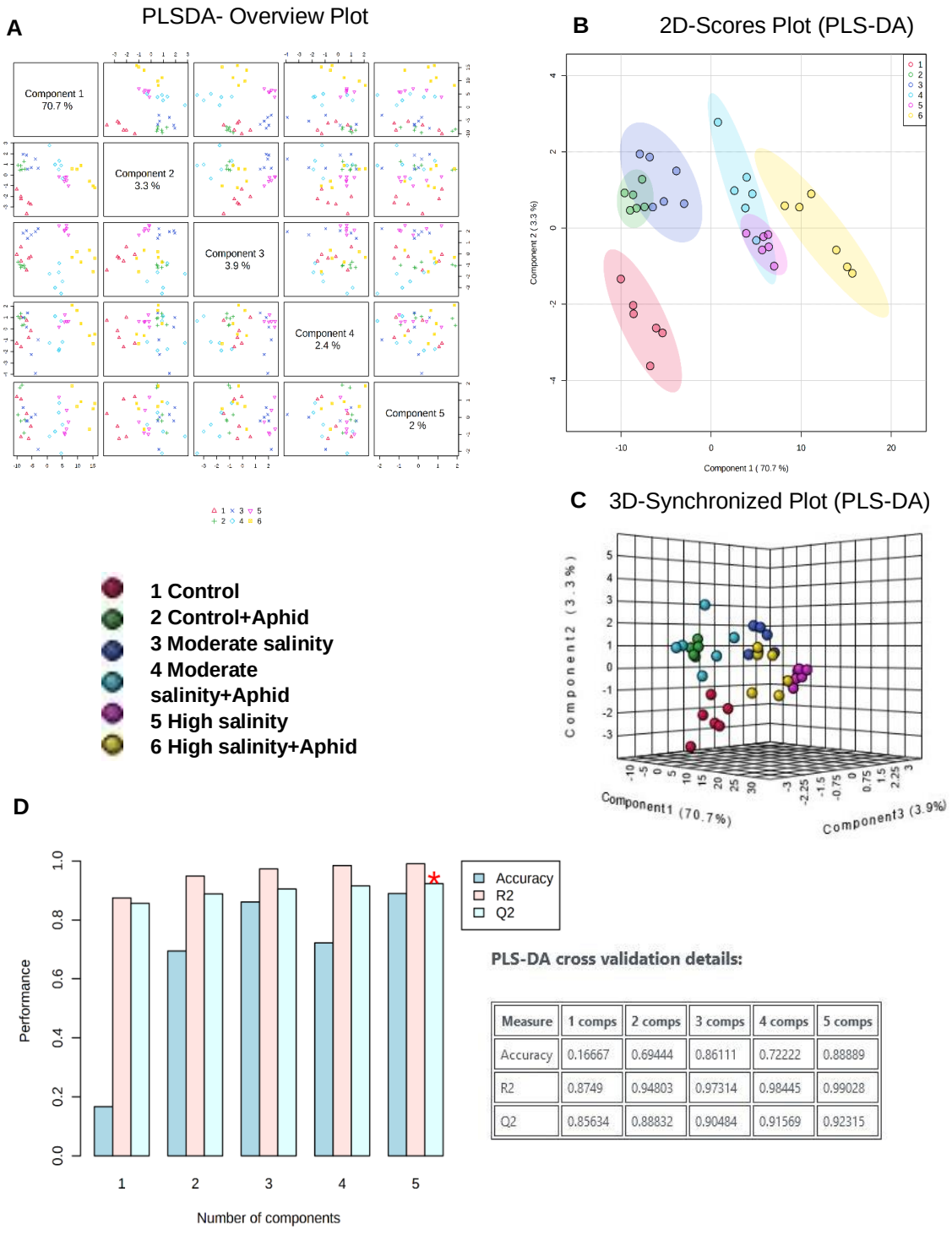


Figure S5: Effect of salinity stress (0 mM NaCl, 75 mM NaCl, 150 mM NaCl) and aphid infestation on the sugar beet plant central metabolites. The heatmap illustrates the hierarchical clustering of central metabolites collected from sugar beet phloem exudates, n=6.



Measure	1 comps	2 comps	3 comps	4 comps	5 comps
Accuracy	0.16667	0.69444	0.86111	0.72222	0.88889
R2	0.8749	0.94803	0.97314	0.98445	0.99028
Q2	0.85634	0.88832	0.90484	0.91569	0.92315

Figure S6: Partial least square discriminant analysis (PLS-DA) of central metabolites profile in sugar beet plant in response to salinity and aphid infestations are Illustrated as 95% confidence intervals for each group as overview plot (A); 2D- scores plot (B); 3D-synchronized plot (C); and PLS-DA performance measurements (D). The displayed results include the accuracy, multiple correlation coefficient R2, and the explained variance in prediction Q2. The red asterisk signifies the highest value among the selected measure (Q2). The color code indicates the treatments for the data points, n= 6.

Drought differentially affects the preference and performance of the beet leaf miner *Pegomya cunicularia* by altering plant chemistry

Shahinoor Rahman*[1], Musrat Zahan Surovy[2], Ilka Vosteen[1], Franz Hadacek[3], and Michael Rostás*[1]

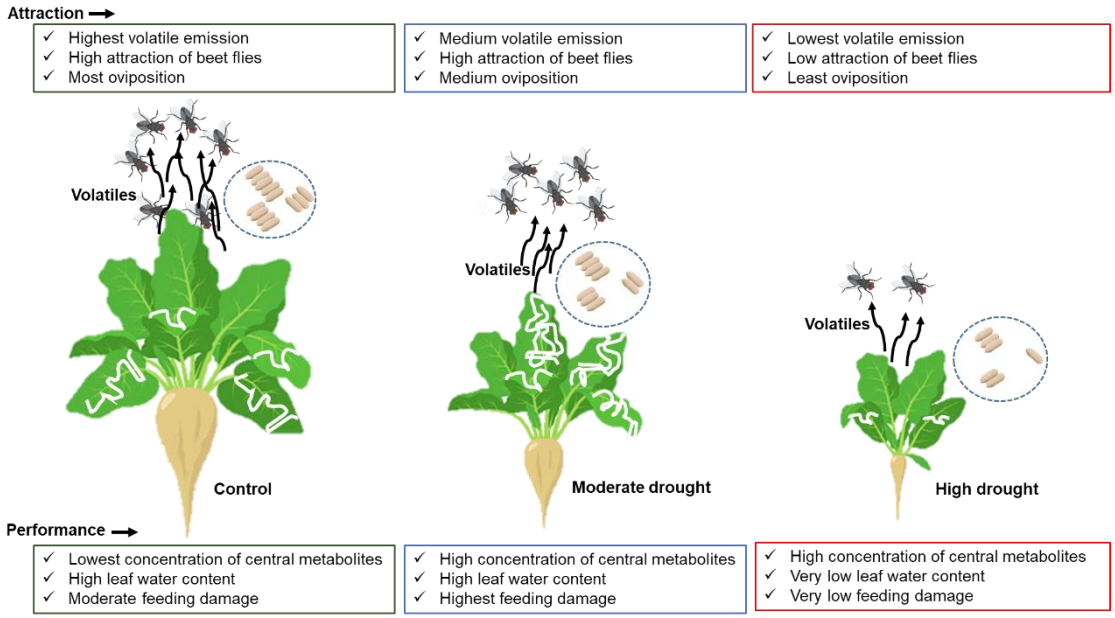
[1]Department of Crop Sciences, Division of Agricultural Entomology, Georg-August-University of Goettingen, Germany

[2]Department of Crop Sciences, Division of Plant Pathology and Crop Protection, Georg-August-University of Goettingen, Germany

[3]Department of Plant Biochemistry, Albrecht-Haller Institute for Plant Sciences, Georg-August-University of Goettingen, Germany

*Correspondence: shahinoor_ent@yahoo.com; michael.rostas@uni-goettingen.de

Graphical abstract



Abstract

Climate change leads to more frequent drought events that can severely impact sugar beet (*Beta vulgaris*) production in Europe. Insects also reduce sugar beet yield but there is little knowledge on the interactions between drought and herbivory. Here we comprehensively investigated how herbivory by the leaf mining fly *Pegomya cunicularia* (Diptera: Anthomyiidae) and two levels of drought alter the morphology and physiology of *B. vulgaris* and whether such changes affect the insect's behaviour and performance. Increasing drought stress led to stunted growth, lower biomass, higher root-to-shoot ratio, reduced leaf area, and fewer leaves. However, leaf water content was not significantly different between controls and moderately drought-stressed plants. Increasing drought stress alone resulted in decreasing photosynthetic capacity measured as chlorophyll fluorescence. In combination with herbivory, however, the strongest negative impact was found at moderate drought levels, which correlated with the most extensive feeding damage. Moderate drought also resulted in a higher number of emerging larvae and enhanced pupal and adult weights. Central metabolites analysis showed that increasing drought increased concentrations of amino acids, organic acids, fatty acids, and sugar metabolites but led to reduced emission of plant volatile organic compounds. This correlated with female flies preferring control plants for oviposition compared to plants experiencing moderate and high drought stress. Flies were also more strongly attracted towards the scent of control plants than to biotic (infested by beet leaf miners) and abiotic (drought) stressed plants in a Y-tube olfactometer. In summary, the present study suggests that moderate drought favours *P. cunicularia* which may lead to negative synergistic effects in sugar beet cultivation.

Keywords: Beet flies, Central metabolites, Chromatogram, Chlorophyll-fluorescence, Drought, GC-MS, Olfactometer, Oviposition, VOCs

Introduction

Drought is the most crucial limiting environmental factor for the sustainable production of crops (Ray et al., 2020). Global climate change alters precipitation patterns and distribution, leading to increased drought events, as recently experienced in central Europe (Boergens et al., 2020). Increased frequency and severity of droughts can have a broad range of impacts on agricultural crops, thus changing insect-plant interactions (Hamann et al., 2021; Lin et al., 2022a).

Drought negatively interferes with plant growth and development, decreasing plant height, shoot-root ratio, biomass, leaf area, leaf water content, etc. (Yang et al., 2021). During periods of drought, plants experience oxidative stress, leading to physiological changes such as alterations in photosynthetic activities (Yao et al., 2018; Zhuang et al., 2020). In response to oxidative damage, plants alter various cellular and molecular processes by accumulating different compatible solutes or osmolytes like sugars, proline, glycine etc. (Anjum et al., 2017; Sharma et al., 2019) to cope with drought stress. As a result, plants impair their metabolism, leading to changes in their nutritional composition (Fàbregas & Fernie, 2019). This could potentially lead to a decrease in the allocation of resources toward developing defense mechanisms against herbivores (Wittstock & Gershenzon, 2002). Additionally, nutritional status of plants, which is important for herbivores plays a significant role in insect growth rates and body mass (Boggs & Freeman, 2005; Mevi-Schütz, Goverde & Erhardt, 2003). Drought may enhance (Staley et al., 2008) or decrease (Staley et al., 2007) the performances of insects and observed patterns are highly inconsistent as the effects of drought on insect performance can depend on the magnitude of drought, specialist and generalist insects, feeding guides, host quality, and the host plant species (Carvajal Acosta et al., 2023; Kuczyk et al., 2021; Shehzad et al., 2021).

Moreover, decreased photosynthetic activity due to oxidative stress limits carbon fixation in drought-stressed plants and affects the amount of carbon that is available for the production of central and secondary metabolites such as anti-feedants, toxins, and volatiles. The biosynthesis of secondary metabolites like plant volatiles depends on primary metabolism (Pott et al. 2019), and plants may respond to carbon shortage by reallocating carbon to specific pathways and functions, which may, for example, result in increased VOC production rates upon

drought stress in some species (Szabó et al., 2020). Volatile emission rates do not only depend on de-novo synthesis and release from storage but, in the case of most alcohols, carbonyls, aldehydes, and oxygenated monoterpenes (compounds low Henry's law volatility constant, for definition see Sander, 2015), also on stomata conductance (Lin et al., 2022b). Drought stress usually results in changes in total VOC emission and blend composition, with increased emission rates of some compounds and reduced emission of other compounds (Catola et al., 2018; Pagadala Damodaram et al., 2021; Salerno et al., 2017; Tariq et al., 2013; Weldegergis et al., 2015). Herbivores use plant volatiles as cues to detect their host plants (Bruce et al., 2005; Bruce & Pickett, 2011), and female insects use these volatile cues for selecting oviposition sites (Honda, 1995). Due to the inducibility of VOC emission, herbivores can further use these compounds to avoid or seek plants that are attacked by con- or heterospecific herbivores (Bezerra et al., 2021). Drought-induced modifications of VOC might alter female oviposition preferences.

Sugar beet (*Beta vulgaris* spp. *vulgaris*) is the primary source of sugar in temperate regions, and European Union (EU) is the world's leading sugar beet producer, with around half of the global production; and in the year 2020-21, Germany is the top producer of sugar from sugar beet among all EU countries (Shahbandeh, 2023). Changing climate patterns, such as an increase in drought, and stricter pesticide regulations, including the ban of neonicotinoids in European countries, have led to an observed increase in pest pressure (Viric Gasparic et al., 2021). Beet leaf miner (*Pegomya cunicularia*) is an economically important sugar beet pest. Its larvae tunnel inside the sugar beet leaves, creating large irregular blotch-shaped mines in the leaves and occasionally causing serious damage to the beet (Michelsen, 1980). Drought might have the potential to alter the ecological adaptations of beet leaf miners that may create conditions more conducive to their reproduction. After the ban of neonicotinoid insecticides, the increase of leaf miners was recently observed, threatening sugar beet production. To date, no comprehensive study has confirmed the effect of drought on sugar beet leaf miners and their potential mechanisms of interaction. In this comprehensive study, we specifically addressed the effects of varying levels of drought on sugar beet leaf miners and aimed to explore the following questions:

(i) How do drought and interactions with beet leaf miners change the profiling of volatiles, and does these volatiles have any effects on the oviposition preferences of beet flies? (ii) How do drought and interactions with leaf miners affect the profiling of plant central metabolites, and is there any relationship that can explain the growth performances of the beet leaf miner?

Materials and Methods

Insects, plants, drought system, and experimental setup

Adults of the beet leaf miner *Pegomya cunicularia* (Diptera: Anthomyiidae) were collected from sugar beet fields near Göttingen, Germany, and artificial mass-reared was carried out to continue the experiments. Detailed information about the methodology of rearing techniques can be found in the supporting information (Figure S1).

Seeds of *B. vulgaris* subspec. *vulgaris* cultivar 'Vasco' (SESVanderHave, Belgium) were sown in plastic trays (54 holes, each 3.5 cm diameter) filled with quartz sand (0.2-0.8 mm). The growing of the seedlings to the whole experiments were carried out in controlled climate conditions (16L:8D photoperiod; light intensity: 130 ± 10 µmol/(s m^2); relative humidity: 65 ± 5 %; temperature: 20 ± 2 °C). Seedlings were grown by supplying tap water for up to eleven days. From twelve days after sowing (DAS) to 23 DAS, seedlings were supplied with half-strength modified Hoagland solution (HS) according to the protocol described in chapter 1.

For all experiments, at 24 DAS, seedlings were transferred to the capillary action-based drought system as described in chapter 1, and drought was implemented from 27 DAS by maintaining three drought levels i) Control: ~40% volumetric water content (VWC) ii) moderate drought: ~16% VWC and iii) High drought ~10% VWC.

Experiment 1: Plant performance

Plant height was measured in three-time points starting at two true leaf stages (26 DAS before the drought was implemented), 36 DAS, and 46 DAS. At 58 DAS plants were harvested, and total biomass, root weight, shoot weight, root length, shoot length, root shoot ratio by weight and length, number of total leaves,

and the average leaf area (LI-3100C Area Meter, LI-COR Biosciences, Germany) were recorded.

Leaf water content

After measuring the plant morphological parameters, leaf disks (3.5 cm diameter) were cut off from two fully expanded leaves of each plant, weighed, and recorded as FW, then dried 72 h at 60°C in a drying chamber (BD-115, Binder, Germany). The dry matter weighed was recorded as DW. Leaf disks were weighed in high precision balance (KC BA 100, Sartorius Micro, Germany). The leaf water content was calculated as the following:

Water content (%) = (FW-DW)/FW*100

Where, FW = Fresh weight of the leaf disk, DW = Dry weight of the leaf disk

Experiment 2: Oviposition preferences of sugar beet fly Pegomya cunicularia

Plants were infested with *P. cunicularia* at 36 DAS by placing four eggs (age ≤ 24h) on the abaxial side of each leaf (2nd two opposite expanded leaves) with a fine brush. In general, it takes five days to emerge as larvae, and 42 days old plants were used to conduct choice and no-choice assays. Two different choice tests were conducted, one with three treatments (Control, Moderate drought, and High drought) in one tent, and another with six different treatments (Control, Control + beet miner, Moderate drought, Moderate drought + beet miner, High drought, and High drought + beet miner) in another tent (60 × 60 × 70 cm). In the case of the no-choice assay, a separate tent was used for each of the six treatments. For each tent, three female *P. cunicularia* (have no plant volatiles experience before, 10 days old-having high potential of oviposition) were released for 24 h, and the eggs were counted. In total, 18 replication were carried out for each of the bioassays.

Experiment 3: Y-tube olfactometer

The olfactometer consisted of a 3.5 cm inner diameter glass Y-tube, each arm 20 cm long, positioned inside a chamber (60 × 60 × 60 cm; Yorbay eBusiness GmbH, Germany) on a green platform inclined at an angle of 8°, and the chamber was homogeneously illuminated with LED (5500K) light. The plants used as

stimuli (combination listed below) were wrapped in oven bags (Bratschlauch, Toppits, Germany), with the open ends of the bags securely sealed to the tubing (6.4 mm ø, Tygon S3 E-3603, Saint-Gobain Ceramics & Plastics, Ohio, USA). Filtered (activated charcoal filter, 400 ccs, Alltech, Deerfield, IL, USA) and humidified air was pushed into each vessel at a rate of 1.0 L per minute, originating from a central in-house compressor.

Plants (42 DAS) and the flies were prepared as described in the “choice and no choice test” for conducting a Y-tube olfactometer assay. Odor pairs were offered to the flies as follows: i) Soil substrate vs control, ii) Moderate drought vs control, iii) High drought vs control, iv) Control + beet miner vs control, v) Moderate drought + beet miner vs control and vi) High drought + beet miner vs control.

A total of 36 flies were tested for one combination. After testing six flies, the position of the odors in the olfactometer was altered. After testing 12 flies, new combinations of plants and a fresh Y-tube olfactometer were utilized. One fly at a single time was placed at the opening of the Y-tube and kept for 3 min to make a choice. A choice was recorded once the flies had entered one of the two odor-permeated arms. To reuse the Y-tube olfactometer, at first, cleaned with demineralization water and then rinsed with 99.5% acetone. After evaporating the solvents, all cleaned glassware was placed in an oven for two hours at 180 °C.

Experiment 4: Analysis of plant volatiles

For the collection of volatiles from six different treatments, plants (42 DAS) were treated as described in the choice tests. The lower part of the six-arm olfactometer (Turlings et al., 2004) was used to collect volatiles, enabling the collection of volatiles from six different treatments. Six replicate days were considered to collect volatiles. Before collecting the volatiles, plants were bagged in polyester foil without plasticizer (Toppits Bratschlauch, Cofresco Frischhalteprodukte GmbH & Co. KG, Germany), and collections of volatiles were carried out for 24 h (from 09:00 to 09:00 the next day). Ten fluorescent lamps (PAR: 130 µmol photons m^{-2} s^{-1} at 3 cm distance from lamps) illuminated the plants during VOC collection for 16 hours during the day and were switched off for 8 hours during night conditions. Dynamic headspace extraction technique was used and analyzed on Agilent Technologies (GC 7890B, MS 5977B) as described

in chapter 1. The software MSD ChemStation with the NIST17 and Wiley11 mass spectral libraries was used to tentatively identify compounds by their mass spectra and retention indices. Compound quantification was achieved by comparing peak areas to the peak area of the internal standard. The calculated retention indices from the experiment and compared through literature indices based on the NIST database were summarized in Table S1.

Experiment 5: Leaf photosynthetic efficiencies

At 40 DAS, four eggs (age ≤ 24 h) were placed on the abaxial side of each leaf (2^{nd} two opposite expanded leaves) with a fine brash. The leaf chlorophyll fluorescence parameters (photosynthetic efficiencies) were measured at 39 DAS (before eggs were placed), 44 DAS, 49 DAS, and 54 DAS. The plants were kept in the dark for 30 minutes before measurement. Photosynthesis yield analyzer (MINI-PAM-II, Heinz Walz GmbH, Germany) was used to measure the chlorophyll fluorescence parameters like Maximum photochemical quantum yield of Photosystem (PS) II (Fv/Fm), the effective photochemical quantum yield of PS II (Φ_{PSII}), and electron transport rate (ETR).

B*eet leaf miner performances*

Daily observations were made to track the timing and percentage of larval emergence from the eggs, which were carefully placed on the leaves. At 55 DAS, leaves were cut at the base of the petiole. Photographs were taken to facilitate later analysis using Image-J software to assess the mined leaf area and placed the leaves in small boxes having a mixture of soil substrate (sand: clay: organic matter; 2:1:1) for pupation. Egg to pupal time was recorded, and pupae were weighed (KC BA 100, Sartorius Micro, Germany) and returned to the boxes until they emerged. After emergence, adult flies were killed with CO_2 and weighed. Pupae to adult time was also recorded.

Experiment 6: Central metabolites analysis

Plants (42 DAS) receiving drought and herbivore treatment were prepared as described in the choice and no choice tests for collecting leaf samples to analyze central metabolites. During the collection of leaf samples, larvae were quickly removed from leaves, cut at the leaf's base, placed into the falcon tube (50 ml), and immediately dipped in liquid nitrogen. All of these works were done

within 30 sec. Then the samples were lyophilized, powdered, and stored at -80°C. About 40 mg powdered samples were weighed (KC BA 100, Sartorius Micro, Germany) into 1.5 ml Eppendorf tube, added 1ml of methanol (99.95 % v/v LC-MS Grade), vortexed for 30 sec., ultra-sonicated for 3 min (VWR-600THD, Malaysia), centrifuged at 20156 g for 10 min (Eppendorf-5425, Germany), and then supernatant transferred to 1.5 ml glass vial. Blank samples were also prepared without leaf material. To each sample, 20 µl adonitol (prepared as 20 ng/µl of 99.95 % LC-MS Grade methanol) was added as an internal standard and then dried with a speed vac at 30°C for 300 min (RVC 2-25 CD plus, Germany). Argon gas was added to each sample to prevent oxidation, and samples were stored overnight at -80°C. The next day, 200 µl methoxyamination reagent (prepared by 20 mg/ml of methoxyamine hydrochloride in pure pyridine) was added to each sample, including blanks. The mixtures were vortexed, short centrifuged, and placed in a shaker for 90 min. After that, 20 µl samples were transferred to a glass insert with a GC-MS glass vial. 20 µl *N*-methyl-*N*-trimethylsilyl-trifluoracetamide (MSTFA) was added to each sample and vortexed for 10 sec. Samples were run with Agilent Technologies (GC: 7890B, MSD: 5977B, USA) fitted with Restek Rtx-5 w/Integra-Guard column (30 m × 0.25 mm ID × 0.25 µm df, USA). PAL autosampler (PAL RSI 85, Switzerland) was used to inject 1µl of samples with a split ratio of 20:1. The oven temp. was held at 70 °C for 2 min and then increased gradually to a final temp. of 325 °C, held for 10 min. Helium (1 ml min^{-1}) was used as the carrier gas. *n*-Alkane standards (C8-C40) were analyzed for determining retention indices. Metabolite derivatives were identified by comparing their mass spectra and retention indices with data bank entries in the Golm Metabolome Database (GMD), Fein BinBase database, and database from our experience with the help of MS-DIAL (ver. 4.8) software. Metabolites were quantified relative to the peak area of the internal standard adonitol. Metabolites labeled as “unknown” were not found in open-source databases but were encountered several times in other plant samples based on our experience.

Statistical analysis

All data were analyzed using the statistics package R-version 4.2.1 integrated with R-Studio Desktop-version 2022.07.02+576 and the

MetaboAnalyst 5.0. Various models were considered for analyzing different types of data, and the most appropriate model was selected based on its assumptions. Detailed information about the statistical analysis can be found in the supporting information.

Results

Effect of drought stress on the performance of sugar beet plant

Drought stress had a negative effect on sugar beet plant height over time (GLMM: Drought: χ^2= 92.19, p < 0.001; Time: χ^2= 281.37, p < 0.001). Similarly, high drought significantly reduced total plant biomass (LMM: $F_{2,6}$= 484.34, p < 0.001), root weight (LMM: $F_{2,6}$= 63.50, p < 0.001), shoot weight (LMM: $F_{2,6}$= 268.07, p < 0.001), and shoot length (Kruskal-Wallis: $\chi^2_{(df\ 2)}$= 47.17, p < 0.001). However, root length (LMM: $F_{2,6}$= 13.49, p = 0.006), root-to-shoot ratio by length (GLMM: $\chi^2_{(df\ 2)}$= 43.79, p < 0.001), and by weight (LMM: $F_{2,6}$= 16.50, p = 0.003) was highest in high-stressed plants. The total number of leaves (Kruskal-Wallis: $\chi^2_{(df\ 2)}$= 45.14, p < 0.001) and the average leaf area (LMM: $F_{2,6}$= 893.11, p < 0.001) significantly reduced in high-stressed plants compared to control plants. These plant morphological characteristics are already evident in moderate drought conditions and become more pronounced under high drought (Figure S2 A-J). Moreover, significantly lowest amount of leaf water was found in high drought-stressed plants compared to control plants (Kruskal-Wallis: $\chi^2_{(df\ 2)}$= 38.36, p < 0.001), but no difference was observed between control and moderate drought plants (Figure S3).

Oviposition and behavioral responses of beet flies

Female flies showed significantly highest number (64) of egg on control plants (GLMM: $\chi^2_{(df\ 2)}$= 44.43, p < 0.001; control vs moderate: p < 0.001, control vs high: p < 0.001, moderate vs high: p < 0.001) compared to moderate (16) and high drought (5) plants (Figure 1A) and in total 85 eggs were recorded on the plants in one tent (choice test) having abiotic treatments. Similarly, in another choice test with all biotic and abiotic treatments, 92 eggs were found over the six treated plants (Figure 1B). Significantly highest number (41) of eggs was found on control plants (GLMM: $\chi^2_{(df\ 2)}$= 77.88, p < 0.001), followed by moderate drought (20) and high drought (8) plants. Interestingly, flies were not so interested

in laying their eggs on plants already infested by beet miners, evidenced by the significantly lowest number of eggs (Figure 1B).

However, in no-choice test showed oviposition significantly varies depending on the treatments (GLMM: $\chi^2_{(df\ 5)}$= 64.58, $p < 0.001$; control vs moderate drought: $p = 0.19$ (ns), control vs high drought: $p < 0.001$, moderate drought vs high drought: $p < 0.001$) (Figure 1C). Interestingly, a significantly lower number of eggs were found on plants infested with beet miners (Figure 1C).

The Y-tube olfactometer test confirmed that flies exhibited significantly higher attraction towards sugar beet plant volatiles in comparison to the soil substrate (Binomial GLM: $\chi^2_{(df\ 1)} = 64.47$, $p < 0.001$) (Figure 1D). A significantly higher attraction was also observed towards control plants in comparison to high drought plants (Binomial GLM: $\chi^2_{(df\ 1)} = 92.92$, $p < 0.0089$), as well as high drought + beet miner plants (Binomial GLM: $\chi^2_{(df\ 1)} = 86.85$, $p < 0.001$). However, no significant differences were found for other odor pairs.

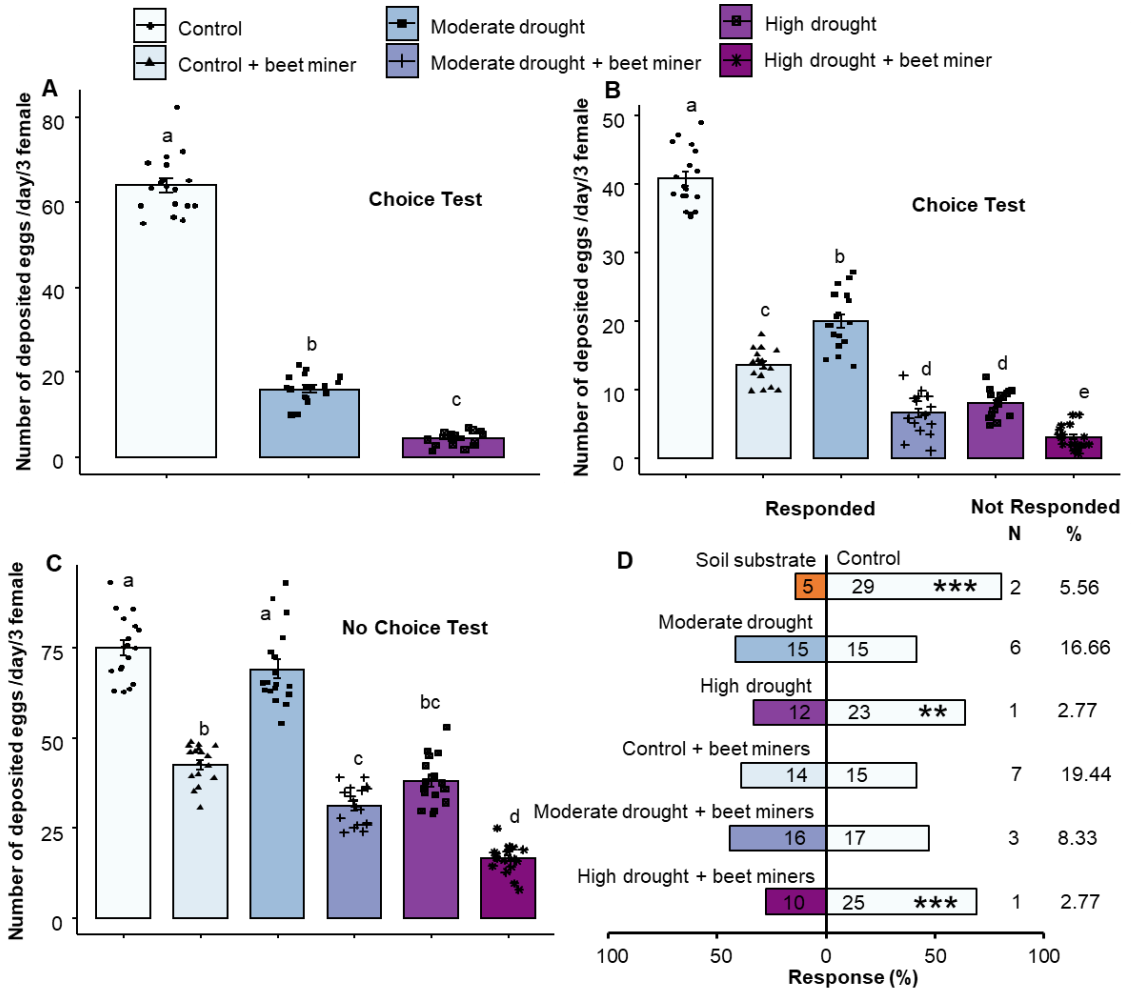


Figure 1: Oviposition and behavioral responses of female beet flies (age: 10 days- high potential of egg laying time, no plant volatile experience before). Plants are 42 days old, having 15 days of drought stresses (starting at 27 days after sowing (DAS)) and 24 h infestation (egg insertion at 36 DAS) time. Choice test having drought treatments in a single tent (Test: GLMM) (A); Choice test having all treatments (drought and beet miner infestation) in a single tent (Test: GLMM) (B); No choice test having all treatments (drought and beet miner infestation) in separate tents (Test: GLMM) (C). For figures A, B, and C, data point represents individual replicates, and different letters ($p \leq 0.05$) indicate significance among treatments, n = 18. Number of flies response in Y-tube olfactometer across different odor sources (Test: Binomial GLM) (D). Asterisks (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$) indicate significance among treatments, n = 36.

Analysis of VOCs

Hierarchical clustering of quantitative and qualitative differences between the volatile blends reveals that the blend emitted from the beet miner-infested control plant is distinct from other treatments (Figure 2A, Table S2). However, when compared to per g FW of leaves, high drought with beet miner-infested plants released a more concentrated blend of VOC (Figure 2B, Table S3). Mostly monoterpenes (trans-2-hexanal, 3-hexanal, octanal, cis-3-hexanyl-1-acetate, 3-carene, 2-ethylhexanol etc.) were released from control plants with lower concentrations, but higher concentrations released from beet miner-infested

plants. Sesquiterpene, namely *γ*-elemene, *β*-guanine, carotol released from beet miner-infested plants with higher concentration compared to moderate drought + beet miner stressed plants; however, in high drought + beet miner plants, no sesquiterpene was detected (Figure 2A, B). Among the blend of these VOCs, they were positively and negatively correlated to each other for eg. D-limonene was highly positively correlated with octanal, trans-2-hexanal, and p-cymen-7-ol, respectively, whereas carotol and *β*-guaiene were negatively correlated (Figure S4).

Drought and beet miner infestation had a significant effect on total VOC emission from the whole plant (LM: Drought: $F_{2,30}$= 26.16, $p < 0.001$; Beet miner: $F_{1,30}$= 15.67, $p < 0.001$; Drought × Beet miner: $F_{2,30}$= 1.18, $p = 0.319$) and also when standardized by per gram fresh weight (LM: Drought: $F_{2,30}$= 9.02, $p <0.001$; Beet miner: $F_{1,30}$= 12.39, $p = 0.0013$; Drought × Beet miner: $F_{2,30}$= 2.59, $p = 0.091$) (Figure 2C). Total VOC emission was higher in high drought with beet miner-infested plants when standardized by per gram fresh weight. However, when compared to the emission of the entire plant, it was found that control plants with beet miner infestation exhibited significantly higher emission levels (Figure 2C).

The Venn diagram (Figure 2D) shows the general pattern whereby many VOCs are found in the control and control + beet miner plants and shared with the other stressed plants. Drought stress emits few numbers (moderate drought: 11, high drought: 10) of VOCs compared to control (23) plants. Interestingly, beet miner-infested plant emits more VOCs than associated treatments (control + beet miner: 31, moderate drought + beet miner: 19, high drought + beet miner: 18) (Figure 2D).

To reduce the dimensionality of the dataset and visualize the relationship among samples, principal component analysis (PCA) was performed. The scores plot between PC 1 and PC 2 shows a clear separation among treatments. PC 1 explained 37.7 % of the total variation, while PC 2 explained 19.5 % variation across the data set (Figure S5 A). This indicates the changes in the volatile profile caused by drought and beet-miner stresses.

To investigate putative differences and identify stress-responsive VOCs, partial least squares-discriminant analysis (PLS-DA) was performed for all treatments. The scores plot explained 34.7% and 20.6% of the total variation in

component 1 and component 2, respectively (Figure S5 B). Based on the variable importance in projection (VIP) score, the top fifteen VOCs were identified, and their changing pattern was presented in Figure S5 C.

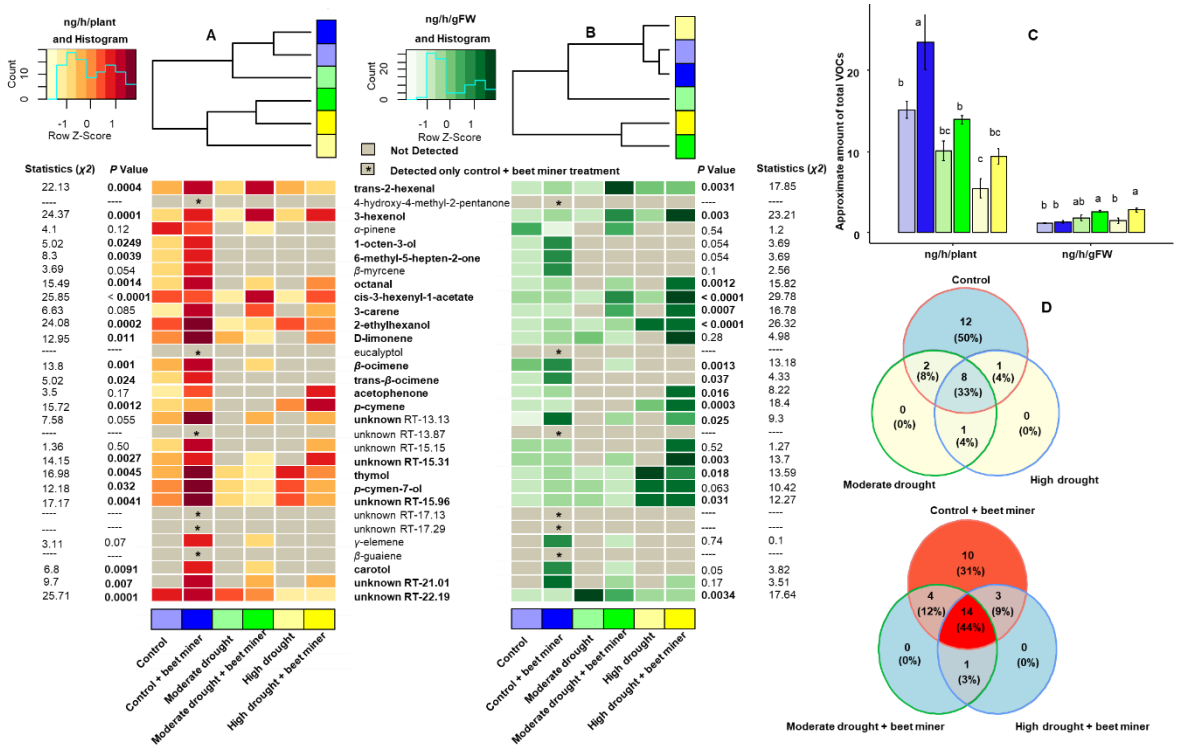


Figure 2: Effect of drought stress and beet miner infestation on volatile emission. Heatmap represents the emission rate of specific volatile organic compounds emission from whole plant (ng/h/plant) (Test: Kruskal-Wallis) (A); and VOC emission standardized by plant fresh weight (ng/h/gFW) (Test: Kruskal-Wallis) (B). Compound names and *P* values in bold indicate significant differences among treatments ($p \leq 0.05$, n = 6). Bar represents the approximate total amount of VOC as expressed (ng/h/plant) and (ng/h/gFW) (LM, 2-way ANOVA) (C); and Venn diagrams showing the number and percentage of VOCs shared among different treatments (D).

Effect of drought and beet miner infestation on leaf photosynthetic efficiencies

Drought stress and beet miner infestation significantly affected the photosynthetic efficiencies in sugar beet leaves over time. The presence of drought stress resulted in a notable decrease in the maximum quantum yield of PS II (Fv/Fm) (LM: $F_{2,408} = 601.66$, $p < 0.001$). This reduction was particularly pronounced in plants exposed to high drought levels over an extended period (LM: $F_{3,408} = 4519.50$, $p < 0.001$). Additionally, beet mining further exacerbated the decrease in photosynthetic efficiencies, with a more pronounced effect observed in moderate drought-stressed plants (LM: $F_{1,408} = 7656.06$, $p < 0.001$) (Figure 3A). Beet miner infestation at moderate drought conditions also significantly reduced the effective photochemical quantum yield (Φ_{PSII}) of PS II

(LM: $F_{1,408}$ = 2015.43, p < 0.001) (Figure 3B) compared to high drought and control conditions. A similar trend was noticed in the measurement of electron transport rate (ETR) (Figure S6). The specific statistical interactions can be found in Table S4.

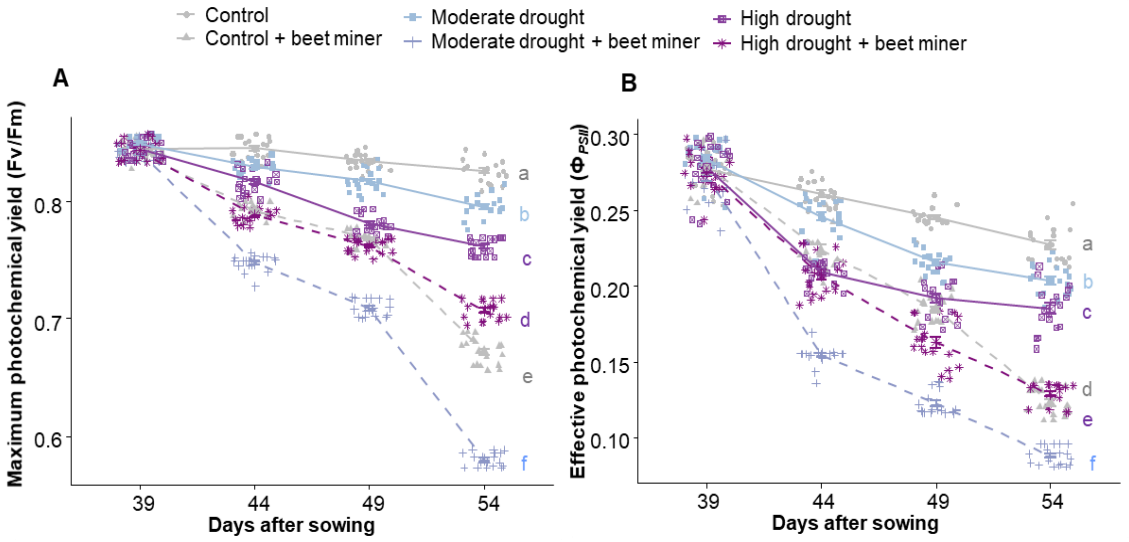


Figure 3: Effect of drought stress and beet miner infestation on the parameter of sugar beet leaf photosynthetic efficiencies (Test: LM 3-way ANOVA) of maximum photochemical quantum yield of photosystem (PS) II (Fv/Fm) (A); and effective photochemical quantum yield of PS II (Φ_{PSII}) (B). Data point represents individual replicates, and different letters ($p \leq 0.05$) indicate significance among treatments, n = 18.

Effect of drought stress on the performance of beet leaf miner

Drought had a significant effect on the number of hatched larvae (Binomial GLM: $\chi^2_{(df\ 2)}$= 20.05, p < 0.001). Significantly highest larvae emerged from moderate drought-stressed plants, and the lowest was observed in high drought-stressed plants (Figure 4A). Similarly, significantly most serious damage was found in terms of leaf area (cm^2) (Figure S7) as well as percent leaf mined area (Kruskal-Wallis: $\chi^2_{(df\ 2)}$= 41.54, p < 0.001) in moderate drought plants (Figure 4B). Moreover, moderate drought favors *P. cunicularia* as evidenced by highest pupal (LMM: $F_{2,14}$= 9.30, p = 0.002) and adult (LMM: $F_{2,14}$= 13.53, p < 0.001) weight (Figure 4C). As the same fashion, in the moderate drought plants, *Pegomya* takes more time to develop as the egg to pupal time (Kruskal-Wallis: $\chi^2_{(df\ 2)}$= 61.98, p < 0.001), pupae to adult time (LMM: $F_{2,14}$= 16.10, p < 0.001) and as well total development time (Kruskal-Wallis: $\chi^2_{(df\ 2)}$= 34.38, p < 0.001) (Figure 4D).

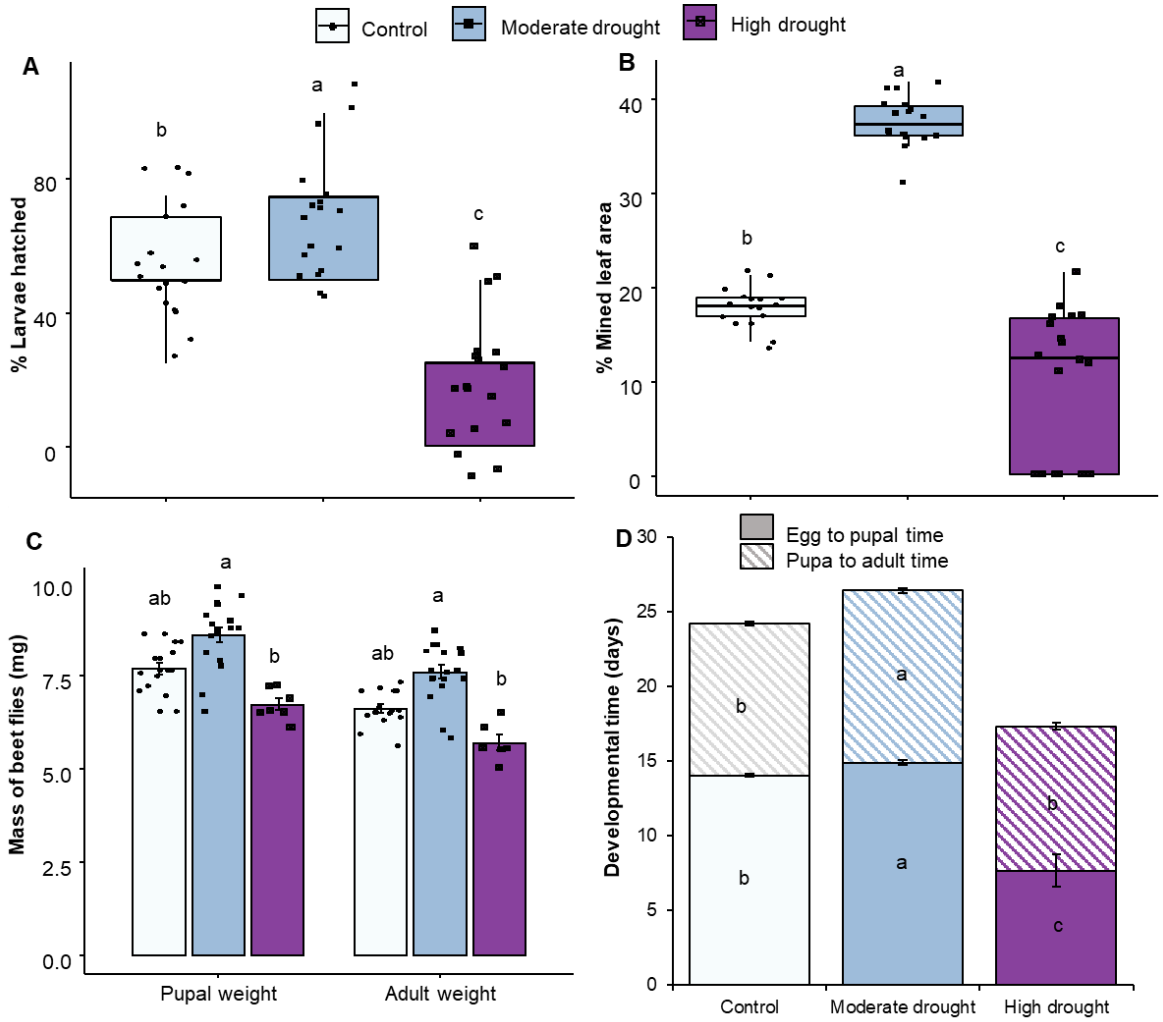


Figure 4: Effects of different levels of drought on the performance of *Pegomya cunicularia* on the percentage of hatched larvae (Binomial GLM) (A); percentage of mined leaf area (Kruskal-Wallis) (B); weight of pupae and adults (Test: LMM) (C); total development time (Egg to pupae and pupae to adult time) (Test: Kruskal-Wallis) (D). Data points in graphs represent individual replicates, and different letters indicate significance among treatments ($p \leq 0.05$), n = 18.

Effect of drought stress and beet miner infestation on central metabolites of sugar beet leaf

A total of 120 metabolites were identified from the leaf tissue that was classified as a major group of metabolites (Figure 5B) and visualized in a circular heatmap (Figure 5A). Hierachical cluster analysis further classified these metabolites into five major groups based on their expression pattern (Figure 5A). Due to drought stress and beet miner infestation, metabolites concentrations were increased significantly. However, some metabolites were significantly upregulated, and some were significantly downregulated based on the comparison of fold change (Figure S8) among different treatments.

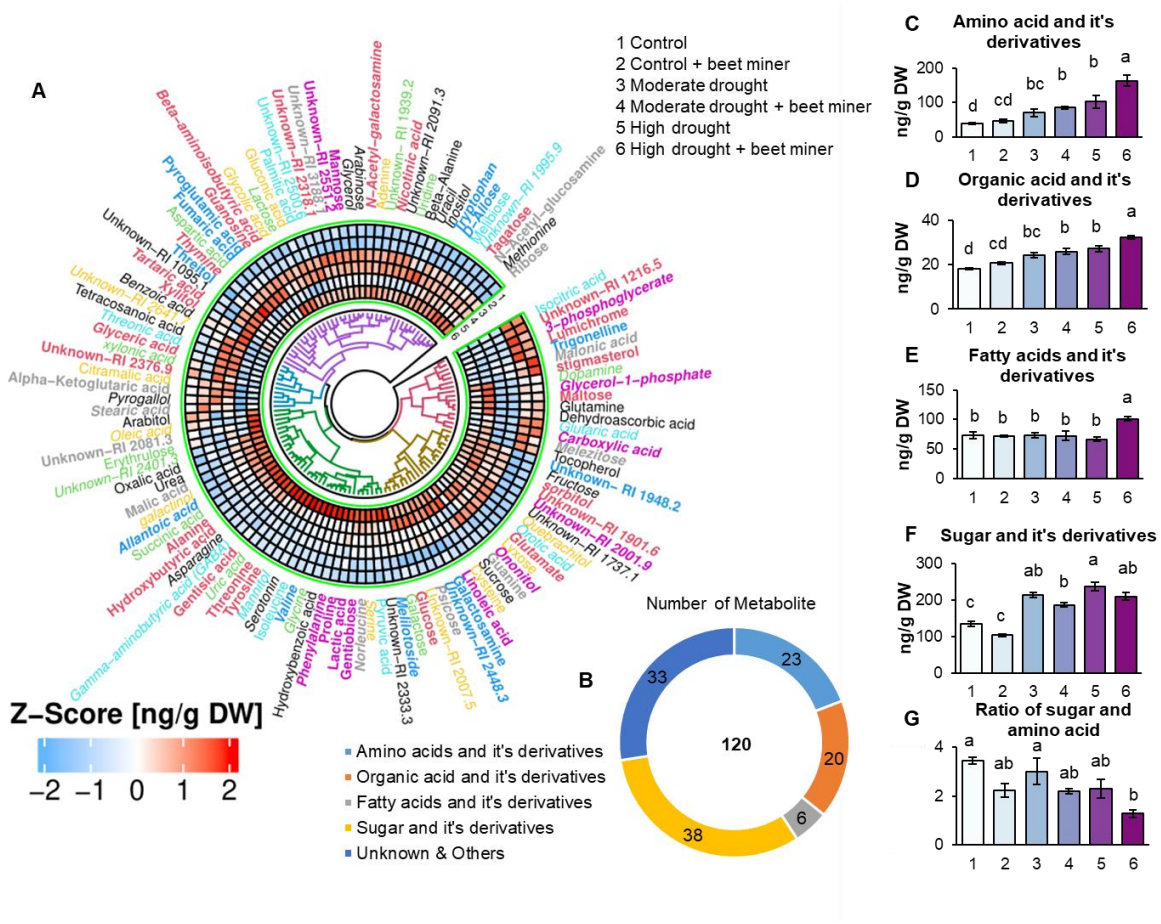


Figure 5: Effect of drought stress and beet miner infestation on sugar beet leaf central metabolites. Circular heatmap and clustering representation of all metabolites found in all different treatments (A); number of metabolites and their derivatives of major classes (B); approximate concentration of amino acids (Test: LM, log10 transformed) (C); organic acids (Test: LM) (D); fatty acids (Test: LM) (E); sugars (Test: LM) (F); and sugar-to-amino acid ratio (Test: LM) (G). Different letters indicate significance among treatments ($p \leq 0.05$), n = 6.

The combination of drought and beet miner infestation led to an increase in amino acid concentration (LM: drought: $F_{2,30} = 44.24$, $p < 0.001$; beet miner: $F_{1,30} = 10.08$, $p = 0.003$; drought × beet miner: $F_{2,30} = 1.33$, $p = 0.27$). However, there was no statistically significant distinction observed between plants subjected to moderate and high levels of drought stress (Figure 5C). Organic acid concentration also followed a similar pattern (LM: drought: $F_{2,30} = 52.06$, $p < 0.001$; beet miner: $F_{1,30} = 14.14$, $p < 0.001$; drought × beet miner: $F_{2,30} = 1.62$, $p = 0.21$) (Figure 5D). Moreover, a significantly high concentration of fatty acid was observed in high drought with leaf miner infestation plants (LM: drought: $F_{2,30} = 3.43$, $p = 0.045$; beet miner: $F_{1,30} = 7.05$, $p = 0.01$; drought × beet miner: $F_{2,30} = 8.67$, $p = 0.001$) (Figure 5E). Drought and leaf miner infestation resulted in

significantly elevated concentrations of sugar metabolites (LM: drought: $F_{2,30}$ = 85.78, $p < 0.001$; beet miner: $F_{1,30}$ = 17.42, $p < 0.001$; drought × beet miner: $F_{2,30}$ = 0.025, p = 0.97). Nevertheless, there was no discernible distinction observed between plants exposed to moderate and high levels of drought stress (Figure 5F). The ratio of sugar to amino acid was significantly higher in control and moderately drought-stressed plants, and slightly lower in plants experiencing high drought stress, although no significant difference was observed among them (LM: drought: $F_{2,30}$ = 5.27, p = 0.01; beet miner: $F_{1,30}$ = 21.30, $p < 0.001$; drought × beet miner: $F_{2,30}$ = 0.05, p = 0.94) (Figure 5G).

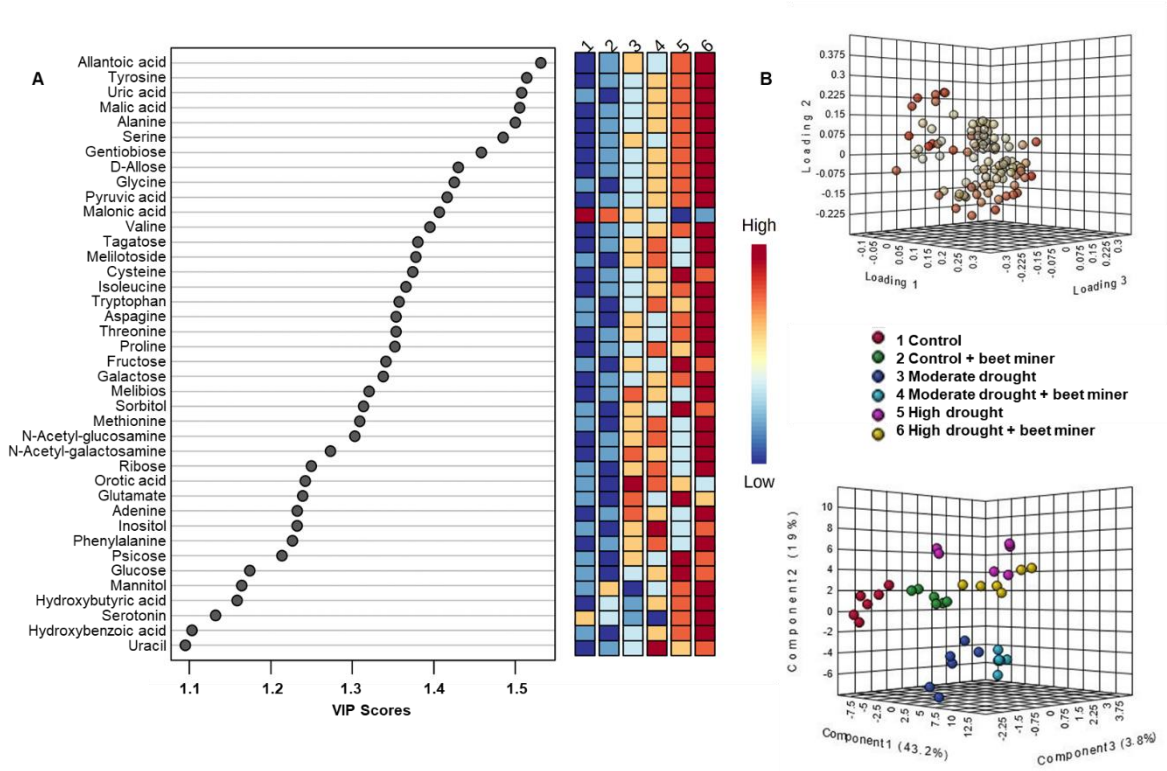


Figure 6: Important central metabolites in response to drought stress and leaf miner infestation were identified by partial least square-discriminate analysis (PLS-DA). Forty top metabolites according to the variable importance of projection (VIP) score to different treatments are shown. Colored boxes indicate the relative approximate concentrations of the corresponding metabolites in each group (A); synchronized 3D plot illustrated the 95% confidence intervals for each group (B), n = 6.

The PLS-DA (partial least square-discriminate analysis) was performed to identify stress-responsive metabolites in sugar beet. Based on VIP (variable importance of projection) score > 1, the top forty metabolites were identified out of 120 metabolites, and their respective concentrations were summarized in Figure 6A. To reduce dimensionality and visualize the relationship among samples, principal component analysis (PCA) of overview plot (Figure S9 A),

biplot of PCA (Figure S9 B), synchronized 3D plot for PCA (Figure S9 C), and synchronized 3D plot for PLS-DA (Figure 6B) was performed. A synchronized 3D plot of PLS-DA shows a clear separation among different treatments. Component 1 explained 43.2 % of the total variation, component 2 explained 19 %, and component 3 explained 3.8% of the variation across the data set (Figure 6B). This indicates the changes of the metabolites profile caused by drought stress and beet miner infestation.

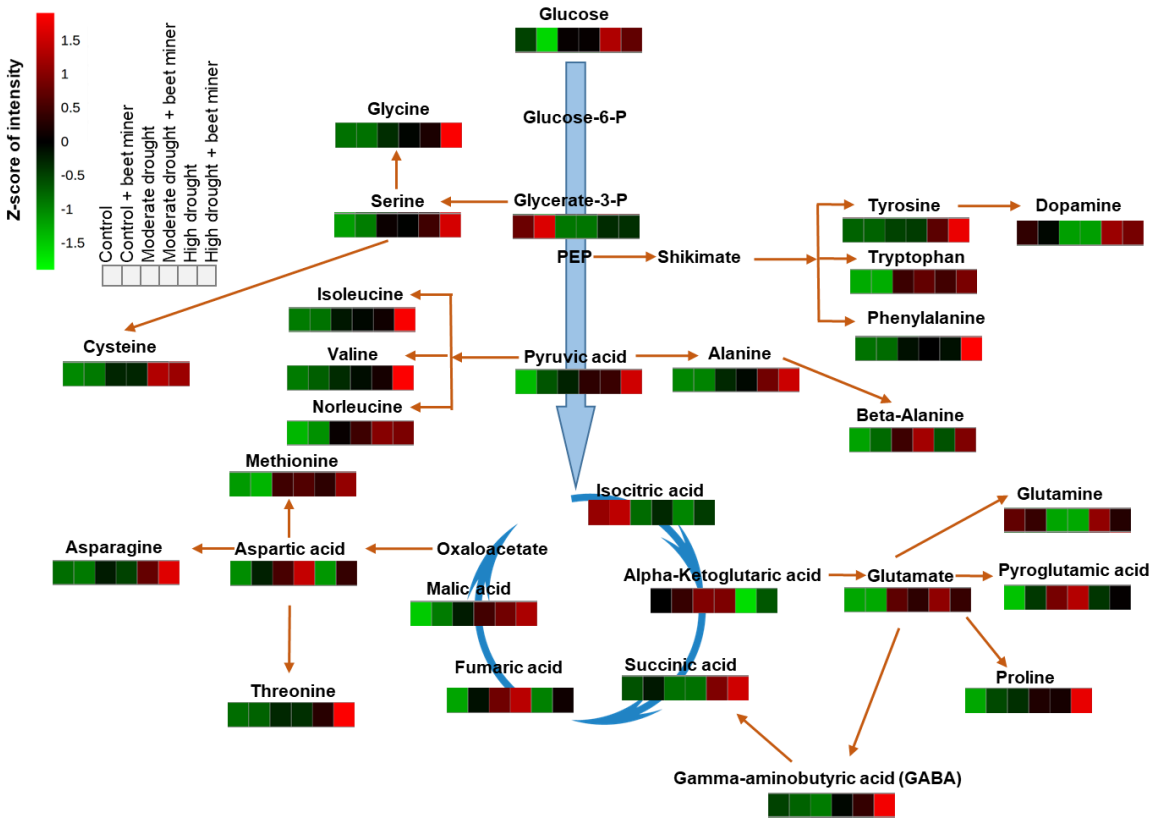


Figure 7: Metabolic changes involved in the amino acid pathway and tricarboxylic acid cycle under drought stress and beet miner infestation. Each heatmap represents the normalized intensity of the corresponding metabolite, n= 6.

Based on the VIP score, primarily amino acids, some organic acids, and some sugars were the most responsive metabolites, and all of these metabolites increased the concentration based on the stress level. Amino acid biosynthesis showed the relative concentration of metabolites (Figure 7), where glucose concentration is higher than in control plants. Amino acids, including glycine, leucine, isoleucine, valine, proline, alanine, asparagine, threonine, and tyrosine, were increased significantly over control plants. However, in the citric acid cycle, the main compound, isocitric acid, and alpha-ketoglutaric acid are higher in control plants (Figure 7).

Discussion

Our study documents that increasing drought led to stunted growth, lower biomass, reduced leaf area, decreased leaf number, low leaf water content, and reduced emission of volatile organic compounds (VOCs). Female flies were more attracted to the scent (VOC) of control plants than plants experiencing drought and infested by beet miners. Additionally, there was a strong preference for ovipositing on control plants. As drought stress intensified, there was a decline in photosynthetic capacity, as indicated by chlorophyll fluorescence parameters. However, when combined with beet miner infestation, the most severe negative impact was observed at moderate drought levels, which coincided with extensive feeding damage. Central metabolites revealed that increasing drought stress elevated the concentrations of amino acids, organic acids, fatty acids, and sugar metabolites but decreased the sugar-to-amino acid ratio, reflecting the higher nutritional food for insects in high-drought plants. Still, moderate drought conditions resulted in better performance of beet leaf miners, as evidenced by the higher emergence of larvae and increased weights of pupae and adults. In the subsequent paragraphs, we discuss in detail how drought stress alters plant morphology and how the infestation of leaf miners alters volatile organic compounds (VOCs), impacting the ability of beet flies to find suitable hosts for oviposition. Furthermore, we explore the alteration of central metabolites due to drought and beet miner infestation and possible links to how moderate drought conditions favour beet miners.

Drought stress causes many physiological and biochemical changes in plants, resulting in significant changes in morphological characteristics. Drought stress modulates the different hormones and various signaling messengers like reactive oxygen species, nitric oxide, cytosolic pH, calcium etc. that increases cation/anion efflux and leads to turgor loss in guard cells resulting in the closure of the stomata (Agurla et al., 2018). Due to the closure of stomata, the photosynthesis rate decreases (Santos et al., 2018), resulting decrease in cell expansion, a smaller leaf area that ultimately affects plant height, and a negative impact on other plant morphological features (Yang et al., 2021). Antagonistic hormone pair involves regulation of the root/shoot ratio, and a decrease in shoot growth combined with an increase in root growth leads to survival under drought

stress (Kurepa & Smalle, 2022). Our study observed drought stress symptoms at moderate levels and became more severe under high drought conditions (Figure S2). However, leaf water content was not significantly lower between control and moderate drought conditions (Figure S3). This stress can increase plants' susceptibility to herbivores by directly impacting their defenses or reducing their attractiveness for insect oviposition.

In addition to the observed morphological traits, the emission patterns of plant VOC can undergo quantitative and qualitative changes in response to drought and interactions with herbivores (Copolovici et al., 2014; Jardine et al., 2015; Pagadala Damodaram et al., 2021; Rering et al., 2020). Female insects may rely on these plant volatile cues to locate suitable hosts for oviposition (Achhami et al., 2021; De Moraes et al., 2001). In our study, we observed that well-watered control plants exhibited an increase in total VOC emission, particularly in the number of volatiles, predominantly monoterpenoids, in comparison to plants subjected to moderate and high drought conditions. VOCs are mainly released through the stomata and the reduced emission of VOCs from drought-stressed plants may be partly the result of low stomatal conductance (Harley, 2013; Lin et al., 2022; Niinemets et al., 2004; Seidl-Adams et al., 2015). The infestation of beet leaf miners in well-watered control plants resulted in a substantial increase in total VOC emission, accompanied by the release of eight VOCs that were not detected in the well-watered control plants without leaf miners. However, in the presence of leaf miners in drought-stressed plants, the concentration of VOC was significantly higher. Y-tube and choice experiments confirmed that beet flies preferred healthy plants without stress. PCA and PLS-DA showed the categorical differentiation of volatile blend among different treatments, and VIP score helps to identify the top most important VOCs, which played a crucial role in the volatile blend. *cis*-*β*-ocimine, *β*-myrcene, 1-octane-3ol, *trans*-*β*-ocimene, *β*-guaiene, eucalyptol, and 4-hydroxy-4-methyl-2-pentanone were emitted in higher amounts in control plants infested by beet leaf miner that might reduce the attraction of beet flies. This may result in creating a distinctive blend of VOC that serves as a cue for female beet flies to locate their preferred site for oviposition. Debiased Sparse Partial Correlation (DSPC) network analysis (Figure S4) helps us to understand how different VOCs are correlated to form a

complex blend of volatiles. Forming a special volatile blend might help female flies to choose a suitable host plant for oviposition. However, the emission of plant VOCs can vary extensively due to abiotic/biotic stresses (Holopainen & Gershenzon, 2010; Shivaramu et al., 2017), elevated (Ebel et al., 1995) or reduced (Tariq et al., 2013), due to drought stress or due to herbivore interactions (Rostás et al., 2006). The blend of plant volatiles also depends on the feeding guild of the herbivores (Dicke et al., 2009), which might depend on the host plant's species and the stress intensity level. Emission rates of total VOC standardized by plant biomass were highest from severely drought-stressed leaf miner-infested plants, suggesting that the production of these potentially important compounds might be upregulated in highly drought-stressed plants upon beet miner infestation to compensate for the decrease in plant biomass.

Due to stress, non-stomatal factors also play an essential role in closing the stomata. For example, Ribulose-1,5-bisphosphate (RuBP) plays a critical role in the photosynthetic assimilation process (Gimenez et al., 1992). Enzyme, Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) also plays a significant role in regulating photosynthesis. It was reported that the activity of RuBisCo had less affected by mild drought. However, a significant decrease was reported in severe drought, and the consequent changes in photochemical and biochemical processes like electron transport rate (ETR) decreased in cotton (Deeba et al., 2012). Shin et al., 2021 found drought affects chlorophyll fluorescence parameters in lettuce, and de Souza et al., 2020 reported Fv/Fm, ΦPSII, ETR were affected due to the infestation of *Spodoptera frigiperda* in maize plants. We found that due to drought and the infestation of beet leaf miners, the chlorophyll fluorescence parameters like Fv/Fm, Φ_{PSII}, ETR were severely affected, which might be due to damage of light-harvesting complex (LHC) protein in chloroplasts (Grewe et al., 2014). More importantly, we observed moderate drought with the infestation of leaf miners affected most in respect of leaf chlorophyll fluorescence parameters compared to control and highly stressed plants due to the higher infestation in moderate drought plants (Figure 3A, B; Figure 4B).

Moderate drought favours the leaf miner, as shown in the percent larvae hatched from eggs, pupal and adult weight. Santiago-Salazar et al., 2022

reported that moderate drought enhances the performance of coffee leaf miner, *Leucoptera coffeella,* and that could be due to the quality of the nutrients available to the leaf miner. In contrast to other studies, leaf miners performed similarly in both control and drought plants, but larval and pupal survival is higher in drought plants (Acidri et al., 2020; Hahn & Maron, 2018; Lenhart et al., 2015). Due to drought stress, exhibit an increase in the concentration of soluble proteins and essential amino acids and might depend due to drought intensity (Franzke & Reinhold, 2011; Sconiers et al., 2020; Sconiers & Eubanks, 2017). Increasing the nutrient availability in drought-stress plants could favor the development of beet leaf miners, reflecting the body weight of adult flies. So, plant central metabolites due to drought might play a vital role in the better survival of the beet leaf miner.

The present study investigated GC-MS-based central metabolite analysis from sugar beet leaves stressed with drought and beet leaf miners to identify abiotic and biotic responsive metabolites. Our analysis identified 120 metabolites, which were categorized into four major groups. Top forty stress-responsive metabolites based on VIP projection and most of the metabolites increase the concentration in drought stress and even more on feed upon the leaf miners. Drought modulates the more significant fold changes (up and down) compared to leaf miner infestation (Figure S8), suggesting biotic stress accumulates fewer metabolites than abiotic stress. We observed that drought stress increased the concentration of all major metabolite groups, including amino acids, organic acids, fatty acids, and sugar metabolites. Reported that an adequate concentration of plant sugar and/or amino acid is essential for making the best blend of nutrients for the better growth and development of herbivores (Body et al., 2019; Chown et al., 2004; Schoonhoven et al., 2005). So, higher sugars and amino acids should generally improve leaf miner performance in high drought-stress plants. In theory, beet leaf miners would be expected to thrive in high drought-stress plants due to the availability of highly nutritious food. However, contrary to this expectation, our study revealed that beet leaf miners performed better in moderate drought-stressed plants than in control or high-stress plants. Most importantly, leaf water content is also essential in maintaining the leaf's nutritional quality and is responsible for the better development of the leaf miners (Loomis, 1997; Wei et al., 2000). Our study found non-significant differences in

leaf water content between control and moderate stress plants and significantly low in high drought plants (Figure S3). Furthermore, we observed a higher sugar-to-amino acid ratio in control plants and those experiencing moderate drought stress. So, our study shows that leaf water content plays a vital role in making a perfect quality nutritional blend for the better growth of beet leaf miners.

Furthermore, accumulating such metabolites occurs due to the alteration of the complex metabolic pathways. Among the different pathways, amino acid biosynthetic pathways provide essential protein building blocks and connect central carbon metabolism to a diversity of secondary metabolism like volatiles. Our investigated amino acid biosynthesis pathways with their respective concentration to different treatments (Figure 7) help us to understand how abiotic and biotic stress alter the metabolite profiles in sugar beet leaves that are ultimately responsible for the emission of plant volatiles. These secondary metabolites, for example, volatiles, are synthesized from a certain central metabolite precursor(s), such as sugars, amino acids, nucleotides, organic acids, and fatty acids, which are essential for maintaining cellular homeostasis and volatiles might act as a defense compound. Our study demonstrated that beet miner-induced plant volatiles inhibits adult flies' oviposition. After biotic infestations, alters in plant metabolism have often been interpreted as a requirement to satisfy the increased demand for energy and carbon skeletons to sustain the direct defense machinery and corresponding physiological adaptations (Kerchev et al., 2012) and might affect volatile emissions and act as an indirect plant defense mechanism.

In conclusion, this comprehensive study suggests that drought stress and beet miner infestation result in a complex reprogramming of primary and secondary metabolism and the emission of plant volatiles that act as plant defense metabolites for oviposition preferences of beet flies. In addition, moderate drought stress having much water content in leaves alters the internal chemistry of sugar beet plants by concentrating metabolites, turning them into nutrient-rich sinks that provide essential energy for the growth and development of beet leaf miners.

Supporting information

Additional supporting information can be found in the supporting information section at the end of this article.

Author contributions

Shahinoor Rahman: conceptualization, conduction of experiments, data collection, data analysis, and writing the first draft. Musrat Zahan Surovy: helps during the conduction of experiments, analysis of metabolome data, and construction of graphical abstract. Ilka Vosteen: conceptualization, manuscript editing, and supervision. Franz Hadacek: method establishment for central metabolite analysis in GC-MS and helps for peak analysis from metabolic samples, review, and editing. Michael Rostás: conceptualization, review, editing, and supervision. The authors declare no conflicts of interest.

References

Achhami, B. B., Reddy, G. V. P., Hofland, M. L., Sherman, J. D., Peterson, R. K. D., & Weaver, D. K. (2021). Plant Volatiles and Oviposition Behavior in the Selection of Barley Cultivars by Wheat Stem Sawfly (Hymenoptera: Cephidae). Environmental Entomology, 50(4), 940–947. https://doi.org/10.1093/ee/nvab035

Acidri, R., Sawai, Y., Sugimoto, Y., Handa, T., Sasagawa, D., Masunaga, T., Yamamoto, S., & Nishihara, E. (2020). Exogenous Kinetin Promotes the Nonenzymatic Antioxidant System and Photosynthetic Activity of Coffee (Coffea arabica L.) Plants Under Cold Stress Conditions. Plants, 9(2), Article 2. https://doi.org/10.3390/plants9020281

Agurla, S., Gahir, S., Munemasa, S., Murata, Y., & Raghavendra, A. S. (2018). Mechanism of Stomatal Closure in Plants Exposed to Drought and Cold Stress. In M. Iwaya-Inoue, M. Sakurai, & M. Uemura (Eds.), Survival Strategies in Extreme Cold and Desiccation: Adaptation Mechanisms and Their Applications (pp. 215–232). Springer. https://doi.org/10.1007/978-981-13-1244-1_12

Anjum, S. A., Ashraf, U., Tanveer, M., Khan, I., Hussain, S., Shahzad, B., Zohaib, A., Abbas, F., Saleem, M. F., Ali, I., & Wang, L. C. (2017). Drought Induced Changes in Growth, Osmolyte Accumulation and Antioxidant Metabolism of Three Maize Hybrids. Frontiers in Plant Science, 8, 69. https://doi.org/10.3389/fpls.2017.00069

Bezerra, R. H. S., Sousa-Souto, L., Santana, A. E. G., & Ambrogi, B. G. (2021). Indirect plant defenses: Volatile organic compounds and extrafloral nectar. Arthropod-Plant Interactions, 15(4), 467–489. https://doi.org/10.1007/s11829-021-09837-1

Body, M. J. A., Casas, J., & Giron, D. (2019). Manipulation of plant primary metabolism by leaf-mining larvae in the race against leaf senescence (p. 777334). bioRxiv. https://doi.org/10.1101/777334

Boergens, E., Güntner, A., Dobslaw, H., & Dahle, C. (2020). Quantifying the Central European Droughts in 2018 and 2019 With GRACE Follow-On. Geophysical Research Letters, 47(14), e2020GL087285. https://doi.org/10.1029/2020GL087285

Boggs, C. L., & Freeman, K. D. (2005). Larval food limitation in butterflies: Effects on adult resource allocation and fitness. Oecologia, 144(3), 353–361. https://doi.org/10.1007/s00442-005-0076-6

Bruce, T. J. A., & Pickett, J. A. (2011). Perception of plant volatile blends by herbivorous insects – Finding the right mix. Phytochemistry, 72(13), 1605–1611. https://doi.org/10.1016/j.phytochem.2011.04.011

Bruce, T. J. A., Wadhams, L. J., & Woodcock, C. M. (2005). Insect host location: A volatile situation. Trends in Plant Science, 10(6), 269–274. https://doi.org/10.1016/j.tplants.2005.04.003

Carvajal Acosta, A. N., Agrawal, A. A., & Mooney, K. (2023). Plant water-use strategies as mediators of herbivore drought response: Ecophysiology, host plant quality and functional traits. Journal of Ecology, 111(3), 687–700. https://doi.org/10.1111/1365-2745.14059

Catola, S., Centritto, M., Cascone, P., Ranieri, A., Loreto, F., Calamai, L., Balestrini, R., & Guerrieri, E. (2018). Effects of single or combined water deficit and aphid attack on tomato volatile organic compound (VOC) emission and plant-plant communication. Environmental and Experimental Botany, 153, 54–62. https://doi.org/10.1016/j.envexpbot.2018.05.001

Chown, S. L., Chown, S., & Nicolson, S. (2004). Insect Physiological Ecology: Mechanisms and Patterns. OUP Oxford.

Copolovici, L., Kännaste, A., Remmel, T., & Niinemets, Ü. (2014). Volatile organic compound emissions from Alnus glutinosa under interacting drought and herbivory stresses. Environmental and Experimental Botany, 100, 55–63. https://doi.org/10.1016/j.envexpbot.2013.12.011

De Moraes, C. M., Mescher, M. C., & Tumlinson, J. H. (2001). Caterpillar-induced nocturnal plant volatiles repel conspecific females. Nature, 410(6828), 577–580. https://doi.org/10.1038/35069058

de Souza, M. W. R., Ferreira, E. A., dos Santos, J. B., Soares, M. A., de Castro e Castro, B. M., & Zanuncio, J. C. (2020). Fluorescence of chlorophyll a in transgenic maize with herbicide application and attacked by Spodoptera frugiperda (Lepidoptera: Noctuidae). Phytoparasitica, 48(4), 567–573. https://doi.org/10.1007/s12600-020-00816-5

Deeba, F., Pandey, A. K., Ranjan, S., Mishra, A., Singh, R., Sharma, Y. K., Shirke, P. A., & Pandey, V. (2012). Physiological and proteomic responses of cotton (Gossypium herbaceum L.) to drought stress. Plant Physiology and Biochemistry, 53, 6–18. https://doi.org/10.1016/j.plaphy.2012.01.002

Dicke, M., van Loon, J. J. A., & Soler, R. (2009). Chemical complexity of volatiles from plants induced by multiple attack. Nature Chemical Biology, 5(5), 317–324. https://doi.org/10.1038/nchembio.169

Ebel, R. C., Mattheis, J. P., & Buchanan, D. A. (1995). Drought stress of apple trees alters leaf emissions of volatile compounds. Physiologia Plantarum, 93(4), 709–712. https://doi.org/10.1111/j.1399-3054.1995.tb05120.x

Fàbregas, N., & Fernie, A. R. (2019). The metabolic response to drought. Journal of Experimental Botany, 70(4), 1077–1085. https://doi.org/10.1093/jxb/ery437

Franzke, A., & Reinhold, K. (2011). Stressing food plants by altering water availability affects grasshopper performance. Ecosphere, 2(7), art85. https://doi.org/10.1890/ES11-00095.1

Gimenez, C., Mitchell, V. J., & Lawlor, D. W. (1992). Regulation of Photosynthetic Rate of Two Sunflower Hybrids under Water Stress. Plant Physiology, 98(2), 516–524. https://doi.org/10.1104/pp.98.2.516

Grewe, S., Ballottari, M., Alcocer, M., D'Andrea, C., Blifernez-Klassen, O., Hankamer, B., Mussgnug, J. H., Bassi, R., & Kruse, O. (2014). Light-Harvesting Complex Protein LHCBM9 Is Critical for Photosystem II Activity and Hydrogen Production in Chlamydomonas reinhardtii[C][W]. The Plant Cell, 26(4), 1598–1611. https://doi.org/10.1105/tpc.114.124198

Hahn, P. G., & Maron, J. L. (2018). Plant water stress and previous herbivore damage affect insect performance. Ecological Entomology, 43(1), 47–54. https://doi.org/10.1111/een.12468

Hamann, E., Blevins, C., Franks, S. J., Jameel, M. I., & Anderson, J. T. (2021). Climate change alters plant–herbivore interactions. New Phytologist, 229(4), 1894–1910. https://doi.org/10.1111/nph.17036

Harley, P. C. (2013). The Roles of Stomatal Conductance and Compound Volatility in Controlling the Emission of Volatile Organic Compounds from Leaves. In Ü. Niinemets & R. K. Monson (Eds.), Biology, Controls and Models of Tree Volatile Organic Compound

Emissions (pp. 181–208). Springer Netherlands. https://doi.org/10.1007/978-94-007-6606-8_7

Holopainen, J. K., & Gershenzon, J. (2010). Multiple stress factors and the emission of plant VOCs. Trends in Plant Science, 15(3), 176–184. https://doi.org/10.1016/j.tplants.2010.01.006

Honda, K. (1995). Chemical basis of differential oviposition by lepidopterous insects. Archives of Insect Biochemistry and Physiology, 30(1), 1–23. https://doi.org/10.1002/arch.940300102

Jardine, K. J., Chambers, J. Q., Holm, J., Jardine, A. B., Fontes, C. G., Zorzanelli, R. F., Meyers, K. T., de Souza, V. F., Garcia, S., Gimenez, B. O., de O. Piva, L. R., Higuchi, N., Artaxo, P., Martin, S., & Manzi, A. O. (2015). Green Leaf Volatile Emissions during High Temperature and Drought Stress in a Central Amazon Rainforest. Plants, 4(3), 678–690. https://doi.org/10.3390/plants4030678

Kerchev, P. I., Fenton, B., Foyer, C. H., & Hancock, R. D. (2012). Plant responses to insect herbivory: Interactions between photosynthesis, reactive oxygen species and hormonal signalling pathways. Plant, Cell & Environment, 35(2), 441–453. https://doi.org/10.1111/j.1365-3040.2011.02399.x

Kuczyk, J., Müller, C., & Fischer, K. (2021). Plant-mediated indirect effects of climate change on an insect herbivore. Basic and Applied Ecology, 53, 100–113. https://doi.org/10.1016/j.baae.2021.03.009

Kurepa, J., & Smalle, J. A. (2022). Auxin/Cytokinin Antagonistic Control of the Shoot/Root Growth Ratio and Its Relevance for Adaptation to Drought and Nutrient Deficiency Stresses. International Journal of Molecular Sciences, 23(4), 1933. https://doi.org/10.3390/ijms23041933

Lenhart, P. A., Eubanks, M. D., & Behmer, S. T. (2015). Water stress in grasslands: Dynamic responses of plants and insect herbivores. Oikos, 124(3), 381–390. https://doi.org/10.1111/oik.01370

Lin, P.-A., Kansman, J., Chuang, W.-P., Robert, C., Erb, M., & Felton, G. W. (2022a). Water availability and plant–herbivore interactions. Journal of Experimental Botany, erac481. https://doi.org/10.1093/jxb/erac481

Lin, P.-A., Chen, Y., Ponce, G., Acevedo, F. E., Lynch, J. P., Anderson, C. T., Ali, J. G., & Felton, G. W. (2022b). Stomata-mediated interactions between plants, herbivores, and the environment. Trends in Plant Science, 27(3), 287–300. https://doi.org/10.1016/j.tplants.2021.08.017

Loomis, R. S. (1997). On the utility of nitrogen in leaves. Proceedings of the National Academy of Sciences, 94(25), 13378–13379. https://doi.org/10.1073/pnas.94.25.13378

Mevi-Schütz, J., Goverde, M., & Erhardt, A. (2003). Effects of fertilization and elevated CO2 on larval food and butterfly nectar amino acid preference in Coenonympha pamphilus L. Behavioral Ecology and Sociobiology, 54(1), 36–43. https://doi.org/10.1007/s00265-003-0601-8

Michelsen, V. (1980). A revision of the beet leaf-miner complex, Pegomya hyoscyami s.lat. (Diptera: Anthomyiidae). Insect Systematics & Evolution, 11(3), 297–309. https://doi.org/10.1163/187631280794824631

Niinemets, Ü., Loreto, F., & Reichstein, M. (2004). Physiological and physicochemical controls on foliar volatile organic compound emissions. Trends in Plant Science, 9(4), 180–186. https://doi.org/10.1016/j.tplants.2004.02.006

Pagadala Damodaram, K. J., Gadad, H. S., Parepally, S. K., Vaddi, S., Ramanna Hunashikatti, L., & Bhat, R. M. (2021). Low moisture stress influences plant volatile emissions affecting herbivore interactions in tomato, Solanum lycopersicum. Ecological Entomology, 46(3), 637–650. https://doi.org/10.1111/een.13012

Pott, D. M., Osorio, S., & Vallarino, J. G. (2019). From Central to Specialized Metabolism: An Overview of Some Secondary Compounds Derived From the Primary Metabolism for

Their Role in Conferring Nutritional and Organoleptic Characteristics to Fruit. Frontiers in Plant Science, 10. https://www.frontiersin.org/articles/10.3389/fpls.2019.00835

Ray, R. L., Ampim, P. A. Y., & Gao, M. (2020). Crop Protection Under Drought Stress. In K. Jabran, S. Florentine, & B. S. Chauhan (Eds.), Crop Protection Under Changing Climate (pp. 145–170). Springer International Publishing. https://doi.org/10.1007/978-3-030-46111-9_6

Rering, C. C., Franco, J. G., Yeater, K. M., & Mallinger, R. E. (2020). Drought stress alters floral volatiles and reduces floral rewards, pollinator activity, and seed set in a global plant. Ecosphere, 11(9), e03254. https://doi.org/10.1002/ecs2.3254

Rostás, M., Ton, J., Mauch-Mani, B., & Turlings, T. C. J. (2006). Fungal Infection Reduces Herbivore-Induced Plant Volatiles of Maize but does not Affect Naïve Parasitoids. Journal of Chemical Ecology, 32(9), 1897–1909. https://doi.org/10.1007/s10886-006-9147-3

Salerno, G., Frati, F., Marino, G., Ederli, L., Pasqualini, S., Loreto, F., Colazza, S., & Centritto, M. (2017). Effects of water stress on emission of volatile organic compounds by Vicia faba, and consequences for attraction of the egg parasitoid Trissolcus basalis. Journal of Pest Science, 90(2), 635–647. https://doi.org/10.1007/s10340-016-0830-z

Sander, R. (2015). Compilation of Henry's law constants (version 4.0) for water as solvent. Atmospheric Chemistry and Physics, 15(8), 4399–4981. https://doi.org/10.5194/acp-15-4399-2015

Santiago-Salazar, C. M., Barrera, J. F., Rojas, J. C., Huerta-Palacios, G., & Escamilla-Prado, E. (2022). Response of a specialist leaf miner insect to the environmental stress of its host plant. Arthropod-Plant Interactions, 16(3), 329–339. https://doi.org/10.1007/s11829-022-09900-5

Santos, V. A. H. F. dos, Ferreira, M. J., Rodrigues, J. V. F. C., Garcia, M. N., Ceron, J. V. B., Nelson, B. W., & Saleska, S. R. (2018). Causes of reduced leaf-level photosynthesis during strong El Niño drought in a Central Amazon forest. Global Change Biology, 24(9), 4266–4279. https://doi.org/10.1111/gcb.14293

Schoonhoven, L. M., Loon, J. J. A. van, & Dicke, M. (2005). Insect-plant biology (2nd ed). Oxford University Press.

Sconiers, W. B., & Eubanks, M. D. (2017). Not all droughts are created equal? The effects of stress severity on insect herbivore abundance. Arthropod-Plant Interactions, 11(1), 45–60. https://doi.org/10.1007/s11829-016-9464-6

Sconiers, W. B., Rowland, D. L., & Eubanks, M. D. (2020). Pulsed drought: The effects of varying water stress on plant physiology and predicting herbivore response. Crop Science, 60(5), 2543–2561. https://doi.org/10.1002/csc2.20235

Seidl-Adams, I., Richter, A., Boomer, K. B., Yoshinaga, N., Degenhardt, J., & Tumlinson, J. H. (2015). Emission of herbivore elicitor-induced sesquiterpenes is regulated by stomatal aperture in maize (Zea mays) seedlings. Plant, Cell & Environment, 38(1), 23–34. https://doi.org/10.1111/pce.12347

Shahbandeh. (2023). *Sugar beet: Sugar production in European countries 2021*. Statista. https://www.statista.com/statistics/1126827/sugar-beet-sugar-production-in-european-countries/

Sharma, A., Shahzad, B., Kumar, V., Kohli, S. K., Sidhu, G. P. S., Bali, A. S., Handa, N., Kapoor, D., Bhardwaj, R., & Zheng, B. (2019). Phytohormones Regulate Accumulation of Osmolytes Under Abiotic Stress. Biomolecules, 9(7), 285. https://doi.org/10.3390/biom9070285

Shehzad, M., Gulzar, A., Staley, J. T., & Tariq, M. (2021). The effects of drought stress and type of fertiliser on generalist and specialist herbivores and their natural enemies. Annals of Applied Biology, 178(2), 377–386. https://doi.org/10.1111/aab.12654

Shin, Y. K., Bhandari, S. R., Jo, J. S., Song, J. W., & Lee, J. G. (2021). Effect of Drought Stress on Chlorophyll Fluorescence Parameters, Phytochemical Contents, and Antioxidant Activities in Lettuce Seedlings. Horticulturae, 7(8), Article 8. https://doi.org/10.3390/horticulturae7080238

Shivaramu, S., Jayanthi, P., Kempraj, V., Anjinappa, R., Nandagopal, B., & Chakravarty, A. (2017). What signals do herbivore-induced plant volatiles provide conspecific herbivores? Arthropod-Plant Interactions. http://dx.doi.org/10.1007/s11829-017-9536-2

Staley, J. T., Mortimer, S. R., & Morecroft, M. D. (2008). Drought impacts on above–belowground interactions: Do effects differ between annual and perennial host species? Basic and Applied Ecology, 9(6), 673–681. https://doi.org/10.1016/j.baae.2007.10.006

Staley, J. T., Mortimer, S. R., Morecroft, M. D., Brown, V. K., & Masters, G. J. (2007). Summer drought alters plant-mediated competition between foliar- and root-feeding insects. Global Change Biology, 13(4), 866–877. https://doi.org/10.1111/j.1365-2486.2007.01338.x

Szabó, K., Zubay, P., & Németh-Zámboriné, É. (2020). What shapes our knowledge of the relationship between water deficiency stress and plant volatiles? Acta Physiologiae Plantarum, 42(8), 130. https://doi.org/10.1007/s11738-020-03120-1

Tariq, M., Wright, D. J., Bruce, T. J. A., & Staley, J. T. (2013). Drought and Root Herbivory Interact to Alter the Response of Above-Ground Parasitoids to Aphid Infested Plants and Associated Plant Volatile Signals. PLOS ONE, 8(7), e69013. https://doi.org/10.1371/journal.pone.0069013

Turlings, T. C. J., Davison, A. C., & TamÒ, C. (2004). A six-arm olfactometer permitting simultaneous observation of insect attraction and odour trapping. Physiological Entomology, 29(1), 45–55. https://doi.org/10.1111/j.1365-3032.2004.0362.x

Viric Gasparic, H., Lemic, D., Drmic, Z., Cacija, M., & Bazok, R. (2021). The Efficacy of Seed Treatments on Major Sugar Beet Pests: Possible Consequences of the Recent Neonicotinoid Ban. Agronomy, 11(7), 1277. https://doi.org/10.3390/agronomy11071277

Wei, J., Zou, L., Kuang, R., & He, L. (2000). Influence of Leaf Tissue Structure on Host Feeding Selection by Pea Leafminer Liriomyza huidobrensis (Diptera: Agromyzidae). Zoological Studies, 6.

Weldegergis, B. T., Zhu, F., Poelman, E. H., & Dicke, M. (2015). Drought stress affects plant metabolites and herbivore preference but not host location by its parasitoids. Oecologia, 177(3), 701–713. https://doi.org/10.1007/s00442-014-3129-x

Wittstock, U., & Gershenzon, J. (2002). Constitutive plant toxins and their role in defense against herbivores and pathogens. Current Opinion in Plant Biology, 5(4), 300–307. https://doi.org/10.1016/S1369-5266(02)00264-9

Yang, X., Lu, M., Wang, Y., Wang, Y., Liu, Z., & Chen, S. (2021). Response Mechanism of Plants to Drought Stress. Horticulturae, 7(3), Article 3. https://doi.org/10.3390/horticulturae7030050

Yao, J., Sun, D., Cen, H., Xu, H., Weng, H., Yuan, F., & He, Y. (2018). Phenotyping of Arabidopsis Drought Stress Response Using Kinetic Chlorophyll Fluorescence and Multicolor Fluorescence Imaging. Frontiers in Plant Science, 9. https://www.frontiersin.org/articles/10.3389/fpls.2018.00603

Zhuang, J., Wang, Y., Chi, Y., Zhou, L., Chen, J., Zhou, W., Song, J., Zhao, N., & Ding, J. (2020). Drought stress strengthens the link between chlorophyll fluorescence parameters and photosynthetic traits. PeerJ, 8, e10046. https://doi.org/10.7717/peerj.10046

Drought differentially affects the preference and performance of the beet leaf miner *Pegomya cunicularia* by altering plant chemistry

Shahinoor Rahman*[1], Musrat Zahan Surovy[2], Ilka Vosteen[1], Franz Hadacek[3], and Michael Rostás*[1]

[1]Department of Crop Sciences, Division of Agricultural Entomology, Georg-August-University of Goettingen, Germany

[2]Department of Crop Sciences, Division of Plant Pathology and Crop Protection, Georg-August-University of Goettingen, Germany

[3]Department of Plant Biochemistry, Albrecht-Haller Institute for Plant Sciences, Georg-August-University of Goettingen, Germany

*Correspondence: shahinoor_ent@yahoo.com; michael.rostas@uni-goettingen.de

Supporting information

Methodology for artificial mass rearing

Plant, insect, and rearing condition

Six-week-old sugar beet plant (*Beta vulgaris*) subspec. *vulgaris* cultivar 'Vasco' (SESVanderHave , Belgium) was used as a host. Beet flies *Pegomya cunicularia* were collected in May 2020 from the sugarbeet field in Göttingen, Germany. The rearing room condition was 16L:8D photoperiod, light intensity 130 ± 10 µmol/(s m^2), relative humidity of 65 ± 5 %, and a temperature of 20 ± 2 °C.

Artificial diet for the flies

Two types of food were supplied at the same time i) Dry food: prepared with skimmed milk powder, dextrose, soya meal, and yeast with the ratio of 10:10:1:1, and ii) Wet food: prepared with honey, soya meal, and yeast with the ratio of 5:5:1, and mix with a drop of water to make it creamy. Water was also provided in a separate glass vial to the flies, and fresh foods were provided every seven days.

Rearing technique

Three separate tents (60 × 60 × 70 cm) were used for successful rearing. One tent was used for oviposition purposes having sugar beet plants, beet flies (male and female), and food for the flies. Generally, a single female fly can lay 20-30 eggs/day, enough for a single plant for the next larval stage. Adult flies were left

them 24 h for oviposition and transferred to the second tent to maintain the larval time. Water was sprayed daily on the mining plants for better performance of the beet leaf miner. The larval period generally takes 10-15 days, and before finishing the larval period (10 days), leaves were cut at the base of the petiole and placed in the tray (10 × 20 × 8 cm) having the mixture of soil substrate (sand:clay:organic matter; 2:1:1) and transferred to the third tent for pupation. It generally takes 10-15 days to come adult flies from pupae, and the pupae were maintained by spraying water on the soil substrate. Finally, the adult flies transferred to the first oviposition tend to continue the rearing process (Figure S1).

Statistical analysis

Experiment 1

Three drought boxes (six plants of each box considered a block) were used to monitor the morphological features of the sugar beet plants, and a total of n= 18 plants were considered for each of the treatments (control, moderate drought, high drought). Repeated measure 2-way ANOVA was performed by generalized linear mixed effect model (GLMM) with glmmTMB function with a log link to Gaussian family distribution for analyzing sugar beet plant height, followed by Tukey HSD posthoc test for multiple comparisons. The model was performed by package “glmmTMB” (Brooks et al., 2017), and assumptions were checked by “DHARMa” (Florian Hartig, 2022) package in –R. Posthoc test was performed by using “emmeans” (Lenth et al., 2023) and “multcomp” (Hothorn et al., 2008) packages. ANOVA for total biomass, root weight, shoot weight, root shoot ratio by weight, root length, and average leaf area were analyzed by linear mixed effect model (LMM) by the package “nlme” (José Pinheiro et al., 2023), followed by Tukey HSD posthoc test by “emmeans” (Lenth et al., 2023) and “multcomp” (Hothorn et al., 2008) packages. ANOVA for shoot length, number of leaves, and sugar beet leaf water content were analyzed by Kruskal-Wallis non-parametric test followed by the Dunn test with “rcompanion” (Mangiafico, 2023) package. ANOVA for root shoot ratio by length was analyzed by generalized linear mixed effect model (GLMM) with “glmmTMB” (Brooks et al., 2017) function followed by Gaussian family distribution, and assumptions were checked by “DHARMa” (Florian Hartig, 2022) package in –R. Tukey HSD posthoc test was performed by

using “emmeans” (Lenth et al., 2023) and “multcomp” (Hothorn et al., 2008) packages.

Experiment 2

The number of eggs laid by *pegomya* fly was counted by choice, and no-choice test (n=18) and statistical analysis (ANOVA) was performed by the generalized linear mixed model (GLMM) with “glmmTMB” (Brooks et al., 2017) function with Conway-maxwell-Poisson (compois) family distribution, followed by Tukey HSD posthoc test for multiple comparisons by used “emmeans” (Lenth et al., 2023) and “multcomp” (Hothorn et al., 2008) packages.

Experiment 3

In a Y-tube olfactometer, the number of *pegomya* beet fly responses was counted, and the statistical analysis was performed by the generalized linear model (GLM) with binomial family distribution, where the number of successes (= all flies that went to the respective odor source) was considered as a one-vector response variable. Chi-square tests were performed to find ANOVA from the respective models.

Experiment 4

Hierarchical clustering was computed for VOCs based on the length of the straight line drawn followed by Euclidean distance with complete linkage. Statistics were performed for individual VOCs, where χ^2 value represents the Kruskal-Wallis non-parametric test (n= 6). 2-way ANOVA was performed to analyze the approximate total volatile organic compound as per plant, and per g FW of leaves, followed by Tukey HSD posthoc test for multiple comparisons. Principal component analysis (PCA) and partial least square-discriminate analysis (PLS-DA), Debiased Sparse Partial Correlation (DSPC) network analysis (n= 6) of VOCs profiles of different treatments were analyzed using MetaboAnalyst 5.0 software packages (https://www.metaboanalyst.ca/).

Experiment 5

Six drought boxes (six plants of each box considered a block) were used for each treatment (control, control + beet miner, moderate drought, moderate drought + beet miner, high drought, high drought + beet miner), resulting in a total of n= 18 plants per treatment. Repeated measure 3-way ANOVA was

performed by the linear model (LM) for analyzing sugar beet leaf chlorophyll fluorescence parameters like maximum photochemical quantum yield of PSII (Fv/Fm), effective photochemical quantum yield of PS II (Φ_{PSII}), and the electron transport rate (ETR), followed by Tukey HSD posthoc test for multiple comparisons. The number of larvae hatched from eggs was analyzed by the generalized linear model (GLM) with binomial family distribution, followed by Tukey HSD posthoc test for multiple comparisons. Mined leaf area, pupal and adult weight, and pupae to adult time were analyzed by the linear mixed effect model (LMM), followed by Tukey HSD posthoc test. Percent mined leaf area, egg to pupal time, and total development time were analyzed by Kruskal-Wallis non-parametric test followed by the Dunn test.

Experiment 6

Metabolomics data sets were obtained from leaf samples (n= 6). A linear model was employed (LM) to analyze total amino acid, organic acid, fatty acid, and sugar metabolites and the sugar-to-amino acid ratio. Subsequently, a two-way ANOVA was conducted, and multiple comparisons were performed using the Tukey HSD posthoc test. The calculations for orgainic acid, fatty acid, sugar content and the sugar-to-amino acid ratio were derived directly from the original dataset, while amino acid was computed after applying a $\log_{10}$ transformation. Circular heatmap and clustering representation of all metabolites were analyzed with software R with Z-score initialization. Other metabolites data were analyzed with MetaboAnalyst 5.0 software packages (https://www.metaboanalyst.ca/). Before the analysis, data were normalized by $\log_{10}$ and auto-scaling (mean-centered and divided by the standard deviation of each variable). Both supervised partial least square-discrimination analysis (PLS-DA), and unsupervised principal component analysis (PCA) method was performed to reduce the dimensionality and visualize the relationship among different treatments. The top forty compounds were identified based on the variable importance of projection (VIP) score. To find out significantly up and down-regulated metabolites, volcano plots were performed.

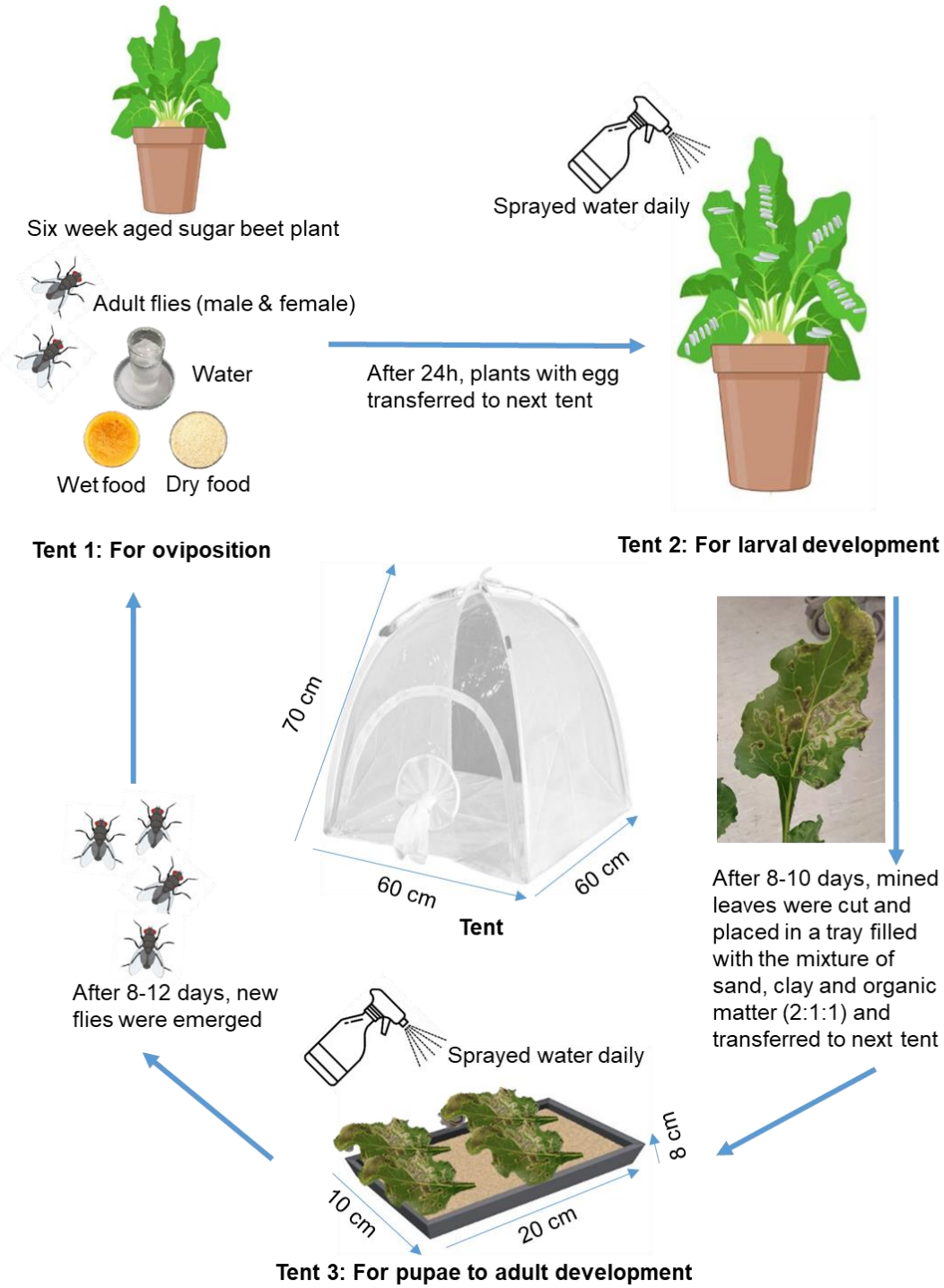


Figure S1: Schematic representation of laboratory method for the rearing of sugarbeet leaf miner *Pegomya cunicularia.*

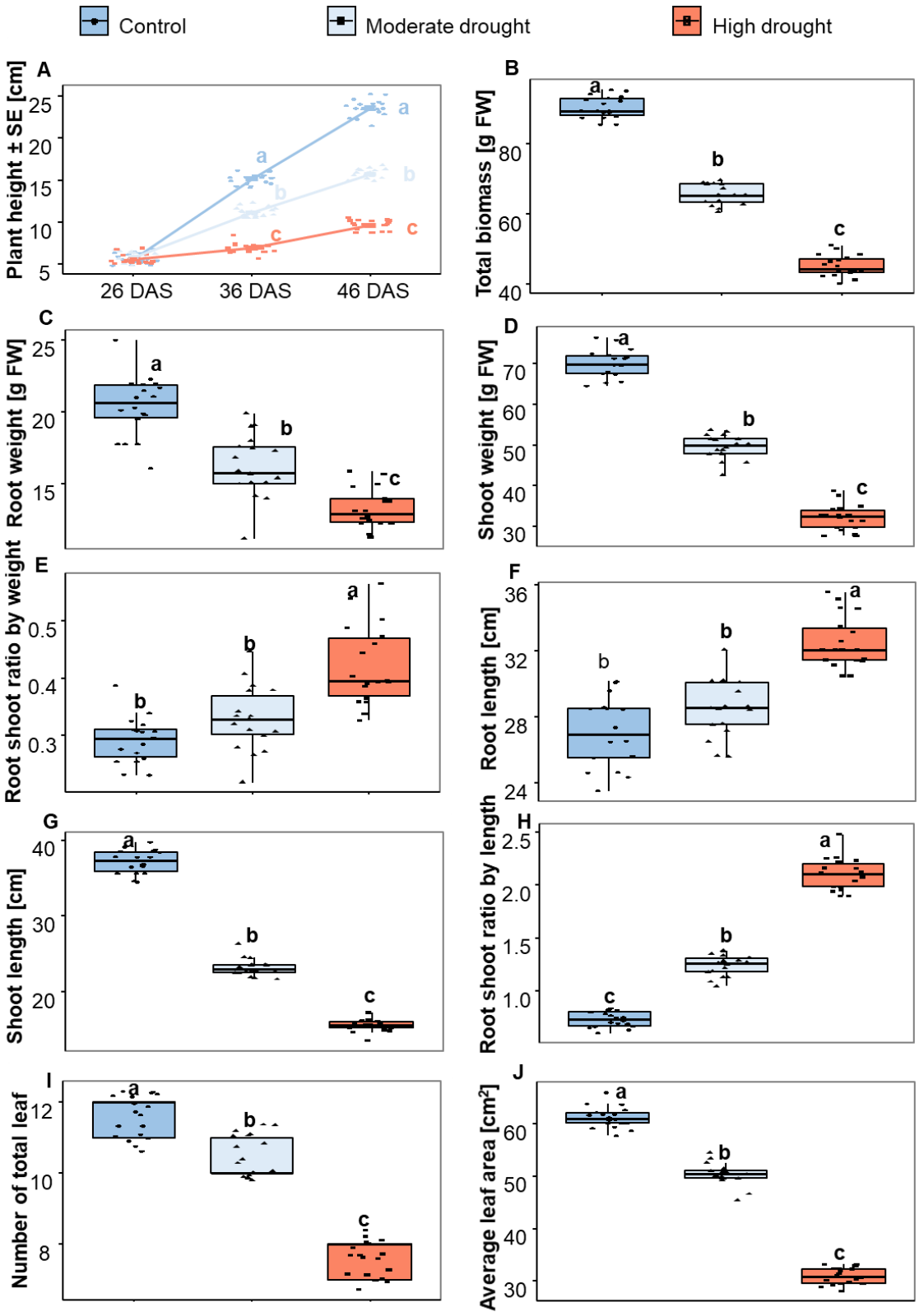


Figure S2: Effect of different magnitude of drought on sugarbeet plant morphological parameters. Plant height (Test: GLMM) (A); Total biomass (Test: LMM) (B); Root weight (Test: LMM) (C); Shoot weight (Test: LMM) (D); Root shoot ratio by weight (Test: LMM) (E); Root length (Test: LMM) (F); Shoot length (Test: Kruskal-Wallis) (G); Root shoot ratio by length (Test: GLMM) (H); Number of leaves (Test: Kruskal-Wallis) (I); Average leaf area (Test: LMM) (J). Data points represent individual replicates, and different letters ($p \leq 0.05$) indicate significance among treatments, n = 18

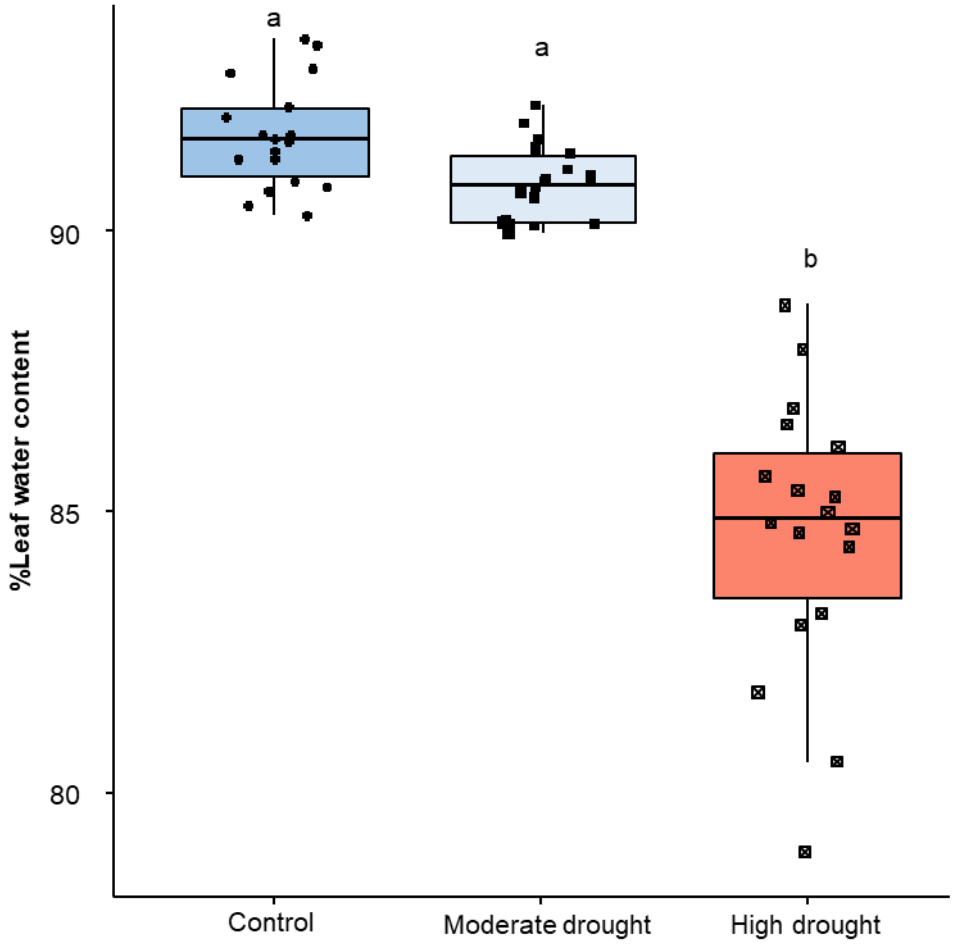


Figure S3: Effect of different magnitude of drought on sugar beet leaf water content (Test: Kruskal-Wallis). Data points represent individual replicates, and different letters ($p \leq 0.05$) indicate significance among treatments, n = 18.

Table S1: Compound detected among different treatments summarized with their retention index from experiment and literature (NIST database)

Compound name	Retention Index Experimental	Retention Index Literature
trans-2-hexenal	801.26	822
4-hydroxy-4-methyl-2-pentanone	845.54	841.5
3-hexenol	856.89	858
α-pinene	936.45	937
1-octen-3-ol	980.61	983
6-methyl-5-hepten-2-one	989.05	998
β-myrcene	991.88	992
octanal	1004.5	1004
cis-3-hexenyl-1-acetate	1007.65	1007
3-carene	1013.31	1012
2-ethylhexanol	1030.01	1029
D-limonene	1032.09	1033
eucalyptol	1035.77	1033
β-ocimene	1039.84	1044
trans-*β*-ocimene	1050.53	1050
acetophenone	1071.3	1073
p-cymene	1089.01	1033
unknown RT-13.13	1118.39	-----
unknown RT-13.87	1156.82	-----
unknown RT-15.15	1225.11	-----
unknown RT-15.31	1234.15	-----
thymol	1250.78	1290
p-cymen-7-ol	1259.88	1278
unknown RT-15.96	1270.26	-----
unknown RT-17.13	1337.57	-----
unknown RT-17.29	1346.91	-----
γ-elemene	1402.27	1410
β-guaiene	1460.35	1479
carotol	1543.16	1594
unknown RT-21.01	1583.39	-----
unknown RT-22.19	1692.64	-----

Table S2: Emission of approximate concentration (ng/h/plant) of specific above-ground VOCs from differently treated sugarbeet plants. CB- = Control without beet miner, CB+ = Control with beet miner, MDB- = Moderate drought without beet miner, MDB+ = Moderate drought with beet miner, HDB- = High drought without beet miner, HDB+ = High drought with beet miner.

Compound	**VOC emission per plant ng/h/plant ± SE**					
	CB-	**CB+**	**MDB-**	**MDB+**	**HDB-**	**HDB+**
trans-2-hexenal	0.08±0.005 abc	0.32±0.21 ab	0.03±0.008 c	0.32±0.08 b	0.09±0.01 abc	0.07±0.02 ac
4-hydroxy-4-methyl-2-pentanone	-----	0.01±0.006	-----	-----	-----	-----
3-hexenol	0.25±0.01 abc	0.88±0.61 abc	0.07±0.01 c	0.91±0.14 a	0.13±0.03 abc	0.75±0.15 ac
α-pinene	0.01±0.002	0.01±0.002	-----	0.001±0.002	-----	-----
1-octen-3-ol	0.03±0.009 b	0.10±0.02 a	-----	-----	-----	-----
6-methyl-5-hepten-2-one	0.04±0.009 b	0.14±0.03 a	-----	-----	-----	-----
β-myrcene	0.01±0.003	0.04±0.01	-----	-----	-----	-----
octanal	0.01±0.001 ab	0.04±0.01 a	-----	0.01±0.002 b	-----	0.02±0.002 a
cis-3-hexenyl-1-acetate	3.97±0.64 ab	4.14±0.13 abc	0.38±0.02 c	5.41±0.47 a	0.79±0.17 ac	4.28±0.05 abc
3-carene	0.06±0.01	0.15±0.05	-----	0.11±0.01	-----	0.08±0.004
2-ethylhexanol	0.19±0.01 ab	0.30±0.06 a	0.07±0.01 c	0.10±0.006 bc	0.21±0.02 ab	0.17±0.01 abc
D-limonene	0.05±0.008 ab	0.09±0.01 b	0.03±0.004ab	0.02±0.0005 a	-----	0.04±0.02 ab
eucalyptol	-----	0.03±0.01	-----	-----	-----	-----
β-ocimene	0.08±0.02 ab	0.27±0.07 a	-----	0.001±0.001 b	-----	-----
trans-β-ocimene	0.04±0.01 b	0.16±0.04 a	-----	-----	-----	-----
acetophenone	0.02±0.005	0.06±0.01	-----	-----	-----	0.06±0.01
p-cymene	0.01±0.001 b	0.03±0.01 b	-----	-----	0.05±0.008 ab	0.10±0.01 a
unknown RT-13.13	0.04±0.01	2.16±1.3	-----	0.18±0.07	-----	0.22±0.07
unknown RT-13.87	-----	0.01±0.007	-----	-----	-----	-----
unknown RT-15.15	0.03±0.01	0.05±0.01	-----	-----	-----	0.04±0.01
unknown RT-15.31	0.08±0.01 ab	0.18±0.06 ab	-----	0.03±0.004 b	-----	0.20±0.04 a
thymol	0.13±0.03 ab	0.43±0.12 a	0.08±0.003ab	0.05±0.02 b	0.31±0.07 a	0.21±0.06 a
p-cymen-7-ol	0.36±0.05 ab	0.68±0.16 a	0.25±0.008ab	0.19±0.03 b	0.41±0.08 ab	0.32±0.10 ab
unknown RT-15.96	0.26±0.03 ab	0.45±0.10 a	0.17±0.007ab	0.12±0.02 b	0.28±0.04 ab	0.22±0.06 ab
unknown RT-17.13	-----	0.02±0.004	-----	-----	-----	-----
unknown RT-17.29	-----	0.02±0.006	-----	-----	-----	-----
γ-elemene	-----	0.02±0.007	-----	0.01±0.001	-----	-----
β-guaiene	-----	0.07±0.1	-----	-----	-----	-----
carotol	-----	0.04±0.01 a	-----	0.001±0.001 b	-----	-----
unknown RT-21.01	-----	0.82±0.43 a	-----	0.02±0.008 b	-----	0.02±0.006 b
unknown RT-22.19	9.40±0.45 a	11.76±0.77 a	9.00±1.21 ab	6.45±0.28 ab	3.14±1.10 b	2.56±0.97b

Table S3: Emission of approximate concentration (ng/h/gFW) of specific above-ground VOCs from differently treated sugarbeet plants. CB- = Control without beet miner, CB+ = Control with beet miner, MDB- = Moderate drought without beet miner, MDB+ = Moderate drought with beet miner, HDB- = High drought without beet miner, HDB+ = High drought with beet miner.

Compound	**VOC emission standardized by plant biomass ng/h/gFW ± SE**					
	CB-	**CB+**	**MDB-**	**MDB+**	**HDB-**	**HDB+**
trans-2-hexenal	0.01±0.0003 c	0.02±0.01 ab	0.01±0.001 c	0.06±0.01 b	0.02±0.004 ab	0.02±0.006 ab
4-hydroxy-4-methyl-2-pentanone	-----	0.001±0.0003	-----	-----	-----	-----
3-hexenol	0.02±0.001 bc	0.05±0.03 abc	0.01±0.002 c	0.17±0.03 ab	0.03±0.009abc	0.22±0.05 a
α-pinene	0.001±0.0001	0.001±0.0001	-----	0.001±0.0003	-----	-----
1-octen-3-ol	0.001±0.0007	0.01±0.001	-----	-----	-----	-----
6-methyl-5-hepten-2-one	0.001±0.0007	0.01±0.001	-----	-----	-----	-----
β-myrcene	0.001±0.0002	0.001±0.0006	-----	-----	-----	-----
octanal	0.001±0.0001 a	0.001±0.0005ab	-----	0.001±0.0003 a	-----	0.01±0.001 b
cis-3-hexenyl-1-acetate	0.30±0.04 abc	0.23±0.005 abc	0.07±0.006 c	1.01±0.11 ab	0.20±0.05 bc	1.27±0.10a
3-carene	0.001±0.0008 c	0.01±0.002 bc	-----	0.02±0.002 ab	-----	0.02±0.001 a
2-ethylhexanol	0.01±0.0009 a	0.02±0.002 a	0.01±0.001 a	0.02±0.0008 ab	0.05±0.009 b	0.05±0.008 b
D-limonene	0.001±0.0006	0.001±0.0008	0.01±0.001	0.001±0.0001	-----	0.01±0.006
eucalyptol	-----	0.00±0.0005	-----	-----	-----	-----
***β*-ocimene**	0.01±0.002 a	0.01±0.003 a	-----	0.001±0.0003 b	-----	-----
trans-*β*-ocimene	0.001±0.001 a	0.01±0.002 b	-----	-----	-----	-----
acetophenone	0.001±0.0004 b	0.001±0.0008ab	-----	-----	-----	0.02±0.005 a
***p*-cymene**	0.001±0.0001 c	0.001±0.0005bc	-----	-----	0.01±0.003 ab	0.03±0.005 a
unknown RT-13.13	0.001±0.0007 a	0.11±0.06 ab	-----	0.03±0.01 ab	-----	0.07±0.02 b
unknown RT-13.87	-----	0.001±0.0003	-----	-----	-----	-----
unknown RT-15.15	0.001±0.0008	0.001±0.0007	-----	-----	-----	0.01±0.003
unknown RT-15.31	0.01±0.001 b	0.01±0.003 b	-----	0.01±0.0009 b	-----	0.06±0.006 a
thymol	0.01±0.002 a	0.02±0.006 ab	0.01±0.001 ab	0.01±0.003 a	0.09±0.02 b	0.07±0.02 ab
p-cymen-7-ol	0.03±0.004	0.04±0.007	0.04±0.004	0.03±0.004	0.11±0.029	0.11±0.03
unknown RT-15.96	0.02±0.003 a	0.02±0.004 ab	0.03±0.002 ab	0.02±0.003 ab	0.07±0.01 b	0.07±0.02 ab
unknown RT-17.13	-----	0.001±0.0001	-----	-----	-----	-----
unknown RT-17.29	-----	0.001±0.0003	-----	-----	-----	-----
γ-elemene	-----	0.001±0.0003	-----	0.001±0.0003	-----	-----
β-guaiene	-----	0.001±0.001	-----	-----	-----	-----
carotol	-----	0.001±0.0007	-----	0.001±0.0002	-----	-----
unknown RT-21.01	-----	0.04±0.02	-----	0.001±0.001	-----	0.001±0.001
unknown RT-22.19	0.70±0.04 bc	0.65±0.03 c	1.63±0.35 a	1.19±0.02 ab	0.84±0.31 abc	0.71±0.20 c

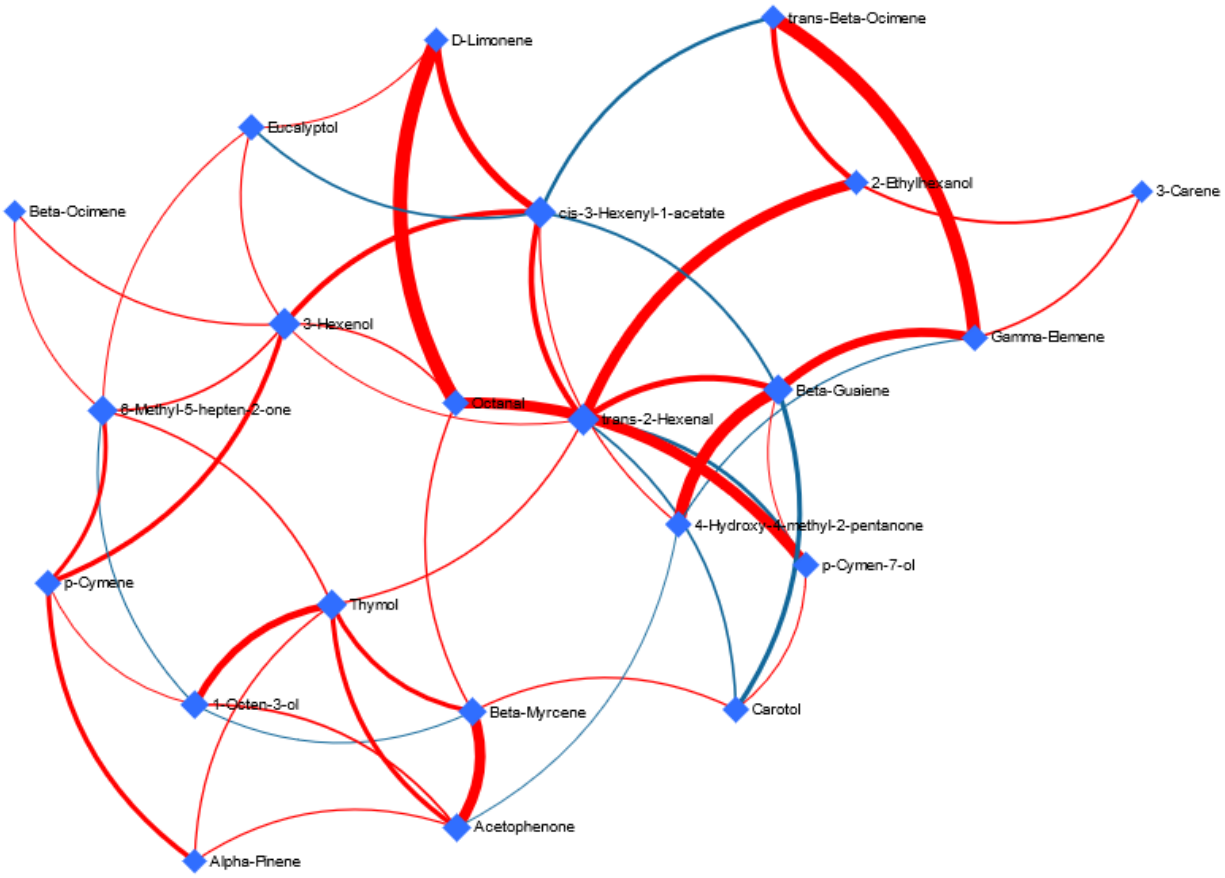


Figure S4: Debiased Sparse Partial Correlation (DSPC) network among different VOCs was performed using MetaboAnalyst 5.0 software packages. Red edges represent positive correlations, while negative correlations are indicated by blue edges. The thickness of the edges reflects the strength of the relationship.

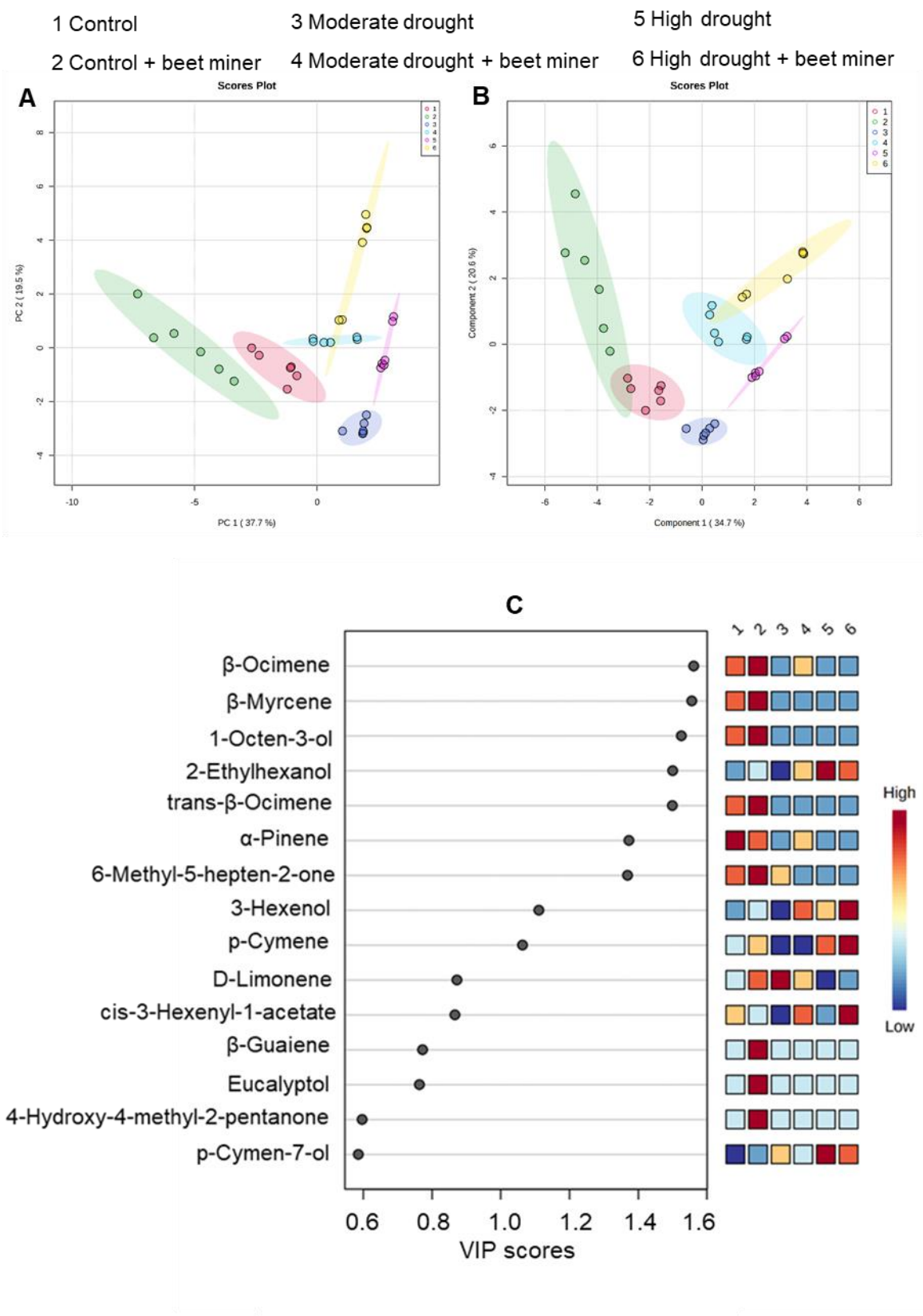


Figure S5: Score plot showing clear clustering of VOCs profiles in sugar beet in response to drought and beet miner stress for both Principal component analysis (PCA) (A); and Partial least square discriminant analysis (PLS-DA) (B). Important VOCs in response to different treatments identified by partial least square-discriminate analysis (PLS-DA). Fifteen top VOCs are shown according to the variable importance of projection (VIP) score to different treatments (C). Colored boxes indicate the relative concentrations of the corresponding VOCs in each group. n= 6.

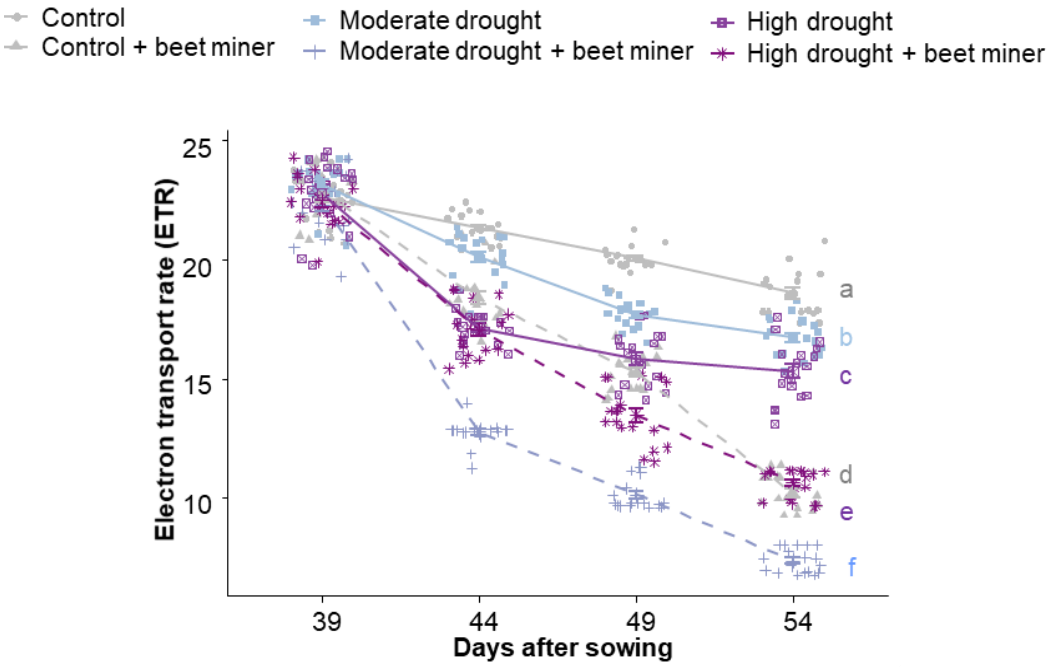
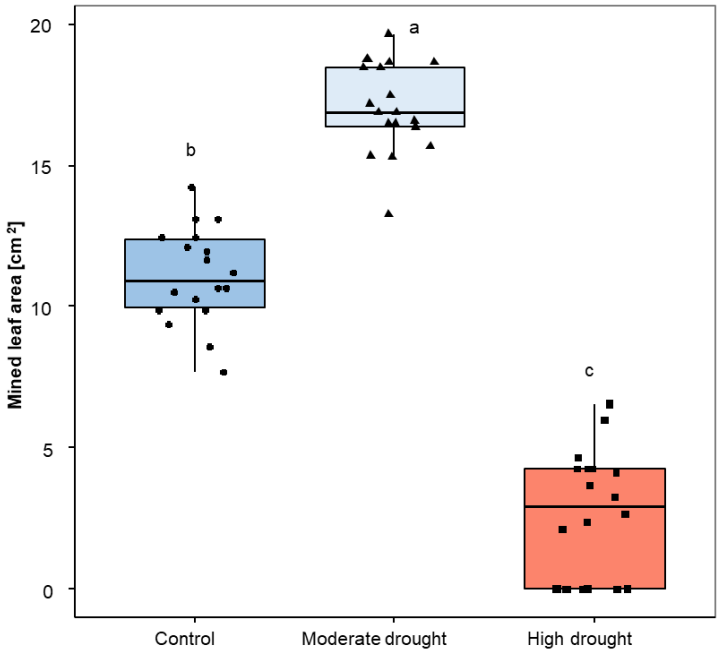


Figure S6: Effect of drought stress and beet miner infestation on the parameter of sugar beet leaf photosynthetic efficiencies of electron transport rate (ETR) (Test: LM 3-way ANOVA). Data point represents individual replicates, and different letters ($p \leq 0.05$) indicate significance among treatments, n = 18.



Supplementary Fig. S7: Effect of different magnitudes of drought on the leaf area infestation by the larvae of *Pegomya cunicularia* (Test: LMM). Data point represents individual replicates, and different letters ($p \leq 0.05$) indicate significance among treatments, n= 18.

Table S4: Statistical interactions of leaf photosynthetic efficiencies parameters

Photosynthetic efficiencies	Factors	Statistics
Maximum quantum yield of PS II (Fv/Fm)	Drought	$F_{2,408} = 601.66$, $p < 0.001$
	Beet miner	$F_{1,408} = 7656.06$, $p < 0.001$
	Time	$F_{3,408} = 4519.50$, $p < 0.001$
	Drought × Beet miner	$F_{2,408} = 898.51$, $p < 0.001$
	Drought × Time	$F_{6,408} = 126.87$, $p < 0.001$
	Beet miner × Time	$F_{3,408} = 1445.90$, $p < 0.001$
	Drought × Beet miner × Time	$F_{6,408} = 161.24$, $p < 0.001$
Quantum yield (Φ_{PSII}) of PS II	Drought	$F_{2,408} = 252.94$, $p < 0.001$
	Beet miner	$F_{1,408} = 2015.43$, $p < 0.001$
	Time	$F_{3,408} = 2069.20$, $p < 0.001$
	Drought × Beet miner	$F_{2,408} = 202.98$, $p < 0.001$
	Drought × Time	$F_{6,408} = 33.75$, $p < 0.001$
	Beet miner × Time	$F_{3,408} = 271.07$, $p < 0.001$
	Drought × Beet miner × Time	$F_{6,408} = 23.87$, $p < 0.001$
Electron transport rate (ETR)	Drought	$F_{2,408} = 251.43$, $p < 0.001$
	Beet miner	$F_{1,408} = 1967.41$, $p < 0.001$
	Time	$F_{3,408} = 2031.44$, $p < 0.001$
	Drought × Beet miner	$F_{2,408} = 195.37$, $p < 0.001$
	Drought × Time	$F_{6,408} = 32.53$, $p < 0.001$
	Beet miner × Time	$F_{3,408} = 270.15$, $p < 0.001$
	Drought × Beet miner × Time	$F_{6,408} = 24.15$, $p < 0.001$

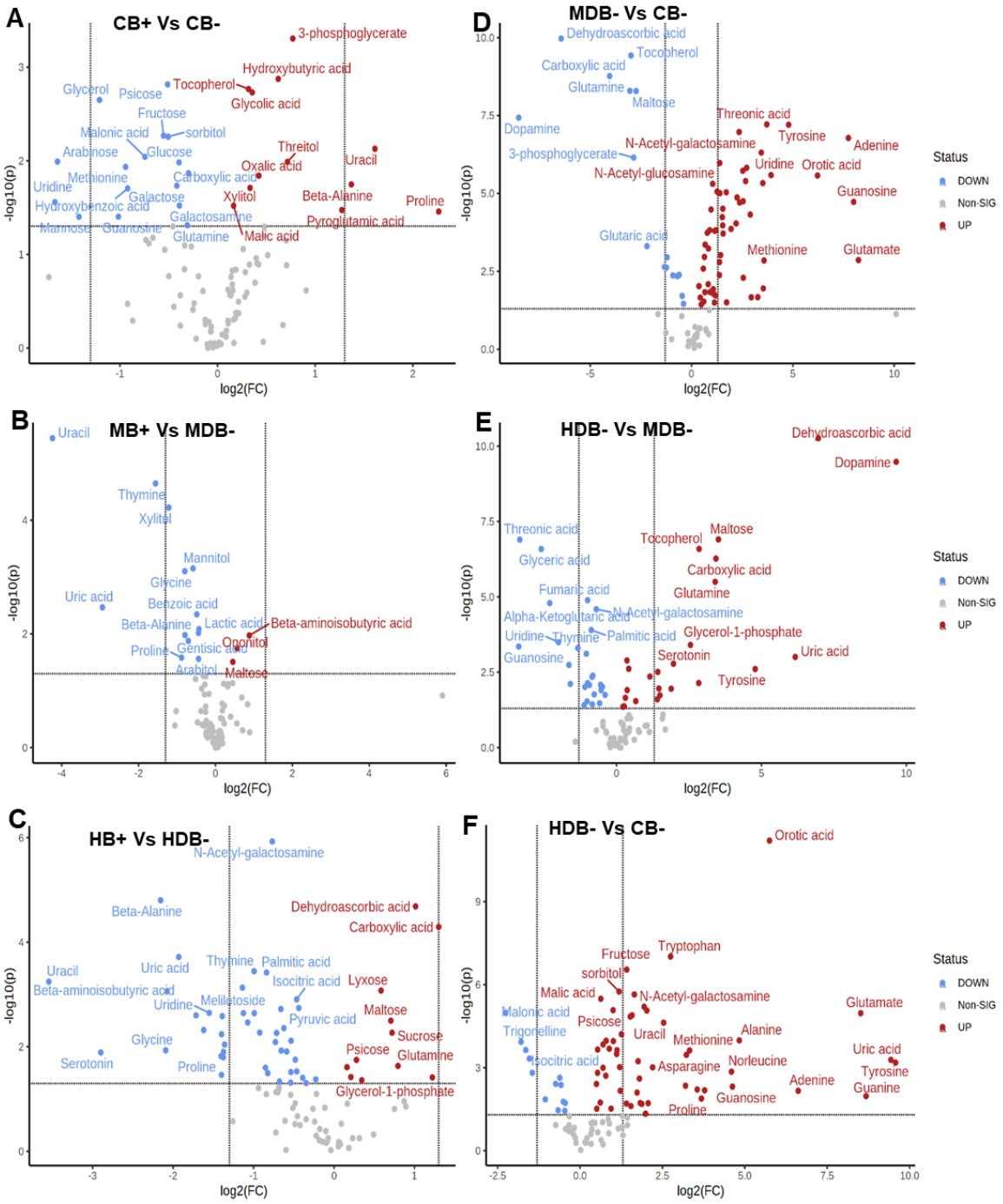


Figure S8: Effect of different magnitude of drought stress and beet miner infestation on central metabolites showing fold changes of up-regulated and down-regulated metabolites. CB- = Control without beet miner, CB+ = Control with beet miner, MDB- = Moderate drought without beet miner, MDB+ = Moderate drought with beet miner, HDB- = High drought without beet miner, HDB+ = High drought with beet miner, n= 6.

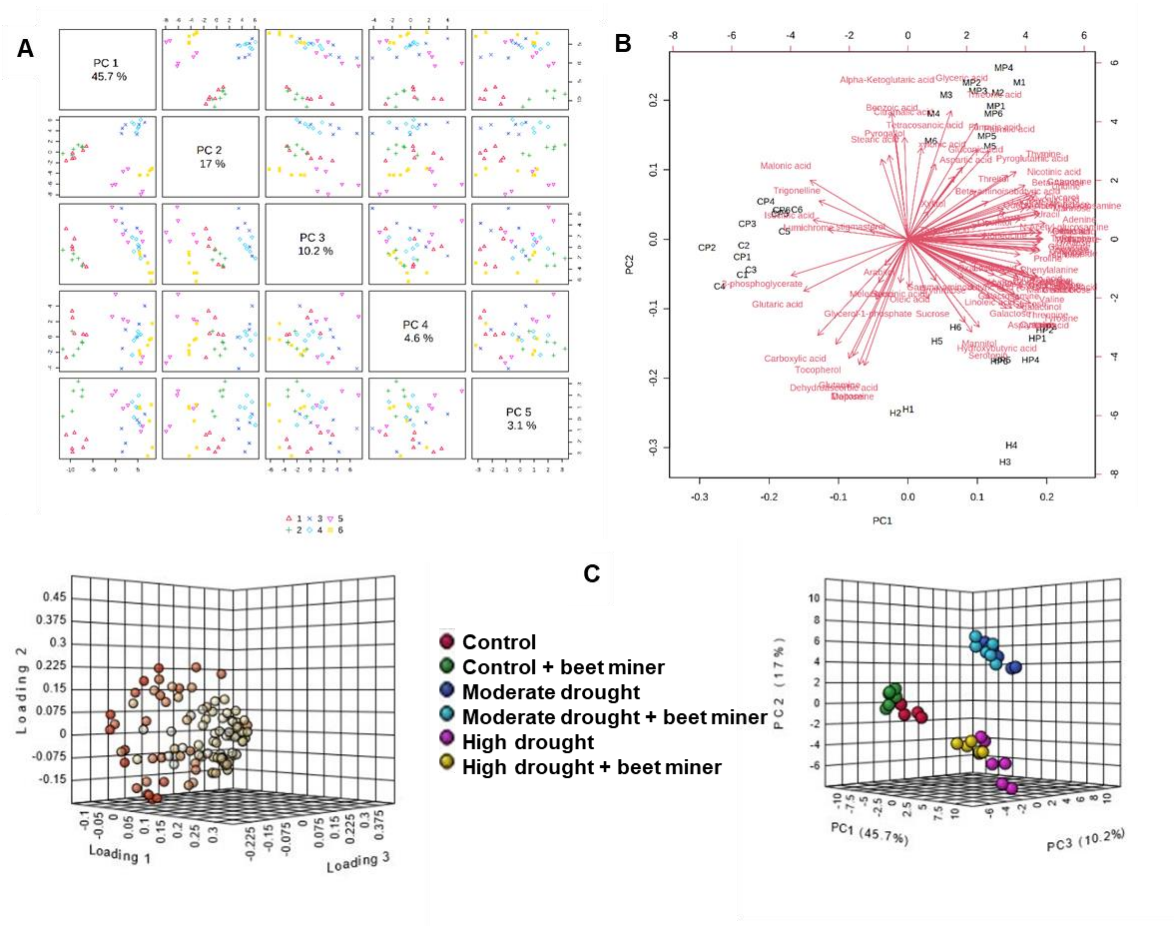


Figure S9: Principal component analysis (PCA) of central metabolites profiles of different treatments. Illustrated are the 95% confidence intervals for each group, n= 6. Overview of PCA (A); biplot of PCA (B); and synchronized 3D plot (C).

References

Brooks, M. E., Kristensen, K., Benthem, K. J. van, Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Mächler, M., & Bolker, B. M. (2017). GlmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. *The R Journal*, *9*(2), 378–400.

Florian Hartig. (2022). *DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models*. https://cran.r-project.org/web/packages/DHARMa/vignettes/DHARMa.html

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous Inference in General Parametric Models. *Biometrical Journal*, *50*(3), 346–363. https://doi.org/10.1002/bimj.200810425

José Pinheiro, J. P., Douglas Bates, Saikat DebRoy, Deepayan Sarkar (up, & R Core Team. (2023). *nlme: Linear and Nonlinear Mixed Effects Models* (3.1-162). https://CRAN.R-project.org/package=nlme

Lenth, R. V., Bolker, B., Buerkner, P., Giné-Vázquez, I., Herve, M., Jung, M., Love, J., Miguez, F., Riebl, H., & Singmann, H. (2023). *emmeans: Estimated Marginal Means, aka Least-Squares Means* (1.8.5). https://CRAN.R-project.org/package=emmeans

Mangiafico, S. (2023). *rcompanion: Functions to Support Extension Education Program Evaluation* (2.4.26). https://CRAN.R-project.org/package=rcompanion

General discussion

The study findings revealed that drought and soil salinity have distinct impacts on the three trophic levels associated with sugar beet. Both stress directly affected the morphological (reduced size, biomass, etc.), physiological (decreased photosynthetic efficiency), and biochemical (changes in phytohormones, central metabolites, VOCs) characteristics of sugar beet plants. These alterations in plant features resulted in either positive effects (e.g. drought promoting aphid and beet leaf miner performance) or negative effects (e.g. salinity affecting aphid performance) on herbivores depending on magnitude of drought and salinity stress. However, parasitoid *Aphidious colemani* at the third trophic level consistently exhibited negative performances due to sugar beet-mediated drought and salinity stress. Moreover, VOC played a significant role in attracting female beet flies to find suitable hosts for oviposition and attracting parasitoids to aphid-infested plants.

Abiotic-biotic stresses in sugar beet performance

Abiotic stresses like drought and salinity severely alter plant growth and morphology, leading to many physiological and biochemical responses. Drought triggers a phytohormonal signaling cascade and induces stomatal closure, resulting in reduced gas exchange and, ultimately, reduced photosynthesis (Ding et al., 2018; Farooq et al., 2009). Drought also interferes with mitosis, and turgor loss inhibits cell elongation, leading to reduced growth, biomass, leaf area, leaf water content etc. (Fahad et al., 2017; Farooq et al., 2009; Yang et al., 2021). Conversely, soil salinity interferes with plant water equilibrium, reduces turgor pressure, and leads to the accumulation of Na+ and Cl- ions while inhibiting K+ and Ca2+ uptake and causing ion toxicity (Arif et al., 2020; Zörb et al., 2019). Ultimately, salinity also negatively impacts plant's morphological features resulting in stunted growth, reduced cell elongation, decreased photosynthetic efficiencies like maximum photochemical quantum yield of PSII (Fv/Fm), metabolic adaptations, etc. (Chele et al., 2021; Munns & Tester, 2008; Shin et al., 2021; van Zelm et al., 2020). Findings showed that drought and salinity stress symptoms were already visible at moderate levels and were even more pronounced in the high level of drought and salinity treatments (Chapters 1, 2, and 3).

Abiotic-biotic stresses in sugar beet phytohormones

Phytohormones are signaling molecules that regulate various cellular activities in plants. In this study (Chapter 2), ABA levels were significantly increased upon salinity stress. ABA modulates stomata conductance and rapid increases in ABA levels are typical for plants subjected to salinity or drought stress to reduce water loss by transpiration (Chen et al., 2020; Huang et al., 2021; Min et al., 2015; Niu et al., 2018; Yu et al., 2020). Similarly, ODPA has a regulatory effect on stomatal conductance under drought- and salinity-stress and regulates ion transport and osmotic adjustment in salinity-stressed plants. As a precursor for JA, OPDA further plays a key role in defense induction against herbivores, including aphids, and increased levels of JA have been reported in sugar beet plants upon aphid infestation. Levels of JAI, which is the main signaling molecule in defense induction by the JA signaling pathway closely resemble JA levels in this study. JA signaling may further play a direct role in plants' responses to salinity stress and an increase in JA levels upon salt stress were found in tobacco, Arabidopsis, and wheat (Chen et al., 2016; Valenzuela et al., 2016; Zhao et al., 2014). Crosstalk between ABA signaling and defense signaling pathways has been reported (Guo et al., 2016; Quais et al., 2020) and some aphid species seem to activate ABA signaling to suppress JA and SA-related defenses (Hillwig et al., 2016; Studham & MacIntosh, 2013). In some plant species, an increase in JA levels can suppress SA-mediated defense responses (Morkunas et al. 2011). However, the relationship between JA and SA in sugar beet defense against aphids is not yet well-characterized. Findings indicated that increased SA levels in aphid-infested sugar beet plants at low and moderate salinity levels after 48 h of aphid feeding, while no differences were observed for the other time points and treatments, suggesting that SA signaling does not play an important role in sugar beet reaction to salinity stress and aphid-infestation. These results suggest that salinity stress enhances JA signaling in aphid-infested sugar beets due to increased levels of the JA-precursor OPDA in salinity-stressed plants, which gets converted to JA upon aphid feeding.

Abiotic-biotic stresses in sugar beet central metabolites

Plants synthesize central metabolites, and a balanced production of these metabolites is necessary for essential functions such as growth and development.

Abiotic stresses, such as drought and soil salinity, induce osmotic stress in plants (Zhu, 2016). As a response, plants modify their central metabolites to cope with these harsh environmental conditions. For example, when plants are exposed to salinity or drought conditions, they can respond by synthesizing and accumulating small molecules such as proline, betaine, trehalose, sugar, and glycine. These compounds aid in balancing the concentration of solutes inside and outside of the cells, thereby maintaining the proper osmotic potential (Abobatta, 2020; Anjum et al., 2017; Hassan et al., 2015; Koyro et al., 2012; Sharma et al., 2019). Similar conditions were also observed in this study, wherein both drought and salinity increased the concentration of amino acids, organic acids, fatty acids, and sugar metabolites (Chapters 2, and 3). This increase in metabolite concentration might partially be the result of reduced water availability caused by osmotic stress. Moreover, abiotic and biotic stressors cause significant upregulation or downregulation of plant metabolites, with the most important metabolites varying depending on the level of abiotic and biotic stress (Chapter 3), suggesting stresses may alter osmoprotectants to maintain cell turgor pressure via osmoregulation, for protection of cellular components (Singh et al., 2022).

Abiotic stress on herbivore performances

Abiotic stressors, such as drought and soil salinity, alter herbivore performance in multiple ways. They can change the quality of host plants, leading to reduced water availability, nutritional imbalances, decreased palatability, and the induction of ion toxicity. Drought stress on aphid performance are variable (Leybourne et al., 2021), indicating that there is no general response of aphids towards drought-stressed host plants. The effects of drought stress on aphids can further depend on the timing and magnitude of the water limitation experienced (Luo & Gilbert, 2022; Tariq et al., 2013) and on specific interactions between aphid species and host plant species (Leybourne et al., 2021; Mewis et al., 2012). This study (Chapter 1) found that the performance and fecundity of individual *Aphis fabae* were highest on plants receiving high drought treatment, intermediate on plants experiencing moderate drought and lowest on well-watered plants. Reasons for enhanced aphid performance on drought-stressed plants could be an increase in the nutritional quality of the drought-stressed plant (Smith et al., 2019), which includes sugar and or/ amino acid concentrations

(Fàbregas & Fernie, 2019; Hale et al., 2003). The findings from Chapter 3 indicate that drought conditions can result in the concentration of amino acids, organic acids, fatty acids, and sugar metabolites. This concentration of nutrients can potentially offer improved nourishment for the development of herbivores, as evidenced by the enhanced performance and growth of beet leaf miners in moderately drought-stressed plants. Moreover, leaf water content is essential in maintaining the leaf's nutritional quality and is also responsible for the better development of the leaf miners (Loomis, 1997; Wei et al., 2000). In this study, non-significant differences in leaf water content between control plants and moderately drought plants were observed, which allows for an adequate concentration of plant sugars and/or amino acids, creating an optimal nutrient blend that promotes the improved growth of beet leaf miners, as observed other leaf miners studies (Body et al., 2019; Chown et al., 2004; Schoonhoven et al., 2005).

On the other hand, salinity also increases the concentration of amino acids, organic acid, and sucrose metabolites found in other plants (Hartzendorf & Rolletschek, 2001; Khan et al., 2020), including sugarbeet roots (Liu et al., 2020) that might have a either positive (Eichele-Nelson et al., 2018; Polack et al., 2011) or negative (Araya et al., 1991) effect on aphid performances. In the same way, findings from Chapter 2 also indicate that increased salinity stress increases the concentration of metabolites but observed adverse effects on the performance and reproduction of individual aphids and aphid population growth. One factor might impact aphid performance is phloem viscosity. Due to the higher concentration of sugars, phloem viscosity might be increased, which might have a negative effect on phloem uptake. Another factor that might affect aphid feeding and performance is the accumulation of toxic ions, such as Na^+, Cl^-, Mg^{2+}, SO_4^{2-}, or HCO_3, in plants growing under saline conditions (Zörb et al., 2019).

Abiotic stress on parasitoid performances

Findings from Chapters 1 and 2, emerging adults of *A. colemani* displayed smaller body size, lower emergence rate, and a male-biased sex ratio when developing in aphids on drought-stressed or salinity-stressed plants. This may be due to the smaller body size of aphid hosts on drought-stressed sugar beets (Rahman et al. unpublished) or smaller body size of aphids in salinity conditions

(Chapter 2). Aphid body size and the size of emerging parasitoids are usually positively correlated, and parasitoid emergence rate can be related to host size and quality (Garratt et al., 2010; Tariq et al., 2013; Yasir Ali et al., 2022). Aphid parasitoids can actively control sex allocation by laying more unfertilized eggs, resulting in more male offspring on poor-quality hosts (Cloutier et al., 1991; Pandey & Singh, 1999). Alternatively, female parasitoids, with their larger body size, may require larger hosts to develop successfully, and female larvae may not have been able to develop successfully in small hosts on drought and salinity-stressed plants.

Abiotic-biotic stresses in sugar beet volatiles and herbivore oviposition and parasitoids preferences

The emission of plant volatiles can vary both in quantity and quality, depending on the biotic and abiotic stress factors involved, and these changes can influence the attraction of natural enemies to herbivore-infested plants (Dicke & Baldwin, 2010; Kugimiya et al., 2010). Female insects may also rely on these plant volatile cues to locate suitable hosts for oviposition (Achhami et al., 2021; De Moraes et al., 2001). In Chapters 1, 2, and 3 of this study, it was observed that control plants exhibited a higher total VOC emission compared to drought and salinity-stressed plants, which can be attributed to the reduced biomass of the stressed plants. But, when plants were infested by herbivores (aphids or beet miners), a consistent increase in VOC emissions was observed. The highest total VOC emissions and a greater number of VOCs are typically observed in control plants that were infested by herbivores. Chapter 3 of the study found that VOC emissions from control plants generally attract more female beet flies than plants experiencing drought. This could be attributed to the higher number of volatile compounds, particularly monoterpenoids, and total higher emission of volatiles in control plants. Interestingly, when sugar beet plants were infested with beet miners, they emitted higher concentrations of additional volatiles, including cis-*β*-ocimine, *β*-myrcene, 1-octane-3ol, trans-*β*-ocimene, *β*-guaiene, eucalyptol, and 4-hydroxy-4-methyl-2-pentanone, which might reduce the attraction of beet flies.

Drought has been shown to affect the foraging success of a parasitoid by altering plant volatile emissions. This resulted in less attractive (Tariq et al., 2013) or even unrecognizable signals (Martini & Stelinski, 2017), but positive (Salerno

et al., 2017) or neutral effects of drought stress on parasitoid attraction have also been reported (Weldegergis et al., 2015). In our study (Chapter 1), aphid infestation on well-watered plants resulted in a strong increase in total VOC emission and in the release of eight VOCs that were not detected in the well-watered controls without aphids. The well-watered aphid-infested plants were the most attractive to the parasitoid *A. colemani*. In the drought-stressed plants, on the other hand, the number of compounds detected and the total emission of volatile compounds per plant were greatly reduced. Not all plant VOCs are perceived by parasitoids (Goelen et al., 2021; Li et al., 2022), but in our study, we found a strong correlation between the total amount of emitted VOCs and the attraction of *A. colemani*. Aphid-infested plants from the well-watered treatment and from the high drought treatments emitted significantly more volatiles and attracted more parasitoids than the uninfested plants subjected to the same watering regime. This shows that, despite the lower overall attraction of *A. colemani* to drought-stressed plants, the parasitoids were still able to discriminate between aphid-infested and non-infested plants under severe drought stress. Similar to our results, *A. colemani* and *Diaeretiella rapae* preferred aphid-infested well-watered Brussels sprouts to aphid-infested drought-stressed plants. Emission rates of allyl isothiocyanate, limonene, and *β*-phellandrene from drought-stressed aphid-infested plants were more similar to the undamaged well-watered controls than to aphid-infested well-watered plants (Tariq et al., 2013), suggesting that reduced differences in emission rates of behaviorally active compounds from infested and undamaged plants under drought stress may reduce parasitoid attraction. Most non-oxygenated monoterpenes emitted by sugar beet (e.g. *β*-pinene, *p*-menthane, *β*-myrcene, 3-carene, D-limonene, *o*-cymene etc.) were released in higher quantities from aphid-infested plants than from undamaged plants belonging to the well-watered and the high drought treatments. Thus, these compounds may be reliable cues for parasitoids to indicate host presence even under drought stress conditions. Notably, most of these compounds were absent or emitted only in low amounts from the aphid-infested plants in the moderate drought treatment, coinciding with low parasitoid attraction.

However, Chapter 2, observed that more parasitoids were attracted to the volatiles emitted by aphid-infested plants compared to undamaged plants, regardless of the salinity treatment. This suggests that the signal encoded in the volatile blend remained intact despite the reduced total emission from aphid-infested plants under severe salinity stress. 1,2,4,5-tetramethyl benzene, 1,3-dimethyl-5-methyl benzene, longifolene, and *β*-caryophyllene were only emitted by aphid-infested plants or in much higher amounts than from the undamaged plants and may thus play an important role as the key components in attracting parasitoids in our system.

Conclusion and future directions

Chapter-1: Drought benefits aphids in three ways- (i) by enhancing plant suitability as hosts, (ii) reducing parasitoids reproduction, and (iii) decreasing emission of parasitoid-attracting volatiles.

Chapter-2: Salinity alters trophic interactions in three ways- (i) by reducing aphid reproduction due to induction of defense phytohormones OPDA, JA, and JAI, (ii) reducing parasitoid performance due to smaller body size of aphids, and (iii) decreasing emission of parasitoid-attracting volatiles, but parasitoid attraction to aphid-infested plants not affected because of some volatiles only emitted upon aphid infestation.

Chapter-3: The preference and performance of beet leaf miners are altered by drought conditions, as indicated by the findings that- (i) beet flies exhibit a host-finding preference for oviposition by distinguishing between infested and uninfested or drought-stressed plants through the detection of volatile cues. (ii) Moderate drought benefits beet miners by improving plant suitability by enhancing nutrients and maintaining leaf water content.

Based on these findings, several potential future research directions could build. Some possible avenues for future research include:

1. Chemical ecology of volatile cues: Identifying and characterizing the specific volatile compounds emitted by infested or stressed plants and exploring their effects on the behavior and physiology of parasitoids and beet flies. Using electrophysiological measurements to investigate the receptor mechanisms of these volatile cues in herbivores and their

natural enemies can shed light on their ecological significance and potential for manipulating herbivore interactions.

2. Unraveling the molecular mechanisms: Conducting transcriptomic and proteomic analyses to understand the molecular responses of plants under abiotic stress and their implications for herbivore interactions. This would involve studying the expression patterns of genes and proteins involved in synthesizing and releasing volatile compounds, defense signaling pathways, and herbivore perception. By unraveling the intricate molecular mechanisms underlying these processes, we can comprehensively understand the chemical ecology of abiotic stress and herbivory.

3. Multitrophic interactions and virus dynamics: Investigate the complex multitrophic interactions involving aphids, host plants, primary parasitoids, hyperparasitoids, predators, and pathogens in the context of virus dynamics. Explore how changes in plant defense responses, herbivore feeding behavior, and natural enemy effectiveness influence virus transmission. Assess the potential for indirect effects, such as altered plant volatiles or defense-related compounds, to influence the behavior and performance of aphids and their associated natural enemies, subsequently affecting virus spread.

References

Abobatta, W. F. (2020). Plant Responses and Tolerance to Combined Salt and Drought Stress. In M. Hasanuzzaman & M. Tanveer (Eds.), Salt and Drought Stress Tolerance in Plants: Signaling Networks and Adaptive Mechanisms (pp. 17–52). Springer International Publishing. https://doi.org/10.1007/978-3-030-40277-8_2

Achhami, B. B., Reddy, G. V. P., Hofland, M. L., Sherman, J. D., Peterson, R. K. D., & Weaver, D. K. (2021). Plant Volatiles and Oviposition Behavior in the Selection of Barley Cultivars by Wheat Stem Sawfly (Hymenoptera: Cephidae). Environmental Entomology, 50(4), 940–947. https://doi.org/10.1093/ee/nvab035

Anjum, S. A., Ashraf, U., Tanveer, M., Khan, I., Hussain, S., Shahzad, B., Zohaib, A., Abbas, F., Saleem, M. F., Ali, I., & Wang, L. C. (2017). Drought Induced Changes in Growth, Osmolyte Accumulation and Antioxidant Metabolism of Three Maize Hybrids. Frontiers in Plant Science, 8, 69. https://doi.org/10.3389/fpls.2017.00069

Araya, F., Abarca, O., Zúñiga, G. E., & J. Corcuera, L. (1991). Effects of NaCl on glycine-betaine and on aphids in cereal seedlings. Phytochemistry, 30(6), 1793–1795. https://doi.org/10.1016/0031-9422(91)85014-Q

Arif, Y., Singh, P., Siddiqui, H., Bajguz, A., & Hayat, S. (2020). Salinity induced physiological and biochemical changes in plants: An omic approach towards salt stress tolerance. Plant Physiology and Biochemistry, 156, 64–77. https://doi.org/10.1016/j.plaphy.2020.08.042

Body, M. J. A., Casas, J., & Giron, D. (2019). Manipulation of plant primary metabolism by leaf-mining larvae in the race against leaf senescence (p. 777334). bioRxiv. https://doi.org/10.1101/777334

Chele, K. H., Tinte, M. M., Piater, L. A., Dubery, I. A., & Tugizimana, F. (2021). Soil Salinity, a Serious Environmental Issue and Plant Responses: A Metabolomics Perspective. Metabolites, 11(11), Article 11. https://doi.org/10.3390/metabo11110724

Chen, K., Li, G.-J., Bressan, R. A., Song, C.-P., Zhu, J.-K., & Zhao, Y. (2020). Abscisic acid dynamics, signaling, and functions in plants. Journal of Integrative Plant Biology, 62(1), 25–54. https://doi.org/10.1111/jipb.12899

Chen, X., Zhang, X., Jia, A., Xu, G., Hu, H., Hu, X., & Hu, L. (2016). Jasmonate mediates salt-induced nicotine biosynthesis in tobacco (Nicotiana tabacum L.). Plant Diversity, 38(2), 118–123. https://doi.org/10.1016/j.pld.2016.06.001

Chown, S. L., Chown, S., & Nicolson, S. (2004). Insect Physiological Ecology: Mechanisms and Patterns. OUP Oxford.

Cloutier, C., Lévesque, C. A., Eaves, D. M., & Mackauer, M. (1991). Maternal adjustment of sex ratio in response to host size in the aphid parasitoid Ephedrus californicus. Canadian Journal of Zoology, 69(6), 1489–1495. https://doi.org/10.1139/z91-208

De Moraes, C. M., Mescher, M. C., & Tumlinson, J. H. (2001). Caterpillar-induced nocturnal plant volatiles repel conspecific females. Nature, 410(6828), 577–580. https://doi.org/10.1038/35069058

Dicke, M., & Baldwin, I. T. (2010). The evolutionary context for herbivore-induced plant volatiles: Beyond the 'cry for help.' Trends in Plant Science, 15(3), 167–175. https://doi.org/10.1016/j.tplants.2009.12.002

Ding, L., Lu, Z., Gao, L., Guo, S., & Shen, Q. (2018). Is Nitrogen a Key Determinant of Water Transport and Photosynthesis in Higher Plants Upon Drought Stress? Frontiers in Plant Science, 9. https://www.frontiersin.org/articles/10.3389/fpls.2018.01143

Eichele-Nelson, J., DeSutter, T., Wick, A. F., Harmon, E. L., & Harmon, J. P. (2018). Salinity Improves Performance and Alters Distribution of Soybean Aphids. Environmental Entomology, 47(4), 875–880. https://doi.org/10.1093/ee/nvy072

Fàbregas, N., & Fernie, A. R. (2019). The metabolic response to drought. Journal of Experimental Botany, 70(4), 1077–1085. https://doi.org/10.1093/jxb/ery437

Fahad, S., Bajwa, A. A., Nazir, U., Anjum, S. A., Farooq, A., Zohaib, A., Sadia, S., Nasim, W., Adkins, S., Saud, S., Ihsan, M. Z., Alharby, H., Wu, C., Wang, D., & Huang, J. (2017). Crop Production under Drought and Heat Stress: Plant Responses and Management Options. Frontiers in Plant Science, 8. https://www.frontiersin.org/articles/10.3389/fpls.2017.01147

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., & Basra, S. M. A. (2009). Plant drought stress: Effects, mechanisms and management. Agronomy for Sustainable Development, 29(1), 185–212. https://doi.org/10.1051/agro:2008021

Garratt, M. P. D., Leather, S. R., & Wright, D. J. (2010). Tritrophic effects of organic and conventional fertilisers on a cereal-aphid-parasitoid system. Entomologia Experimentalis et Applicata, 134(3), 211–219. https://doi.org/10.1111/j.1570-7458.2009.00957.x

Goelen, T., Vuts, J., Sobhy, I. S., Wäckers, F., Caulfield, J. C., Birkett, M. A., Rediers, H., Jacquemyn, H., & Lievens, B. (2021). Identification and application of bacterial volatiles to attract a generalist aphid parasitoid: From laboratory to greenhouse assays. Pest Management Science, 77(2), 930–938. https://doi.org/10.1002/ps.6102

Guo, H., Sun, Y., Peng, X., Wang, Q., Harris, M., & Ge, F. (2016). Up-regulation of abscisic acid signaling pathway facilitates aphid xylem absorption and osmoregulation under drought

stress. Journal of Experimental Botany, 67(3), 681–693. https://doi.org/10.1093/jxb/erv481

Hale, B. K., Bale, J. S., Pritchard, J., Masters, G. J., & Brown, V. K. (2003). Effects of host plant drought stress on the performance of the bird cherry-oat aphid, Rhopalosiphum padi (L.): A mechanistic analysis. Ecological Entomology, 28(6), 666–677. https://doi.org/10.1111/j.1365-2311.2003.00563.x

Hartzendorf, T., & Rolletschek, H. (2001). Effects of NaCl-salinity on amino acid and carbohydrate contents of Phragmites australis. Aquatic Botany, 69(2), 195–208. https://doi.org/10.1016/S0304-3770(01)00138-3

Hassan, M. A., Fuertes, M. M., Sánchez, F. J. R., Vicente, O., & Boscaiu, M. (2015). Effects of Salt and Water Stress on Plant Growth and on Accumulation of Osmolytes and Antioxidant Compounds in Cherry Tomato. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 43(1), Article 1. https://doi.org/10.15835/nbha4319793

Hillwig, M. S., Chiozza, M., Casteel, C. L., Lau, S. T., Hohenstein, J., Hernández, E., Jander, G., & MacIntosh, G. C. (2016). Abscisic acid deficiency increases defence responses against Myzus persicae in Arabidopsis. Molecular Plant Pathology, 17(2), 225–235. https://doi.org/10.1111/mpp.12274

Huang, Y., Zhou, J., Li, Y., Quan, R., Wang, J., Huang, R., & Qin, H. (2021). Salt Stress Promotes Abscisic Acid Accumulation to Affect Cell Proliferation and Expansion of Primary Roots in Rice. International Journal of Molecular Sciences, 22(19), 10892. https://doi.org/10.3390/ijms221910892

Khan, N., Ali, S., Zandi, P., Mehmood, A., Ullah, S., Ikram, M., Ismail, I., Shahid, M. A., & Babar, M. A. (2020). Role of sugars, amino acids and organic acids in improving plant abiotic stress tolerance. Pakistan Journal of Botany, 52(2). https://doi.org/10.30848/PJB2020-2(24)

Koyro, H.-W., Ahmad, P., & Geissler, N. (2012). Abiotic Stress Responses in Plants: An Overview. In P. Ahmad & M. N. V. Prasad (Eds.), Environmental Adaptations and Stress Tolerance of Plants in the Era of Climate Change (pp. 1–28). Springer. https://doi.org/10.1007/978-1-4614-0815-4_1

Kugimiya, S., Shimoda, T., Tabata, J., & Takabayashi, J. (2010). Present or Past Herbivory: A Screening of Volatiles Released from Brassica rapa Under Caterpillar Attacks as Attractants for the Solitary Parasitoid, Cotesia vestalis. Journal of Chemical Ecology, 36(6), 620–628. https://doi.org/10.1007/s10886-010-9802-6

Leybourne, D. J., Preedy, K. F., Valentine, T. A., Bos, J. I. B., & Karley, A. J. (2021). Drought has negative consequences on aphid fitness and plant vigor: Insights from a meta-analysis. Ecology and Evolution, 11(17), 11915–11929. https://doi.org/10.1002/ece3.7957

Lin, P.-A., Chen, Y., Ponce, G., Acevedo, F. E., Lynch, J. P., Anderson, C. T., Ali, J. G., & Felton, G. W. (2022). Stomata-mediated interactions between plants, herbivores, and the environment. Trends in Plant Science, 27(3), 287–300. https://doi.org/10.1016/j.tplants.2021.08.017

Liu, L., Wang, B., Liu, D., Zou, C., Wu, P., Wang, Z., Wang, Y., & Li, C. (2020). Transcriptomic and metabolomic analyses reveal mechanisms of adaptation to salinity in which carbon and nitrogen metabolism is altered in sugar beet roots. BMC Plant Biology, 20(1), 138. https://doi.org/10.1186/s12870-020-02349-9

Loomis, R. S. (1997). On the utility of nitrogen in leaves. Proceedings of the National Academy of Sciences, 94(25), 13378–13379. https://doi.org/10.1073/pnas.94.25.13378

Luo, R., & Gilbert, B. (2022). Timing of short-term drought structures plant–herbivore dynamics. 2022(1). https://doi.org/10.1111/oik.08860

Martini, X., & Stelinski, L. L. (2017). Drought stress affects response of phytopathogen vectors and their parasitoids to infection- and damage-induced plant volatile cues. Ecological Entomology, 42(6), 721–730. https://doi.org/10.1111/een.12439

Mewis, I., Khan, M. A. M., Glawischnig, E., Schreiner, M., & Ulrichs, C. (2012). Water Stress and Aphid Feeding Differentially Influence Metabolite Composition in Arabidopsis thaliana (L.). PLOS ONE, 7(11), e48661. https://doi.org/10.1371/journal.pone.0048661

Min, J.-H., Chung, J.-S., Lee, K.-H., & Kim, C. S. (2015). The CONSTANS-like 4 transcription factor, AtCOL4, positively regulates abiotic stress tolerance through an abscisic acid-dependent manner in Arabidopsis. Journal of Integrative Plant Biology, 57(3), 313–324. https://doi.org/10.1111/jipb.12246

Morkunas, I., Mai, V. C., & Gabryś, B. (2011). Phytohormonal signaling in plant responses to aphid feeding. Acta Physiologiae Plantarum, 33(6), 2057–2073. https://doi.org/10.1007/s11738-011-0751-7

Munns, R., & Tester, M. (2008). Mechanisms of Salinity Tolerance. Annual Review of Plant Biology, 59(1), 651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911

Niu, M., Xie, J., Chen, C., Cao, H., Sun, J., Kong, Q., Shabala, S., Shabala, L., Huang, Y., & Bie, Z. (2018). An early ABA-induced stomatal closure, Na+ sequestration in leaf vein and K+ retention in mesophyll confer salt tissue tolerance in Cucurbita species. Journal of Experimental Botany, 69(20), 4945–4960. https://doi.org/10.1093/jxb/ery251

Pandey, S., & Singh, R. (1999). Host size induced variation in progeny sex ratio of an aphid parasitoid Lysiphlebia mirzai. Entomologia Experimentalis et Applicata, 90(1), 61–67. https://doi.org/10.1046/j.1570-7458.1999.00423.x

Polack, L. A., Pereyra, P. C., & Sarandón, S. J. (2011). Effects of Plant Stress and Habitat Manipulation on Aphid Control in Greenhouse Sweet Peppers. Journal of Sustainable Agriculture, 35(7), 699–725. https://doi.org/10.1080/10440046.2011.606489

Quais, M. K., Munawar, A., Ansari, N. A., Zhou, W.-W., & Zhu, Z.-R. (2020). Interactions between brown planthopper (Nilaparvata lugens) and salinity stressed rice (Oryza sativa) plant are cultivar-specific. Scientific Reports, 10(1), Article 1. https://doi.org/10.1038/s41598-020-64925-1

Salerno, G., Frati, F., Marino, G., Ederli, L., Pasqualini, S., Loreto, F., Colazza, S., & Centritto, M. (2017). Effects of water stress on emission of volatile organic compounds by Vicia faba, and consequences for attraction of the egg parasitoid Trissolcus basalis. Journal of Pest Science, 90(2), 635–647. https://doi.org/10.1007/s10340-016-0830-z

Schoonhoven, L. M., Loon, J. J. A. van, & Dicke, M. (2005). Insect-plant biology (2nd ed). Oxford University Press.

Sharma, A., Shahzad, B., Kumar, V., Kohli, S. K., Sidhu, G. P. S., Bali, A. S., Handa, N., Kapoor, D., Bhardwaj, R., & Zheng, B. (2019). Phytohormones Regulate Accumulation of Osmolytes Under Abiotic Stress. Biomolecules, 9(7), 285. https://doi.org/10.3390/biom9070285

Shin, Y. K., Bhandari, S. R., & Lee, J. G. (2021). Monitoring of Salinity, Temperature, and Drought Stress in Grafted Watermelon Seedlings Using Chlorophyll Fluorescence. Frontiers in Plant Science, 12. https://www.frontiersin.org/articles/10.3389/fpls.2021.786309

Singh, P., Choudhary, K. K., Chaudhary, N., Gupta, S., Sahu, M., Tejaswini, B., & Sarkar, S. (2022). Salt stress resilience in plants mediated through osmolyte accumulation and its crosstalk mechanism with phytohormones. Frontiers in Plant Science, 13, 1006617. https://doi.org/10.3389/fpls.2022.1006617

Smith, M. R., Veneklaas, E., Polania, J., Rao, I. M., Beebe, S. E., & Merchant, A. (2019). Field drought conditions impact yield but not nutritional quality of the seed in common bean (Phaseolus vulgaris L.). PLOS ONE, 14(6), e0217099. https://doi.org/10.1371/journal.pone.0217099

Studham, M. E., & MacIntosh, G. C. (2013). Multiple Phytohormone Signals Control the Transcriptional Response to Soybean Aphid Infestation in Susceptible and Resistant Soybean Plants. Molecular Plant-Microbe Interactions®, 26(1), 116–129. https://doi.org/10.1094/MPMI-05-12-0124-FI

Tariq, M., Wright, D. J., Bruce, T. J. A., & Staley, J. T. (2013). Drought and Root Herbivory Interact to Alter the Response of Above-Ground Parasitoids to Aphid Infested Plants and

Associated Plant Volatile Signals. PLOS ONE, 8(7), e69013. https://doi.org/10.1371/journal.pone.0069013

Valenzuela, C. E., Acevedo-Acevedo, O., Miranda, G. S., Vergara-Barros, P., Holuigue, L., Figueroa, C. R., & Figueroa, P. M. (2016). Salt stress response triggers activation of the jasmonate signaling pathway leading to inhibition of cell elongation in Arabidopsis primary root. Journal of Experimental Botany, 67(14), 4209–4220. https://doi.org/10.1093/jxb/erw202

van Zelm, E., Zhang, Y., & Testerink, C. (2020). Salt Tolerance Mechanisms of Plants. Annual Review of Plant Biology, 71(1), 403–433. https://doi.org/10.1146/annurev-arplant-050718-100005

Wei, J., Zou, L., Kuang, R., & He, L. (2000). Influence of Leaf Tissue Structure on Host Feeding Selection by Pea Leafminer Liriomyza huidobrensis (Diptera: Agromyzidae). Zoological Studies, 6.

Weldegergis, B. T., Zhu, F., Poelman, E. H., & Dicke, M. (2015). Drought stress affects plant metabolites and herbivore preference but not host location by its parasitoids. Oecologia, 177(3), 701–713. https://doi.org/10.1007/s00442-014-3129-x

Yang, X., Lu, M., Wang, Y., Wang, Y., Liu, Z., & Chen, S. (2021). Response Mechanism of Plants to Drought Stress. Horticulturae, 7(3), Article 3. https://doi.org/10.3390/horticulturae7030050

Yasir Ali, M., Lu, Z., Ali, A., Bilal Amir, M., Afaq Ahmed, M., Shahid, S., Liu, T.-X., & Pan, M. (2022). Effects of Plant-Mediated Differences in Aphid Size on Suitability of Its Parasitoid, Aphelinus varipes (Hymenoptera: Aphelinidae). Journal of Economic Entomology, 115(1), 74–80. https://doi.org/10.1093/jee/toab245

Yu, Z., Duan, X., Luo, L., Dai, S., Ding, Z., & Xia, G. (2020). How Plant Hormones Mediate Salt Stress Responses. Trends in Plant Science, 25(11), 1117–1130. https://doi.org/10.1016/j.tplants.2020.06.008

Zhao, Y., Dong, W., Zhang, N., Ai, X., Wang, M., Huang, Z., Xiao, L., & Xia, G. (2014). A Wheat Allene Oxide Cyclase Gene Enhances Salinity Tolerance via Jasmonate Signaling. Plant Physiology, 164(2), 1068–1076. https://doi.org/10.1104/pp.113.227595

Zhu, J.-K. (2016). Abiotic Stress Signaling and Responses in Plants. Cell, 167(2), 313–324. https://doi.org/10.1016/j.cell.2016.08.029

Zörb, C., Geilfus, C.-M., & Dietz, K.-J. (2019). Salinity and crop yield. Plant Biology, 21(S1), 31–38. https://doi.org/10.1111/plb.12884

Acknowledgments

I am immensely grateful and would like to express my deepest appreciation to Prof. Dr. Michael Rostás for allowing me to pursue my Ph.D. studies at the prestigious Georg-August University of Göttingen. Receiving his response to my initial email filled me with a profound sense of gratitude and optimism. I am grateful for his unwavering trust, support, patience, invaluable guidance, and steadfast belief in my potential. His unconditional support and encouragement have created an environment where I can explore and grow as a researcher and an individual. His patience and expert advice, both in my scientific work and career development, have been invaluable to me. His vast knowledge and experience serve as an endless source of inspiration and insight. I am truly privileged to work under his mentorship and strive to embody the qualities of a dedicated and accomplished researcher that he exemplifies.

I am thankful to Prof. Dr. Mark Varrelmann for his generosity in always making time for my project meetings. His willingness to prioritize and allocate his valuable time for our discussions has been instrumental in the progress of my research. I sincerely appreciate his dedication and support, as his availability has allowed for productive and meaningful interactions. I am thankful for his role as a member of my thesis committee and co-referee, as his meticulous evaluation and insightful feedback.

I would like to express my sincere gratitude to Dr. Ilka Vosteen for her invaluable role as my co-supervisor. I am grateful for her guidance in understanding and applying statistical methods to my data and her valuable insights on crafting clear and impactful manuscripts. I am truly grateful for her consistent availability and willingness to provide constructive feedback, significantly contributing to my growth as a researcher.

I am sincerely grateful to Dr. Kerstin Krüger for her kindness and willingness to join my examination committee. Her expertise and contributions have been invaluable in evaluating and assessing my work. I deeply appreciate the time and dedication she has invested in ensuring the successful completion of my Ph.D. defense.

The collective contributions of Dr. Bernd Ulber, Dr. Franz Hadacek, Dr. Mohammad Alhussein, and Dr. Ines Vollhardt have been indispensable in shaping the course of my research. Their unwavering support, collaboration, and guidance have been pivotal in my academic growth. I consider myself fortunate to have worked with such exceptional individuals, and I am sincerely grateful for their contributions to my research journey.

I would like to express my sincere gratitude to the technicians, particularly Jonas Watterott and Ruth Pilot, for their invaluable assistance throughout my Ph.D. research journey. Their support and expertise have been indispensable in the successful execution of my experiments. I would like to extend my heartfelt appreciation to all of my colleagues and members of the Division of Agricultural Entomology for their unwavering support throughout my research journey.

I would like to express my heartfelt acknowledgments to my beloved wife Musrat Zahan Surovy, for her unwavering support, inspiration, and love. Her encouragement and belief in my abilities have propelled me to explore new ideas and push the boundaries of my research. Our shared experiences, conversations, and moments of companionship have provided me with the strength and joy to pursue my academic goals. Her presence in my life has been a blessing, and I am grateful for the continuous encouragement she provides on my research journey.

I am deeply thankful for my parents and my dear younger sister's sacrifices to ensure my success and happiness. Their unconditional love, guidance, and encouragement have been instrumental in shaping the person I am today. I am grateful for their unwavering belief in me, even from a distance, and their constant support in caring for my mental health.

Md Shahinoor Rahman

Grisebachstrasse 6, Division of Agricultural Entomology, University of Goettingen, Germany
shahinoor_ent@yahoo.com
(+49) 1575 4225327
https://www.uni-goettingen.de/en/631071.html
https://www.linkedin.com/in/shahinoor-rahman-75869ba9/
https://www.researchgate.net/profile/Shahinoor-Rahman

Gender: Male **Date of Birth:** 12/10/1990 **Nationality:** Bangladesh

ABOUT ME

Enthusiastic, adaptable, achievement-oriented, and loving to take challenges to achieve successful outcomes. Expertise in the Agricultural crop protection discipline has around 10 years of demonstrated teaching, research, project management, multicultural student/technician & employee supervision, consulting-coordination-communication, problem-solving, and team lead experiences. Enough experience in data processing, agronomic trials, insect/pest/disease/virus monitoring assessment and their biological-chemical-IPM as well development/execution/evaluation of digital application technologies in farmaer's field for smart/digital farming solutions. Interested to work in the international environment for coordination/consultation/supervision of strategic projects focusing on Agricultural crops and pest management.

WORK EXPERIENCES

[17/10/2022-Current] **Research Scientist (PostDoc)**

Agricultural Entomology, University of Goettingen, Germany

Main activities and responsibilities
Teaching & Supervision: MS, BS, intern students, and technicians
Research: Resistance of sugar beet, SBR disease, plant-vector/insect/pest-virus-microbe interactions & chemical ecology

[09/03/2020-29/08/2023] **Research Scientist (Ph.D. Candidate)**

Agricultural Entomology, University of Goettingen, Germany

Main activities and responsibilities
Teaching & Supervision: MS, BS, and intern students
Research: Global climate change, drought, soil Salinity, sugar beet, insect (aphids, beet leaf miners), insect monitoring, parasitoids, greenhouse-lab, chemical ecology, multitrophic interactions
[01/06/2022-30/06/2022] University Teaching Assistant (Part Time)
Activities: Preparation of teaching materials
[25/03/2021-31/12/2021] University Research Assistant (Part Time)
Activities: Monitoring of oilseed rape insect-pests in field

[27/09/2018-15/02/2020] **Research Associate & Team lead**

E-Village Foundation (Phase II), Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Bangladesh

Main activities and responsibilities
[27/09/2018-04/11/2018] Research Fellow at E-Village Foundation (Phase I)
Supervision: MS, BS, technicians, and farmers
Team Lead: Coordination among employees (4 people), digital/smart/precision farming solution, coordination and management of external/internal stakeholders

[12/06/2014-04/11/2018] **University Lecturer & Scientist**

Entomology, EXIM Bank Agricultural University, Bangladesh

Main activities and responsibilities
Teaching & Supervision: BS, and intern students
Research & Collaboration: Research grant acquisition, project management & collaboration focusing crop production & protection

[01/08/2013-11/06/2014] **Research Assistant**

Entomology, BSMRAU, Bangladesh

Main activities and responsibilities
Monitoring insect-pest/virus/disease & crop management in the field of – vegetables /cereals/corn, using biologicals (insects/fungi/bacteria), insecticide/pesticide, and IPM practices

EDUCATION

[09/03/2020-29/08/2023] **Doctor of Philosophy (Ph.D.)**

University of Goettingen, Germany

Field of Study: Agricultural Entomology (chemical ecology)
Dissertation: "The Effect of Global Change on Multitrophic Interactions of Sugar beet"

[01/04/2012-22/04/2014] **Master of Science (MS)**

Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Bangladesh

Field of Study: Agricultural Entomology (IPM/insecticide toxicology)
Thesis: "Chemodynamics of Cypermethrin in Eggplant Ecosystem"
Grade: Excellent (3.90 out of 4.00)

[17/03/2008-07/05/2012] **Bachelor of Science (BS)**

BSMRAU, Bangladesh

Field of Study: Agriculture
Credits: 240
Grade: Excellent (3.63 out of 4.00)

[10/07/2005-26/08/2007] **Higher Secondary Certificate (HSC)**

Govt. Shahid Bulbul College, Bangladesh

Field of Study: Science
Grade: Excellent (4.80 out of 5.00)

[01/01/1997-09/07/2005] **Secondary School Certificate (SSC)**

Pabna Zilla School, Bangladesh

Field of Study: Science
Grade: Distinction (5.00 out of 5.00)

SKILL & EXPERTISE

Chemical Ecology
Assessment of biotic/abiotic stress effects, plant volatiles, phytohormones, metabolomics, plant physiology (Fv/Fm, YPSII, ETR), plant-insect/pest/virus-parasitoid interactions, insect behavior and insect (chemical) ecology, assessment of plant/bacterial/fungal volatiles (liquid-liquid, SPME) etc.

Instrumental Handling
GC-MS, TD-GC-MS, UHPLC-MS/MS, electroantennogram (EAG), electrical penetration graph (EPG), Y-tube/six arm/below-ground olfactometers, Z-stack microscopy, mini PAM II, RTqPCR etc.

Statistical Analysis & Software
Statistics: R, SPSS, statistix 10, metaboanalyst5, prism-GraphPad; **Graph/Figures:** R /microsoft applications/prism GraphPad; **Software:** chem-station, MS-DIAL, image-J, BioRender etc.

Plant & Insect Culture/Entomology
Monitoring of insects/pests/viruses/parasitoids in field-greenhouse-lab, agronomic trials sugarbeet / vegetables /corns, biological control, insect damage assessment/imaging, fungal/ bacterial disease assessment, insect/parasitoid rearing, vertical farming, smart/ precision farming, hydroponics etc.

Others
Multicultural student-technician & employee supervision, team building and leading capabilities, project writing/ management-coordination & cooperation, consultancy, writing-publication-presentation etc.

Language
Bangla (Mother tongue), English (C1), Hindi (B1), Arabic (B1), German (B 2.1)

Driving
German driver's license class B

KEY RESEARCH GRANTS

For Details & Other Research Grants Please Visit Linkedin ID

[2018] **Research Grant as Principal Investigator (PI)**

Exim Bank Agricultural University, Bangladesh

Project: "Effectiveness of different techniques to reduce the residues of insecticide cypermethrin in mango fruits"
Awarding Institution: Ministry of Science & Technology Bangladesh

[2017] **Research Grant as Principal Investigator (PI)**

Exim Bank Agricultural University Bangladesh

Project: "Determination of insecticide residues activities in selected varieties of mango fruits in different regions of Chapainawabganj, Bangladesh"
Awarding Institution: Ministry of Science & Technology Bangladesh

KEY AWARDS

For More Honors & Awards Please Visit Linkedin ID

[2023] **DGaaE Travel Award**
Awarding Institution: German Society for General and Applied Entomology

[2023] **GFA Travel Award**
Awarding Institution: University of Goettingen, Germany

[2012] **Scholarship for Master Studies**
Awarding Institution: BSMRAU, Bangladesh

[2005] **Gold Medel for Outstanding Results in SSC**
Awarding Institution: Pabna Zilla School, Bangladesh

KEY PUBLICATIONS

For Details & Updated Publications Please Visit Research Gate ID

[2023] *microrganisms*
"Suppressive effects of volatile compounds from *Bacillus spp.* on *Magnaporthe oryzae triticum* (MoT) pathotype, causal agent of wheat blast"
Musrat Zahan Surovy, **Shahinoor Rahman**, Michael Rostàs, Tofazzal Islam, Andreas Von Tiedemann DOI: https://doi.org/10.3390/microorganisms11051291

[2022] *Frontiers in Microbiology*
"Dormancy and germination of microsclerotia of *Verticillium longisporum* are regulated by soil bacteria and soil moisture levels but not by nutrients"
Sarenqimuge Sarenqimuge, **Shahinoor Rahman**, Yao Wang, Andreas Von Tiedemann DOI: https://doi.org/10.3389/fmicb.2022.979218

KEY CONFERENCES

For Details & Updated Conferences/Workshops Please Visit Linkedin ID

[2023] *Bozen-Bolzano, Italy*
"German Society for General and Applied Entomology (DGaaE) Congress"
Oral Presentation 1: Drought matters: higher aphid performance and reduced parasitoid attraction to plant volatiles alter tritrophic interactions in sugar beet
Oral Presentation 2: Drought differentially affects the preference and performance of beet leaf miners/flies by altering the plant chemistry of sugar beet

[2017] *Kathmandu, Nepal*
"3rd International South Asian Biotechnology Conference (SABC)"
Poster Presentation: Dissipation and residue pattern of synthetic pyrethroid insecticide in okra crop agro-ecosystem in Bangladesh

KEY MEMBERSHIP

For Details & More Membership/Networks Please Visit Linkedin ID

German Society for General and Applied Entomology (DGaaE), Bangladesh Entomological Society (BES), Bangladesh Association for the Advancement of Sciences (BAAS)

HOBBIES & INTERESTS

Playing Badminton, Cricket; Travelling, Fishing, Photography

RECOMMENDATIONS

Reference-1
Prof. Michael Rostás
Division of Agricultural Entomology
University of Goettingen, Germany
Cell: (+49) 152 58754777
Email: michael.rostas@uni-goettingen.de

Reference-2
Prof. Mark Varrelmann
Institute of Sugar Beet Research
University of Goettingen, Germany
Email: varrelmann@ifz-goettingen.de

Reference-3
Dr. Ilka Vosteen
Division of Agricultural Entomology
University of Goettingen, Germany
Cell: (+49) 178 6965152
Email: ilka.vosteen@uni-goettingen.de

Reference-4
Dr. Kerstin Krüger
Lead Phytopathology
KWS Group, Einbeck, Germany
Email: kerstin.krueger@kws.com

Göttingen, 10/07/2023

Md Shahinoor Rahman

Statutory declaration

I, hereby, declare that this dissertation was undertaken independently and without any unaccredited aid.

Place: Göttingen

Date: 10th July 2023

Md Shahinoor Rahman