Apple flower traits change in response to infection with apple proliferation phytoplasma

TECHNISCHE UNIVERSITÄT DARMSTADT

Doris Maurer[1,2], Jürgen Gross[1,2], Andreas Jürgens[1], Mascha Bischoff[1,2]

[1]Plant Chemical Ecology, Department of Biology, TU Darmstadt, Schnittspahnstrasse 10, 64287 Darmstadt
[2]Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Fruit Crops and Viticulture, Schwabenheimer Strasse 101, 69221 Dossenheim

JKI Julius Kühn-Institut Federal Research Centre for Cultivated Plants

Introduction

One of the most severe economic threats to European fruit farming is apple proliferation, a disease caused by the bacterium *Candidatus* Phytoplasma mali. Typical symptoms include witches broom, enlarged stipules and undersized fruits. However, it is unknown whether apple proliferation affects floral traits. In this study, we compared a number of different floral traits between infected and healthy apple trees (*Malus domestica*). We collected data from naturally infected apple trees in the field and from potted plants (**Fig. 1A**) infected via grafting with avirulent (1/93) and virulent (12/93 and 3/6) phytoplasma strains. For the nectar analysis, we included the model system *Nicotiana tabaccum* (**Fig. 1B**) infected with 1/93 and 12/93 strains for comparison.

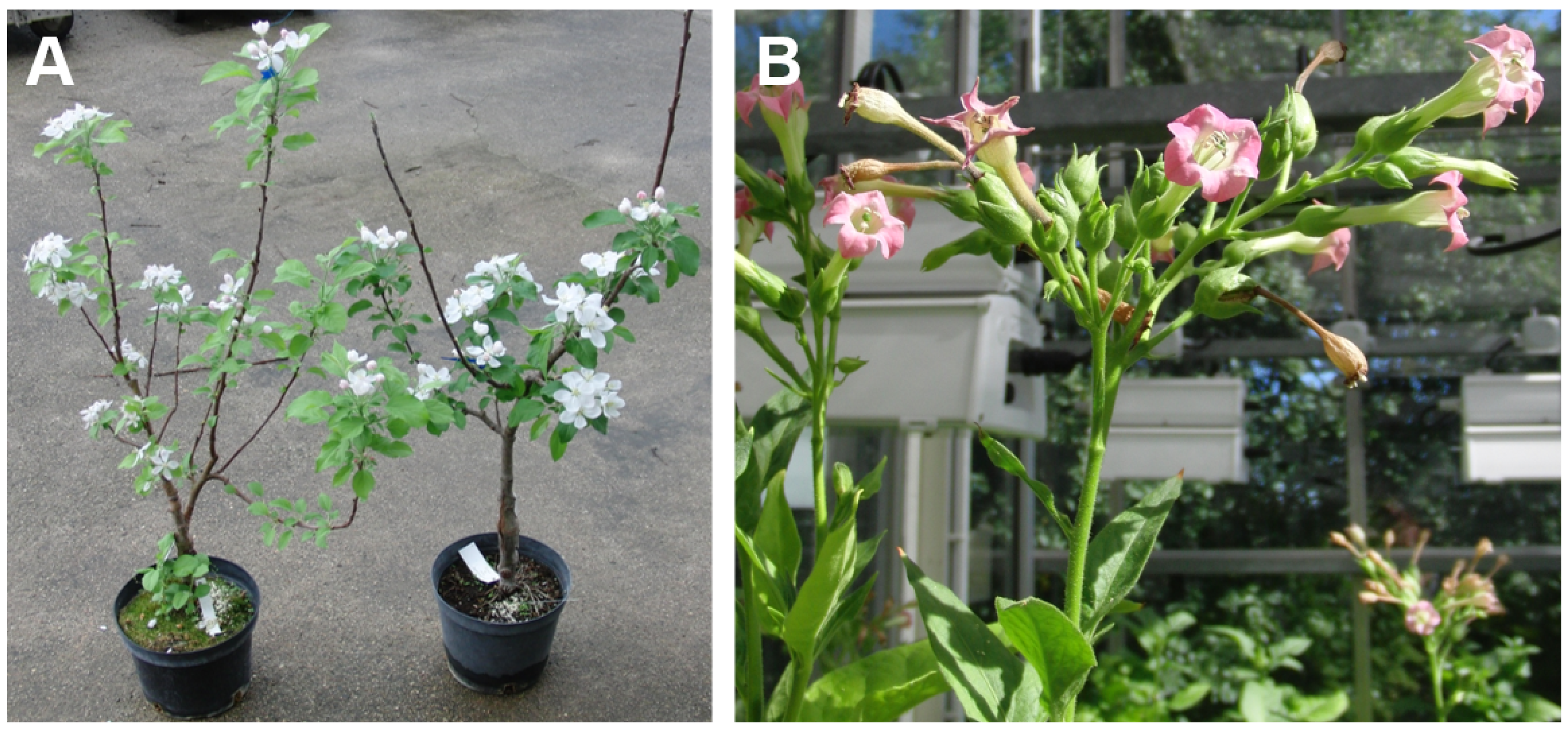

Figure 1A: *Malus domestica* 'Golden Delicious' and **B:** *Nicotiana tabaccum*

Important results

- **Apple trees responded to an increasing bacterial virulence with a decrease of flower number.**
- **Although flower diameter did not change, a higher variability in petal length was observed in infected plants.**
- **Infected plants showed a reduced sucrose concentration in nectar.**
- **Infected plants showed quantitative differences in some floral scent compounds.**

Flower quantity and size

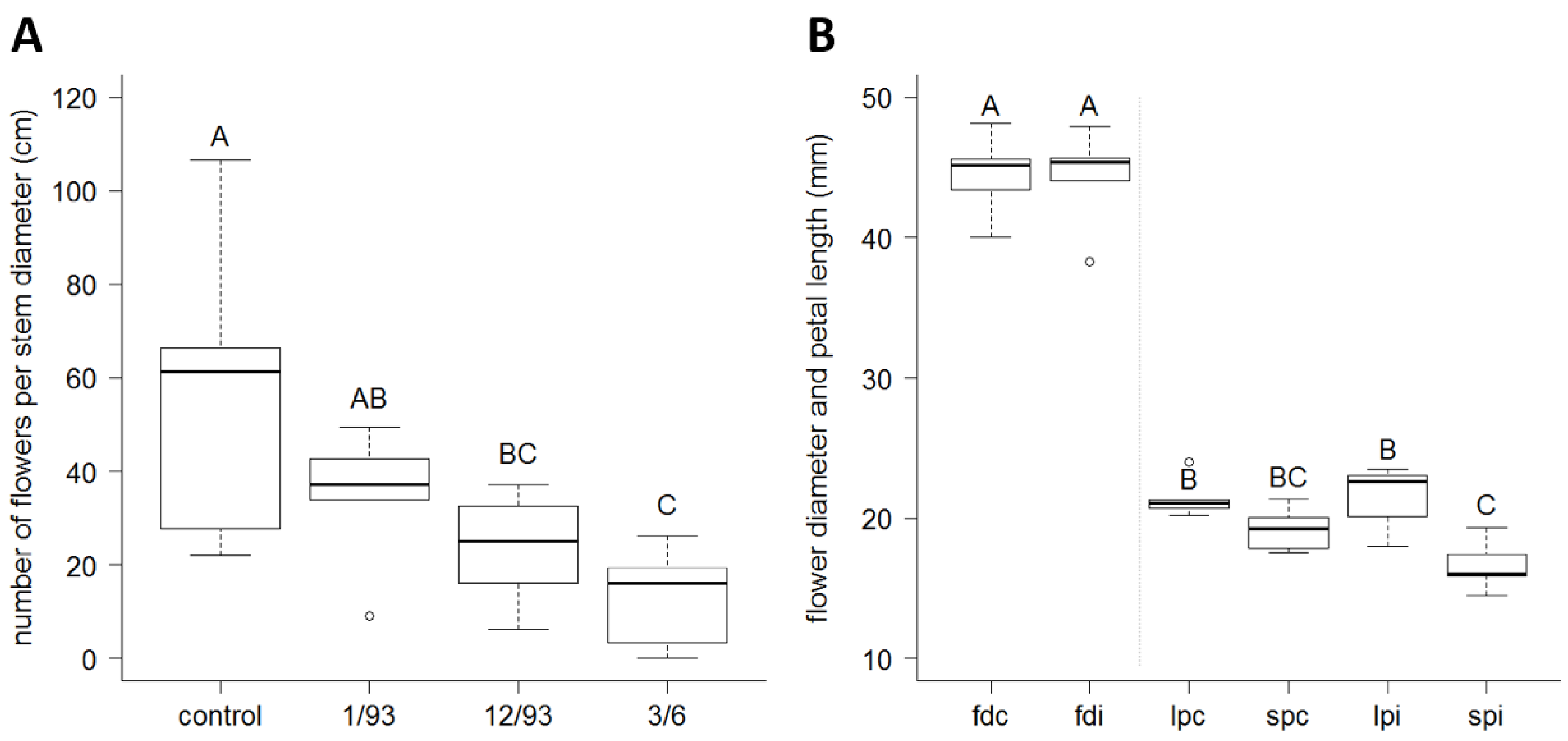

Figure 2A: Number of flowers per stem diameter (cm) of *M. domestica* 'Golden Delicious'. Number of flowers per stem declined with an increasing virulence. Differences in stem diameter between the groups control, 1/93 and 12/93 were insignificant. Group 3/6 showed an increased stem diameter paired with a reduction of flower number. control=healthy trees (N=10), 1/93 = trees infected with avirulent phytoplasma strain (N=6), 12/93 and 3/6 = virulent phytoplasma strains (N=6 each). Kruskal-Wallis test followed by Dunn's test (chi-sqrd.=13.92, df=3, p=0.003) **B:** Average flower diameters and petal lengths. The petal lengths showed a greater variation in infected trees. fdc/fdi=flower diameter control/infected, lpc/spc=longest/shortest petal control, lpi/spi=longest/shortest petal infected. Diameter: N=10, petal length: N=10. Different letters indicate significant differences between groups (Kruskal-Wallis test followed by Dunn's test, $p<0.025$)

Nectar sugar concentration

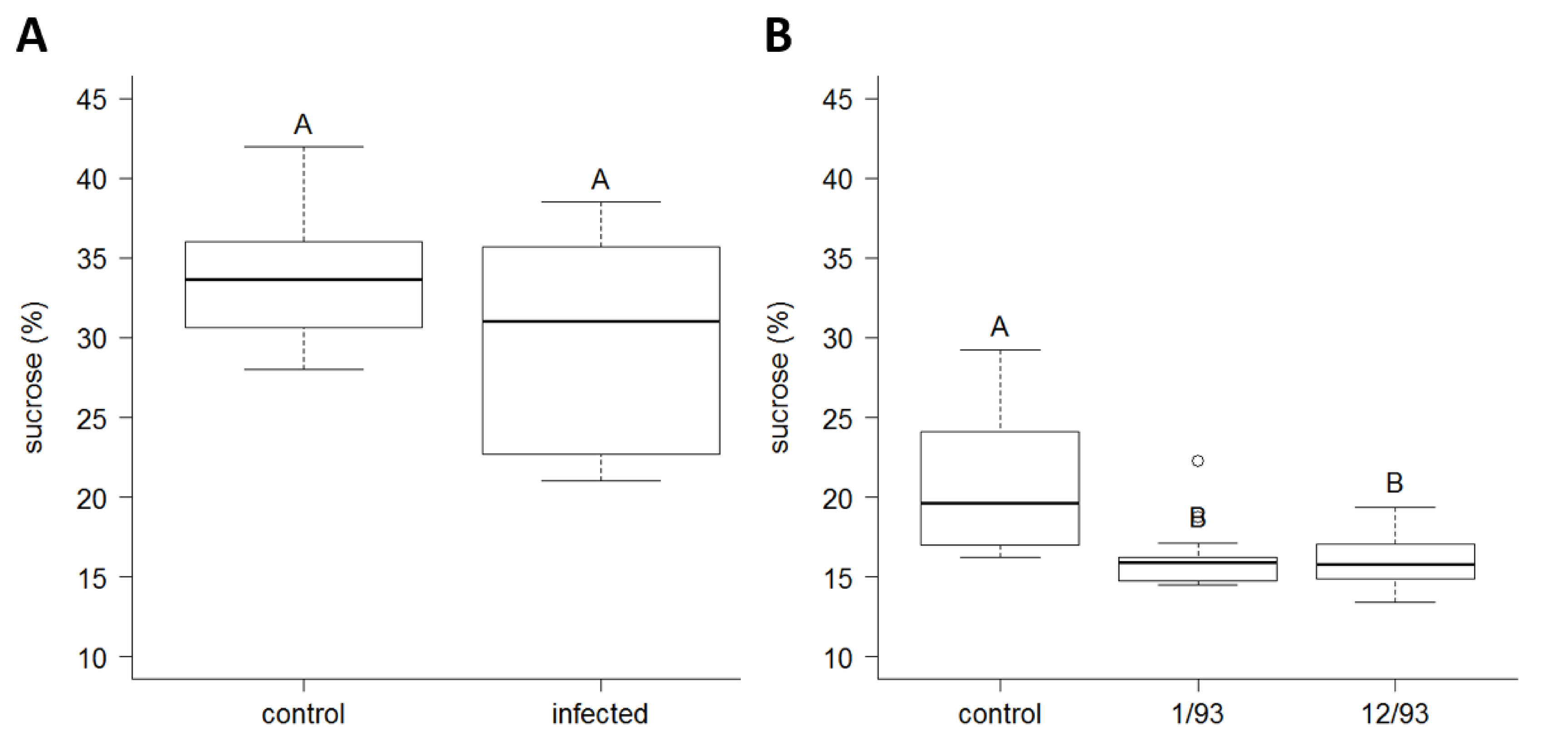

Figure 3A: Refractometer analysis of sucrose concentration in nectar of healthy and infected *M. domestica* 'Topaz'. (N=5 for each group, no sigificant differences). **B:** Refractometer analysis of nectar of *N. tabaccum* showed a significantly reduced sucrose concentration in the infected groups (1/93 and 12/93). N(control)=14, N(1/93)=13, N(12/93)=11. Kruskal-Wallis test followed by Dunn's test, chi-sqrd=14.237, df=2, p=0.0008

Floral scent

Table 1: Comparison of the ten main components (relative amounts %) in the floral bouquet of *M. domestica* 'Golden Delicious'. Collection and analysis of floral scent followed [1]. Classification of compounds according to literature [2]. Asterisks indicate significant changes in compound concentration, Kruskal-Wallis test, $p<0.05$.

Class	Compound	RI	Control (N=10)	1/93 (N=8)	12/93 (N=9)	3/6 (N=8)
Aliphatics	3-Hexenal	798	0,88	0,34	0,66	1,28
	3-Hexen-1-ol	853	7,29	22,96	6,21	7,63
	3-Hexenyl acetate	1006	53,92	52,78	59,02	51,55
	Hexyl acetate	1013	0,09	0,86	0,90	0,65
Benzenoids	Benzaldehyde	960	4,44	4,39	6,93	8,87
	Benzyl alcohol	1040	24,92	13,34	19,52	21,90
	Benzyl acetate*	1162	0,54	0,34	0,98	1,69
	Methyl salicylate	1192	0,15	0,16	0,93	1,05
Terpenes	6-Methyl-5-hepten-2-one*	984	1,92	1,19	0,90	0,21
	Linalool	1099	4,37	2,43	2,37	2,51

increase: Benzaldehyde, Benzyl acetate*, Methyl salicylate

decrease: 6-Methyl-5-heptene-2-one*, Linalool

Conclusion and outlook

We found that the number of flowers and the flower traits of infected apple trees changed in response to the presence of *Candidatus* Phytoplasma mali. As pollinators require visual and olfactory cues to locate flowers, they might be able to discriminate between flowers of infected and healthy plants, which in turn could influence pollination and fruit set. Experiments with pollinators (e.g. bumblebees) including electroantennography measurements and behaviour analysis in flight cages and in the field are under way.

References

[1] Gross, J., Gallinger, J. & Rid, M. (2018). Collection, Identification, and Statistical Analysis of Volatile Organic Compound Patterns Emitted by Phytoplasma Infected Plants. In Musetti, R. and Pagliari, L. (eds.), Phytoplasmas: Methods and Protocols, Methods in Molecular Biology, vol. 1875, Springer Science+Business Media, LLC

[2] Knudsen, J. T., Eriksson, R., Gershenzon, J. & Stahl, B. (2006). Diversity and Distribution of Floral Scent. The Botanical Review, 72(1), 1-120.

Acknowledgements

We are grateful to Leonie Dries, TU Darmstadt, for field assistance and to Kerstin Zikeli, JKI Dossenheim, for excellent lab assistance.

DOI: 10.5073/20180904-151846