Interplay between biotic and abiotic stress in *Arabidopsis* infected with *Xylella fastidiosa*

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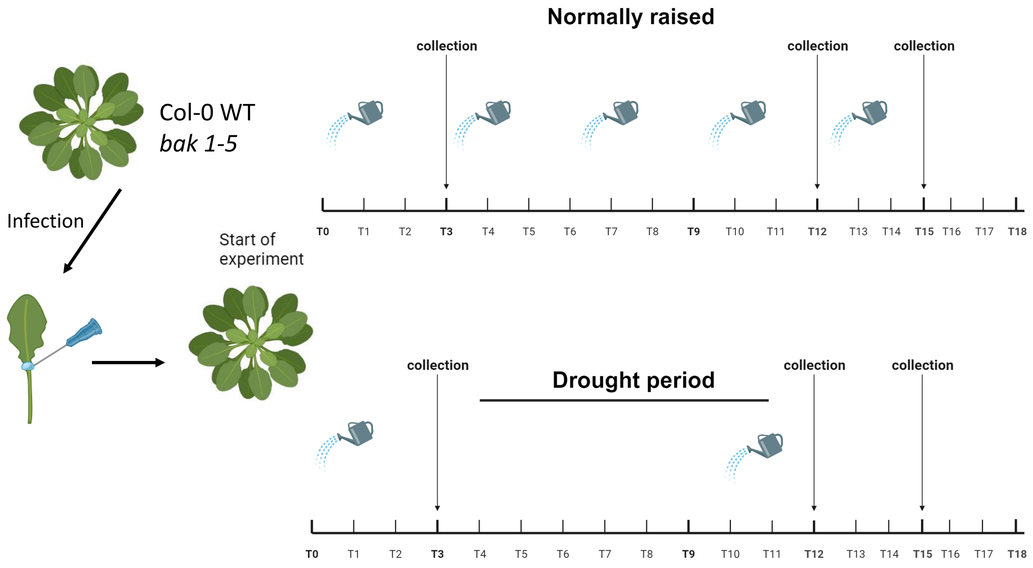
Introduction

Plants are sessile organisms that deal with biotic and abiotic stresses simultaneously. Abiotic stresses such as drought can induce vessel emboly and loss of water potential, particularly at xylem vessel. While these responses are essential for plant survival during drought, some bacteria may evolve to explore these host responses for their own gain. *Xylella fastidiosa,* a vector borne xylem-colonizing bacterial pathogen can infect more than 500 different plant species[1]. In xylem vessels, *Xylella fastidiosa* switches between lifestyles: i) Cells form a biofilm attached to xylem cell walls, which promotes insect transmission, and ii) cells shed from the biofilm, systemically colonizing the plant with and against the transpiration flow. Interestingly, some host species develop disease symptoms reminiscent of drought stress, e.g. leaf scorch, while other infected hosts remain symptomless. The underlining molecular mechanisms, and particularly, the relative importance of the host response to the abiotic stress vs biotic stress for the outcome of bacterial infection success and/or disease development remains largely unexplored. Here, we investigated the effect of drought stress on the establishment and development of symptoms in the genetic model plant *Arabidopsis thaliana* upon infected with *Xylella fastidiosa subsp. fastidiosa (Xff)*Temecula-1 and subsp. *pauca* (*Xfp*) de Donno.

Methodology



Does drought affect symptom development in *Arabidopsis* plants exposed to *Xylella* ?

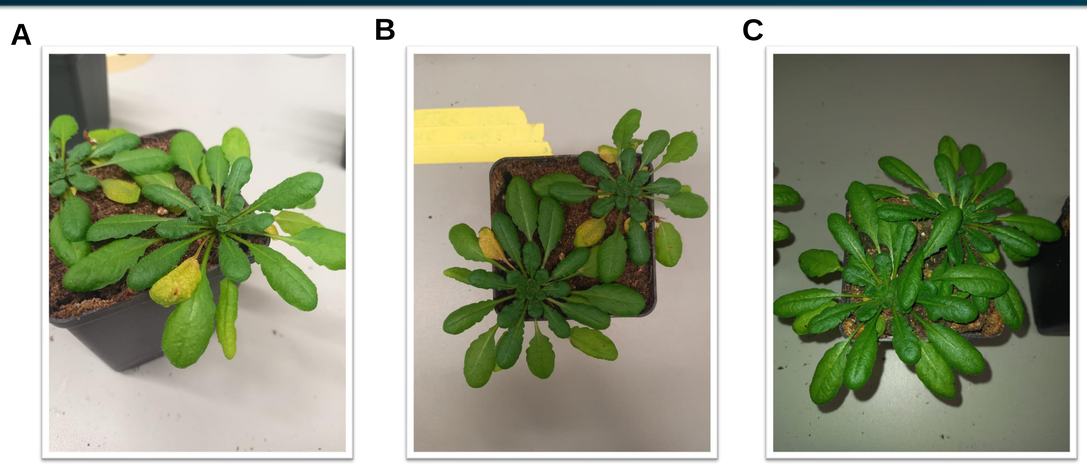


Figure 1. Drought stress enhances leaf scorch symptomatology in leaves infected with *Xff* and *Xfp* but semms not to promote further systemic colonization.
Petioles of 6 weeks old *Arabidopsis thaliana* Col-0 was prick-infected through a 0.5 OD600nm droplet of either *Xff* (**A**), *Xfp* (**B**) or mock (**C**). After infection, plants were watered once and left without watering for 10 days, then watered and scored after 2 days. **A** (15 out of 20) and **B** (17 out of 24) are representative pictures of plants showing leaf scorch like symptoms in comparison with **C** (4 out of 20)**.** Symptoms were constrained to the infected leaves; no macroscopic signs of systemic disease spread were observed.

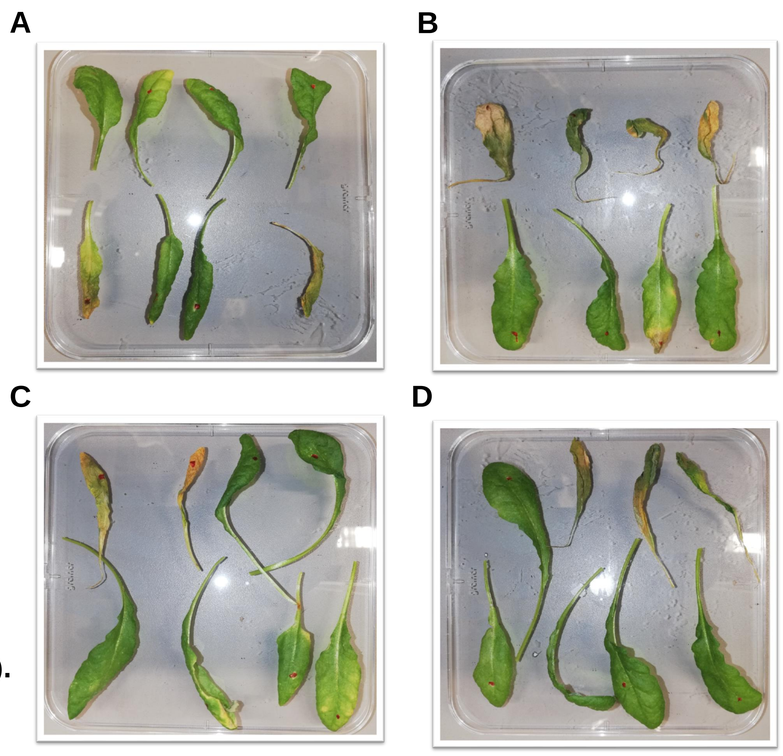


Figure 2. *bak1-5* immuno-compromised mutants show increased signs of leaf scorch.
Arabidopsis thaliana Col-0 was infected and watered as described in Figure 1 or normally watered. **A)** Infection of *bak1-5* and normal watering, **B)** Infection of *bak1-5* and drought stress conditions**, C)** Infection of Col-0 wild type (WT) and normal watering, **D)** Infection of WT and drought stress conditions**.** *bak1-5* mutants showed increased number of symptomatic leaves in comparison to WT, particularly under drought stress, no macroscopic signs of systemic disease spread were observed.

Does drought and the immune system affect loads of *Xylella*?

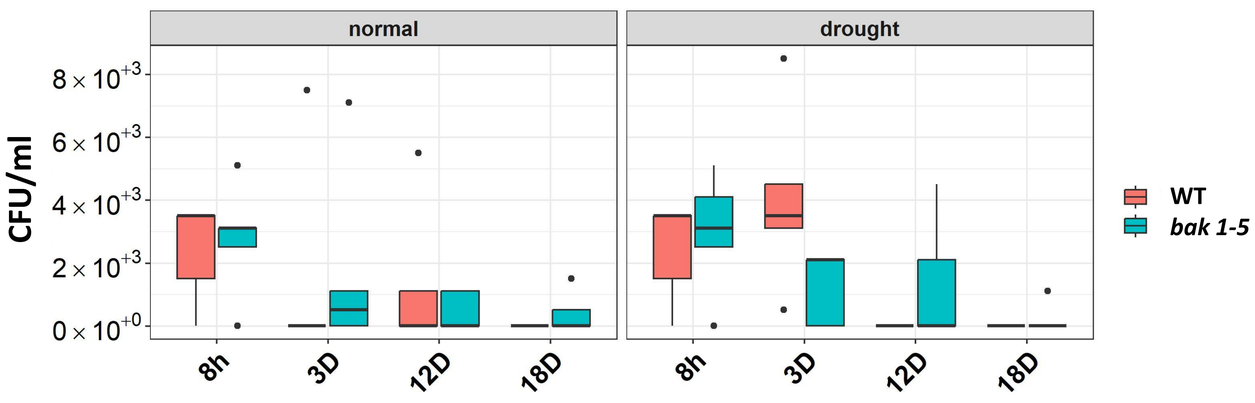


Figure 3. *bak1-5* mutants show slightly higher bacterial loads than Col-0.
Arabidopsis thaliana Col-0 was infected and watered as described in Figure 1. At the indicated time points, 4 infected petioles were collected, surface sterilized and homogenized in PBS 1x, serial dilutions were plated and colonies were counted after at least 14 days. Boxplots represent colony forming units (CFUs) of 4 independently infected plants. Bacterial loads were higher in *bak1-5,* particularly under drought conditions. Independent of normal watering or drought conditions, *Xff* loads decrease over time.

Is *Xylella* expressing known virulence genes in *Arabidopsis*?

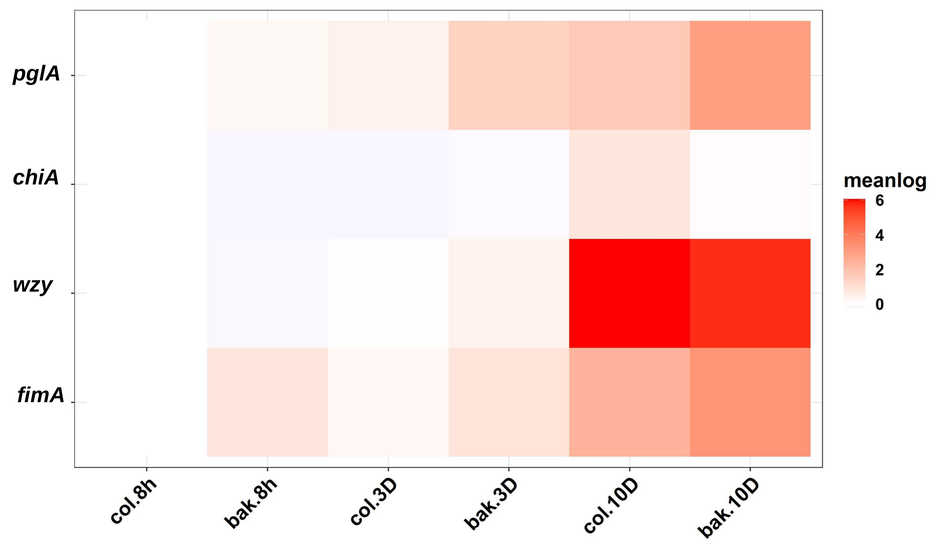


Figure 4. *Xylella fastidiosa* upregulates genes involved in adhesion during early stages of infection of *Arabidopsis thaliana.*
Arabidopsis thaliana Col-0 was infected and watered as described in Figure 1. At the indicated time points, infected petioles were collected for RNA extraction. Selected bacterial genes were amplified by qPCR. Data were analyzed using the Pfaffl method. Gene expression was normalized to Col-0 WT 8 hours after infections. Each colored box represent averages of 4 independently infected plants. Expression of most genes was affected by time, not host genotype. Genes with roles in early infection stages such as *fimA* and *wzy* are highly expressed 10 days after infection.

Conclusions

- Biotic (immune response) and abiotic (drought) effects interact and can be exploited by bacteria to increase colonization.
- *bak1-5* mutants show higher *Xylella fastidiosa* loads both, when normally watered and under drought conditions.
- *Xylella fastidiosa* genes *fimA* and *wzy* are higher expressed during infection over time.
- *Arabidopsis thaliana* Col-0 shows no symptoms of systemic spread and reduced bacterial loads at later infection stages, suggesting levels of resistance to *Xylella fastidiosa* infection.

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References: Landa, B.B., et al. (2022), *Xylella fastidiosa*'s relationships: the bacterium, the host plants, and the plant microbiome. New Phytol, 234: 1598-1605.

Method scheme were created with BioRender.com