Assessing the effectiveness of different *Pseudomonas syringae* pv. *actinidiae* pre-inoculation procedures to promoting disease visual symptoms in adult kiwifruit plants

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Introduction

- The bacterium *Pseudomonas syringae* pv. *actinidiae* (Psa) is the aetiological agent of Kiwifruit Bacterial Canker (KBC).
  - Worldwide pandemic after successive outbreaks since 2008;
  - Disease with greatest impact on the kiwifruit (*Actinidia* spp.[1]) industry.

[1] Commercial cultivars belong to the species *A. chinensis* (var. *deliciosa* and var. *chinensis*) and *A. arguta* (kiwiberry).

- Psa colonizes the hosts mainly through natural openings and wounds (e.g., pruning cuts), rapidly reaching the xylem and phloem → systemic
- Main disease symptoms:

- Favorable environmental conditions to Psa spread:
  - Mild temperatures, high relative humidity, hail and frost events.
- *In planta* experiments under laboratory and field conditions, based on artificial Psa inoculations, follow different procedures.
  - Most utilized methods of Psa artificial inoculation include induction of plant physiological stress, plant wounding and promoting bacterial entrance through natural openings and favoring the environmental conditions for Psa colonization (e.g., humidity).

Objective

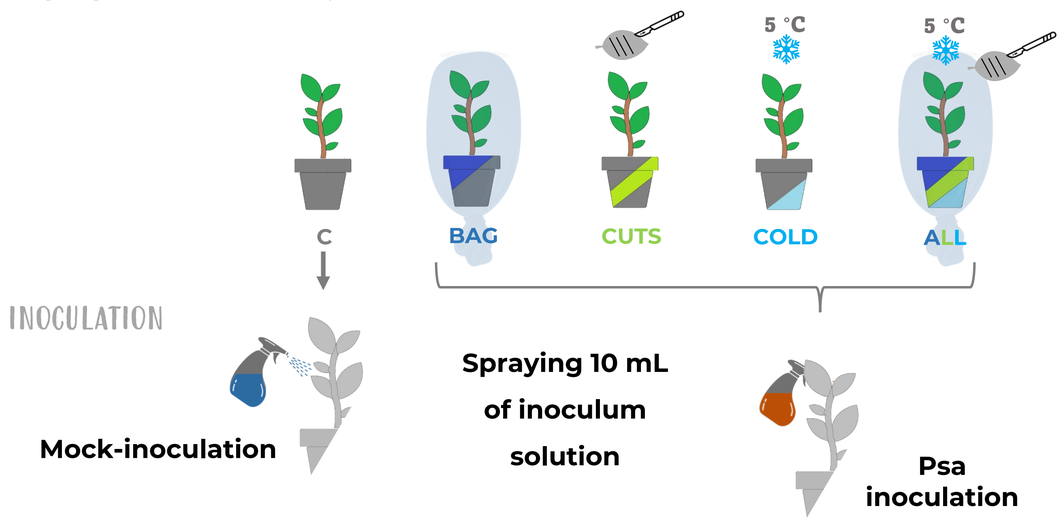
- Assess the effectiveness of Psa pre-inoculation procedures in terms of promoting Psa capacity to cause disease visual symptoms.

Materials & methods

- Testing four pre-inoculation procedures in adult kiwifruit plants artificially inoculated with Psa.
- *A. chinensis* var. *deliciosa* 'Tomuri' plants (6-months old);
  - Grown in 2.5 L pots with peat-based substrate mixed with perlite (3:1 v/v);
  - Grown in a climate chamber for 21 days after Psa-inoculation;
  - Four plants (n = 4) per treatment.
- Inoculum of Psa strain CFBP7286 (10^7 CFU/mL) prepared in Ringer´s solutions.

Pre-inoculation procedures

- [**C**] – mock-inoculation with Ringer´s solution (control);
- [**BAG**] – Plant enclosure in plastic transparent bag for 24 h before and 24 h after inoculation;
- [**CUTS**] – Leaf wounding with three superficial cuts with a blade;
- [**COLD**] – Plant maintained at 5 °C for 24 h before inoculation;
- [**ALL**] – Combination of all procedures above.

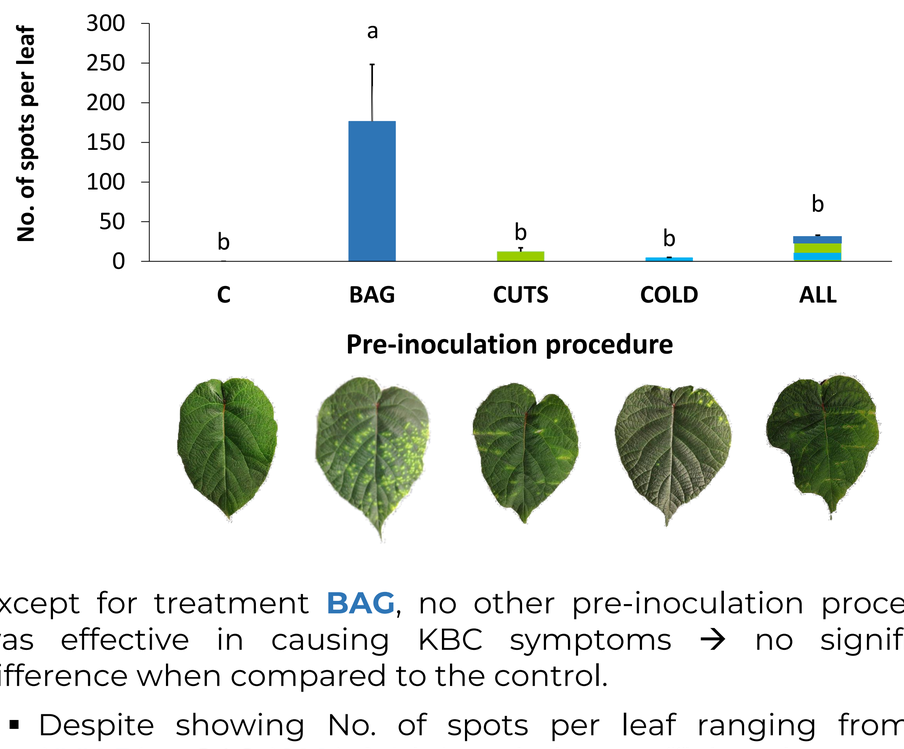


Assessment

- Visual symptoms → number of spots per leaf.

Results and discussion

- Treatment **BAG** strongly increased the capacity of Psa to cause visual symptoms.
  - Plants from **BAG** showed 7 times more leaf spots than plants from all pre-inoculation procedures combined (**ALL**).
  - Maintenance of high-humidity in **BAG** most likely promoted stomatal aperture → natural opening for colonization.



- Except for treatment **BAG**, no other pre-inoculation procedure was effective in causing KBC symptoms → no significant difference when compared to the control.
  - Despite showing No. of spots per leaf ranging from 4.4 (**COLD**) to 24.9 (**ALL**) → high variation coefficient, in general.
  - Use of low temperature (**COLD**) and leaf cutting (**CUTS**) may not be suitable pre-inoculation procedures to promote Psa infection → they may have diminished the efficacy of using bag enclosure, causing low symptom appearance in **ALL**.

Concluding remarks

- The pre-inoculation procedure influences Psa colonization, subsequently affecting the ability of the bacteria to cause KBC symptoms in kiwifruit.
- The results from this study indicate that cautious should be taken when comparing Psa pathogenicity between studies where different (pre-)inoculation procedures have been adopted.
- This work highlights the importance of following optimum procedures of plant artificial inoculation in phytopathology research studies.

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