

Effect of different pre-inoculation procedures on leaf spotting appearance in adult kiwifruit plants artificially inoculated with *Pseudomonas syringae* pv. *actinidiae*

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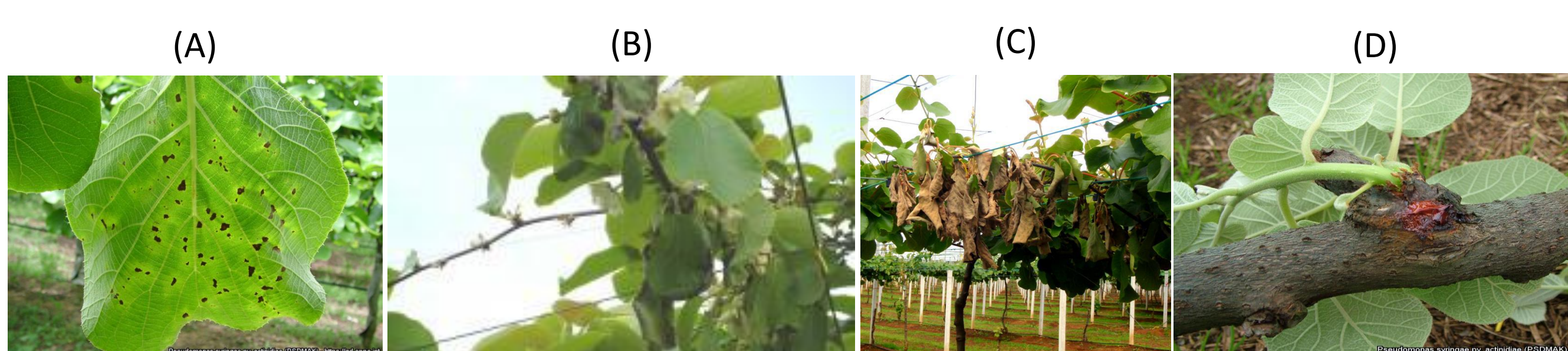
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Introduction

- Kiwifruit Bacterial Canker (KBC) is amongst the most relevant crop diseases due to its devastating impact on kiwifruit (*Actinidia* spp.) production.
 - The disease is caused by the bacterium *Pseudomonas syringae* pv. *actinidiae* (Psa).
- In the field, Psa infection mainly occurs through natural openings and wounds (e.g., pruning cuts).
 - Risk of infection and spread is highly dependent on environmental conditions which favors Psa pathogenicity → mild temperatures, high relative humidity, hail and frost events.
- First visual symptom of KBC consists in leaf spotting appearance (A), which is followed by foliar wilting (B), twig and cane dieback (C) and canker formation with exudates (D), which often leads to plant death.



- Developing KBC control methods often rely on *in planta* experiments which require successful plant infection.
 - A variety of pre-inoculation procedures are frequently used in plant artificial inoculation;
 - Most utilized ones are based on (i) promoting bacterial entrance through natural openings, (ii) favoring the environmental conditions for Psa colonization (e.g., humidity), (iii) plant wounding and (iv) induction of plant physiological stress;
 - Variability in pre-inoculation procedures can influence the infection dynamics → pathogenicity analysis amongst different research studies become very complex.

Objective

- Testing the effect of pre-inoculation procedures in terms of promoting Psa infection success in lignified kiwifruit plants.

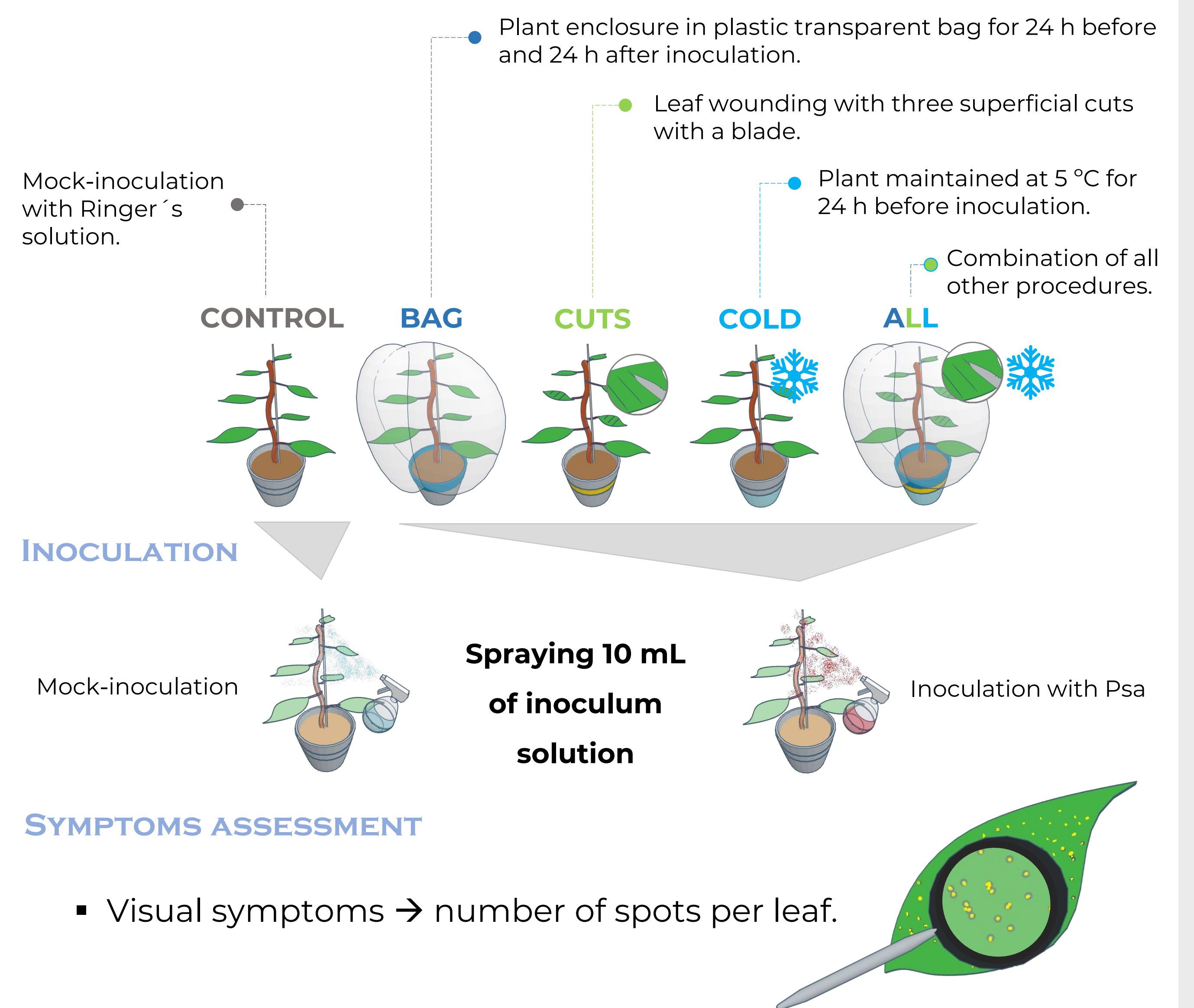
Materials and methods

- Four pre-inoculation procedures were tested in lignified 15-month-old kiwifruit plants artificially inoculated with Psa.
- *A. chinensis* var. *deliciosa* 'Tomuri' plants (commonly cultivated male cultivar).
 - 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 (10⁷ CFU/mL) prepared in Ringer's solutions.

Conclusions

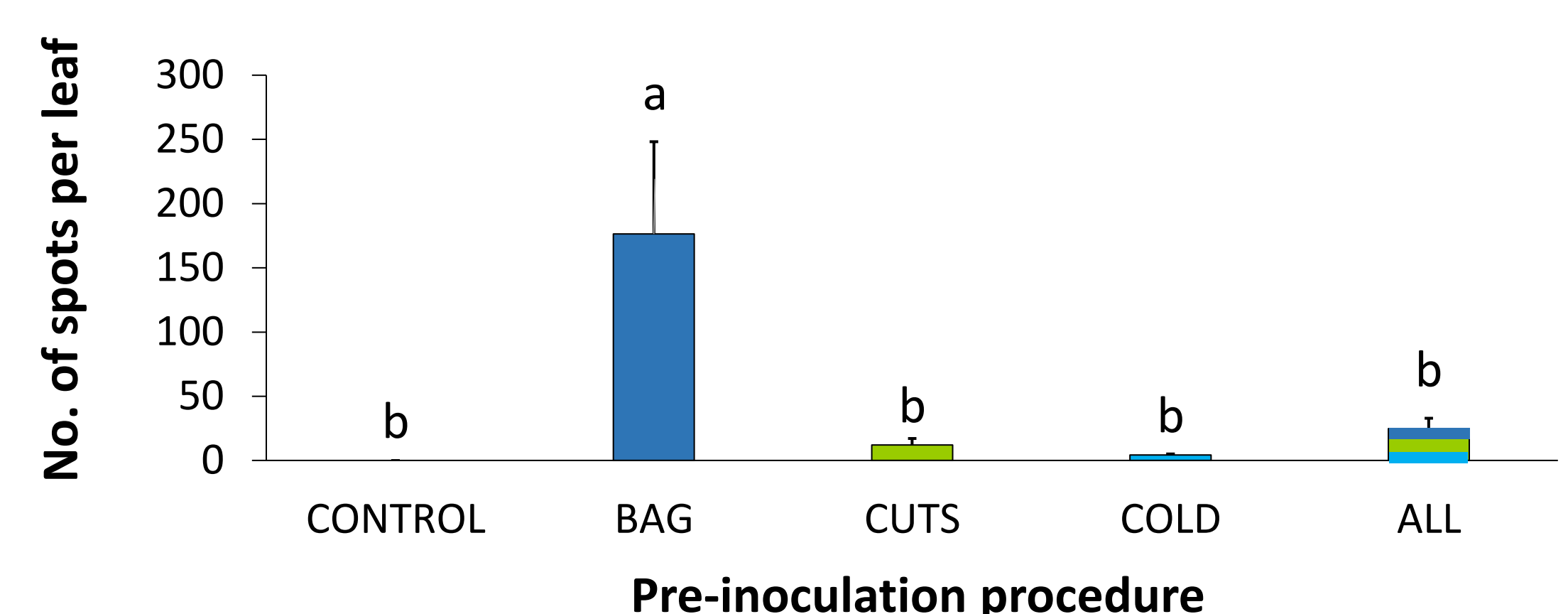
- The pre-inoculation procedure influences Psa colonization, subsequently affecting the ability of the bacteria to cause KBC symptoms in kiwifruit.
- The results from this study indicate that caution should be taken when comparing Psa pathogenicity between research studies in which different (pre-)inoculation procedures have been applied.
- This work highlights the importance of following precise pre-inoculation procedures in plant artificial inoculation with *Actinidia chinensis* – Psa, where symptoms appearance is commonly utilized in disease severity analysis.

PRE-INOCULATION PROCEDURES



Results and discussion

- **BAG** was the treatment with highest efficacy in causing appearance of leaf spots.
 - Plants from **BAG** showed 7.1, 15 and 40 times more leaf spots than plants from **ALL**, **CUTS** and **COLD**, respectively.
 - Treatment **BAG** most likely promoted stomatal aperture through the maintenance of high-humidity → entry point for Psa 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**.

Acknowledgements

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