

Inoculation techniques of the agents of common bacterial blight in *Phaseolus vulgaris*

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STATE OF ART

- Common bacterial blight (CBB) is an endemic disease of common bean (*Phaseolus vulgaris*) caused by *Xanthomonas phaseoli* pv. *phaseoli* (*Xpp*) and *Xanthomonas citri* pv. *fuscans* (*Xcf*).
 - A complete resistance to CBB is lacking and the efficacy of known resistance varies depending on the strain tested.
- Methods for disease assessment and resistance scoring differ by their efficacy in differentiating the virulence of strains and the resistant genotypes.
 - Effective disease phenotyping methods are essential for searching novel resistant genotypes.
- AIM:** To select among 4 different inoculation methods the most effective for virulence discrimination, resistant genotype selection, time efficiency and assessment method.

METHODS & RESULTS

EXPERIMENTAL DESIGN:

Experiments performed on unifoliate leaves at 13 days after sowing.

Used two *Xanthomonas* strains with different virulence:

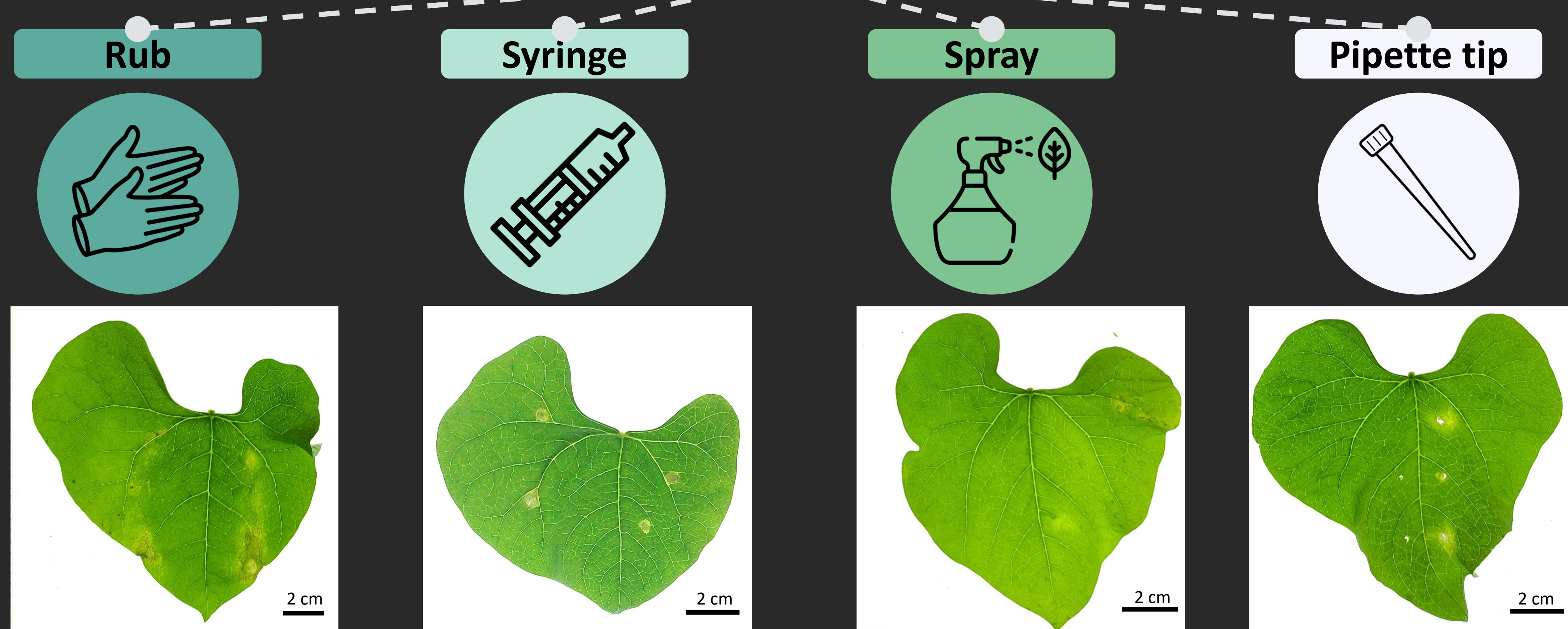
- *Xcf*: USB 749 (more virulent)
- *Xpp*: USB 771.

Tested in two common bean genotypes, one resistant and one susceptible.

The symptom assessment was done by machine learning based imaging (Ilastik) or with a visual scale.

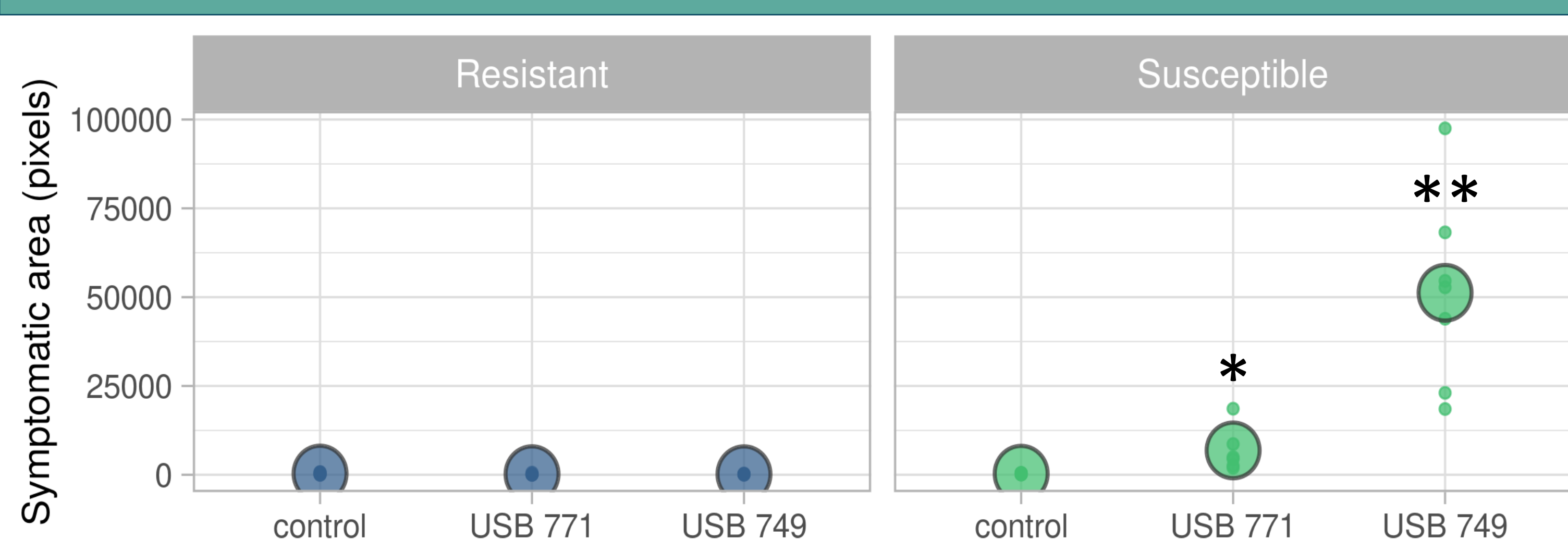
Experiments repeated at least two times.

4 INOCULATION METHODS

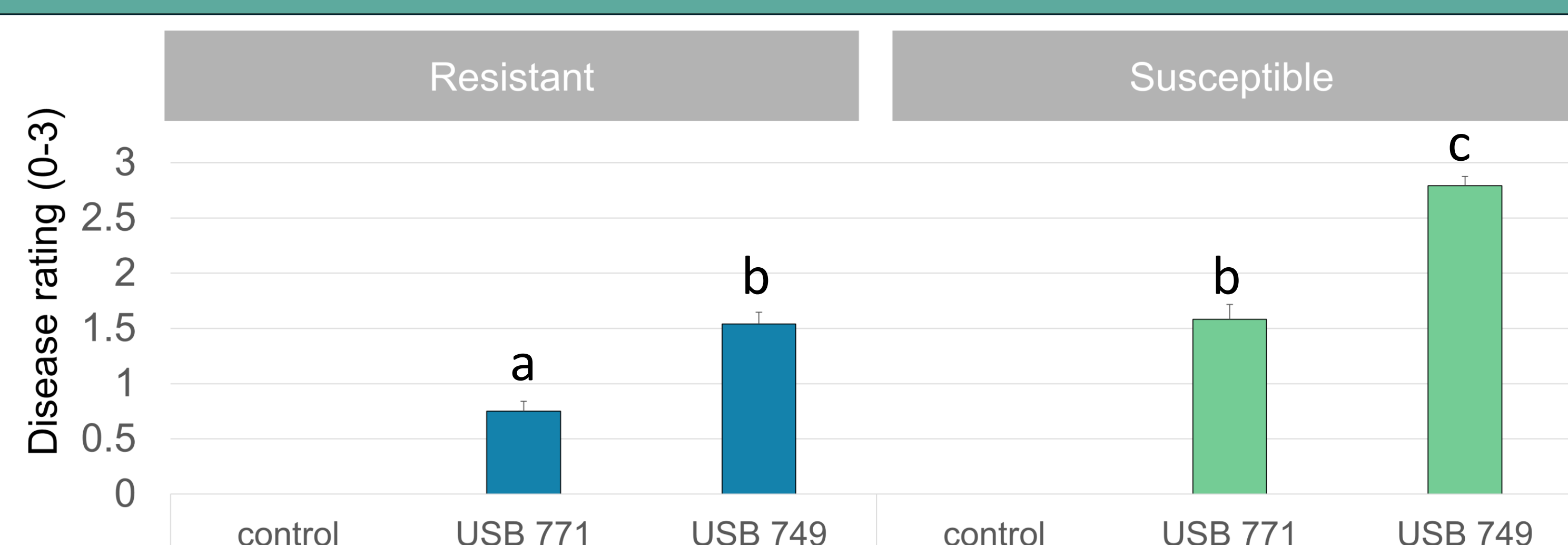


Characteristics	RUB	SYRINGE	SPRAY	TIP
Assessment date - days after inoculum	14	7	14	14
Inoculation time required per plant	30 s	2 min	1 min	20 s
Discrimination of strain virulence	high	high	low	medium
Discrimination of resistant genotype	medium	high	-	medium
Type of assessment	imaging	visual scale	imaging	imaging

RUB



SYRINGE



CONCLUSIONS and PERSPECTIVES

- 2 methods (rub and syringe) were able to discriminate both the strain virulence and the resistant genotype in a short time.
- The syringe method showed some symptoms even on the resistant genotype, which is very useful in discriminating levels of plant resistance, however the inoculation is time consuming and requires a certain level of technical skill.
- Rub is a rapid inoculation method that can be coupled with imaging, however it does not cause any symptoms on resistant genotype.
- Future work: the best methods will be tested on genotypes with different degrees of resistance and used in the breeding process for phenotypic selection.

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References:

- Zapata, M. A proposal for a uniform screening procedure for the greenhouse evaluation of variability of *Xanthomonas axonopodis* pv. *phaseoli* and resistance on leaves of *Phaseolus vulgaris*. *Annual Report-Bean Improvement Cooperative* 49, 213 (2006).
- Foucher, J. *et al.* Improving Common Bacterial Blight Phenotyping by Using Rub Inoculation and Machine Learning: Cheaper, Better, Faster, Stronger. *Phytopathology* 112, 691–699 (2022).
- Lo Cantore, P., Figliuolo, G. & Iacobellis, N. S. Response of traditional cultivars of Fagioli di Sarconi beans to artificial inoculation with common bacterial blight agents. *Phytopathologia mediterranea* (2010).