

# Testing biostimulants for validating the claims: a multi-level analysis

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## Context

Biostimulants products are placed on the market on the basis of their intended effects on crop plants (Regulation (EU) 2019/1009; du Jardin, 2020), referred to as 'claims'. Standardized protocols are required to validate the claims of biostimulant products and to allow their access to the EU market. Which protocols proving efficiency, robustness and relevance to a range of crops still needs to be determined.

## Set up

In order to set up an evaluation platform for biostimulants, a project named 'BioStimTest' has been initiated in Belgium. Organic, inorganic and microbial biostimulant products available on the market are tested on crops for a multi-scale analysis. Laboratory and greenhouse bioassays are compared with plant performance in the field under the temperate climate of Belgium. Improved nutrient use efficiency and drought tolerance are the two biostimulant claims evaluated.

## Objectives

The 'BioStimTest' project consists of setting up bioassays to identify potential correlations between the efficacy data of tests conducted under controlled conditions and the results of field trials. The main goal is to develop claims validating protocols at the laboratory and greenhouse scales which are most predictive of success in the field.

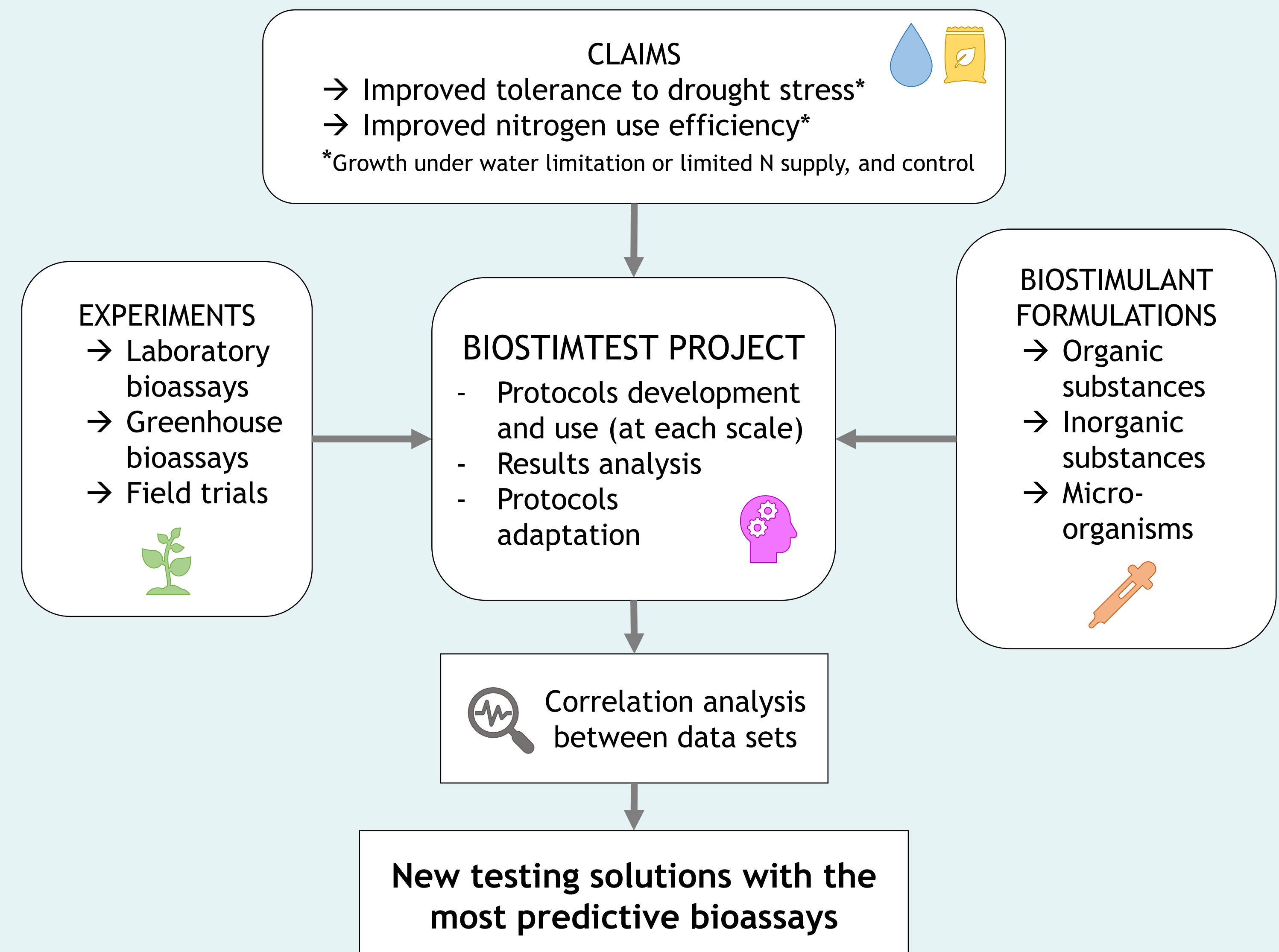


Figure 1 : Home-made mobile rainout shelters for field trials.



Figure 3 : *Arabidopsis thaliana* (var. Col-0) seedlings grown on nutritive gelose in phytostrips.

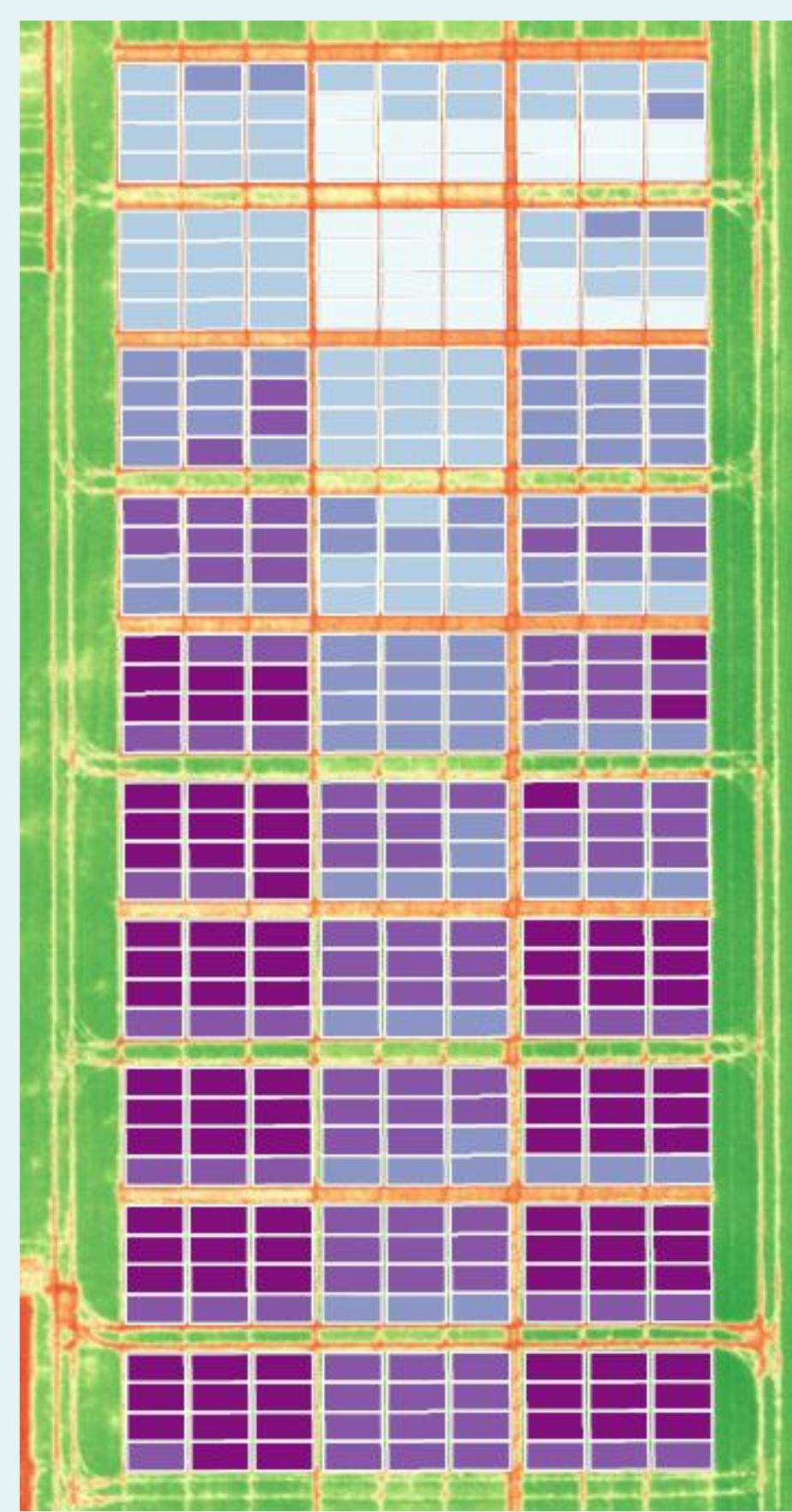


Figure 2 : Multispectral picture of the trials on nitrogen use efficiency in winter wheat (April 2021).

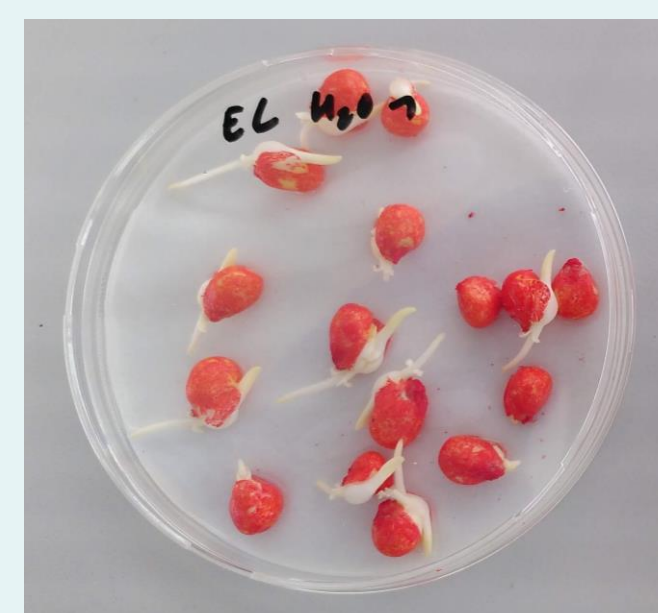


Figure 4 : Maize seeds (var. Elstream) sown in Petri dishes (7 days after sowing).



Figure 5 : Wheat plants (var. Mistral) grown on solid substrate (loam and sand) with three nitrogen fertilization (0, 50 and 100% of the recommended input) (60 days after sowing).



Figure 6 : Tobacco plants (var. Xanthi) grown on solid substrate (loam and sand) and with different water stress levels (64 days after sowing).

## Experiments

### • Biostimulants and claims

A variety of organic, inorganic and microbial commercial biostimulant products were chosen to be tested on several crops. For assessing drought tolerance, home-made mobile rainout shelters (fig. 1) are used for field trials, while greenhouse and laboratory bioassays use different watering regimes and polyethylene glycol 6000 (osmotic agent). For nitrogen use efficiency analyses, different fertilization regimes (field) or contrasted nutrients solutions (laboratory and greenhouse) are applied on crops and plants.

### • Laboratory bioassays

- Germination tests on tomato (*Solanum lycopersicum* L.) and maize (*Zea mays* L.) under osmotic stress (fig. 4), with either treatment of the media or seed priming with a biostimulant : germination kinetics and seedling biomasses;
- Microphenotyping of *Arabidopsis thaliana* L. in phytostrips (Burrell *et al.*, 2017) under nitrogen or osmotic stress (fig.3) : proxy variables of growth parameters (root length and mass, and rosette area);
- Rhizosphere acidification microassay on *A. thaliana*, as an indicator of root metabolic activity, including nitrogen uptake and utilization;
- Hydroponic growing of *A. thaliana* in Araponics™ under nitrogen or osmotic stress : rosette area and fresh and dry masses of shoots and roots.

### • Greenhouse bioassays

Spring wheat (*Triticum aestivum* L.), maize and tobacco (*Nicotiana tabacum* L.) plants are grown in pots in greenhouses (fig. 5 and 6), challenged with nitrogen or water stress, and treated or untreated with biostimulants. Phenotypic traits are measured, related to growth and photosynthesis activity.

### • Field trials

The project includes three growing seasons. From one to three varieties of winter wheat, potato (*Solanum tuberosum* L.) and maize are grown in fields under optimal or stress conditions (water or nitrogen shortage), and treated or untreated with biostimulants. Data sets bear on plant counts (stems, tillers...), times to crop key stages, biomasses measurements, plant health monitoring, crop multispectral analysis with drones (fig. 2), and measurements of qualitative and quantitative harvest traits.

## References

- Regulation (EU) 2019/1009 of the European Parliament and of the Council of 5 June 2019 laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003.
- du Jardin, P. in : Rouphael, Y., du Jardin, P., Brown, P., De Pascale, S. and Colla, G. (ed.), *Biostimulants for sustainable agriculture*, Burleigh Dodds Science Publishing, Cambridge, UK, 2020, (ISBN: 978 1 78676 336 5; [www.bdsublishing.com](http://www.bdsublishing.com)).
- Burrell, T. et al. *The Microphenotron: a robotic miniaturized plant phenotyping platform with diverse applications in chemical biology*. *Plant Methods* 13, 10 (2017). <https://doi.org/10.1186/s13007-017-0158-6>.

## Acknowledgement

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**More about this project ?** Contact the corresponding author or [info@redeb.com](mailto:info@redeb.com) !