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Answering to the need for consistent biostimulants with a measurable impact on plants.

Exploring the performance of targeted, plant-based solutions from Roquette

Biostimulants have an important role to play in helping to realise the potential of crops and improving yields, with estimates suggesting accessible gains in the region of at least 5 - 10%.¹ Defined as stimulating 'natural processes in plants to enhance nutrient uptake and efficiency, crop quality, and tolerance to abiotic stress...'² biostimulants align closely with trends towards greater sustainability. By optimizing nutrient usage, they can enhance the benefits derived from fertilizers, allowing lower application rates. By increasing tolerance to abiotic stress they can make plants more resilient to climatic change and less reliant on crop protection chemicals. Predominantly biological in nature, biostimulants support a smarter approach to cultivation that focuses on optimizing productivity while simultaneously reducing environmental impact. The market reflects this appeal. Estimated at around 1.5 – 2.0 billion USD for 2022, compound annual growth rates (CAGRs) are currently in the region of 10 - 12%.¹

In the US, biostimulants are not yet regulated³; new regulations will come into force in the EU from 2022⁴. It could be argued that this lack of regulation in combination with the inherent variability of unrefined biological products has contributed to a sub-optimal marketplace, suppressing the greater uptake and routine use of biostimulants. Going forward there is a need for high quality, consistent products, of known provenance, with proven efficacy, to safeguard grower confidence and establish stronger cost benefit data.

Roquette, a global leader in the development and manufacture of plant-based ingredients, has developed a range of plant-derived solutions for plant nutrition and protection, collaborating with external partners and experts to refine products for specific applications, conditions and crops. These products include organic biostimulants and fertilizers with well-controlled properties that have delivered proven benefit in trials with crops such as tomatoes, lettuce, cress and turfgrass. In this paper we look at how Roquette processes plant-based feedstocks to make plant-care solutions of exemplary consistency and present trial data demonstrating their performance.



Differentiating biostimulants

In any discussion of biostimulants clarity as to definition is vital. Biostimulants, as the name suggests, act on naturally occuring metabolic and enzymatic processes within the plant to improve yield and vigor. They are not nutritional sources or pesticides, though they may enhance uptake/utility of an existing nutrient supply or stimulate a plant's natural defense system.

In the past, biostimulants were used predominantly by organic growers for relatively high value crops¹, but their capabilities answer directly to intensifying problems for growers. For example, biostimulants can make plants more resilient to cold, heat, drought and flooding, potentially providing a measure of protection against the greater seasonal variability associated with climate change. They also have potential to improve resistance to saline stress, thereby helping to mitigate impact of soil salinization, an environmental issue that has resulted in the loss of millions of acres of farmland in recent decades⁵. Increasingly innovative and targeted products in combination with factors, such as fertilizer price volatility and rising consumer demand for food products grown with minimal environmental impact, provide additional motivation for broader commercial use¹.

Most biostimulants are biological in origin and fall into one of the following categories³:

- Humic substances, such as humic and fulvic acids
- Protein hydrolysates and amino acids
- Seaweed, algae and other plant extracts
- Chitosan (and other biopolymers)
- Inorganic compounds
- Beneficial microbes and microorganisms

The biological nature of biostimulants introduces inherent variability, potentially leading to products of unknown provenance and variable quality, with ill-defined performance, and, most crucially, unproven benefit. Forthcoming EU legislation defines biostimulants on the basis of justified claims i.e. product functionality rather than content, intensifying pressure on manufacturers to demonstrate an effect via trial data⁶. Though demonstrating a specific level of efficacy is not practical, because of the impact of variables ranging from crop variety to soil microbiome to weather, better data and regulation will help to flush out sub-standard products and enable more rigorous product evaluation.

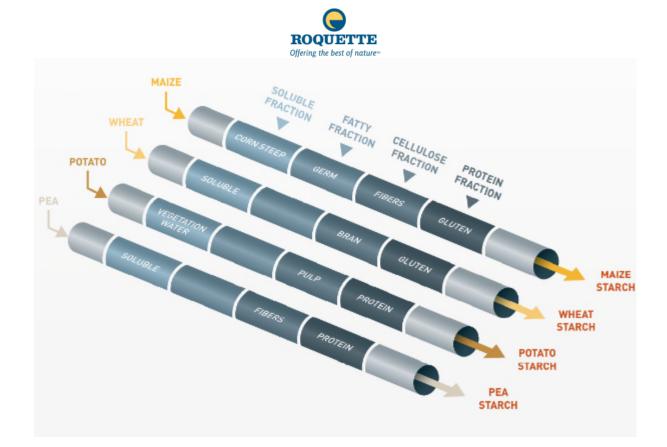


Figure 1: Roquette creates fertilizers/biostimulants and other plant care solutions via wellcontrolled processes of progressive refining that extract highly complex mixtures that deliver consistent performance outcomes.

Roquette produces biostimulants, fertilizers and other plant care solutions by processing crops such as corn, peas, potatoes and wheat using the following steps:

- Solubles extraction, via steeping (extended contact with water)
- Fermentation
- Enzymatic hydrolysis
- Separation, including filtration and centrifugation
- Concentration, notably low temperature drying.

All Roquette products are wholely plant-based, rather than inorganic - a distinction that improves acceptability for many growers - and produced from crops of known provenance, with several OMRI (Organic Materials Review Institute) compliant solutions available for organic growers. Closely controlled, processes prevent feedstock variability translating into product variability, require no solvent, and operate under relatively benign conditions. The resulting fractions are complex mixtures with a low carbon footprint that can be relied upon for highly consistent outcomes.



The following trial data provide supporting evidence of the performance of these products as biostimulants. It is worth noting that the complexity of plant extracts makes it demanding to rigorously demonstrate purely biostimulant effect. For example, gains could be due to the nutritive effect of trace elements. Here dosage levels and controls have been chosen to minimise this likelihood, but it is important to consider this issue in any assessment of biostimulant effect.

Data set 1: Using plant extracts to combat nutritive stress

Trials were carried out in a regulated growth chamber to assess the biostimulant effect of Roquette plant extracts on crops subject to nutritive stress: tomato, lettuce, arabidopsis thaliana, and Lolium Perenne.

Table 1 shows data for tomato plants grown in a hydroponic culture. Each test condition/modality was assessed via a box of 12 plants, all subject to an initial basal dose of a commercial NPK (14/7/26) fertilizer; this formed the negative control. A positive control was established using a commercial biostimulant containing iron and zinc (along with associated chelating ions), phytohormones, inorganic and organic nitrogen and other components. Roquette products A and B were tested at two different concentrations (see table). Results are presented in the form of percentage increase (or decrease), relative to the negative control.

Please note: This and all subsequent datasets was subject to rigorous statistical analysis; further details are available on request. <u>Contact us</u>

		Shoot Biomass (mg)	Root Biomass (mg)	Root Length (cm)	Root Surface (cm ²)	Root Tips Number	Root Forks Number
А	Negative Control 7,0.10 ³ gN/plant	0	0	0	0	0	0
В	Positive Control 1,5.10 ³ gN/plant	298	104	231	148	99	304
С	Roquette A 1,3.10⁵ gN/plant	87	43	92	87	27	54
D	Roquette A 3,3.10⁵ gN/plant	301	165	286	233	83	345
Е	Roquette B 1,3.10⁵ gN/plant	239	141	322	236	49	251
F	Roquette B 3,3.10⁵ gN/plant	140	80	156	126	56	104

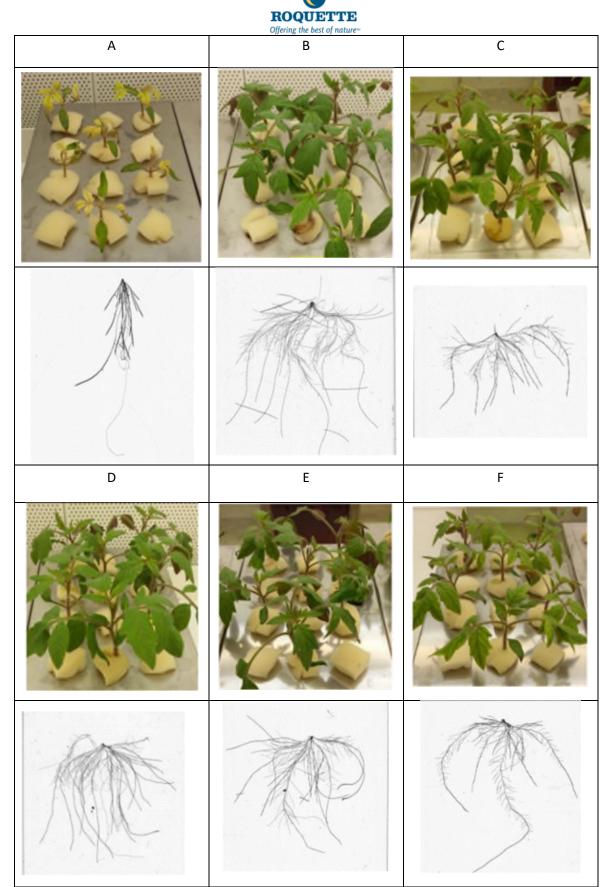


Table 1/Figure 2: Trial data and associated images for tomato plants subject to nutritive stressdemonstrate the ability of Roquette A and B to improve all growth metrics and deliverperformance comparable to a commercial biostimulant at much lower nitrogen loadings.



Roquette A and B deliver similar performance to one another with substantial improvement across all measured metrics. Comparing data at high and low concentrations for each of these products shows that concentration influences effect and that higher concentrations are not necessarily beneficial. More significantly, performance equivalent to the positive control can be achieved at dosage levels, on a nitrogen basis, that are around 10 to 100 times lower.

Table 2 shows data from a comparable trial with lettuce carried out under identical conditions, but using potting soil containing NPK (14/10/8) fertilizer as the growth medium. These trials were conducted with Roquette A only at dosage levels (nitrogen basis) equivalent to those of the commercial biostimulant in the positive control.

		Shoot Fresh Matter (mg)	Shoot Dry Matter (mg)	Root Dry Matter (mg)	Chlorophyll (mmol/m²)
А	Negative Control 2,7.10 ¹ gN/plant	0	0	0	0
В	Positive Control 3,0.10 ⁻³ gN/plant	74	73	287	14
С	Roquette A 3,0.10 ⁻³ gN/plant	38	34	375	8

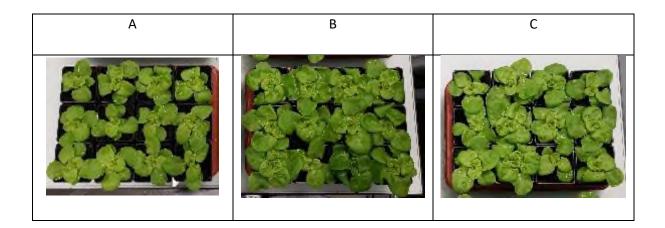


Table 2/Figure 3: Trial data and associated images for lettuce plants subject to nutritive stress demonstrate the ability of Roquette A to improve all growth metrics and deliver performance comparable to a commercial biostimulant.

Here, Roquette A has a positive impact on all the measured metrics, especially root growth, delivering comparable performance to the commercial biostimulant at an equivalent dosage.

Results from a third trial with arabidopsis thaliana, using a third plant extract, Roquette C, are shown in Table 3. Trial conditions were as for the preceding lettuce trial, however, the commercial biostimulant used for the positive control was an alternative product with listed ingredients including microalgae extract and corn steep liquor.



		Main Stem Size (mm)	Shoot Fresh Matter (mg)	Shoot Dry Matter (mg)	Root Dry Matter (mg)	Chlorophyll (mmol/m²)	Leaf Surface (cm ²)
	Negative Control 2,7.10 ¹ gN/plant	0	0	0	0	0	0
В	Positive Control 1,0.10 ⁻¹ gN/plant	600	13	6	322	15	20
	Roquette C Lot1 1,0.10 ⁻ gN/plant	1465	21	18	741	14	28
D	Roquette C Lot2 1,0.10 ⁻¹ gN/plant	1624	56	59	837	10	34

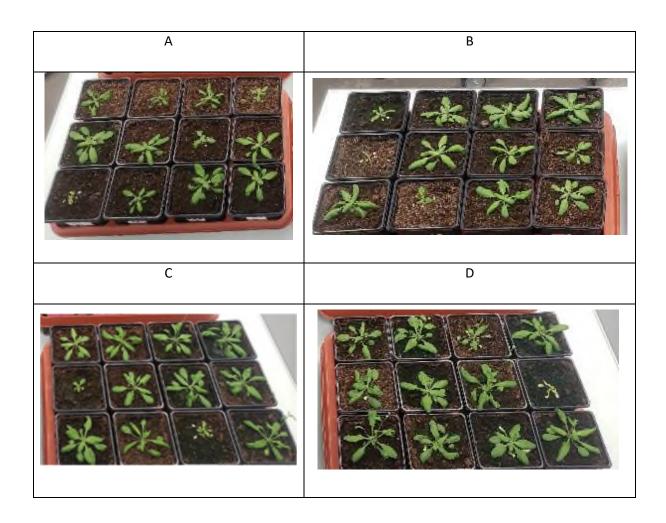


Table 3/Figure 4: Trial data and associated images for arabidopsis thaliana plants subject to nutritive stress demonstrate the ability of Roquette C to improve all growth metrics well beyond the levels obtained with the commercial biostimulant.

Roquette C significantly accelerates the growing cycle of the plant, leading to particularly substantial increases in stem size and root mass at the two week mark relative to both the negative controls and to the commercial biostimulant, when used at equivalent levels.



Table 4 shows data from a final plant extract trial with turf grass (Lolium Perenne) grown in potting soil containing NPK (14/10/8) fertilizer. In this trial each modality was assessed via 3 pots of 50 plants, 150 plants in total per modality, with results gathered four weeks after basal product application. The impact of SOLULYS® 048 (Roquette), a corn protein extract, was assessed relative to the negative control (fertilizer only), across a concentration range spanning two orders of magnitude.

		Maximum Height (cm)	Shoot Fresh Matter (mg)	Shoot Dry Matter (mg)	Root Dry Matter (mg)	Chlorophyll (mmol/m²)
A	Negative Control 5,4.10 ⁻¹ gN/plant	0	0	0	0	0
В	SOLULYS® 048 2,0.10⁴ gN/plant	-2	9	20	-36	-1
С	SOLULYS® 048 2,0.10³gN/plant	-5	27	33	130	0
D	SOLULYS® 0482,0.10 [,] gN/plant	-97	-100	-100	-94	-99

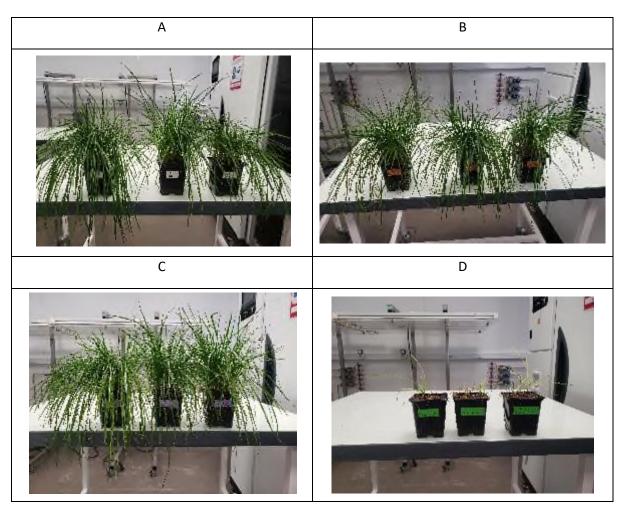


Table 4/Figure 5: Trial data and associated images for turf grass plants (Lolium Perenne) subject to nutritive stress demonstrate the ability of SOLULYS® 048 to improve shoot and root metrics when applied at an appropriate concentration.



SOLULYS[®] 048 has a beneficial effect when dosed at an appropriate concentration, particularly with respect to root volume development. Lower concentrations are relatively ineffective while higher concentrations lead to elemental excess, notably nitrogen, and the plants begin to die. Comparable trials with an alternative species of turfgrass, Festuca Trachyphylla, provided further evidence of the potentially beneficial effect of SOLULYS[®] 048 (data not shown).

Taken together these four sets of data show appreciable evidence of the biostimulant effect of Roquette plant extracts across a variety of crops. Furthermore, strong performance is demonstrated relative to commercial biostimulants on the basis of nitrogen equivalence. Selecting a basis for comparing biostimulant dosages is complicated by the different nature of alternative products; nitrogen was selected due to its relevance as a nutrient. However, is worth noting that the very low nitrogen loadings associated with Roquette product application reduce the likelihood of fertilizer action on the basis of nitrogen addition, though the potential for nutritive input from other elements in the products cannot be completely discounted. Concentration trends within the trials and the extent to which impact varies from species to species illustrates the importance of uniquely optimizing biostimulant use for any given crop.

Data set 2: Using NUTRALYS® H85 to combat nutritive stress in turfgrass

NUTRALYS[®] H85 (Roquette) is a pea protein hydrolysate containing free amino acids and peptides which serves as an organic nitrogen source. Table 5 shows data from a trial to assess the impact of NUTRALYS[®] H85 on turf grass (Lolium Perenne) growth, conducted under identical conditions to those described for the preceding turf grass trial with SOLULYS[®] 048. NUTRALYS[®] H85 dosage rates were assessed across a concentration range spanning two orders of magnitude.

		Maximum Height (cm)	Shoot Fresh Matter (mg)	Shoot Dry Matter (mg)	Root Dry Matter (mg)	Chlorophyll (mmol/m²)
А	Negative Control 5,4.10 ⁻¹ gN/plant	0	0	0	0	0
В	NUTRALYS® H85 2,0.10 ⁻⁴ gN/plant	-3	3	11	50	11
С	NUTRALYS® H85 2,0.10 ⁻³ gN/plant	1	21	32	63	-5
D	NUTRALYS® H85 2,0.10 ⁻² gN/plant	-90	-94	-95	-78	-90



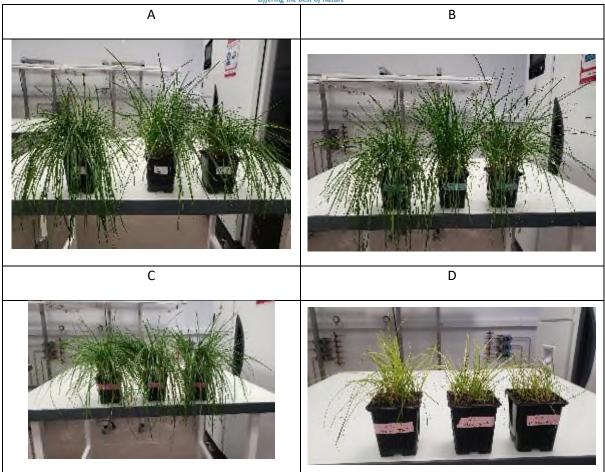


Table 5/Figure 6: Trial data and associated images for turf grass plants (Lolium Perenne) subject to nutritive stress demonstrate the ability of NUTRALYS® H85 to improve shoot and root metrics when applied at an appropriate concentration.

These results are analogous to those reported with SOLULYS[®] 048 (see Table 4). At lower dosages, NUTRALYS[®] H85 leads to substantial improvements in root and shoot biomass but higher levels are detrimental. Table 6 shows data for a parallel trial with Festuca Trachyphylla, an alternative species of turfgrass.

		Maximum Height (cm)	Shoot Fresh Matter (mg)	Shoot Dry Matter (mg)		Chlorophyll (mmol/m²)
A	Negative Control 1,8.10 ⁻¹ gN/plant	0	0	0	0	0
в	NUTRALYS® H85 6,7.10 ⁻⁵ gN/plant	6	24	12	11	11
с	NUTRALYS® H85 6,7.10 ⁻⁴ gN/plant	6	22	9	-14	-13
D	NUTRALYS® H85 6,7.10 ⁻³ gN/plant	-1	-91	-84	-110	-106



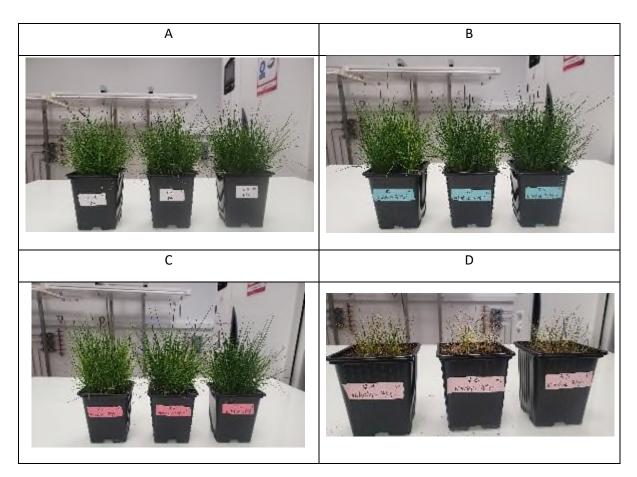


Table 6/Figure 7: Trial data and associated images for turf grass plants (Festuca Trachyphylla) subject to nutritive stress demonstrate the ability of NUTRALYS® H85 to improve all growth metrics when applied at an appropriate concentration.

Here, the lowest dosages are most beneficial resulting in modest improvements to shoot and root development, and chlorophyll levels. Higher dosages are again associated with plant failure. Taken together these results are valuable in illustrating the varied response of different species of the same crop and the need to closely tailor dosage levels to optimize beneficial impact.

Data set 3: Using polyols to combat saline stress

In a final set of trials, a series of polyols were assessed with respect to their ability to combat saline stress. Derived from plant starches extracted from cereal crops, NEOSORB® P60, a sorbitol, and PEARLITOL® are used as humectants, stress reducers and biostimulants; Roquette D is a polyol product in development. Table 7 shows data from a trial with tomato plants grown in a hydroponic culture. Each modality was assessed via 2 boxes of 6 plants, 12 plants per modality in total, with results gathered two weeks after foliar application of the product. All plants received a basal dose of a commercial NPK (18/6/26 + 4 MgO) fertilizer; this formed the negative control. Saline stress was applied by dosing with a sodium chloride solution.



		Shoot Fresh Matter (mg)	Shoot Dry Matter (mg)	Root Fresh Matter (mg)	Root Dry Matter (mg)	Chlorophyll (mmol/m²)
	Negative Control 1,8.10 ⁻² gN/plant	0	0	0	0	0
в	PEARLITOL® (55mM) 0,2 mmol/plant	106	106	69	82	207
	NEOSORB® (55mM) 0,2 mmol/plant	105	101	65	67	116
	Roquette D (55mM) 0,2 mmol/plant	124	312	98	111	135

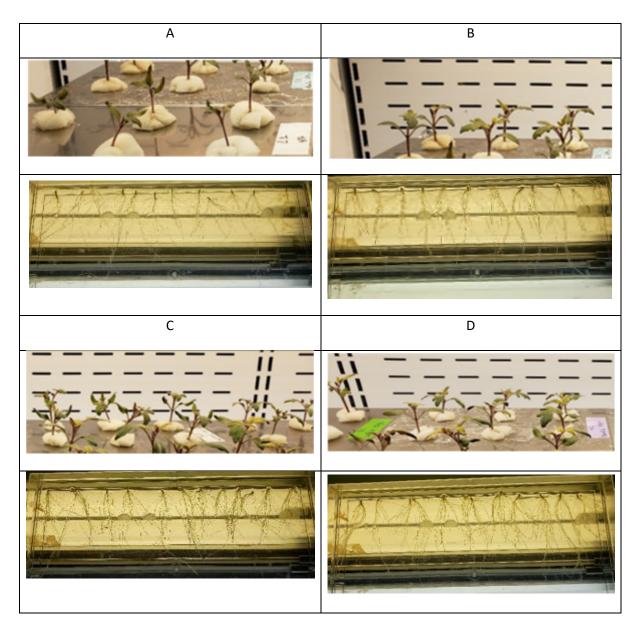


Table 7/Figure 8: Trial data and associated images for tomato plants subject to saline stressdemonstrate the ability of polyols to substantially improve all growth metrics.



All three polyols show a clear benefit with respect to offsetting the impact of saline stress. PEARLITOL[®] is particularly beneficial with respect to chlorophyll levels while Roquette D shows excellent promise in terms of shoot growth.

In conclusion

Biostimulants have the potential to help growers towards greater sustainability and reduced chemical/synthetic fertilizer use, even as envionmental issues such as climate change and soil salinization put pressure on yields and land productivity. The utilization of this potential relies on access to products capable of delivering consistent outcomes. Roquette manufactures a range of plant-derived products for plant care from crops of known provenance using well-controlled processes. The result is products of exemplary quality and consistency with substantial biostimulant potential for a range of crops, as the trial data presented here illustrates. Using these products, growers can robustly explore how biostimulants can help their crops, and be used to address specific issues. Furthermore, Roquette is actively engaged in developing new solutions, working directly in collaboration with customers to help them reap the rewards of this smart, low environmental impact technology.

Talk to us today about biostimulants and the issues you're looking to address: contact us.

A comprehensive plant-derived portfolio for plant care

Roquette has a comprehensive, established portfolio of plant-derived products for plant nutrition and protection. These help customers to provide key nutrients and growth factors, increase nutrient absorption, enhance stress tolerance, improve germination and stimulate growth while reducing the use of synthetic chemicals. Specific products for the formulation of plant nutrition solutions, solid- or liquid-based include:

- SOLULYS[®], GLUTALYS[™], NUTRALYS[®] and TUBERMINE[®]: Derived from corn (SOLULYS[®] and GLUTALYS[™]), peas and potatoes respectively these products are all sources of nitrogen, making them valuable as fertilizers, with various additional features. For example, SOLULYS[®] is OMRI compliant and available as a liquid product, for formulation as a water-based spray or drip.
- **NEOSORB®** and **PEARLITOL®**: These cereal-derived polyols sorbitol and mannitol respectively are highly compatible with other bioactive compounds and used as formulation excipients to help plants resist abiotic stress.
- **Dextrose**: Supplied in liquid or powder form, in a range of free-flowing grades, this nutrient and carrier is particualrly valuable as an energy/carbon source for microbial solutions.
- *Gluconic acid, Sodium Gluconate, BIOSUCCINIUM®:* These organic acids and salts are used as progressive acidifiers and complexing agents to increase the uptake of minerals and metal ions, particularly in neutral and alkaline soils.
- To find out more about these products and how they can help you formulate an optimized plant care solution for your needs < <u>Check our website</u> and <u>contact us</u>>.



References

¹ EBIC 'Economic Overview of the European Biostimulants Market'. Available to view at: <u>https://biostimulants.eu/highlights/economic-overview-of-the-european-biostimulants-market/</u>

² EBIC 'Plant Biostimulants contribute to climate-smart agriculture'. Available to view at: <u>https://biostimulants.eu/issue/plant-biostimulants-contribute-to-climate-smart-agriculture/</u>

³ UMass Extension Greenhouse Crops and Floriculture Program Factsheet 'What are biostimulants?' Oct 2019. Available to view at: <u>https://ag.umass.edu/greenhouse-floriculture/fact-sheets/what-are-biostimulants</u>

⁴ EUR-Lex 'Safe and effective fertlizers on the EU market (from 2022)' Available to view at: <u>https://eur-lex.europa.eu/legal-content/EN/LSU/?uri=CELEX%3A32019R1009</u>

⁵ EOS Blog 'Soil Salinization Causes and How to Prevent and Manage It' available to view at: <u>https://eos.com/blog/soil-salinization/</u>

⁶ M. Ricci et al 'General Principles to Justify Plant Biostimulant Claims' *Front Plant Sci* 16th April 2019. Available to view at: <u>https://www.frontiersin.org/articles/10.3389/fpls.2019.00494/full#T3</u>