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# SEM analysis of the effect of biostimulants on potato yield and nutrient use efficiency

4th NFTN Conference 2026

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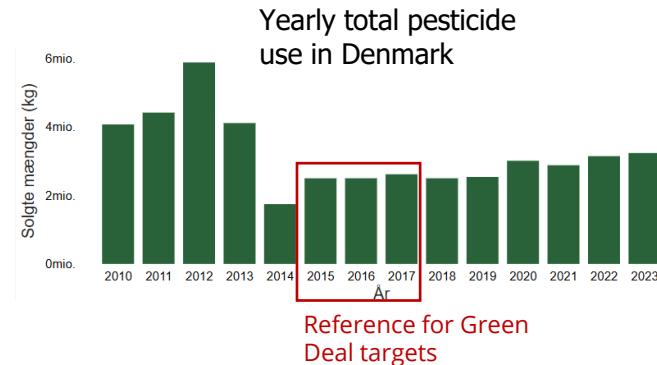
## Why biostimulants?

- There is a **need for sustainable agricultural food production systems** with less input of nutrients and chemicals
- Biostimulants might be **one of several solutions**
- but evidence on the effectiveness of biostimulants remain **scarce and ambiguous**

(Mannino et al., 2025)



**The EU's Green Deal is targeting a reduction of pesticide use and risk by 50% by 2030.**





## What are biostimulants?

According to Regulation (EU) 2019/1009:

- **“Substances, mixtures and micro-organisms (products) that aim solely at improving the plants’ nutrient use efficiency, tolerance to abiotic stress, quality traits or increasing the availability of confined nutrients in the soil or rhizosphere”.**
- **“Products that act in addition to fertilizers, with the aim of optimizing the efficiency of those fertilizers and reducing the nutrient application rates”.**

See more at:





There is a clear **need** for:

- development of **new test tools & protocols**
- addressing the complex, multi-variable **Mode-of Action (MoA)** claims of biostimulants
- via **impartial testing of producer claims** of biostimulant products

Claimed multi-functional benefits of biostimulants

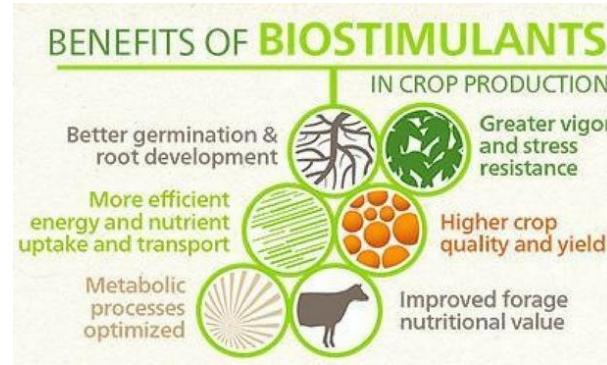


Figure by **Alltech**

1. Better germination & root development
2. More efficient nutrient utilization (NUE)
3. Optimized metabolic processes
4. Greater vigor and stress resistance
5. Higher crop quality and yield
6. Improved nutritional value of the crop



## Aim

- By utilizing a **Structural Equation Model (SEM)** approach for **testing hypotheses of claimed mode-of-actions (MoA)** of the five biostimulants

*Acadian, CropSet, Humifirst, Proradix, and Vesta,*

- we investigated biostimulant effects on **potato yield**, **yield quality**, **nutrient use efficiency (NUE)**, and **soil enzyme activity**.
- We did this by combining data from **two Danish organic potato field trials** conducted within the GUDP-project [BioGrowth](#).



BioGrowth (Ministry of Food, Agriculture & Fisheries GUDP) - Biostimulants for improved growth and quality of field vegetables

Edelenbos, Merete (PI), Kristensen, Hanne Lakkenborg (Deltager), Gebremikael, Mesfin Tsegaye (Deltager)

Institut for Fødevarer - Fødevareteknologi, Institut for Fødevarer - Planter, Fødevarer & Bæredygtighed



# Road map for our SEM approach

1) Producer claims



2) Turning claims into testable endpoints



3) Connect endpoints into a MoA hypothesis network



4) Setup SEM model in line with MoA hypothesis



5 ) Compile trial data



6) Do SEM analysis



7 ) Results

# 1) Producer claims

	Acadian	Crop-Set	Humifirst	Proradix	Vesta
Biostimulant Type	Seaweed extract	Fermentation-based + micronutrients	Humic–fulvic acid	Microbial with biocontrol functions	Microbial
Key composition	Seaweed ( <i>Ascophyllum nodosum</i> ) extracted with potassium hydroxide. The extract contains bioactive acids produced by algae, polysaccharides including fucose, mannitol, monosaccharides, amino acids, organic acids, betaines, and macro- and micro nutrients (Zn, Mg, Fe).	A mixture of yucca palm extract ( <i>Yucca schidigera</i> ), a bacterial fermented extract without alive bacteria, and addition of sulfur sulfate (1.2% S), copper sulfate (0.2% Cu), iron sulfate (0.6% Fe), and manganese (1.5% Mn)	Extract of humic (12 %) and fulvic acids (3 %) made from American Leonardit	Powder containing <i>Pseudomonas</i> sp. DSMZ 13134	Suspension of >5,400 living microorganisms extracted from fermented seaweed, microbial metabolites, and humic extract.
Main Effects	Increased root growth and improved plant establishment. Improved plant vigor and plant growth enhancing yield and crop quality	Improves nutrient uptake and partitioning, and stimulates plant metabolic processes (root growth vegetative growth reproductive growth) through greater availability of essential nutrients. Improves crop vigor yield and marketable yield, including crop uniformity.	Improves soil aggregation and ICEC, and germination and root growth. Improves nutrient uptake (P, Fe, Mn, Zn, Cu). Increases root and plant growth enhancing yields and crop quality	Improves root growth which improves the nutrient use efficiency. Improves plant and crop growth enhancing yield and crop quality. Controls silver scab, and stem canker and black scurf caused by <i>Rhizoctonia solani</i>	Restores soil microbiology and increases root growth. Increases nutrient cycling efficiency and improves nutrient use efficiency. Increase plant vigor and uniformity.
Stress Tolerance Effects	Enhances tolerance to drought and cold stress, linked to upregulation of defence-related genes and pathways in the plant.	Improves performance under in-season stress. The plant extract activates the plant defence mechanisms, increases photosynthetic pigments and gas exchange under drought stress	Increases resistance to drought, salinity, and cold stress as a result of increased enzymatic and non-enzymatic defence, increased compatible solute production, and changed ion balance.	Enhances abiotic stress response via induced systemic resistance induction	Increased tolerance to heat and water stress. Improved photosynthesis.
Reference/link	<a href="https://acadianplanthealth.com/your-crops/vegetables/potatoes/">https://acadianplanthealth.com/your-crops/vegetables/potatoes/</a> <a href="https://explore.azelis.com/en_GB/nor_ag/azelis-seaweed-in-cucumber-tomato">https://explore.azelis.com/en_GB/nor_ag/azelis-seaweed-in-cucumber-tomato</a>	<a href="https://www.bj-agro.de/dlg/crop-set">https://www.bj-agro.de/dlg/crop-set</a> <a href="https://www.alltech.com/en/our-solutions/crop-science/performance">https://www.alltech.com/en/our-solutions/crop-science/performance</a>	<a href="https://www.n-xt.com/en/products/tradecorp-products/humifirst%AE">https://www.n-xt.com/en/products/tradecorp-products/humifirst%AE</a>	<a href="https://en.sourcex-pudera.de/linee/line-plant-protection-product-category/proradix-united-kingdom.html">https://en.sourcex-pudera.de/linee/line-plant-protection-product-category/proradix-united-kingdom.html</a>	<a href="https://sovitse.com/products/">https://sovitse.com/products/</a>



## 2) Turning claims into testable endpoints

Claimed beneficial effect		Endpoint indicator	Biostimulant				
Group	Subgroup		Acadian	CropSet	Humifirst	Proradix	Vesta
Microbial soil health		DHA/BGA			X		X
Root growth		Not assessed	X	X	X	X	X
Plant growth & vigor		Fv/Fm	X	X	X	X	X
Plant stress resistance		Not assessed	X	X	X	X	X
Nutrient use efficiency		PFPn, PFPp		X	X	X	X
Yield		NTTY	X	X	X	X	
Quality	Marketable yield <sup>1</sup>	Prop. marketable <sup>1</sup>		X			
	Yield uniformity	VAR (NTTY)	X		X	X	
	Storage stability	Prop(Tubers > 6 cm) (TS)					
		Total sugar content (SC)					
Number of testable claims in the current study			3	4	5	4	3

<sup>1</sup>Marketable yield = NTTY \* (100 - mechanical damage% + disease damage%) / 100

In the current data, disease damage%  $\gg$  mechanical damage%, and, hence, total disease damage% (**TDD**) was used as endpoint.



# Some definitions

## DHA

"Dehydrogenase enzyme activity (DHA) is considered as the indicator of oxidative activity of soil microorganisms and increases significantly upon application of balanced fertilization."

## BGA

" $\beta$ -glucosidase (BGA) is involved in catalyzing the hydrolysis and biodegradation of various  $\beta$ -glucosides that are present in plant debris. ...  $\beta$ -glucosidase's involvement in C cycling has remarkably facilitated its adoption for soil quality testing."

[https://www.researchgate.net/publication/320388949\\_The\\_biological\\_activities\\_of\\_beta-glucosidase\\_phosphatase\\_and\\_urease\\_as\\_soil\\_quality\\_indicators\\_A\\_review](https://www.researchgate.net/publication/320388949_The_biological_activities_of_beta-glucosidase_phosphatase_and_urease_as_soil_quality_indicators_A_review)

## $F_v/F_m$

" $F_v/F_m$  tests whether or not plant stress affects photosystem II in a dark adapted state."

[https://en.wikipedia.org/wiki/Plant\\_stress\\_measurement](https://en.wikipedia.org/wiki/Plant_stress_measurement)

## PFP

Partial factor productivity

"This is the ratio of crop yield to fertilizer nutrient applied. It indicates the productivity per unit of fertilizer input."

PFPn = PFP for nitrogen

PFPp = PFP for P

## IUE

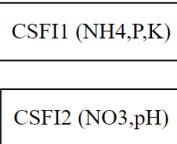
Internal use efficiency

"This is the ratio of crop yield to nutrient content in harvested parts. It indicates the efficiency of economic product formation per unit of removed nutrient. A high IUE means a high yield with low nutrient concentration in harvested parts."

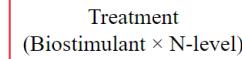
<https://geopard.tech/blog/defining-nutrient-use-efficiency-in-responsible-plant-nutrition/>

### 3) Connect endpoints into a MoA hypothesis network

PCA-derived  
Soil Fertility  
indicators



Soil Fertility



Edaphic capacity

Photosynthetic capacity

Fv/Fm

Crop vigor

Growth

Establishment

BGA

DHA

Storage capacity

Total disease damage

PFPn

PFPp

Yield  
(Net total tubers)

Microbial soil health

Total sugar content

Size class

Yield quality

NUE

Yield quantity



Manifest variable (measurable)



Latent variable (unobservable)

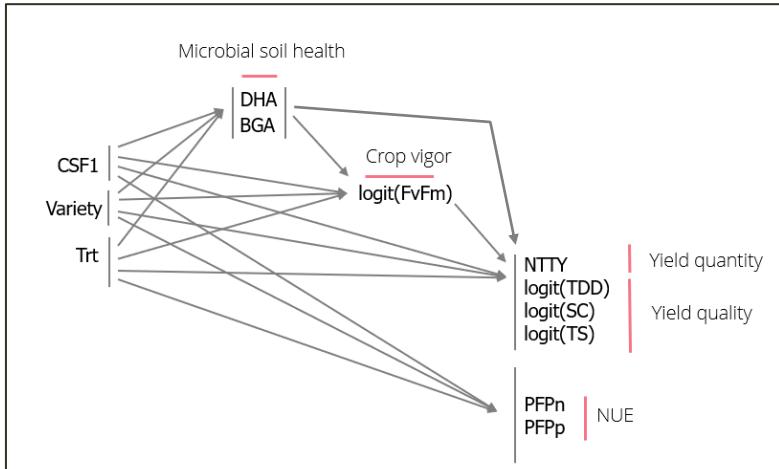
Claims

Harvest





## 4) Setup SEM model in line with MoA hypothesis


$$(1) DHA_i = \beta_{1,0} + \beta_{1,1}CSFI1_i + \beta_{1,2}Variety_i + \beta_{1,3}Trt_i + D_i$$
$$(2) BGA_i = \beta_{2,0} + \beta_{2,1}CSFI1_i + \beta_{2,2}Variety_i + \beta_{2,3}Trt_i + D_i$$
$$(3) \text{logit}(F_v/F_m)_i = \beta_{3,0} + \beta_{3,1}CSFI1_i + \beta_{3,2}Variety_i + \beta_{3,3}DHA_i + \beta_{3,4}BGA_i + \beta_{3,5}Trt_i + D_i$$
$$(4) NTTY_i = \beta_{4,0} + \beta_{4,1}CSFI1_i + \beta_{4,2}Variety_i + \beta_{4,3}DHA_i + \beta_{4,4}BGA_i + \beta_{4,5}\text{logit}(F_v/F_m)_i + \beta_{4,6}Trt_i + D_i$$
$$(5) \text{logit}(TDD)_i = \beta_{5,0} + \beta_{5,1}CSFI1_i + \beta_{5,2}Variety_i + \beta_{5,3}DHA_i + \beta_{5,4}BGA_i + \beta_{5,5}\text{logit}(F_v/F_m)_i + \beta_{5,6}Trt_i + D_i$$
$$(6) \text{logit}(SC)_i = \beta_{6,0} + \beta_{6,1}CSFI1_i + \beta_{6,2}Variety_i + \beta_{6,3}DHA_i + \beta_{6,4}BGA_i + \beta_{6,5}\text{logit}(F_v/F_m)_i + \beta_{6,6}Trt_i + D_i$$
$$(7) \text{logit}(TS)_i = \beta_{7,0} + \beta_{7,1}CSFI1_i + \beta_{7,2}Variety_i + \beta_{7,3}DHA_i + \beta_{7,4}BGA_i + \beta_{7,5}\text{logit}(F_v/F_m)_i + \beta_{7,6}Trt_i + D_i$$
$$(8) PFPn_i = \beta_{8,0} + \beta_{8,1}CSFI1_i + \beta_{8,2}Variety_i + \beta_{8,3}Trt_i + D_i$$
$$(9) PFPp_i = \beta_{9,0} + \beta_{9,1}CSFI1_i + \beta_{9,2}Variety_i + \beta_{9,3}Trt_i + D_i$$

where  $D_i = \text{Year} \times \text{Location} \times \text{Block}_{j[i]} + e_i$

$$\text{Year} \times \text{Location} \times \text{Block}_j \sim N(0, \sigma_b^2)$$
$$e_i \sim N(0, \sigma_e^2)$$



## 5 ) Compile trial data

	<b>Trial 1</b>	<b>Trial 2</b>
<b>Locality</b>	Årslev	Auning
<b>Year</b>	2021	2022
<b>Soil properties</b>	Mean (SD)	
mineral N, kg/ha	27.38 (1.70)	28.35 (6.90)
P, kg/ha	135.98 (18.91)	75.08 (1.86)
pH	6.35 (0.05)	6.19 (0.10)
<b>Variety</b>	Arielle	Belana (Arielle)
<b>N-levels</b>	N100 + N50	N100 + N50
<b>Trial design</b>	2-factorial RCBD <sup>1</sup>	3-factorial RCBD
<b>Factorial structure</b>	<b>Factor 1 (F1):</b> Biostimulant {Control, Acadian, CropSet, Humifirst, Proradix, Vesta, CombiSet}  <b>Factor 2 (F2):</b> Nitrogen level {norm N-level (100%), reduced N-level (50%)}  <b>Factor 3 (F3):</b> Variety {Belana : F1×F2, Arielle : (F1=Control)×F2}	<b>Factor 1 (F1):</b> Biostimulant {Control, Acadian, CropSet, Humifirst, Proradix, Vesta, CombiSet}  <b>Factor 2 (F2):</b> Nitrogen level {norm N-level (100%), reduced N-level (50%)}  <b>Factor 3 (F3):</b> Variety {Belana : F1×F2, Arielle : (F1=Control)×F2}
<b>Effective number of replicates (Ctrl)</b>	3 (3)	4 (8)

<sup>1</sup>RCBD: Randomized Complete Block Design



## 6) Do SEM analysis

	<i>I</i>	<i>II</i>		
	n = 39	n = 67		
		Variety (Trial1/Trial2)		
Arielle	18/0	18/7		
Belana	0/21	0/42		
		N-level (Trial1/Trial2)		
N100	18/21	18/24		
N50	0/0	0/25		
		Overall goodness of fit		
Fisher's C (df)	62,182 (66)	32,04 (36)		
P-value	0,610	0,657		
		Component model goodness of fit		
Response	Marginal R <sup>2</sup>	Conditional R <sup>2</sup>	Marginal R <sup>2</sup>	Conditional R <sup>2</sup>
<i>logit(FvFm)</i>	0,48	0,48	0,35	0,35
<i>NTTY</i>	0,77	0,84	0,75	0,85
<i>logit(TDD)</i>	0,38	0,45	0,48	0,48
<i>DHA</i>	0,66	0,71	0,68	0,79
<i>BGA</i>	0,63	0,63	0,56	0,63
<i>PFPn</i>	0,60	0,69	0,81	0,87
<i>PFPp</i>	0,20	0,41	0,68	0,80
<i>logit(SC)</i>	0,82	0,83	-	-
<i>logit(TS)</i>	0,90	0,90	-	-

# 7 ) Results

Claimed beneficial effect		Endpoint indicator	Estimated Cohen's <i>d</i> effect sizes <sup>3</sup>					Note	
Group	Subgroup		Acadian	CropSet	Humifirst	Proradix	Vesta		
Microbial soil health	DHA	0.47 (N50) <sup>II</sup>	0.05 (N50) <sup>II</sup>	0.23 (N50) <sup>II</sup>	-0.02 (N100) <sup>I</sup>	<b>0.62+</b> (N50) <sup>II</sup>		High is good	
		0.08 (N100) <sup>I</sup>	<b>0.72*</b> (N100) <sup>I</sup>	0.17 (N100) <sup>I</sup>	-0.11 (N100) <sup>I</sup>	-0.15 (N100)			
Plant growth & vigor	Fv/Fm	<b>1.46*</b> (N50) <sup>II</sup>	0.64 (N50) <sup>II</sup>	<b>0.61+</b> (N100) <sup>I</sup>	0.42 (N100) <sup>I</sup>	0.46 (N100) <sup>I</sup>			
Nutrient use efficiency	PFPn	0.36 (N100) <sup>I</sup>	<b>0.68*</b> (N100) <sup>I</sup>	0.08 (N100) <sup>II</sup>	<b>0.60+</b> (N100) <sup>I</sup>	0.15 (N100) <sup>II</sup>			
		0.53 (N100) <sup>I</sup>	<b>1.00*</b> (N100) <sup>I</sup>	0.15 (N50) <sup>II</sup>	<b>0.76*</b> (N50) <sup>II</sup>	0.15 (N100) <sup>II</sup>			
Yield	NTTY	0.29 (N100) <sup>I</sup>	<b>0.62*</b> (N50) <sup>II</sup>	0.21 (N50) <sup>II</sup>	<b>0.70*</b> (N50) <sup>II</sup>	0.16 (N50) <sup>II</sup>			
Quality	Marketable yield <sup>1</sup>	TDD <sup>1</sup>	-0.66 (N50) <sup>II</sup>	<b>-0.69+</b> (N100) <sup>II</sup>	-0.49 (N50) <sup>II</sup>	<b>-1.19+</b> (N50) <sup>II</sup>	-0.40 (N50) <sup>II</sup>	Negative is good	
	Yield uniformity	VAR (NTTY) <sup>2</sup>	1.11 (N100) <sup>I</sup>	<b>0.35+</b> (N50) <sup>II</sup>	0.42 (N50) <sup>II</sup>	0.92 (N100) <sup>II</sup>	0.80 (N100) <sup>I</sup>	Low is good	
	Storage stability (N100) <sup>I</sup>	Prop(Tubers > 6 cm) (TS)	-0.05	0.26	0.05	<b>0.62*</b>	-0.18	High is good	
		Total sugar content (SC)	<b>-0.36*</b>	<b>-0.41*</b>	<b>-0.42*</b>	<b>-0.73***</b>	-0.24	Negative is good	
# met / producer stated claims		p < 0.15	2 / 3 66%	3 / 4 75%	2 / 5 40%	3 / 4 75%	1 / 3 33%		
		Cohen's <i>d</i> >  0.5	2 / 3 66%	4 / 4 100%	1 / 5 20%	3 / 4 75%	1 / 3 33%		
# found / possible beneficial effects		Cohen's <i>d</i> >  0.5	3 / 5 60%	5 / 5 100%	1 / 5 20%	3 / 5 60%	1 / 5 20%		

<sup>+</sup>: p < 0.15, <sup>\*</sup>: p < 0.05, <sup>\*\*</sup>: p < 0.01, <sup>\*\*\*</sup>: p < 0.001

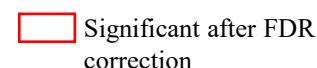
<sup>1</sup>Marketable yield = NTTY \* (100 - mechanical damage% + disease damage%) / 100

In the current data, disease damage% ≫ mechanical damage%, and, hence, total disease damage% (**TDD**) is used as endpoint.

<sup>2</sup>Var(NTTY) is evaluated as  $Sd_{\text{treatment}} / SD_{\text{control}}$

<sup>3</sup>Cohen's *d* =  $\hat{\beta} / SD(y)$ , small effect: *d* = 0.20; medium effect: *d* = 0.50; large effect: *d* = 0.80

 Producer claim

 Significant after FDR correction



## Discussion & Conclusions

- **All five biostimulant products showed at least one significant beneficial treatment effect** at the un-corrected 0.15 significance level.
- However, after false-discovery-rate multiple testing correction, only a single effect remained significant; Proradix on *TS*. This clearly indicates that **statistical power should be a major concern**, when assessing the multi-variable MoA claims of biostimulants.
- **Products varied in how good they met their claims:** we found poor (20-33%; Humifirst, Vesta), moderate (66%; Acadian) to good (75-100%; Proradix and CropSet) agreement between SEM estimates and claims.
- **Significant yield improvements** were found for CropSet and Proradix, only.
- However, significant **improvement in storage stability**, measured as decreased total sugar content (TS), was found in 4 out of 5 products.



## Collaborators



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## Funding



BioGrowth (Ministry of Food, Agriculture & Fisheries GUDP) - Biostimulants for improved growth and quality of field vegetables

Edelenbos, Merete (P), Kristensen, Hanne Lakkenborg (Deltager), Gebremikael, Mesfin Tsegaye (Deltager)

Institut for Fødevarer - Fødevarereteknologi, Institut for Fødevarer - Planter, Fødevarer & Bæredygtighed





# THANK YOU!

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