Serenade (strain QST 713), a unique biological fungicide to enhance potato quality and viability: foundational to regenerative potato production

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Plant microbiome and potato production

Plant microbe interactions play a fundamental role in the establishment of food systems. The microbiome in the soil sets balances for soil health, driving nutrient cycling and enabling plant nutrition. In parallel, soil microbes influence gene expression in the plant by activating resistance genes to increase plant tolerance to pest and diseases. Invisible to our eyes, the microbiome in the soil is fundamental to the productivity of plants and human health (Kendzior, 2022).

The soil microbiome is characterized by its quantitative as well its qualitative abundance. One gram of soil is estimated to contain 1 billion of viable microorganisms representing more than 20 000 species (ESDAC, 2023). Gram positive bacteria of the Bacillus family represent an important functional group of the soil microbiome and they serve as a rich source for the development of biological crop protection products. Serenade is a biological fungicide based on *Bacillus amyloliquefaciens* strain QST 713. In the Netherlands, Serenade is registered with a broad label in more than 50 crops (Ctgb, 2022). The product is widely used, and it holds potential to strengthen crop protection programs either by controlling foliar diseases once applied as foliar fungicide or by suppressing soil borne diseases through in-furrow application at planting.

In The Netherlands potatoes are generally produced in crop rotations with crops like wheat, sugar beet, onions, grass, peas, and corn. Since the last 10 years cover crops with various mixtures of dicot and monocot crops have been established as a standard practice in the integrated cropping systems of the mainstream farmers. Driven by the high capital investments, potatoes represent 25 to 30% of the crop rotation. In case of starch potatoes the crop intensity can go up to 50% of the rotation. Soil borne fungi such as *Rhizoctonia solani*, *Colletotrichum coccodes*, *Verticillium dahliae*, *Helminthosporium solani* and soil borne bacteria such as *Streptomyces scabies* and nematodes such as *Pratylenchus penetrans* cause disease complexes reducing the yield potential of the potatoes (Larkin, 2011, Scholte, 1989).

In the period 2019 – 2022 Bayer CropScience established a large-scale research platform with 50 lead farmers in The Netherlands, potato breeders, processors, and packers with the objective to improve the understanding of Serenade's performance in potatoes through large scale data collection and analysis at farmer locations. The outcomes of this research are summarized in this paper.

Serenade – QST 713 a plant root symbiont

Serenade is based on the natural occurring soil-born bacterium *Bacillus amyloliquefaciens*, strain QST 713. When applied to the soil, germination of QST 713 dormant spores is activated by exudates of the growing plant roots. The spores germinate and vegetative cells multiply building a matrix composed of vegetative cells, exopolysaccharides and proteins on the root surface. This so-called biofilm supports the survival of the *Bacillus amyloliquefaciens* on the root interface and it shields the root tissue from pathogen attack by soil-borne diseases (Marring, 2016, Beauregarda, 2013).

QST 713 enhances root formation and modifies root architecture with an increased fraction of smaller roots. The increased root volume and root surface area support the increased uptake of both macro and micronutrients. Due to the symbiotic relationship between the plant root and QST 713, Serenade modifies the root exudation pattern of plant roots to the benefit of both bacterium and the plant. The bacteria utilize the exudates as an energy and carbon source, while nutrient acquisition by plant roots is stimulated through bacterial sourced chelating agents and enhanced exudation. In parallel microbial metabolites activate innate plant resistance through expression of resistance genes and the activation of the plant defense systems based on jasmonic and salicylic acid pathways (Pieterse, 2014, Preece, 2020).

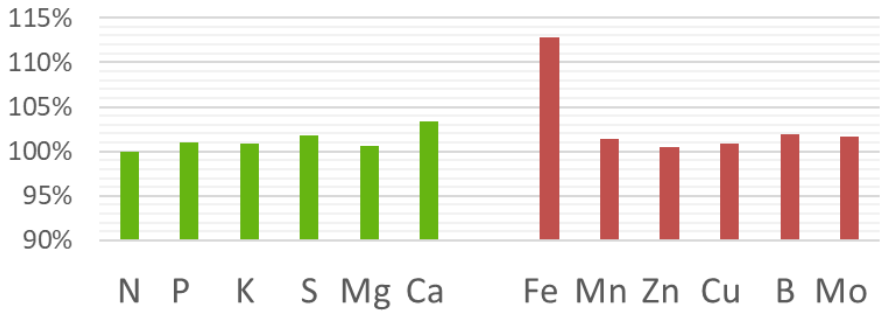

Figure 1. Relative nutrient content of tubers after Serenade treatment compared to standard farmer practice (average from 147 locations).

Co-development platform with lead farmers – from field to the lab

A consortium of 50 advanced potato growers representing seed, ware and starch potatoes acted as the reference for the data generation to monitor the potato's crop performance after the in-furrow application of 5-liter Serenade per ha at planting. In seed potatoes Serenade was added to the fungicide solution of the grower. In ware potatoes Serenade's performance in combination with 50% of the standard fungicide application, was compared to the farmer practice with a 100% fungicide program. In starch potatoes a stand- alone Serenade application was compared to a 100% fungicide program. Depending on the grower practices Serenade was applied in combination with NP starter fertilizers and or nematicides at planting.

The assessments of crop and tuber parameters were based on strip trials comparing the farmer practice with a Serenade modified program each with 6 replicated field samples (= 4,5-meter row length). Sample locations in the field were fixed before planting along those locations with similar yield potential using satellite imaging as a reference (Farm Management tool Boerenbunder, Dacom).

Data related to crop management (variety, date of planting, use of soil treatments at planting, desiccation, history of crop rotation and use of cover crops), crop -, tuber- and weather parameters were quantified and collected. At planting, Eurofins conducted a full soil analysis including microbial biomass. Mid- season the root colonization by QST 713 was monitored using a QST 713 specific qPCR method at Ghent University, Belgium (Mendis, 2018). After harvest the internal and external quality of the tubers (yield, tuber size distribution, dry matter and starch content, external tuber and skin quality, incidence of *Rhizoctonia solani* (= black scurf) and *Streptomyces scabies* (= common scab), number of green potatoes, internal brown spotting) were analyzed at the facilities of the potato breeder Averis. Post-harvest a full nutrient analysis of the whole tubers was conducted by Eurofins.

In the years 2020, 2021 and 2022, subsamples of potatoes taken at harvest, were used to monitor the distribution of glycoalkaloids in the tuber tissues with the MALDI mass spectrometry imaging technology at Bayer CropScience, Monheim, Germany. In 2020 and 2021 subsamples of 6 respectively 20 locations treated with Serenade were stored and replanted to analyze the performance of the Serenade sourced seed potatoes in the succeeding cropping season.

Data analysis and shifts in qualitative parameters

Performance signals of Serenade in large scale farmer trials were masked by the multiple farming practices used e.g., variations in soil type, cropping system and use of multiple potato varieties, seed quality, nutrient, and water management. The statistical analysis of the data set of 147 field trials revealed several interesting shifts in qualitative parameters.

Serenade proved to be a successful colonizer of the potato root system. Throughout the years 2019 and 2021 root colonization varied between 75–100% of the respective potato fields. The success of root colonization was independent from soil type. Since occurrence or lack of root colonization did not explain the observed quality improvements further qPCR analysis were stopped from 2021 onwards.

Serenade reduced the incidence of Streptomyces scab by respectively 3 and 4% in seed and ware potatoes. Rhizoctonia control improved by 1.5% in seed potatoes whereas in both ware and starch potatoes, no significant differences in Rhizoctonia control were observed between the Serenade treatments and the farmer practices using full rates of synthetic fungicides.

Farmer practice (Streptomyces)

Farmer practice + Serenade

Figure 2. Visual appearance of tubers (cultivar Zorba, location Rutten) Serenade treatment compared to standard farmer practice (Streptomyces).

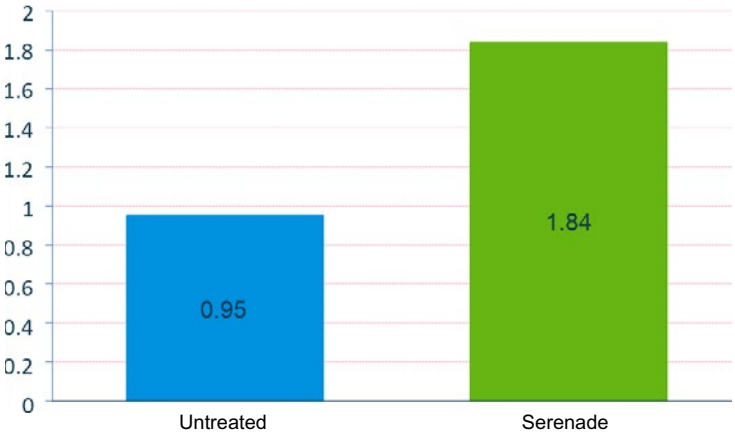

Figure 3. Gloss index of tubers: Serenade compared to untreated tubers measured at an angle of 60 °.

Improved skin quality and stolon root formation

Nutrient analysis of the whole tubers indicated increased levels of especially calcium (Ca) and iron (Fe) (see Figure 1).

Visual assessment of skin quality revealed large differences between tubers sourced from Serenade treatment versus farmer practice. See Figure 2. The reduction in the presence of common scab was associated with tubers having skins with a shiny and smooth appearance.

In the US the improved skin setting could be reproduced in greenhouse studies and associated to the improved formation of stolon roots. Under these controlled conditions soil applied Serenade increased the amount of stolon roots with 110%. Stolons produce many fine roots with a root – soil contact area equivalent to the basal roots. In potatoes, stolon roots play a crucial role in the uptake of non-phloem mobile nutrients such as calcium and micro- nutrients for the developing tubers. The amount of stolon roots is variety driven. Approximately 80% of the calcium content in the tubers is based on uptake by stolon roots (Busse, 2006; Wishart, 2009 & 2013).

The improved skin quality was quantified based on the measurement of light reflection of tubers expressed in a gloss index. Independent from tuber size Serenade treated tubers hold a significant higher gloss index which is attributed to an overall improved suberin deposition in the skin tissues (see Figure 3).

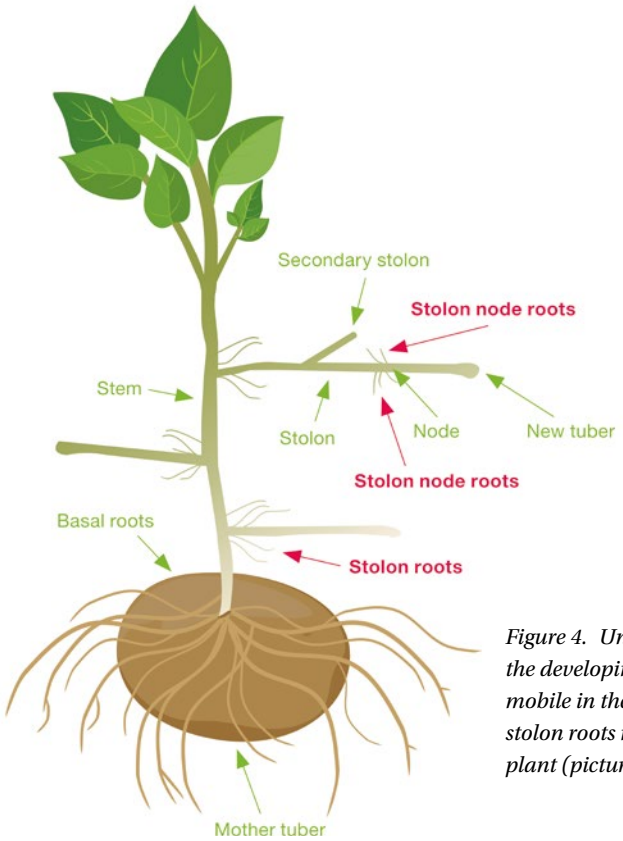

Figure 4. Unnoticed, thin and numerous stolon roots are crucial to feed the developing tubers with nutrients especially those nutrients that are not mobile in the phloem (calcium, manganese). Depending on the variety, stolon roots represent more than 50% of the total root surface of a potato plant (picture modified after Wishart).

Table 1. Relative reduction in greening and formation of α-solanine and α-chaconine in potato tubers of different varieties by Serenade.

Days of storage under UV light	Saprodi	Fontana	Avarna
0 days	5%	5%	5%
7 days	50%	50%	45%
14 days	70%	70%	65%
21 days	80%	80%	80%
28 days	80%	80%	80%

Reduced glycoalkaloid content and tuber sensitivity to light

Upon exposure to light, potato tubers turn green while in parallel the tuber tissue accumulates glycoalkaloids invisible to the human eye. The level of glycoalkaloids is variety dependent and maximum thresholds are set by regulatory authorities to safeguard healthy food (Dhalsamant, 2022). The MALDI Mass Spectrometry Imaging technology was applied to monitor and visualize the influence of Serenade on the distribution of glycoalkaloids in the tuber tissues. Interestingly potatoes formed after the treatment of Serenade accumulate reduced levels of glycoalkaloids whereas they were all concentrated in the skin tissue. In contrast non treated potatoes build naturally high levels of glycoalkaloids in all tuber tissues inclusive of the periderm. Serenade treated potatoes reduced glycoalkaloid formation by 80% when exposed to UV light for a period of 28 days at 21°C. (See table 1). Reduction in glycoalkaloid content was similar for the two major endogenous metabolites e.g. α-solanine and α-chaconine. The observed reductions were independent from the potato cultivars tested. The reduced light sensitivity of Serenade treated tubers is a great attribute to the production of healthy food.

Increased potato vitality

In 2020 and 2021 seed potatoes from respectively 6 and 20 locations were stored and planted in the succeeding year to analyze crop performance. Interestingly potatoes sourced from Serenade treated fields in 2020 delivered an average yield gain versus farmer practice of + 8% in the 2021 growing season. In the dry and hot growing season of 2022 an average yield gain of + 3% was achieved for Serenade sourced potatoes. (See Figure 5a). The lower yield gains in 2022 are most probably a result of the early maturation of the crop induced by the dry spell and high temperatures in the month of August.

Independent research in Denmark confirmed the increased productivity of potatoes after treatment of seed potatoes in the year before with Serenade. In starch potatoes an average yield gain of 7.4% was achieved (see figure 5b).

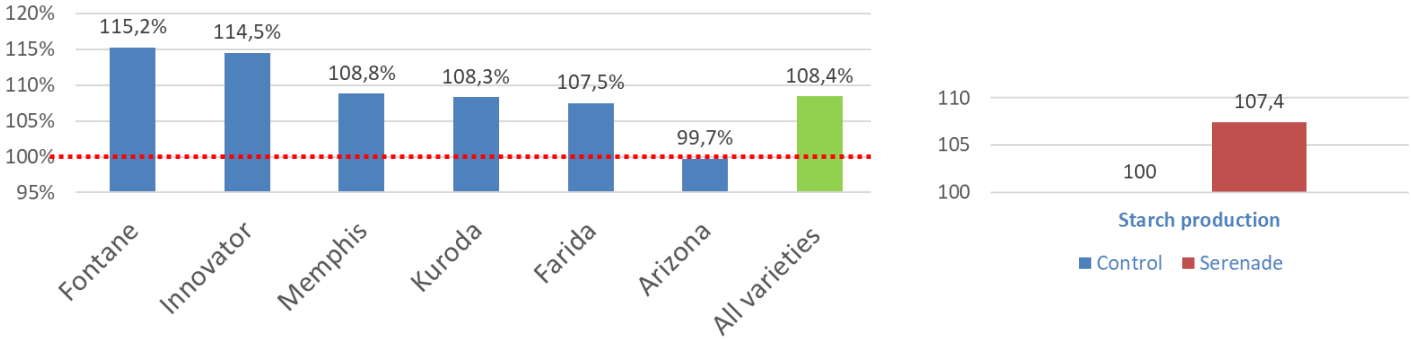

Figure 5 a and b. Carry over effect of Serenade treated seed potatoes (2020) on yield gain (%) in the succeeding year (2021) in respective The Netherlands (left) and Denmark (right).

The increased vitality of seed potatoes sourced from Serenade treated fields could not be explained by the increased nutrient content of the tubers nor to differences associated with the reduced incidence of tuber-borne diseases. The increased potato vitality might be associated to the endophytic properties of strain QST 713 as described in the research paper of Buchholz for other Bacillus species (Buchholz, 2019).

Discussion and outlook

The data generated with the research platform with leading potato growers improved the understanding of the plant root symbiont, *Bacillus amyloliquefaciens*, strain QST 713 to improve potato productivity. Statistical analysis of 147 field trials uncovered favorable functional shifts supporting the control of soil-borne diseases while increasing nutrient content of the tubers along with improved skin setting. Tubers sourced from Serenade treated soil express a low sensitivity to light exposure: greening and accumulation of glycoalkaloids is strongly reduced. The increased vitality of seed potatoes originating from Serenade treated fields increase the yield potential of the succeeding potato crops.

Serenade holds high potential to improve sustainable cropping practices. The product enables substitution of synthetic fungicides reducing the environmental footprint of the potato crop. Serenade will be a product of choice to improve potato quality, to reduce accumulation of glycoalkaloids as it will significantly contribute to sustain the production of healthy food.

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