The VESTA biological soil amendment dramatically shifted microbiota composition inside strawberry roots, altered nutrient dynamics, and increased strawberry plant growth.

By Rajnish Khanna, Ted K. Raab, Siwen Deng, and Devin Coleman-Derr

I-Cultiver, Inc.
www.i-cultiver.com

*To whom correspondence should be addressed.

Telephone: (707) 205-7084
E-mail: raj@i-cultiver.com

Beneficial microbes in fertile and productive soils process organic matter and make nutrients available to plants. Agrobiologic products have the potential to provide benefits to overall health of the plant and the environment, and they cost considerably less to bring to market compared to chemical pesticides and seed traits. Large agrochemical companies are investing heavily in new microbial products with future projections of significant profitability. If these trends hold, the current activities will accelerate formulation of new products in a highly competitive industry. There are three broad classifications of agrobiologic product types: biopesticides, biostimulants, and biofertilizers. The biopesticide market is showing the fastest growth rate as growers turn away from highly toxic pesticides and seek organic options. As the demand for agricultural biologicals grows, the challenges facing the industry are many. Starting with the basic hurdles of product efficacy and performance in diverse growing environments, across a variety of crops, and associated issues of application technology at the field scale, and then leading to the complexity of manufacturing and supply chain, quality management, and regulatory scrutiny with multiple standards in the United States, Europe and Asia. These trends will fundamentally reshape the industry. As we look forward to new product portfolios, it is important to know that this industry is not new. Biological products have been sold and used for decades with remarkable successes in disease suppression, plant growth promotion and yield enhancement. Here we report the impact of a historically proven soil amendment on microbial communities in commercial strawberry fields.

This study was performed to investigate changes in microbial community composition in soil, rhizosphere (associated with root surface) and root endosphere (inside roots) in response to a commercially available soil amendment product, VESTA® (SOBEC Corporation, Fowler, CA). VESTA is a liquid product designed to deliver a broad spectrum of beneficial microbes, associated microbial metabolites, humus derived from microbial fermentation and from natural sources. The application of the soil amendment described in this study has produced substantial yield increases in a variety of plant systems.

Following initial bed establishment in November 2014, a commercial strawberry operation was split in two fields - one with control (untreated) beds and the other with VESTA-treated beds. Replicate samples were collected at four time points between January and August 2015. The samples were subjected to microbial community analysis for bacterial identification, and chemical analyses were performed as described.

Strawberry plants treated with VESTA exhibited increased growth rates (**Fig. 1**) with larger root and crown mass (**Fig. 1A**). Strawberry plants pictured in (**Fig. 1B**) are growing on control (left) and treated (right) beds in May 2015 in Guadalupe, CA. These plants were good representations of the control and treated groups. A U.S. quarter dollar coin was used for size comparison. The diameter of a quarter dollar is 0.955 inches. Control plants and strawberry fruit were smaller in size in comparison to VESTA treated plant and strawberry fruit size. Quantitative analyses of strawberry plant and fruit size are needed to make final determination of the effect of VESTA on growth rate and strawberry yield. Total strawberry harvest was not quantified for this study. The differences in size and plant growth were clearly visible between untreated and treated fields. Fruit color and taste were also notably enhanced, based upon non-quantitative visual and taste inspections.

The biological soil amendment had a substantial impact on the bacterial community structure. At all four-time points tested between Jan and Aug 2015, bacterial community diversity was reduced only in VESTA-treated soil and rhizosphere. These results were unexpected since the amendment was expected to add new organisms to the soil and rhizosphere. Previously, in a separate study with commercially grown romaine and iceberg lettuce, we found that this product significantly decreased the incidence of corky root diseases of lettuce, and substantially reduced the occurrence of Sphingomonadaceae, members of the family linked to the lettuce disease. The decrease in soil and rhizosphere community diversity might be attributed to changes in community structure as VESTA organisms establish themselves in the environment. A shift in soil microbiome community structure can be advantageous by replacing harmful microbes with beneficial organisms. Further studies are needed to confirm this hypothesis.

The product itself is comprised of many plant-colonizing bacterial species. We found that the majority of bacterial species present in VESTA were able to colonize the surrounding soils, rhizosphere and root endosphere. Our study indicated that there is a significant shift in community composition in response to VESTA in soil, rhizosphere and inside strawberry roots. Root communities were most affected in response to VESTA, and the microbiome in VESTA treated roots shifted dramatically even at the first time point and it evolved over time. These dramatic changes in community structure were accompanied by increased root and crown growth as seen in **Fig. 1**. The vigorous root growth in VESTA treated plants appeared to be from enhanced root-proliferation at the crown. It is possible that application of the soil amendment creates plant hormone analogues that stimulate cell division in the root apical meristem.

We speculated that the major shift in root microbial composition and the concurrent increase in growth might be accompanied by measurable changes in nutrient exchange between the soil and the plant. It may also be relevant that the water content of VESTA treated root tissues was uniformly higher after the first time point (T1) throughout most of the strawberry trial (**Fig. 2A)**. As nutrient- and other resources-replete plants rely on expansion of their tissues for growth, higher water contents in tissues should correlate strongly with growth potential. In the next section, we address what we learned from all plant tissues with regards to nutrition.

Plant nutrient pools: The edible portions (strawberry drupes) rely on the concerted efforts of the root zone to mine available nutrients from the soil, and with the assistance of energy generated in leaves by photosynthesis, transport those nutrients eventually to the fruit. We assessed nitrogen (N) and carbon (C) by Carlo Erba elemental analyzer (combustion/GC), ICP-OES (inductively coupled plasma-emission spectrometry) for Ca, Mg, K, Na, P, S and micronutrients following acid (HNO_3-$HClO_4$ digestion), as well as energy-dispersive X-ray fluorescence for additional elements.

Considering leaves first, in this study only the final time point (T4) leaves were analyzed, but soil-N was translocated from the root zone at a quicker rate (or fluxes through soil pools higher) under VESTA treatment. The %N and %C levels were higher in leaves of plants treated with VESTA (**Fig. 2B**). Depending on the fate of plant residues after strawberry harvest (Time point 4), the [C/N] ratios were uniformly lower in VESTA treated plants – an expression of their more rapid growth – and this would result in faster-decomposing litter in minimal-till systems. Uniformly, leaves from plants treated with the soil amendment contained higher levels of plant nutrients tested (Na, Mg, Si, P, S, K, Ca) by the time of strawberry harvest. There are several implications of these altered nutrient pools, for example; experienced growers are aware that controlling root-zone sodium can enhance flavor of the final harvest. Silica has been documented to protect plants from certain classes of pathogens.

Soil nutrient dynamics: Extracting soil with 2M potassium chloride disrupts the exchange complex and loosely-associated nutrients. We analyzed for nitrate and ammonium, the two most common nitrogen-sources for plants. The control soil had significantly higher nitrate. For these soils of the commercial strawberry operation in San Luis Obispo County, we have a mix of sandstone-derived alluvium from the Coast Range, and wind-borne materials. The physical texture of the soil suggested a sandy loam. Such soils would hardly prevent transport of nitrate away from an agricultural operation – this usually is an environmental concern for water quality nearby, as well as a loss-pathway for a valuable resource. The exact mechanism is not known, but some combination of factors must either: **(1) suppress microbial nitrification in soil under VESTA treatment, or (2) stimulate uptake and utilization of soil nitrate.** Nitrate is a very useful nutrient to plants, as it contributes (along with sugars and amino acids) to the osmotic potential of the tissues in which it accumulates. Improved efficiency of plant N uptake and reduced soil nitrate are beneficial to crop production and the environment.

Our analyses of the microbiome composition and plant nutrient pools indicated that the amendment tested here shifts the microbial ecology and benefits the crop system, in this case strawberry. There was a decrease in microbial diversity in soil and a marked change in root microbiome, accompanied by increased root and crown growth and higher water and nutrient levels in the host plant (**Fig. 3**). The current analyses have been limited to bacterial communities. Further studies are needed to determine the role of fungal communities in the soil amendment.

About the authors

Rajnish Khanna, Ph.D., Co-Founder, I-Cultiver, Inc.

Ted Raab, Ph. D., Carnegie Institution for Science, Stanford, CA

Siwen Deng, Plant Gene Expression Center, UC Berkeley

Devin Coleman-Derr, Ph. D., Plant Gene Expression Center, U C Berkeley / USDA

Detailed report of this strawberry study is available at:
http://www.sobecinc.com/uploads/4/4/4/6/44460235/vesta_strawberry_report_ic_secure_030316.pdf

Figure 1. Increased Strawberry Plant Growth. Strawberry plants were planted on the same day in untreated and VESTA treated beds. Plants in VESTA treated beds grew larger in size. Plant growth rate was consistently higher in treated beds.

A. VESTA increased strawberry root and crown size.

B. Control and treated strawberry plants are shown for comparison. A U.S. quarter is shown for scale (diameter 0.955 inches).

Figure 2. VESTA treated plants had increased root water content and leaf nutrients. Values were normalized to dry weight.

A. **Root Water % avg**

Time point	Control	VESTA Treated
T1	**86.4%**	83.6%
T2	83.6%	**86.6%**
T3	83.2%	**87.2%**
T4	84.4%	**85.8%**

B. **%N (in leaves)** **%C (in leaves)**

Time point	Control	VESTA Treated	Control	VESTA Treated
T4	1.61%	**2.06%**	25.0%	**27.6%**

Figure 3. Improved water and nutrient uptake by host plant.