

or millennia, civilizations have extracted indigo dye from plants, creating a product that by the 18th century had become a driver of global economics. But, as the 20th century began, global output of natural indigo fell by over 90% as synthetic indigo took its place. With recent trends toward naturally derived alternatives for synthetic materials in processed foods, consumer goods, and other markets, the \$427-million global market for indigo dye — whose synthetic building blocks include benzene, formaldehyde, and cyanide — represents an opportunity. Natural indigo dye and its parent agricultural crops could benefit from more than 100 years of advances in chemistry, chemical engineering, and agricultural science.

Indigo can be derived from a variety of plants. Leaves of *Persicaria tinctoria* and *Indigofera spp.* contain indoxyl -D-glucoside (plant indican). Shortly after the leaves are harvested, they begin to degrade. As degradation occurs, a native glucosidase enzyme hydrolyzes the indican to form indoxyl, which in turn dimerizes to form indigo. Traditionally, an indigo plant's dye yield has been determined by harvesting the plant, performing an aqueous extraction of indican and indoxyl, precipitating indigo via



chemical manipulation, filtering and drying the crude product, and performing a spectrophotometric analysis in a solvent mixture. This process takes roughly one day and must be replicated multiple times, as there are many opportunities for error. At the commercial scale, the method quickly becomes a limiting factor in carrying out crop enhancement experiments and refining the genetics of the diverse seed stock.

Stony Creek Colors, Inc., a Tennessee-based producer of natural indigo dye, is addressing the lag in natural indigo research and development (R&D) by creating an in-field rapid assay device with funding from a Small Business Technology Transfer grant from the National Science Foundation (NSF). The company's new assay device directly measures the potential dye yield of indigo-bearing plants under different field conditions and throughout their growth cycle to inform seed crop selection, agronomics characteristics, and harvest timing.

method to directly measure the indican content in leaves that overcomes the limitations of conventional techniques. Indican returns a strong fluorescence peak at 302 nm excitation/385 nm emission. Through the NSF grant, the company devised methods to selectively extract indican to the leaf surface and, using a laboratory or handheld surface fluorometer, quantitatively measure the potential dye yield of an indigo plant. Traditionally, leaf-surface fluorometry has focused on chlorophyll fluorescence in a band less populated by highly responsive organic constituents. Stony Creek Colors' novel use of surface fluorometry allows a farmer in the field to assess the harvest-readiness of maturing crops in real time and enables predictive analysis for its indigo processing factory.

CEP

This technology was funded through the NSF Small Business Technology Transfer Program.

T a c a Stony Creek Colors has assembled a CEP.

a genetically diverse seed stock from resources around the world, comprising many varieties and accessions that have not been selectively bred for dye content for over 100 plant genera-