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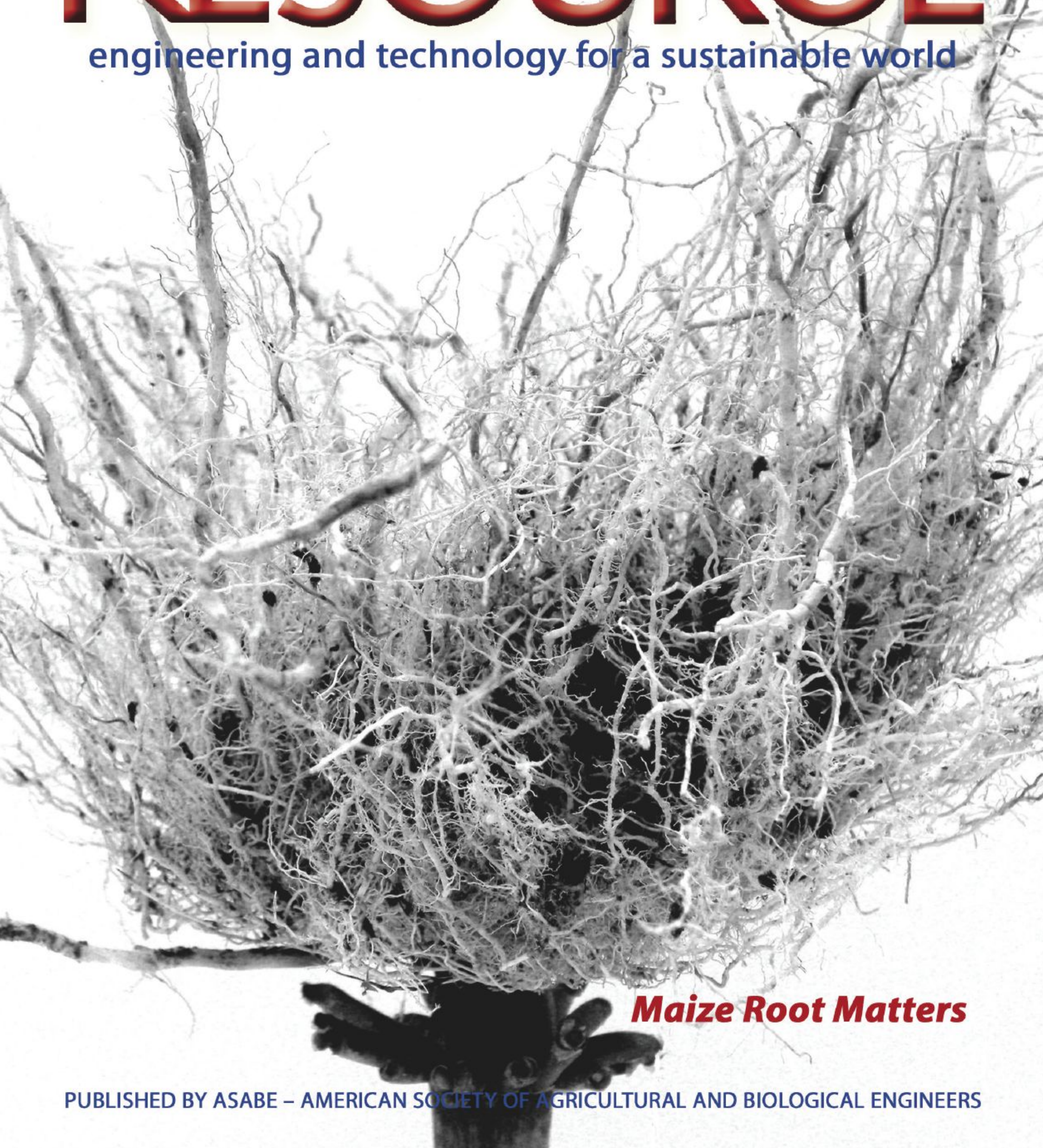

Maize Root Matters

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Tony Grift and Martin Bohn

Maize is one of the world's major crops due to its variety of uses as a human and animal food staple, as well as in nutraceuticals, biofuels, biodegradable plastics, and an astounding number of co-products. The historical yields of corn in the United States remained stable at around 26 bushels per acre (bu ac^{-1}), or 1630 kg ha^{-1}, from the Civil War until 1936. After the Dust Bowl of the 1930s, farmers adopted hybrid seed, which led to an annual increase of 0.8 bu ac^{-1} per year from 1937 to 1955. Following that period, a constant increase of 1.9 bu ac^{-1} per year was established, in part due to mechanization, as well as improved agronomics and genetics. In 2010, a record yield was set at over 160 bu ac^{-1}, passing the magical metric mark of 10,000 kg ha^{-1} or 1 kg m^{-2}. However, the Great Drought of 2012 showed that natural causes can have a devastating effect on maize yield, pushing the average U.S. yield down to a mere 123 bu ac^{-1} (7720 kg ha^{-1}).

The horrors of Haber

One of the most influential factors in the historical grain yield increase was the application of artificial ammonia-based fertilizer through a process invented by Fritz Haber, commonly known as the Haber-Bosch process. Even though Haber was awarded the Nobel Prize in 1918, his true intentions were far from noble. Not only was his process for producing artificial ammonia from air targeted at the development of explosives, he was in fact the main scientist behind the development of chlorine gas and other chemical weapons used in the gruesome trench warfare of World War I. His wife was so disgusted by his war "contributions" that she committed suicide with his service gun. The "father of chemical warfare" soldiered on, though, apparently quite unfazed. Later, attempts were made to strip Haber of his Nobel Prize because of his involvement in the war effort; however, after Haber smartly pointed out that Alfred Nobel himself had made his fortune from the pursuit of war, these voices went silent.

Haber's name may never be detached from chemical warfare, but the food supply of half the world currently relies on the large-scale production of artificial fertilizer that he enabled. Artificial fertilizer was also one of the cornerstones of the Green Revolution, attributed to Nobel Peace Prize Laureate Norman Borlaug. Although the Green Revolution has been a tremendous benefit to humanity, there is now a call for a Second Green Revolution. The most recent projection by the United Nations claims that the current global population of 7 billion will increase to 9.3 billion by 2050 and to 10.1 billion by 2100. To feed that many people, crop yields must increase by 1.3 percent annually under the uncertain conditions of global climate change. Since the Second Green Revolution will not be able to deploy more fertilizer, irrigation, or agricultural land, improvement of crops will be the focus. Crop breeders need to accelerate the development of cultivars that feature high yields and stress resistance, and agricultural engineers can help in this process.

Getting to the root of the matter

In the breeding area, there have been huge advances in genotyping, but very little development in the area of phenotyping—the observation of crop characteristics or traits. This discrepancy has limited the scale of breeding programs for decades, since the phenotyping technology currently used in even the most advanced research programs is primitive at best.

While phenotyping of aboveground plant organs is difficult and tedious, phenotyping the root system is even more cumbersome. One of the obvious reasons is that root systems are hard to study in a non-destructive manner, since instruments that allow studying a root system *in situ* do not exist, and destructive methods require ample labor when conducted in a high-throughput fashion. Although there is no dispute about the importance of the root system for the health, resilience, survivability, and yield potential of any plant, the study of root systems is utterly deficient compared to the study of aboveground plant features. Studying the plant's root system is not merely justified by the intuitive idea that the whole plant should be studied equally intensively; research indicates that, with regard to yield potential, the root might be more important than the aboveground plant!

Time is of the essence

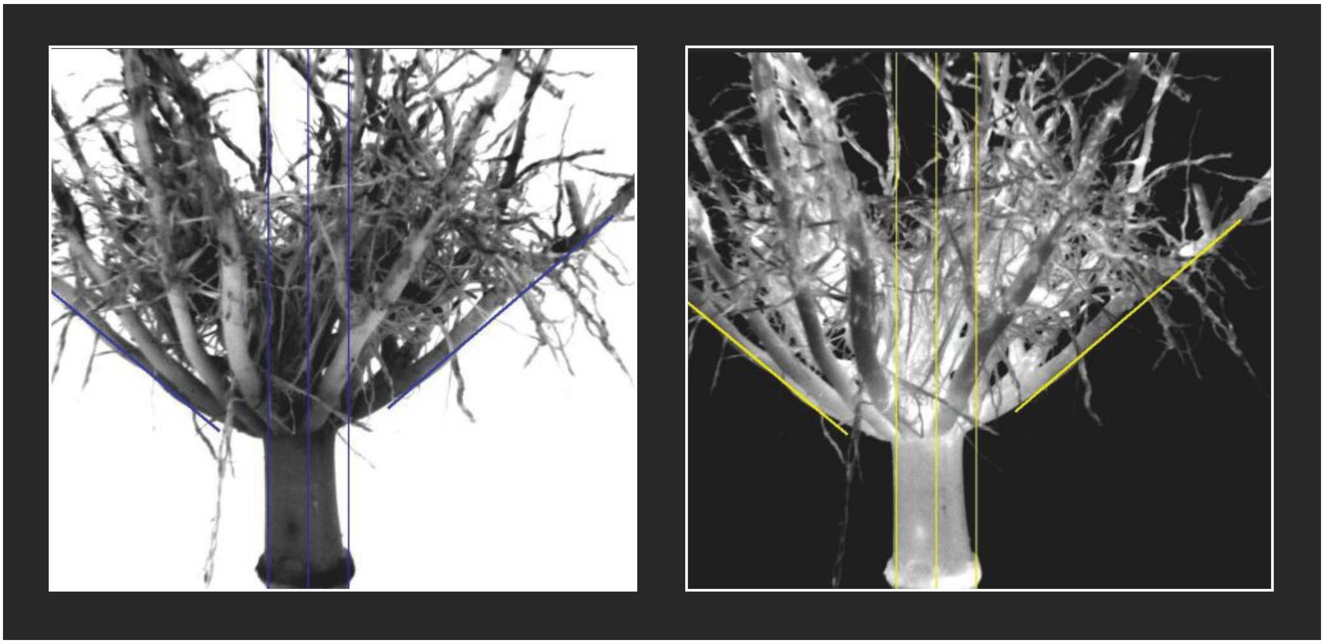
In 2009, Graeme Hammer and his colleagues published a "fast-breaking paper" in *Crop Science* titled "Can changes in canopy and/or root system architecture explain historical maize yield trends in the U.S. Corn Belt?" The authors reasoned that, based on crop models, the root architecture in general and the root angle in particular likely had a more profound impact on the maize yield increases over the past decades than aboveground indicators, such as leaf erectness. If this hypothesis is correct, then the study of the root structure of maize (and potentially other crops) needs to be expanded. However, this can only be done if new techniques are developed that allow high-throughput phenotyping of root systems.

For this purpose, professors in the departments of Agricultural and Biological Engineering and Crop Sciences at the University of Illinois have developed the Corn Root Imaging Box (CRIB), an instrument that can image up to 600 maize roots per day. This throughput is very important, since a typical maize experiment can consist of thousands of plants. The CRIB features highly diffuse illumination, which prevents shadows from obscuring fine root structures. The digital cameras are computer controlled, as is the system that rotates the roots to acquire lateral images. To obtain highly detailed images, the root images are accompanied by a background image that is taken before the root is inserted into the CRIB. By combining the root and background images in a differential manner, high contrast and sharp images are obtained, ready for processing.

Corn Root Imaging Box (CRIB).

Tackling the problem by going backward

One of the most complicated tasks in maize root analysis is measuring the complexity of the root system. Intuitively, a highly complex root system would enable the plant to reach water and nutrients easily, provide plant stability, and adapt to the soil matrix surrounding it. There is, however, no useful definition of root complexity, let alone a standard measurement methodology. Even the terminology varies and includes terms such as architecture, morphology, and structure. In spite of the lack of standards, we quantified the elusive complexity of the roots by calculating their fractal dimension, an index that was first defined by mathematician Benoit Mandelbrot in 1975. Later, in their book *The Algorithmic Beauty of Plants*, Przemyslaw Prusinkiewicz and Aristid Lindenmayer showed how fractals can be used to create artificial plants that have a striking similarity to their natural counterparts. In our research, we worked backward. By assuming that the root system resembles a fractal-like structure, we used a technique termed the "box counting method" to calculate the fractal dimension (a number between



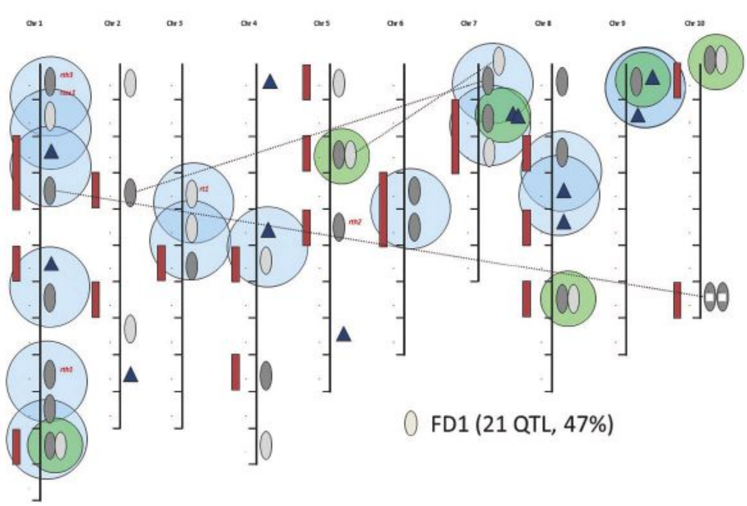
***Left*, a corn root image after background subtraction with computer-generated root angle and stalk diameter. *Right*, corn root inverted.**

1 and 2 for two-dimensional images) of the root images. The results showed that maize roots indeed have fractal properties, such as self-similarity across various scales.

The advantage of maintaining a large repository of root images became evident after Hammer and his colleagues hypothesized that root angle may be an important yield-related trait. In response, a new algorithm was developed to determine the root angle, and it was applied to over 10,000 maize roots virtually overnight. The statistical analysis showed that root complexity was not significantly correlated with root angle, which indicates that these traits respond independently to stresses.

Having measured three root traits (complexity, root angle, and stem diameter), the question remained how to translate these data into information that is useful for maize breeders. This is where the crop scientists come in. Professors and students in the Department of Crop Sciences at the University of Illinois used the measured trait data to produce Quantitative Trait Loci (QTL) maps that highlight areas in the maize genome responsible for the phenotypic expression of the measured traits. This allows directed breeding by selecting plants that have the proper genetic makeup for desirable traits.

The future directions of the program are to develop new measurement techniques, such as a laser-based three-dimensional imaging system, as well as expanding phenotyping to other plant organs, such as the maize ear. The research described here is a shining example of fruitful collaboration that aims to solve an important problem. Even Norman Borlaug would have been proud of our efforts.



Quantitative Trait Loci (QTL) map, in which circled regions indicate which areas in the maize genome are responsible for root complexity as expressed in the fractal dimension.

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