BY MICHAEL J. HEALY, PH.D.

Toxin trail

Four seemingly unrelated events lead to an unexpected scientific discovery

The word serendipity is used to describe the process by which seemingly unrelated observations, conversations and communications unexpectedly lead to what a scientist or inventor considers a eureka moment. I've had only two eureka moments in my career, separated by about 40 years of mundane routine. My latest eureka moment came as the result of a four-part serendipity.

EARLY FINDINGS

In late January 2006, I traveled to Northern Indiana to visit Agdia, a company I'd done work for during the early 1990s. I met Chet Sutula, Ph.D., who founded Agdia in his basement 25 years before and who was proud of becoming one of the world's largest developers and manufacturers of plant pathogen diagnostic test technology, with 50 employees and a six-acre complex of buildings.

At the end of my tour and dinner, almost as an afterthought, Sutula described a test the company was working on to determine a toxin produced by blue-green algae (cyanobacteria) in drinking water. The test was sensitive to 10 parts per billion, and Agdia's goal was to get its sensitivity down to 1 part per billion. Because the test didn't fit the company's standard product line of plant pathogen

Left: Heavy cyanobacterial accumulation near an irrigation pond inlet testing positive for microcystin toxins. Photo: Mike Healy

Above: Heavy build up of terrestrial cyanobacteria (blue-green algae) in a badly damagaed turf area testing positive for microcystin toxins. Photo: Mike Healy

An outbreak of miniring disease on ultradwarf bermudagrass in midsummer on a microcystin toxin-positive course. The exact cause or causes of miniring aren't clear. Photo: Mike Healy

tests, Sutula felt it might need to look at various options to market the test once it was thoroughly validated. I agreed, but didn't immediately see any applications in my field.

And, as Paul Harvey says, the rest of the story. A year later, in early January 2007, I was called to a municipal golf course in Southern Alabama to look at a live oak problem. Live oak trees, on and adjacent to the course, which had been irrigated by spray, were suffering from leaf blighting – but only as far up as the water came in contact with the leaves.

As it turned out, the blighted leaves fell off eventually, and the new leaves replacing them were unaffected. The superintendent thought sediment removal from the irrigation pond the previous summer must have had something to do with the problem. I didn't find any fungal disease on the symptomatic leaves.

RESERVOIRS IN AUSTRALIA

Then, in early May 2007, I was in Dubai. One evening I had dinner at a Mexican restaurant with several people including Jeff Ferney, an expatriate Australian who came to the United Arab Emirates as manager of the landscape maintenance division of a local company based in Abu Dhabi.

During our dinner, Jeff talked about the serious problem Australia was having with a toxin, produced by blue-green algae, found in reservoirs. This was, potentially, a serious human- and livestock-health problem. I remembered my conversation with Sutula in January 2006 and said I would pass on Agdia's contact information to him.

DISEASED GREENS

The final part of my serendipity took place in mid-May 2007. I visited a golf course where a greens-disease problem began on nine holes in the fall of 2006. The symptoms returned in the spring of 2007. As I rode with the golf course superintendent, he said he was having problems with the greens, tees and fairways on these nine holes, which didn't respond to fertilizer as the turf on the adjacent nine did. Those nine holes had never had any greens-related problems, even though they were maintained identically.

I asked about the source of irrigation water for these two nines, assuming the source was the same for both nines.

"Oh no," replied the superintendent. "The nine with no problems has its own irrigation pond and pump station, as does the nine with all the problems."

I asked if the separate sources of irrigation water had been tested. They had been, and the water quality test results showed no distinct differences. The superintendent also indicated he pulled soil samples for nematode and standard soil fertility testing, and these tests also showed no difference between the good and bad nines.

Could there be a toxin being produced in the irrigation pond serving the bad nine that was affecting turf quality, perhaps making a conventional disease more difficult to control or cure? I called Agdia and spoke with the scientist working on the toxin test. The test was a microcystin toxins immunostrip assay, and Heather Chambers, a scientist at Agdia, provided me with more background information about the toxin. Chambers said that while their primary interest in the test was for use in drinking or recreational water testing, articles had been published about its adverse affect on plant growth, even causing root dysfunction.

Agdia agreed to run samples from the good and bad nines, specifically water from each pond, as-irrigated samples from each nine and samples of badly affected turf (in areas with a heavy blue-green algae buildup). The sample test results were startling. The good nine samples all tested negative, while the bad nine samples all tested positive for microcystin toxins. Might microcystin toxins produced by aquatic forms of blue-green algae exist in other golf course irrigation ponds as well?

TESTING THE WATER

During the following months, I requested, and had sent to my laboratory, golf course pond and as-irrigated water samples from 35 golf courses in Florida, Alabama, Georgia, Louisiana and Texas. Microcystin toxin testing of all samples was carried out by Agdia, using its immunostrip assay and, in certain cases, a standard immunoassay. Positive samples came from golf courses in Florida, Alabama, Louisiana and Texas. Four of the courses which tested positive for microcystin toxins had unusual and/or difficult-to-control disease or disease-like problems on their greens. Water samples from more than 50 percent of the ponds tested contained microcystin toxins.

Selected samples archived by freezing were sent to Linda Lawton, Ph.D., at the School of Life Sciences at The Robert Gordon University in Aberdeen, Scotland, for additional testing by conventional analytical techniques. On January 9, 2008, Lawton's lab confirmed the presence of microcystin-LR and microcystin-LA. In addition to analytical capabilities, Lawton is recognized as an expert in the area of microcystin toxins toxicity to plants.

Microcystin toxins are produced by a number of genera of blue-green algae, which almost always grow in an aquatic environment. These toxins are hepatotoxic cyclic heptapeptides. Microcystin-LR is one of the most investigated of these toxins. It has been found in rivers, lakes and ponds throughout much of the world. Factors influencing toxin production and its sometimes immediate production cessation aren't clearly understood.

The major concern about the presence of microcystin toxins is their known mammalian toxicity. Most current water quality standards mandate potable water contains no more than 1 parts per billion and no more than 10 to 20 parts per billion in recreational water. Many countries, along with the World Health Organization, have introduced such standards. Currently, the United States has no microcystin toxins standards, although the U.S. Environmental Protection Agency considers these toxins of interest and is headed toward regulatory authority. The impact of these toxins on plant growth is being examined further.

FUTURE PLANS

So where do I go from here? I've made my findings known to several organizations, including the U.S. EPA, with hope of finding sponsorship for a much larger survey. Until then, I'll continue to seek courses that have difficult-to-control disease or disease-like problems and recommend conventional plant disease diagnosis, along with multiple samples of pond water for microcystin toxins testing. Soon, I plan to have a PowerPoint presentation that can be shown remotely. **GCI**

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Typical cyanobacterial bloom in an irrigation pond. Blooms are pushed by wind or drawn to the induction point of the operating irrigation pump. Photo: Mike Healy