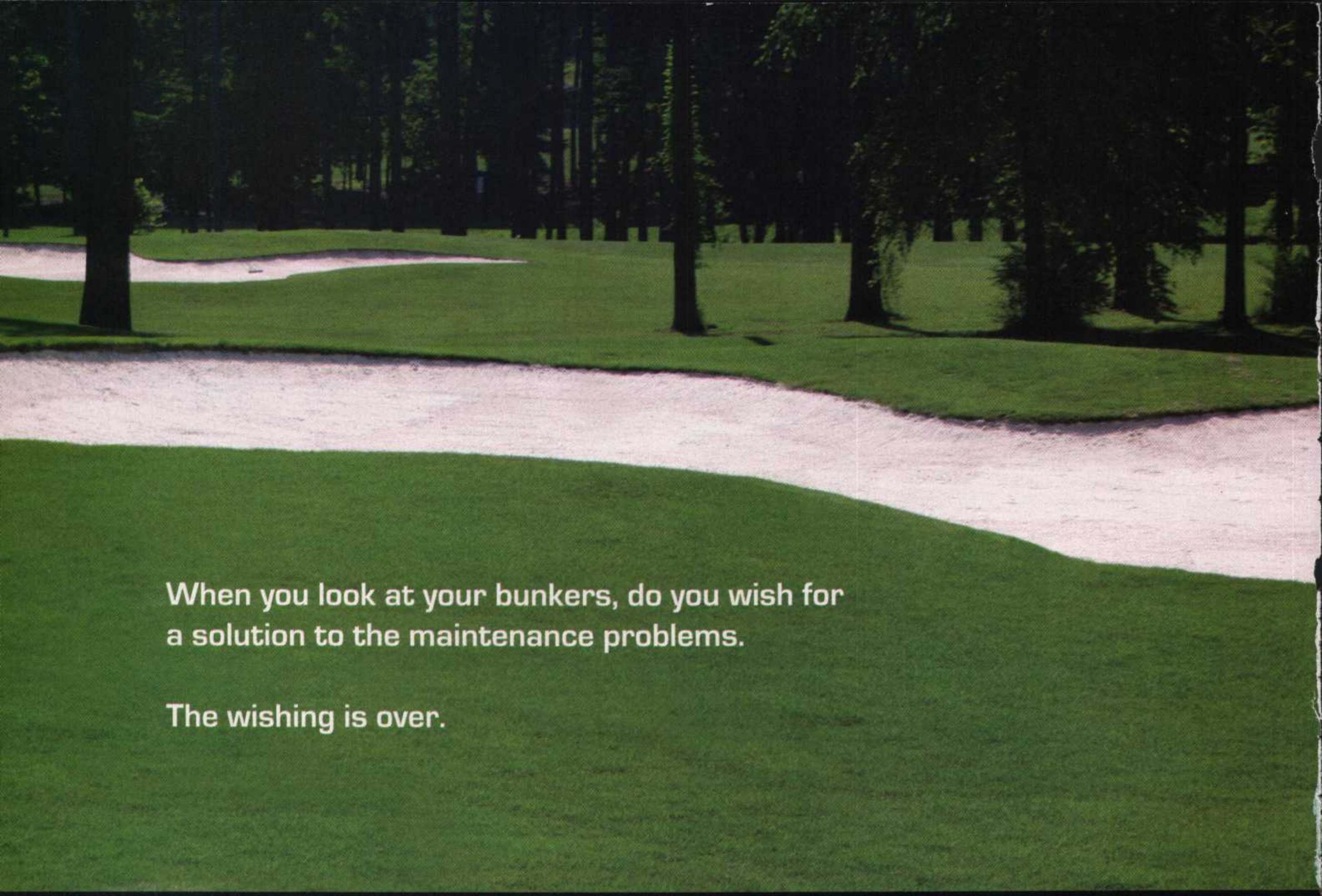

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TURFGRASS TRENDS

NITROGEN ASSIMILATION

Leaf Analysis Offers Window Into Proper Fertilization Schedule

By Zhongchun Jiang

Proper nitrogen (N) management is critical for maintaining high-quality turf while conserving resources and protecting the environment. This is in part because N applications affect turfgrass growth directly and disease susceptibility indirectly. N application can be costly, not only because of the expenditures on N fertilizers. More importantly, costs rise because of the maintenance costs associated with the excessive shoot growth, poor root growth, and high disease susceptibility that often result. Such ill-timed and excessive N applications can potentially cause runoff, ammonia volatilization, nitrate leaching and groundwater contamination.

Turfgrass maintenance professionals have often relied on empirical knowledge-based fertilization programs to decide when and how often to apply N fertilizers. Chlorophyll meters and near infrared reflectance spectroscopy have been used in research to schedule N applications (Rodriquez and Miller, 2000a, 2000b).

These technologies provide the means to estimate the content of total N in turfgrass leaves. However, readings of a chlorophyll meter or a near infrared reflectance spectrometer do not indicate how much N is stored in the vacuoles of leaf cells and how fast the stored, unassimilated N will be assimilated into chlorophylls and other N compounds. The amount of the leaf-stored unassimilated N and the rate of N assimilation must be known before one can attempt to estimate when and how much a turfgrass needs supplemental N. Research on nitrogen assimilation will help turfgrass managers in this regard.

> Here is a surprising discovery: Turfgrass assimilates nitrate at high rates during the night.

Nitrate assimilation, not ammonium

There are numerous brands of N fertilizers with different chemical analyses, but the inorganic N forms that turfgrasses take up from the soil water are only two: ammonium (NH_4^+) and nitrate (NO_3^-) (Figure 1).

In addition to fertilizers such as ammonium sulfate that provide ammonium directly, ammonium is produced through hydrolysis from urea and through mineralization from organic fertilizers and organic matter in the soil. Ammonium is an ion that carries one positive charge, which is attracted to negatively charged soil colloids. When the soil is anaerobic, ammonium stays in the soil and turfgrass roots can absorb it when oxygen becomes available. When the soil is acidic (pH above 8), some of the ammoni-

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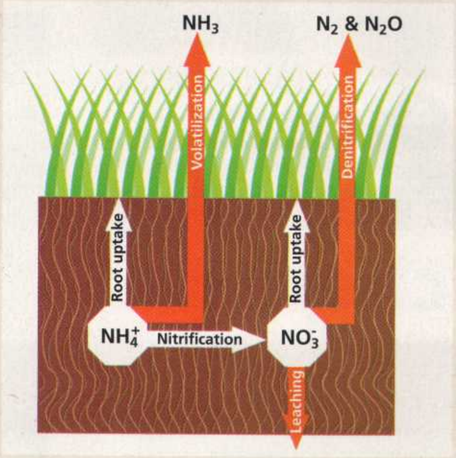
FIGURE 1

Fate of nitrogen fertilizers in turf systems



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um ions become ammonia gas (NH$_3$) and can be lost as ammonia escapes into the atmosphere (Figure 1).

Turf soils are often aerobic and mildly acidic. Under such conditions, most of the ammonium is converted to nitrate through nitrification (Figure 1). Some quick-release fertilizers provide nitrate directly. In general, turfgrass roots see more nitrate ions than ammonium ions, and nitrate is the predominant form of inorganic N available to turfgrass roots. In addition, nitrate is safer to the turfgrass than ammonium because high concentrations of ammonium are toxic to plant cells (Jiang et al., 2001b).

Nitrate can be accumulated at high concentrations in turfgrass leaves without apparent toxicity (Jiang and Hull, 2000).

Nitrate assimilation pathway

Once the roots have absorbed nitrate from the soil water, the turfgrass can store and assimilate nitrate in the roots (Figure 2). Nitrate is stored in the vacuole of the cell. If roots cannot assimilate nitrate at a rate that meets the needs of the turfgrass for N compounds, such as amino acids and proteins, the turfgrass transports nitrate to the leaves and assimilates it there to meet its needs.

In fact, research shows that cool-season turfgrasses cannot assimilate nitrate in roots at a sufficient rate (Jiang and Hull, 1998, 1999, 2000; Jiang et al., 2001a; Bushoven et al., 2002). Leaves are the primary sites of nitrate assimilation in these turfgrass species. For example, in Kentucky bluegrass, leaf blades contribute as much as 96 percent to 98 percent of the plant total nitrate reduction at nitrate concentrations above .15 micromoles (*mM*) or 2.1 parts per million. Efforts can be made to develop new varieties that assimilate nitrate in roots at rates high enough to meet the plant needs. In the meantime, we can practically focus on leaf nitrogen assimilation potential (NAP) alone to ascertain the NAP of the whole turfgrass plant.

In leaf cells, nitrate is first converted to nitrite in a chemical process called oxidation-reduction (Figure 3). Oxidation is the chemical process in which a chemical compound loses one or more electrons, often to the oxygen atom. On the other hand, reduction is the process in which a chemical compound accepts one or more electrons. When nitrate is reduced to nitrite, the N atom in the nitrate ion gains two electrons and the N in the nitrite ion is reduced by the same number. These two electrons come from the reduced form of nicotinamide adenine dinucleotide or NADH. When NADH gives its electrons to nitrate, NADH is oxidized and becomes NAD^+. This oxidized NAD^+ goes into the mitochondrion, during which NAD^+ gains two electrons from carbohydrates and is reduced to NADH. The NADH produced in mitochondria can be exported to the cytosol and be used again to reduce another nitrate ion.

The product of nitrate reduction is nitrite, which is immediately transported into the chloroplast where nitrite is reduced to ammonium (Figure 3). The enzyme nitrite reductase (NiR) catalyze the reduction of nitrite to ammonium. Here, the N atom in nitrite gains six electrons from six molecules of reduced ferredoxin (Fd red). Fd red is produced in the chloroplast only under light.

During the day, if leaves are placed in total darkness, no new Fd red can be produced and the reduction of nitrite to ammonium cannot be sustained. Therefore, on cloudy days, nitrate reduction is slowed and eventually stopped due to nitrite accumulation and feedback inhibition.

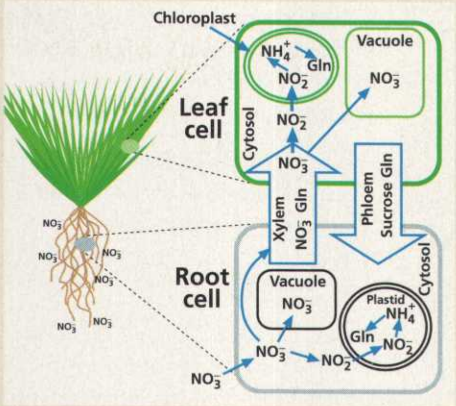
When there is sufficient light, ammonium produced in nitrite reduction and in photorespiration is immediately assimilated into glutamine, one of the 20 amino acids in living organisms. In leaves, ammonium assimilation occurs in chloroplasts. The enzyme glutamine synthetase (GS) catalyzes ammonium assimilation into glutamine. This step is not an oxidation-reduction reaction and no electron donors are needed, but it requires adenosine triphosphate (ATP), the energy currency in living organisms. Glutamine carries the N throughout the plant and donates it to the production of other molecules such as chlorophylls and proteins. Until a new molecule of glutamine is synthesized from ammonium and organic acids, nitrate-N is not assimilated.

Nitrite is a smaller ion than nitrate ion and is toxic to plant cells at high concentrations. Nitrite is also toxic to humans because it binds with hemoglobin to form methemoglobin. Hemoglobin carries oxygen throughout our bodies but methemoglobin does not. When young babies are fed with food or water that contains high concentrations of nitrate, it is converted to nitrite in the gastrointestinal tract. Nitrite is then absorbed into blood, reacting with hemoglobin and interfering with oxygen supply. The baby turns blue, a symptom referred to as blue baby syndrome.

Ammonium is also toxic at high concentrations. In fact, L-phosphinothricin-based herbicides kill plants by inhibiting GS, blocking ammonium assimilation and causing rapid accumulation of ammonium to lethal concentrations in growing leaves. As a result, the plant dies. This effect has been observed in creeping bentgrass (Jiang et al., 2001b).

FIGURE 2

Fate of nitrate in turfgrass plants



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Due to the toxicities of nitrite and ammonium ions, the plant cell must control the reduction of nitrate to nitrite precisely so that it meets its needs for assimilated N forms such as amino acids and proteins while causing no harm to itself.

There are three ways turfgrass leaves can control the nitrate reduction process: do not supply nitrate, do not supply NADH, and/or deactivate the enzyme nitrate reductase (NR) (Figure 3). NR is an enzyme that catalyzes the reaction between nitrate ion and NADH. If leaf samples do not contain active NR, the reduction of nitrate to nitrite cannot occur. Activation and deactivation are the quickest among the three ways. This is why studies on turfgrass NAP have been focused on the enzyme NR.

NAP research findings

To estimate leaf NAP in turfgrasses, the author of this article modified a method used for wheat, barley and corn leaves (Jiang, 1998). This method is *in vivo*, which in Latin means "within a living organism." First, we weighed a small amount of leaf tissue containing the enzyme NR within the live leaves and incubate the leaf sample in an incubation solution that contains nitrate at a saturating concentration. After proper preparation, the leaf tissue will absorb the nitrate rapidly, and the supply of nitrate will not be limiting. As long as there is enough NADH available inside the leaves, the enzyme will reduce nitrate to nitrite. To determine how fast nitrite is produced, the reduction of nitrite to ammonium is prevented in the dark. By determining how much nitrite is produced during a known period of time, NAP is calculated.

NAP studies have revealed the following, among other findings:

- Turfgrass roots have an extremely low NAP (Figure 1) and the reason is because turfgrass roots often contain limited carbohydrates (Jiang et al., 1998).
- Turfgrass leaves are the primary sites for nitrate assimilation (Figure 1, Jiang and Hull, 1999).
- Large amounts of nitrate — unassimilated N — are found in leaves under certain conditions (Jiang and Hull, 2000). When the grass is mowed, the unassimilated N will be lost from the plant. Nitrate content is more important

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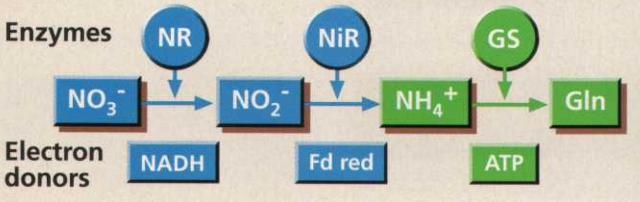
FIGURE 3

Nitrate assimilation pathway



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than total N content of the leaves when predicting fertilization needs.

- Turfgrass assimilates nitrate at high rates during the night (Jiang and Hull, 2000). This discovery is surprising, but it makes sense as turfgrass grows at higher rates during the night and thus needs more assimilated N for growth.
- Turfgrass species differ in NAP. Kentucky bluegrass tends to have greater leaf NAP than perennial ryegrass, tall fescue and bermudagrass (Jiang et al., 2002). Perennial ryegrass tends to have greater leaf NAP than creeping bentgrass (Bushoven et al., 2002).
- Cultivars within the same species differ in NAP. For example, in Kentucky bluegrass, Baron had greater NAP than Princeton 104 when grown at 1 *mM* (Jiang and Hull, 1998). At .1 *mM*, Glade Kentucky bluegrass had a higher NAP than Suffolk (Jiang et al., 2001). In perennial ryegrass, Secretariat had greater NAP than Figaro, Nighthawk, Morning Star, Palmer III, and Linn. In creeping bentgrass, SR 1020 was higher than PennLinks (Bushoven et al., 2002). This new knowledge will help turfgrass breeding programs, enhance N-use efficiency and reduce N fertilizer use.

Future NAP research

Another possible use of NAP knowledge is in the development of models for forecasting turfgrass fertilization needs. Estimating NAP using the *in vivo* method described above is impractical for turfgrass managers. However, we know that leaf NAP is correlated with nitrate concentration and turfgrass variety (Jiang and Hull, 1998). Leaf NAP should also be correlated with environmental variables, such as light intensity and temperature.

When sufficient data is available, it will be possible to construct computer models for predicting NAP based on soil nitrate concentration, turfgrass variety, light, temperature and other factors. By then, turfgrass managers will be able to forecast when and how much to apply N simply by providing the computer program with turfgrass variety and soil data, along with a few readily available weather data.

Jiang is an associate professor of plant science at the State University of New York College of Agriculture and Technology at Cobleskill, N.Y.

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Simulating Real-Life Conditions Provides Valuable Learning Tool

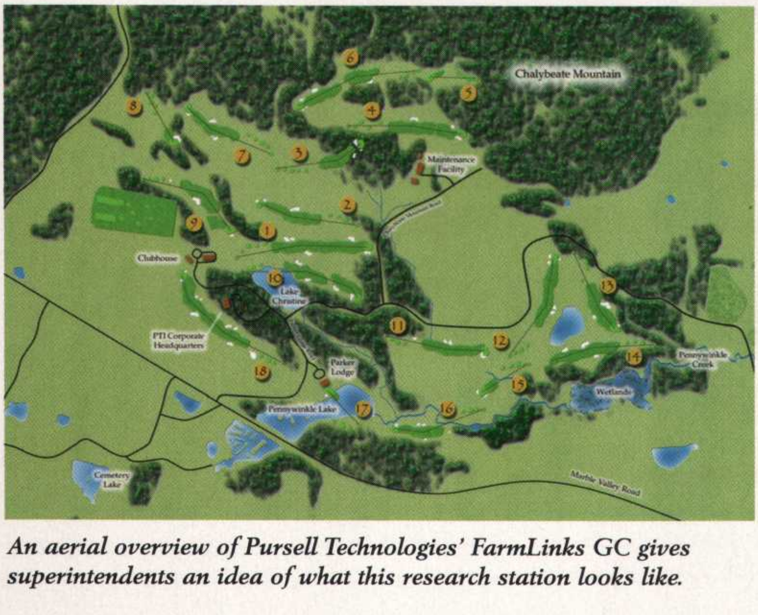
By Jeff Higgins

Much of our everyday knowledge comes from using simulators — driving, flying, surgery — and now superintendents can take the same approach with golf at FarmLinks GC in Sylacauga, Ala.

FarmLinks is a new 18-hole championship golf course developed by Pursell Technologies as a dedicated site for product research and experimentation, as well as environmental studies. Created as an educational "living laboratory," FarmLinks conducts ongoing research on a course that experiences real-world wear and tear.

The company manufactures Polyon controlled-release fertilizer and Precise controlled-released pesticides. The program was originally intended to be a giant test plot for the firm's products. Shortly after its conception, however, the company realized the course's potential for developing other golf-related products, from equipment to seed to greens mixes. Now a number of industry partners use the facility to study and demonstrate their products or prototypes.

Best of all, the knowledge being gained from such tests can ultimately benefit every golfer and superintendent.



An aerial overview of Pursell Technologies' FarmLinks GC gives superintendents an idea of what this research station looks like.

A one-of-a-kind opportunity

Most golf research is conducted on plots at universities or places that mimic real golf course conditions. Many of those facilities don't experience realistic stresses and factors. Because Farmlinks is an actual golf course, it faces the same issues and problems that superintendents and golfers do under real-world conditions.

What's more, researchers literally have the whole place to work with. Typically, other facilities are limited to replicating small test plots, such as 5 feet by 5 feet. At FarmLinks, a whole fairway or entire hole may be dedicated to a certain program of treatment. That way, we're able to compare not only the efficacy of that treatment or product, but also its real-life longevity, economics and other criteria.

The concept provides a nonbiased approach that allows superintendents to evaluate different technologies better. Products can be applied side-by-side to see what happens. That's truly educational, because visiting superintendents can look at the test areas and gauge the differences for themselves.

Visitors also have the luxury of that experience without worrying about golfers hitting on top of them. While it is a real golf course environment physically, we can control the traffic on it.

Evaluating economic impact

We look at applied research in the big picture context, as everything comes back to the economics of maintaining a golf course. When a product is tested, we also study the time and labor involved in using it.

For fertility programs, the plan is to take each hole and dedicate it to a different technology. Our goal is to maintain the turf quality similarly for the entire golf course, with certain variables factored in from hole to hole to provide a larger base of

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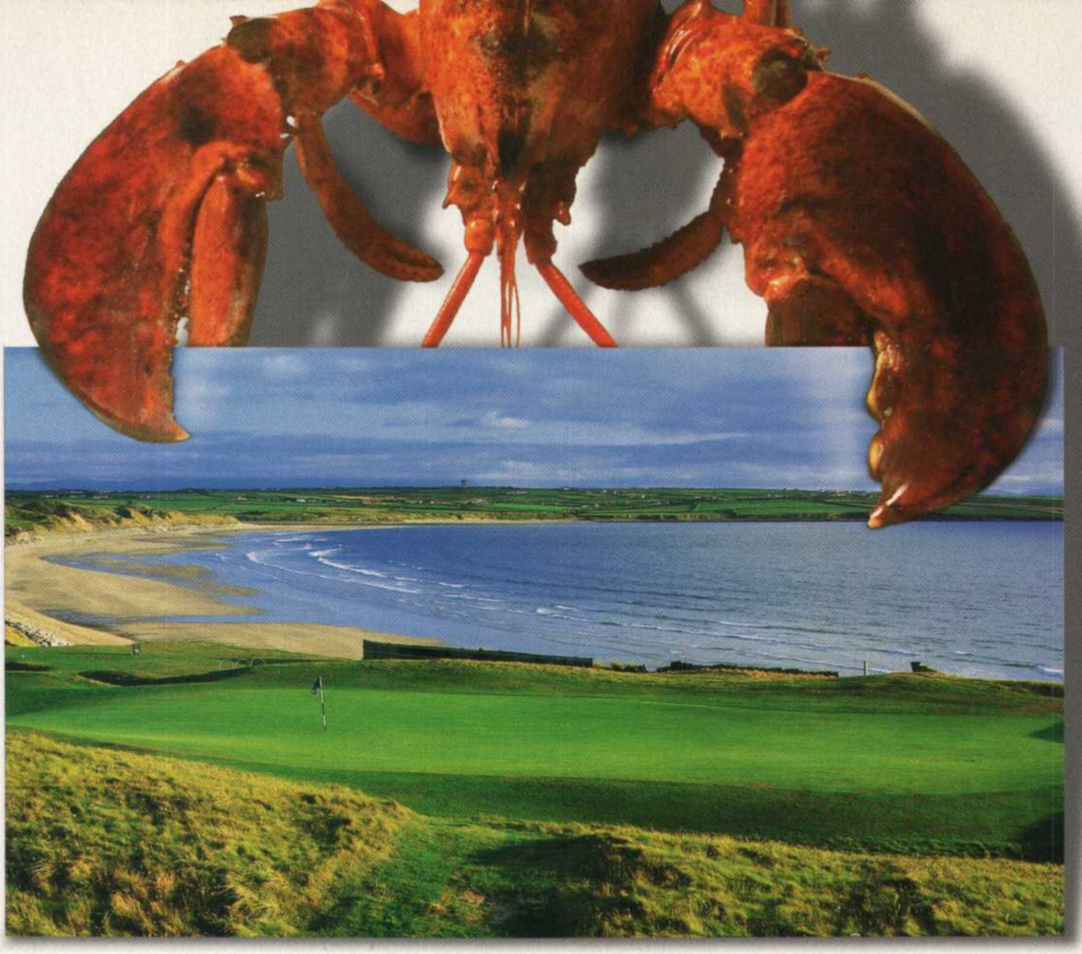

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Farmlinks GC allows numerous different industry companies to conduct their research under real-world conditions.

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research. For example, if we apply a water-soluble product like ammonium sulfate on one hole we may need to apply that fertilizer every three weeks to maintain the same quality of, say hole No. 2, which may use one application of fertilizer all year.

By keeping the turf quality the same throughout, we can then look at how many applications of each product it takes to accomplish that quality. Then there's an overall cost associated with that — labor, equipment, fuel, the product itself — so at the end of the year we can accurately say, "OK, this program cost x dollars a year and this one cost 2x." We're not only looking at just fertilizer performance, we're assessing its economy as well.

Unfortunately, many superintendents today are in a Catch-22 situation — they're told to improve turf quality conditions, but with smaller budgets. In many ways, a superintendent's job is as much about management of money and people as it is agronomics. With the work at FarmLinks, we hope to help superintendents look at their budgets and better manage projects, time or capital.

Every aspect studied

We want to help superintendents learn how to do things more efficiently, and tighter maintenance budgets can affect everything. Having an entire golf course at our disposal opens up countless opportunities to learn about all of those aspects of golf course maintenance. Among the products and practices being researched are pesticides, turfgrass, equipment, irrigation hardware and software, soils and much more.

For example, Toro is working on new technology for an irrigation system that can program and maintain a certain soil moisture level, say 40 percent. Using sensors, the system automatically turns on a certain set of sprinkler heads for that area or zone if soil moisture falls below 40 percent.

During construction, we tried different grow-in treatments, fertility treatments, application timings and rates, and we have some data already.

Syngenta is studying insect, weed and disease control. We are testing different varieties of bentgrasses on putting greens, and for chipping greens we looked at varieties of Ultradwarf bermudagrasses vs. Tifdwarf.

We're evaluating about 25 different active ingredients formulated with our Precise controlled-release pesticide technology. We're looking at the performance of various fungicides, herbicides and insecticides.

All of this research requires a lot of attention to detail. Mark Langner is director of applied research, and he protects the integrity of all studies by ensuring that none of the treatments or processes are compromised. Ironically, a golfer may see some areas that are not in pristine condition, but that's part of research.

A third-party firm was hired to evaluate the course's environmental impact, both during construction and on a recurring basis to measure the effects of routine maintenance. Using eight monitoring stations, specific audits are conducted for water quality (surface and subsurface), erosion, wildlife and plant life. Again, it's all to help the entire golf industry know more about itself.

Experience the future

One of the most exciting aspects of the concept is that it's there for everyone to enjoy and learn from. Each year, more than 1,000 superintendents, academics, golf professionals, students and others play the course and observe product performances and results along the way.

You can preview prototypes of equipment and products before they're available. These are impressive items in operation, and it's not likely you'll see them at a field day or tradeshow.

In addition to seeing new ideas still in development, guests offer their own feedback. It's all like having an insight into the future of turfgrass management technology and getting a head start on your cohorts.

Jeff Higgins is executive director of business development for Pursell Technologies, located in Sylacauga, Ala.