What is ELISA, and Why You Should Care?

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What happens to pesticides after they are applied? It's a tricky question that has multiple implications affecting both those that apply pesticides and those that do not. A person not familiar with pesticide usage might immediately think of the environmental implications such as environmental fate and the affect on non-target organisms. Turfgrass managers who require effective disease control to retain employment might immediately think of the length of efficacy provided. For instance, if one knew that an effective concentration allowed for an additional two weeks of control beyond the recommended interval without reapplying the pesticide then they would be foolish to reapply. Most managers, though, are unwilling or consider it foolish to take that risk without proof the fungicide is present. Thinking ahead to increased pesticide regulation, the time may come where pesticide applications are treated the same as phosphorus fertilizer applications are in Wisconsin. That is to say, a need for the pesticide application must be proven before the application can be made.

There are currently a couple options for measuring the fungicide currently present on and in the plant. Currently the most common method for determining pesticide residues in plants is gas chromatography along with mass spectrometry or flame ionized detection. This method is usually very accurate, but also costly and time consuming (Watanabe *et al.*, 2006). High performance light chromatography is also used for the purpose of measuring fungicide concentration, but cost and time are also a significant drawback. These two methods are usually used by most pesticide labs that investigate pesticide contamination.

A technique that has been developed more recently for detecting pesticide residues in plants and other media is called enzyme-linked immunosorbent assay (ELISA). This is certainly not a new method, as it was initially developed in the 1970's for the rapid detection of parasites in the populations of developing nations (Anonymous, 1976). It is also a technology you have almost certainly been exposed to or are aware of. Probably the most common public use of the ELISA method is with the home pregnancy test (Fletcher, 1986). They are also widely used in pharmaceutical development to detect for increases or decreases in body function in response to different drugs (Bai *et al.*, 2010). Medical research uses ELISA to measure the presence of certain proteins in the blood and other organs (Kaefferlein *et al.*, 2010). A more recent extension of the ELISA method has been to measure pesticide residues in groundwater, on plants, and in food residues (Giersch, 1993; Gabaldon *et al.*, 1999; Shankle *et al.*, 2001).

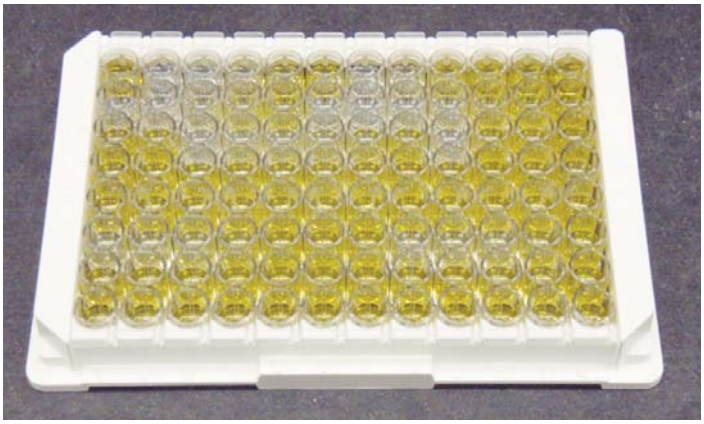

Figure 1: An ELISA test upon completion. The varying colors in the individual wells represent the varying concentrations of the pesticide tested for. This particular test was completed in the Department of Plant Pathology at UW-Madison for the presence of iprodione.

ELISA has also been used extensively in turfgrass research the past twenty years. Identification of fungal species, especially the difficult root diseases, were developed in the early 1990's (Nameth *et al.*, 1990; Fidanza and Dernoeden, 1995). Presence of specific proteins and cytokinin levels in the plant can be measured using ELISA that offer clues into the turfgrass plant's response to stresses (Zhang and Ervin, 2004; Huang and Wang, 2005; Luciani *et al.*, 2007). Detecting endophyte activity is another use of ELISA in turfgrass (Johnson, 1983).

ELISA works in much the same way a vaccine works by taking advantage of the mammalian system's immune response. When a foreign compound enters the body it is met with an immediate response that triggers an immune response. Part of that immune response is the production of cells called antibodies that specifically bind to that compound. These antibodies are long lasting cells that are meant to immediately recognize the presence of the compound again, and it can trigger an immediate and effective response. Specific antibodies are produced for measles and mumps when the vaccine is administered during infancy, and offer protection against these diseases throughout a person's entire life should the disease

enter the body again. These antibodies can be isolated from the blood, and the ELISA method is based on the ability of these antibodies to bind and recognize the foreign compound it was developed for. The amount of binding, and hence the amount of the compound present, can be measured through a chromogenic test (Figure 1).

Antibodies specific to each pesticide (i.e. chlorothalonil, malathion, atrazine) are harvested from a mouse, rat, or rabbit following injection of the pesticide into the animal. These antibodies are then purified and adhered to the bottom of a well plate. Though there are several types of ELISA reactions, the most straightforward is the direct ELISA method. In brief, extract containing pesticide collected from plants or water is placed into the container with the antibodies. Any pesticide present will bind to the antibodies and stick in the container even after washing the unbound solution out. Another set of antibodies specific to the pesticide is then added to the container, but this set has an enzyme attached that will cause the fluid to change colors when it comes in contact with a chromogenic reagent that is added at the end. So basically, the more fungicide present in the extract, the more fungicide-antibody complexes are formed in the container. This leads to a greater binding with the enzyme-linked antibodies, which causes a greater change in the color of the fluid. This change in color is measured using a microplate reader, and results in specific numbers that can be converted to fungicide concentrations.

Why you should care

Admittedly, this seems like a rigorous scientific procedure that has a wide range of uses for university research. But deeper thinking about the procedure reveals a wealth of possibilities that can extend to golf course superintendents. The most obvious uses lie in the realm of pesticide efficacy and especially the length of control provided. This is the basis for research currently underway at the University of Wisconsin by Paul Koch, Dr. Jim Kerns, and Dr. John Stier exploring the rate of degradation of different snow mold fungicides. Using ELISA, superintendents may someday be able to conduct a quick and affordable ELISA test to see if sufficient fungicide remains to delay another dollar spot application. This would offer more comfort and likely significant fungicide savings when compared to the calendar or feel-based methods currently in practice.

Further possibilities include environmental contamination and governmental regulation. Possible regulations that require proof of a "need" to apply pesticides before it can actually be done is a realistic possibility in the future. For those who think this sounds ridiculous, it sounds awfully similar to the need to show a soil is deficient in phosphorus before phosphorus containing fertilizer can be applied.

These sorts of applications are several years off, and it's likely that the most applicable use of ELISA

technology in turfgrass hasn't yet been thought of. But keep an eye out for ELISA in turfgrass, it might just do more than signal a long nine months ahead.

The TDL thanks its supporters

Despite difficult budgets and more difficult decisions in 2010, the TDL continued to receive tremendous support from organizations and individual facilities alike in 2009. In addition to the 74 contract members listed in the November/December 2009 issue of The Grass Roots (Koch, 2009), several organizations offered significant support of the lab. The Wisconsin Golf Course Superintendents Association (WGCSA) offered a gift of $1,000 in support of the lab. The WGCSA also continued to fund the fungicide degradation research ongoing at UW with $8,000 in direct support.

The Northern Great Lakes Golf Course Superintendents Association (NGLGCSA) offered $2,500 in support of both the fungicide degradation research and research investigating the disease resistance of several modern bentgrass cultivars.

For the third year in a row, Dennis Robinson of Horst Distributing has donated the proceeds of Aquatrols 'Turfbucks' program earmarked for research to be presented to the TDL, a gift in excess of $900! All these gifts are instrumental in keeping diagnostic submission fees low while still maintaining the excellent quality of service that the Wisconsin turfgrass industry deserves. Please remember these organizations and companies when considering the benefits of membership or purchasing a product, for without their support our state industry would be much less vibrant.

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