PLANT DISEAS

USING THE SILVER BULLET TO GET TO THE ROOT OF THE PROBLEM

By Dr. Jo Handelsman

One of the greatest challenges of the plant pathologist is to quickly and accurately diagnose disease. We usually start with a sick plant that has some generic symptoms: brown, yellow or wilted leaves, stem lesions, rotting roots. . . . . .In all, not very informative about the causal agent. To identify the agent that is responsible for the symptoms, and prescribe an appropriate treatment, laborious microscopy, culturing, and plant inoculations are used. Often, the diagnosis takes days or weeks to complete. By then, the patient may be dead and may have spread the disease to its neighbors.

Imagine if, instead of these slow methods, we could simply drop the sick plant into a liquid, shake it up, and WHAMMO!!! the liquid would turn a different color depending on which pathogen was present. How quickly we could nip diseases in the bud with the right treatment if we could complete a diagnosis in a few minutes, instead of a few weeks.

Many plant pathologists are developing such rapid diagnostic methods. The major tool that we have available is the monoclonal antibody. This is an antibody, developed in a mouse, that has exquisite specificity for a particular plant pathogen—either viral, bacterial or fungal. Because of their high degree of specificity, monclonal antibodies have been dubbed, "Silver Bullets." In a diagnostic assay, if the silver bullet finds its target, then the assay mixture turns a characteristic color.

Monoclonal antibodies are made by an interesting process. Mammals contain an immune system that involves antibodies that recognize foreign invaders. If an animal is injected with a plant pathogen, it will mount an immune response to the pathogen. This will involve turning on the synthesis of antibodies in specialized cells called "B cells," which are present in the spleen. The problem faced by the plant pathologist is how to separate the antibodies against the plant pathogen from all of the antibodies in the animal. An ingenious solution to this problem was developed in the '70's. A group of scientists found that, instead of trying to purify the antibody, they could purify the individual B cells that produce the antibody of interest, since each B cell can only produce a single antibody. The B cells are fused with cancer cells so that they can be grown in culture in the laboratory. The fused cells secrete the antibody, which can be collected and used to assay for plant pathogens.

Monoclonal antibodies have been called the Silver Bullets for a variety of medical uses. They are being used to tag tiny packets of medicine that are delivered to particular cells in the body, such as cancer cells. By putting such an "address" on the medicine, it can be targeted to the cells of interest and won't go to other cells in the body that it might harm. Monoclonal antibodies are also used to detect miniscule amounts of proteins in the blood that can be indicative of diseases, abnormal conditions, or pregnancy.

Now it is time for the plant pathologists to use the silver bullets to diagnose diseases simply and rapidly. The tests may be so easy to perform that the grower could perform the diagnosis with a kit, and treat the disease accordingly. The next few years will bring exciting changes in the way that plant disease diagnosis is performed, and monclonal antibodies, the silver bullets of modern biology, will play an important role in these changes.

Editor's Note: Dr. Jo Handelsman joined the Plant Pathology Department last year as an Assistant Professor. A native of the metropolitan New York City area, Dr. Handelsman received a B.S. degree from Cornell in 1979 and a Ph.D. from the UW—Madison in molecular biology in 1983. She studies the genetics and biochemistry of specificity and the antagonism of bacterial pathogens.

THE STATE OF THE ART OF TURF DISEASE DIAGNOSIS OR IS THERE DISPAIR IN THE PLANT PATHOGEN DETECTION CLINIC?

By Mary Francis Heimann, O.S.F.

There are two crops that most diagnosticians dread the most. One of these is turf. Diagnosis of turf diseases is almost like Russian roulette; sometimes after hours of work on a specimen we would like a to take a pistol to our heads; provided, of course it is loaded with water. A good drenching washes away sweat and tears of exasperation.

Since this is for the "Grass Roots" publication let me start with the roots. Usually they appear in one of three stages. They are: 1) brown and rotted, 2) white and healthy, and 3) half and half—some dead and some alive. If they fall into category 2, I can continue on to look at the crown. If the roots are rotted, I ask what could have caused this condition. The little fungus chomping away at

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root tissue does not hold up a sign with its name on it. So I make a water mount of the tissue and what do I find: Usually my little banana shaped friends with inside cross walls are there and say "Hi, we're **Fusarium** spores. Try to guess if we have caused this state of affairs or if we are covering up for one of our other fungus friends."

There are bacteria galore upon which some wiggly nematodes are feeding. Or are they feeding just on the bacteria and dead tissue? The answer to this is to act a bit like a traffic cop. I put on the red light, in this case a bit of heat on the slide. This gets them to slow down. They have to be quiet enough to see whether or not they have stylets. (These are little "spears" with which they pierce the root cells.) If I apply too much heat, I cook them, and while the bacteria might like par-boiled nemas for lunch, I don't gain much insight into the problem. If my heating technique has become skilled and I find that the nematodes are without stylets I can cross them from my list of suspects. If they do have stylets, then I have another game to play. It's called threshold guessing or speculation. Are there enough present to have really caused the root problem? To solve this involves a trip up to fourth floor for a conference with Dr. Ann MacGuidwin. In my enthusiasm I may have

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APPLICATIONS OF PLANT DISEASE DETECTION KITS FOR TODAY'S TURF MANAGER

By Dr. Sally A. Miller

One of the many challenges faced by managers and growers of turfgrass, ornamental plants and other high value crops is the timely and accurate diagnosis of plant diseases. Many diseases are caused by highly aggressive pathogens that can cause severe damage within a short time after symptoms are spotted. Other diseases develop more slowly but in the end may be just as devastating. Often, diagnosis is hindered by the absence of 'classic' symptoms. Pathogens may also infect and colonize plants but fail to induce symptoms until environmental conditions favor disease development. Until recently, few analytical tools have been available to assist the turf manager in detecting and diagnosing plant diseases. However, advances in biotechnology during the past decade have made it possible to develop rapid, specific, user-friendly tests for the detection of pathogens in plants, soil, and water. Such tests utilize antibodies in formats designed to take advantage of the unique properties of these 'reagents': their ability to recognize and bind to specific substances such as components of plant pathogens. One of the most popular types of assay is the enzyme-linked immunosorbent assay (ELISA), in which the pathogen-detecting antibodies are tagged with an enzyme. When an appropriate substrate is added to the reaction mixture, a positive reaction is visualized by a color change as substrate is converted to a colored product. There are a number of different types of assay formats that are applicable to plant disease diagnosis. One that has been developed recently is the dipstick assay. In this assay, the reactive end of the dipstick is incubated in the sample extract with the enzyme-tagged antibody solution, washed, then transferred to substrate. A positive reaction is indicated by the deposition of insoluble colored product onto the dipstick. Semi-quantitative results can be obtained by comparing the color of the dipsticks to known standards. Quantitative results are obtained by using an inexpensive, field-adaptible reflectometer that measures the intensity of the color on the dipstick. Dipstick assays can be carried out rapidly, often in a few hours.

Plant disease detection kits should be viewed as tools for managing agronomic practices. They can provide a turf manager with information that will help him or her to make the right choice of disease control remedy. Some of the decisions are: selection of pesticides and timing of pesticide applications, selection of plant varieties, and use of cultural control practices. For the turf manager, the availability of such kits will make it possible to diagnose plant diseases quickly and accurately, often before symptoms are present. Where symptoms are indistinct or confusing, kits can be used to confirm a preliminary diagnosis based on the appearance of the plant and/or signs of the pathogen. Used in conjunction with crop and weather data and forecasting systems, the kits will make it easier to predict the occurrence of disease outbreaks in a variety of crops.

Once diagnostic tests are widely available, they will plan an integral role in crop management. The primary components of the assays, the antibodies, can be produced for most plant pathogens, and in the coming years more and more tests will become available as part of the crop manager's arsenal against plant disease.

Editor's Note: Dr. Sally Miller was raised in Ohio and received her B.Sc. degree in 1976 from Ohio State University with an undergraduate major in biology. Her interests in graduate studies included mechanisms of disease resistance in alfalfa and she graduated with a Ph.D. degree in Plant Pathology from the University of Wisconsin—Madison in 1982. Currently, she is Unit Head of Plant Pathology Research for Agri-Diagnostics Associates, Cinnaminson, NJ. Her responsibilities included the development of diagnostic kits for detection of turf pathogens.

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taken the stairs to fourth floor. After our conference I wait for the elevator. It's easier to ponder what was meant by the "perhaps, perhaps not" answer while standing quietly during my descent back to the clinic.

By what else could the root rot have been caused? Well, **Helminthosporium** can do this but it is terrific at "hide and seek." It hardly ever produces spores for me to see in a water mount of the root tissue. So I set about isolating. However, when looking into the tissue I may have spotted what looks like **Pythium** reproductive spores, as well as something that looked like **Rhizoctonia**. So instead of looking only for **Helminthosporium** I have to allow for these other creatures to grow if they are part or all of the problem. So I pull out several selective media with each medium containing certain "goodies" that each particular fungus likes most.

In the meantime, I move on up to the crown of the plant. Before I concentrate totally on the crown I should mention a fourth stage of affected root tissue. This stage has its partner in the crime of creating turf problems. That partner is the crown of the plant. When these two parts die they do not behave like good dead tissue and make themselves available as food for the decomposers. No, they form a pesky relationship, and become thatch. What does thatch do? Well, it lies there under the grass plant and defies the living roots to try to penetrate. And you and I know what can happen to a shallow rooted grass plant when a bit of stress occurs. It goes "phfff" and crosses over to the enemy thatch to join its forces. It also complicates celar-cut symptom expression.

I return to the crown. It frequently is affected by many organisms. It is a disease of the crown that causes yellow patch, brown patch, Fusarium patch, necrotic ring spot patch, summer patch, ad finitum. I'm certain someday we'll have winter patch, spring patch, blue patch, and, and. . .

How do I know which fungus causes which patch? I examine the crowns and aha, there I find dark runner hyphae with certain characteristics. I conclude I am looking at a case of necrotic ring spot (NRS). But, the hyphae of **Philalophora** are very smiliar, and the hyphae of **Gaeumannomyces** are also very similar, to **Leptosphaeria** which is the causal fungus of NRS. How do I find out with certainty which it is? I can give the isolate to Jana Stewart, because she has tricks at which she is adept and she can get the hyphae to produce spores. These spores will give away the fungus' name. But herein lies a problem. Jana can do this, but the fungus does not eat at a fast food restaurant. It eats slowly and sporulates slowly. Jana might be able to tell me which one it is around Christmas time. So on what basis do I give the client a satisfactory diagnosis? The bases are on other clues such as what we know about the frequency or presence of the other fungi in Wisconsin, on the symptoms and on the background information that the client gives, and on which turf grass species we found which fungus.

Other crown diseases are possible. **Helminthosporium** can be a crown disease as well as one that affects the roots, and one that has a leaf spotting phase. Since only rarely can we find these spores, we most often diagnose this disease on the basis of a number of symptoms along with information supplied by the client. **Rhizoctonia** can attack the crown. This fungus can be readily seen with the microscope. However, sometimes it is present without being the primary culprit. I must also rely on symptoms, both those I see and again, those the client reports.

Leaf diseases are also possible. They are easy! If I look at the fruiting bodies (pycnidia) of **Ascochyta** or **Septoria** on the leaf blade with the use of a dissecting scope they both look exactly the same! However, it **is** easy to distinguish between the two with a compound microscope. Their spores are unique, and very different in character.

Symptoms, signs and "gut feelings" all come into play in diagnosis. It's well to remember that plant disease diagnosis is really often plant pathogen detection. If the specimen is dead, I cannot do an autopsy. Dead tissue is rapidly invaded by secondary organisms that hide or tell the primary pathogen to "get lost." I need diseased, but living tissue with which to work. Another point; if I have a headache, I do not send a wisp of my hair to the doctor. I take my whole self. Very frequently leaf symptoms are simply telling us that something is going wrong somewhere else. An adequate sample is the first step towards successful diagnosis. One or two four-inch squares or round plugs of turf taken from the interface where healthy grass meets affected grass are necessary. A fresh sample, packaged in such a way that the soil has not broken up, resulting in soil particles covering the leaf blades, is also necessary. A few grass leaves torn off and taped to a piece of paper test my since of humor. A "happy" sample keeps the diagnostician happy!

I've touched on a few turf diseases. This has not been meant as a lesson in plant pathology. I've tried to show what happens when a turf specimen enters the lab. If I look at each entry as a problem to be solved it can lead to dispair. If each entry is a challenge to be met, then it is all in a day's fun.

Editor's Note: Mary Francis Heimann is a native of Chilton, Wisconsin. She has an undergraduate major in Horticulture at the University of Wisconsin, as well as a B.A. in Biology from Cardinal Stritch College in Milwaukee. She also received a M.S. in Plant Pathology with a minor in Horticulture from the University of Wisconsin—Madison. Mary Francis has many years of experience as worker and supervisor of greenhouse and grounds at St. Francis Convent in Milwaukee, and four years of teaching Biology on the secondary level. She joined the staff of Plant Pathology as plant disease diagnostician in June, 1977.