Industry News

Monoclonal Antibody Diagnostics in Plant Disease Management. R. K. Lankow, S. H. Woodhead, R. J. Patterson, R. Massey, and G. Schochetman, IGEN, Inc., Rockville, MD 20852

Each year plant pathogens, including fungi, viruses, bacteria, mycoplasmas, and nematodes, are responsible for billions of dollars of economic losses in agricultural commodities. As a result, large sums of money are employed to combat these pathogens. Effective management of plant disease requires that growers obtain timely and accurate information concerning threats to their crops. Information that would be valuable includes inoculum potential for individual pathogens, prevalent pathogen races, the occurrence of appropriate climatic conditions for infections, and the development of pesticide-resistant strains. Equally important is the need to obtain this information as early as possible, preferably prior to the development of symptoms. This information would prove invaluable in predicting impending disease epidemics. Current diagnostic practices in the agricultural industry are not suited to provide this information in a simple and timely fashion.

Existing plant disease diagnostic procedures require trained personnel, well-equipped laboratories, and, above all, time. Diagnosis can consume from a few days, if distinctive spores can be induced and identified, to several months, if various hosts must be inoculated to identify a specific virus.

It is clear that a simple, rapid, sensitive, and specific method for crop disease diagnosis is highly desirable. Antibody-based diagnostics, which are widely used in human clinical diagnosis, offer such an alternative. These diagnostics are based on the specific interaction of antibodies with target organisms. Antibody diagnostics in plant pathology were used initially for the detection of plant viruses and more recently for the detection of fungi, bacteria, spiroplasmas, and nematodes.

Antibodies are a heterogeneous population of proteins that an animal produces in response to a foreign substance (antigen). For approximately 100 years, antibodies for experimental use have been obtained from animals inoculated with the antigen for which an antibody is desired. While production of antibodies by this method is sufficient for many applications, it presents problems that limit the performance of antibody-based diagnostics. The problems with conventional antibodies are 1) the difficulties in obtaining a reproducible source of well-characterized antibodies and 2) the considerable variation in the types and specificity of antibodies produced.

These problems can be overcome by the use of monoclonal antibodies. In contrast to conventional serum-derived antibodies, monoclonal antibodies are homogeneous populations secreted by hybridoma cells that have been produced in the laboratory and grown in culture. Interactions between an antigen and a monoclonal antibody are characterized by greater specificity than those between an antigen and polyclonal antiserum. Because monoclonal antibodies are produced by the hybridoma cells in tissue culture, they can be reproduced consistently in large quantities without the need for reimmunizing animals.

Diagnostic products based on highly specific monoclonal antibodies will provide a valuable source of information to guide crop management decisions. Information that today is unavailable or available only to research specialists will be easily obtained. Many of the time-consuming traditional diagnostic procedures will be supplemented by these rapid and sensitive methods. Well-designed diagnostic products based on highly specific monoclonal antibodies will yield economically important information without the need to isolate or purify pathogens.

The following are some of the more important uses:

Presymptomatic detection of pathogens. Current methods, which are limited largely to research studies, include spore trapping, use of selective media,

baiting techniques, and direct recovery and observation of pathogens. Immunologic tests will allow direct detection and identification of pathogens on foliage, fruit, flowers, seeds, and other propagating materials. In addition, detection of pathogens in soil, water, planting media, and spore traps will be routine. The ease of these methods will permit regular sampling to allow detection of pathogens before infection or prior to symptom expression.

Specific, rapid diagnosis of disease. Rapid confirmation of a visual diagnosis will be most useful when symptoms are sparse, undeveloped, or atypical. Diagnosis with monoclonal antibodies can be accomplished within minutes to hours, whereas traditional methods require days or even months. Because of the exquisite specificity of monoclonal antibodies, primary disease can be diagnosed in tissues that have been invaded by secondary pathogens and saprophytes without having to isolate or purify the primary pathogen.

Quantitation of inoculum. Monoclonal antibody assays can determine the abundance of an organism as well as detect its presence. This task today is labor-intensive, and such data are rarely available to the grower. The only current examples are nematode analysis services, which provide useful information but are limited by the extensive manpower required to conduct the assays. The simplicity of monoclonal antibody tests will enable large numbers of samples to be rapidly examined, allowing significant populations of pathogens to be more accurately located. Additionally, the availability of such assays will allow epidemiologists to determine economic thresholds for pathogens.

Scheduling chemical treatments. The use of chemical disease control products is a major approach to crop disease management. Efficient detection of pathogens will allow more precise application of control agents. Disease progress can be monitored throughout the growing season, allowing farmers to modify their treatment programs to obtain maximum benefit.

<u>Determining species and races</u>. To make certain crop management decisions, such as selecting a crop variety, growers must know the precise identity of pathogens present in a crop or a field. The specificity of monoclonal antibodies can be used to identify a species, pathovar, forma specialis, or race. Changes in pathogen population over time can be monitored with specific immunoassays.

Inspection and certification. Seeds and other propagating materials and commodities intended for import and export are examined for the presence of pathogens. Immunoassays have been shown to be useful for examining large numbers of samples for the presence of key plant pathogens. Commercial availability of monoclonal antibody tests will greatly enhance the ease and specificity of inspection and subsequent certification of plant materials.

Diagnostic tests based on monoclonal antibodies can be tailored to meet the needs of each user. Assays can be designed to allow single determinations in the field or to allow large numbers of samples to be run in a short time in a laboratory. The inherent specificity and simplicity of these assays require minimum sample preparation, and the results are easily interpreted.

Monoclonal antibody diagnostic assays for agriculture will provide data that are not currently available and will allow advances in epidemiological research on pathogen movement, population dynamics, and pathogen distribution. These products will become an integral part of the services provided by extension specialists, consultants, pest control advisors, and industrial plant pathologists and will form the basis of their professional disease management recommendations. These specialists will be able to provide rapid turnaround on key diagnoses and conduct more detailed and specific analyses of grower problems. Agricultural professionals will be able to scout and survey plant pathogen populations as they now monitor insect populations. Monoclonal antibody-based diagnostics will fit closely with established integrated crop management practices, and when applied at the crop production level, diagnostic products will provide an unusually effective tool to enhance the productivity and profitability of modern agriculture.