Technological Advances in Plant Disease Diagnosis

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Plant disease diagnosis is an essential component in managing and preventing losses from plant diseases. Effective diagnosis requires accuracy, reliability, speed, and a statement indicating the causal agent of disease. In addition, diagnosis is most valuable when it is accomplished at an early stage of disease development. However, current services for pathogen identification are commonly provided after commencement of disease. Delay in diagnosis frequently results in the failure of recommended control strategies. Implementation of technological advances in plant disease diagnosis will help to meet the ultimate goal of plant health.

Diagnostic methods. Identification of plant pathogens from a diseased plant follows the guidelines of Koch's postulates to prove an etiologic agent, but a complete fulfillment of the postulates may not be required when a proven method is available for a specific pathogen identification. The common methods of disease diagnosis include symptomatology, microscopy, microbiological techniques, immunoassay, and bioassay techniques. Natural, induced, macroscopic, and microscopic symptoms and signs serve as basic tools for disease diagnosis and pathogen identification.

Available references used for diagnostic purposes frequently explain what to observe but not how to induce those characteristics needed for the diagnosis. Furthermore, methods developed or used for research are often borrowed in a diagnostic application. These methods frequently require evaluation and modification before they can be applied in a diagnostic situation. This type of work consumes much of the diagnostician's time, and the method becomes a personally tailored technique that is not available to other diagnosticians. A refereed, diagnostic methods manual would enhance the function of a laboratory diagnostician. Additionally, a compilation of refereed, "standard" diagnostic methods for a specific pathogen or pathogen-host complex would enhance the reliability of diagnosis.

The tedious and time-consuming procedures of isolation, extraction, purification, and subsequent identification of the target organisms, including pathogenicity tests, are performed when the diagnostic symptoms and signs are unavailable or insufficient for accurate diagnosis. However, additional diagnostic tools are becoming universally accepted as part of a "standard" procedure. The following procedures, once used solely by research specialists, are now available to diagnosticians: selective and differential media; LOPAT test for pathogenic fluorescent pseudomonads; nuclei staining for *Rhizoctonia*; dichotomous, tabular, synoptic, and working keys for *Phytophthora*; use of host range and indicator plants; and enzyme-linked immunosorbent assay (ELISA).

Immunoassay. Immunoassay is an effective tool for pathogen identification. Antibodies can be produced not only against pathogens but against other diagnostic components associated with a disease, such as proteins, polysaccharides, toxins, enzymes, and nucleic acids. Although monoclonal antibody production is a time-consuming process, it is a promising approach for obtaining highly specific and uniform sources of antibodies. Polyclonal antibodies, although more readily available, may show nonspecific activities and variation among antiserum batches. Routine use of immunoassay techniques in a plant disease diagnostic laboratory is possible with readily available antisera (ATCC) along with rapid and simple immunoassay tests (ELISA, dot-blot, dipstick, etc.).

New techniques. In addition to monoclonal antibodies, recent molecular approaches to pathogen identification provide hope for supplementing the existing pathogen identification techniques. Nucleic acid probes (tobacco streak virus-necrotic shock), restriction fragment-length polymorphisms (*Armillaria* spp.), and isozyme analyses (*Xanthomonas campestris* pathovars) for plant disease diagnosis and pathogen identification are still in their infancy as diagnostic tools.

Molecular procedures for pathogen identification are not simple. Detection by nucleic acid hybridization requires generating a specific probe. This involves isolating and possibly cloning a nucleic acid with sequences complementary to and specific for the nucleic acid of the pathogen. The probe must then be labeled with radioisotopes, biotins, enzymes, or fluorescent markers. At this time, lack of suitable markers, other than radioisotopes, is one of the factors limiting the general use of molecular probes.

Electrophoretic analyses of isozymes in a crude extract of soluble pathogen proteins have been applied for fungi, bacteria, and nematode identification by matching the pattern of specific enzymes that are characteristic of the pathogen.

Industry participation. Recent developments by industry in the area of plant disease diagnosis and pathogen identification have facilitated the function of the diagnostic laboratory. Materials, services, and instrumentation provided include: antisera from ATCC (Rockville, MD); reagent kits for bacterial identification from Difco (Detroit, MI); ready-to-use immunoassay-based diagnostic kits and services for bacteria and viruses from Agdia Inc. (Mishawaka, IN); dipstick diagnostic kits for turf diseases from Agri-Diagnostics (Cinnaminson, NJ); aflatoxin detection kits from Neogen Corporation (Lansing, MI) and Agri-Sciences, Inc. (Rolling Hills Estates, CA); an aflatoxin screening test from International Diagnostic Systems Corp. (St. Joseph, MI); and bacterial identification based on analysis of fatty acids using a computer-coupled gas chromatographic system from Microbiol ID, Inc. (Newark, DE).

Limitations. Accurate, rapid, early diagnosis of plant pathogens using new technology still has many limitations. Use and acceptance of diagnostic techniques that require special or costly equipment, materials, and training are limited. Procedures requiring the use of electron microscopy, fluorescent microscopy, gas chromatography, electrophoresis, radioactive materials, virus inclusion-body detection, scarcely available antisera, nucleic acid probes, or computerized keys are limited in their application. These limitations can be minimized, however, through a stronger commitment to diagnostics that includes purchasing equipment for diagnostic use, encouraging the use of existing equipment for diagnostic purposes, and providing better training in unfamiliar techniques for diagnostic personnel.

Conclusion. The service of plant disease diagnosis and pathogen identification may meet its goal of plant health by: 1) development of more accurate and faster simple diagnostic tools, possibly "instant" identification tools for certain pathogens; 2) compilation of refereed methods; 3) provision of services for pathogen detection before disease develops; and 4) cooperation among scientists, industry, and government for a common goal of plant health.