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Chapter 1 : Microbial Plant Pathogens-Detection and Disease Diagnosis: : P. Narayanasamy :

Plants are infected by different microbial pathogens, of which fungal pathogens form the highly evolved and earliest recognized group. The morphological, biological, biochemical and physiological characteristics have been used for the detection, identification and differentiation of fungal pathogens up to species level.

Food and Drug Administration, Washington, D. Nainan, and Harold S. S u s m Springthorpe, and Jason A. Other Foodborne Viruses Syed A. Sattar and Jason A. Mcrtsui and Rarnsey C. Cheung xv xwi Contents of Other Volumes 8. Sattar and Sabah Bidawid Taeniasis and Cysticercosis Zbigniew S. Waterborne and Foodborne Protozoa Ronald Foyer Medical Management Pm1 Prociv Immunodiagnosis of Infections with Cestodes Bruno Gottstein Diagnosis of Toxoplasmosis Alan M. Admm and Debra D. Contents of Other Volumes Selected Plant Toxicants 2. Beier and Herbert N. Glycosides Walter Majak and Miclznel H. Plant Toxicants and Livestock: Prevention and Management Michcrel H. Aspergillus Zojia Kozcrkiewicz Claviceps and Related Fungi Gretchen A. Kuldau and Charles W. Koshinsky, Adrieme Woytowich, and George G. Analytical Methodology for Mycotoxins James K. Immunological Techniques Fur1 S. Chrr xvii xviii Volumes Contents of Other General Identification Features Dmid G. Seafood and Environmental Toxicant Exposures: Fish Toxins Bruce W. Shellfish Chemical Poisoning Lyndon E. Pathogens Transmitted by Seafood Russell P. Epidemiology of Seafood Poisoning Lorn E. Hnnurlond Contents of Other Volumes Reid a d Timothy D. Food and Drug Administration Kim R. Nutritional Toxicology David Kitts Food Additives Lasclo P. Analysis of Aquatic Contaminants Joe W. Agricultural Chemicals Debra L. Browning and Car1 K. Radioactivity in Food and Water Hank Kocol Food Irradiation Hark Kocol Long and Jose E. Food and Hard Foreign Objects: Food, Filth, and Disease: Food Filth and Analytical Methodology: Bristlecorle Enterprises, Denver, Colorado I. Poison Information Centers 10 1. A few organizations have attempted to gather such information and organize it into yearly reports. The American Association of Poison Control Centers and some federal agencies work toward obtaining epidemiological information, while the AAPCC has an active role in assisting with the treatment of exposures. Epidemiological studies assist government and industry in determining package safety, effective treatment measures, conditions of exposure, and frequency of exposure. Studies on bacterial exposures provide information on the type of people most commonly involved. Studies can also tell us which bacterial species are most commonly involved. The symptoms first seen, the onset of symptoms, and the sequelae may also be determined and compared to current norms. The group in the United States most concerned on a daily basis with potential poisonings due to household agents, industrial agents, and biologics including plants and mushrooms is the American Association of Poison Control Centers AAPCC. This is an affiliation of local and regional centers that provides information concerning all aspects of poisoning and often refers patients to treatment centers. This group of affiliated centers is often supported by both government, private funds, and industrial sources. Poison centers were started in the late s, with the first center thought to be in the Chicago area. The idea caught on quickly, and at the peak of the movement there were hundreds of centers throughout the United States. Unfortunately there were few or no standards regarding what might be called a poison center, the type of staff, hours of operation, or information resources. One center may have had a dedicated staff of doctors, pharmacists, and nurses trained specifically in handling poison cases; the next center may just have had a book on toxicology in the emergency room or hospital library. In the Health and Safety Code Sec. This action helped to standardize the activities and the staffs of the various poison centers. The National Clearinghouse for Poison Control Centers initially collected data on poisonings, information on commercial product ingredients, and biological toxic agents. For several years the National Clearinghouse provided product and treatment information to the poison centers who handled day-to-day calls. At first, most poison centers were funded by the hospital in which they were located. As the centers grew in size and number of calls being handled, both city and state governments took on the responsibility of contributing funds. In recent years the local governments have found it very difficult to fund such operations and centers have had to look to private industry for additional funding. Some states with fewer residents may contract with a neighboring state to provide services to its residents. Some states are so

populous that more than one center is funded by the state. A few bacterial exposures are listed in this log, most of which have to do with food poisoning. Regional Centers The number of listed centers has dropped significantly since its peak of more than Many centers have been combined into regional organizations. These regional poison centers provide poison information and telephone management and consultation, collect pertinent data, and delivery professional and public education. Cooperation between regional poison centers and poison treatment facilities is crucial. The regional poison information center, in cooperation with local hospitals, should determine the treatment capabilities of Poison Centers and Bacterial Exposure 3 the treatment facilities of the region and identify and have a working relationship with their analytical toxicology laboratories, emergency departments, critical care wards, medical transportation systems, and extracorporeal elimination capabilities. This should be done for both adults and children. Documentation of these state designations must be in writing unless a state chooses in writing not to designate any poison center or accepts a designation by other political or health jurisdictions. Regional poison information centers should serve a population base of greater than one million people and must receive at least 10, human exposure calls per year. The number of certified regional centers in the United States is now under Certification as a regional center requires the following. Maintenance of a 24 hours per day, days per year service. Providing service to both health care professionals and the public. Having available at least one specialist in poison information in the center at all times. Having a medical director or qualified designee, on call by telephone, at all times. Service should be readily accessible by telephone from all areas within the region. Comprehensive poison information resources and comprehensive toxicology information covering both general and specific aspects of acute and chronic poisoning should be available. The center is required to have a list of on-call poison center specialty consultants. Written operational guidelines, which provide a consistent approach to evaluation, follow-up, and management of toxic exposures should be obtained and maintained. These guidelines must be approved in writing by the medical director of the program. There should be a staff of certified professionals manning the phones at least one has to be a pharmacist or nurse with hours and cases of supervised experience. There should be a hour physician Board Certified consultation service. The Regional Poison Center shall have an ongoing quality assurance program. The regional poison information center must be an institutional member in good standing of the AAPCC. Who Staffs a PoisonCenter? The staffing of poison centers varies considerably. The three professional groups most often involved are physicians, nurses, and pharmacists. Who answers the phones is somewhat dependent on the local labor pool, moneys available, and the types of calls being Spoerke 4 received.

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Extraction of Total RNA Total Nucleic Acid Extraction Reverse Transcription RT Reaction Extraction of Total Nucleic Acids Preparation of Crude Extracts Extraction of Nucleic Acids Probes for Target cDNAs Printing of Oligonucleotide Probes on Membranes Extraction of Nucleic Acids Enriched with the Viroid Dot Blot Hybridization Assay Extraction of DNA from Soils Preparation of Single Aphid Squash Samples This volume aims to provide required information on various techniques for the detection, differentiation and quantification of fungal plant pathogens in infected plants, planting materials, environment, as well as accurate diagnosis of the diseases even in plants and plant materials that may not exhibit any recognizable symptoms of infection. The efficacies of different techniques are compared; their limitations are indicated and the suitability of the techniques for large scale application is highlighted. The important role played by the disease diagnostic centers, plant quarantine and certification programs in providing advice to the growers, prevention of introduction of new diseases and establishment of disease-free nuclear stocks has to be realized and necessary facilities should be provided for their efficient functioning. The estimates of losses made later indicated that about The loss assessments have been made for different types of diseases with different levels of accuracy. However, irrespective of the levels of accuracy, the estimates of losses emphasize the imperative need for measures to be taken urgently to avoid the losses to the extent possible. To achieve this aim, three principles of crop disease management viz. The effectiveness of crop disease management systems P. Fungal pathogens including oomycetes have been responsible for several destructive diseases such as potato late blight, wheat stem rust, rice blast and grapevine downy mildew that have ruined the economy of several countries resulting in famine and migration of millions of humans to other countries to escape starvation and ultimate death. Occurrence of diseases affecting cereals and wine crops has been mentioned in ancient scriptures. But they were attributed to supernatural elements, because of the prevailing superstitious beliefs, religious dogmas and faiths. Plant diseases were considered as God-sent curses as punishment for sins committed, because of lack of scientific and analytical observations. Anton de Bary â€" established in beyond doubt that potato late blight disease was caused by a fungus. He led a distinctive group of students from different countries striving for explorative excellence which unfolded unquestionable conclusions on several diseases like smuts, rusts and downy mildews affecting various crops based on experimentation. His remarkable contributions formed the cornerstone for the future development of modern investigations on fungal, bacterial and viral plant pathogens causing a wide range of diseases affecting crops all over the world. Hence, he is deservingly regarded as the father of Plant Pathology Horsfall and Cowling The pathogen s may gain access to the susceptible host plant species through infected seeds or vegetatively propagated planting materials such as tubers, corms, suckers, setts etc. Furthermore, the pathogen propagules may be present in the soil, water, air, natural vectors and alternative host plant species providing inoculum for newly planted crops. Incidence of diseases not known to occur earlier may be observed from time to time. The nature of the cause of the newly observed disease has to be determined immediately to minimize its further spread. These procedures are time-consuming, labor intensive and often provide inconsistent results and require considerable knowledge of fungal taxonomy. Biochemical and physiological characteristics of the fungus isolated from infected plants have been used for the identification of some fungal pathogens with more certainty compared with isolation-based methods. Antibody-based immunological techniques have been employed for detection, differentiation and quantification of fungal pathogens rapidly. Nucleic acid-based techniques, especially based on polymerase chain reaction PCR have been demonstrated to be more precise, sensitive, rapid and reliable for the detection, differentiation and quantification of fungal pathogens present in symptomatic, as well as in aymptomatic plants and plant materials Chapter 2. Fungal pathogens can survive in the environment in the absence of

susceptible host plant species, as they produce spore forms resistant to adverse conditions. Further many of them, except the obligate pathogens that cause rusts, powdery mildews and downy mildews, are capable leading a saprophytic life using organic substrates present in the soil. Special methods have to be applied for their isolation from soil samples. The techniques based on the biological, immunological and nucleic acid-based characteristics have been employed for the detection and quantification of fungal pathogens in the soil. Airborne fungal spores play a vital role in the spread of the diseases affecting aerial parts of the plants. Alternative host plants including weed and wild plant species have been shown to be important sources of inoculum and they play an important role in the survival and perpetuation of the fungal pathogens in the absence of crop plants. It is essential to detect and quantify the pathogen populations in the alternative hosts that may be near the cropped areas by applying efficient methods. Comparative efficacies of different detection methods are discussed in Chapter 3. Fungal pathogens are known to exist as different formae speciales, varieties, strains, biotypes or races that differ in their virulence pathogenic potential. Suitable efficient techniques have to be employed for characterization of different strains within a morphologic species for the development of effective management systems. The pathogen may produce a new strain that can overcome resistance of newly introduced resistant crop cultivar. Further, emergence of fungicide resistant strains has become a problem of concern for both the grower and the administrators. Constant and consistent monitoring of production of new strains with greater infection potential or higher levels of resistance to chemicals of fungal isolates present in a geographical location is very important to prevent the imminent destruction due to the new strain of the pathogen. Identification of fungicide resistant strains is essentially required for making decisions for the continuation or withdrawal of the fungicide that 4 1 Introduction is being used in the location concerned. Likewise, useful information may be obtained from these studies for the development of varieties that have to be resistant to all strains of the fungal pathogen existing in a geographical location. The information available in this aspect is critically discussed in Chapter 4. Accurate diagnosis of crop diseases represents the culmination of all efforts to detect, differentiate and identify the fungal pathogen isolated from the newly observed disease in a region. The role of disease diagnostic centers DDCs , plant quarantines and certification programs in disease diagnosis is well recognized. Exclusion of fungal pathogens by proper testing by quarantine personnel and provision of disease-free planting materials to the growers by the personnel of certification programs can be expected to have a significant impact on profitable cultivation of crops Chapter 5. The information reflecting extensive literature search is presented in an easily understandable style. It is expected that the various aspects of detection, differentiation, identification and quantification of the fungal pathogens in the plants and environment, as well as the diagnosis of the diseases caused by them presented in this volume, will be highly useful to the researchers, teachers and graduate students in the Departments of Plant Pathology, Microbiology, Plant Protection, Molecular Biology and Plant Breeding. In addition, the extension plant pathologists in disease diagnostic centers and personnel of plant quarantine and certification programs will find the information to have practical utility. Presentation of several protocols appended as appendices in appropriate chapters will assist in selecting the right procedures for reaching their research targets. Uber die Geschlechtsorgane von Peronospora. The sociology of plant pathology. Crop Production and Crop Protection: The concepts of etiology in the history of plant pathology. Memore sur la cause immediate de al carie ou carbon des bles, et de plusieurs autres maladies des plantes, et sur les preservatifs de la carie. Dissertation sur la cause qui corrompt et noircit les grans de ble dans leseps; et sur les moyens de prevenir ces accidents. Chapter 2 Detection of Fungal Pathogens in Plants Abstract Among the microbial plant pathogens, fungus-like and fungal pathogens have well developed thallus consisting of hyphae, asexual and sexual reproductive structures. However, the formae speciales, strains, varieties or biotypes within a morphologic species have to be identified using other characteristics such as pathogenicity, biochemical and immunological properties or nucleotide sequences of the genomic DNA. Isozyme analysis, vegetative compatability group VCG analysis and electrophoretic mobility of cell wall proteins have been shown to be useful for the detection of strains of some fungal pathogens. The usefulness of immunoassays for early detection and precise identification has been significantly enhanced following the development of enzyme-linked immunosorbent assay ELISA and monoclonal antibodies which exhibit greater sensitivity and

specificity compared with isolation based methods which are laborious and time-consuming. Nucleic acid-based diagnostic techniques depending on the variations in the nucleotide sequences of the pathogen DNA have become the preferred ones, because of their greater speed, specificity, sensitivity, reliability, and reproducibility of the results obtained, following the development of polymerase chain reaction PCR. Several variants of PCR and commercial kits for on-site adoption under field conditions, away from the laboratory, are now available, providing the results in a short time. The possibility of detecting two or more pathogens simultaneously has become bright after the development of DNA array technology. A wide range of diagnostic techniques can be applied for detection, identification and quantification of fungal pathogens present in the infected plants, propagative plant materials and postharvest produce. Speed, specificity, sensitivity and cost-effectiveness are the primary factors that may determine the suitability and choice of the diagnostic tests. In many cases, the pathogens may be carried by seeds or propagative planting P. Whatever may be the source of inoculum, the susceptible plant species or crop varieties may exhibit clear visible local symptoms in or on the tissues where infection is initiated. If the pathogen is able to find favorable conditions for further development, systemic symptoms are induced in tissues or organs far away from the point of pathogen entry into the plant. When the symptoms of infection is not expressed externally, it is termed latent infection. Some fungal pathogens infecting unripe fruits do not induce any visible symptom, as they remain dormant. When the fruits begin to ripen, the pathogen proliferates, as the conditions become favorable resulting in the formation of characteristic symptoms. Such infection is known as quiescent which reflects the transient inactive stage of the pathogen. It is essential to recognize infection of plants by microbial pathogens as rapidly as possible, preferably before the appearance of symptoms to eliminate the infected plants or planting materials to avoid disease incidence and to restrict further spread of the disease s. In the case of several diseases, especially those infecting perennial woody plants, the first step to be followed for effective management of crop diseases, is the detection of microbial pathogens and diagnosis of the diseases caused by them, facilitating the elimination of infected plants and clonal materials. Detection of microbial pathogens refers to the process of establishing the consistent presence of a particular target organism s within the plant or in its environments, irrespective of the development of visible symptoms in the plant suspected to be infected by the pathogen s in question. Diagnosis, on the other hand, relates to the identification of the nature and cause of the disease problem under investigation. Pathogens may be able to infect a wide range of plant species or restricted to one or a few host species. Further, they may be either obligate parasites like pathogens causing powdery mildews, downy mildews or rust diseases requiring the presence of living plants for their development during their entire life cycle. On the other hand, most of the 2. They can lead a saprophytic life for short or long periods in the absence of their natural host plants. It is well known that a significant number of fungal pathogens can survive in the soil and infect the plants, when seeds are sown or young seedlings are planted in the pathogen-infested soil. Following infection of plants through roots, disease symptoms appear after a short or long incubation period during which no visible symptom is produced. Likewise, aerial plant parts such as leaves, stems, inflorescence and seeds may be infected by the pathogen propagules carried by wind. Fungal pathogens are disseminated by wind-borne fungal spores or bacterial cells. A comprehensive knowledge is essential on the nature of plant hosts and the manner in which the healthy plants get infected, in order to check the infiltration of host plant environment by different fungal pathogens. Maintenance of plant health to the desired levels is possible, if the presence of the microbial pathogen s in the crop plants, other plant species that can serve as sources of inoculum and other pathogen habitats, is detected, differentiated and quantified. Numerous methods based on biological, biochemical, immunological and molecular characteristics of the fungal plant pathogens have been developed to detect them in different plant sources with varying degrees of accuracy. The usefulness and the limitations of different detection techniques applicable to fungal pathogens are discussed in this chapter. Traditional methods, applied commonly earlier, involve the isolation of the fungal pathogens in suitable standard agar media and studying the cultural characteristics such as colony morphology, color and production of asexual structures like sporangia, conidia, chlamydospores, sclerotia etc. Light microscopes may be used to examine the presence of sporangia, conidia, pycnidia or acervuli.

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