Plant Disease Diagnostic Capabilities and Networks

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Plant Disease Diagnostic Capabilities and Networks

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plant pathogen surveillance, laboratory accreditation, standard operating procedures, phytosanitary, invasive pathogens, crop biosecurity

Abstract

Emerging, re-emerging and endemic plant pathogens continue to challege our ability to safeguard plant health worldwide. Further, globalization, climate change, increased human mobility, and pathogen and vector evolution have combined to increase the spread of invasive plant pathogens. Early and accurate diagnoses and pathogen surveillance on local, regional, and global scales are necessary to predict outbreaks and allow time for development and application of mitigation strategies. Plant disease diagnostic networks have developed worldwide to address the problems of efficient and effective disease diagnosis and pathogen detection, engendering cooperation of institutions and experts within countries and across national borders. Networking maximizes impact in the face of shrinking government investments in agriculture and diminishing human resource capacity in diagnostics and applied pathology. New technologies promise to improve the speed and accuracy of disease diagnostics and pathogen detection. Widespread adoption of standard operating procedures and diagnostic laboratory accreditation serve to build trust and confidence among institutions. Case studies of national, regional, and international diagnostic networks are presented.

INTRODUCTION

Why Diagnostics Matter

Pathogen detection: the identification of microorganisms or their products, e.g., toxins, in any number of substrates including plant tissues, soil, and water

Invasive species: exotic, nonnative organisms that negatively affect the habitats they colonize

Network: a large and distributed group of individuals or organizations that exchange information and work toward a common goal

It is well recognized that threats by invasive pathogens to plants, whether crops, horticultural commodities, or members of natural communities such as forests and grasslands, are increasing as a result of globalization, increased human mobility, climate change, and pathogen and vector evolution (2, 32, 66, 87, 104). Taken in total with damage caused by emerging, re-emerging (e.g., new races, pathotypes, forms resistant to pesticides or antibiotics), and chronic/endemic pathogens, the potential for economic loss is significant in plant systems (94). Although considerations of the economic, social, and environmental consequences of plant diseases have taken a back seat to concerns of infectious diseases of humans and animals, an appreciation of the potential destructiveness of plant diseases is beginning to be realized outside the plant pathology community (13, 49, 53). Food security is threatened in resource-poor countries during disease epidemics in staple crops and income generation from opportunities to exploit new and emerging markets is curtailed (26). In addition, crop failures contribute directly to malnutrition and indirectly to the spread of human infectious diseases and environmental damage as a result of displacement of the rural poor from farms that are no longer productive to crowded urban areas, forests, or marginal lands (2). Further direct effects on human and livestock health are caused by mycotoxins produced by plant pathogenic fungi such as *Aspergillus* and *Fusarium* species, which may contaminate food and feed, consumption of which results in a spectrum of diseases and disorders (28, 35, 46, 67).

Disease diagnosis and pathogen detection are central to our ability to protect crops and natural plant systems, and are the crucial prelude to undertaking prevention and management measures. The concept of integrated pest management (IPM) is predicated on the ability to detect and diagnose diseases and pests, which then informs management decisions. Failures in pathogen detection and disease diagnosis lead directly to inadequate disease control and reductions in crop production and quality, and hence trade. Trade is further negatively impacted by noncompliance with sanitary and phytosanitary (SPS) requirements of importing countries (20, 27, 57, 98, 102).

It is the responsibility of governments to protect agricultural and natural plant systems in their countries from invasive pathogens, while at the same time putting in place measures to prevent their own endemic pathogens from becoming invasive species in others. Although the private sector also has a responsibility to produce clean plant products, safeguarding of agricultural and natural plant systems from introduced pathogens is usually considered a public good, and therefore a role of the government (85). Because plant safeguarding may entail interception and mitigation of pest risk associated with the commodity, accurate and timely diagnosis is critical. There is also demand from producers—farmers, foresters, greenhouse managers, gardeners, etc.—for rapid and accurate diagnoses of pathogens to guide disease management decision making and issue of phytosanitary certificates (59, 61). As a result of the acknowledged shortage of trained field and clinical pathologists (72, 87, 91) and other necessary resources, a general reduction worldwide in government support of agriculture (85), and the inability or unwillingness of the private sector to pay the full cost, it has become necessary to find innovative ways to maximize the delivery of diagnostic services to agriculture. A capable disease diagnostic system or network should be viewed as a fundamental pillar to the development or maintenance of a plant health care service. Diagnoses must be combined with sustained surveillance that includes standardized and quantitative estimates of actual and potential impacts of disease in order to prioritize those requiring urgent attention. Targeted studies are required to determine epidemiological parameters and mechanisms of pathogenicity and virulence to enable the development of control interventions and to determine their efficacy both temporally and spatially (44, 94). Development of coordinated, robust diagnostic networks that share expertise and technical capacity toward a common goal offers a solution to resource limitations and an opportunity to improve the quality and quantity of these services. In 1995, van Halteren (99) called for development of a diagnostic network that would serve national plant protection services in Europe. The network, comprised of interdisciplinary working groups, would develop standardized diagnostic procedures, share expertise, and expedite the adoption or adaptation of new diagnostic techniques. Similar calls for formalization of such networks in other, primarily developed, countries were made early in the beginning of this century, in large part a response to concerns for agricultural biosecurity (6, 29, 64, 66). Furthermore, increased emphasis on agricultural trade as a means of poverty reduction in developing countries has drawn attention to the need for international capacity building and better coordination of diagnostic services (87). Since those initial calls, progress has been made toward the development of regional, national, and international plant diagnostic networks that address phytosanitary and/or disease management needs (38, 65, 72, 85, 90, 92, 100, 108). The goal of this review is to provide a perspective on the current status and future prospects of networks for plant disease diagnostics and pathogen detection worldwide. Although the critical importance of diagnostic services for all crop pests is acknowledged, an analysis of insect pest and weed diagnostics is beyond the scope of this review; however, it can be assumed that many of the issues considered here are relevant to all plant pest diagnostics.

DIAGNOSTICS FOR PLANT SAFEGUARDING AND DISEASE MANAGEMENT

Plant disease diagnosis is the determination of the cause of a disease or syndrome in a plant or plant population, whereas detection refers to the identification of microorganisms or their products, e.g., toxins, in any number of substrates including plant tissues, soil, and water. Surveillance generally refers to the monitoring of plant systems for pathogens and/or diseases. As aptly stated by Stack & Fletcher (91), "...surveillance is the process of searching, detection is the process of finding, and diagnosis is the process of determining and/or verifying what is found." Detection, surveillance, and diagnosis are all necessary components of programs designed to (*a*) safeguard plant systems from invasive pathogens and (*b*) identify causal agents of plant diseases as the first step toward disease management.

Plant Safeguarding and Biosecurity

The responsibility for safeguarding plants against invasive pathogens is held officially by national plant protection organizations (NPPOs). In addition to their regulatory functions, NPPOs conduct pathogen surveillance and pest risk analyses, inspect, treat, and certify export products, inspect and, if necessary, mitigate risks on imports and share information on pathogens and regulations. In the United States, for example, this responsibility is shared by the Department of Agriculture (USDA) and the Department of Homeland Security (DHS), in collaboration with individual state departments of agriculture. Regional organizations such as the North American Plant Protection Organization (NAPPO) and the European and Mediterranean Plant Protection Organization (EPPO) serve a coordinating function among member NPPOs. Official international actions to limit the introduction and spread of plant pathogens are facilitated by the International Plant Protection Convention (IPPC), through the Food and Agriculture Organization (FAO) of the United Nations. Currently 170 countries are signatories to the IPPC (**http://www.fao.org/Legal/TREATIES/004s-e.htm**), forming a de facto network under the umbrella of the IPPC Secretariat and operating under international standards for phytosanitary measures (ISPM). Contracting parties provide information regarding pest status to trading partners (84), using a Web portal for information

Phytosanitary: referring to plant health, particularly freedom from diseases and pests

Plant disease diagnosis: determination of the cause of a disease or syndrome in a plant or plant population

NPPO: national plant protection organization

Pathogen surveillance: the monitoring of plant systems for pathogens and/or diseases

EPPO: European and Mediterranean Plant Protection Organization

IPPC: International Plant Protection Convention

NDR: new disease report

LGU: land grant university

exchange (**https://www.ippc.int/IPP/En/default.jsp**). Surveillance of regulated pathogens is a critical component of plant safeguarding/biosecurity programs (53, 91, 104) and is regularly conducted in countries with sufficient resources (70, 74, 80, 91, 93). Some pathogens considered of high consequence because of their potential use as bioterrorism agents may not be on quarantine lists but should also be monitored (31). The cost of pathogen surveillance can be very high (70), and therefore many developing countries have a poor record of acquiring and updating accurate lists for pests within their borders, limiting their prospects for trade (87, 105).

This is particularly important in Africa, where the number of new disease reports (NDRs) has decreased over the past century in comparison to Europe, where they have dramatically increased (106). Furthermore, an analysis of the introduction of pest taxa and crops affected during the twentieth century was found to be broadly similar for the two continents, whereas the capacity to detect and identify pests was disparate (105). The consequence of this is that many diseases in sub-Saharan Africa simply spread without being recognized or controlled, leading to habitual losses in crop yield and quality. Although there are many national programs in Africa responsibly filing NDRs, in general there is a reliance on international agricultural research centers such as International Institute for Tropical Agriculture (IITA), in collaboration with international partners, including the Global Plant Clinic headquartered in the United Kingdom, to publish these reports. Although this process is almost universally perceived as a helpful intervention, it can occasionally lead to controversy, such as was the case for the diagnosis of banana xanthomonas wilt (BXW), caused by *Xanthomonas vasicola* pv. *musacearum* in Burundi. BXW is a new invasive disease in East Africa and can be misdiagnosed as Panama disease based on symptoms in the field. In this instance, poor in-country expertise prevented effective follow-up on the initial identification, which subsequently led to uncertainty as to the status of the disease and its continued presence in the country. This reinforces the need for active cooperation among governments and their pathologists within and between regions, and with advanced research institutes across the world.

Disease Management Decision Making

Disease diagnosis and surveillance of nonregulated pathogens to inform disease management decision making, particularly pesticide application, is generally outside the purview of NPPOs and is conducted by the private sector and/or public research or extension programs (53). It ranges in complexity from field scouting (12, 48, 51, 71) to large-scale, coordinated monitoring efforts (25, 56, 58, 90). In areas where computers and internet access are readily available and crop production systems are technically advanced, scouting is often combined with weather-based disease predictive systems. The success of such systems requires accurate disease diagnosis and/or pathogen detection, and can result in significant economic benefits, usually accrued from a reduction in pesticide misuse or increased yield and quality resulting from properly timed interventions (12, 48, 71). Portable diagnostic products—such as serologically based lateral flow devices (LFDs) (11, 23, 50, 52, 81, 82)—can facilitate on-the-spot diagnostics during field scouting, or samples may be sent to a public or private laboratory for diagnosis. Digital images can be sent to experts for diagnosis through specific networks or directly to extension personnel and other professionals via email (41, 90, 107). In the United States, most land grant university (LGU) plant diagnostic clinics have formal or informal mechanisms to receive and diagnose digital images of diseased plants.

Diagnostic networks are crucial in conducting large-scale monitoring programs; surveillance may be done by established networks or by networks organized for monitoring a specific pathogen and disbanded after completion of the program. The example of soybean rust illustrates the role of diagnostic networks in

Figure 1

Soybean rust (*Phakopsora pachyrhizi*) sentinel plot in Citra, Florida, with disease foci. Photo by C. Lapaire Harmon.

pathogen detection, diagnosis, and surveillance. *Phakopsora pachyrhizi*, causal agent of soybean rust, was initially listed as a select agent (indicative of a potential bioterrorist threat) before its recent entry into the United States. It is an economically devastating disease in Brazil, causing more than $1 billion in direct yield losses and added costs of fungicides in 2003 alone (109). Soybean rust development is heavily influenced by environmental conditions, plant age, and host species, and the disease has yet to cause significant losses in the United States soybean belt. However, the risk of catastrophic loss led to an almost unprecedented mobilization of the public and private sectors to develop a coordinated approach to meet this challenge (25, 58). Diagnostic tests including a real-time PCR assay (30), an immunofluorescence spore assay (5), and a field-usable lateral flow immunoassay (9) were developed and tested. Surveillance and monitoring were accomplished utilizing a network of sentinel plots (**Figure 1**) and spore traps (**Figure 2**), tied into Web-based reporting and communications. Thousands of farmers and agronomic professionals were trained as first detectors (76). Although a soybean rust epidemic has failed to materialize in the United States to date, the soybean industry has been spared millions of dollars in fungicide costs as a result of the availability of accurate disease forecasting based on pathogen surveillance and environmental data (78).

PCR: polymerase chain reaction

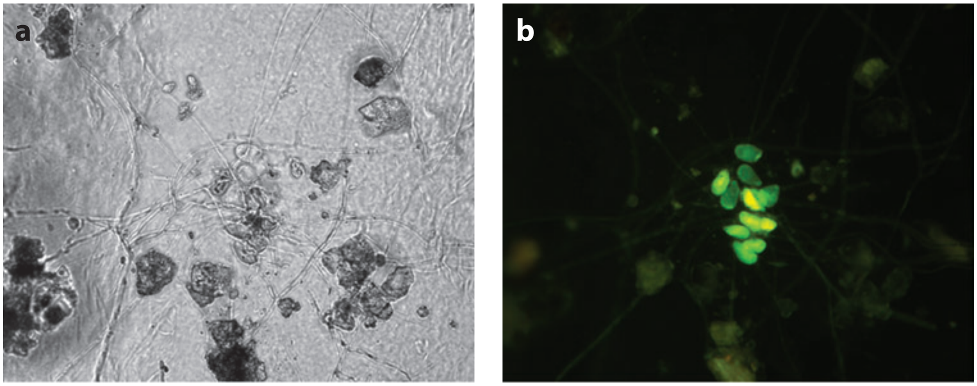

Figure 2

Immunofluorescence assay for urediniospores of the soybean rust pathogen *Phakopsora pachyrhizi* captured on a glass slide in an air sampler. (*a*) Bright field microscopy; (*b*) epifluorescence microscopy. Photographs by F. Baysal-Gurel, The Ohio State University.

DIAGNOSTIC CAPACITY

Human Resources

The quality of plant disease diagnostic services depends on the availability and quality of human capital, infrastructure, and technology. Although all three are not necessary for many routine diagnoses in which symptoms or signs are obvious, at least one must be of high quality to solve all but the simplest diagnostic problems. For example, common and easily recognized diseases are often diagnosed by an astute individual, who may be a trained diagnostician, experienced farmer, extension educator, or consultant familiar with the crop (53). However, unlike human and veterinary medicine, trained practitioners in plant pathology are a relatively rare commodity, and clinicians with appropriate training and access to necessary infrastructure and technology to diagnose the broad range of pathogens afflicting plants are particularly scarce. In a report issued in 2002 (85), Plant Health Australia assessed the status of human resources involved in plant diagnostics, concluding that significant gaps existed in the capacity to diagnose plant diseases and that strategic planning for staff development and succession was needed to ensure sufficient capacity in the future. A significant erosion in plant disease diagnostic capacity also occurred in the United States in the last quarter of the twentieth century as financial support of applied research and extension programs in LGUs dwindled and resources were diverted to fundamental research. Heightened concern for agriculture biosecurity in the United States after the terrorist attacks of 2001 and the anthrax scare was the motivating factor for increased government support of plant diagnostics. Increased investment in diagnostic clinic staffing and infrastructure and the development of the National Plant Diagnostic Network (NPDN) was the result (90). In the United Kingdom, until the late 1980s and early 1990s, the British government funded the Agricultural Development and Advisory Service (ADAS) and CABI to serve diagnostic needs of farmers in the United Kingdom and Commonwealth, respectively (15, 39). Changing government policy since then resulted in the demise of diagnostic services, reductions in expert staff and taxonomic training, and potential loss of valuable reference collections. This critical gap in taxonomy capacity was recognized recently as a serious constraint by a report of the House of Lords Science and Technology Committee (42).

Human resource development in plant diagnostics in the developing world has generally lagged behind that of developed countries,

NPDN: National Plant Diagnostic Network but it also appears to be declining (17, 87, 88, 105). Results of a Phytosanitary Capacity Evaluation administered from 2000–2003 in 36 developing countries showed that understaffing and lack of regular training programs were among the main limiting factors in implementation of the IPPC guidelines in these countries. Physical infrastructure and equipment ranked as lower priorities for enhancement in these surveys (17). However, diagnostic capacity is beginning to increase in India as plant health clinics are being established in the private and public sectors throughout the country (3, 10, 79), although coordination and networking of the clinics remains a goal (60, 89). Local and regional needs in taxonomic expertise, in relation to plant pathogen diagnosis and surveillance, can begin to be addressed by sharing resources and building capacity. BioNET-INTERNATIONAL (BioNET) is a global network launched in 1993 and comprised of locally organized and operated partnerships (LOOPs) networked regionally in the developing world. Capacity building programs within the LOOPs include developing information and communication services, taxonomic training, developing and maintaining culture collections, and developing or adapting new technology for identification (88).

Technology

Although capacity for traditional pathogen identification is generally insufficient to meet needs in both developed and developing countries, the creation of high-tech tools for plant pathogen diagnostics has expanded at a rapid rate (11, 83, 96, 103, 105). As mentioned above, field-ready serological tests such as lateral flow devices (LFDs) are commonly used as diagnostic tools to aid disease management decision making, to back up diagnoses based on symptoms, and as a triage tool to prescreen plants for specified target diseases (23, 45, 68, 87). LFDs and laboratory enzyme-linked immunosorbent assay (ELISA) kits are convenient and easy to use, and have been adopted widely in developed countries. For example, an LFD for *Phytophthora* spp. detection has proven to be of significant value in the United Kingdom for prescreening woody plants for the absence of *P. ramorum/P. kernoviae* at the time of inspection (50, 52). Samples testing positive are sent to a laboratory for follow-up testing with more specific lab tests such as PCR and pathogen culture. Tools developed commercially for use in developed countries are generally too expensive for the same crops in poorer countries. Serodiagnostics are being used in limited situations for specific applications, such as brown rot (*Ralstonia solanacearum*) testing of seed potatoes (75). Adoption of ELISA kits, LFDs, and potentially other assays may expand when they are produced locally or regionally and can therefore be affordably priced. For example, ELISA kits are being developed by India's Ministry of Science and Technology for virus disease diagnosis in high-value crops including ornamentals, fruits, and spices (33).

DNA-based assays, particularly PCR and real-time/quantitative PCR (qPCR), are being adopted in diagnostic laboratories; ordinary PCR testing for many pathogens is now routine and affordable (11, 24, 83, 96, 103) in the developed world. Quantitative PCR is more expensive than PCR as a result of high start-up costs and expensive materials, but it has been adopted for high-consequence pathogens, especially in regulatory circumstances, because of their high sensitivity and specificity (30, 55). Both types of assay, however, are out of reach of all but elite biotechnology laboratories in the developing world. Tests utilizing isothermal amplification techniques (reviewed in 11, 103) may prove to be less costly and thus more appropriate than current DNA-based methods in these countries in the future. It is encouraging that within the field of human health greater strides have been made to recognize the need for a strategy for high-impact diagnostics in the developing world (97). Urdea et al. (97) identified as crucial the need to develop diagnostic tests that can be performed at low-infrastructure sites that serve most of the global population, with support from more advanced labs, in order to identify where treatment is and is not required.

LFD: lateral flow device

ELISA: enzyme-linked immunosorbent assay

qPCR: quantitative (or real-time) PCR; sometimes referred to as RT-PCR

PCR assays, as with serological tests, are limited in the number of pathogens that can be tested at once, and thus a preliminary differential diagnosis must be done to narrow down the possible causes and reduce costs due to unnecessary testing. Considerable emphasis is now being placed on generic platforms such as DNA microarrays and sequencing (DNA barcoding), which will facilitate diagnosis of unknowns. Combinations of serological and nano- and nucleic acid detection technologies almost unimaginable a decade or two ago are under development for human diagnostics and may eventually make their way to plant disease diagnostic applications (18, 24).

Electronic technology is also finding its way into disease diagnostic and pathogen surveillance systems. In addition to digital systems widely available for distance diagnosis, several free forms of software can be used to map and share the presence of diseases. One such example is Google Earth, a GIS system that can be employed by registered users to update pest distribution. An application called RustMapper is being tested to map the distribution of wheat stem rust race UG99 across the world, coordinated by the International Maize and Wheat Improvement Center (CYMMIT) (19). Registered users of plant disease diagnostic network databases such as the University of Florida's Digital Diagnostic and Identification System (DDIS) also use Google Earth to map the distribution of diseases from samples received in network laboratories. In situations where internet access is lacking a novel alternative is to exploit the worldwide adoption of mobile phones to facilitate information exchange (34). The Grameen Foundation has established information services that include basic mobile phones for text messaging and more advanced technologies for increasingly detailed data exchange (4). Systems have been developed for information transfer to facilitate the provision of microfinance schemes and measures to enhance human and animal health. The application Farmer's Friend aims to provide advice on treating common pests and diseases and can include fertilizer recommendations and information on input suppliers, e.g., for resistant planting material or pesticides. A pilot study in collaboration with IITA is underway in Uganda to train rural community members in the use of mobile phones to collect and disseminate information on disease outbreaks and methods for their control. Community members will be distributed in a strategic way to form a geographical network that covers key agroecological zones and potential hot spots for the introduction of a crop disease. This network will be linked to centralized research communities allowing for bidirectional and dynamic flows of information that can be compiled in a SMS database accessible by phone users. The potential to exploit mobile phones to enhance field surveillance of disease outbreaks and the efficacy of recommended control options is massive and will help to bridge the current gap between science and practice. Furthermore, enhanced field surveillance through interventions such as this will permit NPPOs to recognize risks due to disease earlier and to deploy control measures to prevent catastrophic disease epidemics. Information communication technologies are already being deployed for human disease outbreaks (**http://instedd.org/mcp**), humanitarian crises (**http://ushahidi.com/**), and drought warning systems for livestock (**http://cnrit.tamu.edu/lews/description.html**). Moreover, GIS disease distribution maps could be overlaid with other data such as demographics, crop distribution, growing conditions, farming practices, calorific dependency on certain crops, and other biophysical characteristics and trade data. This would facilitate the possibility of predictive sensing of disease spread and risk at various geographic scales and could be used, for example, to determine the likely impact of climate change.

Infrastructure

Visual examination, microscopy, culturing, a few simple biochemical tests, and ELISA are the mainstays for most routine diagnoses in many laboratories. When coupled with diagnostic references such as disease compendia, pathogen-specific manuals, and image databases, these techniques in the hands of trained diagnosticians or specialists are sufficient to provide answers in a reasonable amount of time at a manageable cost. One of the critical components of a modern, highly functioning diagnostic laboratory is access to high-speed internet. Web-enabled microscopes permit laboratories to network with each other and with experts worldwide in real time to identify pathogens and other microorganisms morphologically (90). Many diagnostic networks build and maintain expertise databases within their communications systems to facilitate knowledge sharing. For example, EPPO supports a searchable database on its Web site containing a list of laboratories by country and an expertise list by pathogen (72). Experts can be contacted via email by network members upon entry of an access code. Access to up-to-date reference materials has been facilitated by open internet search engines such as Google (**http://www.google.com**), Google Scholar (**http://www.scholar.google.com**), and Google Book Search (**http://www.books.google.com**). This is particularly useful for giving advice following the diagnosis, as most extension fact sheets, newsletters, and similar materials are now freely available on the internet. Many scientific journals now provide free internet access to articles immediately or within several months to a year after initial release. Subscription databases such as AGRICOLA and BIOSIS can be readily accessed through institutional libraries, and institutions in many developing countries can download articles from hundreds of scientific journals free or at very low cost through AGORA, HINARI, TEEAL, and PERI electronic libraries.

Standardization and communication of laboratory practices and protocols is increasingly important as international commerce requires mechanisms to define and ensure safe and pathogen-free trade. Valid and internationally supported diagnostic methods must be employed to encourage trust in test results. Motivation for development and implementation of a laboratory quality assurance (QA) system may be client-driven or as a result of legislative action or other rule-making authority. Development of standard operating procedures (SOPs) is one step in a process to ensure reliable diagnosis; accreditation of laboratories is a process of assuring quality management within the laboratories. A laboratory must be able to document that procedures are applied in appropriate facilities and infrastructure, using appropriate and properly calibrated instrumentation, and by trained personnel (101). Plant Health Australia (64) noted their strategic plan to establish a diagnostic laboratory network must include a QA framework. Several models for QA exist; one being adopted internationally is ISO 17025 accreditation. Documentation, proficiency demonstration, and calibration and maintenance of instrumentation to the level required by ISO 17025 are time consuming and require resources to dedicate personnel to accomplish the documentation. Most plant diagnostic laboratories lack sufficient funding to accomplish the full accreditation to ISO 17025, but components of the system are applicable to even the most basic laboratories. The application of a flexible scope may be more appropriate than strict adherence to full IOS 17025 for laboratories that must respond to new and/or changing samples or procedures such as those in plant diagnostic laboratories (16). The Danish Plant Directorate described several advantages of the accreditation of plant laboratories, citing improved capability of personnel and increased quality of diagnostic work (95). New Zealand's Plant Health and Environment Laboratory processes the highest level of diagnostic samples and attained ISO 17025 accreditation in 2007 (1). All activities of the laboratory were covered by the QA system, but test types were defined broadly and were accredited just a few at a time. Additional resources were required to achieve even limited accreditation if the laboratory was to remain in service to its clientele. Given the client-based nature of extension plant diagnostic laboratories in the United States, and the large number of laboratories to be accredited,

QA: quality assurance

USDA APHIS: United States Department of Agriculture Animal and Plant Health Inspection Service

a tiered system is being developed, with regulatory accreditation as the highest tier. Laboratories that attain that level will be approved to process samples of a regulatory nature to assist the USDA Animal and Plant Health Inspection Service (USDA APHIS) during periods of sample surge and regional triage. USDA APHIS's federal diagnostics and confirmation laboratories will follow ISO 17025 accreditation to comply with international regulatory QA. Laboratory personnel in the United States Southern Plant Diagnostic Network (SPDN), a regional member of NPDN, acknowledged the value of a minimum standard for laboratory infrastructure with the development of a checklist in 2006 (14). The SPDN list of minimum standards for laboratory infrastructure, EPPO guidelines, and ISO 17025 accreditation standards are all being incorporated into the national System for True and Reliable Diagnostics (STAR-D), a system of tiered accreditation of plant laboratories in the United States in development by NPDN, APHIS, and Cooperative State Research, Education, and Extension Service (CSREES).

In many plant clinics in developing countries, accreditation is a long-term goal at best, as minimum standards are often met in name only, with physical infrastructure and equipment often in need of repair, computers and reference materials outdated, internet access slow and inconsistent, and basic materials and supplies difficult to come by. Our limited surveys of plant pathologists ($n = 41$) in East and West Africa conducted in 2006 and 2007 as part of a capacity analysis for the International Plant Diagnostic Network (IPDN; see below) demonstrated significant gaps in infrastructure throughout these regions. Half or more of the respondents considered core infrastructure components inadequate in their laboratories with the exception of laboratory space, electrical supply, and microscopes (West Africa). Addressing these core gaps, as well as inadequacies in human resources and access to technology in African and other developing countries, is complex but necessary to alleviate deficiencies that indirectly affect the livelihoods of millions of people. This should include enhanced training and networking to maximize capacity, which may include development of centers of excellence, according to national and regional needs and priorities (43, 77).

IITA Ibadan and the Biosciences eastern and central Africa (BecA) hub are examples of how more technically advanced equipment and staff can be pooled to provide a common bioscience research platform for a region. Research hubs such as Ibadan and BecA serve as providers, feeding a network of regional nodes and other laboratories. BecA is ideally suited to this role as it is the first of NEPAD's (New Partnership for Africa's Development; **http://www.nepad.org/**) continent-wide scientific centers of excellence. Its aim is to facilitate the use of cutting-edge science and technology in Africa by the continent's researchers to develop solutions tailored to African problems. The hub and nodes are suited to providing differing levels of diagnostic capacity: genomics, proteomics, immunology, bioinformatics, gene technology, and databases. The capacity for safe handling and storage of microorganisms would be ideal for the diagnostic network. Such facilities would provide a platform for capacity building to disseminate technologies developed to train scientists from both public and private sectors. The aim is for these advanced facilities to promote scientific excellence through links with the international scientific community in a cost-efficient manner that avoids unnecessary duplication of equipment, staff, and materials.

The Kenya Plant Health Inspectorate Services (KEPHIS) has been recognized among the NPPOs in eastern and southern Africa to serve as a center of excellence with the support of the African Union (AU), Inter-African Phytosanitary Council (IAPSC) and various donors (69). The Center for Phytosanitary Excellence (COPE) is funded by the World Trade Organization's Standards and Trade Development Facility (STDF), KEPHIS, and others, and aims to provide technical outreach through national and international collaboration with countries in eastern and southern Africa. Its goals are to strengthen the management of phytosanitary issues to improve plant resource protection and market access for African plant products. At the time of this writing, COPE has just completed the definition of a course curriculum and is in the process of becoming fully operational, but offers great potential for enhanced capacity building within the region. Furthermore, it offers the opportunity to develop active communication networks between national representatives toward a sustained system of phytosanitary capacity and regulation.

PLANT DISEASE DIAGNOSTIC NETWORKS

Although it is not possible to describe all of the networks of plant disease diagnostic laboratories and diagnosticians and other experts operating worldwide, several examples have been selected as case studies to demonstrate the benefits of networks in improving capacity, efficiency, and effectiveness in diagnostics. These networks share the goal of maximizing the ability of countries and multinational regions to improve their ability to prepare for and respond to threats to agricultural productivity and food security posed by plant pathogens, as well as to assist clientele in disease management decision making.

The United States National Plant Diagnostic Network

The USDA Cooperative State Research, Education, and Extension Service (CSREES) responded to homeland security risk assessments in 2001 by developing in 2002 the United States National Plant Diagnostic Network [NPDN (**http://www.npdn.org**)], a consortium of land grant university (and some state departments of agriculture) laboratories in the United States and its territories. The network functions and goals include increasing capacity in all labs to a minimum standard, training diagnostic personnel, standardizing diagnostic protocols, increasing communication between partners such as the CSREES, APHIS, university and state department of agriculture personnel, and training field personnel as first detectors (90). The NPDN is a hub and spoke system, with five regional centers each supporting a radius of state laboratories. Funding, training, administration, and sample surge support are directed from the regional center, and triage diagnosis, diagnostic data, and first detector training occur in the individual states. Diagnostic data are collected centrally in the NPDN National Repository at Purdue University, populating a secure and confidential database that will be investigated for epidemiological research and anomaly detection. The NPDN is standardizing diagnostics at all labs through the development of SOPs for high-risk and select agent pathogens in conjunction with regulatory partners. Nine SOPs for select agents are available on the NPDN Web site and seven more SOPs for select agents and other high-impact pests and pathogens are in development. In addition, diagnosticians are populating an online diagnostic cookbook at **http://wiki.bugwood.org/Diagnosticians_cookbook;** this resource is publicly accessible, but only approved personnel may write to the files, allowing for quality control and review. NPDN labs are involved in the development and testing of new diagnostic methods (47) and work with researchers and the national reference laboratory personnel to improve protocols for regulatory pests. Diagnosticians are trained to process suspect select agents by the USDA APHIS Center for Plant Health Science Technology (CPHST) method development laboratory personnel, increasing NPDN laboratory capability and further standardizing select agent processing. Additional training sessions held each year increase diagnostic personnel capability in techniques (PCR, virus detection, etc.) and specific pathogens and pests (*Fusarium* spp., stramenopiles, etc.). Technical training sessions occur in a traditional laboratory environment and through technological aids such as video-conferencing via AdobeConnect and streaming videos available on the NPDN websites. Further communication of protocols and diagnostic advice occurs between diagnosticians via email listservers, digital diagnostic services such as DDIS (**www.ddis.ifas.ufl.edu**) and PDIS (**www.pdis.org**), NPDN and American

Phytopathological Society (APS) committee work, and networking during regional and national meetings. The NPDN has developed a system of continuous improvement through the promotion of training and standardization opportunities; this system aids in the professional development of both experienced and newly hired diagnosticians and increases the capacity of plant laboratories in the United States.

Mediterranean and European Plant Protection Organization

Since its inception in 1951, the Mediterranean and European Plant Protection Organization (EPPO) has expanded from 15 to 50 member countries. EPPO is an intergovernmental organization responsible for cooperation in plant protection in the European and Mediterranean region. The aim of EPPO is to develop a strategy against the introduction of pests that damage cultivated and wild plants through the harmonization and promotion of appropriate methods for pest control. The need for a standardized scheme for detection and diagnosis of organisms that were harmful to plants was recognized as a priority by EPPO to improve transparency and comparability of data. Furthermore, standardized diagnostic protocols could be used as the basis for training exercises and to resolve trade disputes. By the turn of the last century, diagnostic protocols were developed for 18 quarantine pests (40). Efforts have since intensified with the result that diagnostic protocols have now been produced for 80 regulated pests (73).

Dissemination of information is a major output of EPPO's activities and includes a series of databases, scientific papers, bulletins, reports, and pest alert lists, which can all be accessed via the internet (**http://www.eppo.org**). The aim of EPPO to produce common standards and detailed information on quarantine pests is instrumental to the efficient and precise operation of NPPOs in Europe and the Mediterranean. Furthermore, recognition has been made of the crucial need to employ diagnosticians into NPPOs in the current environment of globalized trade and consumer—as opposed to producer—driven politics (100). The development of systems to harmonize the content of diagnostic protocols supports the issue of plant passports to facilitate trade in plants and plant products while imposing measures against correctly identified quarantine pests (110). To complement EPPO's initiative in compiling an inventory of diagnostic expertise (72), and to enhance cooperation and coordination between NPPOs in Europe and the Mediterranean region, guiding principles have been developed for the establishment of National Reference Laboratories (NRLs) in the European Union (65). The role of NRLs will be to ensure compliance with regulations for plant phytosanitary issues and to fulfill diagnostic responsibilities through more rapid adoption and transfer of diagnostic methodologies. Development of a system of NRLs would also provide a sustainable basis for the maintenance of expertise and reference material required for diagnosis. Key responsibilities of each NRL would be to act as national contact points, provide guidance on collection and handling of samples, provide confirmatory diagnoses, develop and validate diagnostic protocols, and provide training through collaboration with other NRLs. The priority organisms for NRLs to diagnose have been identified as those that are quarantine pests with potentially devastating impacts on plant health and trade, requiring regular testing employing complex diagnostic tests (65).

Plant Pest Management Network: Taiwan

Taiwan is a subtropical island nation with varying terrain, intensive agriculture systems, and an increasing number of agricultural imports raising the risk of introduction of exotic pathogens. Consequently, Taiwan has developed a comprehensive network of university and government diagnostic laboratories, research institutes, and field stations that serve the dual purpose of plant safeguarding and provision of diagnostic services for farmers (108). This vertically integrated network provides a stream of information from farmers to research, extension, and regulatory personnel, with management advice and, if necessary, eradication or other containment programs flowing back to the farmers. The tiered network is set up with the Bureau of Animal and Plant Health Inspection and Quarantine (BAPHIQ) at the apex, serving as a control center and organizing monitoring and surveillance programs, as well as cooperating with other institutions in investigations of high-impact pathogens (and other pests). There are eight regional monitoring centers (seven district agricultural improvement stations plus the Taiwan Tea Experiment Station) responsible for disease discovery, planning of management strategies, and disease reporting. Pathogens are identified by laboratories in four universities and the Taiwan Agricultural Research Institute (TARI). Diagnostic services are provided at 30 stations associated with universities, district agricultural improvement stations, private organizations, TARI, and the Taiwan Agricultural Chemicals and Toxic Substances Research Institute (TACTRI). TACTRI also serves as an information management center for the network. Finally, local governments distribute plant disease information to farmers and work with local farmers and farmer organizations in the event of a potentially damaging disease outbreak. This system provides a means of passive disease monitoring and early notification of the presence of unusual or regulated pathogens through its close interactions with farmers.

Global Plant Clinic

The Global Plant Clinic (GPC) is a consortium of CABI Bioscience, Rothamsted Research, and Central Science Laboratory, United Kingdom. The GPC provides a cost-free diagnostic and advisory service for NPPOs in developing countries that provide diseased plant samples. This service has resulted in the publications of 40 NDRs over the last 8 years. In 2001, the GPC initiated the establishment of mobile plant health clinics in several developing countries (7, 10). Plant health clinics fulfill an advisory role in a cost-efficient and locally operated manner. They occur in public places such as markets on a regular basis where growers routinely arrive with diseased plant samples (**Figure 3**). The clinics offer reliable advice on routine plant health problems affecting any crop and differentiate symptoms due to abiotic and biotic stresses. Many of the plant doctors who manage them are agronomists or extension workers in existing grassroots organizations. Plant doctors are trained in symptom recognition, provision of control recommendations (prescriptions), clinic management, and data collation by GPC and its network of partners. Farmers benefit from the management advice at clinics and in turn provide the doctors with surveillance on new and emerging diseases and of the efficacy of prescriptions to control disease. This direct advice averts losses by quick action, often reducing pesticide use and losses due to disease. Furthermore, new and emerging diseases are identified and can be diagnosed through links to GPC in the United Kingdom. By 2008, 71 plant health clinics were routinely operating in nine developing countries worldwide. In Nicaragua, the plant health clinics have developed into a nationwide network in which technical backup is provided by experts in universities, research centers, diagnostic labs, and regulatory services (22).

The GPC increased awareness of new diseases such as Napier grass stunt in East Africa through the likes of Going Public exercises and the production of fact sheets that clearly demonstrate symptoms and control options (8). A further example of publicity campaigns to help recognize the symptoms of disease and methods for their control was adopted for cassava mosaic disease and banana bacterial wilt in six East African countries in a project coordinated by Catholic Relief Services (CRS) and involving IITA. This project was particularly successful because it recognized the need to develop a network through promotion of collaboration between regional associations and agricultural institutes, country-level agricultural research organizations, and local implementing partners (21). There is a wealth

Figure 3

Ugandan farmers wait to be seen by plant doctors at rural plant clinic in Katine market, Soroti. The clinic is staffed by personnel from the NGO, Soroti Catholic Diocese Integrated Development Organization (SOCADIDO), and extension staff from the Ministry of Agriculture. Photo by Rob Harling of the Global Plant Clinic.

of literature addressing how best to inform farmers, and a range of participatory learning processes have been tested including farmer field schools (44). Although it is recognized that one of the most urgent needs is, and perhaps always has been (97), to remove the major bottleneck between science and practice, it is not within the scope of this review to do justice to the complexity of this issue. Suffice to say that examples such as use of SMS technology and plant health clinics need to be further developed to provide an active interface between extension and farmers. Furthermore, it must be recognized that extension will only be precise and practically relevant if linked to current research knowledge through a direct working relationship with laboratories with the capacity to diagnose new and emerging plant pathogens.

The International Plant Diagnostic Network

The International Plant Diagnostic Network [IPDN (**http://www.intpdn.org**)] was initiated in 2005 with the goal of fostering development of local capacity for diagnostics through establishment of communication and data sharing networks, training in classical and modern diagnostics and research into new diagnostic methods (62). The program is funded by the United States Agency for International Development (USAID) under the Integrated Pest Management Collaborative Research and Development Program (IPM CRSP). The IPDN is modeled after the United States NPDN, and as with that network, is comprised of regional hub and local satellite diagnostic laboratories. Three regional programs have been established to date; in Central America (hub lab in Guatemala coordinated by a private company; Agroexpertos), East Africa (hub lab in Kenya coordinated by Kenya Agricultural Research Institute), and West Africa (hub lab in Benin coordinated by IITA). For each regional program satellite laboratories in three additional countries have been linked to hub laboratories. Each hub and satellite laboratory is linked to several national institutions and laboratories, creating a regional network and by default increased capacity. All of the laboratories have access to the Clinic Information Management System/Digital Distance Identification System (CIMS/DDIS) internet portal developed by the University of Florida (107). This portal allows data entry, storage and retrieval for samples received, sharing of digital images within the system and with outside experts, and storage and retrieval of management recommendations. Sample information remains confidential within a country, or when indicated, between the submitter and the diagnostician. An important objective of the IPDN is training diagnosticians in basic and advanced diagnostic methodologies. Working groups within the regional programs are developing SOPs that will be appropriate for local capacity and validated within the region. Reporting new diseases through international outlets is highly encouraged. For example, the West Africa regional program recently reported for the first time the occurrence of *Ralstonia solanacearum*, the cause of a devastating wilt disease of tomato, throughout Benin (86).

SUMMARY POINTS

1. Globalization of trade, human mobility, climate change, pathogen and vector evolution, and political instability combine to create a global environment that is increasingly fraught with risks to food security and income generation. The social and economic consequences of the failure to recognize, contain, and/or control threatening pathogens require that every effort be made to engage in efficient and effective programs of surveillance, diagnosis, and detection. The fact that threats are many and resources few points to the increasing importance of diagnostic networks that can rapidly and precisely identify causal organisms of disease as a crucial first step towards the deployment of control and/or mitigation strategies. This requires the networking of human and technical capacities from field to basic to advanced laboratories, rapid and secure communications, and exchange of information with governments and policy makers. National and regional plant diagnostic networks have materialized during the past 10–15 years, bolstered by the communications revolution made possible by the internet. Although globalization and free-trade agreements have made it easier for pathogens to cross borders, the internet has opened global avenues for access to databases, communication, and cooperation not thought possible decades ago. Without this innovation we would be unlikely to organize effective networks to meet the challenge of invasive pathogens.
2. The diagnosis of plant diseases and detection of pathogens rely on a diverse range of technologies from traditional taxonomy to advanced molecular methods. Laboratory ELISAs are widely adopted, but these and other advanced diagnostic technologies are often inaccessible to laboratories in the developing world because of their relatively high cost. Field-portable serological assays such as LFDs continue to be adopted for diagnosis of specified pathogens primarily in high value crops. Such devices can be used to confirm

Threatening pathogen: invasive or indigenous pathogen able to cause significant crop losses in yield or quality, resulting in negative health, economic, and/or social consequences the identity of causal agents in disease outbreaks to guide rapid disease management decision making. This technology continues to evolve with pull-through from human and veterinary disease diagnostics, and it is hoped that more sensitive and specific devices are developed for a range of pathogens that can be deployed in the field. Similarly, advanced generic high-throughput platforms for molecular technologies will continue to develop and be more widely adopted as costs decrease and applicability to diagnosis of unknowns increases. Although technology such as DNA barcoding is reliant on the use of PCR, it is relatively simple and cheap, and unlike traditional molecular methods, can be used to diagnose unknown samples to the species level and even enhance the discovery of new species. It is somewhat ironic that as new advanced technologies such as DNA barcoding evolve there is still reliance on more traditional methods such as morphological identification to validate taxonomy. However, this should not be overlooked, as any technology used for diagnoses, irrespective of how technically advanced, will always be dependent on morphological studies. The two disciplines should ideally operate in cohort for any system of diagnostics to remain effective and of practical relevance. This is particularly important bearing in mind that systematics will forever remain a dynamic science, as it reflects the dynamism of natural ecological systems and pathosystems. Systematics is essential to the study and communication of plant pathology, diagnostics, and disease control (63). Therefore, as diagnostic capability through networks is developed, the pivotal role of morphological identification and systematics must not be ignored.

3. As a corollary to the above, it must be noted that capacity in traditional taxonomic methods, as well as in applied plant pathology, is diminishing worldwide. Although the NPDN system in the United States is an excellent model where advice is provided to farmers as a responsibility of academic institutions, such robust systems are in decline or even lost in many developed nations and are yet to materialize in most developing countries where national research programs operate without clear linkages to national extension services. Without the presence of astute individuals in agricultural communities, the ability to discover and document the presence and movement of pathogens is highly compromised if not impossible. Effective diagnostic networks are vertically integrated, from well-trained first responders such as farmers and extension personnel in the field to diagnosticians and specialists capable of providing management advice. Secure and trusted communication must travel in both directions. Internet-based communication networks can facilitate sharing of information horizontally within and between networks nationally, regionally, and globally. The increased capacity generated from this exchange of information can be used to update new disease reports, optimize surveillance strategies, and develop and modify pest risk analyses. This will alert plant quarantine officers of potential new threats and to deploy preemptive control options under the auspices of agreed intergovernmental policies.

4. It is imperative that the impacts of specific crop diseases are assessed and diseases prioritized per crop, country and region, and in relation to key trading partners. Assessment of risk due to each existing and potential plant pathogen will help to develop appropriate contingency plans for sampling strategies in terms of technical requirements and frequency of samples to be analyzed. Sampling must be performed in a cost-efficient and representative manner. Sustained disease surveillance and diagnosis of prevalent pathogens in any single cropping system, with information dissemination via networks, is crucial to plant safeguarding globally.

5. Trust is an essential component of the relationship between the sample submitter (farmer, extension agent, inspector, etc.), the plant disease diagnostician, the government entities with sovereign responsibility for plant safeguarding and government networks with regional responsibility. The availability of and adherence to validated standard operating procedures engender confidence in the validity of the diagnosis. Similarly the production of NDR must be considered as a positive step towards national and regional responsibility in order to support international trade and to enable agriculture to become a driver of development in resource-poor countries. Laboratory accreditation plays a key role, although it is clear that accreditation should be flexible in that the degree to which laboratories should be held to specific standards should depend on their roles within the system.

6. The gap in capacity to deliver plant disease diagnostic services between developed and developing countries is significant. Developing countries consistently lag behind developed ones in creation and adaptation of new technology and networks to enhance delivery of disease diagnostic services. It is foremost the responsibility of governments to support diagnostic capacity within their sovereign borders and to nurture relationships with other neighboring countries and elsewhere in the world, especially where similar crops or diseases exist. Whether by using their own resources or the assistance of development oriented donors, governments need to provide sufficient subsidies to support diagnostic capability as a critical component of a plant health care system. Eventually the private sector may contribute to disease diagnostics but not realistically in the short term, so the responsibility remains primarily with governments to provide support for an impartial diagnostic service. Such a service must provide increased capacity at field level to recognize symptoms of common diseases and extension techniques that are client oriented and adapted to demographic profiles. Where this is not possible because of new and emerging diseases, a system of interconnected laboratories with a range of technologies will increase the precision and speed of diagnosis. Effective delivery of plant disease diagnostic services will not be achieved without substantial growth in human, technological, and infrastructure capacity, including improvements in the supply chain that allow timely access to affordable laboratory materials and reagents. Local or regional production of such materials, including diagnostic assays, will substantially decrease costs and increase laboratory output and reliability. A potential bright spot is the widespread adoption of mobile phones and initiatives to utilize them as platforms for rapid and targeted information exchange related to disease surveillance and management. The prospect of connecting farmers to each other and to plant doctors, extension personnel, and researchers to diagnose plant diseases and exchange advice on management, while also developing a network of eyes on the ground to enhance discovery and monitoring of critically important plant diseases, is truly exciting.

7. Although plant diagnostic services have traditionally been underfunded compared with similar systems for animals and humans, concerns arising in this century for food security have brought needed attention by governments to this arena. As a consequence, networks have been established or strengthened in both developed and developing countries to address phytosanitary, biosecurity, and disease management issues associated with the diagnosis of diseases and detection and surveillance of pathogens. From a network of plant health clinics operating in markets in Nicaragua to coordinated diagnostic laboratories in Europe, Asia, and the United States, these systems share the goal of providing the highest possible level of service to clientele.

FUTURE ISSUES

1. The probability that a particular pathogen will be used as an agent of bioterrorism is low, but the threat is nonetheless real (6). It is much more likely that pathogens capable of causing severe economic and social problems will continue to be introduced inadvertently. NPPOs must make every effort to safeguard plants within their country's borders, but it is also crucial to prepare for these pathogens preemptively, so that they can be managed effectively when they do arrive. An initiative such as "Predict and Prevent" (36), currently directed toward human and animal diseases, would be timely and appropriate for plant systems as well. Such an initiative would involve a global effort in early detection of emerging diseases (identifying hot spots), monitoring pathogen movement, and preparing mitigating responses well in advance. Enhanced networking of diagnostic and research laboratories and expert plant pathologists beyond their own borders and sharing information on a global scale will be necessary to accomplish this goal. Communications tools made possible by the internet are available to enhance interactions among the appropriate partners. The recent NATO project "Tools for Crop Biosecurity" was a foray into cross networking, developing a broad multinational consortium representing the United States, the European Union, and Israel in the Eastern Hemisphere Plant Diagnostic Network (38). Such consortiums have value in bringing like-minded scientists together to promote dialogue and information exchange. It is hoped that such dialogue will form the basis for, or at least encourage, interactions between NPPOs in order to foster formation of official ties with other countries and regions.
2. In this age of shrinking support for agricultural programs worldwide, the issue of sustainability of diagnostic systems and networks, in fact of plant health programs in general, is of utmost concern. All too often, governments and funding agencies do not commit to sustained support of a program, regardless of its success. It is likely that the private sector, at least in the developed world, will need to play a larger role in support of diagnostic networks. This may mean a greater willingness to pay more of the cost for diagnoses and advice, although in the experience of most public diagnostic laboratories, when the private sector is asked to pay more, the sample volume decreases. It is, however, encouraging that protection of plant-based agriculture from invasive pathogen threats has been recognized and investments made in recent years. It will be necessary to continue to stress the need for public support. Donors to programs in developing countries expect that sponsored programs will become institutionalized under public funding or adopted by the private sector. Both alternatives have merit, but the latter is unlikely in the near term at least. Therefore, governments in these countries will need to recognize the importance of both safeguarding natural and agricultural systems and assisting farmers in developing disease management practices that maximize income generation, and prioritize these areas for support. Centers of excellence will provide a means of increasing regional capacity for disease diagnosis and pathogen detection at reasonable cost. It is important that these centers establish strong interconnecting linkages that ultimately tie them directly to farmers. This approach will help to direct their efforts toward the most important issues and thereby maximize their impact across the region.
3. Investments in human resource capacity are desperately needed in both developing and developed countries. The shrinking supply not only of taxonomists but of diagnosticians and plant disease management specialists who are linked directly to farmers erodes

capacity to discover threatening pathogens and meet the challenges of managing them. Graduate programs must continue to train students in applied plant pathology; however their training must encompass not only traditional disease diagnosis and management but provide a good foundation in molecular biology to prepare them to develop, adapt, and/or adopt the new technologies that will become available to agricultural systems in the future.

4. New and exciting diagnostic technology for plant pathogens will continue to develop, as there is sufficient demand for diagnostics in the field of human and veterinary medicine to drive innovation. The number of organizations developing diagnostic technology for plant-based agriculture is small, but nonetheless, selective adaptation of medical technology will take place—the driving factors being applicability and cost (including intellectual property considerations). Many laboratories have adopted ELISA and ordinary PCR for routine use, whereas the highest tier laboratories, including centers of excellence and those responsible for identification of regulated pathogens, are currently using qPCR on a routine basis, usually under appropriate SOPs. Inexpensive field tests such as LFDs should be developed that can precisely diagnose new disease outbreaks to rapidly stimulate decision-making processes for disease management. The next phase in technology adoption is likely to be a move towards generic platform technology such as microarrays. Microarray technology is currently too costly for wide utilization for plant pathogen diagnosis, but as the price for development of diagnostic chips reduces and demand increases, adoption rates will also increase. Local or regional development and manufacture of diagnostic assays in poorer regions of the world will be necessary to keep costs low enough for adoption of the technology. Supply chain improvements that increase the ease of access to and cost of basic diagnostic supplies and reagents are critical to technology adoption as well. The developing world is leading the way in adaptation of mobile phone technology for information exchange, and innovations in this area to enhance disease diagnosis, surveillance, and management may find their way into agricultural production systems in developed countries. Whatever the platform, diagnostic technologies will continue to advance, and the extent of their applications will be driven by ease of use, cost, and the implications of the results they produce. Tests that directly inform a decision, whether it is the implementation of phytosanitary measures, change in cultural practices or application of a particular fungicide, for example, are the most likely to be adopted.

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LITERATURE CITED

1. Alexander BJR, Flynn AR, Gibbons AM, Clover GRG, Herrera VE. 2008. New Zealand perspective on ISO 17025 accreditation of a plant diagnostic laboratory. *EPPO Bull.* 38:172–77
2. Anderson PK, Cunningham AA, Patel NG, Morales FJ, Epstein PR, et al. 2004. Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnological drivers. *Trends Ecol. Evol.* 19:535–44
3. Anonymous. 2007. KVK to open plant health clinic. *The Hindu*, Feb. 26. **http://www.hindu.com/2007/02/26/stories/2007022602912100.htm**
4. AppLab: Transforming lives through innovation in information access. **http://www.grameenfoundation.applab.org/section/index**
5. Baysal-Gurel F, Lewis Ivey ML, Dorrance A, Luster F, Frederick R, et al. 2008. An immunofluorescence assay to detect urediniospores of *Phakopsora pachyrhizi*. *Plant Dis.* 92:1387–93
6. Bénoliel I. 2008. European Commission's green paper on bio-preparedness. See Ref. 37, pp. 135–39
7. Bentley JW, Boa ER. 2004. Community plant health clinic: an original concept for agriculture and farm families. Online. Global Plant Clinic, CABI, Wallingford, UK. **http://www.jefferybentley.com/clinic.pdf**
8. Bentley JW, Boa E, Van Mele P, Almanza J, Vasquez D, Eguino S. 2003. Going Public: a new extension method. *Int. J. Agric. Sust.* 1:8–23
9. BNET. 2006. EnviroLogix introduces its much-anticipated field test for soybean rust. *Business Wire*, Sep. 12. **http://findarticles.com/p/articles/mi_m0EIN/is_2006_Sept_12/ai_n26983534**
10. Boa E. 2007. Plant healthcare for poor farmers: an introduction to the work of the Global Plant Clinic. APSnet Feature, **http://www.apsnet.org/online/feature/clinic/**
11. Boonham N, Glover R, Tomlinson J, Mumford R. 2008. Exploiting generic platform technologies for the detection and identification of plant pathogens. *Eur. J. Plant Pathol.* 121:355–63
12. Bounds RS, Podolsky RH, Hausbeck MK. 2007. Integrating disease thresholds with TOM-CAST for carrot foliar blight management. *Plant Dis.* 91:798–804
13. Brownlie J, Peckham C, Waage J, Woolhouse M, Lyall C, et al. 2006. *Foresight. Infectious Diseases: Preparing for the Future—Future Threats*. London: Office of Science and Innovation
14. Bush E, Hansen M. 2007. Recommendations of the Southern Plant Diagnostic Network (SPDN) Infrastructure Committee. **http://www.intpdn.org/SPDN_PDC_infrastructure.pdf**
15. CABI. 2008. Our history. **http://www.cabi.org/datapage.asp?iDocID=235**
16. Camloh M, Dreo T, Zel J, Ravnikar M. 2008. The flexible scope of accreditation in GMO testing and its applicability to plant pathogen diagnostics. *EPPO Bull.* 38:178–84
17. Canale F. 2003. Phytosanitary capacity evaluation: the tool, its results and its relation to alien invasive species. See Ref. 42a, pp. 186–90
18. Chan PC, Cheung Y, Renneberg R, Seydack M. 2008. New trends in immunoassays. *Adv. Biochem. Engin. Biotechnol.* 109:123–54
19. CIMMYT International Maize and Wheat Improvement Center. 2008. Ug99—RustMapper. **http://www.cimmyt.org/gis/rustmapper/**
20. Coulibaly O, Hell K, Bandyopadhyay R, Hounkponou S, Leslie JF. 2008. Economic impact of aflatoxin contamination in Sub-Saharan Africa. See Ref. 54, pp. 67–76
21. Crop Crisis Control Project. 2008. Objectives. **http://c3project.iita.org/Objectives.aspx**
22. Danielsen S. 2008. Local plant health clinics pave the way for a nation-wide health care system for plants in Nicaragua. *J. Plant Pathol.* 90:S2.25.3 (Abstr.)
23. Danks C, Barker I. 2000. On-site detection of plant pathogens using lateral-flow devices. *EPPO Bull.* 30:421–26

24. Darling JA, Blum MJ. 2007. DNA-based methods for monitoring invasive species: a review and prospectus. *Biol. Invasions* 9:751–65
25. Dorrance AE, Schneider R, Hershman D, Geisler L, Draper M. 2005. Extension response to Asian soybean rust, *Phakopsora pachyrhizi*. *Phytopathology* 95:S144 (Abstr.)
26. FAO. 2006. *The state of food insecurity in the world (SOFI): eradicating world hunger—taking stock ten tears after the world food summit*. Rome, Italy: FAO, UN. **http://www.fao.org/docrep/009/a0750e/a0750e00.htm**
27. Federal Register. 2002. Part V Department of Agriculture, Animal and Plant Health Inspection Service, 7 CFR Part 331,9 CFR Part 121, Agricultural Bioterrorism Protection Act of 2002: Possession, Use and Transfer of Biological Agents and Toxins; Interim Final Rule
28. Fink-Gremmels J. 2008. The impact of mycotoxins in animal feeds. See Ref. 54, pp. 155–68
29. Fletcher J, Stack JP. 2007. Agricultural biosecurity: threats and impacts for plant pathogens. See Ref. 53, pp. 86–94
30. Frederick RD, Snyder CL, Peterson GL, Bonde MR. 2002. Polymerase chain reaction assays for the detection and discrimination of the soybean rust pathogens *Phakopsora pachyrhizi* and *P. meibomiae*. *Phytopathology* 92:217–27
31. Gamliel A. 2008. High consequence plant pathogens. See Ref. 37, pp. 25–36
32. Garrett KA, Dendy SP, Frank EE, Rouse MN, Travers SE. 2006. Climate change effects on plant disease: genomes and ecosystems. *Annu. Rev. Phytopathol.* 44:489–509
33. Global Knowledge Center on Crop Biotechnology. 2006. PK 22: Plant disease diagnostics. Philippines: ISAAA. July 2006
34. Global Mobile Suppliers Association. **http://www.gsacom.com/**
35. Gong YY, Turner PC, Hall AJ, Wild CP. 2008. Aflatoxin exposure and impaired child growth in West Africa: an unexplored international public health burden? See Ref. 54, pp. 53–66
36. Google. 2008. Predict and prevent: identify hot spots and enable rapid response to emerging threats. **http://www.google.org/predict.html**
37. Gullino ML, Fletcher J, Gamliel A, Stack JP, eds. 2008. *Crop Biosecurity: Assuring Our Global Food Supply*. New York: Springer. 148 pp.
38. Gullino ML. 2008. Scope of the project. See Ref. 37, pp. 11–14
39. Hardwick NV. 1998. Whither or wither extension plant pathology? *Plant Path.* 47:379–93 (Br. Soc. for Plant Path. Pres. Address 1997)
40. Harju VA, Henry CM, Cambra M, Janse J, Jeffries C. 2000. Diagnostic protocols for organisms harmful to plants. *EPPO Bull.* 30:365–66
41. Holmes GJ, Brown EA, Ruhl G. 2000. What's a picture worth? The use of modern telecommunications in diagnosing plant diseases. *Plant Dis.* 84:1256–65
42. House of Lords. 2008. Science and Technology Committee, fifth report pf session 2007–2008, Systematics and Taxonomy: follow up. *HL Paper 162*. 386 pp.
42a. IPPC. 2003. Identification of risks and management of invasive alien species using the IPPC framework. *Proc. Worksh. Invasive Alien Species Int. Plant Prot. Conv. Braunschweig, Germany, Sept. 22–26*. Rome: FAO
43. IPPC Secretariat. 2008. Considerations for developing a phytosanitary capacity building strategy for developing countries. *Proc. Open-ended Working Group on Building National Phytosanitary Capacity, Dec. 8–12, Rome*. **https://www.ippc.int/IPP/En/default.jsp**
44. James B, Neuenschwander P, Markham RH, Anderson P, Braun A, et al. 2003. Bridging the gap with the CGIAR Systemwide Program on Integrated Management. In *Integrated Pest Management in the Global Arena*, ed. K Maredia, D Dakouo, D Mota-Sanchez, pp. 419–34. Wallingford, UK: CABI International
45. Ji P, Allen C, Sanchez-Perez A, Yao J, Elphinstone JG, et al. 2007. New diversity of *Ralstonia solanacearum* strains associated with vegetable and ornamental crops in Florida. *Plant Dis.* 91:195–203
46. Jolly PE, Jiang Y, Ellis WO, Sheng-Wang J, Afriye-Gyawu, Phillips TD, Williams JH. 2008. Modulation of the human immune system by aflatoxin. See Ref. 54, pp. 41–52
47. Jurick W, Harmon CL, Marois J, Wright D, Lamour K, et al. 2007. A comparative analysis of diagnostic protocols for detection of the Asian soybean rust pathogen, *Phakopsora pachyrhizi*. *Plant Health Progress* doi:10.1094/PHP-2007-0531-01-RS
48. Keinath AP, Dubose VB, Rathwell PJ. 1996. Efficacy and economics of three fungicide application schedules for early blight control and yield of fresh tomatoes. *Plant Dis.* 80:1277–82

49. King DA, Peckham C, Waage JK, Brownlie J, Woolhouse MEJ. 2006. Infectious diseases: preparing for the future. *Science* 313:1392–93
50. Kox LFF, van Brouwershaven IR, van de Vossenberg BTLH, Van Den Beld HE, Bonants PJM, et al. 2007. Diagnostic values and utility of immunological, morphological and molecular methods for in planta detection of *Phytophthora ramorum*. *Phytopathology* 97:1119–29
51. Krupinski JM, Bailey KL, McMullen MP, Gossen BD, Turkington TK. 2002. Managing disease risk in diversified cropping systems. *Agron. J.* 94:198–209
52. Lane CR, Hobden E, Walker L, Barton VC, Inmana JA, et al. 2007. Evaluation of a rapid diagnostic field test kit for identification of *Phytophthora* species, including *P. ramorum* and *P. kernoviae* at the point of inspection. *Plant Pathol.* 56:828–35
53. Lemon SM, Hamburg MA, Sparling PF, Choffnes ER, Mack A, eds. 2007. *Global Infectious Disease Surveillance and Detection: Assessing the Challenges—Finding Solutions*. Washington, DC: The National Academies Press. 264 pp.
54. Leslie JF, Bandyopadhyay R, Visconti A, eds. 2008. *Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade*. Wallingford, UK: CABI Int. 464 pp.
55. Li W, Hartung JS, Levy L. 2007. Evaluation of DNA amplification methods for improved detection of "*Candidatus* Liberibacter species" associated with citrus huanglongbing. *Plant Dis.* 91:51–58
56. Main CE, Keever T, Holmes GJ, Davis JM. 2001. Forecasting long-rage transport of downy mildew spores and plant disease epidemics. **http://www.apsnet.org/onlne/feature/forecast/**
57. Marasas WFO, Gelderblom WCA, Shephard GS, Vismer HF. 2008. Mycotoxins: a global problem. See Ref. 54, pp. 29–40
58. Magarey RD, O'Hern C, Royer M, El Lissy O. 2005. USDA coordinated framework for soybean rust. *Phytopathology* 95:S144 (Abstr.)
59. McCartney HA, Foster SJ, Farrije BA, Ward E. 2003. Molecular diagnostics for fungal plant pathogens. *Pest Manag. Sci.* 59:129–42 (online: 2003) DOI: 10.1002/ps.575
60. Mehta N. 2008. Development of plant health clinics in India—status, strategies and challenges. *J. Plant Pathol.* 90(S2):51.3 (Abstr.)
61. Miller SA. 1995. Plant disease diagnosis: biotechnological approaches. In *Molecular Methods in Plant Pathology*, ed. RP Singh, US Singh, pp. 461–73, Boca Raton, FL: CRC Press/Lewis Publishers
62. Miller SA, Momol MT, Tolin SA, Gilbertson R, Beed FD, et al. 2006. Development of the International Plant Diagnostic Network (IPDN): a multinational collaboration. *Phytopathology* 96:S79 (Abstr.)
63. Miller SE. 2007. Commentary: DNA barcoding and the renaissance of taxonomy. *Proc. Natl. Acad. Sci. USA* 104:4775–76
64. Moran J, Muirhead I. 2002. Assessment of the current status of the human resources involved in diagnostics for plant insect and disease pests. **http://www.planthealthaustralia.com.au**
65. Müller P. 2008. Development of national reference laboratories in the European Union. *EPPO Bull.* 38:195–97
66. Myerson LA, Reaser JK. 2002. Biosecurity: moving toward a comprehensive approach. *Bioscience* 52:593–600
67. Okioma MN. 2008. The 2004 and 2005 aflatoxin tragedies in Kenya. See Ref. 28, Vol. 11, pp. 127–36
68. Opina N, Miller SA. 2005. Evaluation of immunoassays for detection of *Ralstonia salanacearum*, causal agent of bacterial wilt of tomato and eggplant in the Philippines. *Acta Hortic.* 695:56
69. Otieno W. 2008. Discussion paper for OEWG on National Capacity Building
70. Pearson A. 2008. An independent review of New Zealand's biosecurity surveillance systems—plants. Biosecurity New Zealand. **http://biosecurity.govt.nz/pests-diseases/surveillance-review/plants.htm**
71. Peres NAR, Souza NL, Furtado EL, Timmer LW. 2004. Evaluation of systems for timing of fungicide sprays for control of postbloom fruit drop of citrus in Brazil. *Plant Dis.* 88:731–35
72. Petter F. 2008. Introduction to the EPPO Workshop on quality assurance for plant pest laboratories: EPPO's involvement in quality assurance. *EPPO Bull.* 38:167–68
73. Petter F, Roy AS, Smith I. 2008. International standards for the diagnosis of regulated pests. *Eur. J. Plant Pathol.* 121:331–37

74. Pheloung P. 2005. Contingency planning for pest incursions in Australia. See Ref. 42a, pp. 166–74
75. Priou S, Gutarra L, Aley P. 1999. Highly sensitive detection of *Ralstonia solanacearum* in latently infected potato tubers by postenrichment enzyme-linked immunosorbent assay on nitrocellulose membrane. *EPPO Bull.* 29:117–25
76. Rane KR, Ruhl GE. 2007. NPDN and the purdue plant and pest diagnostic laboratory—partners in protecting Indiana agriculture. *Proc. NPDN Natl. Meet.* **http://www.plantmanagementnetwork.org/proceedings/npdn/2007/posters/36.asp**
77. Ransom L. 2008. Australia—Discussion paper for OEWG on national capacity building
78. Roberts MJ, Schimmelpfennig D, Ashley E, Livingston M. 2006. The value of plant disease early-warning systems. *United States Department of Agriculture Economic Research Service Report 18*, 38 pp. **http://www.ers.usda.gov/publications/err18/err18.pdf**
79. Rohtaki N. 2008. Plant health clinic in P'kula soon. *Express India*, Jun. 28, **http://www.expressindia.com/latest-news/plant-health-clinic-in-pkula-soon/328613/**
80. Royer, M. and Bowers, J. 2007. Plant health: pest identification. United States Department of Agriculture Animal and Plant Health Inspection Service. **http://www.aphis.usda.gov/plant_health/plant_pest_info/pest_detection/index.shtml**
81. Salomone A, Roggero P. 2002. Host range, seed transmission and detection by ELISA and lateral flow of an Italian isolate of Pepino mosaic virus. *J. Plant Pathol.* 84:65–68
82. Salomone A, Mongelli M, Roggero P, Boscia D. 2004. Reliability of detection of citrus tristeza virus by an immunochromatic lateral flow assay in comparison with ELISA. *J. Plant Pathol.* 86:43–48
83. Schaad NW, Frederick R, Shaw J, Schneider WL, Hickson R, et al. 2003. Advances in molecular-based diagnostics in meeting crop biosecurity and phytosanitary issues. *Annu. Rev. Phytopathol.* 42:305–24
84. Secretariat. 2007. Recommendations of the EPPO workshop on pest reporting, Lyon, FR. **http://archives.eppo.org/MEETING/2007_meetings/lyon/Pest_Reporting.htm**
85. Sheldrake R, Williams M, Turner R. 2003. Developing a world class plant pathology diagnostics network. **http://www.planthealthaustralia.com.au**
86. Sikirou R, Beed F, Ezin V, Gbèhounou G, Miller SA, Wydra K. 2009. First report of bacterial wilt of tomato (*Solanum lycopersicum*) caused by *Ralstonia solanacearum* in Benin. *Plant Dis.* 93:549
87. Smith JJ, Waage J, Woodhall JW, Bishop SJ, Spence NJ. 2008. The challenge of providing plant pest diagnostic services for Africa. *Eur. J. Plant Pathol.* 121:365–75
88. Smith R. 2003. BioNet: a regional approach to capacity building in taxonomy for sustainable development. See Ref. 17, pp. 229–33
89. Srivastava MP. 2008. The plant health clinic—a global perspective. *J. Plant Pathol.* 90 S2:25.15 (Abstr.)
90. Stack JP, Cardwell K, Hammerschmidt R, Byrne J, Loria R, et al. 2006. The national plant diagnostic network. *Plant Dis.* 90:128–36
91. Stack JP, Fletcher J. 2007. Crop biosecurity infrastructure for disease surveillance and diagnostics. See Ref. 53, pp. 95–106
92. Stack JP, Hammerschmidt R, Hudler G, Luke E, Bostock R, et al. 2007. National Plant Diagnostic Network: a record of accomplishment. CSREES five-year review. **http://www.npdn.org/Library/ViewDocument.pdf?filetype=pdf&DocumentId=6431**
93. Staff Reporter. 2007. 2007 plant protection survey report. Canadian Food Inspection Agency. **http://www.inspection.gc.ca/english/sci/surv/sit2007e.shtml**
94. Strange RN, Scott PR. 2005. Plant disease: a threat to global food security. *Annu. Rev. Phytopathol.* 43:83–116
95. Thrane C. 2007. Quality assurance in plant health diagnostics—the experience of the Danish Plant Directorate. *Eur. J. Plant Pathol.* 121:339–46
96. Tinivella F, Gullino ML, Stack JP. 2008. The need for diagnostic tools and infrastructure. See Ref. 37, pp. 63–71
97. Urdea M, Penny LA, Olmsted SS, Giovanni MY, Kaspar P, et al. 2006. Requirements for high impact disease diagnostics in the developing world. *Nature* S1:73–79. doi:10.1038/nature05448
98. USDA. 2004. New pest response guidelines *Ralstonia solanacearum* race 3 biovar 2 southern wilt of geranium. USDA, APHIS, PPQ Pest Detection and Management Programs. Riverdale, MD

99. van Halteren P. 1995. A diagnostic network for the EPPO region. *EPPO Bull.* 25:1–4
100. van Halteren P. 2000. Diagnostics and national plant protection organizations. *EPPO Bull.* 30:357–59
101. van Opstal N, ed. 2007. Basic requirements for quality management in plant pest diagnosis laboratories. *EPPO Bull.* 37:580–88
102. Verstraete F. 2008. EU legislation on mycotoxins in food and feed: overview of the decision making process and recent and future developments. See Ref. 54, pp. 77–100
103. Vincelli P, Tisserat N. 2008. Nucleic acid-based pathogen detection in applied plant pathology. *Plant Dis.* 92:660–69
104. Waage JK, Mumford JD. 2008. Agricultural biosecurity. *Philos. Trans. R. Soc. London B* 363:863–76
105. Waage JK, Woodhall JW, Bishop SJ, Smith JJ, Jones DJ, Spence NJ. 2009. Patterns of plant pest introductions in Europe and Africa. *Agric. Sys.* 99(1):1–5
106. Waage JK, Woodhall JW, Bishop SJ, Smith JJ, Jones DJ, Spence NJ. 2006. T15: patterns of new plant disease spread: a plant pathogen database analysis. See Ref. 13
107. Xin J, Beck HW, Halsey LA, Fletcher JH, Zazueta FS, Momol T. 2001. Development of a distance diagnostic and identification system for plant, insect and disease problems. *Appl. Eng. Agric.* 17:561–65
108. Yeh Y. 2008. Current status and development of plant pest information management system in Taiwan. **http://www.agnet.org/library/eb/582/**
109. Yorinori JT, Paiva WM, Frederick RD, Costamilan LM, Bertagnolli PF, et al. 2005. Epidemics of soybean rust (*Phakopsora pachyrhizi*) in Brazil and Paraguay from 2001–2003. *Plant Dis.* 89:675–77
110. Žlof V, Smith IM, McNamara DG. 2000. Protocols for the diagnosis of quarantine pests. *EPPO Bull.* 30:361–63

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Errata

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