ANNUAL REVIEW OF PHYTOPATHOLOGY

VOLUME 33, 1995

HISTORICAL PERSPECTIVES

DIAGNOSIS AND APPRAISAL OF PLANT DISEASE

FUNGI, BACTERIA, MOLLICUTES, NEMATODES, AND VIRUSES AS PLANT PATHOGENS

ABIOTIC STRESS FACTORS

MORPHOLOGY, ANATOMY, AND PHYSIOLOGY

BIOCHEMISTRY AND MOLECULAR BIOLOGY

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BIOLOGICAL, PHYSICAL, AND CHEMICAL CONTROL

ANNUAL REVIEW OF PHYTOPATHOLOGY

VOLUME 33, 1995

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ANNUAL REVIEWS INC.
Palo Alto, California, USA

International Standard Serial Number: 0066–4286
International Standard Book Number: 0–8243–1333-x
Library of Congress Catalog Card Number: 63-8847

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Typesetting by Kachina Typesetting Inc., Tempe, Arizona; John Olson, President; Marty Mullins, Typesetting Coordinator; and by the Annual Reviews Inc. Editorial Staff

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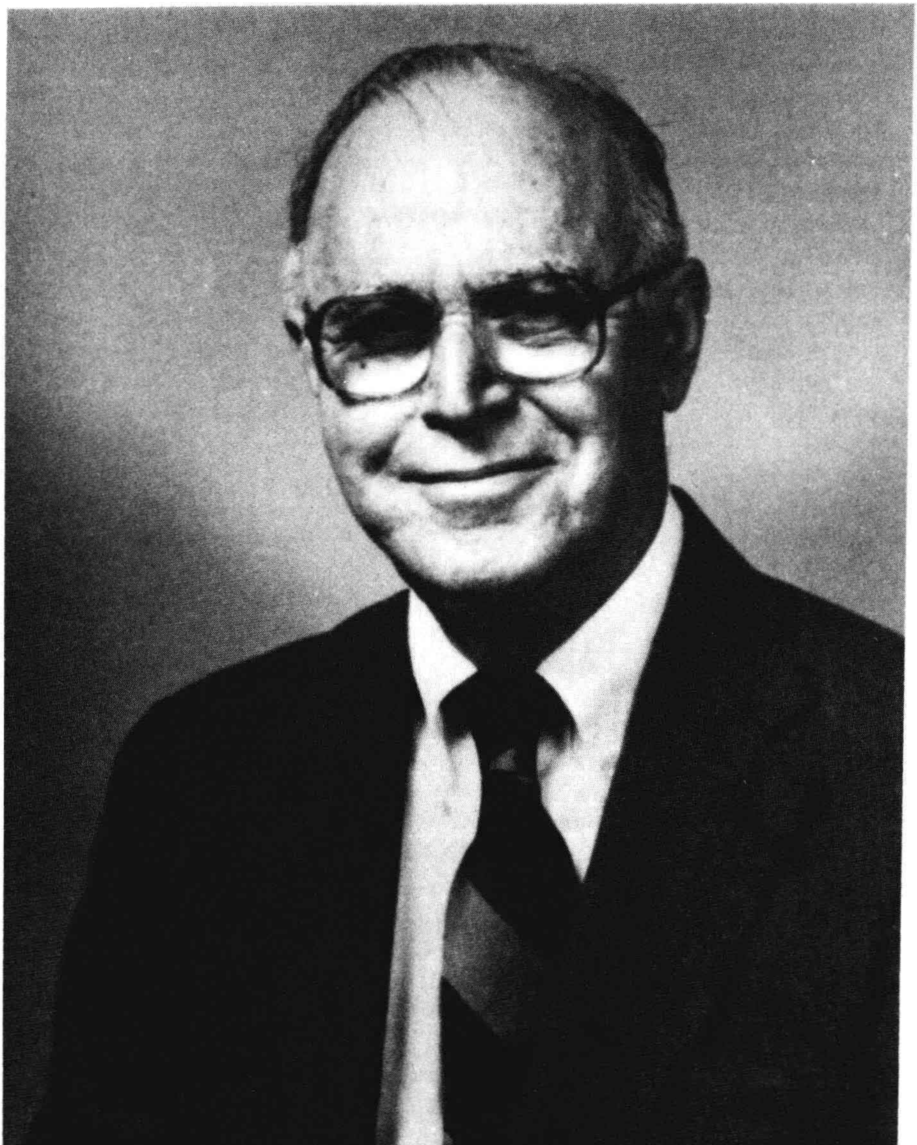

Arthur Kelman

Annual Review of Phytopathology
Volume 33, 1995

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Annu. Rev. Phytopathol. 1995. 33:1–21

CONTRIBUTIONS OF PLANT PATHOLOGY TO THE BIOLOGICAL SCIENCES AND INDUSTRY[1]

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KEY WORDS: viruses as plant pathogens, spiroplasma, ice-nucleation positive bacteria, effect of plant diseases on breeding strategies, Wisconsin fast plants

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ABSTRACT

Research in plant pathology has made major contributions to knowledge of basic biology and genetics of plants and microorganisms as well as to development of new products for industry. Among examples cited are first evidence of the nature of viruses as agents of disease; development of density gradient ultracentrifugation, a powerful tool for research in virology and cell biology; studies on the unique properties of xanthan gum, the extracellular polysaccharide of a plant pathogenic bacterium; development of a method for introduction of beneficial genes into plants via a tumorigenic bacterium minus its tumor-inducing capability; and discovery that epiphytic ice-nucleating bacteria can trigger frost damage in plants.

INTRODUCTION

This is a period when the dark clouds of decreased funding create pressures for reassessment of priorities in both basic and mission-oriented research in the agricultural sciences and land grant universities (22). Members of Con-

[1]Adapted from Chapter 4, "Contributions of Plant Pathology to the Biological Sciences," in *Historical Perspectives in Plant Science*, ed. Kenneth J Fry. 1994. Ames, IA: Iowa State Univ. Press

0066-4286/95/0901-0001$05.00

gress, growers, and representatives of commodity groups as well as public interest groups with special agendas in the area of sustainable agriculture are all questioning the benefits of current research programs and are seeking rapid solutions, not long-term basic studies. Numerous regional and national conferences and workshops have been convened to discuss whether current research objectives are designed to meet the long-term needs of our society. Our professional society (APS) is also seeking to establish priorities for future research in plant pathology and to assess the advances in increasing crop productivity and effective control of plant diseases (14, 40, 59). The specific contributions of plant pathology have also been well documented in recent symposia and reports. Emphasis has been placed on the potential for future progress in effective control of disease that will contribute not only to increased productivity, but also to sustainable agricultural systems and an increased concern for environmental quality (14, 15, 40, 59). However, the specific advances in related basic and applied biological and agricultural sciences as well as certain industries that have received their impetus from studies of plant diseases have not been enumerated or described in detail. Rarely has in-depth consideration been given to the positive contributions of plant pathology to other fields of science and to some industries that have been established directly or indirectly from the studies on plant diseases and their causal agents.

Furthermore, when we introduce students to the study of plant pathology, we usually emphasize how dependent our science is on mathematics, the physical sciences, the basic biological sciences, and to a lesser degree on other agricultural sciences (1). The manner in which these areas impinge on the science and art of plant pathology is reasonably well recognized and documented. The general historical development of the field of plant pathology has been thoroughly reviewed in a large number of texts and articles (37, 43, 61, 68) and in numerous articles in the *Annual Review of Phytopathology*. Thus, this article aims to examine the significant contributions to other disciplines and, to a limited extent, to new industries that have developed from studies on mechanisms of pathogenesis and biology of plant pathogens. Selected examples are cited to indicate how studies on the stresses that diseases impose on plants have provided new insights on plant growth and development. It is proposed that these findings may not have been discovered in investigations solely concerned with normal processes in plants. In evaluating the benefits arising from research in plant pathology, it is important to consider not only those areas viewed to be the normal responsibility of our science, but also to expand our perspectives and boundaries. Thus, the examples selected will serve to illustrate how plant pathology serves as a key contributor to basic understanding of biological systems and the nature of disease in all organisms, and how it contributes to other fields in agriculture and industry in ways not fully recognized.

THE GERM THEORY OF DISEASE

The earliest written records provide vivid evidence of the devastating impact of plant diseases on mankind (11, 43, 61, 68). In the absence of either any explanation for destructive epidemics of plant diseases or adequate means of developing control measures, it was only natural that these outbreaks would be attributed to supernatural forces. In numerous biblical references, outbreaks of crop diseases such as blight, mildews, and rusts were interpreted as manifestations of the wrath of a supreme being and as a punishment for immoral behavior. The Romans offered sacrifices to Robigus, a god who had to be propitiated to prevent the rust that periodically ravaged the wheat fields of Rome and its territories. In the Middle Ages the dread effects of eating bread made from rye infected by the ergot fungus came to be called the Holy Fire and then Saint Anthony's Fire. For several centuries thousands of people in central and western Europe died because of this plant disease. They suffered extreme agony from the hallucinogenic effects and gangrene that resulted from restriction of movement of blood to extremities after ingestion of the various alkaloids present in bread made with ergotized grain (11, 61).

Another pervasive belief was that disease in plants and animals resulted from the machinations of an evil spirit. Thus, in King Lear Shakespeare mentioned the foul fiend, Flibbertigibbet, who "mildewed the white wheat" among other evil deeds (61). As more knowledge was obtained, the concept evolved that diseases resulted mainly from adverse environmental factors; numerous observations indicated close correlations between disease incidence and specific extremes of temperature and moisture as well as adverse soil conditions.

All students of microbiology and general biology have learned about the classic contributions of Robert Koch and Louis Pasteur in establishing the germ theory of disease (9). However, many decades before these discoveries, other scientists interested in the nature of plant diseases had clearly demonstrated that specific microorganisms caused disease in plants. Thus, early investigators are truly the unrecognized heroes of microbiology and medicine. The leaders in science and medicine early in the last century did not accept the fact that the microorganisms present in the diseased tissue of plants and animals actually could be the causal agents. Finally, acceptance of these revolutionary new ideas brought within the grasp of humans the potential ability to control disease in man, animals, and plants and to enhance the quality of life for mankind.

Many scientists contributed to the establishment of the concept that disease in plants could be caused by parasitic microorganisms (37, 61, 68). The first critical experimental evidence that fungi can cause disease in plants was presented in 1803 by IB Prevost in his study of covered smut of wheat, and after a lapse of half a century by HA de Bary. In 1853, at the age of 22, he published a comprehensive paper on the rusts and smuts as causes of disease in plants; this paper has become a classic in the field of plant pathology (33). Note that this publication was issued about two decades before Koch published his classical studies on the anthrax disease of cattle. De Bary was not afraid to take a stand; he characterized as "inexact and based upon illusion" the reports of the two leading distinguished proponents of the concept that fungi in diseased plants arose by spontaneous generation. With the publication of his initial paper and subsequent books and journal articles, de Bary became recognized as a world leader in mycology, and students from many countries came to work with him. Many of his 68 students established centers for research in the new field of plant pathology in the United States and other countries.

One of de Bary's major contributions resulted from his investigations on late blight disease of potato and presentation of evidence that the cause was a fungus. Few other diseases have had as devastating an effect as the major epidemics of this disease in Ireland, western Europe, and the USA in 1845 and in years thereafter (11, 43). It was a source of great concern both politically and scientifically that a plant disease of this magnitude could not be controlled and that no one really knew what the cause was although many theories were proposed. The initial effects in Ireland were death by starvation and disease of over 1 million people and the mass migration to Canada and the United States of another 1.5 million refugees. This was unequivocal evidence of the helplessness of mankind in coping with a disease affecting a major food crop, and it served as a major incentive for research on this and related diseases (11, 43, 61).

In a survey of available major texts on the history of biological sciences, particularly microbiology (9), it is disconcerting that the real pioneers in the establishment of the germ theory of disease are rarely even mentioned. However, it is difficult to believe that the leaders in the effort to establish the germ theory of disease in man were totally unaware of the publications on the nature of disease in plants. In addition, these early studies probably aided in creating the intellectual climate in which the landmark studies of Robert Koch and Louis Pasteur would be acceptable.

Paradoxically, many scientists in Germany, the very country in which the germ theory of disease for plants(fungi), animals, and humans (bacteria) was given solid grounding, were unwilling to accept the evidence that bacteria could cause disease in plants. From this circumstance there arose a very famous controversy (1897–1901) between the leading researcher on bacterial disease of plants in the United States, Erwin F Smith, and a senior German scientist, Alfred Fischer (10). The publicity given to this acrimonious debate undoubtedly hastened acceptance of the evidence that plant disease could be caused by bacteria as well as fungi. It undoubtedly also gave impetus to the developing field of microbiology as a science that would lead to control of the diseases of mankind.

VIRUSES AS AGENTS OF DISEASE

The demonstration that the tobacco mosaic disease was caused by a virus probably had greater impact on other sciences, particularly molecular biology and human medicine, than any other finding in studies on plant diseases (36, 53). In 1882, Adolf Mayer completed the first experiments that conclusively demonstrated that a disease of tobacco, which he named mosaic (TMV), was caused by a transmissible biologic agent. The next key experiments were completed by Dimitri Iwanowski in 1892. In addition to confirming Mayer's work, he completed a critical experiment in which he demonstrated that the causal agent could pass through a filter that prevented the passage of bacteria. Six years later it was Martinus Beijerinck who realized the remarkable nature of TMV and was perhaps the first to suggest that it should be called a "virus." There has been some controversy over which of these early investigators should be designated the first to recognize the novel nature of the causal agent of the tobacco mosaic disease. Recently, Bos (5) proposed that the credit should go to Mayer rather than Iwanowski. Lustig & Levine (50) selected 1892, the date of publication of Iwanowski's paper on TMV, as the starting point for 100 years of virology, but Bos provided valid reasons for setting this date ten years earlier, the publication in 1882 of Mayer's research. This was based on the fact that, two decades after Mayer's work was published, Iwanowski still insisted that TMV was caused by a microorganism small enough (in some resting or spore stage) to pass through filters that prevented the passage of typical bacterial cells.

Three decades were to pass, however, before the true nature of the plant viruses was recognized (5, 50, 53). In 1935, Wendell Stanley, a biochemist working with a group of plant pathologists, reported in *Science* that he could obtain protein crystals from juice from infected plants and that he could reproduce symptoms of TMV with these preparations. In Judson's (36) fascinating text, *The Eight Days of Creation*, he presents a historical account of the seminal discoveries of molecular biology. He evaluated Stanley's contribution as "the most portentous and publicized biological discovery of the decade." The idea that a living, self-reproducing entity could be a crystallizable protein captured the imagination of scientists and laymen alike.

Major advances in research often follow and build on the discovery of new simple techniques. Thus, Stanley's work as well as subsequent studies by many other virologists were greatly dependent on the finding of Francis Holmes, a plant pathologist, that TMV caused hypersensitive reactions, "local lesions" in certain resistant plants. This finding made it possible in each of the purification steps or treatment procedures to determine the concentration of TMV in a given preparation. Although Stanley received great acclaim for his discovery, including the Nobel Prize in chemistry, there was a major flaw in his conclusion that the crystal of TMV was a large protein. Two years after Stanley's paper was published, Bawden & Pirie, in the plant pathology group at the Rothamsted Agricultural Experiment Station in England, published the evidence that TMV was a nucleoprotein (3). Harrison (27) notes with respect to this important paper by Bawden & Pirie that "not only were their observations and conclusions correct, but, more remarkably, the accuracy of their quantitative estimates have scarcely been bettered by more modern techniques after more than fifty years." The ease with which large quantities of TMV could be obtained, its stability, high degree of infectiousness, and related properties made it the model of choice for basic studies in virology. These initial studies of a plant virus opened the door for research in the field of general virology and virus diseases of humans and animals.

The true significance of the presence of RNA as a component of viruses was demonstrated in the elegant experiments reported by Frankel-Conrat & Williams in 1955 and Gierer & Schramm in 1956. They demonstrated independently that the naked RNA of TMV, free of its protein coat, was the infectious agent that entered the cell and initiated replication of new virus. The protein served as a protective shell around the RNA (53).

When James Watson decided to learn about the techniques of crystallography in his work with Francis Crick at the University of Cambridge, he selected TMV as the model structure to be examined initially in X-ray diffraction studies. At the end of about six months on the project, he obtained some first-rate results and observed the helical arrangement of the viral subunits. Thus, his own early observations on a plant virus may have enhanced progress in developing the concept of the helical structure of DNA (36).

Density Gradient Ultracentrifugation

Among other techniques that emerged from studies on plant viruses and that contributed to advancing the field of general virology was the procedure developed by Myron Brakke while he worked with Lindsay Black at the Brooklyn Botanic Garden (6–8). This technique, density gradient centrifugation, was described by Matthews (53) as "one of the most influential developments in virology and molecular biology." Although the method had a high potential for wide application, it did not become widely used until almost a decade after the paper describing the technique was published in 1951. Brakke (7) attributed this delay to the fact that few biochemists read the literature on plant viruses and that initial studies on fractionation of subcellular components were still in their infancy. However, after the value of the technique was recognized by several prominent animal virologists, it became a standard procedure in hundreds of research laboratories (7). This is evident from the numerous citations in the literature that appeared after 1960. In 1970, Beckman Instruments published a bibliography covering the period from 1960–1970 of all the papers in which this technique was used; 100 pages of citations were listed.

Although few other contributions in plant virology compare in importance with these early contributions, studies on plant viruses continue to contribute to the advancement of the fields of general virology and molecular biology (8). Recent studies on the movement of viruses in plant tissues have revealed a remarkable interaction between viruses and host cells that results in modifications in how plasmodesmata function (49). These studies are another example of how continuing research on plant viruses expands our knowledge of normal structure and function in plants.

MYCOPLASMA-LIKE ORGANISMS (MLOs) AND SPIROPLASMAS AS DISEASE AGENTS

For many decades plant pathologists were frustrated in their studies on a large group of "yellows" diseases of plants because they were unable to characterize the nature and structure of viruses presumed to be the causal agents (51, 71). The mystery associated with these diseases was in part resolved when Doi and associates, working in Japan, recognized that wall-less prokaryotes were present in the phloem of plants considered to be affected by yellows viruses (19, 51). Doi's discovery was in part accidental; he was working in an electron microscope facility that was also being used by Kaoru Koshimuzu, a veterinarian. Koshimuzu saw Doi's electron photomicrographs and noted a remarkable similarity of the MLOs in these preparations to the mycoplasma in cells of diseased birds that he was studying. Although it was shown that these organisms in plants were highly sensitive to certain antibiotics, they could not be cultured and to this day, defy the best efforts of all who have tried to do so (46). Thus they continue to be described as mycoplasma-like organisms (MLOs). Although over 300 diseases are now considered to be caused by MLOs, some investigators think even after three decades of study that only a small percentage of the total number of these organisms present in nature has been isolated, identified, and described.

Closely linked to the studies on MLOs was the discovery of a previously undescribed group of organisms, the spiroplasmas, by Robert Davis (16). Davis was attempting to determine the nature of the causal agent of the corn stunt disease and sought other means than the electron microscope to examine the structure of the causal agent. When phase contrast and dark field microscopy were used, he found, to his surprise, that crude extracts from corn stunt plants contained the remarkable tiny spiral-shaped motile cells that he named spiroplasmas (16). Subsequent examination of cultures of the pathogen of citrus stubborn disease revealed that this organism was also a spiroplasma. Since that time a large number of spiroplasmas have been identified; these include pathogens of insects and animals and many different saprophytic forms that appear to be widely distributed on plants. Thus, a new area of scientific specialization has evolved as the study of these strange prokaryotes has been expanding in recent years.

DISCOVERY OF OTHER PREVIOUSLY UNDESCRIBED ORGANISMS OR AGENTS OF DISEASE

In addition to the recent developments in studies on mycoplasma-like organisms, other organisms and pathogens new to science have been discovered in connection with investigations of several diseases in which specific causal agents had not been identified. A full discussion of the research that has followed recognition of these pathogens is beyond the scope of this paper; however, a few examples can be cited

The potato spindle tuber disease is one example of a disease that was presumed to be caused by a virus, but no specific virus had been found in infected plants. The causal agent, a viroid, was discovered by Diener (17, 18). Viroids are unique in that they are the smallest of all known agents of disease; they lack the protein coat that characterizes plant viruses and exist as tiny naked single-stranded RNA molecules. As yet, only a few other diseases of plants have been found to be caused by viroids, and their potential importance as agents of disease in man remains to be determined. However, the discovery of viroids has opened a new area of research on mechanisms of disease induction since viroids do not behave in plant cells in the same manner as viruses and apparently are able to induce changes in metabolism of host cells by interference with gene regulation.

For many years a serious disease of grapevines known as Pierce's disease was thought to be caused by a virus, but, as was the case with potato stunt disease, the specific causal agent could not be isolated or characterized. The causal agent is a fastidious xylem-limited prokaryote now classified in the genus *Xylella* (31). Many different woody plants are susceptible to strains of this pathogen, including some forest trees that show symptoms of decline. Diseases of this type will provide a fertile new area for future study on mechanisms of wilt induction and the relationship of stress factors to disease development in woody plants.

In efforts to explain the appearance of plaques in cultures of a plant pathogenic pseudomonad in the absence of a phage, Stolp (63) discovered a hitherto unknown and unusual bacterium, which he named *Bdellovibrio bacteriovorus*.

The parasitic strains of these remarkable gram-negative bacteria have the unique ability to attach themselves to other bacteria, to bore through their cell walls, multiply, and cause the attacked cells to lyse and thereby release more parasite cells. Here, too, the discovery of previously unreported organisms has indirectly resulted from studies of plant pathogenic organisms. The roles of this pathogen of bacteria in the microbial ecology of soils and as a potential biological control agent are still to be explored fully.

MODIFICATION OF GENERAL MANAGEMENT PRACTICES

Modifications in management practices of both crops and forest trees (11a) have received their impetus from studies on the biology of a number of plant pathogens. Numerous examples can be cited in which standard crop management practices in horticulture and agronomy are governed primarily by an understanding of factors influencing disease development in a given crop. These factors include the sequence in which crops are rotated, methods for weed control, regulation of environmental factors in particular irrigation practices, and control of insects as well as postharvest handling and storage practices. Most of these approaches have been well documented (1, 68).

Under forest conditions direct intervention for control of specific diseases may be very difficult (11a, 29). Thus, application of standard practices that may be effective in the absence of disease or pathogens can result in disaster if imposed on situations where aggressive pathogens may be present. This is well illustrated by the shift in the prevalence and importance of dwarf mistletoe that occurred in certain stands of ponderosa pine in the southwestern USA because the biology of this tree pathogen was not well understood (28). In national forests under the management of the Forest Service, a specific number of seed trees had to be left to insure a more uniform and rapid regeneration of stands after cutting operations. The requirement for seed trees, ostensibly a sound practice in other areas, had a very unfortunate impact on the new stands on thousands of acres. Scattered in the virgin forests of the region was a parasitic seed plant, the dwarf mistletoe (*Arceuthobium*). This parasite has a destructive impact on parasitized trees and results in witches brooms and severe stunting (62). The female plant produces seed that are discharged at a high velocity and can be projected for distances of 30–50 feet from parasitized trees. In many large areas, seed trees that were left were often of no commercial value because of heavy dwarf mistletoe infestations. As a result, the new stands became so severely affected that it will be extremely difficult to salvage them. In sharp contrast, the new stands after clear-cutting by commercial companies in which no seed trees were left were usually free of mistletoe infestations.