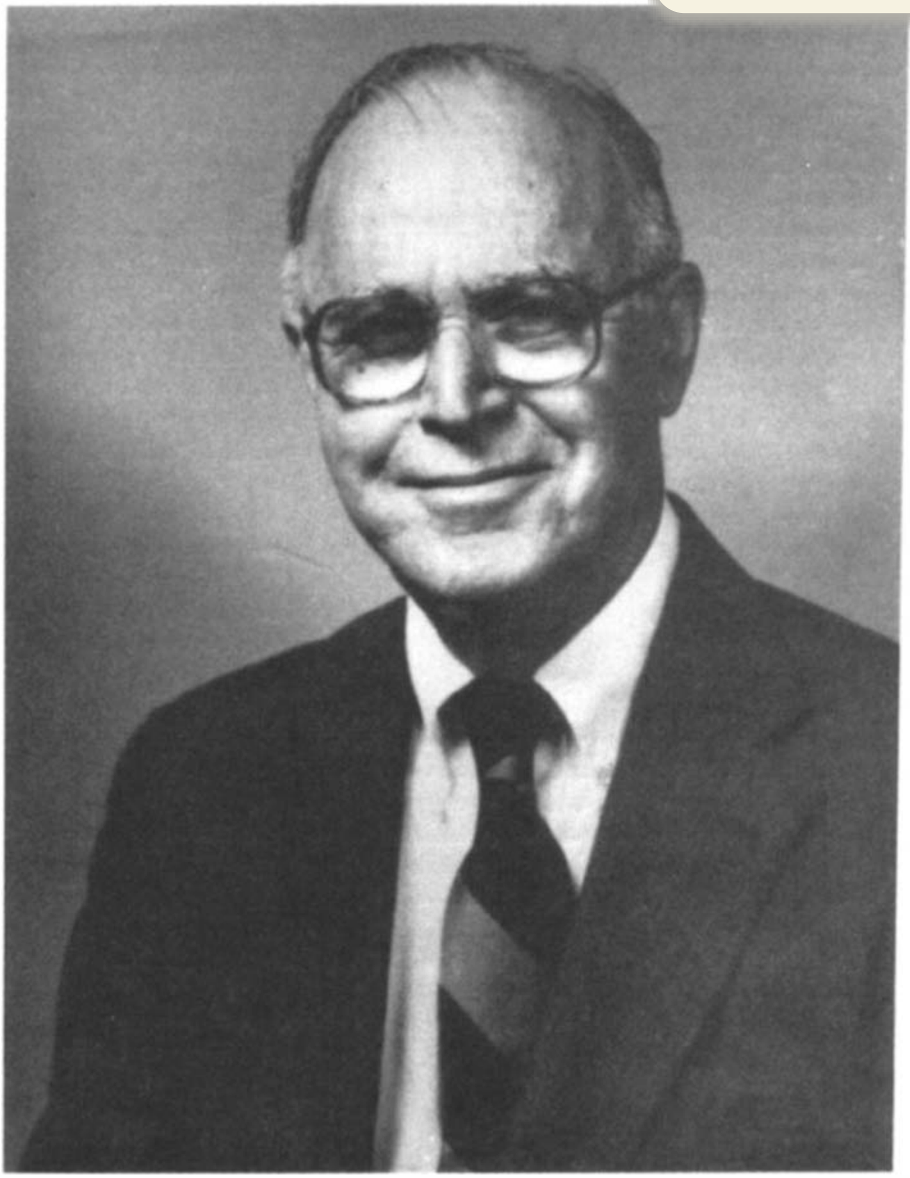

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CONTRIBUTIONS OF PLANT PATHOLOGY TO THE BIOLOGICAL SCIENCES AND INDUSTRY[1]

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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-

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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.

Detailed studies on the biology of the mistletoe have provided the background needed for the development of a new management program to minimize mistletoe infections in the new stands. This experience also emphasized the importance of selecting high-quality pathogen-free seed trees for the regeneration of forest stands and the importance that knowledge of forest pathogens has in establishing acceptable forestry management practices in stands in which diseases are endemic (11a, 29).

PLANT PATHOGENS AS MYCOHERBICIDES

Discovery of a biological control of northern jointvetch (*Aeschynomene virginica* [L.] B.S.P.), a weed in rice fields, by a strain of *Colletotrichum gloeosporiodes* (66) evolved from studies to determine the causal agent of a destructive disease of this weed. The damage from the pathogen was so severe that adequate data in the field experiment to evaluate impact of the weed on rice plantings could not be obtained. Basic studies on the biology of the pathogen indicated that the mechanism for dissemination of spores was not effective. If an effective means of obtaining uniform infection could be developed the possibility existed for use of the fungus as a mycoherbicide. The initial research findings were promising, and a collaborative effort was initiated at the University of Arkansas headed by George E Templeton to develop effective means of inoculation of weed plants under field conditions (65, 66). Subsequently, a commercial product, *Collego®*, was developed that is now used effectively to control this weed. A second product, *DeVine*, has been used effectively in Florida to control stranglervine (*Morrenia odorata* Lindl.) in citrus groves. In this instance, *Phytophthora palmivora* was the pathogen found to be highly effective for use as a mycoherbicide. A number of research and development programs are now exploring other pathogens with weed-host specificity (65).

NOVEL CHEMICAL COMPOUNDS PRODUCED BY PLANT PATHOGENS

Gibberellins

Research on the alteration in physiological processes in diseased plants and the chemicals involved has resulted in the identification and characterization of some very unusual chemicals, many with remarkable physiological effects at incredibly low concentrations. Among these compounds are the gibberellins, which were discovered by a Japanese scientist, E Kurosawa (42), in a study on the disease of rice known as the bakanae or "foolish seedling" disease caused by *Gibberella fujikori* (24, 54). Seedlings affected by the bakanae disease grow faster and much taller than healthy plants. Kurosawa's paper was overlooked until after World War II. Subsequent research resulted in the discovery that gibberellins are formed normally in higher plants as well as by a number of microorganisms (54). At present more than 65 gibberellins have been characterized and an entire field of research developed that is providing new insights into basic aspects of plant growth and development (24). A literature survey for the period from January 1, 1992, to December 30, 1994, revealed a listing of 285 publications on the gibberellins and related compounds. Practical applications involve the use of gibberellic acid and related gibberellins to promote growth, flowering, and germination of seed.

Xanthan Gums

Investigators who studied the complex extracellular polysaccharide of *Xanthomonas campestris,* causal agent of black rot of cabbage and diseases of many other crops, were always impressed with the high viscosity of this capsular material (45, 64). Based in part on the recommendation of Mortimer Starr at the University of California at Davis, the Kelco Company, then engaged in the production of agar from seaweed, explored the possible commercial production and use of the polysaccharide as a viscosifier and suspending agent in oil well–drilling fluids. Extensive studies at the USDA Northern Regional Laboratory in Peoria, Illinois, in cooperation with the Kelco Company, elucidated the structure and promising properties of xanthan gum. Xanthan gums have unusual properties including the ability to maintain their structure under relatively high temperatures (38). The polysaccharide has a high viscosity at low concentrations, is relatively stable over a range in pH levels, and is resistant to various acids and bases (35). Commercialization began in 1964 and has increased rapidly; annual production is now over 100,000 tons. Currently, xanthan gum is incorporated in many different food products such as batters, baked goods, beverages, candy, frozen dairy products, low-calorie foods as a replacement for starch, salad dressing, etc; pharmaceuticals such as cough medicines; and personal care products such as toothpaste, liquid soaps, and shampoos. In addition, it is used extensively in a wide range of industrial applications and in oil fields in drilling fluids and oil recovery processes (38). No investigator who was engaged in the original studies on the extracellular slime of *Xanthomonas campestris* could have possibly envisioned the current industrial and potential future uses of this unique material (45, 64). Few other major industries can point to an origin as unusual as this one involving development of multiple uses of the extracellular slime of a bacterial pathogen of cabbage.

Secondary Metabolites Formed by Infected Plants

Plants respond to infection by synthesizing a number of different secondary metabolites associated with possible resistant reactions. Considerable effort has been invested in the identification and characterization of these compounds. Many of these metabolites also have novel and rare structures that have attracted the interest of chemists (56). These include capsidiol (41), a sesquiterpenoid; isoflavonoids such as pisatin and glyceollin (34); stilbenes such as viniferin (44), casbene (60) and many other unusual compounds with remarkable biological effects at extremely low concentrations. Pimentel (56), in his survey of opportunities for research in chemistry, emphasized the unexploited opportunities to increase knowledge of the chemistry of these secondary metabolites. A few specific examples are described below.

Nematode Egg-Hatching Factor

The soybean cyst nematode is one of the more difficult of plant parasitic nematodes to control. Eggs persist in the cysts formed by the body of the female and can survive for long periods of time in the soil. Roots of host plants were found to release a substance that stimulates hatching of the eggs. The chemical structure of this compound, glycinoeclepin A, has been elucidated. The physiological activity of this compound is truly remarkable. It can induce hatching at concentrations as low as a few parts per trillion (21, 52, 69). Its potential as a control agent or in physiological studies has not yet been exploited, although the compound was recently synthesized (69).

Witchweed Seed Germination Factor

One of the most destructive of all parasitic seed plants is witchweed (*Striga asiatica*). The tiny seeds of witchweed remain dormant in the soil until a compound released by roots of certain plants triggers the germination process. The chemical structure of the compound (strigol) and closely related compounds have now been determined (56, 62). Strigol and its synthetic analogs have a potential for application as possible chemical control compounds and provide a means of gaining an improved understanding of seed germination processes.

Tentoxin

Studies on the nature of several compounds produced by fungi, in particular host-specific toxins such as tentoxin, have provided new knowledge of basic cellular and enzymatic processes in plants that had not been defined previously (20, 25). A full assessment of recent research on tentoxin is beyond the scope of this report, but the scope of this research and new knowledge obtained is very extensive. This is further demonstration of the value of studies on extracellular products of plant pathogenic fungi.

ADVANCES IN PLANT BREEDING AND GENETICS

The evolution of cultivated plants from wild species occurred through a continuous selection in which a broad range of useful characteristics was sought, resulting in plants adapted to regional environments and with desirable characteristics. Throughout this process, plants with relatively high levels of resistance to local diseases and insects were selected either consciously or by chance (26). However, the important discovery by Biffen in 1905 that resistance to stripe rust in wheat was governed by the then recently rediscovered laws of Mendelian inheritance provided a scientific basis for breeding for disease resistance in general (68). Biffen's discovery also called attention to the importance of incorporating disease-resistance factors in programs that had as their primary objectives increasing quality and productivity of crops (25, 68).

Nature of Resistance to a Host-Specific Toxin

No other disease of a food crop ever received publicity in the United States as extensive as that given to the outbreak of the southern corn leaf blight epidemic in 1970 (67). The impact of the disease on the hybrids that carried the Texas cytoplasmic male sterility (Tcms) factor has been well documented. Fortunately, the resources were available for a rapid shift in the breeding lines, the use of Tcms for large-scale production of hybrids was stopped, and the problem that was so threatening initially has been resolved. However, as a result of this outbreak, major advances have been made in our understanding of the relationship between Tcms and susceptibility to *Bipolaris (Helminthosporium) maydis* Race T (47). This was the first major widespread example of non-Mendelian maternal inheritance of disease susceptibility in higher plants (57). The characteristics of male sterility and disease susceptibility are closely linked and are carried in the mitochondria on a 13-kDa polypeptide (URF13). In addition to pathotoxin sensitivity and male sterility, it has been found that plants containing the URF 13 protein are also sensitive to the carbamate insecticide methomyl. Tcms-maize lines are also highly susceptible to another fungus disease, yellow leaf blight caused by *Phyllosticta maydis*. This fungus produces a host-specific toxin with a chemical structure similar to the toxin of the southern corn leaf blight pathogen (47). Chaumont et al (12) recently reported on a study to determine whether introduction of the T-urf13 gene into a species other than corn would also confer sensitivity to the pathotoxin and methomyl, as well as conferring male sterility. Transgenic tobacco plants in which high concentrations of the polypeptide were present also were sensitive to methomyl; however, in these transgenic plants the polypeptide was not solely associated with the mitochondria, but with other organelles as well. Also in transgenic plants expression of URF13 was not correlated with male sterility. Either male sterility is not conferred by the presence of the protein in transgenic tobacco or the polypeptide is not formed in sufficient amounts in the anther cells to affect pollen formation. Additional studies are required to gain a full understanding of the close association between male sterility and sensitivity to toxic moieties such as a fungal pathotoxin and a carbamate insecticide. These and related studies have reemphasized the need for constant vigilance in those breeding programs that result in widespread introduction into any major crops of specific genetic factors.

Wisconsin Fast Plants

In the search for improved procedures to facilitate the breeding program for resistance to pathogens of cabbage and related species of brassicas, Paul Williams at the University of Wisconsin-Madison conceived a novel approach (72). To develop a model plant he decided to capitalize on the ability of certain brassicas to flower quickly and complete their life cycles in a relatively short time. He initiated an ambitious screening program and began to examine a world collection of over 2000 brassicas. Fortunately, he found a few plants with relatively short life cycles. Rapid-cycling strains of *Brassica rapa* and five other related species were developed in an intensive effort that extended over a decade. The product of selection with *B. rapa* was a model plant that flowered 14 days after planting the seed, was about 6 inches tall at maturity, and could complete 10 generations in 1 year. It was soon recognized that these plants developed for the breeding program in disease resistance had qualities that make them very attractive for basic investigations on cell and molecular biology, physiology, genetics, and plant breeding. The demand for seed from these unique plants was so great that in 1982 Williams established a Crucifer Genetics Cooperative that now has on its roster over 1600 scientists in 56 countries examining over 100 specific genetic traits.

Because these plants can be grown in large numbers under fluorescent light (up to 2500 plants per m^2), are easily pollinated, have a large number of variant types, and are self-incompatible, they are very adaptable for classroom projects. With the support of the Educational Materials Development Program of the National Science Foundation, the Wisconsin Fast Plants Program was established; it is now actively designing materials for instruction in various aspects of plant biology for use in classrooms from kindergarten through college. The influence of the complete range of environmental and nutritional factors on the development of plants can be examined readily. The possibility of making crosses and following several generations of plants offers students unparalleled opportunities to design simple experiments and obtain results in one semester. The educational materials developed in connection with the rapid cycling plants now known as Wisconsin Fast Plants are available to elementary, high school, and college teachers. Over 35,000 biology teachers and their students are now participating in the Fast Plants Program (P Williams, personal communication).

Declining enrollments in graduate programs in agriculture, and the plant sciences in particular, are a source of growing concern. The decline in funding and number of available positions are obvious factors to be considered in this concomitant decrease in enrollments. The long-term benefits of Fast Plants and related programs will not only foster learning and demystify subjects such as genetics as an essential component of the education of all students, but may also attract students to careers in the plant sciences.

Genetic Engineering Plants with a Modified Tumor-Inducing Factor

The rewards of basic research on a plant disease often arrive in unanticipated ways. One striking example is illustrated by results of the search for the basic mechanism of tumor induction by the soilborne pathogen, *Agrobacterium tumefaciens*. Hundreds of papers record the efforts of a large number of scientists who struggled for many years to unravel the remarkable process that enables the bacterium, *A. tumefaciens*, to transform plant cells so that they grow in an autonomous fashion (55). However, it is now apparent that the salient experiments to decipher the enigma could only have been completed after the powerful techniques of recombinant DNA were discovered. Much of the credit for these studies on tumor induction goes to Eugene Nester and his coworkers at the University of Washington (55, 58). In particular, the experiments of Mary Dell Chilton and associates (13) provided the definitive data indicating that the tumor-inducing segment of the DNA of the Ti plasmid introduced into the plant cell by the bacterium actually is integrated into the chromosome of the cell. Since the DNA from the bacterium could be engineered in such a way that the genetic factors for tumor induction could be eliminated, a remarkable system became available for introduction of other desirable genes into plants. With the development of new techniques for regeneration of plants that could flower and produce seed carrying introduced genes, the prospect for producing transgenic plants became a reality.

The development of transgenic plants with virus resistance (4, 23, 39) constitutes a milestone in disease control. This resistance is conferred by expression of viral coat protein genes in transgenic crop plants (4). This evolution of basic research findings to an unexpected practical application illustrates how basic research on a bacterial disease has aided in establishment of a new technology. It has already shown its value to plant breeders seeking higher precision in introducing genetic traits not available via standard breeding procedures. The technology can also be used in the development of mutations that will make it possible to analyze the functions of growth hormones and fundamental processes of plant biology (39).

ROLE OF EPIPHYTIC BACTERIA IN FROST INJURY

Abiotic injuries and diseases are generally caused by a broad range of environmental extremes with adverse temperatures and moisture relationships as the major factors. Plant pathologists have developed innovative approaches to minimize adverse effects caused by environmental extremes, misuse of chemicals, mineral deficiency diseases, and injuries resulting from harvesting, shipment, and storage procedures. However, reducing losses from frost damage has been extremely difficult to implement. The discovery of the relationship of epiphytic bacteria with frost injury is another excellent example of how studies in plant pathology lead to unexpected discoveries in the fields of microbiology and to the establishment of new industries.

Many frost-sensitive fruit crops periodically suffer severe damage to blossoms and subsequent loss of fruit that may be measured in terms of millions of dollars annually. If a grant proposal had been prepared to study populations of bacteria on fruit blossoms and leaf surfaces as a means of increasing the understanding of frost damage, it is unlikely that such a grant proposal would have received approval for funding. That epiphytic bacteria influence frost damage to plants was an unexpected outcome from a study of the presumed effect of a plant pathogen on a crop plant under frost conditions. Paul Hoppe, a USDA corn pathologist working at the University of Wisconsin-Madison in the mid-1960s, was evaluating corn lines for resistance to northern leaf blight (*Helminthosporium turcicum*). In one experiment in 1964 his inoculation procedure involved spraying young plants in his field plots with a spore suspension of the causal fungus (32). A water suspension had been prepared by grinding heavily infected leaves on which lesions with abundant sporulation of the fungus were present. A period of low temperatures occurred several days after the inoculations were made (32). An unforeseen result was the fact that severe symptoms of frost injury appeared on the leaves of corn lines that had been sprayed with the spore suspension; damage was markedly less severe on the plants in unsprayed plots. The assumption was made that incipient infections had in some manner predisposed leaves to frost injury. For several years after these initial observations, Hoppe conducted experiments in growth chambers in which he reproduced the same pattern of increased frost injury on plants treated with water suspensions containing powdered infected corn leaves obtained from field plants. However, the specific causal factors in these interactions were not determined. After his retirement the project was continued by Deane Arny and Chris Upper in the Department of Plant Pathology at the University of Wisconsin-Madison. After additional tests with appropriate controls, it became evident that the ice-nucleation factor was an entity present on field-grown corn leaves and not related to the action or products of the fungus (2).

In 1973, Steven Lindow initiated a doctoral program with Upper. Soon he determined that ice-nucleation active (INA^+) bacteria in the nonsterile powdered corn-leaf suspensions were involved in this unique effect (2). He modified an ingenious technique developed by Gabor Vali in Wyoming for use with bacteria to test the ice-nucleating ability of test strains. This involved floating a sheet of aluminum foil on the surface of a refrigerated constant-temperature bath held at -5°C. Droplets of water placed on the sheet at this temperature do not freeze unless ice nuclei are present, while those containing INA^+ bacteria froze quickly. One group of the INA^+ cultures were found to be similar in all of their key reactions with *Pseudomonas syringae* (48). A modification of procedures developed by Lindow was subsequently applied in elegant epidemiological studies on brown spot of bean (30). Insertion of leaves with epiphytic populations of *P. syringae* into test tubes with water at –5°C results in rapid freezing and provides a means of following shifts in populations under different environmental conditions. New understanding of the epidemiology of epiphytic bacterial pathogens has been gained from these studies.

Upon completion of his degree at the University of Wisconsin, Lindow was appointed to the faculty at the University of California, Berkeley, and continued his studies on the ice-nucleating bacteria. In one phase of his research he and coworkers genetically engineered one strain of *P. syringae* from potato so that the gene for ice nucleation was deleted (48). This provided the model strain for testing the hypothesis that INA^- bacteria could be used to prevent frost damage. Since the INA^- strain was a good colonizer of leaf surfaces, the possibility existed that it would prevent the populations of INA^+ strains from increasing on leaves of frost-sensitive plants. On the basis of inoculation tests on a large number of possible host plants, it had been found that the epiphytic INA^+ strain from potato also was not pathogenic on any of a broad range of the crop species tested (48).

At that time Jeremy Rifkin, head of an environmental group, was leading a campaign to prevent the introduction of any genetically modified organism into the environment. As a result of various lawsuits and associated publicity, the experiments to test the genetically modified INA^- strain under field conditions became the most highly publicized field tests in the history of plant pathology, or indeed any of the biological or agricultural sciences. The experiments were delayed almost five years until all the requirements of the US Environmental Protection Agency were satisfied. In cooperation with Lindow, one of the first field experiments was completed by a biotechnology company, Advanced Genetic Sciences. A photograph of Julie Lindeman of Advanced Genetic Sciences in a protective space suit spraying strawberry plants with a suspension of the INA^- bacterium appeared on the front page of the *New York Times* and was shown on many television news reports. In the background of the photograph reporters were visible drinking coffee in apparent unconcern about the presumed dangers of the experiment. When Lindow finally established his field plots, attempts were made to block progress in the experiments by vandalism that involved destruction of test plants and damage to equipment.

Large-scale commercial application of INA^- bacteria as a means of reducing frost damage on a commercial basis is still under development. A naturally occurring ice^- strain of *Pseudomonas fluorescence* is now registered and will be sold commercially as "Blight Ban A506" for frost and fire blight control in 1995 (S Lindow, personal communication). One commercial application has been the use of INA^+ bacteria in a patented product, *Snowmax,* which is now used to improve the efficiency of snow production on ski slopes. An additional application now under study is the use of INA^+ bacteria as an aid in freezing foods more rapidly at higher temperatures than normal (72a).

SUMMARY AND CONCLUSIONS

The above examples illustrate some of the ways in which the study of plant diseases has led to significant advances in other biological sciences and to the establishment of new industries. Many other examples can be cited, and the prospects are high that many similar discoveries will be made in the near future. The powerful tools of molecular biology can be applied to gain an understanding of a host of compounds still not fully characterized that are produced by plant pathogens and by plants in the act of defending themselves against pathogens. The prospects are high that our understanding of the nature of tumorigenesis and differentiation of self and nonself may best be resolved in studies on pathogen-host reactions.

Plant pathologists have played a leading role in the debate about release of genetically engineered organisms (GEMS). Wilson & Lindow (70) have recently summarized the evidence for low risk in properly designed and managed releases and emphasized the potential benefits of research in this area, including the prospects for use of GEMS in bioremediation, basic studies in soil ecology, and biocontrol.

In several instances, long delays occurred in wide application of an innovative technique because of the high degree of insularity that, unfortunately, still separates many agricultural scientists from basic biological scientists. The long delay before the density gradient centrifugation technique of Brakke (7) was widely adopted is a case in point. The conclusive evidence that a microorganism can be the cause of disease in a living organism was ignored for over 50 years by scientists investigating human disease. Medical mycologists initially overlooked the close taxonomic relationship between fungi that cause disease in humans and those that infect plants.

Under our current system of funding and with intense competition for the available funds, there is a natural tendency for young scientists to avoid the research projects that explore new areas in innovative or unconventional ways. A number of the advances cited here exemplify instances in which individuals had the courage to venture into unexplored territory. Thus, the prospects are bright for additional major basic contributions to other biological sciences as well as in the development of new technology and beneficial products of value to agriculture and industry resulting from research in plant pathology.

References have been made to discoveries that resulted from the cooperative efforts of researchers with many different scientific backgrounds and scientists who did not have in-depth training in plant pathology. However, they were intrigued by the advantages of using a plant disease as a model system to gain an understanding of normal plant growth, development, and reproduction.

Thus, in a period of change and stress, plant pathologists have reason to point with pride not only to the significant contributions that our field has made to insure the availability of food and fiber and the beauty of ornamental plants to the world but to other fields of science and industry. We also need to recognize the fundamental importance of investigations into the nature of disease and biology of pathogens. These studies are an aid to development of improved controls as well as a means of advancing our understanding of normal physiology and genetics of plants and the microorganisms that either attack them or exist in nature as helpful saprophytes. Major benefits can continue to accrue to science and humanity by these investigations.

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