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Plant-Microbe Interactions: Life and Death at the Interface

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INTRODUCTION

Interactions between microorganisms and plants have undoubtedly had major effects on the development of civilization since humans began to rely extensively on cultivated crops for food. Indeed, ancient chronicles of famines, plagues, and epidemics show that some of the more serious plant diseases, such as rusts, smuts, and mildews, were recognized soon after the emergence of organized agriculture. Theophrastus (~371 to 287 BC) described disease symptoms on a number of plants used for food and the Romans paid tribute to appease the rust god Robigo.

More recently, plant disease outbreaks have resulted in catastrophic crop failures that have triggered famines and caused major social change. The effects of such epidemics have been particularly devastating in situations such as the Irish potato famine of the 1840s, in which communities depended on a single crop as their primary food source. The potential for serious crop disease epidemics still persists today, as evidenced by recent outbreaks of Victoria blight of oats and southern corn leaf blight. These diseases result from agricultural practices that rely on monoculture crops—planting closely related crop species over wide geographical areas provides, in effect, a large Petri dish for the evolution of increasingly virulent pathogen forms.

In addition to causing food shortages, microbial interactions with plants can directly affect the health of humans and livestock. One notable example is ergot poisoning, caused by toxins in the fruiting bodies of the fungus *Claviceps purpurea*, which can contaminate rye flour. These toxins cause a frightening syndrome typified by hallucinations, burning sensations, miscarriages, gangrene, and, in severe cases, death. The affliction, known as St. Anthony's fire or Holy fire, was most prevalent in the Middle Ages; present day outbreaks are only prevented by the strict cultural and sanitary standards that are now applied in the regulation of grain sales. Further problems resulting from the presence of allergens, carcinogenic compounds, and various mycotoxins in moldy grain, peanuts, and animal feed have recently been shown to affect human health. For readers interested in more fundamental information, a broad coverage of plant pathology is provided in the text by Agrios (1988), and excellent descriptions of the effects of plant diseases on society are given by Large (1940), Carefoot and Sprott (1967), and Schumann (1991).

Disease is not the only outcome of plant–microbe interactions. A number of mutually beneficial relationships between plants and microorganisms affect agricultural productivity and the health of plants in general, and these systems have also been the foci of intensive studies (Stacey et al., 1992; Smith and Read, 1996). In symbiotic relationships, the microorganism assists the plant with nutrient absorption or contributes biochemical activities that the plant lacks. The plant, in turn, contributes photosynthate, to the competitive advantage of the corresponding microbial symbiont in the rhizosphere. By altering the balance of microflora in the rhizosphere, symbiotic associations may also help to protect plants from disease-causing microorganisms.

Exploitation of other beneficial, nonsymbiotic rhizosphere organisms for the biological control of plant diseases is also an important discipline that relies on detailed knowledge not only of specific plant–pathogen interactions, but also on the general ecology of the interacting organisms in the soil. Other forms of biological control of plant disease are also emerging. These range from the use of microbial pathogens of pathogens, to molecular genetic "immunization" strategies, in which the expression of specific pathogen determinants in transgenic plants interferes with various phases of the infection process.

Studying plant–microbe interactions is important for all of these practical reasons, and the rationale behind much of the research on these interactions has, at least distantly, the goal of improved agricultural productivity in mind. However, there are many fundamental spin-offs from these studies that contribute to our understanding of basic plant processes and to the generation of useful tools and techniques for plant biology. One example is provided by the crown-gall disease pathogen *Agrobacterium tumefaciens*, which infects a large number of plant species. Not only is the intimacy of this interaction astounding (see below), but the experimental utility of Agrobacterium-mediated gene transfer is now ubiquitous in plant biology laboratories worldwide. Other examples include the insights into plant signaling processes provided by studies of disease resistance genes and rhizobial Nod factors.

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Interactions between pathogenic bacteria, such as *Pseudomonas* and *Xanthamonas*, and plants also illustrate another broad concept in the biology of plant–microbe interactions—their specificity. In these examples, individual species or strains of bacteria are only capable of interacting with corresponding individual plant species or cultivars. This theme is repeated in many of the reviews in this issue, as is the demonstration of the profound differences in the outcomes of plant–microbe interactions that are conditioned by changes in only a single plant or microbial gene.

This special issue focuses on recent and exciting findings in the biology of plant–microbe interactions. The reviews are organized into four sections. In the first section, Pathogenic Processes, the mechanisms by which the major groups of microbial pathogens gain entry to plants and the nature of the diseases they cause are discussed. In the second section, Resistance Responses, the plant responses provoked by pathogen attack are detailed. The third section, Control of Pathogens, includes reviews focusing on strategies that use microorganisms to combat plant disease, and the fourth section, Symbioses, describes mycorrhizal and nitrogen-fixing symbioses.

In this introductory chapter, we aim to outline the nature of the problems addressed in each section of the special issue, as well as the kinds of organisms involved and the information we can expect to obtain from the various studies. We will also attempt to offer descriptions of a number of important terms and concepts, as well as to introduce the reader to some of the microorganisms involved in the interactions.

PATHOGENIC PROCESSES

Thousands of microorganisms are known to cause plant diseases. The reviews in this section focus primarily on recent advances in our understanding of the mechanisms utilized by the three major categories of disease-causing organisms, viruses and viroids, bacteria and mycoplasma-like organisms, and fungi, to gain entry into plants and, once there, to elicit disease. Nematodes are also considered in this section. Although not strictly microorganisms, nematode infestations cause a number of serious plant diseases. Figure 1 illustrates a small sample of the many agents known to cause disease on tomato, a representative crop species. A more comprehensive list would include a large number of viruses, several bacteria and nematodes, and numerous fungi (Jones et al., 1991).

To successfully colonize a particular host, a microorganism must develop the ability to circumvent defensive barriers elaborated by the plant to prevent infection. Once these barriers are breached, the newly susceptible host then faces selection pressure to develop countermeasures that block invasion by the pathogen. After a novel resistance response has evolved in the plant, the pathogen must again respond with an alternative mechanism that restores virulence. These dynamic and ongoing coevolutionary battles have resulted in the utilization of highly specific and extremely sophisticated attack strategies by the pathogen and equally elaborate defense responses by the host.

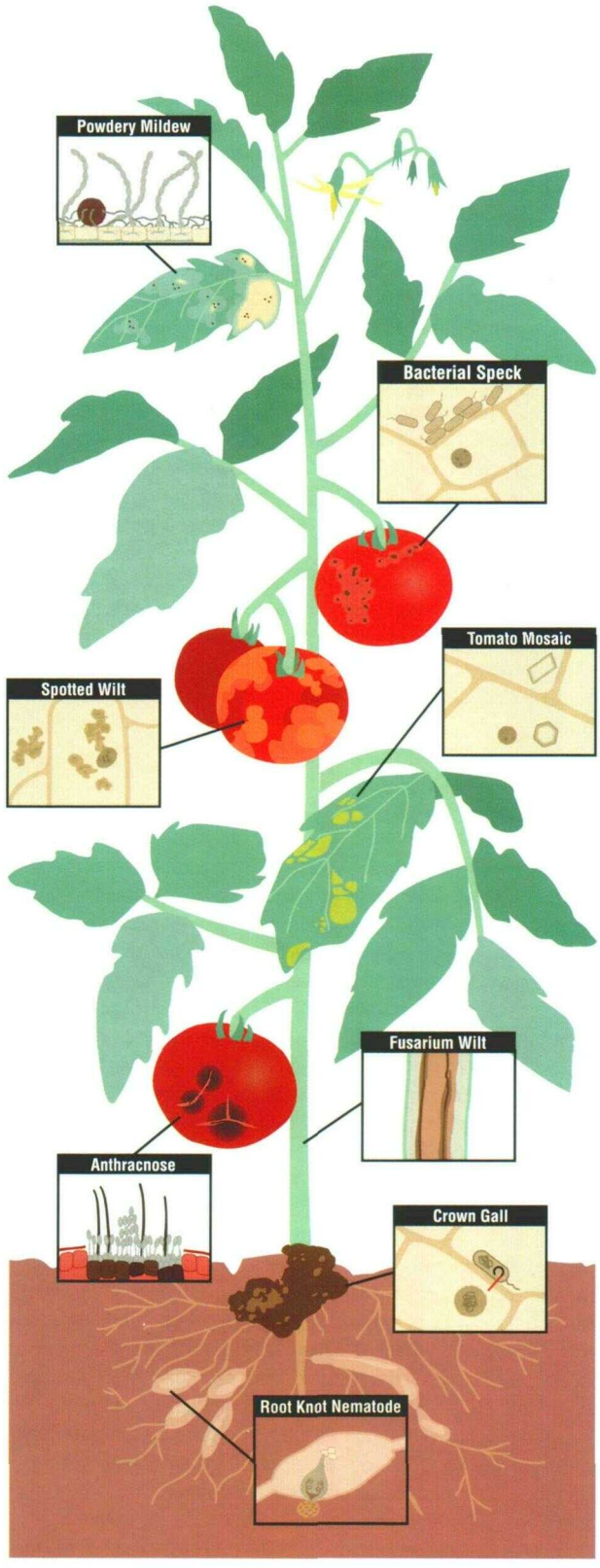
The specificity that can develop during the evolution of plant diseases is most clearly exemplified by the interactions that define relationships between pathogen races or pathovars and host plant cultivars. In this kind of relationship, the appearance of disease symptoms, i.e., "compatibility," is the outcome in most cases and results from the ability of the pathogens to overcome the complexities of the host defense responses. However, "incompatibility," a resistance reaction that prevents or severely retards pathogen growth, may be conditioned by a single interacting gene pair—a host resistance (*R*) gene and a pathogen avirulence (*Avr*) gene (Flor, 1971). In most cases, the *R* and *Avr* genes are dominant, which suggests that the gene products may interact directly as receptor and ligand, but this has yet to be demonstrated conclusively. Although these "gene-for gene" interactions are extremely important components of contemporary investigations of plant pathogens, they form only a subset of the highly specific interactions known to occur during pathogenesis.

Viruses and Their Infection Processes

Viruses were first recognized as pathogenic entities about a century ago. Since that time, considerable effort has been expended to understand their biology: how they gain entry into plants, and once inside, how they coopt host plant processes to replicate and spread systemically throughout the host. Within the past 25 years, enormous progress has been made in the identification and classification of viruses and in the functional characterization of virus-encoded genes. The principal characteristics used in the classification of plant viruses are their genome type and organization, but physicochemical, biochemical, and biological criteria are also important. Over 40 families of plant virus have now been defined, and these are illustrated schematically in Figure 2.

Despite differences in the properties of their genomes, all plant viruses face the same two fundamental challenges during the establishment of systemic infections in their plant hosts. The first necessity is to replicate in the initially infected cells. This is achieved in a wide variety of ways, all relying to some extent on the utilization of host components to complement replication determinants encoded by the virus. The second requirement is for the viruses to move through adjacent plant cells to the vascular system, before spreading throughout the plant. This process also depends on highly specific interactions with host proteins.

Although the viral genes required for replication have been defined, only limited information on the host components involved in these processes is available. Thus, a major future challenge is to identify the host genes that are required for the replication of RNA and DNA viruses and that control the



biochemical processes leading to functional interactions between the viral and host replication determinants. Nevertheless, molecular genetic analyses of the viruses belonging to the groups illustrated in Figure 2 have identified some general principles. For example, many plus-strand RNA viruses appear to utilize components of the host translation apparatus during replication, whereas DNA viruses interact with host DNA replication components (Shaw, 1996).

Further developments in this area should be facilitated by the application of more sophisticated genetic techniques to dissect the host components involved in virus replication. For example, innovative systems have been developed recently in which yeast can be infected with brome mosaic virus (Janda and Ahlquist, 1993). The genetic resolving power of yeast, combined with mutational analyses in plants, should prove fruitful in the future identification of genes required for replication of some plant viruses. A more detailed understanding of viral replication should also provide general insight into plant nucleic acid metabolism, gene expression, and cell biology.

Molecular genetic and biochemical studies over the past few years have provided considerable insight into mechanisms by which viruses establish systemic infections within plants. The review by Carrington et al. provides a thorough synopsis of recent research into virus movement. Studies of cell-to-cell movement have shown that many plant viruses encode dedicated movement proteins (MPs) that facilitate the transport of nucleoprotein complexes and/or virus particles to adjacent cells through modified plasmodesmata. It appears that two distinct plasmodesmatal transport mechanisms are utilized for localized cell-to-cell movement. One movement strategy, which is utilized by Tobamoviruses (see Figure 2), involves increasing the size exclusion limits of plasmodesmata during localized trafficking of nucleoprotein complexes. A second strategy, utilized by Comoviruses and Caulimoviruses (Figure 2), involves large tubular structures composed of MP that appear to facilitate movement of virus particles through enlarged plasmodesmata.

In a striking parallel between endogenous host processes and viral movement, Carrington et al. point to recent findings that the Knotted homeodomain protein of maize can facilitate

Figure 1. Characteristic Diseases of Tomato Caused by Viruses, Bacteria, Fungi, and Nematodes.

A variety of syndromes, ranging from mosaics to galls and tissue necroses, are caused by these pathogens. In some cases, such as powdery mildew, crown gall, or root-knot nematode infection, the symptoms can be used for diagnosis of the particular host–pathogen interaction. In contrast, similar virus mosaics, bacterial necrosis syndromes, and fungal blights are caused by a large number of organisms. In all cases, the disease phenotype is affected to some extent by the specific combination of pathogen isolate, host variety, and environmental conditions. More than 100 tomato diseases are known, descriptions of which may be found in Jones et al. (1991). The figure was produced by Ann Boughton, Thumbnail Graphics, Oklahoma City, OK.

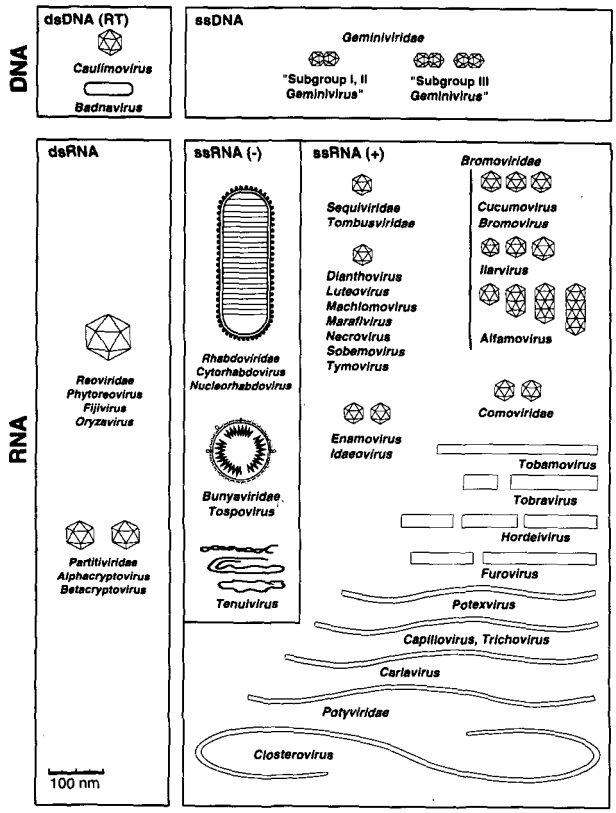


Figure 2. Families of Plant Viruses.

Virus families are designated according to genome composition and organization, particle morphology, and replication strategy. Reprinted from Murphy et al. (1995), with the permission of Springer-Verlag.

the transfer of its own mRNA between cells (Lucas et al., 1995). This observation may impinge on questions concerning the evolutionary origins of viral movement genes and how viruses acquired them in the first place. Although recognizable similarities exist between the MPs of some viruses, many MPs have little obvious sequence similarity. This fact, and the fact that different viral movement strategies have been described, suggests that the acquisition of movement functions may have occurred more than once during evolution.

A growing body of comparative sequence evidence indicates that viruses have evolved via modular recombination to obtain beneficial genes from other viruses. Given this, recombination with host genes, and acquisition of their functions, remains a distinct possibility. Thus, MPs may have evolved from essential plant genes whose products are required for normal function of the host cell. If this proves to be true, some of the diversity evident in viral MPs could be attributed to the independent acquisition of members of different host gene families, which, like Knotted, normally facilitate nucleic acid transport.

In contrast to the information that has accumulated concerning the mechanisms involved in localized movement, long distance transport processes and the roles of host components in virus movement have proven to be much more difficult to dissect. It is clear that the requirements for transit through the vascular system are different from those necessary for localized cell-to-cell movement. With few exceptions, efficient long distance movement requires involvement of functional virus coat protein (CP), which implies that intact virus particles are actually transported. However, only limited information concerning interactions between CP or virus particles and components of the phloem is currently available.

A few host genes that contribute to localized or long distance movement have been identified, but Carrington et al. stress that a more concerted effort needs to be expended in identifying host genes that affect various movement processes. The authors also raise a number of outstanding questions that need to be addressed: Can nascent replication complexes be exported from the cell? Do host-specific interactions of movement complexes contribute to tissue specificity and host range restriction? What mechanisms regulate the temporal aspects of movement? What processes operate during movement into and out of the phloem during long distance transport? We can expect significant progress toward obtaining answers to these questions during the next few years as novel host and viral genetic approaches are combined with more extensive biochemical and cell biological analyses of virus movement in plants.

Mechanisms of Prokaryote Pathogenesis

Many important plant diseases exhibiting a wide range of symptom phenotypes are caused by bacteria and mycoplasma-like organisms (Figure 3). These organisms have diverse life styles, ranging from facultative pathogens such as *Erwinia* and *Pseudomonas*, which can be manipulated in the laboratory, to more fastidious obligate pathogens, such as *Xylella* and mycoplasma-like organisms, which are difficult to culture (Agrios, 1988). The most widespread and destructive losses are caused by Gram negative rod-shaped bacteria of the genera *Erwinia*, *Pseudomonas*, and *Xanthomonas*. Members of these groups have two modes of pathogenesis: biotrophic pathogens kill their host plants relatively slowly, thus allowing maximal opportunity for pathogen replication, whereas necrotrophic pathogens use a brute force strategy that results in rapid tissue death.

Biotrophic bacteria elicit fire blights, wildfire, halo blights, leaf and fruit spots, scalds and yellowing diseases, vascular wilts, scabs, cankers, and galls (Figure 3). Although these bacteria collectively infect a large number of plant species, individual pathovars conform to the gene-for-gene hypothesis in that they have a defined specificity. For example, *P. syringae* pv *glycinea* attacks soybean; however, the pathovar can be further subdivided into "races" on the basis of reactions to different soybean cultivars. In contrast, necrotrophic pathogens generally have broader host ranges than biotrophic bacteria and their pathology is more dependent on environmental conditions that stress the host. Most bacterial

necrotrophs are members of the genera *Erwinia* and *Pseudomonas*, which macerate tissue through the secretion of pectic enzymes, thus causing so-called soft rot diseases (Figure 3).

Alfano and Collmer provide a very informative review of the mechanisms used by biotrophic and necrotrophic bacteria to invade plants and the virulence factors that contribute to their differing pathologies. A particularly exciting finding concerns the clustered *hrp* (hypersensitive response and pathogenicity) genes that are required for the delivery of the *Avr* gene–derived signal from the bacteria into the cells of their host plants. The extensive similarities between *hrp* genes and those of pathogenic animal bacteria are of special interest because they imply that plant and animal pathogens make use of similar virulence strategies (Cotter and Miller, 1996; Pettersson et al., 1996; Zhang and Normark, 1996). The products of the *hrp* genes are thought to form an infection structure that may actually inject plant cells with bacterial components. These novel findings demonstrate that *Avr* gene products may trigger resistance responses from inside host cells, where they would be recognized by the corresponding *R* gene products, rather than from outside the cells as had previously been believed.

In an interesting parallel with population monitoring by some rhizosphere inhabitants, Alfano and Collmer describe evidence that the necrotrophic bacterial pathogens engage in a stealth mode of pathogenesis by lying in wait to express virulence factors until a population of bacteria that is sufficient to overwhelm the host has accumulated. This mob attack response is proposed to be mediated by "quorum sensing" mechanisms in which quantitative autoinduced signals monitor bacterial populations and regulate the release of antibiotic or virulence factors (Moré et al., 1996). Interestingly, similar quorum sensing mechanisms are also thought to have important roles in the survival of rhizosphere bacteria (Pierson et al., 1994).

Many questions are raised by these quorum sensing models. For example, which signal molecules are involved in sensing? Which receptors recognize the signals? How are signals produced by one population of bacteria distinguished from those produced by another? How does quorum sensing contribute to bacterial fitness? Answering these questions may help in the development of transgenic plants which express components that specifically interfere with the quorum sensing mechanisms of disease-causing organisms, or that enhance the fitness of beneficial rhizosphere inhabitants.

In contrast to the diverse lethal syndromes caused by biotrophic and necrotrophic bacteria, the tumor-forming Agrobacteria do not kill cells directly. Instead, characteristic tumors often appear just below the soil level at the crown of

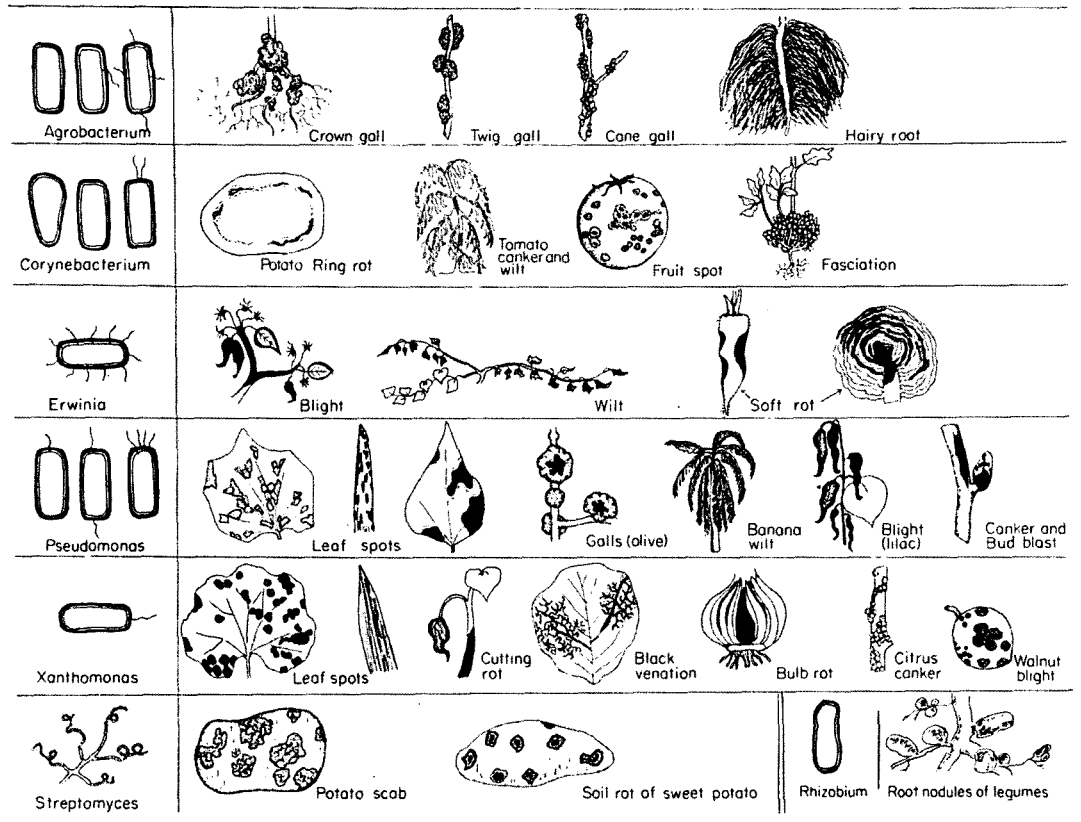


Figure 3. Genera of Bacteria and the Symptoms Elicited in Response to Infection. Reprinted from Agrios (1988), with the permission of Academic Press.

the plant, hence the name crown-gall disease (Figure 3). The most remarkable feature of this disease is that the bacterium engages in genetic engineering of its host. Agrobacterium transforms the plant cell with a DNA fragment, the T-DNA, which encodes genes whose products subvert the plant's biochemistry, causing it to synthesize sugar compounds that the bacteria can metabolize. The specificity of the Agrobacterium–plant interaction again serves to illustrate the finely tuned evolution and the high degree of specificity that can be required for successful pathogenesis.

The review by Sheng and Citovsky provides a contemporary discussion of our knowledge of the events involved in the synthesis, transfer, targeting, and insertion of the Agrobacterium T-DNA into the plant genome. Many of these events appear to be mechanistically related to other plant or bacterial processes. For example, the VirA/VirG transcriptional regulators, which activate the other Agrobacterium virulence (*vir*) genes, are functionally similar to the two-component sensor/signaling systems that mediate a wide range of developmental and environmental responses in bacteria.

T-DNA transfer requires the concerted action of a number of the *vir* genes, and recent evidence suggests that this phase of Agrobacterium pathogenesis is functionally similar to bacterial conjugation (Fullner et al., 1996). Conversely, the targeting of T-DNA to the plant cell nucleus is more analogous to viral movement mechanisms, with Agrobacterium exploiting endogenous macromolecular transport and targeting pathways. There may also be parallels between T-DNA transfer processes and the *hrp*-mediated transfer of Avr signals into plant cells and/or the plasmodesmatal tubules involved in the spread of some viruses, but these possibilities remain to be investigated.

Studies of T-DNA transfer and targeting are bringing conceptual advances in our understanding of both prokaryotic and eukaryotic cell processes. It is expected that ongoing studies of the unique aspects of Agrobacterium-mediated plant transformation will continue to impact research in both of these areas.

Fungal Pathogenic Processes

Numerous species of fungi in each of the major phylogenetic groups cause serious plant diseases. These include lower fungi, such as Plasmodiophoromycetes, Chitridomycetes, and Oomycetes, and higher fungi, such as Ascomycetes, Basidiomycetes and Deuteromycetes (Webster, 1980). Fungal pathogens produce many different fruiting body and spore forms (Figure 4), and their life styles range from obligate parasites, such as the chitrids, downy mildews, and rust fungi, to facultative parasites that are capable of attacking plants only under certain circumstances.

Obligate fungal parasites have established intimate and highly evolved relationships with their plant hosts. During the course of infection, these pathogens engage in many sophisticated but poorly understood activities that redirect nutrient flow in plant tissues and alter the growth and morphology of the plant. Changes in the morphology of the pathogen are also evident during pathogenesis, and these developmental modifications offer great future potential for molecular genetic analyses, biochemical studies, and cell biological investigations of infection. Necrotrophic fungal parasites, such as *Cochliobolus heterostrophus*, are unable to attack living tissue but produce host-specific toxins that kill plant tissue in advance of hyphal invasion.

Knogge's review begins by describing the penetration processes through which fungi gain entry into plant tissue. These are clearly complex events that in many cases involve the secretion of a cocktail of hydrolytic and proteolytic enzymes, the composition of which varies depending on the particular fungus–plant interaction. During penetration, the hydrolytic enzymes and/or plant defense responses generate fragments of fungal and/or plant cell walls. These compounds, which are often oligosaccharides, can elicit broad host range defense responses that slow pathogen ingress. The rapid elicitation of the plant's defense responses mandates that successful pathogenic fungi must have evolved strategies to suppress and/or avoid the responses of potential hosts, and Knogge discusses how such mechanisms, both pathovar specific and broad host range, may have evolved.

Once inside the plant, how do fungi actually cause disease? Symptoms often result from the effects of fungal toxins, the genetics, biosynthesis, and modes of action of which are steadily being resolved. These low molecular weight molecules appear to target critical biochemical pathways, and their action can have pleiotropic effects on plant metabolism. For example, fusicoccin blocks the function of plasma membrane ATPases, thus perturbing the energy status of cells, and tentoxin affects energy transfer in chloroplasts. Fusicoccin and tentoxin affect a broad range of plant species. However, other fungal toxins, the so-called host-selective toxins (HSTs), appear to be specific for individual plant species, and in some cases their effects are mediated by gene-for-gene interactions. Knogge introduces the concept of toxins, describes how they can be used to help understand the basic plant processes they perturb, and provides some models for toxin action in gene-for-gene–mediated interactions.

Knogge also discusses the interactions of *Avr* and *R* genes during pathogenesis. At first glance, the expression of *Avr* genes by pathogens is counterintuitive. Why should a pathogen express a dominant gene that conditions a concerted resistance response by the plant? The answer is that the biological function of the *Avr* genes is not to trigger host defense responses. Instead, the *Avr* gene products are virulence components that have strategic roles in pathogenicity or in pathogen fitness. In fact, any gene expressed by a pathogen could become an *Avr* gene if an *R* gene capable of recognizing its product evolved in the plant host.

Models describing how virulence may be restored after the evolution of successful resistance strategies (i.e., novel *R* genes) by the host are also presented in this review. Among the possibilities are the deletion of *Avr* genes, frame shifts that lead to the production of truncated (and unrecognizable) Avr

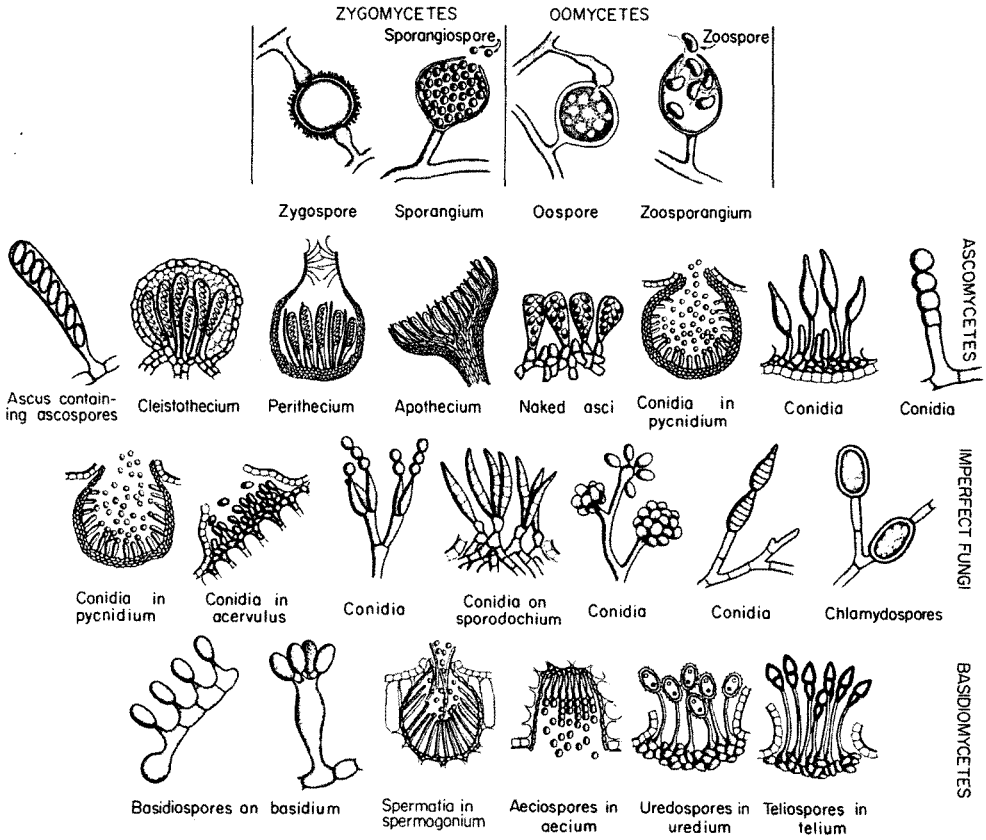


Figure 4. Some Representative Fruiting Structures of the Four Groups of Fungi.

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proteins, and ectopic expression from altered promoters. The time taken for evolution of these modified Avr determinants likely depends on the nature of the originally targeted specificities. If resistance is targeted against indispensable pathogen products, such as those involved in replication, it is likely that the effectiveness of the corresponding *R* gene (its "durability") may be extended.

HSTs were the first compounds shown to confer plant disease specificity. In his review, Walton provides a very informative description of the structure of HSTs and outlines the recent advances in the genetics and biochemistry of their production and function. Single host genes condition sensitivity to HSTs, and the ability of HST-producing fungi to cause disease is strictly correlated with synthesis of the toxin. Thus, HSTs are potent weapons in the arsenals of otherwise relatively weak pathogens. Interestingly, as pointed out by Walton, the majority of known HSTs are produced in two fungal genera, *Cochliobolus* and *Alternaria*.

The most extensive information concerning the mechanism of toxin activity comes from studies of T-toxin, which is produced by *C. heterostrophus*. T-toxin sensitivity is mediated by a chimeric 13-kD protein (URF13), which is localized on the inner mitochondrial membrane. Several studies indicate that in the presence of T-toxin, URF13 forms pores in membranes. It is thought that this property accounts for the catastrophic biochemical and morphological disturbances in T-toxin–sensitive mitochondria. HC-toxin, which is produced by *C. carbonum*, inhibits histone deacetylase, leading to the accumulation of acetylated histones in affected plant nuclei. In another example of the scope of mechanisms that pathogens use to alter and subvert plant metabolism, HC-toxin may actually indirectly modify the transcription of plant genes. Among the genes whose transcription may be affected by the buildup of acetylated histones are those encoding components of the plant's defense response mechanisms.

Given the exquisitely precise targeting of HSTs against a single aspect of host metabolism, how did toxin production evolve? This is a fascinating question, because HST synthesis and delivery are coordinated by surprisingly complex genetic loci in the fungi. For example, genetic analyses discussed by Walton indicate that HC-toxin production in *C. carbonum* requires at least two genes, in addition to that encoding the HC-toxin synthetase enzyme involved in the biosynthesis of the HST. All three genes have been mapped to the same locus, *TOX2*, which turns out to contain at least two physically linked copies of each gene. Conversely, in *C. heterostrophus*, two genes, *Tox1A* and

Tox1B, are required for T-toxin synthesis. These genes map to the same locus, even though they are actually located on different chromosomes. The explanation for this unusual situation is that cosegregation of the two genes is caused by a translocation event.

Walton also introduces what is known of plant resistance to toxins. In a notable achievement, the first plant disease resistance gene to be cloned was *Hm1*, which confers resistance to HC-toxin in maize. *Hm1* was cloned by transposon tagging, and homologs are present in several HC-toxin–insensitive grasses. The gene encodes an NADPH-dependent reductase, HC-toxin reductase, which inactivates HC-toxin through a side chain modification.

Nematode Predation

Parasitic nematodes can also have profound effects on plant metabolism, and in some cases can even affect root architecture (Figure 1). Members of the nematode, or roundworm, phylum represent some of the most abundant multicellular organisms on earth, and it has been estimated that their species diversity even exceeds that of insects. Over 20 nematode genera cause plant diseases (Figure 5), and many more are parasitic on animals (Dropkin, 1989). Other species are free living and feed on microorganisms and organic matter. All of the nematodes that attack plants are obligate parasites, and all possess a hollow feeding stylet that is used to penetrate plant cell walls. Nematodes vary considerably in their external appearance (Figure 5), and morphological variations in their mouthparts, digestive tracts, and reproductive organs form the basis for classification and identification.

Nematodes go through four molts before becoming sexually active, hermaphroditic, or parthenogenic adults. Most plant parasitic nematodes feed exclusively on roots, but a few species feed on foliar tissue and other above-ground parts of ornamental plants; some seed-gall nematodes infect the floral organs of cereals and grasses. Ectoparasitic nematodes feed at root surfaces and can inhibit root development and/or cause lesions around feeding sites. In contrast, endoparasitic nematodes migrate into the root, where they develop into adults and cause gross abnormalities in root development. Feeding injury by both categories of nematodes can cause considerable damage to plants. Moreover, root injury can also increase invasion by facultative fungal pathogens, particularly *Fusarium*, *Verticillium*, and *Pythium* spp, which greatly exacerbate

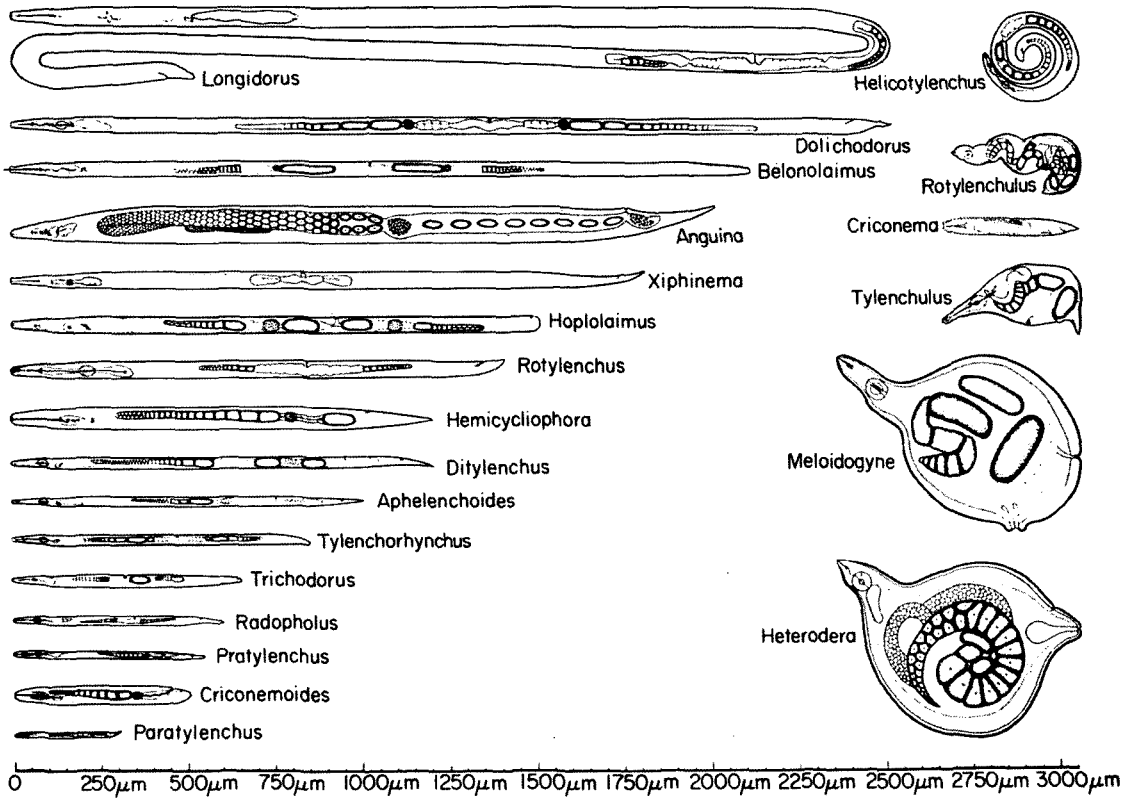

Figure 5. Morphology and Relative Sizes of Plant-Parasitic Nematodes.

Reprinted from Agrios (1988), with the permission of Academic Press.

the damage caused by nematode feeding. In addition, nematodes belonging to the genera *Xiphinema*, *Longidorus*, and *Trichodorus* (Figure 5) are the principal vectors of two major groups of plant viruses that cause many serious diseases.

The sedentary endoparasitic root-knot (*Meloidogyne*) and cyst (*Globodera* and *Heterodera*; see Figure 5) nematodes cause extensive crop losses worldwide, and they affect a large number of dicot species. Williamson and Hussey describe the relationships between these nematodes and plants, and they discuss recent findings regarding parasitic nematode life cycle and pathology. During pathogenesis, root-knot and cyst nematodes establish intimate relationships with their hosts and engage in a series of defined developmental changes as they mature inside plant roots. Concurrently, the development of procambial cells surrounding nematode feeding sites is markedly altered, such that characteristic multinucleate syncytia or giant cells form. These modified plant cells are the source of nutrients for the nematodes.

Williamson and Hussey also describe the changes in plant gene expression that are associated with nematode infection, with a view toward identifying genes whose products may be critical in mediating the infection process. There already is evidence that antisense strategies may prove effective in preventing nematode infection in transgenic plants, and the authors describe other engineered resistance approaches that also show promise. These include the expression of collagenase to dissolve the nematode cuticle, the potential of engineered toxins to kill the invading nematode or the developing giant cell, and the synthesis of novel single chain antibodies to block development of the feeding site.

Arabidopsis is a promising model organism for studying the effects of nematode predation on plant health and development. Arabidopsis is susceptible to root-knot nematodes, and its translucent roots have facilitated high resolution microscopy studies of the infection process. The relative ease with which the infection process can be followed and the genetic tractability of Arabidopsis should facilitate the identification of mutants affected in the infection process. Direct comparisons of the effects of host mutations on nematode development during various stages of pathogenesis are also feasible.

Several dominant *R* genes effective against root-knot and cyst nematodes have been mapped over the past 5 years. Williamson and Hussey describe the ongoing efforts to clone these genes, some of which map to loci containing *R* genes that mediate gene-for-gene interactions between plants and other pathogens. In the future, exciting advances are expected as the genetic utility of Arabidopsis is applied to root-knot nematode pathology. Advances in the genetics of the extensively studied free-living nematode *Caenorhabditis elegans* are also expected to have an impact, as homologies between *Meloidogyne* sp and *C. elegans* genes are identified. Such genes will be extremely useful for probing plant pathogenic nematode development, the cell biology of infection, and the role of the nematode in influencing the formation of the feeding site.

RESISTANCE RESPONSES

Despite the large number of microorganisms capable of causing disease, most plants are resistant to any given pathogen. The defense mechanisms utilized by plants can take many different forms, ranging from passive mechanical or preformed chemical barriers, which provide nonspecific protection against a wide range of organisms, to more active host-specific responses that provide host- or varietal-specific resistance. Genetic studies carried out nearly a century ago first identified *R* genes that were effective against individual pathogen varieties. These genes were immediately employed in breeding programs and have subsequently provided the most cost-effective agronomic basis for disease control in crop plants.

Over time, plant pathologists identified so-called physiological races of pathogens that could circumvent the protection conferred by individual *R* genes. However, not until the detailed studies of Flor (1971) was it realized that *R* genes in the host interact in paired combinations with pathogen *Avr* genes to condition resistance. These gene-for-gene relationships provided the basic underpinning for more efficient utilization of *R* genes in plant breeding, which in turn motivated detailed genetic analyses of host–pathogen interactions. The gene-for-gene hypothesis also provided a rationale for the development of isogenic host and pathogen systems to probe the biochemical and molecular events that occur during interactions leading to the diseased and resistant states. Indeed, during the past decade, numerous viral, bacterial, and fungal avirulence determinants have been cloned. Moreover, genetic characterization of host *R* genes has recently resulted in the isolation of more than 10 genes that collectively condition resistance to each of these groups of pathogens.

A hypersensitive response (HR) that is elaborated in response to invasion by all classes of pathogens is the most common feature associated with active host resistance. In most cases, activation of the HR leads to the death of cells at the infection site, which results in the restriction of the pathogen to small areas immediately surrounding the initially infected cells. At the whole plant level, the HR is manifested as small necrotic lesions. The number of cells affected by the HR is only a small fraction of the total in the plant, so this response obviously contributes to the survival of plants undergoing pathogen attack. Because the HR appears to be so intimately involved in resistance, it is of considerable fundamental and practical interest to identify the events that trigger the response and to define the steps culminating in cell death.

Is cell death actually responsible for resistance or is it a consequence of pathogen attack? That the host may have a major role in controlling the HR is suggested by the identification of mutants, termed lesion mimics, which exhibit a disease- or HR-like phenotype in the absence of pathogens, or in the presence of pathogens that should not trigger the HR (see the cover photograph). Cloning and analysis of the genes conditioning these mutations should help to identify components of signal transduction cascades and ancillary proteins involved in

triggering and/or controlling cell death during the HR and disease. A more complete catalog of these and other mutants should provide answers to important questions concerning the number of signaling pathways that lead to disease resistance, the organization of pathway components, and the potential cross-talk between these components.

Our understanding of disease resistance has undergone explosive growth within the past few years. We now appear to be poised to enter an era of remarkable discovery that should soon lead to the resolution of many outstanding questions concerning disease development. Among these issues are: How are resistance and avirulence specificities generated? How do *R* gene products trigger defense responses? How is the HR initiated and controlled? What are the roles of the various induced compounds in resistance? How are secondary defense responses such as systemic acquired resistance (SAR) elicited and maintained? What is the nature of preformed barriers to pathogen attack? The reviews in the section entitled Resistance Responses discuss recent findings in these areas and suggest future directions for research aimed at answering the questions posed above.

Breeding Strategies and the Genetics of Resistance

The genetics of well-characterized resistance specificities is the subject of the review by Crute and Pink. *R* genes are often organized into linkage groups that may contain separate resistance loci, each with a number of different allelic specificities and each conditioning resistance to a different pathogen. Other examples exist in which *R* genes with functional homology, in that they are directed toward recognition of the same Avr determinant, are found in unrelated host species. These findings suggest that *R* genes are often members of large multigene families that can be conserved in distantly related plant species.

As discussed by Crute and Pink, the complex arrangement of *R* loci facilitates the inheritance of suites of different resistance specificities. However, the organization of these loci also expedites recombination and gene duplications and deletions that can lead to the evolution of novel resistance specificities. In addition, gene dosage effects, nonallelic and epistatic interactions, and host background genotypic factors can all influence the inheritance patterns of *R* genes and can also affect the phenotypes they mediate. Some of these modifying genes are unable to independently condition a resistance response but are essential for the activity of *R* genes. It is possible that this class, like the lesion-mimic genes, forms part of a signal transduction pathway leading from specific recognition determinants (i.e., the *R* gene products) to the genes that actually trigger cell death. Crute and Pink point out that a refined understanding of these genes is essential if they are to be manipulated for the introduction of broader pathogen resistance into a wide range of transgenic crop species.

Technologies that were developed to facilitate gene cloning will assist in plant breeding strategies aimed at the incorporation of cloned resistance-mediating genes into desirable crop varieties. For example, Crute and Pink discuss how marker-aided selection strategies provide a useful tool for adding or "pyramiding" multiple *R* genes into a single crop variety and for the production of isogenic multiline cultivars differing in their resistance specificities. A number of varietal multiline strategies that facilitate deployment of cultivars in a manner designed to maximize the durability of *R* genes are also described. Thus, it is clear that once functional *R* genes can be routinely transferred to crop species, they will be quickly used in breeding programs. It is also obvious that considerable effort is already going into the planning of strategies that will provide greater agronomic flexibility in the deployment of disease-resistant specificities than has been available in the past.

R Gene Structure and Function

The deduced structural properties of cloned *R* gene products are described in the review by Bent. Although only about a dozen *R* genes have been cloned, it is already apparent that these genes can be grouped on the basis of their deduced structural features. Four classes of *R* gene (excluding *Hm1*, which encodes HC-toxin reductase) and two subclasses have been proposed, but it would be surprising if this scheme were not quickly superseded as additional *R* genes are characterized. Among the potentially important *R* gene motifs discussed by Bent are serine/threonine kinase motifs, which imply that the corresponding R proteins may be components of a kinase/phosphatase signaling cascade; leucine-rich-repeat (LRR) motifs, which have been implicated in protein–protein interactions in animal systems; nucleotide binding motifs; and a Drosophila Toll-like motif, which is similar to motifs in proteins with a presumed function in transcriptional regulation. However, these motifs have considerable consensus variation among the isolated *R* genes, which is likely to contribute to resistance specificity.

Although the functional significance of the motifs present in *R* gene products has not been fully resolved, it is expected that each motif has an instrumental role in R protein activity; Bent discusses preliminary indications of what these roles may be. As additional pairs of *R* and *Avr* genes are cloned, the precise functional dissection of their interactions can be performed using site-directed mutagenesis in combination with biochemical analyses. Sequence data from additional *R* genes will also help to define the conserved regions and, conversely, those portions of the proteins that are specific for individual *Avr* determinants. These analyses will provide valuable background information for the rational design of novel resistance specificities.

Downstream Resistance Responses

From the perspective of practical utility, one of the potential pitfalls of focusing only on *R–Avr* interactions is that the transferred *R* genes may not always contribute novel resistance specificities to the transgenic crop. This would happen if downstream components of the signal transduction pathway that leads to induction of the full resistance response were species specific. Hints of downstream species specificity are emerging from recent experiments showing that transfer of *R* genes to closely related species may be accomplished readily but that functional transfer of *R* genes to more distantly related species may be difficult to achieve. Thus, a more detailed dissection of downstream *R* gene interactions and the reactions that confer signaling specificity will be necessary to harness the full potential of transgenic *R* genes in a wide range of crop species.

The biochemical events occurring during the defense response are the subject of reviews by Hammond-Kosack and Jones, Dangl et al., and Ryals et al. Hammond-Kosack and Jones take a broad approach and outline a number of biochemical changes that occur when the HR is triggered by gene-for-gene interactions. Although the biochemical basis for hypersensitivity is unknown, during the HR controls regulating ion flux are compromised, damaging concentrations of reactive oxygen species (ROS) accumulate, and marked changes occur in normal metabolic processes, including the synthesis of salicylic acid (SA). The ROS may also function either to cross-link cell wall components or as toxic substances that attack the pathogen, or both. Moreover, both ROS and SA appear to have roles in signal transduction cascades that coordinate various defense responses in the plant.

Several downstream defense responses described by Hammond-Kosack and Jones that occur in and around the infection site may serve to confine invading pathogens. These include the synthesis of pathogenesis related (PR) proteins and the accumulation of hydroxyproline-rich glycoproteins, which may function in the strengthening of cell walls, and of biosynthetic precursors of callose and suberin, which may be involved in sealing off the infection site. Although these responses have been investigated for many years, there is as yet no clear consensus concerning their direct mechanistic contributions to the overall defense response or to the containment of the invading pathogen. It is possible that many components of the HR are instead part of a general long distance warning system that functions nonspecifically to protect the plant against subsequent pathogen attack.

Other compounds that are synthesized during the defense response also appear to have more indirect effects on resistance. For example, the synthesis of phytoalexins and the roles of these compounds as microbial antagonists have been scrutinized for over 30 years. Although these compounds have demonstrable activity against numerous pathogens in vitro, their in vivo participation in the primary defense response has yet to be convincingly demonstrated. Studies in Arabidopsis suggest that the gene-for-gene resistance response to *Pseudomonas* is not directly affected in phytoalexin-deficient mutants. Thus, phytoalexins may have important roles that affect secondary invasion or disease development by virulent pathogens, rather than a central role in the containment of avirulent pathogens.

Hammond-Kosack and Jones also discuss recent advances in genetic approaches aimed at understanding downstream resistance responses. Screens to identify genes that are required for disease resistance have already resulted in the recovery of genes thought to function in interactions with the primary recognition determinants and those functioning near the upstream portion of the signal transduction pathway. Mutant alleles that affect one or more *R* genes some distance down the signal pathway from the site of Avr perception have also been identified. The cloning and characterization of the genes that these mutations define are eagerly anticipated.

Lesion-Mimic Mutants

Another class of informative mutants, the so-called lesion-mimic mutants, is the focus of the review by Dangl et al. These mutants, many of which have been identified in maize, Arabidopsis, and other species, exhibit disease- or HR-like symptoms in the absence of pathogens or in the presence of pathogens that do not ordinarily trigger the HR (see the cover photograph). The identification of lesion-mimic mutants raises two important questions pertinent to the role of the HR in defense: which events initiate hypersensitivity, and how is the spread of the HR confined? One advantage of using the lesion-mimic mutants to help in answering these questions is that the defense-like responses are triggered in the absence of complications arising from the simultaneous expression of pathogen genes.

Two broad classes of lesion-mimic mutants are described by Dangl et al.: those that appear to spontaneously initiate lesion formation (so-called initiation class mutants) and those that appear to have a defect in constraining lesion expansion (propagation class mutants). The nature of initiation class mutants implies that some early step in the HR, such as a signal receptor or an early component of a signal transduction pathway, is malfunctioning. The fact that some of the initiation class mutations map to known disease resistance loci suggests that they may represent "*R* genes gone bad." These "paranoid" mutant plants may consequently exhibit hair-trigger responses to a variety of minimally stressful environmental cues or injurious insults.

The propagation class lesion-mimic genes could encode negative regulators of developmental cell death pathways, such as those that operate during xylem vessel formation in plants and various developmental pathways in animals. The loss of such negative regulators would imply that inhibitory signals normally leading to the constraint of programmed cell death

in surrounding cells are compromised. This lack of negative regulatory signals could, in turn, lead to a cell death stampede that affects large areas of tissue surrounding the normal target cells. Alternatively, propagation class mutants could be affected in signals that specifically constrain lesion formation during pathogenesis.

A number of compensatory suppressor mutants that condition reversion of lesion mimics have also been identified. These mutants provide a valuable resource for the dissection of disease resistance responses and, possibly, for studies of developmentally programmed cell death pathways, which are poorly understood in plants. Dangl et al. address a number of outstanding issues in these areas. For example, is HR-mediated cell death analogous to the endogenously programmed apoptotic death of some animal cells; or does the HR result from induced membrane leakage and general cellular damage? These possibilities are not mutually exclusive, and it is possible that both kinds of cell death may occur during plant defense responses.

Systemic Acquired Resistance

SAR and the role of SA in triggering this response are covered extensively in the review by Ryals et al. The SAR early warning alert is elicited during necrotic resistance responses against viruses, bacteria, and fungi, and it culminates in the activation of broad spectrum resistance against a large number of biotrophic pathogens. During necrosis, or upon application of SA, normally susceptible tissues develop highly resistant responses during which expression of at least nine families of so-called SAR proteins are elicited. Some of these are similar to the PR proteins described above, and others can contribute to resistance against bacterial and fungal pathogens when expressed individually in transgenic plants.

Among the more informative tools in these studies have been transgenic plants that express the bacterial gene *nahG*, which encodes salicylate dehydroxylase. Plants expressing high levels of this gene (so-called NahG plants) exhibit markedly reduced levels of SA, and both local and systemic resistance responses are compromised to some degree. Crosses between NahG plants and resistance response mutants, such as the lesion-mimic mutants described above, have helped to establish epistatic relationships between different classes of mutants (or transgenics). This approach is helping to unravel the signal transduction pathways that trigger both local and systemic resistance.

Experiments designed to determine whether SA is the translocated signal that leads to SAR in distal organs are also discussed by Ryals et al. Some results suggest that SA is indeed transported throughout the plant. However, grafting experiments with NahG tobacco stem segments, which should destroy SA, suggest that the SAR-inducing signal is able to pass through NahG grafts. Nevertheless, these experiments do demonstrate a direct link between the signals participating in local lesion formation (i.e., the HR) and SAR signal transduction.

Chemical inducers of SAR, developed with a view toward their application in the field, have also been informative in defining steps in the SAR induction pathway. Genetic analyses have revealed that these inhibitors are involved in the same signaling pathway as SA and the lesion-mimic genes but that they act independently or downstream of SA. From the present results, it appears that SA also acts downstream of the lesion response, but that SA-dependent processes may participate in the feedback-regulated suppression of lesion formation.

Preformed Defense Mechanisms

As in human affairs, the best form of disease control is prevention, and most plants have a battery of preformed secondary metabolites that are directly antimicrobial. These secondary compounds were among the first components to be implicated in resistance to plant pathogens. Some of the compounds are biologically active, whereas others are converted to active forms by host enzymes released during infection or injury. However, definitive genetic tests to verify the roles of these compounds in plant disease resistance have been conducted only in a relatively few cases. Osbourn focuses on three classes of preformed compounds: saponins, cyanogenic glycosides, and glucosinolates and on their respective roles as inhibitors of plant disease-causing organisms. To date, the most informative principles are derived from studies of saponins.

The saponins are glycosylated compounds which have been separated into three groups (triterpenoids, steroids, or steroidal glycoalkaniods). The contribution of these compounds to disease resistance has been characterized most extensively in oats and tomato. Some indirect evidence suggests that resistance of oats to the take-all fungus correlates with the presence of avenacin, which is present in oats in a biologically active form. A genetic approach using *Avena strigosa* mutants lacking avenacin should permit a more rigorous assessment of resistance and its correlation with saponin content. The best-characterized tomato saponin is α-tomatine, a steroidal glycoalkaloid, which is also present in a biologically active form. Considerable variation in α-tomatine content exists in tomato varieties, but there is no compelling correlation between α-tomatine levels and varietal-specific resistance to phytopathogenic fungi.

Osbourn also describes the mechanisms that fungal pathogens have evolved to protect themselves against saponin toxicity. For instance, the tomato pathogen *Cladosporium fulvum* appears to prevent the release of α-tomatine from host cells. *Alternaria solani* uses a different strategy to block the effects of saponins. By lowering the pH at the infection site, it is able to reduce saponin toxicity. Moreover, sterol-containing membranes are thought to become porous due to the formation of sterol–saponin complexes. Some Oomycetes (Figure 4) such as *Pythium* and *Phytophthora*, which have membranes

that lack sterols, are thought to avoid the toxic effects of saponin by virtue of their membrane composition.

Biochemical detoxification of saponins is another fungal protective mechanism. Saponin-detoxifying enzymes from *Gaeumannomyces graminis*, *Septoria lycopersicae*, *S. avenae*, and *F. oxysporum* have related sequences, despite their differing specificities in detoxification activity. DNA hybridization analyses show that the corresponding genes are present in a wide range of other pathogenic fungi, so it is possible that the ability to detoxify saponins may contribute to fungal virulence. Clearly, however, additional genetic studies need to be conducted to analyze the determinants required for pathogenicity of these fungi and to determine whether varietal- or host-specific resistance actually correlates with saponin levels.

BIOLOGICAL AND PATHOGEN-MEDIATED DISEASE CONTROL

Hundreds of plant diseases that limit the kinds of crops that can be grown in particular localities have been described over the past century. Even in areas where resistance genes or agronomic strategy control some serious diseases, other plant pathogens often demand the use of costly agrochemicals that affect the environment and raise production costs. It is therefore important to implement multifaceted approaches toward disease control that are based both on a sound knowledge of host–pathogen interactions and the ecological underpinnings that affect them.

Within the past decade, numerous advances in the molecular genetic interdiction of virus diseases have been made, and steps toward the biological control of soilborne pathogens have also been taken. These novel approaches are beginning to provide the information necessary to permit rational agronomic responses as evolving pathogens overcome contemporary control strategies. The reviews by Baulcombe, Nuss, and Handelsman and Stabb describe developments in three emerging areas that show promise for the future development of novel approaches toward biological disease control.

Pathogen-Derived Resistance and the Control of Virus Disease

The control of virus diseases has traditionally relied on the utilization of *R* genes, on cultural practices that reduce disease spread, and on production of pathogen-free plants. However, a seminal advance in biological control was made in 1929, when McKinney described a resistance phenomenon that he called cross-protection. These experiments showed that tobacco could be protected from infection by a severe strain of TMV by previous inoculation with mild strains. Although a few notable studies demonstrated the potential of the strategy (Fulton, 1986), cross-protection did not become widely used. This was primarily because of the labor required to infect plants with live virus and the concern that mild virus strains used to protect one crop may cause serious diseases on others.

More than 50 years later, these two problems were finally circumvented when Beachy and coworkers expressed viral genes in transgenic plants (Powell-Abel et al., 1986). These experiments demonstrated that tobacco expressing the TMV CP gene was resistant to TMV infection. The resistance was specific for TMV and provided a considerable level of protection. Other studies quickly verified that CP-mediated protection was generally applicable to a large number of plant viruses. Subsequently, genes other than those encoding CPs were shown to confer effective protection against infection. These important findings opened up a practical approach with vast implications for disease control that is being applied rapidly to produce disease-resistant cultivars of various crop plants (e.g., see Tricoli et al., 1995). However, despite the rapid commercial adaptation of the strategy, an understanding of the mechanisms through which interference with the infection process is mediated by viral transgenes has developed more slowly (Scholthof et al., 1993; Lomonossoff, 1995). In his review, Baulcombe describes recent findings that are beginning to explain the fundamental basis for pathogen-derived resistance (PDR) and the insights these findings are providing for other aspects of plant biology.

It is apparent that a number of different mechanisms mediate PDR, although it is not yet clear exactly how these operate. The first cases cited by Baulcombe appear to function at the protein level. Several examples that require the expression of functional CP have been reported, and it has been proposed that the small amounts of endogenously expressed CP may inhibit virion disassembly in the initially infected cells. However, other evidence indicates that long distance movement may also be suppressed, which suggests that CP transgenes may also interfere with phloem transport.

Dysfunctional MPs expressed in transgenic plants, but not those encoding functional MPs, also interfere with cell-to-cell movement. Interestingly, limited trials using these mutated MP derivatives suggest that they condition broader resistance than that obtained through CP-mediated PDR. It is therefore possible that the abnormal MPs bind to and block conserved plasmodesmatal components that are required for cell-to-cell movement of these viruses.

A second major form of resistance appears to operate at the level of nucleic acids, and this phenomenon shares many features with cosuppression, or silencing, of nonviral transgenes. Data from recently published experiments clearly show that plants transformed with nonviral genes are protected from viruses that express homologs of these genes. Baulcombe discusses several models that predict how these effects may be mediated, including the possibility that sequence specificity may be conferred through antisense RNA fragments and/or by methylation of the transgenes. However, several contradictory results have been reported, and it is clear that additional experiments need to be carried out to clarify the mechanism(s)

involved in this phenomenon. Nevertheless, these examples serve once again to demonstrate a situation in which studies of plant–pathogen interactions impinge on fundamental questions in plant biology. A more complete understanding of gene silencing may have implications for studies of mRNA turnover and other aspects of gene regulation that could be exploited to provide broader protection against virus diseases.

Hypovirus-Mediated Control of Fungal Pathogens

In addition to losses in cultivated crops, diseases of forest and urban trees have also resulted in costly environmental and ecological disasters. The most notable example is chestnut blight, which caused the demise of the North American chestnut throughout its entire range. Prior to the appearance of the blight in 1904, chestnuts constituted ~25% of the hardwood forest from Maine to Alabama, or about four billion trees. By 1940, essentially all of the trees within this region were killed by the fungus *Cryphonectria parasitica,* due to its ability to invade the cambium and girdle the tree. The remnants now exist only as shrubs that have emerged from old stumps, and even these are periodically killed by recurring attacks of the fungus. The decline of the chestnut affected many aspects of life for the inhabitants of the Appalachians; for example, the timber was valuable for lumber and furniture, and the nuts provided significant income. The blight has also had a major impact on the wild life of the region because it destroyed an important food source for many animal species.

The active sexual stage of *C. parasitica* ensures the continuous reassortment of virulence genes and the rapid evolution of novel pathogenicity determinants. This propensity is confounded by the long generation time of the chestnut, which precludes the rapid evolution of effective resistance to the pathogen. Fortunately, a virus-like double-stranded RNA that infects *C. parasitica* and attenuates its pathogenicity on chestnut has been identified. This "hypovirus" can be transmitted to different strains of the fungus during hyphal fusion (anastomosis). However, the hypovirus would be more useful as a disease-control agent if it could be efficiently transmitted in fungal ascospores or conidia (see Figure 4).

In his review, Nuss describes the properties of the hypovirus and discusses the mechanisms through which the virulence of infected fungi may be attenuated. The review focuses on the results of experiments using a biologically active cDNA hypovirus derivative that has been integrated into the fungal genome. Experiments with these fungi have enabled the abnormal colony phenotypes that appear after hypovirus infection to be dissected from the attenuation of virulence. Additional experiments demonstrating that the hypovirus host range can be extended to closely related fungal pathogens have permitted comparative analyses of hypovirus transformation, cytoplasmic replication, effects on virulence, and asexual conidiospore production. These experiments further our understanding of the requirements for hypovirus transmission and the mechanisms through which its attenuation of fungal virulence is mediated.

Of particular interest is Nuss's analysis of changes in fungal gene expression during infection by the hypovirus. In addition to facilitating the identification of a number of differentially expressed genes, these experiments have implicated a G-protein–mediated signaling cascade in the attenuation of virulence. These results support a mechanistic model in which virulence attenuation is affected by a G-protein/adenylcyclase signaling pathway that transmits extracellular host signals from the hyphal surface to the nucleus.

This hypothesis provides important clues for understanding the processes through which phytopathogenic fungi recognize external cues and how this recognition affects virulence. It also suggests a potential route toward the identification of key host ligands that activate fungal virulence genes. Moreover, a striking gene silencing response was also reported after *C. parasitica* was transformed with sense derivatives of the G-protein gene. This response is reminiscent of the RNA-mediated transgene silencing that operates during PDR and indicates that cosuppression phenomena are not restricted to plants.

Biological Control of Soilborne Pathogens

Plants and pathogens do not exist in a vacuum, and many interactions among plants, microbes, and local environments can affect the outcome of their associations. Rhizosphere communities are among the most diverse ecological niches on the planet: competition for nutrients among the rhizosphere microflora is fierce, and plant root exudates can play important roles in influencing the species diversity and population levels of microbes in the soil. Can this competition be rationally exploited, and can harmful microbe populations be manipulated through careful application of more beneficial microbes?

Many attempts have been made over the past century to do just this. However, success has been variable, and progress in developing general principles for biocontrol has been slow, primarily due to the vast array of interactions between plants and the rhizosphere microflora. This complexity and the fluctuating microenvironments that exist within the rhizosphere have confounded the design of experiments aimed at identifying the key factors that influence host effects on microbial competition. Developing realistic biocontrol strategies by manipulating plant–microbe interactions requires a comprehensive understanding of the genetic determinants and environmental factors that affect rhizosphere biology at or on the surface of the root. Consequently, in their review Handelsman and Stabb stress the importance of genetic approaches to address the complexity of rhizosphere interactions and their roles in disease suppressive effects.

The authors also provide a synopsis of recent developments in the biocontrol of root pathogens. They focus on the practical utility of *Bacillus* and *Pseudomonas* bacteria (Figure 3), and two fungi, *Trichoderma* and *Gliocladium,* as biocontrol agents for take-all, Oomycete root infections, and damping-off diseases (which are caused by *Pythium* and related

organisms). Ongoing studies on the production, nature, and regulation of antimicrobial compounds in biocontrol processes are also discussed in the review. Of particular interest are the quorum sensing mechanisms used by *Pseudomonas* sp to regulate the synthesis and coordinated release of antibiotics in response to increases in other microbial populations in the rhizosphere (Moré et al., 1996). This strategy is similar to mechanisms that are utilized to coordinate expression of virulence functions during infection by necrotrophic pathogens.

Another interesting biocontrol strategy discussed by Handelsman and Stabb involves hyperparasitism, or the highly specific ability of one fungal species to parasitize another. Organisms utilizing this approach provide interesting paradigms that may well be relevant to fungal parasitism of higher plants. Molecular genetic approaches using gene disruption techniques should enable refined analyses of infection processes occurring during hyperparasitism that may have practical utility for the use of *Trichoderma* and *Gliocladium* in disease control. Such studies may also provide insight into common signaling events that occur within the rhizosphere and at the surface of host plant leaves during fungal infection.

Even if rational control strategies based on antibiotic production by soil microorganisms are achieved, a major concern is that endogenous genes for resistance, which are used by the biocontrol agents for protection against their own antibiotics, may be transferred to soil pathogens. This scenario was already played out when resistance to the herbicide agrocin-84 was transferred from the biocontrol agent *Agrobacterium radiobacter* to the pathogen *A. tumefaciens*. Consequently, additional areas for future research include the mechanisms that have evolved to protect antibiotic producers and how transfer of the corresponding genes between soil microbes may occur.

More fundamental questions concerning rhizosphere ecology remain to be answered. How do rhizosphere inhabitants respond to root exudates and surface determinants? Which of their traits contribute to root colonization and to biocontrol efficacy? What are the host responses that occur during colonization by beneficial microorganisms? Can we deliberately engineer or select microbes with superior biocontrol properties and can host varieties be selected that provide more hospitable environments for biocontrol agents? The development of more extensive genetic and mathematical tools will help to answer these questions and will permit a more profound understanding of the strategies used in disease suppression, the resistance of pathogens to suppression, and the genetic and ecological bases for interactions among biocontrol agents, plant pathogens, and their host plants.

SYMBIOSES

Among the favorable plant–microbe interactions that have been studied in the greatest detail are those in which bacteria or fungi enter into mutually beneficial symbioses with higher plants (Stacey et al., 1992; Smith and Read, 1996). As is the case for the majority of plant–pathogen interactions, symbioses are characterized both by their complexity and by their specificity; they are also of enormous importance for global agricultural productivity. Moreover, symbioses provide model systems for studying fundamental plant and/or microbial processes, such as signal perception and transduction, control of the cell cycle, and cellular differentiation.

In most nitrogen-fixing symbioses, soil bacteria of the unrelated genera *Rhizobium* and *Frankia* induce cell divisions in fully differentiated (and quiescent) cells in the root cortex or pericycle of plants in the families Rosaceae and Leguminosae. Bacteria enter the root and migrate, intercellularly or intracellularly, toward these foci of dividing plant cells. As cell division continues and the nascent structures mature into nodules, the bacteria differentiate into forms that are capable of fixing nitrogen (i.e., reducing gaseous N_2 to compounds such as ammonia). The fixed nitrogen is transported throughout the plant and, in return, the bacteria are supplied with photosynthate and a protected environment in which to divide.

In contrast to the restricted phylogenetic distribution of nitrogen-fixing symbioses, mycorrhizal associations are almost ubiquitous, and the effects of these associations on plant fitness and health, and on the ability of plants to grow productively in suboptimal environments are profound. Mycorrhizas are intimate associations between plant roots and certain soil fungi, and are typified by outgrowths of fungal hyphae from mycorrhizal roots. These hyphae serve to increase the absorptive surface area of the root, thus facilitating the uptake of nutrients and minerals, particularly phosphorus (which is limiting for plant growth in many soil types). Mycorrhizal associations may also help to protect roots from infection by pathogenic organisms in the soil.

Are mycorrhizal and nitrogen-fixing symbioses related? Although the microorganisms and plants involved in the different symbioses are quite distinct, it is clear that these symbioses do, in fact, share a number of similar features. For example, infection by both kinds of nitrogen-fixing bacteria, and by mycorrhizal fungi, triggers similar, but limited subsets of the plant's defense responses. This implies that, between them, the symbionts must be capable of modulating these responses. Furthermore, there is evidence that Nod factors, which mediate the specificity of legume–*Rhizobium* interactions, can also stimulate mycorrhizal formation. After infection, the developmental program of nodule formation is controlled by the plant in both kinds of symbioses. In fact, nodules formed on *Parasponia*, the only nonlegume known to form nitrogen-fixing associations with rhizobia, are developmentally more similar to those formed on actinorhizal plants by *Frankia* than they are to nodules on legumes.

However, the most compelling evidence for common steps in the different symbioses is that some plant mutants, initially identified through their inability to enter into symbiotic relationships with nitrogen-fixing bacteria, are also refractory to infection by mycorrhizal fungi. Although these mutations may define genes with housekeeping roles in the manipulation or maintenance of the symbiotic state, the fact that they can affect very early events in both kinds of symbiosis implies that their products may be more directly involved with symbiont

recognition. By identifying and cloning the genes controlling these symbioses, it should be possible to define the molecular mechanisms through which their specificity is mediated and the plant defense responses are restricted. This may be achieved through the use of model legume species, such as *Medicago truncatula* and *Lotus japonicus*, which are more amenable to molecular genetic manipulations than many of the other legumes. One of the most pressing questions concerns the nature of the specific plant receptor molecules. Are they similar to the *R* gene products that mediate the specific recognition of pathogens in gene-for-gene interactions?

Genetic approaches have also proven useful in the identification of *Rhizobium* genes whose products are required for symbiosis. However, these approaches are in their infancy for *Frankia* and are not yet feasible for mycorrhizal fungi, many of which are unculturable. Nevertheless, as comparative approaches help to highlight the similarities between different symbiotic strategies, it may be possible to use information derived from the analysis of one kind of relationship to help in the understanding of the other.

Mycorrhizal Symbioses

The importance of mycorrhizal associations was recognized when difficulties in transplanting forest trees to new soils were encountered. The new environments lacked the appropriate fungal species, and the trees were not able to thrive without them. Mycorrhizal associations are now known to be important for a wide variety of cultivated and native plants and form an essential component of their ecology. These associations can take on a number of different morphologies but they fall into two broad categories (Smith and Read, 1996). In endomycorrhizal associations, such as arbuscular mycorrhizas (AM), the mycorrhizal fungus penetrates root cells in response to specific signals from the plant. In the cortical cells the fungi differentiate nutrient exchange structures, termed arbuscules. These are anatomically similar to the haustoria (feeding structures) formed by pathogenic fungi, although their function is very different. Gross changes in root morphology are not generally seen in these symbioses, although subcellular modifications are extensive. By contrast, in ectomycorrhizal symbioses fungi grow within the cortical cell walls and their hyphae form a sheath around the root.

In her review, Gianinazzi-Pearson focuses on AM symbioses by providing a detailed analysis of the changes in biochemistry and gene expression that accompany their establishment. She discusses what is known of the exchange of nutrients at the interface between the plant and fungal symbionts, including the recent identification of a plant hexose transporter gene whose expression increases markedly in cortical cells containing arbuscles (Harrison, 1996).

Gianinazzi-Pearson also describes the current state of the art of mycorrhizal genetics, with descriptions of two classes of plant mutant that are affected in their ability to establish AM symbioses. Myc^{-1} mutants do not support fungal penetration or growth and are likely blocked at a very early stage of the symbiosis. However, some aspects of external fungal differentiation are supported by Myc^{-1} mutants, implying that initial signaling between the plant and the fungus is unaffected. Myc^{-2} mutants support the early phases of fungal penetration and growth, but arbuscles do not develop. Interestingly, neither class of mutant appears to have defects in its responses to pathogens, fungal, or otherwise.

Rhizobial Symbioses

Signals between plant and microbial symbionts also feature prominently in Long's review, in which she describes the early phases of the *Rhizobium*–legume symbiosis. In this system, as in mycorrhizal symbioses, specific signals from the plant trigger increases in the rhizosphere populations of certain rhizobial strains. These rhizobia, in turn, secrete lipochitooligosaccharide molecules, termed Nod factors, that initiate the developmental program in the appropriate plant species that leads to infection and nodule formation. Long describes the structure of Nod factors and outlines the genetic bases of their formation and exquisite specificity. She also discusses the experimental utility of recently reported synthetic versions.

Early signal transduction mechanisms that may be important in plant responses to Nod factors are also discussed by Long. For example, there is correlative evidence that membrane depolarization and waves of Ca^{2+} in root hairs mediate Nod factor responses. These events are likely to occur in other rapid signal transduction cascades in plants, and Long points out the general utility of the legume–*Rhizobium* system in this regard. How the early signaling events trigger the cortical cell divisions that form the prenodule is not known, but it is likely that changes in the relative levels or activities of auxin and cytokinins are involved. Indeed, one of the legume genes induced soon after rhizobial infection of roots, *ENOD40*, has recently been shown to encode a short peptide that modifies plant responses to auxin (van de Sande et al., 1996). It has even been suggested that Nod factors themselves may be related to plant hormones, and Long provides a thoughtful analysis of this somewhat contentious hypothesis.

Actinorhizal Symbioses

In their review, Pawlowski and Bisseling compare the features of the legume–*Rhizobium* symbiosis with those of the actinorhizal symbioses that develop between plants and nitrogen-fixing Gram positive bacteria in the genus *Frankia*. Despite the profound differences in the bacterial species involved, many aspects of these symbioses appear to be shared, including plant control over infection processes and nodule ontogeny. Some defense-related genes are induced in actinorhizal plants during *Frankia* infection, as they are in legumes. However, most actinorhizal plants are herbaceous shrubs and, as such, are not readily amenable to the kinds

of molecular genetic manipulations that have been so informative in other symbioses. It will be most interesting to determine whether any of the plant genes with a critical role in legume nodule initiation are also involved in the establishment of actinorhizal symbioses.

One significant difference between legume and actinorhizal nodules is the site at which root cells become reactivated to form nodule primordia. In rhizobial symbioses, cortical cells are activated to reenter the cell cycle, whereas in actinorhizal symbioses, pericycle cells are activated. The early phases of actinorhizal nodule initiation are therefore quite similar to lateral root initiation, and Pawlowski and Bisseling hypotheisize that actinorhizal nodules may have coopted this developmental program during their evolution.

More fundamental aspects of the evolutionary origin of symbiotic associations are also addressed in this review. Although such questions are hard to answer, Pawlowski and Bisseling address the following: Did symbioses evolve from pathogenic interactions, or are they entirely distinct? Why are nitrogen-fixing symbioses restricted to members of two plant families, whereas mycorrhizal associations are so widespread? Answers to these questions are of considerable practical value and may provide fundamental insight into the evolution and operation of many plant–microbe interactions.

CONCLUDING REMARKS

The rapid progress that continues to be made in our elucidation of plant–microbe interactions is contributing to major advances in our understanding of plant and microbe cell biology and biochemistry. On the pathogen side, studies reported in this issue provide illuminating information concerning the evolution of virulence, the signaling processes involved in symbiotic relationships, pathogenesis, and resistance responses, and the nature of protective mechanisms relevant to the ecology and survival of nonpathogenic microbes.

On the plant side, studies of plant–microbe interactions have led to the development of a wide range of model systems that can be used to probe normal cell biological processes. For example, the recent suggestion that plant pathogenic bacteria export virulence factors directly into plant cells is an exciting concept that impacts investigations of both plant and animal diseases. Similarly, identifying the host components that are involved in plant virus replication and movement should prove helpful for understanding the intricacies of plant nucleic acid metabolism. The same systems are also providing information about the role of the cytoskeleton in macromolecular targeting and the potential developmental impact of diffusion gradients that may be created by cell-to-cell transport of these macromolecules.

The practical utility of understanding plant–microbe interactions is obvious. For example, as the bases for the astounding specificity that is typical of most host resistance responses are unravelled, it should become possible to genetically engineer crop plants that express novel or altered specificities and therefore exhibit broad spectrum resistance to disease. Disease control strategies based on a more detailed understanding of the intricate relationships among roots, pathogens, and beneficial microbes in the rhizosphere are also forthcoming. Strategies hinging on the deployment of decoy molecules that contribute to shifts in the expression of pathogen virulence genes or that affect the ability of rhizosphere pathogens to compete with benign soil organisms could well be realized.

It has been enormously satisfying to participate in the formulation and editing of this Special Issue of THE PLANT CELL. Collectively, the reviews in this issue offer an outstanding analysis of our current knowledge of plant–microbe interactions and the plant and microbial processes upon which they depend. The insight gained from these analyses are already impacting studies of plant development and responses to the environment. We fully anticipate that you will enjoy reading these reviews as much as we have enjoyed editing them!

REFERENCES

Agrios, G.N. (1988). Plant Pathology (San Diego: Academic Press).

Carefoot, G.L., and Sprott, E.R. (1967). Famine on the Wind (Chicago: Rand McNally and Co.).

Cotter, P.A., and Miller, J.F. (1996). Triggering bacterial virulence. Science **273,** 1183–1184.

Dropkin, V.H. (1989). Nematology (London: John Wiley and Sons).

Flor, H.H. (1971). The current status of the gene-for-gene concept. Annu. Rev. Phytopathol. **9,** 275–296.

Fullner, K.J., Lara, J.C., and Nester, E.W. (1996). Pilus assembly by *Agrobacterium* T-DNA transfer genes. Science **273,** 1107–1109.

Fulton, R.W. (1986). Practices and precautions in the use of cross-protection for virus disease control. Annu. Rev. Phytopathol. **24,** 67–81.

Harrison, M.J. (1996). A sugar transporter from *Medicago truncatula*: Altered expression patterns in roots during vesicular-arbuscular (VA) mycorrhizal associations. Plant J. **9,** 491–503.

Janda, M., and Ahlquist, P. (1993). RNA-dependent replication transcription and persistence of brome mosaic virus RNA replicons in *Saccharomyces cerevisiae.* Cell **72,** 961–970.

Jones, J.B., Jones, J.P., Stall, R.E., and Zitter, T.A. (1991). Compendium of Tomato Diseases (St. Paul, MN: APS Press).

Large, C.E. (1940). Advance of the Fungi (New York: Dover Publications).

Lomonossoff, G.P. (1995). Pathogen-derived resistance to plant viruses. Annu. Rev. Phytopathol. **33,** 323–343.

Lucas, W.J., Bouche-Pillon, S., Jackson, D.P., Nguyen, L., Baker, L., Ding, B., and Hake, S. (1995). Selective trafficking of Knotted1 homeodomain protein and its mRNA through plasmodesmata. Science **270,** 1980–1983.

Moré, M.I., Finger, L.D., Stryker, J.L., Fuqua, C., Eberhard, A., and Winans, S.C. (1996). Enzymatic synthesis of a quorum-sensing autoinducer through use of defined substrates. Science **272,** 1655–1658.

Murphy, F.A., Fauquet, C.M., Bishop, D.H.L., Gabriel, S.A., Jarvis, A.W., Martelli, G.P., Mayo, M.A., and Summers, M.D. (1995). Virus

Taxonomy: Sixth Report of the International Committee on Taxonomy of Viruses. (New York: Springer–Verlag).

Pettersson, J., Nordfelth, R., Dubinina, E., Bergman, T., Gustafsson, M., Magnusson, E., and Wolf-Watz, H. (1996). Modulation of virulence factor expression by pathogen target cell contact. Science **273,** 1231–1233.

Pierson III, L.S., Keppenne, V.D., and Wood, D.W. (1994). Phenazine antibiotic production in *Pseudomonas aureofaciens* 30-84 is regulated by *PhzR* in response to cell density. J. Bacteriology **176,** 3966–3974.

Powell-Abel, P., Nelson, R.S., De, B., Hoffmann, N., Rogers, S.G., Fraley, R.T., and Beachy, R.N. (1986). Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. Science **232,** 738–743.

Scholthof, K.-B., Scholthof, H.B., and Jackson, A.O. (1993). Control of plant virus diseases by pathogen-derived resistance in transgenic plants. Plant Physiol. **102,** 7–12.

Schumann, G.L. (1991). Plant Diseases: Their Biology and Social Impact (St. Paul, MN: APS Press).

Shaw, J.G. (1996). Plant viruses. In Fundamentals of Virology, B.N. Fields, D.M Knipe, and P.M Howley, eds (Philadelphia: Lippincott-Raven), pp. 367–400.

Smith, S., and Read, D.J. (1996). Mycorrhizal Symbiosis (London: Academic Press).

Stacey, G., Burris, R.H., and Evans, H.J. (1992). Biological Nitrogen Fixation (New York: Chapman and Hall).

Tricoli, D.M., Carney, K.J., Russell, P.F., McMaster, R.J., Groff, D.W. Hadden, K.C., Himmel, P.T., Hubbard, J.P., Boeshore, M.L., and Quemada, H.D. (1995). Field evaluation of transgenic squash containing single or multiple virus coat protein gene constructs for resistance to cucumber mosaic virus, watermelon mosaic virus 2 and zucchini yellow mosaic virus. Bio/Technology **13,** 1458–1465.

van de Sande, K., Pawlowski, K., Czaja, I., Wieneke, U., Schell, J., Schmidt, J., Walden, R., Matvienko, M., Wellink, J., van Kammen, A., Franssen, H., and Bisseling, T. (1996). Modification of phytohormone response by a peptide encoded by *ENOD40* of legumes and a nonlegume. Science **273,** 370–373.

Webster, J. (1980). Introduction to Fungi (Cambridge: Cambridge University Press).

Zhang, J.P., and Normark, S. (1996). Induction of gene expression in *E. coli* after pilus-mediated adherence. Science **231,** 1234–1236.