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How Plant-Pathogenic Bacteria Survive

For nearly as long as it has been recognized that bacteria incite plant diseases, it has been known that these pathogens do not have spores adapted to carry them over long unfavorable periods. Thus, we have lived for a long time with the question of how, in fact, do pathogenic bacteria survive? They may survive for years; of that we have plenty of unwelcome evidence. We have wondered, also, about the sudden increase of some diseases in the field—how can surviving cells account for all of this disease?

During the last two decades we have begun to understand what is happening in the field and more about the surviving bacterial cell itself. We have come to realize that these pathogens may have special relationships with plants they attack and sometimes also with plants they do not damage. How these matters affect survival is the main theme of this article. Particular emphasis is given to the increase of pathogens associated with healthy plants—the chief recent discovery that has so enlarged our perspective of the epidemiology of bacterial diseases.

The survival time for plant-pathogenic bacteria is the most diminished time in the life cycle. With numbers of pathogen cells at ebb, it would seem to be the best period to apply control measures effectively. However, the pathogen cell during the survival period is least vulnerable to treatments aimed at its eradication. Nevertheless, this phase of the life cycle bears much more careful study than it has received. This is a worthwhile objective with the bacterial diseases, because one of the traditional ways of disease control—the use of chemical inhibitors—generally is not effective. Most reliance has come to be placed on the use of "pathogen-free" seed stocks, resistant varieties, and sanitation. Ordinarily these methods are satisfactory, but sometimes they may be inadequate or not available.

With most annual agricultural plants, pathogenic bacteria survive from season to season with seed or propagative parts, in debris from plants that have been diseased, or in soil. Some also survive on or in living crop or weed plants. This is common with perennial woody species; in our region, for example, small cankers are the main source of the spring inoculum for the fire blight pathogen of apple. The bacterial pathogens that incite yellows diseases survive within vascular tissues of perennial plants. These pathogens are the wall-less bacteria (mycoplasmas, including spiroplasmas) and the walled bacteria that are cultured with difficulty or not at all (rickettsias). The yellows diseases and the comparatively few pathogens that survive with insects are not of concern here. Emphasis is on bacteria belonging to these genera: *Agrobacterium, Corynebacterium, Erwinia, Pseudomonas,* and *Xanthomonas.*

Fig. 1. *Pseudomonas glycinea* lesions on soybean leaf are water-soaked and beginning to become necrotic. This is the pathogenic phase of the pathogen's life cycle, and nearly all of the cells that survive from season to season are from this phase.

Before taking up matters relating directly to survival, it is worthwhile to consider sources of pathogen cells that provide the survivors. Therefore, let us consider the life cycle.

Phases of the Life Cycle

The *pathogenic phase* of the life cycle is the chief trial of the grower—it produces the losses and the leaf spots, wilts, cankers, and other manifestations of disease. With a leaf-spotting bacterium, such as *Pseudomonas glycinea*, which incites soybean blight, the largest pathogen populations develop shortly after onset of the disease, during the water-soaking stage (Fig. 1). In a week or so, lesions become entirely necrotic and dry. This is accompanied by a decline in viable pathogen cells; we noted a drop of about 100-fold in 3 weeks. Even so, large amounts of dried lesion-bearing debris can be present in the field at the close of the season, and it always is potential inoculum the next season. For diseases of annual plants, the pathogenic phase provides nearly all of the cells that survive from season to season, in debris and in other "protected positions," such as with seed and propagative parts. If disease is plentiful, the carry-over is likely to be plentiful, but this does not always mean there will be disease the following season.

The *resident* or *epiphytic phase* of the life cycle was first described about 20 years ago and is now known to be important in the epidemiology of some diseases. Most investigators agree that epiphytic growth means an increase in pathogen cells on apparently healthy external parts of the shoot (leaves, buds, pods, etc.). It has been shown, for example, that sizable populations of a pathogen may develop on the healthy host leaf; this does not mean that the leaf will become diseased, but it may. Similarly, it is becoming evident that some pathogens may be able to multiply on or near the healthy root, a subject mentioned below.

I use the term "resident phase" in the life cycle of a pathogen if multiplication is associated with the healthy shoot or root.

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Some prefer the term "latent" for pathogens within the plant. Terminology is still evolving, but however it eventually settles, it now is clear that an external resident phase in the life cycle of a shoot pathogen can be significant in the epidemiology of the disease incited by that pathogen. In pathogen-host combinations where this phase exists, sizable numbers of surviving pathogen cells may be produced.

Although sporeless, bacterial pathogens may live for years in the *survival phase*. Cells surviving for a long time have been little studied; they probably differ structurally as well as metabolically from actively growing cells. Metabolism must be close to a standstill. Such cells are called hypobiotic. In general, hypobiotic cells are more resistant to inhibitors and other harmful conditions than are actively growing ones. Active plant-pathogenic bacteria are particularly sensitive to drying; the reverse is true for hypobiotic cells. The hypobiotic cell arrives in its diminished state slowly; it is formed gradually as the lesion grows older and the season progresses. This cell probably is surrounded and protected by chemicals produced by the cell, the plant, and their interaction. If these materials and the cell remain dry, the continuity of the pathogen to the next season is assured.

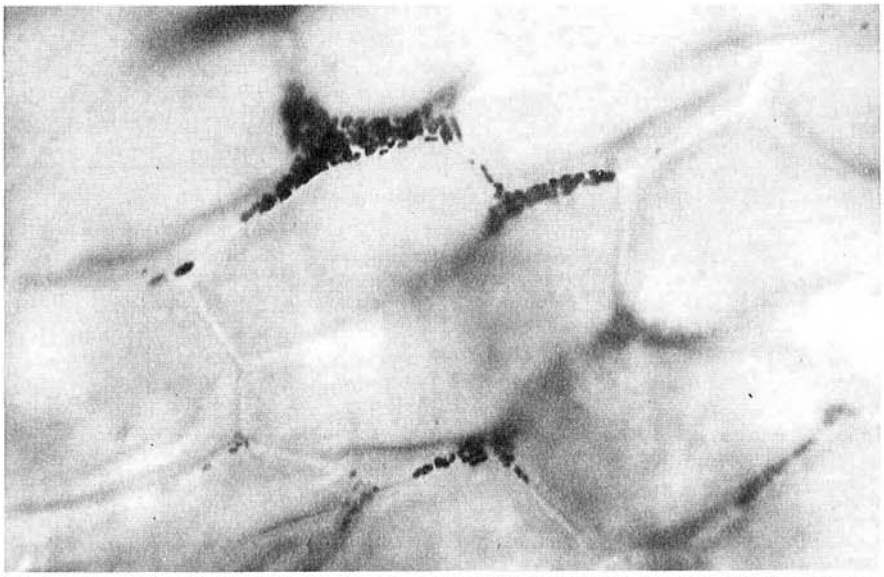


Fig. 2. Nonpathogenic bacteria growing on a healthy seedling cucumber leaf. Progenitors of these bacteria were applied to the seed; the cells migrated to the leaf and multiplied. Similar tests have been made with pathogens.

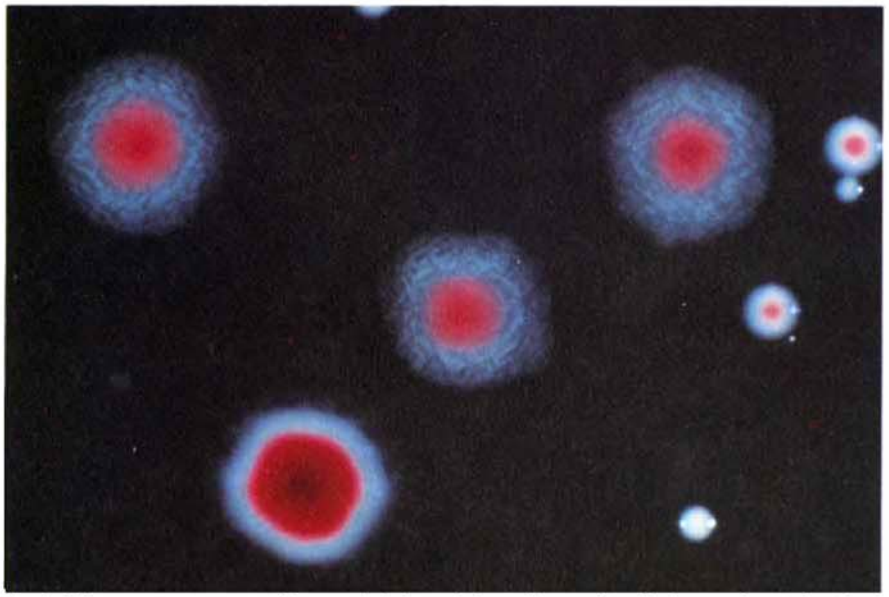


Fig. 3. When grown on a medium containing a tetrazolium salt, which some bacteria alter to form a red substance, colonies of *Pseudomonas lachrymans*, a pathogen of cucumber, have pink centers and crinkled peripheries; without the salt, colonies are white. When associated with many other bacteria in natural habitats, the pathogen can be reliably identified by use of this medium. (Photograph by A. deLange)

The *saprophytic phase*, that time in which the pathogen multiplies on dead material, seems to have little importance in providing surviving pathogen cells in nature. Saprophytism usually has been presumed to take place in the soil, but pathogenic bacteria appear to be poor competitors with other organisms there and in most natural situations, as discussed below. Exceptions may be the weak pathogens of parenchymatous tissue, *P. aeruginosa* and *P. marginalis*. These species are related to *P. fluorescens*, which is found widely in nature.

New Tools, New Insights

For many years persons studying life cycles of bacterial pathogens were at a disadvantage, compared with their mycological colleagues, for two reasons. First, stained bacterial pathogen cells usually look about the same under the microscope as the cells of nonpathogenic bacteria with which they are often associated in nature (Fig. 2). Second, in vitro colonies of pathogens on most agar media cannot be distinguished with certainty from those of associated bacteria, particularly if the numbers and types of the associates are large. During the last 20 years new tools have helped reduce these uncertainties and have provided many new insights. Pathogen cells in natural material can now be "tagged" and identified under the microscope or in culture. Media have been devised that permit the selection and recognition of pathogen colonies, despite an abundance of associated bacteria (Fig. 3). In this connection, the use of virulent mutants of pathogens that are resistant to antibiotics have told us much about the activities of pathogens in the field.

Bacteria and the Healthy Shoot

In the early years of microbiology it was not unusual to suppose that bacteria isolated from plant surfaces were actively growing there. In 1911, the great American plant pathologist E. F. Smith cautioned that nonpathogenic bacteria on plants often were mistaken for pathogens in culture attempts. He called the unwanted organisms "habitual residents or bacterial epiphytes, so to speak." Years passed before it was actually demonstrated that nonpathogenic bacteria did indeed multiply on the plant surface. In 1963, *Xanthomonas vesicatoria* was shown to multiply on healthy tomato seedling shoots.

In these studies, a given number of pathogen cells was applied to a seed and multiplication demonstrated by noting increased cell numbers on the healthy shoot. Plants were grown in a humid atmosphere in chambers that excluded microflora carried in air and by arthropods. Obtaining direct evidence of multiplication on healthy plants in the field is complicated because pathogens

may be spread some distance from existing lesions during wind-rain storms, by arthropods and other animals, or by aerosols. Usually, the bacterium in question is assumed to have multiplied if assays of the healthy plant surface indicate increasing pathogen numbers with time.

P. syringae, which damages shoot tissues of many herbaceous and woody plants, has been reported more often than any other pathogen as having an external resident phase. It is probably a common nonpathogenic resident on leaves of many plants. Only certain tissues are damaged in some plants; for example, the leaves at the boot stage of wheat. The pathogen is carried by seed, multiplies on healthy seedling leaves, survives there, and in some way reaches the boot leaves. Another example is our finding *P. syringae* on healthy soybean plants in the field, where it apparently multiplied in buds; progressive lesions did not form when leaves were inoculated, but inoculation did produce disease on cotyledons and hypocotyls. It is noteworthy that leaves carrying populations of this bacterium are especially subject to frost damage. This is because *P. syringae* acts as a nucleus for ice formation. If the bacterium is not present, tissues are not damaged by temperatures several degrees below the freezing point.

A resident phase of a crop pathogen on a weed plant may be of consequence in epidemiology in the crop nearby. For example, *P. syringae* multiplied on leaves of a weed, hairy vetch, that survived the winter under Wisconsin's snows. In the spring, the pathogen was transferred from the weed by wind-rain storms to the nearby bean crop to initiate disease. Similarly, *X. phaseoli*, which incites bean common blight, multiplied on leaves of nonhost crop and common weed species, where it survived for at least 3 weeks.

Moist conditions for at least part of the day appear to be essential for the growth of all types of epiphytic flora, which in the tropics ranges from procaryotes to sizable seed plants. Evidence suggests that nearly all bacterial residents of leaves in temperate zones, including pathogens, die as soon as they become dry. Sunshine also is inimical. Some bacteria, however, do remain alive in "protected positions" and are able to resume activity as soon as water is present and other conditions are favorable. Refuge may be for hours or days. The substomatal cavity first was suspected as a survival location, but there is little proof for this. Evidence now suggests that bacteria survive regularly in the lumen of either broken or intact empty trichomes on leaves (Fig. 4).

Considering the diverse morphology of leaves and other plant parts, there probably are other types of protected positions. Possibly, bacteria enter and leave places of sanctuary by swimming, as discussed below. In our region, where there usually are daily periods when leaves are wet, diurnal fluctuations of kinds and numbers of leaf bacteria are likely, depending on length of moist periods, relative humidity, exposure to sunshine, and other factors.

Epiphytic growth of pathogens has been most studied on leaves, but other plant organs may serve as multiplication and survival sites. In our laboratory, for example, we found that pseudomonad pathogens of cucumber, soybean, and bean multiplied on and among bud parts of host plants, and recently it was discovered that two xanthomonad pathogens of bean are disseminated from buds of bean plants in the field. The bud appears to be an ideal protected survival site—usually it is moist longer and its parts are less subjected to sunlight than other shoot organs. Survival in buds may be for short times with annual plants and from

Fig. 4. Trichomes on a young soybean leaf. Bacteria in water enter either broken or intact hollow trichomes and survive in the lumen after leaves dry.

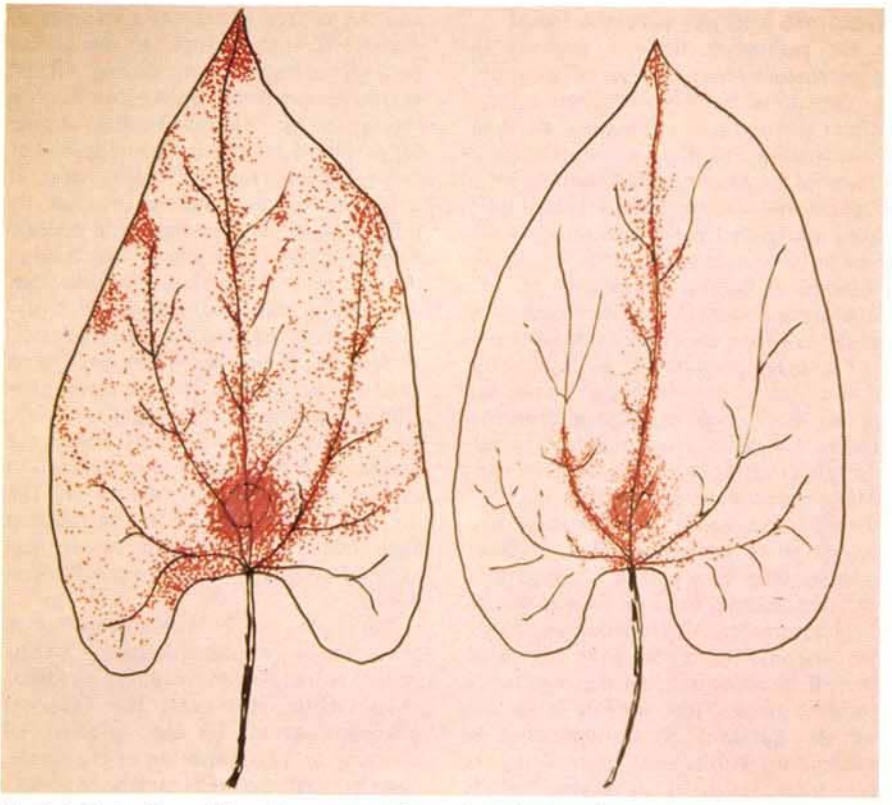


Fig. 5. Distribution of *Pseudomonas syringae* (red dots) on bean leaves (right) after 24 hours and (left) after 48 hours. Bacteria were applied in a small area in the main vein depression near the petiole (hatched circle). Leaves were held horizontally in moist chambers. Pathogen distribution was determined by pressing leaves momentarily on an agar medium and allowing colonies to develop.

season to season with perennials. As the bud unfolds, the harbored bacteria may be distributed among the emerging plant parts, and resident or pathogenic development can follow. In California, the pear flower is the site of a resident phase of the fire blight pathogen. There is utility in this discovery, because bactericidal sprays need begin only after the pathogen is detected in flowers.

In the field, the Erwinia soft rot pathogens often damage underground storage organs and fleshy plant stems near the ground line. These bacteria also have been found on the lower leaves and elsewhere on the shoots of some plants. Determining if these organisms grow on shoot tissues is difficult because of splashing rain or other transfer agencies. Nevertheless, it is possible they do multiply on shoot surfaces, perhaps only during one period of the plant life cycle.

Systemic pathogens are another type of bacteria found on plant surfaces. For example, *P. solanacearum*, which multiplies in the vascular tissues of a variety of plants, may be exuded from stomates prior to leaf wilting. It also may reach the root surface via the root-rootlet juncture. Whereas a systemic pathogen of this type may have an internal resident phase, which may be more or less extended, an external phase would be difficult to prove. In fact, with some pathogen-host combinations, determining all of the places where multiplication occurs may be impossible.

Bacteria and the Healthy Root

Do pathogenic bacteria multiply in association with healthy roots in nature, as they do with the shoot? There is little doubt that the root surface and the area nearby support a diverse nonpathogenic bacterial flora in soil, a fact that has been recognized for some 70 years. I consider it quite likely that pathogens multiply (as well as survive, as discussed below) in the rhizosphere, but this is technically difficult to prove and the evidence is not conclusive as far as I have been able to determine.

Pathogen position in the root zone does change, however. For example, the potato mother seed piece is considered to be the source of contamination of the daughter tubers by *Erwinia* spp. Movement between the two may be owing to an active multiplication and migration of pathogen cells associated with roots, or to activities of arthropods or other animals, or to the flow of rain or irrigation water. Also, one cannot be sure that bacteria in the root zone originated there if the shoot is diseased or supports a resident phase. Thus, the jury is still out on the question of multiplication in association with healthy roots. If it does take place, survivors for varying periods of time undoubtedly would be produced.

Survival Among Enemies

In nature, the pathogen is rarely alone. Probably the only time it is free or relatively free from negative (and possibly positive) influences of nearby microorganisms is during the early stages of disease, the time when it is multiplying most rapidly. The capability of extracting nutrients from the living plant is the unique possession that temporarily frees the pathogen from constraints likely to be present otherwise. Constraints may take many forms; one, the actual degradation of the pathogen cell, is stressed here in connection with survival.

Many chemical and physical factors influence the activities of associated microorganisms as well as those of the pathogen. Water is especially important, because the pathogen can be demolished by other organisms when moisture is present. For example, if debris carrying surviving pathogen cells is moistened, both debris and cells are likely to be consumed by associated organisms for the nutrients they contain. Depending on the natural situation, bacteria, fungi, protozoa, nematodes, arthropods, and other small organisms may take part in the degradation, either directly or indirectly. In most situations, pathogens seem to be poor competitors. This probably relates to the fact that among bacteria, pathogens do not grow so well on minimal substrates as do many nonpathogens.

Survival with Seeds

Survival of pathogenic bacteria with seed and propagative parts is widespread and the source of serious epidemics of diseases of annual crops. As few as five pathogen-infested seeds among 10,000 bean seeds can result in a substantial crop loss, provided conditions are favorable for development of disease and spread of the pathogen. On the other hand, if conditions are not suitable, even heavily infested seed may produce little disease. For example, with heavily inoculated soybean seed in our field tests one year, cotyledons were badly blighted. Cotyledons were shed as usual during a period of dry, bright weather. These conditions prevailed for another 3 weeks. During the rest of the season, little blight was seen, even though conditions then were favorable for the disease. Our explanation is that, with the shedding of the cotyledons and with the dry period that followed, the pathogen never was transferred to the shoot from cotyledon debris.

Pathogens may be within as well as on the seed or propagative parts. Debris should be removed by cleaning procedures if possible. However, the internal pathogen usually is not affected by cleaning or by application of chemicals. Internal pathogen cells may be in vessels of the seed coat or be associated with the embryo or other seed parts. Similarly, vascular pathogens may be buried within sizable vegetative propagative parts. Surviving pathogen cells—and they may be few in number—gradually become metabolically active and begin to divide as the plant starts to grow. High pathogen populations may entirely prevent germination, the emerged seedling itself may become diseased, or epiphytic growth may be initiated.

There is evidence showing that pathogens associated with seed in storage often die before the seed does. This probably depends, however, on storage conditions, position of the pathogen, and other factors. Consequently, predicting when a pathogen would be eradicated from a given seedlot would be difficult. Hot water treatment of seed has been used widely for killing pathogens, with varying degrees of success. In our laboratory, studies have shown that pathogens are killed with storage at a relative humidity of 75% but some seeds are not. More studies are needed to exploit differences between survival of pathogens and of seed or propagative parts.

Survival in Soil

There is much evidence to show that plant-pathogenic bacteria survive from season to season in soil, but it is difficult to explain exactly what is happening or how they are surviving. Microflora in soil are believed to be in a quiescent state most of the time—they do not grow unless nutrients reach them. In this respect, soil is energy-deficient. If, for example, dry leaf debris happens to be incorporated, it and the pathogen it may carry soon become moist, nearby microorganisms multiply, and decomposition follows.

Yet, pathogens *do* survive in soil. How is this to be explained? There are two possible reasons. The first has to do with the actual location of survivors. Clearly, hypobiotic cells set deeply within hard-to-decompose tissues would not be readily accessible to degradation. Pathogen cells within a horny tomato stem would be an example. Therefore, practices that encourage the decomposition of crop residues, such as adding nitrogen and organic material, fragmenting and burying pathogen-bearing debris, and allowing time for decomposition to take place during warm, moist seasons, should reduce carry-over. Also, rotations with crops that do not permit pathogen increase would allow more time for decomposition of pathogen-bearing debris.

The second way in which pathogens may survive in soil was first suggested in 1944, when it was reported that two leaf pathogens of tobacco overwintered in the rhizosphere of living, nonhost weed plants. Since then, there have been many reports of isolating pathogens of the shoot from the roots of host and nonhost plants, but determining the source of these bacteria is difficult. Whatever the source, it is now clear that pathogens may at least survive in association with apparently healthy roots. Thus, survival

would be expected as long as living, suitable roots of crop or weed plants were available for survival or for growth, if this also occurs. There is evidence that this survival mechanism prevails with Erwinia soft rot of potato, and I would look for it with other root-infecting pathogens, especially those with a wide host range.

Motility and Adherence

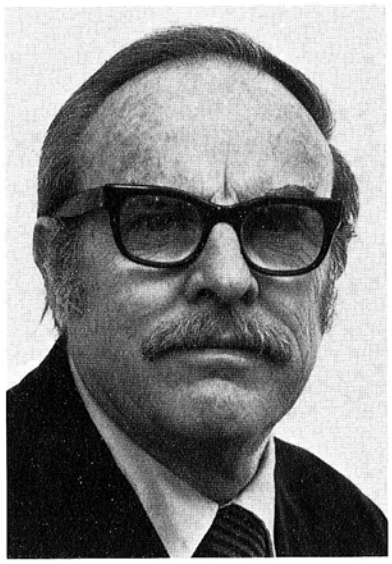
Most pathogenic bacteria are motile. An activity that requires so much energy surely has ecologic significance, and plant pathologists are beginning to study how motility influences pathogen life cycles. Motility of nonpathogenic bacteria is purposeful, in a way—toward nutrients and away from harmful agents. Motility is very rapid with some plant pathogens. For example, we found that in 20 minutes *P. lachrymans* swam, with an accompanying multiplication, from a water suspension 4 mm into a capillary tube containing nutrients. Motility was the main cause of movement of *P. syringae* from a point on a bean leaf (Fig. 5). Motility on the leaf could increase survival if cells swam into a wound to result in infection, or if cells reached a protected survival site. The fire blight pathogen of apple recently was reported to be attracted by apple nectar extracts; this probably is a fitness factor, because the pathogen attacks flowers. As we sharpen our tools and devote more effort to the study of motility of pathogens, we anticipate that much useful information about life cycles will be acquired.

The common property of bacteria in water to stick to solid surfaces also appears to have some survival value for plant pathogens. Both pathogenic and nonpathogenic bacteria adhere to leaves, and this appears to take place widely in nature. Adherence is nonselective and completed in minutes, and adhered bacteria are not readily removed by washing with water. Survival on the leaf would be favored if adherence takes place at sheltered sites. On the other hand, there probably is a far greater chance for the large numbers of pathogens produced in and disseminated from leaf lesions in the field to be adhered at exposed leaf sites and to die. Adherence of pathogens and other bacteria to leaves likely is the result of coevolution of plants and bacteria. An advantage to the plant is that most potential pathogen cells are taken out of circulation. Disease is a comparatively rare event.

With Understanding . . . and Luck

The story that I have tried to tell is that plant-pathogenic bacteria appear to be much more dependent on associations with living and dead plant tissues than previously suspected. These bacteria, even though they do not form spores and are frail things by themselves, are well adapted for surviving with living plants, especially seed or propagative parts, or with crop debris that is not moist. It now appears that some shoot pathogens survive in the rhizosphere of healthy host and nonhost plants and perhaps multiply there. The reverse relationship may hold for some of the pathogens that damage plant tissues below ground; they, too, appear able to survive on healthy shoots and may multiply there as well. In this connection, there are reports that nonpathogenic bacteria that multiplied on the shoot also increased in the root zone. Indeed, in our laboratory some years ago, a collection of nonpathogenic cucumber shoot residents were found to have nutrient requirements similar to those described for nonpathogenic rhizosphere bacteria. In view of these observations, we may ultimately find that pathogenic as well as nonpathogenic bacteria are intimately associated with many parts of the plant, even though we now think of pathogens as root, shoot, or systemic organisms.

This possibility, plus the finding that some pathogens of the shoot are capable of multiplying and surviving on the healthy shoot, is not making bacterial diseases any easier to control, but we now are able to understand some of our failures and to direct efforts better. I hold firmly to the proposition that through understanding, along with some luck and inspiration, we will be able to deal more effectively with that difficult group of plant pathogens—the bacteria. Moreover, it will be fun to pry into these secrets.



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