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BACTERIAL AND PHYTOPLASMA DISEASES AND THEIR CONTROL

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29.1 Introduction

In many ways, bacterial diseases of plants are similar to those caused by fungi. Both kinds of organism often produce enzymes which break down pectin, cellulose and other plant cell components. Both can produce toxins which affect plant tissues. The disease symptoms induced may also be very similar. Both bacteria and fungi can cause leaf spots, wilts, soft rots, galls and other hypertrophies. However, there are also some important differences between bacterial and fungal plant diseases. Some of these will be described before giving detailed descriptions of a few important bacterial diseases.

Bacterial plant pathogens are not organised into tissues and, with the exception of *Streptomyces* spp., exist only as single cells. Consequently there is no possibility of special structures being formed for specific purposes. For example, there are no special structures which help to spread bacteria from a diseased plant to a healthy plant, nor are there any desiccation-resistant resting cells capable of long range dispersal in air currents. In contrast, fungi have developed many such structures to ensure that reproductive propagules spread and survive dispersal. Although most bacterial pathogens are motile, they move over only short distances (e.g. over the surface of a leaf). To spread from a diseased to a healthy plant, they require the intervention of some outside agency. This is usually rain or overhead irrigation water. When a water droplet hits a leaf

it disintegrates and many very small droplets bounce back into the atmosphere and become airborne. If cells of a plant pathogenic bacterium are present on the leaf surface, some will become incorporated into the small droplets and they also will become airborne. In this way pathogenic bacteria can spread from diseased to healthy plants. Wind-blown rain is even more effective in spreading bacterial pathogens because more energy is involved, more small droplets are formed and they are carried further. Other methods of spread are by seed, by infected planting material, by insects and nematodes, by moving soil and moving water and by mechanical means. In all cases, the bacteria themselves provide no energy to promote their spread.

Let us now consider what happens when plant pathogenic bacteria arrive on the surface of a healthy plant. Unlike many fungi, they cannot exert enough pressure to force their way through the outside protective cover of plants. The only way they can enter is through wounds or natural openings such a stomata, lenticels, hydathodes (water pores), nectaries and leaf scars. Motility is important at this stage because a bacterium can only reach a natural opening if it is motile. In some cases (perhaps all) it is clear that a chemotactic response is involved. The bacteria move up a concentration gradient of chemicals exuded from stomata, hydathodes etc. Non-motile bacteria such as *Clavibacter* spp. are frequently transmitted mechanically, for example by cutting knives in *C. michiganensis* subsp. *sepedonicus* on potato and *C. michiganensis* subsp. *insidiosus* on lucerne (*Medicago sativa*). The bacteria are not only spread mechanically from a diseased to a healthy plant, but also deposited on a wounded surface through which infection can occur. Other bacteria such as *Rathayibacter* (e.g. *R. toxicus*) depend on nematodes for transport to, and inoculation of, susceptible tissue. If the nematode is controlled, the bacterial disease will also be controlled.

Following infection, plant pathogenic bacteria must grow inside the plant. However, most plants are resistant to most pathogenic bacteria. Disease is a very special situation where the plant's defence mechanisms are not activated to a level sufficient to prevent bacterial growth. Only recently has the basis of the plant–pathogen interaction been partly revealed. When large numbers (say 10^8 mL^{-1}) of cells of a non-pathogenic bacterium are inoculated onto a plant, the plant does not respond and bacterial numbers slowly fall over several days because they cannot obtain nutrients needed for growth and multiplication. In contrast, when the same number of pathogenic bacteria are inoculated onto the plant, there is a violent reaction by the plant, known as the hypersensitive reaction. Bacterial numbers rise initially but then fall dramatically. This only occurs when a plant is resistant to the pathogen. Clearly there is a difference between pathogenic and non-pathogenic bacteria.

Some of the bacterial genes that induce the hypersensitive reaction have been cloned. There seem to be at least two distinct types, avirulent (*avr*) genes which are highly specific for different cultivars of a susceptible species and hypersensitive response and pathogenicity (*hrp*) genes. The relationship between these two types is not fully understood. In many phytopathogenic bacteria, the *hrp* gene cluster encodes proteins which permit the secretion of other proteins, including those produced by *avr* genes. Recent evidence indicates the Avr proteins are transported into the plant cell where they are recognised by plant resistance gene proteins, if present. This sets up a cascade reaction and leads rapidly to the hypersensitive reaction which involves the death of the plant cells and also of the invading bacteria. This is the basis for the specificity of the gene-for-gene reaction which determines one form of resistance of plants to pathogens. If a resistance gene is not present in the plant, the pathogen will not be recognised and disease will ensue. The *hrp* genes seem to have another function

and are essential for pathogenicity in Gram-negative bacteria (except *Agrobacterium*). A 34.7 kD protein called $harpin_{pss}$ is secreted by *hrpZ* in *Pseudomonas syringae* pv. syringae and can elicit a non-specific hypersensitive necrosis. In contrast, *avr* genes are not essential for pathogenicity, in most cases. In fact, loss of an *avr* gene can convert an avirulent strain into a virulent one on a particular cultivar. Today, this is a very exciting field of research and our understanding of pathogenicity and resistance is increasing rapidly.

When considering control measures, it is essential to know how the pathogen is spread and how it survives from one crop to the next. For example, if the pathogen is seed-borne, it will probably be easier to ensure a supply of healthy seed than to control the disease in the field. Chemical sprays have only rarely proved effective in controlling bacterial diseases. One exception is the use of the antibiotic streptomycin in America and New Zealand to control fireblight of pome fruit caused by *Erwinia amylovora.* However, streptomycin resistant strains of the pathogen have appeared and are no longer effectively controlled. In Australia, the use of antibiotics of medical value to control plant diseases is discouraged. Disease resistance is an important method of control for bacterial diseases but sometimes resistance breaks down when temperatures are high. The development of new virulent strains that can attack previously resistant cultivars does not seem to be such a common phenomenon with bacteria as with fungi, although it does occur. Other methods of control include biological control, control through sanitation and control by cultural methods such as crop rotation. The most appropriate method of control will depend on the nature of the disease and on the crop that is affected.

Although phytoplasmas are thought to cause disease in more than 200 species of plants including important food and fibre crops, the number of phytoplasmas involved is still not known. The main impediment to their study has been the inability to grow them in culture. In contrast to our knowledge of other plant pathogens, we know little about how phytoplasmas cause disease. Until recently, phytoplasmas could be studied only on the basis of biological properties such as disease symptoms, plant host range and vector specificity. Phytoplasmas can now be detected and identified using molecular tests and this has greatly increased our capacity to identify them in plant and insect samples collected in the field. In Australia, the number of phytoplasmas involved in different diseases is still not clearly resolved. However, modern diagnostic tests have facilitated the identification of diseased plant material and the subsequent restriction of its movement within and between countries. This is the best method of controlling phytoplasma diseases.

29.2 Some important bacterial diseases in Australia

Many diseases caused by bacteria and phytoplasmas occur in Australia. Some of these will be discussed in this chapter. The examples given were chosen to show the wide range of symptoms that can result from infection and also to illustrate the various strategies for disease control that have been adopted. Many other diseases caused by bacteria and phytoplasmas occur in Australia and these are discussed by Fahy and Persley (1983). *The ideal method of controlling a disease is to exclude the pathogen from regions where it does not occur. The importance of quarantine in achieving this goal cannot be over-emphasised.* Important bacterial diseases of quarantine importance to Australia are listed and some are briefly described.

Crown gall of stone fruit

Crown gall of stone fruit is caused by the soil-inhabiting bacterium *Agrobacterium rhizogenes* and, less frequently, *A. tumefaciens*. Affected plants have large swellings or galls on the main stem, just below soil level, and on the tap root (Fig. 29.1). Galls may also be present on side roots but they probably do not cause much damage.

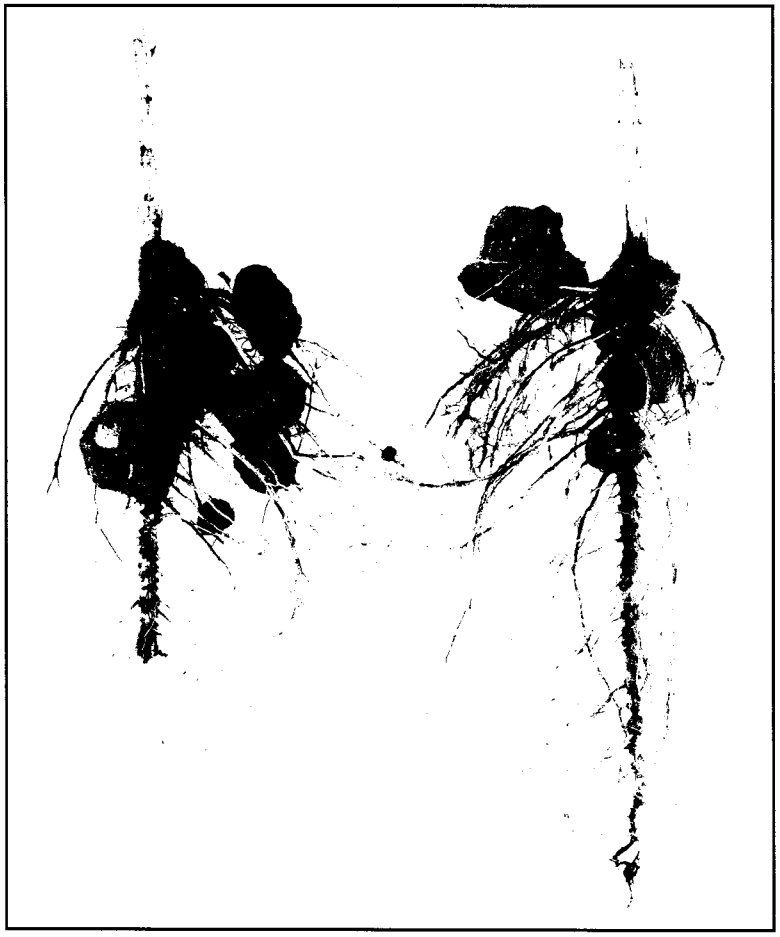
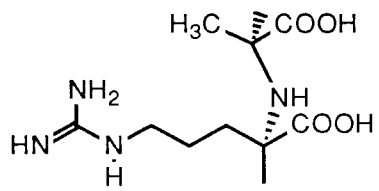


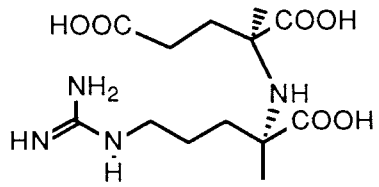
Figure 29.1 Crown gall on stone fruit.

The pathogens are really natural genetic engineers because, following wounding, plant cells are 'transformed'. A small piece of DNA is transferred from a bacterial cell to a plant cell where it encodes the synthesis of plant growth hormones and **opines**. The growth hormones are auxins and cytokinins which stimulate the plant cells to grow and divide to produce the characteristic galls. Opines form a group of compounds found nowhere else in the plant kingdom. They consist of two common plant substances joined together in an unusual way. Examples include octopine which combines arginine and pyruvic acid, nopaline, a combination of arginine and α-ketoglutaric acid and agrocinopine A where sucrose and arabinose are joined by a phosphodiester bond (Fig. 29.2). Pathogenicity in agrobacteria is dependent on the presence of a large plasmid, the tumour-inducing plasmid (pTi). Several kinds of pTi have been described and are usually defined by their opine characteristics. So there are octopine plasmids, nopaline plasmids etc. Only part of a pTi, the transfer DNA (T-DNA), is transferred to a plant cell where the opines are synthesised. If octopine is synthesised, bacteria containing an octopine plasmid can also catabolise octopine but not nopaline. The converse also applies. Most strains induce the synthesis of

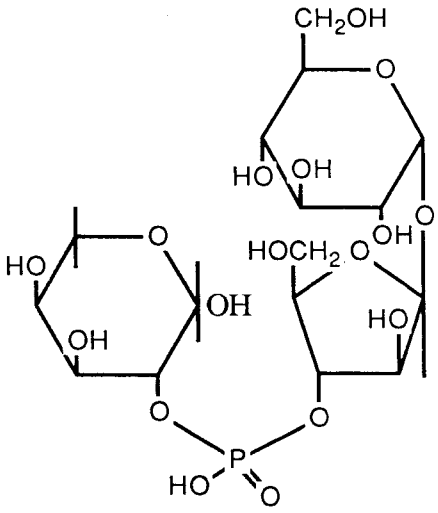
more than one opine and the corresponding catabolic genes are always present on pTi but not on the T-DNA. Most non-pathogens and soil bacteria cannot utilise opines. The biological sophistication of the system is apparent. Agrobacteria live on and around plant roots. If wounding occurs, T-DNA is transferred to a plant cell where it directs the synthesis of plant hormones leading to gall formation and of opines which provide a more or less reserved source of nutrients for the inciting bacteria. As a result, they have a great advantage over competing organisms. A further sophistication is that some opines also induce conjugation and pTi transfer between bacteria. Not only do pathogens have a considerable ecological advantage over non-pathogens lacking pTi, but the latter are converted to pathogens. The system is not perfect, however, because some strains of *Pseudomonas* can also catabolise opines and use them as sources of carbon and nitrogen, as can some fungi.



OCTOPINE



NOPALINE



AGROCINOPINE A

Figure 29.2 Chemical structures of the opines, octopine, nopaline and agrocinopine A.

Control of crown gall of stone fruit is achieved by a biological method. Planting material is dipped in a cell suspension of a non-pathogenic strain of *A. rhizogenes*. The original biocontrol agent was strain K84 but it has now been replaced (at least in Australia) by strain K1026, a genetically engineered derivative of strain K84. Nearly 100 per cent control is achieved if properly applied (Table 29.1). Both strains produce an antibiotic or bacteriocin called agrocin 84 (Fig. 29.3) which inhibits the growth of pathogenic agrobacteria containing a nopaline Ti plasmid. Fortunately, strains carrying a nopaline Ti plasmid are the main cause of crown gall of stone fruit. Most other pathogens and soil-inhabiting bacteria are unaffected. The remarkable specificity is an uptake phenomenon. Susceptible strains possess an agrocin 84 permease gene whereas resistant strains do not. In fact, the 'normal' function of the permease gene is for the uptake of an opine called agrocinopine A (Fig. 29.2) but it also transports into the cell agrocin 84, which inhibits growth by preventing DNA replication. The permease gene, which is responsible for uptake of agrocin 84, is located on the nopaline Ti plasmid and this explains why only pathogens are sensitive. The specificity is highly desirable because no non-target organisms are affected and no ecological damage is possible. Biological control is now practised in most countries where stone fruit are grown.

Table 29.1 Biological control of crown gall on almond seedlings by strains K84 and K1026 of *Agrobacterium rhizogenes*.

Treatment[1]	Mean number of galls per plant
Water	46.33
K84	0.20
K1026	0.67

[1] Soil was inoculated with a pathogenic strain of *A. rhizogenes*. Two days later, almond seedlings were dipped in water or in bacterial cell suspensions of strains K84 or K1026 before planting in the inoculated soil (15 replicates per treatment).

A threatened breakdown of biological control was first reported from Greece. Agrocin 84 synthesis is directed by genes located on another plasmid, present in strain K84. This plasmid, pAgK84, was being transferred from strain K84 to pathogenic strains, which as a result, became immune to agrocin 84. To prevent a breakdown in the efficacy of biological control, pAgK84 was made transfer deficient (Tra$^-$) by deleting most of the genes involved in the transfer process. The new strain K1026 is just as efficient as strain K84 in producing agrocin 84 and in controlling crown gall (Table 29.1). It is used widely in Australia and was the first genetically engineered organism in the world to be released for commercial use.

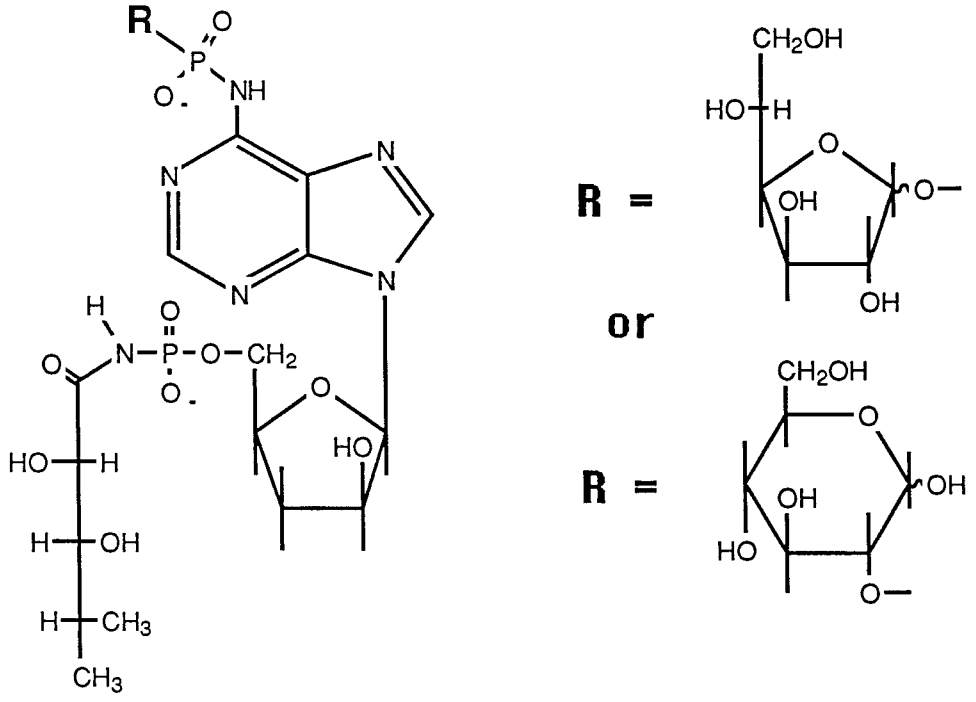


Figure 29.3 Chemical structure of agrocin 84. (Courtesy of M.E. Tate.)

Crown gall of grapevine

Crown gall of grapevine is caused by another species of *Agrobacterium*, *A. vitis*, but the process of crown gall induction is the same as that described above. However, *A. vitis* differs from *A. rhizogenes* and *A. tumefaciens* in several characteristics, including the fact that it lives systemically in the vascular tissue

of its host. The disease takes two distinct forms. The more destructive form is cane gall which occurs in countries with low winter temperatures. Canes and trunks are damaged by frost and this allows the systemic bacteria to transform wounded cells. In the following spring, masses of galls appear along the length of the canes and even on the trunks. In warmer areas, such as Australia, galls are largely confined to the bases of cuttings used for propagation or to major graft wounds. They are particularly noticeable when a new cultivar has been top-grafted on to established vines in a vineyard. Surprisingly, galls do not develop on pruning wounds.

Agrobacterium vitis is resistant to agrocin 84 and is therefore not subject to efficient biological control by strains K84 and K1026. It does not survive for long periods in soil but can be detected in plant debris in soil for at least two years. The main source of infection is planting material, not soil. This means that the strategy to control crown gall of grapevines is quite different from that for crown gall of stone fruit. The first essential is to ensure that planting material is healthy. This can be achieved in two ways. First, and the more reliable, is to propagate from shoot tips using tissue culture. Shoot tip propagated vines of some cultivars are now commercially available and, so far, have remained free of the pathogen when planted into soil not previously used for grapevine cultivation. It is essential that both stock and scion be propagated in this way. The second method of obtaining healthy propagating material is to use heat therapy of dormant cuttings. Generally, 50°C for 30 minutes is recommended but complete eradication of *A. vitis* from infected cuttings is not always achieved. Heat treatment is probably a satisfactory method of disease control for normal propagation of grapevine in areas where severe frosts do not occur. Crown gall in the nursery is dramatically reduced and establishment of plants is usually improved. Once a vineyard is established in such areas, later infections are probably of little importance unless top-grafting is practised. Heat treatment is not considered satisfactory for the establishment of disease-free nuclear stocks for widespread distribution and propagation. Nor is it satisfactory for normal propagation in areas subject to severe frosts where zero infection is desirable.

Once disease-free planting material has been obtained, it is important to prevent recontamination for as long as possible. Details of the recontamination process are not fully defined but there is ample evidence to suggest that if healthy vines are planted into old vineyard soil containing contaminated plant debris, the new plants will become infected. Many laboratories around the world are trying to find methods of preventing this. A promising approach is through biological control using a non-pathogenic strain of *A. vitis* which was isolated in South Africa. Dipping planting material in a bacterial suspension of this strain not only prevents gall development on cuttings but also markedly reduces systemic colonisation. This treatment is not yet a commercial recommendation.

Soft-rot of potatoes and other vegetables

The main causes of bacterial soft-rot of potato and other vegetables are *Erwinia carotovora* subsp. *carotovora*, *E. carotovora* subsp. *atroseptica* and *E. chrysanthemi*. *Erwinia carotovora* subsp. *carotovora* is widely distributed and is the most common cause of soft-rot in Australia. *Erwinia carotovora* subsp. *atroseptica* can be considered a low temperature variant and is an important pathogen of potatoes in countries such as Scotland which have a cool climate. In contrast, *E. chrysanthemi* causes most damage at 30°C or higher.

All these organisms produce a soft, wet rot resulting from the production of pectolytic enzymes. The cell-cementing pectins are destroyed and the tissues

collapse. This can be quite spectacular when potato tubers in storage are affected. They can become a structureless wet mass with an unforgettable smell. Plants growing in the field can also be affected by both subspecies of *E. carotovora* and here, rotting of the stems is a common feature (e.g. blackleg of potato) although under dry conditions, plants may be stunted, wilted or desiccated.

There are no simple measures to control soft-rot but recent ecological studies, mainly in Scotland and the USA, have defined the conditions which favour disease development and this suggests measures to reduce infection. Although soft-rotting bacteria seem to be ubiquitous, having been found in soil, streams, rivers, lakes, reservoirs, ditches, even the sea, there is good evidence that latent infection of planting material is important. Pathogenic bacteria are frequently present on the surface of seed potato tubers but lenticel infection is probably more important because the bacteria can readily survive in lenticels from one growing season to the next. Roguing of infected plants is still an important method of controlling blackleg by reducing the inoculum load in seed tubers.

Free moisture is another important factor promoting infection. Not only does it allow bacterial motility but it also reduces oxygen levels leading to semi-anaerobic conditions which are ideal for infection. Because *Erwinia* species are facultative anaerobes, they remain very active under these conditions but the ability of the host to resist infection is reduced. It has been reported that pectic enzymes from *Erwinia* species only macerate tissue under anaerobic conditions. It is very important therefore to prevent the occurrence of free moisture. If tubers are washed, they must be dried before storage and marketing. Also water condensation on tubers in storage must be prevented.

Bacterial wilt of potato and other crops

Bacterial wilt of potato and other crops is caused by *Ralstonia solanacearum* (previously named *Pseudomonas solanacearum*). The species consists of a large number of divergent strains which have been divided into biovars, based on biochemical reactions as described in Chapter 6, and races, based on host range within cultivars of a species. Biovars and races do not coincide except for biovar 2 and race 3 which appear to be very similar, if not identical. Strains in this group are largely responsible for bacterial wilt of potato in temperate areas of Australia. Leaves suddenly wilt, without previous yellowing and if the stem is cut at ground level, a whitish exudate may be seen on the cut surface. A more reliable diagnosis is to suspend the cut stem in water. A characteristic white cloudy exudate in water is a useful presumptive diagnosis. On the tubers, a wet decay occurs at the point of attachment to the stolon and at the eyes. If infected tubers are cut open, a light-brown ring will be seen surrounding the vascular tissue. Milky fluid may be squeezed from this discoloured area. Tomato can also be infected by biovar 2 strains.

In tropical and subtropical areas, many crops, including tobacco, tomato and ginger are affected by wilt caused by different strains of *R. solanacearum*. Biovar 3 strains have a very wide host range and are the most prevalent and destructive strains in tropical and subtropical Australia. The pathogen is an important limiting factor in the cultivation of potato and other vegetables in these areas.

Ralstonia solanacearum can be readily grown in culture. Tetrazolium chloride (TZC) medium is usually used for initial isolation. Growth on this and other media containing at least 0.5% glucose or sucrose is characteristic. Some colonies spread over the surface of the plate whereas others are much more restricted. TZC is also useful in distinguishing pathogenic forms from non-

pathogens (or weak pathogens). Pathogenic colonies are white with a pink centre, whereas those of non-pathogenic strains are deep red. In culture, pathogens readily convert to non-pathogens by a process known as phenotypic conversion, the regulatory genes for which have recently been cloned.

At least some biovars and races of *R. solanacearum* can survive for long periods in soil but weeds are probably important in this respect, both as symptomless hosts and also as non-hosts whose rhizospheres can be colonised. Details of the method of penetration have not been described but wounding and nematode infection are known to increase disease incidence. It is not clear how the bacteria penetrate the vascular tissues where they grow and multiply. Production of indole acetic acid and cytokinins are important in pathogenicity by inducing growth of new meristematic tissue in the xylem parenchyma which re-routes nutrients into infected xylem. Hyperplasia and hypertrophy ultimately lead to the crushing of young tracheids with a resultant rapid increase in sugars and amino acids at the infection court. Virulent pathogenicity is also dependent on the production of pectinases and cellulases by the pathogen.

R. solanacearum can be spread in many ways, including root to root contact, movement in soil and of soil and water, dissemination by farm implements, pruning and, most importantly, by movement of infected planting material.

In temperate regions, because biovar 2 strains do not survive well in soil, bacterial wilt of potato is largely controlled by ensuring that tubers used for planting are free of the pathogen. This is not always easy to achieve and the incidence of disease appears to be increasing, at least in certain areas. Latently infected potato tubers (i.e. showing no visual evidence of infection) are a major source of infection in southern Australia where farmers use uncertified seed or even one-off certified seed from infested land. This is a problem in New South Wales, Victoria and South Australia and the disease is introduced into new land all too frequently. Reliable tests are needed to screen seed potatoes for the presence of *R. solanacearum* biovar 2. Some very promising molecular tools are now becoming available to test for the presence of specific biovars and strains of *R. solanacearum*. In the tropics and subtropics, rotation combined with disease resistance is practised in crops such as tobacco where resistant cultivars are available. This has to be combined with strict management to control nematodes and weeds and to destroy stubble. However, these measures are not adequate to control potato wilt in hot areas. In some tropical countries, control is achieved by in vitro micropropagation followed by tuber multiplication in pathogen-free soil. A strict seed certification scheme is also required.

Bacterial blight of peas

Bacterial blight of peas is caused by *Pseudomonas syringae* pv. pisi and *P. syringae* pv. syringae. *P. syringae* pv. pisi is seed-borne and causes considerable, if sporadic, economic damage especially during spring and summer. A recent estimate of loss in Australia was $13m in one year. In addition to direct losses, export markets can be lost if seed is infected because many countries have an embargo on the importation of infected seed. *P. syringae* pv. syringae is a less virulent pathogen but can cause severe disease following hail damage or under excessively wet conditions. It is not normally seed-borne but is present as part of the normal leaf-surface flora of healthy plants. The two pathogens can be distinguished serologically or by inoculating stems of the susceptible pea cultivars, Rovar and Blue Prussian. Inoculation with *P. syringae* pv. pisi produces a water-soaked lesion which spreads from the site of inoculation, whereas

inoculation with *P. syringae* pv. syringae results in extensive necrotic lesions and stunting of the inoculated plants.

In the field, all above-ground parts may be affected but symptoms on stems and stipules are most characteristic. Stem infection usually appears 2–4 cm above soil level and spreads upwards towards the growing point and outwards into the stipules in a fan-like pattern. Lesions are at first water-soaked but soon turn olive green then brown. Lesions on leaves and stipules also turn brown and then become papery in texture. Pods may be infected and this can lead to seed infection, starting at the micropyle end and then into the seed coat where the bacteria may remain alive but dormant for at least three years. When sown, infected seeds give rise to various proportions of infected seedlings depending to some extent on the soil moisture content. From a few infected plants, pathogenic bacteria are readily spread through a crop by the splashing of wind-blown rain and hail. Secondary infection may be through stomata but mechanical injury by hail or wind-blown particles greatly increases the level of infection. Severe outbreaks of bacterial blight frequently follow frost damage and the ice-nucleating ability of both pathovars is probably important in this regard. Tissue damaged by ice crystal formation is readily invaded by the two pathogens. In severe cases of bacterial blight, plants wither and die and large, usually randomly distributed, patches may develop in crops as a result of seed-borne infection. Spread and development of the disease is checked by warm, dry weather.

Various methods of disease control have been tried, including the use of resistant cultivars. However, *P. syringae* pv. pisi is very variable and seven different races have so far been identified. The most common race in Australia is race 6 to which there are no resistant cultivars. Streptomycin treatment of both seed and growing crops has also been tested and has proved effective in reducing disease incidence but is not economic. *The only satisfactory method of controlling bacterial blight of peas is to ensure that seeds are free of infection.* This is not easy to achieve because infected seed usually shows no symptoms and seed saved from an apparently healthy crop may be infected. Various procedures have been developed to test for the presence of seed-borne infection. In Australia, a bacterial blight testing service is available to growers in New South Wales, South Australia and Victoria. It involves soaking samples of seed in water and testing for the presence of pathogenic bacteria by a sensitive ELISA technique (see Chapter 11 for details). Routine procedures for sampling are still being developed and the minimum level of infection that can be detected is not yet clear. Seed infection of 0.01% has been reported to lead to a major epidemic. Combining seed testing with the testing of growing crops and seed certification may achieve satisfactory disease control.

Bacterial blight of cotton

Cotton growing in Australia is largely confined to New South Wales (74%) and Queensland (26%). The crop is normally planted in late September and October (spring), grown over summer and harvested in April and May (autumn). In the areas where cotton is grown, there is substantial summer rainfall (200 mm of rain during the growing season is common) and the warm moist conditions are ideal for disease spread and development. Bacterial blight of cotton, caused by *Xanthomonas campestris* pv. malvacearum, was first recorded in Australia in 1923 and was for many years considered to be the most important disease of cotton in Australia. Angular water-soaked leaf spots are the most characteristic feature of the disease. Lesions may extend into veins which become blackened