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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.
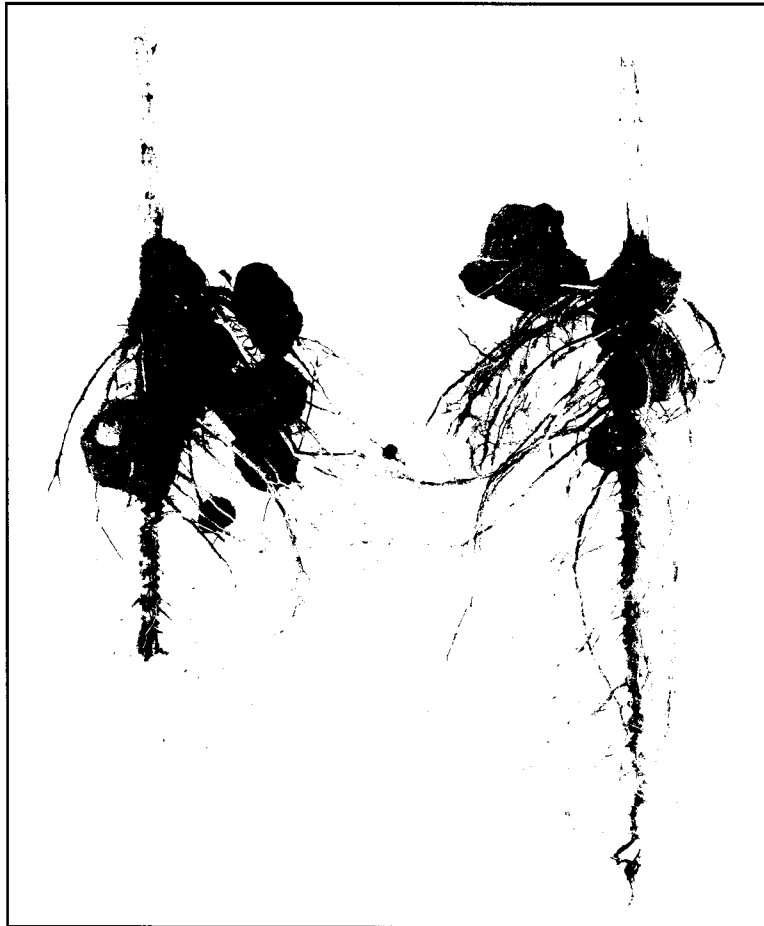

Figure 29.1   Crown gall on stone fruit.

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.
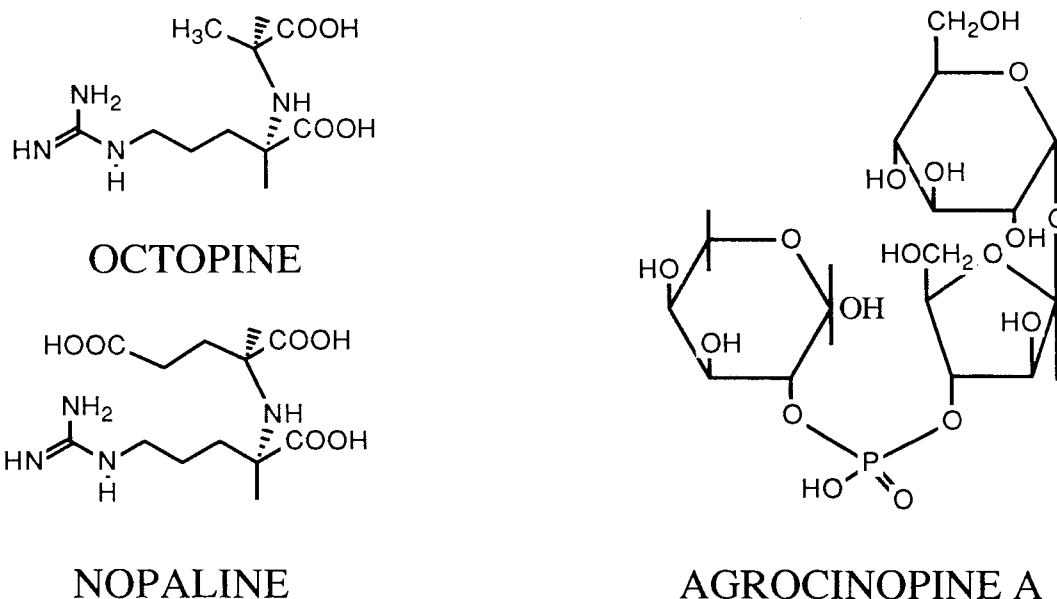

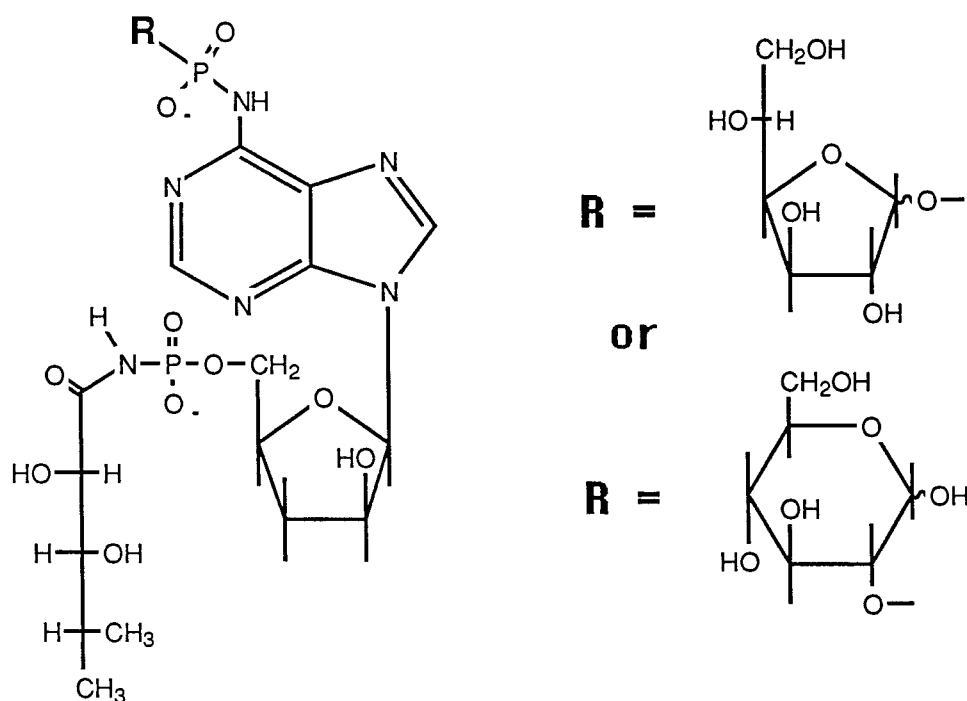**Figure 29.2** Chemical structures of the opines, octopine, nopaline and agrocinopine A.

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

and premature defoliation results. Boll infections also occur and may prevent maturation.

In the 1980s, regular surveys of cotton crops showed that Race 18 of the pathogen, which is highly virulent on the popular Deltapine cultivars, was predominant in Australia and also that seed infection was a major factor in the epidemiology of the disease. Seed could become infected in the field or during the ginning process where the fibre is removed from the seed. Dust from gins may contain 2×10^7 pathogenic bacteria per gram. It was also established that the pathogen may survive from one season to the next on crop residues in soil.

In 1985, in response to severe losses by growers, a scheme was developed and implemented to reduce the level of seed infection. The scheme included the following:

  (i)   the isolation of cultivar nurseries from susceptible, commercial crops,
  (ii) the use of copper sprays in nurseries to reduce the spread of the disease early in the season,
  (iii) the location of seed production in drier western areas of New South Wales,
  (iv) the rejection of seed crops on the basis of blight severity (13 out of 30 pure seed Deltapine crops were rejected in the first year),
  (v)   the processing (ginning) of cotton from immune cultivars prior to the processing of susceptible cultivars from which planting seed is to be produced to reduce seed contamination in gins,
  (vi) the storage of seed for one season prior to planting.

The success of the scheme is demonstrated in Table 29.2. In six years, seed infection was reduced to a very low level and disease incidence on bolls was reduced from 23.76% to 0.29%.

Table 29.2.   Incidence (%) of bacterial blight of cotton in the susceptible cultivar Deltapine following the introduction of a healthy seed scheme. (Data provided by Stephen J. Allen, NSW Agriculture.)

Season	84/85	85/86	86/87	87/88	88/89	89/90	90/91
In seed	3.30	12.00	2.10	0.95	0.25	0.29	0.016
On seedlings	3.00	10.13	17.11	2.67	0.21	0.35	0.04
On bolls	23.76	19.56	19.68	22.66	7.96	8.02	0.29

Following the introduction of the seed certification scheme, new cultivars resistant to *X. campestris* pv. malvacearum were released in 1985/86 and now account for approximately 95% of the cotton grown in Australia. From being the most serious disease of cotton in Australia, bacterial blight is now of much less importance, although it is always possible that the pathogen will mutate and become virulent on the new resistant cultivars. Alternatively, new races of the pathogen could be introduced from abroad. To reduce this possibility, new quarantine measures have been introduced to reduce the chance of symptomless epiphytic transfer with imported seed. Steps have also been taken to reduce the chance of introducing the bacterium on crop residues attached to second-hand machinery imported into Australia. The control of bacterial blight of cotton is an excellent example of how growers, administrators and scientists can combine to achieve an important goal.

Leaf scald of sugarcane

Leaf scald is one of the most devastating diseases of sugarcane. It is present in more than 50 countries and occurs in most sugarcane-growing districts of Australia. It was first recorded in Australia in 1926 and was probably introduced on clonal planting material at a time when quarantine restrictions were inadequate.

Leaf scald of sugarcane is caused by *Xanthomonas albilineans*. As the specific name indicates, white lines are a characteristic feature of the disease. They appear on the younger leaves and are associated with major veins where the bacteria grow. As the disease progresses, leaves curl inwards and the leaf tips become scalded. Internally, affected stalks show bright to dark red streaks due to necrosis of the vascular bundles. Sometimes, blocks of apparently healthy cane suddenly die. The disease may also have a latent phase when young shoots on an infected plant seem to outgrow the disease and appear to recover. If used for propagation, such shoots will carry the pathogen and disease symptoms may reappear at any time. Latent infection poses a major problem for quarantine inspectors. Highly specific and sensitive serological tests have been developed to distinguish healthy from diseased vegetative propagating setts.

The characteristic chlorosis is caused by a family of toxins known as albicidins, which are produced by the pathogenic bacteria. Albicidins do not affect mature chloroplasts but only immature protoplastids which do not produce chlorophyll if exposed to albicidins. Toxin production is required for pathogenicity because toxin deficient mutants are non-pathogenic. Studies on the mode of action of albicidins show that the primary target is prokaryotic DNA replication. As chloroplasts are of prokaryotic origin, they are susceptible, as are many species of bacteria, including *Escherichia coli.*

Leaf scald is transmitted mainly by infected cuttings and mechanically by implements which cut stalks or stools. Infected cuttings are an important means of spreading the disease over long distances. Within a crop, the disease may be spread by cane knives and mechanical harvesters. There is no conclusive evidence that it can be spread by wind-blown rain, although the disease is more prevalent after cyclones.

Xanthomonas albilineans has a fairly narrow host range, being restricted to sugarcane and a few other members of the Poaceae which could provide a long-term source of infection. Sweet corn is very susceptible but natural infection has not been observed. It can be very useful in pathogenicity tests.

One approach to controlling leaf scald is to ensure that planting material is disease free. A technique for the elimination of *X. albilineans* from planting material has been developed. It consists of soaking setts in water at ambient temperature for at least 24 hours, followed by hot-water treatment for 3 hours at 50°C. Treatment enables the cultivation of moderately susceptible cultivars in areas where leaf scald occurs. A second approach is to grow resistant cultivars but unfortunately, they are not as productive as some susceptible cultivars. A new approach is being taken by research workers at the University of Queensland. It involves the genetic engineering of sugarcane. The strategy is to find genes that will inactivate the bacterial toxin and introduce them into high-yielding leaf scald susceptible cultivars. If the gene is active in the plant, it should destroy the toxin and make the plant resistant to the disease. Many bacteria are susceptible to albicidins and by exposing various species to the toxins, strains were found that were resistant. Genes conferring resistance were cloned and their mode of action investigated. Those that inactivated the toxins were engineered to ensure that they would be expressed in sugarcane and then transferred to

sugarcane by means of a DNA 'gun' or micro-projectile. Recent data indicate that susceptible cultivars have been made resistant by this method. It is a method that could have wide application in the control of bacterial diseases of plants.

Ratoon stunting of sugarcane

Ratoon stunting of sugarcane is a major disease and is present in all sugar-growing districts of Australia. In years of below-normal rainfall, losses may reach 30% in individual plantings. It was first described in Australia in 1955 and was thought to be caused by a virus. However, the evidence is now conclusive that it is caused by the small, slow-growing Gram positive bacterium *Clavibacter xyli* subsp. *xyli* which is present in the xylem of infected plants. It has fastidious nutritional requirements and a special medium is required for its isolation and growth. A closely related bacterium, *C. xyli* subsp. *cynodontis*, can infect sugarcane following inoculation but does not cause symptoms.

Symptoms of ratoon stunting are rather ill-defined and include stunting and general unthriftiness, especially during drought conditions. Internal symptoms are more characteristic. In mature cane, vascular bundles at the lower part of the nodes are discoloured red, orange or yellow. The discoloured strands are seen as dots, commas and other straight or curved shapes up to 3 mm long. *Clavibacter xyli* subsp. *xyli* can be detected by phase contrast microscopy of expressed vascular sap. Alternatively, serological methods can be used to detect bacteria in infected cane. The bacteria are easily transmitted mechanically by cutting implements used during harvesting and in the preparation of planting material.

The most important method of control is to ensure that planting material is healthy. Heat treatment can eradicate *C. xyli* subsp. *xyli* from infected cane and this is widely practised. Hot water at 50°C for 2 hours, hot air at 58°C for 8 hours or aerated steam at 52°C for 4 hours may be used, but total eradication is rarely achieved in commercial practice. Sanitation is also important to prevent spread by cutting implements. Resistant or tolerant cultivars are available but the most desirable commercial cultivars are susceptible. A breeding and selection program is under way.

An interesting research program in the United States is attempting to develop *C. xyli* subsp. *cynodontis* as a biological agent to control vascular pathogens and insects. The bacterium colonises the vascular tissues of many plant species but induces few, if any, symptoms. Various genes encoding the synthesis of insect toxins and antibiotics have been introduced into the bacterial cell and early results indicate that both vascular pathogens and insects can be controlled by the genetically manipulated organism.

Annual ryegrass toxicity and related diseases

Annual ryegrass toxicity (ARGT) often results in fatal poisoning of livestock. The problem occurs in South Australia, where it has been known since 1956, Western Australia and South Africa. A related disease occurs in New South Wales and South Australia. A family of toxins called corynetoxins is produced by the Gram positive bacterium *Rathayibacter toxicus* (previously named *Clavibacter toxicus*) which has a nematode vector, *Anguina funesta*, that infects annual ryegrass, *Lolium rigidum*. All stock, including sheep, cattle, horses and goats are susceptible to the toxin which is among the most lethal toxins produced in nature. In Western Australia, 88,186 sheep were killed in one year. It seems likely that humans are also susceptible and precautions should be taken when handling toxic samples. Only mature pasture plants are toxic and death of stock may start about three weeks before the pasture dries off. In addition to the direct

losses caused by the disease, there are important indirect losses due to reduced stocking rates, abortions, pasture treatments and the cost of daily stock inspections.

Early symptoms of corynetoxin poisoning in sheep can be observed by making the animals run for one to two hundred metres. The first symptom is usually loss of coordination in the hind legs, which causes affected animals to lag behind the rest. They may also show a high-stepping gait, with head held high and arched back. They may then fall to the ground and have convulsions with the head arched back and limbs extended. In the final stages, the animals lie on their sides and make walking motions with their legs, sweeping bare the ground beneath. ARGT can be confused with perennial ryegrass staggers (caused by a toxin produced by an endophytic fungus in the grass), botulism and tetanus. However, animals affected by ARGT may have periods of apparent recovery, and this distinguishes ARGT from these other diseases.

During winter, before seed heads emerge, the nematodes move to ryegrass plants and invade developing ovaries which, as a result, develop into galls that can be distinguished from healthy seed (Fig. 29.4). The nematodes multiply inside galls during flowering and the second stage larvae remain within the galls until the following winter. Galls fall to the ground and under moist soil conditions rot and nematodes are released to repeat the cycle. This life cycle is quite independent of the toxin-producing bacteria and nematode galls are not toxic. When bacteria are present, bacterial cells become attached to a nematode when it moves across the soil surface and are carried into the immature seeds with the nematode. As a gall develops, bacteria multiply rapidly, filling the cavity and smothering the nematodes. When moist conditions prevail, a bright orange exudate may be seen. Some toxin is present when seed heads emerge and the level increases until the plants dry off. There is strong evidence that toxin production is associated with bacteriophage infection of the bacteria.
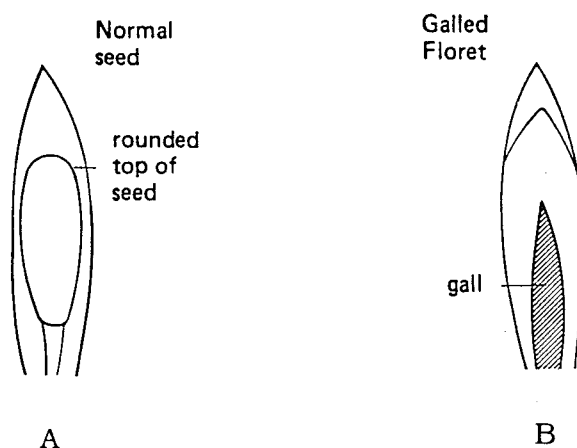

Figure 29.4 Annual ryegrass toxicity caused by the bacterium *Rathayibacter toxicus*. (A) Healthy round-topped seed which is almost as wide as the husk. (B) Infected seed with a gall half as wide as the husk with a pointed top in place of the seed. (From McLeod, Cregan and Doughty, 1982.)

Rathayibacter toxicus can be difficult to isolate from plant material because it grows slowly on artificial media and is often overgrown by saprophytic bacteria. Moreover, the toxigenic bacteria infected with bacteriophage grow even more slowly with the result that non-toxigenic bacteria are usually isolated. *R. toxicus* can be identified serologically and, if non-toxigenic, by resistance to the

bacteriophage. It has not been possible to establish pathogenicity of *R. toxicus* grown in pure culture, presumably because non-toxigenic strains were used.

Long-term management is the best method to control ARGT. The most important thing for a farmer to know is whether or not the farm is affected. A routine testing service for ARGT is based in South Australia but is available to farmers in other States. There is a pre-flowering test based on ELISA that is applied before toxin levels are high enough to cause damage. The test detects the bacterium, *R. toxicus*, in ryegrass seed heads emerging from the boot. If the test is positive, stock will be poisoned later in the season in 50% of affected paddocks, unless steps are taken to minimise toxin production. When the bacterium is detected in early spring, the ryegrass should be grazed out three weeks before the pasture dries off. If this cannot be achieved, the ryegrass should be dried before flowering by mowing or by spraying with a herbicide. The paddocks should be grazed out as soon as possible and should not be used for hay. Stock on suspect pasture need to be inspected daily. The inspections should begin about two weeks before the seed heads dry off and should continue for about eight weeks. If symptoms of poisoning occur, stock should be moved to a 'safe' paddock.

Alternatively, mature ryegrass may be tested. This test can detect both the nematode and bacterium in dry ryegrass or hay. The test is very sensitive and nematodes can be detected several years before the bacterial population is high enough to poison stock. The test can be used to diagnose ARGT if stock show signs of poisoning and also to locate and identify safe paddocks where stock may graze.

It is generally easier to control the host than the nematode or the bacterium. Two successive years of low ryegrass numbers in a crop (ideally, less than 5 plants per m^2) will drastically reduce nematode numbers and usually prevent stock losses in the following two years of pasture. This can be achieved by judicious use of herbicides. Unfortunately, herbicide-resistant ryegrass is now widespread and new methods of control are needed. These include the use of nematode-resistant ryegrass, biological control of the nematode vector by means of fungi such as *Dilophospora* sp. and development of vaccines to protect the animals.

In 1990, a new livestock problem caused the deaths of cattle grazing dried-off pasture along flood plains of the Bogan River in north-western New South Wales. The condition was later detected on 31 properties along the flood plains of a number of rivers in the Darling River catchment area. The problem was known locally as flood plain staggers. The localities where stock were poisoned, were flooded or received heavy rain during winter and pastures were dominated by blown grass, *Agrostis avenacea*. An orange, crystalline material resembling dried bacterial slime was observed on very large, distorted seed heads of *A. avenacea*. From this material, *R. toxicus* was isolated along with the bacteriophage associated with toxin production in ARGT. A nematode was also present in the distorted seed heads but, although it was identified as an *Anguina* species, it was different from *A. funesta*.

The discovery of a bacterium and nematode complex on *A. avenacea* associated with poisoning of stock, prompted a further investigation of a livestock problem known as Stewart's Range syndrome, 1,000 km away in the south-east of South Australia. The problem had been known, but not the cause, for more than twenty years. The dominant grass in the area was *Polypogon monspeliensis*. Again, *R. toxicus* along with its bacteriophage were isolated and the same nematode vector as in NSW was also present. It seems likely that the two diseases are the same and both are now called flood plain staggers. There can be little doubt that ARGT and flood plain staggers are both caused by *R. toxicus* and transmitted by

nematodes. It is much easier to control ARGT than flood plain staggers which occurs in non-arable country.

29.3 Bacterial diseases not present in Australia but of potential importance

There are several bacterial diseases of potential importance to Australian agriculture but which are not present in Australia (Table 29.3). The recent introduction of *Ralstonia solanacearum* race 2, the cause of the very destructive Moko disease of banana, indicates how vulnerable we are. *R. solanacearum* race 2 was introduced into Cairns, North Queensland on *Heliconia*, an ornamental host related to banana. Fortunately, it was detected early, identified using RFLP analysis and eradicated by the prompt application of stringent control measures. This was probably the first application of molecular methods to identify an introduced plant pathogen that could have caused havoc to an Australian primary industry. The importance of having available the facilities and skills necessary for this kind of work cannot be over emphasised.

Table 29.3   Some bacterial diseases of quarantine importance to Australia. (Based on Hayward, 1993.)

Pathogen	Disease/host	Records in Australia	Present status
Clavibacter michiganensis subsp. *sepedonicus*	Ring rot of potato	None confirmed	–
Xanthomonas campestris pv. citri	Citrus canker	Northern Territory (1912)	Eradicated
		Cocos (Keeling) Islands (1981)	Eradicated
		Thursday Island, Torres Strait (1984)	Eradicated
		Near Darwin (NT) (1991)	?Eradicated
		Christmas Island, Indian Ocean (1985)	Not eradicated
Ralstonia solanacearum race 2	Moko disease of banana	Cairns (north Qld) (1989)	Eradicated
Ralstonia solanacearum biovar [1]	Bacterial wilt of *Eucalyptus* spp.	None confirmed	–
Pseudomonas syzygii	Sumatra disease of cloves (and a few other Myrtaceae)	None confirmed	–
'*Pseudomonas celebensis*' [1]	Blood disease of banana	None confirmed	–
Pseudomonas sp. (near to *R. solanacearum*)	Bugtok or tapurok of cooking bananas	None confirmed	–
Erwinia amylovora	Fireblight of apple and pear	Victoria and South Australia (1997)	Eradicated
Erwinia stewartii	Bacterial wilt of maize	None confirmed	–
Xylella fastidiosa	Pierce's disease of grapevine etc.	None confirmed	–
Xylophilus ampelinus	Bacterial blight of grapevine	None confirmed	–
Liberobacter (trivial name for phloem-limited bacterium)	Citrus greening	None confirmed	–

[1] Not a valid name.

Citrus canker caused by *Xanthomonas campestris* pv. citri is another disease that has been recorded in Australia but has not become established on the mainland, although it is now endemic on Christmas Island, an Australian territory in the Indian Ocean. The disease could cause considerable damage to citrus crops in some areas.

Perhaps the greatest threat of all is from fireblight of apples and pears caused by *Erwinia amylovora*. Considerable work has been done on the epidemiology of the disease and it is clear that if it became established in Australia, it would cause very serious damage to our pome fruit industry. Fireblight was recorded in New Zealand in 1919 (the first record outside North America) but surprisingly has appeared in Australia only in 1997 on *Cotoneaster* bushes in the Melbourne and Adelaide Botanic Gardens. Infected plants have been destroyed and the disease has not been detected in commercial fruit growing districts.

29.4 Some important diseases caused by phytoplasmas in Australia

Phytoplasmas affect a range of crop, ornamental and weed plants causing diseases such as legume little leaf, witches' broom of lucerne, big bud of tomato, Australian grapevine yellows, green petal of strawberry, strawberry mycoplasma yellows, yellow crinkle of papaya (pawpaw), dieback of papaya, mosaic of papaya, bunchy top of peanut, little leaf of sweet potato, purple top wilt of potato, virescence (greening of flowers) of choko and sesame and phyllody (greening of petals) of foxglove. Some of these diseases are considered to be economically important and are described below.

Big bud of tomato

Big bud of tomato (TBB) was first described in 1933 as a virus disease and affected plants were reported as having a 'dense, tufted habit with an enlarged bladder-like calyx'. It was reported to occur on tobacco, tomato and other species throughout Australia. The leafhopper *Orosius argentatus* was found to be a vector and it was noted that both the disease and its vector had a wide host range. TBB was reported to be associated with a phytoplasma in 1969, only at that time phytoplasmas were referred to as 'mycoplasma-like organisms'. *Orosius argentatus* was confirmed as a vector of TBB during serious outbreaks in Victoria in the 1970s. It has long been the view that the big bud phytoplasma is also the causal agent of witches' broom of lucerne (LWB), purple top wilt of potato and little leaf of legume (LLL) but until recently, there was no way to confirm this. In 1974, it was reported that TBB, LWB and LLL could be distinguished on the basis of comparative symptomatology and incubation period of the pathogen in certain hosts. A severe and a mild strain of LWB were also studied and the severe strain was indistinguishable from LLL, while the mild strain gave very different symptoms in all host plants tested and could not be transmitted by *O. argentatus*. This work highlighted the need for a specific phytoplasma probe that could distinguish between phytoplasmas. In 1995, using a molecular diagnostic test based on the polymerase chain reaction, it was shown that in Australia, a phytoplasma detected in over 50 host plant species with yellows, phyllody or little leaf symptoms was the TBB type phytoplasma. This phytoplasma belongs to the faba bean phyllody (FBP) strain cluster which includes representatives from Thailand. The closest relatives from temperate regions of the northern hemisphere are those belonging to the western-X-disease cluster. In Australia, research so far has shown that the only phytoplasmas that do not belong to the FBP strain cluster are those associated with dieback in papaya and yellows in grapevine.

Dieback, yellow crinkle and mosaic of papaya

In Australia, papaya (*Carica papaya*) is affected by yellow crinkle, mosaic and dieback. Dieback is the most serious. It has been known since 1922 and frequently causes losses from 10 to 100% of trees in southern and central Queensland. A typical symptom is a bunched appearance of the inner crown leaves, with one or more of these leaves shrivelling and dying. The larger crown leaves rapidly develop chlorosis, then necrosis. The entire crown dies within 1 to 4 weeks and the stem gradually dies back from the top. Despite decades of research, it has been shown only recently that the disease is associated with a phytoplasma which belongs to the stolbur strain cluster which is a sub-group of the aster yellows strain cluster. The insect vector of dieback is unknown and there is currently no effective control measure.

Phytoplasmas associated with yellow crinkle of papaya have been found in phloem cells using electron microscopy of ultrathin sections. The causal agent was transmitted by dodder (*Cuscuta* sp.) from diseased papaya to tomato, white clover and thornapple (*Datura stramonium*) in all of which it caused symptoms typical of phytoplasma diseases. Periodic epidemics of yellow crinkle occur, usually after hot, dry weather which favours the breeding and movement of leafhoppers. Transmission of yellow crinkle by the leafhopper *O. argentatus* has not been clearly demonstrated and it is probable that other leafhoppers can also act as vectors. The initial symptoms of yellow crinkle are a pronounced yellowing of leaves about halfway up the canopy, accompanied by bending of the petioles. The crown leaves develop thin, translucent areas along the margins and between the main veins. These areas become necrotic and tattered as growth proceeds. The crown leaves eventually become claw-like in appearance and the older leaves dry and fall, leaving the tree a bare pole with a few stunted leaves at the top. Floral parts may show phyllody and proliferation of axillary leaves, which are strap-like and thickened.

Mosaic appears sporadically and is generally the least important of the three diseases of papaya but is mentioned here because of its relationship to yellow crinkle. The etiology of the disease is still not clearly understood, although recently there has been strong evidence that it is associated with a phytoplasma. Young leaves of plants affected by mosaic are stunted and yellow with translucent areas around the margins. Affected plants are often stunted and produce multiple side shoots. In field-grown papaya, it is often difficult to distinguish mosaic from yellow crinkle but water-soaked streaks on the petioles and upper portion of the stem and reduced or absent latex flow are characteristic of mosaic. A distinguishing feature of yellow crinkle is phyllody, which is not observed in papaya with mosaic disease. The phytoplasmas associated with yellow crinkle and mosaic are indistinguishable from each other and from the tobacco big bud phytoplasma. It is possible, however, that mosaic results from a disease complex that may include the yellow crinkle phytoplasma but also some other as yet unknown factor.

Australian grapevine yellows

Australian grapevine yellows was first recorded in 1975 and a phytoplasma etiology was suspected after symptom regression following tetracycline treatment. Phytoplasma-like bodies were also observed in electron microscopy of ultrathin sections of phloem from affected grapevines. Diseased grapevines show yellowing and downward curling of leaves on stunted shoots that do not harden off and remain rubbery. Shoot tips stop growing and die and bunches shrivel and fall. Cultivars producing grapes for premium white wines are affected and most

damage has been found in Chardonnay and Riesling cultivars but symptoms are also seen on Traminer, Montils and Semillon. The disease is now present in nearly every viticultural district of Australia and a survey in 1980 showed the highest incidence of disease in the irrigation areas of the Riverland (from Waikerie to Renmark, South Australia), Sunraysia (centred around Mildura, Victoria), the Murrumbidgee (centred in Griffith, NSW) and in the Hunter Valley, NSW. During the 1980s, average crop losses of 10% were observed in vineyards of Chardonnay and Riesling. In some vineyards, 5% of all vines were diseased and severely diseased vines showed yield reductions of 40-50%. Recently, another type of symptom which appears towards the end of the growing season has been recorded. These 'late season' symptoms are back curling of leaves which remain on the vine well after symptomless leaves have fallen. The relationship between these symptoms and the grapevine yellows phytoplasma is not yet known.

Australian grapevine yellows is similar in appearance to other grapevine yellows diseases and to flavescence dorée, an important disease of grapevine in parts of France and Italy. Serological tests and molecular tools have been used to study the genetic relatedness of these pathogens. The Australian grapevine yellows phytoplasma is not related to the flavescence dorée phytoplasma and is most similar to, but not identical with, the phytoplasmas associated with grapevine yellows from Italy and Germany (Vergilbungskrankheit) and bois noir which occurs in parts of France and Spain. These phytoplasmas belong to the stolbur strain cluster which form a subgroup within the aster yellows cluster. The Australian grapevine yellows phytoplasma is indistinguishable from that causing papaya dieback which is surprising given that the two crops rarely overlap geographically. The insect vectors of these two diseases have not yet been identified and the key to this mystery may lie in their identification. It is not known whether the phytoplasma associated with Australian grapevine yellows was originally introduced with infected European vine stocks, or whether it arose from an indigenous phytoplasma within Australasia. Its uniqueness when compared with European or American grapevine yellows phytoplasmas indicates that it is not a recent introduction from these areas.

29.5 Further reading

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