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Infection and Systemic Invasion of Deciduous Fruit Trees by *Pseudomonas syringae* in South Africa

Deciduous stone and pome fruit crops grown in the temperate and Mediterranean regions of the world are attacked by at least four pathovars of *Pseudomonas syringae* (2): pv. *syringae*, pv. *morsprunorum*, pv. *papulans*, and pv. *persicae*. Globally, *P. s.* pv. *syringae* is regarded as the most important of these pathogens. In South Africa, *P. s.* pv. *syringae* causes canker of stone fruit, apple blister bark, and pear blossom blast. In other countries, *P. s.* pv. *morsprunorum* causes leaf spots and cankers on cherries and other stone fruit crops (4), but in South Africa it is virtually restricted to cherries. *P. s.* pv. *papulans* elicits blister spot on apple fruit (3). This disease has not yet been seen in South Africa, but the apple cultivar Mutsu, which is highly susceptible to the pathogen, has recently been imported into the country. Finally, *P. s.* pv. *persicae* is a pathogen of peach (7) and has killed more than a million trees in France.

It is difficult to translate the damage caused by *P. s.* pv. *syringae* and *P. s.* pv. *morsprunorum* on deciduous fruit trees into monetary terms. The severity of the damage varies from subtle, almost undetectable effects to rapid death of many trees in some nurseries and orchards. In South Africa, bacterial canker is one of the most important diseases of stone fruit crops, and annual damage probably exceeds $10 million (U.S.).

Several features of the diseases caused by *P. s.* pv. *syringae* and *P. s.* pv. *morsprunorum* on deciduous fruit trees in South Africa are puzzling. Canker, in particular, appears to be more severe than elsewhere in the world. The reason for this is obscure, but we suspect that a combination of several predisposing components, particularly climatic and soil factors, favors disease expression. Most researchers regard *P. syringae* as a weak pathogen that causes disease only when the host is stressed. The organism is an excellent opportunist by virtue of its ability to colonize the foliar surface epiphytically and then to spread systemically through the tree. Systemic invasion is of particular concern when one considers that deciduous fruit trees are propagated by grafting vegetative material onto rootstocks.

The behavior of *P. s.* pv. *morsprunorum* and *P. s.* pv. *syringae* on deciduous fruit trees appears to be very much the same. To simplify the discussion, the emphasis of our presentation falls on systemic invasion of host trees by *P. s.* pv. *syringae*. We briefly describe certain of the symptoms attributed to this pathovar, discuss some predisposing factors, and propose a modified life cycle for bacterial canker of stone fruit.

General Features of the Diseases

The type of symptom depends on the particular cultivar, plant part invaded, strain of pathogen, and nature of the predisposing factors. On stone fruit, cankers caused by *P. s.* pv. *syringae* develop typically at the bud union (Fig. 1A and B); in pruning wounds (Fig. 1C),

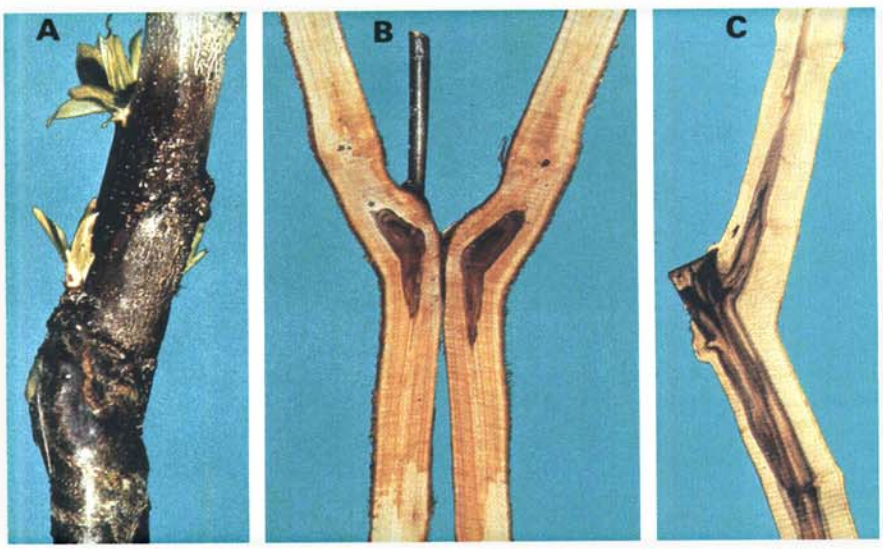


Fig. 1. Bacterial canker caused by *Pseudomonas syringae* at the graft union on young stone fruit trees. (A) External appearance of canker. (B) Longitudinal section showing discoloration of wood due to an infected, nonviable bud inserted laterally on the rootstock. However, a second but noninfected bud positioned at the same time grew and the new growth subsequently compartmentalized the diseased region. (C) Pattern of wood discoloration indicates that the pathogen had gained entry through a pruning wound.

Fig. 2. Symptoms of bacterial canker on stone fruit trees. (A) Cankers on peach trunk. (B) Gum exuded from apricot branch. (C) Dieback of twig on plum tree during middle of summer. (D and E) Cankered plum trees showing (D) early and (E) more advanced stages of dieback. (F) Suckers developing from the rootstock of an otherwise dead tree.

including those made during removal of suckers; or at the base of infected spurs. Cankers on mature trees are conspicuous (Fig. 2A) and usually exude gum (Fig. 2B), especially early in the growing season. Terminal shoots or twigs of a cankered tree often die back (Fig. 2C). If girdled by a canker, the diseased branch or trunk dies within weeks (Fig. 2D and E). The root system of a diseased tree usually remains healthy, however, and suckers develop in the crown region (Fig. 2F). The pathogen might also be present in dormant leaf and flower buds. Infected dormant buds are often killed, but some invaded buds open normally in spring, only to collapse in early summer. Leaves from these buds wilt, and fruit tend to dry out. In contrast, leaves and flowers arising from other infected buds may remain symptomless. If blossom infection occurs, cankers subsequently form on twigs and spurs, and the dead flowers typically remain attached to trees.

P. s. pv. *syringae* causes somewhat different diseases on pome fruit crops. Bacterial blister bark of apple is typified by a raised tan area on the bark and a dried-out epidermal layer (Fig. 3) that may flake off to expose underlying necrotic tissue. Fruit spurs of apple trees with this disease are often blighted. Pear blossom blast is favored by cold (0–12 C), wet weather. Two types of blossom infections occur (9): blast of blossom trusses and development of lesions on the inside of the calyx cup.

Infection via Natural Openings

Leaves. Crosse (6) pioneered studies on the establishment of epiphytic populations of pathogenic *P. syringae* on the surface of seemingly healthy leaves of deciduous fruit trees. These epiphytes already are present in the buds and colonize new leaves as they emerge in spring. Population levels fluctuate dramatically from day to day and might

Fig. 3. Blister bark of apple after infection by *Pseudomonas syringae* pv. *syringae*.

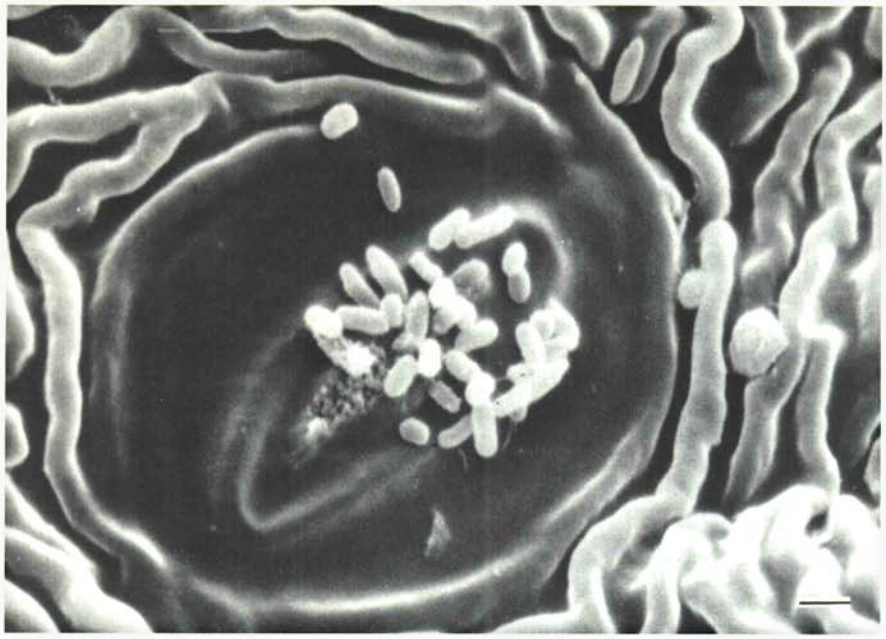


Fig. 4. Scanning electron micrograph of cells of *Pseudomonas syringae* pv. *syringae* extruded from a systemically invaded stomatal chamber on a plum leaf. Scale bar = 1 μm.

Fig. 5. Water-soaked veins infected by *Pseudomonas syringae* pv. *morsprunorum* on a cherry leaf.

Fig. 6. Seed transmission of *Pseudomonas syringae* pv. *syringae*. (Left) Symptomless control peach seedling and (right) diseased seedling developing from naturally infected seed.

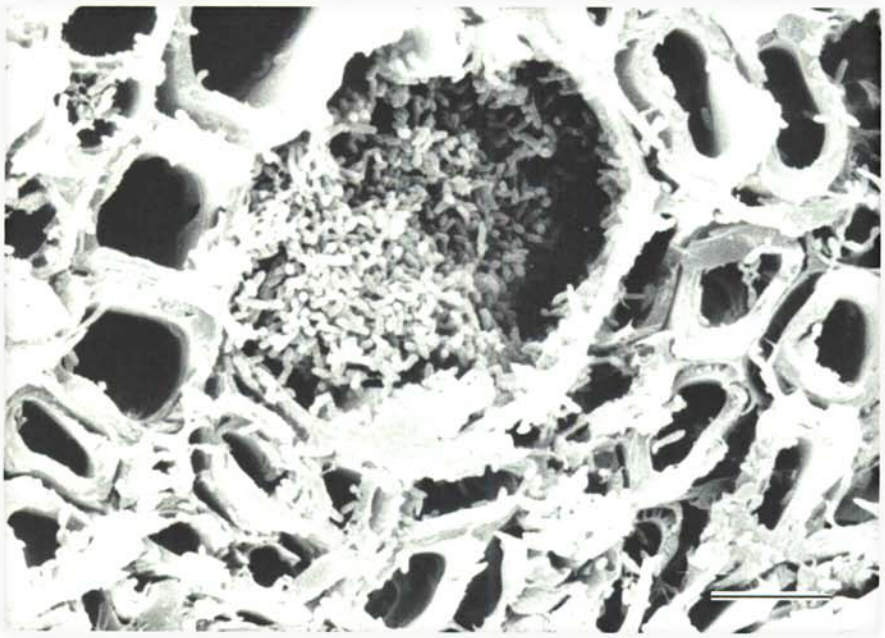


Fig. 7. Scanning electron micrograph showing *Pseudomonas syringae* pv. *syringae* in xylem tissue of a systemically invaded plum shoot. Scale bar = 10 μm.

even change within hours (8). Early in the season, periods of frequent rainfall and high humidity, cool temperatures, and wind favor infection and dispersal of the pathogen. Secondary dispersal during the growing season ensures that inoculum is available throughout the orchard. Bacterial activity subsequently declines during the dry, hot summer months, then increases in autumn. Despite the common presence of the pathogen on leaf surfaces, leaf spots develop rarely, if at all, in South African orchards. However, symptoms on leaves can appear under experimental conditions, depending on the method of inoculation. Symptom development is favored by injuring leaves or allowing tissue to become water-soaked during application of inoculum containing large numbers of the pathogen.

Epiphytic populations of *P. syringae* are not restricted to the external surface of leaves. When we used scanning electron microscopy to study invasion of apple leaves by *P. s.* pv. *syringae* (12), we found that the pathogen entered these leaves through stomata. We postulate that substomatal chambers subsequently serve as protected sites that enable the pathogen to survive adverse atmospheric conditions during warm, dry spells. Surprisingly, pear stomata appeared to be unimportant as points of entry. Instead, *P. s.* pv. *syringae* infects pear leaves through the open bases of damaged trichomes and possibly also through microscopic fissures in depressions of the cuticle (10).

After gaining entry through stomata, *P. s.* pv. *syringae* colonizes the intercellular spaces of the spongy parenchyma. When uninvaded substomatal chambers are reached, bacteria can multiply profusely, and masses of these cells are extruded through stomata (Fig. 4). It is possible that epiphytic populations of the pathogen are constantly replenished in this way. Irrespective of the mode of entrance of leaf tissue, evidence obtained by scanning electron microscopy suggests that the pathogen probably moves through the parenchyma of the bundle sheath into the vascular system of a minor vein. Upon entering a main vein, aggressive strains of the pathogen seem to be virtually assured of passage to axillary buds and to the twig supporting the leaf, thereby promoting long-term survival of the pathogen. In one of our experiments (15), water-soaked, infected veins (Fig. 5) developed on new cherry leaves arising out of axillary buds of leaves sprayed the previous spring with a suspension of *P. s.* pv. *morsprunorum*. Control trees remained healthy.

In England, most cankers caused by *P. s.* pv. *morsprunorum* on cherry trees originate through the leaf scars on fruiting spurs and extension shoots during autumn (6). We have not been able to substantiate these findings under

South African conditions. Instead, we believe that *P. s.* pv. *morsprunorum* or *P. s.* pv. *syringae* probably reaches axillary buds by systemic spread well before leaf fall occurs. Cankers subsequently appear at the base of invaded buds.

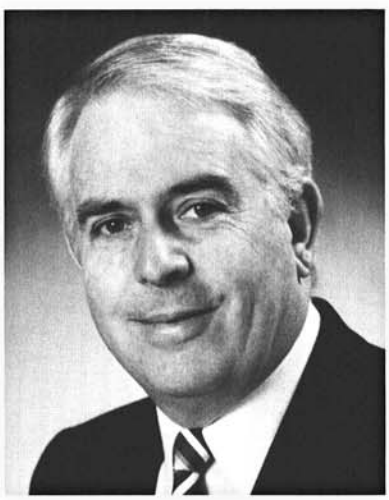
Blossoms and seed. Blossoms of some cultivars are more readily infected than those of others. Inherent biochemical factors and differences in blossom morphology as depicted by pear and apple blossoms (11,12) are two of many factors determining whether infection will occur and how much damage follows successful infection. The stigmatic papillae of both hosts are colonized. However, the tight circular arrangement of the stamens and abundant stylar trichomes associated with apple blossoms seem to prevent bacteria from contacting the hypanthium. As can be expected, the exposed nectariferous region on pear blossoms favors *P. s.* pv. *syringae*. More specifically, the pathogen appears to enter through nectarthodes (resembling stomata) and nectar-secreting hairs (resembling glandular trichomes) of the cultivar Packham's Triumph. After entry, localized necrotic lesions, typical of blossom blast, develop in the hypanthium within 2 days. Since primary infection of apple nectariferous tissue does not occur, it is difficult to distinguish between localized pathological necrosis of styles and natural deterioration that follows anthesis.

Cherry blossoms inoculated with an aggressive strain of *P. s.* pv. *morsprunorum* are usually killed (13). Surviving infected blossoms give rise to infected fruit that have typical dark, sunken necrotic lesions at or near the distal ends. We hypothesized that the pathogen colonizing the stigma moves to the developing ovule through the style (we have seen *P. s.* pv. *syringae* within styles of apple but have no direct evidence that *P. s.* pv. *morsprunorum* occurs in stylar tissue of cherry). One month after blossoms are inoculated with *P. s.* pv. *morsprunorum*, bacterial cells can be detected in the pericarp of developing fruit. Furthermore, the presence of the pathogen in sclereids of the stony endocarp (17) led us to believe that the pathogen might be transmitted to seed. We subsequently found proof for this, but with *P. s.* pv. *syringae* in peach.

Local nurserymen reported that several batches of peach seeds collected in autumn, then stratified in cold storage, germinated poorly when planted in fertilized wood shavings during the following spring of 1988. Many of the seedlings that did develop had necrotic lesions (Fig. 6). We isolated *P. s.* pv. *syringae* from these lesions and from seeds that had failed to develop. Koch's postulates were satisfied with several representative isolates. It is possible that some symptomless rootstocks might shelter latent populations of the pathogen originally present in seed. To our knowledge, transmission of a bacterial pathogen to seed of a deciduous fruit crop has not yet been reported.

Systemic spread in shoots. An aggressive strain of *P. s.* pv. *syringae* inoculated into plum petioles in spring also spreads to the xylem and other elements of leaf veins (16). The pathogen multiplied and was exuded from stomata of these invaded leaves. In orchards, internal migration of the pathogen from stems and shoots to leaves might compensate for epiphytic populations lost during unfavorable conditions. Pockets of bacteria can readily be seen in invaded stems (Fig. 7).

More recently, we investigated systemic spread and pathogenicity of different strains of *P. s.* pv. *syringae* in several plum and apple cultivars. Standardized bacterial suspension was injected into the internode immediately above the petiole of the fifth fully expanded leaf of a vegetative shoot early in summer. The extent of shoot invasion (Fig. 8) in each host-pathogen combination was determined after 2 months. *P. s.* pv. *syringae* generally spreads much



Martin J. Hattingh

Dr. Hattingh is associate professor of plant pathology at the University of Stellenbosch in South Africa. He obtained his D.Sc. degree in microbiology at this university in 1969. He subsequently spent sabbaticals in the departments of plant pathology of the University of Illinois and Cornell University. His research interests have been in several bacterial diseases of major crops grown in South Africa. This work is being funded mainly by the Council for Scientific and Industrial Research. He also teaches a course in phytobacteriology.

Isabel M. M. Roos

Dr. Roos is a researcher employed by the Fruit and Fruit Technology Research Institute in Stellenbosch, South Africa. She received a Ph.D. (Agric.) degree from the University of Stellenbosch on bacterial canker of stone fruit trees. At present she is using molecular biological methods to determine the mechanism of pathogenesis of this disease. The research forms part of an extensive program being funded by the South African fruit industry.

E. Lucienne Mansvelt

Dr. Mansvelt, also employed by the Fruit and Fruit Technology Research Institute in Stellenbosch, obtained a Ph.D. (Agric.) degree from the University of Stellenbosch on a thesis "Epidemiology of bacterial diseases of pome fruit trees caused by *Pseudomonas syringae* pv. *syringae*." She is currently investigating bacteriophages of *P. s.* pv. *syringae* and *P. s.* pv. *morsprunorum*.

further from the site of introduction in plum than in apple shoots. In addition, most strains caused necrotic lesions exceeding 1% of the plum shoot length, whereas lesions on apple shoots were inconspicuous or absent. This strengthens the view that apple is an inhospitable host and that disease is not likely to develop in apple orchards unless trees are severely stressed or cultural practices favor dissemination and establishment of the pathogen.

Manifestation of host-pathogen interactions on plum cultivars appears to be more varied. Four extremes can be mentioned, but many intermediate reactions occur as well: 1) no growth of the pathogen and no symptoms, 2) systemic spread but no symptoms, 3) pathogen confined to the lesion (canker), and 4) extensive spread of pathogen beyond the canker. The nature of the response depends on both the pathogen strain and the host cultivar. For example, one of the *P. s.* pv. *syringae* test strains spread systemically in cultivar Songold without causing appreciable cankers. The same strain spread much less in cultivar Laetitia but resulted in more prominent lesions. We conclude that differential interactions occur between strains of *P. s.* pv. *syringae* and plum cultivars and probably also other stone fruit cultivars. Our results are consistent with the general assumption (6) that populations of *P. syringae* in cankers on deciduous fruit trees decline during summer. However, the rate of decline and level of the population inside the tree at the end of summer seem to depend on the particular host-pathogen combination.

Problems and Prospects for Disease Control

In the past, programs to control bacterial canker of stone fruit have met with mixed success, as can be expected if the subtle nature of the disease and the excellent ability of the pathogens to survive on the surface and inside the host are considered. The modified life cycle that we propose for bacterial canker (Fig. 9) illustrates the ramifications of this intricate disease.

Trees with latent infections probably occur in most of the major fruit-producing regions of the world. If crucial predisposing factors can be avoided or counteracted, however, canker is unlikely to be a serious problem. At present, this seems to be the most logical approach for management of the disease. Some of the stress factors that have been recognized in the United States and elsewhere include freeze damage, wounds, nematode damage, and dual infections of *P. s.* pv. *syringae* and plant-pathogenic fungi such as *Cytospora* and *Nectria*.

Financial opportunities within expanding markets tempt some growers to establish new orchards under marginal conditions. Trees are particularly susceptible in some sandy soils, in waterlogged soils that drain poorly, and during prolonged periods of drought. Also, the dormancy requirements of many stone and pome fruit cultivars are not properly met in regions that experience mild winters. In South Africa, most deciduous fruit trees do not defoliate before the middle of June (comparable with December in the Northern Hemisphere). Some local producers apply copper sprays in early winter to achieve defoliation, but this practice is not encouraged by professional extension services. The abrupt, abnormal loss of leaves disrupts the active root development that continues during this period when soil temperatures are still relatively high. Trees then become stressed, and canker development is enhanced.

Major outbreaks of bacterial canker in young orchards are often attributed to poor horticultural practices. The situation is undoubtedly aggravated if the pathogen has been introduced uniformly into young nursery trees through infected buds or rootstocks.

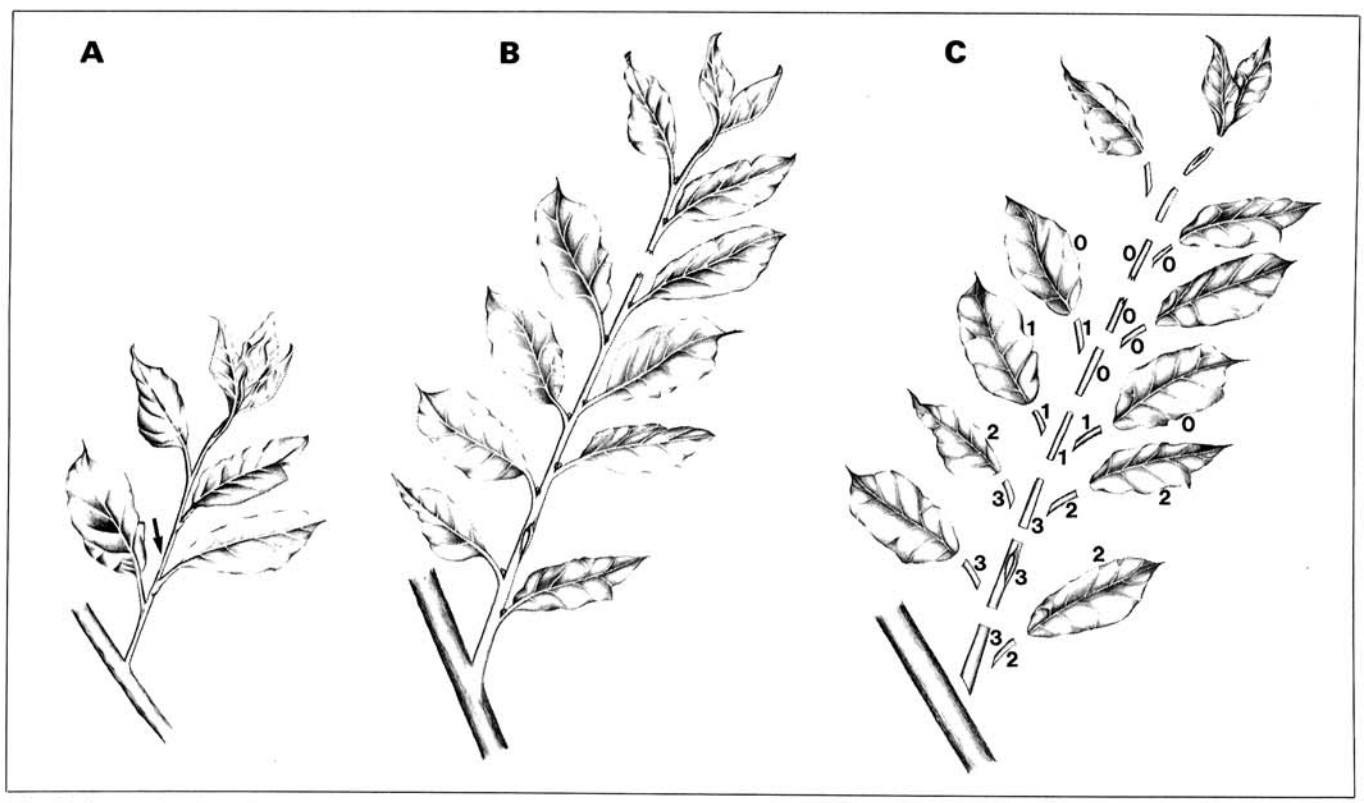


Fig. 8. Determination of systemic spread of *Pseudomonas syringae* in apple and plum shoots. (A) Arrow indicates point of needle injection of pathogen (10^8 cfu/ml). (B) Shoot after 2 months, before being processed. Note canker at site of inoculation. (C) Leaves were removed and the shoot cut into 2-cm segments starting from the point of attachment to the main stem and extending 20 cm beyond the site of injection. Each figure represents the number of colony-forming units of the pathogen (0 = nil, 1 = ≤10, 2 = >10-200, and 3 = >200) present in a sampling unit as determined by dilution plating. The average value of the total scores of at least four individual shoots of a particular host-pathogen combination was taken to indicate the extent of systemic spread.

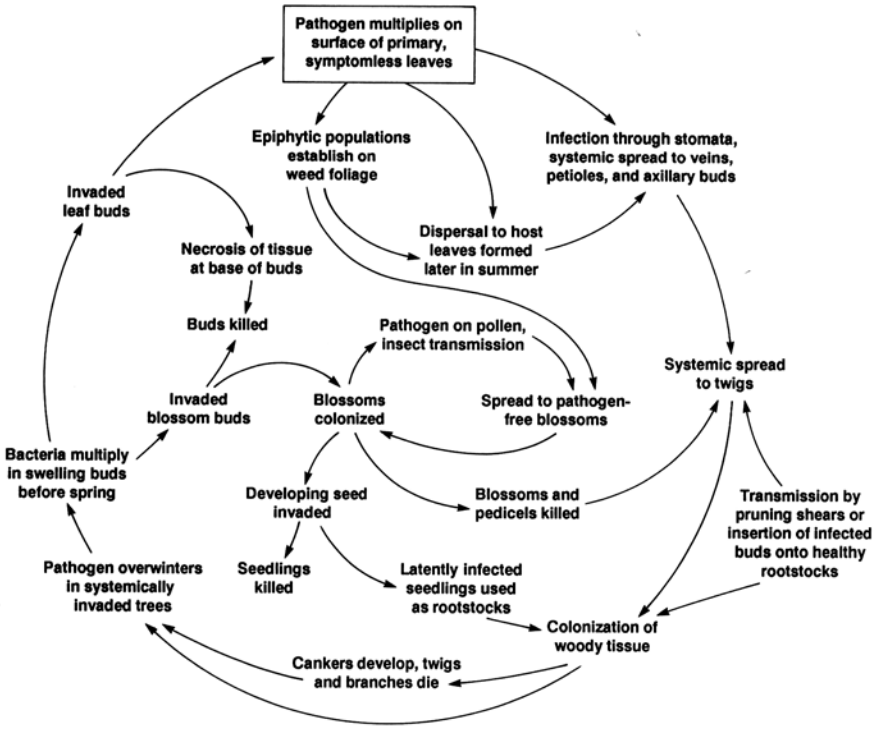


Fig. 9. Life cycle of bacterial canker of stone fruit trees caused by *Pseudomonas syringae*.

These trees might not become diseased in nurseries where optimal growth conditions can be maintained with little difficulty. The trees are severely stressed during transplanting, however, and therefore are more prone to disease development until they have become established in their new environment.

Chemical control of bacterial canker in Europe and North America is based primarily on protective sprays with fixed copper or Bordeaux mixture in autumn and in spring before blossoming (1). However, chemical control of the disease in South Africa has been a complete failure. This is not surprising if one considers the heterogeneous nature and versatility of the pathogens (14), especially their outstanding ability to populate symptomless leaves and to invade host trees systemically.

Future attempts to manage bacterial canker more effectively will place greater emphasis on selection and breeding for disease resistance and on the correction of factors that tend to aggravate disease. To achieve this, more information is required on the nature of the host-pathogen interactions. Genetics and molecular biology should be used to determine how genes on chromosomes or plasmids confer pathogenesis and virulence traits (5). A thorough knowledge of the genetics of the pathogen might lead to the eventual development of effective biological control agents or modification of the host to incorporate resistance.

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