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PSEUDOMONAS SYRINGAE: DISEASE AND ICE NUCLEATION ACTIVITY

Editor's note: Because of the recent awareness of the seriousness of Pseudomonas syringae in the nursery industry, and because of the many unanswered questions, this special report is rather technical and indepth. It is intended as a review of what is known and what is unknown and as a basis for continued research and development of controls.

Plant Symptoms Caused by *Pseudomonas syringae*

A variety of symptoms are associated with woody plants infected by *Pseudomonas syringae* pv. *syringae* a bacterium distributed widely on plants. Apparently, kinds of symptoms and symptom development are dependent upon the species of plant infected, plant part infected, the infecting strain *of Pseudomonas syringae*, and the environment. More than one symptom can occur simultaneously on a plant.

Plant species, plant part, *Pseudomonas syringae* strain, and environment determine symptoms and severity.

Symptoms: A) *Flower blast* where flowers and/or flower buds turn brown to black. B) *Dead dormant buds*, especially common on cherries and apricots. C) *Necrotic leaf spots* - entire clusters of younger, expanding leaves may be killed on filbert trees. D) *Discolored and or blackened leaf veins and petioles* resulting from systemic invasion and infection. E) *Spots and blisters on fruit*. F) *Shoot tip dieback* appears as dead, blackened twig tissue extending downward for some distance from the tip (very common on maples and other seedlings). G) *Stem cankers*, depressed areas in the bark, become darkened in color with age. A gummy

substance is often exuded from cankers on fruiting and flowering stone fruits (This symptom is characteristically referred to as "gummosis"-see reference 8). If the cankers continue to enlarge, they may girdle the stem and subsequently cause the death of a branch or the entire plant. When the outer tissues of the canker area are cut away, the underneath tissue typically exhibits a reddish brown discoloration. This discoloration may also occur as vertical streaks associated with the vascular tissue.

Tip dieback was the most common symptom observed on the 40 woody deciduous plants collected in Pacific Northwest nurseries (11); *Pseudomonas syringae* was isolated from all plants having the tip dieback symptom. Plants most commonly and most severely affected were maple, dogwood, filbert, blueberry, magnolia, lilac, oriental pear, aspen, and linden. However, since our survey of nurseries in Oregon and Washington was not exhaustive and was conducted over only a one year period, woody plant species in addition to these are likely susceptible to this pathogenic bacterium. Even so, these observations illustrate the widespread nature of this disease.

Pseudomonas syringae symptoms suggest toxin involvement. Syringomycin, a toxin produced by *Pseudomonas syringae*, has been isolated and chemically characterized (22, 33). At first, this toxin was thought to be produced by all virulent strains and therefore responsible for tissue damage. Since then, studies have shown that there is a poor correlation between production of this toxin and virulence (66). We (4) isolated numerous naturally occurring pathogenic strains which produced no detectable amounts of syringomycin in vitro. Conceivably, toxins other than syringomycin might be produced in the infected tissue. The characteristic symptoms caused by *Pseudomonas syringae* strongly suggest toxin involvement, and may in fact, contribute an important part to the disease syndrome, but no one has isolated other toxins at this time.

Severity of *Pseudomonas syringae* to Plants

There seems to be no consensus about the severity of diseases caused by *Pseudomonas syringae*. Experiences of researchers have been variable relative to the amount of damage caused by strains of this pathogen. Most researchers consider *Pseudomonas syringae* a weak pathogen, an opportunist that capitalizes on a host that has been weakened by some predisposing condition.

Severity of *Pseudomonas syringae* is related to other predisposing factors. Entire plants may be killed.

English (9) reported that peach trees planted in sandy soil in the San Joaquin Valley were highly susceptible to *Pseudomonas syringae* and could be killed the first year after planting. van Meteren of the Netherlands (personal communication) stated that whole Forsythia plants could be destroyed during the spring of the second growing season if night frosts occurred. Isolations from Forsythia tissues showed total infection of most parts during the whole year. Luisetti, while visiting our lab in 1985, indicated that *Pseudomonas syringae* pv. *persicae* had killed more than 1 million peach trees in France that were under 5 years of age. He thinks pv. *persicae* is a mutant or variant of pv. *syringae*. A paper from Greece (69) reported that a new bacterium closely related to *Pseudomonas syringae* caused severe damage to filbert trees, killing branches and

entire trees. Blossom blast can seriously reduce fruit yields, as illustrated by the report about pear blossom blast in South Africa (56).

Predisposing Factors Increase Severity

A number of factors have been reported to make plants more susceptible to infection. The foremost factor is freeze damage. Freezing wounds the plant allowing the pathogen entrance and destroys plant cells; the cell contents become a source of nutrients for the pathogen (81). Numerous workers have reported that symptom development in the field was related to cold temperatures (39, 65, 86). Similar observations have been made in Oregon. For example, Bailey Nursery reported that Sugar Maple and Linden were severely attacked by *Pseudomonas syringae* after a spring frost. Klaus at Michigan State University also states (personal communication) that *Pseudomonas syringae* is a serious problem on cherry trees in Michigan because the trees are subjected to severe cold weather. Ironically, many strains of *Pseudomonas syringae* act as ice nucleating agents: They catalyze the formation of ice crystals on and in the plant tissues. Their presence on the plant serves to raise the freezing temperature above that at which sensitive plant tissues would normally freeze (51). Most frost-sensitive plants have no significant mechanism of frost tolerance and must be protected from -ice formation to avoid frost injury (51). In the absence of this protection, ice crystals formed in or on sensitive plants rapidly spread intercellularly and intracellularly, causing mechanical disruption of cell membranes and subsequent death. Many of the *Pseudomonas syringae* strains which we isolated from the woody plants in Oregon and Washington were ice nucleation active (INA), but the INA characteristic was variable, depending in part upon the host plant from which the strains of *Pseudomonas syringae* were isolated (4).

Wounding: Wounding of any kind seems to play a significant role in most of these infections, whether mechanical wounds or wounds from such things as frost injury. Wounds predispose trees to blossom blight and bacterial canker (8, 11, 17, 51, 70 81, 86). As already mentioned, pruning wounds aid infection by not only *Pseudomonas syringae*, but also *Cytospora* and *Nectria* (76).

Crosse (15) showed that leaf scars are points of entry on cherry trees in England. However, Cameron (8) could not obtain infection through leaf scars of cherry trees grown in western Oregon. In contrast, Klos at Michigan (personal communication) said they were able to get infection of cherry by knocking leaves off in the fall and inoculating the leaf scars. Early reports about the disease pointed out that susceptibility was highest after leaf fall (90), but this could be a function of dormant tissues being more susceptible (4, 22) rather than necessarily related to leaf scar infection.

Plant Dormancy: Dormancy may also predispose susceptible trees to damage from *Pseudomonas syringae* (90). Dormant peach trees were reportedly more susceptible to the disease than active ones (19).

Soil Factors: Factors such as soil pH, mineral nutrition, etc. may predispose trees to *Pseudomonas syringae* infection.

Dual Infections Increase Severity: Data from studies of *Pseudomonas syringae* show that disease severity is greater when the plant is attacked by more than one pathogen. *Cvtospora* and *Pseudomonas syringae* together were far more serious than alone in studies reported from Hungary (76). Nematode damage to trees has been cited as another predisposing factor to *Pseudomonas syringae* infections in California (English, personal communication and reference 29): bacterial canker was more severe on trees parasitized by nematodes.

In some cases, diagnosis of *Pseudomonas syringae* symptoms on maple in Oregon nurseries has been complicated by the occurrence of verticillium wilt in the trees being examined. In maple, symptoms of each disease can be similar, and dual infections have been observed. The relative contribution of each pathogen to the total disease impact upon the tree is unknown.

In Hungary, *Cytospora cincta* and *Pseudomonas syringae* frequently attack peach trees simultaneously (76). The data showed that the two pathogens caused more damage together than individually. *Cytospora* infections did not occur unless open wounds were available; wounds caused by pruning were especially vulnerable.

Although we have no data relative to dual infections of *Nectria* and *Pseudomonas syringae*, *Nectria cinnabarina* does cause cankers and death of maple trees (Moore and Leben, unpublished data).

Ornamental crab apple trees may represent another host affected by a dual infection of *Pseudomonas syringae* and *Phytophthora syringae*. *Phytophthora syringae* is a fungus recently shown by Linderman (personal communication) to be a serious winter disease of several ornamental shrubs and trees in western Oregon. It causes a blackening of the tissues much like we have described for *Pseudomonas syringae*.

An odor of sour sap accompanies some of the severe *Pseudomonas syringae* infections of ornamental trees (Baca and Moore, unpublished). Sour sap has been observed commonly with bacterial canker of peach and peach tree short-life syndrome (87, 93). Often there is such a mixture of microorganisms associated with these severe infections that it is difficult to pinpoint a single causal agent.

Nematode-infected peach trees in the San Joaquin Valley were more highly susceptible to *Pseudomonas syringae* infections (19, 29). Soil fumigation with a nematicide reduced *Pseudomonas syringae* damage.

Disease Severity in the Northwest: Reports from northwest nurseries provide examples of the variableness of both the severity and host range of *Pseudomonas syringae*.

One northwest nursery reported that 30% of 90,000 Linden trees showed severe symptoms in the spring of 1982. Another nursery reported that their Linden trees were also severely diseased. In contrast, a third nursery reported that the disease was very serious on Japanese Lilac and two cultivars of red maple, Red Sunset and October Glory, but they observed no problem on Linden trees. (Does this differential attack on nursery trees indicate that some strains of the pathogen are host specific and only attack certain tree species?)

A fourth northwest nursery reported the disease on Laburnum trees (Golden Chain Tree) and Bradford pear trees, with 90% of the latter being killed. At a fifth nursery, damage was light to moderate, but mostly light and primarily on the leaves of Thundercloud and Newport plums and on Norway, Red, and Silver maples.

The first leaf symptoms appeared at the start of the second week of May and again the first part of June. In both cases, symptom development was associated with wet, cool weather. Twig die-back symptoms and actual tree mortality did not appear until late summer. This late appearance of decline symptoms may have been caused by, or at least linked with, Verticillium wilt, a serious fungal disease of maple trees.

Classification of *Pseudomonas Syringae* Isolates

Pathogen Variability and Genetics: Numerous studies report that variations occur among strains of *Pseudomonas syringae* relative to their pathogenicity (4, 28, 68, 91, 35, 64, 73). For example, Gross et al (35) in Washington reported 50% of 82 strains of *Pseudomonas syringae* were pathogenic to immature pear and sweet cherry fruit. Values of 85% of the strains being pathogenic were reported by workers in Michigan (42). In England, Garrett (32) reported that no single isolate would infect all hosts recorded as being susceptible to *Pseudomonas syringae*.

Our own studies in Oregon (4) showed that not all our isolates would infect immature fruit of yellow pear tomato or induce a hypersensitive response in tobacco. Some of the isolates infected inoculated cherry seedlings and sudan grass, but others did not (3). This differential response may or may not represent host specificity, but it certainly shows that infectiousness varies among isolates. Similar work out of N. Carolina (28) showed that young apple seedlings were the most susceptible assay host of several tested. Over 95% of their strains were infectious using this system, whereas 80% or less were pathogenic with other tests. They also concluded from foliar inoculations of peach, apricot, nectarine, and plum cultivars with three *Pseudomonas syringae* pv. *syringae* strains (isolated from stone fruit hosts) that these strains varied more in levels of virulence than ability to infect specific hosts.

Pathogenicity is only one of a multiple set of phenotypic traits that characterize **Pseudomonas syringae**. Not only can they be variable in pathogenicity, but they also can be variable relative to ice nucleation (4) and biochemical-physiological tests used for identification (4, 73, 77). Given this variability, it is imperative that a large number of strains be studied before making general conclusions.

> ***Pseudomonas syringae* strains differ in pathogenicity, ice nucleation activity, and response to identification tests.**

Phenotypic variation is but a reflection of genotypic variation. Work is now underway at several labs on the genetics of these bacteria. Genetic work to date includes research to identify the gene involved in ice nucleation (50), insertion elements (58), toxins and virulence and avirulence

factors (66), but little work has been done to my knowledge regarding mapping of the chromosome of *Pseudomonas syringae*.

Pathogenic strains of *Pseudomonas syringae* could possibly be modified by genetic engineering.

An interesting aspect of the genetic research from Mills' lab at Oregon State University (58) is the discovery that insertion elements (small pieces of DNA) move back and forth from the plasmid to the chromosome. Not only does this event provide opportunity for new traits via genetic recombination, but insertion of this element into a particular gene can inactivate or modify the gene, thus a phenotypic trait such as pathogenicity could be altered or lost. The significance of this finding to the wild type population in nature is unknown, but it certainly could provide a mechanism to insure variation among strains.

Some strains of *Pseudomonas syringae* will nly grow on certain isolation media. This further complicates identification.

Isolations were made from a wide variety of woody plant cultivars from Oregon and Washington which showed symptoms suspected to be caused by *Pseudomonas syringae*. The efficacy of a selective medium developed by Sasser (personal communication) for isolation of *Pseudomonas syringae* was tested, but some strains known to be *Pseudomonas syringae* failed to grow on this medium. Sasser's medium was therefore discarded in favor of the more general Kings Medium B (37). Recipes for two other selective media have since been published (6, 60), but their effectiveness in the Pacific Northwest is unknown.

We isolated a large number of bacterial strains from 42 cultivars of diseased woody plants representing 13 families (11). Of the 558 bacteria isolated, 466 (79%) were fluorescent. Of the 466, 303 (65%) were oxidase negative and arginine dihydrolase negative. Fluorescent pseudomonades with these two phenotypic traits have traditionally been called *Pseudomonas syringae*. Unfortunately, these tests tell us nothing about whether the isolates are pathogenic.

Testing this large number of isolates for the ability to infect such a diverse group of plant cultivars represented a commitment of time and resources beyond our means. We elected to try a shorter method and use the tobacco -hypersensitivity bioassay to determine which isolates were pathogenic. Two hundred ninety seven of our bacterial isolates induced a HR, and 252 of these isolates were fluorescent, oxidase negative, and arginine dihydrolase negative--phenotypic traits of *Pseudomonas syringae*. Over 50% of these isolates were also ice nucleators (Baca, 1987).

***Pseudomonas syringae* isolates may cause a hypersensitive response but not be pathogenic.**

The ability of a bacterium to cause a hypersensitive response (HR) in tobacco leaves was described as highly correlated with pathogenicity of the bacterium by Klement et al. (38). However, Endert and Richie (28) reported a considerably lower correlation between HR and pathogenicity and found that an apple and pear hypocotyl inoculation test gave a better

correlation. Because of this controversy, we also inoculated the immature fruit from the yellow pear tomato (10) as a back-up test. Then, in 1987, Willis et al. (89) generated two Tn5

non-pathogenic mutants of *Pseudomonas syringae* pv. *syringae* which failed to elicit any disease symptoms on either the pods or leaves of bean cultivars (which were susceptible to the parent strain of these two mutants). Yet these two mutants produce the typical hypersensitive response shown by the parent strain when infiltrated into the leaves of tobacco. **The data suggest that the gene(s) for pathogenicity are different than those for the hypersensitive response (HR), which means that greater care will be needed in using the HR as an indicator of pathogenicity.**

There are relatively few characteristics that distinguish one pathovar of *Pseudomonas syringae* from another.

Classification of isolates in our research to a specific pathovar proved to be rather chaotic (77). There are relatively few characteristics that distinguish between pathovars of *Pseudomonas syringae*, and the variability in tests among our isolates was so great that no sense could be made out of the data (Baca and Moore, unpublished data). This heterogeneity among isolates has also been noted by others (3, 4, 10, 21, 28, 32, 34, 35, 47, 55, 68). Roos and Hattingh (73) reported that the phenotypic heterogeneity of the *Pseudomonas syringae* pv. *syringae* strains which they studied appeared to be even more extensive than that indicated by other diagnostic schemes.

Epiphytes are bacteria that live saprophytically on the surface of plant parts without necessarily parasitizing the tree and causing disease. Some *Pseudomonas syringae* are epiphytes.

Lindow et al. (49) isolated *Pseudomonas syringae* strains from numerous nonsymptomatic plants, showing that this bacterium survived as an epiphyte without causing disease. Since the isolated strains were from nonsymptomatic plants, they concluded that the strains were unknown pathovars of *Pseudomonas syringae*.

Our collection of isolates may indeed contain several pathovars, but the methodology for identification and classification must improve before we will attempt to name them. Application of restriction fragment length polymorphism analysis of an isolate's DNA may prove to be a more accurate tool for identification of *Pseudomonas syringae* (21). Using this method, Denny (21) reported considerable heterogeneity among strains of *Pseudomonas syringae* pv. *syringae*, whereas strains of *Pseudomonas syringae* pv. tomato exhibited rather tight homogeneity. *Pseudomonas syringae* pv. tomato may be an example of specific adaptation of a strain to a host.

Pathogenicity Tests / Classification

Inoculations are required to determine experimentally the host range of particular isolates or the resistance/susceptibility of plant selections in a breeding program. Wounding of these plants at the time of inoculation typically enhances infection. However, there is no consensus among

scientists who study *Pseudomonas syringae* as to the best method for determining the ability of isolates to infect plants or the host range of an isolate. Numerous attempts have been made to fmd an acceptable host(s) and method (28, 47, 64, 73). In general, methods of inoculation typically include use of the following plant parts or some variation thereof: A) immature fruit, B) green shoots of woody plants or young seedlings and C) woody tissues.

Davis and English (19 and English, personal communication) make a tangential wound through the cambium of current season or second and third year-old Prunus wood grown in California, then fill the wound with bacteria from a syringe. To be successful, inoculations in the field must be made in December or January. This timing results in maximum extension of the canker by April. For best canker development on trees in greenhouse studies, they placed the trees briefly at 25C then at 12-15C for a week.

Inoculation of wounded, dormant plants caused most severe development of disease.

Garrett (32) reported that although cankers would develop from inoculations made anytime from August to March in England, it was those inoculations made from December through January (the middle of the dormant season) that ultimately caused the longest cankers to develop. She reiterated the point that trees were usually immune to infection during most of the growing season. To inoculate trees being screened for resistance to *Pseudomonas syringae* and *Pseudomonas morsprunorum*, Garrett wounded the stems with a scalpel and deposited a few drops of inoculum (10(8)cells/ml) into the wound. High humidity was maintained by wrapping the wound with polythene tape.

Endert and Ritchie (27) also found that wounding and inoculation of peach trees in February resulted in more severe canker symptoms than October inoculations. February infections were also associated with necrosis of fruit and shoot buds and delayed budbreak. No disease developed following inoculation of leaf scars in October and the population of the marked *Pseudomonas syringae* strain used for inoculum declined to nondetectable levels.

Attempts to find a bioassay procedure to determine pathogenicity of *Pseudomonas syringae* isolates are encouraging.

Recent attempts to fmd a uniform bioassay procedure to determine the pathogenicity of an isolate have been encouraging. Endert and Richie (28) inoculated etiolated hypocotyls of apple and pear seedlings and showed high correlation with peach tree inoculations in the greenhouse. Roos and Hattingh (73) used Endert and Richie's hypocotyl bioassay as a preliminary screen to test the pathogenicity of various strains of *Pseudomonas syringae* isolated from trees and weeds in pome and stone fruit orchards in South Africa. Two year old apple, pear, and cherry trees were inoculated with selected strains *of Pseudomonas syringae*.

To compare the reliability of the several types of bioassays, Lindemann and Suslow (47) injected a number of heterologous pathovars of *Pseudomonas syringae* into immature fruits, green shoots or seedlings, and woody tissues. They reported that Lovell peach seedlings were a very reliable

indicator of pathogenicity and free of heterologous responses. All known pathogenic strains induced lesions within four days following inoculation. Nemaguard seedlings were less susceptible than Lovell. Interestingly, Zehr et al. (93) reported that Lovell were much less susceptible than Nemaguard rootstock to peach tree short-life in South Carolina field tests. It may be unfair to compare these reports since one is a lab/greenhouse test and the other a field test. The short-life syndrome is characterized by a complicated mixture of causal factors. Lastly, the strains of *Pseudomonas syringae* used in these tests may be quite different. Burr and Katz (7) reported, for example, that the *Pseudomonas syringae* strains they obtained from DeVey in California were much more virulent than any of their isolates from New York.

Sources and Survival of *Pseudomonas syringae* Inoculum

Historically, identification of the primary source of inoculum has been a crucial step towards developing a workable strategy for control of plant diseases. Once the source is identified, attempts are made to avoid, exclude, or eradicate this inoculum. Several potential sources of *Pseudomonas syringae* are discussed individually below, but the relative contribution of each source to disease development remains unknown. This ignorance, among other things, lends credence to the conclusion of Gross et al. (35) that our knowledge of the ecology of *Pseudomonas syringae* is poor.

Buds: Buds are considered a major overwintering site of *Pseudomonas syringae* (45). This bacterium was detected inside apparently healthy apple and pear buds during growing and dormant seasons over a 2-year period in South Africa (57). Apple budwood contained more *Pseudomonas syringae* than pear budwood. However, none to very few bacteria were detected in some cultivars such as Granny Smith, indicating that buds of some cultivars are less hospitable to the bacterium. Roos and Hattingh (72) also isolated pathogenic *Pseudomonas syringae* from many apparently healthy buds of stone fruit trees; a higher percentage of active, expanding buds contained the pathogens than did dormant buds. In New York, *Pseudomonas syringae* pathovars were isolated from the interior of diseased as well as healthy buds of apples, but not from the outer bud scales (7). In contrast, Cameron (9) reported that most cherry bud infections with *Pseudomonas syringae* in Oregon orchards did not take place through leaf scars but apparently originated at the bases of the outside bud scales and then spread throughout the base of the bud rather than though the avenue of leaf scars reported by Crosse (15). This disagreement among researchers may be due to the uniqueness of anatomy and physiology of buds of different plant species, and potentially different environmental conditions at their geographic location.

Cankers: Holdover cankers from a previous year's infection have long been thought to be the primary source of inoculum (59). However, this can't be the only source of primary inoculum since the nursery trees we examined in our survey were usually too young to have such established cankers and were usually isolated from larger trees.

Systemic Invasion: An early report (Cameron, 1970) strongly indicated that *Pseudomonas syringae* could be isolated from interior tissues of fruiting cherry trees; most of these trees typically had 3 to 6 cankers, but two showed no visible symptoms. Bacteria were isolated as far as 20 feet from any obviously diseased tissue. The highest bacterial counts were obtained from the trunk, roots, and lower scaffold limbs. However, of nearly 10,000 isolates examined, fewer

than 10% were identified as *Pseudomonas syringae*. This indicates that many other bacteria also reside in the plant interior. A preliminary survey of orchard trees at Hood River recently showed that *Pseudomonas syringae* could be isolated from extruded vascular sap of cherry and pear trees, especially from the roots (Whitesides and Spotts, personal communication). The work is not far enough along to know the frequency of occurrence of systemically invaded trees within an orchard or how widespread the invasions are across orchards. Work with rooted grape cuttings in Oregon (31) has also demonstrated that *Pseudomonas syringae* can be transported upwards through the vascular system of the cutting.

Recent work in South Africa using antibiotic-resistant bacteria (both *Pseudomonas syringae* and *Xanthomonas campestris* pv. *pruni*) and scanning electron microscopy has demonstrated conclusively that the bacteria introduced into leaves and leaf petioles can invade leaves and shoots of plum and cherry trees and cause disease (26, 74, 75). In some cases, disease symptoms appeared the following spring.

INA *Pseudomonas syringae* have also been isolated from root xylem of alfalfa grown in Canada (30). When alfalfa plants were inoculated with these isolates prior to a freeze test, plant survival was reduced as compared to control plants or plants inoculated with an INA-minus strain.

Symptomless, systemic infections challenge the idea of controlling *Pseudomonas syringae* by applying protective bactericide sprays.

Latent Infections: Latent *Pseudomonas syringae* infections have been suspected to occur, but the evidence for such has been limited. The recent work out of South Africa using a marked strain of *Pseudomonas syringae* showed that the bacterium could enter plant tissues during the current growing season, but symptoms did not develop in the invaded tissues until the following growing season (74, 75), thus indicating that latent infections can occur. The isolation of pathogenic *Pseudomonas syringae* from the vascular tissues of systemically inhabited, but symptomless, trees also suggests that latent infections can occur (refer to the above section on systemic infections).

Establishment of *Pseudomonas syringae* inside symptomless tissues during the summer could represent a very important source of primary inoculum. Systemic invasion of tissues by *Pseudomonas syringae*, therefore, assumes considerable ecological significance and obviously poses a significant challenge to the idea of controlling the disease by applying protective bactericide sprays to the tree surface. Cameron (10) states that such sprays have been ineffective in about half the cases and were only 10-80% effective in the other half.

Epiphytes: *Pseudomonas syringae* also exists as an epiphyte on the surfaces of many plants, and as such, is in a position to cause infection should the right environmental conditions develop (79). Crosse (16, 17) demonstrated for the first time in 1959 that phytopathogenic bacteria could be a component of this microbial community. Leben (44) was one of the first to investigate epiphytic *Pseudomonas syringae* on soybean plants. Dowler and Weaver (24) showed that these bacteria survived on healthy peach trees without causing disease, and Lindow et al. (49) isolated epiphytic *Pseudomonas syringae* that were ice nucleation active from 95 symptomless plant species, indicating that the epiphytic stage phase is widespread.

Monitoring epiphytic *Pseudomonas syringae* populations associated with nursery trees in Oregon showed that the population increased rapidly and peaked during the first two to three weeks after bud break (4, 55, 62). The population declined during the summer, increased a small amount in October, and was often undetectable during December through February. The work of Gross et al. (34) at Prosser, Washington and Hood River, Oregon showed that population trends on apple trees were the same in both states. However, Baca did observe that these curves could vary considerably depending upon which plant species was monitored (4), a fact also observed by Malvick (55).

The observed decrease in epiphytic populations of *Pseudomonas syringae* during some periods of the year raised a question of whether the epiphyte could survive long periods on the plant, or if the population was replenished regularly from adjacent colonized plants. Recent work from our laboratory (55) has demonstrated that an antibiotic-resistant strain of *Pseudomonas syringae* could be recovered from symptomless maple trees for up to 10 months, showing that a particular strain of *Pseudomonas syringae* can survive successfully over the summer and winter. These findings suggest that the epiphytic *Pseudomonas syringae* may be important for survival and secondary spread during the growing season, but they may not be important relative to survival and overwintering of primary inoculum.

Weeds and Grasses: Work reported from California (see 19) was the first to implicate weeds as hosts for *Pseudomonas syringae*. Since then, workers in Michigan (42), Oregon (3), Poland (53) and South Africa (71) have reported that *Pseudomonas syringae* could be isolated during the growing season from weeds or grasses.

Baca et al. (3, unpublished data) observed that several fields of Sudan grass cover-crops used in Oregon Nurseries showed symptoms suspiciously like those caused by bacteria. Isolations made in late Oct. from these infected leaves yielded populations in excess of 10(8)/g of tissue. The fields of sudan grass were disked to chop up the grass before replanting with seeds of winter rye grass. During the winter, *Pseudomonas syringae* could be recovered from newly emerged rye grass at populations of 10(6)/g of tissue. The new rye grass may have become infested by *Pseudomonas syringae* which survived on the disked sudan grass or from the rye seed itself since *Pseudomonas syringae* was recovered from seed-lots of rye grass grown in the Willamette Valley. In contrast, *no Pseudomonas syringae* were recovered from several seed-lots of grass produced in California. Infested roadside grasses around the nurseries also carried high populations of the pathogen.

The significance of grasses as overwintering hosts for *Pseudomonas syringae* is unknown.

The significance of these grasses as overwintering hosts for *Pseudomonas syringae* is unknown. As mentioned above, we have shown (55) that a marked strain of *Pseudomonas syringae* could survive epiphytically for 10 months on inoculated maple trees. However, lateral and upward movement of this marked strain from inoculated plants to adjacent noninoculated plants or onto agar plates was very limited.

Soil as a source of inoculum: It is generally accepted that *Pseudomonas syringae* survives poorly in soil, but neither the soil phase nor the potential survival of *Pseudomonas syringae* on roots (the rhizosphere) have been studied in any depth. Valleau et al. (82) isolated *Pseudomonas syringae* pv. *tabaci* (close relative to *Pseudomonas syringae* pv. *syringae*) from roots of tobacco, oats, wheat, rye and several weed species in Kentucky. Knoche et al. (40) recently isolated *Pseudomonas syringae* pv. *tabaci* from the rhizosphere of tobacco and witchgrass and from soil in Wisconsin; they demonstrated that wild type strains of pv. *tabaci* were competent colonizers of wheat roots. Survival of *Pseudomonas syringae* pv. tomato was associated with tomato seed, briefly with host tissue returned to the soil, and weed hosts (54).

Dissemination of *Pseudomonas syringae* Inoculum

The threat of disease from pathogen movement is illustrated in the report by Luisetti (52, personal communication) who showed that *Pseudomonas syringae* pv. *persica* spread readily from one point in a French orchard to another. Luisetti inoculated 12 trees in the center of a 1 Ha orchard with this pathogen, and the bacteria were recovered from the leaves of adjacent symptomless trees in 2-3 months. Within one year, 60% of the trees were showing symptoms. The mechanism of dissemination was not determined.

Pseudomonas syringae can be moved from place to place by various means such as wind and rain (17, 55), insects which carry inoculum from cankers (91), through infested budwood (25; 53, only 10% of grafted buds survived; 56, 57) and transportation of infested nursery stock. The potential spread of the pathogen through mechanical equipment and pruning tools has received little study, but nonsterile pruning tools would seem a logical means of dispersal.

Aerosols: Ice nucleating *Pseudomonas syringae* have been recovered from the ocean (51) and the air canopy above bean fields (46). However, neither the pathogenicity of these isolates nor their duration of survival in the relatively harsh environment of the aerosol have been determined.

Aerosol deposition of *Pseudomonas syringae* did not occur very far from the inoculum source.

Andersen and Lindow (2) positioned petri plates of media at the border of citrus groves and at uniform distances inward towards the center of the grove. Lids were removed from the petri plates for 30 minutes at each location to capture *Pseudomonas syringae* deposited from the air. INA bacteria were detected most frequently at the border of the grove when weeds or other crops bearing *Pseudomonas syringae* were adjacent to the grove. During the winter months, bacterial populations on navel oranges were inversely correlated with the downward distance from vegetation bearing high populations of INA *Pseudomonas syringae*; frost damage to the fruit was also highest on the exterior of the groves adjacent to this vegetation and decreased with distance towards the center of the grove. Groves free of weeds or bordering next to other groves showed no correlation of bacterial populations with location of the plates in the grove. This indicates that a source of *Pseudomonas syringae* external to the grove was important to aerosol transmission of the INA bacteria, but that deposition did not occur very far into the grove.

Mechanical harvesting of alfalfa fields next to the citrus groves also greatly increased the number of INA bacteria captured on the petri plates of agar (ibid 2). This finding agrees with Lindeman et al. (46) published report that plants can serve as a source of airborne *Pseudomonas syringae* and demonstrates additionally that mechanical shearing of colonized plant tissue can generate aerosols of *Pseudomonas syringae*.

Malvick and Moore (55, 62) could detect but few *Pseudomonas syringae* strains with an Anderson air sampler (1) positioned next to a maple tree nursery. There was only very limited vertical and lateral movement of a marked strain of *Pseudomonas syringae* from tree to tree or to and from grass, and such displacement and movement required that the plant tissue be bombarded by water droplets either from overhead sprinkler irrigation or heavy wind-blown rains.

Host Specificity of *Pseudomonas Syringae*

Pseudonionas syringae pv. *syringae* has a wide host range. The wide-spread presence of epiphytic phases of *Pseudomonas syringae* may cause problems in interpreting how broad the host range really is. As Lindow et al. (49) pointed out, many of the strains that he examined were isolated from nonsymptomatic plants.

***Pseudomonas syringae* has a broad host range.**

A study of the pathogenic potential of different strains and pathovars of *Pseudomonas syringae* on fruit trees demonstrated that response to infection depended upon the host-pathogen combination (73). Three *Pseudomonas syringae* pv. *syringae* test strains effectively colonized apple, cherry, pear, and plum tissue and caused cankers on all but plum. None of the three test strains caused symptoms on plum shoots, even though they were present in high numbers in plum tissues. Two strains of *Pseudomonas syringae* pv. *morsprunorum* caused cankers only on cherry, but they appeared to be weaker pathogens than *Pseudomonas syringae* pv. *syringae*. Roos and Hattingh (73) propose that each fruit cultivar supports a heterogeneous population of *Pseudomonas syringae* pv. *syringae*, and that some of these strains are more virulent on other host trees. And, with a particular host-pathogen combination, symptoms develop only if environmental conditions favor a particular strain or if the tree is severely stressed.

In Oregon, Baca found that Sango Kaku and Oshi Beni, cultivars of Japanese Maples, were exceptionally susceptible to *Pseudomonas syringae*. Bradford pear was also badly damaged by this pathogen as were Magnolia trees, both of which are not very cold hardy. This differential susceptibility of woody plants was also reported for different cultivars of wheat which exhibited varying degrees of susceptibility to a variety of *Pseudomonas syringae* strains (79). Despite the implications of host specific pathogens, definitive experiments have not been done to prove whether this susceptibility is related to specificity of a particular strain of *Pseudomonas syringae* for that host.

Controls

A variety of methods have been tested for control of *Pseudomonas syringae* in commercial plantings and include chemical means, biological control with microbial antagonists, cultural management schemes, and host resistance. Efforts have been targeted primarily for either control of disease or to reduce the risk of frost damage from ice nucleation active *Pseudomonas syringae*. However, the results of these control efforts have often been erratic, again indicating that there is much we don't yet know about the epidemiology and ecology of *Pseudomonas syringae*.

Chemical Controls

Copper compounds were reportedly used successfully to reduce *Pseudomonas syringae* pv. *morsprunorum* infections on montmorency sour cherry (63) and to reduce frost injury induced by INA *Pseudomonas syringae* (51). Kocide (10 lb/100 gallons of water) applied with Triton X for control of *Pseudomonas syringae* pv. *syringae* was reported to last longer than 3 months on dormant trees in a heavy rainfall area of western Oregon (Alan Elliott, Carlton Plant Co., personal communication). Similarly, copper Kocide 101 (10 lb/100 gal) plus Nufilm 17 16 oz/100 gal was used successfully for control of *Pseudomonas syringae* pv. *syringae* on a variety of nursery trees at Bailey Nursery (Todd Erickson, personal communication). Bailey Nursery has developed a successful spray schedule based on A) alternating applications of streptomycin with applications of fixed coppers to reduce the risk of selecting for copper-resistant or streptomycin-resistant mutants of *Pseudomonas syringae* that might occur if only one bactericide were used repeatedly (20, 80, 92), and B) timing sprays to avoid phytotoxicity to sensitive tissues. At Bailey's, they apply Agristrep once anytime during August and Kocide once anytime during the month of October. Kocide is applied again anytime after January 1 up to bud break. To avoid copper phytotoxicity, Agristrep is applied to trees when trees begin to break bud. A combination copper-streptomycin spray schedule was also used to control pear blossom blast in California (5).

Some growers in Oregon have reported (personal communication) poor control of *Pseudomonas syringae* following use of either copper or streptomycin sprays. Because fixed copper sprays gave sporadic disease control of bacterial canker in sweet cherry, Cameron (10) speculated that the poor control was due in large part to systemic infections.

Improved control of *Pseudomonas syringae* pv. tomato (the pathovar that causes bacterial spot of tomato) has been reported when a mixture of copper and either Maneb or Mancozeb (fungicides) were applied (14, 67). Apparently, this mixture produces a copper carbamate which is more effective than copper alone. To my knowledge, this mixture has not been tested for control of *Pseudomonas syringae* on woody nursery crops or fruit trees. Since Maneb and Mancozeb are fungicides, their application with a copper might provide better protection against dual infections involving *Pseudomonas syringae* and fungi such as *Cytospora* or *Nectria.*

A final consideration for improved chemical spray control is the report of Jardine and Stephens (41) regarding a predictive system for timing chemical applications to control *Pseudomonas syringae* pv. tomato. Stagewise multiple linear regression techniques were used to identify those meteorological and biological variables useful in predicting bacterial speck symptom development cause by this bacterium. Encouragingly, their model accounted for 85% of the observed variation in the population for the years used in model development. In 1984, the equation correctly predicted values above or below the preselected threshold for spray application 12 of 12 times and resulted in three fewer sprays than used in a calendar spray schedule.

Better disease control was also reported at some planting sites in California following soil fumigation which reduced the populations of *Criconemoides xenoplax*, a plant parasitic nematode that parasitizes peach trees and predisposes them to damage by *Pseudomonas syringae* (29, 61). In contrast, Weaver et. al. (87) reported that soil populations of the nematode *Macroposthonia xenoplax* differed significantly among the rootstocks they examined, but were not correlated with disease incidence. The interaction of planting site relative to fumigation and the presence of nematodes showed that disease severity was greater on sandy soils than clay soils; fumigation had little effect on disease incidence of plants grown in clay soil (61, 93).

Zehr et al. (93) recommended soil fumigation before planting and at two-year intervals after planting to control the nematode, *Criconemoides xenoplax*, which also provided protection against peach tree short-life. Shortlife is a term used to define a complex syndrome of peaches which is poorly understood, but in addition to bacterial canker, includes damage from more than one pathogen. The severity of short-life is affected by a number of important predisposing factors such as nematodes, cold injury, pruning time, pythiaceous fungi and acid soils (see also references in citation 93). However, tree vigor and losses were also affected by the choice of rootstock as well as by nematode control; Lovell rootstock was recommended in problem orchard sites because Nemaguard rootstock was unsatisfactory even when used with fumigation.

Biological Control with Bacterial Antagonists

Efforts at biological control have been directed almost entirely at frost control using bacterial antagonists to prevent buildup of ice nucleation active (INA) populations of *Pseudomonas syringae* (48, 51). Through recombinant DNA technology, Lindow and colleagues (50) have removed the gene that is responsible for ice nucleation from one of these INA positive strains, thus disarming the bacterium and allowing its use as a biocontrol agent for frost damage control. Other tests for biocontrol of frost damage have utilized *Pseudomonas syringae* strains chemically mutated to the INA-minus state as well as naturally occurring INA-minus bacterial antagonists; both types gave positive results. Application of these antagonists prior to bud break or at seeding allowed the antagonist to obtain a dominant competitive position in the leaf habitat, prevent colonization by INA-positive *Pseudomonas syringae*, and thereby provide frost protection. Kocide and streptomycin applications also reduced the population of INA *Pseudomonas syringae* and provided frost control.

Frost control was not achieved by applying Lindow's antagonists or chemical bactericides in field tests on apple and pear orchards in Washington (35). These workers propose that there is an intrinsic ice nucleation active molecule within plant tissue not related to the INA bacteria.

Cultural Management

Attempts to alter susceptibility of trees to bacterial canker and/or peach tree short-life disease have included a variety of approaches, each of which has offered some benefit even though the benefit might be localized to a particular area or soil type. For example, liming the soil at the planting site reportedly promoted peach tree growth and vigor (93), and Weaver et. al. (84) felt that alteration of the soil pH affected the susceptibility of peach to *Pseudomonas syringae*.

The nature of the soil at the planting site can effect tree susceptibility in an indirect way. Clay soils typically were free of nematodes in South Carolina and losses from peach tree short-life in these soils were minimal (93). Other soil amendments of iron (18) and calcium and magnesium (88) reportedly affect tree susceptibility to bacterial canker and short-life. Weaver et al. (85) also measured the elemental content of dormant peach trees to determine if susceptibility was related to nutrition; accumulation during dormancy of above average concentrations of calcium in the twigs was correlated with increased susceptibility to cold injury and bacterial canker. Weaver postulated that the increase in elemental concentration of calcium in the twig may be an indicator that the trees had been predisposed. Plants receiving no iron developed the longest bacterial cankers, and in the zero and low rate of iron *Pseudomonas syringae* was recovered farther from the inoculation site and after a longer time following inoculation than in other rates of iron (18). Early work out of California (61) showed that nutrient levels were lower in leaves of peaches whose roots were parasitized by *Criconemoides xenoplax*, and water stress was also greater. Development of the root system of these trees was greatly reduced compared to the nonparasitized controls, and bacterial canker development was greatest on the trees suffering from nematode infections.

Since peach tree short-life is more of a problem on old peach orchard soils, Chandler and Daniell (12) examined the effect of leachates from these soils, and from roots of potted peaches grown in these soils, on plant growth and bacterial canker development. Seedling growth was reduced in old peach soil and pot-soil leachates. After inoculation with *Pseudomonas syringae*, the longest cankers developed on seedlings growing in leachates from the pots than any other treatment. They hypothesize that some water soluble substance from dead peach roots was taken up by the trees which predisposed them to bacterial canker and peach tree short-life.

Pruning in the fall and early winter also predisposed the trees to more severe damage from *Pseudomonas syringae* infections and the short-life syndrome (13, 83, 87, 90).

Davis and English (19) reported that cankers from *Pseudomonas syringae* infections were longer when twigs were pruned from trees in December than when pruned in January or February. In those cases where trees may be threatened by Cytospora and *Pseudomonas syringae*, Rozsnyay (personal communication) recommended pruning in early spring when trees are practically resistant to *Cytospora*.

Burning of *Pseudomonas syringae* cankers on limbs of stone fruit trees in New Zealand with a hand-held propane burner reportedly cauterized the tissues and limited further spread of the canker so that the branch or trunk did not become girdled and killed (36). The living tissue surrounding the burned canker area callused quickly forming an effective barrier against reinfection, and within 2 years the treated limb showed little evidence of the infection. Cauterization was reportedly rapid and easy to use in the orchard, and most cankers were controlled by a single treatment. The method was tested successfully on apricots, sweet cherries and peaches. This method is part of a total control strategy that also includes a spray program of autumn and winter bordeaux sprays followed by spring sprays of streptomycin. Curiously, I have not heard of the cauterization method being used in orchards in the U.S.

Contaminated budwood (25, 53) is another problem which must be circumvented to reduce the incidence of bacterial canker. Refer to the earlier section regarding survival of *Pseudomonas syringae* in buds for further information.

Plant Resistance

Breeding for resistance is a slow process with woody trees, both because of the time involved for tree growth and the threat of *Pseudomonas syringae* to adapt genetically and infect the new germplasm. However, some successful control of *Pseudomonas syringae* has been realized using plant germplasm resistant to this pathogen. In Oregon, the rootstock F12-1 is reportedly quite resistant to *Pseudomonas syringae*. Unfortunately, the cultivars of sweet cherry are not resistant. But, by budding the scion high on the F12-1 rootstock, growers have been able to avoid total death of the tree and can start anew with a well established rootstock in those cases where disease is severe and the diseased scion tissue must be removed or pruned severely.

Schmidle (78) observed that some cultivars of sour cherry in Germany were resistant to bacterial canker caused by *Pseudomonas syringae*. In England, Garrett (32) has a program to select rootstocks resistant to *Pseudomonas syringae* pv. *mosprunorum*, but this program will likely be phased out since the research station has been closed. Similarly, Weaver et al. (87) screened various peach seeding rootstocks for susceptibility to bacterial canker and found significant differences in incidence and severity of bacterial canker symptoms among six peach rootstock selections. Soil populations of the nematode *Macroposthonia xenoplax* also differed significantly among rootstocks, but were not correlated with disease incidence. In contrast, Zehr et al. (93), as noted above, found that peach tree short-life resistance of Lovell rootstock was correlated with nematode resistance. Nemaguard or Elberta rootstocks were highly susceptible to the nematode and short-life, and some losses occurred even in fumigated soil.

Summary of Disease Control Recommendations

To obtain good disease control, it seems imperative to recognize the correlation between susceptibility to bacterial canker caused by *Pseudomonas syringae* and those factors which weaken the tree. Both bacterial canker and peach tree short-life involve similar interacting factors. Accordingly, Zehr et al. (93) recommended pre- and post planting fumigation of soils carrying threatening populations of parasitic nematodes, use of quality nursery stock with rootstocks resistant to *Pseudomonas syringae* and nematodes, attention to soil pH, good weed

control, and proper timing of pruning. In addition, the choice of planting site is important as is protection from frost injury where possible through biological, chemical, and physical means. Choose appropriate chemical sprays and apply them properly, especially where dual infections of *Pseudomonas syringae* and fungi such as *Cytospora* and *Nectria* occur. Cauterization used to limit bacterial canker -development on stone fruits in New Zealand should be investigated where appropriate.

For additional information on *Pseudomonas syringae*, please see: Ornamentals Northwest 11(1):22, July 1987, and Ornamentals Northwest 5(6)20-21, Nov 1981.

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