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Title: Distribution and Characterization of Ice Nucleation Active Strains of Pseudomonas Syringae From Diseased Woody Plants and Grasses

Abstract approved: _____
Dr. Larry Moore

In a recent survey, it was observed that many woody plant species grown in Pacific Northwest nurseries exhibited disease symptoms typical of a bacterial infection and Pseudomonas syringae was commonly isolated from these tissues. The distribution of the ice nucleation phenotype among P. syringae strains recovered from these infected woody hosts was examined. More than eighty-five percent of the P. syringae strains from linden, lilac, dogwood and oriental magnolia samples were ice nucleation active (INA) at 5°C; 76% of the P. syringae strains from aspen were INA at -5°C; but only 30% of the P. syringae strains from Japanese pear and 24% of the red maple strains were active ice nucleators at this temperature.

The P. syringae strains isolated from these seven plant hosts were variable relative to their ability to induce a hypersensitive response in tobacco leaves and their ability to induce pathogenic changes when injected into immature tomato fruits. The range in

hypersensitivity response by P. syringae strains isolated from a particular host varied from 100% in aspen strains to 57% in Japanese pear while the range in potential pathogenic ability on tomato fruit varied from 100% in aspen to 36% in saucer magnolia.

In November 1983, tissue samples were also obtained from fields of diseased sudan grass used as green manure, from fields of symptomless cereal rye grass grown as cover crops as well as from roadside grass species growing around the perimeter of nursery production areas. Large populations of pathogenic and INA strains of P. syringae were isolated from these grass strains with populations of fluorescent pseudomonads exceeding 10^9 cfu/g fresh tissue from sudan samples whereas populations of 10^6 cfu/g were obtained from cereal rye grass and roadside grass samples. Eighty-one randomly selected strains from these isolations were tested using the LOPAT determination scheme for fluorescent pseudomonad identification. Fifty-eight of the 81 strains (72%) were similar to P. syringae, whereas 34 (59%) of the 58 strains were ice nucleation active at -5°C. Thirty-one of the 58 strains induced a hypersensitive response in tobacco leaves, and 29 (50%) were pathogenic to green fruit of tomato. Several P. syringae strains isolated from sudan and cereal rye grass were pathogenic when inoculated to greenhouse grown sudan seedlings; however, none of the strains tested were pathogenic to cereal rye grass seedlings in the greenhouse. Three of six P. syringae strains tested were also pathogenic to young shoots and leaves of peach trees maintained in a greenhouse chamber at high humidity.

DISTRIBUTION AND CHARACTERIZATION OF ICE NUCLEATION ACTIVE STRAINS OF *PSEUDOMONAS SYRINGAE* FROM DISEASED WOODY PLANTS AND GRASSES

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This thesis is dedicated to the loving memory of my father, Ernest Baca, and my father-in-law, John A. Datesman, my family, my loving wife, Karen, and my daughter, Laurel Melissa.

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INTRODUCTION

Pseudomonas syringae, a motile, aerobic, fluorescent pseudomonad, is a phytopathogen with a host range in excess of 80 plant species (21). Plant species infected by this bacterial pathogen include herbaceous as well as deciduous woody plants. Symptom expression is variable and may be influenced by many factors, including but not limited to, plant species or variety, environmental conditions (especially temperature and humidity), growth stage of the plant, and virulence of the pathogen (3). Symptom expression may include tip-dieback of stems, cankers on trunk and stems; leaf spots, blasts of buds (dead bud), leaves, and shorts; floral blasts, spotting of fruit and gummosis (10). *P. syringae* has also been found as an epiphyte on a wide variety of plants, including non-host plants, such as rye grass, sow thistle and hairy vetch (20,22).

Strains of *P. syringae* van Hall (22), *Erwinia herbiola* Lohnis (Dye) (22), and *P. fluorescens* (Migula) (22), have been shown to induce ice formation and subsequent frost injury to susceptible plant species at temperatures higher than normally expected. Frost injury incited by *P. syringae* has been implicated as a predisposing factor to infection in many plants including pea, pear, peach, poplar and apricot (23). It has been reported by Lindow (19) that on average only one ice nucleus is expressed, in a population of

300-1000 bacteria, with the potential for ice nucleation activity. A single ice nucleus is currently thought to be sufficient to initiate ice formation and subsequent frost injury to an entire leaf, fruit, flower, or even groups of leaves or flowers depending on the degree of restriction of ice propagation within a plant (21).

In the Pacific Northwest beginning in 1979 nursery operators and inspectors described symptoms on many woody plant species that were similar to P. syringae infections, and they reported that the disease was occurring more frequently and with greater severity. Episodes of light frost were reported to have preceded initial symptom expression particularly involving aspen (Populus tremuloides) and magnolia (Magnolia soulangiana). Many varieties of woody plants are grown in Pacific Northwest nurseries for shipment to markets in the midwest where episodes of light frost occur during the early spring. A survey by Canfield et al (in press) established that pathogenic P. syringae isolates were widely distributed within woody decideous nursery plants in Washington and Oregon nurseries.

In the past, little work has been published about diseases occurring in woody nursery plants caused by P. syringae nor has the source of P. syringae inoculum been identified. Arsenijevic (1), in Yugoslavia reported that strains of P. syringae isolated from herbaceous hosts such as sudan grass (Sorghum sudanense) were pathogenic to woody plants and vice-versa. Latorie and Jones, in Michigan, established that P. syringae pathogenic to sweet and sour

cherry exists on weed and plant refuse within the nursery environment. Lindemann in 1982 reported that populations of pathogenic P. syringae could be isolated from non-host plants and such plant canopies could be sources of airborne bacteria, including ice nucleation bacteria.

This research was designed to determine i) the distribution of INA phenotype within populations of P. syringae isolated from selected woody nursery plants, ii) the association of the INA phenotype with pathogenicity to green fruit of yellow pear tomato, and the hypersensitivity response in tobacco leaves, and iii) the distribution of INA P. syringae within indigenous and cultivated grass species within the nursery environment.

CHAPTER 1

DISTRIBUTION AND CHARACTERIZATION OF ICE NUCLEATION *PSEUDOMONAS SYRINGAE* FROM DISEASED WOODY NURSERY PLANTS

ABSTRACT

Infections incited by *Pseudomonas syringae* have been reported by nursery operators in many woody plants grown in Pacific Northwest nurseries. In several cases, aspen and magnolia, nursery operators reported that episodes of light freezing temperatures (0°C to -5%C) preceded or were associated with initial symptom development. Because ice nucleation induced by *P. syringae* has been implicated as a predisposing factor to infection of other woody hosts, the association of the ice nucleation phenotype with *P. syringae* strains recovered from infected woody hosts was examined. Eighty-five percent or more of the strains isolated from linden, lilac, dogwood, and oriental magnolia were ice nucleation active (INA) at -5°C; 76% of the strains from aspen were active ice nucleators; but only 30% of the strains from Japanese pear and 24% of those from red maple were active ice nucleators at -5°C. The *P. syringae* strains recovered from these seven woody hosts were variable relative to the induction of a hypersensitive response in tobacco and the ability to infect green fruit of yellow pear tomato. The range in hypersensitivity response varied from 100% in aspen to 54% in J. pear, and in pathogenicity from 100% in aspen to 36% in saucer magnolia.

INTRODUCTION

In recent years, infection by Pseudomonas syringae of many woody plants grown in Pacific Northwest nurseries has increased both in severity and frequency of occurrence. A survey conducted to determine the distribution of Pseudomonas syringae among plants in 32 nurseries showed that populations of pathogenic strains of P. syringae could be recovered from 40 of 44 plant species representing 13 plant families (Canfield et al., in press). Nursery operators and state nursery inspectors reported that in magnolia and aspen, light freezing temperatures (0 to -5°C) preceded or were associated with initial symptom development.

It has been shown that certain strains of P. syringae van Hall (22), P. fluorescens Migula (22), and Erwinia herbicola (Lohnis) Dye (22), can initiate ice nucleation activity (INA) at temperatures higher than would normally be expected. Frost injury and subsequent damage in susceptible plants frequently occurs (23). This damage to plant tissues incited by INA P. syringae has been reported to predispose apricot, poplar, sour cherry, and peach, to infection by P. syringae (23). Although epiphytic INA strains of P. syringae are apparently widely distributed in nature on healthy plants, no pathogenicity studies were reported for these strains (22).

These seven woody hosts were also selected since the majority of these plant species are grown in the Pacific Northwest for shipment to midwest markets. Therefore, colonization of these plants by INA P. syringae may increase their susceptibility to

frost injury due to induced ice nucleation and subsequent disease by P. syringae or by other opportunistic pathogens.

The purpose of this research was to determine (i) the distribution of the ice nucleation active phenotype within populations of P. syringae recovered from seven different species of infected woody plants, and (ii) the association of ice nucleation activity with the ability to incite a hypersensitive response in tobacco or incite pathogenic changes on green fruit of yellow pear tomato.

MATERIALS AND METHODS

Source and Maintenance of bacterial strains. The P. syringae strains used in this study were isolated from diseased tissues of woody plants during a 1982-1983 survey. Strains from seven plant species representing seven plant families included aspen (Populus tremmuloides), lilac (Syringa vulgaris), linden (Tilia cordata), saucer magnolia (Magnolia soulangiana), Japanese pear (Pyrus spp.), dogwood (Cornus florida), and Maple (Acer rubrum). All strains isolated in this survey were stored as working cultures on slants of potato dextrose agar amended with 0.5% (wt./vol.) $CaCo_3$ as a buffering agent. For long term storage, bacteria in a mixture of sterile glycerol and water 30%/70% (vol./vol.) were stored in 1 ml aliquots at -70°C.

Characterization of strains. Strains were characterized as P. syringae by the methods of Canfield et al. Tests included oxidase and arginine dihydrolase reaction (29), and hypersensitivity response in tobacco leaves Nicotiana tabacum cv. White burley. Further characterization included pathogenicity in green fruit of yellow pear tomato Lycopersicon asculentum cv. yellow pear (4), and ice nucleation activity (22).

Inoculum preparation. Inoculum was prepared by streaking the bacteria on slants of King's Medium B (KB medium) and incubating slants at 24°C for 48 hrs. Aqueous suspension of cells were used in testing for INA, pathogenicity and hypersensitivity (22, 4, 14).

Ice nucleation activity. The ice nucleation activity was determined by the freeze drip method of Vali as modified by Lindow

(22). Slants of KB medium were streaked with each bacterial strain and grown for 48 hours at 24°C, scraped into 3 ml. sterile water blanks and vortexed for 30 min. The ability of each strain to induce ice formation at -5°C was determined by pipetting ten-10 1 drops of each bacterial suspension onto a paraffin coated sheet of aluminum foil. The foil sheet was then floated on a 50:50 solution of propylene glycol and water. This solution was maintained at -5°C ± 1°C by an Excal 300 controlled temperature bath. Tests for ice nucleation activity were performed using a 10^9 cfu/ml cell suspension. The freezing of at least one of ten drops within 30 seconds was considered positive for this test.

Pathogenicity to green tomato fruit. The ability of these strains to induce pathogenic changes in green fruit of yellow pear tomato (Lycopersicon esculentum Mills) was tested by the method of Cameron (4). Surface sterilized green tomato fruit were given three injections of 0.1 ml per fruit of a 10^6 cfu/ml suspension and inoculated fruit placed into a plastic box with wetted paper towels to increase relative humidity. Fruit were observed for pathogenic changes after 7 days.

Hypersensitivity response. The ability of each strain to induce a hypersensitivity response in tobacco (Nicotinia tabacum cv. White Barley) was tested by infiltrating 0.1 ml of a 10^9 cfu/ml suspension into leaves of tobacco and observing after 24 hours for white collapsed areas (16, 29).

RESULTS

Out of 150 strains of putative P. syringae tested from lilac, linden, dogwood, magnolia, Japanese pear, and red maples, 62% (93/150) were active as ice nucleators at -5°C. However, the proportion of INA strains of P. syringae strains varied according to the host from which they were isolated (Fig. 1). The percentage of INA strains isolated from each plant species varied from 24% to 94% and the plant families could be grouped into two classes (high and low) according to the percentage of INA strains recovered from each host. The first group included linden, lilac, dogwood, magnolia and aspen, all of which yielded a high percentage of INA strains (94-76%). The second group included strains from Japanese pear and red maple which exhibited a much lower frequency of ice nucleation activity (30% and 24%, respectively).

Strains of INA P. syringae from different hosts were also variable relative to inducing a hypersensitive response (HR) in tobacco leaves and in infecting green fruit of yellow pear tomato (Fig. 2). A mean of 85% (79/93) of all INA P. syringae strains induced a HR in tobacco, whereas only 69% (64/93) of these strains were pathogenic on tomato fruit. All strains from aspen were INA positive, pathogenic on tomato fruit, and induced a HR. In contrast, the INA and HR percentages were identical for P. syringae strains isolated from lilac and dogwood (Fig. 3), while 85% and 70% of the lilac and dogwood strains, respectively, were pathogenic on tomato. For linden (94% INA) and magnolia (86% INA), 88% and 80% were HR positive, respectively. However, the percentage of these

strains pathogenic on tomato fruit was much lower (70% and 36%, respectively).

Whereas the percentage of strains exhibiting INA activity was higher than the percentage of strains producing either an HR or pathogenic reaction on tomato from strains isolated from lilac, linden, magnolia, dogwood and aspen, the reverse was true for strains from Japanese pear and red maple. For P. syringae strains from Japanese pear (30% INA), 57% of the strains induced an HR in tobacco and 50% were pathogenic on tomato. Only 4 of 47 strains isolated from red maple (24% INA) were P. syringae types, all four strains induced the HR in tobacco and three induced pathogenic changes in green tomato fruit.

Combining a positive response for either the HR or pathogenicity in tomato as a measure of potential pathogenicity, the range of variation between INA strains and pathogenic strains is more easily observed. In three hosts linden 100% INA, lilac 93% INA and dogwood 95% INA, the proportion of strains potentially pathogenic were similar ie. linden 93%, lilac 93% and dogwood 89%. This variation was greater in aspen 95% INA and 76% pathogenic, and saucer magnolia 75% INA and 88% pathogenic. This variation was greatest in maple and J. pear where 30 and 24% respectively were INA while 60% and 77% (respectively) of these strains were pathogenic in tomato fruit or induced a hypersensitive response in tobacco.

DISCUSSION

Strains of INA *P. syringae* were the predominant species of bacterial ice nucleators recovered from these infected hosts. Several ice nucleation active strains of fluorescent pseudomonads resembling *P. fluorescens* (oxidase and arginine dihydrolase positive) and an occasional non-fluorescent bacterial colony resembling *Erwinia herbicola* were isolated, but they were always a minor component of the INA species in a sample. This is consistent with observations by Lindow (22) and other researchers (11) on the distribution of INA bacteria in nature. Although *P. syringae* strains were recovered from blighted rhododendron blossoms, none were pathogenic in tomato fruit, induced a hypersensitive response in tobacco, or were INA.

Because the distribution of INA *P. syringae* was variable among hosts, it may be that the host plant influences whether a strain of *P. syringae* is an ice nucleator. The variability of occurrence of INA strains was particularly noticeable from the several hosts selected for this study. The low percentages of INA strains isolated from maple (Fig. 1) may have been due to an earlier sampling time than the other hosts. The maple strains were isolated in December and January (1981, 1982) while the rest were obtained from March to July 1982. However, the number of INA *P. syringae* strains isolated from Japanese pear at the later date was also low.

Along with the variation observed in the distribution of the INA characteristic, variations in the hypersensitivity response in

tobacco and pathogenicity to green tomato fruit were also observed among strains of P. syringae isolated from different hosts (Figure 2). Although the data suggests that the variability between strains relative to INA, HR, and pathogenicity traits may have been influenced by the host plant from which they were isolated, additional strains need to be isolated from these hosts at different times of the year before generalizing that different species of plants affect the phenotype of P. syringae.

Aspen was the only plant host where all P. syringae strains were INA and were also positive for both pathogenicity in tomato and hypersensitive response in tobacco. This finding is of interest to us because one of the two reports of frost episodes preceding symptom development in Pacific Northwest nurseries involved aspen. The propagation of aspen cultivars in some nurseries in the PNW has been reduced, due to the susceptibility of this species to P. syringae infection. The losses in rooted cuttings in the greenhouse and liners in the field due to P. syringae infections have made this species uneconomical to produce for many nursery operators.

Based on our findings we suggest that ice nucleation ability be considered a virulence factor which can contribute to pathogenesis, but does not confer pathogenicity by its presence. We also find that not all strains of P. syringae in the nursery environment are pathogens by our methods or ice nucleators at -5°C. Finally, the presence of potentially pathogenic strains of P. syringae was detected on the majority of woody plant species

tested. The predominance of INA strains within this population increases the risk of frost injury and disease occurring on plant material shipped to markets where conditions of temperature and humidity make episodes of light frost more likely.

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Distribution of INA Pseudomonas syringae isolated from infected woody nursery plants from Pacific Northwest Nurseries.

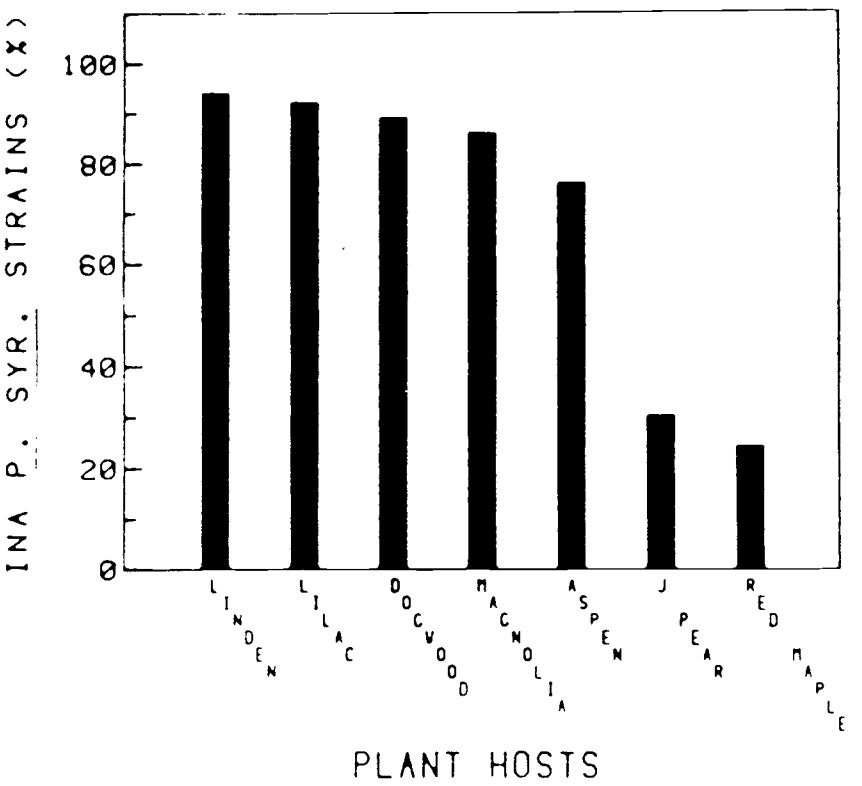

Figure 1. Proportion of INA P. syringae to total fluorescent pseudomonads tested. Number of strains tested per host include: Lilac-14, Linden-17, dogwood-18, aspen-21, magnolia-16, Japanese pear-47, and Red maple-17. Red maples sampled during December 1981 to January 1982, all other strains isolated from February 1982 to July 1982.

Association of INA phenotype in P. syringae strains isolated from woody plants with the ability to induce a hypersensitive response in tobacco and pathogenicity in green fruit of yellow pear tomato.

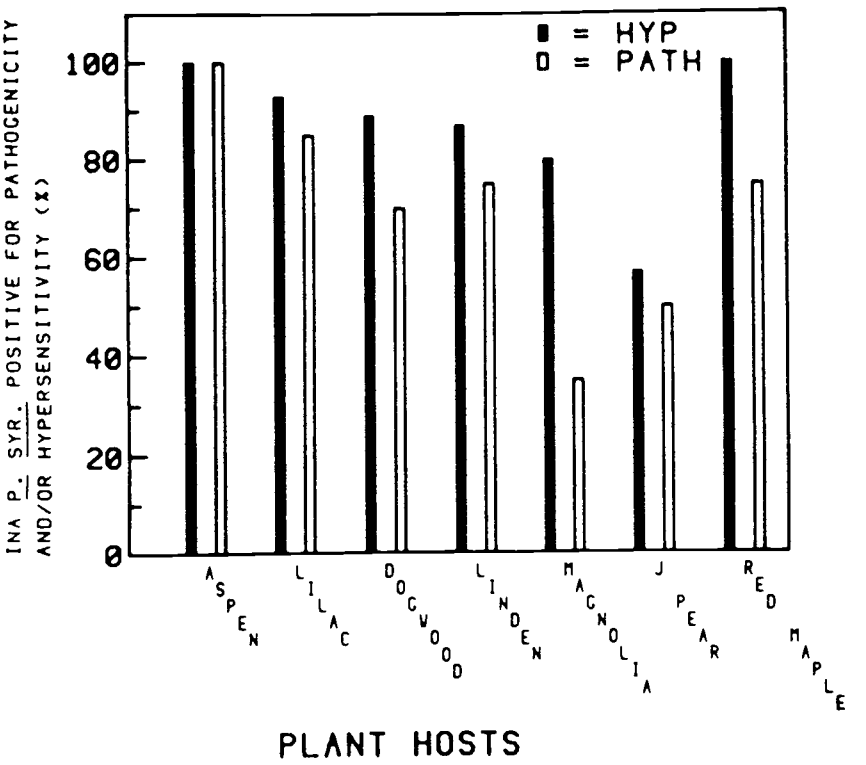

Figure 2. Percent of strains per host expressing either the hypersensitive response in tobacco or pathogenicity in green fruit of yellow pear tomato relative to the total number of fluorescent pseudomonads tested.

Comparison of P. syringae strains isolated from infected woody plants with ice nucleation ability and potential pathogenicity.

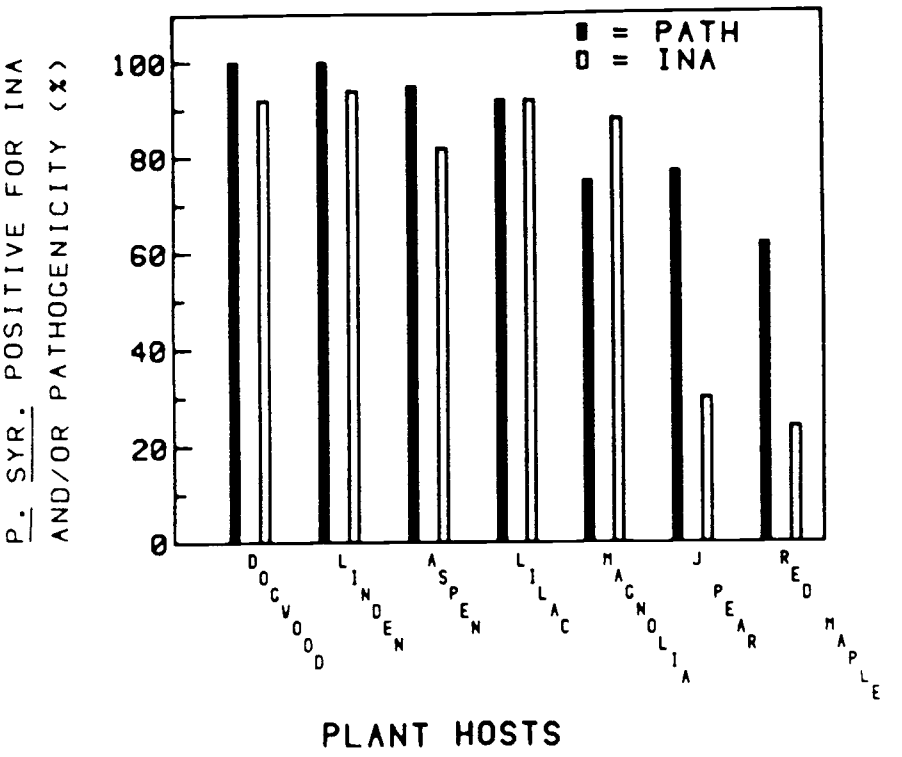

Figure 3. Combined index of pathogenicity (pathogenic and/or HR) versus INA. Percent of strains for each host testing positive for either trait relative to the total number of fluorescent pseudomonads tested.

CHAPTER 2

ICE NUCLEATION ACTIVE *PSEUDOMONAS SYRINGAE* ISOLATED FROM DISEASED AND SYMPTOMLESS GRASSES IN THE PACIFIC NORTHWEST

ABSTRACT

High populations of *Pseudomonas syringae* were isolated from naturally occurring grass species on the edge of nursery production areas and from sudan and cereal rye grass used as green manure and cover crops, respectively. Leaf samples obtained from diseased sudan and symptomless cereal rye grass and roadside grass species in November yielded large populations of *P. syringae* that were pathogenic on tomato fruit and ice nucleation active. Populations approaching 10^9 cfu/g of fresh tissue were isolated from sudan grass samples, whereas populations of 10^6 cfu/g were obtained from rye grass and roadside grass samples. Eighty-one randomly selected strains from these isolations were tested and fifty-eight of the 81 strains (72%) were similar to *P. syringae*. Of these 58 *P. syringae*, 34 (59%) were ice nucleation active at -5°C. Thirty-one of the *P. syringae* strains (54%) induced a hypersensitive response in tobacco leaves, and 29 (50%) were pathogenic on green fruit of yellow pear tomato. Several *P. syringae* strains from sudan grass and rye grass were pathogenic on greenhouse grown sudan grass seedlings; however, none of the strains tested were pathogenic on rye grass. Three of six *P. syringae* strains tested were also pathogenic on leaves of peach trees in the greenhouse.

INTRODUCTION

The nursery industry in Oregon is a major producer of woody ornamental and fruit trees in the United States, the majority of which are shipped to markets outside Oregon. During the past few years the industry has experienced an increase in both the incidence and severity of a bacterial infection affecting many deciduous woody plant species. Strains of Pseudomonas syringae were detected on 40 of 44 infected woody plant species surveyed, and more than 50% of these strains were pathogenic on green fruit of yellow pear tomato. Greater than 60% of these P. syringae were also ice nucleation active (INA) at -5°C (Baca et al. in press). However, not all INA strains were pathogenic on green tomato fruit. Conversely, not all of the pathogenic strains induced ice formation at this temperature.

Strains of INA P. syringae are widely distributed in nature, occuring on both herbaceous as well as deciduous plant species (22). No information is available, however, regarding where and how P. syringae overwinters in nurseries that produce woody plants. In some woody plants, populations of P. syringae are often undetectable on dormant tissues during the winter and early spring (Gross, Moore, unpublished). Burr, (2) however, reported that low populations of P. syringae could be isolated from dormant buds on some cultivars of apple trees but not from other cultivars. Furthermore, high populations of P. syringae can develop quickly after bud break (10).

Populations of P. syringae decreased to undetectable levels on most of seven woody plant species during the winter months in Oregon including samples of stem and bud tissue (Baca, Moore, unpublished data). Therefore, sources exterior to the woody plants might be involved as the primary source of inoculum in spring.

Several studies have demonstrated that P. syringae can exist as an epiphyte on weeds, grasses, apparently healthy plants, and on several non-host plants (19, 20). These sources are then possible reservoirs of P. syringae inoculum. Arsenijevic (1) showed that P. syringae isolated from sudan grass (Sorghum sudanense) in Yugoslavia could infect woody plants, and that strains of P. syringae isolated from woody hosts were pathogenic to sudan grass, however, this has not been documented in the United States.

Sudan grass is commonly planted as a green manure crop in many nurseries in the Pacific Northwest and then plowed under the soil in late October. Cereal rye grass (Secale cercales), a non-host of P. syringae, is subsequently planted to these fields and between rows of nursery stock as a winter cover crop. In some nurseries, strips of sudan grass are left unplowed between rows of nursery stock so machinery can be moved throughout the production area during the wet winter months.

Because sudan grass and cereal rye grass have been implicated as potential sources of P. syringae inoculum, and are currently used in the cultural management schemes of Pacific Northwest nurseries, we investigated i) the potential of these grass species for harboring P. syringae; ii) for the distribution of potential

pathogenic and ice nucleation active strains of P. syringae among these grasses, and iii) the cross-infectivity of selected grass strains to woody plant hosts.

MATERIALS AND METHODS

Selection and maintenance of bacterial strains. A total of 300 bacteria which fluoresced on King's Medium B (KB) were purified by repeated streaking on this medium until colonies from two successive streakings remained pure in colony morphology. Eighty-one working strains were selected at random from the 300, and were maintained at 4°C on slants of potato dextrose agar amended with 0.5% wt./vol. calcium carbonate ($CaCO_3$), a buffering agent, and 0.1% chloranthalonil to prevent accidental fungal contamination. Permanent collections of the P. syringae strains were prepared immediately to reduce the phenotypic variations we had observed during subculturing. Bacteria for permanent storage were grown on slants of KB medium for 48 hours at 24°C and suspended in 3 ml of sterile glycerol and distilled water solution (30% to 70% vol./vol.). Duplicate 1 ml aliquots of these suspensions were stored at -70°C.

Sampling procedures. Leaf samples of grasses from four nursery sites within the Willamette Valley of Western Oregon were collected during November 1983, placed in plastic bags and transported to the laboratory on ice. The samples were held at 4°C until processed. All samples were processed within 24 hrs by placing 1 g of fresh plant material into 9 ml sterile distilled water. Samples were allowed to soak for 30 min, vortexed for 15-30 seconds, and serially diluted. Aliquots (0.1 ml ea) were spread on two plates of King's Medium B and incubated for 48 hrs at 24°C before counting colonies that fluoresced under near ultra violet light (350 nm). Isolated colonies representative of all different colony

morphologies were selected at random for further characterization.

Biochemical and pathogenicity tests. Eighty-five strains from sudan, cereal rye, and roadside grasses along with four known strains of P. syringae were characterized by 1) LOPAT (Levan production, oxidase reaction, pectase, arginine dihydrolase, tobacco hypersensitivity) (29), and 2) pathogenicity to green fruit of yellow pear tomato (4). A cell suspension of approximately 10^6 cfu/ml of each strain was used as inoculum in pathogenicity studies on green tomato fruit of yellow pear tomato (Lycopersicon esculentum Mills), on young shoots of peach (Prunus persica), and on greenhouse grown seedlings of sudan (Sorghum sudanense) and cereal rye (Secale cercale). Inoculations to peach and grass seedlings were made by puncturing the plant leaves with a 23 gauge needle, a drop of the 10^6 cfu/ml suspension placed over the injury and the plants placed in an enclosed plastic tent with a relative humidity of greater than 80% for 7 days. Tomato fruit were injected with 0.1 ml of a 10^6 cfu/ml at three sites on each fruit and the inoculated fruits were incubated in a plastic covered crisper to maintain high relative humidity.

Ice nucleation activity. All strains were tested for ice nucleation activity at -5°C (22). A suspension of approximately 10^8 cfu/ml was used to test for ice nucleation activity by the freeze drop method of Vali as modified by Lindow (22). Activity was determined only at -5°C. The test for INA was recorded as positive if one of 10 drops of the bacterial suspension froze within 30 seconds.

RESULTS

Distribution of P. syringae on grass species in Oregon nurseries. Populations of fluorescent pseudomonads were isolated from all grass samples collected at four nursery sites in Western Oregon. Mean populations averaged greater than 10^6 cfu/mg fresh tissue in both the cereal rye grass and roadside grass samples, and exceeded 10^9 cfu/mg in standing fields of sudan grass (Fig. 1).

Biochemical and physiological tests. The 81 selected fluorescent pseudomonads varied from one another when characterized. For example, only 59/81 (73%) of these pseudomonads were identified as putative P. syringae by their negative reactions to oxidase and arginine dihydrolase. Fifty-nine percent of the strains isolated from sudan grass, 81% of the cereal rye strains, and 95% of the roadside grass strains were identified as putative P. syringae. Of the 24 sudan grass strains that resembled P. syringae in oxidase and arginine dihydrolase reactions, 92% were levan positive, 71% induced a hypersensitivity reaction in tobacco leaves and all were pectate negative. One strain from cereal rye grass was identical to the reference strains of P. syringae, except that it was arginine dihydrolase positive. Within the 21 strains isolated from cereal rye grass that resembled P. syringae in oxidase and arginine dihydrolase reactions, 71% were levan positive, and 71% induced a hypersensitive response in tobacco leaves. All strains were pectate negative. In the roadside grass strains the variations from the control strains was not as prominent. For instance, 95% of those strains resembling P. syringae were levan positive and all

of these strains induced a hypersensitive response in tobacco and were pectate negative.

Ice nucleation activity. Thirty-five of the 59 (59%) fluorescent pseudomonads identified as P. syringae were ice nucleators at -5°C, but the percentage of P. syringae strains from each grass species active as ice nuclei ranged from 42 to 72% (Table 2). For example, 15/24 of the sudan grass strains (63%) were active ice nuclei, 7/17 (42%) of the rye grass strains, and 13/18 (72%) of the strains from roadside grasses were active ice nucleators.

Pathogenicity of P. syringae strains. All 59 P. syringae strains were tested for the ability to induce water soaking and necrosis in immature green fruits of yellow pear tomato, however variations were observed between the grass species in the percent of strains with this ability. Of the P. syringae strains isolated from sudan grass, rye grass and roadside grass, 58, 47, and 61% respectively, produced disease in tomato fruit. Responses were similar to the known P. syringae strains included as controls. Infection of sterile water into control tomato fruit induced no changes from noninfected green fruit.

Two strains of P. syringae from each grass species were tested further for pathogenicity or cross-infectivity to sudan grass, rye grass and young leaves of peach trees in the greenhouse (Table 4). Both strains from sudan grass induced water-soaked symptoms which progressed to necrosis in both the sudan grass seedlings and peach leaves. Injections of sterile water as controls into peach leaves did not promote any necrotic changes. Only one strain from

rye grass was pathogenic to both sudan and peach leaves. Both roadside grass strains were pathogenic on sudan grass but were not tested on peach leaves. None of the strains from grasses induced necrotic changes in rye grass seedlings.

DISCUSSION

Pathogenic and ice nucleation active strains of P. syringae exist at high population levels on the cultivated grasses and on grasses bordering the nursery site, but the traits of pathogenicity and ice nucleation activity were variable among the strains. Strains of P. syringae isolated from diseased sudan grass were able to infect greenhouse grown sudan seedlings causing disease symptoms identical to symptoms observed in the field. Populations of total fluorescent bacteria from diseased sudan grass were 30 times higher than on the other two grasses. Disease symptoms were not evident on either rye or roadside grass species. Over 50% of the fluorescent pseudomonads from diseased sudan grass samples were of the P. syringae phenotype. In contrast, 83% and 93% of the fluorescent pseudomonads isolated from symptomless cereal rye and roadside grasses, respectively, were of the P. syringae type.

The use of sudan and cereal rye grass species as alternate green manure and cover crops within the nursery boundaries may act as reservoirs of inoculum of P. syringae. Likewise, the borders of grass around the nursery may contribute to the spread of P. syringae throughout the nursery and surrounding fields and both should be considered in the disease management program of nurseries producing woody nursery stock, and possibly herbaceous plants.

These findings suggest that P. syringae from these grass species might initiate diseases early in the spring. It has been shown by researchers like Gross, Moore and others that populations of P. syringae are low to undetectable on many woody plant species

during the extremes of winter and in some woody hosts during the hot days of summer. The near absence of P. syringae from these woody tissues during the winter followed by a rapid increase in the spring, may be due to the P. syringae being transferred from the adjacent grasses to the trees. Moreover, P. syringae may also exist in the interior of buds as demonstrated by Burr (2) and Cameron (3), however, this has been shown for relatively few woody plant species and was not observed in this study. In our sampling of twigs and dormant buds, the intact buds were cut and soaked to obtain interior as well as exterior bacterial colonizers. However, we could detect no indication that P. syringae was colonizing the interior of these buds.

Although selected strains of the P. syringae isolated from the three grass species studied incited pathogenic changes in greenhouse grown sudan grass as well as in young leaves of peach trees, none of the strains produced disease changes in greenhouse grown cereal rye grass. This is consistent with the findings of Lindemann (20) that rye grass is able to be colonized by P. syringae but is not susceptible to infections by P. syringae. Despite the nonsusceptibility of rye grass, it still harbors epiphytic populations of pathogenic/INA P. syringae which may serve as an inoculum source for adjacent woody plants. Of greatest import to the nursery industry is the apparent demonstration of cross-infectivity of these strains between grasses and woody hosts. Further work is needed to definitively establish that these P. syringae from herbaceous hosts can infect woody plants and vice-

versa. We are now testing the hypothesis that P. syringae is involved in a cycle of infection and re-infection between certain grass species cultivated in the nursery environment and adjacent woody plants.

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Table 1. Mean[a] population of *Pseudomonas syringae* per gram of fresh tissue from three grass species collected from Oregon nurseries during November 1983.

Host	# ob sites	Mean population cfu/g fresh tissue	Symptom
Sudan grass	3	$> 1.6 \times 10^9$	leaf spot
Cereal rye grass	3	5.6×10^6	asymptomatic
Roadside grass spp	3	5.4×10^4	asymptomatic

[a] Average population from 3 nursery sites.

[b] Plate count of fluorescent pseudomonads vs. non fluorescent bacterial from 10 fold dilution series of initial tissue samples.

Table 2. Characterization of fluorescent pseudomonads isolated from three grass species collected from 3 Pacific Northwest nurseries during November 1983.

Characteristics	Controls	Source of Strains		
LOPAT	Reference strains[a]	Sudan	C. Rye	Roadside
Number strains tested	4	24	17	18
Levan production	75%	92%	71%	95%
Oxidase (negative)	100%	100%	100%	100%
Pectate (negative)	100%	100%	100%	100%
Arginine dihydrolase (negative)	100%	100%	95%[b]	100%
Tobacco hypersensitivity (positive)	100%	71%	71%	95%

[a] Two *Pseudomonas syringae* strains were obtained from L. Moore and S. Sule.

[b] One strain arginine dihydrolase positive.

Table 3. Ice nucleation activity of Pseudomonas syringae strains isolated from three grass species in Pacific Northwest nurseries during November 1983.

Source of *P. syringae* strains	Number of strains tested	Ice nucleation activity (%)[a]	Total fluorescent pseudomonads (%)[b]
Reference strains[c]	4	100	100
Sudan grass	24	63	37
Cereal rye grass	17	42	33
Roadside grass spp[d]	18	72	68

[a] Calculated by INA/total *P. syringae* x 100.

[b] Calculated by INA/total fluorescent pseudomonads x 100.

[c] Received two strains *P. syringae* from L. Moore and S. Sule, respectively.

[d] Taxonomic identification of grass species inconclusive.

Table 4. Pathogenicity testing of P. syringae strains isolated from grass species in Oregon nurseries November 1983.

Pathogenicity tests	Reference	Source of strains: Sudan grass[a]	C. Rye grass[c]	Roadside grass spp.[e]	Sterile H_2O Controls
Green tomato fruit	100%	58%	47%	61%	-
Sudan grass seedlings	+[b]	+	+	+	-
Cereal rye grass seedlings	-[d]	-	+	NT[f]	-
Peach leaves	+	+	+	NT	-

[a] Four strains of P. syringae tested, two strains positive.

[b] (+) sign denotes at least one pathogenic event.

[c] Two strains of P. syringae tested, one strain positive.

[d] (-) sign denotes non-pathogenic.

[e] Two strains of P. syringae tested two strains positive.

[f] Not tested.

CONCLUSION

Significant progress has been made toward understanding the life cycle of Pseudomonas syringae and the association of this bacterial phytopathogen with many woody plant species in Pacific Northwest nurseries.

A survey of 44 diseased plant species isolated from over 30 nurseries showed that populations of P. syringae colonized most of the plant hosts tested, and that the distribution of P. syringae among these plants was variable (Canfield et al, in press). More than 50% of the fluorescent pseudomonads isolated and selected at random were P. syringae strains, which agrees with early studies on the distribution of P. syringae in nature by Lindow. However, Lindow's studies were concerned with epiphytic colonization and no determinations of pathogenicity of the isolated P. syringae were conducted.

Populations of P. syringae from seven different host varied in the percentages of strains active as ice nucleators at -5°C. Greater than 85% of the fluorescent pseudomonads isolated from Aspen, lilac, linden, dogwood, and magnolia spp. were active ice nucleators, whereas less than 30% of the P. syringae obtained from Japanese pear and maple sp. were active INA at this same temperature. It is interesting that the majority of P. syringae strains isolated from aspen and magnolia were active ice nucleators. These two plant species were the same species reported by nursery operators to be associated with frost induced disease symptoms. The predominance of the INA phenotype on these

susceptible plant hosts may explain in part the severity of disease expression and the frequency of occurrence of infection. It is not known why this sudden and widespread outbreak of disease occurred.

Ice nucleation activity and pathogenicity of randomly selected strains, as determined by induction of a hypersensitive response in tobacco and pathogenic changes in inoculated green fruit of tomato, bore no relationship to the host species from which they were obtained. Additionally, the pathogenic ability of a strain was not dependent on the ability of a strain to induce ice formation at -5°C. Many strains were isolated in which the ice nucleation ability was absent while they were able to incite pathogenic changes. Conversely, strains without pathogenic ability were isolated that were active ice nucleators. A few strains of P. syringae were neither pathogenic nor active ice nucleators.

It was observed in the seven plant families tested in greater detail that those hosts colonized by higher population of P. syringae ie. aspen, lilac, linden, dogwood, and magnolia, also contained relatively higher percentages of INA, and pathogenic P. syringae. This is contrasted by the lower percentage of INA P. syringae found on Japanese pear and red maple which were less colonized by fluorescent pseudomonads.

A survey of sudan grass and cereal rye grass plantings from four nursery sites as well as samples of grasses growing naturally around the perimeter of these nursery fields yielded large populations of fluorescent pseudomonads exceeding 1×10^9 cfu/g from sudan grass and 1×10^6 cfu/g from cereal rye and roadside

grass samples. Greater than 50% of these strains were P. syringae and of these P. syringae 50% or greater were pathogenic on tomato and/or ice nucleators at -5°C. These population levels and percentages were similar to those levels seen on woody hosts, however, these levels were obtained at a time when resident populations of P. syringae on the dormant woody tissues were declining in number or undetectable by plate counts. It is therefore possible that these grass strains may act as reservoirs of inoculum of P. syringae at a time when growth and survival of P. syringae on woody hosts is compromised by temperature and other environmental conditions.

Selected strains of P. syringae from these grass species induced pathogenic changes when infiltrated into sudan grass seedlings and when injected into young shoots and leaves of peach trees in the greenhouse, but no strains were pathogenic to cereal rye grass seedlings. However, cereal rye grass may still act as a reservoir of P. syringae because of the large populations obtained from samples of this grass. It is therefore hypothesized that a relationship may exist between P. syringae strains colonizing herbaceous grasses and weeds during the winter months and with woody plants in adjacent fields. These strains may subsequently infest and infect susceptible woody plants in close proximity to these grasses.

BIBLIOGRAPHY

1. Arsenijevic, M. 1973. Response of some herbaceous and woody plants to Pseudomonas syringae van Hall isolates of various origin. ISPP Working party on "Pseudomonas syringae" group. pp. 69066. Angers, France.

2. Burr, T. J., and B. H. Katz. 1984. Overwintering and distribution pattern of Pseudomonas syringae pv. populans and pv. syringae in apple buds. Plant Disease 68:383-385.

3. Cameron, H. R. 1962. Tech. Bull. Ore. Agric. Exp. Stn. 66, 64pp.

4. Cameron, H. R. 1970. Pseudomonas content of cherry trees. Phytopathology 60:1343-1346.

5. Canfield, M. L., S. Baca and L. W. Moore. 1985. Isolation of Pseudomonas syringae from 40 cultivars of diseased woody plants in Pacific Northwest nurseries. Plant Disease (in press).

6. Dowler, W. M. and D. J. Weaver. 1975. Isolation and characterization of fluorescent pseudomonads from apparently healthy peach trees. Phytopathology 65:233-236.

7. Endert, Elke and David F. Ritchie. 1984. Detection of pathogenicity, measurement of virulence, and determination of strain variation in Pseudomonas syringae pv. syringae. Plant Disease 68:677-680.

8. English, H. and J. R. Davis. 1960. The source of inoculum for bacterial canker and blast of stone fruit trees. (Abstr.) Phytopathology 50:634.

9. English, Harley, T. E. Devay, and J. M. Ogawa. 1980. Bacterial canker and blast of deciduous Fruits. Division of Ag. Science. University of California Leaflet 2155.

10. Gross, D. C. and R. A. Spotts. 1984. Pseudomonas--What can we do about it. Proceedings of the Oregon Horticultural Society, Volume 75:24-28.

11. Gross, D. C., Y. S. Cody., E. L. Proebsting, Jr., G. K. Radamaker and R. A. Spotts. 1983. Ecotypes and pathogenicity of ice nucleation-active Pseudomonas syringae isolated from deciduous fruit tree orchards. Phytopathology 74:241-248.

12. Hirano, S. S., E. A. Maher, A. Kelman and C. D. Upper. 1978. Ice nucleation activity of fluorescent plant pathogenic pseudomonads. p. 717-724. In Station de Pathologie Vegetale et Phytobacteriologie (ed.). Proc. 4th Int. Conf. Plant Path. Bact. Gilbert-Clarey, France.

13. Hirano, S. S., E. A. Maher, S. E. Lindow, A. Kelman, and C. D. Upper. 1978a. Phytopathology News 12(9), 176.

14. Klement, Z. 1963. Rapid detection of the pathogenicity of phytopathogenic pseudomonads. Nature (Lond.) 199:299-300.

15. Klement, Z. 1974. Apoplexy of apricots. III. Relationship of winter frost and the bacterial canker and die-back of apricots. Acta Phytopathol. Acad. Sci. Hung. 9:35-45.

16. Klement, Z., D. S. Rozsnyay and M. Arsenijevic. 1974. Apoplexy of apricots. II. Relationship of winter frost and the bacterial canker and dieback of apricots. Acta Phytopathology Acad. Sci. Hung. 9:35-45.

17. Lansade, J. A. 1946. Recherches sur le chancre de peuplier en France. Ann. Epiphyt. 12:23-31.

18. Latorre, B. A. and A. L. Jones. 1979. Pseudomonas morsprunorum, the cause of bacterial canker of sour cherry in Michigan, and its epiphytic association with P. syringae. Phytopathology 69:335-339.

19. Latorre, B. A., and A. L. Jones. 1979. Evaluation of weeds and plant refuse as potential sources of inoculum of Pseudomonas syringae in bacterial canker of cherry. Phytopathology 69:1122-1125.

20. Lindemann, J., H. A. Constantinidou, W. R. Barchet, and C. D. Upper. 1982. Plants as sources of airborne bacteria, including ice nucleation-active bacteria. Appl. Environ. Microbiol. 44:1059-1063.

21. Lindow, S. E. 1983. The role of bacterial ice nucleation in frost injury to plants. Ann. Rev. Phytopathology 21:363-84.

22. Lindow, S. E., D. C. Arny and C. D. Upper. 1978. Distribution of ice nucleation-active bacteria on plants in nature. Apl. Environm. Microbiol. 36:831-838.

23. Lindow, S. E., D. C. Arny and C. D. Upper. 1982. Bacterial ice nucleation: a factor in frost injury to plants. Plant Physiol. 70:1084-1089.

24. Maki, L. R. and K. J. Willoughby. 1978. Bacteria as biogenic sources of freezing nuclei. J. Applied. Meteorol. 17:1049-1053.

25. Panagopoulos, C. G. and J. E. Crosse. 1964. Frost injury as a predisposing factor in blossom blight of pear caused by Pseudomonas syringae van Hall. Nature (Lond.) 202:1352.

26. Paulin, J. P. and J. Luisetti. 1978. Proc. Int. Conf. Plant Path. Bact., 4th, 1978 Vol. 11, pp. 725-733.

27. Paulin, J. P. and Luisetti, J. 1978. Ice nucleation activity among phytopathogenic bacteria. pp. 725-731 in: Proc. 4th Int. Conf. Plant Pathogenic Bact., August 27-September 2, 1978. Station de Pathologie Vegetale et Phytobacteriologie, ed. Gilbert-Clarey, France, 978 pp.

28. Sabet, K. A. 1953. Studies on the bacterial dieback and canker disease of poplar III: Freezing in relation to the disease. Ann. Appl. Biol. 40:645-650.

29. Schaad, N. W. ed. 1980. Laboratory guide for identification of plant pathogenic bacteria. American Phytopathological Society. St. Paul, MN. 72 pp.

30. Weaver, D. J. 1978. Interaction of Pseudomonas syringae and freezing in bacterial canker on excised peach twigs. Phytopathology 68:1460-1463.

31. Weaver, D. J., C. F. Gonzales, and H. English. 1981. Ice nucleation by Pseudomonas syringae associated with canker production in peach. Phytopath. 71:109-112.

32. Zeller, V. W. and A. Schmidle. 1979. The effect of frost on the infection by Pseudomonas syringae von Hall on leaves of sour cherries (Prunus cerasus). Nachrichtenbl. Deut. Pflanzenschutzd. 31:97-99.