ETIOLOGY AND EPIDEMIOLOGY OF BACTERIAL BLIGHT OF RED RASPBERRY IN BRITISH COLUMBIA

by

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Abstract

Pseudomonas syringae van Hall was recovered from 31 of 32 samples of red raspberry tissue showing typical symptoms of bacterial blight. Of the 99 isolates recovered, 85 were physiologically-typical, P. syringae isolates, three did not produce the fluorescent pigment, six did not utilize lactate and five did not produce toxin as determined by the Geotrichum candidum bioassay. When a suspension of 10^7 CFU/ml was sprayed on the leaves of 6-week-old raspberry plants, 42 of 48 isolates caused necrosis within 4 days. No other bacterium recovered from the diseased raspberry tissue was pathogenic to raspberry in greenhouse tests.

P. syringae isolates remained viable and retained their toxin-producing ability when stored for one year on nutrient glycerol agar at $5^{\circ}C$. Identical isolates stored on nutrient agar at $5^{\circ}C$ or in sterile distilled water at room temperature either did not survive or lost their toxin-producing ability. There seemed to be a relationship between toxin-producing ability and an isolate's virulence as about 50% of toxin-producing isolates were rated pathogenic and 35% weakly pathogenic while none of the non-toxin-producing isolates were rated pathogenic and 60% were rated weakly pathogenic.

A scheme was devised for rapid identification of P. syringae from raspberry tissue. An isolate was determined to be P. syringae if it produced a distinctive raised mucoid colony on nutrient sucrose agar, produced a fluorescent pigment, was oxidase negative and reacted in drop agglutination

tests with an antiserum prepared against P. syringae. These tests could be done within three days. This scheme was used to study the overwintering site of P. syringae on raspberry. P. syringae was found to naturally populate 25-75% of raspberry buds during the winter months. Populations of 10^4 CFU/six bud sample were most common in the buds that contained P. syringae. During the spring and summer months, P. syringae survived as an epiphyte on raspberry leaves both in the field and in the greenhouse trials. P. syringae was also shown to cause brownish-red spots surrounded by yellow halos on the leaves of raspberry during the summer months.

About 90% of the P. syringae isolates from raspberry were ice nucleation active. Raspberry plants that had been sprayed with a suspension of 10^7 CFU/ml and then held at $-2^{o}C$ for 4 hours developed symptoms similar to those of bacterial blight within 12 hours of the freeze treatment.

In preliminary tests, raspberry cultivars showed varying degrees of resistance to P. syringae infection. Raspberry cultivar Chilcotin showed greatest resistance and cultivar Malling Leo showed greatest susceptibility to Pseudomonas syringae infection. Three different species of bacteria were found in the normal microflora of the raspberry that were antagonistic to P. syringae in vitro.

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INTRODUCTION

Bacterial blight of red raspberry, Rubus idaeus L. (hereafter referred to as raspberry) was first observed in British Columbia (B.C.) in the spring of 1964 in a commercial field in the Lower Mainland area (79). The symptoms of the disease were a sudden wilting and blackening of new shoots, cane tips and young laterals. In 1967, Pepin et al. (79) reported isolation of a bacterium from diseased tissue that would produce similar symptoms on raspberry under greenhouse conditions. The bacterium was identified as Pseudomonas syringae van Hall although it did not produce a fluorescent pigment or acid from glucose or sucrose, typical characteristics of P. syringae (32). By April of 1972, "...some blight was found in every one-year-old field examined in Matsqui, Abbotsford and Yarrow areas with serious damage evident in two fields" (68). The symptoms of the disease were most noticeable during April and May but were not evident during the summer months. They sometimes reappeared in the fall with the return of cool temperatures and increased rainfall. Disease occurence was erratic. It was reported to be widespread in 1968, 1970, 1972 and 1973 (12,14,68,70) but no damage was attributed to the disease in the other years since its discovery. There seemed to be a reduced amount of lateral bud emergence in raspberry plantings where blight had been seen. While a causal relationship has not been established, economic losses were attributed to the dead bud problem in 1968, 1972 and 1973 (13,15,17) the same years when blight was a problem.

Unfortunately, bacterial isolations were not done in the years since 1967 and all subsequent reports were based only on observation of symptoms. Monetary losses due solely to bacterial blight are therefore difficult to estimate

because the disease was never the only problem in any one growing season. However, in the 4 years when bacterial blight was reported to be widespread actual yields were well below estimated yields while actual yields approximated estimated yields in years when blight was not a problem (Table 1).

The objectives of this study, therefore, were:

1. to establish the causal organism of bacterial blight,
2. to investigate the sites inhabited by the bacterium during the winter and summer and establish a disease cycle,
3. to determine the relationship of bacterial blight to the dead bud syndrome, and
4. to study possible controls for the disease.

This thesis is divided into two parts. The etiology of the disease is examined in Part I and the epidemiology in Part II.

Table 1. Comparison of estimated versus actual yields of raspberry fruit from 1966-1973*

Year	Yield in kilograms	
	Estimated	Actual
1966	7.3×10^6	7,556,244
1967	7.8×10^6	7,685,463
+1968	5.9×10^6	5,595,446
1969	6.4×10^6	6,171,800
+1970	7.3×10^6	4,820,100
1971	4.3×10^6	4,636,364
+1972	6.4×10^6	4,970,455
+1973	6.4×10^6	5,000,000

*Information obtained from Horticulture Newsletters, British Columbia Department of Agriculture, Victoria, B.C.

+Years when bacterial blight was reported to be widespread

PART I

Etiology of Raspberry in B.C.

Literature Review

A bacterial blight of raspberry was first reported by Detmers in Ohio in 1891 (29). Laterals and flowers were infected by an unidentified bacterium. Detmers reported that he sent a sample to T.J. Burrill who identified the disease as "pear blight" noting that raspberry in Illinois was also infected by the disease. Pear blight, now commonly known as fireblight, was later found to be caused by Erwinia amylovora (Burrill) Winslow et al. (60).

In 1932, Lehman (59) reported that E. amylovora was responsible for necrosis of raspberry laterals in North Carolina. Infected leaves had dead margins bordered by brown, water-soaked tissues. White droplets of bacterial ooze were observed on necrotic tissues. Bacterial isolates recovered from these diseased plants were not pathogenic to apple, which is commonly infected by E. amylovora. Apple isolates of E. amylovora did not infect raspberry. The inability to cross-infect suggests that strains of E. amylovora exist which have different pathogenic capabilities on different hosts.

Starr et al. (94) studied an outbreak of fireblight in plantings of the raspberry cultivar (cv) Latham in Maine. The infection started in the flowers or leaves and spread downward into the stem or started in the stem and moved upward into the leaves or growing tips. Infected flowers and growing tips turned purple then became curved and necrotic. Petioles and veins of leaves turned purplish-black and adjacent leaf tissue became brown. Drops of bacterial ooze exuded from infected tissue. Blisters full of bacteria developed in the young bark. Bacteria isolated from infected tissue were identified as E. amylovora. As in the previous example, raspberry isolates of E. amylovora did not infect apple and apple isolates did not infect raspberry. Raspberry

plants, cv Newburg, and wild raspberry plants (Rubus strigosus Michx.) growing near the diseased Latham planting were not infected by this bacterium.

E. amylovora was implicated as the cause of fireblight on several cultivars of thornless blackberry (Rubus sp. hybrid) in Illinois in 1977 (82). The symptoms observed on blackberry were similar to those described for raspberry except that the blackberry fruits were also infected. Infected fruits became dry, brown, hard and remained attached to the pedicel. Different cultivars showed varying degrees of susceptibility to the disease. Neither apple nor pear plants could be infected with these blackberry strains. Apple and pear isolates of E. amylovora did not infect raspberry or blackberry plants.

Another bacterial blight of raspberry was reported from B.C. in 1964 (79). Within a few years the disease had become fairly widespread in the Lower Mainland area (68). Typical symptoms included brown water-soaked spots on leaves, petioles, internodes of young shoots and developing laterals. Spots enlarged and blackened. Brown streaks extended from the blackened tips into the vascular tissues. Entire laterals often blackened and died. Occasionally new growth was killed to ground level. In fields where blight was a problem an unusually high percentage of buds failed to open in the spring. The causal organism was identified as Pseudomonas syringae although the isolates recovered did not produce a fluorescent pigment and did not produce acid from glucose and sucrose, which are common characteristics of P. syringae.

Another report of a raspberry disease caused by P. syringae appeared in 1977 (80). In Yugoslavia, this bacterium was isolated from raspberry plants with halo-spotting disease. Typical symptoms were reported to be oily-appearing spots at the ends of veins which enlarged and became necrotic surrounded by yellow halos. Bacterial ooze was noted on the underside of

leaves below the spots. The morphological and biochemical characteristics reported were similar to those expected for P. syringae (32).

Except for the Yugoslavian report, the published descriptions of the symptoms of fireblight and bacterial blight of raspberry were very similar. Symptoms of both initially appeared as water-soaked lesions which became necrotic as the tissues dried out. Curling of infected shoot tips and laterals was characteristic of both diseases. However, neither the infection of flowers nor the production of bacterial ooze was reported for bacterial blight.

The similarity of symptoms caused in many hosts by P. syringae and E. amylovora has led to misidentification of the incitant of a disease in the past. In England, Billing et al. (9) found both P. syringae and E. amylovora on pear trees as incitants of pear blast and fireblight, respectively. These two diseases could not be distinguished on the basis of symptoms. Isolations were necessary to determine which organism was responsible for the symptoms observed. An epiphytotic of blossom and twig blight assumed to be fireblight on the basis of symptoms occurred in the Saanich Peninsula of Vancouver Island (64). Attempts to isolate E. amylovora from diseased trees failed and P. syringae was found instead. Similarly, fireblight had been reported as a common disease in Chile (19) but, when isolations were attempted, only P. syringae could be recovered from infected tissues. Subsequent surveys failed to detect E. amylovora anywhere in the country. In Alberta, both E. amylovora and P. syringae were recovered from blighted raspberry plants near Edmonton (44). P. syringae alone was isolated from diseased raspberry near Wainwright. Pathogenicity tests were not reported. The relationship of the two bacteria to the disease is currently being investigated.

The ideal isolation medium for determining the causal agent of bacterial blight of raspberry should allow for the growth of P. syringae and E. amylovora but inhibit the saprophytic microflora. Morphology of the colonies of these two genera should be sufficiently different so that each can be distinguished from the other. King's Medium B (KMB) has been used for the isolation of P. syringae (50). This medium enhances the production of the fluorescent pigment normally synthesized by this bacterium. Colonies of E. amylovora or a non-fluorescent isolate of P. syringae would not be distinctive on this medium. Also, it is difficult to distinguish fluorescent P. syringae colonies from the other fluorescent pseudomonads, commonly found as saprophytes on plant tissues (56). Consequently King's Medium B would not be a suitable selective medium in this study.

Moustafa et al. (67) described a partially selective medium for P. syringae. The medium contained proline which enhances fluorescence, and manganese sulfate which is toxic to non-pathogenic bacteria associated with infected tissue. Recovery rates for P. syringae on this medium were not reported nor was the growth of Erwinia species tested.

Miller and Schroth (66) developed a complex medium which was selective for Erwinia species but which permitted only slow growth of 12 species of Pseudomonas. Subsequently Cuppels and Kelman (28) showed that the medium became increasingly toxic to all bacteria as it aged. Because the medium is time-consuming to prepare, has to be used immediately and does not allow for growth of Pseudomonas species it would not be a desirable medium to use in this case.

Kado and Heskett (47) proposed several media for the isolation of plant pathogenic bacteria. A recovery rate of 77% for E. amylovora was attained on their D3 medium compared to recovery rates on a standard medium. No pseudomonads grew on this medium. Their D4 medium was reported to be absolutely selective for P. syringae but only a 6% recovery rate was attained. All other bacteria were inhibited on this medium. Otta (73) found that in liquid form the D4 medium could be used as an enrichment medium. Tissue to be sampled was homogenized, put in the medium, and incubated for 24 hours. P. syringae, if present, would multiply and was detected by streaking some of the broth culture onto KMB. This medium would be useful if the natural populations of P. syringae were so low compared to the saprophytic population that enrichment would be required prior to plating. Neither D3 nor D4 medium allowed the growth of both P. syringae and E. amylovora.

Nutrient sucrose agar (NSA) has been used by Garrett et al. (41) for the isolation of P. syringae from fruit trees. The medium contained 5% sucrose which is utilized by both P. syringae and E. amylovora to produce levan. Levan production results in the formation of distinctive raised mucoid colonies by both bacteria. The addition of crystal violet (CVSA) made the medium more selective as gram-positive plant saprophytes are inhibited (25). This medium has been used by several workers for isolation of P. syringae (36,42).

Crosse and Goodman (27) found that by increasing the sucrose concentration to 40% a greater percentage of the background microflora was reduced and after 60 hours of incubation, pits formed on the surface of the E. amylovora colonies. They did not report the characteristics of P. syringae on the medium.

The best medium for isolation of bacteria from raspberry tissues would probably be NSA. Both E. amylovora and P. syringae form distinctive colonies on this medium. The addition of more sucrose, manganese sulfate or crystal violet might also prove beneficial as all have been reported to reduce numbers of other bacteria without interfering with the growth of the suspected incitants.

Once a levan-forming colony has been isolated, its identification requires several tests. To identify an isolate as E. amylovora the following tests are usually done: check for formation of distinctive colony type on Miller-Schroth sorbitol medium (MSS) (66), presence of peritrichous flagella (65), absence of polypectate gel pitting (45), production of a hypersensitive reaction in tobacco (51).

Identification of a bacterial isolate as P. syringae is complicated by differing concepts of what constitutes the species. The dilemma involving the separation of the fluorescent pseudomonads into distinct species was summarized by Lelliot et al. (61): "The fluorescent pseudomonads are divided into some 70 species whose pathogenicity on different hosts appears to be the only grounds for maintaining them as separate species. The method of classification generally used has little predictive merit taxonomically, it is cumbersome and often impracticable in diagnostic work." These workers proposed a determinative scheme to divide the phytopathogenic fluorescent pseudomonads into five groups based on the isolate's oxidase reaction, ability to rot potato, presence of arginine dihydrolase, levan formation from sucrose and production of a hypersensitive reaction in tobacco. P. syringae gave negative reactions for the first three tests and positive results for the last two.

Sands, Schroth and Hildebrand (87) divided the phytopathogenic pseudomonads into four groups with Groups I and II containing the fluorescent species. Group I included pseudomonads that do not possess arginine dihydrolase, do produce a hypersensitive reaction in tobacco and do produce a fluorescent pigment. Of 62 nomenspecies that fell in this category the authors proposed that the group be divided into two species, oxidase positive strains designated as Pseudomonas cichorii (Swingle) Stapp and oxidase negative strains as P. syringae, and that different strains from different hosts be designated as pathotypes until their status could be determined. Hildebrand and Schroth (45) later revised their original scheme and recognized 11 different species of oxidase negative phytopathogenic, fluorescent pseudomonads. An isolate is considered P. syringae if it is oxidase negative, does not pit polypectate gel medium and does utilize lactate.

Doudoroff and Palleroni (60) establish five criteria for identification. To be P. syringae an isolate must neither accumulate poly-ß-hydroxybutyrate as an intracellular carbon reserve nor possess arginine dihydrolase. It must produce a fluorescent pigment, be pathogenic, and be oxidase negative. In this treatment, 42 nomenspecies have been "...provisionally included in this species [P. syringae] which may be synonyms, biotypes, pathotypes, or varieties and some of which may even deserve independent specific rank".

Dye et al. (35), however, found this treatment "inadequate from a plant pathologist's viewpoint because it does not provide a nomenclature that expresses the phytopathogenic individuality of these bacteria". They proposed that 18 of 42 nomenspecies lumped into P. syringae by Doudoroff and Palleroni be retained as species pending further research and the remaining 24

nomenspecies be discarded because the species names are "illegitimate, synonyms of an accepted species, represent a culture that has obviously been misidentified or represents a culture that is not found in a recognized culture collection".

In order to satisfy all of the schemes, 10 different tests must be done to a bacterial culture to identify it as P. syringae (Table 2). This is further complicated by the variability of results for some tests. Isolates that do not produce a fluorescent pigment have been reported (79, J.E. DeVay, personal communication). Sands et al. (87) found only 8 of 12 P. syringae isolates that they examined utilized lactate. About 10% of P. syringae isolates tested by Doudoroff and Palleroni (60) did not produce levan when grown on NSA. Also, the similarity of P. syringae to E. amylovora in these tests (Table 2) emphasizes the importance of having a selective medium which permits distinguishing these species.

Another consideration of any etiological study is the development of a reliable method for testing pathogenicity of an isolate. Bacteria must be introduced into plant tissues in some way to cause symptoms. Kiraly et al. (51) pointed out that concentrations used are critical. If too few bacteria are inoculated into the plant, no symptoms result even if the bacteria are pathogenic. If too many bacteria are used, hypersensitive reactions often occur which are easily confused with disease symptoms.

To induce shoot dieback they suggested pricking stems of plants with a needle and rubbing the wound with a suspension of 5×10^6 bacterial cells/ml or injection of a similar suspension into shoots. To simulate leaf spot diseases they suggested spraying the undersurface of leaves with either a

Table 2. Typical reactions of Pseudomonas syringae and Erwinia amylovora to the biochemical tests normally used for identification of P. syringae

TESTS	Pseudomonas syringae	Erwinia amylovora
Intracellular accumulation of poly-β-hydroxybutyrate	-	not reported
Oxidase reaction	-	-
Tobacco hypersensitivity	+	+
Levan production from sucrose	+	+
Fluorescent pigment production	+	-
Ability to pit polypectate gels	-	-
Arginine dihydrolase	-	-
Lactate utilization	+	-
Ability to rot potato	-	-
Pathogenic to raspberry	+	variable

suspension of greater than 5 x 10^6 cells/ml from a low pressure sprayer or a suspension of less than 5 x 10^6 cells/ml from an atomizer providing a pressure of 1.5 kg/cm^2.

The ability of P. syringae to cause disease has been associated with the production of the antibiotic syringomycin by this organism. Backman and DeVay (3) isolated syringomycin from P. syringae grown on potato dextrose broth (PDB) containing 4% casamino acids and found that it caused damage to peach leaves similar to the symptoms produced when live bacterial suspensions of P. syringae were sprayed on peach leaves. Gross and DeVay (42) found that the toxin could reproduce the symptoms of holcus spot disease of maize caused by P. syringae.

A simple bioassay was developed to check for toxin production (30). An isolate was spotted in the center of a plate of PDA with 4% casamino acids, allowed to incubate for 6 days and then sprayed with a suspension of Geotrichum candidum Pers. ex Links. A zone of inhibition surrounding the bacteria indicated toxin production. DeVay et al. (30) consistently found P. syringae isolates to produce this toxin. They used this test as one of their criteria for identification of an isolate as P. syringae. However, isolates that do not produce toxin but are identical to P. syringae in all other biochemical tests and are pathogenic to peach seedlings have been reported (4,74) so this test may not be absolutely reliable for determination of pathogenicity. If pathogenic B.C. isolates produce this toxin and if this toxin can be shown to be responsible for symptom production it may be unnecessary to conduct greenhouse pathogenicity tests. The simple bioassay only may be required to determine an isolate's pathogenic capabilities.

Maintenance of a bacterial isolate in a stable, pathogenic condition is essential to any long term study. P. syringae cultures have been suspected of losing their virulence in storage by several workers (80, H.S. Pepin, personal communication). Various storage methods have been employed. P. syringae has commonly been stored on nutrient agar (NA) (72,79) or nutrient glycerol agar (NGA) (36,41). Lyophilization has been used for long term storage (31). No data on virulence of isolates stored in these ways was reported.

DeVay and Schnathorst (31) reported successful storage of P. syringae in sterile glass distilled water at 10°C. After 20 months in storage no loss of viability or pathogenicity to peach was noted. Storage of isolates on KMB was discouraged by Otta (72) because he found that "wild type" smooth colony cultures converted more rapidly to atypical rough colony forms on this medium than on NA or NA with 1% dextrose.

The storage of E. amylovora has not posed the same problem. Viable, virulent cultures have been maintained on modified Emerson's medium for over 2 years (81).

In summary, the incitant of bacterial blight of raspberry in B.C. has not been absolutely established. The symptoms of the disease are very similar to those described for fireblight of raspberry caused by E. amylovora. The pathogenic bacterium isolated from diseased raspberry plants in B.C. was identified as P. syringae although it differed from typical strains of this species in several important aspects. The organism gave results to biochemical tests similar to those expected for E. amylovora. The purpose of this study was, therefore, to establish what the incitant of bacterial blight

of raspberry in B.C. is. Thus the objectives were:

1. to find an isolation medium suitable for this study,
2. to establish criteria for identification of the incitant to species,
3. to develop a reliable technique for testing pathogenicity of a suspected pathogen, and
4. to determine a method for maintaining a bacterial culture in a pathogenic condition for long periods of time.

MATERIALS AND METHODS

SOURCE AND STORAGE OF CULTURES

All cultures used in this study that were received from other sources are listed in Table 3. Cultures were routinely stored on nutrient glycerol agar NGA (41) at $4^{o}C$.

SELECTIVE MEDIA EVALUATION

P. syringae isolate Ps-1 was inoculated onto KMB plates. After 48 hours cells were taken up in sterile distilled water (SDW) and the concentration of bacteria adjusted to 5×10^{9} colony forming units (CFU)/ml. Aliquots of serial dilutions of this suspension were plated on each of the following: (1) NSA (41), (2) NSA containing 6% crystal violet (CVSA) (25), (3) CVSA with .25% manganese sulfate (MCVSA) as prepared by Ercolani et al. (36), (4) Miller and Schroth's sorbitol Erwinia medium (MSS) (66), and (5) Crosse and Goodman's 40% sucrose medium (CGSA) (27). Numbers and characteristics of colonies growing on each of the five media were noted after 3 days. This experiment was repeated on three separate occasions. A suspension of E. amylovora isolate, Ea-1, grown on NA, was similarly prepared and dilution-plated on NSA, CVSA, CGSA and MSS. This experiment was repeated twice.

To determine the selectivity of the media, six dormant raspberry buds that had been sprayed with a suspension of 10^{8} CFU/ml of P. syringae were ground in 3 ml of SDW using a sterile mortar and pestle. The resulting solution was dilution plated on NSA, CVSA and CGSM. Types and numbers of each type of bacteria growing on each medium were recorded.

Table 3. Authentic cultures received from other sources

Number	Species	Host	Source	Source No.
Ps-1	*P. syringae*	Lilac	D.J. Hagedorn, Madison, Wisconsin	N.G.*
Ps-2	*P. syringae*	Forsythia	R.J. Copeman, Vancouver, B.C.	N.G.
Ps-3	*P. syringae*	Forsythia	R.J. Copeman, Vancouver, B.C.	N.G.
Ps-4	*P. syringae*	Forsythia	R.J. Copeman, Vancouver, B.C.	N.G.
Ps-5	*P. syringae*	Lima bean	D.J. Hagedorn, Madison, Wisconsin	N.G.
Ps-6	*P. syringae*	Pear	M.P. Starr, Davis, California	ICPB PS-269
Ps-7	*P. syringae*	Bean	D.J. Hagedorn, Madison, Wisconsin	N.G.
Ps-8	*P. syringae*	Almond	J.E. DeVay, Davis, California	B-15+
Ps-9	*P. syringae*	Pear	J.E. DeVay, Davis, California	B-301
Ps-10	*P. syringae*	Peach	J.E. DeVay, Davis, California	B-3A
Ps-11	*P. syringae*	Unknown	J.E. DeVay, Davis, California	P-359
Ps-12	*P. syringae*	Almond	J.E. DeVay, Davis, California	B-15
Ps-13	*P. syringae*	Peach	J.E. DeVay, Davis, California	B-3A
Ea-1	*E. amylovora*	Pear	W.G. Bonn, Harrow, Ontario	E2017P
Ec-1	*E. carotovora* var *carotovora*	Potato	R.J. Copeman, Vancouver, B.C.	755
Pp-1	*P. phaseolicola*	Bean	D.J. Hagedorn, Madison, Wisconsin	N.G.
Pm-1	*P. marginalis*	Unknown	A. Kelman, Madison, Wisconsin	PM6

*Source number not given

Otta's modification (73) of Kado and Heskett's D4 medium was tested as an enrichment medium. One ml of a 10^6 CFU/ml suspension of isolate Ps-1 or the ground inoculated raspberry bud suspension was dispensed into 9 ml of liquid D4 medium, incubated for 24 hours and dilution-plated on NSA. The raspberry bud suspension was also dilution-plated immediately on NSA. After 2 days on NSA, numbers of each different colony type were recorded.

ISOLATION AND GROUPING OF BACTERIA RECOVERED FROM DISEASED RASPBERRY

In the spring and summer of 1976, raspberry plants showing symptoms associated with bacterial blight from the plots at the Vancouver Research Station's Substation at Clearbrook (hereafter referred to as the Clearbrook Substation) were sampled. Tissues from 31 different cultivars or selections were collected from May 7 to July 18 on 11 different sampling dates. Approximately 0.5 g of tissue from the healthy/necrotic interface was crushed in 3 ml SDW using a sterile mortar and pestle. This suspension was then serially diluted and plated on NSA. After 48 hours representatives of every colony type present on the plates were restreaked on NSA to insure purity. After 48 hours incubation, colony morphology was noted and isolates were streaked onto KMB. The oxidase reaction (45) and fluorescent pigment production checked under ultraviolet light were done after 24 hours. All isolates were also tested for toxin production using the Geotrichum candidum bioassay (30). Cells of the bacterium to be tested were transferred to the center of a petri dish of potato dextrose agar (PDA) containing 4% casamino acids. After 6 days incubation, G. candidum was sprayed onto the plate. Twenty-four hours later the clear zone of inhibition from the edge of the bacterial growth to the

start of the fungal growth was measured. Isolates were put into groups based on their colony type and reactions to these tests.

Isolates having characteristics similar to E. amylovora (non-fluorescent on KMB, mucoid colonies on NSA, and oxidase negative) were streaked onto CGSA. Colonies were checked after 48 and 64 hours for the distinctive pits which form on the surface of E. amylovora and P. syringae isolates.

PATHOGENICITY TESTS

Raspberry plants of the cv Willamette were grown from root cuttings in the greenhouse, fertilized every ten days with 20-20-20, and used for pathogenicity tests when the plants were 6 to 10 weeks old. Representative isolates from each of the groups were tested for pathogenicity. The isolates to be tested were grown for 48 hours on KMB. For stem prick inoculations the stem of a raspberry plant was pricked with a sterile needle near the growing tip. Some of the bacteria from the KMB plate were smeared in the wound. Inoculated plants were placed in a mist chamber in the greenhouse at 16-23^{o}C. The plants were checked after 10 and 14 days for systemic necrosis spreading from the point of inoculation. For leaf spray inoculations, bacteria from a 48-hour KMB plate were suspended in SDW and adjusted spectrophotometrically to 10^{7} CFU/ml. The suspension was sprayed from a hand sprayer onto the underside of the leaves. Sterile water was sprayed on similar plants as controls. The tissue appeared water-soaked after spraying. The plants were placed in a mist chamber as described for the stem prick inoculations and checked for necrosis spreading from the sprayed areas after 4 and 7 days. Diseased tissues from several plants showing symptoms were ground in SDW and dilution-plated on NSA

in an attempt to reisolate the bacterium that had been inoculated into the plants.

IDENTIFICATION OF ISOLATES IN PATHOGENIC GROUPS

Isolates in the pathogenic groups were tested for the following characteristics: Gram reaction as recommended by Bradbury (10), production of hypersensitive reaction in tobacco, Nicotiana tabacum L. (51), utilization of lactate (85), ability to pit polypectate gel medium (45), presence of arginine dihydrolase tested using Thornley's 2A medium (95). These tests were also done on the known cultures listed in Table 3.

SURVEY OF BACTERIAL BLIGHT IN COMMERCIAL FIELDS

In the summer of 1976, raspberry tissues showing typical blight symptoms were taken from the Dyck and Hoogie farms, two commercial raspberry plantings near Clearbrook, B.C. Necrotic tissues from raspberry plants showing atypical symptoms were also taken from the Maddocks farm near Richmond, B.C. and the Reynolds farm on Westham Island, B.C. The tissues were ground and plated on NSA as described. Raised, mucoid colonies were selected and tested to see if they were P. syringae.

STORAGE OF BACTERIA

The bacteria isolated from diseased raspberry and identified as P. syringae were stored in each of the following ways: (1) on NA at $5^{o}C$, (2) on NGA at $5^{o}C$, and (3) as a turbid suspension in SDW at room temperature ($23-30^{o}C$). After 1 year, isolates were streaked onto NSA to check for survival. Each

isolate was also tested for toxin production using the G. candidum bioassay. Cultures of representative isolates Ps-205 and Ps-346, stored 1 year on NGA and in water, were tested for pathogenicity using the leaf spray inoculation method previously described.

Two raspberry isolates, Ps-346 and Ps-248, stored on NGA for 1 year were put into water storage. After 2 and 3 months storage, 0.1 ml of these water stocks was dilution-plated on NSA. Twelve single colonies of each culture were selected and tested in the G. candidum bioassay to determine what percentage of the bacteria in water retained the ability to produce toxin. Six of these single colony cultures that had lost their toxin-producing ability after 3 months storage in water were transferred to an NGA slant and tested after 1 month to determine if reversion to a toxin-producing form would occur.

RESULTS

SELECTIVE MEDIA EVALUATION

Colony descriptions and the average number of bacteria recovered from a 5 x 10^9 suspension of bacteria on each medium were recorded (Table 4). Using recovery of bacteria on NSA as a standard, recovery rates were found to be highest on CVSA and CGSA for both species. On NSA E. amylovora colonies appeared slimy and had clear margins while P. syringae colonies appeared dry and the margins were not clear (Fig. 1). The colonies are difficult to distinguish in these photographs but are more easily differentiated on the plates. On CVSA the two species are indistinguishable (Fig. 2). The addition of 0.25% manganese sulfate to CVSA caused a precipitate to form even if the manganese sulfate was sterilized separately and then added to the agar component just before pouring into plates. For this reason, growth of E. amylovora on this medium was not tested. No bacteria grew on the MSS medium. E. amylovora colonies were smaller than those of P. syringae on CGSA. Also pits formed on the surface of E. amylovora colonies after 48 hours on this medium. Pits formed on the surface of P. syringae colonies but not until after 60 hours of incubation.

Several morphologically different types of bacteria were recovered from the dilutions of the ground bud suspensions on both CVSA and NSA (Fig. 3). The growth of a slimy, yellow-pigmented bacterium, commonly recovered from raspberry tissue, was not inhibited on any of the media tested.

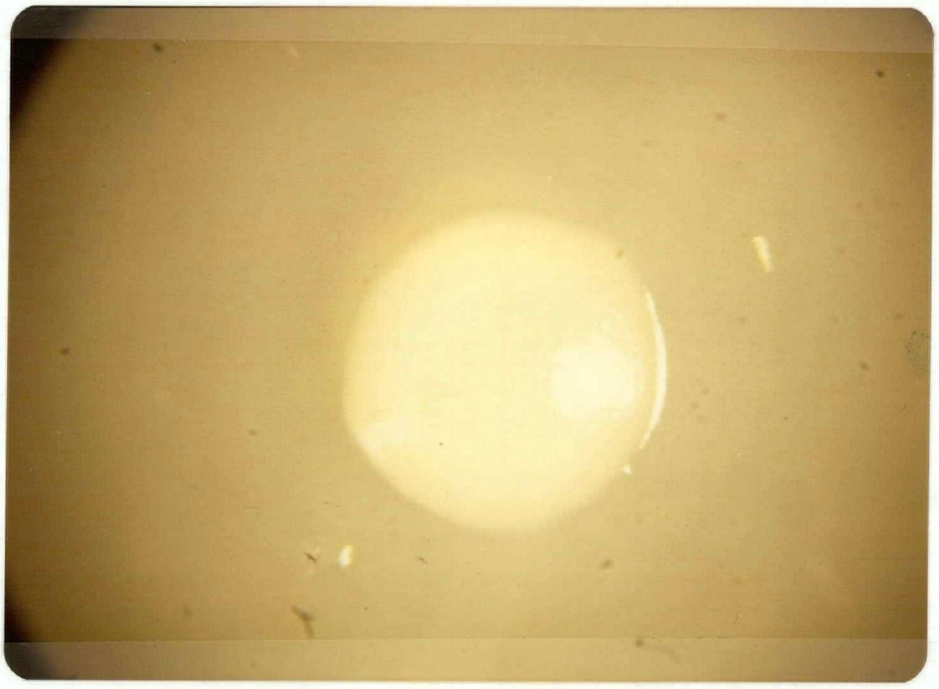
P. syringae was easily recovered on NSA when it was first grown alone in liquid D4 medium. However, when liquid D4 medium was inoculated with the

Table 4. Recovery rates for Pseudomonas syringae and Erwinia amylovora on selective media after 72 hrs incubation

Media	Colony Description	Number of Bacteria Recovered*	Recovery Rate
P. syringae			
NSA (standard)	raised, greenish-white opaque, mucoid, dry, 4 mm	50	100%
MVSA	raised, purple, opaque, mucoid, 4 mm	8	16%
CVSA	raised, purple, opaque, mucoid, 4 mm	20	40%
CGSA	raised, light purple, clear, mucoid, pits on surface after 60 hrs.	20	40%
MSS	no growth	0	0%
E. amylovora			
NSA (standard)	raised, white, opaque center with clear margin, mucoid, slimy, 5 mm	50	100%
CVSA	raised, purple, mucoid, 5 mm	30	60%
CGSA	raised, light purple, mucoid, clear 1-2 mm, pits on surface after 48 hrs.	30	60%
MSS	no growth	0	0%

*Number of bacteria recovered from a 10^{-8} dilution of a 5 x 10^{9} suspension of the bacterium being tested. Each number represents the average on three replicate plates in three experiments for P. syringae and in two experiments for E. amylovora.

a



b

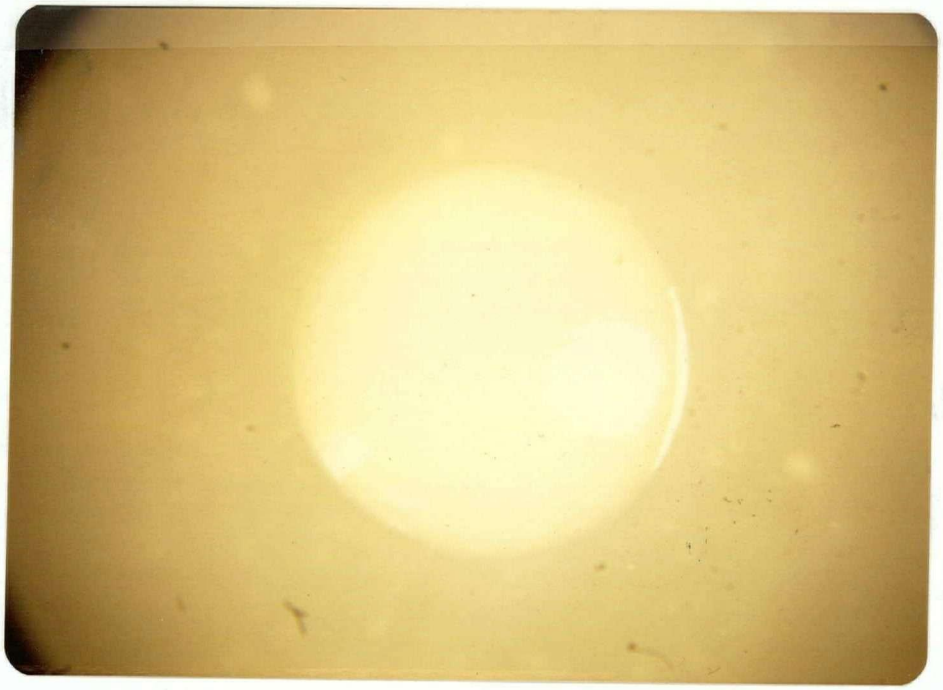


Fig. 1 Comparison of Pseudomonas syringae colony (a) to Erwinia amylovora colony (b) on NSA (6 x magnification)

a

b

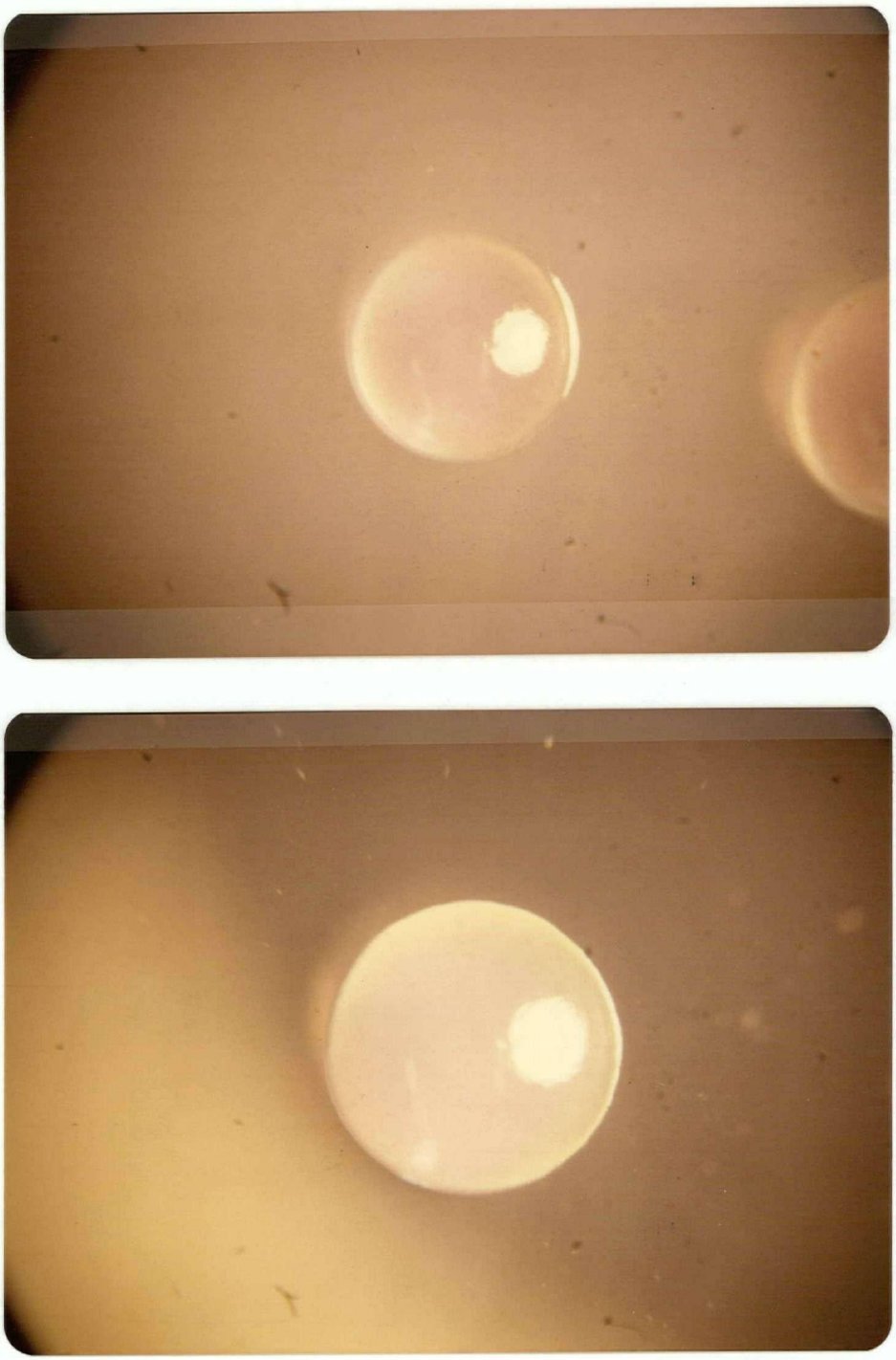


Fig. 2 Comparison of Pseudomonas syringae colony (a) with Erwinia amylovora colony (b) both grown on CVSA (6 x magnification).

a

b

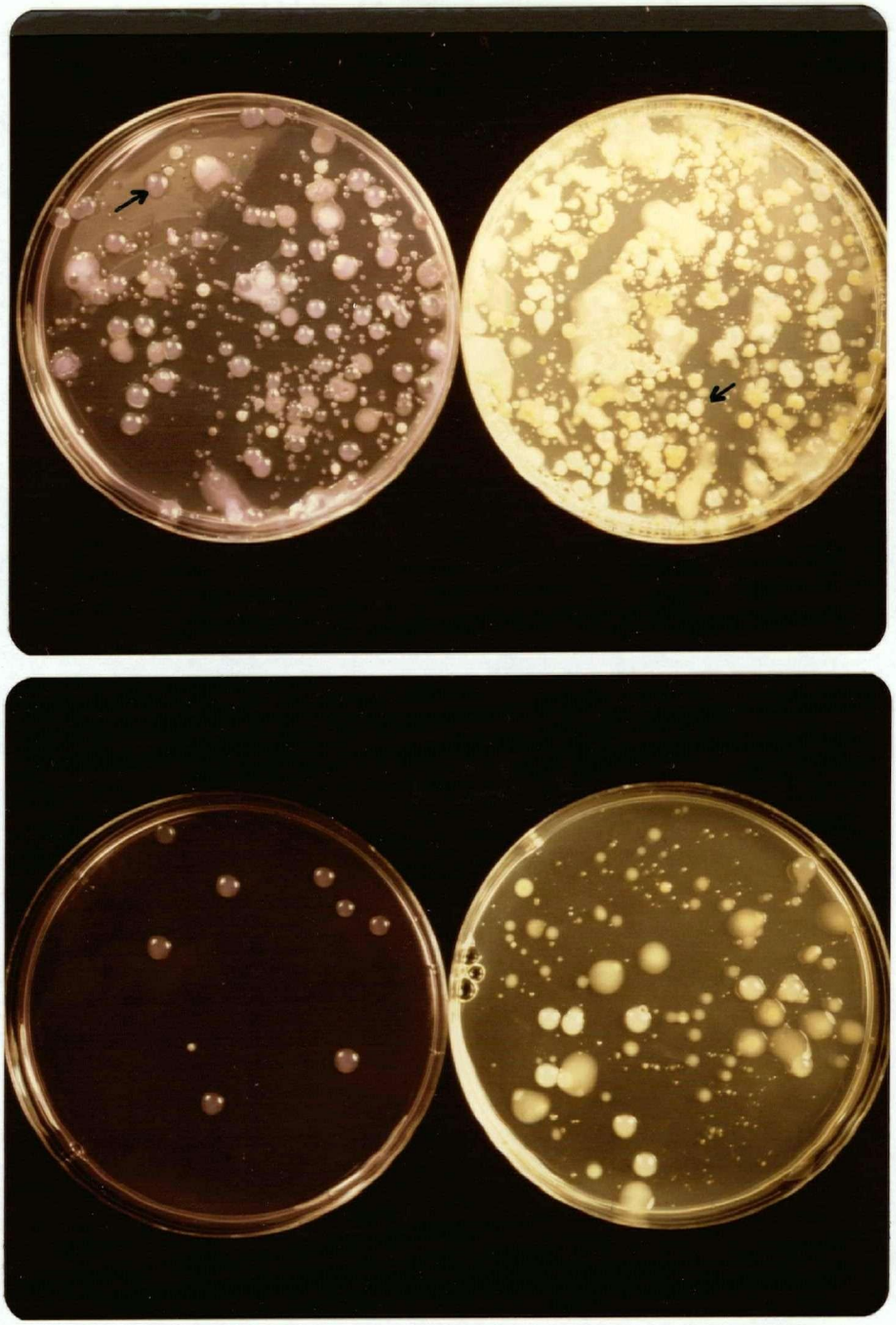


Fig. 3 Recovery of Pseudomonas syringae colonies (arrows) on CVSA (left) and NSA (right) from ground raspberry bud suspensions. Saprophytes were commonly present in these isolations (a) while pure cultures of P. syringae on CVSA were rarely seen (b).

ground raspberry bud suspension known to contain P. syringae, incubated for 24 hours and 1 ml of this medium dilution-plated on NSA, no P. syringae was recovered because the slimy, yellow-pigmented bacterium completely overgrew any other bacteria on the plates.

GROUPING OF BACTERIA RECOVERED FROM DISEASED RASPBERRY TISSUE

From the diseased raspberry samples, 174 bacteria were isolated. These were divided into eight types based on colony morphology on NSA, oxidase reaction, fluorescent pigment production and G. candidum inhibition (Table 5). Most of the bacteria recovered fell into Type I. An example of infected tissue from which Type I isolates were recovered is shown in Fig. 4. This sample shows symptoms typical of those associated with bacterial blight. Bacteria in Types IV and V had characteristics similar to E. amylovora. When these isolates were streaked onto CGSM, Type V isolates did not grow. Type IV isolates grew on this medium and pits formed on the surface of the colonies when they were 64 hours old.

PATHOGENICITY TESTS

Most isolates in Type I were pathogenic to raspberry in greenhouse tests (Table 6). Stem-prick inoculations with these isolates resulted in vascular blackening within 10 days that spread away from the point of inoculation (Fig. 5). Leaf spray inoculations caused necrosis of tissue directly hit by the spray and adjacent tissue. Often a yellow halo would form around the necrotic area (Fig. 5). These symptoms usually developed within 4 days. Isolates of Type II were either weakly or non-pathogenic. No other bacterium in the other types was pathogenic to raspberry. Bacteria, which formed raised, mucoid

Table 5. Types of bacteria isolated from blighted raspberry

Type	Colony Description on NSA	Fluorescent Pigment Production	Oxidase Reaction	*Geotrichum candidum* inhibition	Number isolated in type
I	raised, mucoid, greenish	+	-	+	91
II	raised, mucoid, greenish	+	-	-	5
III	raised, mucoid, greenish, very large	+	+	-	5
IV	raised, mucoid, white	-	-	+	3
V	raised, white, clear, slimy	-	-	-	4
VI	flat, white	+	+	-	4
VII	flat, reddish	-	+	-	7
VIII	flat, yellow or white	-	-	-	55

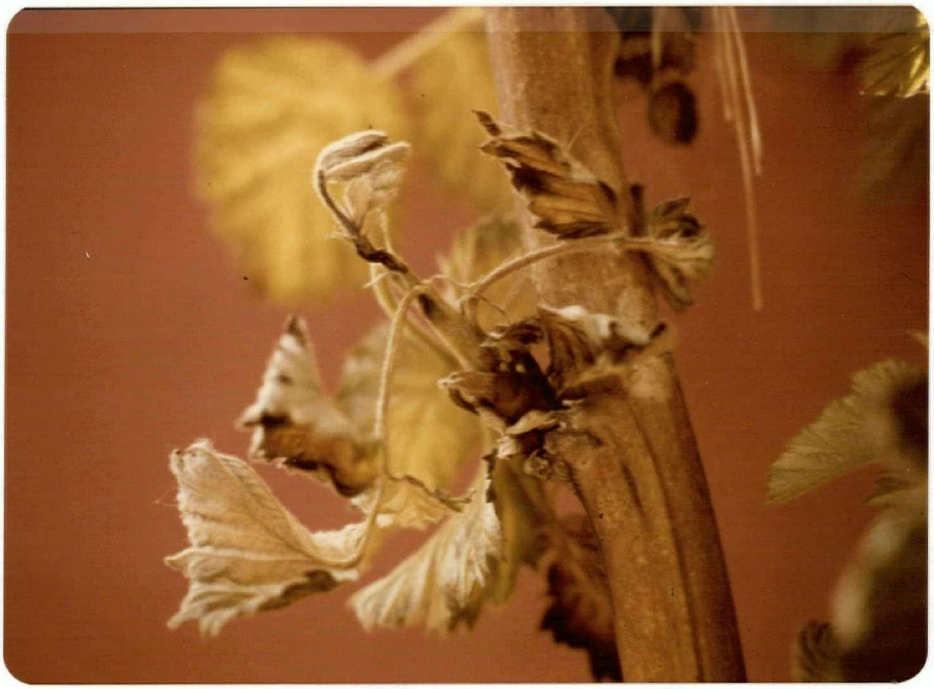


Fig. 4 Raspberry lateral showing typical symptoms of bacterial blight. Type I bacteria were isolated from this tissue.

Table 6. The pathogenicity to raspberry of different types of bacteria isolated from blighted raspberries

Type	Total Number of Isolates Tested	Number that were		
		Pathogenic	Weakly Pathogenic	Non-Pathogenic
I	48	25	17	6
II	5	0	3	2
III	5	0	0	5
IV	3	0	0	3
V	3	0	0	3
VI	3	0	0	3
VII	3	0	0	3
VIII	10	0	0	10

a

b

Fig. 5 a. Blackening of petioles following stem prick inoculation with Pseudomonas syringae b. Necrosis surrounded by yellow halo on leaf following leaf spray inoculation with a 10^7 CFU/ml suspension of P. syringae.

colonies on NSA, were oxidase negative and produced a fluorescent pigment, were recovered from each raspberry plant showing symptoms in these pathogenicity tests.

IDENTIFICATION OF PATHOGENIC ISOLATES

Further characterization of the pathogenic types I and II indicated at least 85 of 91 isolates of Type I and four of five isolates of Type II were *P. syringae* (Table 7). Type IV isolates were non-fluorescent *P. syringae* and not *E. amylovora*.

All biochemical tests done on the *P. syringae* cultures received from other sources (Table 2) gave expected results with the following exceptions. Isolates Ps-1 and Ps-8 did not utilize lactate. Ps-2 and Ps-4 did not produce levan from sucrose. No toxin was produced by isolates Ps-1, Ps-2, Ps-3, Ps-4, Ps-5, Ps-7, Ps-11, Ps-12 or Ps-13. None of the authentic cultures were pathogenic to raspberry (Table 7).

SURVEY OF BACTERIAL BLIGHT IN COMMERCIAL FIELDS

Three of four samples of blighted plant material taken from the Dyck farm near Clearbrook, B.C. contained Type I *P. syringae* isolates. Both samples from the Hoogie farm in the same area contained Type I *P. syringae*. Blight was evident but not widespread in these fields. Only five of 17 samples of necrotic tissue from the Maddock farm in Richmond, B.C. contained *P. syringae*. No *P. syringae* was isolated from the three samples from the Reynolds farm on Westham Island, B.C.

Table 7 Characteristics of Types I, II, IV and authentic Pseudomonas syringae isolates

Type	Gram Reaction	Hypersensitivity	Lactate Utilization	Polypectate Gel Pitting	Presence of Arginine Dihydrolase	Pathogenicity to Raspberry
I	0/91*	91/91	85/91	0/91	0/91	42/48
II	0/5	4/5	4/5	0/5	0/5	3/5
IV	0/3	3/3	3/3	0/3	0/3	0/3
Authentic P. syringae	0/3	13/13	11/13	0/13	0/13	0/13

*fractions represent ratio of isolates giving positive reactions to total number of isolates tested

STORAGE OF BACTERIA

Isolates survived 1 year stored on NGA at 5°C or in water at room temperature. Five of seven isolates survived when stored on NA at 5°C (Table 8). Isolates that survived on NGA and NA retained their ability to produce toxin as demonstrated in the G. candidum bioassay while the same isolates stored in water could not produce toxin (Fig. 6). Ps-205 and Ps-248, stored on NGA at 5°C for 1 year, were still pathogenic to raspberry. The identical isolates stored in water were not pathogenic after 1 year in storage.

After toxin-producing isolates Ps-346 and Ps-505 had been stored in water for 2 months, some single colony cultures of each had lost their toxin-producing ability (Table 9). After 3 months in water storage, none of the single colony cultures produced toxin. After 1 month on NGA, none of six non-toxin-producing single colony cultures of Ps-346 had reverted to a toxin-producing form.

Table 8. The effect of the method of storage on the toxin-producing ability of Pseudomonas syringae isolated from raspberry

Isolate Number	Zone of Inhibition of G. candidum in mm.			
	within 2 weeks of isolation	one year after isolation		
		stored on NA at 5°C	stored on NGA at 5°C	stored in water at room temp.
205	5*	no growth	4	0
223	0	0	0	0
231	8	no growth	6	0
248	3	4	5	0
327	8	4	5	0
368	3	3	4	0
505	5	3	4	0

*average of the distance from the margin of the bacterial growth to the edge of the zone of inhibition in three replications.

a b

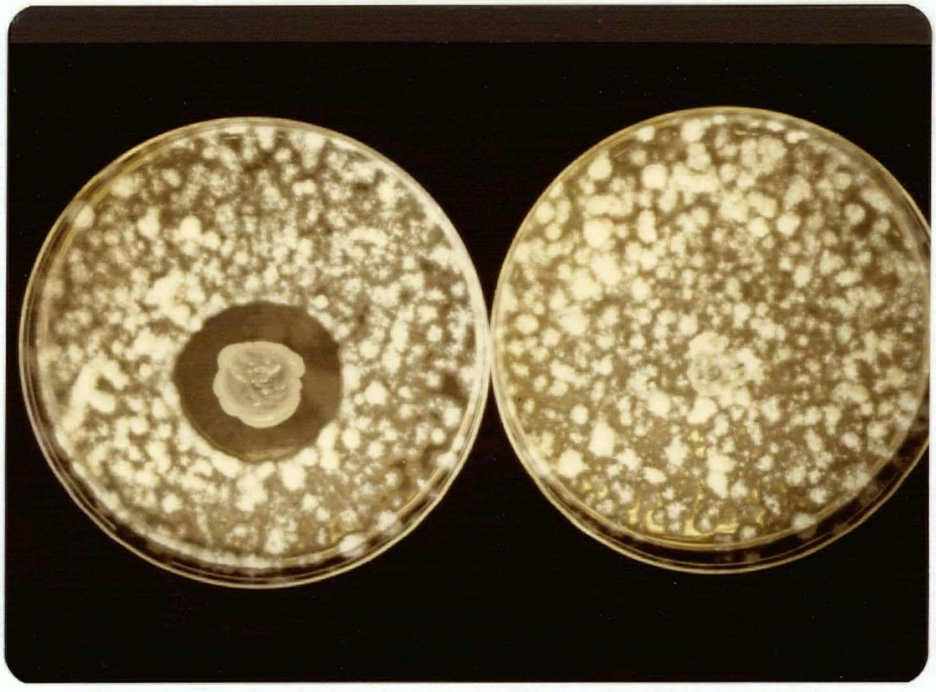


Fig. 6 Effect of storage method on toxin-producing ability of Pseudomonas syringae, Ps-248 as demonstrated by the Geotrichum candidum bioassay. a) Isolate stored for one year on NGA at 5°C and b) identical isolate stored in water at room temperature.

Table 9. Toxin-producing ability of Pseudomonas syringae single-cell isolates, Ps-346 and Ps-505 after 2 and 3 months in water storage

	Original toxin-producing ability*	After 2 months water storage	After 3 months water storage
Ps-346	4 mm	10/12+	1/12
Ps-505	5 mm	6/12	0/12

*zone of inhibition as determined in the Geotrichum candidum bioassay done immediately after isolation from raspberry

+fraction of isolates retaining toxin-producing ability

DISCUSSION

E. amylovora and *P. syringae* both grew well on NSA forming distinctive raised colonies on this medium that could be distinguished from one another and from other bacterial components of the raspberry microflora. A saprophytic pseudomonad was frequently recovered which formed colonies similar to *P. syringae* on NSA but this pseudomonad was oxidase positive and could be easily distinguished from *P. syringae* by this biochemical test. Additions to NSA did not improve its performance. When crystal violet was added to the medium, *P. syringae* colonies could not be distinguished from those of *E. amylovora*. The addition of crystal violet also reduced the number of other bacteria recovered but because there was some question about the identity of the incitant it was felt that it should not be added so that a more representative microflora could be isolated and tested for pathogenicity. This additive also reduced the numbers of *P. syringae*.

The addition of manganese sulfate to CVSA caused a precipitate to form. It also reduced the numbers of *P. syringae* that could be recovered. For these reasons it was not tested further as a selective medium. Kado and Heskett's liquid D4 medium could not be used as an enrichment medium in this study because other bacteria in the raspberry microflora were not inhibited and overgrew the *P. syringae* present.

Of the 174 bacterial isolates recovered from blighted raspberry tissue, 91 belonged in Type I. Eighty-five of these isolates could be identified as *P. syringae* by any of the identification schemes currently in use. Six of the isolates did not utilize lactate. They gave results similar to *P. syringae* in all other biochemical tests and were pathogenic to raspberry in greenhouse

tests. Because only the identification scheme proposed by Hildebrand and Schroth (35) considers lactate utilization necessary for identification as P. syringae and because two authentic P. syringae cultures were also negative for lactate utilization, these six isolates were considered to be P. syringae.

Four of the five Type II isolates were also positively identified as P. syringae in spite of the fact that the isolates did not produce toxin as determined by the G. candidum bioassay. This concurs with the finding of non-toxin-producing pathogenic isolates of P. syringae by other workers (4,74). The fifth isolate of this group caused no hypersensitivity reaction in tobacco. Because most of the P. syringae identification schemes considered this ability essential, this fifth isolate was not considered to be P. syringae. Non-fluorescent isolates of P. syringae were recovered in this survey confirming the initial report of Pepin et al. (79). However the isolates in the present survey differed from those found by Pepin et al. in that they were not pathogenic to raspberry in greenhouse tests.

The leaf spray inoculation was preferred to the stem prick method for pathogenicity tests because fewer bacteria were required so the spray more closely approximated populations that would be expected in nature (51). Also symptoms developed sooner with the leaf spray technique.

Pathogenic P. syringae isolates were recovered from 22 of 31 cultivars or selections of raspberry showing blight symptoms in 31 out of 40 isolation attempts. Eight of the nine failures to recover P. syringae occurred during a 2-week period of dry weather in June, 1976. The samples taken were dry, a condition not conducive to isolation of bacteria.

The recovery of P. syringae from most of the diseased raspberry samples indicated this organism was probably responsible for the blight seen on the

Dyck and Hoogie farms. Blight was not widespread in these fields and probably had little effect on yields in these plantings. The low recovery rate of P. syringae from the Maddocks farm and its absence from the Reynolds farm indicated bacterial blight was probably not the problem. Checks for P. syringae were only made in these few fields because bacterial blight was not a problem in the Lower Mainland Area in 1976 or 1977, the period of this study.

The best method of storage of P. syringae was on NGA at 5°C as both viability and toxin-producing ability were maintained for at least 1 year without transfers. On NA, the isolates did not remain viable for 1 year and in water the isolates lost their toxin-producing ability within 3 months.

Whether toxin production is essential for pathogenicity could not be clearly established. Only half of the toxin-producing Type I isolates were strongly pathogenic in greenhouse tests. The remainder were weakly pathogenic or non-pathogenic even though they produced toxin. About 50% of the non-toxin-producing isolates in Type II were weakly pathogenic. An isolate's ability to produce toxin seems to improve its pathogenic capabilities but is not essential for pathogenicity. The effect of the purified toxin on raspberry plants would be required to establish a role for the toxin in pathogenesis.

No E. amylovora was isolated from raspberry during this investigation and no other bacterium isolated from blighted plants caused symptoms on raspberry in pathogenicity tests. Typical, fluorescent isolates of P. syringae are therefore confirmed as the causal agent of bacterial blight of red raspberry in B.C. Now that the incitant of bacterial blight has been established and techniques for its isolation, identification and storage have been determined, a study of the epidemiology of the disease can be undertaken.

PART II

EPIDEMIOLOGY OF BACTERIAL BLIGHT OF RED RASPBERRY IN B.C.

LITERATURE REVIEW

Confirmation of Pseudomonas syringae as the incitant of bacterial blight of raspberry in B.C. made it possible to begin an investigation of the epidemiology of the disease. Very little was known about the disease cycle at the beginning of this study. Neither the source of the primary inoculum nor the oversummering site for the causal agent were known. The set of conditions required for the initiation of the disease had not been established. It was felt that the study of these factors might explain why the disease was widespread and damaging some years and absent in others. It was also thought that the connection between bacterial blight and the dead bud syndrome could be determined. Moreover it was felt that a more effective control measure might be found once the disease cycle was understood.

An investigation of the disease cycle would be greatly facilitated by the development of a quick, reliable technique for detecting P. syringae. Techniques involving the use of selective media, bacteriophages or serological methods are currently used for rapid detection of phytopathogenic bacteria. Selective media have been successfully developed for many plant pathogenic bacteria (28,66,83), but no reliable medium has been developed for P. syringae as was discussed in Part I of this thesis. Another method of rapid detection is based on the ability of bacteriophages to selectively lyse all isolates of one species of bacteria. Baldwin and Goodman (6) used phage to detect Erwinia amylovora in apple buds. Klement (53) discovered phages in Corynebacterium flaccumfaciens (Hedges) Dowson and Xanthomonas phaseoli (Erw. Smith) Dowson that would only lyse cells of the species from which they were found. He later reported a phage of Xanthomonas vesicatoria (Doidge) Dowson that was

specific for isolates recovered from tomato (54). A different phage lysed cells of X. vesicatoria isolates from pepper. Klement (52) found phage of P. syringae from bean but this phage also lysed isolates of other species of Pseudomonas. Crosse and Garret (26) also found non-specific phage which attacked P. syringae from cherry and plum. Billing (8) found phage useful for identification of P. syringae from several different hosts but only when used with other biochemical tests.

The most promising method for quick detection of plant pathogenic bacteria involves the use of serological techniques. In studies of the relationships among several Pseudomonas species, Friedman (40) found that an antiserum prepared against unfixed, unheated P. syringae cells reacted in immunodiffusion tests with three of four isolates of P. syringae and with three isolates of P. fluorescens Migula. This early work indicated this technique might not be species-specific. Otta and English (74) successfully prepared an antiserum, against sonicated cells that was useful for P. syringae detection. They differentiated 10 serotypes of P. syringae from isolates from 20 different hosts. Two strains tested had been isolated from raspberry and both belonged to the same serotype although host of origin did not usually correlate with serotype. A rabbit was injected with a mixture of seven different serotypes and the antiserum obtained reacted with any strain of P. syringae from any host. The antiserum also reacted in double diffusion tests with other species of Pseudomonas, all of which are now considered to be P. syringae by Doudorff and Palleroni (32).

The fluorescent antibody stain (FAS) technique was a further development of the serological detection method. Paton (78) used this technique to detect

P. syringae on turnips. Antiserum against P. syringae was conjugated with fluorescein isothiocyanate (FITC), a dye that fluoresces under high intensity light. This method, which allows for the visualization of single cells under the microscope, is more sensitive than the other serological techniques which depend on macroscopic observation of the precipitate formed when bacterial cells react with the antiserum.

Once a quick, reliable method for detection of P. syringae has been perfected, the epidemiological study can be started. The first consideration of this study is the source of the primary inoculum. Isolates of P. syringae which cause bacterial speck of tomato were isolated from rhizospheres of weed species near the tomato fields (89). However, Schuster and Coyne (91) stated that P. syringae was incapable of persisting in a free state in the soil. Hoitink et al. (46) in Wisconsin found that P. syringae, cause of brown spot of bean, could be detected in the soil from August to March, but they could not recover the bacteria from the soil in late spring or early summer when the beans were infected with the pathogen. Later, Ercolani et al. (36) found that this bean pathogen overwintered as an epiphyte on the leaves of hairy vetch, a common weed along bean field borders. The bacteria from the vetch were spashed by rain onto the bean crop in the spring.

The primary inoculum for some diseases caused by P. syringae was found on the host plants. P. syringae was found to cause cankers in which it overwintered on peach, plum, apricot, sweet and sour cherry, almond, pear and nectarine (18,22). Cameron (18) found viable P. syringae in the bark, buds and vascular tissue of cherry trees. Crosse (24) reported live P. syringae overwintered in the dead buds of cherry and plum trees. Leben et al. (58)

found that sudden outbreaks of blast on citrus, almond and pear incited by P. syringae followed a night of frost or a hail storm in the spring indicating that the primary inoculum was present on the host before environmental conditions occurred which predisposed the plants to infection.

In B.C. overwintering of P. syringae has not been investigated. In the first report of bacterial blight of raspberry in B.C., the infected raspberries were found to be in close proximity to a lilac infected with P. syringae. It seems possible that the bacterium might have overwintered on the lilac and spread into the raspberry crop in the spring. The presence of P. syringae in the soils of raspberry fields in B.C. has never been investigated. Cankers have not been observed on raspberry plants with bacterial blight. In addition, no check for P. syringae in the buds, bark, or vascular tissue of raspberry plants has been undertaken. The most likely strategy for overwintering would be for the bacterium to establish itself on the host plant. A raspberry produces biennial fruiting canes from perennial roots. Because new shoots are sent up from the roots every spring, new canes and one-year-old canes are together throughout the growing season. If the bacteria pass from the old canes and become established on the young canes, the organism could easily overwinter on these canes and be readily available for infection of new canes in the spring.

During the drier summer months in B.C., bacterial blight was not evident. P. syringae survived as a non-symptom-causing resident on other plants on which it is pathogenic (56). English and Davis (37) isolated P. syringae from healthy leaves, fruits, and twigs of peach and almond during the summer months. Crosse (25) found populations of P. mors-prunonum Wormald (considered

to be P. syringae by Doudoroff and Palleroni (32)) as high as 10^7 bacteria/ cm^2 on healthy cherry leaves. Leben et al. (58) found P. syringae to colonize healthy buds of bean plants from which it spread onto unfurling leaves. No check has been made for P. syringae on healthy raspberry plants.

Many plants infected by P. syringae develop leaf spots as part of the disease syndrome. On the leaves of fruit trees P. syringae caused two different kinds of infections (18). On peach, plum, cherry and apricot, leaf spots started as dark green water-soaked angular spots about 1-2 mm in diameter. The spots turned red or brown and were usually surrounded by a yellow or reddish-brown halo. The spots became necrotic and in the case of peach, plum and cherry, the necrotic tissue became dry, brittle and eventually dropped out producing a shot-hole effect. The second type of leaf infection, common on pear, began as a distinct necrotic spot but spread rapidly over the entire leaf area. P. syringae was easily isolated from both of these types of spots during the spring but as the leaf matured and the necrotic tissue dried isolations became increasingly difficult. P. syringae also caused necrotic spots surrounded by yellow halos on corn, bean, wheat and wild grass species (49,71,77).

In 1973, Rancovic and Sutic (80) reported that raspberry plants were infected with halo-spotting disease. Water-soaked spots appeared at the ends of leaf veins, enlarged, became necrotic and were surrounded by yellow halos. P. syringae was shown to be the cause of these symptoms. Leaf spots have not been reported on raspberry in B.C.

Bacterial blight was a problem in 1968, 1970, 1972 and 1973 but has not been a problem in the Lower Mainland Area of B.C. since then. To explain this

erratic incidence it is essential to consider the three components which interact in pathogenesis: the incitant, the host plant and the environment. Changes that have occurred in any one of these factors over the years may account for the erratic disease incidence.

There are two possible changes that may have occurred to the incitant. The first is that P. syringae has disappeared from raspberry fields in B.C. as suddenly as it appeared. This suspicion is encouraged by the absence of blight symptoms in recent years. A population study should be done to confirm whether or not P. syringae is currently present in the raspberry fields. Secondly, a change in the genetic makeup of the pathogen may have occurred. Such changes are not uncommon in bacteria. All isolates of Erwinia amylovora recovered from an apple orchard were found to be non-pathogenic (6). It was postulated that the isolates had changed genetically in some way as outbreaks of fireblight caused by E. amylovora had been common in this orchard in years prior to that study. Streptomycin-resistant mutants of P. syringae have been found in apricot orchards (98). Both pathogenicity and the ability to resist antibiotics have been attributed to the incorporation of an epichromosomal factor, known as a plasmid, into the genetic material of the bacterium. The pathogenicity of some human bacterial pathogens has been shown to be determined by plasmids (84,93). Panagopolous et al. (76) speculated that plasmids were responsible for pathogenicity of plant pathogens also. Gall-inducing ability was shown to be determined by large plasmids in Agrobacterium tumefaciens (Smith and Townsend) Conn (96). Lai et al. (55) showed that plasmids, responsible for antibiotic resistance, were lost spontaneously from the plant pathogenic bacteria Xanthomonas vesicatoria in storage. If plasmids

are responsible for the pathogenicity of P. syringae and if these plasmids are relatively easily acquired and lost to the natural population of P. syringae, variability in the incidence of disease would be expected.

The susceptibility of a host plant to infection of P. syringae is dependent on several factors most important of which is the genetic makeup of the host plant. Different raspberry cultivars displayed varying susceptibility to infection by E. amylovora (94) and P. syringae (79). In the Fraser Valley in B.C. no change has occurred that could account for the erratic incidence of the disease. Willamette was the most commonly grown cultivar in the years when blight was a problem and it remains the most commonly grown cultivar today (H.A. Daubeny, personal communication).

The rate of fertilizer application is another factor that affects host susceptibility. Karlen et al. (48) found that the incidence of chocolate spot of corn caused by P. syringae was most severe when the crop was not fertilized. Additions of 160 lb/acre of nitrogen resulted in a slight decrease in infection while addition of 40 lb/acre of potassium almost eliminated the disease. English et al. (39) found that bacterial canker caused by P. syringae killed 85% of the peach trees grown in soils with low levels of nitrogen. When the soil was adequately fertilized by adding nitrogen, potassium and phosphorus, only 13% of the trees died. If manure and moderate amounts of nitrogen were added 39% of the trees were killed. It was concluded that some fertilization decreased the damage due to P. syringae infection but high levels of nitrogen were actually detrimental to the trees. In B.C., bacterial blight was most severe in fields that had been heavily fertilized with chicken manure (16). Since this observation was made, the indiscriminate use of chicken

manure to increase nitrogen content in the soils of raspberry fields has been discouraged (69).

The ease with which plant tissues can be penetrated by the causal organism is another factor affecting the host plant's susceptibility. All plant pathogenic bacteria enter host tissue through natural openings or wounds (51). The natural plant openings utilized by bacteria include lenticels, hydathodes and stomates. Wounds due to cultural practices or natural causes more commonly provide entrance for bacteria. Common raspberry cultivation practices include tying canes to wires strung along the plant rows to support the canes. When it is windy, the canes rub against the wires and the bark is removed at these points. Natural wounding occurs when wind-blown dust scratches the surface of leaves. Wind-blown rain causes water-soaking of leaves. Xanthomonas malvacearum, Pseudomonas tabaci (Wolf and Foster) Stevens and P. mors-prunorum are known to enter host tissue through water-soaked areas (23). Water-soaked leaf tissue was commonly seen in the raspberry fields in B.C. during periods of wet, windy weather. Leaf scars resulting from natural leaf fall in autumn provided entry for P. syringae into cherry trees which resulted in canker formation (21). No changes have occurred in B.C. to any of these mechanisms that would discourage the chances of P. syringae entering plant tissue and account for the decline of the disease.

Variations in environmental conditions from year to year may account for the erratic incidence of the disease. Several attempts have been made to determine the environmental conditions required by P. syringae for disease initiation. English and Davis (38) found peach tree infection by P. syringae more likely to occur at 12°C than at 28°C. Cameron (18) noted infection

of sweet cherry was most common during cool, wet periods in the spring in Oregon. In California leaf spot and canker of citrus was much more common in wet years (70). Schmidle and Zeller (88) reported temperatures of 15-25°C accompanied by a 12-hour period of 100% relative humidity were most conducive to initiation of sour cherry blast. Crosse (23) found that windy, wet periods were responsible for epiphytotics of cherry leaf spot and spur wilt caused by P. syringae. In B.C., such wet, cool, windy periods are recorded almost every spring and fall.

There is a possibility that initiation of bacterial blight of raspberry depends on freezing temperatures. Sudden outbreaks of diseases caused by P. syringae have often followed a night of frost. Wormald (99) reported an outbreak of blossom blight of pear after a late frost in May. Baker (5) and Leben et al. (58) observed a similar phenomenon. Panagopoulos and Crosse (75) reported that P. syringae was universally distributed on pear trees as the dominant component of surface microflora. They speculated that freeze injury resulted in a "lifting of the skin of the receptacles and pedicels due to separation of the hypoderm from underlying cortical cells". This injury allowed the entry of the bacteria into plant tissue followed by the initiation of disease.

Recent investigations have implicated P. syringae as the cause of freeze injury and not merely its beneficiary. In 1974, Maki et al. (63) found that drops of sterile water or suspensions of most bacteria froze between -9 and -17°C while drops of suspensions of P. syringae isolated from alder leaves froze at temperatures of -1.8 to -3.8°C. Concentrations of at least 10^4 P. syringae cells per drop were required for this effect to occur. They

proposed that one bacterium in 10,000 was able to seed ice crystal formation. Arny et al. (2) found that the ability of P. syringae to cause ice nucleation also depended on the medium on which the cells had been grown. When corn plants, sprayed with either P. syringae or Erwinia herbicola (Lohnis) Dye, were placed in a mist chamber for 24 hours and then placed in a freeze chamber, freeze injury occurred at -3 to -4°C. No damage was observed on control plants sprayed with sterile distilled water. They speculated that either bacterium, when present on plant tissues, could act as a nucleus for ice crystal formation. Because water expands on freezing, plant cells ruptured and damage occurred. Attempts were made to determine if P. syringae cells had to be alive in order to be ice nucleation active. Cells were treated with dyes, antibiotics, chemicals or were heat-killed or sonicated (63). Only treatment with the antibiotics polymyxin B and streptomycin sulfate effectively reduced the numbers of viable bacteria in a suspension without destroying the ice nucleation ability. Weaver (98) has recently shown that bacterial canker on peach is a result of the interaction of P. syringae and freeze injury. Cankers developed on excised peach twigs that were inoculated with P. syringae, held at -10°C for 36 hours and then kept at 15°C for 10 days. Neither inoculation with P. syringae or the freeze treatment alone caused canker formation.

If the isolates of P. syringae in B.C. possess this ice nucleation ability it follows that raspberry plants infested with P. syringae may be more susceptible to freeze injury than raspberry plants free of P. syringae. It may even be that freeze injury to the plant, facilitated by the presence of P. syringae is responsible for the symptoms associated with bacterial blight. Also this ice nucleation ability may provide an explanation for the dead bud

syndrome which is most likely to occur in fields where bacterial blight has been a problem.

Present recommendations for control of bacterial blight of raspberry in B.C. are not effective. The British Columbia Department of Agriculture recommends that fixed copper or Bordeaux mixture sprays be applied at bud-burst followed by three more sprays at 14-day intervals for control of bacterial blight of raspberry (3). Satisfactory control of the disease was not achieved using this schedule (17,70). The development of a better control method for bacterial blight would therefore be very desirable.

Other diseases caused by P. syringae have not been effectively controlled with Bordeaux or fixed copper sprays (18,22,34). Better results have been realized with streptomycin sulfate sprays. Crosse (24) reported control of the leaf spot but not the canker phase of bacterial canker of cherry with this spray. Dye (34) found streptomycin sulfate sprays provided good control for stone fruit blast caused by P. syringae. Bethel et al. (7) found streptomycin-fixed copper sprays to be very effective for control of pear blossom blast also caused by P. syringae. There are disadvantages for the use of this antibiotic for control. It is expensive and bacteria have been known to develop resistance to it. Streptomycin had been successfully used to control fireblight of pears in California until an epiphytotic broke out in 1970 which was found to be caused by streptomycin-resistant strains (66). Pathogenic, streptomycin-resistant P. syringae strains were recovered from apricot fruit lesions and leaf surfaces in New Zealand (100). Sands and McIntyre (86) have reported good control of P. syringae infection of pear with aqueous sprays of sodium tartrate. The advantages of using this organic salt for control were that it was biodegradable, inexpensive, and was not phytotoxic to pear.

Attempts have been made to control P. syringae or closely related species biologically with antagonistic bacteria. Crosse (25) isolated a bacterium with characteristics similar to Erwinia species from cherry trees. This bacterium reduced the incidence and severity of leaf scar infections caused by P. mors-prunorum when it was sprayed on the tree prior to inoculations with this pathogen. The population of the antagonist on the fruit trees was increased by spraying bacterial suspensions on the trees but the population dropped off rapidly and natural equilibrium was reestablished within a few days. Crosse proposed that competition for space and nutrients was the basis of this antagonism. Dowler (33) reported inhibition of P. syringae isolated from peach trees by a fluorescent, non-pathogenic, gram-negative bacteria the same size as P. syringae. In mixed cultures at 25°C the saprophyte markedly inhibited the pathogen. When equal numbers of both antagonist and pathogen were inoculated into peach trees at 25°C the saprophyte was recovered in greater numbers than the pathogen after 48 hours. Also infection was not as severe when peach seedlings were inoculated with both antagonist and pathogen as when the seedlings were inoculated with the pathogen alone. Leben (57) isolated three bacterial strains from the seed coats of soybeans that produced toxicants that diffused in agar and inhibited P. glycinea Coerper (now considered P. syringae by Doudoroff and Palleroni (32)) which infected soybean seedlings. Of seedlings developing from seeds naturally infected with P. glycinea, between 7 and 17% were infected when the seeds were treated with the antagonist while 46-98% of the seedlings were infected if the seeds were not treated.

There is also hope that genetic resistance against bacterial blight may provide control. Crosse (22) reported that cherry cv Napoleon was more susceptible to bacterial canker than cherry cv Frogmore. Plums cv Victoria was also found to be susceptible to P. syringae infection while cv Purple Egg was highly resistant. Different wheat cultivars also showed varying resistance to P. syringae (103). Glenlea was highly resistant to infection while cv Era was moderately susceptible. Pepin et al. (79) found that cv Latham was susceptible while Newburgh and Viking cultivars were most resistant to bacterial blight of red raspberry in B.C. Starr (94) also observed cv Latham to be susceptible and cv Newburgh to be resistant to fireblight infection of red raspberry. This similarity in reaction might indicate some raspberry cultivars are generally resistant or susceptible to bacterial infections. Because new cultivars of raspberry are being developed in B.C. in a breeding program, a method of screening the new cultivars for resistance to bacterial blight is needed.

In summary, bacterial blight of raspberry is an erratic, uncontrolled disease which appears in the spring and/or fall of some years. How and where P. syringae survives the rest of the year is not known. Environmental conditions may prove to be very important for disease initiation because of the unique ice nucleation ability of strains of this bacterium found elsewhere. Therefore the objectives of this study were:

1. to develop a technique for rapid detection of P. syringae on red raspberry so that the overwintering and oversummering sites for this bacterium can be located,
2. to investigate the ice nucleation ability of local P. syringae isolates and determine the effect of this ability on the disease syndrome, and
3. to evaluate some potential control measures for this disease.

MATERIALS AND METHODS

CULTURE MAINTENANCE

Authentic cultures used in this study are listed in Table 3 (Part I). Cultures were stored on nutrient glycerol agar (NGA) at 5°C. Unless otherwise stated, P. syringae cultures were grown on King's Medium B (KMB) for 48 hours at 27°C before use in tests.

ANTISERUM PRODUCTION AND TESTING OF SEROLOGICAL METHODS

P. syringae culture Ps-346, recovered from a blighted raspberry from the Clearbrook Substation of the Vancouver Research Station of Agriculture Canada, was grown on nutrient agar (NA) for 48 hours. The cells were glutaraldehyde-fixed by the procedure of Allan and Kelman (20) and then adjusted to 10^9 cells/ml. One ml of this suspension was mixed with 1 ml of Freud's incomplete adjuvant (Difco, Detroit, Michigan) and injected intramuscularly into a 2 kg New Zealand rabbit. The rabbit was thereafter injected with 1 ml of the same suspension at weekly intervals for 4 weeks. Prior to the first injection and 2 weeks after the last weekly injection, 20 ml of blood was collected by bleeding from the ear vein of the rabbit (105). The blood was allowed to clot overnight at 5°C, the serum collected and centrifuged at 12,000 g for 20 minutes in an International Equipment Co. centrifuge, Model HT.

The titre of the antiserum was determined by drop agglutination. A suspension of 10^8 colony-forming units (CFU)/ml of the homologous culture Ps-346 was prepared in 0.01M buffered saline (PBS), pH 7.2. The antiserum was diluted 1:10 and then serially-diluted 1:2 ten times with PBS. One drop of the bacterial suspension was mixed with one drop of each concentration of antiserum in a plastic petri plate. For controls, one drop of PBS was tested

with each concentration of antiserum and with the bacterial suspension. The plate was placed in a humid chamber at room temperature and checked for agglutination under 6 times magnification after 2, 4 and 8 hours. The denominator of the highest dilution that still reacted with the bacterial suspension was taken as the titre of the antiserum. Because the titre was high enough for routine testing after the four weekly injections the rabbit was thereafter bled once every month and injected two weeks after each bleeding.

To determine the specificity of the antiserum PBS suspensions of 10^7 and 10^8 CFU/ml of each of Ps-346, Ps-205, P. phaseolicola (Burkholder) Dowson (Pp-1) and P. marginalis (Brown) Stevens (Pm-1) which had been grown on NGA were made. One drop of each concentration of bacteria was mixed with one drop of a 1:80 dilution of each of the normal and anti-Ps-346 serum in PBS as above and checked after 4 hours for agglutination.

Because the antiserum reacted with isolate Pp-1, the antiserum was cross-absorbed. Ten NA plates were inoculated with Pp-1 and incubated for 48 hours. Cells were taken up in 100 ml of PBS and centrifuged at 12,000 g for 20 minutes. The supernatant was discarded and the cells were taken up in 8 ml of PBS. Of the resulting suspension 0.7 ml were added to each of four small test tubes containing 0.1 ml of antiserum each. The tubes were held at $50^{\circ}C$ in a waterbath for 2 hours, centrifuged and the supernatant transferred to a clean test tube (97). The supernatant was cross-absorbed two more times using 0.6 ml of bacterial suspension each time. The supernatants were pooled giving about 8 ml of a 1:20 dilution of cross-absorbed antiserum.

The specificity of this cross-absorbed antiserum was determined by testing the following authentic isolates with the antiserum in drop agglutination:

Ps-1, Ps-2, Ps-3, Ps-4, Ps-5, Ps-6, Ps-7, Ps-8, Ps-9, Ps-10, Ps-11, Ps-12, Ps-13, Pm-1, Pp-1, Erwinia amylovora (Ea-1), and Erwinia carotovora var carotovora Dye (Ec-1). Also tested were 51 isolates biochemically identified as P. syringae, 5 unidentified pseudomonads, and 41 other bacteria, all isolated from diseased raspberry plants from the Clearbrook Substation in 1976. Six P. syringae isolates from diseased raspberry plants from Richmond were also tested.

Fluorescein isothiocyanate (FITC) was conjugated to the cross-absorbed antiserum as described by Allan and Kelman (104). Suspensions of bacterial isolates Ps-346, Pp-1, and Pm-1 and 10 non-pseudomonad bacteria, commonly isolated from diseased raspberry from the Clearbrook Substation, were smeared in 5 mm diameter circles etched on glass microscope slides. The slides were air-dryed, heat-fixed, and stained with a 1:1 dilution of the congugated antiserum in PBS for 30 minutes. The slides were rinsed in PBS and viewed with a Zeiss Universal microscope equipped for incident fluorescent microscopy using a Neofluar 40 objective, excitation filter BG-12, and barrier filter 50. Smears containing fluorescing bacterial cells were noted.

MONITORING OVERWINTERING POPULATIONS OF P. SYRINGAE IN RASPBERRY BUDS

Populations of P. syringae on raspberry buds from the Clearbrook Substation were monitored throughout the winter of 1976-1977. The sample area consisted of two 17-m rows, 2 m apart. Each row was divided into six 2-m sections of raspberry plants with 1-m spaces between sections. Each section contained six raspberry plants cv Willamette with eight canes per plant. Because no bacterial blight had been seen in this plot during the 1976 growing

season, dormant canes in six randomly selected sections were sprayed to runoff with a suspension of 5×10^8 CFU/ml of Ps-346 from a hand sprayer on November 19. Plants in the other sections were sprayed with water.

Before and immediately after spraying, and at monthly intervals thereafter until March, two buds were selected at random from each plant and the 12 buds from each section were grouped. These 12 buds were divided into two six-bud samples and each sample was ground in 3 ml of sterile distilled water (SDW) using a sterile mortar and pestle. Two ml of the resulting solution were serially diluted and plated on nutrient sucrose agar (NSA). After 2 days raised, mucoid colonies were counted. Representative colonies were streaked on NSA to ensure purity and then transferred to KMB. After 24 hr incubation the oxidase reaction and production of a fluorescent pigment were checked. Each isolate was then transferred to a NGA slant. After 48 hours incubation, a loop of the culture was transferred to 2 ml of PBS and tested in drop agglutination with a 1:80 dilution of the cross-absorbed antiserum prepared against Ps-346.

SUMMER SURVIVAL OF P. SYRINGAE

Healthy leaves were obtained from raspberry plants cv Willamette from the Clearbrook Substation on four sampling dates during the summer of 1977. Seven two-leaf samples (each approximately 1 g) were ground in SDW and dilution-plated on NSA. After 48 hours, raised, mucoid colonies were selected and characterized as described above. The pathogenicity of four of these isolates was tested by spraying a bacterial suspension of 10^8 CFU/ml on the leaves of 6-week-old rapsberry plants cv Willamette. After spraying, the plant

tissue appeared water-soaked. The plants were then placed in a mist chamber in the greenhouse and checked for symptoms after 7 days.

To determine how long P. syringae could survive on the raspberry leaves under greenhouse conditions a suspension of 10^8 CFU/ml of isolate Ps-346 was sprayed to runoff on four, 6-week-old raspberry plants cv Willamette. Plant tissues did not appear water-soaked after spraying. After 4 and 6 weeks, two leaves from each plant were ground and plated as was done with the healthy leaf samples. After incubation raised, mucoid colonies were counted.

In the spring and summer of 1977, leaf spots were observed on red raspberry plants at the Clearbrook Substation. To determine if these spots were caused by P. syringae, samples of leaves with spots were collected. A sterile 5 mm diameter cork-borer was used to cut discs of tissue that included a leaf spot and some surrounding healthy tissue. Eighteen, 20-disc samples were ground and dilution-plated on NSA. After 48 hours incubation, colonies suspected to be P. syringae were selected and characterized as described above. A suspension of 2×10^8 CFU/ml of each of three cultures characterized as P. syringae recovered from the leaf spot samples were each sprayed on two 6-week-old raspberry plants cv Willamette. The tissues did not appear water-soaked after spraying. The plants were placed in a humid chamber and checked for leaf spots 10 days later. Discs of tissue surrounding the spots that did develop were cut from the plants and isolations were made as described above. The colonies obtained were compared to those inoculated into the plant to fulfill Koch's postulates.

ICE NUCLEATION ABILITY

Two P. syringae cultures, Ps-282 and Ps-346, isolated from diseased raspberry plants in the summer of 1976, and one Erwinia carotovora culture, Ec-1, from potato were extensively tested for ice nuclation ability (INA). The isolates were grown on KMB and NGA for 1, 2, 4 and 7 days. Cells were suspended in SDW and adjusted to 10^9 CFU/ml. Dilutions were made to obtain suspensions of 10^5, 10^6, and 10^7 CFU/ml in SDW. A piece of aluminum foil was coated with a 1% solution of paraffin in xylene (62). The xylene was allowed to evaporate in a drying oven at 60°C. The coated aluminum foil was floated in a waterbath containing ethylene glycol (1:2 Prestone antifreeze to water) held at -5°C. A 10 µl droplet of each concentration from each medium for the three bacterial isolates and a sterile water control were placed on the foil. After 1 minute the drops were checked for freezing. This was repeated using the same suspension to confirm results.

Subsequently, 48 other P. syringae cultures isolated in the spring and summer of 1976 from the diseased raspberries, 28 P. syringae cultures isolated during the winter of 1976-1977 from dormant buds, 5 pseudomonads from raspberry and 10 other species of bacteria were grown on NGA for 4 days. Suspensions of 10^5 and 10^7 CFU/ml were prepared and tested as above.

Ps-346 was grown on NGA for 4 days, suspended in SDW and adjusted to 10^7 CFU/ml. This suspension was tested for INA before and after heat treating it for 20 minutes at 121°C. A 4-day-old NGA culture of Ps-346 was exposed to formalin vapors for 20 minutes. (To confirm that all bacteria were killed, a loop of cells was streaked on a fresh NSA plate and no growth was noted after 48 hours.) The killed bacteria from the plate were suspended in SDW and adjusted to 10^7 cells/ml. The INA of the suspension was then tested.

Four 6-week-old raspberry plants cv Willamette were sprayed with a suspension of 10^7 CFU/ml of Ps-346 or SDW. The plants were put into a growth chamber at $-2^{\circ}C$ for 4 hours and then returned to the greenhouse. After 24 hours, injury to the plants was recorded.

In an attempt to correlate frequency of frosts with disease incidence, the numbers of nights during which temperatures of $-1.8^{\circ}C$ or lower were recorded at the Abbotsford Airport, 2 km from the Clearbrook Substation, between March 25 and May 31 for the years 1973-1978 were obtained. The period between March 25 and May 31 was chosen because the raspberry buds have usually broken dormancy by March 25 and the disease usually was noticeable in April and May.

SURVEY FOR BACTERIA ANTAGONISTIC TO P. SYRINGAE

Several different bacteria representing the normal microflora on raspberry plants isolated from commercial raspberry plantings in the Clearbrook-Abbotsford area in the summer of 1975 were obtained from Dr. R.J. Copeman. These bacteria along with P. syringae isolates from Forsythia, were tested to determine if they could inhibit P. syringae from raspberry. Bacterial cells from a 24-hour NA culture of Ps-346 were suspended in 2 ml SDW. From this a suspension of 10^5 CFU/ml was prepared and 0.1 ml was spread on a NA plate and allowed to dry. One sterile 6-mm filter paper disc was saturated with a suspension containing 10^7 CFU/ml of each of the suspected antagonists and then placed immediately on the lawn of Ps-346. Five discs were placed on each plate. After the plates were incubated for 3 days at $27^{\circ}C$, the zone of inhibition from the edge of the disc to the beginning of the bacterial growth was measured.

CULTIVAR SUSCEPTIBILITY TRIALS

Several cultivars of raspberry were tested for their susceptibility to P. syringae infection in greenhouse tests. Root cuttings of cultivars Haida, Malling Promise, Malling Leo, Meeker, and Willamette and the recently developed cultivars Chilcotin, Nootka and Skeena were obtained from the Agriculture Canada Red Raspberry Breeding Program in B.C. Three leaves on each of three 6-week-old plants were sprayed with a direct stream of a suspension of 10^7 CFU/ml of Ps-346 from a hand sprayer. One plant of each cultivar was similarly sprayed with sterile water. Leaves appeared water-soaked where they had been sprayed. Inoculated plants were placed in a mist chamber in the greenhouse for 7 days at which time they were rated for symptoms. Ratings were based on the number of sprayed leaves that showed symptoms and the severity of these symptoms expressed according to the following scheme:

0 - no leaves infected

1 - one leaf slightly infected

2 - one or two leaves infected with necrosis at areas directly sprayed

3 - at least two leaves infected with necrosis spreading from the area directly sprayed

4 - all sprayed leaves infected with necrosis spreading from the area directly sprayed.

These trials were repeated at least once for each cultivar.

RESULTS

ANTISERUM PRODUCTION AND TESTING

The antiserum obtained after four weekly injections had a titre of 5,120 as determined in drop agglutination tests. Subsequent bleedings for 3 months had an identical titre but after that time the titre dropped to 2,560 and the rabbit was sacrificed. No agglutination occurred when either the bacterial suspension or the antiserum alone was mixed with PBS. Agglutination did occur with both P. syringae isolates and the P. phaseolicola isolate but not with the P. marginalis isolate. The normal rabbit serum did not react with any of these four isolates (Fig. 7).

After cross-absorption with P. phaseolicola, the Ps-346 antiserum no longer reacted with P. phaseolicola but still reacted with Ps-346 (Fig. 8). It reacted with all 51 P. syringae isolates recovered from diseased raspberries from the Clearbrook substation (Table 10). Three of these isolates did not produce a fluorescent pigment when grown on KMB. Of the six Richmond P. syringae isolates tested, four reacted strongly, one reacted weakly and one did not react with the antiserum. Authentic P. syringae isolates from other hosts from California and Wisconsin reacted strongly with the antiserum. None of the other 41 bacteria tested reacted with the antiserum.

Bacterial cells stained strongly with FITC-conjugated antiserum in the smears of Ps-346, and weakly with P. phaseolicola and one of the 10 other bacteria commonly found on diseased raspberry in the summer of 1976 (Fig. 9). The photomicrographs in Fig. 9 were all taken with identical exposure times and magnifications. Cells of P. marginalis and the other nine bacteria were not stained. The other bacterium that stained was rod-shaped with slightly

a

b

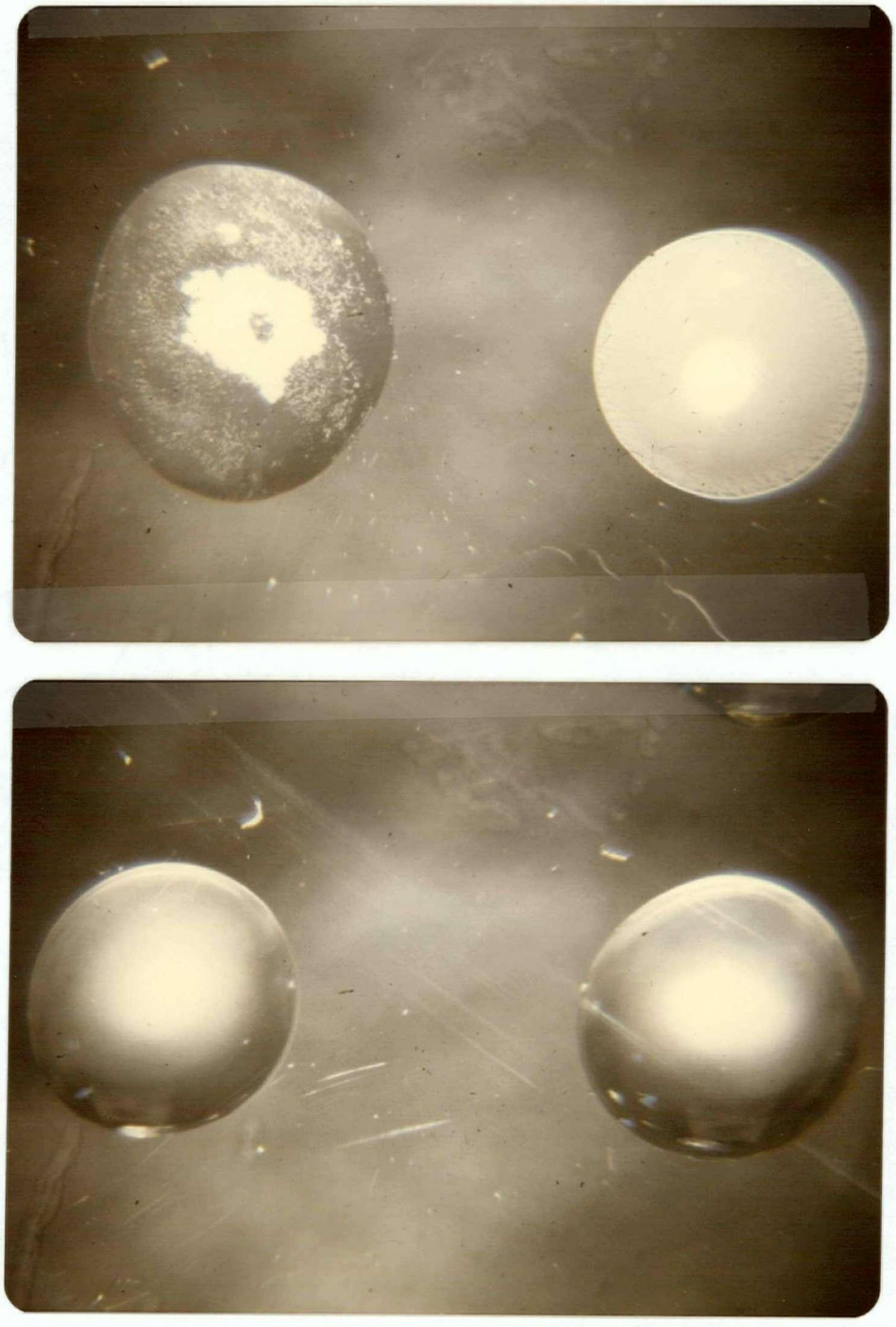


Fig. 7 Agglutination reaction of Pseudomonas syringae antiserum (left) and normal antiserum (right) with homologous culture Ps-346 (a) and Pseudomonas marginalis (b). 6 x magnification.

a

b

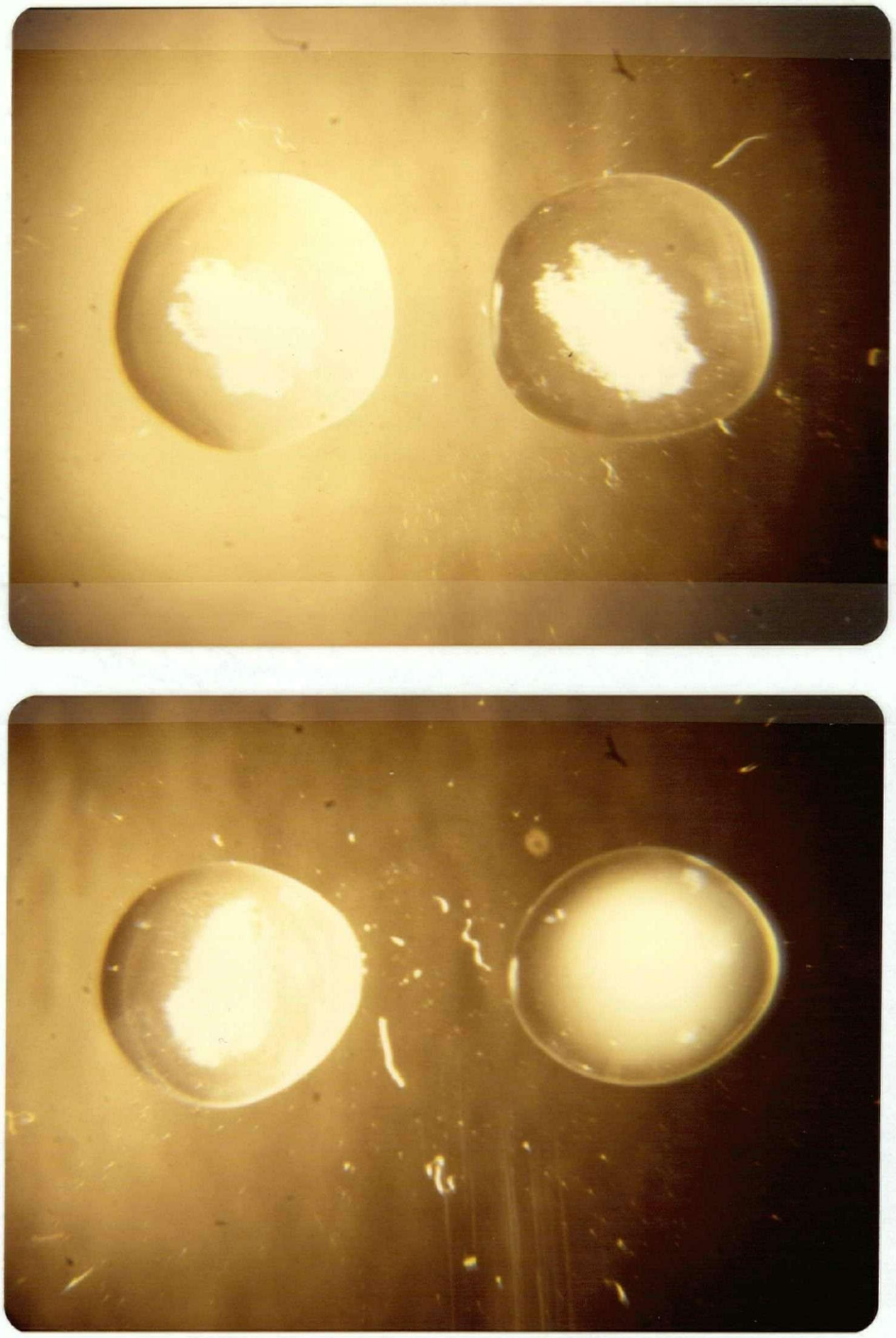


Fig. 8 Agglutination reaction of Pseudomonas syringae, Ps-346 (left) and Pseudomonas phaseolicola, Pp-1 (right) with Ps-346 antiserum (a) and Ps-346 antiserum that had been cross-absorbed with P. phaseolicola (b). 6 x magnification.

Table 10. Reactions of various bacterial isolates with cross-absorbed *Pseudomonas syringae*, Ps-346 antiserum

Isolate tested	Number of Isolates Producing Each Reaction Type		
	Strong	Weak	No Reaction
P. syringae (raspberry, Clearbrook, B.C.)	51	0	0
P. syringae (raspberry, Richmond, B.C.)	4	1	1
Ps-1 Lilac, Wisconsin	1	0	0
Ps-2 *Forsythia*, B.C.	0	1	0
Ps-3 *Forsythia*, B.C.	0	0	1
Ps-4 *Forsythia*, B.C.	0	0	1
Ps-5 Lima bean, Wisconsin	1	0	0
Ps-6 Pear, Wisconsin	1	0	0
Ps-7 Bean, Wisconsin	1	0	0
Ps-8 Almond, California	1	0	0
Ps-9 Pear, California	1	0	0
Ps-10 Peach, California	1	0	0
Other pseudomonads (raspberry, Clearbrook, B.C.)	0	0	5
Other bacteria (raspberry, Clearbrook, B.C.)	0	0	41
Erwinia carotovora, Ec-1, potato, B.C.	0	0	1
Erwinia amylovora, Ea-1, pear, Ontario	0	0	1

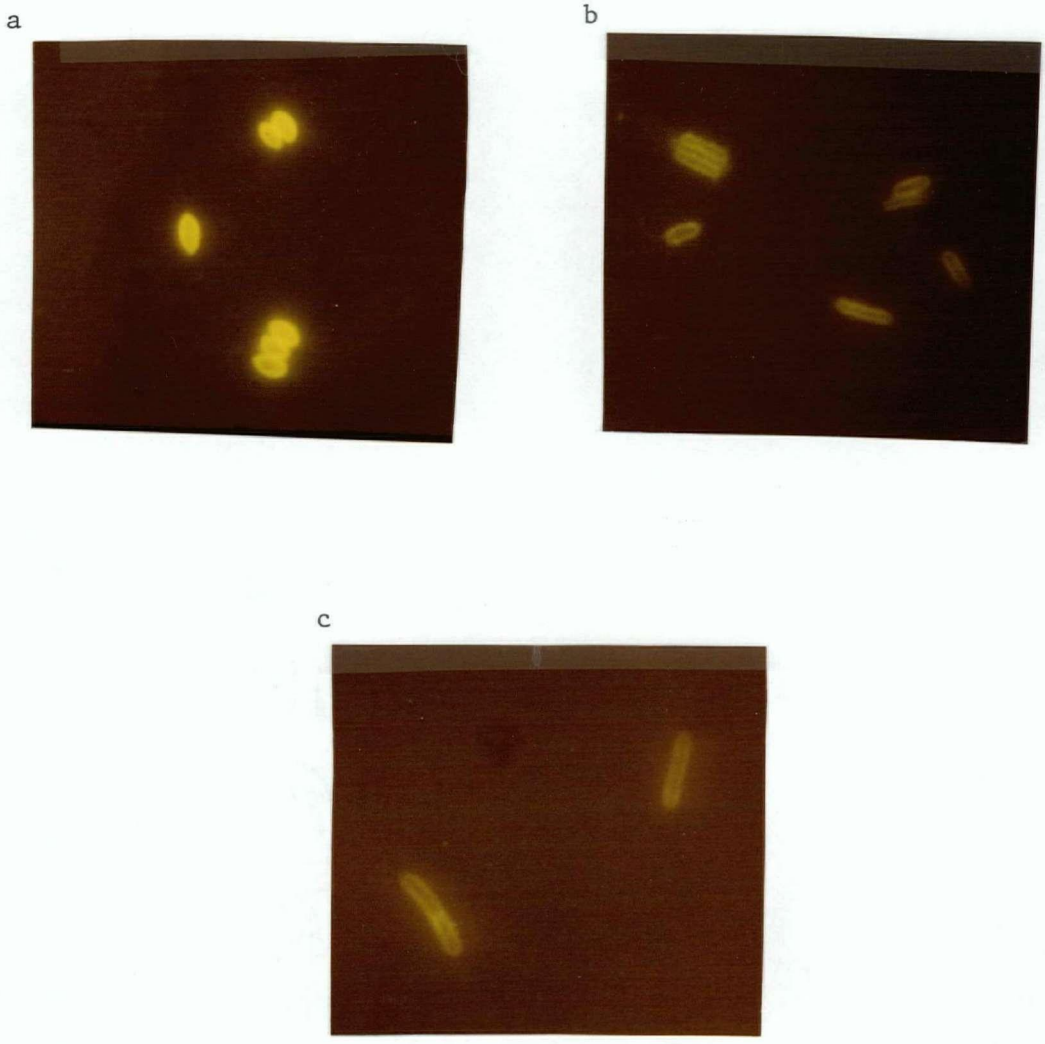


Fig. 9 Bacterial cells stained with FITC-conjugated, Ps-346 antiserum that had been cross-absorbed with Pseudomonas phaseolicola. a) Pseudomonas syringae, Ps-346 (b) P. phaseolicola (c) unidentified bacterium commonly isolated from raspberry (400 x magnification, 2 sec exposures).

larger cells than P. syringae. When grown on NSA, this bacterium produced large, mucoid colonies that were almost clear. This bacterium did not react with the cross-absorbed Ps-346 antiserum in drop agglutination tests.

OVERWINTERING POPULATIONS OF P. SYRINGAE IN RASPBERRY BUDS

P. syringae was recovered from about 30% of the six-bud samples from the dormant raspberry canes before they were sprayed with the bacterial suspension (Fig. 10). In the samples that did contain P. syringae, the mode (the number of bacteria that was most commonly recovered from the buds) was 10^4. In the sections that were sprayed with bacteria, 92% of the bud samples contained P. syringae with a mode of 10^6 CFU per sample immediately after spray. By December 17, one month later the mode had returned to 10^4 CFU per sample and remained at this level for the rest of the winter. The natural populations of P. syringae in the water-sprayed buds were similar to the populations in the buds sprayed with the bacterial suspension from January 14 to March 18.

Of 196 P. syringae isolates recovered from the bud samples, 190 were oxidase negative, produced a fluorescent pigment and did react in drop agglutination tests with the Ps-346 antiserum. The remaining six isolates were oxidase negative, produced a fluorescent pigment but reacted weakly or not at all with the antiserum. These isolates, when subsequently subjected to additional biochemical tests, were identified as P. syringae.

SUMMER SURVIVAL OF P. SYRINGAE

P. syringae survived as an epiphyte on raspberry leaves in greenhouse tests. Approximately 10^3 CFU/leaf could be recovered from healthy raspberry

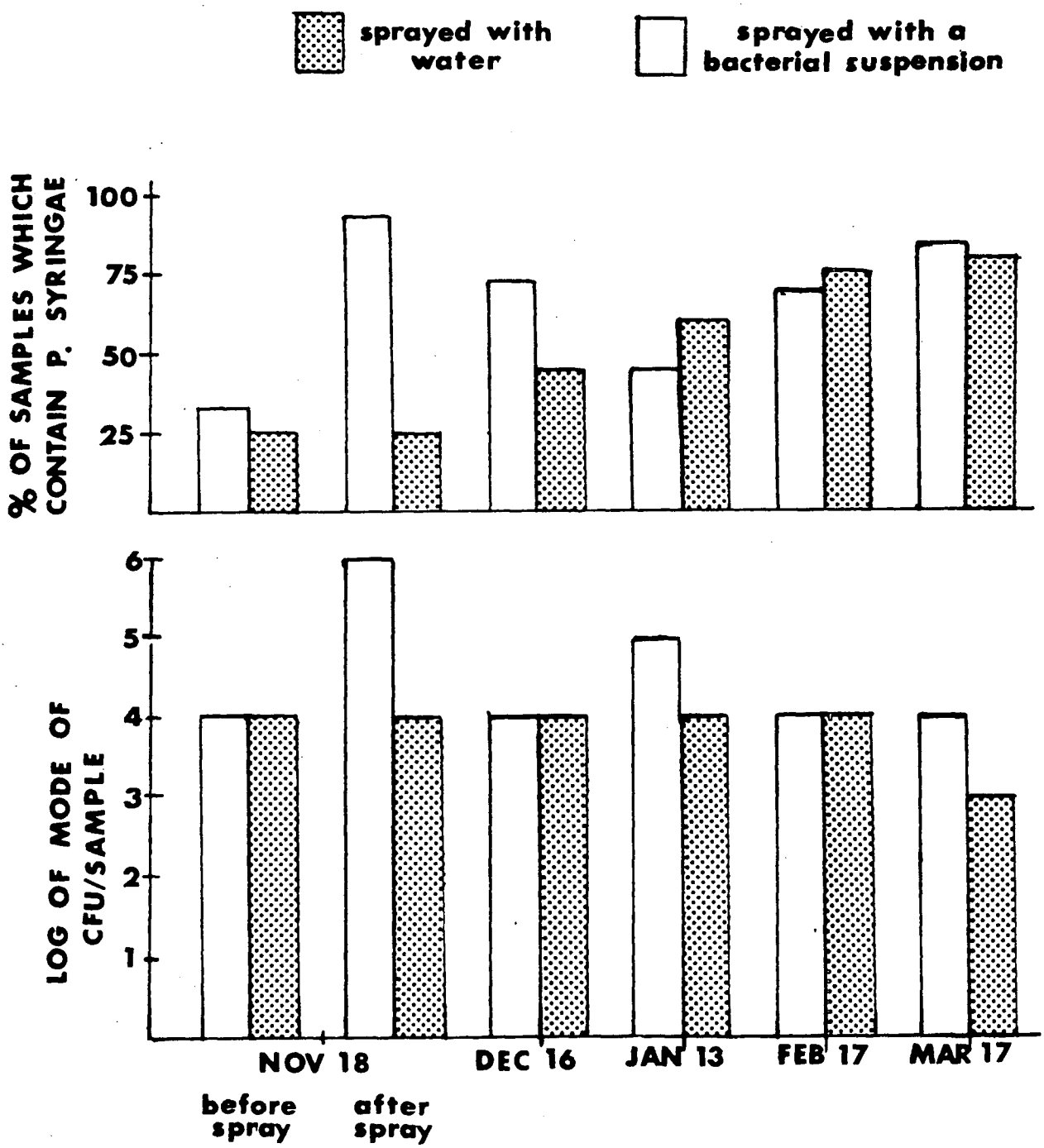


Fig. 10 Populations of Pseudomonas syringae recovered from raspberry buds throughout the winter of 1976-1977. Top graph gives percentage of six-bud samples that contained P. syringae on each sampling date. Bottom graph shows the log of the most common number of bacteria (the mode) recovered per six-bud sample on each sampling date.

leaves 4 and 6 weeks after these plants had been sprayed with a suspension of 10^8 CFU/ml. Similarly, P. syringae was recovered from four of seven healthy leaf samples from raspberry plants in the field. An average of 10^4 CFU/leaf were recovered from those samples containing P. syringae. Four representative isolates caused symptoms on raspberry plants in greenhouse pathogenicity tests.

P. syringae was recovered from 16 of 18 leaf spot samples. Leaf spots from which P. syringae was isolated (Fig. 11) were typically 2 to 5 mm in diameter with brownish-red centers surrounded by yellow halos. These spots would enlarge, turn brown and often coalesce with nearby spots. Isolates recovered from field leaf samples when inoculated onto young raspberry plants produced symptoms similar to those on the natural material (Fig. 12) except that curling and savoying of the young expanding leaves in the greenhouse tests were also noted. Typical P. syringae isolates were recovered from these inoculated leaves.

ICE NUCLEATION ACTIVITY

The ability of a P. syringae isolate to cause ice nucleation depended on several factors but Erwinia carotovora (included as a control) was never found to be ice nucleation active (Fig. 13). Culture medium effected ice nucleation ability (INA) as a suspension of 10^7 CFU/ml of Ps-346 grown on NGA was nucleation active while a concentration of 10^9 CFU/ml was required for INA if the bacteria were grown on KMB (Fig. 14). The age of the culture was also important. A suspension of 10^7 CFU/ml of a 4-day-old culture grown on NGA was nucleation active while a suspension of 10^9 CFU/ml was required if the

a

b

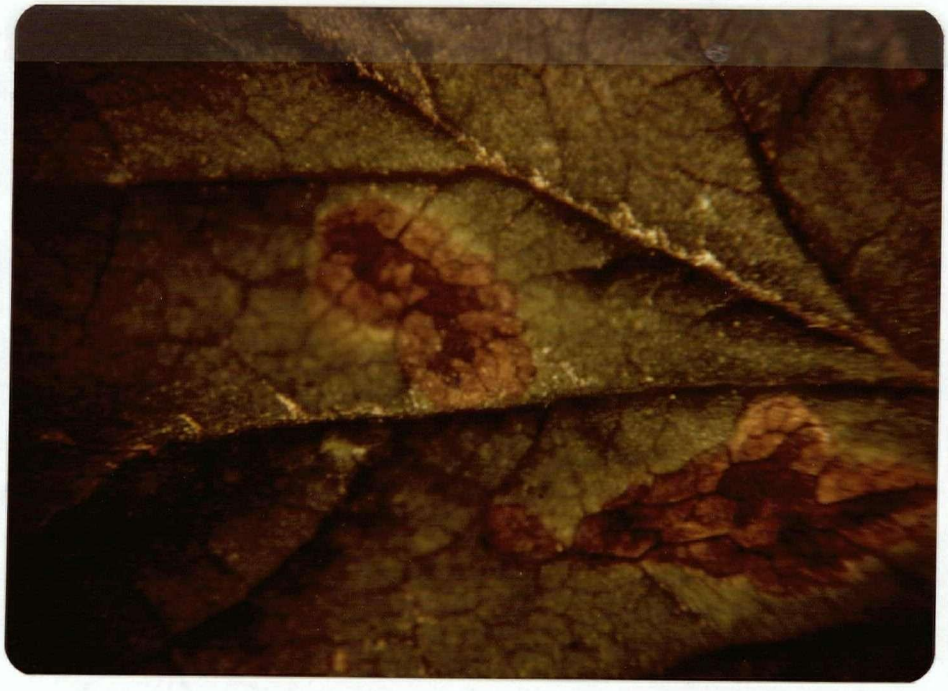


Fig. 11 Raspberry leaf from a naturally-infected plant from the Clearbrook Substation showing typical leaf spots (a) and leaf spots magnified 30 x (b).

a

b

c

Fig. 12 Leaf spots from naturally-infected raspberry plants at the Clearbrook Substation from which Pseudomonas syringae was isolated (a. and b.) and leaf spots on a six-week-old raspberry plant four days after spray inoculation with P. syringae under greenhouse conditions (c).

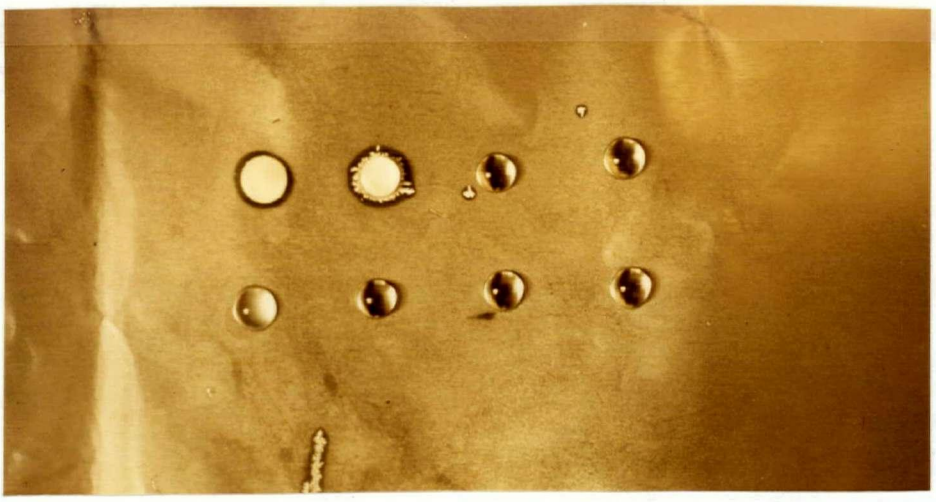


Fig. 13 Ice nucleation activity test showed Pseudomonas syringae, Ps-346 (top) to be ice nucleation active and Erwinia carotovora to be inactive (bottom). Concentrations of bacteria/ml were (from left to right) 10^9, 10^7, 10^5 and SDW.

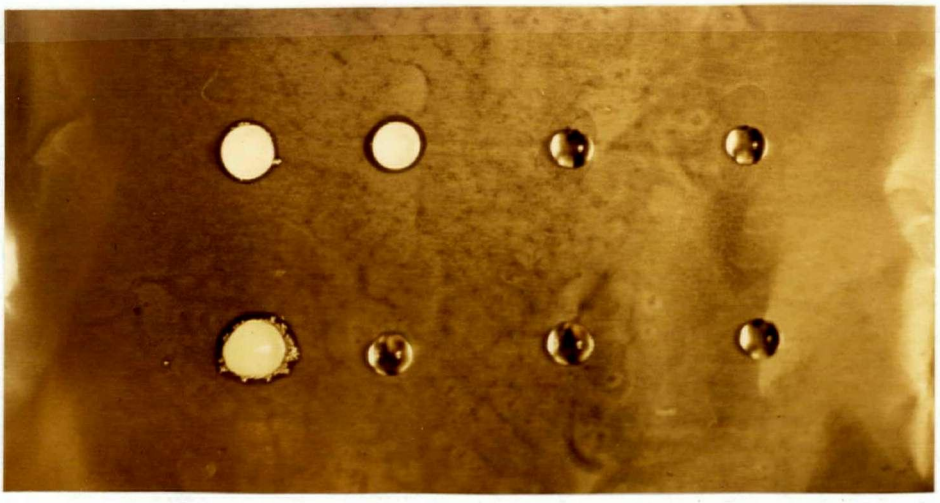


Fig. 14 Effect of culture medium on ice nucleation activity. Four-day-old culture grown on NGA (top) and on KMB (bottom). Concentrations of bacteria/ml were (from left to right) 10^9, 10^7, 10^5 and SDW.

culture was 2 days old (Fig. 15). INA also varied among cultures. Ps-205 and Ps-346 were similar in their response to all factors except that 10 times fewer Ps-205 bacteria were required to induce ice nucleation as Ps-346 (Fig. 16).

Of 48 P. syringae cultures recovered from blighted red raspberry plants in the spring and summer of 1976, 43 were ice nucleation active. Of 28 P. syringae cultures recovered from buds in the winter of 1976-1977, 22 were ice nucleation active. No other bacteria from red raspberry were ice nucleation active in these tests.

A suspension of 10^7 CFU/ml of P. syringae was ice nucleation active before but not after heat-treatment. The formaldehyde-killed bacterial suspension was also ice nucleation inactive.

When raspberry plants were sprayed with Ps-346, placed at $-2^{\circ}C$ for 4 hours and then left in the greenhouse, necrosis was evident on the plants by 12 hours after inoculation. Stems and leaves turned brown and eventually became blackened. The symptoms were similar to those typical of bacterial blight except the tissues became very dry. Plants that were sprayed with water and then given the 4-hour freeze treatment did not develop these symptoms.

Temperatures of $-1.8^{\circ}C$ or lower were recorded two or more times in the years when blight was a problem (Table 11). In the years when blight was not seen one or no frosts were recorded except in 1965 and 1975 when six and five such freezing periods were recorded respectively. Temperatures of $-3.5^{\circ}C$ or lower were only noted once in this 16 year period, in 1975.

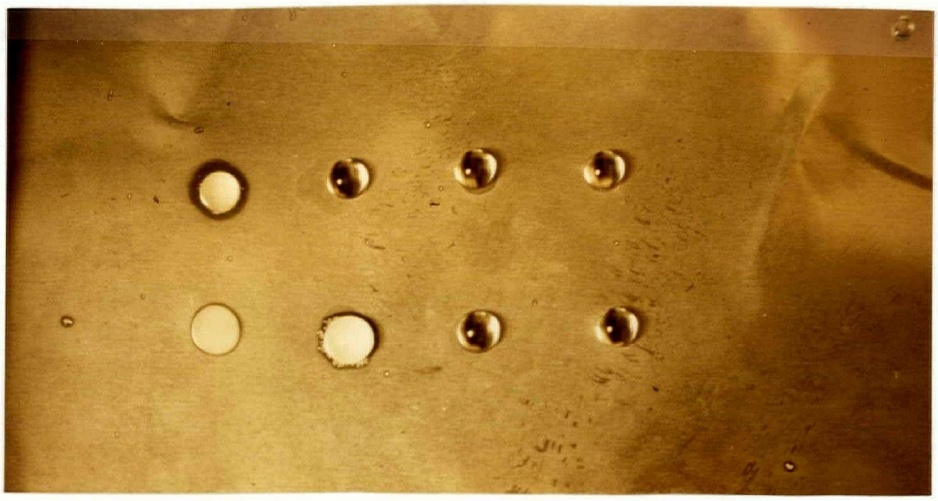


Fig. 15 Effect of culture age on ice nucleation activity of Pseudomonas syringae, Ps-346 cells grown on NGA for two days (top) and four days (bottom). Concentrations of bacteria/ml were (from left to right) 10^9, 10^7, 10^5 and SDW.

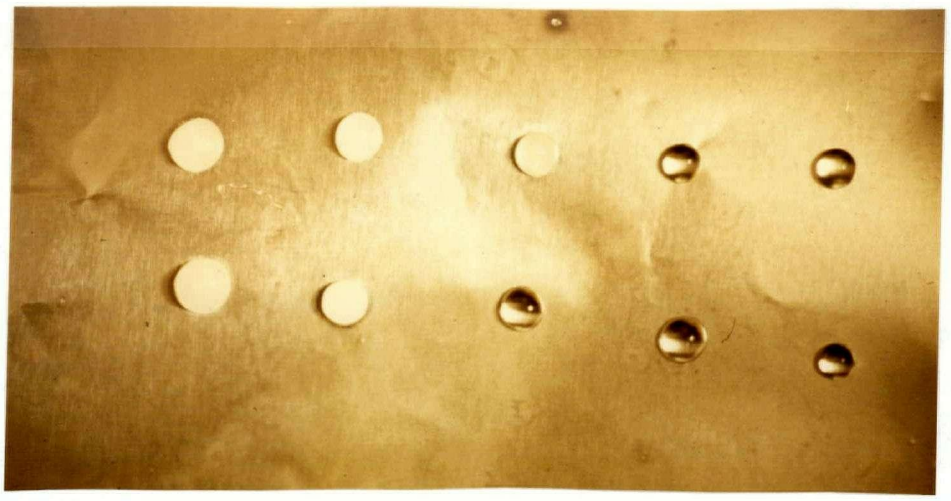


Fig. 16 Differing ice nucleation ability of suspensions of two different Pseudomonas syringae strains, Ps-205 (top) and Ps-346 (bottom) both grown on NGA for four days. Concentrations of bacteria/ml were (from left to right) 10^9, 10^7, 10^6, 10^5 and SDW.

Table 11. Numbers of nights during which temperatures of -1.8°C or lower were recorded in the years since bacterial blight of raspberry was first seen

Year	Number of nights temperatures of -1.8°C were recorded*
1963	1
+1964	3
1965	6
1966	0
1967	1
‡1968	3
1969	0
‡1970	6
1971	1
‡1972	2
‡1973	2
1974	1
1975	5
1976	0
1977	1
1978	0

*Data from Abbotsford Airport taken from Monthly Records of Canada Meterological Information, Fisheries and Environment Canada
+first year bacterial blight was seen in the Lower Fraser River Valley
‡years when bacterial blight was reported to be widespread

BACTERIA ANTAGONISTIC TO *P. SYRINGAE*

Three bacteria from the normal flora of the raspberry were found to be antagonistic to Ps-346. An 8-mm zone of inhibition surrounded isolate UN-1 on a lawn of Ps-346 (Fig. 17). Isolates UN-2 and Ps-2 caused 3-mm zones of inhibition on a Ps-346 lawn.

CULTIVAR SUSCEPTIBILITY TRIALS

Raspberry cultivars seemed to differ in their susceptibility to *P. syringae* infection (Table 12). Although definite differences could not be established in this preliminary study there was a continuum in susceptibility from Malling Leo which was rated most susceptible to Chilcotin which was most resistant (Figs. 18 and 19).

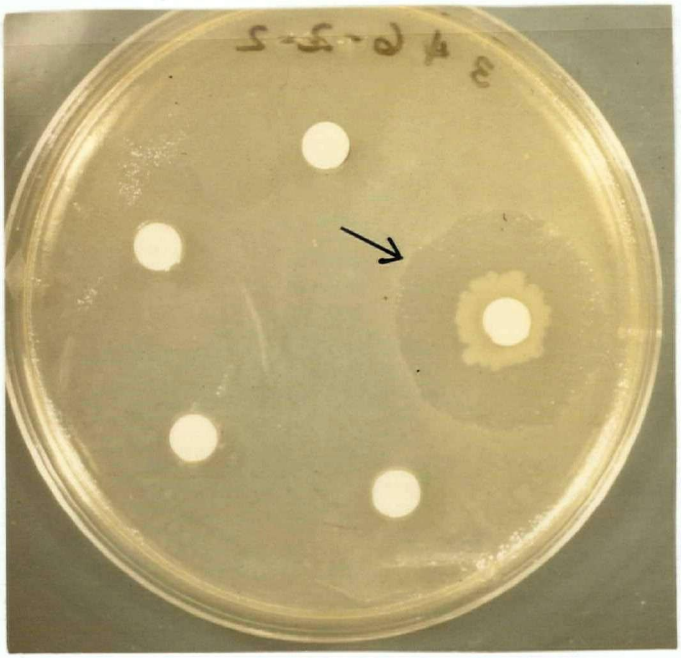


Fig. 17 Zone of inhibition of growth of Pseudomonas syringae, Ps-346 (arrow) by an unidentified bacterium from raspberry.

Table 12 Ratings of raspberry cultivars for susceptibility to *Pseudomonas syringae* infection

Cultivar	Trial Date					
	Nov. 17	Feb. 11	Feb. 17	March 2	March 13	Mean
Haida		1.5*			1.3	1.4
Malling Promise			1.8	1.8		1.8
Malling Leo			3.0	2.3	2.3	2.5
Meeker	2.2	2.0			2.0	2.1
Willamette	1.7			1.5	2.3	1.8
Chilcotin		1.3			.6	1.0
Nootka			1.3		2.0	1.7
Skeena	1.3	2.0		1.5	2.6	1.9

*ratings vary from 0-resistant to 4-highly susceptible

a

b

Fig. 18 a. Symptoms on raspberry cultivar Malling Leo caused by spray inoculations with Pseudomonas syringae (left) and water (right). b. Symptoms on raspberry cultivar Willamette caused by spray inoculation with water (left) and P. syringae (right).

a

b

Fig. 19 Symptoms and corresponding disease ratings for raspberry cultivars. a. Meeker (left) rating - 3 and Willamette (right) rating - 2. b. Skeena (left) rating - 2 and Chilcotin (right) rating - 1.

DISCUSSION

The cross-absorbed antiserum developed against isolate Ps-346 proved to be a valuable tool for the detection of P. syringae from diseased raspberry tissues from the Clearbrook area. All of the isolates recovered in the summer of 1976 and identified as P. syringae in biochemical tests reacted in drop agglutination tests with the antiserum. Of the P. syringae isolates recovered from raspberry buds during the winter of 1976-1977, 97% reacted with the antiserum. The usefulness of this antiserum for detection of P. syringae from other hosts or from other areas is not certain. The antiserum reacted with all authentic cultures from California and Wisconsin which had been isolated from a variety of hosts. However, this antiserum reacted strongly with only four of six raspberry isolates from Richmond, B.C. and did not react strongly with any of the three Forsythia isolates from B.C. These data indicate that there may be serologically different strains of P. syringae present in B.C. This is not unexpected since Otta and English (74) found 10 different serotypes of P. syringae from various hosts. Immunodiffusional analysis with antiserum prepared against other isolates of P. syringae would be required to confirm this suspicion.

Because all of the P. syringae isolates that did not react with the antiserum produced a fluorescent pigment and were oxidase negative, the scheme given in Fig. 20 can be used in future work for quick detection of P. syringae. With this scheme, detection of the typical P. syringae isolates, which accounted for greater than 90% of the isolates recovered, can be completed in 2 days and the remaining atypical isolates can be identified in

an additional 2 days. Before the antiserum was employed, 6 to 10 days were required to identify an isolate as P. syringae. One reservation remains, however, due to the finding by Pepin et al. (79) of pathogenic P. syringae isolates that did not produce a fluorescent pigment. In this current study such isolates were found and all of them reacted with the antiserum so they would be detected by the scheme in Fig. 20. Problems would arise if isolates are found in the future that do not react with the antiserum and do not produce fluorescent pigment.

The FAS technique could not be routinely used for the detection of P. syringae from raspberry. Cells of an unidentified saprophytic bacterium commonly present in the microflora of raspberry were non-specifically stained. Although the unknown bacterium had cells larger than those of P. syringae and did not stain as brightly it was still difficult to differentiate these cells from P. syringae cells. Cross-absorption of the antiserum with the unknown was not attempted because P. phaseolicola cells still stained faintly with the FITC-conjugated antiserum cross-absorbed with P. phaseolicola. The increased sensitivity of the FAS technique proved to be its downfall here. Even slight reactions between antiserum and bacterial cell give positive results. In the drop agglutination method, however, greater reaction of antiserum with bacterial cells must occur for the formation of the large precipitate needed for visualization at 6 x magnification. The drop agglutination technique was therefore preferred in this epidemiological study.

The recovery of P. syringae from the buds of dormant raspberry canes before they were sprayed with a bacterial suspension indicated P. syringae naturally inhabits the buds of raspberry plants. The high populations of P.

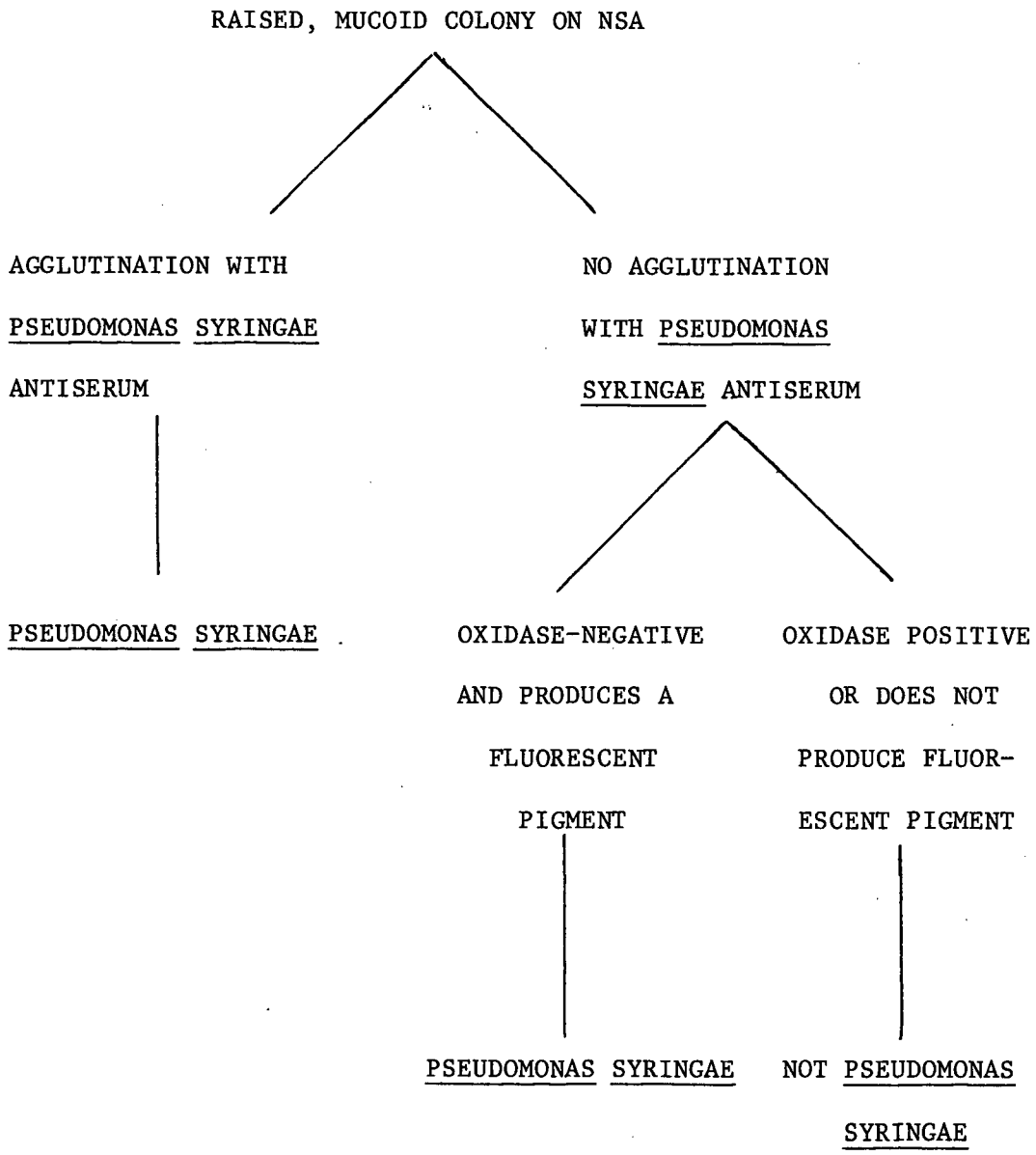


Fig. 20 Scheme for rapid detection of Pseudomonas syringae from raspberry.

syringae in the buds after spraying with the bacterial suspension quickly dropped off to a level equal to the natural populations in the water-sprayed buds. This would suggest that there is an optimum number of P. syringae which can be naturally supported in the buds. Cameron (18) and Crosse (24) also found P. syringae to overwinter in the buds of Rosaceous hosts. It was surprising to find P. syringae overwintering in the raspberry buds here because very little bacterial blight was seen in the fields in the two years prior to monitoring the buds and it had been suspected that no P. syringae would be found naturally in the fields. The incitant of bacterial blight therefore is not dependent on the presence of an alternate host for overwintering. This finding does not preclude the possibility that the infected lilac was the original source of inoculum. From the raspberry buds the bacteria are readily available for infection of the laterals that develop from the buds.

The isolation of pathogenic P. syringae from healthy leaves throughout the summer indicated the pathogen is present on the raspberry during this time although no symptoms are evident. This concurs with the finding of P. syringae existing as an epiphyte on the leaves of peach and almond (37). That populations of 10^3 CFU/leaf of P. syringae survived on raspberry leaves for at least 6 weeks in greenhouse tests is further evidence that a stable population can be maintained on apparently healthy leaves.

The leaf spot symptom first observed on raspberry in the summer of 1976 was shown to be caused by P. syringae. While these spots could be found throughout the summer, recovery of bacteria from them proved to be more difficult after a dry period. The 2 of 18 attempts to isolate P. syringae from leaf spots that failed were done on leaves collected during a dry period

in July. Crosse (22) also found isolations from leaf spots to be more difficult when the leaf became mature and the necrotic tissue became dry. This leaf spot phase is an important part of the disease cycle because it provides a means for the bacterium to survive the dry, hot summer in a place where it is readily available to cause infection in the fall when more suitable environmental conditions return. The bacteria can also be rain-splashed from the leaves onto the developing buds of the first year canes and the disease cycle is complete (Fig. 21).

While it is possible to propose a disease cycle, the reasons why the disease is so erratic still remain a mystery. Very little change has occurred in the host plant. The same cultivar of raspberry is grown now as was grown in the years when the disease was a problem. No cultural practices or natural processes that might have changed the degree of wounding of the host plant have occurred during these years. Chicken manure is no longer applied to raspberry fields in large quantities which may somewhat reduce the susceptibility of the host to disease but this alone cannot account for the disappearance of bacterial blight. No marked change in weather patterns has occurred over the years. Wet, cool periods are recorded every spring and fall in B.C. There is indication that freezing temperatures do have an effect on the occurrence of blight. Arny et al. (2) found that damage to corn plants sprayed with P. syringae occurred at temperatures of -3.5 to -4°C. Temperatures in this range were recorded only one night in B.C. during the period of March 25 to May 30 in the years 1963 to 1978. A temperature of -4°C was recorded in 1975, a year when blight was not a problem. Maki et al. (63) found suspensions of P. syringae would freeze from -1.8 to -3°C in

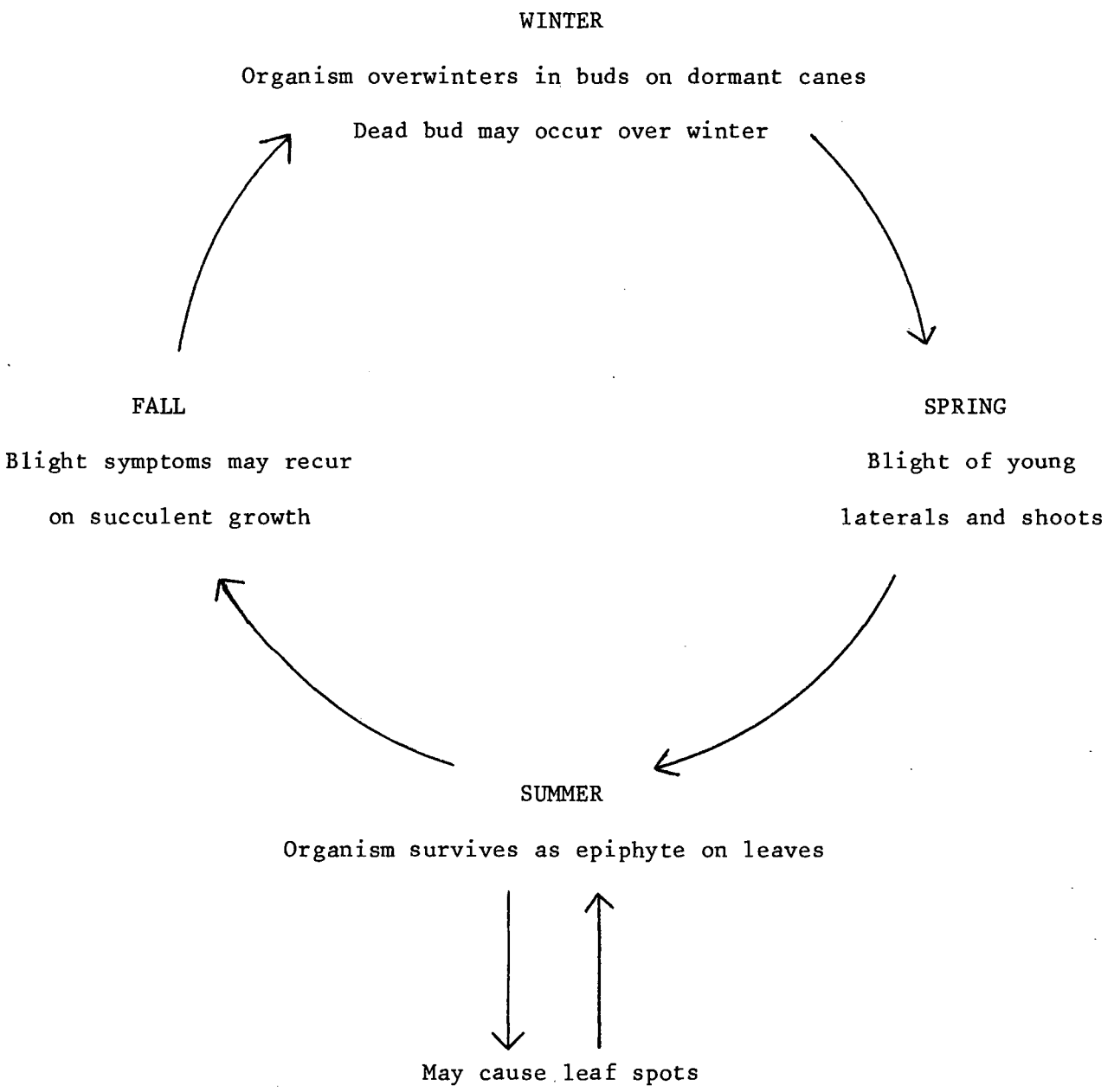


Fig. 21 Proposed disease cycle of bacterial blight of raspberry caused by Pseudomonas syringae.

laboratory tests and in a preliminary test in this current study damage to young raspberry plants sprayed with P. syringae was recorded after the plants had been exposed to -2°C for 4 hours. In years when blight was a problem (1968, 1970, 1972, 1973) temperatures of -2°C or lower were recorded two or more times between March 25 and May 31. One or no such frosts occurred in the other years when bacterial blight was not noticed except in 1965 and 1975 when temperatures below -2°C were recorded six and five times respectively. So except for these 2 years a higher number of frosts were recorded in years when blight was a problem than in years when it was not seen. Further experimentation must be done to first establish absolutely the role of freeze injury caused by P. syringae in bacterial blight and second, the exact conditions required for ice nucleation (concentration of bacteria needed, the exact temperature and time of exposure to this temperature).

There is one other possible explanation for the change in the incidence of bacterial blight. The incitant may have changed genetically over the years to a form that is not highly virulent. This supposition is based on the fact that it was difficult to produce symptoms on plants when conducting pathogenicity tests in this study even with cultures freshly isolated from diseased raspberry plants. In the original work done on this disease, Pepin et al. (79) did not have problems reproducing symptoms in greenhouse tests when freshly isolated cultures of P. syringae were used. Also in the Geotrichum candidum bioassay raspberry strains of P. syringae from B.C. usually caused zones of inhibition ranging up to 10-mm wide while isolates from various hosts tested by Gross (41) in California produced inhibition zones ranging up to 20 mm. It

may be that highly virulent isolates of P. syringae were on the infected lilac found in the first field infected with bacterial blight. If its virulence was due to a plasmid, as has been suggested for other pathogenic bacteria (55), it would have been passed to the natural population of P. syringae on the raspberry. The plasmid may have been lost from the isolates in the field as they have a tendency to be lost from the bacteria while in storage (76).

The role of P. syringae in the dead bud syndrome could not be exactly established. The ice nucleation ability of P. syringae provides an explanation for the syndrome but further experimentation in the laboratory must be done to confirm that the presence of P. syringae in buds exposed to freezing temperatures increases the chance of bud death. Since the populations of P. syringae in the raspberry buds were monitored over the winter of 1976-1977, it was hoped that a relationship between bacterial population levels and the number of dead buds in the spring could be found. However, spur blight, caused by Didymella aplanata (Niessl) Sacc. was also prevalent and could also have been responsible for bud deaths. It was therefore not possible to ascertain the cause of the bud deaths seen. Only under controlled environment conditions using raspberry canes free of spur blight can the relationship of dead bud and the ice nucleation ability of P. syringae be established.

No chemical control methods were attempted during this study because there was no natural outbreak of the disease in the field and because of the difficulty of infecting plants in the greenhouse. If a natural outbreak does occur it would be interesting to attempt to control the disease with sodium tartrate as suggested by Sands and McIntyre (86).

The bacteria found in this study that were antagonistic to P. syringae have potential in control of bacterial blight. Because they were a natural component of the raspberry microflora, attempts to increase the populations of the antagonists by spraying suspensions onto host plants pose no threat to the health of the host plant. Even if the natural populations of the antagonists cannot be increased for long periods of time as Crosse (25) found, there is still hope for control because the antagonists produce substances that diffused out into the agar medium. If these substances are produced in liquid cultures it may be possible that the substances could be sprayed on the plants. Further experimentation must be done to test these possibilities.

Some cultivars were found to be more resistant to P. syringae infection than others. This confirms the report by Pepin et al. (79). Chilcotin showed less susceptibility to infection that most cultivars. Its Newburgh parent was also found to be resistant in tests done by Pepin et al. (79). Nootka was found to display a susceptibility similar to its Willamette parent. The results obtained in these cultivar trials must be accepted with caution because of the difficulty of obtaining infection in greenhouse trials and the small number of times these trials were replicated. Observations on infection of the cultivars during natural infection in the field must be made before meaningful conclusions about their relative resistances can be made.

Many questions remain unanswered but they will remain so until an epiphytotic of bacterial blight is again seen in the fields, an event enlightening to the academic but disastrous in the short term to the farmer.

THESIS SUMMARY

1. Typical P. syringae isolates were confirmed as the causal agent of bacterial blight of raspberry in B.C.
2. Nutrient sucrose agar was found to be the best medium for isolation of P. syringae from raspberry tissues.
3. The biochemical tests that are most useful for identification of an isolate as P. syringae are gram reaction, oxidase test, arginine dihydrolase test, polypectate gel pitting ability, fluorescent pigment production, tobacco hypersensitivity, toxin production bioassay, lactate utilization and production of levan when grown on sucrose medium. P. syringae isolates typically gave negative results for the first four tests and positive results for the last five although some raspberry isolates were found that did not produce a fluorescent pigment, could not utilize lactate or did not produce a toxin.
4. For pathogenicity testing, the leaf-spray inoculation technique was preferred to the stem-prick inoculation because symptoms developed sooner and fewer bacteria were required to initiate disease.
5. The ability to produce toxin improved an isolate's pathogenic capabilities but this ability was not essential for pathogenicity.
6. The best method for long-term storage of P. syringae isolates was on nutrient glycerol agar slants at $5^{o}C$. Isolates remained viable and pathogenic for 1 year when stored in this manner.
7. An antiserum prepared against P. syringae was useful for quick detection of this organism from raspberry especially when used in conjunction with

tests for levan production on a sucrose medium, fluorescent pigment production and oxidase reaction. Three days were required for completion of these tests.

8. P. syringae was found to naturally populate 25-75% of raspberry buds during the winter months. Populations of about 10^4 CFU/six bud sample are most common in buds that do contain bacteria.
9. During the spring and summer months P. syringae was found to survive as an epiphyte on leaves of raspberry plants in the field. In greenhouse tests, P. syringae survived on healthy raspberry leaves for at least 6 weeks. P. syringae was also shown to cause brown spots surrounded by yellow halos on the leaves of raspberry during this time.
10. About 90% of P. syringae isolates from raspberry were ice nucleation active. Raspberry plants that had been sprayed with a P. syringae suspension and then held at $-2^{\circ}C$ for 4 hours developed symptoms similar to those of bacterial blight within 12 hours of the freeze treatment.
11. Bacteria were found in the normal microflora of the raspberry that were antagonistic to P. syringae in vitro and may have potential for control of bacterial blight.
12. In preliminary tests, raspberry cultivars showed varying degrees of susceptibility to P. syringae infection indicating some control of bacterial blight might be achieved by growing resistant varieties.

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