Bacterial leaf and stem blight of safflower in Montana : its epidemiology, sources of resistance and inheritance of resistance
by Darrel Lee Jacobs

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Plant Pathology
Montana State University

Abstract:
Safflower (Carthamus tinctorius) is an important alternate crop grown in the dryland areas of north central, south central and eastern Montana, northwest South Dakota and western North Dakota.

During the 1978 growing season, a severe leaf necrosis and stem blight of safflower occurred. Symptoms included the development of irregular, reddish-brown, necrotic lesions on leaves and bracts, and brown necrotic lesions on stems and petioles. During periods of warm weather, necrotic lesions turned white to milky colored. The disease was determined to be incited by a bacterium resembling Pseudomonas syringae based upon: consistent isolation of a fluores- cent pseudomonad from infected tissue; successful inoculation of seedlings with the isolated bacterium; reisolation from inoculated tissue which exhibited symptoms similar to those observed in the field and identification of the bacterium based on phenotypical tests.

Safflower seed, produced in Montana, was infested with P. syringae and the pathogen was transmitted to the aerial parts of the seedling. In addition, P. syringae was isolated from weeds, plant debris and soil suggesting these may be possible sources of inoculum for bacterial blight of safflower.

A greenhouse method was developed to screen safflower varieties or lines for resistance to P. syringae; subsequently, many varieties and lines were found to vary in resistance. Several commercial varieties of safflower exhibiting some degree of resistance included: Sidwill, Hartman and Rehbein. A commercially val- uable level of resistance was present in 88-74-2, N-4051-1 and N-1-1-5.

No specific mode of inheritance of disease resistance was discerned; however, results suggested that resistance to P. syringae was heritable. The segregation of progenies into a range of disease index classes suggested the character of inheritance to be polygenic.

Considering the apparent ubiquity of P. syringae an integrated approach appears to be the best means of controlling bacterial blight of safflower. The following control measures were recommended: 1. resistant varieties; 2. planting disease free seed; 3. avoiding production of other susceptible crops in the same rotation; and 4. growing susceptible safflower only once every 4-5 years on the same field.

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BACTERIAL LEAF AND STEM BLIGHT OF SAFFLOWER IN MONTANA: ITS EPIDEMIOLOGY, SOURCES OF RESISTANCE AND INHERITANCE OF RESISTANCE

by

DARREL LEE JACOBS

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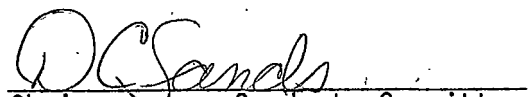

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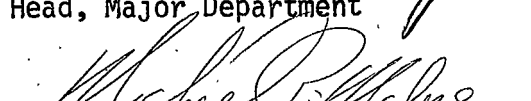

Graduate Dean

Montana State University
Bozeman, Montana

August, 1982

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ABSTRACT

Safflower (Carthamus tinctorius) is an important alternate crop grown in the dryland areas of north central, south central and eastern Montana, northwest South Dakota and western North Dakota. During the 1978 growing season, a severe leaf necrosis and stem blight of safflower occurred. Symptoms included the development of irregular, reddish-brown, necrotic lesions on leaves and bracts, and brown necrotic lesions on stems and petioles. During periods of warm weather, necrotic lesions turned white to milky colored. The disease was determined to be incited by a bacterium resembling Pseudomonas syringae based upon: consistent isolation of a fluorescent pseudomonad from infected tissue; successful inoculation of seedlings with the isolated bacterium; reisolation from inoculated tissue which exhibited symptoms similar to those observed in the field and identification of the bacterium based on phenotypical tests.

Safflower seed, produced in Montana, was infested with P. syringae and the pathogen was transmitted to the aerial parts of the seedling. In addition, P. syringae was isolated from weeds, plant debris and soil suggesting these may be possible sources of inoculum for bacterial blight of safflower.

A greenhouse method was developed to screen safflower varieties or lines for resistance to P. syringae; subsequently, many varieties and lines were found to vary in resistance. Several commercial varieties of safflower exhibiting some degree of resistance included: Sidwill, Hartman and Rehbein. A commercially valuable level of resistance was present in 88-74-2, N-4051-1 and N-1-1-5.

No specific mode of inheritance of disease resistance was discerned; however, results suggested that resistance to P. syringae was heritable. The segregation of F_2 progenies into a range of disease index classes suggested the character of inheritance to be polygenic.

Considering the apparent ubiquity of P. syringae an integrated approach appears to be the best means of controlling bacterial blight of safflower. The following control measures were recommended: 1. resistant varieties; 2. planting disease free seed; 3. avoiding production of other susceptible crops in the same rotation; and 4. growing susceptible safflower only once every 4-5 years on the same field.

CHAPTER 1

BACTERIAL LEAF AND STEM BLIGHT OF SAFFLOWER

Introduction:

Pseudomonas syringae is the incitant of bacterial leaf and stem blight of safflower in Montana, South Dakota and North Dakota. The disease on safflower was first described in California in 1964 (18). However, Klisiewicz et al. (36) described a similar disease in northern California in 1963, caused by an unnamed but apparently similar pseudomonad. *P. syringae* is a ubiquitous bacterium found throughout the world and as a group has been reported pathogenic on at least 40 different plant genera (66) including lilac, bean, cherries, sorghum, wheat, peas, tomato, corn, safflower, etc. The taxonomic difficulty presented by this species is that certain host range limitations do exist for specific strains, however, clear cut physiological differences are not yet known.

Pseudomonas syringae is a fluorescent, oxidase negative rod (0.7 - 1.2 by 1.5 - 3.0um) with polar multitrichous flagella (13). Considerable effort has been put forth in many plant systems to understand *P. syringae's* epidemiology, to determine its taxonomic status and to devise control measures.

The objectives of this study are to investigate the following areas of bacterial blight of safflower in Montana:

1. to identify and describe the principal incitant of leaf and stem blight of safflower.

2. to determine sources of primary inoculum.
3. to determine the mode of transmission.
4. to develop a greenhouse seedling screen for safflower resistance to P. syringae.
5. to determine sources of genetic resistance.
6. to determine the mode of genetic inheritance of safflower resistance to P. syringae.

Symptoms of Bacterial Blight of Safflower:

During periods of cool, moist conditions (18, 36), safflower may become severely infected with P. syringae resulting in the development of irregular, reddish-brown, necrotic lesions on leaves (18, 22, 36). In addition, dark brown to black water-soaked lesions may develop on stems and leaf petioles (22, 36). Safflower in the rosette stage exhibits necrotic streaks and spots on the leaves and a dark nearly black necrosis in pithy tissues of stems and roots which may result in death of the plant (18, 22). Disease development is inhibited during periods of dry, warm weather conditions, afterwhich, infected plants usually recover.

Inoculation and Screening Techniques for P. syringae Resistance:

The various methods described for inoculating P. syringae onto various hosts for purposes of screening for varietal resistance are similar. Inoculum was produced by growing the bacterium on a rich, bacteriological medium conducive for growth, i.e.

nutrient dextrose agar, King's medium B, tryptic soy agar, etc., for 24-48 hours at room temperature (5, 7, 26, 72). The resulting culture then was suspended in sterile water (5, 7, 26, 72). The bacterial suspension was standardized at a specific optical density with a Bausch and Lomb Spectronic 20 colorimeter (5, 26) to obtain a specific concentration of bacterial cells/ml of water. Seedlings were inoculated by spraying inoculum against the underside of the leaf with a DeVilbiss atomizer attached to an air line at 15-20 psi (5, 26, 72). After inoculation, plants were either placed in a mist chamber for a few hours and then transferred to a greenhouse bench or transferred directly to a greenhouse bench without exposure to a high humidity chamber (26, 72). Disease reactions were recorded approximately a week after inoculation.

Taxonomy of P. syringae:

A taxonomic problem exists in regard to the phytopathogenic, fluorescent pseudomonads. The nomenclatural confusion, illustrated by the listing of over 60 nomenspecies of phytopathogenic, fluorescent pseudomonads in the 7th ed. of Bergey's Manual to the listing of only 2 species in the 8th ed. of Bergey's Manual, has created quite a controversy among plant pathologists (14). The definition of P. syringae according to Doudoroff and Palleroni (13) essentially followed the recommendations of Sands et al. (58).

Sands et al. (58) proposed that most fluorescent, phytopathogenic nomenspecies, according to the 7th ed. of Bergey's Manual be placed under the group P. syringae. The basis for this recommendation was the impossibility to distinguish the nomenspecies listed on a phenotypic basis. The major source of phenotypic characterization was based upon nutritional tests; i.e. ability to utilize various substrates for growth.

The confusion surrounding the taxonomy of fluorescent, phytopathogenic psuedomonads has been largely attributed to early plant pathologists naming many species on the basis of host specificity and the type of symptoms elicited (13). However, as Hildebrand (28) suggested, the host is serving as a complex medium for the pathogen and this complex medium supports the growth of only a select few pathogens. This would indicate that different pathogens, based on host specificity, should differ nutritionally. Furthermore, many investigators have shown host specificity among the ecotypes of P. syringae (9, 16, 20, 56). Therefore, routine recognition of species through nutritional screening may be feasible if the appropriate nutritional tests are discovered and utilized.

Future studies may help to clarify taxonomy and nomenclature of the nomenspecies and pathotypes included in P. syringae. Numerical analysis suggests there may be a clustering of strains

around certain nomenspecies (58). Nucleic acid homology studies (50, 52) also have shown that some species goups were sufficiently closely related to each other to be united into large nucleic acid homology groups. Therefore, besides nutritional tests, other physiological tests such as phage typing, serological comparison, disc-gel electrophoresis and nucleic acid homology should be utilized to identify and classify species within the P. syringae group.

Sources and Dissemination of P. syringae:

There have been many reports describing sources of P. syringae inoculum in different host systems including soil-borne inoculum, epiphytic populations* on weeds or crops and seed-borne inoculum. P. syringae has been reported as being ubiquitous* in soils and as usually occuring in the rhizosphere* of various plants (15, 60, 68). Furthermore, it has been suggested (60, 68) that soil-borne inoculum is adequate for initiation of disease epidemics. Epiphytic populations of P. syringae on host and non-host plants also may be a source of disease-inciting inoculum (8, 15, 60). Ercolani et al. (17) established a correlation between large epiphytic populations of P. syringae on hairy vetch

* see Appendix B for description of term.

and subsequent outbreaks of bacterial brown spot of beans in adjacent fields. Weeds also were suggested as a source of P. syringae inoculum for bacterial canker of stone fruit trees (15), blast of pears (70) and bacterial canker of cherry (40). In addition, Latorre et al. (40) suggested plant refuse as a source of primary inoculum for bacterial canker of cherry and that P. syringae could over-winter on weeds in Michigan. However, Hoitink et al. (30), in contrast to Ercolani (17), failed to isolate P. syringae from weeds surrounding diseased bean fields and reported that infected seed was the principal source of inoculum. Furthermore, Fryda et al. (19) reported P. syringae moving from infected wheat seeds to the aerial parts of the seedling and becoming part of the epiphytic microflora, thus, showing seed-borne P. syringae as a possible source of inoculum for bacterial blight of wheat. Grogan et al. (23) and Kennedy (31) also have reported that bacterial infected seed may be the major source of primary inoculum in other pathogenic pseudomonad-host systems.

Many means of bacterial inoculum dissemination have been reported including wind-blown soil and cultivation practices. However, rainstorms accompanied with wind is probably the chief means of dissemination of P. syringae from plant to plant or field to field. Ercolani et al. (17) reported P. syringae as being

spread from weeds to bean fields during rainstorms. In addition, several investigators have indicated that a succession of wind - rainstorms may result in bacterial blights of epidemic proportions (10, 69, 71). Venette et al. (69) reported P. glycinea as surviving in rain aerosols for over 8 hours under laboratory conditions, providing ample time for infections to occur. In addition, Langhans et al. (38) reported that dissemination by rain may be the major mode of introducing and spreading P. syringae in cereal fields. P. syringae also was isolated from rainstorms at altitudes ranging from 500 to 5,000 feet (38).

Relation of P. syringae Within Tissues and Cells:

P. syringae occurs naturally on leaf and stem tissue of safflower (18, 36). In addition, Hoitink et al. (30) and Langhans et al. (38) reported the isolation of P. syringae from the surface of bean and cereal seeds respectively. Leben et al. (43) found P. syringae capable of colonizing the bud and inside surface of stipules and spreading eventually to the leaves of beans. P. syringae enters the plant via stomata, hydathodes or wounds (53, 72) and subsequently multiplies within the intercellular spaces of the leaf (11, 72). Magyarosy et al. (44) reported that P. phaseolicola decreased the rate of photosynthesis in infected bean leaves by destruction of chloroplast membranes.

There have been suggestions that the production of a toxin, syringomycin (SR), by isolates of P. syringae, may be correlated to pathogenesis (12, 24, 30, 63). DeVay et al. (12) ascribed a major role for SR in the bacterial canker disease of peach and Hoitink et al. (30) found that all isolates of P. syringae from bean that caused bacterial brown spot also produced SR. However, several workers have reported there was no positive correlation between SR production and pathogenicity (3, 49, 55).

Resistance:

The ultrastructural and physiological basis for the resistance of plants infected by P. syringae has not been well defined. Plants inoculated with incompatible bacteria results in a hypersensitive reaction (HR) (34). The main characteristics of the HR in the host are that the cells of the tissues containing the bacteria lose their turgor, collapse and become necrotic resulting in localization of the pathogen (35). Recent evidence suggests attachment of bacterial cells to host cell walls as being an initial step in the induction of a HR. Incompatible bacteria are reported to attach readily to host cell walls followed by envelopment from material arising from the host cell (4, 21, 61). In contrast, Daub et al. (11) reported that envelopment of compatible and incompatible bacteria in the intercellular spaces occurred

only rarely in both resistant and susceptible bean leaves. Alosi et al. (2) also suggested the envelopment response in beans as being nonspecific.

However, differences in regard to symptom expression, time of symptom development and multiplication of bacteria in various host-bacteria combinations; i.e. imcompatible and compatible pathogens inoculated into susceptible and resistant hosts, have been observed (6, 11, 24, 46, 64). Incompatible and compatible pseudomonads multiplied rapidly in the intercellular spaces for the first few hours (11, 24, 56). In contrast, multiplication of compatible bacteria in a susceptible host continued with final populations reaching 10^4 times the original cell density with subsequent typical disease expression (11, 24, 56). The multiplication rate of compatible bacteria in resistant hosts was slower as compared to the multiplication rate in susceptible hosts with the length of the growth phase being the same (11). However, several investigators have reported that the duration of the growth phase was shorter in resistant hosts that in susceptible hosts (6, 46, 64). In either case, final bacterial populations are least in the resistant host.

Control of P. syringae:

Various measures have been tried to control leaf-spotting

bacterial diseases without complete success. Since the seed of a number of annual plants (i.e. bean, soybean and wheat) is an important mode for survival and dispersal of P. syringae (19, 23, 30, 31), the use of disease free seed is an important control measure. Disease free seed may be obtained by either production of seed in areas where the pathogen does not develop or by use of chemical seed treatments. However, control methods for P. syringae aimed at breaking the pathogen-seed association would not be as useful if the pathogen survived season to season in plant debris, if it possessed a resident phase on weeds and crops, or if it is soil-borne. At least a three year crop rotation also has been suggested (1) for control of bacterial blights in order to decompose crop residue and thereby eliminate soil-borne inoculum. However, this measure also may not be successful if the pathogen exists as a resident phase on weeds. Copper containing bactericides used as a foliar spray may reduce the severity of bacterial blight (1), although, it may be economically unfeasible. The development of resistant varieties to P. syringae appears to the the best means to control bacterial blight of safflower. Resistant or tolerant varieties have been described in other P. syringae - plant systems (5, 47, 54, 59, 72). For example, Otta (47) and Scharen et al. (59) have reported resistant varieties of both winter and spring wheats to P. syringae. In addition, Dr. J.W. Bergman (personal

communication) has reported that the safflower varieties 'Sidwill', 'Hartman' and 'Rehbein' possess at least moderate resistance to *P. syringae*.

CHAPTER 2

BACTERIAL LEAF SPOT AND STEM BLIGHT OF SAFFLOWER IN MONTANA AND ITS EPIDEMIOLOGY

Safflower (Carthamus tinctorius) was first introduced into Montana in the 1920's. Once established, it is more drought tolerant than other annual crops. Safflower is important in dryland crop rotation practices with dryland cereals to break disease cycles and to control weeds that build up in a strict small grain rotation. Safflower production also is helpful in the dryland areas having saline seeps as safflower is deep rooted and has a long growing season both of which are beneficial in extracting surplus soil moisture from the contributing recharge areas of the seeps. The development of safflower as an alternate crop for Montana has progressed significantly as evidenced by the production of 180,000 acres in 1979.

During the 1978 growing season in which cool, moist conditions prevailed a severe leaf necrosis and stem blight of safflower occurred. Symptoms included the development of irregular, reddish-brown, necrotic lesions on leaves and bracts, and brown, necrotic lesions on stems and petioles. In addition, safflower in the rosette stage exhibited dark water-soaked lesions on leaves. Erwin et al. (18) and Klisiewicz et al.(36) described similar symptoms on safflower infected with Pseudomonas syringae. With the onset of dry, warm weather, disease development was in-

hibited and necrotic lesions turned white to milky colored. However, with the onslaught of rain and cool temperatures in July disease development recurred spreading from the lower infected leaves of the plant to the upper portions of the plant. Erwin et al. (18) first completely described bacterial leaf and stem blight of safflower caused by P. syringae in 1964. Klisiewicz et al. (36) described bacterial blight of safflower caused by an unnamed pseudomonad in a report in 1963. However, no information regarding the epidemiology of the pathogen was provided to aid in devising possible control measures.

The principal objectives of this study were to identify and describe the incitant of leaf and stem blight of safflower in Montana; to determine the sources of primary inoculum; and to determine the mode of transmission. In addition, possible control measures are discussed.

Materials and Methods:

Isolation of P. syringae from safflower plants and pathogenicity tests.

Isolations were made from the edge of necrotic lesions cut from leaves and stems of safflower plants located at the Eastern Agricultural Research Center, Montana Agricultural Experiment Station, Sidney, Mt. (MAES). Samples were surface sterilized for 1 minute in 0.5% sodium hypochlorite and washed 3 times in sterile

distilled water. Samples then were placed directly on BCBRVB agar plates (King's medium B (MB) (32) to which 100 ppm of Cycloheximide, 500 ppm of Benlate (50% active), 10 ppm of Bacitracin, 6 ppm of Vancomycin and 0.5 ppm of Rifampicin was added after autoclaving) or alternately placed into a test tube containing 1 ml of sterile deionized water. After 4 hours incubation, the test tube suspension was streaked onto BCBRVB agar plates. The BCBRVB plates were examined for bacterial colonies after 2 days incubation at room temperature. Each isolate was examined for oxidase activity (37), arginine dihydrolase (67), production of a fluorescent pigment (32) and ability to induce a hypersensitive reaction in tobacco leaves (33)*. Fluorescent isolates that gave a negative reaction for the first two tests were regarded as potentially P. syringae.

Two methods showing pathogenicity were utilized. With the first method, seeds of the S-208 variety were surface sterilized before planting in pots containing sterilized soil. The pots were placed on a greenhouse bench and carefully watered with sterile water. Two week old safflower plants were inoculated by wounding the leaves with carborundum and subsequently spraying the leaves to run-off with a deVilbiss atomizer containing a bacterial

* see Appendix A for complete description of tests.

suspension. The bacterium used in the suspension was grown on MB for 24 hours and then suspended in sterile deionized water at a concentration of about 6 X 10^7 cells/ml. Plants inoculated with sterile water served as a control. Inoculated and noninoculated plants were placed in separate incubation chambers at 95 - 100% relative humidity and at a temperature of 21 C for 2 - 3 days; afterwhich, the plants were placed on the greenhouse bench. Disease reactions were recorded 7 days after inoculation.

The second method involved vacuum infiltrating leaves of three week old safflower plants of S-208 (susceptible) and Sidwill (resistant) varieties with a 10^8 or 10^4 cells/ml bacterial suspension. Bacterial suspensions were prepared as described above The aerial portions of plants were immersed in the proper dilutions of inocula contained in 500 ml beakers and vacuum infiltrated at 15 cm Hg. After 2 minutes the vacuum was released suddenly and the procedure repeated. After immersion in the suspension, the plants were rinsed under running water and placed on a greenhouse bench. Safflower leaves were sampled on days 0, 1, 2, 3, 5, 7 and 9 for bacterial population determinations. Bacterial populations in leaves were monitored by removing four 7 mm diameter disks from each of the 2 primary leaves per plant. Each group of 8 disks was ground in 1 ml of phosphate buffer (0.05 M, pH 6.5). The slurry was diluted with 9.0 ml of buffer followed by standard $\log_{10}$

serial dilutions in sterile phosphate buffer. At each dilution three 0.1 ml samples were spread on BCBRVB agar plates and incubated for 3 days at 24 C. The data reported are the means of three replications.

Identification.

Bacteria, isolated from naturally infected safflower plants, were identified based upon the criteria of Doudoroff and Palleroni (13). The wide range of biochemical and physiological tests utilized were those outlined by Stanier et al. (65) and Shinde et al. (62)*. The only modification of standard tests was in the standard mineral base for the nutritional tests. The following minerals were added to 1 liter of distilled water: $(NH_4)H_2PO_4$, 1.0 g; KCl, 0.2 g; $MgSO_4$ $7H_2O$, 0.2 g.

Isolation from seed and seed transmission.

Isolations were obtained from 3 seed lots of each variety 'Sidwill' and 'S-208' which had been produced at MAES. Each seed lot consisted of 100 seeds. Seeds were plated directly onto BCBRVB agar plates or surface sterilized for 10 minutes. Prior to placement onto agar plates, surface sterilized seed was crushed with sterile pliers to determine if the pathogen was borne in-

* see Appendix A for complete description of tests.

ternally in the seed. Isolates obtained were determined to be potentially P. syringae based on the results of the oxidase test, the arginine dihydrolase test and the ability to induce a hypersensitive reaction in tobacco leaves.

Seed of the variety 'S-208' was surface sterilized, placed on MB and allowed to germinate. Germinated seeds which were not contaminated with any bacteria or fungi were aseptically transplanted into pots. Non-surface sterilized seed also was planted in pots. In addition, seeds were inoculated with P. syringae isolated from diseased safflower leaves. The bacterial suspension was prepared as described above at a concentration of 1×10^8 cells/ml. Seeds were placed in the suspension for 10 minutes; afterwhich, the seed was air dried at room temperature and planted into pots. Pots of all treatments (4 seeds per 8 cm diameter pot) contained sterilized soil. Each pot was separated on a greenhouse bench and watered carefully with sterile water. Soil sterilization was completed by autoclaving a 1 inch layer of soil for 3 hours at 121 C and 30 psi. Five days after emergence, seedlings were excised aseptically above the soil line and placed in 5 ml of sterile deionized water. After 4 hours, five 0.5 ml samples of each suspension were pipetted onto separate plates of BCBRVB, spread with a L-shaped rod and incubated for 3 days at room temperature. All fluorescent bacterial isolates, obtained from each 0.5 ml sample,

were determined to be potentially P. syringae based on the results of the oxidase test, the arginine dihydrolase test and the ability to induce a hypersensitive reaction in tobacco leaves.

Isolation of P. syringae from weeds, plant refuse and soil.

Approximately 10 g fresh weight leaf samples of symptomless weeds (dicotyledons and monocotyledons) were collected from weedy borders adjacent to a safflower field at MAES. Approximately 15 g fresh weight samples of semi-decomposed safflower debris and 20 random soil samples (20 grams) also were collected. The leaf and debris samples were placed in flasks containing sterile distilled water for 4 hours. One gram from each of the soil samples was thoroughly mixed in 100 ml sterile water in a Waring blendor and allowed to settle for 20 minutes. The wash water of the leaf, plant refuse and soil samples were diluted in a $\log_{10}$ series with sterile water and 0.1 ml portions were spread on the surface of BCBRVB agar plates. Samples were collected from a field at MAES that had been continuously cropped with safflower since 1961. Collection dates were June, July and August 1978 and June, July and August 1979. Fluorescent isolates were determined to be potentially P. syringae based on the results of the oxidase test, the arginine dihydrolase test and the ability to induce a hypersensitive reaction in tobacco leaves.

Results:

Isolation of *P. syringae* from safflower plants and pathogenicity tests.

Both methods employed to isolate the pathogen from margins of lesions on stems and leaves of infected safflower plants consistently yielded cultures of fluorescent, oxidase negative bacterial colonies. In addition, the bacterial isolates were negative for arginine dihydrolase and positive for induction of hypersensitivity in tobacco.

Since most leaf-blighting pseudomonads exhibit the above characteristics (29, 51, 58), a typical isolate was selected for completing pathogenicity tests. Symptoms developed on 80% of the spray inoculated plants within 7 days after inoculation. However, the leaf lesions that developed were not as severe as those symptoms noted in the field and lesions did not develop on the stems. Uninoculated control plants, otherwise treated similarly, did not become infected. The pathogenic bacterium was reisolated from lesions on inoculated plants; hence, the completion of Koch's postulates.

The second method utilized to show pathogenicity was completed to insure that the 'symptoms' expressed by spraying leaves with the bacterial inoculum were not in fact a hypersensitive reaction which may be induced at high inoculum concentrations (35,

56). When P. syringae was infiltrated into leaves of the susceptible and resistant host, little or no lag phase* was observed (Figure 1). Doubling times* of bacteria during the exponential phase* (24 hours) in the two hosts at both inoculum concentrations were similar (Table 1). However, doubling times during the transition phase, the period between the exponential growth phase and the stationary phase*, in the susceptible host (S-208) were approximately half the doubling times observed in the resistant host (Sidwill) at both inoculum concentration of 3.4×10^6 and 3.3×10^7 bacterial cells/8 leaf disks were observed in the resistant and susceptible hosts respectively.

Identification.

The pseudomonads isolated from infected safflower plants were phenotypically similar to Pseudomonas syringae in the majority of the characterization tests utilized and described by Doudoroff and Palleroni (13) for this bacterium (Table 2). Results of the additional tests including aesculin hydrolysis, tartrate utilization and L-isoleucine utilization provided further positive evidence that the safflower isolates were of the P. syringae group (39, 58).

* see Appendix B for description of terms.

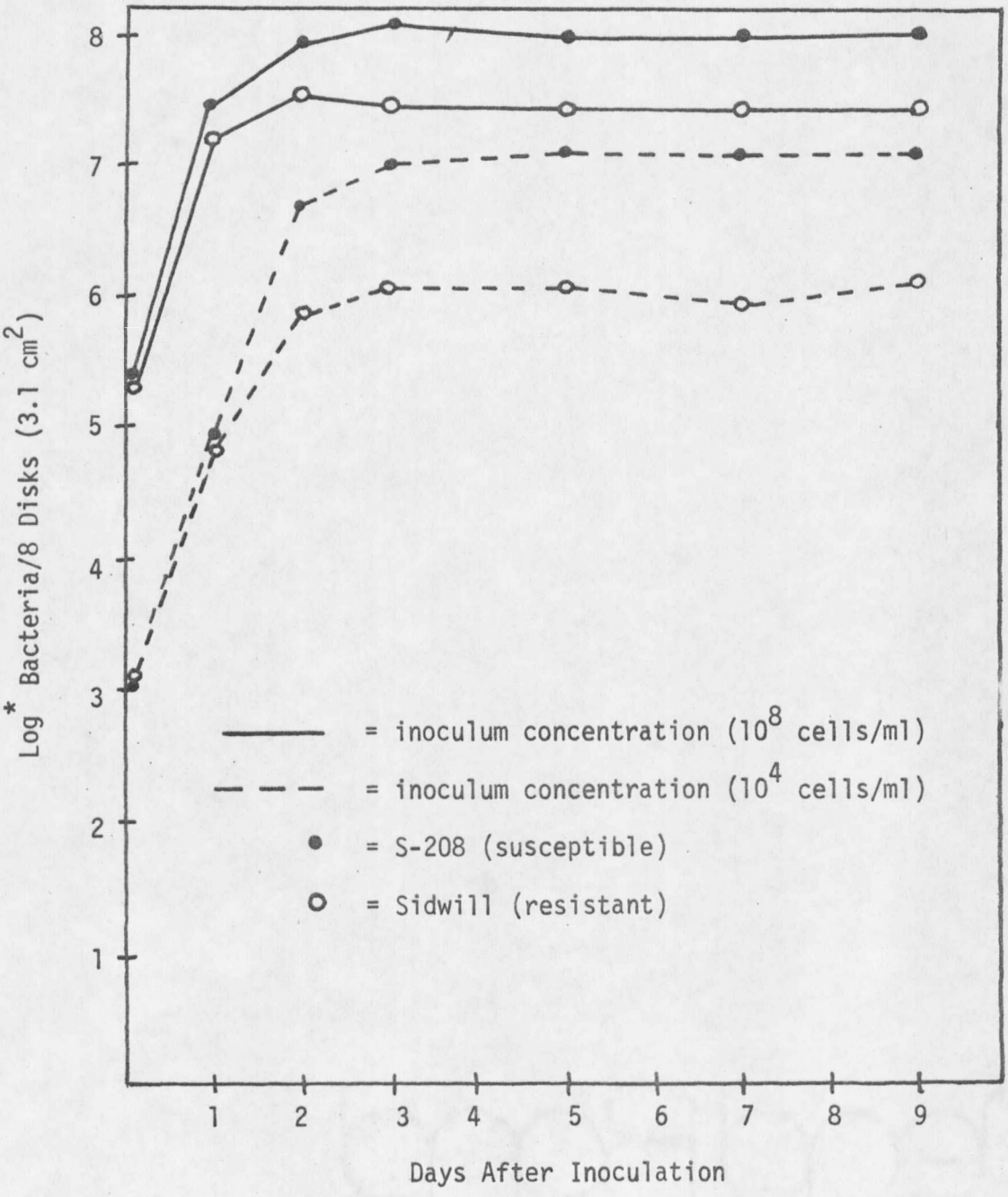

Figure 1. Multiplication of P. syringae in leaves of susceptible and resistant safflower varieties.

* - Log = 10^n, where n= 1, 2, 3 9

Table 1. Doubling times of P. syringae in resistant (Sidwill) and susceptible (S-208) safflower varieties during the exponential phase and the transition period

	Exponential Phase		Transition Period	
Variety	10^8 cells/ml	10^4 cells/ml	10^8 cells/ml	10^4 cells/ml
S-208	3.48[a]	3.95	13.3	4.28
Sidwill	3.93	4.23	23.5	8.0

a. doubling time - hours

Isolation from seed and seed transmission.

Pseudomonas syringae was isolated from 7.3% and 3.6% of the seed which had not been surface sterilized of the varieties S-208 and Sidwill respectively (Table 3). Whereas, no P. syringae was isolated from seed that had been surface sterilized. Langhans et al. (38) and Otta (48) recently reported P. syringae being isolated from wheat seed; but, they did not report if contaminated seed would result in infection of seedlings. However, in this study P. syringae was recovered from the aerial parts of S-208 seedlings grown from non-surface sterilized seed and seed inoculated with P. syringae. No P. syringae isolates were obtained from seedlings which had been grown from surface sterilized seed. Only 2.6% of the healthy S-208 seedlings grown from non-surface sterilized seed yielded isolates of P. syringae as opposed to more

Table 2. Comparison of biochemical and physiological characteristics of Pseudomonas syringae and the pseudomonads isolated from naturally infected safflower in Montana

Test	Safflower Isolates (%)[a]	P. syringae[d]
Motility	100 +[b]	+
Fluorescent	100 +	+
Pyocyanine	100 -	-
Growth at 41 C	100 -	-
Levan formation	96.8 +	c
Arginine dihydrolase	100 -	-
Oxidase reaction	100 -	-
Denitrification	100 -	-
Gelatin hydrolysis	100 +	c
Starch hydrolysis	100 -	-[e]
Aesculin hydrolysis	96.8 +	+[e]
Carbon Substrate:		
Glucose	100 +	+
Trehalose	81.3 -	-
2-Ketogluconate	84.3 -	-
meso-Inositol	100 +	c
L-Valine	81.3 -	-
B-Alanine	100 -	-
DL-Arginine	78.1 +	c[e]
Tartrate	100 -	-[f]
L-Isoleucine	100 -	-

a - % based on 32 isolates collected from infected safflower leaves.
b - + = positive; - = negative.
c - positive for more than 10% but less than 90% of strains tested.
d - Data from Doudoroff and Palleroni (14).
e - Data from Latorre and Jones (39).
f - Data from Sands et al. (58).

than half of the seedlings grown from inoculated S-208 seed yielded P. syringae (Table 3). The low percentage of seedlings that

Table 3. Percent seed yielding P. syringae and transmission of P. syringae from the seed to the aerial parts of the plant.

Seed Treatment	% seed with P. syringae[a]		Seedlings with P. syringae (%)[b]
	Sidwill	S-208	
Sterilized Seed	0.0	0.0	0.0
Non-sterilized Seed	3.6	7.3	2.6
Inoculated Seed	*	*	52.6

a - based on 3 replications, 100 seeds/replication.
b - based on 3 replications, 50 seedlings/replication; variety - S-208.
* - % of seed with P. syringae not determined.

yielded P. syringae from non-surface sterilized seed in contrast to seedlings grown from inoculated seed probably was due to the low amount of natural infection present on the non-sterilized seed (i.e. 7.3%).

Isolation of P. syringae from weeds, plant refuse and soil.

During the 1978 growing season, weeds, plant refuse and soil yielded isolates of P. syringae (Table 4). However, very few P. syringae isolates could be isolated from the various sources during the 1979 growing season (Table 4). This presumably was due to the relatively dry, hot conditions which prevailed during the

Table 4. Percentage of *P. syringae* of total fluorescent Psuedomonads obtained from weeds[a], plant debris[b], and soil[c]

Source of Isolates	1978			1979		
	June	July	August	June	July	August
Weeds	41	33	25	2	0	3
Plant Debris	5	1	0	15	2	4
Soil	2	3	1	1	0	3

a - based on 100 fluorescent bacterial isolates randomly selected.
b - based on 50 fluorescent bacterial isolates randomly selected.
c - based on 100 fluorescent bacterial isolates randomly selected.

1979 growing season as opposed to the cool, moist conditions that favor *P. syringae* development which prevailed throughout the 1978 season. In most cases, fluorescent pseudomonads were the predominant organism isolated on BCBRVB from all sources.

Discussion:

As a result of consistent isolations of fluorescent pseudomonads from infected safflower tissue, pathogenicity tests involving the isolated pathogen and subsequent identification of the bacterium based on phenotypical tests (Table 2), bacterial leaf spot and stem blight (BLSSB) of safflower has been determined to be incited by a bacterium resembling *P. syringae*. Furthermore, several investigators have reported that pathogenicity of

P. syringae within a host is dependent upon the ability of the pathogen to grow from an initial low population (10^3 - 10^4 cells/ml) to a final high population (10^7 - 10^9 cells/ml) within the host (24, 35, 46, 56). With an initial concentration of approximately 2.9 X 10^3 cells/8 leaf disks in both the resistant and susceptible host, a final concentration of 3.4 X 10^6 and 3.3 X 10^7 cells/8 leaf disks was observed in the resistant and susceptible hosts respectively 9 days after inoculation. Therefore, these results are consistent with those previously reported concerning pathogenicity. BLSSB of safflower is considered the major disease problem affecting safflower production in Montana during periods of cool, wet weather. During the 1978 growing season in which rainstorms and cool temperatures prevailed, safflower was severely infected throughout eastern Montana and western North Dakota. In some areas, e.g. Williston, N.D., severe stunting resulted with concomitant losses in yield. It appears periodic wind-rain storms throughout the growing season is important in inducing plant injury and in spreading inoculum (10, 17, 71) resulting in severe bacterial blights. Furthermore, Langhans et al. (38) reported that dissemination by rain may be the major mode of introducing and spreading P. syringae in cereal fields in Montana based on the isolation of P. syringae from rainstorms at altitudes ranging from 500 to 5,000 feet. It is worth noting that during the 1979 growing

season in which rain and cool temperatures did not prevail no blight outbreaks occurred in Montana. A similar observation was described by Daft and Leben with bacterial blight of soybeans (10).

This investigation indicates that safflower seed is infested with P. syringae and that the pathogen can be transmitted to the aerial parts of the seedling. Furthermore, it was determined that P. syringae occurred on the seed surface and was not borne internally. The seedlings grown from non-surface sterilized and inoculated seed in which P. syringae was isolated exhibited no symptoms, therefore, suggesting P. syringae can exist on healthy safflower plants in a "resident phase". These results indicate that seed-borne P. syringae is a primary source of inoculum for BLSSB of safflower.

P. syringae was isolated from weeds, plant debris and soil during 1978 and 1979. However, a higher percentage of P. syringae isolates were obtained in 1978 than in 1979. This may be due to the ability of saprophytic pseudomonads to out-compete the pathogenic pseudomonads during adverse environmental conditions which were prevalent in 1979 as opposed to 1978. The ability of saprophytic pseudomonads to utilize a greater variety of substrates than plant pathogenic pseudomonads (57) may be related to the competitive ability of the saprophytic pseudomonads. In either case, the results suggests that weeds, plant debris and soil may be

possible sources of inoculum for BLSSB incited by P. syringae. In addition, P. syringae has been reported as being isolated from wheat and barley (47, 59) which may provide yet another source of inoculum during wind-rain storms. Because isolates of P. syringae from these sources were not tested for pathogenicity to safflower, the importance of these sources are not clear. However, Latorre and Jones (40) reported that 56-95% of oxidase negative and green fluorescent pseudomonads isolated from weeds and plant refuse were pathogenic. Based on field observations mentioned above and reports to other workers (10, 17, 38, 69, 71) wind-rainstorms may be the major mode of disseminating P. syringae from weeds to safflower, from soil to safflower, from safflower to safflower or from other crops to safflower.

Results from seed transmission studies and from isolation studies indicate that seeds, weeds, plant debris or soil may be possible sources of primary inoculum. Therefore, disease free seed produced in arid areas may be an important control measure. Walker and Patel (72) reported halo blight on beans had been reduced to a minor disease by use of seed produced in semi-arid regions. However, Leben (42) reported that methods aimed at breaking pathogen-seed association would not be useful if the pathogen survived from season to season in plant debris, if it possessed a resident phase on weeds or if it is soil-borne. On

the other hand, the use of disease-free safflower seed, produced in Arizona, has provided a greater vigor to S-208 seedlings as opposed to those grown from Montana produced seed (27). This added vigor could result in enabling seedlings to better resist an invasion by P. syringae from other sources. In addition, the increased vigor may result in an earlier maturation date. Considering the apparent ubiquity of P. syringae, an integrated approach appears to be the best means of controlling bacterial blight of safflower with the development of resistant varieties playing a major role. The safflower varieties, 'Sidwill', 'Rehbein' and 'Hartman' developed at MAES possess at least moderate resistance to P. syringae (personal communication with Dr. J.W. Bergman).

The following control measures are recommended for control of bacterial leaf spot and stem blight of safflower:

1. using resistant varieties.
2. planting disease free seed.
3. avoiding production of other susceptible crops in the same rotation.
4. growing susceptible safflower only once every 4-5 years on the same field.

Information regarding the epidemiology of the pathogen is essential in devising possible control measures. This investigation determined that seeds, weeds, safflower debris, soil and other crops may be possible sources of primary inoculum. However, this

study did not test isolates of P. syringae from these sources for pathogenicity to safflower. Additional investigation in this respect is essential to fully understand the epidemiology of the disease. If only P. syringae isolated from safflower seed was capable of infecting safflower, theoretically, disease free seed would control the disease. However, if P. syringae from other sources were capable of infecting safflower, the need of resistant varieties would be imperative. Therefore, the determination of the pathogenicity of P. syringae isolated from all sources is required to adequately suggest control measures to integrate with disease resistance.

The main method of all plant disease control involves the elimination or reduction in the amount or effectiveness of the inoculum. This can be accomplished by use of resistant varieties and disease free seed or by proper rotation practices. However, the use of chemical seed treatments for disease control should not be overlooked for controlling disease. Since seed infestation may lead to serious disease outbreaks in the field, additional effort is required in studying this aspect of disease control. New seed treatment chemicals for control of disease are being developed continually; thus, research to screen potential chemical seed treatments must be continually conducted to effectively incorporate this possible method of control into a well rounded disease control

program. Chemical seed treatments may be an effective disease control measure.

CHAPTER 3

SOURCES OF RESISTANCE AND INHERITANCE OF RESISTANCE TO BACTERIAL LEAF SPOT AND STEM BLIGHT OF SAFFLOWER

Safflower (Carthamus tinctorius) is an important alternate crop grown in dryland areas of north central, south central and eastern Montana, northwest South Dakota and western North Dakota. It is drought resistant and important in crop rotation practices with dryland cereals to break cereal disease and insect cycles and in weed control. The development of safflower as an alternate crop for Montana has progressed significantly as evidenced by the 180,000 acres of safflower produced in 1979. Bacterial leaf spot and stem blight (BLSSB) of safflower, incited by Pseudomonas syringae, is considered the major disease problem affecting safflower production in Montana, South Dakota and North Dakota.

Studies, reported herein, have incicated that safflower seed produced in Montana was infested with P. syringae and that the pathogen was transmitted to the aerial parts of the seedling. In addition, P. syringae was isolated from weeds, plant debris and soil from June through August 1978 and 1979. These results suggested that seeds, weeds, plant debris and soil may be possible sources of inoculum for BLSSB of safflower. Furthermore, several investigators have reported that wind-rainstorms throughout the growing season was important in disseminating P. syringae from inoculum sources to field crops resulting in severe bacterial blights

(10, 17, 38, 71). Considering the apparent ubiquity of P. syringae and the lack of an effective bactericide, the development of resistant varieties appears to be the best means of controlling bacterial blight of safflower.

The following study was undertaken to develop a greenhouse seedling screen for safflower resistance, to determine sources of resistance and to determine the mode of genetic inheritance of safflower resistance to P. syringae.

Materials and Methods:

Two methods of inoculation were developed to screen safflower seedlings for resistance to P. syringae. The first method involved the injection of three week old safflower seedlings with a bacterial suspension of 10^7 cells/ml with a syringe and needle approximately 1 cm above the soil line. A drop of inoculum was formed at the tip of the needle before insertion through the stem, afterwhich, another drop of inoculum was formed and the needle was withdrawn back through the stem. Inoculation with sterile deionized water served as a control. Inoculated seedlings were placed immediately in a growth chamber at 95-100% relative humidity and 20 C. Disease reactions were read 7 days after inoculation. Seedlings were classified into five reaction types (0-4) indicating an increasing degree of stem discoloration and susceptibility.

The second method of inoculation utilized in the development of a large scale seedling screen for safflower resistance to P. syringae in the greenhouse involved watersoaking leaves of two week old safflower plants with a bacterial suspension of 10^4 cells/ml. In addition, this leaf inoculation method was used in determining the mode of genetic inheritance of safflower resistance to P. syringae. The apparatus utilized consisted of tongue seizing forceps, soft rubber stoppers, hypodermic needle and syringe (25). The leaf to be inoculated was held firmly between the rubber stoppers mounted on the forceps. The bacterial suspension in the syringe was forced through the needle into a cavity formed in one of the rubber stoppers and then through the stomata into the leaf mesophyll cells resulting in a watersoaked area. Watersoaking safflower leaves with sterile deionized water served as the control. Inoculated seedlings were placed on the greenhouse bench at 21 to 25 C and disease reactions were read 7 days after inoculation. Plants were given disease index numbers according to the following classes: 0 = no infection; 1 = few small lesions; 2 = many small lesions; 3 = coalescence of lesions; 4 = large necrotic lesion.

Regarding both of the inoculation procedures described, seeds of each variety were sown in 15 X 21 inch flats. Fourteen seedlings in each flat served as controls while the remaining 100 seedlings were inoculated with the bacterial suspension. The

culture used in this study was isolated from a safflower leaf collected at the Eastern Agricultural Research Center, Montana Agricultural Experiment Station, Sidney, Mt. (MAES) in 1978. Its physiological and biochemical reactions were characteristic of those described for P. syringae. The inoculum was prepared by suspending a 24 hour culture grown on King's medium B (32) in sterile deionized water. The inoculum was kept in an ice-water bath until all inoculations were completed.

The commercial varieties and lines tested were obtained or developed at MAES. In addition, F_2 populations were obtained from crosses performed at MAES. Inoculation of F_2 progeny was accomplished by use of the leaf inoculation method described above. S-208 was used as the susceptible control for comparisons in all inoculation experiments performed to insure occurrence of adequate infection or to detect virulence changes in the isolate used for inoculation.

Results:

Method of inoculation.

In most cases the reactions obtained from the stem inoculation method correlated closely to the observed field reactions (Table 5). However, the stem inoculation reactions of the varieties UC-1, P-1, US-10, N-8 and Partial Hull were notably different from

Table 5. Field and greenhouse reactions (stem and leaf inoculation of commercial safflower varieties and lines to P. syringae

Variety or Line	Disease Index		
	Stem[a] Inoculation	Leaf[b] Inoculation	Field[c] Reactions
UC-1	2.64	3.94	4.50[d]
P-1	1.50	3.90	4.50
US-10	2.52	3.92	4.25
N-10	3.15	3.70	4.25
Gila	3.12	3.72	4.00
S-208	3.22	3.50	4.00
Partial Hull	2.11	3.56	4.00
Dart	2.87	3.52	4.00
N-8	1.83	3.43	3.75
P-2	3.21	3.49	3.75
Frio	2.91	3.10	3.50
Rio	2.52	2.85	3.50
87-42-3	2.73	3.11	3.50
87-14-8	2.87	3.20	3.50
Royal	2.50	2.97	3.25
Biggs	2.24	2.91	3.25
PCM-1	2.83	3.10	3.25

a. based on a scale of 0-4 (0 = resistant, no necrosis; 4 = susceptible, extreme necrosis).

b. based on a scale of 0-4 (0 = resistant, no infection; 4 = susceptible, large necrotic lesion).

c. based on field readings recorded in 1975 at MAES; 2 replications. Rated on a scale of 0-9 (0 = resistant; 9 = susceptible).

d. field ratings were divided by two for comparison to the 0-4 scale of the inoculation methods.

Table 5. (Cont.) Field and greenhouse reactions (stem and leaf inoculation of commercial safflower varieties and lines to *P. syringae*

Variety or Line	Disease Index		
	Stem[a] Inoculation	Leaf[b] Inoculation	Field[c] Reactions
87-14-6	2.44	2.70	3.00[d]
87-14-B	2.49	2.58	3.00
88-26-B	2.31	2.80	3.00
88-74-2	2.40	2.37	2.75
Sidwill	2.24	2.30	2.50
N-4051	2.90	1.98	2.25
Cargill 1653	3.22	3.50	-
88-45-4	2.67	3.08	-
AC-1	2.16	2.78	-
C. paloestinus	2.34	2.71	-
S-202	3.13	2.55	-
N-1-1-5	2.44	2.12	-
Ute	-	-	3.25
PCM-2	-	-	3.25
Rehbein	-	-	2.50
Hartman	-	-	1.75

a. based on a scale of 0-4 (0 = resistant, no necrosis; 4 = susceptible, extreme necrosis).

b. based on a scale of 0-4 (0 = resistant, no infection; 4 = susceptible, large necrotic lesion).

c. based on field readings recorded in 1975 at MAES; 2 replications. Rated on a scale of 0-9 (0 = resistant; 9 = susceptible).

d. field ratings were divided by two for comparison to the 0-4 scale of the inoculation methods.

those reactions recorded under field conditions in that they gave "false" resistance readings. The reactions obtained from the leaf inoculation method were comparable to those obtained in the field (Table 5). Controls did not exhibit any disease reactions.

To determine the optimum conditions to inoculate safflower seedlings for detecting disease resistance, the affect of inoculum concentration and age of seedlings were studied. Two three and four week old seedlings of the varieties S-208 (susceptible) and Sidwill (resistant) were inoculated with 10^3, 10^4, and 10^5 cells/ml by the leaf inoculation method (Table 6). Disease reactions of S-208 and Sidwill were significantly different at all inoculum concentrations tested depending upon the age of the seedling as indicated in Table 6. However, the disease reactions of two week old seedlings inoculated with 10^4 cells/ml correlated most closely with those disease reactions observed under field conditions. The disease reactions of S-208 and Sidwill were 3.5 and 2.3 respectively while disease reactions observed in the field for S-208 and Sidwill were 4.0 and 2.8 respectively.

Sources of resistance

The distribution of the 29 safflower varieties or lines tested by the leaf inoculation method among the five seedling reaction types were as follows (Table 5):

Table 6. The effect of inoculum concentration and age on disease reactions of safflower seedlings inoculated by the leaf method[a]

Age (weeks)	Variety	Inoculum Conc. (cells/ml) 10^3	10^4	10^5
2	S-208	1.3[b]	3.5*	4.0
	Sidwill	1.0	2.3	3.5
3	S-208	0.9*	2.4*	2.6*
	Sidwill	0.0	1.0	1.3
4	S-208	0.0	0.5	1.2*
	Sidwill	0.0	0.0	0.0

a. based on 3 replications, 20 seedlings/ replication.

b. disease reactions based on a scale of 0-4 (0 = resistant, 4 = susceptible).

* pairs of means which are significantly different (P = 0.05).

highly resistant - 0.0 to 0.4 (0), 0 cultivars

resistant - 0.5 to 1.4 (1), 0 cultivars

moderately resistant - 1.5 to 2.4 (2), 4 cultivars

moderately susceptible - 2.5 to 3.4 (3), 16 cultivars

susceptible - 3.5 to 4.0 (4), 9 cultivars

The most resistant tested varieties or lines to P. syringae were Sidwill, N-4051, 88-74-2 and N-1-1-5 with no highly resistant lines

of safflower being found. the variety 'Hartman', not tested by the leaf inoculation method, had been observed in the field to be more resistant than other varieties or lines tested (Table 5).

Genetics of resistance.

Seven days after inoculation, F_2 plants were classified readily into their respective disease reaction types (i.e. 0-4). The heterologous reaction types exhibited by 100 plants of each safflower line '87-42-3' and 87-14-6', which were used as parents in crosses with other safflower varieties or lines, indicated a segregating population for disease resistance (Tables 7 and 8). The homologous reaction types of varieties or lines crossed to either 87-42-3 or 87-14-6 indicated there was little segregation within these parent populations with the exception of the line 87-26-B.

Segregation of resistant and susceptible plants in the F_2 generation of progeny resulting from crosses of 87-42-3 with the susceptible varieties 'Frio' and 'AC-1' satisfactorily fit hypothetical 1 resistant:3 susceptible (1R:3S) ratios (Table 7). The 1R:3S ratios suggests that resistance of each parent is conditioned by a single recessive gene pair. In contrast, the number of resistant to susceptible plants in the F_2 generation of the segregating cross between 87-42-3 and the susceptible varieties 'Cargill 1653', 'S-208' and 'US-10' satisfactorily fit a 3R:1S ratio. This

Table 7. Reaction types of F_2 progenies from crosses between safflower line 87-42-3 and various safflower varieties when inoculated with P. syringae in the greenhouse

Progeny or Variety	No. of Plants	% of Plants in Disease Index				
		0	1	2	3	4
87-42-3	100		2	11	82	5
Frio	30				73	27
*Frio/87-42-3	200			28	58	14
Rio	30			20	80	
Rio/87-42-3	200		2	36	53	9
Cargill 1653	30				37	63
**Cargill 1653/87-42-3	200			35	42	23
S-208	30				53	47
**S-208/87-42-3	200			19	56	25
P-2	30				60	40
P-2/87-42-3	200			14	69	17
AC-1	30			27	73	
*87-42-3/AC-1	200			24	65	11
US-10	30				10	90
**87-42-3/US-10	200			19	57	24
N-4051-1	30			60	40	
87-42-3/N-4051-1	200			39	49	12

* - Expected ratio (1R:3S); Chi square probability, 0.0-0.9
Reaction types - 0, 1, 2 = R; 3, 4 = S

** - Expected ratio (3R:1S); Chi square probability, 0.0-0.9
Reaction types - 0, 1, 2, 3 = R; 4 = S

Table 8. Reaction types of F_2 progenies from crosses between safflower line 87-14-6 and various safflower varieties when inoculated with P. syringae in the greenhouse.

Progeny or Variety	No. of Plants	% of Plants in Disease Index 0	1	2	3	4
87-14-6	100		8	17	72	3
Frio	30				67	33
*Frio/87-14-6	200		1	21	62	16
Dart	30				60	40
**Dart/87-14-6	200		2	17	54	27
AC-1	30			20	80	
AC-1/87-14-6	200			39	53	8
P-1	30				27	73
**P-1/87-14-6	200			10	68	22
87-26-B	30		10	17	70	3
87-26-B/87-14-6	200		10	38	49	3
S-208	30				60	40
*87-14-6/S-208	200			25	44	31
N-10	30				37	63
87-14-6/N-10	200			47	39	14
PCM-1	30				80	20
87-14-6/PCM-1	200			31	51	18

* - Expected ratio (1R:3S); Chi square probability, 0.0-0.9
Reaction types - 0, 1, 2 = R; 3, 4 = S

** - Expected ratio (3R:1S); Chi square probability, 0.0-0.9
Reaction types - 0, 1, 2, 3 = R; 4 = S

ratio suggests that resistance of each parent is conditioned by a single dominant gene pair (Table 7). F_2 progeny, resulting from crosses between 87-42-3 and the susceptible varieties 'Rio', 'P-2' and 'N-4051-1', segregated into a range of disease index classes (i.e. R:S ratios did not fit simple genetic ratios) indicating inheritance was polygenic or complex.

Similar resistant:susceptible ratios, as those described above, were obtained from the F_2 progeny resulting from crosses of 87-14-6 with various susceptible varieties (Table 8). However, a 1R:3S ratio resulted from crossing 87-14-6 to S-208 in contrast to the 3R:1S ratio which resulted from crossing 87-42-3 to S-208.

Discussion:

In certain varieties, the stem inoculation method did not correlate to disease reactions recorded under field conditions. The lack of correlation may be due to a difference in stem versus foliar resistance as the field reactions were recorded according to foliar symptoms and not to stem reactions.

The consistency between seedling reactions in the greenhouse inoculated by the leaf method and plant reactions to P. syringae in the field indicates the usefulness of utilizing this method to screen safflower varieties or lines for resistance. Inoculation of 2 week old safflower seedlings with 10^4 cells/ml appears optimum to

screen safflower plants for resistance. However, depending upon the virulence of the isolate used for inoculation, the concentration of the inoculum may have to be adjusted accordingly. In addition, the possibility of the existence of different virulence types of P. syringae should not be overlooked while screening lines for resistance. One virulence type may elicit a susceptible disease reaction in a particular line while another virulence type may cause a resistant disease reaction in the same line. Walker and Patel (72) warned breeders to be wary of assays involving a restricted number of isolates based on their discovery of 2 races of P. phaseolicola which caused different disease reactions on the same bean variety. Therefore, it may be desirable to use inocula consisting of a mixture of P. syringae isolates or to use a series of inocula consisting of numerous single isolates.

Safflower varieties and lines vary in susceptibility to P. syringae. Most of the safflower lines commonly grown in the production areas of Montana, North Dakota and South Dakota are susceptible, with the exception of Sidwill and Rehbein which are moderately resistant. In addition, the variety 'Hartman' exhibits a higher degree of resistance than any other commercially available variety according to field observations (personal communication with Dr. J.W. Bergman). Furthermore, a commercially valuable level of resistance was present in 88-74-2, N-4051-1 and N-1-1-5 (Table 5).

The source of resistance in 88-74-2, Hartman, Sidwill and Rehbein was obtained from a bulk population of the world collection of safflower consisting of 555 safflower introductions. This bulk population has been cropped continuously in the same field since 1961 at MAES, thus, allowing natural selection to occur. The possible diverse origins of these resistant entries suggest that several different genetic sources of resistance may be present in the material, although this has not been verified by appropriate genetic analyses.

The purpose of this investigation was not only to devise a greenhouse seedling screen and determine sources of resistance, but, also to determine in what manner BLSSB of safflower was inherited. If F_2 progeny resulting from crosses involving either 87-14-6 or 87-42-3 with a particular variety inherited resistance in a similar manner, the resulting resistant:susceptible ratios would be the same. However, this did not occur as evidenced by the contrasting ratios which resulted when 87-14-6 and 87-42-3 were crossed to S-208 (i.e. 1R:3S and 3R:1S respectively). In addition, when either 87-14-6 or 87-42-3 were crossed to several susceptible varieties no one ratio resulted as might be expected if disease resistance were inherited in a simple Mendelian manner. These results may be due in part to the apparent segregation within the 87-14-6 parent population and the 87-42-3 parent population (Tables

7 and 8). Although no specific mode of inheritance was discerned, the results suggests that resistance to P. syringae is heritable as evidenced by the percentage of F_2 progeny of most crosses being more resistant than the susceptible parent. The segregation of F_2 progenies into a range of disease index classes suggested the character of inheritance to be polygenic.

Extensive research is needed in the area of understanding and developing resistant varieties of safflower. As mentioned earlier, different virulence types of P. syringae may exist in various areas of safflower production. Therefore, a safflower variety may be resistant in one production area while susceptible in another production area. The isolation of P. syringae from safflower grown in various production areas throughout Montana, North Dakota and South Dakota and subsequent inoculation of several different safflower varieties with these isolates is necessary to determine whether virulence types exist.

Even though various lines and varieties have been identified as being moderately resistant to P. syringae, the identification of distinctly different genetic sources of resistance has not been determined. The identification of different sources of resistance would be invaluable to the safflower breeder as it would afford him the opportunity to build P. syringae-resistant varieties with multiple genes for resistance. The utilization of all avail-

able resistant genes would provide long-term protection against the rapid development of virulent isolates of P. syringae to current resistant varieties.

Further extensive investigation is necessary to determine the possible existence of different virulence types of P. syringae and the identification of distinctly different sources of P. syringae resistance in order to markedly facilitate safflower breeding programs and to prevent a possible P. syringae epidemic when conducive environmental conditions prevail for disease development.

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APPENDIXES

APPENDIX A

Description of biochemical and physiological tests:

Motility - The presence of flagella were regarded as indicating motility. A loopful of bacteria, grown on King's B medium was gently touched to a slide which had a drop of distilled water placed upon it. The bacterial suspension on the slide was allowed to air dry. The bacterial smear was stained with a mixture of reagents I, II and III for 10 minutes; afterwhich, the slide was gently rinsed off with running tap water. The bacteria then were counter-stained with 1% methylene blue for 5 to 10 minutes; afterwhich, the slide was washed in water and air dried. The bacteria were examined under oil immersion for the presence of flagella.

Reagents: I - 1.2 g basic fuchsin in 100 ml ethanol
II - 3 % tanic acid
III - 1.5% NaCl

Pigment production (fluorescence) - Fluorescent pigment production by pseudomonads was determined by streak inoculation on King's B medium (1) plates. Cultures were incubated at 27 C and examined for fluorescent pigmentation under ultraviolet light (366 nm) after 1, 2 and 3 days.

Growth at 41 C - Tubes of Difco yeast extract (5.0 g/l) were observed for growth after inoculation and incubation at 41 C.

Arginine dihydrolase production - Approximately 5.0 ml of Thornley's arginine medium 2A (3) were sterilized by autoclaving in screw capped test tubes. The pH was adjusted to 6.8 with NaOH. Tubes were stab inoculated with 48 hour old cultures and incubated at 27 C. The tubes were sealed with 1% sterile melted agar. The anaerobic formation of alkali from arginine was detected by a change in color after 4 days incubation. A change in color to red indicated a positive reaction.

Levan formation - Nutrient agar plates containing 5% w/v sucrose were streaked with bacterial isolates. Three to five days after inoculation isolates that produced large white, convexed, mucoid colonies were considered to be levan producers.

Oxidase production - A loopful of bacteria from 24 hour old cultures grown on King's B medium (1) was smeared on filter paper previously soaked with a 1.0% w/v aqueous solution of N, N'-dimethyl-p-phenylene-diamine with a platinum loop. Production of a dark purple color within 10 seconds indicated the presence of oxidase.

Nitrate Reduction (nitrate, NO_3^- to nitrite, NO_2^-) - Tubes containing KNO_3, 5.0 g/l; peptone, 5.0 g/l; yeast extract, 3.0 g/l and agar, 3.0 g/l were inoculated with 24 hour old cultures. After inoculation, the tubes were incubated for 5 days.

Test for nitrite: to a 0.5 ml culture add 1 drop each of sulfanilic acid and naphthylamine HCl. A positive test results in a pink reaction. If nitrite test is negative, check for presence of nitrates.

Test for nitrate: to 2 drops of culture add 1 drop of diphenylamine. A positive test results in a blue reaction.

Reagents: Sulfanilic acid - 0.8% sulfanilic acid in 5N acetic acid.

naphthylamine HCl - 0.5% naphthylamine dissolved in 5N acetic acid.

diphenylamine - add 100 ml of concentrated H_2SO_4 to 200 ml distilled water, afterwards, add 0.5 g diphenylamine and stir until dissolved.

Aesculin hydrolysis - A medium containing peptone, 10.0 g/l; aesculin, 1.0 g/l and ferric citrate, 0.5 g/l was dispensed into tubes and autoclaved. Tubes were inoculated and incubated for 14 days at 25 C. A change in the medium color to dark brown or black was regarded as indicating utilization of aesculin.

Gelatin hydrolysis - A medium containing yeast extract, 3.0 g/l; peptone, 5.0 g/l and gelatin 120.0 g/l was warmed to approximately 50 C and dispensed into tubes. After autoclaving, tubes were stab inoculated with 24 hour old cultures and incubated at 27 C. After 2 weeks, tubes were cooled to 4 C for 30 minutes. Gelatin hydrolysis was recorded as positive if the medium flowed readily in tubes after tilting.

Starch hydrolysis - Starch hydrolysis was determined by streak inoculation of starch agar plates. The starch agar consisted of tryptone, 10.0 g/l; yeast extract, 10.0 g/l; K_2HPO_4, 5.0 g/l; soluble starch, 3.0 g/l and agar, 1.5 g/l. After incubation for 4 and 10 days at 27 C, the plates were flooded with dilute iodine solution (i.e. iodine crystals, 1.0 g; potassium iodide, 2.0 g and water, 300 ml). Clear zones around colonies indicated B-amylase activity.

Carbon source utilization - Substrates were filter sterilized (Millipore filtration) before adding to an autoclaved standard mineral base containing $(NH_4)H_2PO_4$, 1.0 g/l; KCl, 0.2 g/l and $MgSO_4$, 0.2 g/l and Noble agar, 12.0 g/l. The organic and amino acids were added at a 0.1% w/v final concentration while the sugars and sugar alcohols were added at a 0.2% w/v final concentration to the above medium. The pH was adjusted to 7.2. Sixteen bacterial isolates were patched onto each plate by replica plating methods (2) and incubated at 27 C. Growth was compared to inoculated plates which contained no added carbon source.

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APPENDIX B

Description of terms:

Doubling time - represents the average generation time of the culture as a whole, usually determined by doubling of the microbial mass in the culture.

Epiphytic population - bacterial populations that are observed on or isolated from the surface of healthy, living plants.

Exponential phase - period during the growth cycle of a population in which growth increases at an exponential rate.

Lag phase - period after inoculation of a population before growth begins.

Rhizosphere - the soil zone or ecologic habitat around plant roots subject to the specific influence of the plant roots.

Stationary phase - period during the growth cycle of a population in which growth ceases.

Ubiquitous - present, or seeming to be present everywhere; existing everywhere.

Jacobs, D. L.
Bacterial leaf and stem blight of safflower in Montana