MONTANA STATE UNIVERSITY

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
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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 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

A thesis submitted in partial fulfillment
of the requirements for the degree

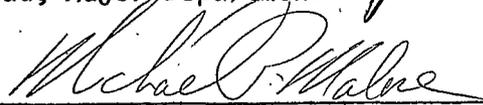of

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in

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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.

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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 re-
    sistance 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), saf-
flower 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 resist-
ance 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 ex-

posure 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 phytopatho-

genic, fluorescent pseudomonads. The nomenclatural confusion,

illustrated by the listing of over 60 nomenspecies of phytopatho-

genic, 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 com-
parison, 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 be-
tween 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 surviv-
ing 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 tol-
erant 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 produc-
tion of 180,000 acres in 1979.

During the 1978 growing season in which cool, moist con-
ditions prevailed a severe leaf necrosis and stem blight of
safflower occurred. Symptoms included the development of irreg-
ular, 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 Cyclohexi-

mide, 500 ppm of Benlate (50% active), 10 ppm of Bacitracin, 6 ppm

of Vancomycin and 0.5 ppm of Rifampicin was added after autoclav-

ing) 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 pig-

ment (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 steri-

lized 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 6×10^7 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 hyper-
sensitive 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 trans-
planted 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 pre-
pared as described above at a concentration of 1 X 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 com-
pleted 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 de-
ionized water. After 4 hours, five 0.5 ml samples of each suspen-
sion 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 symptom-less 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 suscep-
tible 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 tran-
sition 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 X 10^6 and
3.3 X 10^7 bacterial cells/8 leaf disks were observed in the resist-
ant 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 utiliza-
tion and L-isoleucine utilization provided further positive evi-
dence that the safflower isolates were of the P. syringae group
(39, 58).

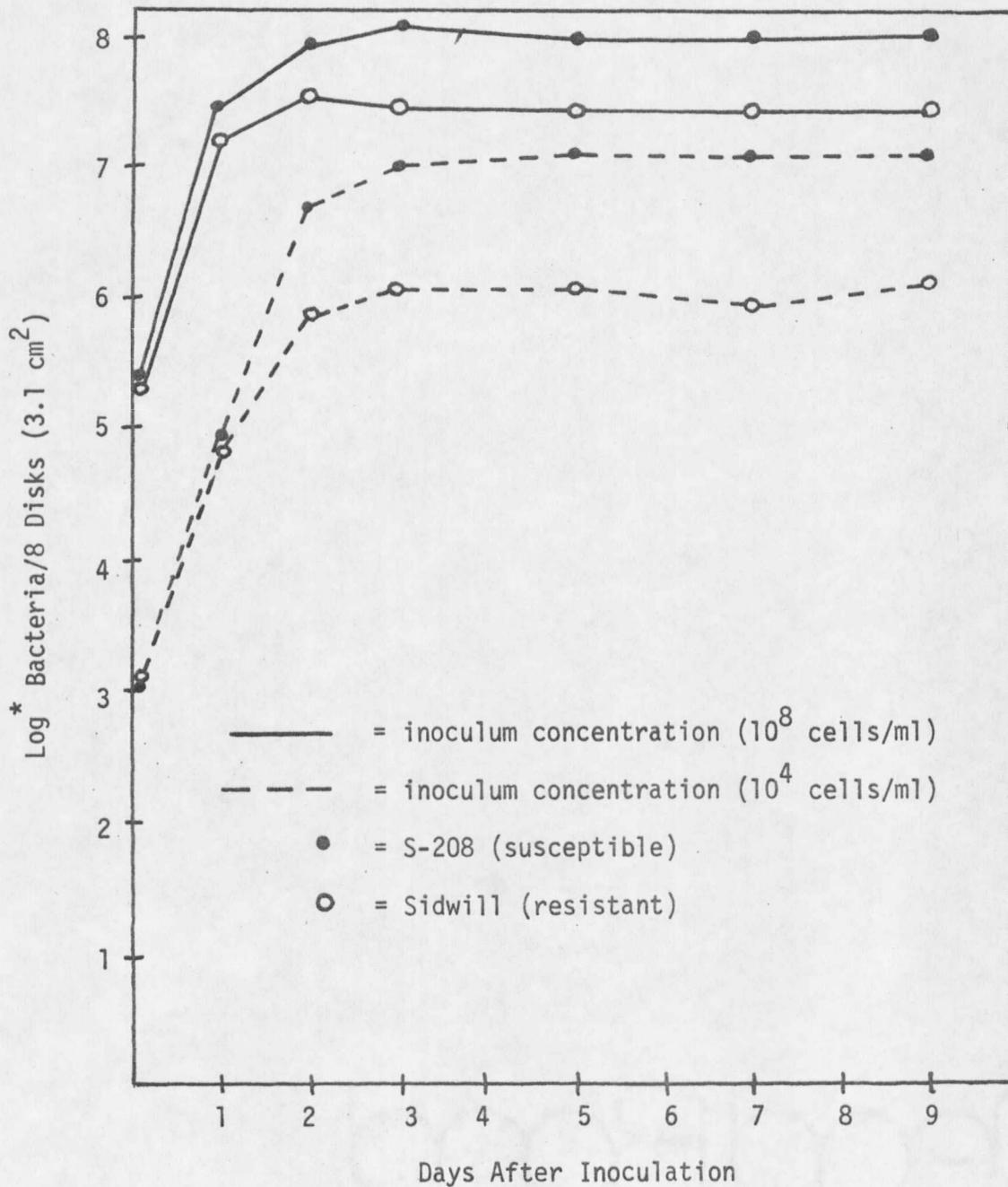* see Appendix B for description of terms.

Figure 1. Multiplication of <u>P. syringae</u> 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

Variety	Exponential Phase		Transition Period	
	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 <u>Pseudomonas</u> <u>syringae</u> 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 -	-
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 -	-[e]
L-Isoleucine	100 -	-[f]

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 yield-

ed 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