

CHEMICAL CONTROL OF BEAN COMMON
AND FUSCOUS BACTERIAL BLIGHTS

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ABSTRACT

CHEMICAL CONTROL OF BEAN COMMON AND FUSCOUS BACTERIAL BLIGHTS

BY

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Several chemical formulations were tested as controls of foliage infection by Xanthomonas phaseoli (Xp) and Xanthomonas phaseoli var. fuscans (Xpf), the incitants of bean common and fuscous bacterial blights, respectively. Bactericidal activity of each chemical was initially determined in vitro by measuring the inhibition of blight bacteria grown in liquid culture containing various chemical concentrations. The most potent chemical formulations were then tested in the field against secondary spread of blight infection; two spray schedules were compared, the first spray schedule (early) was initiated 2 weeks before the second spray schedule (late). Kocide 101 (83% Cupric hydroxide) applied on both spray schedules and Bunema (40% Potassium N-hydroxymethyl-N-methyldithiocarbamate) applied on the early spray schedule provided the best foliage control of Xp and Xpf. Pod infection was not significantly reduced by any of the chemical treatments, and no yield increase was realized.

Populations of blight bacteria present internally in healthy leaf tissue, after secondary spread, were monitored in the field. High levels of Xp and Xpf were detected several days before symptom expression. Non-pathogenic bacteria of varied morphologies were also detected in

apparently healthy tissue.

Three Xp and four Xpf isolates were tested for tolerance to each of six chemicals by a disc assay method. The isolates demonstrated a wide range of tolerance to individual chemicals; no isolate demonstrated tolerance to all six chemicals. In general, the Xpf isolates were more tolerant to copper-containing compounds than the Xp isolates.

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AND FUSCOUS BACTERIAL BLIGHTS

By

David M. Weller

A handwritten signature in cursive script that reads "David M. Weller". The signature is written in black ink and is positioned below the printed name.

A THESIS

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To my wife Suellen

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TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	iii
LIST OF TABLES	v
LIST OF FIGURES	vi
INTRODUCTION AND LITERATURE REVIEW	1
MATERIALS AND METHODS	9
General	9
Inhibition studies in liquid culture	10
Disc assay	11
Chemical uptake	12
Field study of chemicals applied early and late	13
Data collection	15
Monitoring bacterial populations in healthy tissue	16
Cooperative field study	16
RESULTS	19
General chemical screening	19
Chemical combinations	23
Uptake experiment	25
Field study of chemicals applied early and late	27
Foliate infection	27
Pod infection	29
Yield	29
Monitoring of bacterial populations in healthy tissue	29
Cooperative Field Experiment	36
Phytotoxicity	38
Chemical tolerance of various blight isolates	40
DISCUSSION	48
SUMMARY	59
LIST OF REFERENCES	62

LIST OF TABLES

Table	Page
1. End point determination of bactericidal and bacteriostatic action of various chemicals against <u>Xp</u> and <u>Xpf</u> strains as determined by bacterial inhibition in liquid culture	21
2. Inhibitory activity of Agri-Strep 500 against <u>Xp</u> and <u>Xpf</u>	22
3. Comparison of residual and systemic activities of various chemicals on bean leaf tissue	26
4. The effect of various chemical sprays on leaf and pod infection of Navy (pea) beans by <u>Xp</u> and <u>Xpf</u> evaluated at the 5% level	30-31
5. The effect of various chemical sprays on leaf and pod infection of Navy (pea) beans by <u>Xp</u> and <u>Xpf</u> evaluated at the 1% level	32-33
6. Effect of various chemical treatments on yield of Navy (pea) beans infected with <u>Xp</u> and <u>Xpf</u>	34
7. Levels of pathogenic (P) and non-pathogenic (NP) bacteria internally present in symptomless bean leaf tissue from control plots	37
8. The effect of chemical treatments on foliage infection and yield of Navy (pea) beans in the cooperative field experiment	39
9. Disc assay comparison of responses to various chemicals by <u>Xp</u> , <u>Xpf</u> and <u>Pp</u> isolates	44

LIST OF FIGURES

Figure	Page
1. Growth of <u>Xp 11</u> , as determined by optical density in liquid media containing 10 ppm of various chemicals or chemical combinations; A = Agri-Strep 500, B = Bunema, F = For-Cop-80 NC, H = HPMTS, I = Isobac.....	24
2. Isolate response to Agri-Strep 500 (500 ppm) by the disc assay method	45
3. Isolate response to Bunema (500 ppm) by the disc assay method	45
4. Isolate response to HPMTS (500 ppm) by the disc assay method	46
5. Isolate response to Isobac (500 ppm) by the disc assay method	46
6. Isolate response to Kocide 101 (600 ppm) by the disc assay method	47

INTRODUCTION AND LITERATURE REVIEW

Bacterial diseases constitute major production-limiting factors of beans (Phaseolus vulgaris L.) in many places throughout the world. The most serious of these diseases are common blight (incited by Xanthomonas phaseoli (E.F.S.) Dows), fuscous blight (incited by X. phaseoli var. fuscans (Burk.) Starr & Burk.) and halo blight (incited by Pseudomonas phaseolicola (Burk.) Dows). While this study concerns primarily common and fuscous blights, halo blight is occasionally mentioned because of its similarity to the other two diseases. All three bacteria possess similar infection patterns, and on certain bean types such as colored and snap, induce quite similar symptoms. Isolations are usually necessary to determine the causal agent in such bean types. Halo blight is infrequently found in the field infecting Navy (pea) beans; common and fuscous blights are the primary bacterial problems on Navy beans.

X. phaseoli (Xp) and X. phaseoli var. fuscans (Xpf) are both gram negative motile rods, 0.87 by 1.9 microns with one polar flagellum. The colonial morphologies of both are characterized by lemon yellow, smooth, circular colonies (3). The only factor which distinguishes the two is a diffusible water-soluble brown pigment produced by Xpf in certain agar media (2).

Xp and Xpf have been reported from many bean-growing areas of the world including the United States, Canada, Russia, Yugoslavia, New Zealand, and Chile (50). The two bacteria are frequently found together in the same field or on the same plant and the relative importance of each may vary from year to year (46). Bacterial isolations

are necessary to distinguish between the two.

Common and fuscous blights are especially important in Michigan, which produces approximately 50% of all dry edible beans and 90% of all Navy (pea) beans in the U.S.

Yield losses attributable to each pathogen are difficult to estimate because of similarity in symptom expression. Furthermore, halo blight may also be present which makes it even more difficult to ascertain yield losses in the colored and snap bean types. In 1969, at least 75% of Michigan's 650,000 acres of Navy beans were damaged by common and fuscous blights, with yield reductions of 10-20% (35). In the Dominican Republic, common blight was reported in 1971 as the most important bean disease (45). Sutton and Wallen reported 60% of Navy bean fields in Southwest Ontario in 1962 infected with fuscous blight (49).

Xp and Xpf induce disease symptoms on leaves, pods, and seeds. Leaf symptoms appear as water-soaked spots which enlarge irregularly; frequently, adjacent lesions coalesce. Lesions consist of necrotic tissue bordered by a narrow band of lemon-yellow tissue. Pod lesions appear as water-soaked spots which gradually enlarge and become dark and slightly sunken. Yellow bacterial ooze can often be found in the lesions. Seed infection is characterized by several different symptoms (40). If infection occurs early in pod development, the seed may be shriveled. Infection in later stages of pod development results in symptoms ranging from slight hilum darkening to discoloring of part or all of the seed coat. In Navy (pea) beans the seed coat becomes yellow.

Primary infection is usually established through the planting of

infected seed. Young plants which develop from infected seed bear lesions on the cotyledons or primary leaves; bacterial ooze may accumulate if environmental conditions are favorable. Such infected plants serve as primary inoculum sources for secondary spread to surrounding plants (50). Secondary spread may occur in several ways, primarily from rain accompanied by wind (48). Rain splash dispersal is especially effective when heavy rain causes water soaking of leaf tissue. Insects have also been reported to spread inoculum (37). Men, machinery and animals moving through fields while the plants are wet also disperse bacteria.

Xp and Xpf enter leaves through natural openings such as stomata and hydathodes or through wounds. Severe blight outbreaks have been reported following hail storms (50). The bacteria then invade intercellular spaces and cause a gradual dissolution of the middle lamella. Later, as the host cells disintegrate a pocket of bacteria develops. Burkholder (5) reported that the bacteria are systemic and move through the vascular system from the leaf into the stem. This report remains unconfirmed since Burkholder's original study is the only time that systemic movement of Xp and Xpf has been investigated. The seed becomes infected when the bacteria invades the suture of the pod and then passes into the funiculus and through the raphe and is finally harbored under the seed coat.

Epiphytotics of Xp and Xpf are usually found in areas where adequate precipitation and warm temperatures are common during the growing season. If rainfall is low or absent, secondary spread is limited. For this reason Xp and Xpf are rarely problems in areas such as California, Idaho,

Washington, and Oregon; however, these diseases are perennial problems in Michigan, Wisconsin, and Nebraska.

Numerous approaches for the control of common and fuscous blights have been used. Plant breeding has met with only limited success in that no commercial variety is resistant to the disease though some varieties show tolerance. Two Great Northern varieties "Tara" and "Jules" are reported as having moderate and high tolerance, respectively, to common blight (8,9). Tolerance is associated with late maturity (10).

The most effective approach for control of Xp and Xpf at the present time is through the planting of disease-free seed. In Michigan, there are two sources of such seed: a) seed grown in the arid western states of Idaho, California, Oregon, and Washington without overhead irrigation, and b) Michigan certified seed which is only two generations removed from breeder seed grown in the West. Certified seed is grown in Michigan due to the high cost of Western grown seed and secondly since the Western producers can not meet the demands of Michigan's large bean acreage. Western grown seed is also of lower quality because the low humidity under which it is produced results in greater mechanical damage. In order to qualify for certification in Michigan, seed must pass rigid standards established by the Michigan Crop Improvement Association (1).

Despite the effectiveness of planting clean seed to control disease, one disadvantage exists. Such seed is in limited supply and many acres, especially in Michigan, are planted with locally-grown, blighted seed. In Michigan this type of seed is usually one year away from certification and much of it home-grown. As a result interest has been renewed in

the use of chemicals for the control of common and fuscous blights.

Chemical control of Xp and Xpf appears likely because of the excellent control of halo blight achieved with chemicals, especially copper-containing compounds, applied as foliage sprays (14,22,33,41,42). Xp and Xpf have similar infection and dispersal patterns as Pp; they might be expected to react to chemical treatment similar to the halo blight organism. Unfortunately, chemical control of Xp and Xpf has not proven as apparent as with halo blight, especially in Michigan.

Numerous types of chemicals have been tested throughout the years for control of common and fuscous blights. Dimond and Stoddard from 1948 to 1952 controlled common blights in the greenhouse with systemic compounds such as 2-(4-morpholinyl)-ethylphenyl ketone, benzoic acid, sorbic acid, auramine, and various derivatives of salicylic acid applied as drenches to the soil (16,17,18). Field tests were not conducted.

Streptomycin was shown to be translocated from stems to leaves and inhibited symptom expression of halo and common blights in the leaves when applied as a 1% mixture with melted lanolin to kidney bean stems. On the other hand, movement of streptomycin from leaves to stems and fruits or absorption from the soil was not demonstrated (31,32,36). Although effective in greenhouse studies, streptomycin has given marginal blight control in field studies. Marlatt (28) showed no effective control of common blight on pinto beans by 1000 ppm sprays of streptomycin sulfate, but Saettler (38) obtained limited control of common and fuscous blights on Navy (pea) beans with 400 ppm sprays.

Copper-containing compounds have been the most widely studied of the

possible chemical controls for common and fuscous blights. Edgerton and Moreland (20) tested Bordeaux mixture on common blight in 1913 with limited success. However, in 1934 Christow (6) obtained good control with the same material. Burke and Starr (4) in 1948 found that Bordeaux mixture as a dust and spray (12.75%), puratized spray (5%) and cuprox dust (copper oxychloride) were the best treatments on hail-damaged plants. However, none of the treatments gave complete control.

More recent research with modern copper formulations and non-metallic organic bactericides have yielded some promising results; however, even in these instances, results are conflicting. In 1969 Dickens and Oshima (15) obtained good control of secondary spread of common blight on snap beans with 2 applications of O-Cop-53 (53% copper sulfate) (3 lb/100 gal water). In 1971 they (34) obtained near complete control of blight on snap beans with 2 applications of Oxy-Cop-8L (8% copper ammonium carbonate) (.75 gal/100 gal water) and O-Cop-53 (3 lb/100 gal water). Personal communications with Dickens and Oshima in 1973 revealed that they obtained complete control of common blight on snap beans with one spray of Copper-Count-N (8% copper ammonium carbonate) (.5 gal/acre) and Bunema (1.5 gal/acre); on pinto beans they controlled the same disease with 3 applications of Copper-Count-N (.75 gal/acre). In all the experiments by Dickens and Oshima disease control was based on foliage infection and no yield increases were reported. North Dakota has an extension recommendation of Kocide 101 (2-3 lb/acre) and Oxy-Cop 8L (0.33-0.75 gal/acre) for control of common and fuscous blights (7).

In contrast to the Colorado results, Michigan research on Navy

(pea) beans has yielded much less impressive data. Only occasional statistically significant reductions of leaf and pod infection were reported; effective control and yield increases were not obtained. Saettler and Potter in 1967 (41) screened several copper-containing compounds and reported a 50% decrease in common blight on the foliage with Kocide 101. Saettler in 1970 (38) reported only a very small decrease in pod infection by Xp and Xpf with streptomycin sulfate and UNI-G-454. On the other hand, unpublished studies by Saettler in 1972 showed good control of leaf infection with For-Cop-80 NC and moderate control with HPMTS and streptomycin sulfate. In 1973, however, the same treatments proved ineffective. Such inconsistent results have discouraged recommendations of chemicals for common and fuscous blight control in Michigan.

The difference in disease control obtained with sprays in Michigan and Colorado have raised several questions about chemical control of common and fuscous blights. Why are chemical treatments effective in Colorado but ineffective in Michigan? An even more important question is whether chemicals can effectively control secondary spread of common and fuscous blights in Michigan and if so, is such control economically feasible?

The purpose of this study was to attempt to answer these questions in relation to the Navy (pea) bean, the main bean type grown in Michigan.

In trying to find an effective chemical control, numerous chemicals and combinations of chemicals were screened in the laboratory for their relative bactericidal activity against Xp and Xpf. Compounds selected for screening were those previously tested in Michigan or mentioned in

the literature. Chemicals which demonstrated potential in laboratory tests were then examined more extensively in the field.

MATERIALS AND METHODS

A. GENERAL

The following isolates of Xp and Xpf used in laboratory and field experiments were obtained from stock cultures maintained at 5 C in sterile distilled water: Xp 11, Xp 816, Xp 15, Xp 22, Xpf CIAT-A, Xpf 16, Xpf 3, Xpf 28. The strains were grown on yeast extract calcium carbonate agar, (YCA=10 gm Difco yeast extract, 2.5 gm calcium carbonate, 15 gm Difco agar in 1000 ml distilled water); the bacteria usually remained viable until the agar dried up. No more than five transfers were made past the stock culture stage in order to maintain genetic stability in the isolates. The strains of Xp and Xpf were periodically checked for virulence using a seedling injection technique (39). All of the blight isolates were from Michigan except Xp 816 and Xpf CIATA-A which were from Nebraska and Colombia, respectively.

Strains used as assay organisms for laboratory screening of chemicals were grown in 125 ml flasks containing 50 ml of buffered yeast extract liquid medium (BYE=10 gm yeast extract per 1000 ml, 0.01 M phosphate buffer, pH 7.2). The flasks were inoculated with one loopful of bacteria suspended in a 1 ml sterile water blank. Sufficient growth was obtained within 24 hr after seeding for use as inoculum.

Chemicals tested in the laboratory and the field were commercial formulations alone or combinations of such formulations. Stock chemicals were stored at 25C in manufacturer's containers. Prior to each laboratory experiment, stock formulations were diluted in sterile BYE

or distilled water to appropriate concentrations. Chemicals tested in the field were suspended in tap water.

The following chemicals were subject to initial screening in the laboratory:

Agri- Strep 500- (53.3% Streptomycin sulfate), Merck and Co. Inc.

Bunema - (40% Potassium N-hydroxymethyl -N-methyldithiocarbamate),
Buckman Laboratories, Inc.

Commercial Bleach - (5.25% Sodium hypochlorite), Patterson
Laboratories, Inc.

For-Cop-80 NC - (8% Copper from copper ammonium carbonate),
Forshaw Chemical, Inc.

HPMTS - (100% 2-hydroxypropyl methane thiosulfonate), Buckman
Laboratories, Inc.

Isobac 20 - (20% Mono sodium salt of 2, 2' methylenebis 3, 4,
6-trichlorophenol), Nationwide Chemical Corporation.

Kocide 101 - (83% Cupric Hydroxide), Kennecott Copper Corporation

Nabac 25 EC - (25% 2, 2'-methylenebis 3, 4, 6-trichlorophenol),
Nationwide Chemical Corporation.

Oxine - (2% Chlorine dioxide), Lilly Products.

B. INHIBITION STUDIES IN LIQUID CULTURE

Chemical inhibition experiments were carried out in 25 x 150 mm culture tubes. A measured amount of BYE was added to the tube and autoclaved; appropriate amounts of chemical diluted in sterile BYE were then added to yield a total volume of 25 ml BYE with a known chemical

concentration. The pH of the solution at each test concentration was measured and consistently fell between 6.5 and 7.5, except with Oxine where the pH levels were slightly above 8.0 for concentrations of 1000-5000 ppm. The culture tubes were inoculated with approximately 3×10^8 bacteria and were incubated on a rotary shaker at room temperature. To determine the amount of growth, optical density was measured with a Spectronic 20 colorimeter set at 620 nm wavelength, or visual estimates of turbidity were made.

Forty-eight hours after inoculation, four drops from the inoculated tubes were transferred to fresh culture tubes containing 25 ml of BYE in order to check for bacteriostatic activity by the chemical. This transfer resulted in a 1/125 dilution of the original chemical concentration. Each chemical at each concentration was replicated three times. All stock chemicals were used without sterilization, except for Agri-Strep 500, which was dissolved in distilled water and sterilized through a fritted glass filter. These same procedures were also used for a second experiment in which various chemical combinations were compared.

C. DISC ASSAY

A disc assay was adapted from the methods of Thornberry and other previous researchers (47,27) in order to assay for the presence of bactericides in tissue samples and to compare chemical tolerance of blight isolates. Ten ml of autoclaved water agar (15 gm agar per 1000 ml distilled water) were pipetted into petri plates and designated, bottom agar. Four ml portions of soft BYE agar (10 gm agar per 1000 ml

BYE) were autoclaved in 13 X 10 mm test tubes and designated, top agar. As needed the top agar was melted and held at 45 C in a water bath. One ml of a 3×10^7 bacteria per ml suspension was added to the top agar and then layered on the plates of bottom agar. Filter paper discs (Schleicher and Schuell) of 12.7 mm diam were either dipped in chemical preparations, or the preparations were pipetted onto the discs. Three discs were placed on each plate and the diameter of inhibition zones were measured across the center of the disc 48 hrs later.

D. CHEMICAL UPTAKE

An attempt was made to assay for HMPTS, Bunema, Isobac, Agri-Strep 500, For-Cop-80 NC and Kocide 101 at various time intervals after their application to bean leaves in order to determine their relative persistence in and on the leaves. Of the above chemicals only streptomycin is known to be absorbed and translocated in bean leaf tissue (31); the fixed coppers are protectants and easily detected on the leaf surface but never systemic. HPMTS, Bunema and Isobac are not known as systemics, however, slight tissue penetration by the chemicals might be possible and such activity would be useful in disease control.

Navy (pea) beans ('Gratiot' cultivar) were grown in the greenhouse for approximately 4 weeks. Twenty-five ml aliquots of 1000 or 2000 ppm solutions were sprayed on the 4 plants in each pot using a hand spray applicator. The volume applied was adequate to cover all secondary leaves to the point of run off. Each pot represented one replication

and each chemical treatment at each concentration was replicated 3 times. On three consecutive days after spraying, twenty leaflets per replication were sacrificed; the leaflets were divided into washed and unwashed groups. In a procedure modified from Crossan (11), all leaflets of the washed group were held under running distilled water for 30 sec. and the surface was gently rubbed. The unwashed group of leaflets was not rinsed. One tissue disc, 23 mm in diameter was cut from each of 10 leaflets. The ten discs were combined and then ground in a glass tissue grinder with 1 ml of sterile distilled water and 0.08 ml was applied to filter paper discs which were placed on agar, seeded with Xp 11.

E. FIELD STUDY OF CHEMICALS APPLIED EARLY AND LATE

Field experiments were carried out during the summer of 1974 at the Botany and Plant Pathology Farm, M.S.U. A 400 X 56 ft block of Navy (pea) beans ('Gratiot' var) was mechanically planted on 15 June with 28 inches between rows and 2 inches between plants within the row. After seedling emergence the field was divided into 8 ranges, 45 ft long, east to west, with 3 feet of unplanted row between the ranges. Each range consisted of 9 separate treatment replications, and each replication consisted of 2 adjacent rows. Each treatment replication was bordered by a 'spreader' row on both sides, which were inoculated with Xp and Xpf and thus served as the source of inoculum for secondary spread. Early and late treatments were placed in alternating ranges.

'Spreader' rows were inoculated with Xp 15, Xp 11, Xp 22; Xpf 16, Xpf 28, and Xpf 3 on 27 July using a Knapsack sprayer; inocula were prepared in water at a concentration of 10^6 cells/ml and applied at a

rate of 15 ml per lineal ft of row. In order to insure successful blight development the spreader rows were re-inoculated on 30 July with a power sprayer operating at 210 psi and at a rate of 20 ml per lineal ft using the same isolates and concentration. With power inoculation, the foliage became water-soaked thus assuring that bacteria were in the leaf tissue.

The following chemicals were applied at a rate of 73.2 gal spray material per acre with ppm based on manufacturer's indicated percent active ingredient:

Agri-Strep 500, 1000 ppm (1.084 lb/acre)

Agri-Strep 500 + Bunema, 500 ppm + 2000 ppm (.542 lb + .366 gal/acre)

Agri-Strep 500 + For-Cop-80 NC, 500 ppm + 500 ppm (.542 lb + .458 gal/acre)

Agri-Strep 500 + Isobac, 500 ppm + 100 ppm (.542 lb + 4.685 oz/acre)

Bunema, 4000 ppm (.732 gal/acre)

Bunema + Isobac, 2000 ppm + 100 ppm (.366 gal + 4.685 oz/acre)

For-Cop-80 NC, 1000 ppm (.915 gal/acre)

HPMTS, 2500 ppm (.183 gal/acre)

Isobac, 200 ppm (9.370 oz/acre)

Kocide 101, 3000 ppm (2.206 lb/acre)

Nabac 25 EC, 156 ppm (5.856 oz/acre) .

Each chemical was applied as an early treatment and a late treatment.

Early treatments were applied on 7/25, 8/1, 8/8, 8/15, 8/25, 9/7/74;

late treatments were applied on 8/9, 8/16, 8/25, 9/7/74. Applications were generally at 7 day intervals except between 8/15-9/7, during which time the intervals were increased in order to allow irrigation and leaf

data collection. Late treatments simulated the normal spray pattern used by this laboratory in previous years, in which sprays were initiated when visible symptoms appeared in the spreader rows. Early treatments were initiated two weeks before late sprays. The purpose behind using two spray schedules was to test the hypothesis that better disease control would result if foliage protection was present earlier in the growing season.

Spray applications were made with a Knapsack sprayer. After each replication was sprayed, the sprayer was repressurized to capacity to ensure uniform flow rate. All sprays were applied mid-morning in order to avoid the heat of the afternoon, when the plants are most susceptible to chemical phytotoxicity. To enhance symptom expression on the plots, overhead irrigation was set up on 8/22 and the field was watered for a total of 10 hr over a 3 day period. On 8/25 an increase in symptoms was noted.

Data collection

Data were collected by four methods: (1) visual evaluation based on a 0-4 scale, in which twenty locations per treatment replication were rated, (2) blight lesions were counted on leaflets from 8 plants per replication between 9/1 and 9/4/74, (3) pod lesions were counted on ten plants per replication between 9/12 and 9/17/74, and (4) six lineal meters of plants in each replication were harvested on 10/22/74 and threshed in order to determine yield. All leaf infection data were collected before the last spray application. The pods were in a mixed

state of maturity with most in the green-fleshy or yellow-fleshy stage and some in the yellow dried stage.

Monitoring bacterial populations in healthy tissue

An attempt was made to monitor bacterial populations present in symptomless leaf tissue. After spreader rows were inoculated, leaf tissue samples from plants in the control rows were taken at several times in order to determine when blight was spreading from the spreader rows. Ten discs of tissue 10 mm in diameter were taken from each of 5 leaves. The discs were then surface sterilized, (2.125% sodium hypochlorite for 3 min) and ground with 10 ml sterile distilled water in a glass tissue grinder. Serial dilutions were made in distilled water, plated in BYE agar, and colonies were counted after 3 days. Since selective media for Xp and Xpf are unavailable, all blight-suspect colonies were transferred from the dilution plates and streaked on fresh YCA plates. The isolated colonies were then tested for pathogenicity via the seedling injection technique (39).

F. COOPERATIVE FIELD STUDY

A second field study was run at the Botany and Plant Pathology Farm in cooperation with Dr. H. S. Potter, Extension Plant Pathologist at M.S.U. The following treatments were tested as possible chemical protectants for common, fuscous, and halo blights on three bean cultivars, Navy (Gratiot), Red Kidney (Manitou) and Snap (Cascade):

Citcop 4E (48% copper salts of fatty and rosin acids),
9600 ppm (2 qt/acre)

Citcop 4E + Copper sulfate (53% metallic copper), 9600 ppm +
500 ppm 2 qt + 2 lb/acre

Dithane M-22 Special (80% Maneb) + Kocide 101, 3200 ppm
+ 4200 (1 lb + 1 lb/acre)

Nabac 25 EC, 625 ppm (0.5 pt/acre)

Nabac 25 EC + Kocide 101, 625 ppm + 4200 ppm (0.5 pt + 1 lb/acre)

1451 (variation of Kocide 101, 57% metallic copper), 5500 ppm
(2 lb/acre)

1501 (variation of Kocide 101, 57% metallic copper), 5500
ppm (2 lb/acre)

All chemical sprays were applied at a rate of 25 gal per acre with a boom sprayer operating at 40 psi pressure and moving at 4 mph. Three flat fan nozzles were used per row with one on the top of the row and one on either side of 12 in drops. Eight applications were made on a 7-10 day schedule beginning on 7/25 and ending on 9/19/74.

The plot was planted on 6/21/74 and consisted of 42 rows, each approximately 120 ft long; the plot was divided into 3 blocks with each treatment replicated once in each block. Each treatment consisted of four rows, 2 rows of snap beans in the middle and one row of Navy (pea) beans on one side and one row of kidney beans on the other.

The kidney and snap beans were inoculated with Pp using a Knapsack sprayer on 7/15/74. The Navy beans were power-inoculated on 30 July with the same methods and isolates of Xp and Xpf as used in the other field inoculation. In this thesis only data from the Navy beans are considered because symptoms caused by Pp in the snap and kidney beans could not be distinguished from that caused by Xp and Xpf.

Foliage infection was rated on 9/10/74 using the Horsfall and Barratt method (23). Nine lineal feet of each replication for each bean type was harvested on 10/27/74 for yield.

RESULTS

A. GENERAL CHEMICAL SCREENING

Results of experiments with 9 chemicals which were screened in liquid culture for bactericidal and bacteriostatic activities against Xp and Xpf are summarized in Table 1. Xp 15, Xp 11, Xp 16, Xpf 816, Xpf CIAT-A, and Xpf 3 were used individually as inocula and the end point of bactericidal activity was the concentration at which all isolates were inhibited. A constant inoculum concentration (3×10^8 cells/ml) was maintained, since preliminary studies showed marked variation in biocidal effects of the chemicals when inoculum concentration was not standardized.

The non-metallic organic compounds, HPMTS, Bunema, Isobac and Nabac 25 EC were the most toxic of the chemicals and were bactericidal between 50-100 ppm. The copper compounds, For-Cop-80 NC and Kocide 101 were less toxic, and exhibited bactericidal action between 250-500 ppm. Relatively high concentrations of Bleach (NaClO) and Oxine (ClO_2) were needed for bactericidal activity. The difference in activity between For-Cop-80 NC and Kocide 101 was actually less than indicated by Table 1. The concentration for For-Cop-80 NC was based on metallic copper content, whereas the concentration of Kocide 101 was based on copper hydroxide as the active ingredient. When the Kocide 101 concentration was recalculated on the basis of metallic copper, bactericidal activity occurred at 325 ppm. Also, Kocide 101 is a wettable powder and may quickly fall out of aqueous suspension compared to the more water-soluble For-Cop-80 NC.

In vitro activity of Isobac and Nabac 25 EC also probably differed less than indicated since Isobac is the sodium salt of Nabac and consequently more water-soluble than Nabac 25 EC.

Despite the known effectiveness of streptomycin sulfate as a bactericidal agent, no end point was determined for Agri-Strep 500 in this study. The only consistent quality of the material in its inhibitory action was its variability. Table 2 shows one experiment with Agri-Strep 500; the same pattern was found at lower concentrations. The pattern basically shows that Xp and Xpf were inhibited at a certain concentration, yet sometimes not at a higher concentration. Table 2 shows that 2000-5000 ppm Agri-Strep 500 was almost always sufficient to kill all the bacteria inoculated into the culture tubes. However, the few tubes with higher concentrations of Agri-Strep 500 that became turbid probably received cells that were resistant (Table 2). This type of resistance is probably similar to that encountered in E. coli where resistance to streptomycin does not develop in a stepwise manner but mutants for high, medium or low resistance are equally probable (12). The resistant Xp and Xpf cells were naturally present in the population since the inoculum was not previously exposed to streptomycin sulfate.

On the basis of the screening experiments, Bleach and Oxine were eliminated from further testing due to lack of sufficient bactericidal activity. Of the nine chemicals tested Isobac and Bunema showed the best bactericidal activity. Oxine, Bleach and Isobac have never been field-tested for control of Xp or Xpf in the past.

Table 1.--End point determination of bactericidal and bacteriostatic action of various chemicals against Xp and Xpf strains as determined by bacterial inhibition in liquid culture^a.

Chemicals	Chemical concentrations (ppm) ^b									
	10	50	100	250	500	1000	2000	3000	4000	
Agri-Strep 500				variable ^c						
Bleach										X ^e
Bunema		X								
For-Cop-80 NC ^d			0	X						
HPMTS			X							
Isobac		X								
Kocide 101 ^d					X					
Nabac 25 EC		0	X							
Oxine					0					X

^aXp 15, Xp 11, Xp 816, Xpf 16, Xpf CIAT-A, Xpf 3, were used individually as inocula; 96 hr after inoculation 4 drops were transferred from the inoculated tubes to fresh tubes. Value is concentration at which all isolates were inhibited.

^bConcentration based on manufacturer's labelled active ingredient.

^cConstant end point not obtained.

^dKocide 101 (ppm) concentration based on copper hydroxide as active ingredient; For-Cop-80 NC (ppm) concentration based on metallic copper as active ingredient.

^e0=Bacteriostatic action (inoculated tubes remained clear, transfer tubes became turbid).

X=Bactericidal action (both inoculated tubes and transfer tubes remain clear).

Table 2.--Inhibitory activity of Agri-Strep 500 against Xp and Xpf^a.

Isolate	Replication	Agri-Strep 500 (ppm)				
		1000	2000	3000	4000	5000
<u>Xp</u> 11	1	.085	.000	.000	.010	.010
	2	.100	.050	.000	.031	.000
<u>Xp</u> 15	1	.025	.070	.010	.000	.025
	2	.120	.000	.000	.000	.000
<u>Xpf</u> CIAT-A	1	.180	.000	.100	.100	.000
	2	.160	.030	.000	.070	.000
<u>Xpf</u> 3	1	.130	.050	.000	.000	.000
	2	.000	.000	.000	.000	.000

^aOptical density readings at 96 hr. after inoculation measured by Spectronic 20 at 620 nm.

B. CHEMICAL COMBINATIONS

The possibility that chemical combinations might be more toxic to blight bacteria than individual chemicals was studied. Agri-Strep 500, For-Cop-80 NC, HPMTS, Bunema, and Isobac were compared in all possible dual combinations. In chemical combinations one-half of the chemical concentration of each chemical was used (Figure 1).

The combinations of Bunema-Isobac, Agri-Strep 500-Isobac, and Agri-Strep 500-Bunema had greater toxicity than any of the chemicals alone (Fig. 1). However, the combinations of Bunema-Isobac and Agri-Strep 500-Isobac were more toxic than Agri-Strep 500-Bunema since the two former allowed no growth even after 3 days of incubation whereas the later combination did.

A second group of combinations, Agri-Strep 500-HPMTS, Isobac-HPMTS, For-Cop-80 NC-Isobac, and Bunema-HPMTS showed greater inhibitory activity than one of their parent chemicals, but less activity than the other. In one case Agri-Strep 500-For-Cop-80 NC combination was as inhibitory as Agri-Strep 500 alone but better than For-Cop-80 NC alone. All other combinations were less inhibitory than each compound alone (Fig. 1).

The enhanced inhibitory action of some chemical combinations as compared to individual chemicals may be due to an increase in the number of modes of action against the bacteria.

The results indicate that certain chemical combinations can lead to enhanced activity as compared to parent compounds; however, randomly mixing chemicals will not ensure greater activity by the combination than the parent compounds.

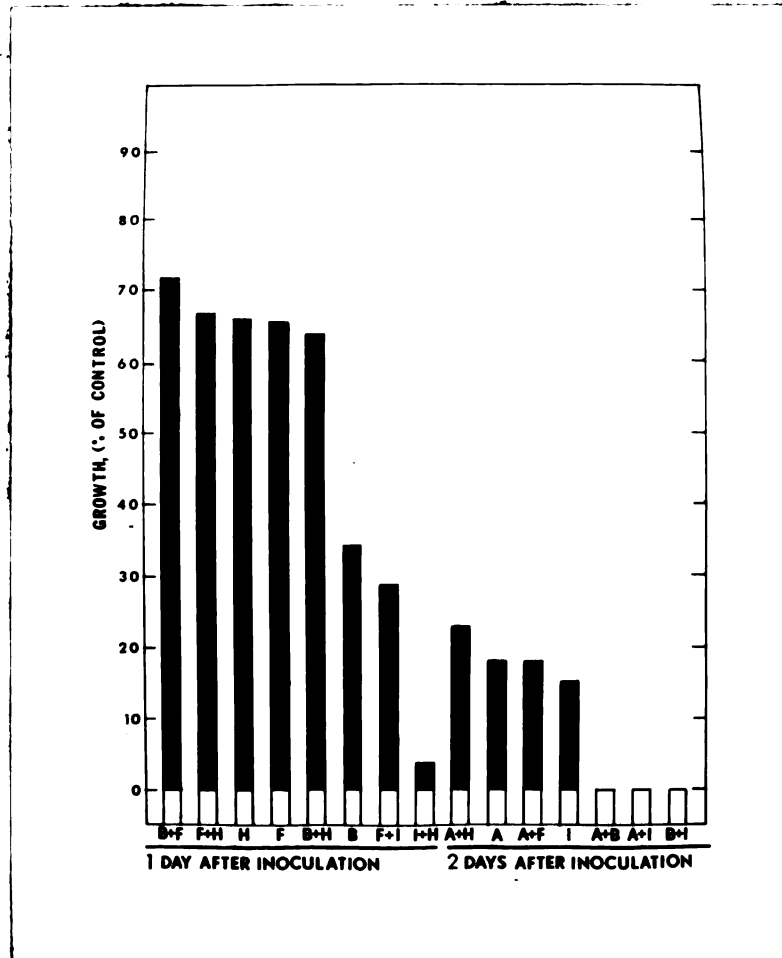
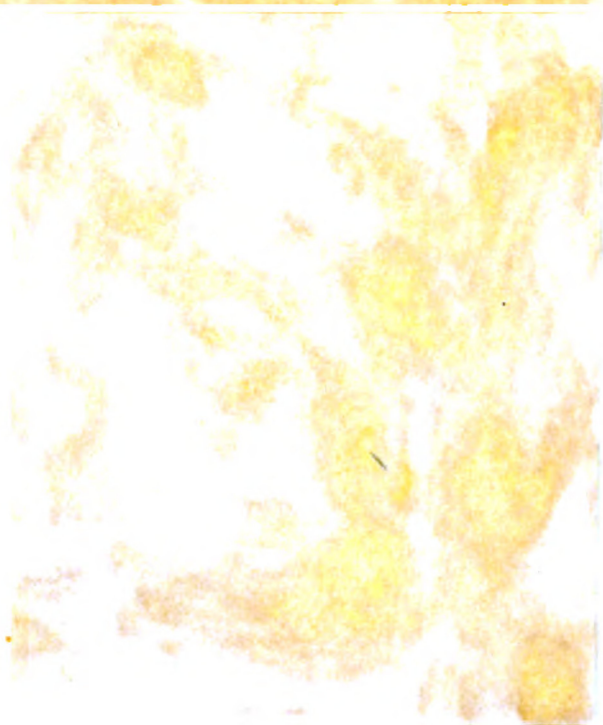
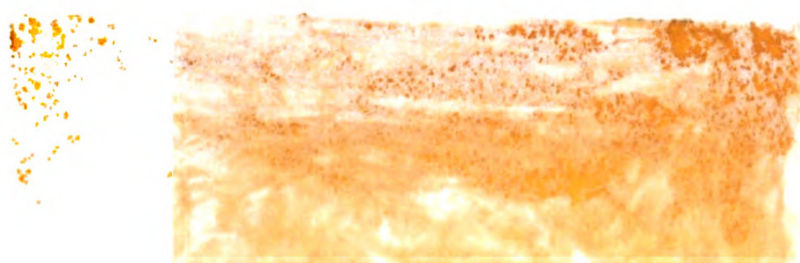


Figure 1. -- Growth of Xp 11, as determined by optical density, in liquid media containing 10 ppm of various chemicals or chemical combinations; A = Agri-Strep 500, B = Bunema, F = For-Cop-80 NC, H = HPMTS, I = Isobac.



C. UPTAKE EXPERIMENT

The purpose of the uptake experiment was to determine the relative persistence of Agri-Strep 500, Bunema, Isobac, HPMTS, For-Cop-80 NC and Kocide 101 on the leaf surface, and to detect possible local systemic activity by Bunema, Isobac and HPMTS. Standard curves for each chemical were established which related chemical concentration to zone size using the disc assay method. The sensitivity of the assay standards for each chemical differed; Agri-Strep 500 was detected as low as 40 ppm, Isobac at 80 ppm, For-Cop-80 NC at 200 ppm, Kocide 101 at 300 ppm, HPMTS at 60 ppm and Bunema at 400 ppm. Although the standards were established by diluting the chemicals in distilled water, ground leaf tissue did not interfere with zone size when tissue extracts were added to the diluted chemicals.

The chemicals were compared on the basis of ppm active ingredient recoverable as estimated by the bio-assay (Table 3). Agri-Strep 500 was recovered at the highest concentration, and therefore, showed the best residual activity of the tested chemicals. As expected Agri-Strep 500 was detected internally as had been demonstrated in the past (31). The copper compounds also demonstrated good residual quality over the three day period and were easily recovered. These 3 chemicals demonstrated that the system could be used to detect active chemicals on the leaf surface. HPMTS, Bunema, and Isobac were not detected internally or externally as residues. The inability to detect Bunema could be due to the low sensitivity of the bio-assay. However, this appears unlikely in the

Table 3.--Comparison of residual and systemic activities of various chemicals on bean leaf tissue.

Chemical	Concentration applied (ppm)	Leaflet processing ^a	Chemical Concentration estimated by bio-assay after spraying		
			day 1	day 2	day 3
Agri-Strep 500	1000	W	240 ^b	240	100
		UW	2100	1300	580
	2000	W	700	330	120
		UW	2100	1500	2000
Bunema	1000	W	0	0	0
		UW	0	0	0
	2000	W	0	0	0
		UW	0	0	0
For-Cop-80 NC	1000	W	0	0	0
		UW	390	280	350
	2000	W	0	0	0
		UW	1300	800	450
HPMTS	1000	W	0	0	0
		UW	0	0	0
	2000	W	0	0	0
		UW	0	0	0
Isobac	1000	W	0	0	0
		UW	0	0	0
	2000	W	0	0	0
		UW	0	0	0
Kocide 101	1000	W	0	0	0
		UW	0	0	0
	2000	W	0	0	0
		UW	900	430	LOST

^aW=washed, UW=unwashed

^bChemical concentration (ppm active ingredient) recovered from 10 discs ground with 1 ml sterile water.

case of ISOBAC and HPMTS since their sensitivity of detection was comparable to Agri-Strep 500 and superior to FOR-Cop-80 NC. It appears, then, that HPMTS and Isobac are less persistent on the leaf surface than Agri-Strep 500 or the coppers and suggest rapid loss of activity by these 2 chemicals.

In order to determine the speed of the deactivation of Isobac, Bunema, and HPMTS the experiment was repeated with these 3 chemicals. Samples were taken at 2 and 24 hr after spraying and assayed by the disc method. No inhibitory activity was detected with any of the chemicals at either sample time, thus suggesting the possibility of rapid deactivation on the leaf surface. However, the presence of an unknown factor which could interfere with the assay method for Isobac, HPMTS, and Bunema could also be involved. If indeed rapid deactivation of the 3 chemicals does take place the potential of these chemicals in the field would definitely be limited.

D. FIELD STUDY OF CHEMICALS APPLIED EARLY AND LATE

Foliage infection

Chemicals applied as both early and late sprays significantly reduced foliage infection evaluated by Duncan's Multiple Range Test (19). On the basis of % leaflet infection, 10 treatments significantly reduced infection at the 5% level. Three groups of chemicals could be recognized (Table 4). Seven treatments, For-Cop-80 NC (early,late), Agri-Strep 500-Isobac (late), HPMTS (early), Agri-Strep 500-For-Cop-80 NC (early),

Bunema (late) and Isobac (early) were all statistically similar, and effected the lowest degree of disease reduction; Kocide 101 (early) was statistically different from the above treatments and demonstrated the best control; Bunema (early) and Kocide 101 (late) were statistically similar to both of the above groups.

On the basis of number of lesions per leaflet, a similar pattern of effective treatments was recognized except that Agri-Strep 500-Isobac (late and Bunema (late) were eliminated as effective treatments (Table 4). However, in contrast to the first rating method, all eight treatments fell into one group and thus reduced infection equally.

With visual evaluations, at the 5% level of testing, only 6 treatments significantly reduced leaf infection (Table 4); however, one of them Bunema-Isobac (early) was not significantly different by the other 2 rating methods. For-Cop-80 NC (late), Bunema (early, late), and Bunema-Isobac (early) were the least effective treatments, Kocide 101 (early) was statistically the best treatment. Kocide 101 (late) was intermediate in disease control and statistically equal to both of the above two groups.

Only 3 treatments, Kocide 101 (early and late) and Bunema (early), were effective at disease control when data were tested at the 1% level of significance (Table 5). By each infection rating all three treatments were statistically similar in the degree of disease reduction. However, based on gross mean values Kocide 101 (early) was consistently the best treatment.

Foliage infection ratings indicated that early sprays were more

effective in reducing $\bar{X}_p$ and $\bar{X}_{pf}$ than late sprays. At the 5% significance level, 6 of 11 chemicals gave control as early sprays, but only 4 of 11 gave control as late sprays, when rated by % leaflet infection. On the basis of No lesions per leaflet, 6 of 11 early sprays gave control compared to 2 of 11 late sprays. However, on the basis of visual ratings an equal number, 3 of 11 early and 3 of 11 late sprays, of treatments reduced blight. The enhanced activity of early sprays was further supported by 1% level testing which showed that Bunema was effective only as an early spray.

Pod Infection

No treatment significantly reduced infection of bean pods by $\bar{X}_p$ and $\bar{X}_{pf}$ (Table 4 and 5). However, gross mean values of pod infection data showed that Kocide 101 (early) was the best control (Table 4); Kocide 101 was also the best control of foliage infection.

Yield

None of the treatments significantly increased yield (Table 6). Yield samples taken from the early spray plots except those of Isobac, Bunema-Isobac, Agri-Strep 500-Bunema, were slightly lower than expected since plants in one replication of these had delayed development and poor pod set. In addition, one of the Bunema (early) replications received cultivation damage and thus contained fewer plants.

Monitoring of bacterial populations in healthy tissue

Bacterial populations in symptomless leaf tissue from control row plants were monitored, beginning just after the spreader rows were inoculated. The purpose was to detect when blight bacteria became

Table 4.--The effect of various chemical sprays on leaf and pod infection of Navy (pea) beans by Xp and Xpf evaluated at the 5% level^a.

Chemical	Concentration (ppm)	Spray Schedule	Leaf Infection			Pod Infection ^e	
			%leaflet infection ^c	No. lesions per leaflet	Visual evaluation ^d	% pod infection	
Agri-Strep 500	1000	E	21.9 a-c	0.37 a-b	26.1 a	3.14 a	
		L	24.8 a	0.39 a	23.0 a-m	3.73 a	
Agri-Strep 500 + Bunema	500 + 2000	E	18.6 b-i	0.31 a-g	22.6 a-n	4.12 a	
		L	20.4 a-e	0.34 a-f	24.1 a-f	4.08 a	
Agri-Strep 500 + For-Cop-80 NC	500 + 500	E	14.8 e-t	0.22 d-p	23.1 a-k	2.85 a	
		L	19.2 a-h	0.29 a-h	23.1 a-l	4.16 a	
Agri-Strep 500 + Isobac	500 + 100	E	19.8 a-g	0.27 a-i	24.8 a-c	3.14 a	
		L	16.6 c-n	0.23 c-o	23.3 a-j	4.36 a	
Bunema	4000	E	10.4 n-u	0.14 i-p	18.1 n-u	3.75 a	
		L	15.6 c-p	0.25 c-n	19.8 d-t	4.17 a	
Bunema + Isobac	2000 + 100	E	17.6 b-l	0.26 a-l	21.0 c-r	4.56 a	
		L	21.6 a-d	0.34 a-f	24.0 a-g	4.71 a	

^aValues followed by any common letter are not statistically different at the 5% level (Duncan's Multiple Range Test).

^bE=early spray, L=late spray.

^cEight plants per replication sampled.

^dSum of twenty ratings divided by 2; rating scale 0-4.

^eTen plants per replication sampled.

"Table 4 (cont'd)"

For-Cop-80 NC	1000	E	15.4 d-g	0.20 g-p	21.1 b-q	3.16 a
		L	15.1 e-s	0.21 d-p	19.8 d-t	3.15 a
HPMTS	2500	E	15.3 d-r	0.21 d-p	22.1 a-o	4.60 a
		L	18.0 b-k	0.25 c-n	21.5 a-p	4.43 a
Isobac	200	E	15.9 c-o	0.22 d-p	23.5 a-i	4.50 a
		L	17.4 b-m	0.26 a-l	23.6 a-h	6.10 a
Kocide 101	3000	E	8.1 u	0.09 p	12.6 v	1.91 a
		L	11.9 j-u	0.17 h-p	15.6 s-v	3.32 a
Nabac 25 EC	154	E	18.1 b-j	0.26 a-l	24.5 a-e	4.80 a
		L	20.1 a-f	0.34 a-f	24.5 a-e	6.32 a
Control			23.1 a-b	0.36 a-c	26.0 a-b	5.86 a

^aValues followed by any common letter are not statistically different at the 5% level (Duncan's Multiple Range Test).

^bE=early spray, L=late spray.

^cEight plants per replication sampled.

^dSum of twenty ratings divided by 2; rating scale 0-4.

^eTen plants per replication sampled.

Table 5.--The effect of various chemical sprays on leaf and pod infection of Navy (pea) beans by Xp and Xpf evaluated at the 1% level^a.

Chemical	Concentration (ppm)	Spray schedule ^b	Leaf Infection		Pod infection ^e	
			% leaflet infection ^c	No. lesions per leaflet	Visual evaluations ^d	% pods infection
Agri-Strep 500	1000	E	21.9 a-c	0.37 a-b	26.1 a	3.14 a
		L	24.8 a	0.39 a	23.0 a-m	3.73 a
Agri-Strep 500 + Bunema	500 + 2000	E	18.6 a-i	0.31 a-g	22.6 a-n	4.12 a
		L	20.4 a-e	0.34 a-f	24.1 a-f	4.08 a
Agri-Strep 500 + For-Cop-80 NC	500 + 500	E	14.8 b-o	0.22 a-i	23.1 a-k	2.85 a
		L	19.2 a-h	0.29 a-h	23.1 a-l	4.16 a
Agri-Strep 500 + Isobac	500 + 100	E	19.8 a-g	0.27 a-i	24.8 a-c	3.14 a
		L	16.6 a-n	0.23 a-i	23.3 a-j	4.36 a
Bunema	4000	E	10.4 j-o	0.14 g-i	18.1 d-u	3.75 a
		L	15.6 b-o	0.25 a-i	19.8 a-t	4.17 a
Bunema + Isobac	2000 + 100	E	17.6 a-l	0.26 a-i	21.0 a-r	4.56 a
		L	21.6 a-d	0.34 a-f	24.0 a-g	4.71 a

^aValues followed by any common letter are not statistically different at the 1% level (Duncan's Multiple Range Test).

^bE=early spray, L=late spray.

^cEight plants per replication sampled.

^dSum of twenty ratings divided by 2; rating scale 0-4.

^eTen plants per replication sampled.

"Table 5 (cont'd)"

For-Cop-80 NC	1000	E	15.4 b-o	0.20 b-i	21.1 a-q	3.16 a
		L	15.1 b-o	0.21 a-i	19.8 a-t	3.51 a
HPMTS	2500	E	15.3 b-o	0.21 a-i	22.1 a-o	4.60 a
		L	18.0 a-k	0.25 a-i	21.5 a-p	4.43 a
Isobac	200	E	15.9 b-o	0.22 a-i	23.5 a-i	4.50 a
		L	17.4 a-m	0.26 a-i	23.6 a-h	6.10 a
Kocide 101	3000	E	08.1 o	0.09 i	12.6 u	1.91 a
		L	11.9 e-o	0.17 d-i	15.6 p-u	3.32 a
Nabac 25 EC	154	E	18.1 a-j	0.26 a-i	24.5 a-d	4.80 a
		L	20.1 a-f	0.34 a-f	24.5 a-d	6.32 a
Control			23.1 a-b	0.36 a-c	26.0 a-b	5.86 a

^aValues followed by any common letter are not statistically different at the 1% level (Duncan's Multiple Range Test).

^bE=early spray, L=late spray.

^cEight plants per replication sampled.

^dSum of twenty ratings divided by 2; rating scale 0-4.

^eTen plants per replication sampled.

Table 6.--Effect of various chemical treatments on yield of Navy (pea) beans infected with Xp and Xpf.

Chemical	Concentration (ppm)	Yield ^b	
		Early spray schedule ^a	Late spray schedule
Agri-Strep 500	1000	578.1 a	606.1 a
Agri-Strep 500 + Bunema	500 2000	659.2 a	615.9 a
Agri-Strep 500 + For-Cop-80 NC	500 500	481.1 a	588.7 a
Agri-Strep 500 + Isobac	500 100	656.1 a	679.1 a
Bunema	4000	628.3 a	725.5 a
Bunema + Isobac	2000 100	575.1 a	605.6 a
For-Cop-80 NC	1000	353.6 a	524.2 a
HPMTS	2500	479.5 a	546.8 a
Isobac	200	627.5 a	609.8 a
Kocide 101	3000	525.3 a	611.4 a
Nabac 25 EC	154	496.5 a	662.6 a
Control	---	593.3 a	

^aValues followed by any common letter are not statistically different at the 5% level (Duncan's Multiple Range Test).

^bGm seed per 6 lineal meter per replication; average of 3 replications.

established in healthy leaf tissue as a result of secondary spread (Table 7). No attempt was made to distinguish between Xp and Xpf on the agar plates. Very few or no blight bacteria were detected on 8/1, 8/7, and 8/12/74. On 8/15, an increase in the population was detected in replication 4. Blight populations increased suddenly in tissue samples assayed on 8/20/74. The sudden increase in Xp and Xpf may have been due to either a mass spread of bacteria from the spreader rows or sudden multiplication of undetectable levels of Xp and Xpf residing in the tissue as the result of earlier secondary spread. After 8/20/74, the Xp-Xpf population increased steadily.

All samples were taken from healthy symptomless leaves; visibly-infected leaves were purposely avoided. The control plants were generally symptomless and showed only scattered yellowing up to 8/22. However, after this time the foliage developed increased numbers of typical blight lesions.

In addition to Xp and Xpf, numerous bacteria were detected in healthy tissue which appeared to be non-pathogenic by the seedling injection technique (39). The bacteria were of several morphologies and species. The most prevalent colony type produced smooth, dark yellow colonies on YCA.

No attempt was made to classify the bacteria. Most of the colonial types consistently appeared throughout the sampling period. The levels of these bacteria were low until 8/12, when their levels increased. Between 8/1 and 8/15 the non-pathogens were more predominant than Xp and Xpf in symptomless leaf tissue; after this time Xp and Xpf levels

increased rapidly and predominated. The range of non-pathogen levels varied little among samples taken at the same time, and never exceeded a 10-fold difference except in one case, on 8/27 when about a 100 fold difference occurred. Generally these non-pathogens appear to be uniformly distributed in healthy bean leaf tissue.

Several fungi were occasionally isolated from the tissues and are not included in Table 7, because of their low isolation frequency. It would appear that there is very little fungus colonization of internal bean leaf tissue.

Isolation of Xp and Xpf from bean leaf tissue was hampered considerably due to the absence of a selective medium. Nevertheless, relatively high levels of Xp-Xpf were detected in symptomless leaves. The levels of Xp and Xpf per unit area of tissue were probably higher than presented, since 5 discs were pooled for a sample and the probability of more than one or 2 discs having bacteria was low. A second important point is that blight bacteria were detected almost 2 weeks before typical blight symptoms developed.

E. COOPERATIVE FIELD EXPERIMENT

Chemical treatments significantly reduced foliage infection by Xp and Xpf (Table 8). At the 5% level, Dithane M-22 Special, 1451, Citop 4E + Copper sulfate and Nabac 25 EC + Kocide 101 were statistically comparable in disease reduction. Nabac 25 EC and 1501 gave comparable control as the above treatments; however, 1501 was statistically better than Nabac 25 EC. Analysis at the 1% level demonstrated that all

Table 7.--Levels of pathogenic (P) and non-pathogenic (NP) bacteria internally present in symptomless bean leaf tissue from control plots^{a,b,c}

Replication	Bacterial cells per 25 cm ² of symptomless leaf tissue at indicated sampling date															
	8/1		8/7		8/12		8/15		8/20		8/24		8/27			
	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP
1	0	23	0	11	0	610	1	23	220	265	0	32	0	68		
2	0	2	2	7	0	15	0	57	2595	175	0	28	0	55		
3	0	10	0	12	1	6	0	345	1090	45	1700	0	1650	0		
4	0	27	0	16	0	14	10	73	15	9	0	190	55500	7000		
5	-	-	0	9	0	4	0	59	-	-	2085	0	20700	600		

^aEach replication represents 10 discs; 2 discs (diam.=18mm) taken from each of 5 randomly selected symptomless leaves.

^bP=pathogen (Xp or Xpf), NP=non-pathogen, determined by seedling injection technique.

^cSpreader rows were inoculated on 30 July.

treatments, except Nabac 25 EC, reduced blight significantly, and were equally effective. Infection was more severe in this study than in the early-late field study because treatment rows rather than spreader rows were power-inoculated. The cooperative field study indicates that good control of Xp-Xpf can be obtained with chemical sprays, particularly sprays of copper compounds even under a very heavy inoculum load.

Seed samples taken from the various treatment plots of the cooperative study indicated no significant increase in yield due to any treatment (Table 8).

F. PHYTOTOXICITY

Foliage phytotoxicity was noted with For-Cop-80 NC and Kocide 101; For-Cop-80 NC was the more phytotoxic copper formulation. For-Cop-80 NC damage developed after the second spray and symptoms appeared as irregular brown areas on the upper leaf surface, somewhat resembling air pollution damage. On the lower leaf surface, veins developed brown necrotic streaks of varying lengths. Subsequently, the leaves became puckered and remained somewhat smaller than leaves on control plants. Overall damage in the For-Cop-80 NC late plots was less than in the early plots since fewer sprays were applied. Phytotoxicity interfered with accurate data collection in both early and late plots. When For-Cop-80 NC was combined with Agri-Strep 500, symptoms were less severe than when For-Cop-80 NC was sprayed alone. Early and late applications of Kocide 101 caused symptoms only on the leaf undersurface which appeared as dark streaks on the veins.

Table 8.--The effect of chemical treatments on foliage infection and yield of Navy (pea) beans in the cooperative field experiment.

Chemical	Rate per acre	% foliage infection ^a	Significance level		Significance level	
			5%	1%	5%	1%
Citcop 4E	2 qts	48.0 ^b	bc	b	a	a
Citcop 4E + Copper sulfate	2 qts 2 lbs	46.0	bc	b	a	a
Dithane M22 + Kocide 101	1 lb 1 lb	52.0	bc	b	a	a
Nabac 25 EC	1/2 pt	56.0	b	a	a	a
Nabac 25 EC + Kocide 101	1/2 pt 1 lb	46.0	bc	b	a	a
1451	2 lb	42.0	bc	b	a	a
1510	2 lb	37.0	c	b	a	a
Control		76.5	a	a	a	a

^aConversion from Barratt - Horsfall ratings (Elanco Conversion Tables for Barratt-Horsfall Rating Numbers).

^bValues followed by any common letter are not statistically different (Duncan's Multiple Range Test).

Navy (pea) beans treated with coppers in the cooperative experiment also showed phytotoxic symptoms similar to those of Kocide 101; in addition, some upper leaf surface browning also developed.

Phytotoxicity due to For-Cop-80 NC has not been reported previously; however, Saettler and Potter reported phytotoxicity with TC-90 (42). In the present study, copper phytotoxicity may have been associated with the unusually dry environmental conditions during the growing season which resulted in moisture-stressed plants. Greenhouse grown plants sprayed in the morning and exposed to the afternoon sunlight at a temperature of 85 F, in early June, showed that For-Cop-80 NC at 1000 ppm was phytotoxic to Navy beans in less than 24 hr. Symptoms appeared as large patches of darkened green, sunken, tissue. After 3 days, the leaves became necrotic and abscised. Kocide 101 at 2000 ppm caused no apparent damage under the same conditions.

G. CHEMICAL TOLERANCE OF VARIOUS BLIGHT ISOLATES

Disc assay demonstrated that isolates Xp 15, Xp 11, Xp 816, Xpf 16, Xpf CIAT-A, Xpf 3, Xpf 28 and Pp 13-S differ widely in their response to Agri-Strep 500, Isobac, For-Cop-80 NC, Kocide 101, HPMTS and Bunema (Fig. 2-6). The differences were expressed both in size and type of inhibition zone. Two qualitatively different zone types were observed. The first type was most common and consisted of a completely cleared zone surrounded by a lawn of heavy bacterial growth (Fig. 5C). The second type was less prevalent and was characterized by a completely cleared area, bordered on the exterior by a ring of medium-heavy growth

and then the normal lawn of heavy growth (Fig. 5B). The ring of lighter growth, which will be referred to as the ring of partial inhibition, usually became visible several hours after the normal bacterial lawn. If the plates were incubated longer than 2 days, the distinction between the two areas became vague. The ring of partial inhibition results from a slight inhibitory effect of the chemical which slowed bacterial multiplication in that area. This ring denoted a semi-tolerant reaction on the part of the bacteria in the ring, but their response was simply delayed. Possibly, isolates showing this zone type possess additional sites of action to a particular chemical not present in other isolates. The ring of partial inhibition was unique for individual chemical-isolate interactions and no isolate showed the response to all chemicals. However, Xp CIAT-A developed this ring in response to all chemicals except Isobac and Bunema (Fig. 2D-6D), and the development was more common with Xpf isolates (8 cases) than with Xp isolates (2 cases). Size of the ring of partial inhibition varied greatly depending on isolate-chemical combinations. For example, a simple narrow ring inside the outer lawn occurred with the Xpf CIAT-A-Agri-Strep 500 combination (Fig. 2D), whereas a ring of partial inhibition of over 30 mm diameter with no cleared zone occurred with the Xpf 16-Kocide 101 combination (Fig. 6E). For quantitative comparisons only completely cleared areas of the zones were measured; the smaller the zone diameter the more tolerant is the isolate to a particular chemical. If two isolates were statistically the same but one had a ring of partial inhibition, the isolate with the ring of partial inhibition was considered less tolerant to the chemical. An example

of this was with Agri-Strep 500 (Fig. 2) where Pp 13-S, Xpf 16, Xp 816 and Xpf 3 all had equal zone size but only Xpf 3 developed the ring of partial inhibition and, therefore, was considered less tolerant.

Response of Xpf 3 to Bunema was not considered in the analysis of that chemical since no accurate measurement of zone size could be made due to the large number of resistant colonies that developed throughout the cleared area (Fig. 3G). The zone pattern that developed suggested that most of the cells were quite sensitive to the chemical but, that there were also many mutant cells resistant to the chemical as well.

Duncan's test demonstrated that isolates differed statistically in tolerance to the chemicals (Table 9). Except for the copper chemicals, Xp and Xpf did not differ in their overall chemical tolerance. However, Xpf isolates were generally more tolerant to For-Cop-80 NC and Kocide 101 than Xp isolates. This was most evident with For-Cop-80 NC where the smallest 3 zone sizes developed when Xpf 16, Xpf 28, and Xpf CIAT-A were seeded on the plates; however, Xpf 3 was least tolerant. The Xp isolates were intermediate in response. Greater Xpf copper tolerance was less evident with Kocide 101 where Xpf 16 and Xpf 28 were the most tolerant isolates but Xp 15 was more tolerant than Xpf CIAT-A.

Certain isolates were tolerant to several chemicals; however, this did not apply to all the chemicals. Statistically, Xpf CIAT-A gave the smallest zones with Agri-Strep 500, second smallest with Isobac and HPMTS, moderate size with the 2 coppers, but largest with Bunema (Fig. 2-6D). Similarly Pp 13-S gave the smallest zone against Isobac and HPMTS, second smallest with Bunema and intermediate size with the

remaining chemicals (Fig 2-6H).

Of particular interest was the wide range of isolate response to the coppers (Fig 6). Considering only Xp and Xpf isolates, the largest zone was double that of the smallest with each copper. This wide range is interesting considering the non-specific mode of toxic action of coppers, which are generally thought to tie up proteins and enzymes. This suggests that unique cell wall or membrane properties may regulate the entrance of copper ions for each isolate.

Table 9.--Disc assay comparison of responses to various chemicals by Xp, Xpf and Pp isolates. ^{a,b,c}

Chemicals						
Isolate	Agri-Strep 500 (500 ppm)	Bunema (500 ppm)	For-Cop-80 NC (500 ppm)	HPMTS (500 ppm)	Isobac (500 ppm)	Kocide 101 (600 ppm)
Xp 15	34.8 a	<u>15.4 g</u>	24.0 b	29.0 cdef	19.1 f	18.2 e
Xp 11	34.4 ab	21.7 c	23.0 bcd	31.4 b	<u>21.0 e</u>	22.4 b
Xp 816	27.8 defg	20.8 cd	23.2 bc	29.5 cde	<u>30.3 a</u>	21.5 bc
Xpf 16	28.2 de	<u>20.6 cde</u>	<u>17.7 h</u>	29.7 c	28.9 b	<u>13.0 h</u>
Xpf CIAT-A	<u>25.5 h</u>	29.8 a	<u>20.0 f</u>	<u>28.1 g</u>	17.8 g	<u>17.9 ef</u>
Xpf 28	29.2 c	23.3 b	19.1 fg	32.6 a	23.0 d	16.4 g
Xpf 3	<u>28.3 d</u>	-----	38.0 a	29.5 cd	<u>24.8 c</u>	26.3 a
Pp 13 - S	27.8 defg	18.5 f	22.0 cde	18.0 h	13.0 h	21.2 cd

^aValues followed by any common letters are not statistically different at the 1% level (Duncan's Multiple Range Test).

^bFilter paper discs were dipped into chemical solutions.

^cUnderlined entries indicate presence of ring of partial inhibition.

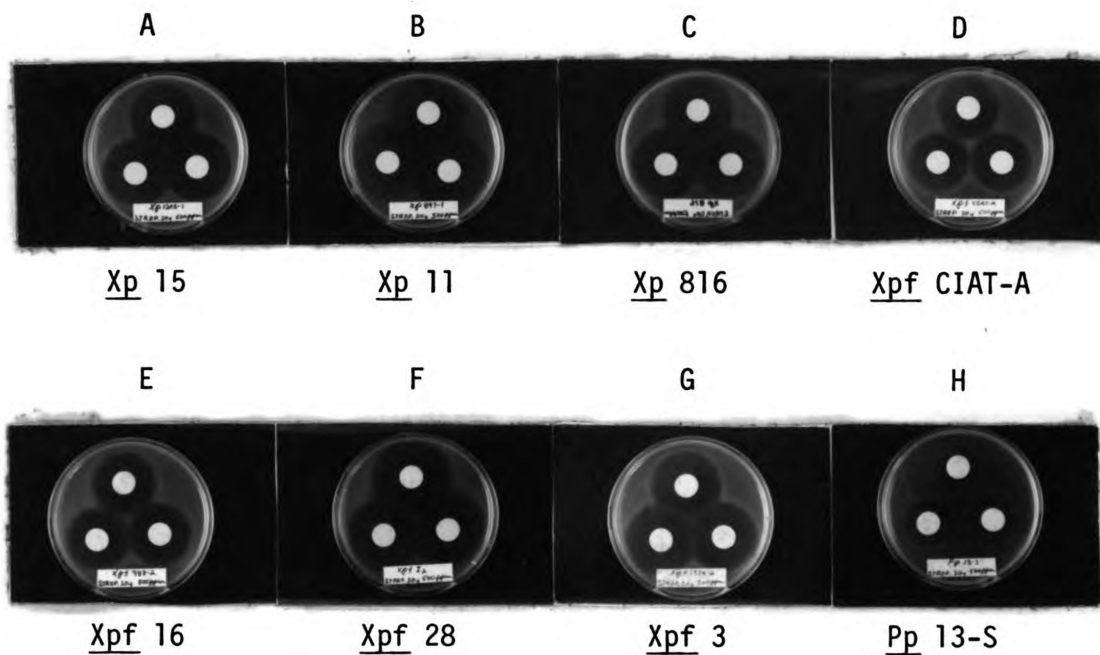


Figure 2. -- Isolate response to Agri-Strep 500 (500 ppm) by the disc assay method.

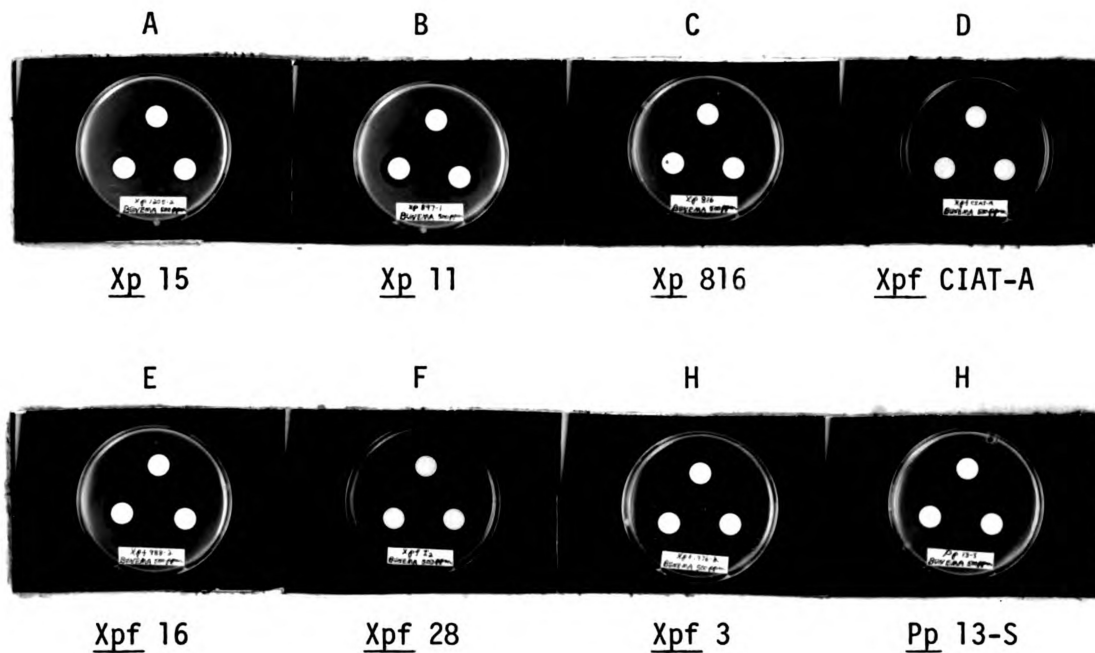


Figure 3. -- Isolate response to Bunema (500 ppm) by the disc assay method.



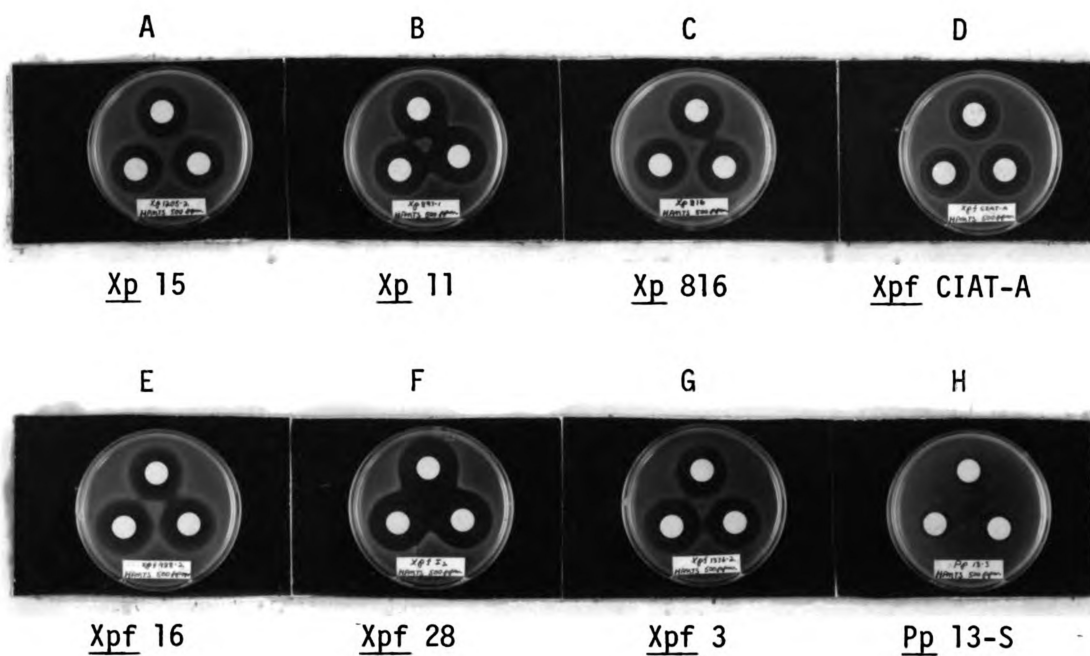


Figure 4. -- Isolate response to HPMTS (500 ppm) by the disc assay method.

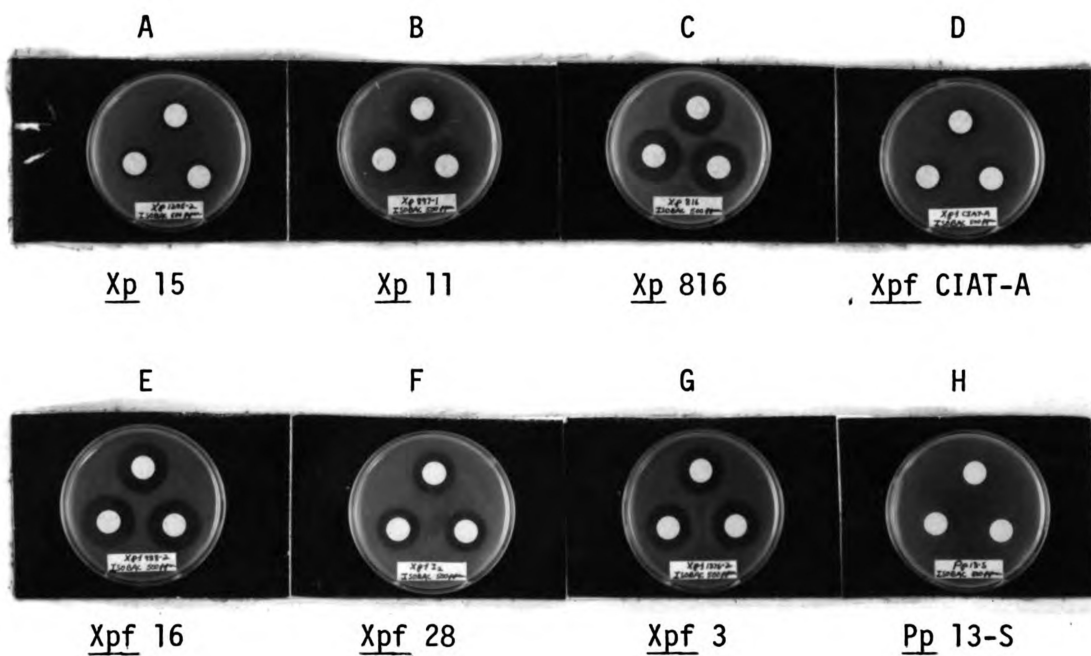


Figure 5. -- Isolate response to Isobac (500 ppm) by the disc assay method.



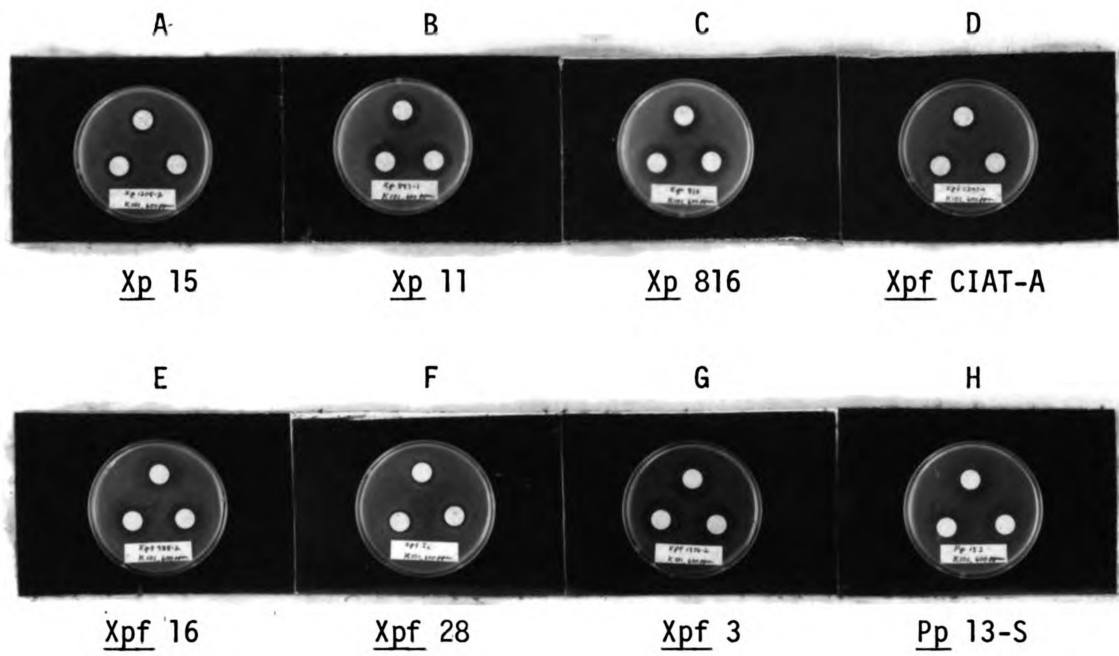


Figure 6. -- Isolate response to Kocide 101 (600 ppm) by the disc assay method.



DISCUSSION

Studies on chemical control of bean common and fuscous blights in Michigan and Colorado have produced conflicting results as to the feasibility of chemical sprays in controlling these serious seed borne diseases. The purpose of this study was to determine whether the near complete blight control reported by Colorado researchers is possible with Navy (pea) beans in Michigan and secondly to explain the conflicting results from Michigan and Colorado.

Laboratory screening permitted chemicals and chemical combinations to be compared on the basis of toxicity to Xp and Xpf. Results showed that chemicals previously field tested in Michigan were all good bactericides and thus demonstrated a potential for Xp and Xpf control in the field. Therefore, Xp and Xpf chemical insensitivity could be eliminated as a reason for the limited control experienced in Michigan. Secondly, based on in vitro assay, four chemical combinations were shown to have increased toxicity over their parent compounds and thus were included in the field testing. The assay thus offered a means to consider a wide variety of combinations without laborious field trials.

Field efficacy and in vitro activity were not well correlated, suggesting that other factors besides toxicity are important for field control of bean blight. Clearly, Isobac, HPMTS, Nabac, and the other combinations which were very toxic in vitro, failed to demonstrate the same toxicity in the field, thus yielding little or no control. One possible reason for the failure of Isobac and HPMTS to provide good



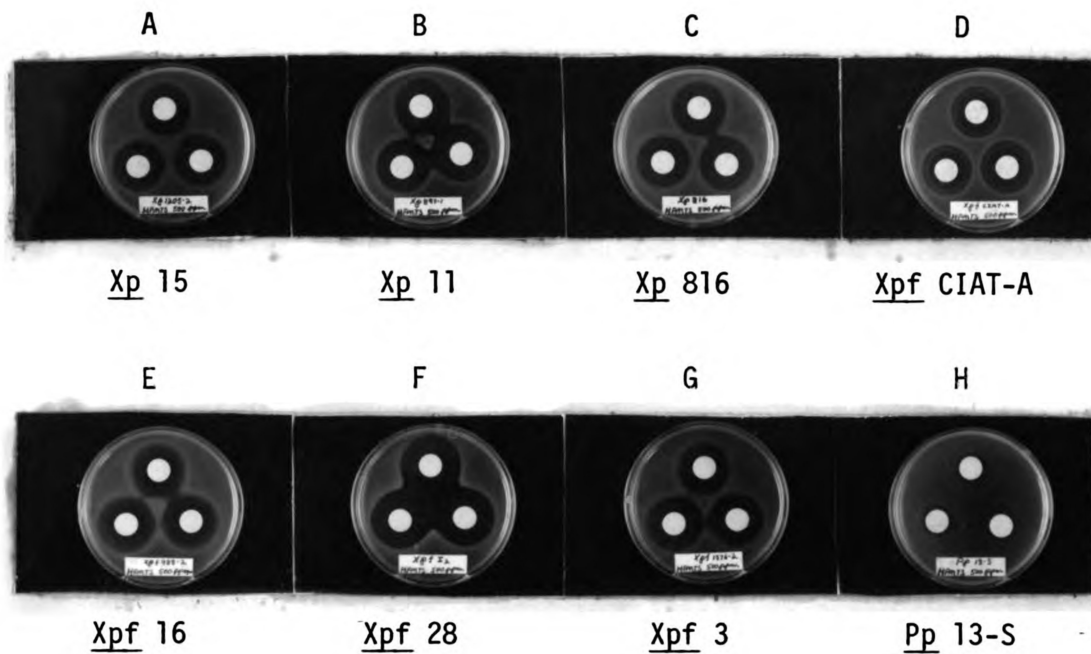


Figure 4. -- Isolate response to HPMTS (500 ppm) by the disc assay method.

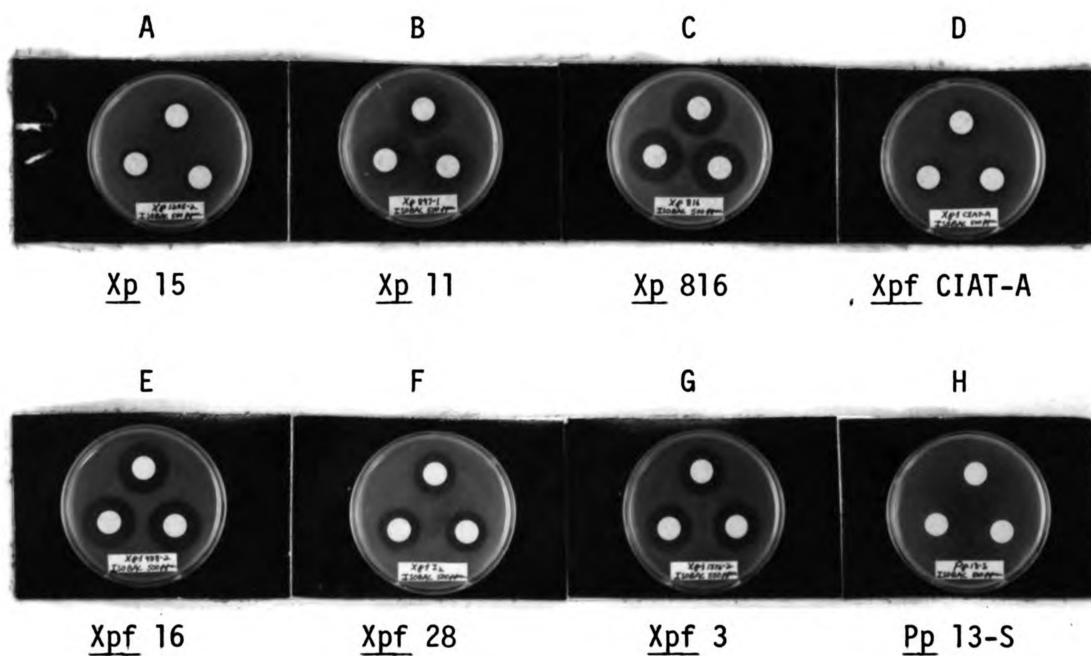


Figure 5. -- Isolate response to Isobac (500 ppm) by the disc assay method.



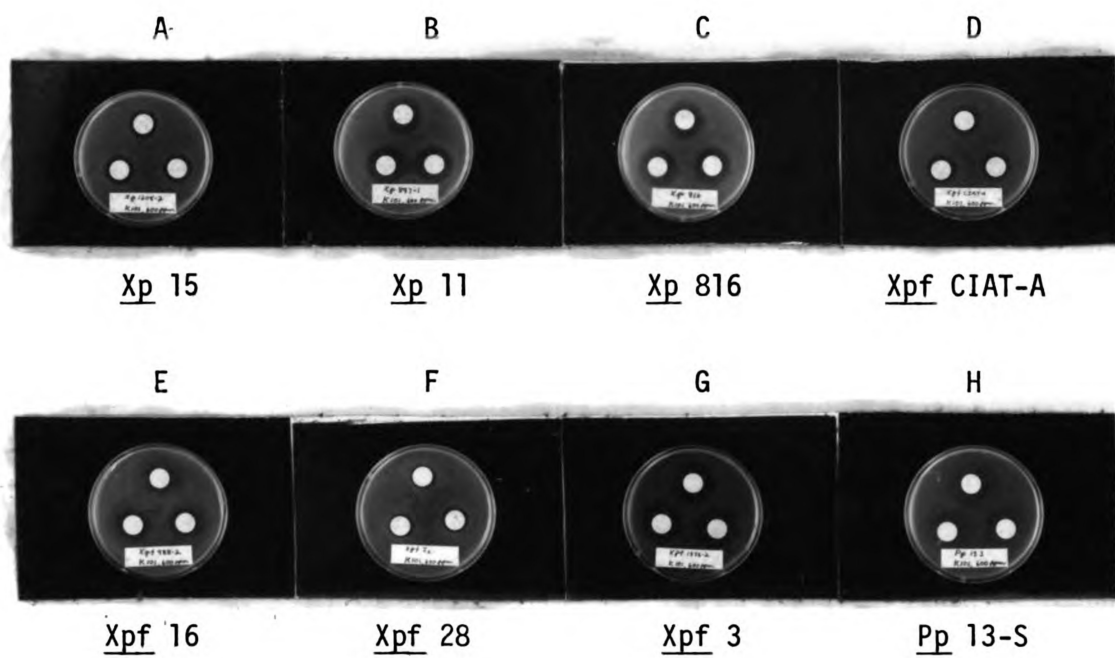


Figure 6. -- Isolate response to Kocide 101 (600 ppm) by the disc assay method.



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Field efficacy and in vitro activity were not well correlated, suggesting that other factors besides toxicity are important for field control of bean blight. Clearly, Isobac, HPMTS, Nabac, and the other combinations which were very toxic in vitro, failed to demonstrate the same toxicity in the field, thus yielding little or no control. One possible reason for the failure of Isobac and HPMTS to provide good

control is their poor residual qualities and possibly photolytic breakdown. Both chemicals are relatively water-soluble and their relative abilities to remain active on the leaf surfaces were inferior when compared to For-Cop-80 NC, Agri-Strep 500 or Kocide 101. Only in the case of Bunema were high toxicity in vitro and field effectiveness correlated. Kocide 101 which was least bactericidal in the in vitro studies was most effective in the field and this is probably linked to its good residual activity.

Because of the past field performance of Agri-Strep 500, For-Cop-80 NC, Nabac 25 EC, Bunema and HPMTS, despite their effectiveness in cultural studies, it was suggested that field effectiveness might be increased by applying these chemicals to field plots earlier than in previous studies. The early application of sprays was attempted with the hope that early spraying would enhance blight inhibition. Dickens and Oshima (34) found that sprays applied "before secondary spread" gave 10% better control than those applied "after secondary spread". Late spraying approximated the procedures used by Saettler in previous research, where spraying was initiated only after symptoms became evident in the spreader rows. The late spray was able to provide protection against secondary spread which occurred after appearance of definite symptoms but offered no protection against early spread occurring before major symptom expression. Once Xp and Xpf became internally established none of the chemicals except Agri-Strep 500 could inhibit the bacteria since only streptomycin is systemic. Thus, eradication of internal populations of Xp and Xpf by the other chemicals would be impossible.

The early spray schedule presupposed the possibility that such early inoculum can have a significant effect on the total amount of blight in a given season. Early inocula would be available from lesions that had developed before spray initiation. The amount of inocula from this source could be sizeable since, in past field experiments and also in this study, spreader rows developed a "fair" blight rating before late spray initiation. In a commercial farmer's field, spread from these initial lesions would probably be greater since the farmer would be less able to identify blight symptoms. Lesions in lower bean leaves are not readily visible, and blight may not be detected until the infection spreads to the upper foliage. A second possible source of the early inoculum could be pockets of bacteria in symptomless leaf tissue. Field monitoring established that high populations of Xp and Xpf can exist in symptomless bean tissue. There is the possibility that the bacteria can move to the surface of such symptomless tissue and become available for dispersal. Probing insects feeding on such infected tissue could also pick up bacteria. It is generally believed that infected tissue is not contagious until a water-soaked area appears; however there are no data to support this. A third possible source of early inoculum could be from Xp and Xpf growing epiphytically on leaf surfaces without causing infection. Such a "resident phase" of pathogenic bacteria was first recognized by Leben (24) who showed that Xanthomonas vesicatoria multiplied on the surface of tomato seedlings without causing infection, when the plants were grown from surface-infested seed. Mew and Kennedy (29) reported that Pseudomonas glycinea increased 1000 fold on the surface of

symptomless leaves 10 days after inoculation in the greenhouse. Similarly Leben (25) reported a 150 fold increase in the surface population of P. glycinea 4 days after inoculation; this was 3 days before symptom expression. "Resident phases" have been reported for other bacteria associated not only with leaves but also with buds and flowers, such as with P. lachrymans in cucumber buds (13) and Erwinia amylovora on pear leaves (30). Leben has suggested that surface growth may be important as a means of rapid inoculum build-up (26). Early blight spread in the early-late field experiment was indicated by the presence of Xp and Xpf on 8/7/74, in the control rows, before late spraying began 2 days later. Early sprays initiated two weeks before late sprays provided protection against the early inoculum.

The idea that chemicals could be more effective when applied as early sprays was only partially confirmed in the present study. Although statistically significant differences between early and late applications of the same chemical were not shown, some chemicals provided statistical disease control only when applied early, therefore indirectly supporting the idea. The best example of this was Bunema where 1% level testing revealed effectiveness only when used as an early spray. Moreover at the 5% level, of 10 statistically significant treatments, with % leaflet infection, 6 were early and 4 late; No. lesions per leaflet was similar, with 6 early treatments and 2 late treatments being significant. Although Kocide 101 (early and late) were both statistically similar, the early spray consistently showed lower mean levels of infection in all three foliage evaluations. Early and late comparisons might have shown

greater differences if optimum conditions for blight development had occurred in the field. The lack of rain may have reduced early inoculum spread, therefore decreasing the effect of the early inoculum on the total blight development over the entire season.

Results from both field experiments demonstrate that considerable reduction of foliage blight is possible with chemical sprays. The degree of control was greater than that demonstrated in previous field studies in Michigan. On the other hand, the present results are not as impressive as those reported from Colorado where only 2 or 3 sprays completely eliminated common blight in Pinto beans (34). The best treatment in the early-late experiment was Kocide 101 used as an early spray and it reduced infection about 66%. In the cooperative study, the Kocide-like formulation, 1501, reduced infection by 50%. These studies show that copper formulations, particularly Kocide 101, are good chemicals for Xp-Xpf control under Michigan conditions. The failure of For-Cop-80 NC, another copper formulation, to provide control equal to Kocide 101 may be due to phytotoxicity, which made it difficult to take accurate disease ratings. In spite of the phytotoxicity For-Cop-80 NC statistically controlled disease in all foliage evaluations at the 5% level. Bunema was also quite effective when used early in the season. These results are similar to those of Dickens and Oshima who have reported Copper-Count-N, Oxy-Cop-8L, O-Cop 53 and Bunema effective at blight control. Secondly, the results are correlated with recommendations of the North Dakota Extension Service of 1974 for the use of Kocide 101 for common and fuscous blight control. On the basis of

previous and present studies, both in Michigan and Colorado, it is apparent that in general, copper chemicals provide the best foliage control of Xp and Xpf followed by Bunema. Under Michigan conditions, good foliage control is possible when Kocide or Bunema are used early in the season as protective sprays.

The primary reason chemicals are sprayed to control bean blight is to increase yield, regardless of whether foliage or pod infection is decreased. If no significant yield increase occurs then it is not economical for the farmer to spray. Dickens and Oshima (34) reported excellent foliage control of blight, but do not mention yield in any of their studies. Saettler reported no significant yield increase due to spraying. The present study confirms that chemical control of Xp and Xpf on Navy (pea) beans in Michigan is not accompanied by an increase yield. Based on results thus far chemical control of bean common and fuscous blights in Michigan, therefore, appears to be economically unfeasible and of no demonstrated value for the commercial farmer. These results question the usefulness of spray recommendations for Xp and Xpf in North Dakota. On the other hand, chemical sprays for blight may be feasible and useful in fields producing certified seed. In such fields, even low levels of blight can result in loss of seed certification. Chemicals might reduce the chances for blight and subsequent loss of certification if used as protectants beginning early in the season.

The inability to increase yield as a result of foliage disease control, even under heavy inoculum levels suggests that Navy (pea) bean plants are able to sustain considerable loss in photosynthetic tissue

without affecting the amount of storage carbohydrates translocated to the developing pods. This may be explained in part by the fact that blight develops relatively late in the growing season and by that time much of the storage carbohydrate has already been transported to the pods. This was evident in the early-late plots where severe blight symptoms appeared in late August when the pods were nearly fully developed.

There are several possible reasons to explain the discrepancy between results from Michigan and Colorado relative to chemical control of Xp and Xpf. One reason is that environmental conditions in Colorado are less conducive to blight than the warm, humid growing season in Michigan. Therefore, blight severity is probably not as intense in Colorado as in Michigan. A second reason is that Colorado studies have been conducted only on common blight control whereas Michigan studies, including the present study, have been conducted on both common and fuscous blight control. Ekpo (21) showed that Xpf generally is a more virulent group of pathogens with a greater tendency for secondary spread than Xp; Xpf is more virulent to the pinto bean (Ouray) than to the Navy bean (Sanilac and Seafarer). Xpf isolates tested in this study were generally more copper-tolerant than the Xp isolates, therefore, field screening of copper-compounds might be more intense in Michigan because of the absence of Xpf isolates in Colorado research. A final reason to account for the discrepancy in chemical control between the 2 regions is that Michigan blight control studies routinely include at least 3 Xp and 3 Xpf isolates in spreader row inoculations, to ensure the presence of a wide pathogenic potential.

Dickens and Oshima (34) do not indicate the number of isolates used, however, their reports suggest that only a couple, at most, were involved. The use of several isolates for inoculation of spreader rows allows one to compare chemical efficacy against a wide range of pathogen virulence; fewer isolates would narrow the genetic complexity encountered by the treatments.

In vitro comparisons of Xp and Xpf isolates to various chemicals demonstrated the presence of varying degrees of chemical tolerance. Tolerance to chemicals was both isolate and chemical-specific; high tolerance to one chemical did not ensure tolerance to all chemicals. Differing modes of action by each chemical are probably responsible for this phenomenon. Xpf CIAT-A was an unusual isolate which showed high tolerance to several chemicals, Agri-Strep 500, Isobac and HPMTS. The different isolate-chemical responses are important relative to field testing of chemicals for blight control and further supports the need for multi-isolate inoculations suggested above. Chemicals tested against a wide spectrum of blight isolates would model the actual condition present in nature where blight strains differ among fields. Field testing of chemicals against only one isolate may present a distorted picture as to the true field efficacy of a chemical, since such strains could be either very chemical-tolerant or susceptible. As an example of this, if only one isolate such as Xpf 3 were used then the degree of control would probably appear much better with copper compounds than if isolate Xpf 16 were used.

No attempt was made to relate the isolate's chemical tolerance to

virulence. However, comparisons between the disc assay and results of Ekpo (21) show that Xpf CIAT-A possesses high tolerance to several chemicals and high virulence. Xpf 16 possesses high copper tolerance and intermediate virulence. Such isolates would have major impact on blight control programs.

The fact that halo, common, and fuscous blights are all seed-borne diseases with similarities in disease cycle makes it difficult to explain why common and fuscous blights are not as easily controlled by copper sprays as halo blight. This difference suggested that perhaps Pp is more sensitive to bactericides than Xp or Xpf. However, in vitro studies demonstrated that Pp (one isolate) does not possess greater chemical sensitivity than Xp or Xpf. In fact Pp demonstrated high tolerance against several chemicals and was completely tolerant to Isobac. This suggests that other factors account for effective Pp control in the field.

Non-pathogenic bacteria were consistently found internally in symptomless bean leaf tissue from plants in the control rows. The population of such bacteria was fairly constant except in the last sample, on 8/27/74. The role of these non-pathogenic bacteria in bean blight development is not known, although they could possibly exert an antagonistic effect on blight bacteria and delay or prevent lesion development by Xp and Xpf. Such a possibility is supported by the work of Scherff (44), who reported isolation of "yellow bacteria" which had an antagonistic effect on P. glycinea populations on the surface of soybean leaves. The yellow bacteria significantly reduced the populations of P. glycinea when both were sprayed on the leaves at a ratio of 1 yellow bacterium to 4 P.

glycinea. Scherff suggested that the non-pathogens may determine whether pathogenic bacteria remain in a resident phase or become infectious and cause lesion formation. The predominant non-pathogenic bacteria isolated from healthy Navy (pea) bean leaves in this study were also yellow bacteria and possessed characteristics similar to Scherff's isolates, in that they were rods which produce a water insoluble yellow pigment. However, the Navy (pea) bean yellow bacteria were present internally in healthy tissue, probably in substomatal cavities, whereas Scherff's isolates were from leaf surfaces.

Although the three foliage evaluation methods correlated in the number of statistically significant treatments at the 1% level of testing, the three methods differed in the number of statistically significant treatments when tested at the 5% level. Visual evaluations rated symptoms present only on the outermost leaves of the plant, since these are the only ones visible by viewing the rows from overhead. On the other hand, % leaflet infection rated the degree of infection not only of the unshaded surface leaves, but also infections on the more sheltered internal leaves. Number of lesions per leaflet evaluated chemicals on their ability to limit spread on a leaf once the bacteria became established. Thus the effectiveness of a chemical against blight can vary depending on how infection is rated.

In summary, three conclusions have been generated by this study on the chemical control of common and fuscous bacterial blights in Navy (pea) beans.

- 1) Good foliage control of Xp and Xpf is possible in Michigan using Kocide 101 and Bunema applied as early sprays.
- 2) Foliage control by chemicals does not increase yield even under heavy inoculum loads.
- 3) Xp and Xpf strains demonstrate varying degrees of susceptibility and tolerance to various chemicals and such responses should be considered when screening chemicals in the field for blight control.

SUMMARY

This study had two main objectives: (1) to find a chemical treatment which would provide good consistent control of secondary spread of Xp and Xpf in Navy beans and (2) to determine why chemical control of blight is more effective in Colorado than in Michigan.

The relative toxicity of 9 bactericides was measured by growing blight bacteria in liquid culture with various chemical concentrations; Agri-Strep 500, Isobac, HPMTS, Bunema, For-Cop-80 NC, Kocide 101, and Nabac 25 EC showed high toxicity to blight bacteria in vitro and, therefore, were tested in the field. Chemical combinations of Agri-Strep 500-Isobac, Bunema-Isobac, Agri-Strep 500-Bunema, and Agri-Strep 500-For-Cop-80 NC were also tested in the field. Each chemical or chemical combination was applied on two spray schedules, early and late; the early spray schedule consisted of 6 spray applications with 2 sprays occurring 2 weeks prior to initiating the 4 late sprays. Foliage infection was rated by 3 methods, % leaflet infection, number lesions per leaflet, and sight evaluation. Only Kocide 101 (early and late) and Bunema (early) provided good control and were statistically significant when data was tested at the 1% level of significance (Duncan's Multiple Range Test). Kocide 101 (early) was the best treatment and resulted in a 66% disease reduction. In a second field study two different Kocide formulations provided up to 50% reduction of blight. It is apparent, then, that quite good control of Xp and Xpf is possible under Michigan conditions.

Despite foliage control of Xp and Xpf, no yield increases resulted; this suggests that blight damage following secondary spread may not be important as a yield limiting factor in Navy (pea) beans.

Symptomless leaf tissue from control plots was sampled to determine when Xp and Xpf spread from the inoculated spreader rows to uninoculated control plots. These samples revealed the presence of large populations of blight bacteria internally in leaf tissue several days before symptoms developed in the control plots. It was suggested that these pre-symptom infections may play a significant role in early blight spread. High populations of non-pathogenic bacteria were also detected during the sampling, the most prevalent morphological type produced large yellow colonies. Such epiphytic bacteria could have some epidemiological importance in blight development.

The tolerances of 3 Xp and 4 Xpf isolates to Agri-Strep 500, Bunema, For-Cop-80 NC, HPMTS, Isobac, and Kocide 101 were compared by a disc assay method. Filter paper discs were dipped into a chemical solution and placed on petri plates seeded with the blight isolates. Size of inhibition zones revealed a wide tolerance range among isolates in response to a particular chemical. Two different zone types were identified; the main type consisted of a clear area surrounded by a heavy lawn of growth; the second type consisted of a ring of partial clearing between the completely cleared area and the outermost lawn of heavy growth. No isolate demonstrated high tolerance, to all of the chemicals; however, Xpf CIAT-A possessed high tolerance to all chemicals except Bunema. Three of the 4 Xpf isolates demonstrated greater tolerance to copper-

containing compounds than the Xp isolates. In general, the disc assay comparisons suggested that blight isolates used in field screening of chemicals might influence results, depending on the sensitivity of the isolate to a particular chemical.

The contrasting results between Michigan and Colorado may be due to differences in environment and experimental procedures. Michigan has more suitable environmental conditions for blight than does Colorado. Also, Colorado studies are conducted on snap and pinto beans, whereas Michigan studies are conducted on Navy (pea) beans. Colorado studies do not include Xpf as inoculum in their spray trials, whereas Michigan studies do. Xpf has been shown to be of greater virulence than Xp; Xpf may also possess higher copper tolerance. Colorado researchers inoculate with only 1 or 2 Xp isolates whereas in Michigan, a total of 6 Xp-Xpf isolates are used; smaller number of isolates would reduce the genetic complexity, which a chemical spray must confront. Therefore, chemicals screened for blight control in Michigan are tested under more stringent conditions than in Colorado.

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