PATHOGENIC VARIATION IN COMMON (XANTHOMONAS PHASEOLI) AND FUSCOUS (XANTHOMONAS PHASEOLI VAR. FUSCANS) BACTERIAL BLIGHTS OF BEAN (PHASEOLUS VULGARIS L.)

Dissertation for the Degree of Ph. D.
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PATHOGENIC VARIATION IN COMMON (XANTHOMONAS PHASEOLI) AND FUSCOUS (XANTHOMONAS PHASEOLI VAR. FUSCANS) BACTERIAL BLIGHTS OF BEAN (PHASEOLUS VULGARIS L.)

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Ephraim J. A. Ekpo

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Major professor

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ABSTRACT

PATHOGENIC VARIATION IN COMMON (XANTHOMONAS PHASEOLI) AND FUSCOUS (XANTHOMONAS PHASEOLI VAR. FUSCANS) BACTERIAL BLIGHTS OF BEAN (PHASEOLUS VULGARIS L.)

By

Ephraim J. A. Ekpo

Pathogenic variation was examined in isolates of Xanthomonas phaseoli (Xp) and Xanthomonas phaseoli var. fuscans (Xpf), the causal agents of common and fuscous blights of beans, respectively. A total of eight isolates of Xp and seven isolates of Xpf were selected from different geographical regions represented by Michigan and Nebraska in the U.S.A., Uganda in Africa, Guatemala in Central America, and Colombia in South America. Virulence of isolates was compared on thirteen commercial bean varieties (Phaseolus spp.) and two cowpea varieties (Vigna unguiculata) at different stages of plant growth. The methods of seed infiltration, multiple needle inoculation, leaf incision, water soaking of leaves, and excised pod inoculation procedures were examined in comparative pathogenicity tests using a standard inoculum concentration of 2.8×10^7 cells/ml. Results were reproducible with leaf incision, water-soaking and excised pod techniques. Isolates were separated into different virulence groups or strains based on qualitative and quantitative differences in disease reactions.

Plant age was important in disease development; the more virulent isolates of both bacteria incited necrosis on both young and old leaves while the less virulent isolates incited symptoms which were restricted primarily to young succulent tissues. Some isolates that were slightly virulent on plants in the vegetative stage of growth became more virulent when plants entered the reproductive phase of growth. Active multiplication within the tissue was not always accompanied by symptom development. For example, isolates Xp21 and Xp23 actively multiplied in leaves of tolerant G.N. Tara without the production of visible disease symptoms. Mixed isolates of Xp and Xpf were inoculated into Manitou and G.N. Jules leaves and re-isolated from diseased tissue in varying proportions. Symptom development with such composite inocula was sometimes more severe than infection with individual isolates thus indicating compatible co-existence of both bacteria in the same plant tissue. A new cell phenotype was isolated from tissues inoculated with either several isolates of the same bacterium or a mixture of Xp and Xpf isolates. G.N. Tara and P.I. 207262 which are reported as tolerant to Xp by previous workers were shown to be susceptible to some isolates of Xp and Xpf included in the study. Isolates exhibited serological variability but such variability was not easily correlated to the observed pathogenic variation among the isolates. The implications of the findings are discussed in relation to bean breeding programs.

PATHOGENIC VARIATION IN COMMON (*XANTHOMONAS PHASEOLI*) AND FUSCOUS (*XANTHOMONAS PHASEOLI* VAR. *FUSCANS*) BACTERIAL BLIGHTS OF BEAN (*PHASEOLUS VULGARIS* L.)

By

Ephraim J. A. Ekpo

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Botany and Plant Pathology

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To my parents

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INTRODUCTION AND LITERATURE REVIEW

Common blight caused by Xanthomonas phaseoli (E.F.S.) Dows and fuscous blight caused by Xanthomonas phaseoli var. fuscans (Burkh.) Starr & Burkh. are bacterial diseases of major importance in bean production. Plant pathologists consider common and fuscous blights to be two of the most devastating seed-borne diseases of dry edible and green beans in many production areas in the world (48, 50, 69). The prevalence of common blight in most cases parallels that of fuscous blight and both have been reported from Australia (4, 40), Russia (20), Yugoslavia (61, 62), Michigan, U.S.A. (3), and Uganda (34). The causal agents can be recovered from pods, seeds, and vegetative tissue of bean (69).

Incidence and severity of blight on bean may vary from year to year depending on weather conditions, locality, and cultural practices. In some cases, bean seed production is restricted to dry areas where the blight problem is less severe. In Nebraska, for example, most of the planted bean seed is produced in disease-free, semi-arid areas of Idaho to eliminate seed-borne infection (14). Occasionally, however, crop losses do occur when environmental conditions

are favorable for bacterial development and spread. X. phaseoli (Xp) and X. phaseoli var. fuscans (Xpf) are known to overwinter on infected bean straw which is source of inoculum that can infect the new bean crop (69).

Widespread destruction of beans by bacterial blights often necessitates the use of chemical protectants to prevent spread of the disease organisms. Oshima and Dickens (44) report appreciable control (88.6-91.5%) of secondary spread of common blight in Colorado snap and pinto beans using copper sprays. In navy bean production, however, there are no satisfactory chemical control measures. Growers have used copper bactericides for several years as protective sprays, but the control of secondary spread is not always satisfactory (16, 45). Chemical control by seed treatment (25) is also reported to be generally ineffective for control of internally-borne blight pathogens.

Absence of immunity to common and fuscous blights further underscores the role of blight in bean production; extensive screening for disease resistance in greenhouse and field tests revealed no immunity among commercial bean cultivars (69). Tolerant commercial cultivars have only recently been made available with the introduction of late maturing Great Northern Tara (G.N. Tara) and Great Northern Jules (G.N. Jules), in Nebraska (5, 10, 11). The future of world bean production depends largely on the discovery and successful incorporation of tolerant germ-plasm into acceptable high yielding blight-susceptible bean varieties.

The literature is rather scanty on estimates of economic damage caused by Xp and Xpf. Seventy-five percent of the fields in New York were affected and serious losses occurred in 1918 (69). During the same period, Burkholder (7) estimated losses due to common blight at 3-8% and in 1930, Zaumeyer (68) recorded an average loss of 10% for the entire U.S. In 1936, the U.S. suffered an estimated loss of 3,400,000 pounds of snap beans and about 34,700,000 pounds of dry beans (69). Andersen (3) estimated a blight loss of $3.5 million to growers in three Michigan counties in 1951, and Zaumeyer et al. (69) reported a loss of about $1.0 million in Nebraska in 1953. Yield reduction in Russia was 37% in 1958 and 65% in 1959 (33). In similar studies, Zaumeyer et al. (70) estimated losses due to common and halo blight diseases at about 22-28% in garden beans and about 25-30% in field beans. Reductions of about 30-40% in total yields were attributed to common and fuscous blights in Michigan (3). Recently, Coyne et al. (12) have reported that common blight and bean wilt will often cause large reductions in yields and quality of beans grown in Nebraska. Andersen (2) also reported that 75% of the seed from Colorado, Montana, Nebraska, and Wyoming was infected with fuscous blight. The importance of bacterial blight in commercial bean production remains to be established for developing countries where protein-bean diet is being emphasized in nutrition studies.

The pathological histology of Xp and Xpf is similar; under natural conditions the bacteria enters leaves through

stomata or wounds and then invade the intercellular spaces (30), causing a gradual dissolution of the middle lamella (69). Later the cells begin to disintegrate with the formation of bacterial pockets. Stem infection occurs either through the stomata of the hypocotyl and epicotyl, through the vascular connections leading from the leaf, through mechanical wounds and insect damage, or from infected cotyledons (34, 69). In severe cases, bacteria and the dead disintegrating tissue in the xylem vessels may cause a wilting of the plant, either by plugging the vessels or by the disintegration of the cell wall. The pathogen is harbored below the seed coat (69); it enters the pod sutures from the vascular system of the pedicel and then passes into the funiculus and through the raphe leading into the seed coat. The micropyle also serves as a portal of entry into the seed.

Studies suggest a great deal of similarity in sympotomatology between common (Xp) and fuscous (Xpf) blights. This similarity often makes their separation in the field impossible. Typical symptoms consist of necrotic lesions bordered by narrow bands of yellow tissue or chlorotic halos (Figure 1). In greenhouse and field inoculations, however, Zaumeyer (69) reported more severe symptoms on plants infected with Xpf than on plants infected with Xp. In culture, the two pathogens are easily separated on the basis of pigment production; Xp produces a non-diffusible yellow pigment on certain media whereas Xpf produces a

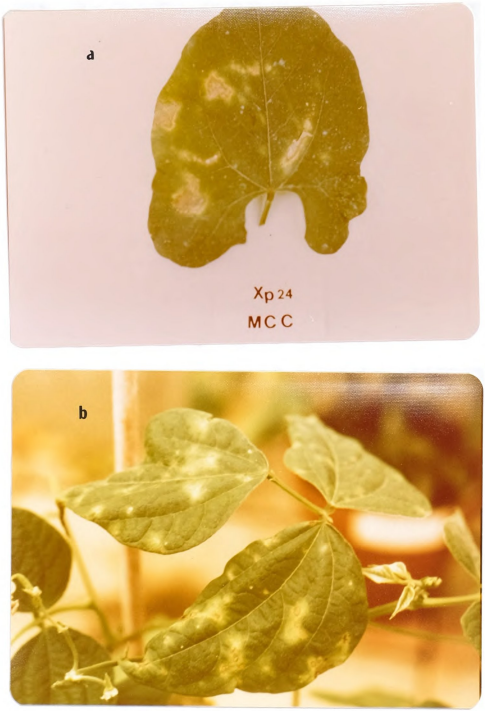


Figure 1.--Typical blight symptoms on (a) primary and (b) trifoliolate leaves of MCC bean 14 days after inoculation with Xp24.

diffusible brown pigment. The primary role of these melanin-like pigments in pathogenicity has not been conclusively established but Burkholder (8) in a comparative study of the bacterial diseases of the bean suggested that pigment production was a factor in the severity of stem splitting on susceptible hosts. Leakey (34) also suggests that there is perhaps a tendency for pigment-producing Xanthomonas spp. to be of greater virulence than non-pigment producing types. Separation of Xp and Xpf based on phage typing differentiates the two pathogens into two lysotypes. Klement et al. (31) isolated a bacteriophage specific for Xpf from infected bean seed and Katznelson and others (27) isolated the specific phage for Xp. These phages only lyse their homologous hosts, thus indicating a high degree of specificity. Evidence regarding the serology of Xp and Xpf is fragmentary. Elrod and Braun (18) point out that the two groups of pathogen may belong to the same serotype.

Xp and Xpf share a number of diagnostic characteristics. According to Dye (17), the cells are non-sporing, gram-negative, strictly aerobic, monotrichous rods. The cells are medium sized with rounded ends occurring singly or in pairs. They measure on the average about 1.9-2.3μ x 0.87-0.98μ (8); they are non-capsulated. Nutritionally there is considerable homology between them as well; in weakly-buffered media, acid is produced in small amounts from many carbohydrates and glucose metabolism is strictly oxidative. They are generally characterized as

high-temperature pathogens in contrast to Pseudomonas phaseolicola, the causal agent of halo blight of beans (34). They are seed-borne, both internally and externally (34,69) and are capable of being transmitted long distances. Basu et al. (6), in a study of survival of Xanthomonas in bean seed, demonstrated ability of Xpf to survive in seed for three years at 20-35 C. Survival of Xp in dry leaf thrash for up to 18 months was demonstrated by Sabet and Ishag (49). Thus survival of the blight pathogens between crops may either occur in or on seeds, or through crop debris, particularly in areas with mild weather.

Xp as a group has been studied more extensively than Xpf. In most cases, studies of the two groups have emphasized host range and varietal resistance rather than pathogenic variation among isolates within the groups. The existence of pathogenic variation among populations of phytopathogenic bacteria may be a common phenomenon. Schroth et al. (52) described the occurrence of pathogenic and nutritional variation in the bean halo blight group of fluorescent pseudomonads. Thyr (64) in a study of virulence of Corynebacterium michiganense from six geographical areas, reported significant differences among seven isolates which differed in degrees of aggressiveness. He suggested the inclusion of highly virulent pathotypes in tomato breeding programs to maintain an acceptable level of resistance. In a related study, Strider and Lucas (60) emphasized the need for a specialized inoculation technique in order to

demonstrate the existence of variation in virulence. They used a knife stabbing procedure (59) and demonstrated that variation in virulence did exist in C. michiganense. Gerarda Perlasca (47) in a study of California isolates of Pseudomonas syringae pathogenic on stone fruit trees, reported considerable differences in isolate pathogenicity or virulence on different hosts. In more recent studies, Shuster and Coyne (55) reported new, highly virulent strains of common blight bacteria in isolates from Colombian dry bean seed. This finding suggests that additional pathogenic variation may be discovered as other blight isolates from diverse origins are studied. Little or no information is available regarding the existence of pathogenic variation in Xpf. Because of the economic importance of common and fuscous bacterial blights, it was decided to study comparative virulence in order to forestall possible resistance-breeding-complications involving pathogenic variations in isolates of both bacterial pathogens.

The present study has focused on determining whether Xp and Xpf isolates from different geographical origins (labeled as different isolates in the present system of numerical nomenclature) possess the same pathogenicity or whether they are heterogeneous in their pathogenic spectrum. It is hoped that the information gained from such study will be of immediate practical importance in meaningful bean breeding endeavors.

Previous studies of pathogenic variation have been limited to foliage infection based on one method of inoculation, a water-soaking technique (54). Additional inoculation procedures could be useful for scoring virulence on both leaves and pods, and they have been included in this study. The host plant is a complex diagnostic medium and disease reactions, which result after it is inoculated with a blight isolate, reflect the physiological properties (genetic potential) of the isolate. Isolates that have a similar host range and produce identical host reactions are usually placed in the same virulence class. However, we recognize that it is difficult to differentiate isolates on the basis of "complete" host range. Accordingly, the pathogenicity spectrum and tissue reaction (symptom development) on an arbitrary sample of commercial bean varieties have been used as an index of virulence. Qualitative and quantitative aspects of symptom development serve to differentiate the isolates of both Xp and Xpf into virulence groups or strains. Mixed-infection studies with a combination of several isolates of each group and combinations of both Xp and Xpf in appropriate ratios have been included to investigate the hitherto unreported interactions or co-existence of both bacteria, in the same tissue, in relation to intensity of disease.

The objectives of the present investigation, then, were: (a) to compare different inoculation techniques in order to develop a useful system(s) for comparative

pathogenicity studies; (b) to compare qualitative and quantitative aspects of symptom development of Xp and Xpf isolates from different geographical regions; (c) to follow population trends in tolerant and susceptible bean tissues; and (d) to relate the implications of the findings to breeding programs to develop blight-resistant bean varieties.

MATERIALS, METHODS, AND PRELIMINARY RESULTS

Origin of Isolates: Pathogenicity tests were conducted with 8 isolates of Xp and 7 isolates of Xpf using different cultivars of Phaseolus vulgaris, L (common bean), P. coccineus (Scarlet Runner), P. acutifolius (Tepary bean), and Vigna unguiculata (cowpeas). Isolates from infected bean seeds were selected from bacterial cultures maintained by Dr. A. W. Saettler (Department of Botany and Plant Pathology, Michigan State University) and by Dr. M. L. Schuster (Nebraska Agricultural Experimental Station, Nebraska) (Table 1) and their pathogenic behavior on bean varieties has been recorded by various workers (Table 2).

Culture Media: Yeast extract-calcium carbonate-agar (YCA) (1000 ml distilled water, 10 gm yeast extract (Difco), 15 gm bacto agar, and 2.5 gm $CaCO_3$) was used throughout the study. There was no observable change in pathogenicity of individual isolates on this medium during the period of investigation as confirmed by disease reactions in susceptible Manitou bean periodically inoculated with transferred cultures. Re-isolates from infected leaves were not used for comparative studies since, according to M. Goto (23),

TABLE 1.--Isolates of Xanthomonas phaseoli and Xanthomonas phaseoli var. fuscans used in comparative pathogenicity studies.

Isolate (Xp)	Origin	Isolate (Xpf)	Origin
12 (879-2)	Michigan	16 (988-2)	Michigan
15 (1205-2)	Michigan	18 (1101-2)	Michigan
21 (C_7)	Colombia	19 (1253-1)	Michigan
22 (Pinto)	Colorado	28	Idaho
23 (S)	Nebraska	29	Nebraska
24 (C_6)	Colombia	844	Guatemala
25 (BBL-25)	Canada	Ciat A	Colombia
U2	Uganda		

isolates with relatively low virulence show high virulence when re-isolated from infected leaves, and re-inoculated into healthy tissues.

Bean Differentials: The bean varieties were selected on the basis of their reported susceptibility or tolerance to isolates of Xp and Xpf (Table 2). Test plants for foliage inoculations were grown under field, greenhouse and growth chamber conditions. Plants for pod inoculations were grown in the field using commercial production practices. In the growth chamber, plants were grown in vermiculite medium in 4.5-inch (11.5 cm) diameter, 32 oz wax-lined cardboard cartons while greenhouse plants were grown in soil contained in either 10-inch earthen pots or 4.5 inch cartons. During November-May, daylight was supplemented 14 hr/day with

TABLE 2.--Bean varieties and their reported reactions to Xp and Xpf.

Bean Variety	Blight Bacteria	Disease Reaction[a] (foliage)	Worker
Sanilac	Xpf	S	Wallen, et al. (66)
Great Northern (G.N.) #1 Sel. 27	Xp	T	Coyne, et al. (12)
G.N. Tara	Xp & Xpf	R, T	Coyne & Schuster (10) Arp, Gregory (5)
G.N. Jules	Xp	T	Coyne & Schuster (11)
Tepary (P. acutifolius) Acc. #10	Xp & Xpf	R	Schuster (54)
P. I. 207262 (Colombia)	Xp	T	Coyne et al. (12)
Manitou	Xp & Xpf	S	Arp Gregory et al. (5)
Scarlet Runner (P. coccineus)	Xpf	R	Burkholder and Bullard (9)
Mich-Cal-Cranberry (MCC)	Xp & Xpf	S	
Seafarer	Xp & Xpf	S	
Red Mexican U.I.#3	Xp & Xpf	S	Saettler (unpublished)
Ouray	Xp & Xpf	S	
Idaho Refugee	Xp & Xpf	S	

[a] T = tolerant; R = resistant; S = susceptible

fluorescent bulbs. Temperature was maintained at 27 ± 2 C and plants were watered with nutrient solution (sequestrene, Fe as metallic 1.8 ppm and Rapid-Gro, 1 teaspoon/2 liters of water) alternated with tap water. Disease-free seed was used for all plantings. At inoculation, plants were examined and selected for uniformity of growth and freedom from damage. Plant age at inoculation varied with individual experiment but was uniform for all isolates in any particular study.

Inoculum Preparation: Bacterial suspensions were prepared from 48 hour cultures grown at room temperature (25 ± 1 C). Cells were scraped from agar surface into sterile distilled water and mixed thoroughly to produce a homogeneous suspension. Unless otherwise stated, all isolates were used at a concentration of 2.8×10^7 cells/ml as determined by standard turbidimetric and dilution plate techniques.

Inoculation Procedures: Pathogenic variation among isolates of the same species is common and its analysis demands standardized, reliable, and convenient devices for scoring virulence. Pathogenic variation is not unique among isolates of Xanthomonas pathogens. It has been reported for the bean halo blight group of fluorescent pseudomonads (53). Different investigators have used various inoculation techniques which make it difficult to directly compare their results. Most of the previous work on Xp and Xpf has been

based on the use of intact plants (55, 56). This is a useful approach when screening for field tolerance but intact plants sometimes have limited utility in the greenhouse and growth chambers where space is a factor. Under these conditions, intact plants are not only inconvenient and tedious to use in the numbers required for genetic analysis, but they are also variable in infectivity (susceptibility) to a degree that large replications are necessary to ensure statistical reliability. To avoid the problem of using intact plants, it is necessary that observations be obtained from the same plant tissue(s) each time. Additionally, it would be necessary to compare results in tissues of the same age since plant age is an important factor in disease development.

Comparative pathogenicity in these studies required an inoculation procedure that circumvented morphological barriers, such as cuticle, by placing the bacteria directly in the tissue where they may encounter host resistance factors such as phytoalexins or naturally-occurring compounds that may be toxic to the pathogen. Such an inoculation technique also reveals which bean varieties exhibit "true" genetic resistance or tolerance to invading pathogens. The use of excised plant tissues for the study of bacterial disease reactions has been reported. Klement et al. (32) used excised bean pods while studying the defense reactions induced by phytopathogenic bacteria. Perlasca (47) also used excised bean pods in his study of relationships among

isolates of Pseudomonas syringae pathogenic on stone fruit trees. More recently, Starr and Douglas (58) have reported the use of excised bean pods as a sufficiently reliable, sensitive and convenient technique for scoring virulence of phytopathogenic bacteria. Until the present study, the utility of this assay technique has not been employed in the study of pathogenic variation among isolates of common and fuscous blight bacteria. Previous workers (11, 55, 56) have maintained strict adherence to the use of intact plants purported to simulate natural situations in the field. However, neither the use of intact plants nor excised material actually simulates natural infection since both methods involve artificial inoculation.

In the present study, my interest was not in the ability of the bacterial isolates to overcome external morphological barriers but in whether an isolate was able to multiply and cause measurable disease within host tissue. The following inoculation procedures were therefore examined as possible techniques for scoring the pathogenic potential of the isolates.

(a) Seed Infiltration: Although there is no study regarding the actual biology of seed resistance to infection in nature, it became desirable, in the present study, to explore the utility of seed infiltration in comparative pathogenicity study in the greenhouse. Disease-free seeds selected for uniformity of size and absence of cracks were

pre-soaked in bacterial suspensions for different periods of time and dried on paper towels before planting in vermiculite medium. If germination and emergence occurred, plants were scored for presence of lesions on cotyledons, cotyledonary node and primary leaves. The result of pre-soaking seeds for 10 minutes is given in Table 3. In some cases there was 100% seedling emergence with little or no lesion development while in other cases pre-emergence rotting complicated the results. Because of the great variability in results associated with the same isolate/seed combination in three trials, this inoculation technique was not employed in succeeding experiments.

TABLE 3.--Infection from soaking seed for 10 minutes.[a,b]

		Xp 12		Xpf 16	
Bean Variety	Expt.#	# Germinated	# Blighted	# Germinated	# Blighted
Tara	I	14	0	15	12
	II	15	4	12	8
	III	15	0	10	0
MCC	I	15	0	10	0
	II	11	5	14	10
	III	9	1	7	0

[a]Readings were taken 16 days after planting

[b]15 seeds were planted in each test

(b) Multiple Needle Technique: Leaves were supported on a sponge saturated with bacterial suspension and pierced with a multiple needle inoculator (flower arranging frog containing 66 number 1 pins) previously wetted with bacterial suspension. By applying a small pressure to the inoculated area, bacterial suspension was forced through the puncture and the inoculum was spread over the punctured surface by the forefinger. Controls consisted of leaves punctured dry and leaves punctured and "inoculated" with sterile distilled water.

The utility of this technique was limited mainly to primary leaves and the results (Table 4) were not reproducible in preliminary studies using Xp 12 and Xpf 16. The inadequacy of the method was caused primarily by the difficulty of controlling the amount of inoculum coming into contact with the punctured surface. This method also required a large number of plants for sufficient replication. Needle punture was too severe on small trifoliolate leaves which sometimes collapsed following inoculation with water. This method was therefore considered unsuitable for reliable comparison of isolates.

(c) Leaf Incision Technique: This was a modification of a leaf-clipping technique designed to evaluate resistance of rice varieties to Xanthomonas oryzae (28). The procedure used in the present study consisted of incising bean leaf lamina with a pair of dissecting

TABLE 4.--Pathogenicity of Xp 12 and Xpf 16 using multiple needle inoculation technique.

Bean Variety[a]	Expt.#	% Necrosis[b]	
		Xp 12	Xpf 16
Tara	I	0.0	25.3
	II	10.7	15.0
	III	0.0	45.1
MCC	I	15.0	15.8
	II	0.0	60.5
	III	40.3	25.0

[a]Plants were 20 days old at inoculation

[b]Readings are averages of 6 replicates

scissors previously dipped in bacterial suspension. Disease symptoms developed within 5-7 days after inoculation. In susceptible reactions, water-soaked lesions readily developed from the cut surface and advanced away from the incision. The tolerant or resistant reaction was characterized by mere browning of the cut edges, but no yellowing. Quantitative and qualitative disease-reaction-differences were easily demonstrated in terms of lesion size and rate of spread of chlorotic halo which preceeded the advancing necrotic lesion (Figure 2). Consistent results in lesion size were obtained with inoculations performed at any point along the leaf blade. However, the position of the leaf was critical during the incision operation. Unless the blade was held horizontally, excess inoculum accumulated at

the far end of the incision and resulted in varying lesion shapes and sizes making quantitative measurements difficult. Therefore, leaves were held in a horizontal position to achieve uniform inoculum distribution along the incised surface. The incision method was useful for comparative studies since (a) more than one isolate could be studied on the same leaf or leaflet (Figure 2), (b) this method reduced the number of plants required for replication, and (c) less inoculum was required for inoculation (as many as three incisions could be effected with a single dip of the scissors into the inoculum suspension). The main disadvantage of the incision method was the possibility of predisposition of wounded tissue to other low grade parasites. The utility of the procedure was therefore limited to greenhouse and growth chamber studies.

(d) Excised Pod Technique: Green pods were harvested from field-grown plants when the seeds were approximately 50% of full size. Pods were surface-decontaminated by washing in running tap water for 10 minutes and then by rinsing in sterile distilled water. Pods were examined for freedom from injury and selected for uniformity in size and maturity. To prevent cross-infection during the inoculation procedure, 6-10 pods were randomly distributed into sterile 14.5 cm diameter petri dish moist-chambers lined with moist paper towel. Pods were pricked while still immersed in bacterial suspension

contained in steam sterilized plastic containers. Depending on pod size (variable with individual bean variety), 5-10 points were pricked to a depth of 2 mm using a flame-sterilized dissecting needle. Approximately 6.1×10^4 cells were introduced into the pod at each needle prick as determined by serial dilution plate method. Inoculations were restricted to one side of the pod. The pods were then placed side by side in the moist chambers with the inoculated side up and incubated at laboratory temperature (25 ± 2 C). Pods were examined after 2-7 days, and observations made of lesion types. Lesion classes were quite discrete so that no difficulty was encountered in classifying them (Figure 3). Quantitative measurements of lesion sizes were also made.

(e) Leaf Water Soaking Techniques: Attempts were made to simulate natural conditions of infection by gently spraying inoculum to the point of run off from leaf surfaces. This treatment caused neither internal soaking of the tissues nor wounding of leaf surfaces so that bacteria would be required to enter through natural opening such as stomata. Though this procedure seems desirable, consistent results were not obtained in repeated trials and the method was therefore rejected. A more conventional water-soaking technique as described by Schuster (54) was then examined with several modifications. Plants were inspected to eliminate abnormal plants not in the desired physiological

Figure 2.--Symptoms on primary leaves of MCC bean 16 days after incision-inoculation with Xp15, Xp22, Xp24, and Xp 26.

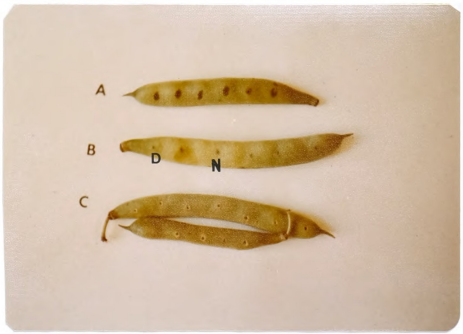


Figure 3.--Symptom types on green pods of P.I. 207262; (A) = 'W' type; (B) = 'N' and 'D' types; and (C) = 'WC' type.

stage of development. Generally, plants were either in the vegetative stage, or in the reproductive stage at inoculation. The leaf stage of the plants was noted and disease reactions were scored on only those tissues present at the time of inoculation. Although time consuming, it was necessary, for purposes of uniformity, to individually spray-inoculate the leaves. Leaves were held against the palm and inoculated on the lower surface from a distance of about 2 cm using a sprayer attached to a compressed air line at 17 p.s.i. This treatment produced visible water-soaked spots (approx. 0.5-1.0 mm diam.). In early studies, plants were given 48 hours of 100% humidity treatment after inoculation but this practice was discontinued in subsequent experiments as discussed elsewhere. Typical blight symptoms (Figure 1) were obtained in repeated trials. The main disadvantage of this inoculation procedure was related to the length of time required for full symptom expression. It usually took 14-21 days for optimum symptom development. However, the method proved very efficient and reliable for testing the reaction of bean varieties to blight bacteria under field and greenhouse conditions.

EXPERIMENTS AND RESULTS

Comparative pathogenicity of isolates of Xp and Xpf in bean leaves assayed by leaf-incision technique

The pathogenicity of Xp and Xpf isolates was studied under growth chamber and greenhouse conditions using the leaf-incision method. The experimental design involved a half-leaf inoculation procedure which permitted up to four isolates to be tested on a single primary leaf. This procedure eliminated plant to plant and leaf to leaf variations in susceptibility.

Growth chamber inoculation. Inoculations with standard inoculum were generally performed within two hours of inoculum preparation between 2:00 p.m. and 4:00 p.m. After 10 days, leaves were scored for disease reactions. Further information was obtained by similar inoculations on the first and second trifoliolate leaves. An incomplete block design allowed comparison of three isolates on the same trifoliolate leaf at different leaflet positions. Disease readings were taken on trifoliolate leaves seven days after inoculation.

Greenhouse Inoculation. Primary leaves were inoculated and plants were incubated at 100% R.H. for 48 hours. Disease reactions were recorded seven days later. In all cases, plants were 14 days old when primary leaves were inoculated and 28 days old when trifoliolate leaves were studied. Each experiment was repeated twice and the mean readings were calculated for each isolate/tissue combination.

The results of growth chamber studies are given in Tables 5 and 6. Disease reactions were scored in terms of lesion size and symptom type. No isolate incited the same symptom type and all tested host differentials; symptom type was dependent upon the specific isolate/host combination. None of the host systems gave a uniform reaction with all isolates. In general, the more pathogenic (virulent) isolates incited 'D' and 'E' symptom types while less virulent isolates incited 'A,' 'C' and 'D' types. The 'B' type of reaction was observed primarily in Idaho Refugee and G.N. #1 Sel. 27 varieties (Table 6). Lesion size varied with isolates from 0-1.7 mm in MCC, 0-2.1 mm in Tara, 0-2.4 mm in Idaho Refugee, to 0-3.2 mm in G.N. Sel 27.

In the greenhouse studies (Table 7), disease reaction different qualitatively (lesion type) and quantitatively (lesion size and number). Percent infection based on the proportion of total inoculations showing pathogenic symptoms varied from 0-100% in most isolate/host

TABLE 5.--Comparative virulence of Xp and Xpf isolates after incision-inoculation of primary leaves.[a]

Isolate	Bean variety							
	MCC		G.N. Tara		Idaho Refugee		G.N.#1, Sel.27	
	Lesion (mm)[b]	Symptom[c]	Lesion (mm)	Symptom	Lesion (mm)	Symptom	Lesion (mm)	Symptom
Xp 12	0.3±.1	D	0.9±.3	D,E	1.8±.1	D	2.5±.2	D,E
Xp 15	1.7±.3	E	0	C	1.6±.3	D,E	1.4±.1	D
Xp 21	0	C	0	C	0.2±.1	A,D	2.0±.6	D
Xp 22	0.7±.1	D,E	0	A	0.2±.1	A,D	0	A
Xp 24	1.4±.2	D	1.7±.1	E	2.1±.3	E	2.6±.2	D
Xp 25	0	C	0	A	1.2±.4	D,E	0	A
Xpf 16	1.5±.3	E	0	C	1.6±.2	E	1.9±.5	D
Xpf 18	1.3±.1	E	0.8±.2	D,E	1.0±.4	D,E	2.4±.2	E
Xpf 19	1.7±.1	D	0	C	0.8±.3	A,E	2.0±.4	D,E
Xpf 28	1.1±.5	E	0	C	0	A	1.6±.3	D
Xpf 29	0.6±.2	D	2.1±.3	D	0.6±.2	A,D	2.0±.7	D
Control(H_2O)	0	A	0	A	0	A	0	A

[a]Plants were incubated 10 days in growth chamber at 27C and 15 hr. photoperiod.

[b]Mean of two tests each of 24 inoculations/isolate.

[c]Letters: A = no symptom; B = grayish-brown lesion without yellow halo; C = yellowing not associated with measurable lesion; D = brown necrotic lesion associated with restricted yellow halo < 2mm from lesion edge; E = like 'D' but with spreading yellow halo > 2 mm from lesion edge.

TABLE 6.--Comparative virulence of Xp and Xpf isolates after incision-inoculation of 1st and 2nd trifoliolate leaves.[a]

Isolate	Bean variety: MCC		G.N. Tara		Idaho Refugee		G.N.#1, Sel.27	
	Lesion (mm)[b]	Symptom[c]	Lesion (mm)	Symptom	Lesion (mm)	Symptom	Lesion (mm)	Symptom
Xp 12	0	A,C	0.7±.1	D	1.7±.3	B,C,D	1.4±.2	B
Xp 15	0	A,C	0.6±.2	D	2.1±.7	D,E	1.6±.3	B
Xp 21	0	A,C	0	C	0.7±.1	C,D,E	1.2±.1	B
Xp 22	0	A,C	0	A	1.3±.5	B,C,D	0	A
Xp 24	0	A,C	1.2±.3	D	2.4±.4	C,E	0	A
Xp 25	0	A	0	A	1.3±.1	B,D,E	0	A
Xpf 16	0	C	1.3±.2	D	1.4±.3	E	2.8±.7	B
Xpf 18	0	C	1.5±.1	D	0.8±.1	E	3.2±.4	B
Xpf 19	0	C	0	A	0.4±.1	E	1.8±.1	D
Xpf 28	0	C	1.5±.5	D	0.7±.2	B,D	0.7±.2	B
Xpf 29	0	A	0.8±.3	B	1.9±.8	C,D	1.2±.1	B
Control (H_2O)	0	A	0	A	0	A	0	A

[a]Plants were incubated seven days in growth chamber at 27C and 15 hr. photoperiod.

[b]Mean of two tests each of 24 inoculations/isolate.

[c]Letters: A = no symptom; B = grayish-brown lesion without yellow halo; C = yellowing not associated with measurable lesion; D = brown necrotic lesion associated with restricted yellow halo < 2 mm from lesion edge; E = like 'D' but with spreading yellow halo > 2mm from lesion edge.

TABLE 7.--Comparative virulence of Xp and Xpf isolates after incision-inoculation of primary leaves.[a]

Isolate	Bean variety								
	MCC			Idaho Refugee			Tepary		
	Lesion (mm)[b]	Symptom[c]	% Infection[d]	Lesion (mm)	Symptom	% Infection	Lesion (mm)	Symptom	% Infection
Xp 12	1.3±.3	D,E	100	1.2±.1	D	100	0	A	0
Xp 15	3.6±.8	E	100	3.2±.6	E	100	0	A	0
Xp 21	0	C	100	0	C	100	0	A	0
Xp 22	1.2±.2	D	63	1.1±.2	D	100	0	A	0
Xp 24	3.3±.5	E	100	2.6±.7	E	100	0	A	0
Xp 25	0	A	0	0.7±.1	D	75	0	A	0
Xpf 16	1.7±.3	E	100	2.3±.5	E	100	0	A	0
Xpf 18	1.5±.1	D	100	2.4±.3	E	100	0	A	0
Xpf 19	0.6±.1	C,D	100	2.5±.2	E	100	0	A	0
Xpf 28	0	A	0	0.5±.1	D	25	0	A	0
Xpf 29	0.4±.3	C,D	25	0.6±.1	D	56	0	A	0
Xpf Ciat A	2.0±.1	E	100	2.6±.3	E	100	0	A	0
Control(H_2O)	0	A	0	0	A	0	0	A	0

TABLE 7.--continued.

Isolate	Bean variety								
	G.N. Tara			G.N.#1, Sel.27			G.N. Jules		
	Lesion (mm)	Symptom	% Infection	Lesion (mm)	Symptom	% Infection	Lesion (mm)	Symptom	% Infection
Xp 12	1.8±.2	D	84	0.7±.1	B	25	0.7±.1	D	38
Xp 15	3.2±.6	E	100	1.5±.2	D,E	100	1.4±.3	D	100
Xp 21	0	C	56	0	A	0	0	C	25
Xp 22	1.5±.2	D	81	0.8±.2	B	50	0	C	25
Xp 24	1.7±.3	B	88	1.0±.3	B,D	100	0	A	0
Xp 25	0	A	0	0	A	0	0	A	0
Xpf 16	1.8±.4	E	100	2.6±.7	E	100	0.8±.1	D	100
Xpf 18	1.4±.1	E	100	1.3±.1	D	100	1.0±.2	D,E	87
Xpf 19	1.6±.2	D,E	100	1.3±.3	D	100	0.8±.1	D,E	100
Xpf 28	0	C	81	0	A	0	0	A	0
Xpf 29	0.5±.4	D	50	0.8±.4	C,D	100	0	C	50
Xpf Ciat A	3.0±.7	E	100	3.0±.3	E	100	1.5±.3	E	100
Control (H_2O)	0	A	0	0	A	0	0	A	0

[a]Plants were incubated 14 days in the greenhouse at 24±1C and 14 hr. photoperiod.
[b]Mean of 32 inoculations/isolate.
[c]Letters: A = no symptom; B = grayish brown lesion without yellow halo; C = yellowing not associated with measurable lesion; D = brown necrotic lesion associated with restricted yellow halo < 2mm from lesion edge; E = like 'D' but with spreading yellow halo > 2 mm from lesion edge.
[d]% infection was based on the proportion of total inoculations with disease symptoms.

combinations. Lesion sizes and symptom types varied with individual isolates on any particular host, and reactions of any particular isolate varied from one host to another.

Effect of humidity on symptom development in beans inoculated with isolates of common blight

It is common practice, in pathogenicity tests, to subject test plants to a high humidity environment before and immediately following inoculation. In field studies, Coyne et al. (12) furrow-irrigated plants immediately after inoculation with common blight and wilt bacteria in order to create a favorable microclimate for disease development. They reported excellent infection and spread of the disease under repeated irrigations. Zaumeyer (68) in a series of experiments confined plants in a moist chamber for 24 hours before and 24 hours after inoculation with Bacterium (Xanthomonas) phaseoli. Infection was obtained without premoisture treatment when plants were held in an infection chamber for 24 hours after inoculation. He suggested that post-inoculation moisture treatment was a factor in disease development.

Seeds were planted in sterile soil in the greenhouse. Four weeks after planting, plants were inoculated by the water-soaking method. One set of plants (2 plants/pot and 3 replicates per treatment) was immediately placed in mist chamber for 48 hours; another set was maintained at the same temperature (27 ± 2 C) but without moisture

treatment. Disease reactions were noted three weeks after inoculation on all plants (Table 9) but time required for expression of first observable symptoms was scored for Xp21, Xp23, Xp24, XpU-2 and a mixture of the 4 Xps on four varieties (Table 8). The experiment was repeated under identical conditions.

The effect of humidity on time of symptom expression is shown in Table 8. Infection generally began either as small discrete chlorotic and necrotic spots or as water-soaked spots all of which eventually developed into the typical blight lesions with necrosis and chlorosis as observed in a susceptible-isolate-host combination. The water soaking reaction was the most virulent reaction (most compatible pathological combination) which always resulted in large lesions and was observed only on susceptible-isolate-host systems. Necrotic flecks and chlorotic spots were observed on both susceptible and tolerant hosts. The flecking response is a hypersensitive response characteristic of a highly incompatible host-parasite relationship. The flecks covered most of the leaf surface particularly on primary leaves of susceptible varieties and usually led to a general chlorosis of the entire leaf.

Moisture seemed important during the latent or incubation period between inoculation and symptom expression. Specifically, moisture appeared to reduce the incubation period in several isolate/host combinations (Xp23--and XpU-2--MCC systems) and gave rise to a more uniform

TABLE 8.--Effect of humidity on time (days) required for visible host/pathogen interaction following water-soaking with bacteria.[a,b]

Isolates	Plants in mist chamber (48hr)				Plants on bench			
	MCC	Manitou	P.I. 207262	Tepary	MCC	Manitou	P.I. 207262	Tepary
Xp 21	4(CS)	4(CS)	3(NF)	4(NF)	4(CS)	4(CS)	3(NF)	4(CS)
Xp 23	4(CS)	4(CS)	3(NF)	4(NF)	7(CS)	4(CS)	7(CS)	3($^{NS}_{CS}$)
Xp 24	4($^{NS}_{CS}$)	4(NS)	3(NF)	5(NF)	4(CS)	3(W)	7($^{NF}_{CS}$)	4(NS)
XpU-2	4(NS)	4($^{W}_{CS}$)	3(NF)	3(NF)	7($^{W}_{CS}$)	5(CS)	4(W)	5($^{NS}_{CS}$)
Xp (21+23+ 24+U2)	4(CS)	4($^{W}_{CS}$)	3(NF)	3(NF)	4(CS)	3(W)	5(CS)	3(CS)

[a]Interaction recorded as CS = chlorotic spots on a few leaves; NS = necrotic spots on a few leaves; NF = necrotic fleck mainly on primary leaves; W = water-soaked lesions with bacterial ooze.

[b]Data are for one experiment.

TABLE 9.--Effect of humidity on symptom development in beans inoculated with isolates of Xp.

Variety	Disease reaction[a] with isolates															
	Xp12		Xp15		Xp21		Xp22		Xp23		Xp24		Xp25		XpU2	
	A[b]	B[c]	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Manitou	Se	Se	Se	Se	Mod	Mod	Mod	Mod	Mod	Mod	Se	Se	Se	Se	Se	Se
MCC	Mod	Mod	Mod	Mod	Mod	Sl	Sl	Sl	Mod	Mod	Se	Se	Mod	Mod	Se	Se
P.I.207262	Sl	Sl	Sl	Sl	Sl	Sl	T	T	T	T	Sl	Sl	Sl	Sl	Sl	Sl
Tepary	T-Sl	T-Sl	T	T	Sl	Sl	T	T	Mod	Mod	T	T	T	T	T	T
G.N.#1, Sel.27	T-Sl	Sl	Sl	Mod	Mod	Sl	Sl	Sl	T	T	Sl	Sl	Mod	Sl	Mod	Mod
G.N. Tara	Sl	Sl	Sl	Sl	Sl	Sl	Sl	Sl	T	T	Sl	Sl	Sl	Sl	Mod	Mod
G.N. Jules	Sl	Sl	Sl	Sl	Sl	T-Sl	T	T	T	T	T	T	T-Sl	Sl	Mod	Mod

[a]Reactions are for two experiments with three replicates each; T = tolerant, no visible symptoms; Sl = slightly susceptible, few chlorotic and necrotic spots on < 20% of inoculated leaf; Mod = moderately susceptible, moderate chlorotic and necrotic lesions, > 20 < 50% of leaf affected; Se = severely susceptible, many large necrotic lesions sometimes associated with wilting and defoliation, > 50% of inoculated leaf affected.

[b]Plants were placed in mist chamber for 48 hours after water-soaking.

[c]Plants were kept at same greenhouse temperature (27 ± 2 C) and photoperiod (14 hr) without moisture treatment.

reaction time in each variety regardless of the challenged isolate. The latent periods were more variable when plants were not given post-moisture treatment following inoculation. In general, the latent period of each isolate varied with the bean variety. There was no direct relationship between the reaction time and the reaction type.

Moisture treatment following water-soaking inoculation was not necessary for disease development (Table 8). Also, symptom type and severity of disease were not influenced by moisture as long as all plants were maintained at the same temperature. Moisture also had no apparent effect on the virulence of the isolates. It was apparent that once bacteria were placed in the intercellular spaces of well-watered plants, the infection process proceeded independently of external moisture treatment. Therefore, moisture treatment was omitted in all subsequent experiments.

Effect of inoculum concentration on infection efficiency of X. phaseoli and X. phaseoli var. fuscans in a susceptible bean

Klement et al. (32) observed that the time of symptom development with phytopathogenic bacteria in bean pod infection was independent of inoculum level and was regulated by the rate of bacterial multiplication. On the other hand, the intensity of the reaction of host tissues depended on the inoculum level. In the present study,

Manitou bean leaves (particularly young trifoliolate leaves) were uniformly susceptible to all 15 isolates of Xp and Xpf when the inoculum contained 2.8×10^7 cells/ml. With such a high inoculum density, it was difficult to detect any pathogenic variation among isolates of both pathogens. In order to use Manitou seedlings as a functional differential host, an infectivity experiment was conducted to study the comparative virulence of isolates based on the general problem of the relationship of inoculum density to successful development of disease reaction.

Three serial dilutions (2.8×10^7, 2.8×10^5 and 2.8×10^3 cells/ml) of inocula were prepared from 48-hour cultures. Eighteen-day-old Manitou seedlings (2 plants/pot) maintained in the greenhouse (28 ± 2 C and 14 hours daylight) were inoculated with each suspension using the modified water-soaking procedure. Inoculated plants (3 replicates per treatment) were returned to greenhouse conditions. Disease reactions and percent leaf infection were recorded 14 days after inoculation (Table 10).

A direct relationship was evident between the necrotic potential (virulence) and inoculum density for all isolates except Xpf28 and Xpf29. The slight virulence associated with Xp21 and Xp23 at 2.8×10^7 cells/ml was completely lost at 2.8×10^3 and 2.8×10^5 cells/ml. The dose-response relationship was quite different for Xpf28 and Xpf29. In this case, a higher pathogenic capability was expressed at 2.8×10^3 cell/ml than at 2.8×10^5 or 2.8×10^7 cells/ml. The slight

TABLE 10.--Effect of inoculum concentration on disease development in 18-day-old Manitou bean.[a]

Isolate	Inoculum concentration					
	2.8×10^3 cells/ml		2.8×10^5 cells/ml		2.8×10^7 cells/ml	
	Disease reaction[b]	% infection[c]	Disease reaction	% infection	Disease reaction	% infection
Xp 12	Sl	5.0	Mod	30.0	Se	75.0
Xp 15	Sl	12.0	Mod-Se	55.0	Se	85.0
Xp 21	T	0.0	T	0.0	Sl	10.0
Xp 22	Sl	5.0	Sl	12.0	Mod	25.0
Xp 23	T	0.0	T	0.0	Sl	10.0
Xp 24	Sl	10.0	Mod	45.0	Se	80.0
Xp 25	Sl	15.0	Sl	15.0	Se	65.0
Xp U2	Sl-Mod	20.0	Mod-Se	55.0	Se	80.0
Xpf 16	Sl	15.0	Se	55.0	Se	80.0
Xpf 18	Sl-Mod	22.0	Mod	45.0	Se	80.0
Xpf 19	T	0.0	Mod	25.0	Se	60.0
Xpf 28	Sl-Mod	20.0	Sl	12.0	Sl	5.0
Xpf 29	Sl	5.0	T-Sl	2.0	T	0.0
Xpf 844	Mod	25.0	Se	75.0	Se	90.0
Xpf Ciat A	Sl	10.0	Mod	45.0	Se	85.0

[a]Reactions were based on 6 replicates each of two plants.

[b]Sl = slightly susceptible; Mod = moderately susceptible; Se = severely susceptible; T = tolerant.

[c]% infection = mean percentage of inoculated foliage diseased 14 days after inoculation at 28 ± 2C.

disease reaction observed in Xpf29/tissue combination at 2.8 x 10^3 cells/ml was completely lost at inoculum density of 2.8 x 10^7 cells/ml.

The isolates differed in terms of disease reaction and percent infection. Sometimes isolates incited identical disease reactions but differed quantitatively in terms of total necrosis. The existence of pathogenic variation among the tested isolates was more evident at low inoculum than at high inoculum densities. The data strongly suggest the existence of pathogenic heterogeneity among isolates of X. phaseoli and X. phaseoli var. fuscans. Based on the quantitative response, three virulent classes were arbitrarily recognized; the most virulent Group I comprised Xpl5, XpU-2, Xp24, Xpfl6, Xpf844, and Xpf Ciat A. Group II with moderate virulence comprised Xpl2 and Xpfl9, while the least virulent Group III contained Xp21, Xp23, Xp25, Xpf28 and Xpf29.

Effect of leaf and plant age on the development of common and fuscuous blights of bean

In the preliminary studies, it was commonly observed that leaves on the same inoculated plant developed different sizes and numbers of lesions. This indicated that leaf age and plant age should be examined in a study of pathogenic variation in Xp and Xpf. The assumption was that if isolates were homogeneous with respect to disease reaction-factors, then the disease reactions should be similar or

identical in comparable tissues of the same age; however, if isolates were heterogeneous, then they should differ with respect to the degree of virulence and symptom type.

The virulence of Xpf isolates was studied using plants of the same age (28 days at inoculation); each plant, however, possessed leaves of different maturities. At this stage the plants were in a 5-leaf stage; the primary leaf and first trifoliolate leaves were fully expanded, the second trifoliolate leaves were still expanding, the third trifoliolate leaves were just unfolding, while the fourth trifoliolate leaves were completely unfolded. Disease reactions were compared on all leaves present at the time of water-soaking inoculation.

For the comparison of isolates of Xanthomonas phaseoli, plants of different ages were grown (staggered planting) and inoculated simultaneously using water-soaking procedure. The three age groups (all vegetative) included 10 days, 20 days, and 30 days. Ten-day-old plants were in the primary leaf stage; plants 20 days at inoculation possessed fully open first and second trifoliolate leaves and partially unfolded third trifoliolate leaves; 30-day-old plants were in their fourth trifoliolate-leaf stage with the youngest leaf partially unfurled. In this case, disease reactions were studied on the primary, first and second trifoliolate leaves.

Seedlings were grown from disease-free seeds of MCC and Manitou (for isolates of Xpf) and Manitou (for isolates

of Xp) beans. Seedlings were grown at the rate of 2 plants per pot of compost soil at 27 ± 2 C and 14 hour photoperiod. Selection for uniformity in leaf stage and leaf size and freedom from malformation was done at the time of inoculation. Leaves were numbered from the base of the plant upward. Inoculation was made with a 48-hour culture of isolates scraped into sterile distilled water. All leaves were individually sprayed with the bacterial suspension on the lower surface to cause visible water soaking. Water soaking was progressively more difficult to effect as leaf age increased. Longer exposure times and a reduction in distance between the spray nozzle and tissues ensured adequate water-soaking of older tissues. Plants were placed at 27 ± 2C and observed daily for symptom development. Final disease rating was done 14 days after inoculation.

Time of first appearance of disease reaction in infection with isolates of Xp is shown in Table 11. Successful infection was evidence by the occurrence of water-soaked lesions associated with bacterial ooze. With all isolate/tissue combinations, the reaction time increased with leaf age. A minumum reaction time of three days was found for all isolates in 10-day-old primary leaves. Typical disease reactions were not observed in 30-day-old primary leaves regardless of isolate; in 20-day-old primary leaves, disease reactions were produced only by Xp24 and XpU-2 at 5 days. The reaction time in first and second trifoliolate leaves varied from 3-6 days depending on the

TABLE 11.--Effect of leaf and plant age on the development of common blight in Manitou bean.

Plant age and leaf position[c]	Disease rating (DR)[a] and reaction time (RT)[b] with															
	Xp12		Xp15		Xp21		Xp22		Xp23		Xp24		Xp25		XpU2	
	DR	RT	DR	RT	DR	RT	DR	RT	DR	RT	DR	RT	DR	RT	DR	RT
30 days																
1	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
2	0	-	2	5	0	-	0	-	0	-	2	4	0	-	2	5
3	1	4	4	4	1	4	0	-	0	-	5	4	1	4	5	4
20 days																
1	0	-	0	-	0	-	0	-	0	-	1	5	0	-	1	5
2	4	4	5	4	1	5	1	6	0	-	5	4	1	44	4	4
3	5	3	5	3	2	4	3	3	2	5	5	3	3	4	5	3
10 days																
	4	3	5	3	3	3	1	3	2	5	5	3	3	4	5	3
Mean DR	2.0		3.0		1.0		0.7		0.4		3.3		1.3		3.1	

[a](DR) = average of 2 experiments each of 6 plants/isolate; 0 = no symptom; 1 = 20% necrosis; 2 = 20-40% necrosis; 3 = 40-60% necrosis; 4 = 60-80% necrosis; 5 = >80% necrosis on inoculated tissue.

[b](RT) = days to 1st appearance of water-soaked lesions; data were based on 6 leaves.

[c]Leaf position explained in text.

leaf-isolate combination. Isolates Xp22, and Xp23 failed to incite typical disease reactions even at 14 days after inoculation on comparable leaves of 30-day-old plants.

The disease reactions were scored using disease index classes ranging from 0-5 as follows: 0 = no visible disease symptoms; 1 = necrosis on less than 20% of inoculated tissue; 2 = necrosis on 20-40% of tissue; 3 = necrosis covering 40-60% of tissue; 4 = necrosis covering 60-80% of tissue; and 5 = necrosis on more than 80% of tissue, often accompanied by wilting and defoliation. The youngest leaf on any plant of any age was most susceptible to both Xp and Xpf blight isolates (Tables 11 and 12, respectively. Comparable leaves in different age groups reacted differently to isolates of Xp; leaves on 30-day-old plants were the least susceptible while those on 10-day-old plants were the most susceptible (Figure 4). The isolates were quite heterogenous in their reaction phenotypes; they markedly differed on the basis of their reaction in comparable tissues of the same age group and in the same tissues of varying ages. Based on the mean disease reading for all plant ages (Table 11), four virulence groups were easily identified for Xp isolates. Virulence Group I comprised Xp15, Xp24, and XpU2 with very similar and sometimes identical disease reactions. These isolates were the most virulent on all three plant-age groups and were associated with mean disease ratings of 3.0, 3.3, and 3.1, respectively. Xp12 with a mean disease rating of 2.0 was less virulent than members

TABLE 12.--Effect of leaf age on development of fuscous blight in MCC and Manitou beans.[a]

Leaf position[c]	Disease rating[b] associated with isolate						
	Xpf16	Xpf18	Xpf19	Xpf28	Xpf29	Xpf844	Xpf Ciat A
MCC							
1	0	0	0	0	0	1	1
2	1	1	0	0	0	2	1
3	2	2	1	0	1	4	3
4	5	4	4	1	1	5	5
5	5	5	5	2	3	5	5
Mean score	2.6	2.4	2.0	0.6	1.0	3.4	3.0
Manitou							
1	1	1	1	0	0	1	1
2	1	2	1	0	1	4	3
3	4	4	4	1	1	5	5
4	5	5	5	4	3	5	5
5	5	5	5	5	3	5	5
Mean Score	3.2	3.4	3.2	2.0	1.6	4.0	3.8

[a]Plants were 28 days at inoculation.

[b]Data are for 12 plants individually scored.

[c]Leaves were numbered upward beginning with primary leaf.

Figure 4.--Effect of leaf age (A) and plant age (B) on symptom development in Manitou bean inoculated at 10, 20, and 30 days after planting.

of virulence Group I, but more virulent than the remaining isolates; Xpl2 constituted a single member of virulence Group II. Xp2l and Xp25 with disease ratings of 1.0 and 1.3, respectively, were similar in their reaction and were put in virulence Group III, while Xp22 and Xp23 fell into Class IV, the least virulent group with disease scores of 0.7 and 0.4, respectively.

On the basis of disease ratings obtained on Manitou and MCC beans, three virulent groups or strains were recognized among isolates of Xpf (Table 12). The most virulent Group I consisted of Xpf844 and Xpf Ciat A, each with a mean disease score of 3.4 and 3.0 in MCC and 4.0 and 3.8 in Manitou, respectively. Group II comprised Xpf 16, Xpfl8, and Xpf 19, each with disease scores of 2.6, 2.4, and 2.0 in MCC and 3.2, 3.4, and 3.2 in Manitou, respectively. The third and least virulent group comprised Xpf28 and Xpf29, each with a disease rating of 0.6 and 1.0 in MCC and 2.6 and 1.6 in Manitou, respectively.

In all cases, the most virulent isolates were able to incite disease reactions in both old and young plant tissues; infection with less virulent isolates was restricted primarily to the young, more succulent tissues of the plant. Separation of isolates into virulence groups was easier on older tissues than on younger tissues where disease reactions tended to be uniform for all tested isolates.

Comparative virulence of Xp and Xpf blight isolates in *Phaseolus* spp. inoculated at vegetative and reproductive stages of development

Reports relating to the effect of plant age on susceptibility to infection by blight bacteria are inconsistent. Goss (22) found that older leaves were more susceptible than younger leaves while Patel and Walker (46) reported that the younger leaves were more susceptible. In both cases, the workers failed to consider the physiological stage of the plant as related to disease reaction. In recent studies, Coyne *et al.* (14) reported that susceptibility or tolerance of beans to common blight depends greatly upon the developmental stage of the plant; plants are more susceptible when in reproductive stage than when in the vegetative stage. They suggest that, in a breeding program, it is important to select plants which exhibit high tolerance during pod development, when the plants are most susceptible. Their suggestion was based upon disease reaction ratings of two bean lines, G.N. Nebraska #1, Sel. 27 (late) and the nearly-isogenic G.N. Nebraska #1, Sel. 27 (early), which were in vegetative and reproductive stages of growth at inoculation. In essence, the major source of variation in disease reaction may be plant age, but one of the previous workers actually compared disease reactions in the vegetative and reproductive stages of the same bean line in a standardized environment. In order to test the claim that bean plants are more susceptible in the reproductive

stage than in the vegetative stage of growth, a sequential inoculation of plants (seeded at the same time) and/or simultaneous inoculation of plants (employing staggered planting) at different physiological stages under identical experimental conditions is necessary.

In the present study, staggered planting technique was employed. The reaction of different bean varieties to Xp and Xpf isolates in vegetative and reproductive stages of development was studied with seven varieties: G.N. Tara, MCC, Manitou, Tepary (only vegetative in greenhouse), G.N. Jules, P.I. 207262, and G.N. #1 Sel. 27. Plants in the vegetative stage (21 days after seeding) and plants in the reproductive stage (35 days after planting) were inoculated with standard inoculum (2.8×10^7 cells/ml) using leaf-water-soaking technique. Inoculated plants were placed on greenhouse benches at 27 ± 2 C and 14 hours photoperiod and watered alternately with tap water and fertilizer solution (sequestrene, Fe as metallic 1.8 ppm and Rapid gro, 1 teaspoon per 2 liters of water). Symptoms were scored on comparable inoculated leaves three weeks after inoculation.

There were differences in the reaction pattern of host varieties to infection by isolates; some isolates incited similar or identical disease reactions regardless of the developmental stage of plant at inoculation, whereas other isolates sometimes became slightly more virulent on plants in the reproductive stage (Tables 13 and 14). These observations suggest the existence of pathotypes with varying

TABLE 13.--Disease reactions of Phaseolus spp. in vegetative (V) and reproductive (R) stages of growth to isolates of X. phaseoli.

	Disease reaction[a,b] with isolate															
	Xp12		Xp15		Xp21		Xp22		Xp23		Xp24		Xp25		XpU2	
Host	V	R	V	R	V	R	V	R	V	R	V	R	V	R	V	R
G.N. Tara	Sl	Sl	Mod	Se	Sl	Sl	Mod	Mod	T	T	Sl	Sl	T	T	Se	Mod-Se
G.N. Jules	T	T	Sl	Sl	Sl	T-Sl	T	T	T	T	T	T	T	Sl	Mod	Mod
G.N.#1,Sel.27	T	T	Se	Se	Sl	Sl-Mod	Sl	Sl	T	T	Sl	Sl	Sl	Sl	Se	Mod-Se
MCC	Mod	Mod	Mod	Mod	Sl	Sl	Sl	Sl	Sl	Mod	Mod	Se	Mod	Mod	Mod	Se
Manitou	Se	Se	Se	Se	Mod	Sl-Mod	Se	Mod	Mod	Mod	Se	Se	Se	Se	Se	Se
Tepary (P. acutifolius)	H-Sl	-[c]	H	-	Sl	-	T	-	Mod	-	T	-	H-Sl	-	T	-
P.I.207262	T	Sl	T-Sl	Sl	T	T	T	T	T	T	Sl	Sl	T	T-Sl	Sl	Sl

[a]Reactions are based on two tests of three replicates each.

[b]Letters: T = tolerant, no visible symptoms; H = hypersensitive, few necrotic flecks; Sl = slightly susceptible, <20% of inoculated leaf necrotic; Mod = moderately susceptible, > 20 < 50% of inoculated leaf necrotic; Se = severely susceptible, >50% of inoculated leaf necrotic.

[c]Tepary did not flower under greenhouse conditions.

TABLE 14.--Disease reactions of Phaseolus spp. in vegetative (V) and reproductive (R) stages of growth to isolates of X. phaseoli var. fuscans.

	Disease reaction[a,b] with isolate													
	Xpf16		Xpf18		Xpf19		Xpf28		Xpf29		Xpf844		Xpf Ciat A	
Host	V	R	V	R	V	R	V	R	V	R	V	R	V	R
G.N. Tara	Se	Se	Mod-Se	Se	Mod	Se	Sl	Sl	Sl	Sl	Se	Se	Se	Se
G.N. Jules	Mod	Se	Mod	Mod	Mod	Se	Sl	Mod	Sl	Mod	Se	Se	Se	Se
G.N.#1,Sel.27	Se	Se	Mod	Mod	Mod	Se	T	Sl	Sl	Mod	Se	Se	Se	Se
MCC	Mod	Mod	Mod	Mod	Mod	Sl-Mod	Sl	T-Sl	Sl	T-Sl	Se	Se	Se	Se
Manitou	Se	Se	Se	Se	Se	Se	Se	Mod	Se	Mod	Se	Se	Se	Se
Tepary (P. acutifolius)	Mod	-[c]	H	-	Sl	-	T	-	H	-	H	-	Mod	-
P.I.207262	Sl-Mod	Mod	Mod	Mod	T	T-Sl	T	T	T	T	Mod	Se	Mod	Se

[a]Reactions are based on two tests of three replicates each.

[b]Letters: T = tolerant, no visible symptoms; H = hypersensitive, few necrotic flecks; Sl = slightly susceptible, <20% of inoculated leaf necrotic; Mod = moderately susceptible, > 20 < 50% of inoculated leaf necrotic; Se = severely susceptible, >50% of inoculated leaf necrotic.

[c]Tepary did not flower under greenhouse conditions.

virulence which may be affected by the stage of plant development. The change in susceptibility or tolerance with plant maturity depended on the specific host/isolate combination. For example G.N. Jules and P.I. 207262 were uniform in their reaction to individual isolates of Xp (Table 13) both in vegetative and reproductive stages of growth. However, these varieties were more susceptible to most isolates of Xpf (Table 14) in the reproductive than in the vegetative stages of growth. This general pattern did not hold for a variety such as Manitou. For example, the greater virulence of Xp22, Xp21, and Xpf 28, and Xpf29 associated with the vegetative growth was absent at the reproductive stage of plant growth. It was difficult to explain the decrease in virulence during the reproductive stage of the Manitou variety. In general, the results in Tables 13 and 14 showed that depending on the host/isolate combination, some isolates which appeared slightly pathogenic during the vegetative stage of plant growth became more pathogenic during the reproductive stage. The data confirm the report by Coyne et al. (14) that many lines may have a moderate level of tolerance in the vegetative stage but become susceptible during the reproductive stage of development. A comparison of disease reactions on all seven differential hosts indicates the existence of pathogenic variation among the tested isolates of Xp and Xpf (Tables 13 and 14).

Effect of mixed inoculum of Xp and Xpf on disease development in bean plants assayed by leaf-incision and water-soaking techniques

In a survey of internally-borne bacterial diseases in Michigan Navy (pea) beans, Saettler and Perry (51) reported that both Xp and Xpf were found together in about 50% of the seed lots. However, there is no report regarding the co-existence of both bacteria in leaf tissues artificially inoculated with a mixture of both pathogens. Previous workers have always studied infection using individual isolates of either X. phaseoli or X. phaseoli var. fuscans. In so doing, they have overlooked the possible aspect of multiple infection with several isolates of each bacterial type, as well as mixed infection with a combination of common and fuscous blight isolates. Pompeu and Crowder (48) reported that resistance of dry bean lines to Xp is conditioned by a few genes; resistant and susceptible lines have different genes for reaction. Coyne et al. (14) and Pompeu et al. (48) in isolated studies reported that reaction to Xp is inherited quantitatively and that lines can be developed with greater resistance than the parents. Artificial inoculations using several isolates of the same species or a mixture of Xp and Xpf, have a direct bearing in resistance-breeding programs since infection involving different pathogen genotypes can very readily reveal which plant entries have the desirable horizontal (quantitative) resistance. The present study examined interactions

resulting from the co-existence of many pathogen cell types in the same tissue in relation to disease development. Two inoculation procedures were used:

(a) Leaf-Incision technique: Bean varieties G.N. Jules, G.N. Tara, Manitou, MCC and Tepary, were grown in vermiculite-peat mixture (3 parts vermiculite: 1 part peat) in the growth chamber at 26 C and 14 hour photoperiod. Suspensions of seven Xp and seven Xpf isolates (Table 15) were prepared from 48 hour YCA-cultures. Mixed inocula were prepared by combining appropriate suspensions in equal volumes so that each composite inoculum contained seven cell types. Xp composite inoculum was combined with Xpf composite inoculum in a 1:1 (vol/vol) ratio and mixed thoroughly to obtain an Xp-Xpf inoculum containing 14 cell types. Prepared in this way, each inoculum type contained approximately 10^7 cells/ml. Inoculation was performed on 12-day-old plants (2 seedlings/carton) using the half-leaf method on uniform primary leaves. Inoculations with Xp12, Xpf18 and distilled water were included as controls. Plants were placed in the growth chamber at post-inoculation temperature of 26 C, watered with nutrient solution, and examined for type and size of lesion 10 days after inoculation.

(b) Water-Soaking inoculation: Bean varieties were grown in compost soil in the greenhouse. Plants were thinned to two plants per pot and kept adequately watered with nutrient solution. Inocula were prepared as described

TABLE 15.--Effect of mixed inoculum of Xp and Xpf on development of symptoms in bean varieties assayed by leaf-incision technique.[a]

Isolate	Variety									
	G.N. Jules		G.N. Tara		Manitou		MCC		Tepary	
	Lesion size (mm)[b]	Symp.[c]	Lesion size (mm)	Symp.	Lesion size (mm)	Symp.	Lesion size (mm)	Symp.	Lesion size (mm)	Symp.
Xpl2 (Control)	1.4	D	3.6	E	2.8	E	3.5	E	0.0	C
7Xps[d]	2.3	E	4.0	E	3.5	E	4.6	E	0.0	C
Xpf18 (Control)	2.0	E	3.2	E	3.2	E	3.6	E	0.0	C
7Xpfs[e]	2.8	E	3.8	E	4.2	E	4.6	E	0.0	C
7Xps + 7Xpfs	3.8	E	4.3	E	3.4	E	5.2	E	0.0	A
H_2O (Control)	0.0	A	0.0	A	0.0	A	0.0	A	0.0	A

[a]Experiment was done in growth chamber at 26C and 14 hr. photoperiod.

[b]Lesions were measured 10 days after inoculation; figures are mean of 24 lesions/ treatment.

[c]Letters: A = no symptom; B = brown necrotic lesion with no yellowing; C = yellowing not associated with measurable lesion; D = brown necrotic lesion associated with restricted chlorotic halo 2 mm from lesion edge; E = necrotic lesion associated with spreading halo 2mm from lesion edge.

[d]7Xps = Xpl2 + Xpl5 + Xp22 + Xp23 + Xp24 + Xp25 + XpU2.

[e]7Xpfs = Xpfl6 + Xpfl8 + Xpfl9 + Xpf28 + Xpf29 + Xpf844 + Xpf Ciat A.

above and plants were inoculated 31 days after planting using the water-soaking procedure. At the time of inoculation, all plants except Tepary were in the reproductive stage. Inoculations with Xpl2, Xpfl8 and distilled water were included as controls. The experiment was run in triplicate and disease reactions were rated three weeks after inoculation.

The effect of mixed infection on disease development using leaf-incision technique is presented in Table 15. Infection was more severe with multiple or mixed isolates of Xp and Xpf than with Xpl2 and Xpfl8 alone. Composite inocula of seven Xps consistently produced larger lesions on all susceptible varieties than did Xpl2 alone. The same observation held for the seven Xpfs inocula as compared to isolate Xpfl8. In most cases, a mixture of seven Xps and seven Xpfs resulted in slightly larger lesions. Co-existence of multiple isolates did not result in any apparent change in symptom type. All inoculum/host combinations (except Xpl2/Jules) produced necrotic lesions associated with spreading chlorotic halo > 2 mm from the lesion edge. Xpl2/G.N. Jules system resulted in small necrotic areas associated with restricted halos. Tepary was tolerant not only to Xpl2 and Xpfl8 but also to all composite inocula; symptoms consisted of a general yellowing of tissue.

Disease reactions obtained with the water-soaking technique are shown in Table 16. The results were quite

TABLE 16.--Effect of mixed inoculum of Xp and Xpf on disease reaction in bean varieties assayed by water-soaking technique.[a]

Isolate	Disease reaction[b] in variety				
	G.N. Jules	G.N. Tara	Manitou	MCC	Tepary
Xpl2(Control)	T	Sl	Se	Mod	T
7Xps[c]	Sl	Se	Se	Se	Sl
Xpf18(Control)	Mod	Se	Se	Mod	T
7Xpfs[d]	Se	Se	Se	Se	Sl
7Xps + 7Xpfs	Mod	Se	Se	Se	H
H_2O(Control)	T	T	T	T	T

[a]Experiment was performed in the greenhouse at 28 ± 2 C and 14 hr. photoperiod.

[b]Letters: T = tolerant, no visible sumptoms; H = hypersensitive, small necrotic flecks on inoculated leaf; Sl = slightly susceptible, small necrotic and chlorotic lesions on <20% of inoculated leaf; Mod = moderately susceptible, moderate to large necrotic lesions on > 20 < 50% of inoculated leaf; Se = severely susceptible, large necrotic lesions on > 50% of inoculated leaf.

[c]7Xps = Xpl2 + Xpl5 + Xp22 + Xp23 + Xp24 + Xp25 + XpU2.

[d]7Xpfs = Xpfl6 + Xpfl8 + Xpfl9 + Xpf28 + Xpf29 + Xpf844 + Xpf Ciat A.

similar to those obtained with leaf-incision. There was no evidence of antagonism among isolates in the composite inocula since disease intensity was not reduced. With this inoculation procedure, Tepary, G.N. Jules and G.N. #1, Sel. 27 were tolerant to Xpl2; Tepary and G.N. Jules were slightly susceptible while G.N. #1, Sel. 27 was moderately susceptible to composite inocula of seven Xps. Tepary was tolerant to Xpf18 but slightly susceptible to a mixture of seven Xpfs. In general, mixed infection with composite inocula was more severe than with either Xpl2 or Xpf18.

Comparative virulence of X. phaseoli and X phaseoli var. fuscans isolates in bean pods

Pod infection was studied in seven varieties of Phaseolus spp. including G.N. Jules, G.N. Tara, G.N. #1, Sel. 27, Tepary, Scarlet Runner (P. coccineus), MCC, and P.I. 207262. The plants were grown at the Michigan State University Farm using commercial production practices. Pods were harvested when still green and about half-filled. They were washed in running tap water to remove sand and debris and any chemical residue from the surface, and rinsed in sterile distilled water. After drying on sterile paper towel, pods were carefully needle-inoculated with appropriate suspensions of blight isolates. Bacterial suspension was introduced into the pod tissue by wounding the tissue with a needle. Control pods were inoculated with sterile distilled water. Following inoculation, the bacterial

suspension diffused in the mesocarp covering an area ranging from 0.5-1.0 mm depending on the bean variety. In most cases, the water soaked spots disappeared after 5-10 minutes and the affected tissue regained its original color following absorption of the inoculum suspension. All samples were incubated in 14 cm diam.-petri dish moist-chambers at room temperature (25 ± 1 C). Pods were examined six days after inoculation and disease reactions were compared both qualitatively and quantitatively on the basis of lesion type (Figure 3) and lesion size, respectively. Lesion size was determined by measuring two diameters at right angles to each other. Measurements were performed only on green looking pods; yellow pods in the senescent stage were discarded at the time of measurement to eliminate a potential source of variability among isolates.

Disease reactions were evident in most isolate/pod combinations 2-3 days after inoculation but final readings were obtained six days after inoculation. The reactions were categorized into five symptom classes as follows:

'D' = dry needle hole showing no necrosis;
'WC' = dry needle hole with white chlorotic halo, tissue not macerated;
'W' = water-soaked light to dark green necrotic lesion with or without bacterial ooze, tissue macerated;
'N' = necrotic, dry-looking brown lesion, tissue not macerated;
'B' = wet looking dark brown necrotic lesion, tissue macerated.

The 'W' class of lesions was indicative of the virulence potential of the isolates in a susceptible isolate-pod-combination; class 'B' was regarded as a more advanced state of 'W'. It was difficult to determine the nature of 'WC' and 'N' lesion types but their occurrence indicated further variability in disease reaction. The isolate/pod combination resulting in 'D' symptom class indicated a tolerant pathological relationship similar to water-inoculation controls. Based on the total number of inoculations, the distribution of the percent lesion types (Table 17) showed a wide spectrum of variation depending on the host/isolate combination. Among isolates of X. phaseoli, Xpl2, Xpl5, Xp24 and Xp25 consistently caused a higher percentage of the 'W' lesion type than Xp21 and Xp22. The 'WC' lesion type was uncommon and was incited mainly by Xp21. Among isolates of Xpf, infection with Xpf16, Xpf18 and Xpf19 consistently resulted in higher percentages of the 'W' reaction type than did infection with Xpf28 and Xpf29. None of the compared Xpf isolates incited the 'WC' lesion type.

Table 18 gives the quantitative estimate of necrosis incited by isolates of Xp in the tested bean varieties. The data show that isolates markedly varied in their invasive ability on the same and different hosts. The quantitative virulence of Xpl5 and Xp24 was significantly greater than that of Xpl2, Xp21, Xp22 and Xp25 at the 5% level. Variation in virulence was also observed in

TABLE 17.--Lesion types resulting from inoculation of bean pods with Xp and Xpf isolates.[a]

	Bean variety																			
	MCC					Tepary					Scarlet Runner					P.I.207262				
	% lesion type[b,c]					% lesion type					% lesion type					% lesion type				
Isolate	WC	W	N	B	D	WC	W	N	B	D	WC	W	N	B	D	WC	W	N	B	D
Xp12		78	14	8			29	71				46		54			100			
Xp15		100					66	34				100					93	3		
Xp21	65	35				70	11		19		48	8	38	6		50		27	18	5
Xp22		100					56		44		65			25	10		88			12
Xp24		100					28		72			56		44			100			
Xp25		83	17				63		37			32	7	61			24	16		60
Xpf16		68	32				67		33			71		29			100			
Xpf18		95	5				12		88			73	13	12	2		90	10		
Xpf19		68	32				22		78			94		6			85	6		9
Xpf28					100		25	75				18		34	48		4	2		94
Xpf29		11	24		65		20	72		8			32		68		10	17		73
Control (H_2O)			1		99					100										100

[a]Pods were incubated at 25 ± 1 C.

[b]Lesion type: D = dry needle holes, no necrosis; WC = dry needle holes with white chlorotic halo, tissue not macerated; W = water-soaked light to dark green necrotic lesion with or without bacterial ooze, tissue macerated; N = necrotic dry-looking brown lesion, tissue not macerated; B = wet-looking dark brown necrotic lesion, tissue macerated.

[c]Percentages were based on 150 needle punctures examined.

TABLE 17.--continued.

Isolate	Bean variety														
	G.N. Tara					G.N. Jules					G.N.#1,Sel.27				
	WC	W	N	B	D	WC	W	N	B	D	WC	W	N	B	D
Xp12		80	20					100				63	37		
Xp15		77		23			10	90				79	21		
Xp21	20	35	45				13	87			48	34	18		
Xp22		77	15	8			33	67				63	37		
Xp24		68	32				11	89			8	46	28	18	
Xp25		57	43					100				54	46		
Xpf16		100						50		50		88	5	7	
Xpf18		34	66				48	52				59	41		
Xpf19		91	9				41			59		100			
Xpf28		48			52			100				22	21		57
Xpf29		21	64	15			25			75		23	21		56
Control(H_2O)					100					100			1		99

[a]Pods were incubated at 25 ± 1 C.

[b]Lesion type: D = dry needle holes, no secrosis; WC = dry needle holds with white chlorotic halo, tissue not macerated; W = water-soaked light to dark green necrotic lesion with or without bacterial ooze, tissue macerated; N = necrotic dry-looking brown lesion, tissue not macerated; B = wet-looking dark brown necrotic lesion, tissue macerated.

[c]Percentages were based on 150 needle punctures examined.

TABLE 18.--Lesion size (mm) in bean pods following infection by Xp isolates.[x,y]

Isolate	Variety						
	G.N. Jules	G.N. Tara	G.N.#1, Sel.27	MCC	Tepary	Scarlet Runner	P.I.207262
Xp12	0.4 h[z]	1.3 g	1.6 fg	1.4 g	1.6 fg	3.4 ab	1.8 ef
Xp15	0.5 h	2.4 d	3.0 c	2.6 d	3.6 ab	3.4 ab	2.4 d
Xp21	1.2 g	2.0 ef	1.2 g	2.4 d	1.4 g	2.8 c	1.6 fg
Xp22	1.6 fg	2.4 d	1.7 f	2.2 de	1.8 ef	2.3 de	1.8 ef
Xp24	0.5 h	2.0 ef	2.4 d	2.7 cd	2.6 d	3.8 a	1.5 fg
Xp25	0.5 h	2.3 de	1.5 fg	1.5 fg	2.5 d	3.2 bc	1.4 g

[x]Pods were inoculated while still green and incubated 6 days; temperature 25 ± 1 C.

[y]Average of 100 lesions/variety.

[z]Mean separation by Duncan's multiple range test, 5% level.

isolates of Xpf (Table 19). Two virulence groups were recognized; Group I with greater virulence contained Xpf16, Xpf18 and Xpf19 and the less virulent Group II contained Xpf28 and Xpf29.

Pathogenic variability in X. phaseoli and X. phaseoli var. fuscans in field-grown bean plants

Previous greenhouse studies based on leaf-incision and water-soaking inoculation procedures have consistently shown considerable variation in the virulence of Xp and Xpf isolates. Laboratory infection of green pods also indicated qualitative as well as quantitative differences among isolates of these bacteria. To establish and confirm existence of pathogenic variation among isolates of these bacteria, it was desirable to compare, in an appropriately designed field experiment, representative isolates of common and fuscous blight bacteria originating from different geographical zones.

Four isolates each of Xp and Xpf, representing different major bean-growing regions were employed in this study. The isolates included XpU2 from Uganda, Xp21 and Xpf Ciat A from Colombia, Xp23 and Xpf29 from Nebraska, Xp15 and Xpf16 from Michigan, and Xpf844 from Guatemala. Isolates were established from diseased seed and were maintained on YCA for a period of three years. Pathogenic comparisons were performed on eight commercial bean varieties. Susceptible varieties included Sanilac,

TABLE 19.--Lesion size (mm) in bean pods following infection by Xpf isolates.[x,y]

Isolate	Variety						
	G.N. Jules	G.N. Tara	G.N.#1, Sel.27	MCC	Tepary	Scarlet Runner	P.I.207262
Xpf16	1.0 fg[z]	1.2 ef	1.7 de	2.5 bc	1.8 d	3.8 a	2.6 b
Xpf18	0.8 fg	2.0 cd	2.3 bc	1.3 ef	2.0 cd	2.8 b	2.3 bc
Xpf19	0.6 gh	1.3 ef	2.4 bc	1.8 d	1.4 def	2.7 b	2.6 b
Xpf28	0.5 h	1.1 efg	0.7 g	0.0 h	1.9 cd	2.9 b	1.3 ef
Xpf29	0.4 h	1.2 ef	0.4 h	0.7 g	1.6 de	1.2 ef	1.3 ef

[x]Pods were inoculated while still green and incubated 6 days; temperature 25 ±1 C.

[y]Average of 100 lesions/variety.

[z]Mean separation by Duncan's multiple range test, 5% level.

Seafarer, MCC, Manitou, Ouray and Red Mexican U.I.#3, tolerant varieties were G.N. #1, Sel. 27 and G.N. Tara. Seedlings were grown from disease-free seed. A randomized split-plot design, consisting of three replications was used with main plots consisting of isolates and sub-plots bean varieties. Each sub-plot contained two single rows, 10 feet long and spaced 28 inches apart. Each sub-plot was isolated from the other by a compact row of corn planted to buffer and check spread of bacteria from one plot to another. Bacterial inocula were prepared from 48 hour-old YCA cultures in tap water and diluted to 10^6 cells/ml. Primary infection was established in one row (spreader row) in each sub-plot by individually water-soaking leaves with appropriate bacterial suspension using a hand operated atomizer. Control rows were sprayed with tap water only. At the time of inoculation (38 days after seeding), Sanilac, Seafarer, MCC, Red Mexican U.I.#3, Tara and Ouray were in the blossom stage of development while G.N. #1, Sel. 27 and Manitou were in the vegetative stage. Initial disease readings were taken 21 days after inoculation and final disease reactions were taken 35 days after inoculation. The virulence of isolates was compared using the following criteria: (a) primary infection on inoculated rows (Figure 5) described as severely susceptible (Se), moderately susceptible (Mod), slightly susceptible (Sl),

T

Sl

Figure 5.--Disease reaction classes in field-grown beans inoculated with blight bacteria; T = tolerant, Sl = slightly susceptible; Mod = moderately susceptible, Se = severely susceptible.

Mod

Se

Figure 5.--continued.

or tolerant (T); (b) secondary spread to adjacent non-inoculated rows, based on the number of lesions/plant; (c) pod infection on spreader (inoculated) row; (d) seed infection/100 gm wt of seed from spreader row; and (e) yield reduction relative to control. Quantitative measurements were analyzed statistically to establish significant differences resulting from differential virulence of isolates.

Table 20 summarizes disease reactions on spreader rows and shows the intensity of secondary spread of the disease to adjacent tester rows in infections with isolates of Xp. The isolates differed not only in the host reaction types (amount of necrosis) but also in the amount of secondary spread to adjacent rows 28 inches away. Based on the amount of necrosis, XpU2 was the most virulent isolate followed by Xp15. Xp21 and Xp23 were the least virulent on the same varieties giving, in most cases, a tolerant reaction. XpU2 and Xp15 could not be differentiated in their reaction in Sanilac, Seafarer, MCC and G.N. Tara. However, they differed in their reaction in Manitou, Red Mexican U.I.#3 and Ouray where XpU2 was consistently more virulent than Xp15. Secondary infection was dependent upon primary infection and varied among isolates. In all compatible combinations, secondary spread was more effective with Xp15 and XpU2 than with Xp23 and Xp21. Infection with Xp23 usually resulted in no secondary spread since primary infection was very slight.

TABLE 20.--Reaction of beans (Phaseolus vulgaris) to Michigan (Xp15), Ugandan (XpU2), Colombian (Xp21), and Nebraska (Xp23) isolates of X. phaseoli.

	Disease reaction[b]								
	Xp15		Xp21		Xp23		XpU2		Control (H_2O)
Variety[a]	P.I.[c]	S.I.[d]	P.I.	S.I.	P.I.	S.I.	P.I.	S.I.	P.I.
Sanilac	Sl-Mod	+	T-Sl	+	T-Sl	-	Mod	+	T
Seafarer	Mod	+	Sl	+	T	-	Mod	+	T
MCC	Sl	-	T-Sl	-	T-Sl	-	Sl	-	T
G.N.#1,Sel.27	T	-	T	-	T-Sl	-	T	-	T
Manitou	Sl	-	Sl	-	T	-	Mod	+	T
Red Mexican U.I.#3	Mod	++	Sl	+	Sl-Mod	+	Se	++	T
G.N. Tara	T-Sl	-	T	-			T-Sl	-	T
Ouray	Mod	+	Sl	+			Se	++	T

[a]All varieties except G.N.#1,Sel.27 and Manitou were in blossom stage at innoculation.

[b]Letters: T = tolerant, no visible symptoms; Sl = slightly susceptible, small discrete lesions (<5mm), <30% of tissue affected; Mod = moderately susceptible, moderate to large lesions (5-10mm), > 30 < 60% of tissue affected; Se = severely susceptible, large coalescing lesions (>10mm) sometimes associated with wilting and defoliation; 60% of tissue affected.

[c]P.I. = primary infection in inoculated rows based on 3 replicates of 25 plants each.

[d]S.I. = secondary infection in adjacent uninoculated rows (28" apart); (-) = no secondary spread; (+) = secondary spread, <5 lesions/plant; (++) = secondary spread, > 5 < 10 lesions/plant; (+++) = secondary spread, > 10 lesions/plant.

Xpf16, Xpf Ciat A and Xpf 844 were quite virulent and tended to be identical in their disease reactions in the same bean varieties (Table 21). The slight variation in disease reaction observed in MCC, G.N. #1, Sel. 27 and Manitou was not distinct enough to differentiate these isolates into virulent classes. Xpf29 was less virulent than Xpf16, Xpf844 and Xpf Ciat A. Primary infection with Xpf16, Xpf844 and Xpf Ciat A resulted in more secondary spread than infection with Xpf29. As noted for Xp, secondary spread in infection with isolates of Xpf was dependent upon the level of primary infection.

Virulence of Xp and Xpf isolates were distinct (Tables 20 and 21). Of interest was the susceptibility of G.N. #1, Sel. 27 and G.N. Tara to isolates of Xpf and their tolerance to isolates of Xp. In general, isolates of Xpf resulted in higher disease reactions and more secondary spread than did isolates of Xp in the same bean varieties.

Table 22 shows pod infection data. There was slight variation among isolates but in general, number of lesions per pod was very low. The data on seed infection of Sanilac bean is shown in Table 23. The number of diseased seeds was lower in infection with isolates of Xp than with Xpf isolates. Statistical analysis of data showed no difference among isolates of Xp; among isolates of Xpf, Xpf16 was similar to Xpf844 and Xpf Ciat A but differed significantly from Xpf29.

TABLE 21.--Reaction of beans (Phaseolus vulgaris) to Michigan (Xpf16), Guatemala (Xpf844), Nebraska (Xpf29), and Colombian (Xpf Ciat A) isolates of X. phaseoli var. fuscans.

	Disease reaction[b]								
	Xpf16		Xpf29		Xpf844		Xpf Ciat A		Control(H_2O)
Variety[a]	P.I.[c]	S.I.[d]	P.I.	S.I.	P.I.	S.I.	P.I.	S.I.	P.I.
Sanilac	Se	++	Mod	+	Se	++	Se	+++	T
Seafarer	Se	++	Mod	+	Se	++	Se	+++	T
MCC	Sl-Mod	+	T-Sl	-	Mod-Se	+	Mod	+	T
G.N.#1,Sel.27	Sl-Mod	+	T-Sl	-	Mod	+	Sl-Mod	+	T
Manitou	Mod-Se	+	Mod	+	Se	++	Se	++	T
Red Mexican #U.I.3	Se	++	Mod	+	Se	+++	Se	+++	T
G.N. Tara	Mod	+			Mod	+	Mod	++	T
Ouray	Se	+++			Se	+++	Se	+++	T

[a]All varieties except G.N.#1,Sel.27 and Manitou were in blossom stage at inoculation.

[b]Letters: T = tolerant, no visible symptoms; Sl = slightly susceptible, small discrete lesions (<5mm), <30% of tissue affected; Mod = moderately susceptible, moderate to large lesions (5-10mm), > 30 < 60% of tissue affected; Se = severely susceptible, large coalescing lesions (>10mm) sometimes associated with wilting and defoliation; >60% of tissue affected.

[c]P.I. = primary infection in inoculated rows based on 3 replicates of 25 plants each.

[d]S.I. = secondary infection in adjacent uninoculated rows (28" apart); (-) = no secondary spread; (+) = secondary spread, < 5 lesions/plant; (++) = secondary spread, > 5 < 10 lesions/plant; (+++) = secondary spread, > 10 lesions/plant.

TABLE 22.--Pod infection (no. lesions/pod) in beans (Phaseolus vulgaris) inoculated with isolates of Xp and Xpf.

Variety[a]	No. lesions[b]								
	Xp15	Xp21	Xp23	XpU2	Xpf16	Xpf29	Xpf844	Xpf Ciat A	Control (H_2O)
Sanilac	0.12	0.04	0.0	0.03	0.14	0.05	0.21	0.29	0.0
Seafarer	0.12	0.17	0.17	0.14	0.48	0.04	0.22	0.13	0.0
MCC	0.0	0.0	0.0	0.0	0.01	0.0	0.0	0.0	0.0
G.N.#1,Sel. 27	0.0	0.0	0.0	0.01	0.0	0.0	0.0	0.03	0.0
Red Mexican U.I.#3	0.48	0.55	0.19	0.28	0.06	0.09	0.06	0.09	0.01
G.N. Tara	0.0	0.0		0.02	0.08		0.04	0.02	0.0
Ouray	0.01	0.01		0.09	0.14		0.26	0.22	0.0

[a]All varieties except G.N.#1,Sel.27 and Manitou were in blossom stage at inoculation.

[b]Average of 400 pods/variety.

TABLE 23.--Seed infection in field-grown Sanilac bean inoculated with isolates of Xp and Xpf.[x]

Isolate	Seed infection[y]
XpU2	0.4 b[z]
Xp15	0.4 b
Xp21	1.4 b
Xp23	0.0 b
Xpf16	12.1 a
Xpf844	5.2 ab
Xpf Ciat A	6.4 ab
Xpf29	0.0 b
Control(H_2O)	0.0 b

[x]Plants were in blossom stage at inoculation.

[y]Number of diseased seeds/100 gm. wt. (samples ranged from 150-200 gm. wt.)

[z]Mean separation by Duncan's multiple range test, 5% level.

The relationship between disease reactions and yield (seed weight) was studied in two varieties: Sanilac and Red Mexican U.I.#3 (Table 24). Variation in yield due to the differential virulence of isolates was significant at 5% level. The difference between the 'check' mean and each isolate mean was significant in both bean varieties. On Sanilac, Xp21 and Xp15 were similar in virulence but differed from both XpU2 and Xp23; Xpf16 and Xpf Ciat A were similar in virulence and differed only slightly from Xpf844 but significantly from Xp29. The following schematic diagram illustrates the virulent groups observed on Sanilac:

Xpf16		XpU2		Xp15		
=	≥	=	>	Xp21	>	Xp23
Xpf Ciat A		Xpf844		Xpf29		
I				II		III

A comparison of isolate means on Red Mexican showed that Xp15, Xp21 and Xp23 were similar in virulence but they differed significantly from XpU2. Among isolates of Xpf, Xpf844 was similar to Xpf Ciat A but differed significantly from Xpf16 and Xpf29. Schematically, the following virulence scale was proposed for isolates of Xp and Xpf on Red Mexican:

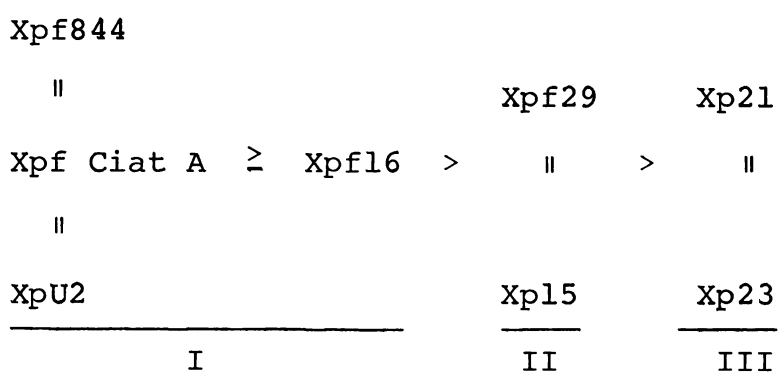
TABLE 24.--Effect of Xp and Xpf isolates on yield (seed weight) of field-grown Sanilac and Red Mexican U.I.#3 beans.[x]

	Seed weight (gm)[y]	
Isolate	Sanilac	Red Mexican U.I.#3
XpU2	96.7 cde[z]	99.4 de
Xp15	112.8 bc	123.5 bc
Xp21	117.1 bc	141.7 b
Xp23	134.4 b	143.5 b
Xpf16	78.6 de	117.3 cd
Xpf844	88.0 cde	92.3 e
Xpf Ciat A	72.1 e	105.3 cde
Xpf29	103.3 bcd	126.6 bc
Control(H_2O)	184.9 a	187.5 a

[x]Plants were inoculated in the blossom stage.

[y]Average of 3 replicates with 10 plants each.

[z]Mean separation by Duncan's multiple range test, 5% level.



In general, disease was more severe with most isolates of Xpf than with isolates of Xp on both Sanilac and Red Mexican U.I.#3.

Population trends of Xp and Xpf isolates in tolerant and susceptible bean cultivars as related to disease reaction

The ability of a phytopathogen to grow and multiply in the host is often considered as a compatible pathogen/host relationship which involves the recognition of the host by the pathogen. Proponents of the so-called 'gene-for-gene' theory argue that an incompatible relationship results from the lack of recognition of the host by the pathogen. Studies by previous workers (56) have demonstrated the existence of pathotypes among isolates of Xp. However, we do not know whether the ability of the pathogen to recognize and concomitantly multiply in one host, and its inability to recognize and multiply in another host, is what accounts for the compatible and incompatible relationship with the hosts. Leben (35) has reported the recovery of Xanthomonas vesicatoria from non-diseased tomato seedlings; the lack of internal multiplication and invasion of

the tissue by the pathogen may explain lack of disease development. Stall and Cook (52) related hypersensitivity and susceptibility in pepper to bacterial concentration; the concentration of X. vesicatoria associated with necrosis in hypersensitive pepper tissues was lower than that in susceptible tissues. Their observation suggests that the ability of bacteria to grow and multiply is a factor in the pathogenicity or virulence of the bacteria in the susceptible pepper. The present investigation concerns the population trends of X. phaseoli and X. phaseoli var. fuscans isolates relative to symptom development in several commercial bean varieties.

Populations were determined in G.N. Jules, Manitou and G.N. Tara. The first part of the experiment involved infection with individual isolates of Xp: Xp21, Xp23, Xp24 and XpU2. These isolates were selected because of their reported pathogenic variation in Phaseolus spp. (56). The second study concerned mixed infection with: (a) composite inocula containing Xp21, Xp23, Xp24 and XpU2, and (b) a mixture of seven isolates of Xp and seven isolates of Xpf. Manitou was selected as a suitable host because it is reported to be susceptible to both Xp and Xpf (5) while G.N. Tara and G.N. Jules were chosen because of their previously reported tolerance to both Xp and Xpf (5, 10) and Xp (11), respectively.

Bacterial cells washed from plates of two-day-old YCA cultures were suspended in distilled water at a standard

concentration of 2.8×10^7 cells/ml, or at a lower concentration of 2.8×10^4 cells/ml. In mixed inoculations, individual isolate suspensions were prepared and combined in equal volumes (10 ml/isolate) to give a multi-component or composite inoculum. The suspension was vigorously shaken to mix the cells and produce a uniform inoculum which, in the case of 7 Xps + 7 Xpfs, yielded a 1 Xp: 1Xpf cell ratio on YCA-dilution-plate counts in repeated trials.

Disease-free seeds were planted (2 seedlings/carton) in steamed compost soil contained in 32 oz. wax-lined cardboard cartons in the greenhouse at 28 ± 2 C and 14 hours photoperiod. All leaves were inoculated by the water-soaking method within 1-2 hours after inoculum preparation two weeks after planting (vegetative period). Multiplication in mixed infection with four isolates of Xp was studied in first trifoliolate leaves of G.N. Tara, while population trends in infection with individual isolates were examined in first trifoliolate and primary leaves of G.N. Tara and Manitou, respectively. Multiple infection with pooled inoculum of Xp and Xpf isolates was studied in first trifoliolate leaves of Manitou and G.N. Jules.

Populations of viable bacterial cells were determined from ten 9 mm-diameter leaf discs from three inoculated leaves selected at random. Discs were surface sterilized in 2.6% NaOCl for 10-15 seconds followed by

rinsing in three changes of sterile distilled water. The discs were placed in 5 ml of sterile distilled water and thoroughly comminuted in a glass tissue grinder. Tenfold serial dilutions of ground suspensions were made in sterile distilled water, and 0.1 ml aliquots pipetted into empty sterile Petri plates. YCA previously cooled to about 45 C was poured into each plate (about 15 ml/plate) and mixed with the suspension by gently swirling the plate in two directions. Plates were incubated at 25 C and colonies were generally counted after 3-5 days of incubation in individual infections and after 5-7 days in mixed infection with Xp + Xpf.

Population after inoculation (Figure 6) resembled that of a typical bacterial growth-curve. Four phases of growth were recognized as follows: (a) a short lag phase (0-24 hr.); (b) the logarithmic or exponential phase; (c) the stationary phase; and (d) the decline or death phase. No host/isolate specificity was apparent although 1.5-2 times as many viable XpU2 cells were sometimes recovered as compared to Xp21, Xp23 and Xp24. The isolates multiplied at different rates particularly during exponential and decline phases but the growth patterns were quite similar at the stationary growth phase. The growth curve generated by the composite inoculum of the four isolates in multiple infection closely resembled those of the individual isolates, thus showing no apparent existence of antagonism or complementarity among the isolates in growth requirements.

Figure 6.--Population trends of Xp isolates in trifoliolate leaves of G.N. Tara inoculated with 2.8×10^7 cells/ml.

Figure 7.--Dilution plate used for isolating bacteria from G.N. Tara leaves 12 days after inoculation with a mixture of Xp21, Xp23, Xp24, and XpU2; (A) = large colonies of Xp; (B) = small colonies of orange-yellow new phenotype (XpE); and (C) = grayish white colonies of resident bacteria.

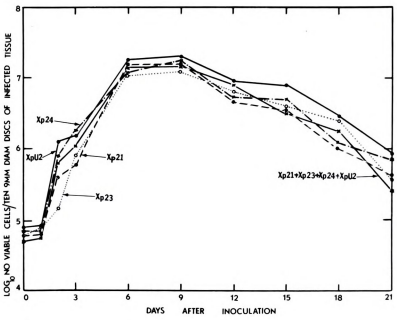


Figure 6

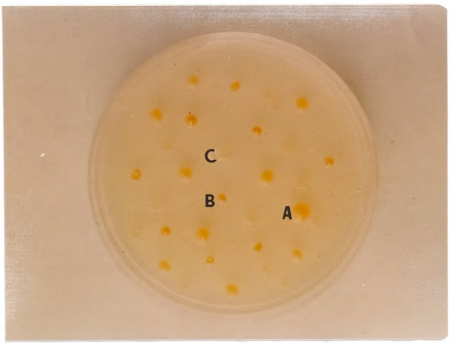


Figure 7

Symptoms on G.N. Tara differed with each isolate. No symptoms developed up to four days after inoculation: the first disease reactions were chlorotic spots developed with Xp24 and XpU2, five days after inoculation. Necrotic lesions developed at six days with Xp24 and XpU2 and after 21 days, XpU2 lesions developed into large necrotic lesions (> 5 mm diam) to give a 'severely susceptible' (Se) reaction-type. Lesions with Xp24 remained restricted in size (< 3 mm diam) and gave rise to a 'slightly-susceptible' (Sl) reaction type. Xp21 initially produced a general chlorosis 7-8 days after inoculation; at 21 days, a 'Sl' reaction type was associated with Xp21 while a 'tolerant' (T) reaction associated with neither chlorosis nor necrosis, was observed in Xp23/Tara combination. Symptoms were delayed with composite inoculum (9 days) but the final disease reaction was 'Se'.

A new orange yellow phenotype (designated XpE, Figure 13) was recovered from G.N. Tara leaves inoculated with the composite inoculum. The occurrence of the 'new' type was first observed nine days after inoculation; the frequency of isolation generally ranged for 0-5 colonies per plate. XpE appeared to differ from the parental types (Xp21, Xp23, Xp24, XpU2) in its growth requirements; it grew very slowly on YCA but very actively in yeast extract liquid medium. In initial tests, it was highly pathogenic in susceptible Manitou but it seemed to belong to a serotype different from that of the parental types

(Figure 13). Periodic inoculation of Manitou seedlings with transferred cultures of XpE maintained on YCA indicated apparent decrease in pathogenic potential with a concomitant increase in ability to grow in YCA.

Multiplication pattern in Manitou inoculated with low inoculum concentration (10^4 cells/ml) is shown in Figure 8. The growth pattern also resembled the general pattern observed for these isolates in G.N. Tara. The more virulent XpU2 and Xp24 showed an enhanced growth rate over the less virulent Xp21 and Xp23 during the early stages of infection; more advanced stages of infection sometimes yielded more viable cells of the less virulent Xp21. The least number of viable cells was recovered from Xp23-infected leaves. In all cases, no symptoms were observed up to 14 days after inoculation; the first indication of disease (a general chlorosis associated with a few necrotic areas) was associated with XpU2 and Xp24, 16 days after inoculation. At the low inoculum density, plants inoculated with Xp21 and Xp23 were not different from water controls. However, in all cases, the population of viable cells rose rapidly from a level of about 10^2 cells one hour after inoculation, to a high population level of about 10^7 cells/ten 9 mm-diameter leaf discs, 16 days after inoculation.

The populations of Xp and Xpf in mixed infection of Manitou and G.N. Jules (Figure 9 and 10, respectively) were similar over 21 days, increasing rapidly one hour after

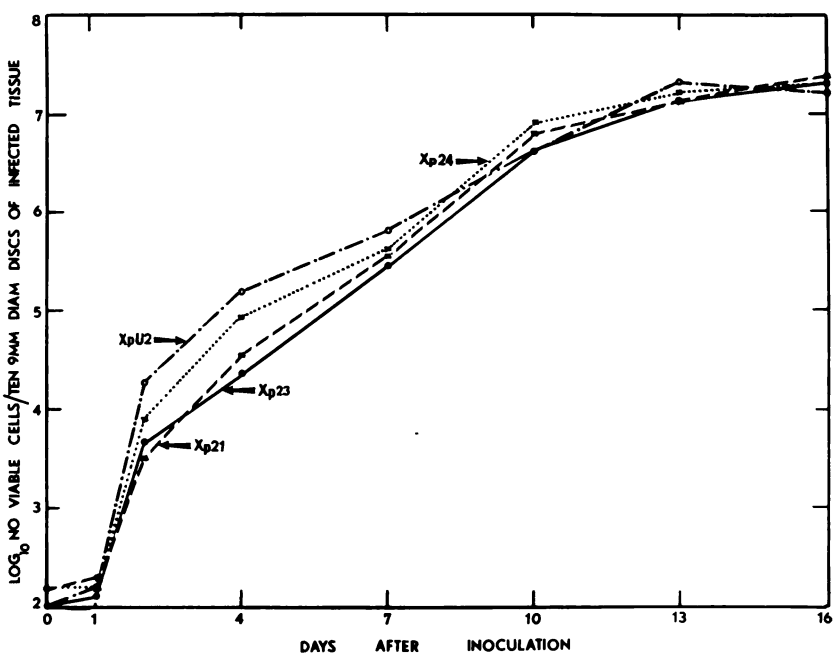


Figure 8.--Population trends of Xp isolates in primary leaves of Manitou bean inoculated with 2.8×10^4 cells/ml.

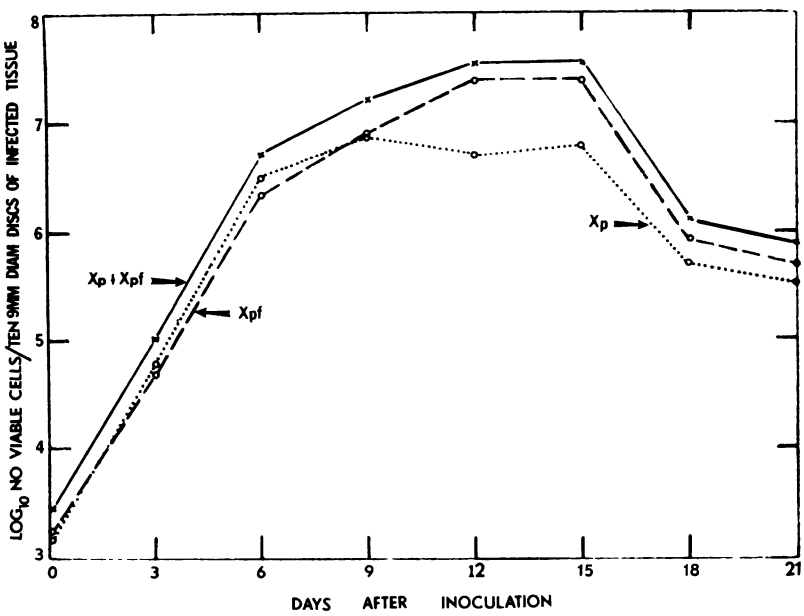


Figure 9.--Effect of mixed infection on the number of viable Xp and Xpf cells in diseased Manitou leaves.

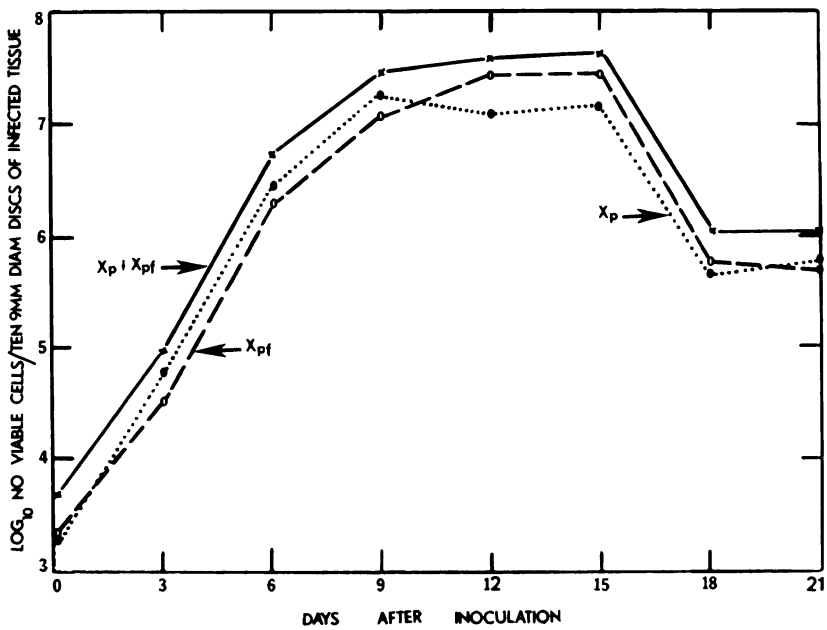


Figure 10.--Effect of mixed infection on the number of viable Xp and Xpf cells in diseased G.N. Jules leaves.

inoculation to a peak 15 days later followed by a decline through 21 days. The growth curves indicated differential growth rates between Xp and Xpf in mixed infection. Between 0-9 days (in G.N. Jules, Figure 10), and 0-6 days (in Manitou, Figure 9), the total population of viable cells was composed of twice as many Xp as Xpf; at nine days, the total population of viable cells consisted of about equal numbers of Xp and Xpf in Manitou; between 12 and 15 days, Xp and Xpf were recovered at a frequency of 1 Xp: 4 Xpf in Manitou and 1 Xp: 3 Xpf in G.N. Jules; but at 18 days, the population of viable cells comprised Xp and Xpf in a 1:1 ratio in G.N. Jules and twice as many Xpf as Xp in Manitou. Xpf exhibited a typical stationary phase of growth in both bean varieties; a gradual decline phase immediately followed the exponential growth phase in Xp/host combinations. In most cases, 1.5-2 times as many total viable cells were recovered from the less susceptible or 'tolerant' G.N. Jules as from the susceptible Manitou bean. The disease reaction in Manitou was 'Se' (more than 80% of inoculated leaves necrotic) and 'Mod' (30-40% of tissue affected) in G.N. Jules, at 21 days, although G.N. Jules contained higher populations of viable cells than did Manitou throughout the period of determination.

There was a gradual build-up of a new orange-yellow phenotype among mixed populations of Xp and Xpf nine days after inoculation in both Manitou and G.N. Jules; the frequency of occurrence increased in later stages of

infection to a maximum 18 days after inoculation. The sudden appearance of the new cell type was concomitant with a rapid decline in recovery of both Xp and Xpf; the new phenotype was not observed in tissues inoculated with sterile distilled water. Growth of the new phenotype was very limited on YCA. The small discrete colonies remained hard and depressed at the center. Subsequent transfers on YCA failed to generate measurable growth and growth in yeast extract liquid medium was negative. The pathogenicity of the 'new' cell type could not be verified because of insufficient inoculum supply; the phenotype was subsequently lost in culture.

Pathogenicity of isolates of X. phaseoli and X. phaseoli var. fuscans in cowpeas (Vigna unguiculata)

It has hitherto been observed that X. phaseoli is pathogenic on Phaseolus vulgaris but non-pathogenic on Vigna spp. (personal communications with Dr. N. Vakili, U.S.D.A. Research Plant Pathologist, Puerto Rico) whereas X. vignicola is pathogenic on both hosts. The development of yellow halo in association with lesions distinguishes X. phaseoli from X. vignicola (Vakili unpublished). We consistently observed chlorotic halos around lesions incited by all isolates of Xp and Xpf in P. vulgaris. Pathogenicity of Xp and Xpf originating from different ecological zones was determined on Vigna unguiculata.

Cultivars Purple Hull Southern Pea and Mississippi Silver Pea, were grown in steam-sterilized compost soil in the greenhouse at 27 ± 2 C. Inoculum consisting of individual isolates of Xp and Xpf (Table 25) was prepared from 48-hour old YCA cultures and adjusted to standard concentration. Seedlings were inoculated at primary leaf nodes 14 days after planting using a seedling injection procedure (50) and incubated on greenhouse benches.

Xp23 was highly pathogenic on Purple Hull Southern Pea (Table 25). Infected plants were stunted and wilted and extensive necrotic lesions on the stem were associated with brownish-yellow bacterial ooze indicative of active multiplication and tissue invasion. All other isolates were non-pathogenic on this host; the needle-injection punctures were dry-looking and showed no observable evidence of browning or ooze production. Except for Xp23, the isolates were not readily differentiated based on their reaction in Southern Pea. Host reaction types were more evident on Mississippi Silver Pea; this host was slightly susceptible to Xp15 and Xpf18; moderately susceptible to Xp21, Xpf16 and Xpf Ciat A; severely susceptible to Xp23 and Xpf844; and resistant to Xp12, Xp22, Xp24, Xp25, XpU2, Xpf19, Xpf28 and Xpf29.

Serological studies of Xp and Xpf insolates

Xp and Xpf comprise a large number of isolates that cannot be distinguished from one another by symptomatology

TABLE 25.--Pathogenicity of X. phaseoli and X. phaseoli var. fuscans isolates in cowpea (Vigna unguiculata) inoculated by seedling injection technique.[a]

Variety	Disease reaction with[a]															
	Xp 12	Xp 15	Xp 21	Xp 22	Xp 23	Xp 24	Xp 25	Xp U2	Xpf 16	Xpf 18	Xpf 19	Xpf 28	Xpf 29	Xpf 844	Xpf CiatA	Control (H_2O)
Purple Hull	-	-	-	-	+++	-	-	-	-	-	-	-	-	-	-	-
Southern Pea	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Mississippi	-	+	++	-	+++	-	-	-	++	+	-	-	-	+++	++	-
Silver Pea	0/6	8/8	7/7	0/7	7/7	0/8	0/7	0/7	8/8	5/8	0/8	0/8	0/8	8/8	8/8	0/6

[a]Plants were incubated 28 days at 27 ± 2 C and 14 hours photoperiod.

[b]Reaction: (-) = negative, dry needle holes; (+) = slight, wet necrotic lesion<5mm from injection hole; (++) = moderate, wet necrotic lesion>5<10mm long; (+++) = severe, wet oozing necrotic lesion >10mm and sometimes associated with wilting. The ratio represents number of plants infected (numerator) to total number of plants inoculated (denominator).

in the field. Significant variation, however, exists among isolates within the same group and between isolates of Xp and Xpf in their necrotic or disease potential. Phage typing and pigment production are additional methods used to identify and differentiate the two closely-related groups of Xanthomonas species. Both Xp and Xpf produce a non-diffusable yellow pigment on culture but Xpf also produces a brown diffusable pigment on certain high carbohydrate media. Each group belongs to a different lysotype (27, 31). Phage typing and pigment production are useful only for separating Xp from Xpf and not for separating isolates within the same group. Prior to the report by Schuster and Coyne (55), a pathological homogeneity was supposed for isolates of Xp; they first reported the existence of pathogenic variability among Xp isolates. Our broad collection of Xp and Xpf isolates from wide geographical locations not only confirms their findings but further shows that there exist pathotypes among isolates of Xpf as well. Knowledge on the serology of these groups of bacteria is still incomplete; previous investigators (18) have suggested that Xp and Xpf may belong to the same serotype but no definitive studies have been carried out to justify such claims. Pathogenic variation in the studied isolates strongly suggests the probable existence of serological heterogeneity in the two groups of blight bacteria. The purpose of this study was to provide further information on the serological identities and relationships among phenotypically-identical

isolates within a group and between isolates of the two groups, and in addition, an attempt was made to correlate the findings to the differential pathogenicity on commercial bean varieties.

Eight isolates of Xp and seven isolates of Xpf were included in the study. Stock cultures were grown on YCA and antigens consisting of living cells ("0" antigen) were prepared from polysaccharide-free cultures; polysaccharide production is reported to interfere with agglutination tests when cultures are grown on high carbohydrate medium (18). The elimination of most of these interfering polysaccharidic antigens was achieved by growing cells in buffered yeast extract (10 gm yeast extract/l PO_4 buffer, pH 6.9). The phosphate buffer used was .01M Na_2HPO_4 - KH_2PO_4. Antigen samples were harvested in the logarithmic phase of growth, usually 12-18 hours after incubation with continuous shaking. The cells were sedimented by centrifuging at room temperature for 20 min. at 10,000 g to free them from growth medium and pigments. The sediment was resuspended and washed at least two times with physiological buffered (PO_4 buffer pH6.9) saline (0.85% NaCl w/v) by centrifuging. The final titer was adjusted turbidemitrically to 10^9 - 10^{10} cells/ml.

The antisera were prepared in rabbits and provided through the courtesy of Dr. A. W. Saettler (Department of Botany and Plant Pathology, Michigan State University). They included antisera prepared against Xp15, Xp24, Xpf16

and Xpf28. The rabbits were immunized by five intravenous injections with live cells and 4-5 blood samples were obtained by ear bleeding at 5-7 day intervals following the last injection. The last sample with the highest antibody titer was the test serum and was preserved in sodium azide (500 ppm NaN_3), stored in the freezer, and used as needed.

Serological relationships were tested by cross-agglutination, agglutinin-absorption and immunodiffusion techniques.

For cross-agglutination, bacterial suspensions were compared at antisera dilutions up to 1:2560; one drop of antigen was added to a drop of anti-serum in a spot depression slide. The antigen and antiserum were mixed and allowed to react undisturbed for 10-20 minutes at 25 ± 1 C; antiserum plus buffered saline and antigen plus buffered saline were included as controls. Slides were examined microscopically using obliquely-transmitted light and reaction end points were recorded (Table 26).

In general, titers (Table 26) were highest with Xp antisera and Xp isolates than with Xpf isolates. With Xpf antisera the reverse was obtained. The wide range of reaction end points suggest heterogeneity in antigenic action of the tested isolates. Reactions between homologous antigen/antisera combinations gave higher titer end points than did certain reaction between heterologous pairs. Certain heterologous combinations equalled

TABLE 26.--Cross-agglutination relationships of isolates of Xp and Xpf.

Antiserum for	Dilution end point with isolate[a]														
	Xp 12	Xp 15	Xp 21	Xp 22	Xp 23	Xp 24	Xp 25	Xp U2	Xpf 16	Xpf 18	Xpf 19	Xpf 28	Xpf 29	Xpf 844	Xpf CiatA
Xp15	1/1280	1/1280	1/320	1/320	1/10	1/1280	1/640	1/1280	1/640	1/640	1/160	1/640	1/640	1/640	1/640
Xp24	1/1280	1/1280	1/1280	1/640	1/160	1/1280	1/1280	1/1280	1/640	1/640	1/320	1/640	1/640	1/640	1/640
Xpf16	1/320	1/320	1/160	1/640	1/80	1/120	1/320	1/640	1/1280	1/1280	1/640	1/1280	1/1280	1/640	1/1280
Xpf 28	1/320	1/640	1/80	1/160	NR[b]	1/640	1/320	1/640	1/1280	1/1280	1/640	1/1280	1/1280	1/1280	1/1280

[a]End points are based on results of 3 experiments.

[b]NR = no reaction.

homologous combinations. Cross agglutination of Xpl2, Xpl5, Xp24 and XpU2 against antisera for Xpl5 and Xp24 indicated that similarities exist; the antisera agglutinated these isolates to a titer of 1:1280. Isolates Xp2l, Xp22 and Xp25 were intermediate. The lowest dilution end points of 1:10 (with antiserum for Xpl5) and 1:160 (with antiserum for Xp24) was obtained with Xp23. End points with antisera for Xpfl6 and Xpf28 were markedly lower than those obtained with antisera for Xpl5 and Xp24; they varied from 1:640 for XpU2 to 1:l20 for Xp24. Again the reaction of Xp23 was quite different fromthose of the other isolates of Xp; no agglutination was observed with antiserum for Xpf28 while a titer as low as 1:60 was obtained with antiserum against Xpfl6.

The cross-agglutination reactions of Xpf isolates except Xpfl9 and possibly Xpf844 were quite similar with all antisera; the titer was 1:640 to 1:1280 for Xpfl6 and Xpf28 antisera and 1:640 for Xpl5 and Xp24 antisera. Xpf isolates except Xpfl9 were not differentiated by cross agglutination test. The reaction of Xpfl9 was quite different; the antisera against Xpfl6 and Xpf28 agglutinated this isolate to a titer of 1:640 while antisera against Xpl5 and Xp24 gave titer end points of 1:160 and 1:320, respectively.

The lack of adequate differentiation of isolates by cross agglutination reaction made it desirable to further compare them by agglutinin-absorption procedure (Table 27).

TABLE 27.--Serological relationships of Xp and Xpf isolates by slide agglutination-absorption technique.[a]

Antiserum (1:10) for	Absorbed with	Agglutinated with antigen[b]														
		Xp 12	Xp 15	Xp 21	Xp 22	Xp 23	Xp 24	Xp 25	Xp U2	Xpf 16	Xpf 18	Xpf 19	Xpf 28	Xpf 29	Xpf 844	Xpf Ciat A
Xp15	-	+++	+++	+	++	+	+++	++	+++	++	++	+	++	++	++	++
	Xp21	++	++	-	+	-	+	++	++	++	+	+	+	+	++	++
	XpU2	+	++	-	+	-	-	-	-	-	-	-	++	+	++	++
Xp24	-	+++	+++	+++	++	+	+++	+++	+++	++	++	+	++	++	++	++
	Xp12	-	+	-	+	-	+	+	+	+	-	+	+	-	++	++
	Xp15	-	-	-	-	-	+	-	-	-	-	++	+	-	++	++
	Xp21	-	++	-	++	-	+++	-	+++	++	+	++	+	+	++	++
	Xp22	-	-	-	-	-	+	-	-	+	-	++	+	-	++	++
	Xp23	-	++	-	++	-	++	+	+	++	++	++	++	++	++	++
	XpU2	-	+	-	-	-	+	-	-	+	-	++	+	-	++	++
Xpf16	-	+	+	+	++	+	+	+	++	+++	+++	++	+++	+++	+++	+++
	Xp21	+	+	-	+	-	+	+	+	+	++	+	+	+	+++	+++
	Xp22	++	-	+	-	-	-	-	-	+	+	++	++	++	++	++
Xpf28	-	++	++	+	++	-	++	++	++	+++	++	++	+++	+++	+++	+++
	Xp21	++	+	-	++	-	+	++	++	++	++	+	+	+	+++	+++

[a]Data are for one experiment performed in triplicate.

[b]Antigen = 18 hour-old live cells (2.8×10^9 cells/ml); (-) = no reaction; (+) = weak agglutination; (++) = moderate agglutination; (+++) = strong agglutination.

Antisera were absorbed overnight at 4 C with excess antigen in test tubes. The supernatant was transferred to sterile plastic tubes and centrifuged to remove all agglutination products. The supernatant was then cross-reacted with freshly prepared antigens (10^9 cells/ml) in depression slides.

There was greater similarity among isolates of the same group and a greater serological difference between isolates of Xp and Xpf. Among Xp isolates, Xp15, Xp22 and XpU2 were quite similar in their agglutinin-absorption reaction; Xp21 and Xp23 were very similar; and Xp12, Xp24 and Xp25 were each different from the others. Among isolates of Xpf, Xpf844 and Xpf Ciat A were very similar in their reaction, Xpf18 and Xpf29 were similar; and Xpf16, Xpf19 and Xpf28 were quite different from the others. The results thus suggested the existence of serotypes among isolates of Xp and Xpf. The observed serological relationships among isolates of the same group and between isolates of Xp and Xpf was further tested on immunodiffusion plates.

The immunodiffusion agar medium contained 8.5 gm purified agar, 10 ml 1% Orange G (dye) and 500 ppm NaN_3 per liter of buffered saline (0.85% w/v NaCl). The medium was steamed to dissolve the agar and then dispensed into unscratched 9 cm diam. plastic Petri plates at the rate of 15 ml/plate. In each plate, six antigenic wells equidistant from the central antiserum well were dug out using previously prepared patterns. Antigens (2.8×10^{10} cells/ml) consisting

of 18-hour-old cultures ('0' antigens) were used. When antisera were previously absorbed, the absorption was done with excess homologous antigens in test tubes at 25 ± 1 C overnight. The supernatants were separated from the pellets and further purified by centrifugation. The supernatant-antiserum or unabsorbed antiserum was placed in the central well and reacted with antigens contained in outside wells.

The difference between the number of bands associated with unabsorbed antisera and antisera previously absorbed with homologous antigens (Table 28) indicate the number of antigenic constituents common to each isolate pair. The complete elimination of precipitin bands (except for Xpf29) associated with isolates of Xpf and the reduction in the total number of bands obtained with isolates of Xp following previous homologous absorption of Xpf28 antiserum, showed the serological affinity of Xpf28 to the other Xpf isolates and the more distant relatedness of Xpf28 to Xp isolates (Figure 11). Xpf16 was less related to Xp isolates than was Xpf28; Xpf16 shared only one specific antigen with Xp12 and XpU2, two antigens with Xp22, Xp24 and Xp25, and no specific antigens with Xp15, Xp21 and Xp23 (Figure 12). Absorption of Xp15 antiserum with Xp15 was even more interesting; the bands associated with unabsorbed antiserum were completely eliminated by previous absorption with the homologous antigen (Figure 13). Complete absorption of Xp24 antiserum with Xp24 and Xpf28

TABLE 28.--Serological reactions of isolates of Xp and Xpf by immunodiffusion technique.[a]

Antiserum for	Absorbed with	Reacted with antigen[b]														
		Xp 12	Xp 15	Xp 21	Xp 22	Xp 23	Xp 24	Xp 25	Xp U2	Xpf 16	Xpf 18	Xpf 19	Xpf 28	Xpf 29	Xpf 844	Xpf Ciat A
Xp15	-	5	5(1)	2	4(1)	1	3	4(1)	4(1)	4	3	3	5	4	3	4
	Xp15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Xp24	-	6(1)	7(1)	1	6(1)	1	2	2(1)	5	5	4	3	5	5	4	3
	Xp24	1	1	1	2	1	2	1	1	-	-	-	1	1	-	-
Xpf16	-	3	3	1	4	2	4	4	3	5	4	3	4	5	3	4
	Xpf16	2	3	1	2	2	2	2	2	-	2	2	1	1	1	1
Xpf28	-	4	4	2	3	-	4	3	4	4	3	3	3	4	4(1)	3
	Xpf28	1	2	-	1	-	1	1	1	-	-	-	1	1	-	-

[a]Reaction was scored as number of precipitin bands counted from photographic films; the bracketed numbers indicate the occurrence of an arc-shaped band very close to antigen well.

[b]Antigen = 18 hour-old cells, 2.8×10^{10} cells/ml.

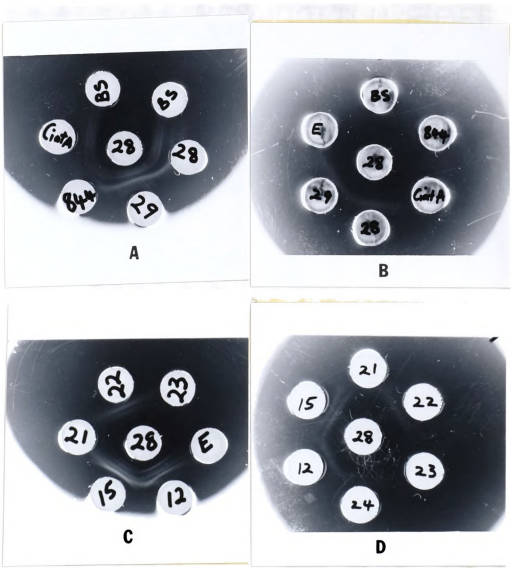


Figure 11.--Immunodiffusion patterns obtained from the reaction of Xpf28 antiserum (central well) with Xpf isolates before (A), and after (B) homologous absorption of antiserum and, reaction of Xpf28 antiserum (central well) with Xp isolates before (C), and after (D) absorption with Xpf28.

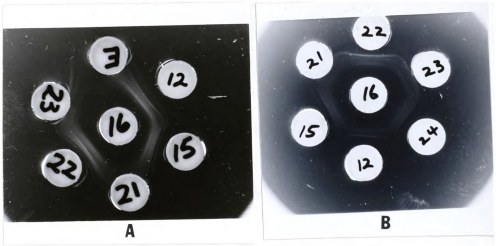


Figure 12.--Immunodiffusion patterns obtained from the reaction of Xpf16 antiserum (central well) with Xp isolates before (A) and after (B) homologous absorption of antiserum with Xpf16.

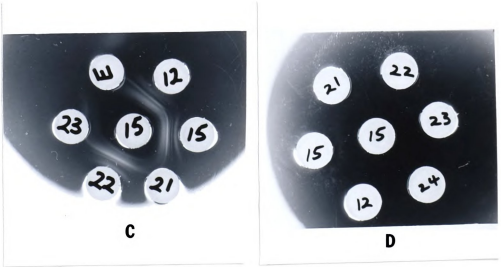
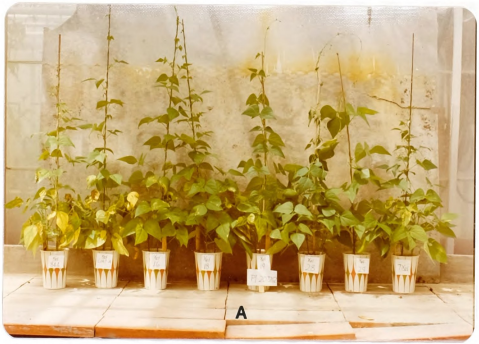


Figure 13.--Immunodiffusion patterns obtained from the reaction of Xpl5 antiserum (central well) with Xp isolates before (C) and after (D) homologous absorption of antiserum with Xpl5.

antiserum with Xpf28 in repeated trials could not be effected but the high number of percipitin bands obtained with heterologous antigens were either completely eliminated or effectively reduced to 1 or 2 in most cases after previous absorption with homologous antigen. It is possible that Xp24 and Xpf28 isolates used in this study are not identical with isolates used for antiserum preparation. Xpl5 and Xp24 antisera produced arc-shaped bands very close to antigen wells of isolates Xpl2, Xpl5, Xp22, Xp25 and XpU2. In general, there were marked differences in the number of bands associated with individual isolates; in most cases, the highest number of bands were obtained with homologous antigen/antiserum pairs but sometimes heterologous pairs gave rise to more bands than did homologous pairs (e.g., Xpf28 antiserum/Xpf28 antigen = 3 bands whereas Xpf28 antiserum/Xpf29 antigen = 4 bands).



A

B

Figure 14.--Disease reaction (A) and reaction categories (B) of P.I.207262 inoculated with Xpf isolates.

DISCUSSION

This study has examined in detail the existence of pathogenic variation among isolates of Xanthomonas phaseoli first observed by Schuster et al. (55). Their studies, however, concerned four isolates of common blight assayed by water-soaking procedure only, but the present study was broadened to include isolates of both X. phaseoli and X. phaseoli var. fuscans originating from distinct ecological bean producing areas represented by Nebraska, Michigan and Idaho in the U.S.A.; Colombia in South America; Uganda in Africa; and Guatemala in Central America. By comparing the pathogenicity of these representative isolates on selected commercial varieties of Phaseolus spp. and Vigna anguiculata using different inoculation techniques detailed in Material and Methods, I have marshalled an evidence for the existence of pathogenic variation in both Xanthomonas phaseoli and X. phaseoli var. fuscans.

Attempts to more closely simulate natural conditions of infection were made by gently spraying the inoculum to run-off onto leaf surfaces; this treatment neither caused wounding of leaf surfaces nor internal soaking of tissues so that cell penetration of the tissue would be through

stomata. This inoculation procedure, although desirable, proved unsuitable since uniform infection was not obtained consistently. Results were consistent when inoculum was physically placed in the cellular environment; this was achieved by pin-prick inoculation of submerged pods, leaf-incision procedure, and water-soaking of leaves. The placement of cells inside the tissue has the advantage of ensuring each cell type an equal opportunity for infection. The method directly compared the physiological ability of each isolate to invade the cellular environment following intromission into cellular spaces; such ability is a reflection of the genetic potential which involves pathogen nutrition, induction of gene products or enzymes, and the elaboration of metabolites for cell growth and multiplication. Theoretically, the reaction phenotypes in inoculated tissues under identical conditions would be expected to be uniform or identical if reaction factors in the isolates were homogeneous; on the contrary, however, a heterogeneous population of isolates should separate into distinct phenotypes on the basis of their reaction with a physiologically-dynamic intracellular environment. Phenotypic reaction types were identified in this study. In addition to adequately standardizing disease reaction conditions, these inoculation procedures have some practical utility in screening for resistance, since active biochemical resistance can readily be distinguished from a conditional rather passive morphological resistance exhibited by some plants.

Leaf clipping has been used for study of other bacterial diseases. Winstead and Kelman (67) used clippings technique to evaluate resistance to Pseudomonas solanacearum and McCarter et al. (42) in field studies reported spread of P. solanacearum and tobacco mosaic virus (TMV) from diseased to healthy tomato plants by clipping with rotary mower. Recently, Kauffman et al. (28) have reported the use of clipping procedure for inoculating rice, Oryza sativa with Xanthomonas oryzae. However, until the present study, bean pathologists have not explored the utility of leaf-clipping and leaf-incision techniques in screening for varietal resistance and pathogenic variability among isolates of bean blight bacteria. In the present study, leaf-incision inoculation allowed a reliable qualitative and quantitative evaluation of infection and the separation of isolates into virulent groups or strains; disease indices from leaf to leaf and from plant to plant showed little or no variation and the large number of replications gave valid disease readings.

Pod infection was not only useful in demonstrating the existence of pathogenic variation but also provided additional information on varietal susceptibility or resistance of commercial hosts to infection by blight isolates. Resistances associated with vegetative tissues of some bean varieties were not necessarily expressed during pod infection. For example, leaves of P.I.207262 were resistant to infection by Xp21, Xp22 and Xp23 but these

isolates incited measurable lesions in inoculated pods. This observation indicates that the infection process in the leaf may be quite different from that in the pod and mandates the use of leaf as well as pod inoculations in studies designed to screen for resistance in commercial bean varieties. A disease may be overt in one part of a plant and latent in another part; for example, Xanthomonas vesicatoria, the cause of bacterial spot of tomato and pepper, has been isolated from the interior of symptomless fruits on plants that showed evidence of infection on leaves (35). To be acceptable, a bean variety should be resistant both in its vegetative and reproductive stages of growth.

Water-soaking procedure was more difficult to effect than either the pin-prick inoculation of pods or the leaf-incision procedure; physical characteristics of the leaf such as number of stomata and degree of internal cellular barriers, affected the rate of penetration, but in most leaves the flooding took place readily. At 17 p.s.i., water-soaking was easily effected without causing evident physical damage to the inoculated tissue; severe disease reactions on susceptible bean varieties were produced both in field and greenhouse experiments and varietal reactions induced were highly reproducible.

Qualitative and quantitative characterization of isolates based on their pathogenic reactions in commercial bean varieties separated the isolates arbitrarily into

virulence-phenotypic groups. Among isolates of X. phaseoli, XpU2, Xpl5 and Xp24 were the most virulent isolates constituting Group I; Xpl2 and Xp25 expressed intermediate virulence and were placed in Group II; and Xp21, Xp22 and Xp23, with the least virulence were put in Group III. Similarly, three virulence groups were constructed for isolates of X. phaseoli var. fuscans; the pooled data on infectivity placed Xpf844 and Xpf Ciat A in the most virulent Group I; Xpfl6, Xpfl8 and Xpfl9 belonged to the intermediate virulence--Group II; while Xpf28 and Xpf29 constituted the least virulent Group III.

In considering the reasons for the observed differential virulence, a number of alternative hypotheses were entertained. Firstly, the differential pathogenicity might result from either 'long association' by some isolates or 'strange contact' by some isolates with the tested commercial bean varieties. If this hypothesis is true, then high virulence may be a consequence of long exposure and adaptability to a particular host while avirulence may result from short-term contact with the host tissue. Whether virulence preceeded avirulence or vice versa needs genetic verification but under this theory it is reasonable to speculate that one pathogenic level evolved from the other in a stepwise manner.

The second hypothesis assumes that what accounts for the observed pathogenic variation may involve interactions between the isolates and the normal resident microflora;

complementary or antagonistic interactions may specify the type and level of disease. During in vivo multiplication studies, four resident phenotypes ranging in colour from red, brown and purple to chocolate white were often isolated from field-grown, healthy-looking beans (David Weller, personal communications); their interaction in mixed infection with individual isolates was not examined but they were apparently non-pathogenic when individually tested on susceptible hosts.

The third hypothesis relates virulence to the ability of the isolate to recognize the tissue microenvironment. In genetic terms, this hypothesis requires that for an isolate to be virulent, it must possess an 'early gene' whose product or function can recognize the cellular environment and cause the production of specific degradative enzymes which act on host metabolites and result in successful infection. In contradistinction, an avirulent phenotype may result from non-recognition of the micro-environment, a probable consequence of loss or change of function of pathogen gene products. Under this hypothesis, it is reasonable to speculate that different host systems require different pathogen gene products for the establishment of compatibility relationships. A large number of early genes would convey an extensive virulence whereas a small number of such genes would specify a limited virulence to the isolate. The 'wild' phenotype (exemplified by Xpf844, Xpf Ciat A, Xp15 and XpU2) may belong to the former

genotype whereas a 'mutant' phenotype (typified by Xp21, Xp23, Xp22, Xpf28 and Xpf29) might represent the latter genotype. Under this model, virulence is a function of early and late gene products; early gene product in the absence of late gene product (degradative product) results in avirulent phenotype or latent infection. The isolation of the gene loci specifying the production of functional pathogenic factors requires a detailed knowledge of the genetic map of a standard blight isolate and is a subject for further investigation.

The final hypothesis holds that the ability of an isolate to actively multiply in a host is of secondary rather than primary importance in infection bionomics; in the in vivo multiplication studies, such ability did not necessarily determine the ability to cause visible disease symptoms. Multiplication may involve a symbiotic relationship in which case the pathogen actively synthesizes the precursors of its progeny without adversely upsetting the physiology or gross anatomy of the host. This type of multiplication results in a tolerant reaction phenotype as in the case of Xp23/Tara combination. If, however, multiplication is accompanied by the production of infection factors, then infection ensues; in this case, a high rate of multiplication will effectively enhance disease severity since the amount of degradative factors would be directly proportional to bacterial cell concentration. The different levels of disease incited in the

universal suscept, Manitou, may be related to the tissue. The development of necrosis or pathogenic relationship in a susceptible isolate-host combination is seen as a morbid bite-the-hand-that-feeds existence of isolate while no symptom expression associated with active multiplication in tolerant varieties is a symbiotic or commensal relationship. If pathogenicity in the latter parasitic system is based on the so-called gene-for-gene relationship, then some genetically-controlled biochemical deficiencies emanating from some functional gene mutation may account for the observed variation in virulence among Xp and Xpf isolates toward the host.

The delayed symptom expression in primary leaves of Manitou inoculated with a low inoculum density (2.8 x 10^4 cells/ml) may be associated with a bacteriostatic action postulated by Allington et al. (1); when Manitou leaves were inoculated with 10^7 cells/ml, necrotic lesions were observed in about 7-8 days after inoculation. Perhaps, the more concentrated inoculum increased too rapidly to a necrosis level before any bacteriostasis developed. Leben and co-workers (37) observed that a low inoculum concentration resulted in lower population maxima and delayed symptom expression in soybean. The ability of an isolate to multiply to a high population level without observable disease reaction may occur under conditions less exacting than those required for infection. The invasion of a plant by a pathogen not accompanied by symptom development, is in

accordance with the idea that parasitism and pathogenicity are not necessarily related. The invasion of symptomless carrier plants, however, has important epidemiological consequences since such hosts, through normal cultural operations or through the activities of ubiquitous insects may serve as a primary source of inoculum for nearby susceptible hosts and an important over-wintering source (survival value) for the next season crop. This poses a major threat to control programs because, if conditions favorable for infection and disease development prevail for a prolonged period, a sudden outbreak of bacterial blight may originate from previously healthy-looking plants and spread to adjacent rows.

Active bacterial multiplication associated with no visual infection has been reported for other phytopathogenic bacteria. Leben (35) reports active multiplication of X. vesicatoria on tomato seedlings without apparent effect on seedlings; English and co-workers (19) and Crosse (15) also isolated bacterial pathogens (Pseudomonas syringae) from non-diseased hosts such as almond, peach and cherry trees. Kennedy (29) has shown that Pseudomonas glycinea, the cause of leaf spot of soybean, is often present within host stems during the growing season, but without profuse multiplication and without evidence of systematic infection. Thomas and others (63) in another study demonstrated the presence of Xanthomonas phaseoli and Corynebacterium flacumfaciens, the cause of bean common

blight and wilt, respectively, in the stems of apparently healthy pinto bean plants. Gaumann (21) terms this type of infection "inapparent" or "symptomless" and Leben (36) describes the host/pathogen combination as a "resident phase" during the life cycle of the pathogen in which active growth and multiplication are not necessarily followed by signs and symptom expression. What accounts for the absence of disease symptoms in a host which actively supports pathogen multiplication is not known, but I submit that such "latent infections" result from some genetically controlled alteration in essential functions of parasite which specify compatible pathogenic interactions. Surface sterilization of leaves followed by serial washings in sterile distilled water strongly support an endophytic rather than epiphytic association of Xp23 and Xp21 with the symptomless tolerant G.N. Tara bean.

Leaf age and plant age affected the virulence of some of the tested isolates. The pooled data on infectivity of isolates as affected by plant age and leaf age (Tables 11, 12) separated the isolates into virulence phenotypes. It is possible that developmental alterations in the metabolism of older tissues may lead to the production of toxic products which inactivate degradative enzymes or functions of the pathogen. Dye (17) points out that X. phaseoli and X. phaseoli var. fuscans utilize similar metabolites in culture; however, a successful in vitro utilization of metabolites does not necessarily imply that

metabolism of the same compounds would be effected in vivo. In vivo metabolism may require regulatory factors in a physiologically-dynamic host tissue. Johnson (26) points out that the amount of polymorphic variation at the enzyme loci of natural populations is quite high. Enzyme polymorphisms increase adaptiveness by providing a means of metabolically compensating for a varying environment; thus individuals with multiple molecular forms of an enzyme may be capable of coping with different cellular environments. Whether enzyme polymorphism at several gene loci is responsible for the observed pathogenic variation among isolates of Xp and Xpf awaits verification by detailed biochemical experimentation in conjunction with a detailed map of the genome.

An increase in tolerance with age of leaf has been reported in cucumber against the angular leafspot pathogen, Pseudomonas lachrymans (65) and in tomato against X. vesicatori (43). In cucumber it was correlated with a decrease in amino nitrogen and in tomato it was associated with a decrease in total and soluble nitrogen. In bean leaves, Patel and Walker (46) point out that no single amino acid showed a pattern of change with aging that could be associated with high susceptibility of younger leaves and the high tolerance of the older leaves. Their studies, however, did not involve plants in different physiological stages. The present study showed that bean plants were more susceptible to blight infection in the reproductive than in the vegetative stage of growth; some isolates with

a suppressed pathogenic potential at the vegetative stage of plant growth showed enhanced virulence when plants entered the flowering or reproductive phase (Tables 13 and 14). Xp21, Xp22, Xpf28 and Xpf29 were, however, more pathogenic on Manitou when in the vegetative than when in the reproductive stage of growth.

Multiple infections with several isolates each of Xp or Xpf and mixed infection with combined Xp and Xpf isolates have not been reported in literature. This study examined this aspect of infection and the results indicate that several isolates of the same bacterium as well as a misture of isolates of Xp and Xpf can compatibly co-exist and cause disease in the same plant tissue. Mixed infection with Xp and Xpf isolates indicated no biocidal or biostatic effect of one cell type upon another. Both phenotypes were recovered from infected tissue but at varying frequencies on culture media. Symptom-wise, virulence complementation was not observed in multiple infections with isolates of the same Xanthomonas sp. or in infected plants inoculated with isolates of Xp and Xpf. The results, however, did not preclude the possibility that complementary biological functions do exist among isolates; studies on synthetic media may reveal their existence. In mixed inoculations, proportionately fewer of the total population of cells comprised Xp in later infection thus indicating a less saprophytic ability of Xp or a reduced competitive ability in mixed infection with Xpf. The rapid

decline in viable population of Xp might be a result of rapid depletion in synthesis precursor pool more actively competed for and accumulated by Xpf cells.

The recovery of a new phenotype in mixed infections was interesting and noteworthy; it pointed to a possible means for the evolution of new isolates or cell types which may complicate blight-control programs. The sudden appearance of the 'new' type was concomitant with a rapid decline in the total population of Xp and Xpf thus suggesting a higher selective saprophytic advantage for the new phenotype over that of the parental types. The absence of the new type in water-inoculated control plants indicated that the new type was not a normal resident of leaf surface or intercellular leaf spaces.

Comparative studies in field infections with isolates of X. phaseoli and X. phaseoli var. fuscans indicated that the two groups may differ in moisture requirement for infection. Coyne et al. (12) obtained excellent infection in field trials with X. phaseoli and reported rapid spread from inoculated plants to the surrounding bean crop causing severe foliage damage and reduction in yield. In the present study, spread occurred under natural conditions without the introduction of irrigation water, a likely source of injury for intromission of bacteria into succulent bean tissues. The practice of spraying inoculated plants with water at 180 pounds' pressure at two-week intervals (12) may account for the frequently reported spread of disease

from a local primary source of inoculum to healthy surrounding plants. Under natural conditions of rain and insect damage (notably from members of the Order Orthoptera, Coleoptera and Homoptera), personal observations showed little spread from inoculated spreader rows to adjacent tester rows, 28 inches apart. However, such spread appeared more efficient and common with isolates of Xpf than with Xp isolates; the observed differences in the degree of secondary spread had a functional relationship with the amount of primary infection on the inoculated spreader rows. The yield losses accompanying infection with most isolates of Xpf were higher than those caused by isolates of Xp. The unusually hot, dry weather during the growing season could account for the reduced virulence of Xp isolates in field tests as compared to their pathogenic efficiency in greenhouse and growth chamber studies where plants were adequately watered. The observation suggests the importance of water potential or turgor pressure in infection bionomics. Isolates of Xpf appeared to be indifferent to moisture stress and showed no apparent attenuation in their pathogenic potential under the same conditions in the field.

It is a truism that weather determines whether a compatible pathogen-host relationship will develop into disease whereas climate is an important indicator of the probable limits of spread and the destructive potential of the pathogen. Humidity is one of the weather factors

important in bean blight infection. In greenhouse studies where plants were kept well watered, moisture was not important in disease development once the bacteria had been placed in the cellular space. The pooled data from field and greenhouse studies, however, indicate that disease forecasting based exclusively on weather predictions will yield little positive results if the population and composition of the bacterial isolates to be encountered are not included in such studies.

The result of serology studies presented interpretative difficulties but the data generally substantiate isolate differentiation. Isolates shared some antigenic components in common but the different titer end-points and precipitin bands indicated the existence of serotypes among isolates of Xp and Xpf; the serological relationship was closer between isolates within a group than between isolates of Xp and isolates of Xpf. We do not know the primary significance of antigenicity in pathogenesis of blight; the observed variability in antigenic constituents in relation to pathogenic variation among isolates of blight bacteria awaits further verification. Strider (59) and Strider and Lucas (60) demonstrated no serological difference in isolates of Corynebacterium michiganense although these isolates varied in virulence. In Pseudomonas lachrymans Lucas and Grogan (39) reported that little or no difference could be detected in the virulence of smooth and rough isolates although serological variability was reported

among isolates of the same pathogen (38). Guthrie (24) reported no serological differences in Pseudomonas phaseolicola races 1 and 2.

The earlier reports regarding the tolerant or resistant reactions of various bean varieties to Xp and Xpf (Table 2) needs to be re-examined. The present study has shown that some of these varieties, for example, P.I. 207262 and G.N. Tara, are susceptible to certain isolates of both groups of bacteria (Figure 14), thus suggesting the existence of host-specialized pathotypes in Xanthomonas pathogenic on beans. This study emphasizes the importance of using breeding material with different germplasms in crosses aimed at evolving a variety with desirable horizontal resistance. It is equally essential to include representative isolates of a given geographical area in resistance screening programs for effective evaluation.

At the present time, little or no success has been achieved with chemical protectants for controlling bean blights; immediate hope rests with use of disease-free seed under proper cultural practices, but long term hope rests in the development of resistant and tolerant varieties. However, while the outlook for successful exploitation of resistance in control of bacterial blight of beans looks promising and fascinating, the studies required to effect it are rather complex and require new techniques and ingenuity. Further research in this area requires a more thorough understanding of the behavior of blight pathogens

in nature, their existence and distribution, survival, genetics, and population dynamics, both in the presence and absence of the hosts and vectors. The biology and virulence of these pathogens in nature on a world basis constitute a challenging approach for phytopathologists engaged in breeding bean for resistance. This study suggests an urgent need for a comprehensive centralized collection of all reported isolates of Xanthomonas phaseoli and X. phaseoli var. fuscans that would be readily available to all laboratories engaged in bean crop management and bacterial blight studies.

SUMMARY

This study examined the existence of pathogenic variation in Xanthomonas phaseoli (Xp) and X. phaseoli var. fuscans (Xpf), the incitants of common and fuscous blights of beans, respectively. Eight isolates of Xp and seven isolates of Xpf were selected from bean production areas located in different geographical regions, and were maintained at room temperature (25 ± 1 C) on YCA throughout the period of investigation. Pathogenic variation was studied on 13 commercial bean varieties selected on the basis of their reported tolerance or susceptibility to common and fuscous blight pathogens. Different inoculation techniques were examined; inoculation by seed infiltration and multiple needle leaf-puncture methods were less satisisfactory than leaf-incision, leaf water-soaking and excised pod techniques. Inoculum consisted of 48 hour YCA bacterial cultures suspended in sterile distilled water and adjusted to a standard concentration of 2.8×10^7 cells/ml using turbidimetric procedure.

The results of leaf-incision inoculation for pathogenicity tests under growth chamber and greenhouse conditions indicated that disease reactions associated with

individual isolates differed qualitatively and quantitatively. The quantitative differences were based on mean lesion size. Post-inoculation moisture treatment was not necessary for optimum symptom development but did shorten the latent period between inoculation and symptom expression.

Isolates differed in their ability to cause disease at different inoculum levels. Using serial dilutions of inocula, the tested isolates were easily separated into three virulent classes based on their necrotic potential on susceptible Manitou bean. Pathogenic variation among isolates was more evident at low inoculum than at high inoculum concentrations. The data suggest the existence of pathogenic heterogeneity among the compared isolates.

Leaf age and plant age had a marked effect on the virulence of isolates. The most virulent isolates were able to incite disease reactions in both old and young plant tissues; infection with less virulent isolates was restricted primarily to the young, more succulent tissues of the plant. Separation of isolates into virulent groups or strains was more evident on older tissues than on younger tissues where disease reactions tended to be uniform for all tested isolates. The developmental stage of the plant was important in disease development. Depending on the host/isolate combination, some isolates which appeared slightly pathogenic during the vegetative stage of plant growth became more virulent during the reproductive stage. The

disease reactions suggest the existence of pathogenic variation among isolates of Xp and Xpf.

Several isolates of Xp or Xpf and a mixture of isolates of both bacteria co-existed in the same tissue without any evidence of antagonism. In most cases, mixed infection with composite inocula resulted in more severe infection than when individual isolates were used. Mixed infections with composite inocula generated "new" cell phenotypes which were not observed in control plants similarly inoculated with water. The occurrence of the new phenotypes suggests a possible source of new isolates of blight bacteria.

Isolates differed in their ability to cause necrotic reactions in excised bean pods. Some isolates that were not pathogenic on leaf tissues caused measurable lesions on excised pods of the same variety. Statistical analysis indicated significant differences in quantitative virulence of the tested isolates. In the field-grown beans, marked differences were also noted in disease reactons incited by isolates. Statistical comparisons of yield reductions associated with individual isolates separated the isolates into three virulent groups or strains. In general, disease was more severe with isolates of Xpf than with isolates of Xp on both Sanilac and Red Mexican U.I.#3 in field tests. The isolates also showed marked variation in virulence on Vigna unguiculata; Xp23 was highly pathogenic on Purple Hull Southern Pea, but all other isolates were non-pathogenic

on this host. Pathotypes were more evident on Mississippi Silver Pea where three virulent classes were evident.

The population variation with time (colony counts) of Xp21, Xp23, Xp24 and XpU2 in tolerant G.N. Tara bean resembled a typical bacterial growth-curve (Figure 6) with a lag phase, exponential growth phase, stationary, and decline phases of growth. Xp24 and XpU2 incited disease symptoms, but active growth of Xp21 and Xp23 was not accompanied by symptom production. The result indicates that active bacterial multiplication in cell tissue may not necessarily result in disease development. A low inoculum concentration of 10^4 cells/ml resulted in delayed symptom expression in a susceptible Manitou tissue which was readily diseased at 10^7 cells/ml level. Serological variability was observed among isolates of Xp and Xpf, but such variability could not be directly correlated to the observed pathogenic variation among these isolates.

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