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EFFECT OF HOST GENOTYPE ON MULTIPLICATION, DISTRIBUTION AND SURVIVAL OF BEAN COMMON BLIGHT BACTERIA (XANTHOMONAS PHASEOLI)

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has been accepted towards fulfillment
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Alfred W. Saettler
Major professor

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EFFECT OF HOST GENOTYPE ON MULTIPLICATION, DISTRIBUTION AND SURVIVAL OF BEAN COMMON BLIGHT BACTERIA (*XANTHOMONAS PHASEOLI*)

By

Claudio R. Cafati

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ABSTRACT

EFFECT OF HOST GENOTYPE ON MULTIPLICATION, DISTRIBUTION AND SURVIVAL OF BEAN COMMON BLIGHT BACTERIA (*XANTHOMONAS PHASEOLI*)

By

Claudio R. Cafati

Naturally-occurring rifampin-resistant mutants of common blight bacteria (Xanthomonas phaseoli, R15-1) and of fuscous blight bacteria (*X. phaseoli* var *fuscans*, R17) have been used as an antibiotic selective system in epidemiological studies. A wide range of *Phaseolus* germplasm was examined for their reaction to Xp and for their ability to support population build-ups of blight bacteria. Results indicate that most of the germplasm sources being utilized in bean breeding programs throughout the world may be potential "symptomless carriers" of the common blight pathogen.

Multiplication and distribution patterns of Xp (R15-1) in resistant (Tepary beans — *P. acutifolius* - P597 and Arizona-Buff), moderately-resistant (MSU-51319 and G.N. Valley), and common blight susceptible (Seafarer and Tuscola) bean genotypes, were

studied in 1977 and 1978. While bacterial grown patterns were similar in and on leaves of moderately-resistant and susceptible cultivars, maximum bacterial populations were generally lower in the former, particularly during the reproductive stage of plant development. High R15-1 populations were detected in non-inoculated, symptom-free leaves of both susceptible and moderately-resistant genotypes. X. phaseoli populations in and on pods of susceptible and moderately-resistant genotypes were initially similar, but approximately eight days after inoculation, populations on the moderately-resistant genotypes reached stationary phase and most bacteria were epiphytic. In Tepary bean (Arizona-Buff), bacterial populations remained at relatively low levels in both leaves and pods, although detectable levels of R15-1 occurred 20 days after inoculation; however, no disease symptoms developed. In the resistant Tepary genotype, bacteria were detected consistently only in inoculated primary leaves. In moderately-resistant G.N. Valley, bacteria were recovered from primary, first, second and 3rd + 4th trifoliolate leaves and also from stems. In susceptible Tuscola, bacteria were isolated from primary, first, second, 3rd + 4th, 5th and 6th trifoliolate leaves; and from stem and roots. High bacterial populations and symptoms were detected first in the older leaves and later in the younger leaves, from the primary to the 5th trifoliolate leaves in the susceptible, from the primary to the 2nd trifoliolate leaves in the moderately-resistant. No symptoms and very low densities of bacteria were detected in inoculated and 1st trifoliolate leaves of resistant Tepary. Tepary bean continues to

be the best source of blight resistance presently available.

Results of greenhouse and field studies indicate that leaves of susceptible and resistant bean genotypes and non-host plants may support epiphytic multiplication of Xp and that the bacteria may possess a resident phase in its life cycle. Xp and Xpf survived and retained pathogenicity after two years storage in the laboratory in dry infected tissue samples of susceptible and resistant plant species; however, neither isolates were detected in similar samples maintained on or buried in field soil at three different Michigan localities for the period November 1977 to June 1978. It is unlikely that leaf debris plays an important role in between-season survival of these pathogens under environmental conditions present in Michigan.

Pods of resistant and susceptible bean genotypes inoculated by scratching the dorsal suture with the needle of s syringe containing the bacterial suspension developed different disease reactions; however, seeds with and without disease symptoms of both susceptible and resistant genotypes, carried internal blight infection. The data suggest that tests to detect seed borne bacterial blight should be a component in certified, blight-free bean seed production programs of all dry bean cultivars.

Ultrastructural evidence suggests that attachment of Xp occurs in the intercellular spaces of leaves of blight-resistant Tepary bean (Arizona-Buff).

To my wife and daughters

To my parents

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TABLE OF CONTENTS

Page

LIST OF TABLES vi

LIST OF FIGURES ix

GENERAL INTRODUCTION AND LITERATURE REVIEW 1

LITERATURE CITED 11

PART I. PRELIMINARY STUDIES 16

1.1 Isolation rifampin-reistant mutants of Xanthomonas phaseoli (Xp) and Xanthomonas phaseoli var. fuscans (Xpf) 16

1.2 Epiphytic growth of Xp on susceptible and resistant plant tissue 18

1.3 Population trends of Xanthomonas phaseoli (Xp) in bean germplasm as related to disease reaction 21

Literature Cited 35

PART II. EFFECT OF HOST GENOTYPE ON MULTIPLICATION AND DISTRIBUTION OF BEAN COMMON BLIGHT BACTERIA (XANTHOMONAS PHASEOLI) 38

INTRODUCTION 38

MATERIALS AND METHODS 41

Host Genotypes 41
Bacterial Isolate 41
Experimental Plots 42
Inoculation Technique 42
Isolation Procedures 42
Evaluation of Disease Reaction 43
Statistical Analysis 44

RESULTS 45

Multiplication of Xp in and on Leaves and Pods of Resistant, Moderately-Resistant and Susceptible Bean Genotypes 45

Page

Leaf populations, 1977 45
Pod populations, 1977 49
Leaf populations, 1978 56
Pod populations, 1978 60
Multiplication, Movement and Distribution of Xp in Resistant, Moderately-Resistant and Susceptible Bean Genotypes 64

DISCUSSION 68

LITERATURE CITED 74

PART III. SURVIVAL AND TRANSMISSION OF BEAN BLIGHT BACTERIA (XANTHOMONAS PHASEOLI AND X. PHASEOLI VAR. FUSCANS) IN TISSUES OF SUSCEPTIBLE AND RESISTANT PLANT SPECIES 78

INTRODUCTION 78

MATERIALS AND METHODS 82

Bacterial Isolates 82
Inoculation Techniques 82
Determination of Bacterial Populations 83
Survival in Leaf Tissues 83
Secondary Spread 84
Seed Transmission 85
I. Greenhouse study 85
II. Field study 86

RESULTS 88

Multiplication of Xp (R15-1) in Leaves of Beans and Non-Host Species 88
Secondary Spread of Xp from Resistant to Susceptible Bean Genotypes 88
Reciprocal Secondary Spread of Xp Between Susceptible Bean Cultivar to Weeds 91
Survival of Xp (R15-1) and Xpf (R17) in Dry Tissues of Susceptible and Resistant Bean Genotypes and Non-Host Species 91
Survival and Seed Transmission of Xp (R15-1) in Seed of Resistant and Susceptible Bean Genotypes 98

DISCUSSION 107

LITERATURE CITED 111

APPENDIX 114

Preliminary Ultrastructural Evidence for Immobilization of Xanthomonas phaseoli in Tepary Bean (Phaseolus acutifolius) Leaves 114
Tepary bean (resistant) 116
Tuscola bean (susceptible) 116
Literature Cited 124

LIST OF TABLES

Table — Page

PART I. PRELIMINARY STUDIES

1. Relative population levels of Xanthomonas phaseoli (R15-1) on several host and non-host species as determined by leaf-prints on YCA-rifampin media. 20

2. Germplasm used a sources of disease-, insect-, and nematode-resistance by the major Bean Breeding Programs 23

3. Reactions of various disease-, insect-, and nematode-resistant bean germplasm to Xanthomonas phaseoli 26

4. Population trends of Xanthomonas phaseoli (R15-1 mutant) in trifoliolate leaves of resistant (45-1 and 29-BK, Phaseolus coccineus lines) and susceptible (Searfarer, P. vulgaris) bean genotypes 28

5. Population trends of Xanthomonas phaseoli (R15-1 mutant) in trifoliolate leaves of resistant (Tepary bean, P. acutifolius, and PI 207262) and susceptible (Seafarer) bean genotypes 29

6. Population levels of Xanthomonas phaseoli (R15-1 mutant) in plants of resistant (Tepary), moderately-resistant (MSU-51319), and susceptible (Seafarer) bean genotypes 31

7. Recovery of Xanthomonas phaseoli (R15-1 mutant) 21 days after inoculation of primary leaves in resistant and susceptible bean genotypes 33

PART II. EFFECT OF HOST GENOTYPE ON MULTIPLICATION AND DISTRIBUTION OF BEAN COMMON BLIGHT BACTERIA (XANTHOMONAS PHASEOLI)

1. Population trends in Xanthomonas phaseoli (R15-1 mutant) in and on trifoliolate leaves of moderately-resistant (MSU-51319) and susceptible (Seafarer) bean genotypes 48

Table Page

2. Population trends of Xanthomonas phaseoli (R15-1 mutant) in and on trifoliolate leaves of resistant (P597, P. acutifolius) and susceptible (Seafarer) bean genotypes 52

3. Population trends of Xanthomonas phaseoli (R15-1 mutant) in and on pods of moderately-resistant (MSU-51319) and susceptible (Seafarer) bean genotypes 55

4. Population trends of Xanthomonas phaseoli (R15-1 mutant) in and on trifoliolate leaves of highly resistant (Tepary), moderately-resistant (G.N. Valley) and susceptible (Tuscola) bean genotypes 59

5. Population trends of Xanthomonas phaseoli (R15-1 mutant) in and on pods of highly resistant (Tepary Arizona-Buff), moderately-resistant (G.N. Valley) and susceptible (Tuscola) bean genotypes 63

6. Population levels of Xanthomonas phaseoli (R15-1 mutant) in resistant (Tepary Arizona-Buff), moderately-resistant (G.N. Valley) and susceptible (Tuscola) bean genotypes 66

7. Recovery of Xanthomonas phaseoli (R15-1 mutant) from various plant parts following inoculation of the primary leaves of resistant (Tepary), moderately-resistant (G.N. Valley) and susceptible (Tuscola) bean genotypes 67

PART III. SURVIVAL AND TRANSMISSION OF BEAN BLIGHT BACTERIA (XANTHOMONAS PHASEOLI AND X. PHASEOLI VAR. FUSCANS) IN TISSUES OF SUSCEPTIBLE AND RESISTANT PLANT SPECIES

1. Population trends of Xanthomonas phaseoli (R15-1 mutant) in leaves of different plant species 89

2. Secondary spread of Xanthomonas phaseoli (R15-1 mutant) from inoculated resistant (MSU-51319) to non-inoculated susceptible (Tuscola) bean genotypes 90

3. Reciprocal secondary spread of Xanthomonas phaseoli (R15-1 mutant) between susceptible bean genotype (Tuscola) to Chenopodium alba ("lambsquarters") and Amaranthus retroflexus ("pig weed") under field conditions 92

4. Population levels of R15-1 (Xanthomonas phaseoli) and R17 (X. phaseoli var fuscans) in dry leaf tissue of greenhouse-grown plants 93

Table Page

5. Population levels of Rl5-1 (Xanthomonas phaseoli) and Rl7 (X. phaseoli var fuscans) in dry leaf tissue of field-grown bean genotypes 96

6. Recovery of Xpf (Rl7 mutant) from dry infected leaf tissue of different bean genotypes stored at room temperature 97

7. Recovery of Xp (Rl5-1 mutant) from seeds harvested from symptomless pods on plants of three bean genotypes inoculated at different stages of plant development 99

8. Incidence of blight bacteria in seed harvested from greenhouse grown plants inoculated with X. phaseoli . . . 105

9. Incidence of blight bacteria in seed harvested from field grown plants inoculated with X. phaseoli 106

LIST OF FIGURES

Figure — Page

PART I. PRELIMINARY STUDIES

1. Population trends of Xanthomonas phaseoli (R15-1 in and on trifoliolate leaves of moderately-resistant (MSU-51319) and susceptible (Seafarer) bean genotypes. Twenty-three day old plants (3rd and 4th trifoliolate leaves) were inoculated to run-off with a $1.0x10^7$ cells/ml suspension of R15-1 at day 0. Values are average of three replications. 46

2. Population trends of Xanthomonas phaseoli (R15-1 mutant) in and on trifoliolate leaves of resistant (P597, P. acutifolius) and susceptible (Seafarer) bean genotypes. Thirty-six day-old plants (3rd and 4th trifoliolate leaves) were inoculated to run-off with a $1.0x10^7$ cells/ml suspension of R15-1 at day 0. Values are average of three replications. 50

3. Population trends of Xanthomonas phaseoli (R15-1 mutant) in and on pods of moderately-resistant (MSU-51319) and susceptible (Seafarer) bean genotypes. Pods (flat-pod stage) were inoculated by gentle spraying to run-off with a $1.0x10^7$ cells/ml suspension of R15-1 at day 0. Values are average of three replications 53

4. Population trends of Xanthomonas phaseoli (R15-1 mutant) in and on trifoliolate leaves of resistant (Tepary, Arizona-Buff), moderately-resistant (G.N. Valley) and susceptible (Tuscola) bean genotypes. Twenty-five day-old plants (2nd and 3rd trifoliolate leaves) were inoculated to run-off with a $1.0x10^7$ cells/ml suspension of R15-1 at day 0. Values are average of three replications. 57

Figure Page

5. Population trends of Xanthomonas phaseoli (R15-1 mutant) in and on pods of resistant (Tepary, Arizona-Buff), moderately-resistant (G.N. Valley) and susceptible (Tuscola) bean genotypes. Pods (flat-pod stage) were inoculated by gentle spryaing to run-off with a $1.0x10^7$ cell/ml suspension of R15-1 at day 0. Values are average of three replications. 61

PART III. SURVIVAL AND TRANSMISSION OF BEAN BLIGHT BACTERIA (XANTHOMONAS PHASEOLI AND X. PHASEOLI VAR. FUSCANS) IN TISSUES OF SUSCEPTIBLE AND RESISTANT PLANT SPECIES

1. Recovery of R15-1 (Xp) from dry leaf tissue, maintained in field soil or stored under laboratory conditions, on YCA supplemented with 15 µg/ml rifampin (R), 100 µg/ml cycloheximide (C), and 100 µg/ml PCNB (P). Photographs taken after six days incubation at room temperature 95

2. Disease symptoms on mature pods of Tepary (Arizona-Buff), G.N. Valley, and Tuscola bean genotypes. Pods at the flat green stage of development were inoculated by scratching the dorsal suture with a syringe containing $1.0x10^7$ cells/ml of R15-1 (Xp mutant). 100

3. Seed obtained from pods of Tepary (Arizona-Buff), G.N. Valley, and Tuscola bean genotypes, inoculated at the flat green pod stage of plant development by scratching the dorsal suture of the pods with a syringe containing $1.0x10^7$ cells/ml of R15-1 (Xp mutant). 102

APPENDIX

1-5. Transmission electron micrographs of spongy mesophyll cells in resistant Tepary bean (Phaseolus acutifolius) leaves showing the interaction of host cell wall and common blight bacteria (Xanthomonas phaseoli) 119

1. Cross section of bacteria at three hours after inoculation. Note group of bacteria aligned close to the host cell walls. (x 12 000) 119

2. Cross section of bacterium in intercellular space three hours after inoculation. Note loose fibrillar material on the host cell wall in close proximity to the bacterium (arrow). (x 60 000) 119

Page

3,4,5. Cross sections of bacteria attached to host cell wall matrix 18 hours after inoculation. Note matrix attached to bacterial cell (arrows). (Fig. 3 and 5 x 32 000; Fig. 4 x 25 000). 119

6. Cross section of intercellular space 96 hours after inoculation. Structure (arrow) that resembles those typically reported to completely encapsulate bacteria (x 52 000) 119

7-8. Transmission electron micrographs of spongy mesophyll cells in Xanthomonas blight susceptible cv. Tuscola (Phaseolus vulgaris) leaves 120

7. Bacteria in susceptible host 18 hours after inoculation. Note large number of bacterial cells (x 16 000). 120

8. Bacteria in the lesion area of susceptible host genotype eight days after inoculation (x 3 200) 120

GENERAL INTRODUCTION AND LITERATURE REVIEW

Common and fuscous blight caused by Xanthomonas phaseoli (E.F. Smith) Dowson (Xp) and X. phaseoli var. fuscans (Burk.) Starr and Burk (Xpf) respectively, are among the most serious seed-borne bacterial diseases of dry edible and green beans throughout the world.

These bacteria are distributed worldwide and continue to be serious production-limiting factors for dry beans in many areas of Latin America, as well as in the humid Great Lakes regions of the U.S. and Canada (34).

Chemical control measures available at the present time are not entirely satisfactory. Although practical short-term control is possible through the use of disease-free seed grown under irrigation in dry areas, effective long-term control depends upon development of resistant cultivars. Considerable effort has been directed toward finding resistant germplasm useful to breeding (27, 14) and absence of immunity to common and fuscous blights further underscores the role of blight in bean production.

Burkholder (4) was the first to conduct extensive screening of bean germplasm for resistance to common blight; he found that none of the cultivars tested were immune, although some differed in disease severity. In 1946 Burkholder and Bullard (5) reported varietal

susceptibility trials to Xpf; all cultivars tested were susceptible except two, which showed a low level of resistance. Coyne et al (15) tested an extensive collection of plant introductions of Phaseolus species and varieties and breeding lines of Phaseolus vulgaris to natural field infections in Nebraska and subsequently rescreened apparently tolerant selections in the greenhouse. Twelve PI accessions were highly tolerant under both tests, although none were fully immune; those that were highly tolerant were also of late maturity.

Major programs of breeding Xanthomonas resistance into dry edible bean types were not initiated until 10-15 years ago. Programs of this type are located at the University of Nebraska, Michigan State University-USDA, Puerto Rico-USDA, Canada, CIAT (Colombia) and Cornell University.

It has long been recognized that certain accessions of Tepary bean (Phaseolus acutifolius) are resistant to bacterial blight (27), but the interspecific cross can only be made with P. vulgaris as the female and by employing embryo culture. In early attempts four fertile F_1 plants were obtained by Honma (25); F_3 bulk populations from the F_2 plants tested by inoculation showed a normal distribution of reaction grades. Great Northern Nebraska No. 1 originated from one of these families.

Great Northern Nebraska No. 1 has been recognized as possessing good resistance to blight bacteria and a selection made within it, sel. 27 (27) has been used in breeding improved Great Northern varieties Tara (8), Jules (9), and Valley (11). The inheritance of

the disease reaction to X. phaseoli has been reported by several workers. In crosses between Great Northern 1140 and Great Northern Nebraska No. 1 selection 27, Coyne et al (16), found that the latter contributed several genes for resistance to the hybrid. In advanced self-pollinated or back-cross generations, the pattern of segregation suggested polygenic inheritance for resistance. Pompeu and Crowder (33) reported that resistance was conditioned by several partially dominant genes in crosses between two bligth susceptible and two blight resistant bean cultivars. This character was quantitative and highly heritable.

Coyne and Schuster (13) reported that PI 207262 (Colombia) possesses different genes for resistance to Xp Nebraska isolate than GN Nebraska 1, sel. 27. In crosses between PI 207292 x GN 1140, nearly complete dominance for a tolerant disease reaction and early-flowering (maturity) was observed in the F_1; a bimodal distribution of disease reaction ratings was noted in the F_2 (10). This is the first report of qualitative genetic control of reaction to Xp in Phaseolus vulgaris L.

Two collections of P. coccineus reported as resistant to X. phaseoli according to Coyne et al (15), might also be used as sources of resistance for incorporation in P. vulgaris since the two species are easily intercrossed. Vakili recently reported (46) that scarlet runner beans (Phaseolus coccineus L.) possess higher level of resistance than dry beans (P. vulgaris L.) to various diseases and pests under greenhouse and field conditions. For this reason a breeding scheme was set up to select plants with the widest and

highest levels of resistance to a number of diseases, including bacterial blight.

The presence of pathogenic variation in Xp was initially suggested in 1956 by Smale and Worley (42) who detected pathogenic differences in individual colonies of stock Xp cultures. Definitive evidence that variation is present in Xp isolates from various geographical areas was presented by Schuster et al in Nebraska (39, 40, 41). Ekpo (20) and Saettler and Ekpo (35) confirmed the existence of pathogenic variation in Xp and extended the existence of such variation to Xpf. In a recent study at CIAT (6) greenhouse experiments were done to determine if pathogenic variation of common bacterial blight was due to distinct races or to variations in isolate virulence. Six cultivars with different degrees of resistance or susceptibility were inoculated with isolates from Latin America and the United States. Virulence between isolates and resistance of the cultivars varied, but there was no interaction at the P=0.05 level between isolates and cultivars to imply the presence of races. In a further study, they found that isolates from different locations in the Americas were as virulent as Xp 123 from Colombia. The authors suggested the use of the most highly virulent local isolate of the blight pathogen when evaluating germplasm for resistance.

That bean leaves of different ages are not equally susceptible to Xp infection has been known for some time. Goss (22) reported that as leaf age increased, Xp susceptibility also increased. On the other hand, Patel and Walker (32), noted that the youngest

rather than the oldest leaves were the most susceptible. Both of these studies were made with plants in the vegetative stage of development, however, and did not simulate a natural field situation of Xp disease development.

In the field, Xanthomonas bacterial blights become most visible at or just following the blossom stage, generally, symptoms are observed initially on the lower, older leaves. Secondary spread of the pathogen occurs most rapidly after this time. The importance of evaluating breeding material in various stages of growth was emphasized by Coyne et al (13, 14, 17) who determined that plants of both susceptible and moderately resistant lines were more susceptible to Xp when in a reproductive stage of development. Increased susceptibility to Xp and Xpf when plants are in reproductive as compared to vegetative stage of development was recently reported to be a common phenomenon by Saettler and Ekpo (35).

Even though developmental stage is important when evaluating breeding material for Xanthomonas resistance, previous work on inheritance of Xp tolerance based on inoculation of vegetative plants is not negated (25, 33). In a recent study by Yoshii et al (49), Phaseolus germplasm was screened for field reaction to Colombian isolates of Xp. Two P. acutifolius lines, PI 169932 and Tepary Nebraska Accession No. 10, had the highest degree of resistance because there were no foliage or pod symptoms. None of four thousand P. vulgaris entries tested were free from blight symptoms and foliage reaction was not correlated with pod reaction or growth habit. Coyne and Schuster (12) suggested that bean blight reactions

may be due to a recombination of different genes controlling the response of different plant parts to bacterial infection and emphasized the importance of obtaining resistance in both leaves and pods to this pathogen.

Epiphytic survival and multiplication on surfaces of host and non-host plants has been described for several plant pathogenic bacteria (19, 21, 23, 26, 28, 29, 30, 36, 43). Several workers have shown that symptomless tissue of bean cultivars and lines possessing different levels of resistance (1, 14, 20) or susceptibility (20, 45, 48) can contain detectable levels of Xp and Xpf. The increase of the pathogen in the absence of symptoms may be of epidemiological importance by serving to build up inoculum for secondary spread. It has been suggested that additional research studies be initiated on the epi- and endophytic phases of Xp and Xpf relative to resistant and susceptible tissues (34).

Under natural conditions Xp and Xpf enter the leaves through natural openings such as stomata and hydatodes or through wounds. The bacteria invade the intercellular spaces, causing a gradual dissolution of the middle lamella. Later the cells begin to disintegrate with the formation of bacterial pockets (52). Systemic movement of Xp was first noted by Barlow (2) in 1904 and later studied by Burkholder (3). Histological studies by Zaumeyer (50, 51) indicated that bacteria may enter the stem through the stomata of the hypocotyl end epicotyl through the vascular elements leading from the leaf, and from infected cotyledons. According to Burkholder the behavior of Xp after infecting the vascular system of the host

plant depends upon environmental conditions and on the variety of beans. In many instances, no external lesions or death of the plant parts occur until blossom time, or even after pod set. Burkholder also stated that one of the most important points in the behavior of the blight bacteria is their ability to enter the pods through the vascular system and infect the seeds without causing lesions on the pod surface.

In a recent investigation, Weller (47) studied the multiplication of blight bacteria in field-grown Navy beans by monitoring populations from seedling till the early reproductive stage. All seeding parts of seedlings grown from infected seeds became colonized by blight bacteria immediately after germination. Multiplication of R10 and Ra (Xpf and Xp rifampin-resistant mutants, respectively) was described by a series of growth curves displaced over time, each leaf becoming colonized as it differentiated from the main stem. Maximal bacterial populations and symptoms were detected first in the older leaves and later in the younger leaves. The spread of R10 and Ra was facilitated by rain, bud colonization, and systemic movement. According to the author, the pattern of bacterial multiplication offers an explanation for the late appearance of field symptoms typical of bacterial blights. No study has been conducted on the movement and distribution of blight bacteria relative to tolerant or resistant bean germplasm.

Transport of a bacterial pathogen in seed is an important means of survival and dissemination in time and space. Infection sites of seed-borne bacterial pathogens may be in contaminating

trash, as surface infection (i.e., limited colonization of the seed coat or hilum area) or as a deep-seated infection (colonization of the embryo or other internal structures). It has been demonstrated that the bacterial blight organism is harbored below the seed coat (52); bacteria apparently enter the sutures of the pods from the vascular system of the pedicel and then pass into the funiculus and through the raphe leading into the seed. The micropyle also serves as a point of entry into the seed. The bacteria either remain in the seed coat or pass into the region of the cotyledons during seed germination.

Both, externally and internally infected seed have been mentioned as important sources of primary inoculum and dissemination of blight bacteria (34). For these reasons disease control is based on seed certification programs to maintain clean seed stocks. Copeland et al (7) have described the process of producing certified bean seed from breeder and foundation seed stocks. Certified seed raised from foundation seed is certified only after the crop has been inspected and the resulting seed tested for the presence of bacterial blight organisms. There is no doubt that such programmes have been successful in reducing seed infection by the bacterial pathogens; nevertheless, outbreak of common and fuscous blights persist and some fields are rejected annually for certification.

In a recent study on the ecology of Xp and Xpf in Navy beans, Weller (47) reported that seeds externally infested with blight bacteria were shown to be an important source of primary inocula and 14% of commercial Navy bean seed lots were so contaminated.

Symptomless seed internally bearing Xp and Xpf was identified as potential primary inocula sources and seeds with symptoms were always associated with visibly-infected pods.

Coyne et al (18) reported that Xp infected seed (internal infection) was detected in susceptible varieties but was not detected in tolerant lines. Nevertheless, the authors reported that seed transmission of Pseudomonas phaseolicola has been found in some halo blight tolerant beans and has caused serious problems to seed producers. The possible transmission of common and fucous blight bacteria in seed of resistant bean germplasm is important relative to breeding and certification programs, and emphasizes the need for research in this area.

How the blight organisms overwinter, especially in the northern states where the winters are long and temperatures low, has been variously argued. Zaumeyer (52) stated that there are several plants other than beans that are susceptible to Xp, and in the south, where a succession of crops is grown throughout the year, the perpetuation of the organism by a series of different hosts is not improbable.

Muncie (31) isolated the blight organism from diseased bean stubble that remained in the field over winter. Zaumeyer (50) related observations that lend support to the possible overwintering in the field. Schuster (37, 38) reported overwintering of Xp and Xpf in refuse of beans and in two weeds in Nebraska. On the other hand, there are also reports of non-overwintering of Xp and Xpf. Hedges (24), at Arlington-Virginia, placed lima bean leaves infected with Xp into pots in the fall and kept them buried during the winter. The next

spring the author was unable to obtain any infection on snap beans planted in these pots. Sutton and Wallen (44), reported that Xp and Xpf were not isolated from fields known to be infected with either one or both of the pathogens. In a more recent study, Weller (47) reports that Xpf and Xp isolates were not detected in bean stem and leaf tissue which was buried in, or laid on, field soil during the winters following the 1975, 1976 and 1977 growing seasons.

The overall purpose of this investigation was to study the effect of host genotype on multiplication, movement, distribution and survival of bean blight bacteria. It is hoped that the information obtained from the study will improve our understanding of and provide further insight into the ecology of Xanthomonas blight as related to the host-parasite relationship, and will contribute practical information for bean blight breeding programs.

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PART I

PRELIMINARY STUDIES

1.1. Isolation of rifampin-resistant mutants of Xanthomonas phaseoli (Xp) and Xanthomonas phaseoli var. fuscans (Xpf).

1.2. Epiphytic growth of Xp and Xpf on susceptible and resistant plant tissue.

1.3. Population trends of Xanthomonas phaseoli in bean germplasm as related to disease reaction.

1.1. Isolation of rifampin-resistant mutants of Xanthomonas phaseoli (Xp) and Xanthomonas phaseoli var. fuscans (Xpf).

The lack of efficient culture medium selective for bean blight bacteria and the presence of heterogenous populations of microorganisms (fungi and bacteria) growing saprophytically on the surface of field-grown beans, suggested the possible utility of antibiotic-resistance mutants for the study of plant pathogenic bacteria.

Antibiotic-resistance has been used only relatively recently in the study of diseases caused by plant pathogenic bacteria. Lewis and Goodman (19) used streptomycin resistant Erwinia amylovora to study mode of penetration and movement of fire blight bacteria in apple leaf and stem tissue. Similar types of mutants were used by Gowda and Goodman (10) to permit selective isolation of E. amylovora from shoot, stem and root of apple. Stall and Cook (14) studied the multiplication of Xanthomonas vesicatoria in susceptible and resistant peppers with a streptomycin-resistant mutant of Xanthomonas oryzae for studying pathogen ecology and as a method for detecting the presence of the bacteria in rice seed. Gardner and Kado (9) studied systemic movement of Erwinia rubifaciens in walnuts by the use of a double mutant resistant to rifampicin and neomycin. More recently rifampin resistance has been used in ecological studies of Agrobacterium tumefaciens (1, 21), and Pseudomonas coronofaciens the causal agent of halo blight of rye (4). Wellwer and Saettler (28) reported the usefulness of rifampin mutants as tools for studying Xp and Xpf under field conditions.

The objective of this preliminary study was the development of rifampin-resistant Xp and Xpf to be used in an antibiotic selective system for qualitative and quantitative ecological studies.

Naturally-occurring rifampin-resistant mutants were selected from wild type isolates Xp 15 and Xpf 17 (highly virulent Michigan isolates) by spreading 10^8 cells on plates of YCA (YCA, 10 gm yeast extract, 2.5 gm $CaCO_3$, 15 gm agar of 1000 ml distilled water) supplemented with 50 µg/ml rifampin.

Mutants were first screened based on colony characteristics and growth in culture media (YCA) and standard physiological test for Xanthomonas (6, 28). Also, mutants and wild types were compared serologically using the Ouchterlony technique (14).

Pathogenicity was tested under greenhouse conditions by: 1) injecting a bacterial suspension ($5.0x10^7$ cells/ml) into stems of 12-day-old Seafarer and Manitou bean seedlings and 2) spraying the undersurfaces of the leaves to a watersoaked appearance with a bacterial suspension of $1.0x10^7$ cells/ml.

Two selected mutants one each of Xp and Xpf were also compared with their respective wild types for growth and pathogenicity, by gently spraying the undersurfaces of field-grown navy bean leaves (cultivar Seafarer) with a bacterial suspension of $1.0x10^7$ cells/ml and recording symptom development. The isolates retained their rifampin-resistant phenotypes after repeated subculturing on YCA in the absence of rifampin and retained pathogenicity as tested by inoculations to beans. The isolates were resistant to greater than 250 µg/ml rifampin.

Isolates Rl5-1 (Xp) and Rl7 (Xpf) were identical to the respective wild types in cultural, physiological, serological, and pathological tests and were selected for use in detailed epidemiological studies.

1.2. Epiphytic growth of Xp on susceptible and resistant plant tissue.

A preliminary study was undertaken to determine whether susceptible and resistant leaf tissues are inherently capable of supporting epiphytic growth of bean blight bacteria.

Plants of bacterial blight resistant (Phaseolus acutifolius, P597 - CIAT and Arizona-Buff) and susceptible navy bean cultivars Seafarer and Tuscola (Phaseolus vulgaris); soybean cv. Hark (Glycine max), cowpea cv. Mississippi Silver Pea (Vigna unguiculata), corn WG 4A (Zea mays), sugar beet US-20 (Beta vulgaris), and two common weeds, lambsquarters (Chenopodium alba) and pigweed (Amaranthus retroflexus), were grown in the growth chamber with air temperature maintained at 25 C and 16 hours photoperiod.

To prepare the inocula, Rl5-1 (Xp mutant) was grown at room temperature (24 $\pm$ 1C) for 48 hours, suspended in sterile-distilled water and adjusted turbidimetrically to 2.0×10^8 viable cells/ml. Bacterial suspensions were lightly sprayed by means of a DeVilbiss atomizer to run-off on the lower and upper surfaces of the leaves, without producing watersoaking. Bean plants possessed fully expanded second trifoliolate leaves and weed plants were at the vegetative stage of growth.

Growth of Rl5-1 on leaves was determined at intervals after inoculation using leaf-impression cultures. Direct leaf-prints were made by gently pressing the upper and lower surface of leaves onto plates of YCA supplemented with 50 µg/ml rifampin and 25 µg/ml cycloheximide, for one minute. Bacterial growth was evaluated by estimating the percentage of the leaf-print area covered with bacteria after 72 hours of incubation at room temperature.

High bacterial populations were detected on leaves of susceptible bean cultivars Seafarer and Tuscola, the resistant genotype P597 (Phaseolus acutifolius) and on the leaves of soybean (cultivar Hark), cowpea (cultivar Mississippi Silver pea), sugar beet (US-20) and pigweed, although in the five last species populations tended to decline about 12 days after inoculation (Table 1). On corn (WG-4A) and lambquarters the bacterial population tended to decline on the third day after inoculation, but remained at detectable levels 18 to 21 days after inoculation, respectively; at that time leaves of lambsquarters were almost senescent. Bacteria were detected both, on the upper and lower surfaces of the leaves, although at higher concentrations on the lower surface. Pathogenicity of Rl5-1 isolated 21 days after inoculation, from each of the different materials under study, was tested by inoculating a susceptible bean cultivar. No change in the virulence of the isolate was observed.

The increase of a pathogen in the absence of symptoms in susceptible and resistant tissue may be of epidemiological importance by serving to build up inoculum prior to infection or as a source of

TABLE 1. Relative population levels of *Xanthomonas phaseoli* (R15-1) on several host and non-host species as determined by leaf-prints on YCA-rifampin media[1].

Species	Days after Inoculation								
	1 hour	1	3	6	9	12	15	18	21
Seafarer (*P. vulgaris*)	++++[2]	++++	++++	++++	++++	+++	(+++)*	(+++)	(+++)
Tuscola (*P. vulgaris*)	++++	++++	++++	++++	++++	+++	(+++)	(+++)	(+++)
P597 (*P. acutifolius*)	++++	++++	++++	+++	++	++	++	++	+
Hark (*Glycine max*)	++++	++++	++++	++	++	+	+	+	+
M.S.P. (*Vigna unguiculata*)	++++	++++	++++	+++	+++	++	++	+	+
WG 4A (*Zea mays*)	++++	++	+	+	+	+	+	+	-
US-20 (*Beta vulgaris*)	++++	+++	++	++	++	+	+	+	+
Chenopodium alba	++++	++	+	+	+	+	+	+	+
Amaranthus retroflexus	++++	++++	++++	++	+	+	+	+	+

[1] Leaves were inoculated to run-off with a 2×10^8 cells/ml suspension of R15-1.

[2] ++++ = 75% of the leaf print area covered with bacterial growth after 72 hours of incubation; +++ = 50-75%; ++ = 25-50%; + = 25%. The estimates are the average of three experiments.

* Parenthesis indicates presence of macroscopic disease symptoms.

inoculum for secondary spread and, also could provide pathogen cells which survived unfavorable times. Leben (16) suggested, on the basis of work with Xanthomonas vesicatoria, that pathogenic bacteria possessed a "resident phase" in their life cycle, and this was defined as the capacity for multiplication on the surface parts of healthy tissue. Since then, several studies have confirmed that a number of bacterial plant pathogens possess a resident phase, which may be associated with leaves, buds or flowers of host and non-host plants (5, 8, 11, 13, 15, 23). More recently, Leben (17) proposed to expand the term resident to include all types of associations of microflora with healthy plants, including the surface and interior plants, above and below ground.

The results obtained in this preliminary study suggest that leaves of susceptible and resistant bean genotypes and non-host plants may support epiphytic multiplication of blight bacteria and that the bacteria may possess a resident phase in its life cycle. It remains to be determined to what extent the epiphytic capability of bean blight bacteria is epidemiologically important for the disease under field conditions.

1.3 Population trends of Xanthomonas phaseoli (Xp) in bean germplasm as related to disease reaction.

Because Xanthomonas blight bacteria are capable of colonizing bean plants without the production of symptoms, we decided to examine a wide range of Phaseolus germplasm relative to their reactions to

Xp and as to their ability to support the population build-up of Xp. Germplasm sources selected for study (Table 2) represent important sources of resistance to diseases, insects, and nematodes; such sources are being utilized in most of the major dry bean breeding programs throughout the world (12).

Six experiments were conducted under controlled conditions at the Botany and Plant Pathology Greenhouse, Michigan State University, East Lansing, Michigan, during February through June 1978. Navy bean cultivar Seafarer was used as susceptible check in all the experiments. Plants were grown in a standard soil mixture in 16 cm diameter clay pots and watered alternately as needed with Rapid-Gro (1 teaspoon per 2 liters of water) and tap water. Temperature was maintained at 27 $\pm$ 2 C and daylight was supplemented with 14 hours of fluorescent lighting.

A spontaneous mutant (R15-1) of Xanthomonas phaseoli resistant to 50 ppm rifampin obtained by conventional selective plating methods and found to possess virulence equivalent to the parental wild type (Xp 15, high virulent Michigan isolate) was used in these experiments.

Inoculum was prepared from two day-old YCA (YCA: 10 g yeast extract, 2.5 g calcium carbonate, and 15 g agar per 1000 ml distilled water) cultures incubated at room temperature (24 $\pm$ 1 C). Bacterial cells were washed from plates and suspended in sterile distilled water. Plants were inoculated when the first trifoliolate leaves were fully expanded by gentle spraying from a DeVilbiss sprayer to run-off with a 1.0×10^7 cells/ml bacterial suspension, on the lower and upper surfaces of the leaves with no visible water soaking. This

TABLE 2. Germplasm used as sources of disease-, insect-, and nematode-resistance by the major Bean Breeding Programs.

Germplasm	Institution	Origin	Resistances(s)
Cornell 49-242[1]	Cornell Univ.	Venezuela	Anthracnose (*Collectotrichum lindemuthianum*)
Mexico[1]	MITA-Puerto Rico	Mexico	Rust (*Uromyces phaseoli*)
Ecuador 299[1]	MITA-Puerto Rico	Ecuador	Rust (*Uromyces phaseoli*)
PI 300659[1]	INIA-Chile	Chile	White mold (*Sclerotinia sclerotiorum*)
PI 325596[2]	Univ. of Nebraska	U.S.A.	White mold (*Sclerotinia sclerotiorum*)
PI 203958[1]	Cornell Univ.	Mexico	White mold (*Sclerotinia sclerotiorum*) Root-rot (*Fusarium* spp.)
PI 311212[2]	Cornell Univ.	Mexico	Root-rot (*Fusarium* spp.)
PI 150414[1]	Univ. of Nebraska	El Salvador	Halo blight (*Pseudomonas phaseolicola*)
G.N.N.#1 sel. 27[1]	Univ. of Nebraska	U.S.A.	Bacterial blights (*Xanthomonas phaseoli*, *Pseudomonas phaseolicola*)
PI 207262[1]	CIAT-Colombia	Colombia	Common blight (*Xanthomonas phaseoli*)
Tepary A-Arizona Buff[3]	Bureau of Indian Affairs	U.S.A.	*Xanthomonas* blights
Tepary B-Arizona Buff[3]	Phoenix-Arizona	U.S.A.	*Xanthomonas* blights
PI 165421[2]	Univ. of Nebraska	U.S.A.	Common blight (*Xanthomonas phaseoli*)
41-1[2]	MITA-Puerto Rico	Puerto Rico	Bacterial blights, others
29-BK[2]	MITA-Puerto Rico	Puerto Rico	Bacterial blights, others
Idaho Refugee[1]	Univ. of Idaho	U.S.A.	Bean common mosaic virus, *Empoasca* spp.
ICA-Tui[1]	CIAT-Colombia	Colombia	Leaf hoppers (Empoasca spp.)
PI 165435[1]	USDA-Prosser	Mexico	Root-knot nematode (*Meloidogyne* spp.)
Alabama Pale 1[1]	Univ. of Alabama	U.S.A.	Root-knot nematode (*Meloidogyne* spp.)
PI 313709[1]	Univ. of Alabama	Mexico	Root-knot nematode (*Meloidogyne* spp.)

[1] *Phaseolus vulgaris*; [2] *Phaseolus coccineus*; [3] *Phaseolus acutifolius*.

procedure deposited about 0.021 ml inoculum/cm^2 of leaf area as determined by weighing leaflets of known area before and after inoculation.

Multiplication of Rl5-1 was followed at intervals after inoculation during the vegetative and early reproductive stage of growth of plants, except for P. coccineus lines that did not flower under greenhouse conditions. The number of viable bacterial cells was determined from a sample of ten leaflets, randomly sampled from different inoculated leaves at each assay period, by grinding them with 50 ml of 0.01 M phosphate buffer, pH 7.2, with a mortar and pestle. After appropriate serial dilutions, suspensions were plated on YCA supplemented with 50 µg/ml rifampin and 25 µg/ml cycloheximide; colonies were counted after four days of incubation at room temperature. Populations of blight bacteria are expressed on the basis of number of colony forming units (CFU) per 100 cm^2 leaf tissue (approximate average area of one leaf).

In order to study systemic colonization within different bean genotypes, seedlings with primary leaves fully expanded of resistant Tepary, moderately resistant MSU-51319 and susceptible Seafarer, were inoculated by watersoaking an area of one cm diameter in the center of the lower surfaces with a 5.0×10^7 cells/ml suspension of Rl5-1 mutant. Successive leaves on the main axis were subsequently assayed for the presence of the mutant; samples consisted of 15 leaflets per replication. At the end of the assay period, sections of stems, previously surface sterilized (five minutes in 2.5% NaOCl and rinsed in sterile distilled water), were assayed following the

same procedure described above for leaf samples. Populations of bacteria are expressed on the basis of CFU per gram fresh weight of tissue.

The genotypes were evaluated for disease reaction on a total plant basis according to the following scale: 0.0 = no visible symptoms of the disease; 1.0 = a few blight lesions, 5% leaf infection; 2.0 = 5.-10% leaf infection; 3.0 = 10-20% leaf infection, lesions large and spreading; 4.0 = 25-50% leaf infection, many lesions coalescing; 5.0 = 50-100% leaf infection, numerous plants dead.

Where statistical analysis was performed, the data were transformed to common logarithm, and analyzed as a split-plot design. Genotypes were considered as the whole plot factor and were arranged in a randomized complete block design with three replications. The sub-plot factor was time. Significant differences among treatments were estimated using least significant ranges (L.S.R.) obtained from Tukey's w-procedure (25).

The data presented in Table 3 summarize the results of three experiments with diverse bean germplasm related to multiplication pattern of R15-1 and disease reaction. The results showed that in almost all genotypes, bean blight bacteria multiplied rapidly until eight days after inoculation reaching population levels of 10^7 to 10^8 cells per leaf; the only exceptions were G.N. Nebr. No. 1, sel. 27, PI 165421, and PI 325596 in which relatively lower levels of bacteria were obtained. No disease symptoms were observed at this time in any of the genotypes, even though the leaves contained high

TABLE 3. Reactions of various disease-, insect-, and nematode-resistant bean germplasm to Xanthomonas phaseoli[4].

Germplasm	CFU/100 cm^2 leaf area[2] + Disease Reaction (DR)[3] Days after inoculation							
	1	DR	8	DR	16	DR	24[4]	DR
Cornell 49242	$5.6x10^4$	0.0	$5.1x10^7$	0.0	$4.2x10^8$	0.5	$2.5x10^7$	1.0
Mexico 309	$2.1x10^5$	0.0	$1.0x10^8$	0.0	$5.4x10^8$	1.5	$1.0x10^8$	3.0
Ecuador 299	$4.3x10^4$	0.0	$7.5x10^7$	0.0	$8.1x10^8$	1.0	$1.5x10^6$	1.0
PI 300659	$5.3x10^4$	0.0	$1.1x10^8$	0.0	$6.2x10^8$	0.5	$1.1x10^7$	0.5
PI 325596	$1.3x10^4$	0.0	$6.5x10^6$	0.0	$2.1x10^7$	0.0	$2.5x10^7$	0.2
PI 203958	$5.2x10^4$	0.0	$6.1x10^7$	0.0	$8.3x10^7$	0.2	$1.5x10^7$	0.4
PI 311212	$6.8x10^4$	0.0	$6.4x10^7$	0.0	$1.2x10^8$	0.0	$4.0x10^6$	1.0
PI 150414	$1.0x10^5$	0.0	$6.7x10^7$	0.0	$2.7x10^8$	2.0	$6.9x10^6$	2.0
G.N. Neb. #1, sel. 27	$2.6x10^4$	0.0	$7.9x10^6$	0.0	$1.3x10^7$	0.0	$3.0x10^5$	0.5
PI 165421	$5.6x10^4$	0.0	$4.4x10^6$	0.0	$1.5x10^7$	0.0	$2.3x10^5$	0.1
Idaho Refugee	$2.5x10^4$	0.0	$2.8x10^7$	0.0	$1.3x10^8$	0.8	$7.0x10^7$	1.2
ICA-Tui	$1.2x10^5$	0.0	$6.8x10^7$	0.0	$1.5x10^8$	0.5	$1.7x10^7$	1.0
PI 165435	$3.3x10^4$	0.0	$7.5x10^7$	0.0	$3.9x10^8$	0.3	$3.6x10^7$	0.5
Alabama Pole #1	$5.9x10^4$	0.0	$2.5x10^8$	0.0	$1.5x10^8$	2.2	$3.4x10^7$	2.8
PI 313709	$7.5x10^4$	0.0	$9.5x10^7$	0.0	$1.0x10^8$	0.8	$5.3x10^7$	1.0
Seafarer	$4.3x10^4$	0.0	$1.2x10^8$	0.0	$4.5x10^8$	2.0	$8.4x10^7$	3.0

(1) Plants with the first trifoliolate leaf fully expanded were inoculated to run-off with a $1.0x10^7$ cells/ml suspension of R15-1 (Xp mutant).

(2) Values are average of three replications.

(3) Disease Reaction (DR) were made on a total plant basis according to the scale 0 = no visible symptoms; 1 = a few blight lesions, 5% leaf infection; 2 = 5-10% leaf infection; 3 = 10-20% leaf infection, lesions large and spreading; 4 = 25-50% leaf infection, many lesions coalescing; and 5 = 50-100% leaf infection, most plants dead.

(4) Only inoculated leaves (primary + 1st trifoliolate) were sampled at 1, 8, and 16 days after inoculation; only non-inoculated leaves were sampled at 24 days after inoculation.

population levels of R15-1. Although bacteria continued to multiply slowly, populations tended to reach a stationary phase 16 days after inoculation.

R15-1 was recovered from non-inoculated leaves of all of the germplasm sources, although a few or no visible blight symptoms were present at that time.

Population trends of R15-1 and disease reactions in inoculated trifoliolate leaves of two P. coccineus lines, as compared with susceptible cultivar Seafarer, are presented in Table 4. The analysis of variance of the data indicated significant differences at 1% level for genotype, time, and genotye/time interactions. Bacterial growth patterns were similar in resistant and susceptible leaves until five days after inoculation, although maximal bacterial populations were lower in the former. At this time populations in the resistant genotypes reached stationary phase and at day 15 started to decline. Populations in Seafarer continued to increase, although slowly until 15 days after inoculation, remaining stable at day 20. Statistically there were no significant differences between the two P. coccineus lines in levels of bacterial populations, at any assay period and both were significantly different from Seafarer after the fifth day. Even though relatively high bacterial concentrations were determined in leaves of the P. coccineus lines, no visible disease symptoms were observed throughout the experiment.

Population trends and disease reaction in trifoliolate leaves of resistant Tepary bean and PI 207262, and susceptible Seafarer bean genotypes, are presented in Table 5. The analysis of variance

TABLE 4. Population trends of Xanthomonas phaseoli (R15-1 mutant) in trifoliolate leaves of resistant (45-1 and 29-BK, Phaseolus coccineus lines) and susceptible (Seafarer, P. vulgaris) bean genotypes[1].

Genotype	CFU/100 cm^2 leaf area[2] Days after inoculation									
	1		5		10		15		20	
45-1	1.2×10^5	a*	5.8×10^6	a	2.7×10^7	a	5.5×10^6	a	3.6×10^6	a
D.R.[3]	0.0		0.0		0.0		0.0		0.0	
29-BK	1.1×10^5	a	1.2×10^7	ab	1.1×10^7	a	5.9×10^6	a	2.5×10^6	a
D.R.	0.0		0.0		0.0		0.0		0.0	
Seafarer	9.3×10^5	a	3.0×10^7	b	1.9×10^8	b	2.4×10^8	b	1.4×10^8	b
D.R.	0.0		0.0		0.2		1.0		2.5	

[1] Trifoliolate leaves were inoculated to run-off with a 1×10^7 cells/ml suspension of R15-1 at day 0.

[2] Values are average of three replications.

[3] Disease Reaction, D.R.: for description see Table 3.

* Means in the same column with the same letter are not significantly different at $\alpha = 0.5$ level by Tukey's w-procedure.

TABLE 5. Population trends of Xanthomonas phaseoli (R15-1 mutant) in trifoliolate leaves of resistant (Tepary bean, P. acutifolius, and PI 207262) and susceptible (Seafarer) bean genotypes[1].

Genotype	CFU/100 cm^2 leaf area[2] Days after inoculation									
	1		5		10		15		20	
Tepary A (Arizona-Brown)	6.5×10^4	a*	1.8×10^5	a	1.3×10^5	a	1.2×10^5	a	4.6×10^4	a
D.R.[3]	0.0		0.0		0.0		0.0		0.0	
Tepary B (Arizona-White)	5.5×10^4	a	1.7×10^5	a	1.3×10^5	a	1.1×10^5	a	1.9×10^4	a
D.R.	0.0		0.0		0.0		0.0		0.0	
PI 207262	6.5×10^5	b	1.9×10^6	b	8.0×10^6	b	1.9×10^7	b	8.0×10^7	b
D.R.	0.0		0.0		0.0		0.0		0.2	
Seafarer	1.3×10^5	a	1.8×10^6	b	3.6×10^7	c	2.7×10^8	c	5.5×10^8	c
D.R.	0.0		0.0		0.0		0.5		2.5	

[1] Trifoliolate leaves were inoculated to run-off with a 1×10^7 cells/ml suspension of R15-1 at day 0.

[2] Values are the average of three replications.

[3] Diesease Reaction (D.R.): for description see Table 3.

* Means in the same column with the same letter are not significantly different at $\alpha = 0.5$ level by Tukey's w-procedure.

of the data showed highly significant differences for genotype, time and genotype/time interactions. Differences in bacterial growth patterns in the genotypes were evident early after inoculation. In both of the Tepary lines, A and B, R15-1 populations increased slightly between one and five days, when maximum bacterial concentrations of $1.8x10^5$ and $1.7x10^5$ cells per leaf respectively, were recorded; populations then remained in stationary phase and began to decline 15 days after inoculation. On the contrary, multiplication patterns of R15-1 in leaves of PI 207292 and Seafarer showed a continuous increase throughout the assay periods, although the rate of increase was slower in the former, resulting in maximal bacterial populations at day 20 of $8.0x10^7$ and $5.5x10^8$, respectively. Statistically there were not significant differences between the two Tepary lines in levels of bacterial populations at any assay period; both were statistically different from 207262 and Seafarer throughout the experiment, except at day 1; and PI 207262 from Seafarer since day 10.

At 20 days after inoculation, no bacterial blight symptoms were observed on either of the Tepary beans, and only slight symptoms were observed on PI 207262.

Population levels of R15-1 in leaves of resistant (Tepary), moderaltely-resistant (MSU-51319), and susceptible (Seafarer) bean genotypes, at different assay periods after inoculation of the primary leaves (seedling stage), are presented in Table 6. In Tepary, bacteria were detected only in inoculated primary leaves; in genotypes with intermediate levels of resistance, bacteria were

TABLE 6. Population levels of Xanthomonas phaseoli (R15-1 mutant) in plants of resistant (Tepary), moderately-resistant (MSU-51319), and susceptible (Seafarer) bean genotypes[1].

Genotype	CFU/g of fresh tissue[2] Days after inoculation 1	6	12	18	24
Tepary (Arizona-Buff)					
Primary	3.2×10^5	1.6×10^7	1.5×10^7	7.9×10^6	1.9×10^6
MSU-51319					
Primary	6.0×10^5	1.7×10^8	2.3×10^8	(1.9×10^8)*	(7.6×10^7)
1st Trifoliolate	0.0	1.1×10^4	2.0×10^7	1.7×10^7	(1.4×10^7)
2nd Trifoliolate	0.0	0.0	0.0	0.0	1.9×10^6
3rd Trifoliolate	0.0	0.0	0.0	0.0	0.0
Seafarer					
Primary	7.3×10^5	2.1×10^8	(6.1×10^8)	(4.3×10^8)	(3.7×10^8)
1st Trifoliolate	0.0	8.5×10^4	6.0×10^7	(2.2×10^7)	(6.0×10^5)
2nd Trifoliolate	0.0	0.0	2.3×10^5	5.0×10^6	(1.6×10^6)
3rd Trifoliolate	0.0	0.0	0.0	0.0	1.2×10^5

[1] Primary leaves were inoculated by watersoaking an area of one cm diameter in the center of the leaf with a 5.0×10^7 cells/ml suspension of Xp mutant (R15-1).

[2] Values are average of three replications.

* Parenthesis indicate presence of visible disease symptoms.

recovered from the primary, first and second trifoliolate leaves, as well as from internodes between primary and secondary leaves (Table 7). In the susceptible genotype bacteria were recovered from primary, first, second, and third trifoliolate leaves and from all internodes between the primary and third trifoliolate leaves. No systemic symptoms were recorded in Tepary, and only a brown necrotic reaction that sharply delimited the inoculated area from healthy tissue on the primary leaves was evident. Blight symptoms were present on inoculated primary leaves of MSU-51319 and Seafarer, and systemic symptoms on first, and on first and second trifoliolate leaves respectively.

These studies indicate that most of the germplasm sources being utilized in bean breeding programs throughout the world may be potential "symptomless carriers" of the common blight pathogen Xanthomonas phaseoli. The data confirms previous claims that large populations of bacteria may develop in inoculated leaves of lines and cultivars with intermediate levels of resistance, although different disease reactions develop as compared with susceptible ones (3, 7, 22). Valladares et al (26) and Yoshii et al (29), reported that low populations of blight bacteria can exist in Tepary beans in the absence of visible symptoms, although no quantitative data were given to the observations.

An important finding in these preliminary studies is the fact that Xp can systemically-colonize the uninoculated leaves of all germplasm sources, frequently with the development of little or no visible disease symptoms, and that the systemic movement of the

TABLE 7. Recovery of Xanthomonas phaseoli (R15-1 mutant) 24 days after inoculation of primary leaves in resistant and susceptible bean genotypes [1].

Genotype	Recovery of R15-1 in various plant parts [2]						
	Primary Leaves	Stem 1	1st Trifoliolate	Stem 2	2nd Trifoliolate	Stem 3	3rd Trifoliolate
Tepary Bean	+	-	-	-	-	-	-
MSU-51319	+	+	+	+	+	-	-
Seafarer	+	+	+	+	+	+	+

[1] Plants were inoculated at the seedling stage by watersoaking an area of one cm diameter in the center of the primary leaves with a 5.0×10^7 cells/ml suspension of Xp mutant (R15-1).

[2] Data were taken from five single plant-replications.

bacteria may be affected by the host genotype.

Several studies on phytopathogenic bacteria have indicated that inoculum may be available for dissemination early in the course of the disease even before symptoms are evident. This has been reported for Pseudomonas glycinea in soybeans (18), X. pruni in peach (20), and recently for Xp and Xpf in susceptible navy bean cultivars (27). The results presented here suggest that this may also be the case in blight resistant bean genotypes, that support an epiphytic growth of the bacteria.

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LITERATURE CITED

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PART II

EFFECT OF HOST GENOTYPE ON MULTIPLICATION AND DISTRIBUTION OF BEAN COMMON BLIGHT BACTERIA (*XANTHOMONAS PHASEOLI*)

INTRODUCTION

Because certain phytopathogenic bacteria can multiply not only in their natural homologous hoast but also in quite unrelated heterologous plants, it is difficult to define host range and host specificity. Several workers have studied the population trends of plant pathogenic bacteria in resistant and susceptible tissues. Generally pathogens multiply rapidly to high levels in host plants in which they induce disease, (compatible or homologous relationships); however pathogens multiply at the same rate but attain lower population levels at the stationary phase in non-host plants (incompatible or heterologous relationships) (16).

In an early study, Allington and Chamberlain (1) reported active multiplication of Pseudomonas glycinea and Xanthomonas phaseoli in leaves of resistant bean and soybean varieties. They suggested that ability of some pathogens to live and multiply in resistant hosts, under natural conditions, may influence the dynamics of disease resistance and adaptation of pathogens to new hosts. Stall and Cook (29) related hypersensitivity in pepper to bacterial concentration; the level of Xanthomonas vesicatoria cells associated with necrosis in hypersensitive pepper tissues was lower than that in susceptible tissue. Their observation suggested that

ability of bacteria to grow and multiply is a factor in pathogenicity.

Grosse (8) compared leaf-surface populations of Pseudomonas morsprunorum in two cherry varieties. Populations of bacteria were consistently higher on the leaf surface of the susceptible variety than on the resistant one. Mew and Kennedy (19) reported that race specificity of Pseudomonas glycinea correlates with the resident phase of the bacteria on leaf surfaces of soybean, and P. glycinea populations increased only on susceptible leaves.

The relationship between in vivo bacterial populations and plant pathogenicity was studied by Young (34), who inoculated leaves of Phaseolus vulgaris with pathogenic pseudomonads and non-pathogenic bacteria, and compared rates of multiplication at stationary phase. Multiplication of the homologous pathogen P. phaseolicola suggested that inhibitory factors did not regulate the behavior of the bacteria; heterologous pathogens multiplied at lower rates and to lower stationary phase population levels, and non-pathogens remained in stasis or declined slowly. Similar results were reported by Omer and Wood (21). P. phaseolicola introduced into leaves of bean cultivars multiplied logarithmically for three to five days reaching much higher populations in the susceptible cultivar than in the resistant cultivar, where the bacteria multiplied less rapidly. Bacteria introduced into the cotyledonary node, moved more rapidly upwards than downwards, and moved rapidly and further in susceptible than in resistant bean cultivars.

Few studies have been conducted on the population dynamics of Xanthomonas blight bacteria in susceptible and resistant bean

genotypes. Coyne et al (6) and Ekpo and Saettler (12), reported that different blight isolates multiplied to similar levels in both susceptible and moderately resistant Great Northern bean cultivars, although different disease reactions developed. Coyne et al (6) noted that plants inoculated in the vegetative stage exhibited higher levels of resistance and lower bacterial populations than plants inoculated at the reproductive stage. The importance of studying the reaction of the plants at different stages of development and at different intervals after inoculation, was emphasized.

Almost all of the studies relative to population trends of phytopathogenic bacteria have looked only at multiplication of the organism in and/or on inoculated susceptible and resistant tissues. No comparative study has been conducted on bean blight bacteria relative to the movement and distribution of the pathogen in susceptible and resistant genotypes.

This investigation compares multiplication and distribution patterns of Xanthomonas phaseoli in bean genotypes possessing different levels of disease resistance.

MATERIALS AND METHODS

The experiments were conducted under field conditions at the Botany and Plant Pathology Research Farm, Michigan State University, East Lansing, Michigan, during the summers of 1977 and 1978.

Host Genotypes

Common blight susceptible navy bean cultivars Seafarer and Tuscola; moderately-resistant MSU-51319 (MSU breeding line) and G.N. Valley; and, resistant Tepary beans (Phaseolus acutifolius), P597 (CIAT) and Arizona-Buff, were used throught the studies.

Bacterial Isolate

A spontaneous bacterial mutant resistant to rifampin was isolated from cells of Xanthomonas phaseoli isolate 15 (highly virulent Michigan isolate) growing on YCA (YCA: 10 g yeast extract, 15 g agar and 2.5 g calcium carbonate per 1000 ml glass distilled water), supplemented with 50 µg/ml rifampin. R15-1 mutant was morphologically, pathologically and serologically indistinguishable from wild-type rifampin-sensitive Xp 15. The mutant retained its rifampin-resistant phenotype after repeated subculturing on YCA in the absence of rifampin and retained pathogenicity as tested by host inoculations.

Bacteria were grown on YCA and stored in dry infested tissue at 4 C and in 40% v/v aqueous glycerol at -10 C.

Experimental Plots

Disease-free seeds of the different host genotypes were planted by hand in three-row plots of 3 m length with 50 cm between rows. There were three replications of each treatment in all experiments.

Inoculation Technique

Suspensions of R15-1 were prepared from 48-hour-old cultures on YCA plates by rinsing the bacteria off the agar surface and suspending in sterile distilled water.

Plants were inoculated by gentle spraying the lower and upper surfaces of primary leaves, trifoliolate leaves or pods to runoff with a Knapsack sprayer, using a bacterial suspension containing 1 to 5.0×10^7 cells/ml. Leaves or pods were not watersoaked during inoculation. This procedure deposited about 0.021 ml inoculum/cm^2 of leaf area as determined by weighing leaflets of known area before and after inoculation.

Isolation Procedures

The number of viable bacterial cells (CFU) was determined on samples of 21 inoculated trifoliolate leaves or 21 pods, randomly sampled from each replication at each assay period by lightly shaking the samples in 100 ml of 0.01 M phosphate buffer, pH 7.2 (surface populations) and by homogenizing in the same amount of buffer in a Waring blender (internal populations). After appropriate serial dilutions, suspensions were plated on YCA supplemented with

50 µg/ml rifampin and 25 µg/ml cycloheximide; colonies were counted after four days of incubation at room temperature.

Bacterial multiplication and movement within different bean genotypes, were studied in inoculated seedlings possessing fully expanded primary leaves. Successive leaves on the main axis were subsequently assayed for the presence of the mutant; samples consisted of 15 leaflets per replication. At reproductive stage (well filled-plump pods) five single plants from each replication were taken and assayed for the presence of the mutant in roots and stems, following the same procedure used for leaves and pods; tissues were previously surface sterilized (5 minutes in 2.5% NaOCl) and rinsed in sterile distilled water.

Populations of blight bacteria are expressed on the basis of number of colony forming units (CFU) per 100 cm^2 leaf tissue (approximate average area of one leaf) or per 10 cm^2 pod tissue (approximate average area of one pod).

Evaluation of Disease Reaction

The genotypes were evaluated for disease reactions on both a foliage and on a pod basis. For foliage reactions: 0.0 = no disease; 1.0 = a few blight lesions, 5% leaf infection in the row; 2.0 = 5-10% infection in the row; 3.0 = 10-20% leaf infection, lesions large and spreading; 4.0 = 25-50% leaf infection, many lesions coalescing; 5.0 = 50-100% leaf infection, numerous plants dead.

For pod reactions: 0.0 = no pod lesions observed; 1.0 = <10 pod lesions in the row; 2.0 = >10 pod lesions in the row; 3.0 = >10

pod lesions in the row and visible infection of upper and lower suture. These rating scales have been used by Saettler and Adams (24) in evaluating breeding lines for Xp and Xpf resistance.

Statistical Analysis

The data were transformed to common logarithm, and analyzed as a split-plot design. Genotypes were considered as the whole plot factor and were arranged in a randomized complete block design with three replications. The sub-plot factor was time. Significant differences among treatments were estimated using least significant ranges (L.S.R.) obtained from Tukey's w-procedure (30).

RESULTS

Multiplication of Xp in and on Leaves and Pods of Resistant, Moderately-Resistant and Susceptible Bean Genotypes

Leaf populations, 1977

Population trends of Xp in and on leaves of moderately-resistant MSU-51319 and susceptible Seafarer cultivar, are shown in Figure 1 and Table 1. Population trends resembled a typical bacterial growth-curve with a three-day lag phase; six-day logarithmic or exponential growth phase with a mean generation (or doubling) time of 17.1 and 15.1 hours for MSU-51319 and Seafarer respectively; and, a stationary phase where bacterial populations remained stable or declined slowly (Fig. 1). Surface populations of R15-1 followed a pattern similar to total populations and ranged from 7.6 to 26.1% and 2.6 to 18.7% of the total population in MSU-51319 and Seafarer respectively. Although maximal bacterial populations were lower in the moderately-resistant MSU-51319, primarily during flower stage of development (Table 1), analysis of variance indicated no significant differences for genotype and genotype/time interactions. In both genotypes, symptoms developed during the flower stage of development, about the time when maximum bacterial populations per leaf were obtained. Disease in MSU-51319 developed later and to a

FIGURE 1. Population trends of Xanthomonas phaseoli (R15-1 mutant) in and on trifoliolate leaves of moderately-resistant (MSU-51319) and susceptible (Seafarer) bean genotypes. Twenty-three day old plants (3rd and 4th trifoliolate leaves) were inoculated to run-off with a 1.0×10^7 cells/ml suspension of R15-1 at day 0. Values are average of three replications.

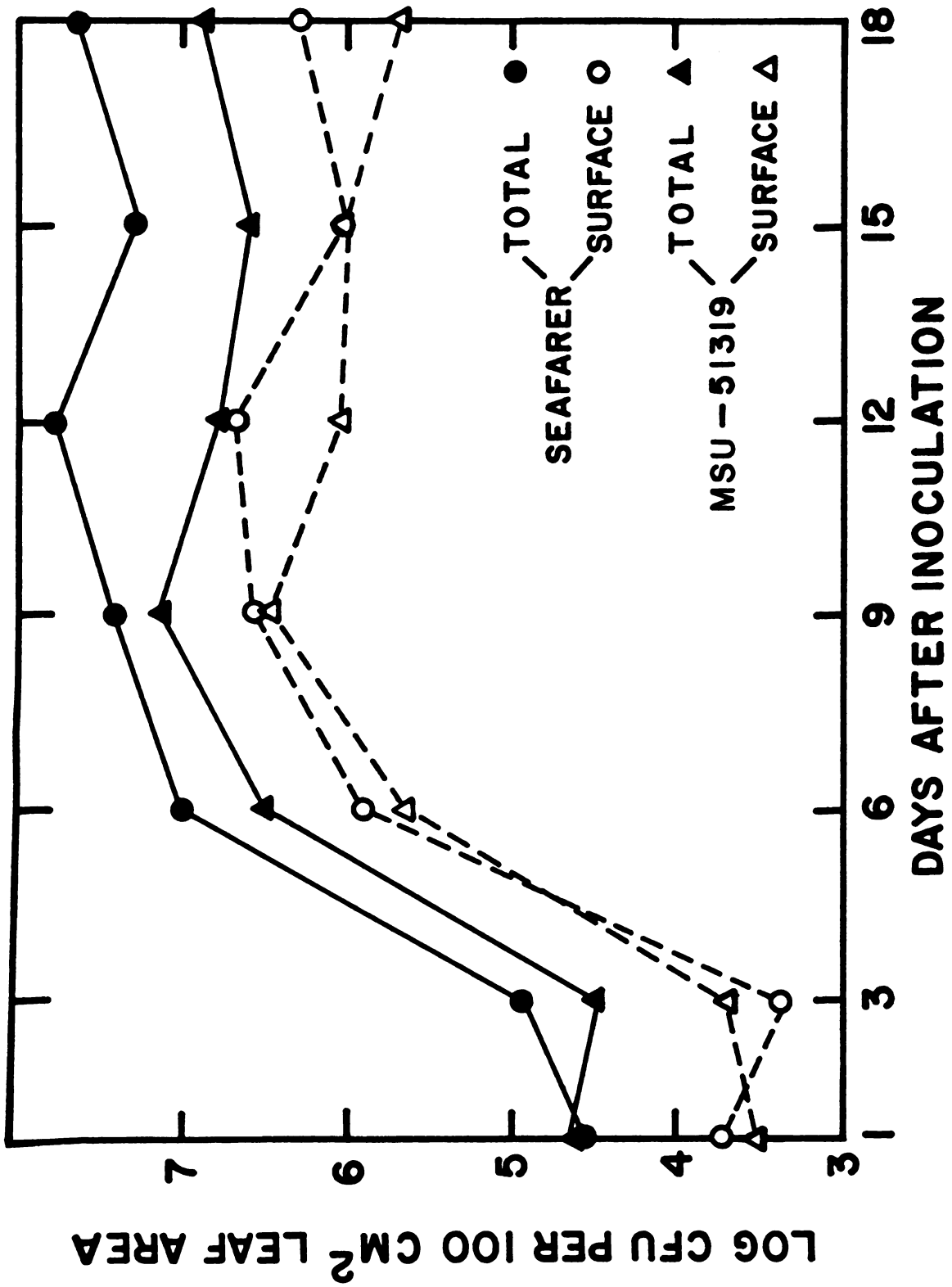
LOG CFU PER 100 CM² LEAF AREA
7
6
5
4
3
1
3
6
9
12
15
18
DAYS AFTER INOCULATION
SEAFARER
TOTAL ●
SURFACE ○
MSU-51319
TOTAL ▲
SURFACE △

TABLE 1. Population trends of Xanthomonas phaseoli (R15-1 mutant) in and on trifoliolate leaves of moderately-resistant (MSU-51319) and susceptible (Seafarer) bean genotypes.

Genotype	CFU/100 cm^2 Leaf Area[1] Days after Inoculation[2] 1	3	6	9	12	15	18
MSU-51319							
Surface	$3.7x10^3$	$4.7x10^3$	$5.2x10^5$	$6.0x10^6$	$1.4x10^6$	$1.2x10^6$	$5.8x10^5$
Total	$4.3x10^4$	$3.1x10^4$	$3.4x10^6$	$2.3x10^7$	$8.3x10^6$	$6.1x10^6$	$7.6x10^6$
S.G.[3]	Vegetative	Vegetative	Vegetative	Bud	Flower	Flower	Flower
D.R.[4]	0.0	0.0	0.0	0.0	0.0	0.0	0.3
Seafarer							
Surface	$5.9x10^3$	$2.3x10^3$	$1.0x10^6$	$5.8x10^6$	$4.7x10^6$	$1.0x10^6$	$2.0x10^6$
Total	$4.0x10^4$	$9.0x10^4$	$1.3x10^7$	$3.1x10^7$	$5.9x10^7$	$1.9x10^7$	$5.0x10^7$
S.G.	Vegetative	Vegetative	Vegetative	Bud	Flower	Flower	Flower
D.R.	0.0	0.0	0.0	0.0	1.0	2.3	3.0

(1) Values are average of three replications.

(2) 23 day-old plants (3rd and 4th trifoliolate leaves) were inoculated to run-off with a $1.0x10^7$ cells/ml suspension of R15-1 at day 0.

(3) S.G. = Stage of Growth.

(4) D.R. = Disease Reaction: 0.0 = No disease; 1.0 = a few blight lesions, 5% leaf infection in the row; 2.0 = 5-10% leaf infection in the row; 3.0 = 10-20% leaf infection, lesions large and spreading; 4.0 = 25-50% leaf infection, many lesions coalescing; 5.0 = 50-100% leaf infection, numerous plants dead.

lesser degree than Seafarer.

Population trends in and on leaves of P597 and Seafarer were similar until 16 to 20 days after inoculation (Fig. 2). Maximum bacterial populations were recorded in the resistant genotype at 16 days after inoculation, populations then gradually declined; in susceptible Seafarer populations continued to increase. There were statistically significant differences in levels of total bacterial populations between genotypes at the end of the assay period, when plants were at the reproductive stage of development (Table 2). Xp populations on leaf surfaces of both genotypes varied throughout the experiment, ranging from 4.3 to 27% of the total population on P597 and from 1.0 to 16.7% on Seafarer. There were no visible disease symptoms on plants of P597.

Pod populations, 1977

Population trends of Xp in and on pods of MSU-51319 and Seafarer are shown in Figure 3 and Table 3. Analysis of variance indicated significant differences for time, genotype and genotype/time interactions. Bacterial growth patterns (Fig. 3) in both genotypes exhibited four-day lag phase, after which the patterns differed. In MSU-51319 Xp exhibited a four-day exponential growth phase with a mean generation time of 23.7 hours; bacterial cell concentrations reached the maximum level at eight days after inoculation, after which there was a stationary phase and a slow decline in populations. In the susceptible genotype, bacteria remained in an exponential growth phase eight days; the bacteria entered a stationary phase at 12 days after inoculation and

FIGURE 2. Population trends of Xanthomonas phaseoli (R15-1 mutant) in and on trifoliolate leaves of resistant (P597, P. acutifolius) and susceptible (Seafarer) bean genotypes. Thirty-six day-old plants (3rd and 4th trifoliolate leaves) were inoculated to run-off with a 1.0×10^7 cells/ml suspension of R15-1 at day 0. Values are average of three replications.

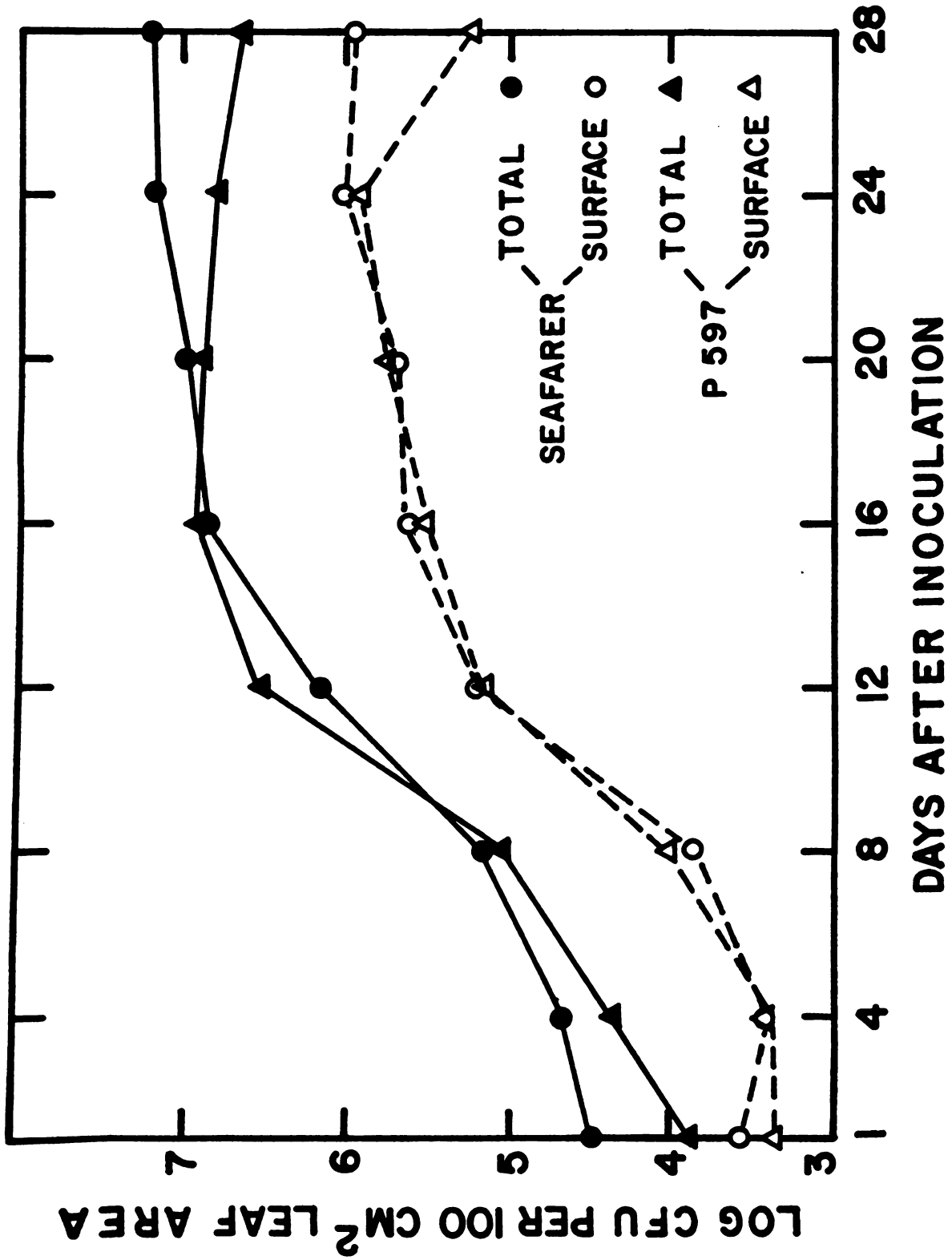
LOG CFU PER 100 CM² LEAF AREA
DAYS AFTER INOCULATION
SEAFARER
TOTAL ●
SURFACE ○
P 597
TOTAL ▲
SURFACE △
3
4
5
6
7
1
4
8
12
16
20
24
28

TABLE 2. Population trends of Xanthomonas phaseoli (R15-1 mutant) in and on trifoliolage leaves of resistant (P597, P. acutifolius) and susceptible (Seafarer) bean genotypes.

Genotype	CFU/100 cm^2 Leaf Area[1] Days after Inoculation[2] 1	4	8	12	16	20	24	28
P597								
Surface	$2.3x10^3$	$3.1x10^3$	$1.2x10^4$	$1.5x10^5$	$5.4x10^5$	$5.4x10^5$	$9.8x10^5$	$1.9x10^5$
Total	$8.5x10^3$ a*	$2.3x10^4$ a	$1.3x10^5$ a	$3.5x10^6$ a	$8.7x10^6$ a	$8.4x10^6$ a	$7.0x10^6$ a	$3.4x10^6$a
S.G.[3]	Vegetative	Vegetative	Vegetative	Vegetative	Bud	Flower	Flat Pod	Pod
D.R.[4]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Seafarer								
Surface	$9.0x10^3$	$3.0x10^3$	$7.2x10^3$	$1.9x10^5$	$5.1x10^5$	$4.7x10^5$	$1.8x10^6$	$2.8x10^6$
Total	$5.4x10^4$ b	$6.0x10^4$ a	$1.6x10^5$ a	$1.8x10^6$ a	$8.7x10^6$ a	$9.7x10^6$ a	$1.6x10^7$ a	$2.5x10^7$b
S.G.	Vegetative	Vegetative	Vegetative	Bud	Flower	Flat Pod	Pod	Pod
D.R.	0.0	0.0	0.0	0.0	1.3	2.3	3.2	3.2

[1] Values are average of three replications.

[2] 36 day-old plants (3rd and 4th trifoliolate leaves) were inoculated to run-off with a $1.0x10^7$ cells/ml suspension of R15-1 at day 0.

[3] S.G. = Stage of Growth.

[4] D.R. = Disease Reaction: for description see Table 1.

*Means in the same column with the same letter are not different at $\alpha = .05$ level by Tukey's w-procedure.

FIGURE 3. Population trends of Xanthomonas phaseoli (R15-1 mutant) in and on pods of moderately-resistant (MSU-51319) and susceptible (Seafarer) bean genotypes. Pods (flat-pod stage) were inoculated by gentle spraying to run-off with a 1.0×10^7 cells/ml suspension of R15-1 at day 0. Values are average of three replications.

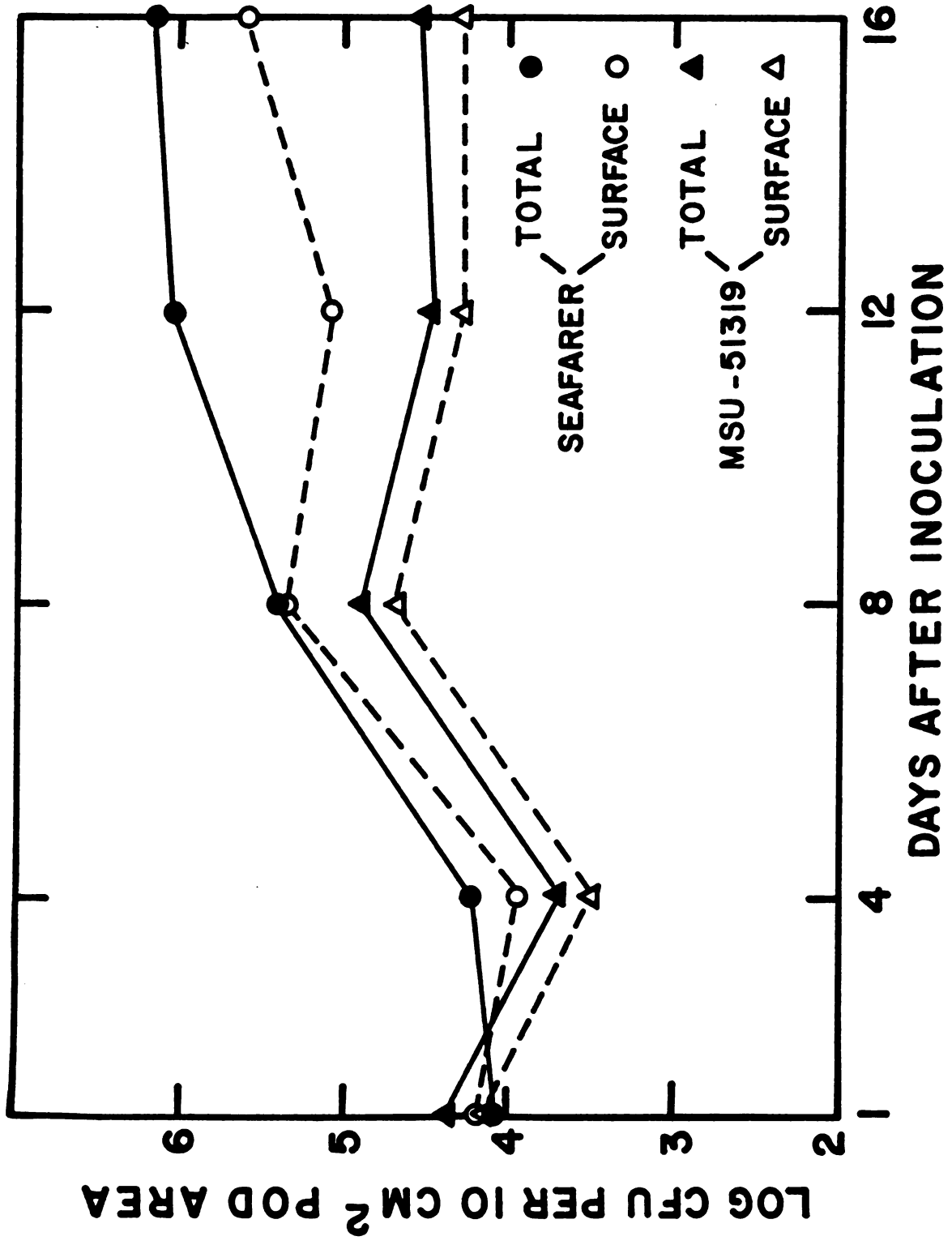
LOG CFU PER 10 CM² POD AREA
2
3
4
5
6
1
4
8
12
16
DAYS AFTER INOCULATION
SEAFARER
TOTAL
SURFACE
MSU-51319
TOTAL
SURFACE

TABLE 3. Population trends of Xanthomonas phaseoli (R15-1 mutant) in and on pods of moderately-resistant (MSU-51319) and susceptible (Seafarer) bean genotype.

Genotype	CFU/10 cm^2 Pod Area[1] Days after Inoculation[2] 1	4	8	12	16
MSU-51319					
Surface	$1.5x10^4$	$3.2x10^3$	$6.0x10^4$	$2.4x10^4$	$3.1x10^4$
Total	$2.5x10^4$ a*	$5.1x10^3$ a	$8.5x10^4$ a	$3.5x10^4$ a	$5.1x10^4$ a
S.G.[3]	Flat green pod	Flat green pod	Plump green pod	Plump green pod	Well-filled plump pod
D.R.[4]	0.0	0.0	0.0	0.0	0.3
Seafarer					
Surface	$1.6x10^4$	$1.2x10^4$	$2.5x10^5$	$1.3x10^5$	$5.9x10^5$
Total	$2.6x10^4$ a	$1.9x10^4$ a	$2.9x10^5$ a	$1.2x10^6$ b	$2.1x10^6$ b
S.G.	Flat green pod	Flat green pod	Plump green pod	Plump green pod	Well-filled plump pod
D.R.	0.0	0.0	0.0	0.0	0.0

[1] Values are average of three replications

[2] Pods (flat pod stage) were inoculated by gentle spraying to run-off with a $1.0x10^7$ cells/ml suspension of R15-1 at day 0.

[3] S.G. = Stage of Growth.

[4] D.R. = Disease Reaction: 0.0 = no pods lesions observed; 1.0 = ≤10 pod lesions in the row; 2.0 = >10 pod lesions in the row; 3.0 = >10 pod lesions in the row and visible infection of upper pod suture.

*Means in the same column with the same letter are not different at α= .05 level by Tukey's w-procedure.

populations continued to increase slowly. There were statistically significant differences in population levels between the genotypes, at 12 and 16 days after inoculation (Table 2). Surface population of Xp on pods of MSU-51319 were similar to the total populations, ranging from 60% to 70% throughout the assay period of the total populations. In susceptible Seafarer, surface populations ranged from 61.5% at day one to 86.2% at day eight and then decreased to 28% of the total population at day 16. Symptoms were first observed in Seafarer at 12 days after inoculation; disease symptoms in MSU-51319 were observed only at the end of the assay period and to a much lower degree (Table 3).

Leaf populations, 1978

Multiplication of Xp in and on leaves of resistant Tepary (Arizona-Buff), moderately-resistant G.N. Valley, and susceptible Tuscola bean genotypes, are shown in Figure 4 and Table 4. Analysis of variance of the data indicated highly significant differences for genotype, time and genotype interactions.

Differences in bacterial growth patterns in the genotypes were evident shortly after inoculation (Fig. 4). In Tepary bean, Xp populations increased slightly during the first days after inoculation and then the populations remained in stationary phase throughout the rest of the experiment. In G.N. Valley bacterial populations showed a four-day lag phase, then increased exponentially for eight days with a mean generation time of 15.2 hours, reached maximum levels at day 12, after which the bacteria entered a stationary phase. No lag phase was evident in Tuscola; bacterial

FIGURE 4. Population trends of Xanthomonas phaseoli (R15-1 mutant) in and on trifoliolate leaves of resistant (Tepary, Arizona-Buff), moderately-resistant (G.N. Valley) and susceptible (Tuscola) bean genotypes. Twenty-five day-old plants (2nd and 3rd trifoliolate leaves) were inoculated to run-off with a 1.0×10^7 cells/ml suspension of R15-1 at day 0. Values are average of three replications

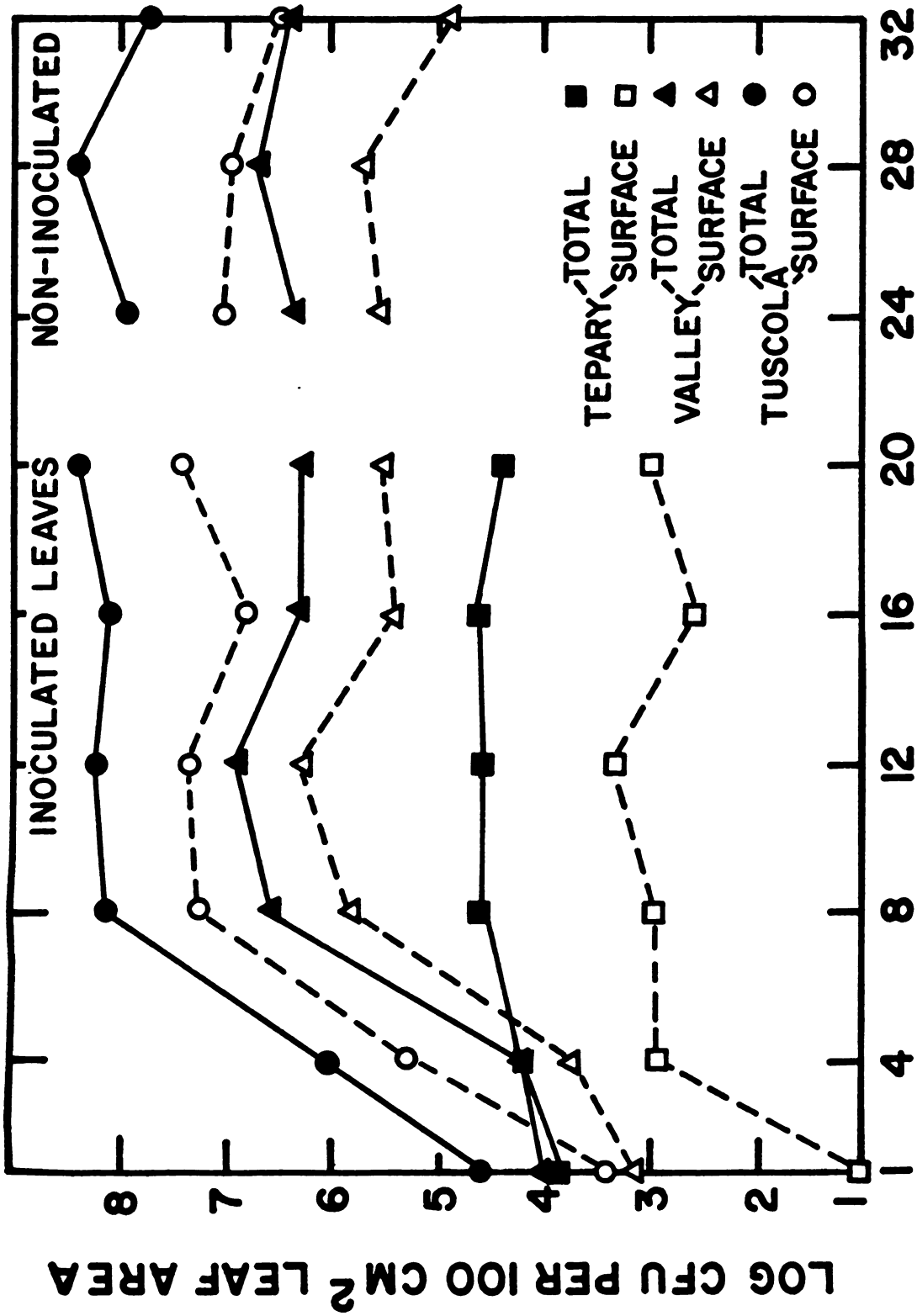
INOCULATED LEAVES
NON-INOCULATED
TEPARY TOTAL
SURFACE
VALLEY TOTAL
SURFACE
TUSCOLA TOTAL
SURFACE
LOG CFU PER 100 CM² LEAF AREA
DAYS AFTER INOCULATION
1
2
3
4
5
6
7
8
4
8
12
16
20
24
28
32

TABLE 4. Population trends of Xanthomonas phaseoli (R15-1 mutant) in and on trifolioate leaves of highly resistant (Tepary), moderately-resistant (G.N. Valley) and susceptible (Tuscola) bean genotypes.

Genotype	CFU/100 cm^2 Leaf Area[1] Days after Inoculation[2]								
	1	4	8	12	16	20	(24)[3]	(24)	(34)
Tepary (Arizona-Buff)									
Surface	$1.0x10^1$	$9.2x10^2$	$1.3x10^3$	$2.8x10^3$	$5.8x10^2$	$2.4x10^3$	$<1.0x10^1$	0.0	0.0
Total	$8.8x10^3$a*	$1.8x10^4$a	$3.9x10^4$a	$4.1x10^4$a	$5.7x10^4$a	$3.2x10^4$a	$<1.0x10^1$	0.0	0.0
S.G.[4]	Veg.	Veg.	Veg.	Veg.	Veg.	Bud	Flower	Flower	Small pod
D.R.[5]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
G. N. Valley									
Surface	$1.5x10^3$	$5.6x10^3$	$6.9x10^5$	$3.2x10^6$	$4.0x10^5$	$3.6x10^5$	$3.9x10^5$	$6.6x10^5$	$1.0x10^5$
Total	$1.0x10^4$ab	$1.9x10^4$a	$3.9x10^6$b	$1.3x10^7$b	$2.6x10^6$b	$2.2x10^6$b	$3.1x10^6$	$6.1x10^6$	$5.1x10^6$
S.G.	Veg.	Veg.	Veg.	Veg.	Bud	Flower	Small pod	Pod	Pod
D.R.	0.0	0.0	0.0	0.0	0.0	0.3	0.5	1.2	1.5
Tuscola									
Surface	$3.2x10^3$	$1.8x10^5$	$1.8x10^7$	$2.2x10^7$	$9.2x10^6$	$2.7x10^7$	$1.1x10^7$	$1.1x10^7$	$4.3x10^6$
Total	$3.7x10^4$b	$1.2x10^6$b	$1.3x10^8$c	$1.7x10^8$c	$1.4x10^8$c	$2.5x10^8$c	$8.6x10^7$	$4.6x10^8$	$6.9x10^7$
S.G.	Veg.	Veg.	Veg.	Veg.	Bud	Flower	Small pod	Pod	Pod
D.R.	0.0	0.0	0.0	0.0	1.2	2.7	3.0	3.5	3.8

[1] Values are average of three replications. [2] 25 day-old plants (2nd and 3rd trifoliolate leaves) were inoculated to run-off with a $1.0x10^7$ cells/ml suspensions of R15-1 at day 0. [3] Indicates tissue samples from non-inoculated leaves. All other samples are tissue samples from inoculated leaves. [4] S.G. = Stage of Growth. [5] D.R. = Disease Reaction: for description see Table 1.

*Means in the same column with the same letter are not different at $\alpha = 0.5$ level by Tukey's w-procedure.

population increased exponentially until eight-days after inoculation, with a mean generation time of 16.4 hours, and then populations attained stationary phase.

There were statistically significant differences in population levels between the genotypes throughout the assay periods, as shown in Table 4. Bacterial populations detected at the surface of the leaves showed growth patterns similar to total populations, ranging from about 1% to 7.5% on Tepary, 15% to 28% on G.N. Valley, and from 6.6% to 15.0% on Tuscola of the total population. Bacterial populations detected in and on uninoculated leaves of G.N. Valley and Tuscola were similar to those previously found in and on inoculated tissue. Practically no bacteria were detected in non-inoculated leaves of Tepary bean. Also, Xp was found only on pod samples of G.N. Valley and Tuscola at 1.0×10^3 and 1.8×10^4 CFU per pod respectively.

Disease symptoms were initially recorded in Tuscola at the early flower stage of plant development; symptoms in G.N. Valley developed later and to a lesser degree, and no symptoms were observed in Tepary (Table 4).

Pod populations, 1978

Population trends of Xp in and on pods of the three genotypes are presented in Figure 5 and Table 5. Analysis of variance indicated significant differences at 1% level for genotype, time, and genotype/time interactions. Bacterial growth patterns were similar in G.N. Valley and Tuscola, although levels of Xp populations were generally lower in the former (Fig. 5). After a four-day lag phase, bacteria

FIGURE 5. Population trends of Xanthomonas phaseoli (R15-1 mutant) in and on pods of resistant (Tepary, Arizona-Buff), moderately-resistant (G.N. Valley) and susceptible (Tuscola) bean genotypes. Pods (flat-pod stage) were inoculated by gentle spraying to run-off with a 1.0×10^7 cells/ml suspension of R15-1 at day 0. Values are average of three replications.

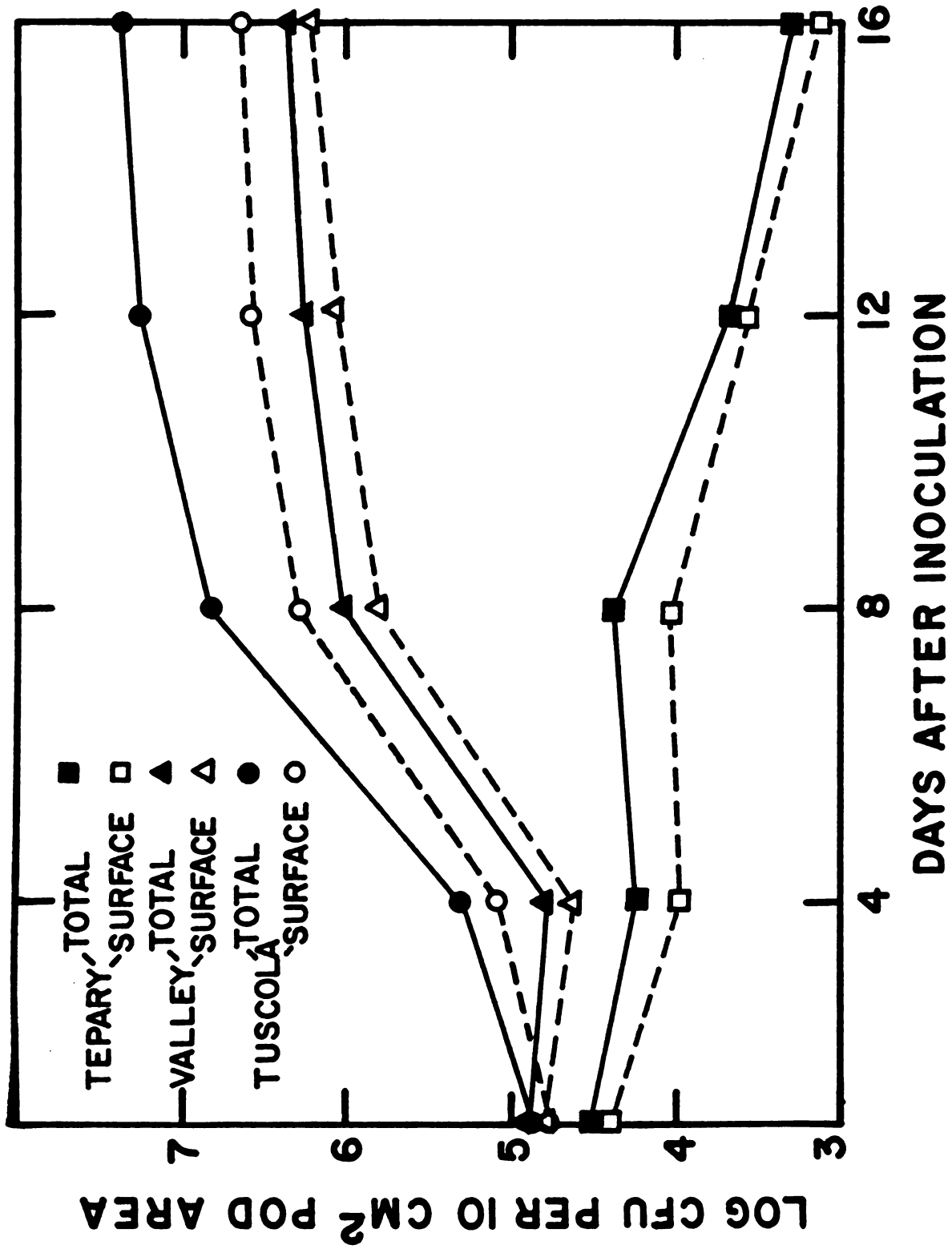
LOG CFU PER 10 CM² POD AREA
7
6
5
4
3
4
8
12
16
DAYS AFTER INOCULATION
TEPARY, TOTAL
TEPARY, SURFACE
VALLEY, TOTAL
VALLEY, SURFACE
TUSCOLA, TOTAL
TUSCOLA, SURFACE

TABLE 5. Population trends of Xanthomonas phaseoli (R15-1 mutant) in and on pods of highly resistant (Tepary Arizona-Buff), moderately-resistant (G.N. Valley) and susceptible (Tuscola) bean genotypes.

Genotype	CFU/10 cm^2 Pod Area[1] Days after Inoculation[2] 1	4	8	12	16
Tepary					
Surface	$2.6x10^4$	$1.5x10^4$	$2.0x10^4$	$4.7x10^3$	$1.5x10^3$
Total	$2.5x10^4$ a*	$2.1x10^4$ a	$2.7x10^4$ a	$5.8x10^3$ a	$2.1x10^3$ a
S.G.[3]	Flat green pod	Flat green pod	Flat green pod	Plump green pod	Well-filled plump pod
D.R.[4]	0.0	0.0	0.0	0.0	0.0
G.N. Valley					
Surface	$7.0x10^4$	$4.2x10^4$	$6.6x10^5$	$1.2x10^6$	$2.0x10^6$
Total	$8.3x10^4$ a	$5.9x10^4$ a	$1.3x10^6$ b	$1.9x10^6$ b	$2.5x10^6$ b
S.G.	Flat green pod	Flat green pod	Plump green pod	Plump green pod	Well-filled plump pod
D.R.	0.0	0.0	0.0	0.0	0.2
Tuscola					
Surface	$6.1x10^4$	$1.2x10^5$	$2.0x10^6$	$4.0x10^6$	$5.1x10^6$
Total	$7.9x10^4$ a	$2.2x10^5$ b	$7.3x10^6$ c	$1.9x10^7$ c	$2.3x10^7$ c
S.G.	Flat green pod	Flat green pod	Plump green pod	Plump green pod	Well-filled plump pod
D.R.	0.0	0.0	0.0	0.5	2.8

[1]Values are average of three replications. [2]Pods (flat pod stage) were inoculated by gentle spraying to run-off with a $1.0x10^7$ cells/ml suspension of R15-1 at day 0. [3]S.G. = Stage of Growth.
[4]D.R. = Disease Reaction: for description see Table 3.
*Means in the same column with the same letter are not different at $\alpha = 0.5$ level by Tukey's w-procedure.

multiplied exponentially for four days with mean generation times of 21.2 hours and 19.0 hours for G.N. Valley and Tuscola respectively. At day eight, Xp populations entered stationary phase in G.N. Valley, but continued to increase slowly in Tuscola. Bacterial population growth in Tepary bean remained in stationary phase, and started to gradually decline eight days after inoculation. There were statistically significant differences in bacterial populations between the genotypes, beginning four days after inoculation (Table 5).

In Tepary and G.N. Valley genotypes leaf surface populations of Xp represented a high percent of the total populations ranging from 71.4% to 81% and 50.8% to 84% respectively. In susceptible Tuscola genotype, leaf surface populations of Xp were high until day four but represent less than 30% of the total population for the remainder of the experimental period.

No Xanthomonas blight symptoms were observed on pods of Tepary bean; only few blight lesions were seen on pods of G.N. Valley at the end of the experiment.

Multiplication, Movement and Distribution of Xp in Resistant, Moderately-Resistant and Susceptible Bean Genotypes

Multiplication and movement of Xp (R15-1 mutant) were studied in inoculated seedlings of the different bean genotypes, throughout the growing season of 1978. Population levels of Xp from primary leaves until early reproductive stage, representing bacterial multiplication on individual leaves as differentiated from the main axis, and symptom

expression, are presented in Table 6. In the resistant Tepary genotype, Xp was detected consistently only in inoculated primary leaves. In moderately-resistant G.N. Valley, bacteria were recovered from primary, first, second and 3rd and 4th trifoliolate leaves. In susceptible Tuscola, bacteria were isolated from primary, first, second, 3rd & 4th, 5th and 6th trifoliolate leaves. High bacterial populations and symptoms were detected first in the older leaves and later in the younger ones, from the primary to the second trifoliolate leaves in G.N. Valley and from the primary to the fifth trifoliolate leaves in Tuscola. No symptoms and low levels of bacteria were detected in inoculated and first trifoliolate leaves in resistant Tepary.

Bacteria systemically colonized stems of G.N. Valley and Tuscola plants but not of Tepary bean (Table 7). Xp populations recovered from mature plants of G.N. Valley and Tuscola were 1.2×10^2 and 6.5×10^4 CFU per g of stem tissue respectively; no visible evidences of infection were observed on stems of either genotypes. Bacteria were recovered only from the roots of Tuscola, although not consistently and at low population levels.

All attempts to isolate Xp from flowers of the different genotypes throughout the growing season were negative. Xp bacteria were recovered only from the pods and seeds with visible symptoms of the Tuscola and G.N. Valley varieties.

TABLE 6. Population levels of Xanthomonas phaseoli (R15-1 mutant) in resistant (Tepary Arizona-Buff), moderately-resistant (G.N. Valley) and susceptible (Tuscola) bean genotypes.

Genotype	CFU/100 cm^2 Leaf Area[1] Days after Inoculation[2]									
	1	4	7	10	14	18	22	26	30	34
Tepary										
Primary leaves	$9.3x10^3$	$3.3x10^5$	$2.8x10^6$	$1.8x10^6$	$3.4x10^6$	$3.1x10^4$	$1.0x10^4$	ab[3]	ab	ab
First trifoliolate	-	-	$1.0x10^1$	$1.0x10^1$	$<1.0x10^1$	$<1.0x10^1$	0.0	0.0	0.0	ab
G.N. Valley										
Primary leaves	$8.1x10^3$	$2.3x10^4$	$1.1x10^6$	$5.8x10^5$	$2.3x10^6$	$(3.0x10^5)$*	$(1.6x10^5)$	ab	ab	ab
First trifoliolate	-	-	$6.4x10^3$	$6.9x10^4$	$1.2x10^6$	$8.7x10^5$	$1.0x10^6$	$7.3x10^6$	$(1.9x10^5)$	ab
2nd trif.	-	-	-	-	$7.8x10^4$	$2.4x10^4$	$1.5x10^6$	$2.0x10^4$	$4.3x10^5$	$(5.3x10^5)$
3rd & 4th trif.	-	-	-	-	-	0.0	0.0	0.0	0.0	$1.3x10^2$
Tuscola										
Primary leaves	$1.5x10^4$	$5.6x10^5$	$4.8x10^6$	$9.0x10^6$	$1.8x10^7$	$(2.6x10^7)$	$(1.8x10^7)$	ab	ab	ab
First trifoliolate	-	-	$6.7x10^5$	$5.9x10^6$	$1.6x10^7$	$2.3x10^7$	$(2.2x10^7)$	$(4.0x10^7)$	$(3.2x10^7)$	ab
2nd trif.	-	-	-	-	$1.1x10^6$	$4.8x10^6$	$1.5x10^6$	$(4.0x10^6)$	$(6.7x10^5)$	$(1.0x10^6)$
3rd & 4th trif.	-	-	-	-	-	$2.1x10^3$	$3.2x10^5$	$1.4x10^5$	$(1.5x10^7)$	$(1.0x10^5)$
5th trif.	-	-	-	-	-	-	$4.7x10^2$	$7.0x10^3$	$1.8x10^4$	$(1.3x10^7)$
6th trif.	-	-	-	-	-	-	-	0.0	$6.7x10^3$	$1.7x10^5$

[1] Values are average of three replications.

[2] 14 day-old plants (seedling stage) were inoculated by gentle spraying the primary leaves to run-off with a $1.0x10^7$ cells/ml suspension of R15-1.

[3] ab = abscised

*Parenthesis indicate presence of macroscopic disease symptoms.

TABLE 7. Recovery of Xanthomonas phaseoli (R15-1 mutant) from various plant parts following inoculation of the primary leaves of resistant (Tepary), moderately-resistant (G.N. Valley) and susceptible (Tuscola) bean genotypes [1].

Genotype	Bacterial Growth on YCA-Rifampin Media[2]										
	Root	Primary leaves	First trif.	2nd trif.	3rd & 4th trif.	5th trif.	6th trif.	Stems[3]	Flowers	Pods[4]	Seeds
Tepary	-	+	+	-	-	-	-	-	-	-	-
G.N. Valley	-	+	+	+	+	-	-	+	-	+	+
Tuscola	+	+	+	+	+	+	+	+	-	+	+

[1] 14 day-old plants (seedling stage) were inoculated by gentle spraying the primary leaves to run-off with a 1.0x10 cells/ml suspension of R15-1.

[2] Data were taken from five single plant-replications.

[3] Bacteria isolated from surface sterilized stems.

[4] Bacteria only recovered from pods and seeds with visible blight symptoms.

DISCUSSION

Multiplication and distribution of Xanthomonas phaseoli (R15-1 rifampin-resistant isolate) in resistant, moderately-resistant and susceptible bean genotypes during vegetative and reproductive stages of growth of the plants, were studied under field conditions during the growing seasons of 1977 and 1978.

In general, growth curves in and on leaves of susceptible and resistant bean genotypes resembled a typical bacterial growth curve, with a three to four-day-lag phase, followed by a logarithmic or exponential phase that varied in the different experiments between four and 12 days, and a stationary phase. Mean generation times during exponential growth ranged from 15.1 to 21.2 hours in leaves and from 23.7 to 36.6 hours in pods. While bacterial growth patterns were similar in and on leaves and pods of moderately-resistant and susceptible genotypes, maximum bacterial populations were generally lower in the former, particularly during the reproductive stage of plant development. At this time, bacterial growth in resistant genotypes showed an abrupt termination of the exponential phase, the population then declined and remained stable. In susceptible genotypes, bacterial growth continued to increase but at a lower rate.

Growth patterns of Xp in tissues of resistant and susceptible genotypes, in general agree with those reported for this (6, 11, 25) and for other phytopathogenic bacteria (4, 8, 10, 16, 19, 29), where pathogen populations increase after inoculation irrespective of the host genotype, but the increases are less in the resistant than in the susceptible tissues.

However bacterial growth patterns in Tepary Arizona-Buff were different in that Xp was able to survive in inoculated tissues for relatively long periods of time and remained at stationary phase or declined only slowly after inoculation. While it has been previously suggested that Xp may grown in Tepary bean (31, 33), this is the first report that shows population trends of the bacteria under field conditions.

Population trends of blight bacteria on the surface of resistant and susceptible bean leaves were similar to total populations and except for Tepary Arizona-Buff, large numbers of bacteria were available for dissemination early in the infection process. The behavior of blight bacterial populations at the surface of pod tissues was noticeable; in resistant genotypes, epiphytic bacteria averaged greater than 65% of the total population throughout the assay period. In blight susceptible genotypes, most of the bacterial populations were internal after 12 days.

Both external and internal factors have been suggested to affect multiplication of bacteria in and on leaves and pod tissues of resistant cultivars (7, 9, 17, 19). On the other hand, independent genetic control of the differential reaction of foliage and pods of

bean to Pseudomonas phaseolicola (14) and to Xanthomonas phaseoli (5, 7) has been reported. The importance of obtaining resistance in both leaves and pods to these pathogens has been emphasized (6).

Our results clearly indicate that even though large populations of Xp grew in and on inoculated leaves and pods of moderately-resistant bean genotypes, the disease reactions on the resistant genotypes were less than those on the susceptible genotypes. The visible appearance of blight symptoms in the genotypes coincided closely with the transition from exponential to stationary phase of growth for the blight bacteria and after a minimal bacterial population was reached; however the incubation period was longer in resistant than in susceptible tissue. High Xp populations were also detected in non-inoculated symptom-free leaves of both blight susceptible and moderately-resistant genotypes, which suggests that Xp may possess a 'resident phase' of growth in both. Such a growth phase suggests that inoculum for secondary spread of Xp may occur in the absence of visible disease symptoms. No visible Xanthomonas blight symptoms were ever found on leaves of pods of Tepary beans during this study, results that agree with previous reports (31, 33).

According to Lyon and Wood (18), after bacteria enter leaves through stomata, the populations may: 1) increase little if at all, and the leaf tissue is apparently not damaged, at least macroscopically; 2) increase, but with no visible damage to the leaves; 3) increase over a short period and then decrease or remain more or less constant. This is associated with death of protoplast in an acute local reaction of tissue containing the bacteria, the

'hypersensitive' reaction; 4) number of bacteria increase over a period considerably longer than in 3) and reach higher levels, visible damage to leaves is delayed but damage is much more extensive, the typical susceptible reaction.

In this study we simulated natural conditions of infection by gently spraying the inoculum to runoff onto leaf surfaces, without wounding or internal soaking of the tissue. Under these conditions, the behavior of the bacteria in Tepary bean would fall into the alternative 2) described by Lyon and Wood. Nevertheless, we also observed in previous greenhouse experiments (Part 1, 3.3), that a characteristic hypersensitive reaction was produced when blight bacteria were infiltrated (watersoaked) into leaf tissue of Tepary bean. Under these circumstances, alternative 3) reflects the interaction.

The behavior of Xp in genotypes with intermediate levels of resistance, suggests an alternative explanation. Even though bacteria increased exponentially over a period and reached high population values, the maximal bacterial populations were lower than in blight susceptible genotypes and disease symptoms were clearly delayed. This interaction appears to be different from the typical susceptible reaction suggested in alternative 4) above.

Little is known about the factor or factors that prevent bacterial multiplication in the absence of visible responses or how bacteria can multiply and not cause visible symptoms. Understandably, pathologists have been more concerned with the susceptible reaction and with the visible damage of the hyper-sensitive reaction associated

with resistance (18). According to Young (34), theories to account for the different behavior of bacteria have particular characteristics which allow them to multiply in plant tissue (23), and those in which preformed inhibitors in the plnat (15) or a post-infection reaction by the plant selectivity inhibits bacterial multiplication (20, 26). More recently, a phenomenon of attachment and envelopment of incompatible and/or saprophytic bacteria by plant cell walls has been reported in several systems (13, 22, 27, 28) and has been suggested as a major host defense mechanism against bacteria (27, 28).

Than Xanthomonas phaseoli may move systemically in infected bean plants was previously reported by Barlow (2), Burkholder (3), and Zaumeyer (35, 36). Burkholder suggested that such systemic movement may be affected by environmental conditions and by the host plant. Recently Weller (32) determined that all above and below ground portions of seedlings grown from internally infected seeds were colonized by the blight bacteria immediately after germination. Spread of the bacteria in the expanded leaf canopy was facilitated by rain, bud colonization, and systemic movement. According to Weller, the overall rate at which bean plants are colonized is strongly affected by the growth rate of the bacterial population on each infected leaf.

Our preliminary greenhouse experiments (Part 1, 3.3), indicated that systemic colonization of bean plants by Xp was affected by host genotype. The present results obtained in field studies further support these previous findings and are consistent with the general pattern of bean blight colonization of susceptible plants as reported

by Weller (32). Patterns of Xp colonization of moderately-resistant genotypes were similar to those in susceptible genotypes, although in the moderately-resistant genotypes the bacteria moved at a slower rate, levels of inoculum for systemic and rain-splashing spread were lower, and the bacteria were recovered closer to the primary site of infection. In the resistant Tepary genotype, bacteria were consistently detected only in the inoculated primary leaves and no colonization occurred beyond this point. Additional studies are necessary to determine the mechanisms involved in Tepary which limit the multiplication and spread of blight bacteria.

The detection of high bacterial populations in moderately-resistant commercial cultivars may be important to bean breeders and seed producers. Hertofore, plant pathologists and breeders have selected for disease resistance on the basis of symptom development and severity. Bean breeding programs directed to the development of Xanthomonas blight resistance should include tests to monitor the leaf, pod, and seed population levels of the pathogen. Tepary bean (Phaseolus acutifolius) continues to be the best source of blight resistance presently available; certain accessions possessed the highest foliage and pod resistance of the germplasm tested to a range of Xanthomonas blight isolates, and also have shown resistance to systemic colonization by the bacteria. Breeding programs should emphasize the transfer of this resistance to Phaseolus vulgaris through interspecific hybridization.

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PART III

SURVIVAL AND TRANSMISSION OF BEAN BLIGHT BACTERIA (*XANTHOMONAS PHASEOLI* AND *X. PHASEOLI* VAR. *FUSCANS*) IN TISSUES OF SUSCEPTIBLE AND RESISTANT PLANT SPECIES

INTRODUCTION

Bacterial survival has been studied extensively in numerous groups, however little information is available relative to survival of plant pathogenic bacteria. A better understanding of the means of survival and mechanism of transmission of phytopathogenic bacteria are important elements in any effort to improve the control of these pathogens.

Although plant pathogenic bacteria are non-sporeforming, many are tolerant to desiccation and survive in a state of reduced metabolism and decreased sensitivity to environmental variables (hypobiosis), for relatively long periods (17). It has been suggested that many bacterial pathogens survive well under some conditions if they are in aggregates and protected by bacterial exudate (ooze), substances commonly found in infected cankers, living or dead plant parts, and in seeds.

Debris from diseased plants have been always considered a possible source for seasonal carryover of the pathogens. Recent studies have also shown that phytopathogenic bacteria can survive in protected positions on healthy leaves of host as well as non-host plants (2, 4, 5, 6, 13, 20, 23). The ability of plant pathogenic bacteria to grow epiphytically on susceptible and resistant plant

tissue may be of epidemiological importance by serving to build up inoculum prior to infection; such growth may provide pathogen cells for dissemination and season-to-season survival (17). Ercolani et al (6) recovered Pseudomonas syringae throughout the year from leaf surfaces of healthy Vicia villosa (hairy vetch), and correlated natural outbreaks of bean brown spot with the epiphytes on non-susceptible hairy vetch. Isaka (12) reported that Xanthomonas oryzae was able to overwinter in association with various weed plants growing in rice fields, and Laub and Stall (15) suggested that X. vesicatoria may be disseminated to weed plants, survive as a resident through the summer period and serve as source of inoculum to tomato and pepper plants. Recently, Latorre and Jones (14) reported Pseudomonas syringae isolated from weeds would infect sour cherry leaves; suggesting weed populations of P. syringae were a source of inoculum for bacterial canker.

Little information is available on the possible role of weed plants in the survival and dissemination of Xp and Xpf. Gardner (7) and Sabet et al (21) suggested that different isolates of Xp may infect a number of weeds under natural conditions, and Schuster (25, 26) reported that Xp overwintered in bean and weed refuses under Nebraska field conditions.

Presence of plant pathogenic bacteria in seeds is an important means of survival and dissemination in time and space; most seed-borne bacteria survive as long as the seed remains viable (27). Seed transmission is the primary means for dissemination of the bean bacterial diseases. Walker and Patel (29), Guthrie et al

(9), and Wallen and Sutton (30) have indicated that low infection levels of Pseudomonas phaseolicola or Xanthomonas phaseoli in bean seeds are capable of initiating heavy field infections and crop losses under epiphytotic conditions. Grogan and Kimble (8) reported that P. phaseolicola was transmitted through bean seeds harvested from a field where the disease was not detected during the growing season.

Internally-infected seed has been mentioned as the main source of primary inoculum in Xanthomonas bean bacterial blights (22, 35). Zaumeyer (33, 35) indicated that X. phaseoli may cause a systemic invasion of the bean plant under certain conditions, and that the bacteria may pass into the developing seed through the vascular system of the plant, without producing visible symptoms. The blight organism may also enter the pod cavity either via stomata of the pod or by breaking through the vascular tissue of the pod suture, the bacteria then pass into the funiculus and the raphe or the micropyle leading into the seed.

It has been mentioned that P. phaseolicola was found in some halo blight resistant beans and has caused serious problems to seed producers (3). Our field experiments (Part II) showed that Xp can multiply to high population levels in and on leaves and pods, and systemically colonize bean genotypes with intermediate levels of resistance. It was therefore desirable to further investigate the possible seed transmission of Xp in resistant bean genotypes.

This study was primarily concerned with: (i) survival and field-overwintering of Xp and Xpf in leaf tissue of susceptible and

resistant bean genotypes and of non-host species; (ii) secondary spread of Xp between blight susceptible and resistant bean cultivars, and weeds; (iii) transmission of Xp in seeds of bean genotypes with different levels of disease resistance.

MATERIAL AND METHODS

This study included experiments conducted under field and greenhouse conditions. Field experiments were done at the Botany and Plant Pathology Research Farm, Michigan State University, East Lansing, Michigan, during the 1977 and 1978 growing seasons. In greenhouse experiments plants were grown at 27 ± 2 C, and in day light supplemented with 14 hours of fluorescent lighting, in a standard soil mixture in 16 cm diameter clay pots and watered alternately as needed with Rapid-Gro (1 teaspoon per 2 liters of water) and tap water.

Bacterial Isolates

Naturally-occurring rifampin-resistant mutants, R15-1 of Xanthomonas phaseoli and R17 of X. phaeoli var. fuscans, were obtained by concentional selective plating methods (18) and found to possess virulence equivalent to the parental wild types (Xp 15 and Xpf 17, Michigan isolates).

Inoculation Techniques

Bacterial cells were washed from plates of two-day-old YCA (YCA: 10 g yeast extract, 2.5 g calcium carbonate, and 15 g agar per 1000 ml distilled water) cutures incubated at room temperature (24 ± 1 C)

and suspended in sterile-distilled water at concentrations of $1x10^7$ to $5x10^7$ cells/ml of Rl5-1 or Rl7. Inoculum was applied to plants in the vegetative stage of development by gentle spraying with a DeVilbiss sprayer (in the greenhouse) or with a Knapsack sprayer (in the field); inoculum was applied to run-off on the lower and upper leaf surfaces or by watersoaking the leaves (24).

Determination of Bacterial Populations

Multiplication and spread of Rl5-1 was monitored at intervals after inoculation. Populations of viable bacterial cells were assayed from ten or 21 randomly sampled leaflets replicated three times, by homogenizing the tissue in 0.01 M phosphate buffer, pH 7.2. After appropriate serial dilutions, suspensions were plated on YCA medium supplemented with 50 µg/ml rifampin adn 25 µg/ml cycloheximide. Colonies were counted after four days incubation at room temperature. Populations of blight bacteria were expressed on the basis of number of colony forming units (CFU) per 100 cm^2 leaf tissue (approximate average area of one leaf), or CFU per gram dry weight of tissue.

Survival in Leaf Tissues

Infected materials for overwinter studies were obtained from infected leaf tissues of bean genotypes grown in the greenhouse and the field. Leaves were harvested 22 days after inoculation and dried at room temperature. Uniform weighed samples of pulverized dried tissue were wrapped in fine mesh nylon bags tied with a nylon string. Initial numbers of viable bacterial cells of Rl5-1 or Rl7 per g of dry tissue were determined at the same time. In November

1977, samples (three replications for each material) were placed outside at three localities: Saginaw, St. Louis and East Lansing (Michigan). Tissue samples were placed: (a) on the soil surface, and (b) 20 cm below soil surface.

Tissue samples were retrieved during June 1978 and returned to the laboratory. To assay for viable blight bacteria, portions of each tissue sample were homogenized in phosphate buffer and after appropriate serial dilutions, suspensions were plated on YCA supplemented with 150 µg/ml rifampin, 100 µg/ml cycloheximide, and 100 µg/ml pentachloronitrobenzene (PCNB). Other portions of the sample were initially incubated in BYE (BYE: 5 g yeast extract in 1000 ml 0.01 M phosphate buffer pH 7.2) supplemented with the above chemicals and subsequently plated on solid media and also infiltrated into bean leaves. Comparison tissue samples were maintained at room temperature and assayed for viable bacteria at six month intervals over a two year period.

Secondary Spread

Alternating ten meter-long rows of MSU-51319 (moderately-resistant) and Tuscola (susceptible) bean genotypes, were planted using commercial planting procedures with 50 cm between the rows. When plants possessed fully expanded second trifoliolate leaves, the rows of MSU-51319 were inoculated by spraying with a suspension of R15-1. In a separate experiment, ten rows of cultivar Tuscola were planted following the above specifications.

When bean plants were at the vegetative stage of growth (second trifoliolate leaf fully expanded), selected plots of the

Tuscola plants and of weed plants (Chenopodium alba and Amaranthus retroflexus), growing between and in the bean rows, were inoculated as above with R15-1.

Multiplication and spread of R15-1, from inoculated MSU-51319 to Tuscola in the first experiment, and from inoculated beans to weeds and from inoculated weeds to bean plants in the second, were monitored at intervals after inoculation. Bacterial populations were determined as described before. R15-1 growth on the leaf surface of weeds was also detected using leaf-impression cultures ("leaf-print") (16) on the rifampin-selective medium.

Seed Transmission

I. Greenhouse study. Xanthomonas resistant Tepary bean Arizona-Buff (Phaseolus acutifolius): moderately-resistant W-117 (USDA, Puerto Rico), and susceptible Seafarer navy bean cultivar, were used in this experiment. When plants were at the flat green stage of development, approximately 50 pods of each genotype were inoculated by scratching along part of the dorsal suture with the needle of a sterile syringe containing 5.0×10^7 cells/ml of R15-1.

At normal maturity the pods were removed and the seeds separated into those without visible symptoms, and those showing some type of visible symptoms of blight infection. Internally-borne blight bacteria were isolated from individual surface-sterilized (3 minutes in 2.5% NaOCl and rinsed twice in sterile-distilled water) seeds, in both, solid (YCA-R: 10 g yeast extract, 2.5 g calcium carbonate, 50 mg rifampin, 25 mg cycloheximide, 15 g agar in 1000 ml distilled water), and liquid (BYE-R: 10 g yeast extract,

50 mg rifampin, 25 mg cycloheximide in 1000 ml .01 M phosphate buffer, pH 7.2) selective media. Individual seeds were first placed hilum down directly on YCA-R and incubated 18 hours at room temperature, and then transferred to 7 ml test tubes containing 3 ml of BYE-R for 48 hours shaker incubation. Bacteria from tubes with turbidity were streaked on YCA-R to confirm the presence of Rl5-1 mutant. In this way, each individual seed was checked for internal blight infection by two methods.

The total number of seeds with visible blight symptoms and symptomless seeds tested for each genotype are presented in Table 8.

II. Field study. The following bean genotypes were selected for field studies on the basis of their reported reactions to bacterial blight: resistant Tepary bean Arizona-Buff, moderately-resistant Great Northern Nebraska #1 selection 27, G.N. Valley, G.N. Jules and MSU-51319, and susceptible Tuscola.

At the flat green stage of plant development, approximately 200 pods of each genotype were inoculated with a bacterial suspension containing 1.0×10^7 cells/ml of Rl5-1, following the same procedures for inoculum preparation and inoculation technique as in the greenhouse study. Pods were collected at normal maturity, and the seeds separated into those with visible symptoms and those without symptoms. Internally-borne bacteria were isolated from individual-surface sterilized seeds using the techniques described previously.

The total number of seeds with visible blight symptoms and symptomless seeds tested for each genotype are presented in Table 9.

In a separate field study plants of Tepary (Arizona-Buff), G.N. Valley, and Tuscola were inoculated by gentle spraying to run-off with a 1.0×10^7 cells/ml suspension of R15-1 at different stages of plant development, viz. seedling stage, third trifoliolate stage, blossom stage, and small flat pod stage. Pods were harvested at normal maturity and internal seed infection assayed by direct plating of seeds on the rifampin-selective medium or after 48 hours incubation in BYE-R.

RESULTS

Multiplication of Xp (R15-1) in Leaves of Beans and Non-Host Species

Growth patterns of Xp after gentle spray inoculation of susceptible (Tuscola), moderately-resistant (W-117) bean genotypes, to non-host species soybean (cv. Hark) and lambsquarters (Chenopodium alba), are shown in Table 1. While bacterial growth patterns were similar in both bean genotypes, maximum bacterial populations were generally lower in the moderately-resistant W-117, particularly during the reproductive stage of plant development; different disease reactions developed on the genotypes. Xp populations remained stable in leaves of soybean until eight days after inoculation; then declined at 12 days and again stabilized until the end of the experiment. Bacterial populations in leaves of lambsquarters remained more or less stable throughout the assay period, although at lower population levels as compared with soybean. No visible disease reaction was observed in leaves of soybean and lambsquarters throughout the experiment.

Secondary Spread of Xp from Resistant to Susceptible Bean Genotypes

Xp was initially detected in non-inoculated leaves of Tuscola plants ten days after the MSU-41319 plants were inoculated (Table 2). Recovery of the bacteria in susceptible Tuscola followed several

TABLE 1. Population trends of Xanthomonas phaseoli (R15-1 mutant) in leaves of different plant species[1].

Genotype	CFU/100 cm^2 Leaf Area[2] Days after Inoculation 1	4	8	12	16	18
W-117 (P. vulgaris)	$3.5x10^6$	$1.1x10^7$	$9.5x10^7$	$1.2x10^8$	$6.0x10^7$	$1.4x10^7$
D.R.	0.0	0.0	0.0	0.0	0.2	0.5
Tuscola (P. vulgaris)	$2.6x10^6$	$5.0x10^7$	$3.1x10^8$	$7.5x10^8$	$1.6x10^8$	$6.5x10^8$
D.R.	0.0	0.0	0.0	0.0	2.2	3.0
Hark (Glycine max)	$3.0x10^5$	$1.5x10^5$	$2.2x10^5$	$5.0x10^4$	$1.4x10^4$	$1.4x10^4$
Lambsquarters (Chenopodium alba)	$6.0x10^4$	$3.3x10^3$	$3.0x10^3$	$3.6x10^3$	$1.4x10^3$	$1.8x10^3$

(1) Leaves of 20 day-old greenhouse-grown plants were inoculated to run-off with a $1.0x10^7$ cells/ml suspension of R15-1 at day 0.

(2) Average of three replications.

(3) D.R. = Disease Reactions were made on a total plant basis according to the scale 0.0 = no diseaase; 1.0 = a few blight lesions, 5% leaf infection in the row; 2.0 = 5-10% leaf infection in the row; 3.0 = 10-20% leaf infection, lesions large and spreading; 4.0 = 25-50% leaf infection, many lesions coalescing; 5.0 = 50-100% leaf infection, numerous plants dead.

TABLE 2. Secondary spread of Xanthomonas phaseoli (R15-1 mutant) from inoculated [1] resistant (MSU-51319) to non-inoculated susceptible (Tuscola) bean genotypes.

Genotype	Replication	CFU/100 cm^2 Leaf Area Days after Inoculation 1	5	10	14	20	26	32
MSU-51319								
(inoculated)	1	$8.2x10^3$	$9.6x10^5$	$1.9x10^6$	$5.3x10^5$	$5.6x10^6$	$1.1x10^6$	$6.5x10^6$
	2	$6.8x10^3$	$4.2x10^5$	$5.7x10^5$	$2.2x10^6$	$6.5x10^5$	$6.4x10^5$	$1.3x10^6$
	3	$8.9x10^3$	$6.8x10^4$	$8.4x10^5$	$7.7x10^5$	$1.6x10^5$	$7.7x10^5$	$1.9x10^5$
	4	$6.0x10^3$	$3.4x10^5$	$6.0x10^5$	$1.2x10^6$	$5.6x10^6$	$4.4x10^5$	$1.4x10^6$
	$\bar{x}$	$7.5x10^3$	$4.5x10^5$	$9.8x10^5$	$1.2x10^6$	$3.0x10^6$	$7.4x10^5$	$8.8x10^5$
D.R. [2]		0.0	0.0	0.0	0.0	0.2	0.3	0.5
Tuscola								
(non-inoculated)	1	0.0	0.0	$1.5x10^3$	$2.0x10^3$	$2.7x10^6$	$5.3x10^5$	$2.4x10^5$
	2	0.0	0.0	$5.7x10^4$	$1.1x10^4$	$7.5x10^4$	$4.0x10^4$	$1.0x10^5$
	3	0.0	0.0	$3.1x10^4$	$1.3x10^6$	$1.1x10^5$	$1.0x10^4$	$1.2x10^6$
	4	0.0	0.0	$2.6x10^2$	$1.2x10^4$	$2.3x10^5$	$1.6x10^4$	$8.0x10^5$
	$\bar{x}$	0.0	0.0	$2.2x10^4$	$3.3x10^5$	$7.8x10^5$	$1.5x10^5$	$5.9x10^5$
D.R.		0.0	0.0	0.0	0.0	0.3	1.5	2.8

[1] 28 day-old plants were gently sprayed (2nd and 3rd trifoliolate leaves) to run-off with a $5.0x10^7$ cells/ml suspension of R15-1 at day 0.

[2] D.R. = Disease Reaction: for description see Table 1.

days of heavy rains. At that time, bacterial populations in the moderately-resistant MSU-51319 were about 10^6 cells per leaf, but no visible disease symptoms were visible. Population trends of Xp in both genotypes followed similar growth patterns, although different disease reactions developed. At the end of the assay period (32 days after inoculation of MSU-51319), Tuscola exhibited typical blight symptoms (10-20% leaf infection in the row, lesions large and spreading), while MSU-51319 showed a few very scattered blight lesions on the lower leaves.

Reciprocal Secondary Spread of Xp Between Susceptible Bean Cultivar to Weeds

Xp multiplied in inoculated leaves of weed and bean plants, although at lower growth rates in the former (Table 3). A substantial proportion of the total Xp population on weeds was detected on leaf surfaces as determined by direct leaf prints on the rifampin-selective medium. Reciprocal secondary spread was first detected after several days of heavy rains, 12 days after inoculation, when Xp had reached exponential growth in both bean and weed leaves, with average population levels of 7.5×10^7 and 1.4×10^6 bacterial cells per leaf, respectively.

Survival of Xp (R15-1) and Xpf (R17) in Dry Tissues of Susceptible and Resistant Bean Genotypes and Non-Host Species

High population levels of Xp and Xpf were recovered 36 days after leaf inoculation on all of the species studied (Table 4). Xp and Xpf isolated from each of the dry tissue samples were

TABLE 3. Reciprocal secondary spread of Xanthomonas phaseoli (R15-1 mutant) between susceptible bean genotype (Tuscola) to Chenopodium alba ("lambsquarters") and Amaranthus retroflexus ("pig weed") under field conditons.

	Replication	CFU/100 cm^2 Leaf Area Days after Inoculation(1)				
		1	6	12	18	24
Tuscola	1	$2.7x10^4$	$1.0x10^6$	($7.8x10^7$)*	($1.1x10^8$)	($5.0x10^7$)
(inoculated)	2	$1.7x10^4$	$9.2x10^5$	($8.4x10^7$)	($1.2x10^8$)	($1.8x10^8$)
	3	$1.6x10^4$	$1.2x10^6$	($9.8x10^7$)	($1.2x10^8$)	($4.6x10^7$)
	4	$1.1x10^4$	$1.3x10^6$	($3.9x10^7$)	($7.5x10^7$)	($7.6x10^7$)
	$\bar{x}$	$1.8x10^4$	$1.1x10^6$	$7.5x10^7$	$1.1x10^8$	$6.8x10^7$
Weeds	1	0.0	0.0	$3.7x10^2$	$2.1x10^5$	$2.5x10^3$
(non-inoculated)	2	0.0	0.0	0.0	$3.1x10^4$	$3.9x10^3$
	3	0.0	0.0	$4.2x10^2$	$7.2x10^4$	$3.7x10^3$
	4	0.0	0.0	$8.4x10^2$	$1.1x10^4$	$1.4x10^3$
	$\bar{x}$	0.0	0.0	$4.1x10^2$	$8.1x10^4$	$2.9x10^3$
Weeds	1	$1.4x10^3$	$7.7x10^4$	$1.2x10^6$	$2.7x10^4$	$7.6x10^4$
(inoculated)	2	$3.4x10^3$	$1.0x10^5$	$1.3x10^6$	$2.5x10^4$	$5.5x10^4$
	3	$6.8x10^2$	$6.9x10^4$	$1.3x10^6$	$3.8x10^4$	$6.3x10^4$
	4	$3.4x10^2$	$1.1x10^5$	$1.6x10^6$	$1.8x10^4$	$4.2x10^4$
	$\bar{x}$	$2.2x10^3$	$8.9x10^4$	$1.4x10^6$	$2.7x10^4$	$5.9x10^4$
Tuscola	1	0.0	0.0	$3.0x10^4$	($1.8x10^7$)	($7.2x10^7$)
(non-inoculated)	2	0.0	0.0	$3.2x10^4$	($1.2x10^7$)	($7.5x10^6$)
	3	0.0	0.0	$3.1x10^4$	($1.1x10^7$)	($2.8x10^7$)
	4	0.0	0.0	$1.7x10^4$	$1.6x10^5$	($2.9x10^7$)
	$\bar{x}$	0.0	0.0	$2.8x10^7$	$1.0x10^7$	($5.1x10^7$)

(1) Plants were inoculated at day 0 by gentle spraying to run-off with a $1.0x10^7$ cells/ml suspension of R15-1.

*Parenthesis indicate presence of macroscopic disease symptoms.

TABLE 4. Population levels of R15-1 (Xanthomonas phaseoli) and R17 (X. phaseoli var. fuscans) in dry leaf tissue of greenhouse-grown plants[1].

Species[2]		CFU/g of Dry Leaf Tissue[3]	
		R15-1	R17
Tepary (Arizona-Buff)	(P. acutifolius)	9.4×10^7	1.2×10^7
P597	(P. acutifolius)	6.4×10^8	1.7×10^7
MSU-51319	(P. vulgaris)	1.4×10^8	6.1×10^7
Tara	(P. vulgaris)	6.8×10^8	5.8×10^7
Seafarer	(P. vulgaris)	6.2×10^8	6.0×10^7
Hark	(Glycine max)	6.0×10^8	6.1×10^7
M.S.P.	(Vigna unguiculata)	2.2×10^7	1.5×10^7
U.S. 20	(Beta vulgaris)	3.2×10^5	1.0×10^4
WG 4A	(Zea mays)	1.2×10^5	3.3×10^3
Lambsquarters	(Chenopodium alba)	2.0×10^6	1.8×10^6
Pig weed	(Amaranthus retroflexus)	2.5×10^6	1.0×10^4
Black nightshade	(Solanum nigrum)	1.2×10^6	7.5×10^3
Rag weed	(Ambrosia artemisiifolia)	3.6×10^4	2.0×10^4
Barnyard grass	(Echinochloa crusgalli)	2.1×10^4	6.7×10^4

[1] 30 to 35 day-old plants were inoculated by water-soaking leaf tissue with a 5×10^7 cells/ml suspension of R15-1 and R17.

[2] Inoculated leaves were harvested 22 days after inoculation and dried at room temperature (22-24 C).

[3] The number of viable bacterial cells (CFU) of R15-1 and R17 was determined in dry leaf tissues after 14 days storage at room temperature.

pathogenic as tested by host inoculations. Weighed samples of these dry leaf tissues and tissues obtained from field-grown plants (Table 5), were utilized to study between-season bacterial survival.

Neither Xp (Rl5-1) nor Xpf (Rl7) was detected in any of the dry leaf tissue samples maintained on or buried in field soil at three different localities from November 1977 to June 1978. Samples were colonized by a heterogenous population of soil microorganisms, predominantly bacteria and fungi, as determined by plating serial dilutions of the samples on YCA medium. These microorganisms were inhibited in YCA supplemented with rifampin, cycloheximide, and PCNB, but allowed the growth of Rl5-1 and Rl7 (rifampin-resistant mutants of Xp and Xpf, respectively) as determined by plating serial dilutions of similar tissue samples stored under laboratory conditions (Fig. 1). Both bacterial isolates have survived and retained pathogenicity after two years storage in the laboratory of dry infected tissue samples of susceptible and resistant bean genotypes. Population levels of Rl7 recovered from these samples tested at six month intervals over a two year period, are shown in Table 6.

Xp was recovered from samples of infected plants left standing in the field following the 1978 growing season in October, November and December 1978, but no bacteria were recovered in January, February, March, and May 1979.

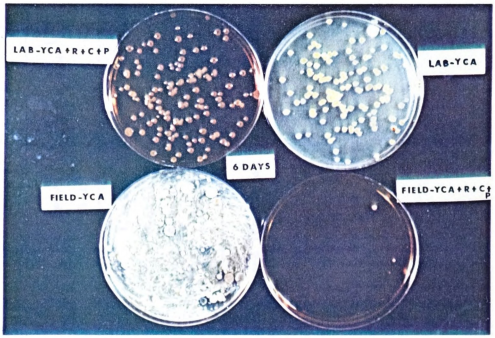


FIGURE 1. Recovery of R15-1 (Xp) from dry leaf tissue, maintained in field soil or stored under laboratory conditions, on YCA supplemented with 15 µg/ml rifampin (R), 100 µg/ml cycloheximide (C), and 100 µg/ml PCNB (P). Photographs taken after six days incubation at room temperature.

TABLE 5. Population levels of R15-1 (Xanthomonas phaseoli) and R17 (X. phaseoli var. fuscans) in dry leaf tissue of field-grown bean genotypes[1].

Genotype[2]	Disease Reaction[4]	CFU/g dry Leaf Tissue[3]	
		R15-1	R17
P597	R	3.3×10^3	3.0×10^3
MSU-51319	MR	8.6×10^3	5.0×10^3
Tara	MR	8.7×10^4	2.3×10^5
Seafarer	S	1.8×10^6	3.2×10^6

[1] 23 day-old plants (3rd and 4th trifoliolate leaves) were inoculated to run-off with a 5.0×10^7 cells/ml suspension of R15-1 (Xp mutant) and R17 (Xpf mutant).

[2] Leaves were harvested when plants possessed well-filled plump pods and dried at room temperature (22-24 C).

[3] The number of viable bacterial cells (CFU) of R15-1 and R17 was determined in dry leaf tissues after 18 days storage at room temperature. Values are average of three replications.

[4] Disease Reaction: R = resistant; MR = moderately resistant; S = susceptible.

TABLE 6. Recovery of Xpf (R17 mutant) from dry infected leaf tissue of different bean genotypes stored at room temperature[1].

Genotype	D.R.[4]	CFU/g Dry Wt of Tissue[2]				
		Initial Bacterial Population	Months after Storage[3] 6	12	18	24
Tepary (Arizona-Buff)	R	1.0×10^5	8.9×10^4	9.3×10^4	9.0×10^4	5.5×10^4
G.N. Nebr. #1, sel. 27	MR	5.5×10^7	3.5×10^7	5.9×10^7	4.0×10^7	5.3×10^7
MSU-51319	MR	4.0×10^7	2.8×10^7	4.6×10^6	3.5×10^6	3.6×10^6
Seafarer	S	2.8×10^7	2.0×10^7	2.6×10^7	1.6×10^7	1.5×10^7

(1)
Trifoliolate leaves of greenhouse-grown plants were inoculated by water-soaking the tissue with a 1.0×10^8 cells/ml suspension of R17. Inoculated leaves were harvested 22 days after inoculation, dried at room temperature (25 $\pm$ 1 C) and the initial bacterial population determined.

(2)
Values are average of four replications.

(3)
Pathogenicity tests were conducted after each storage period.

(4)
D.R. = Disease Reaction: R = resistant; MR = moderately resistant; S = susceptible.

Survival and Seed Transmission of Xp (R15-1) in Seed of Resistant and Susceptible Bean Genotypes

Xp was only recovered from seed samples (seeds with no symptoms) harvested from symptomless pods of field-grown plants inoculated by gentle spraying the bacterial suspension at the small-flat pod stage of plant development (Table 7). G.N. Valley and Tuscola exhibited similar levels of surface bacterial populations but susceptible Tuscola showed the highest incidence of internal seed infection. Relatively few bacteria were externally detected in seed samples from Tepary bean.

When plants of resistant and susceptible bean genotypes were inoculated by scratching the dorsal suture of the pods at the flat green stage with a syringe containing the bacterial suspensions, different disease reactions were observed. At normal maturity, only pods of the susceptible Tuscola exhibited typical Xanthomonas blight symptoms extending beyond the inoculated areas; G.N. Nebr. No. 1 sel. 27, G.N. Jules, G.N. Valley, MSU-51319, and W-117 all showed brown-necrotic reactions and sometimes few and small watersoaked zones around the scratches; only a light-brown necrotic reaction was observed on the inoculated pods of Tepary bean (Figure 2). Seeds with different degrees of Xanthomonas blight symptoms and seeds with no visible symptoms, were harvested from inoculated pods of all the bean genotypes (Figure 3). In both experiments, Xp (R15-1) was recovered from about 40 to 50% of seeds exhibiting any type of visible symptoms of internal blight infection in resistant genotypes, and from about 70% in susceptible bean genotypes. Also, Xp was

TABLE 7. Recovery of Xp (R15-1 mutant) from seeds harvested from symptomless pods on plants of three bean genotypes inoculated at different stages of plant development[1].

Genotype	D.R.[5]	CFU/100 Seeds Stage of Plant Development				
					Small Flat Pods	
		Primary Leaves	3rd Trifoliolate	Flowers	Surface[2]	Internal[3]
Tepary (Arizona-Buff)	R	-[4]	-	-	$<1.0 \times 10^1$	-
G.N. Valley	MR	-	-	-	2.1×10^5	1.6×10^3
Tuscola	S	-	-	-	1.0×10^5	1.2×10^4

[1] Plants were inoculated by gentle spraying to run-off with a 1.0×10^7 cells/ml suspension of R15-1 at the respective stage of plant development.

[2] Population of R15-1 on the surface of seed samples (100 symptomless seeds) was determined by shaking the seed in 0.01 M phosphate buffer (pH 7.2) for one minute, and plating on YCA + rifampin (50 µg/ml) and cycloheximide (25 µg/ml).

[3] Internal population was determined by grinding surface sterilized seeds (3 minutes in 2.5% NaOCl) in 0.01 M phosphate buffer (pH 7.2) and plating on the rifampin-selective media.

[4] No bacterial growth was detected in surface sterilized seeds (3 minutes in 2.5% NaOCl) when seeds were either plated directly on the rifampin-selective media or after 48 hours incubation of the samples in BYE + 50 µg/ml rifampin and 25 µg/ml cycloheximide. Values are average of three replications. The experiment was repeated three times.

[5] (D.R.) Disease Reaction: R = resistant; MR = moderately-resistant; S = susceptible

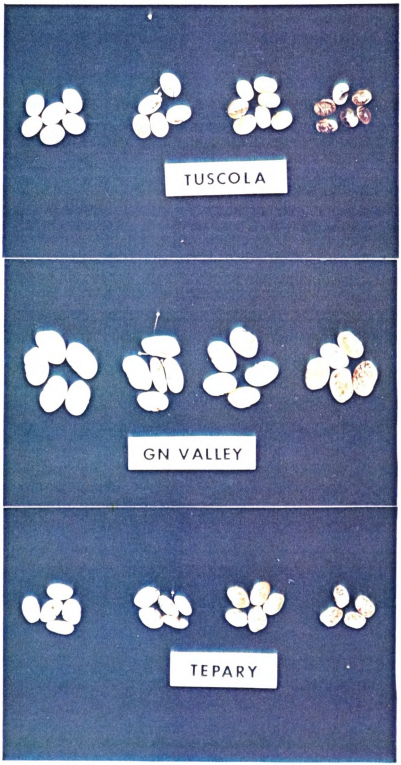
FIGURE 2. Disease symptoms on mature pods of Tepary (Arizona-Buff), G.N. Valley, and Tuscola bean genotypes. Pods at the flat green stage of development were inoculated by scratching the dorsal suture with a syringe containing 1.0×10^7 cells/ml of R15-1 (Xp mutant).


TUSCOLA


GN VALLEY


TEPARY

FIGURE 3. Seed obtained from pods of Tepary (Arizona-Buff), G.N. Valley, and Tuscola bean genotypes, inoculated at the flat green pod stage of plant development by scratching the dorsal suture of the pods with a syringe containing 1.0×10^7 cells/ml of R15-1 (Xp mutant).


TUSCOLA
GN VALLEY
TEPARY

recovered from 5.4, 6.5 and 11.8% of symptomless seeds in the genotypes Tepary, W-117, and Seafarer, respectively, in the greenhouse experiment (Table 8); and from 1.3, 2.0, 1.9, 1.3, 2.0, and 10.4% in the genotypes Tepary, G.N. Nebr. No. 1 sel. 27, G.N. Valley, G.N. Jules, MSU-51319, and Tuscola, respectively, in the field experiment (Table 9).

TABLE 8. Incidence of blight bacteria in seed harvested from greenhouse grown plants inoculated with X. phaseoli (1).

Genotype	D.R.(3)	Internally Infected Seeds(2)			
		Seeds with Visible Symptoms		Seeds with No Symptoms	
		Infected/Total	%	Infected/Total	%
Tepary (Arizona-Buff)	R	23/50	46.0	9/168	5.4
W-117	MR	25/50	50.0	11/158	6.5
Seafarer	S	34/50	68.0	17/144	11.8

(1)
Approximately 50 pods on greenhouse-grown plants of each genotype were inoculated by scratching the dorsal suture with the needle of a syringe containing 1.0×10^8 cells/ml of R15-1 (Xp mutant).

(2)
Bacteria were isolated from individual surface-sterilized (3 minutes in 2.5% NaOCl) seeds. Seeds were first placed hilum down directly into rifampin-selective media for 18 hours and followed by 48 hours incubation in liquid rifampin media.

(3)
D.R. = Disease Reaction: R = resistant; MR = moderately-resistant; S = susceptible.

TABLE 9. Incidence of blight bacteria in seed harvested from field grown plants inoculated with X. phaseoli[1].

Genotype	D.R.[3]	Internally Infected Seeds[2]			
		Seeds with Visible Symptoms		Seeds with No Symptoms	
		Infected/Total	%	Infected/Total	%
G.N. Nebr. #1 Sel. 27	MR	42/100	42	6/303	2.0
G.N. Jules	MR	51/100	51	8/420	1.9
G.N. Valley	MR	46/100	46	5/396	1.3
MSU-51319	MR	42/100	42	7/350	2.0
Tepary (Arizona-Buff	R	40/100	40	6/450	1.3
Tuscola	S	70/100	70	32/307	10.4

(1)
Approximately 200 pods on field-grown plants of each genotype were inoculated by scratching the dorsal suture with the needle of a sterile syringe containing 1.0×10^7 cells/ml of R15-1 (Xp mutant).

(2)
Bacteria were isolated from individual surface-sterilized (3 minutes in 2.5% NaOCl) seeds. Seeds were first placed hilum down directly into rifampin-selective media for 18 hours and followed by 48 hours incubation in liquid rifampin media.

(3)
(D.R.) = Disease Reaction: R = resistant; MR = moderately-resistant; S = susceptible.

DISCUSSION

Results of these studies further support our previous findings (Part 1, 1.2) that leaves of susceptible and resistant bean genotypes and non-host plants may support epiphytic multiplication of bean blight bacteria. Several workers have reported plant pathogenic bacteria surviving on healthy host and non-host tissues (2, 4, 5, 6, 13, 20, 23).

The data on secondary spreas of Xp between blight resistant and susceptible beans, and weed plants indicated that inoculum was available for dissemination early after colonization of the plants, suggesting that secondary spread, primarily due to rain splashing, occurs in the field prior to symptom expression. Such an epidemiological pattern suggests that moderately-resistant bean cultivars could serve as 'symptomless carriers' of bean blight bacteria.

While epiphytic growth of Xp on weed plants has been previously suggested (7, 21), our results indicate that the bacteria may be a resident on weed species. The inherent ability of leaves of host and non-host species to support epiphytic growth of blight bacteria may be of relative importance under Michigan bean growing conditions. In Latin America, particularly in the tropics, however,

environmental conditions allow more than one successive crop during the year; also, beans are frequently cultivated in association with other crops and heavy weed infestations are common problems in bean fields.

Survival of phytopathogenic bacteria associated with plant residues in soil is well recognized (17, 27) and debris from diseased plants have been always considered a possible source for seasonal carryover. Circumstantial evidence suggested that infected plant refuses may play a role in long-term survival of bean blight bacteria (10, 34) and Schuster (25, 26) reported overwintering of Xp and Xpf in bean and weed refuses in Nebraska. However, there are also reports of none overwintering of these pathogens (11, 28, 31). The results of our study indicate that infected leaves of host and non-host plants, whether on the soil surface or buried below ground, do not allow between-season survival or Xp and Xpf in Michigan.

Several factors may influence the survival of plant pathogenic bacteria in nature. It has been reported that maintenance under dry conditions commonly favors bacterial survival in plant residues (17, 26, 27, 32), mainly because in these tissues bacterial cells remain in a reduced state of metabolism (17). Bacterial exudates (ooze) have also been mentioned as playing an important role in the survival of the pathogens by preventing desiccation and affording protection. Wilson et al (32) reported that the longevity of Xp was considerably enhanced when bacteria were stored in exudate under different conditions of temperature, but failed to survive at high relative humidities. According to the authors, at high

relative humidity, the hygroscopic properties of the exudates would permit the retention of sufficient moisture to allow metabolic activity sufficient to exhaust the available reserve nutrients. On the other hand, Patrick (19) found a great abundance of microorganisms among the soil flora, capable of antagonizing most of the bacterial pathogens, and reported that Xanthomonas species were the most sensitive group. Obligately parasitic bdellovibrios, predations protozoa, and free living nematodes, have also been mentioned as influencing bacterial survival in nature (27).

Moist conditions in the soil after placing the tissue samples during the fall, probably allowed an increased activity of microorganisms; and it seems likely that blight bacteria were unable to stand the competition and gradually lost viability. The bacteria survived in dry tissues stored under laboratory conditions, where essentially no competing microorganisms could grow. The possibility that variation in survivability may be present in Xp and Xpf should not be overlooked in interpreting these results.

In an early study, Burkholder (1) reported that bean seeds infected with common blight bacteria were obtained from symptomless pods. Recently, Weller (31) reported symptomless navy bean seed containing low population levels of blight bacteria, and that such seed was produced not only in visibly infected pods but also in symptomless pods. Results of our studies indicated that Xp was only recovered from seeds harvested from symptomless pods when plants were inoculated at the small-flat pod stage of development. This suggests that seed infection was primarily influenced by pod

colonization. Pods of Tepary bean exhibited the highest level of resistance and only few bacteria were recovered from the seed surfaces. Seed of moderately-resistant G.N. Valley and susceptible Tuscola exhibited similar levels of external contamination but internal seed infection was higher in the susceptible genotype. Pods of resistant and susceptible bean genotypes inoculated by scratching the dorsal suture with the needle of a syringe containing the bacterial suspension developed different disease reactions; however, seeds with and without disease symptoms of both susceptible and resistant genotypes, carried internal blight infection although at highest levels in the former.

Transmission of bean blight bacteria in symptomless seed of both resistant and susceptible genotypes, suggests that tests to detect seed-borne bacterial blight should be a component in certified, blight-free bean seed production programs of all dry bean cultivars.

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APPENDIX

Preliminary Ultrastructural Evidence for Immobilization of *Xanthomonas phaseoli* in Tepary Bean (*Phaseolus acutifolius*) Leaves

Common blight, caused by *Xanthomonas phaseoli* (Xp) is considered one of the most serious seed-borne diseases of dry edible and green beans in many production areas throughout the world (2, 7). Although practical short-term control is possible through the use of disease-free seed and crop rotation, long-term control depends on the development of disease resistant cultivars. Considerable effort has been directed toward finding resistant germplasm useful to breeding (2, 5, 7) and until now only certain accessions of Tepary bean (*Phaseolus acutifolius*) exhibit the highest levels of resistance to a range of *Xanthomonas* blight isolates.

Our greenhouse and field studies compared multiplication and distribution patterns of Xp (Rl5-1 rifampin-resistant isolate) in bean genotypes possessing different levels of disease resistance, and the results confirmed previous findings. Although Xp was able to survive in inoculated leaf tissues of Tepary bean (Arizona-Buff) for relatively long periods of time, bacterial populations remained at stationary phase and then declined. Also, Tepary exhibited resistance to systemic colonization, and *Xanthomonas* blight symptoms were never observed throughout the experiments.

Elucidation of the physiological-biochemical bases for disease resistance in plant has remained as elusive a goal for researchers concerned with bacterial diseases as it has for those concerned with other pathogens. Attachment and envelopment (8) or immobilization (3) of incompatible and/or saprophytic bacteria by plant cell walls

appears to be a general phenomenon and has been reported in several host-bacteria systems (1, 3, 6, 8, 9). Such phenomena have been proposed as a major host defense mechanism against bacteria (3, 9). Recently Sequeira (8) suggested that this phenomenon may also occur in resistant host-parasite combinations.

Xanthomonas-resistant Tepary bean (Arizona-Buff) and susceptible cv. Tuscola plants were greenhouse-grown in a standard soil mix and inoculated when the first trifoliolate leaf was fully expanded, 22 days after planting. Leaves were inoculated with 10^8 cells/ml suspension of Xp (R15-1 rifampin-resistant isolate), on the lower surfaces using a sprayer attached to a compressed air line (17 p.s.i.). As a result, the inoculation sites (approx. 0.5-1.0 mm diameter) of both resistant and susceptible leaves retained a watersoaked appearance for approximately three hours.

At one, three, six and 18 hours, and four and eight days after inoculation, leaf samples were taken and prepared for transmission electron microscope (TEM). Sections one mm square were cut from inoculated areas of the leaf and placed in 5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) at 4 C. The tissues were then washed twice with the same buffer during a one-hour period and post-fixed overnight in 1% osmium tetroxide in 0.1 M phosphate buffer at 4 C. The material was then washed, dehydrated in an ethanol series, and left overnight in 100% ethanol. After a transition period through acetone, sections were finally embedded in a mixture of Epon-Araldite/ ERL Epoxy Resine (4). Ultrathin sections were stained in uranyl acetate and then in lead citrate. Sections were examined with a

Philips 300 TEM, operated at 60 kV.

Tepary bean (resistant)

Three hours after inoculation, most bacteria in leaves of resistant Tepary bean were found in the intercellular spaces in close proximity to host cell walls (Fig. 1). Some loose fibrillar material, possibly released from the bean cell walls, were observed at that time between cell wall and the bacterium (Fig. 2). Attachment of Xp bacteria to host cell walls was detected 18 hours after inoculation (Fig. 3, 4, and 5). Macroscopically (48 hours after inoculation) the infiltrated sites possessed a clearly defined necrotic border between the watersoaked area and the surrounding green healthy tissue; by 72 to 96 hours all of the inoculated area was brown and necrotic; at the ultrastructural level, complete disruption of cellular organelles was observed. There was no evidence of multiplication by the immobilized bacteria, and complete encapsulation as described in tobacco leaves (3, 8) was never observed. However, "blister-like" structures were frequently found after 24 hours only in the cell walls of inoculated Tepary bean leaves (Fig. 6). Attachment occurred before observing hypersensitivity, which agrees with other bacteria/host cell wall interactions (3, 8).

Tuscola bean (susceptible)

In leaves of susceptible cultivar Tuscola, watersoaking reappeared at the infiltrated sites 24 hours after inoculation; by 96 hours the watersoaking had expanded beyond the inoculated sites and a faint chlorosis was observed at the upper leaf surfaces. Eight days

after inoculation typical Xanthomonas blight symptoms developed. At the ultrastructural level, no attachment of the bacteria to host cell walls was observed and Xp was seen to divide soon after inoculation. At 18 hours, large numbers of bacteria were observed in the intercellular spaces (Fig. 7), and eight days after inoculation complete disorganization of the cytoplasms and host cells collapse was evident (Fig. 8).

The interaction between bacteria and tobacco mesophyll cell walls and their relation to the various types of resistant reactions which occur in tobacco leaves, have been described in detail by Sequeira et al (8). Attachment of incompatible strains of P. solanacearum to tobacco mesophyll cell walls appeared to be an essential step in the process that lead to HR. According to the authors, the attachment process may essentially be a "recognition" phenomenon that may involve interactions between specific constituents of the bacterial cell walls and binding sites on the host cell walls. Sing and Schroth (9) reported active immobilization of saprophytic bacteria in the intercellular spaces of bean leaves; immobilization was not observed when a pathogenic bacteria, Pseudomonas phaseolicola, was infiltrated into leaves of susceptible bean plants. The authors suggested that bean lectins may be involved in the attachment and encapsulation processes. Recently, Roebuck et al (6) reported that most P. phaseolicola cells "appeared to be attached" to host cell walls after infiltration of the bacteria in the intercellular spaces of leaves of a halo blight resistant bean cultivar. They suggested that bacterial attachment to host cell walls may allow the transfer of some

factor between bacteria and the host protoplast which may trigger an HR. According to Goodman et al (3) "it is under conditions of low inoculum doses, which are more likely to occur in nature, that the active process of bacterial immobilization assumes real importance as a resistance mechanism".

This study indicates that attachment of Xp, a bean pathogen, occurs in the intercellular spaces of leaves of blight-resistant Tepary bean (Arizona-Buff). Further investigation is required to determine whether the phenomenon is directly or indirectly involved in the resistance response of if other defense mechanisms are also operating in this host-pathogen interaction.

Abbreviations used in Figures: B, bacterium; Cl, chloroplast; W, cell wall

Figures 1 to 5. Transmission electron micrographs of spongy mesophyll cells in resistant Tepary bean (Phaseolus acutifolius) leaves showing the interaction of host cell wall and common blight bacteria (Xanthomonas phaseoli).

Figure 1. Cross section of bacteria at three hours after inoculation. Note group of bacteria aligned close to the host cell walls. (x 12 500).

Figure 2. Cross section of bacterium in intercellular space three hours after inoculation. Note loose fibrillar material on the host cell wall in close proximity to the bacterium (arrow). (x 60 000).

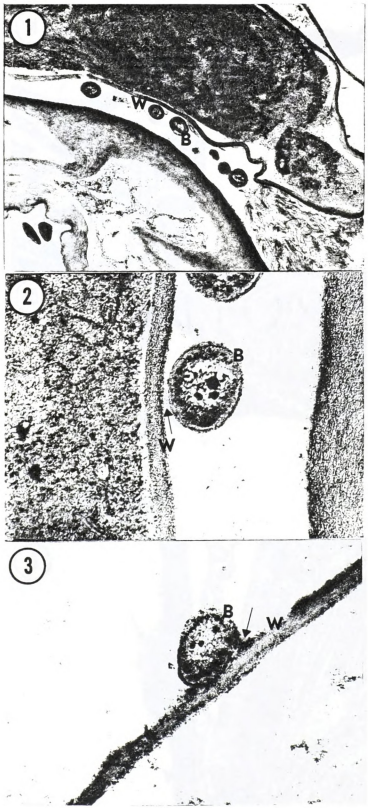
Figures 3, 4, and 5. Cross sections of bacteria attached to host cell wall matrix 18 hours after inoculation. Note matrix attached to bacterial cell (arrows). (Fig. 3 and 5 x 32 000; Fig. 4 x 25 000).

Figure 6. Cross section of intercellular space 96 hours after inoculation. Structure (arrow) that resembles those typically reported to completely encapsulate bacteria (x 52 000).

Figure 7 and 8. Transmission electron micrographs of spongy mesophyll cells in Xanthomonas blight susceptible cv. Tuscola (Phaseolus vulgaris) leaves.

Figure 7. Bacteria in susceptible host 18 hours after inoculation. Note large number of bacterial cells (x 16 000).

Figure 8. Bacteria in the lesion area of susceptible host genotype eight days after inoculation (x 3 200).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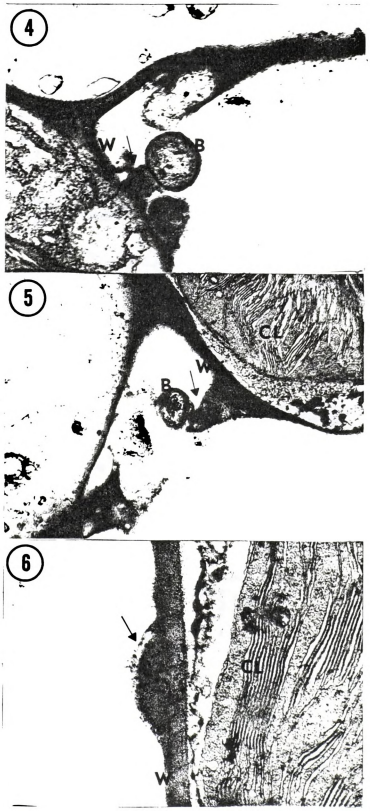
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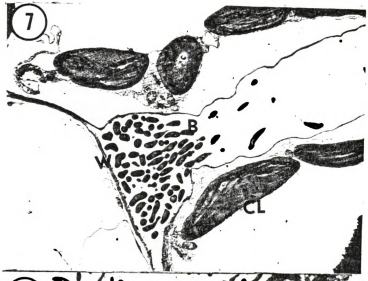
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8
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