This is to certify that the

thesis entitled

Pseudomonas lachrymans Resistance
In Cucumber

presented by

James Michael Dessert

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Horticulture

Major professor

Date 5/13/81

O-7639

PSEUDOMONAS LACHRYMANS RESISTANCE IN CUCUMBER

By

James Michael Dessert

A DISSERTATION

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

DOCTOR OF PHILOSOPHY

Department of Horticulture

1981

ABSTRACT

PSEUDOMONAS LACHRYMANS RESISTANCE IN CUCUMBER

By

James Michael Dessert

Cucumber (Cucumis sativus L.) lines resistant to angular leafspot caused by the bacterium Pseudomonas lachrymans react to infection by developing necrotic lesions (NH lesion type) which lack the chlorotic halo characteristic of the susceptible reaction (CH lesion type). This research was conducted to determine the inheritance of the NH lesion reaction in cucumber and its importance as a component of resistance to P. lachrymans. Resistant pickling cucumber lines MSU 9402 and Gy 14A were crossed with the susceptible lines SMR 18 and National Pickling and genetic analysis of the F_1, F_2, BC, and F_3 populations of these crosses indicated the NH lesion type is controlled by a single recessive gene. The role of the NH lesion type in P. lachrymans resistance was evaluated based upon field disease severity ratings and P. lachrymans population levels on the foliage of F_3 lines from the cross between National Pickling and MSU 9402. The F_3 lines were genetically homogeneous for either the NH or CH lesion type. Lesion type was determined to be a major component of resistance to P. lachrymans. Disease severity on CH F_3 lines was significantly greater than on NH F_3 lines; and, 87 percent of total genotypic variance of F_3 lines was due to differences in lesion type. Broad sense heritability (H^2) for disease severity among F_3 lines within each lesion type was medium to low (.1-.3). Bacterial populations on CH lines were 100 times greater than populations on NH lines and were closely associated with lesions. The development of bacterial populations and lesions over time on parental and F_1 lines was monitored in greenhouse experiments. Bacterial populations on resistant MSU 9402 and susceptible National Pickling increased logarithmically at similar rates after inoculation, but the peak population was lower on MSU 9402. The NH and CH lesions developed similarly over time and were first distinguishable four to five days after inoculation.

ACKNOWLEDGEMENTS

My appreciation and thanks to Dr. L. R. Baker, my major advisor throughout most of my Ph.D. program who taught me much about vegetable breeding and advised me throughout this study. I also appreciate the help and especially the encouragement of Dr. J. F. Fobes, my current major advisor.

Thanks also to other guidance committee members: Dr. M. L. Lacy whose lab facilities I used throughout my research, Dr. D. W. Fulbright, especially for the help with the bacteriological aspects of my research, Dr. A. H. Ellingboe for suggestions and interest in my work and Dr. K. C. Sink and Dr. H. C. Price, especially for their critique and conversation.

So many others must also be thanked: Mr. Dick Crum, Nancy Glandon, Horticulture and Botony field staff and fellow students.

Warmest thanks to my family: especially to my wife, Krista, for her help with the computer analysis and editing, and to my parents who encouraged and supported me for so many years throughout my education.

Guidance Committee:

The journal-article format was adopted for this dissertation in accordance with departmental and university requirements. Chapter I was prepared for submission to *Euphytica*. Chapter II was prepared for submission to *Phytopathology*.

TABLE OF CONTENTS

Page

LIST OF TABLES v

LIST OF FIGURES vii

INTRODUCTION 1

CHAPTER I

INHERITANCE OF LESION TYPE REACTION TO PSEUDOMONAS LACHRYMANS IN CUCUMBER 3

Introduction 3
Materials and Methods 5
Results and Discussion 7
Literature Cited 19

CHAPTER II

CUCUMBER LESION TYPE IN RESPONSE TO PSEUDOMONAS LACHRYMANS INFECTION AND ITS INFLUENCE ON DISEASE SEVERITY AND BACTERIAL POPULATIONS . . 21

Introduction 22
Materials and Methods 25
Results 32
Discussion 57
Literature Cited 64

LIST OF TABLES

Table Page

CHAPTER I

1. Lesion type frequencies of parental cucumber lines and F_1 populations in response to *P. lachrymans* inoculation as determined by greenhouse seedling tests 9

2. Lesion type frequencies of F_2 and backcross populations of cucumber in response to *P. lachrymans* inoculation as determined by greenhouse seedling tests, with Chi squares and probabilities for goodness of fit to apparent ratios 11

3. Frequencies of F_3 families (MSU 9402 x National Pickling) homogeneous and segregating for lesion type in response to *P. lachrymans* inoculation as determined by greenhouse seedling tests, with Chi squares and probabilities for goodness of fit to a 1:2:1 ratio 12

4. Lesion type frequencies in 22 segregating F_3 families of the cross MSU 9402 x National Pickling as determined by greenhouse seedling tests, with Chi squares[w] and probabilities for goodness of fit to a 1:3 ratio 13

5. Lesion type frequencies of resistant x resistant and susceptible x susceptible cucumber crosses in response to *P. lachrymans* inoculation as determined by greenhouse seedling tests . 14

6. Lesion type frequencies of parental cucumber lines and F_1, F_2, and testcross populations in response to *P. lachrymans* inoculation in field plantings, with Chi squares and probabilities for goodness of fit to apparent ratios 16

CHAPTER II

1. Mean squares from analysis of variance for disease severity ratings on F_3 lines homogeneous for CH or NH lesion types in field planting 1. At evaluation, plants had 12-16 nodes on the main axis 33

Table Page

2. Mean disease severity ratings on parental, F_1 and F_3 lines in field planting 1. Individual plants were rated at the 12-16 node stage for disease severity. Disease rated on a 1-10 scale where 1 = no symptoms and 10 = lesions covering 50 percent or more of leaf tissue 35

3. Mean squares from analysis of variance for disease severity ratings on F_3 lines homogeneous for CH or NH lesion types and over three growth stages^x in field planting 2 36

4. Mean ratings of F_3 lines at specific growth stages in field planting 2 for disease severity, lesions per leaf and $\log_{10}$ CFU/cm^2 leaf tissue. Disease severity ratings based on a scale of 1-10 where 1 = no symptoms and 10 = 50 percent or more necrotic leaf area 37

5. Simple correlation coefficients between disease severity ratings and total necrotic lesion number as rated on individual plants at the 10 node stage in field planting 2 39

6. Simple correlation coefficients of mean number CFU/cm^2 with mean disease severity ratings and mean number of lesions per leaf on F_3 lines in field planting 2 41

7. Mean ratings of parental and F_1 lines at specific growth stages in field planting 2 for disease severity, lesions per leaf and $\log_{10}$ CFU/cm^2 leaf tissue 42

8. Genotypic variances of F_3 lines for disease severity partitioned into variance due to lesion type and due to F_3 lines nested within type. $V_T + V_{L(T)}$ = total genotypic variance . . 43

9. Genotypic and environmental (error) variance components and broad sense heritability estimates of F_3 lines within CH and NH lesion type classes for disease severity in field plantings 1 and 2 45

10. $\log_{10}$ mean number CFU/cm^2 at lesion or non-lesion leaf sites on parental and F_1 lines 46

11. Simple correlation coefficients of the number CFU/cm^2 with the number of lesions/cm^2 in individual leaf samples of greenhouse grown plants 11 days after leaf infiltration with approximately 4.5×10^3 CFU/ml inoculum 56

LIST OF FIGURES

Figure Page

CHAPTER II

1. Multiplication of Pseudomonas lachrymans in leaves of resistant MSU 9402 (9402), susceptible National Pickling (NP) and 9402 x NP. The second leaf of three-leaf plants was infiltrated with 1.45×10^5 CFU/ml using a Devilbis atomizer at time = 0. Values are averages of two experiments, three replications per experiment 48

2. Multiplication of P. lachrymans in leaves of resistant MSU 9402 (9402), susceptible National Pickling (NP) and 9402 x NP. The second leaf of three-leaf plants was infiltrated with 1.16×10^3 CFU/ml using a Devilbis atomizer at time = 0. Values are means of three replications, two plants per replication 51

3. Necrotic and total lesion number/cm^2 and $\log_{10}$ CFU/cm^2 on MSU 9402 (9), National Pickling (N) and on MSU 9402 x National Pickling (9 x N) at day 11 after bacterial infiltration. Second leaf of three-leaf plants was infiltrated with 1.8×10^3, 5.0×10^3 and 7.1×10^3 CFU/ml for experiments 1-3 respectively. Values are means of four replications. Means of each character within same experiment with same letter are not significantly different at $p = .05$ by Duncan's Multiple Range Test 54

INTRODUCTION

Pickling cucumbers are a major vegetable crop in the United States. Approximately 125,000 acres of pickling cucumbers are grown in the U.S. Michigan is the leading production state producing 25 percent of the total crop. The average yield in Michigan is 150-220 bu/acre, and 95 percent of the cucumber acreage in Michigan is mechanically harvested. Cucumber culture is often very intensive, including high plant density and overhead irrigation. Under such intensive growing conditions, yields of 400 bushels per acre are common.

Disease management is essential to insure the vigorous uniform growth necessary for high yields. Angular leafspot caused by *Pseudomonas lachrymans*[a] is the current major disease of Michigan cucumbers. Cucumber scab, caused by the fungus *Cladosporium cucumerinum* and cucumber mosaic, a viral disease, were major diseases of cucumbers in Michigan until controlled by incorporation of high levels of resistance to these diseases into Michigan adapted cucumber cultivars.

Angular leafspot infects cucumber foliage, causing limited, angular lesions. Cucumber fruit may also become infected. The disease may cause serious yield and quality losses, especially during warm, wet weather under intensive growing conditions. Angular leafspot is

---

[a] *Pseudomonas syringae* pathovar *lachrymans* is the current correct name of this bacterium according to the *International Journal of Systematic Bacteriology*, 1980. However, the more familiar term, *Pseudomonas lachrymans*, will be used throughout this dissertation.

partially controlled by copper containing chemical sprays; however, genetic resistance would provide more efficient and complete control.

Some *P. lachrymans* resistant cultivars have been developed, but transfer of resistance into adapted varieties has been difficult and inefficient because the mode of inheritance and nature of resistance is unclear. Chand and Walker (1964) reported that resistance in cucumber to *P. lachrymans* is multigenic. When resistant lines are infected with *P. lachrymans* they develop fewer and smaller lesions than susceptible lines. In addition, the appearance of lesions on resistant lines is different than on susceptible lines. Susceptible lines typically develop orange-brown necrotic lesions surrounded by chlorotic halos (CH lesions). Lesions on resistant lines are typically white or tan with no or very slight chlorotic halo (NH lesion). This difference in lesion type is easily distinguished on inoculated seedlings. Furthermore, preliminary studies indicate the NH lesion type appears to be associated with resistance to *P. lachrymans*. If NH lesion type is found to be simply inherited and closely associated with *P. lachrymans* resistance, then selection for the NH lesion type in a seedling population would increase the efficiency of developing adapted cultivars resistant to *P. lachrymans*.

Therefore, the objectives of this research were to: 1) determine the genetic control of lesion type in response to *P. lachrymans* inoculation of the pickling cucumber, and 2) evaluate lesion type as a component of resistance to *P. lachrymans*.

CHAPTER I

INHERITANCE OF LESION TYPE REACTION TO *PSEUDOMONAS LACHRYMANS* IN CUCUMBER

INHERITANCE OF LESION TYPE REACTION
TO *PSEUDOMONAS LACHRYMANS* IN CUCUMBER

Abstract

Cucumber (*Cucumis sativus*) lines resistant to angular leafspot caused by *Pseudomonas lachrymans* react to an infection by developing necrotic lesions that lack the chlorotic halo characteristic of the susceptible reaction. The inheritance of the non-halo lesion reaction was studied in crosses between resistant lines MSU 9402 and Gy 14A, and susceptable cultivars Wisc. SMR 18 and National Pickling. Genetic analysis of the F_1, F_2, backcross, and F_3 populations revealed that the non-halo lesion type, associated with resistance, is controlled by a single recessive gene. This character appears to be an important component of resistance to *P. lachrymans*.

Introduction

Angular leafspot caused by *Pseudomonas lachrymans* (Smith and Bryan) Carsner causes serious yield losses to pickling cucumber (*Cucumis sativus* L.) crops in northern U.S. and Canadian production areas (Kennedy and Alcorn, 1980). Early symptoms include both water-soaked, vein-limited angular lesions on the foliage and circular, water-soaked lesions on the fruit. As the disease progresses, the necrotic centers of the foliar lesions become dry and may fall out. Lesions on the fruit often crack open and exude amber-colored bacterial ooze (Wiles and Walker, 1951). Chand et al. (1963) reported that many fruit infected with *P. lachrymans* were misshapen, developing into "crooks," thus reducing the quality of the harvested crop.

W. C. Barnes (1966) reported that South Carolina breeding lines had a high degree of resistance to *P. lachrymans*. He noted that P.I. 197087 from India was undoubtedly the source of resistance. Since then, cultivars with varying levels of resistance have been developed. Pohronezny et al. (1977) found that *P. lachrymans* caused significantly lower yield losses in resistant cultivars than in susceptible ones when tested under artificially inoculated field conditions.

Because of difficulty in reproducing disease screening results and the unclear genetic control of resistance, the development of new resistant cultivars has been difficult. Breeding programs have used seedling screening programs to increase the ease, efficiency, and reliability of selection programs (de Zeeuw et al., 1971; de Ponti, 1975; Haley et al., 1977). Chand and Walker (1964) reported that resistance to *P. lachrymans* in the accession P.I. 169400 from Turkey was multigenic in crosses with two susceptible Wisconsin cultivars, SMR 15 and SMR 18.

Resistant cultivars develop fewer and smaller lesions when infected with *P. lachrymans*. In addition, lesions on susceptible cultivars become surrounded by conspicuous chlorotic halos, whereas lesions on resistant cultivars have little or no chlorotic halo development. Haley and Palmer (1977) found high positive correlations between cucumber cultivars that produced halos surrounding lesions and severe foliar symptoms. In response to *P. lachrymans* inoculation, the non-halo lesion type appears to be an important component of resistance in cucumber. This character is also easily identified for selection in cucumber breeding programs. The objective of the present study was to determine the genetic control of lesion type in response to P. lachrymans in the pickling cucumber.

Materials and Methods

Parents and Crosses

Two pickling cucumber lines resistant to P. lachrymans, MSU 9402 (9402) and Gy 14A, were crossed with two susceptible cultivars National Pickling (NP) and Wisconsin SMR 18. These parental lines were selected on the basis of disease severity ratings in replicated field trials. Lines Gy 14A, and especially 9402, repeatedly exhibit low disease severity under the epiphytotic conditions of MSU angular leafspot field trials. Gy 14A, a gynoecious line (Clemson University), is commonly a parent in F_1 hybrid cultivars. The line, MSU 9402 is of value chiefly as a source of resistance to P. lachrymans. National Pickling and SMR 18, older cultivars (Michigan State University and the University of Wisconsin, respectively) are highly susceptible to P. lachrymans. All lines were inbred and screened for their reaction to P. lachrymans prior to this study. Reciprocal F_1's and F_2, and backcross populations were produced. In addition, a random group of 9402 x NP F_2 plants were selfed to produce F_3 families.

Pathogen

A single colony isolate (MSU #5) of P. lachrymans was used throughout this study. This isolate was originally supplied by C. A. John, J. H. Heinz Co., Bowling Green, Ohio. A total of six isolates from Michigan, Wisconsin, and Ohio were compared for pathogenicity and virulence on several pickling cucumber lines. No differential pathogenicity was observed, but small differences in virulence were noted. Likewise, Van Gundy and Walker (1957) reported six other isolates of *P. lachrymans* uniform in pathogenicity. Isolate #5 was selected because of its relatively high virulence. As previously observed by Keen et al. (1967), a series of inoculations and re-isolations from cucumber did not noticeably increase the virulence of these isolates.

Isolates were stored either on yeast dextrose carbonate agar slants or in sterile distilled water at 4-5°C. The isolates were transferred on a regular basis to maintain viability. Prior to inoculation, the bacteria were increased for 48 hours in the HK broth of Husain and Kelman (1958), as modified by Keen et al. (1967). Immediately before inoculation, the cultures were diluted with deionized water and adjusted turbidimetrically using a Baush and Lomb Spectronic 20 colorimeter to an optical density of .30 absorbance units at 620 nm. This approximated 2 x 10^8 colony forming units (CFU)/ml.

Greenhouse inoculation technique

Seeds were sown in wooden flats containing a greenhouse soil mix and fertilized once with a water soluble (20-20-20) fertilizer to provide 300 ppm N. Flats were placed on a bottom-heated bench (30°C soil temperature) to insure uniform rapid germination. Parental lines were included in each flat to standardize reaction types. Plants were inoculated at the 'candle' stage (first true leaf just emerging) 8-10 days after sowing. Inoculum was atomized onto the underside of the cotyledons with a Devilbis atomizer held at a distance of 10-15 cm. Inoculations were made between 8:00 a.m. and 12:00 noon and flats were immediately placed in an unlighted incubator at 21-24°C with 95-100 percent relative humidity. After a 48 hour incubation period, flats were removed to a greenhouse maintained at approximately 18°C night and 24°C day temperature. Each seedling was visually classified for its lesion type reaction to *P. lachrymans* 7-9 days after inoculation.

Field studies

Field plots of parental, F_1, F_2 and backcross generations were sown in rows spaced 1.3 meters. Plants were thinned to a within row spacing of approximately 10 cm. Standard Michigan cultural practices were used. Inocula were increased and prepared as specified for the greenhouse studies. At the five-leaf stage, plants were lightly sprayed with a suspension of *P. lachrymans* (10^7 CFU/ml.) using a backpack sprayer. A second inoculation was made 1 week later. Plots were frequently irrigated lightly to ensure optimum conditions for disease development. Typical angular lesions developed approximately 5 days after the first inoculation. Lesion type was classified 10 days after the second inoculation.

Results and Discussion

Disease symptomology

In greenhouse studies, cotyledons of susceptible lines NP and SMR 18 developed rounded or irregularly shaped, orange-brown necrotic lesions surrounded by chlorotic halos. Lesion diameter varied from 1-10 mm, and the surrounding chlorotic halo ranged from 1-5 mm in width. Moreover, diffuse chlorosis often developed between lesions. This symptom will be termed the "chlorotic halo" (CH) lesion type reaction.

Cotyledons of resistant MSU 9402 and Gy 14A developed white or tan necrotic lesions of similar or smaller diameter, with very slight or no chlorotic halo development. This resistant symptomology will be termed the "non-halo" (NH) lesion type reaction. Both lesion types developed in 5-7 days after removal from the incubation chamber. Within 72 hours, they were seen as water-soaked, sunken areas of 1-2 mm diameter on the lower side of the cotyledons. Small chlorotic spots formed on the upper surface opposite the water-soaked area. These water-soaked areas then expanded and developed into the CH or NH lesion type, depending on the cucumber genotype. The NH reaction resembled hypersensitivity, but was distinguished from it in its development. Typical hypersensitive lesions develop within 24-48 hours after inoculation, preceding development of lesions on susceptible plants by 1-2 days (Müller, 1959; Klement et al., 1964). Furthermore, hypersensitive reactions are typically not preceded by water soaking of the leaf tissue (Klement and Goodman, 1967). NH lesions, on the other hand, developed over 3-5 days, along the same time course as CH lesions. In some cases, water soaking preceded the necrosis of the NH reaction. Resistant and susceptible plants developed similar numbers of lesions on inoculated cotyledons. Upon inoculation of true leaves in the field, the two lesion types developed as in seedling tests, except they often became vein limited and angular in shape. In contrast to the seedling tests, NH genotypes in the field developed fewer observed lesions than CH genotypes.

Inheritance studies

In greenhouse studies most F_1 seedlings of resistant and susceptible crosses developed CH lesions, indicating that the CH was dominant to the NH reaction (Table 1). Some misclassification was evident in the F_1 as in the parental generation. Progeny tests indicated that off-types were genetically similar to the expected. The intensity of the

Table 1. Lesion type frequencies of parental cucumber lines and F_1 populations in response to *P. lachrymans* inoculation as determined by greenhouse seedling tests.

No. individual tests	Pedigree	Total no. of plants tested	Observed ratio		Expected ratio	
			NH[x]	CH[y]	NH	CH
	PARENTS					
5	9402	76	76	0	1	0
6	Gy 14A	90	85	5	1	0
8	SMR 18	119	1	118	0	1
7	NP	97	1	96	0	1
	F_1					
10	9402 x NP	201	1	200	0	1
2	NP x 9402	62	2	60	0	1
2	9402 x SMR 18	36	2	34	0	1
1	SMR 18 x 9402	12	1	11	0	1
4	SMR 18 x Gy 14A	86	8	78	0	1
1	Gy 14A x SMR 18	50	1	49	0	1
1	NP x Gy 14A	18	0	18	0	1
1	Gy 14A x NP	62	0	62	0	1

[x] Number of plants with non-halo lesion type.

[y] Number of plants with chlorotic halo lesion type.

CH reaction, though variable, was not always as great in the F_1 generation as the susceptible parent. This was possibly due to incomplete dominance of genes controlling the CH reaction and/or due to the interaction with the genetic background.

All F_2 populations, except Gy 14A x NP fit the ratio of 1NH:3CH (Table 2). Chi square analysis of the F_2 plants from the cross Gy 14A x NP gave a P value which exceeded .05 due to an excess of NH types. This low P value is most likely due to misclassification, especially since the reciprocal F_2 population (NP x Gy 14A) had an excess of CH reaction individuals. All progenies from testcrosses to resistant parents segregated 1NH:1CH (Table 2) whereas backcrosses to susceptible parents resulted in only CH progeny (Table 2).

The F_3 families from the cross 9402 x NP could be classified into three distinct groups, as follows: CH, segregating, and NH. The phenotypes of the F_3 families gave an acceptable fit to the ratio of 1CH:2 segregating:1NH; although, there was some excess of true breeding CH families (Table 3). Within family segregation ratios were determined for the 22 segregating F_3 families. Most families gave a good fit to the expected ratio of 1NH:3CH. Pooling the data from the 22 segregating F_3 populations yielded a total of 97 NH and 315 CH plants, P = .4-.5 for a 1:3 ratio (Table 4). Observed segregation ratios indicated single gene control of lesion type with the NH reaction recessive to the CH reaction.

The F_1 and F_2 populations from crosses between the two resistant parents, 9402 and Gy 14A, were all resistant. In addition, there were no resistant segregates in the F_2 populations of the cross between the two susceptible parents NP and SMR 18 (Table 5). Lack of segregation

Table 2. Lesion type frequencies of F_2 and backcross populations of cucumber in response to *P. lachrymans* inoculation as determined by greenhouse seedling tests, with Chi squares and probabilities for goodness of fit to apparent ratios.

No. individual tests	Pedigree	Total no. plants tested	Observed ratio NH[y]	Observed ratio CH[z]	Expected ratio NH	Expected ratio CH	x^2	P value
2	(9402 x NP)-1	110	24	86	1	3	.594	.4-.5
10	(NP x 9402)-1	287	63	224	1	3	1.423	.2-.3
3	(SMR 18 x 9402)-1	172	46	126	1	3	.316	.5-.6
4	(SMR 18 x 14)-1	165	45	120	1	3	.455	.5
1	(GY 14A x NP)-1	52	20	32	1	3	5.026	.025
1	(NP x GY 14A)-1	67	15	52	1	3	.244	.1-.2
Pooled[w] values		853	213	640	1	3	.0004	.98
3	(NP x 9402) 9402	115	56	59	1	1	.078	.7-.8
3	(SMR 18 x 9402) 9402	156	79	77	1	1	.026	.8-.9
1	Gy 14A(SMR 18 x Gy 14A)	32	17	15	1	1	.125	.7-.8
2	(SMR 18 x Gy 14A)Gy 14A	113	61	52	1	1	.717	.3-.4
1	(NP x Gy 14A)Gy 14A	60	30	30	1	1	0	1
Pooled[x] values		476	243	233	1	1	.210	.6-.7
2	NP (NP x 9402)	86	0	86	0	1	-	-
2	SMR 18(SMR 18 x 9402)	36	4	32	0	1	-	-
1	(SMR 18 x 9402)SMR 18	20	2	18	0	1	-	-
1	(SMR 18 x Gy 14A)SMR 18	13	0	13	0	1	-	-
1	(NP x Gy 14A) NP	17	0	17	0	1	-	-
Pooled values		172	6	166	0	1	-	-

[w] x^2heterogeneity - 8.057 (p = .1-.2)

[x] x^2heterogeneity = 0.736 (p = .95)

[y] Number of plants with non-halo lesion type.

[z] Number of plants with chlorotic-halo lesion type.

Table 3. Frequencies of F_3 families (MSU 9402 x National Pickling) homogeneous and segregating for lesion type in response to *P. lachrymans* inoculation as determined by greenhouse seedling tests, with Chi squares and probabilities for goodness of fit to a 1:2:1 ratio.

	Class				
	NH[x]	seg[y]	CH[z]	X^2	P value
NP x 9402	8	22	14	1.636	.4-.5

[x] Number of F_3 families homogeneous for non-halo lesion type.

[y] Number of F_3 families segregating for lesion type.

[z] Number of F_3 families homogeneous for chlorotic-halo lesion type.

Table 4. Lesion type frequencies in 22 segregating F_3 families of the cross MSU 9402 x National Pickling as determined by greenhouse seedling tests, with Chi squares[w] and probabilities for goodness of fit to a 1:3 ratio.

Number of plants per F_3 family			χ^{2}[w]	P-value
NH[y]	CH[z]	Total		
5	20	25	.12	.7-.8
3	13	16	.08	.7-.8
4	7	11	.27	.6-.7
3	13	16	.08	.7-.8
2	22	24	4.08	.025-.05
8	15	23	.71	.3-.4
5	21	26	.21	.6-.7
5	13	18	.00	1
8	19	27	.11	.7-.8
3	9	12	.00	1
1	6	7	.05	.8-.9
1	4	5	.00	1
5	16	21	.00	1
7	12	19	.86	.3-.4
6	14	20	.07	.7-.8
2	13	15	.56	.4-.5
3	13	16	.08	.7-.8
5	14	19	.00	1
5	14	19	.00	1
6	23	29	.10	.7-.8
6	16	22	.00	1
4	18	22	.24	.6-.7
Pooled[x] values				
97	315	412	.47	.4-.5

[w] Yates correction for continuity (Yates, 1934).

[x] χ^{2} heterogeneity = 11.41 (p = .95).

[y] Number of plants with non-halo lesion type.

[z] Number of plants with chlorotic-halo lesion type.

Table 5. Lesion type frequencies of resistant x resistant and susceptible x susceptible cucumber crosses in response to *P. lachrymans* inoculation as determined by greenhouse seedling tests.

Pedigree	Generation	Total no. plants tested	Observed ratio		Expected ratio	
			NH[x]	CH[y]	NH	CH
Gy 14A x 9402	F_1	11	11	0	1	0
(Gy 14A x 9402)-1	F_2	49	48	1	1	0
SMR 18 x NP	F_1	13	0	13	0	1
(SMR 18 x NP)-1	F_2	39	0	39	0	1

[x] Number of plants with non-halo lesion type.

[y] Number of plants with chlorotic-halo lesion type.

indicated that lesion type is conditioned by the same allele in all parental lines of this study. This was also suggested by the resistant x susceptible crosses where a single gene controlling lesion type was indicated by data from all the crosses.

Field studies

Cucumber breeding programs have screened seedlings under controlled conditions for resistance to _P. lachrymans_. The relationship of these seedling tests to the lesion reaction on mature plants, especially in the field, in unclear. To elucidate this relationship, segregating generations and their parents were inoculated and evaluated for reaction to _P. lachrymans_ in the field. These field studies resulted in similar segregation patterns for the F_2 and testcross generations as did the greenhouse seedling tests (Table 6). Therefore these data confer that foliar lesion type in response to _P. lachrymans_ inoculation is monogenic with the NH lesion type recessive to the CH lesion type.

Variation of lesion size on both cotyledons and true leaves was evident within parental and segregating CH and NH classes. Because of this variation, even within homogeneous parental lines, lesion size was not considered in the classification of lesion type. Halo intensity and size also varied in the CH class, but in this case variation was much more evident in segregating generations. These slight differences in chlorotic halo intensity and size might be classified with a more refined system than the visual rating used in this study. Classification for lesion type was usually distinct in segregating populations of reciprocal crosses between 9402 and NP. However, classification for lesion type in other crosses, especially involving Gy 14A, required

Table 6. Lesion type frequencies of parental cucumber lines and F_1, F_2, and testcross populations in response to *P. lachrymans* inoculation in field plantings, with Chi squares and probabilities for goodness of fit to apparent ratios.

Pedigree	Total no. plants tested	Observed ratio NH[x]	CH[y]	Expected ratio NH	CH	X^2	P value
<u>Parents</u>							
9402	48	48	0	1	0	-	-
Gy 14A	17	17	0	1	0	-	-
NP	54	0	54	0	1	-	-
SMR 18	40	0	40	0	1	-	-
F_1							
NP x 9402	41	0	41	0	1	-	-
9402 x NP	33	0	33	0	1	-	-
Gy 14A x SMR 18	53	0	53	0	1	-	-
F_2							
(NP x 9402)-1	116	27	89	1	3	.184	.5-.75
(9402 x NP)-1	45	10	35	1	3	.185	.5-.75
(SMR 18 x 9402)-1	95	20	75	1	3	.789	.3-.4
(SMR 18 x Gy 14A)-1	123	29	94	1	3	.133	.7-.8
<u>Testcross</u>							
(NP x 9402) 9402	150	74	76	1	1	.027	.8-.9
(SMR 18 x 9402) 9402	133	55	78	1	1	3.977	.025-.050
(SMR 18 x Gy 14A) Gy 14A	59	26	33	1	1	.830	.3-.4
Gy 14A (SMR 18 x Gy 14A)	47	24	23	1	1	.021	.8-.9

[x] Number of plants with non-halo lesion type.

[y] Number of plants with chlorotic-halo lesion type.

meticulous observation of the lesion reaction for proper classification. This might be explained by the observation that 9402, and especially Gy 14A, infrequently expressed a slight chlorotic halo of less than 1 mm width surrounding an otherwise typical NH lesion.

Variability in intensity of the chlorotic halo could probably be reduced by closer control of environmental factors, especially temperature. Van Gundy and Walker (1957) found severity of angular leafspot in cucumber to be dependent on both host nutrition and temperature. Patel and Walker (1963), working with halo blight of bean caused by Pseudomonas phaseolicola, found that the size of the chlorotic halo around lesions increased as temperatures decreased from 28° to 16°C.

Cucumber foliage color sometimes interfered with lesion type classification in both seedlings and mature plants. Susceptible parents, SMR 18 and NP, had a lighter green foliage color which is representative of northern U.S. pickling cucumber genetic backgrounds. The resistant parents, MSU 9402, and Gy 14A, are lines derived from southern U.S. breeding programs which are characterized by darker green foliage. In segregating populations, foliage color displayed an apparent continuous segregation pattern which tended to either mask or enhance chlorotic halo development depending on the intensity of green foliage color.

Chand and Walker (1964) concluded that P. lachrymans resistance in P.I. 169400 appeared to behave as a multigenic factor. Segregation patterns of the F_2 and testcross populations would not fit any simple genetic ratio. Our study has determined that the lesion type reaction in response to P. lachrymans infection is controlled by a single gene, where the NH lesion type associated with resistance, is recessive to the CH lesion type reaction. This apparent discrepancy can be simply resolved: Chand and Walker (1964) based their inheritance study on a disease index incorporating lesion number and size, whereas out study focused on lesion type. Lesion number and size in response to P. lachrymans infection may indeed be controlled by two or more genes as their study indicated. Their disease index approximated the total disease reaction, where lesion type is more likely a single component of resistance. From our observations and the work of Haley and Palmer (1977), the NH reaction is directly associated with low disease severity. It is easily selected for by using the seedling screening procedure emphasized in this study. We suggest that this character confers a very useful degree of resistance to P. lachrymans; other loci interact to effect varying levels of resistance. We propose nh as the symbol for this recessive gene conditioning the non-halo lesion type reaction to P. lachrymans in cucumber.

1. Barnes, W. C. 1966. Development of multiple disease resistant hybrid cucumbers. Proc. Amer. Soc. Hort. Sci. 89:390-393.

2. Chand, J. N., E. K. Wade, and J. C. Walker. 1963. Sprays for control of angular leafspot or cucumber in Wisconsin. I. Plant Dis. Reptr. 47:94-95.

3. Chand, J. N. and J. C. Walker. 1964. Inheritance of resistance to angular leafspot of cucumber. Phytopathology. 54:51-53.

4. dePonti, O. M. B., A. C. van der Giessen, H. Inggamer. 1975. Inpasse in het Toetsen op resistentie tegen bakterievlekkenziekte (Pseudomonas lachrymans) in augurk doorbroken! Overdruk uit Zaadbelangen. 123-127.

5. de Zeeuw, D. J., R. Crum, J. E. Wilson, L. R. Baker. 1971. Screening cucumber seedlings for angular leafspot resistance. HortScience. 6:276 (Abstr.).

6. Haley, A. B. and M. J. Palmer. 1977. Resistance of cucumber to angular leafspot disease--correlation of greenhouse and field reactions. Pickling Crops Research, Univ. of Wisc., Madison.

7. Husain, A., and A. Kelman. 1958. Relation of slime production to mechanism of wilting and pathogenicity of Pseudomonas solanacearum. Phytopathology. 48:155-165.

8. Keen, N. T., P. H. Williams, and J. C. Walker. 1967. Protease of Pseudomonas lachrymans in relation to cucumber angular leafspot. Phytopathology. 57:263-271.

9. Kennedy, B. W. and S. M. Alcorn. 1980. Estimates of U.S. crop losses to procaryote plant pathogens. Plant Disease. 64:674-676.

10. Klement, Z., G. L. Farkas and L. Loviekovich. 1964. Hypersensitive reaction induced by phytopathogenic bacteria in the tobacco leaf. Phytopathology. 54:474-477.

11. Klement, Z., and R. N. Goodman. 1967. The hypersensitive reaction by bacterial plant pathogens. Ann. Rev. Phytopath. 5:17-44.

12. Müller, K. O. 1959. Hypersensitivity. p. 469-519. In J. G. Horsefall and A. E. Dimond (ed.). Plant pathology, an advanced treatise, Vol. 1. Academic Press, New York.

13. Patel, P. N. and J. C. Walker. 1963. Relation of air temperature and age and nutrition of the host to the development of halo and common bacterial blights of bean. Phytopathology. 53:407-411.

14. Pohronezny, K., P. O. Larsen, D. A. Emmatty, and J. D. Farley. 1977. Field studies of yield losses in pickling cucumber due to angular leafspot. Plant Dis. Reptr. 61:386-390.

15. Van Gundy, S. D. and J. C. Walker. 1957. Relation of temperature and host nutrition to angular leafspot of cucumber. Phytopathology. 47:615-619.

16. Wiles, A. B. and J. C. Walker. 1951. The relation of *Pseudomonas lachrymans* to cucumber fruits and seeds. Phytopathology. 41:1059-1064.

17. Yates, F. 1934. Contingency tables involving small numbers and the X^2 test. J. Roy. Stat. Soc. Suppl. 1:217-235.

CHAPTER II

CUCUMBER LESION TYPE IN RESPONSE TO *PSEUDOMONAS LACHRYMANS* INFECTION AND ITS INFLUENCE ON DISEASE SEVERITY AND BACTERIAL POPULATIONS

CUCUMBER LESION TYPE IN RESPONSE TO PSEUDOMONAS LACHRYMANS INFECTION AND ITS INFLUENCE ON DISEASE SEVERITY AND BACTERIAL POPULATIONS

Abstract

Resistance to *Pseudomonas lachrymans* in cucumber (*Cucumis sativus* L.) is a multigenic character. Lesion type on cucumber foliage in response to *P. lachrymans* infection is controlled by a single gene. Susceptible lines develop orange-brown necrotic lesions surrounded by chlorotic halos (CH lesion type). Lesions also develop on resistant lines and are typically white or tan with very slight or no chlorotic halo (NH lesion type). The purpose of this study was to evaluate lesion type as a component of resistance to *P. lachrymans*. The role of lesion type in *P. lachrymans* resistance was evaluated based upon field disease severity ratings and *P. lachrymans* populations on the foliage of F_3 lines. These lines were genetically homogeneous for either the NH or CH lesion type. The F_3 lines were selected from a cross between resistant line MSU 9402, and the susceptible cultivar, National Pickling. In addition, the development of bacterial populations and lesions over time on parental and F_1 lines was monitored in greenhouse experiments. The disease severity ratings for CH lesion type F_3 lines were generally twice that for NH F_3 lines. The degree of resistance on NH lines approached that of the resistant parent. Eighty-seven percent of the total genotypic variance was due to lesion type. Broad sense heritability estimates in disease severity among F_3 lines within each lesion type were moderately low (.1-.3). In field experiments, the average

bacterial population on CH lines was 100 times that on NH lines. Bacterial populations on cucumber foliage was closely associated with specific lesion sites. Bacterial populations on the resistant parent MSU 9402 increased logarithmically at similar rates to those on the susceptible parent National Pickling, but peak populations were lower. Lesions on both MSU 9402 and National Pickling first appeared 2-3 days after inoculation. Differences in lesion type were distinguishable 5 days after inoculation. Based upon the results of this research, the NH lesion type in response to *P. lachrymans* infection is a major component of resistance to *P. lachrymans*.

Introduction

Angular leafspot of cucumber (*Cucumis sativus* L.), caused by *Pseudomonas lachrymans* (Smith and Bryan) Carsner, is a serious problem of pickling cucumbers grown in humid production areas. Initial *P. lachrymans* infestations of cucumber crops usually are from seed borne bacteria or from overwintered bacteria on plant debris (6, 24, 33, 37). Secondary dispersal throughout the crop is predominantly by rain splash or mechanical means (3, 7, 19, 24, 33). Infection takes place through the stomata, wounds, or other openings in the epidermis. The disease initially appears as small, water-soaked spots which later evolve into characteristic vein-limited angular lesions.

Sources of resistance to *P. lachrymans* have been identified (2, 9). Some resistant cultivars have been developed and are in common use. Use of resistant cultivars results in significantly reduced yield losses under epidemic conditions (30). Chand and Walker (9) reported that disease resistance in cucumber, based on lesion number and size, was quantitatively inherited. Resistant cultivars developed fewer and smaller lesions when infected with _P. lachrymans_.

The type of lesions on resistant lines differs from that on susceptible lines. Susceptible lines typically develop orange-brown necrotic lesions surrounded by chlorotic halos ("CH" chlorotic-halo lesion type). Lesions on resistant lines are typically white or tan with very slight or no chlorotic halo ("NH" non-halo lesion type). The lesion type in reaction to _P. lachrymans_ infection has been found to be controlled by a single gene (15). The NH lesion type reaction associated with resistance, is recessive to the CH lesion type reaction. The NH reaction has been correlated with low disease severity (20) and appears to be an important component of resistance to _P. lachrymans_.

A similar lesion type is also associated with resistance to _Pseudomonas phaseolicola_ in common bean (_Phaseolus vulgaris_). Jensen and Goss (23) noted that small, inconspicuous brown circular spots, lacking the typical chlorotic halo, occurred on inoculated leaves of some bean cultivars found to be resistant in field tests. Several studies on the inheritance of resistance to _P. phaseolicola_ followed (13, 32, 35). Schuster (32) and Walker and Patel (35) defined resistance to race 1 of _P. phaseolicola_ on the basis of lesion type and the absence of systemic chlorosis. After a second race of _P. phaseolicola_ was discovered, Patel and Walker described a tolerant reaction (also based on lesion type) to both races and reported that the tolerant reaction was under independent genetic control (28, 29). Coyne, Schuster, and Gallegos (11) first reported that the foliar lesion reaction and systemic chlorosis reaction were separately controlled by two linked genes. Later, two other components of resistance to _P. phaseolicola_, the pod reaction and a wilting reaction of primary leaves, were found to be controlled by independent major genes (10, 21).

Relatively low bacterial populations in host tissue have been associated with resistance to bacterial diseases (1, 14, 18, 26, 27, 31, 34). Homologous bacteria typically multiply logarithmically in susceptible host tissue to population levels of 10^7-10^9 colony forming units (CFU)/cm^2. Bacteria multiply in resistant tissue at similar or slightly slower rates, but reach a lower maximum population. The bacterial population maxima in resistant tissue are typically 10-100 times less than in susceptible tissue. Chand and Walker (8) studied the multiplication of _P_. _lachrymans_ in resistant cucumber lines PI 169400, susceptible cultivar SMR 18, and their F_1 progeny. Large differences were found in bacterial population levels among these lines at all sampling times after inoculation. At maximum, the bacterial population in susceptible lines SMR 18 was 300 times that in resistant PI 169400. The bacterial population in F_1 plants was intermediate but close to the resistant parent. The number and size of lesions in the resistant parent and F_1 plants were also correspondingly lower.

The purpose of the present study was to evaluate lesion type as a component of resistance to _P_. _lachrymans_. The specific objectives were to:

1. Examine the interrelationship between lesion type (CH or NH) and disease severity in the field.
2. Define the relationship between _P_. _lachrymans_ populations and foliar lesions on cucumber.

Materials and Methods

Bacterial Isolates

An isolate of *P. lachrymans*, D_1, originally supplied by O.M.B. dePonti of Instituut voor de Veredeling van Tuinbouwgewassen (IVT), Wageningen was used for lesion type classification and greenhouse conducted experiments. A spontaneous mutant (NR 22) resistant to 500 ug/ml nalidixic acid (NA) and 50 ug/ml rifampin was used for field studies. Use of NR 22 allowed the selective isolation of this isolate on antibiotic-containing media, greatly reducing bacterial contamination.

The *P. lachrymans* isolate, NR 22, was derived from isolate D_1 using selective plating methods. Isolates were initially selected for resistance to NA using the gradient plate technique of W. Szybalski (4). Petri plates were poured with two layers of Kings B agar, adjusted to pH 7.4 with 1N NaOH to prevent precipitation of nalidixic acid. The bottom layer, supplemented with 1 mg/ml NA was allowed to harden with the plate in a slanted position. A second layer of unsupplemented Kings B medium was poured with the Petri plate in the normal horizontal position to just cover the highest part of the slanted layer. This technique established a uniform concentration gradient of the antibiotic. Resistant mutants were obtained by plating a 24 hour culture of approximately 10^9 bacteria over the freshly poured bilayer gradient plates. As colonies appeared at the lower concentration of NA, they were continually streaked towards the highest concentration. Only NA resistant cells were able to form colonies at the higher antibiotic concentrations. Colonies growing at the higher concentrations were subcultured onto Petri plates of Kings B agar (pH 7.4) containing 500 ug/ml NA.

Approximately 10^9 cells of P. lachrymans previously selected for NA resistance were plated on Kings B medium (pH 7.2) supplemented with 50 ug/ml rifampin (Weller and Saettler, 1978). The gradient plate technique was not necessary in order to obtain resistant isolates. Several colonies per plate appeared, and these rifampin resistant colonies were subcultured.

Selected isolates were checked for resistance to both antibiotics by plating on Kings B agar pH 7.4 + 50 ug/ml NA + 50 ug/ml rifampin (KBNR media). The observed colony type and growth rate on Kings B was not distinguishable from the parent isolate, D_1. In addition, colonies were checked for oxidase reaction and flourescence under ultraviolet light prior to testing for virulence on cucumber.

Virulence of resistant isolates was tested using the following two inoculation techniques. Cucumber cotyledons were inoculated with a bacterial suspension of 10^7 colony forming units (CFU)/ml using the "multiple toothpick" inoculation procedure described below; additionally, first and second-true leaves were injected with 10^5 CFU/ml using a hypodermic syringe. In both cases, virulence was rated according to lesion size and severity of chlorotic halo on the cucumber cultivar, National Pickling. Antibiotic resistant isolates were compared to D_1 and other wild type isolates. Only slight differences in virulence were observed among resistant isolates. The NR 22 isolate was selected for use in this study because it appeared identical to the parental isolate, D_1, in all aspects other than antibiotic resistance. Single colonies of NR 22 were sequentially transferred three times on KBNR media prior to field inoculations, insuring inoculum uniformity. Bacterial isolates were maintained for long term storage in sterile distilled water at 4°C or on Kings B Petri plates at 4°C for up to 2 weeks. Prior to all inoculations, bacteria were increased for 24 hours in the modified liquid medium of Husain and Kelman (22), as modified by Keen et al. (25). Immediately before inoculations, bacterial cultures were diluted with phosphate buffer (0.01M, pH 7.2) and adjusted turbidimetrically using a Bausch and Lomb Spectronic 20 colorimeter, and by serial dilution as needed to obtain the desired inoculum concentration. Inoculum concentration was checked with dilution plate counts taken immediately prior to inoculation.

Lesion Type Classification

Prior to the field and/or greenhouse experiments, parental, F_1, and F_3 generations were each classified according to lesion type in response to *P. lachrymans* infection. A multiple puncture inoculation technique was used. Inoculations were made with eight round, wooden toothpicks fastened with a rubber band. Advantages of this inoculation technique included the ability to clearly distinguish between lesion types and a lack of escapes. The disposability of the toothpicks was especially advantageous when testing various isolates of the pathogen for virulence on cucumber. The underside of cotyledons of 10-day-old cucumber seedlings were punctured with toothpicks dipped in a suspension of 10^8 CFU/ml. Cotyledons were supported with the index finger during inoculation. For inoculation, seed was sown in flats of greenhouse soil mix and fertilized once with 20-20-20 water soluble fertilizer to provide 300 ppm N. Seeded flats were placed on a greenhouse bench supplemented with bottom heat (30°C soil temperature) to ensure rapid, uniform germination. The plants were inoculated 10 days after sowing, when the first true leaf was just emerging. Seedling flats were moved to a greenhouse bench after inoculation. Each seedling was classified for lesion type 7 days after inoculation. All inoculated plants of National Pickling and reciprocal F_1 plants developed typical CH lesions. All inoculated plants of MSU 9402 developed typical NH lesions.

Selection of F_3 Lines

When studying the effect of a single character, such as lesion type, it is necessary to differentiate between the specific character effects and effects due to other traits. To permit differentiation, two groups of F_3 lines were chosen for field studies. These lines were genetically homogeneous for either the NH or CH lesion type. The F_3 lines were selected from a cross between the *P. lachrymans* resistant line, MSU 9402 (9402), and the susceptible cultivar National Pickling (NP). Both parental lines were inbred several generations prior to this study. Since lesion type is controlled by a single gene, three types of F_3 lines appear in a ratio of 1:2:1, respectively: (1) homogeneous for the NH lesion type, (2) segregating, or (3) homogeneous for the CH lesion type. Due to the random segregation of other genes, selected groups of F_3 lines only have lesion type as a common distinguishing character.

Field Studies

Disease severity and bacterial populations were monitored in parental, F_1, and F_3 populations in two field plantings (in late summer, 1980) at the Botany and Plant Pathology research farm, Michigan State University, East Lansing, Michigan. Seed for two field plantings were germinated in the greenhouse in 6 cm peat pots and transplanted two weeks later into the field with 45 cm square spacing. Field planting 1 (planted July 17, 1980) including 24 lines; 8 NH lines, 12 CH lines, reciprocal F_1's and parents. Field planting 2 (planted August 18), included 14 lines: five randomly selected NH and CH lines, reciprocal F_1's and parental lines. A randomized complete block design with 12 and 6 single plant replications was used for field plantings 1 and 2, respectively. Two direct seeded border rows were planted around each field plot 2 weeks prior to transplanting. Standard Michigan cultural practices were used.

Each field planting was inoculated with approximately 10^8 CFU/ml. Inoculum was misted on the foliage to run off with a hand pump back pack sprayer. Care was taken not to infiltrate any leaf tissue with the inoculum. Inoculations were made in the a.m. while foliage was wet with dew or after a light irrigation. After inoculations, field plantings were frequently lightly irrigated to ensure good angular leafspot development.

Field planting 1 was inoculated August 11 and 15 at the 10-14 leaf stage. Typical angular lesions appeared August 14. Field planting 2 was inoculated August 23 and September 6 at the 2-3 leaf stage and first symptoms appeared August 28.

Field plantings were rated for foliar disease severity on a relative scale of 1-10, the score of 1 denoting no visible lesions, and 10 denoting severe infection including coalescing of lesions and tearing of leaf tissue on 50% or more of the foliage. Both lesion types, with or without chlorotic halos, were treated equally in rating disease severity. In field planting 2, the actual number of lesions per plant was also counted and the number of lesions per leaf was calculated.

The disease severity scores for F_3 lines in field planting 1 and the mean severity scores (over three growth stages) for F_3 lines in field planting 2 were partitioned into variance components due to lesion type (V_T), and line within type ($V_{L(T)}$). The $V_T + V_{L(T)}$ is equivalent to the total genetic variance for disease severity within these populations. Variance components were claculated by equating mean square values from the analysis of variance for type, and for line nested within type to their expected mean squares and solving for the specific variance component.

Genotypic and error variances for disease severity were calculated for each class of F_3 lines. These variance components were calculated by equating the appropriate expected mean squares to the error mean square and the mean square for F_3 lines. Heritability of disease severity within each lesion class was computed using the formula:

$$\text{Heritability} = V_G/(V_G + V_E)$$

where V_G and V_E are the genotypic and error variances, respectively. This formula estimates heritability both in the broad sense and on a per plot basis.

Bacterial populations in CH and NH classes were estimated in both field plantings using conventional serial dilution plate count methods. All plants were sampled at least one week after inoculation in order to allow bacterial populations to develop and equilibrate. Systematically selected leaf discs were removed from individual plants and cooled with ice until grinding. Each sample of tissue was ground in 0.01 M phosphate buffer, pH 7.2. Ten-fold serial dilutions of ground suspensions were made in buffer, and 0.1 ml aliquots were pipetted onto Petri plates of Kings B medium supplemented with 50 ug/ml rifampin, 400 ug/ml nalidixic acid and 50 ug/ml cycloheximide. Colonies were counted after three days incubation at approximately 22°C.

Greenhouse Studies

Lesion number and bacterial population were monitored on parental and F_1 populations grown under greenhouse conditions. Cucumber seeds were sown in an artificial peat lite medium in 6 cm peat pots and transplanted 14 days later into 11 cm clay pots. Seedlings were fertilized with 20-20-20 at 300 ppm N after germination and transplanting. Plants were grown under fluorescent lighting at approximately 18°C night and 24°C day temperatures. All experiments were conducted in the winter and early spring. Since minimal contamination occurred in the greenhouse, antibiotic resistance and selective plating was not necessary. Therefore, _P_. _lachrymans_ isolate D_1 was used. In all experiments, the second leaf of plants of uniform leaf size and development was inoculated. Plants were inoculated by thoroughly infiltrating the lower leaf surface with a bacterial suspension. A Devilbis atomizer held at 2 cm from the lower leaf surface was used for infiltration. Infiltration was facilitated by covering the plants with a plastic tent the evening prior to inoculation. Leaf tissue appeared completely watersoaked after infiltration. Inoculated leaves were washed with a stream of distilled water and the plants were placed on greenhouse benches. Bacterial populations were monitored in time course experiments at 24-48 hour intervals after inoculation by removing 1.15 cm diameter leaf discs (#8 corkborer) in a systematic pattern. Leaf samples were taken immediately after watersoaking disappeared and up to 6 days after inoculation. The samples were ground in buffer and the suspensions serially diluted and plated on unsupplemented Kings B medium. Colonies were counted after 3 days incubation at approximately 22°C to estimate CFU/cm^2 of leaf tissue.

With low inoculum concentrations (10^3 cells/ml), specific lesions developed on inoculated leaves. These were especially visible on the upper leaf surface. An 11.34 cm^2 disc (1½" cookie cutter) was removed from the center of the leaf 11 days after inoculation and the number of lesions on the disc were counted. The disc was then ground in buffer and CFU/cm^2 was estimated as above.

Results

Field Studies

No angular leafspot symptoms were present prior to inoculation. All cucumber lines in field plantings 1 and 2 developed typical angular lesions after inoculation. Both the resistant line 9402, and F_3 lines within the NH lesion class developed typical NH lesions without a surrounding chlorotic halo. NP, reciprocal F_1 lines and CH class F_3 lines produced CH lesions, easily identified by the presence of a chlorotic halo surrounding the lesion. Chlorotic halo intensity on the F_1 lines was sometimes less than that on NP.

Disease severity in field planting 1 was evaluated (measured on an entire plant basis) on August 21, 10 days after inoculation when plants were at the 11-16 node stage. Analysis of variance for disease severity scores revealed highly significant differences both between the NH and CH lesion type classes and among lines within each lesion class (Table 1). The mean severity score of the NH class (4.02) fell between the mid-parent value (4.79) and the resistant parent 9402 value (2.50).

Table 1. Mean squares from analysis of variance for disease severity ratings on F_3 lines homogeneous for CH or NH lesion types in field planting 1. At evaluation, plants had 12-16 nodes on main axis.

Source	Degrees of Freedom	Mean Square
Replication	11	4.30
Lesion type	1	324.90**
Line within type	18	9.58**
Error	207	1.99

**Significant at .01 level.

The mean severity score for the CH class (6.40) fell between the mid-parent value and the susceptible parent, NP (7.08). There was some overlap of disease severity ratings between classes. 9402 had significantly lower disease severity ratings than either reciprocal F_1 plants or NP (Table 2). F_1 scores fell above the mid-parent value indicating a degree of dominance for susceptibility.

Experimental lines in field planting 2 were evaluated for disease severity at three time periods. The initial disease severity evaluation, at the 3 node stage, was made on the first and second leaves August 31, eight days after inoculation. Leaves above the second node were evaluated at the 6 node stage; and leaves above node five and on laterals were evaluated at the 10 node stage.

As in field planting 1, differences between lesion type classes were significant (Table 3). There were also differences among lines within each lesion class at all growth stages. At the 3 and 10 node stage no differences among lines within either type were found. Lesion type x time interaction was significant. The mean disease severity of CH lines increased slightly over time; whereas, severity ratings on NH lines decreased with maturity (Table 4).

The mean disease severity ranking of individual F_3 lines within the NH class in field planting 2 corresponded closely to the ranking of these same lines in field planting 1. F_3 line N3 in both field planting 1 and 2 had the most severe symptoms of all the NH lines. In contrast to the NH lines, interplot rankings did not correspond closely for the CH lines.

The total number of lesions per leaf and lesions $\geq$ 5mm diameter were counted at the 10 and 14 node stage, respectively (Table 4).

Table 2. Mean disease severity ratings on parental, F$_1$, and F$_3$ lines in field planting 1. Individual plants were rated at the 12-16 node stage for disease severity. Disease was rated on a 1-10 scale where 1 = no symptoms and 10 = lesions covering 50 percent or more of leaf tissue.

Parental, F$_1$ lines**	9402	9402 x NP	NP x 9402	NP
$\bar{X}$ severity	2.50a	6.83b	6.75b	7.08b

F$_3$ lines

**

NH lines**	N4	N6	N1	N5	N2	N8	N7	N3	$\bar{X}$
$\bar{X}$ severity^x	2.58a	3.08ab	4.00bc	4.08bc	4.08bc	4.15bc	4.92bc	5.17bc	4.02

CH lines**	C7	C6	C11	C2	C5	C12	C8	C3	C1	C10	C4	C9	$\bar{X}$
$\bar{X}$ severity	4.75a	5.42ab	5.50ab	6.08bc	6.08bc	6.08bc	6.42bcd	6.83cde	7.00cde	7.33de	7.50de	7.75e	6.40

**Highly significant differences (.01 level) among lines and between lesion classes as determined by analysis of variance.

[x] Mean separations at .05 level by Duncan's Multiple Range Test.
Within each row, means followed by the same letter are not significantly different as determined by Duncan's Multiple Range Test (.05 level).

Table 3. Mean squares from analysis of variance for disease severity ratings on F_3 lines homogeneous for CH or NH lesion types and over three growth stages[x] in field planting 2.

Source	Degrees of Freedom	Mean square
Replication	5	2.52
Lesion Type	1	206.94**
Line (Type)	8	4.98**
Error (a)	45	1.50
Time	2	5.22*
Type x Time	2	9.44**
Time x Line (Type)	16	1.75 n.s.
Error (b)	97	1.35

*, ** Significant at the 0.05 and 0.01 probability levels, respectively.

[x] 3, 6, and 10 node stage: approximate number of nodes on main axis.

Table 4. Mean ratings of F_3 lines at specific growth stages in field planting 2 for disease severity, lesions per leaf and $\log_{10}$ CFU/cm^2 leaf tissue. Disease severity ratings based on a scale of 1-10 where 1 = no symptoms and 10 = 50 percent or more necrotic leaf area.

F_3 Lines	Disease severity ratings[u]				Necrotic lesions/leaf[u] Total	Necrotic lesions/leaf[u] ≥ 5mm	log CFU[v] cm^2 leaf tissue
NH Lines	3 nodes[w]	6 nodes	10 nodes	$\bar{X}$	10 nodes	14 nodes	11 nodes
N1	2.67a	1.67a[x]	1.33a	1.89a	1.67a	0.90a	3.97a
N2	3.00a	2.50a	2.00a	2.50ab	3.25a	1.12a	4.31a
N3	3.67a	4.00b	1.83a	3.17b	3.28a	1.18a	5.50a
N4	3.50a	1.67a	1.33a	2.17ab	1.12a	0.52a	4.87a
N5	2.50a	3.00ab	2.00a	2.50ab	3.20a	0.42a	3.62a
$\bar{X}$ column	3.07	2.57	1.70	2.44	2.50	0.83	4.45
CH Lines							
C1	4.67a	6.00b	4.83a	5.17b	12.28a	3.23a	6.71a
C2	4.33a	3.67a	4.00a	4.00a	13.25a	3.25a	6.35a
C3	4.67a	3.83a	4.67a	4.06a	10.83a	2.47a	6.24a
C4	4.87a	4.33a	4.50a	4.56ab	10.22a	2.50a	6.22a
C5	5.00a	5.00ab	5.50a	5.17b	17.23a	4.87b	6.79a
$\bar{X}$ column	4.50	4.57	4.70	4.59	12.76	3.26	6.46
F value[y] Nested Line	n.s.	**	n.s.	**	n.s.	**	n.s.
F value[z] Type	**	**	**	**	**	**	**

[u] Disease severity and lesion number data are means of six single plant replications.

[v] CFU/cm^2--colony forming units/cm^2 (means of three replications).

[w] Growth stage--approximate number of nodes on main axis.

[x] Within each lesion class and each column, means followed by the same letter are not significantly different as determined by Duncan Multiple Range Test (.05 level).

[y] Scores of lines within CH or NH types significantly different at .01 level (**) or were not significant (n.s.) by Analysis of Variance F Test.

[z] Scores of NH lines significantly different from CH lines at the .01 significance level of Analysis of Variance F Test.

Lesions on leaves one and two were not evaluated. Again, there was a large difference in both the total number of lesions and the number of lesions $\geq$ 5mm diameter between lesion classes. There was not a difference in total lesion number among lines within each lesion type. Only within the CH type was there a significant difference in number of lesions $\geq$ 5mm diameter.

Total lesions were counted primarily to validate the subjective disease severity ratings. The number of lesions $\geq$ 5mm were also evaluated to determine if there was a difference in number of large lesions, in addition to total lesions, between lesion classes. Thirty-three and twenty-six percent of total lesions were $\geq$ 5mm diameter in NH and CH F_3 lines, respectively. This difference was not significant (T test).

Correlation coefficients were calculated for individual plant disease severity ratings and the number of lesions per leaf at the 10 node stage. Correlation coefficients were .67 and .58 for NH and CH populations, respectively. The pooled correlation coefficient for the two populations was .81 (Table 5).

Colony forming units per cm^2 of leaf tissue in F_3 lines were estimated from 3.8 cm diameter leaf discs. A single sample was taken from leaf five or six on three plants per line (blocks 1-3) and repeated each day for 3 days. As with disease severity estimations and lesion counts, there was a large difference in bacterial populations between lesion classes. The mean bacterial population of CH F_3 lines was 100-fold greater than the mean bacterial population in NH F_3 lines. There were not significant differences in bacterial populations among lines within each type. Bacterial populations were highly correlated

Table 5. Simple correlation coefficients between disease severity ratings and total necrotic lesion number as rated on individual plants at the 10 node stage in field planting 2.

NH[x] F_3 lines	CH[y] F_3 lines	Pooled F_3 lines
.665**	.575**	.806**

**Significant at .01 level.

[x] Non-halo lesion type.

[y] Chlorotic-halo lesion type.

with mean severity and lesion number among CH lines, but poorly correlated among NH lines (Table 6).

In addition to the F_3 lines, parental and reciprocal F_1 lines were evaluated in field planting 2 for disease severity, lesion number and bacterial population (Table 7). All ratings of 9402 were less than half those of NP. The F_1 ratings, relative to those in field planting 1, were lower and fell very close to mid-parent values. In no case were there significant differences in disease severity, lesion number, or bacterial populations between reciprocal F_1 lines. Unlike NH class F_3 lines, the interaction between growth stage and the resistant parent 9402 was not significant. However, the same trend was reflected: disease severity ratings on 9402 decreased with increasing maturity.

In field planting 2, mean ratings of NH class F_3 lines for severity, lesion number, and CFU/cm^2 were very close to those of the resistant parent 9402. Mean rating for CH class F_3 lines ranged from mid-parent values to values for National Pickling. In field planting 2, there were large differences between the highest NH class values and lowest CH class values for disease severity, lesion number, and bacterial populations (Table 4). Some overlap may have occurred if all CH lines of field planting 1 had been included in field planting 2. None of the three lowest ranked CH lines in field planting 1 were included among the randomly selected CH lines in field planting 2.

The disease severity scores for F_3 lines in field planting 1 and the mean severity scores (over three growth stages) for F_3 lines in field planting 2 were partitioned into variance components due to lesion type (V_T), and line within type ($V_{L(T)}$) (Table 8). Based upon the equation V_T / (V_T + $V_{L(T)}$), 81 and 93% of the genetic variance for

Table 6. Simple correlation coefficients of mean number CFU/cm^2 with mean disease severity ratings and mean number of lesions per leaf on F_3 lines in field planting 2.

	Disease severity		Necrotic lesions/leaf	
	3-10 node stage	10 node stage	Total	$\geq$ 5mm dia.
CH Class F_3 lines	.878*	.794+	.797+	.858*
NH Class F_3 lines	.808+	.102 n.s.	.281 n.s.	.512 n.s.
All F_3 lines	.890**	.861**	.892**	.928**

+, *, **, n.s.: Significant at the .10, .05, .01 level and not significant, respectively.

Table 7. Mean ratings of parental and F_1 lines at specific growth stages in field planting 2 for disease severity, lesions per leaf and $\log_{10}$ CFU/cm^2 leaf tissue.

	Disease severity ratings[u]				Necrotic Lesions/leaf[u] Total	≥5 mm	log CFU/cm^2[v]
Line	3 nodes[w]	6 nodes	10 nodes	$\bar{X}$	10 nodes	14 nodes	11 nodes
9402	4.17a[x]	2.50a	1.67a	2.78a	3.28a	1.30a	4.39
9XN	4.00a	4.17b	5.00b	4.39b	14.82bc	2.52a	6.07
NX9	4.83a	3.83b	3.83b	4.17b	9.03ab	2.07a	6.70
NP	5.50a	5.67b	5.33b	5.50c	18.92c	4.68b	6.33
F value[y]	--	--	--	**	*	**	n.s.

[u] Disease severity and lesion number data are means of six single plant replications. Disease severity rates on scale of 1-10 where 1 = no symptoms and 10 = 50 percent or more necrotic leaf area.

[v] CFU/cm^2--means of three replications. Estimated from three 3.8 cm diameter leaf discs per replication. Samples were from leaf five or six on three plants per line (blocks 1-3). Sampling was repeated each day for 3 days.

[w] Growth stage: approximate number of nodes on main axis.

[x] Within each column, means followed by the same letter are not significantly different as determined by Duncan's Multiple Range Test (.05 level).

[y] Mean scores among lines within each column are significantly different at the .05 level (*), .01 level (**) or not significantly different (n.s.) by F test. Growth stage or line x growth stage not significant for disease severity ratings as determined by F test.

Table 8. Genotypic variances of F_3 lines for disease severity partitioned into variance due to lesion type and due to F_3 lines nested within type. $V_T + V_{L(T)}$ = total genotypic variance.

	V_{Type}	$V_{Line(Type)}$	$V_T/(V_T + V_{L(T)})$
Planting 1[u]	2.74	.63	.81
Planting 2[v]	2.16	.17	.93

[u] Field planting 1: Variance calculations from disease severity rated once at approximately the 11-16 node stage.

[v] Field planting 2: Variance calculations from mean of three ratings at the 3, 6, and 10 node stage.

disease severity was attributed to differences between lesion types in field planting 1 and 2, respectively.

Heritability values for disease severity within each F_3 lesion type class ranged from .10 to .30 (Table 9). Within each field planting, heritability was higher among CH lines than among NH lines. The heritability estimates for field planting 1 were possibly more accurate than field planting 2 due to the larger sample size.

Leaf tissue was sampled a second time from 9402, 9402 x NP, and NP to determine the relationship between bacterial populations and lesions. A split-plot design was used with cultivar as the main plot, and sample types (with or without lesions) as the sub-plots. Each experimental unit homogenized for bacterial population estimations consisted of nine 0.9 cm diameter discs--three from each of three randomly selected plants per line in this study. Each sub-plot experimental unit, with or without lesions, was taken from the same individual leaves. At isolation, plants were 12 nodes in length. All samples were taken from leaves five, six, or seven. Samples without lesions were taken from green tissue without visible necrosis. For samples with lesions, each leaf disc included a single lesion of approximately 3 mm in diameter. The average bacterial population at lesions was 1000 times that of non-lesion areas (Table 10). The bacterial population at lesion sites on NP and 9402 x NP was 10 times as great as the bacterial population at lesion sites on 9402. No significant difference in bacterial populations was found among lines in non-lesioned sites.

Greenhouse Studies

Infiltration of cucumber leaves with 1.5×10^5 CFU/ml resulted in an initial population of about 2.8×10^2 CFU/cm^2 (Figure 1). Bacteria

Table 9. Genotypic and environmental (error) variance components and broad sense heritability estimates of F_3 lines within CH and NH lesion type classes for disease severity in field plantings 1 and 2.

F_3 lines	Exp. No.	V_G	V_E	H^2
N 1-8	1^x	.52	2.56	.17
C 1-12	1	.70	1.66	.30
N 1-5	2^y	.12	1.13	.10
C 1-5	2	.23	1.53	.13

[x] Field planting 1: variance calculations from disease severity rated once at approximately the 11-16 node stage, 12 replications.

[y] Field planting 2: variance calculations from mean of three ratings at the 3, 6, and 10 node stage, 6 replications.

Table 10. Log_{10} mean number of CFU/cm^2 at lesion or non-lesion leaf sites^z on parental and F_1 lines.

	Main Plots*			Sub-plots**
	9402	9402 x NP	NP	$\bar{X}$ All Lines
+ Lesion	5.90a^y	6.98b	7.04b	6.64
- Lesion	3.33a	2.90a	3.74a	3.32
$\bar{X}$	4.61a	4.94ab	5.39b	

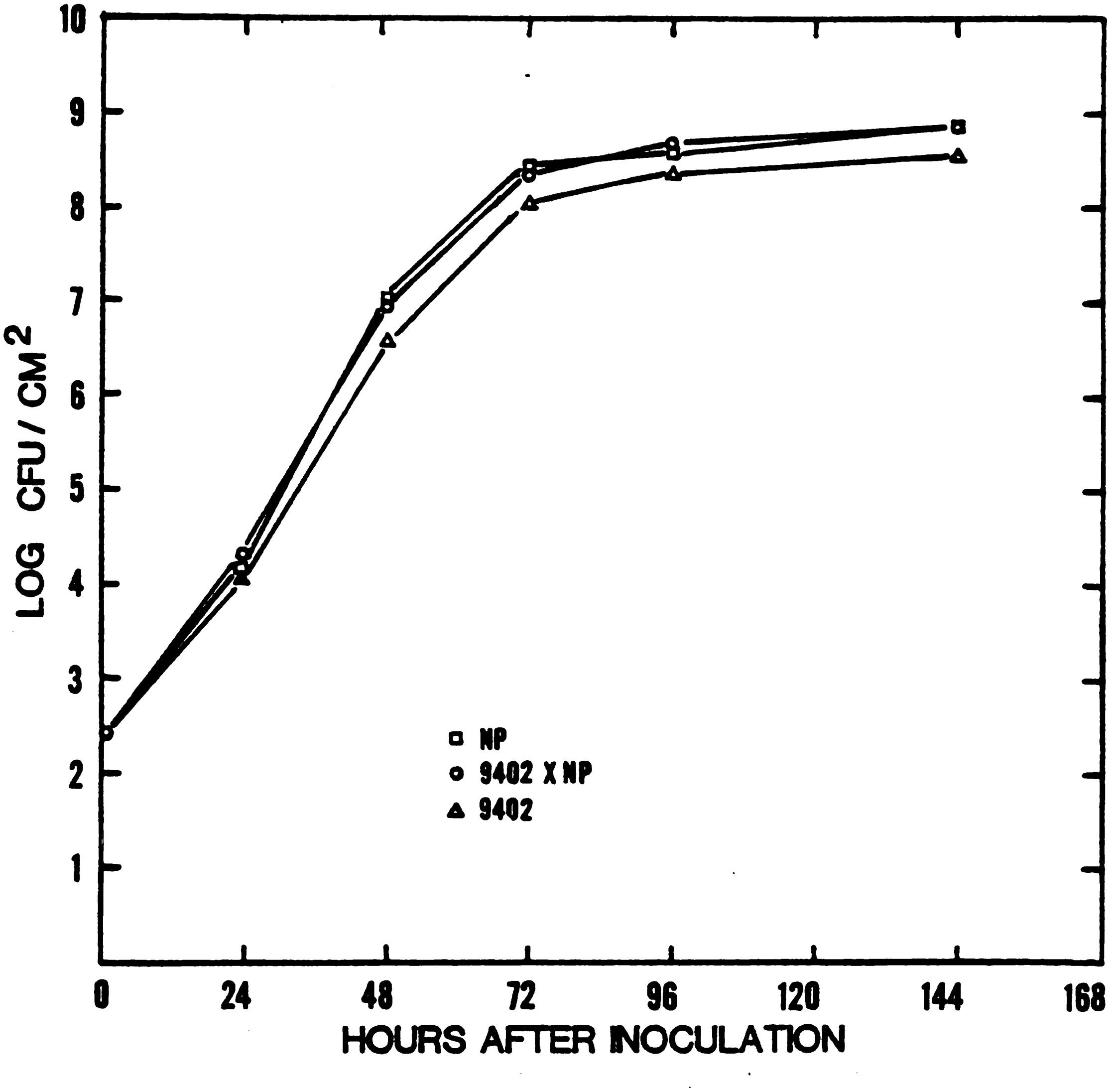
*Significant difference between lines by F test; lines x sample type (+ or - lesion) not significant.

**Highly significant difference between samples with or without lesions.

[y] Cultivar means followed by the same letter are not significantly different as determined by Duncan's Multiple Range Test.

[z] Leaf tissue was sampled with .9 cm diameter discs containing either a single lesion or no lesion. Leaves 5, 6 or 7 were sampled at the 12 node growth stage. Corresponding sub-plot samples (+ or - lesions) were from the same leaf in all cases.

Figure 1. Multiplication of Pseudomonas lachrymans in leaves of resistant MSU 9402 (9402), susceptible National Pickling (NP) and 9402 x NP. The second leaf of three-leaf plants was infiltrated with 1.45 x 10^5 CFU/ml using a Devilbis atomizer at time = 0. Values are averages of two experiments, three replications per experiment.

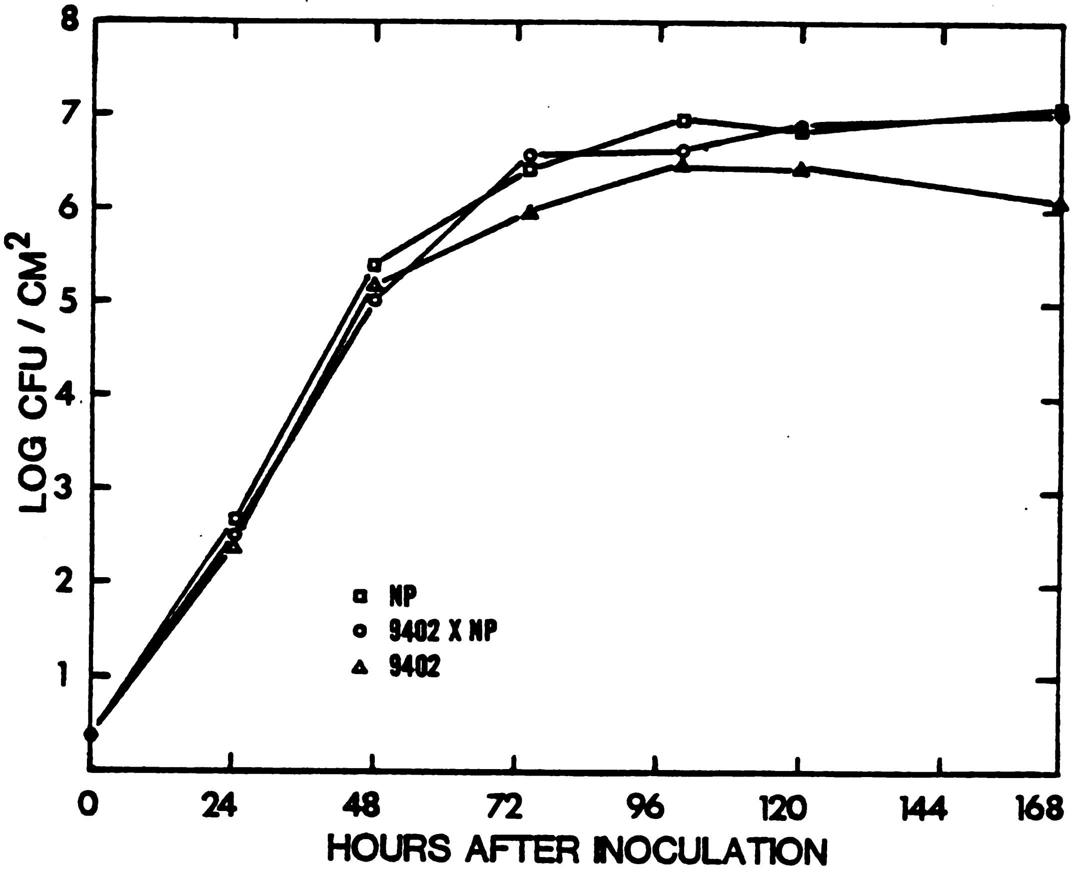

LOG CFU / CM²
10
9
8
7
6
5
4
3
2
1
0
24
48
72
96
120
144
168
HOURS AFTER INOCULATION
□ NP
○ 9402 X NP
△ 9402

in NP and 9402 x NP increased in a typical log phase manner with no apparent lag time. The log phase ended 48-72 hours after inoculation and the bacterial population leveled out at approximately 4.26 x 10^6 CFU/cm^2 until 144 hours after inoculation. Bacteria on 9402 multiplied at a slightly lower rate and leveled off at 2.3 x 10^8 CFU/cm^2: a peak population two-fold less than NP and 9402 x NP. Similarly, when both parental and F_1 lines were infiltrated with a lower inoculum concentration (1.2 x 10^3 CFU/ml), bacteria in 9402 multiplied at the same rate as NP and 9402 x NP (Figure 2). Bacterial populations in all lines increased for 48 hours at a rate almost identical to that observed with the higher inoculum concentration. After 48 hours, bacterial populations leveled off to approximately 6 x 10^6 CFU/cm^2. Regardless of inoculum concentration, peak bacterial populations (at 96 hours) were greater than the initial population by a factor of 2 x 10^6 (Figures 1 and 2).

When plants were infiltrated with the higher inoculum concentration, pinpoint water-soaked spots first appeared on NP and 9402 x NP 48-72 hours after inoculation. Similar pinpoint spots appeared on 9402 72 hours after inoculation. By 96 hours, water-soaked areas expanded, coalesced, and collapsed into irregular necrotic areas between major veins. This occurred on all lines, but less necrosis was observed on 9402. A slight, general chlorosis was observed on NP and 9402 x NP at 96 hours post-inoculation, which intensified by 120 hours. At approximately 120-144 hours, necrotic tissue began to dehydrate on all lines.

Lesions developed over a similar time course for both high and low inoculum concentrations, however, lesions did not coalesce with the latter. At the lower inoculum concentration, lesions in all three lines first appeared at 48 hours as water-soaked, pinpoint spots. By

Figure 2. Multiplication of _P. lachrymans_ in leaves of resistant MSU 9402 (9402), susceptible National Pickling (NP) and 9402 x NP. The second leaf of three-leaf plants was infiltrated with 1.16 x 10^3 CFU/ml using a Devilbis atomizer at time = 0. Values are means of three replications, two plants per replication.


LOG CFU / CM^2
8
7
6
5
4
3
2
1
0
24
48
72
96
120
144
168
HOURS AFTER INOCULATION
□ NP
○ 9402 X NP
△ 9402

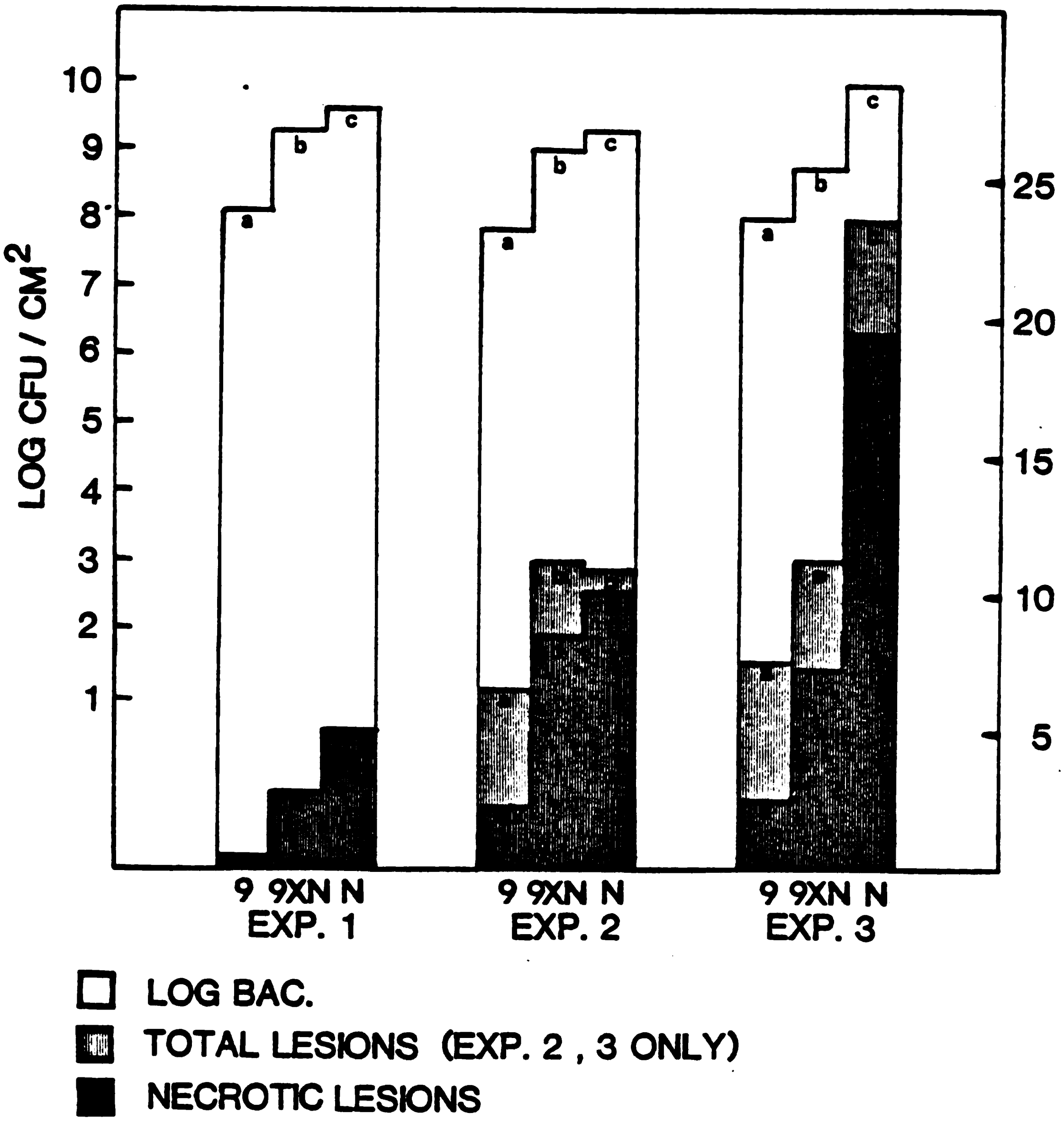
96 hours all lesions were light green round spots, 1mm in diameter with a 0.2mm pinpoint necrotic center. From 120 to 168 hours after inoculation, lesions on NP and 9402 x NP increased to approximately 2-3mm diameter with the periphery of the necrosis turning an orange-brown color. Lesions on 9402 rarely enlarged beyond 1mm in diameter, and remained a light green color. The white, necrotic centers on 9402 enlarged slightly or not at all. In the greenhouse environment, lesions of both classes were not vein-limited as is typical in the field.

The number of necrotic lesions per 20 cm^2 of leaf tissue was counted at 96 hours after inoculation with 1.2×10^3 CFU/ml. The mean number of ncrotic lesions/20 cm^2 was: NP = 42.3, 9402 x NP = 28.3, and 9402 = 3.8.

At 192 hours after inoculation, three .48 cm diameter discs containing a single lesion or none were taken from each plant. The isolations were replicated twice with three plants per replications. With one lesion per disc, the mean number of CFU/cm^2 was: NP = 2.5×10^7, 9402 x NP = 1.8×10^7, and 9402 = 4.1×10^6. All samples without lesions had less than 10^4 CFU/cm^2.

Colony forming units, necrotic lesions and total lesions per cm^2 of leaf tissue 11 days after infiltration for three experiments were determined (Figure 3). Inoculum concentrations for experiments one, two and three were 1.8×10^3, 5.0×10^3, and 7.1×10^3 CFU/ml, respectively. Individual necrotic lesions developed after inoculation in these experiments similar to the inoculations with 1.2×10^3 CFU/ml described above. Lesions on 9402 x NP ranged in intensity both among experiments and among plants within an experiment. In experiment one, lesions on the hybrid were as intense as those on National Pickling.

Figure 3. Necrotic and total lesion number/cm^2 and $\log_{10}$ CFU/cm^2 on MSU 9402 (9), National Pickling (N) and on MSU 9402 x National Pickling (9 x N) at day 11 after bacterial infiltration. Second leaf of three-leaf plants was infiltrated with 1.8×10^3, 5.0×10^3 and 7.1×10^3 CFU/ml for experiments 1-3 respectively. Values are means of four replications. Means of each character within same experiment with same letter are not significantly different at p = .05 by Duncan's Multiple Range Test.


LOG CFU / CM²
10
9
8
7
6
5
4
3
2
1
LESION NO. / CM²
25
20
15
10
5
a
b
c
9 9XN N
EXP. 1
9 9XN N
EXP. 2
9 9XN N
EXP. 3
LOG BAC.
TOTAL LESIONS (EXP. 2 , 3 ONLY)
NECROTIC LESIONS

In experiments two and three, there was more variability in F_1 lesion intensity. F_1 lesions ranged from identical to NP to only slightly more conspicuous than 9402. Necrotic lesions on MSU 9402 were uniformly light green with pinpoint white, necrotic centers.

Bacterial populations were significantly different among lines in experiments one and two ($p = .01$) and in experiment three ($p = .05$). Average bacterial populations over all three experiments were: NP = 3.4×10^9, 9402 x NP = 8.9×10^8, and 9402 = 8.5×10^7. In these experiments, the mean bacterial populations in NP were 40 fold greater than the population in 9402 eleven days after inoculation. This difference among lines was much greater than the 2-10 fold difference seen at four days after inoculation (Figures 1 and 2). There were also large differences in the number of lesions among lines, with the F_1 lines usually intermediate (Figure 3). Lesions counted as necrotic had typical necrotic centers. Total lesions were counted by holding leaf discs up to a background light. This technique permitted non-necrotic, immature lesions to be easily identified. The percentage of the total lesions that was necrotic was calculated from experiments two and three. Only 36% of the total lesions on 9402 were the larger necrotic lesions; whereas 69 and 88% were necrotic on 9402 x NP and NP, respectively. Correlation coefficients between the number of bacteria and number of necrotic and total lesions across all three lines, were highly significant at .71 and .85, respectively (Table 11). Within lines 9402 and 9402 x NP there was little if any correlation between number of bacteria and lesion number.

Table 11. Simple correlation coefficients of the number CFU/cm^2 with the number of lesions/cm^2 in individual leaf samples of greenhouse grown plants 11 days after leaf infiltration with approximately 4.5 x 10^3 CFU/ml inoculum.

Line	Necrotic Lesions	Total Lesions
MSU 9402	-.240 n.s.	-.221 n.s.
9402 x NP	-.435 n.s.	.534 n.s.
National Pickling	.613 *	.885 **
All Lines	.711 **	.852 **

*, **, n.s.: Significant at the .05, .01 level and not significant, respectively.

Discussion

Compared to National Pickling (CH lesion type) MSU 9402 (NH lesion type) showed high levels of resistance to Pseudomonas lachrymans in both field and greenhouse environments. The F_1 plants were intermediate in disease severity, but biased towards the susceptible parent, National Pickling. There was not a significant difference in disease severity between reciprocal F_1 lines which indicated no maternal effects for resistance to P. lachrymans.

Based upon a previous inheritance study, a single gene controls the expression of lesion type (15). In this study, the importance of this single gene as a component of resistance to P. lachrymans was determined by a comparative study of F_3 lines homogeneous for either the NH or CH lesion type. The F_3 lines were used to ascertain the effect of lesion type on disease resistance since the parental lines had different genetic backgrounds. The F_3 lines were selected at the seedling stage only on the basis of lesion type, and unlinked genes were assumed to segregate randomly between the two lesion classes. Differences in disease severity between the two groups of F_3 lines were due to lesion type.

The disease severity of NH lines remained consistenlty lower than CH lines throughout several growth stages. Other studies have shown that resistance to bacterial diseases varies with maturity (8). Disease ratings of CH F_3 lines, NP, and F_1 lines were relatively stable over three growth stages; in contrast, disease ratings of NH F_3 lines and MSU 9402 decreased with increasing maturity.

In order to quantify the importance of the lesion type as a component of resistance to _P. lachrymans_, genotypic variance of disease severity for both field experiments was partitioned into variance components due to lesion type and due to F_3 lines nested within type. Eighty one and ninety-three percent of the total genotypic variance in field plantings 1 and 2 were accounted for respectively by lesion type, indicating that NH lesion type is a major component of resistance to _P. lachrymans_.

Heritability estimates among F_3 lines within each lesion class were medium to low (.1-.3). Even so, heritability estimates on a per plot basis of this magnitude indicate there is sufficient genetic variance within each lesion class to successfully select for higher levels of resistance within each lesion class. The importance of other genes controlling resistance to _P. lachrymans_ is also indicated by the overlap of disease severity ratings between the two classes.

Cucumber lines, homozygous for the CH lesion type, with a genetic background conferring high levels of resistance may have more resistance to _P. lachrymans_ than a homozygous NH line with a background conferring low level resistance. In field plantings 1 and 2, several NH and CH F_3 lines had disease severity ratings close to the parental extremes indicating that only a few (less than four) additional genes condition the reaction to _P. lachrymans_.

Some caution should be used in extrapolating these results beyond this F_3 population. These quantitative estimations illustrate that lesion type, controlled by a single gene, is a major component of resistance to _P. lachrymans_ and that the interaction of minor genes can modify the level of resistance. An analogous situation has been reported for resistance to bacterial blight in cotton, caused by Xanthomonas malvacearum, where minor genes influence the expression of major genes for resistance (17).

Differences in disease severity between CH and NH lesion types were also reflected in foliar bacterial populations. Bacterial populations on F_3 lines of the CH lesion class were, on the average, 100-fold those on NH F_3 lines. These differences between lesion type classes indicated that the gene coding for the NH lesion type limits bacterial multiplication in addition to reducing disease severity under epiphytotic conditions.

In field studies, cucumber lines were monitored for bacterial population levels 16 days or more after inoculation; when they were assumed to be beyond the logarithmic phase of population increase and at a steady state. Greenhouse experiments were initiated to compare growth of bacteria and lesion development among parental and F_1 lines and to determine the association of bacterial populations with lesions.

In greenhouse experiments, bacterial populations in resistant MSU 9402 increased logarithmically after inoculation at a similar or slightly lower rate relative to bacteria in National Pickling and F_1 plants. Beyond the logarithmic stage, and up to 8 days after inoculation, there were only small differences in bacterial population levels, even when leaves were inoculated with a low bacterial concentration of 1.2×10^3 CFU/ml. In three other greenhouse experiments, 11 days after inoculation with an average of 4.6×10^3 CFU/ml, bacterial populations on NP averaged 40 fold those on 9402, and four fold those on 9402 x NP. It appears that under these greenhouse conditions, P. lachrymans populations continued to increase slowly beyond the log phase. Using a similar inoculation procedure, Chand and Walker (8) found that P. lachrymans multiplied slower in leaf tissue of the resistant cucumber lines PI 169400 than in the susceptible line Wisconsin SMR 18. Peak bacterial populations were reached in both lines 4 days after inoculation. In contrast to this research, Chand and Walker (8) found large differences in bacterial populations. At 4 days after inoculation, there were about 300 times as many bacteria in SMR 18 as in PI 169400. Bacterial populations in the F_1 lines were only twice the population of the resistant line. In this study, bacterial populations in the F_1 were closer to those in the susceptible parent.

Other bacterial plant pathogens have been shown to multiply in resistant tissue at the same (1, 16, 31, 34) or at slower rates (14, 18, 27) relative to susceptible tissue. With most bacterial/host systems studied, maximum population levels in susceptible tissue are typically 100-1000 fold those in resistant tissue (14, 27, 31, 34). In several cases where large differences in bacterial populations were reported, resistance was described as hypersensitive (27, 34). The NH lesion resistance is not hypersensitive. NH lesions develop over the same time course as the CH lesion type and often is preceded by water soaking (15). Small differences in bacterial population levels have been reported for "tolerant" or moderately resistant lines (5, 12, 31).

These studies with parental and F_1 lines also illustrated that under greenhouse conditions CH and NH lesions developed over the same time-course. Differences between lesion types gradually became evident once they became necrotic (120 hours after inoculation). Necrotic lesions on 9402 were typically light colored and restricted in size while those on NP were larger, orange-brown in color and surrounded by a chlorotic halo. Lesions on plants under greenhouse environmental conditions never enlarged to become vein-limited. Differences in lesion type were also indicated by the percentage of lesions that became necrotic. At 11 days after inoculation of greenhouse plants, 88, 69, and 36% of the total lesions were necrotic on NP, 9402 x NP, and 9402, respectively.

Bacterial populations were found to be closely associated with lesion sites. Means of bacterial populations on F_3 lines in field plantings were highly correlated (.86 to .93) with mean disease severity and lesion number. Examining each lesion class separately, bacterial population levels and disease severity or lesion number were highly correlated within the CH lesion class, but poorly correlated within the NH lesion class. Correlations of bacterial populations with lesion number on individual samples under greenhouse conditions also reflected this pattern. There were high correlations between bacterial populations and lesion numbers on National Pickling, but these were poorly correlated within samples from 9402 or 9402 x NP. Bacterial populations at specific lesions on plants in the field were 1000 times greater than the populations at non-lesion sites of the same leaves. The line 9402 had significantly fewer bacteria than 9402 x NP and NP at lesions of the same size. At non-lesion areas on the same leaves, there was little difference in bacterial populations among lines. A similar association of bacteria with lesions was also observed in the greenhouse where bacterial populations at lesions on all lines were greater than 4×10^6 CFU/cm^2 and populations at non-lesion sites on the same leaves were less than 10^4 CFU/cm^2. Differences in bacterial population levels among these experimental lines were primarily due to differences in bacterial number at specific lesions and differences in lesion number. There may have been bacterial population differences at non-lesion sites, but population levels at these sites were very low relative to those at lesions. The poor correlation of bacterial populations and lesion numbers on foliage of 9402 and NH F_3 lesion classes might have been due to the small number of lesions on these lines which would increase the relatively importance of bacterial populations at non-lesion areas.

Even though bacterial multiplication in MSU 9402 is limited relative to National Pickling, P. lachrymans still mutliplied to high levels in this resistant line. This is also true for other bacterial-host systems (5, 31, 34). Haas and Rotem (19) suggested that high numbers of bacteria are not of particular importance epidemiologically. They noted that once the initial inoculum is present in a crop, the epiphytic population of the pathogen is not a limiting factor in epidemic development.

The multiplication of the bacterium in the host has another implication. Coyne et al. (12) found large populations of Xanthomonas phaseoli developed on tolerant lines of Phaseolus vulgaris. Wellhausen (36) noted that virulence of some bacterial pathogens has been shown to increase during passage through a tolerant host and suggested that seed of tolerant cultivars should be saved only from plants that are free of bacteria. As with the Xanthomonads, there are varying degrees of virulence among strains of Pseudomonads. Cultivars resistant to specific strains were later found susceptible to more virulent strains (35).
We have, however, observed only slight differences in virulence among tested P. lachrymans isolates. Since first introduced in 1966, cucumber

cultivars resistant to *P. lachrymans* have remained resistant to all *P. lachrymans* isolates encountered, indicating that the bacterium has not had a tendency to mutate towards higher levels of virulence. However, this fact does not preclude the possibility of a new highly virulent strain appearing in the future. Because *P. lachrymans* is often seedborne, we suggest that special attention should be given to the control of *P. lachrymans* in cucumber seed production fields.

The NH lesion type on cucumber in response to *P. lachrymans* infection is associated with relatively low levels of bacterial multiplication. Lesion size and number on NH lines is also limited and disease severity in the field is significantly reduced. Minor genes interact to affect varying levels of resistance to *P. lachrymans*. In summary, lesion type in response to *P. lachrymans* infection is a major component of resistance to this pathogen.

Literature Cited

1. Allington, W. C., and D. W. Chamberlain. 1949. Trends in the population of pathogenic bacteria within leaf tissue of susceptible and immune plant species. Phytopathology. 39:656-660.

2. Barnes, W. C. 1966. Development of multiple disease resistant hybrid cucumbers. Proc. Amer. Soc. Hort. Sci. 89:390-393.

3. Beecher, F. S., and S. P. Doolittle. 1950. Dissemination of angular leafspot of cucumber. Plant Dis. Reptr. 34:382-383.

4. Bryson, V., and W. Szybalski. 1952. Microbial selection. Science. 116:45-51.

5. Cafati, C. R., and A. W. Saettler. 1980. Effect of host on multiplication and distribution of bean common blight bacteria. Phytopathology. 70:675-679.

6. Carsner, E. 1917. Do the bacteria of angular leafspot of cucumber overwinter on the seed? Phytopathology. 7:61-62.

7. Carsner, E. 1918. Angular leafspot of cucumber: dissemination, overwintering, and control. J. Agric. Res. 15:201-220.

8. Chand, J. N. and J. C. Walker. 1964. Relation of age of leaf and varietal resistance to bacterial multiplication in cucumbers inoculated with Pseudomonas lachrymans. Phytopathology. 54: 49-50.

9. Chand, J. N. and J. C. Walker. 1964. Inheritance of resistance to angular leafspot of cucumber. Phytopathology. 54:51-53.

10. Coyne, D. P. and M. L. Schuster. 1974. A morphological and physiological component genetic approach to breeding for tolerance to the bacterial pathogens, Pseudomonas phaseolicola and Xanthomonas phaseoli. Ann. Rep. Bean Imp. Coop. 17:23-24.

11. Coyne, D. P., M. L. Schuster and C. C. Gallegos B. 1971. Inheritance and linkage of the halo blight systemic chlorosis and leaf watersoaked reaction in Phaseolus vulgaris variety crosses. Plant Dis. Reptr. 55:203-207.

12. Coyne, D. P., M. L. Schuster, and K. Hill. 1973. Genetic control of reaction to common blight bacterium in bean (Phaseolus vulgaris) as influenced by plant age and bacterial multiplication. J. Amer. Soc. Hort. Sci. 98:94-99.

13. Coyne, D. P., M. L. Schuster, and L. Shaughnessy. 1966. Inheritance of reaction to halo blight and common blight bacteria in a Phaseolus vulgaris variety cross. Plant Dis. Reptr. 50: 29-32.

14. Daub, M. E. and D. J. Hagedorn. 1979. Resistance of Phaseolus line WBR 133 to Pseudomonas syringae. Phytopathology. 69: 946-951.

15. Dessert, J. M., L. R. Baker, and J. F. Fobes. 1981. Inheritance of reaction to Pseudomonas lachrymans in cucumber. In Review.

16. Diachum, S. and J. Troutman. 1954. Multiplication of Pseudomonas tabaci in leaves of burley tobacco, Nicotiana longiflora, and hybrids. Phytopathology. 44:186-187.

17. El-Zik, E. M. and L. S. Bird. 1970. Effectiveness of specific genes and gene combinations in conferring resistance to race of Xanthomonas malvacearum in upland cotton. Phytopathology. 60: 441-447.

18. Ercolani, G. L. and J. E. Crosse. 1966. The growth of Pseudomonas phaseolicola and related plant pathogens in vivo. J. Gen. Microbiol. 45:429-439.

19. Haas, J. H. and J. Rotem. 1976. Pseudomonas lachrymans inoculum on infected cucumber leaves subjected to dew- and rain-type wetting. Phytopathology. 66:1219-1223.

20. Haley, A. B. and M. J. Palmer. 1977. Resistance of cucumber to angular leafspot disease--correlation of greenhouse and field reactions. Pickling Crops Research, Univ. of Wisconsin, Madison.

21. Hill, K., D. P. Coyne, and M. L. Schuster. 1972. Leaf, pod, and systemic chlorosis reactions in Phaseolus vulgaris to halo blight controlled by different genes. J. Amer. Soc. Hort. Sci. 97:494-498.

22. Husain, A. and A. Kelman. 1958. Relation of slime production to mechanism of wilting and pathogenicity of Pseudomonas solanacearum. Phytopathology. 48:155-165.

23. Jensen, J. H. and R. W. Goss. 1942. Physiological resistance to halo blight in beans. Phytopathology. 32:246-253.

24. Jones, L. R., S. P. Doolittle, and W. W. Gilbert. 1921. Field tests with cucumber leafspot and anthracnose. Phytopathology. 11:298-299.

25. Keen, N. T., P. H. Williams, and J. C. Walker. 1967. Protease of Pseudomonas lachrymans in relation to cucumber angular leafspot. Phytopathology. 57:263-271.

26. Lyon, F. and R. K. S. Wood. 1976. The hypersensitive reaction and other responses of bean leaves to bacteria. Ann. Bot. 40: 479-491.

27. Omer, M. E. H. and R. K. S. Wood. 1969. Growth of *Pseudomonas phaseolicola* in susceptible and in resistant bean plants. *Ann. Appl. Biol.* 63:103-116.

28. Patel, P. N. and J. C. Walker. 1965. Resistance in *Phaseolus* to halo blight. *Phytopathology*. 55:889-894.

29. Patel, P. N. and J. C. Walker. 1966. Inheritance of tolerance to halo blight in bean. *Phytopathology*. 56:681-682.

30. Pohronezny, K., P. O. Larsen, D. A. Emmatty, and J. D. Farley. 1977. Field studies of yield losses in pickling cucumber due to angular leafspot. *Plant Dis. Reptr*. 61:386-390.

31. Scharen, A. L. 1959. Comparative population trends of *Xanthomonas phaseoli* in susceptible, field tolerant, and resistant hosts. *Phytopathology*. 49:425-428.

32. Schuster, M. L. 1950. A genetic study of halo blight reaction in *Phaseolus vulgaris*. *Phytopathology*. 40:604-612.

33. Smith, E. F. and M. K. Bryan. 1915. Angular leafspot of cucumber. *J. Agr. Res*. 5:465-476.

34. Stall, R. E. and A. A. Cook. 1966. Multiplication of *Xanthomonas vesicatoria* and lesion development in resistant and susceptible pepper. *Phytopathology*. 56:1152-1154.

35. Walker, J. C. and P. N. Patel. 1964. Inheritance of resistance to halo blight of bean. *Phytopathology*. 54:952-954.

36. Wellhausen, E. J. 1937. Effect of the genetic constitution of the host on the virulence of *Phytomonas stewarti*. *Phytopathology*. 27:1070-1089.

37. Wiles, A. B. and J. C. Walker. 1952. Epidemiology and control of angular leafspot in cucumber. *Phytopathology*. 42:105-108.