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GENETICS OF RESISTANCE TO CORYNEBACTERIUM
MICHIGANENSE IN LYCOPERSICON.

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GENETICS OF RESISTANCE TO
CORYNEBACTERIUM MICHIGANENSE IN
LYCOPERSICON

By

Jan de Jong

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ABSTRACT

GENETICS OF RESISTANCE TO CORYNEBACTERIUM MICHIGANENSE IN LYCOPERSICON

By

Jan de Jong

The tomato disease, bacterial canker, is caused by Corynebacterium michiganense (E. F. Sm.) H. L. Jens. Inheritance of resistance to the pathogen was studied from (1) a half diallel cross with 4 resistant and 2 susceptible cultivars, and from (2) F₂ and backcross generations derived from selected crosses from the above. Various methods of inoculation of seedlings with this bacterium were studied.

Stem inoculation was proven most reliable in readily differentiating resistant and susceptible plants. Seedling resistance was shown to be related to resistance of the mature plant.

Analysis of the half diallel showed that differences existed in general and specific combining ability for resistance in the resistant and susceptible cultivars.

Resistance to isolate H could best be explained by 4 genes. A combination of one recessive gene (a) and 3 dominant genes (B, C, D) controlled resistance in Lycopersicon esculentum cv. Bulgaria 12. Resistance in the L. pimpinellifolium cvs. A 129 and A 134 was controlled

by the same genes, but the presence of the dominant allele $\underline{D}^2$ enhanced the resistance of these cultivars and their F_1 hybrids.

Resistance in L. hirsutum PI 251305 was controlled by the genes AAbbccdd and the recessive gene x. A modifier gene F was found to be present.

Susceptible cultivars of L. esculentum that carry the dominant allele $\underline{C}^2$ transmitted a higher level of resistance to its progeny than cultivars with either the C or c allele.

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INTRODUCTION

Bacterial canker caused by Corynebacterium michiganense (E.F.Sm.) H. L. Jens., was first found in a greenhouse of tomatoes (Lycopersicon esculentum Mill.) near Grand Rapids, Mich. (35). The disease is still being reported in the tomato growing areas of the world, with losses up to 80% of the crop (41). Initially control measures were aimed at cultural practices, attempting to prevent the spread of the bacteria in the field through quarantine and treatment of infected seed.

Symptoms of the disease in the greenhouse are best described by Bryan (6). Most characteristic is the unilateral wilting of the leaves. The cotyledonary leaves of infected seedlings first show wilt, followed by browning and shriveling. Later the true leaves wilt and shrivel, but the petioles of the shriveled leaves remain attached to the stem. The first wilting appears during the hottest part of the day but initially the plant recovers at night. The vascular bundles of the stem are discolored (brown) and in the advanced stage the stem cracks open and a canker develops. The seedlings show stunting and sometimes develop curved tips. With further development of the disease the whole pith of the stem becomes brown and loses its structure (6).

In the field, the primary symptoms are the browning of the leaves and internal discoloration. The cankers, common in inoculated seedlings in the greenhouse, are not always a typical symptom in the field. The symptoms on the fruit do not always appear, as they are highly dependent upon rain or overhead irrigation. Fruit symptoms are known as bird's eye spot; brown centers with a characteristic white halo, ranging in size from 3 to 6 mm.

Resistance in Lycopersicon pimpinellifolium Mill. was reported in 1944 (1). Elenkov (8) incorporated the resistance of L. pimpinellifolium into L. esculentum cultivar Bulgaria 12. Fruits of Bulgaria 12 were too small for acceptance in the USA but the cultivar has been utilized by breeders as a source of resistance to C. michiganense. Another source of resistance, L. hirsutum Humb. and Bonpl. PI 251305 (17) has not been used in breeding.

Isolates of C. michiganense differ in virulence (43,49). Seedlings of the resistant cultivars succumb to the disease when inoculated with the virulent isolate cm 21, however, it is not known how the mature plant reacts to this isolate. Therefore, there is a need to compare mature plant resistance against seedling resistance, using the virulent isolate.

Resistance was reported to be inherited as a dominant character (8). This was confirmed by Laterrot (25) who noticed that hybrids of susceptible tomato cultivars with Bulgaria 12 are as resistant as Bulgaria (12).

Thyr (49) suggested polygenic control of resistance. Apart from these observations there are no published data on the inheritance of resistance to C. michiganense. Information on the genetics of the resistance of tomato to C. michiganense could improve the efficiency of breeding programs aimed at incorporating resistance into L. esculentum.

The purposes of this study are;

- (1) The development of a fast and reliable screening method for detecting resistance to C. michiganense.
- (2) A determination of an acceptable level of resistance in L. esculentum.
- (3) Investigation of the genetic basis of resistance.

REVIEW OF LITERATURE

Smith, who originally called the disease the "Grand Rapids disease", named the causal bacterium Bacterium michiganense. The name of the disease was changed to bacterial canker in 1920 (37). The organism has since been referred to as Pseudomonas michiganense, Aplanobacter michiganense, Phytomonas michiganense and now as Corynebacterium michiganense (19).

The bacterium is disseminated by water, tools and man, and enters host plants primarily through wounds (28). Though it has never been proved that wounding is the only mode of entrance, most inoculation methods employ wounding to secure fast and uniform infection. Bacteria have been recovered from stomatal chambers after the leaves were sprayed with a bacterial suspension in water. Trichomes, both broken and entire, are thought to be an important point of entry of the bacterium, but subsequent spread through the plant is slow (2, 21). For example, 30 days after inoculating the leaf the bacterium had only reached the petiole (21). It has been suggested that minute rifts in the cuticle of the fruit or broken hairs provide an opportunity for bacteria to penetrate the epidermis of the fruit.

C.michiganense is a xylem invader and Thyer (44) has

reported that as low as 5 cells of the bacterium introduced directly into the xylem can cause infection. The initial infection in the plant and the subsequent movement of the bacteria takes place in the spiral vessel elements of the primary xylem (31). The bacteria move downward first, followed by upward movement in the xylem. Subsequently, when the bacteria break through the cell walls, they move laterally, forming pockets of bacteria in the surrounding tissue. The bacteria do not move longitudinally in the phloem (31).

Since C. michiganense is primarily a xylem invader, the most successful inoculation techniques are those which introduce the pathogen directly into the xylem (45). Inoculation of the root, stem, petiole and leaf have been reported (1, 2, 17, 20, 21).

Root inoculation involves wounding the root system and exposing of the wounded surface to bacteria. The roots are pruned while they are in the inoculum (20), or the plant is dipped in inoculum after the roots have been cut back (38, 42). Other workers placed a bacterial suspension into the soil around young seedlings and then cut the roots at 4 points about an inch from the plant (31). Root inoculations have given variable results (22, 31).

Stem inoculations are made in several ways. The commonly used method involves piercing the stem with an infected needle. Ark (1) using this method obtained only 2 infected out of 50 inoculated plants. Strider (42), pierced the stem at the base of the cotyledonary leaves of 2 and 4

week old seedlings with a root canal knife dipped in inoculum, and obtained infection from 96% to 100%. Seedlings have also been successfully infected by cutting the tip of the stem with a contaminated knife (1). An often used and successful method is cutting a petiole with a contaminated knife (31, 45).

Hassan et al (17) screened for resistance using symptoms of the cotyledons and found a relationship between susceptibility, as determined by the reaction to root and/or stem inoculations, and the number of spots produced per unit area on the cotyledons. A well defined bacterial concentration and a standardized pressure of application was required for the cotyledonary method of screening, since increased concentration and pressure of application resulted in a higher rating of susceptibility. Suspensions of bacteria have been sprayed on leaves with varying degrees of success, Ark (1) kept seedlings in mist chambers prior to spraying with a heavy suspension of C. michiganense and failed to obtain infection. However, atomization of a bacterial suspension from a distance of 35 cm on seedlings kept at high humidity, resulted in infection of the leaves (21, 2). Layne (23) sprayed leaves of seedlings kept in high humidity with and without injuring the trichomes and obtained blister like lesions on both, however, the number of blisters tripled with injury of the trichomes. No systemic infection was reported. Smith (36) noticed bacteria in the stomata after spraying bacteria on the plant, without obtaining systemic infection.

Scoring resistance is based on one or more symptoms

of the disease. Thyr (47) who does not advocate using the length of the canker as a measure of resistance to C. michiganense, favored the length of the vascular discoloration, expressed as the percentage of stem length beyond the point of inoculation. Initial wilting as a measure of resistance is not advisable as L. pimpinellifolium, which is a source of resistance to bacterial canker, has a tendency to show initial wilting followed by a complete recovery. Thyr (49) reported vascular discoloration as being superior to stunting in the determination of susceptibility since the lag between vascular discoloration and stunting was not the same for all cultivar and isolate combinations. Other workers have used the degree of advanced wilting as a measure of resistance (22, 42).

In screening, the experimental conditions have to be carefully monitored including plant age, inoculum concentration and temperature (2, 14, 20, 24, 45).

Optimal concentration of the inoculum depends on the method of inoculation. With foliar application, 10^6 or more cells/cm³ are necessary for infection (2). Piercing the stem with a rootcanal file dipped in a bacterial suspension requires a concentration of 10^7 to 10^9 cells/cm³ (14). If introduced directly into the xylem a minimum of 5 bacterial cells will infect seedlings and possibly only one cell is enough to infect the plant (44). Part of the difference in the various concentrations needed for infection may be explained by the effectiveness of the various inoculation methods in introducing the bacteria directly into the xylem. Bacteria,

especially in concentrations lower than 10^5 cells/cm³, die very rapidly in aqueous suspensions (40, 44).

Symptoms take longer to appear in older plants (20, 42). Inoculation of 5 week old tomato plants (22 cm tall), produced the largest difference in disease rating between susceptible and resistant plants (14), however, this was not the same for all varieties. L. hirsutum, (P.I. 251305) was most resistant at 4 weeks, L. pimpinellifolium, (P.I. 340905) at 5 weeks and L. esculentum cv Bulgaria 12 at 6 weeks.

A day temperature of 24° C and a night temperature of 18° C is optimal for maximal distinction between resistant and susceptible plants, however, the temperature requirement differed with tomato accessions (14). It was generally confirmed that the disease progressed most rapidly under conditions most favorable to the host, but that slightly less than optimal conditions gave maximum separation between susceptible and resistant plants (4, 14).

C. michiganense is potentially a devastating organism to the tomato. The bacterium may overwinter in the soil (3, 11). In addition, certain weeds are reported to be host-plants. Solanum douglasii, the perennial nightshade (18), Solanum mammosum L. (36), and recently Solanum triflorum Nutt. (48) have been found to host pathogen. Hassan et al (17) induced symptoms of the cotyledons on a range of Solanaceous plants, but did not reisolate the bacteria from those plants. When their roots were inoculated only the Lycopersicon and Solanum species died or wilted but the

Capsicum species, were not affected. Volcani et al (51) described a new leaf and fruit spot disease of pepper caused by C. michiganense.

Bacterial canker is seedborne, although only 1% or less of the seeds in infested fruits carry the pathogen (20). Bacteria have been observed in the seedcoat cells but not in the endosperm or the embryo (29). The current recommended seed treatment is either fermentation at 18° C for 96 hours, or soaking of the dry seeds in a 0.6 solution of acetic acid for 24 hours (41). Recently, this method was slightly modified (50).

Direct seeding of the tomato has helped to cut losses drastically in California (16). Experimental data on the effect of bacterial canker on the yield of tomato is presented by Emmatty & John (9). Early inoculation, up to 2 weeks after transplanting, resulted in a high mortality rate and consequently lowered the yield per plant. Inoculation as late as 4 to 6 weeks after transplanting caused significant reduction in yield of the susceptible cultivar H6, as compared to H2990, a resistant tomato cultivar.

Varying degrees of resistance to C. michiganense in L. esculentum have been noted since 1937 (28). However none of the commercial varieties tested were immune. The resistance in some L. pimpinellifolium lines is superior to that in L. esculentum in that after showing slight wilting following inoculation, the plants recover completely (8, -1).

Elenkov (8), without presenting supporting data, reported resistance to be inherited as a dominant character. A level of resistance equivalent to that of the resistant cultivar 8/12 is expressed in the hybrids of 8/12 with the susceptible tomato cultivars Monalbo and Porphyre (25). Polygenic control of the reaction to C. michiganense has also been suggested (49).

Investigations into genetics of pathogenic bacteria - hostplant relationships are limited, probably due to pathogen variability. Pseudomonas phaseolicola (Burk.) Dows., the cause of halo blight in beans is believed to have differentiated into 2 races. Resistance to race one, as found in the Red Mexican U.I.3 cultivar of Phaseolus vulgaris L. is inherited as a monogenic dominant character (52). Schuster (34), using a host of different genetic constitution and possibly a different isolate of the bacterium reported 2 recessive factors controlling resistance.

Resistance to Xanthomonas campestris (Pammel) Dows., the cause of black rot in cabbage was found to be determined by one major gene f whose expression in the heterozygous condition is influenced by 2 modifier genes. Ratios in crosses between resistant and susceptible plants therefore depend not only on the constitution of the major gene f but also on the differences at either the a or b locus. Discovery of other modifier genes was not excluded (55).

The resistance to bacterial wilt of maize, caused by Phytomonas stewartii (E.F. Sm.) Bergey et al appears to

be controlled by 3 dominant, independently inherited genes, and supplementary factors are thought to be involved (54).

Bacterial variability has been demonstrated in Phytomonas stewartii (53). Successive passage through susceptible maize decreased virulence while passages through resistant maize increased virulence. Initially, this could be explained by selection of the existing variation in mixtures of virulent and avirulent bacteria. Lincoln (26) observed the same phenomenon, however, in single cell cultures, with the exception that cultures passing through the resistant line retained their virulence without appreciable change. He notes that the work with single cell cultures did not differ from earlier results with colony or mass inoculations. Mutations at an estimated rate of between 1 to 20,000 and 1 to 800,000 supposedly furnished a source of variability great enough for this variation in virulence.

Clonal variation in C. michiganense is well known. Ark (1) described color mutants and variants that were pathogenic to tomato in varying degrees. A gradual loss of virulence has been reported when the pathogen was grown on artificial media (37, 20). Color and colony form has been linked to degree of virulence. Yellow and white forms that were repeatedly obtained from the pink were markedly more virulent than the parent culture (12). The high rate of mutation and the known recombination capability of bacteria through conjugation, transformation and transduction can explain the numerous strains and the continuous shift in virulence.

Low temperature storage of bacteria, keeping down the biological activity, could result in stable strains. Strider (38) kept cultures of C. michiganense on nutrient agar at 2°C for 30 months without loss of virulence. Infected tomato stem pieces were frozen for 5 months after which test inoculations showed that the virulence was not lessened (20).

Difference in virulence between isolates of C. michiganense was described by Strider (43) but no variation in virulence was found among single colony subcultures within isolates. The effect on virulence of successive passages through resistant or susceptible hosts has not been studied in C. michiganense.

Isolates of C. michiganense that differ in pathogenicity do not affect Lycopersicon accessions in the same manner. Isolate 829-S was more virulent on L. esculentum than isolate cm 4, as measured with vascular discoloration and stunting, however, isolate cm 4 was more pathogenic than 829-S on L. pimpinellifolium and L. hirsutum. This isolate accession interaction was insignificant when only L. esculentum was studied (49).

MATERIALS AND METHODS

Parental

Six tomato cultivars, selected on the basis of their reaction to C. michiganense, were crossed in a half diallel crossing scheme.

The cultivars were:

A 129	(P.I. 344102, L. pimpinellifolium, resistant)
A 134	(Utah 737 , L. pimpinellifolium, resistant)
Bulgaria 12 (	, L. esculentum , resistant)
Earliana (	, L. esculentum , susceptible)
MSU 72-279 (	, L. esculentum , susceptible)
P.I. 251305 (	, L. hirsutum , resistant)

Cultivars A 129 and A 134 were obtained from Dr. Bill Thyr, USDA, Reno, Nevada. With the exception of L. hirsutum, all plants were selfed one generation prior to hybridization.

A single plant from each cultivar was used for the 6-parent half diallel crossing, and was vegetatively propagated for the backcrosses. The F_2 generation consisted of the bulked seed from 6 F_1 plants grown in the field. Six reciprocal combinations were included to determine maternal effects. Due to unilateral incompatibility (27) of L. hirsutum all crosses with this genotype were made with L. hirsutum as the male parent.

In addition to the cultivars mentioned above, the cultivars or F_1 hybrids; Saturn, $Sl_3 \times$ Farthest North, P.I. 340905, G 14565 and Rapids were used for inoculation experiments. The resistance to C. michiganense of mature plants of the cultivars Bulgaria 12, MH-1, Earliana, Saturn and G 14565 was measured in a yield trial.

Pathogen

Originally, 5 isolates of C. michiganense were available: cm 3, cm 15 and cm 21 from Dr. Bill Thyr, isolate F from Dr. James Farley O.S.U., Wooster, Ohio and isolate H from H. J. Heinz Co., Bowling Green, Ohio. Isolate H was reported (Emmatty, D.A. personal correspondence) to be identical to cm 15, but in this study it was more virulent. Isolates H and cm 21 were used for the genetic study.

The isolates were stored in the refrigerator at 4°C and once a year they were passed through a susceptible cultivar. The inoculum was prepared from a nutrient broth culture which was continuously agitated for 4 days at room temperature. Prior to inoculation the bacterial concentration was determined by making a cell count with a hymacytometer.

Methods

Seeds were planted in vermiculite and seedlings were transplanted 2 weeks later into flats. Tests were conducted throughout the year in the greenhouse at 19°C or higher, or in growth chambers at a constant 19°C. Due to the

slow growth of L. hirsutum it was necessary to sow these seeds 3 days earlier than the seed of other cultivars.

Three inoculation methods were used in this study.

- A. Stem-inoculation. Two to 3 weeks after transplanting the seedling tops were clipped off 1 cm above the cotyledons and a drop of inoculum was applied directly on the clipped stem. To compensate for the small cotyledons of the cultivars of L. pimpinellifolium the tops were clipped above the first true leaf while for the tops of L. hirsutum were clipped above the second true leaf. When inoculating with the less virulent isolates, plants were reinoculated 25 days after the first inoculation by clipping the tops of the plants above the first leaf and applying a drop of inoculum on the clipped stem.
- B. Petiole-inoculation. The petiole of the first true leaf of 4 week old seedlings was severed 3 mm from the stem with scissors which had been dipped into the inoculum.
- C. Root-inoculation. Roots of 2 week old seedlings were cut 1 cm below the hypocotyls, then the de-rooted plant was dipped in inoculum for one minute and planted into flats.

Randomized block and split plot designs were used for this study. Cultivars were the main treatment and inoculation methods were the subtreatments. Unless mentioned elsewhere 3 replications were used with 7 plants of each entry per replication.

For the diallel test, each replication consisted of 21 F_1 and parental entries in 3 flats. Seven seedlings of

each entry were planted in a row with the seedlings spaced at intervals of 4.7 cm. To correct for variation between flats within replicates one row of the cultivar Earliana was planted in every flat. Four replicates were grown in the greenhouse and 2 in separate growth chambers. The results were analysed according to Griffing (15) model 1, method 2.

For the inheritance study, 7 plants of the P_1 , P_2 and F_1 , 28 plants of the F_2 , 10 plants of the BC to P_1 , and 11 plants of the BC to P_2 were grown in one flat as one replicate. Six to 12 replicates were used.

The data were statistically interpreted by means of variance analysis. "T" tests, or when appropriate, Duncans multiple range tests were used to compare the means.

Greenhouse plants were rated for disease development several times during the experiment using the following scale: 0 = The growing point has succumbed to the disease. 1 = Extensive wilting, large cankers or stunted growth. 2 = Plant approaches normal size, but shows some wilting symptoms. 3 = Healthy seedling with no apparent symptoms of the disease.

Where vascular discoloration was measured it was expressed as a percentage of the total stem length beyond the point of inoculation for petiole inoculated plants. If the seedling was decapitated for stem-inoculation the vascular discoloration was measured as a percentage of the total stem length of the tallest sprout.

The yield trial for determining the resistance of mature plants consisted of 3 replications of 5 cultivars and

2 isolates in a split plot design with isolates as the main treatment and cultivars as the subtreatments. Guard rows were planted around the main treatment plots, which were separated by a 4 meter wide alley. Five plants of each entry were harvested weekly.

The number and weight of the mature fruit, recorded on a weekly basis, were used as a measure of resistance.

SCREENING TECHNIQUES

Preliminary tests were made to determine the most rapid and reliable method of inoculation under greenhouse conditions, and to learn which isolates of the pathogen produced the most uniform results.

Resistant and susceptible tomato cultivars were inoculated with 5 isolates of C. michiganense by cutting the roots of 2 week old seedlings below the hypocotyls, and dipping the stems in a water suspended inoculum. Although cultivars A 134, A 129 and Bulgaria 12 have been reported to be resistant to C. michiganense (45, 47), results reported here (Table 1) do not agree with Thyr's observations. The virulent isolate, cm 21, killed all cultivars although in resistant lines the isolate required a longer incubation period. The resistant cultivars A 129 and A 134 did not succumb to isolate H as did the susceptible cultivars, therefore isolate H was selected for further studies.

Since the preliminary study did not readily differentiate between resistant and susceptible plants, a study was made to evaluate various inoculation methods. Isolates H and cm 21, resistant cultivars A 129, Bulgaria 12, and the susceptible cultivar Rapids were used. Inoculum was prepared from nutrient agar plates or nutrient broth shake cultures.

Table 1. Effect of root inoculation of 5 isolates of C. michiganense on 12 plants each of 6 tomato cultivars.

Cultivar	Days after inoculation	Number of diseased plants				
		cm3	cm15	H	F	cm21
A 129	12				1	1
	16				5	5
	19				6	5
	24			3	11	8
Bulgaria 12	12		X			3
	16		X	3	7	9
	19	1	X	6	9	12
	24	2	X	11	12	12
Rapids	12		X		2	2
	16	1	X	8	10	11
	19	1	X	11	12	12
	24	2	X	11	12	12
S1 ₃ x FN	12		X		3	
	16	3	X	4	11	9
	19	2	X	8	11	12
	24	3	X	8	11	12
PI 340905	12				2	8
	16			4	9	10
	19		1	4	9	12
	24		1	7	11	12
A 134	12			1		2
	16			2	3	7
	19			2	7	9
	24			5	10	11

X = not planted

The concentration of inoculum was adjusted to 4×10^8 bacteria / cm^3 . A randomized block design with 2 replications of 10 to 14 plants of each entry was used. The inoculation methods were as follows:

- I. Two week old tomato seedlings which had their roots cut 1 cm below the hypocotyl, were dipped into the inoculum for a minute and planted in flats.
- II. The petiole of the first true leaf of 4 week old seedlings was severed 3 mm from the stem, with scissors dipped in inoculum.
- III. A suspension of bacteria was atomized from a distance of 10 cm onto 3 week old seedlings, until they were dripping wet.
- IV. Carborundum powder was dusted on the cotyledons of 3 week old seedlings and gently rubbed with foam pads which had been dipped in inoculum.
- V. Inoculum grown for 5 days on nutrient agar was suspended in water. Seeds of the cultivar Rapids were left to soak in the suspension for 72 hours prior to planting in flats.
- VI. Control. As method III, plants were atomized with distilled water.

The results of the various inoculation methods are presented in Table 2. Method I resulted in the breakdown of resistance of A 129 and B 12, since plants from both cultivars succumbed to isolates H and cm 21 after 19 days. Method II showed the greatest difference in disease rating between resistant and susceptible cultivars with isolate H.

Table 2. Percent of wilted seedlings of 3 tomato cultivars inoculated by 6 methods with 2 isolates of C. michiganense.

Inoculation method	Days after inoculation	Cultivar					
		A 129		Bulgaria 12		Rapids	
		Isolate		Isolate		Isolate	
		cm 21	H	cm 21	H	cm 21	H
		%	%	%	%	%	%
Root dip	8	0	0	0	0	0	0
	13	0	0	4	4	25	25
	19	61	18	32	32	64	79
Petiole first leaf	8	0	0	0	0	0	0
	13	43	0	15	5	29	50
	19	81	5	85	14	86	100
Atomization	8	76	86	100	100	100	90
Abrasion of cotyledons	8	100	100	100	100	100	100
Seed soak	8	not planted		not planted		0	0
	13					0	0
	19					0	0
Control	8	0	0	0	0	0	0
	13	0	0	0	0	0	0
	19	0	0	0	0	0	0

Inoculation with isolate cm 21 produced no difference between resistant and susceptible cultivars. Method III: Seedlings of all cultivars showed 1 mm size white blisters on the cotyledons. The seedling failed to wilt and the disease did not become systemic. Method IV: Injuring the cotyledons prior to applying inoculum resulted in numerous blisters on the cotyledons. The white blisters, associated with infection, later darkened and coalesced, followed by dropping of the leaves. There was no systemic infection.

The seedlings of Method V did not develop any symptoms. Probably the bacteria were inviable by the time the root and plumule emerged: bacteria in dilution of 10^5 cells/cm³ lose viability between 24 and 168 hours (44), or the pathogen was unable to penetrate the seedling.

No symptoms were observed on the control plants.

Qualification of the term "resistance" is necessitated by the different responses of the resistant varieties to isolates and inoculation methods. Regardless of inoculation technique, A 129 was not resistant to isolate cm 21 or to isolate H when inoculated on the roots, or when receiving a foliar spray. A 129 was resistant to isolate H when petiole inoculated. Therefore, petiole inoculation was investigated further.

Pine et al (31) noted that bacteria moved from 10 to 45 mm in one day and suggested that bacteria may be drawn into the vessels following petiole inoculation. This introduces non-genetic variation. If the bacteria could be

allowed to spread in the plant and reach the growing point of all plants at the same time, the non-genetic variation could be reduced. By cutting the growing point 10 mm above an axillary bud, the bacteria would reach the bud in a day, or 6 days before the bud breaks.

Petiole inoculation was compared to stem inoculation on 4 cultivars to determine which method of inoculation would minimize the experimental and environmental variation. If cultivars are assumed to be pure lines all variation within a cultivar would be non-genetic. The number of days between wilting of the first and the last plant is considered to be a valid estimate of the non genetic variation. The following methods were used:

- I. Control, no inoculation.
- II. The growing points of 5 week old seedlings were clipped .5 to 1 cm above the first true leaf, and a drop of inoculum was applied to the decapitated stem.
- III. The first true leaf was removed 3 mm from the stem, with scissors dipped into the inoculum.
- IV. The petiole of the 2nd or 3rd emerging leaf was removed as in Method III.

Isolates cm 21 and F were used in a split plot design, with 3 replicates of 7 plants of each genotype. One replicate of the control seedlings of the cultivar MH-1 died prior to inoculation. Therefore, only 2 replications of MH-1 were used for the analysis of variance.

The results for isolate cm 21 are presented in Table 3.

The mean number of days between the appearance of symptoms on the first and the last plant was 3.8, 7.2, and 9.3 days respectively for Methods II, III and IV. No wilting was observed in the control. Method II showed minimal environmental variation and was therefore used for the genetic studies.

Inoculation with isolate F caused wilting 23 days after inoculation in 3% of the seedlings of Bulgaria 12, 16% in Earliana, 40% in MH-1 and none in A 134. The scoring of wilting symptoms per se with this isolate would introduce a variation as plants within a susceptible variety could be rated either susceptible or resistant. Therefore, all the seedlings were cut transversely at 2 mm intervals from the growing point toward the root to determine the percentage of the vascular discoloration (Table 3). A significant difference between cultivars was noted, with Method II producing the best differentiation between resistant and susceptible cultivars (Table 3). However, the coefficient of variation of individual measurements was 51%, which made this method of screening undesirable for individual plants.

Table 3. Evaluation of 3 inoculation methods with 2 isolates of C. michiganense on 4 cultivars of tomato.

Cultivar	Days between wilting of first and last seedling inoculated with isolate cm 21			Percent discoloration in seedlings inoculated with isolate F		
	II	III	IV	II	III	IV
	Stem	Petiole 1st leaf	Petiole 2nd or 3rd leaf	Stem	Petiole 1st leaf	Petiole 2nd or 3rd leaf
	Days	Days	Days	%	%	%
MH-1	5.7	7.3	10.0	94	73	68
Earliana	3.3	4.0	10.7	74	54	47
Bulgaria 12	3.3	6.7	7.7	5	7	6
A 134	<u>2.7</u>	<u>10.7</u>	<u>8.7</u>	<u>0</u>	<u>0</u>	<u>0</u>
Mean	3.8	7.2	9.3	43	34	31
	LSD (.05) = 3.1 days			LSD (.05) = 8%		

ENVIRONMENTAL INFLUENCES

Temperature and light.

A large variation in the degree of resistance perhaps due to environmental effects was noted for inoculated seedlings. Since this study was carried out in the greenhouse, temperature and light variation may have affected the disease rating. To determine the effect of varying environmental conditions on the disease rating, seedlings grown and inoculated at constant temperature and light were compared with those grown and inoculated in the greenhouse.

Two replicates of the hybrids and parents of the diallel cross were grown, following inoculation in 2 growth-chambers, at $19^{\circ}\text{C} \pm 1$ with a 13 hour day. Two other replicates were kept in the greenhouse under natural light with a minimum temperature of 10°C and a maximum temperature reaching 30° on sunny days. Twenty six hybrids and parents were planted, with 7 plants per entry per replicate. The seedlings were stem-inoculated with isolate H, 32 days after seeding, and scored for their resistance 23, 32, and 40 days later.

Progenies resulting from hybridization of resistant plants were resistant at both temperatures. The results in Table 4 are of the susceptible entries; the F_1 hybrids of

Table 4. The cumulative mean score of disease resistance of 7 plants for F₁ hybrids and parents grown in the greenhouse and in growth chambers.

Hybrid or parent	Growth chamber				Greenhouse			
	Days after inoculation				Days after inoculation			
	23	32	40	mean	23	32	40	mean
MSU 72-279 x A 129	33	33	22	29.3*	28	17	18	21
Earliana x A 129	40	37	38	38.3	28	24	34	28.7
MSU 72-279 x A 134	38	31	20	29.7	20	14	18	17.3
B-12 x Earliana	35	29	30	31.3	25	24	27	25.3
Earliana x B-12	32	30	31	31.0	28	20	26	24.7
Earliana	8	3	0	3.7	6	2	0	2.7
MSU 72-279 x B-12	26	17	15	19.3	20	14	13	15.7
B-12 x MSU 72-279	14	11	12	12.3	25	15	17	19
A 129 x MSU 72-279	33	20	18	23.6	19	11	10	13.3
A 134 x MSU 72-279	31	25	17	24.3	18	11	13	14
MSU 72-279 x Earliana	13	4	1	6.0	14	2	1	5.7
MSU 72-279	9	0	0	3.0	18	2	0	6.7
MSU 72-279 x <u>L. hirsutum</u>	15	10	7	10.7	24	10	11	15.0
Earliana x A 134	38	38	41	39.0	34	30	39	34.3

* The higher the scale, the greater the resistance.

LSD (for means within progenies) (.05) = 4.6
(.01) = 6.2

LSD (for each entry) (.05) = 4.9
(.01) = 6.5

resistant x susceptible cultivars and the susceptible parents.

The disease rating of individual entries was effected by the time of observation. The rating of the F₁ hybrids that involved Earliana as the susceptible parent generally did not change from one rating to the next, while most F₁ hybrids of MSU 72-279 were rated less resistant at the 2nd or 3rd reading (Table 4). The variable environment of the greenhouse generally decreased resistance more than the constant temperature in the growth chamber, with the exception of the F₁ hybrids of Bulgaria 12 x MSU 72-279 and MSU 72-279 x L. hirsutum where the resistance is higher in the greenhouse as compared to the growth chamber.

It is concluded that the resistance of seedlings may be affected by experimental factors. However, the overall resistance relationship between genotypes within an environment or observation date did not change.

Inoculum concentration

Thyr (44) reported loss in viability of C. michiganense in aqueous solutions. This may affect the inoculum load between the first and the last seedling inoculated in a large experiment. To determine the effect of reduced inoculum load on the disease rating, seedlings of the cultivars Rapids, Bulgaria 12, and A 129 were petiole inoculated with isolates cm 21 and H, cultured on nutrient agar plates. Prior to inoculation the agar was placed in distilled water and mixed with a blender. The concentrations

Table 5. Number of days from inoculation until 50% of the plants showed wilting.

Cultivar	Isolate	Concentration of inoculum		
		8×10^8	8×10^7	8×10^6
		Days	Days	Days
Rapids	cm 21	14.5	13.5	15.5
	H	18.0	18.0	18.0
Bulgaria 12	cm 21	17.0	20.5	15.0
	H	—	—	—
A 129	cm 21	14.0	13.0	15.0
	H	—	—	—

— = Less than 50% wilting observed 33 days after inoculation

were adjusted to 8×10^8 , 8×10^7 , and 8×10^6 bacteria / cm^3 . A randomized block design with 2 replicates and 14 plants per treatment was used. The number of wilted plants was recorded on alternate days. Wilting, the first symptom to be expressed by the diseased seedling, was considered to be most indicative of the concentration effect. The results are expressed as number of days following inoculation, when 50% of the plants showed signs of wilting (Table 5).

Rapids seedlings inoculated with 3 concentrations of isolate H were wilted 18 days after inoculation, while the seedlings of Bulgaria 12 and A 129 recovered from the initial wilting following inoculation.

All seedlings wilted when inoculated with cm 21. Concentration difference did not affect the time between inoculation and wilting of the seedlings, suggesting that at the level used, 100 x changes in the concentration of the inoculum did not change the reaction of the seedling to the pathogen.

CRITERIA FOR RESISTANCE

In previous experiments 2 symptoms were used to indicate susceptibility wilting and vascular discoloration. These symptoms were inadequate, since some of the susceptible plants failed to wilt and the degree of the vascular discoloration was variable. Other symptoms commonly associated with the diseased seedlings are stunted growth and cracks in the stem or petiole, commonly called cankers. In the following experiment the manifestation of bacterial canker of tomato was studied with the purpose of determining the best possible symptom for differentiating between resistant and susceptible reactions.

Twenty-five day old seedlings of the cultivars A 134, Bulgaria 12 and Spartan Red 8 were petiole inoculated with isolates cm 21 and F. The bacterial concentration was adjusted to 15×10^8 cells / cm^3 and 3×10^6 cells / cm^3 ; a 500 x dilution. The experiment was replicated 3 times with 7 plants per treatment. The wilting and occurrence of cankers were recorded daily.

Canker size, plant height, and the percentage of vascular discoloration were recorded at the termination of the study. The concentration of the inoculum did not have an effect on the reaction of the cultivars, therefore, the data

for concentration were pooled.

Wilting

Seedlings of susceptible and resistant cultivars inoculated with isolate cm 21 wilted and died 25 days after inoculation (Table 6). Wilting did not occur on all plants inoculated with isolate F, but seedlings of the susceptible cultivar Spartan Red 8 differed significantly in wilting from those of the resistant cultivars A 134 and Bulgaria 12. It appears that the resistance of a cultivar may be determined by the percent of wilted plants, but not the resistance of individual plants (Table 6).

Table 6. The reaction, 25 days after petiole-inoculation, of 3 cultivars of tomato to 2 isolates of C. michiganense.

Cultivar	Percent plants with cankers (isolate F)	Percent wilted plants (isolate F)	Percent wilted plants (isolate cm 21)
	%	%	%
Spartan Red 8	74	43	100
Bulgaria 12	17	7	97
A 134	4	3	93
LSD (.05) :	20%	10%	10%

Cankers

Seedlings inoculated with isolate cm 21 succumbed prior to canker development. Inoculation with isolate F caused canker to develop on 74% of Spartan Red 8 seedlings, while only 43% of the seedlings showed wilting (Table 6). Cultivars can be classified as resistant or susceptible, based on the mean number of plants showing canker on the stem (Table 6), however, the determination of resistance of individual plants was not always possible since many plants did not develop canker.

Vascular discoloration

The percent of vascular discoloration in Spartan Red 8 ranged from 0 to 100%, while for Bulgaria 12 the range was from 0 to 16%. Inoculation at the 3 leaf stage of Bulgaria 12 showed a range of discoloration from 0 to 42%, and for the Spartan Red 8 from 6 to 100%. The coefficient of variation for Spartan Red 8 is 52%, which makes discoloration unsuitable for the determination of the resistance of individual plants, but the mean score may be used for the determination of the resistance of a cultivar (Table 5).

Thyr (47), using mean ratings between cultivars, has shown that the size of the canker was positively correlated with the degree of vascular discoloration. A similar observation was noted in this study, however, within a cultivar no relationship was found.

Stunting

Stem growth of the Spartan Red 8 seedlings, beyond the point of inoculation, ranged from 2 to 43 mm, with a mean of 15 mm and a coefficient of variation of 74%. For Bulgaria 12 the growth ranged from 10 to 72 mm with a mean of 27 mm and a coefficient of variation of 46%.

A significant correlation ($r = .80$) between stunting and vascular discoloration was noted for Spartan Red 8. There was no relationship between the size of the canker and plant size ($r = .20$).

Apparently, each of the described symptoms alone does not appear to be a reliable indicator of the reaction of individual plants to the pathogen. The criteria for resistance used in the genetic analysis included wilting, stunting and the presence of canker (Figure 1).

0= Death of the growing point.

1= Extensive wilting. stunted growth, or large cankers (3 mm or larger).

2= Plant approaching normal size with some wilting of leaves or leaflets.

3= Healthy seedlings with no apparent symptoms of the disease.

Wounds of 2 mm or smaller at the point of inoculation may occur.

1. Disease ratings based on symptoms of plants
inoculated with C. michiganense:

- A. Plant showing one wilted leaf; Rating #2
- B. Plant showing progressive wilting of leaves;
Rating #1
- C. Plant with wilted leaves and dead growing
point; Rating #10
- D. Plant showing several wilted leaves, growing
point alive; Rating #1
- E. Plant showing unilateral wilting of a leaf;
Rating #2
- F. Plant with stem canker; Rating #1

Figure 1



RESISTANCE OF MATURE PLANTS

In the summer of 1973. 5 cultivars of tomato with variable resistance to C. michiganense were planted in the field at intervals of 91 cm in rows, 152 cm apart. Bulgaria 12 is characterized by small fruits and resistance to C. michiganense which was derived from L. pimpinellifolium (8). G-14565 is a resistant cherry tomato (46). Earliana is a susceptible cultivar whose hybrids with L. pimpinellifolium remained healthy when inoculated with a combination of isolates of the pathogen (8). MH-1 is susceptible to C. michiganense. Saturn is resistant to bacterial wilt caused by Pseudomonas solanacearum E. F. Sm. Thyr (personal communication) has reported that resistance to P. solanacearum provides some protection to C. michiganense.

The plants of each genotype were petiole inoculated at anthesis with isolate F or cm 21. The mean fruit weight and the number of fruits were recorded weekly during the harvest season, except for G-14565, where due to small fruits, the total fruit weight was determined at the end of the season. Measurement of the vascular discoloration in plants sampled from the guard rows at various times during the growing season showed that the plants were infected with C. michiganense.

Fruit number was not affected by infection with either isolate, however, fruit size was reduced. Table 7 shows the reduction in the mean fruit weight. With the exception of Bulgaria 12 the mean fruit weight was reduced by isolate cm 21. This reduction became significant after the second harvest. Only MH-1 showed a significant decrease in the mean fruit weight when inoculated with the less virulent isolate F.

Isolate F did not affect the total fruit yield of any of the cultivars, while cultivars inoculated with isolate cm 21 showed an average reduction in yield of 17% (Table 8). The absence of a isolate x cultivar interaction suggests there was no cultivar difference in their reaction to isolate F and cm 21. However, F tests performed on the yield of individual cultivars showed that MH-1, inoculated with cm 21, yielded significantly less than the control.

It is apparent that MH-1 was the most susceptible cultivar in this study, with Earliana and Saturn showing a low level of tolerance to the pathogen. The resistance of Bulgaria 12 was superior since the mean fruit weight of this variety was not reduced when infected with the virulent isolate cm 21. It would appear that the seedling resistance as shown by Bulgaria 12 in greenhouse tests could be used as a criterium for selecting resistant plants in a segregating population.

Table 7. The mean fruit weight of 4 cultivars inoculated with isolate F or cm 21.

Cultivar	Mean fruit weight in grams		
	Control	F	cm 21
Bulgaria 12	42	41	38
MH 1	179	150	97
Earliana	119	130	87
Saturn	122	113	81

Means joined by a common line are not different from each other (.05)

Table 8. The yield of 5 cultivars of tomato inoculated at anthesis with isolate F or cm 21 and control.

Cultivar	Total yield per plant in kg		
	Control	F	cm 21
Bulgaria 12	8.2	8.6	7.6
MH 1	8.1	7.8	4.7
Earliana	6.9	7.6	6.1
G 14565	3.6	3.9	3.6
Saturn	6.9	7.7	5.6
mean	6.9	7.2	5.7

Figures joined by a common line are not different from each other (.05)

Mature plants vs seedling resistance.

Four week old seedlings of the cultivars Earliana, Bulgaria 12, Saturn, MH-1 and G-14565 were grown in the greenhouse following stem-inoculation with isolate H. The disease ratings, on the scale 0 to 3 are presented in Table 9. Earliana, which showed a low degree of tolerance as a mature plant was as susceptible as MH-1 in the seedling stage, 23 days after inoculation. Saturn showed a higher tolerance than either MH-1 or Earliana. Bulgaria 12 was resistant in the seedling stage. Thirty two days after inoculation the resistance of Saturn diminished while Bulgaria 12 and G-14564 remained resistant.

It is apparent that the seedling screening technique used may be reliable, however small differences in resistance may go undetected especially when scored 30 days, or later after inoculation.

Table 9. Disease rating of 5 cultivars of tomato stem-inoculated with isolate H in the seedling stage and with isolate F or cm 21 at anthesis in the field.

Cultivar	Rating 23 days after stem-inoculation with isolate H				Rating 32 days after stem inoculation with isolate H				Resistance rating	
	3	2	1	0	3	2	1	0	Seedling	Mature plant
	Number of plants				Number of plants					
Bulgaria 12	21				21				resistant	resistant
G 14565	21				21				resistant	resistant*
Earliana			15	6			1	20	susceptible	low tolerance
Saturn		9	12				3	19	low tolerance	low tolerance
MH 1			17	4				21	susceptible	susceptible

* Based on absence of symptoms on foliage

DIALLEL ANALYSIS

Estimates of general and specific combining ability were made using a half diallel hybridization scheme. Six reciprocal crosses were made to determine maternal effects.

The diallel was analysed according to Griffings (15) model 1 (selected genotypes), method 2 (parent and one set of F_1 's included) which restricts inferences to the cultivars included in the experiment. The mean values for F_1 hybrids and parents are presented in Table 10. The error based on the cultivar x replication interaction and not only the within cultivar error as suggested by Griffing, was used. Since a highly significant F ratio was obtained for cultivars (Table 11) the null hypothesis that the entries are identical was rejected and the analysis for combining ability was performed (Table 12). General (GCA) and specific (SCA) combining ability were significant ($P = .01$). The estimation of the GCA and SCA is presented in the Tables 13 and 14.

General combining ability compares the performance of each parent with other parents, thus positive values denote desirable performance. The restriction that $\sum_i g_i = 0$ is imposed where g_i is the GCA of the i th parent. A difference in GCA between the 2 susceptible parents Earliana and MSU 72-279 suggests that MSU 72-279 transmits its susceptibility much more readily to all of its hybrids than Earliana.

Specific combining ability measures the deviation of the heterozygote from the average performance of the homozygotes. The restriction imposed on the SCA is $\sum_j s_{ij} + s_{ii} = 0$, where s_{ij} is the SCA of the ij th genotype and s_{ii} the SCA term assigned to the self pollinated progeny of cultivar i . The SCA associated with Earliana indicates that its hybrids with L. pimpinellifolium (A 129 and A 134) and L. hirsutum are considerably more resistant than would be expected where resistance was governed by additive gene action alone. If hybrids were the desired product, the combination of Earliana x L. hirsutum would give superior resistance. The hybrids of MSU 72-279 with Bulgaria 12 and L. hirsutum are less resistant than expected from their average performance.

It is notable that both the GCA and the SCA of the cultivars A 134 and A 129 do not differ significantly suggesting identical genes for resistance.

Reciprocal differences

Means for resistance to isolate H of all parents and their hybrids are presented in Table 10. No difference was observed between the cross of Bulgaria 12 with A 129 and its reciprocal. Hybrids of Bulgaria 12 with susceptible cultivars MSU 72-279 and Earliana also do not differ

Table 10. F₁ hybrid and parent mean values for resistance to isolate H.

Paternal parent	Maternal parent					<u>L.</u> <u>hirsutum</u>
	Bulg. 12	MSU 72-279	Earliana	A 129	A 134	
Bulg. 12	2.88 a	.83 fg	1.75 d	2.92 a		
MSU 72-279	.74 g	.0 h		.76 g	.78 g	
Earliana	1.95 dc	.05 h	.0 h			
A 129	2.90 a	1.33 e	2.29 cb	3.0 a	2.98 a	
A 134	2.98 a	1.14 ef	2.24 c	3.0 a	2.93 a	
<u>L. hirsutum</u>	2.69 a	.48 g	2.69 a	2.83 a	2.62 ab	2.69 a

Means with the same letter do not differ significantly ($P=.05$) as determined by Duncan's multiple range test.

LSD (.05) = .33

LSD (.01) = .44

Table 11. Variance analysis for individual observations of disease resistance for the 15 F₁ hybrids and 6 parental lines of the half diallel hybridization scheme.

Source	df	MS	F	P
Cultivars	20	52.39	88.8	.01
Replicates	5	2.58	4.37	
Cultivars x Rep.	100	.59		
Error (Within cult.)	709*	.327		

* The df are less than expected due to the loss of a few plants.

Table 12. Combining ability analysis for resistance to C. michiganense isolate H.

Source	df	MS	F	P
GCA	5	4.4513	318	.01
SCA	15	.1794	12.8	.01
Error	100	.014		

Table 13. General combining ability for resistance to C. michiganense isolate H.

	Cultivar					
	Bulg. 12	MSU 72-279	Earliana	A 129	A 134	<u>L.</u> <u>hirsutum</u>
GCA	.40	- 1.26	- .57	.57	.50	.36

LSD (.05) = .116

LSD (.01) = .155

Table 14. Specific combining ability for resistance to C. michiganense isolate H.

Paternal parents	Maternal parents					
	Bulg. 12	MSU 72-279	Earliana	A 129	A 134	<u>L.</u> <u>hirsutum</u>
Bulg. 12	.. .10					
MSU 72-279	- .38 de	- .54				
Earliana	.15 bc	- .09 bcd	- .82			
A 129	- .04 bcd	.05 bcd	.32 b	- .11		
A 134	..10 bc	- .07 bcd	.34 b	- .04 bcd	- .05	
<u>L. hirsutum</u>	- .04 bcd	- .60 e	.93 a	- .07 bcd	- .22 de	0.0

Numbers with the same letter do not differ (.05) as determined by Duncan's multiple range test.

LSD (parents) (.05) = .23 (.01) = .31

LSD (hybrids) (.05) = .31 (.01) = .41

from their reciprocals. A significant difference ($P < .05$) between reciprocals was observed in the crosses of MSU 72-279 with A 134. A higher degree of resistance was noted when MSU 72-279 was used as the female parent.

Four weeks after planting the seedlings of the cross A 134 x MSU 72-279 and A 129 x MSU 72-279 were considerably smaller than those of the reciprocals probably due to the smaller seed of A 134 and A 129, which has a smaller embryo and cotyledons.

The resistance of seedlings increases with their size (14), which may explain why the smaller seedlings of the cross L. pimpinellifolium x L. esculentum are more susceptible than the larger reciprocals.

The hybrids of the crosses between L. pimpinellifolium (A 134 and A 129) and L. esculentum (MSU 72-279 and Earliana), evaluated for GCA and SCA were all made with L. esculentum as the female.

Crosses to L. hirsutum could only be made with L. hirsutum as the male parent (27).

THE INHERITANCE OF RESISTANCE

Seedlings with disease rating of 3 were considered resistant, while those rated 2, 1, or 0 were classified as susceptible. The reaction to the pathogen was genetically controlled, however within a genotype the following causes of variation were observed:

- (1) The method of inoculation affected the disease rating. Cultivars resistant to stem-inoculation succumbed when root-inoculated (Table 2).
- (2) Mature plants, in contrast with seedlings generally did not die after inoculation with isolate cm 21. Within the seedling stage, reaction to the pathogen had been reported to be dependent on plant size (20, 42).
- (3) Isolates differed in virulence (Tables 1 and 2).

These observations suggest that resistance is not absolute but needs to be qualified with each isolate, inoculation method and plant-age. The interaction between host and pathogen is further influenced by the environmental and experimental conditions such as temperature, light, nutrition, and water (20), seedling size (20, 42), inoculum load, (even though 100 fold deviations of 10^8 cells / cm^3 did not effect the rating), seedling injury at the time of inoculation, and scoring errors. The errors caused by these, and

possibly other factors as well, can be very large as illustrated by the large coefficient of variation for vascular discoloration and stunting reported earlier in the study.

The possibility remains that variation observed within a cultivar is due to heterozygosity. Although the tomato is classified as self pollinating, natural outcrossing has been reported at a frequency of 0.5 to 4% (33). If an average outcrossing of 2% per generation is assumed, 4% ($2 + 1 + .5 + .25 + \dots$) of the loci are expected to be in heterozygous condition. The possible error caused by this deviation is small compared to the environmental and experimental errors.

In order to measure bacterial movement in the stem, the 4 nodes at the apex of 10 - 12 node plants from the resistant cultivars and F_1 hybrids were sampled. All plants were stem inoculated below the first true leaf with isolate H. Four plants of each cultivar were used for the sampling. The nodes were assayed on seedlings of the susceptible cultivar MSU 72-279. The bacterium was recovered from the 2nd or 3rd intermode of F_1 plants of Earliana x Bulgaria 12. No bacterium was recovered from the sections of Bulgaria 12, A 129, L. hirsutum and Earliana x L. hirsutum. Most seedlings of Earliana x Bulgaria 12 showed visible symptoms of the disease, but the 4 plants selected for this experiment did not. Apparently, the resistant plants of Earliana x Bulgaria 12 were infested with the pathogen without visible symptoms.

The probability of misclassifying plants of the 'more' susceptible genotype is less than for those of the 'less' susceptible genotypes. Therefore, in the latter genotype, there is the possibility that a greater number may be classified as resistant. For example. Plants with the genotype of Earliana AAbbc²C²dd will not be misclassified as frequently as the genotype AaBbc²CDd (F₁ hybrid of Earliana x Bulgaria 12). Similarly it is also possible for misclassification to occur when classifying resistant plants. Weighting procedures have been introduced to correct the F₂, BC to P₁, and BC to P₂ for the misclassifications in the parent and F₁ generation as follows:

(A) Weighting for misclassification of the resistant parent:

$$R_w = R_o + \left(\frac{\text{No. resistant plants in segregating population}}{\text{No. resistant plants in resistant parent}} \times \text{No. misclassified plants of resistant parent} \right)$$

with R_w = weighted number of resistant plants in the segregating population,

and R_o = the observed number of resistant plants in the segregating population.

(B) Weighting for misclassification in the F₁ hybrid generation, F₁ is susceptible but some plants are misclassified as resistant:

$$R_w = R_o - \left(\frac{\text{No. resistant plants in F}_1 \text{ generation}}{\text{Total No. plants in F}_1 \text{ generation}} \times \text{frequency F}_1 \text{ phenotype in segregating generation} \times \text{total No. plants in segregating generation} \right)$$

Based on the information obtained from the diallel hybridization scheme and the progenies of the cross Bulgaria 12 x MSU 72-279, it appears that a 4-gene system approximately describes the overall genetic basis of resistance to isolate H of C. michiganense. The model encompasses the following observations:

- (a) The F_1 hybrid of a susceptible x resistant parent is susceptible, suggesting that resistance is a recessive trait.
- (b) A low level of resistance is conferred to the F_1 hybrids of susceptible x resistant parents, possible because of incomplete dominance of the genes controlling susceptibility.
- (c) The susceptible parents, and possibly the resistant parents, may possess different genes for resistance or susceptibility, which could account for the differences observed in the resistance of their hybrids.

The weighted F_2 ratio of the cross Bulgaria 12 (P_1) x MSU 72-279 (P_2) (Table 15) fits most closely to the 27 resistant (R): 229 susceptible (S) ratio expected from a 4-gene model with the genotype aaB-C-D determining resistance. The backcross to the resistant parent yielded 1 R to 1 S, while the backcross to the susceptible parent resulted in a 0: 1, resistant: susceptible ratio. The proposed genotypes are: aaBBCCDD for P_1 , AAbbccdd for P_2 and AaBbCcDd for the F_1 . A/a is the major gene pair for resistance with AA determining susceptibility, whereas aa

Table 15. Chi-square test for 4-gene model for resistance to isolate H in the F₂, BC to P₁, and BC to P₂ of the cross Bulgaria 12 (P₁) x MSU 72-279 (P₂).

Generation		Days after inoculation	Observed segregation				Expected segregation				X ²	P
			Unweighted R	S	Weighted R	S	Number R	S	Ratio R	S		
Bulgaria 12	(P ₁)	32	77	7			84	0	.1 : 0			
MSU 72-279	(P ₂)		0	80			0	80	0 : 1			
F ₁			5	67			0	72	0 : 1			
F ₂			36	292	35	293	35	293	27 : 229		.001	.90 - .95
BC to P ₁			48	59	49	58	54	53	1 : 1		.76	.30 - .50
BC to P ₂			4	115	4	115	0	119	0 : 1			
Bulgaria 12	(P ₁)	40	81	3			84	0	1 : 0			
MSU 72-279	(P ₂)		0	80			0	80	0 : 1			
F ₁			2	70			0	72	0 : 1			
F ₂			38	290	38	290	35	293	27 : 229		.29	.50 - .70
BC to P ₁			55	52	56	51	54	53	1 : 1		.14	.70 - .80
BC to P ₂			1	118	1	118	0	119	0 : 1			

Table 15 (continued)

Generation		Days after inoculation	Observed segregation				Expected segregation				X ²	P
			Unweighted		Weighted		Number		Ratio			
			R	S	R	S	R	S	R	S		
Bulgaria 12	(P ₁)	47	82	2			84	0	1 : 0			
MSU 72-279	(P ₂)		0	80			0	80	0 : 1			
F ₁			2	70			0	72	0 : 1			
F ₂			32	296	31	297	35	293	27 : 229		.51	.30 - .50
BC to P ₁			55	52	55	52	54	53	1 : 1		.08	.70 - .80
BC to P ₂			0	119	0	119	0	119	0 : 1			
Bulgaria 12	(P ₁)	60	82	2			84	0	1 : 0			
MSU 72-279	(P ₂)		0	80			0	80	0 : 1			
F ₁			3	69			0	72	0 : 1			
F ₂			38	290	36	292	35	293	27 : 229		.03	.80 - .90
BC to P ₁			64	43	64	43	54	53	1 : 1		3.72	.05 - .10
BC to P ₂			0	119	0	119	0	119	0 : 1			

determines resistance when combined with B-C-D. The dominance of A is incomplete, allowing for intermediate levels of resistance. B, C, and D are 3 genes with incomplete dominance. The AaB-C-D- genotypes represent a continuum of intermediate resistant types, whose differences in resistance can only be detected with refined techniques and isolates of appropriate virulence.

Bulgaria 12 x MSU 72-279

Seven plants of the resistant parent were initially classified as susceptible (Table 15). After stem inoculation with isolate H, the remaining leaves wilt for 2 weeks prior to recovery. Probably due to the sampling errors described earlier 2 seedlings failed to recover from the initial reaction, while 5 recovered between the 32nd and 47th day. At each observation similar misclassification as observed in the parents may be expected in the segregating population, thus allowing for weighting of the ratio based on the observed parental deviations.

Plants of the F_1 generation are intermediate in resistance and may also be misclassified. Phenotypes in the segregating populations with F_1 genotypes are subjected to misclassification and are, therefore, weighted based on variations in the F_1 generation.

The weighting for the number of resistant plants in the progenies of the cross Bulgaria 12 (P_1) x MSU 72-279 (P_2) is as follows (Table 15, 32 days after inoculation).

F_2 : $R_w = 36 + (36/77 \times 7) - (5/72 \times 54/256 \times 328) = 34.5$
 BC to P_1 : $R_w = 48 + (48/77 \times 7) - (5/72 \times 8/16 \times 107) = 48.7$
 BC to P_2 : $R_w = 4 + (4/77 \times 7) - (5/72 \times 1/16 \times 119) = 3.5$
 with $54/256 =$ the frequency of the F_1 phenotype ($AaB-C-D-$)

in the F_2

$8/16 =$ the frequency of the F_1 phenotype in the BC to P_1

$1/16 =$ the frequency of the F_1 phenotype in the BC to P_2

Since the genotype aaBBCCDD is assigned to Bulgaria 12 and AAbbccdd to MSU 72-279, the genotype of the F_1 is AaBbCcDd, or AaB-C-D-. Plants having this genotype are not as susceptible as MSU 72-279 due to the incomplete dominance of A and the dominant alleles of genes B, C and D.

The observed and expected segregation ratios for the P_1 , P_2 , F_1 , F_2 , BC to P_1 , and BC to P_2 are presented in Table 15. A good fit to the proposed model is shown at the 32, 40, and 47 day observations. At the 60 day observation, some of the plants of the BC to P_1 that were previously susceptible were classified as resistant possibly because the seedlings shedded their lower leaves which were the only ones diseased and had been used to determine susceptibility.

Bulgaria 12 x Earliana

F_1 hybrids of the cross Bulgaria 12 (P_1) were susceptible but significantly ($P < .01$) less than hybrids of Bulgaria 12 x MSU 72-279 (Table 10). It appears that the genotype of Earliana, although susceptible, differs from the genotype of MSU 72-279. The increased resistance of the F_1

and the higher number of resistant plants in the F_2 is explainable by the presence of genes which increase the resistance of plants heterozygous for A. An allele of C and c, called C^2 , with dominance in the order $C^2 > \underline{C} > \underline{c}$ is proposed to confer resistance to plants of the genotype AaBBC²-D-. The genotype AAbbC²C²dd is proposed for Earliana. The F_2 (Table 16) fits the ratio 54 resistant: 202 susceptible ($P = .80$) with resistance controlled by the genotypes aaB-C²-D-, aaB-CCD-, and AaBBC²-D-. The observed and expected frequencies of the P_1 , P_2 , F_1 , F_2 , BC to P_1 , and BC to P_2 are presented in Table 16.

Table 16. Chi-square test for 4-gene model for resistance to isolate H in the F_2 , BC to P_1 , and BC to P_2 of the cross Bulgaria 12 (P_1) x Earliana (P_2).

Generation		Days after inoculation	Observed segregation				Expected segregation				χ^2	P
			Unweighted R	Unweighted S	Weighted R	Weighted S	Number R	Number S	Ratio R	Ratio S		
Bulgaria 12	(P_1)	32	72	10			82	0	1 : 0			
Earliana	(P_2)		0	75			0	75	0 : 1			
F_1			24	58			0	82	0 : 1			
F_2			78	253	71	260	70	261	54 : 202		.02	.80 - .90
BC to P_1			82	50	79	53	83	49	10 : 6		.40	.50 - .70
BC to P_2			10	110	6	114	0	120	0 : 1			
Bulgaria 12	(P_1)	40	77	5			82	0	1 : 0			
Earliana	(P_2)		0	75			0	75	0 : 1			
F_1			22	60			0	82	0 : 1			
F_2			83	248	72	259	70	261	54 : 202		.16	.80 - .90
BC to P_1			86	46	87	45	83	49	10 : 6		.30	.50 - .70
BC to P_2			7	113	3	117	0	120	0 : 1			

Table 16 (continued)

			Observed segregation				Expected segregation				X ² P	
			Unweighted		Weighted		Number		Ratio			
Generation		Days after inoculation	R	S	R	S	R	S	R	S		
Bulgaria 12	(P ₁)	47	77	5			82	0	1 : 0			
Earliana	(P ₂)		0	75			0	75	0 : 1			
F ₁			13	69			0	82	0 : 1			
F ₂			60	271	55	276	70	261	54 : 202		4.07	.02 - .05
BC to P ₁			91	41	90	42	83	49	10 : 6		1.82	.10 - .20
BC to P ₂			2	118	0	120	0	120	0 : 1			
Bulgaria 12	(P ₁)	93	78	4			82	0	1 : 0			
Earliana	(P ₂)		0	75			0	75	0 : 1			
F ₁			12	70			0	82	0 : 1			
F ₂			55	276	49	282	70	261	54 : 202		8.0	.001 - .01
BC to P ₁			91	41	89	43	83	49	10 : 6		1.36	.20 - .30
BC to P ₂			1	119	0	120	0	120	0 : 1			

The weighting factor as based on the reaction of the parental populations and the F_1 hybrid is similar to those described earlier. The F_1 phenotype is represented by the genotypes AaBbC² - (or CC) D- which occur at a frequency of 48/256 in the F_2 .

Data from the 47 and 93 day observation do not fit the proposed model as resistance in the F_2 continued to break down, possibly due to a delayed reaction of certain genotypes to the pathogen.

MSU 72-279 x A 134

F_1 hybrids of the cross MSU 72-279 (P_1) x A 134 (P_2) are less susceptible ($P = .05$) than the F_1 hybrids of Bulgaria 12 x MSU 72-279 (Table 10), suggesting that A 134 transmits a higher level of resistance to its progeny than Bulgaria 12.

The higher resistance of A 134 is explained by the presence of an allele of gene D in A 134 with an order of dominance of D² > D > d. Similar to C², D² increases the resistance of plants heterozygous for A, so that the genotype of AaBBC-D² - are assumed to be resistant.

The proposed genotype for MSU 72-279 is AAbbccdd and aaBBCCD²D² for A 134. The weighted F_2 ratio (Table 17) fits to the expected ratio of 45 resistant: 211 susceptible ($P = .30$) with aaB-C-D²- and AaBBC-D² controlling resistance. The BC to P_1 and P_2 weighted ratios give an acceptable fit to the expected 0 resistant: 1 susceptible and 3 resistant: 1 susceptible ratios respectively.

Table 17. Chi-square test for 4-gene model for resistance to isolate H, 51 days after inoculation in the F_2 , BC to P_1 , and BC to P_2 of the cross MSU 72-279 (P_1) $\times$ A 134 (P_2).

		Observed segregation				Expected segregation				X ²	P
		Unweighted		Weighted		Number		Ratio			
Generation		R	S	R	S	R	S	R	S		
MSU 72-279	(P ₁)	0	56			0	56	0 : 1			
A 134	(P ₂)	56	0			56	0	1 : 0			
F ₁		11	45			0	56	0 : 1			
F ₂		49	175	43	181	39	185	45 : 211		.49	.30 - .50
BC to P ₁		3	85	2	86	0	88	0 : 1			
BC to P ₂		67	13	63	17	60	20	3 : 1		.60	.30 - .50

Earliana x A 134

F_1 hybrids of the cross Earliana (P_1) x A 134 (P_2) have shown a high level of resistance to isolate H (Table 10). A combination of dominant genes in the F_1 provides a level of resistance, however misclassification may put a proportion of the F_1 hybrids in the susceptible class. A minimum weighting factor is obtained by assuming that under the conditions of this test the F_1 is resistant.

The proposed genotypes for Earliana and A 134 are $AAbbc^2C^2dd$ and $aaBBCCD^2D^2$ respectively. If the F_1 $AaBbC^2CD^2d$ is classified as resistant, then the susceptible genotypes are $AA-- -- --$, $-- bb -- --$ and $-- -- -- dd$ with a frequency of 144/256. The ratio of resistant to susceptible in the F_2 is therefore expected to be 112 : 144. The weighted segregation ratio for 44 days after inoculation shows a good fit to a 4 gene model (Table 18). The genotypes of the BC to P_2 are all expected to be resistant, whereas, in the BC to P_1 the genotypes $AaBbC^2C^2D^2d$ and $AaBbC^2CD^2d$, with a combined frequency of 2/16, are resistant.

With a resistant F_1 , weighting will increase the number of resistant plants in the segregating populations. For the F_2 (Table 18, day 44) the weighted number of resistant plants is:

$$R_w = 80 + (12/55 \times 72/256 \times 223) = 94$$

with 12/55 = number of susceptible F_1 hybrid plants of total

72/256 = frequency of the F_1 phenotype ($AaB-C^2CD^2-$
 $AaB-CCD^2-$)

223 = total number of plants in the F_2 .

Table 18. Chi-square test for 4-gene model for resistance to isolate H in the F_2 , BC to P_1 , and BC to P_2 of the cross Earliana (P_1) x A 134 (P_2).

Generation		Days after inoculation	Observed segregation				Expected segregation				X^2	P
			Unweighted R	S	Weighted R	S	Number R	S	Ratio R	S		
Earliana	(P_1)	33	0	56			0	56	0 : 1			
A 134	(P_2)		56	0			56	0	1 : 0			
F_1			42	13			55	0	1 : 0			
F_2			95	128	110	113	98	125	112 : 144		2.61	.10 - .20
BC to P_1			9	72	11	70	10	71	1 : 7		.11	.70 - .80
BC to P_2			74	12	84	2	86	0	1 : 0			
Earliana	(P_1)	44	0	56			0	56	0 : 1			
A 134			56	0			56	0	1 : 0			
F_1			43	12			55	0	1 : 0			
F_2			80	143	94	129	98	125	112 : 144		.29	.30 - .50
BC to P_1			5	76	7	74	10	71	1 : 7		1.12	.20 - .30
BC to P_2			68	18	77	9	86	0	1 : 0			

This experiment was repeated after isolate H lost some of its virulence. The plants were rated 67 days after inoculation to assure maximum expression of the disease. The complete resistance of the F_1 hybrid (Table 19) suggests that the Aa is resistant and only the AA genotype is susceptible. However, the data shows a poor fit to a 3 resistant: 1 susceptible ratio in the F_2 ($\chi^2 = 2.32$ $P = .10$).

Another possibility is that with a less virulent pathogen, resistance may be controlled by genes at the B, C and D loci only, independent of the genes at locus A. The proposed model assumes resistance to be enhanced if the B, C or D loci are occupied by the dominant alleles. If the genotypes -- bb -- dd and -- bb -- D²d confer susceptibility, a 208 resistant: 48 susceptible ratio can be expected in the F_2 . The BC to P_1 will yield only resistant plants, whereas a 1 resistant: 1 susceptible ratio is expected in the BC to P_2 . Table 19 shows that this model fits the observed ratios.

The action of the B, C, and D genes on the pathogen may also explain the differences observed in the level of resistance as reported in otherwise susceptible cultivars (43, 49).

MSU 72-279 x A 129

Results shown in Table 13 and 14 suggested similarity in the gene action of the L. pimpinellifolium cultivars, A 129 and A 134. Since the F_1 mean values for resistance do

Table 19. Segregation ratios and Chi-square test for resistance to isolate H, 54 days after inoculation, of progenies of Earliana (P_1) x A 134 (P_2).

Generation		Observed segregation		Expected segregation		χ^2	P.
		R	S	Number R S	Ratio R S		
Earliana	(P_1)	0	42	0 42	0 : 1		
A 134	(P_2)	42	0	42 0	1 : 0		
F_1		133	33	135 31	208 : 48	.15	.50 - .70
BC to P_1		66	0	66 0	1 : 0		
BC to P_2		26	34	30 30	1 : 1	1.07	.30 - .50

not differ significantly in the crosses of A 129 and A 134 with a common parent (Table 19), it is presumed that the genotypes of A 134 and A 129 are similar.

In the cross of MSU 72-279 (P_1) x A 129 (P_2) identical ratios to those of MSU 72-279 x A 134 were expected. The weighted and expected ratios are presented in Table 20. The weighted F_2 ratio fits the expected ($P = .50$), but the backcross ratio shows a poor fit ($P = .001$). Sampling in the smaller backcross population, together with the large weighting factor may have effected the segregation ratios.

Crosses between resistant parents

Progenies resulting from the crossing of resistant plants performed as expected with complete resistance in all generations, supporting the hypothesis that the genotypes of the resistant parents: Bulgaria 12, A 129, and A 134 may be identical in their resistance to C. michiganense isolate H.

Crosses between susceptible parents

Progenies resulting from the cross MSU 72-279 x Earliana were susceptible to isolate H.

Table 20. Chi-square test for 4-gene model for resistance to isolate H 34 days after inoculation in the F_2 , BC to P_1 , and BC to P_2 of the cross MSU 72-279 (P_1) x A 129 (P_2).

Generation		Observed segregation				Expected segregation				X ²	P
		Unweighted		Weighted		Number		Ratio			
		R	S	R	S	R	S	R	S		
MSU 72-279	(P ₁)	5	66			0	71	0 : 1			
A 129	(P ₂)	75	1			76	0	1 : 0			
F ₁		33	43			0	76	0 : 1			
F ₂		79	220	49	250	53	246	45 : 211		.37	.50 - .70
BC to P ₁		19	100	8	111	0	119	0 : 1			
BC to P ₂		75	35	61	49	83	27	3 : 1		22.4	< .001

Crosses with L. hirsutum

The gene model for L. hirsutum is based on the following observations;

- (1) F_1 plants of Earliana x L. hirsutum have shown as high a level of resistance to isolate H as F_1 plants of Bulgaria 12 x L. hirsutum and L. pimpinellifolium x L. hirsutum, while F_1 plants of 72-279 x L. hirsutum were very susceptible.
- (2) Increased resistance was observed in plants of the F_2 , BC to P_1 and BC to P_2 of A 134 x L. hirsutum, inoculated with the virulent isolate cm 21.

Based on these observations the following hypotheses were made:

- (1) Genes for resistance in L. hirsutum differ from L. esculentum or L. pimpinellifolium, which allows for transgressive segregation in the F_2 . The gene pair xx controls resistance in L. hirsutum, while XX is present in L. pimpinellifolium and L. esculentum. Gene X shows incomplete dominance.
- (2) The resistance of the Xx genotype is determined by genes C and F, with C²-F- conferring resistance to the Xx genotype. The genes C² and F show complementary gene action in their action on the Xx genotype.

Earliana x L. hirsutum

F_1 progeny of the cross Earliana (P_1) x L. hirsutum (P_2) suggests a significant specific combining ability

($P < .01$). The interaction of gene $\underline{C^2}$ of Earliana with gene $\underline{F}$ of L. hirsutum is suggested to allow for this phenomenon.

Two gene pairs, $\underline{XX}$ and $\underline{ff}$ are added to the genotype of Earliana; $\underline{AAbbc^2C^2dd}$. The genotype of L. hirsutum is assumed to be $\underline{xxAAbbccddFF}$. Therefore the genotype of the F_1 hybrid of Earliana x L. hirsutum is $\underline{XxAAbbc^2C^2ddFf}$. Since only the genes $\underline{X}$, $\underline{C}$, and $\underline{F}$ will be segregating the ratios expected are from the 3-gene model. The genotype $\underline{xx -- --}$ and $\underline{Xx C^2-F-}$ are assumed to be resistant, with $\underline{XX C^2-F-}$ giving a higher level of tolerance.

The progression from resistance to susceptibility is gradual, even within a specific genotype as noted by the change in resistance over a period of time of many F_1 plants. Plants with the genotype $\underline{XXC^2-F-}$ may ultimately be rated susceptible. However, 50 days after re-inoculation only 50% of this genotype may have shown the disease. The resistant genotypes are therefore $\underline{xx -- --}$, $\underline{Xx C^2-F-}$ and $\frac{1}{2}$ ($\underline{XX C^2-F-}$), with an expected frequency of 38.5/64 in the F_2 of 3/8 in the BC of P_1 , and of 3/4 on the BC to P_2 . The weighted data show a good fit to these ratios ($P = .70$, Table 21).

MSU 72-279 x L. hirsutum

F_1 hybrids of MSU 72-279 (P_1) x L. hirsutum (P_2) were susceptible (Tables 10 and 22). The genotype of MSU 72-279 was $\underline{AAbbccdd}$, to which $\underline{XX}$ and $\underline{ff}$ are added, while $\underline{xxAAbbccddFF}$ was proposed for L. hirsutum. Since the action

Table 21. Chi-square test for 3 gene model for resistance to isolate H, 50 days after re-inoculation in the F₂, BC to P₁, and BC to P₂ of the cross Earliana (P₁) x L. hirsutum (P₂).

Generation		Observed segregation				Expected segregation				X ²	P
		Unweighted		Weighted		Number		Ratio			
		R	S	R	S	R	S	R	S		
Earliana	(P ₁)	0	35			0	35	0 : 1			
L. hirsutum	(P ₂)	32	1			33	0	1 : 0			
F ₁		31	4			35	0	1 : 0			
F ₂		75	56	81	50	79	52	39 : 25	.13	.70 - .80	
BC to P ₁		16	34	18	32	19	31	3 : 5	.08	.70 - .80	
BC to P ₂		38	17	40	15	41	14	3 : 1	.09	.70 - .80	

of F is dependent upon the presence of gene C², only gene X is expected to show segregation. Therefore the ratios of resistant: susceptible plants in this cross are not expected to be significantly different from the monogenic ratios of 1 : 3, 0 : 1, and 1 : 1,. A good fit ($P = .50$) to these ratios was obtained (Table 22).

Bulgaria 12 x L. hirsutum

Resistance was observed in the F_1 hybrids of the cross Bulgaria 12 (P_1) x L. hirsutum when stem inoculated with isolate H (Table 10).

The assumed genotypes are XXaaBBCCDDff for Bulgaria 12 and xxAAbbccddFF for L. Hirsutum. In the absence of allele C² the effect of gene F is not detectable. The F_1 genotype can therefore be presented as XxAaB-C-D-. With partial dominance of both the X and A gene, a high level of resistance is expected in this hybrid, but when inoculated with a virulant isolate or under sever conditions this genotype is expected to be susceptible. Root inoculation was tried, but the variance in the disease rating of the parent was too large.

Table 22. Chi-square test for 1 gene model for resistance to isolate H, 50 days after re-inoculation, in the F₂, BC to P₁, and BC to P₂ of the cross MSU 72-279 x L. hirsutum.

Generation		Observed segregation				Expected segregation				X ²	P
		Unweighted		Weighted		Number		Ratio			
		R	S	R	S	R	S	R	S		
MSU 72-279	(P ₁)	0	34			0	34	0 : 1			
<u>L. hirsutum</u>	(P ₂)	32	3			35	0	1 : 0			
F ₁		16	19			0	35	0 : 1			
F ₂		57	82	33	106	35	104	1 : 3		.15	.50 - .70
BC to P ₁		8	42	0	48	0	48	0 : 1			
BC to P ₂		34	23	26	31	29	28	1 : 1		.44	.50 - .70

Bulgaria 12 x L. hirsutum, A 134 x L. hirsutum, and Bulgaria 12 x A 134, inoculated with isolate cm 21

To learn whether phenotypes with higher degree of resistance than either parent are possible, the above crosses were petiole inoculated with the virulent isolate cm 21.

All progenies of Bulgaria 12 x 134 were susceptible, while the resistance in the F_2 and backcrosses of Bulgaria 12 x L. hirsutum did not exceed the resistance of L. hirsutum (Table 23). It was noted that L. hirsutum, when petiole inoculated with cm 21, appeared to be slightly more resistant than either Bulgaria 12 or A 134 (Table 23, 24).

Evidence for transgressive segregation for resistance was found in progenies of the cross A 134 x L. hirsutum, (Table 24) suggesting that genes for resistance in these cultivars were not the same.

Table 23. Segregation for resistance in the F_2 , BC to P_1 , and BC to P_2 of the cross Bulgaria 12 (P_1) $\times$ *L. hirsutum* (P_2) after petiole-inoculation with isolate cm 21.

Generation			Observed segregation		
			R	S	
			3	(2 + 1)	0
		Days after inoculation			
Bulgaria 12	(P_1)	32	—	9	33
<i>L. hirsutum</i>	(P_2)		3	29	11
F_1			—	13	29
F_2			7	102	104
BC to P_1			—	20	22
BC to P_2			3	21	17
Bulgaria 12	(P_1)	58	—	—	42
<i>L. hirsutum</i>	(P_2)		2	11	29
F_1			—	4	38
F_2			2	35	176
BC to P_1			—	1	41
BC to P_2			1	8	32

Table 24. Segregation for resistance in the F_2 , BC to P_1 , and BC to P_2 of the cross A 134 (P_1) x L. hirsutum (P_2) after petiole-inoculation with isolate cm 21.

Generation			Observed segregation		
			R	S	
			3	(2 + 1)	0
Days after inoculation					
A 134	(P_1)	32	—	20	22
<u>L. hirsutum</u>	(P_2)		—	28	14
F_1			1	19	22
F_2			4	83	120
BC to P_1			1	19	22
BC to P_2			1	16	25
A 134	(P_1)	58	—	2	40
<u>L. hirsutum</u>	(P_2)		—	12	30
F_1			—	5	37
F_2			5	38	164
BC to P_1			1	7	34
BC to P_2			1	8	33

SUMMARY AND DISCUSSION

When screening populations segregating for resistance to Corynebacterium michiganense a rapid and reliable inoculation technique is needed. From the several techniques tested. Inoculation of the petiole was found to give better differentiation of resistant and susceptible plants than did inoculation of the root or leaf. When petiole-inoculation was compared with stem-inoculation the latter showed the lowest environmental variation and was therefore used for the inheritance study. Stem-inoculation involved clipping the stems of 4 week old seedlings 1 cm above the cotyledonary leaves with scissors which had been dipped in inoculum.

Petiole inoculation, and to a lesser degree, stem inoculation failed to produce uniform infection, resulting in variation in the expression of the disease. The segregation ratios were therefore based on the presence, and not the degree, of wilting and stunting, or the size of the cankers. Plants free of all these symptoms were rated resistant. The coefficient of variation for vascular discoloration on individual plants was calculated to be 50%, which in part may be explained by the histopathology of the host.

At each node, part of the vascular system of the stem (leaf traces) is deflected into the leaf (9). Several

traces go into a petiole of one leaf. Even though the stem bundles are variously interconnected the pathogen does not move freely laterally (31). This combined with the rapid longitudinal movement of the bacteria and the random distribution of the pathogen in the bundles of the cut petiole, may explain the large variation in the vascular discoloration. If a leaftrace of the first leaf originates from an infected bundle, the leaf will be diseased and further upward movement depends on how fast the pathogen moves into the vessel cells. The pathogen may also have been deflected into the leaf trace of the second or third leaf which allowed the pathogen to move upwards faster. By cutting the stem of the seedling with scissors dipped in inoculum the pathogen spread across the entire before longitudinal movement could set in. This may have reduced the experimental error.

In cotton the rate of multiplication of Xanthomonas malvacearum (E.F. Sm.) Dows. in the leaves of resistant and susceptible cultivars was similar for the first 2 days, but afterwards the rate was less in resistant leaves (30). The initial wilting observed in resistant tomato cultivars suggests that there is a initial increase of the pathogen as in cotton, after which resistance slows the multiplication of the bacteria. Thyr (47) has presented data which showed that C. michiganense multiplies at a lower rate in resistant cultivars and suggests that suppression of the multiplication may be an important factor in resistance.

Perhaps, as suggested by Thyr (47), resistance in Lycopersicon cultivars used in this study may be the result of reduced bacterial multiplication, resulting in retarded movement of the pathogen in the plant rather than the breakdown of bacterial substances toxic to the plant (37, 39).

The inheritance of resistance to isolate H of C. michiganense appears to be determined by 4 genes. A combination of recessive gene a and 3 dominant genes, B, C, and D, controls resistance. Three alleles of genes C and D are involved. Incomplete dominance of gene A is expressed in plants bearing the Aa genotype only in the presence of genes c² or d². Resistance to the less virulent isolates appears to be determined by the B, C, or D genes.

The 4-gene model with triple alleles for C and D provides for a continuum of intermediate resistant genotypes with small differences in resistance which makes it difficult to separate and when combined with a large error term suggests quantitative inheritance (49). The 4-gene model parallels with the observations of Laterrot (25) who reported resistance as dominant based on the reaction of the F₁ hybrid. This is possible with either a weak isolate or a parent with the c² or d² gene.

The resistance of Bulgaria 12, A 129, and A 134 could be explained by the same model, however the resistance of L. hirsutum requires an additional gene X, with xx controlling resistance. Plants with the genotype Xx are resistant when the gene c² with the modifier F is present.

As the virulence of isolate H decreased with time, a longer period was required for the symptoms to appear and re-inoculation accelerated the expression of symptoms in the susceptible parent. Variation in the virulence of isolates of C. michiganense has been reported (43, 49). The virulent isolates cm 21 caused death of seedlings resistant to isolate H. However, cultivars resistant to isolate H in the seedling stage did not decrease in fruit weight as compared to susceptible cultivars when inoculated with isolate cm 21 at anthesis. Thyr (49) described 7 pathotypes ranging from a low to a high pathogenicity. As previously reported (5) with other pathogenic bacteria, it is possible that there may be similar variation in the resistance of the host. The pairing of genes for resistance in the host with genes for virulence in the pathogen was first reported by Flor (12). With the range of virulence observed one might consider the existence of a range of genes for resistance in the host, more than that suggested in this study.

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