THE GENETICS OF RUST REACTION TO SPECIFIC COLLECTIONS OF RUST, MELAMPSORA LINI (PERS.) LEV., IN CERTAIN FLAX CROSSES

by

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ABSTRACT

Four varieties of flax (C.I. 1225, C.I. 1223, C.I. 1219 and C.I. 1218) were studied for reaction to three physiologic races of flax rust. Each of the four varieties was crossed with the tester varieties Bison, Dakota, Bombay and Crystal. Segregation of the resulting F_2 and F_3 populations was analyzed for reaction to each of the three races of rust (D-8, D-10 and 41). Results of the analysis were as follows:

1. A gene governing an immune reaction to all three races in C.I. 1225. This gene is allelic to the (L) gene assumed to be carried by Crystal.
2. Two genes governing an immune or resistant reaction to all three races in C.I. 1223. One of these genes is allelic to the (M) gene assumed to be carried by Dakota. The second gene is allelic to the (N) gene assumed to be carried by Bombay.
3. A gene governing an immune reaction to all three races in C.I. 1219. This gene was not allelic to any of the genes assumed to be carried by the tester parents.
4. Two genes governing an immune reaction to all three races in C.I. 1218. These two genes are different from the (M) and (N) genes assumed to be carried by Dakota and Bombay respectively. The relationship of these two genes to the gene or genes carried by Crystal is uncertain.

INTRODUCTION

Cultivated flax, Linum usitatissimum L., is the only one of about one hundred species of the genus Linum (47) that is cultivated. The chief useful products of the crop are the linseed oil, which is used in the paint and varnish industry, and the fiber which is used in the manufacture of linen. The flax rust fungus, Melampsora lini (Pers.) Lev., has been found to exist in all areas of the world where flax is cultivated, namely, North America, the Argentine, Russia, Europe, India, Australia and New Zealand. The disease, commonly known as 'flax rust', has occasionally been very destructive to the flax crop due to its occurrence in epidemic proportions. Several such epidemics have been reported in the United States between the years 1928 and 1951. In India the disease is responsible for an average annual reduction in yield of about 28% (34).

However, losses in yield due to rust have been reduced considerably in recent years, chiefly as a result of a systematic approach to the breeding of resistant varieties brought about by a knowledge of the physiologic races existing in a region and of the genes for resistance possessed by available host varieties.

The importance of the role that physiologic races have played in North America is illustrated in a number of varieties grown in the North Central States between the years

1931 and 1951. Bison, released as a wilt and rust resistant variety in 1926, has been susceptible to all collections of flax rust made in North America since 1931. Koto which was developed as a replacement for Bison, succumbed to rust while being increased for distribution in 1927. Dakota, Renew, Custer and Arrow were released as resistant varieties in 1946, but in 1948 races attacking these varieties were observed in North-western Minnesota and Eastern North Dakota. By 1952, the varieties B 5128, Marine, Redwood, Rocket and Sheyenne had replaced Dakota.

Melampsora lini (Pers.)Lev. differs from Puccinia graminis Eriks. and Henn., in that it is autoecious. The possible origin of new races through hybridization and genetic recombination, therefore, seems unlimited. That there are extensive recombinations in the sexual stage of M. lini was shown by Flor (13). He isolated 64 races from the F_2 progeny of a cross between race 22 from South America and race 24 from the United States. Of these, 62 were previously unknown, and some were more virulent on certain varieties of flax than either parental race.

In view of the complexity of the problem, it is imperative that new sources of resistance be explored and classified to provide for the eventuality of new races of flax rust found to be virulent on varieties now considered resistant. It is well known that varieties which are resistant in one region may be susceptible in another. It is also known

that South American races are widely virulent (18), attacking most of the varieties resistant in Australia, Europe and North America. It is therefore possible that introductions from South America could be valuable sources of resistance enabling plant breeders to deal more effectively with changes in the races prevalent in North America. This study was undertaken to determine the mode of inheritance of rust reaction in four flax varieties of Argentine origin.

LITERATURE REVIEW

I. The Host

1. Origin. Cultivated flax, Linum usitatissimum L. is an important commercial crop. According to Schilling (39) it originated through human selection from the wild species, L. angustifolium Huds., which is prevalent throughout the Mediterranean region and is the only species with which it can be crossed readily. Vavilov (42) maintained that cultivated flax was polyphyletic in origin.

2. Chromosome Number. Ray (35) reported haploid chromosome numbers of 8, 9, 10, 14 and 15 in 36 species of the genus Linum. There are conflicting reports as to the chromosome number in Linum usitatissimum. Several workers (6) report the haploid chromosome numbers of n = 16. However, many other workers report (5) the chromosome number of n = 15.

3. Sources of Rust Resistance. Varieties highly resistant in one region may be susceptible in another. Bison, universally susceptible to races in North America, Europe and South America, has been found to be immune in Australia (18). Bombay, which is susceptible to Australian races, was found to be immune to races prevalent in the Argentine (18). Twenty races isolated from European collections of flax rust resemble North American races in pathogenicity (18).

II. The Pathogen

1. History and Host Specialization. Persoon (32) described a rust fungus on L. catharticum and L. usitatissimum

in 1801. He named it Uredo minata B. lini. Leveille (28) transferred it to the genus Melampsora and called it Melampsora lini.

Arthur (2) demonstrated that Melampsora lini (Pers.) Lev. is eu-autoecious. While it is known that the rust of cultivated flax can perpetuate itself readily in the absence of any wild species of Linum, it is possible that some of them may serve to increase the inoculum. Arthur (2), Pethybridge et al. (33) and Miss Hart (22) obtained successful infection of L. lewisii, L. angustifolium and L. rigidum respectively with telial material from cultivated flax. Arthur (2) reports three additional species, L. breweri, L. congestum and L. Drymaroides, as hosts of Melampsora lini in California. Palm (31) from cross inoculation studies concluded that rust on cultivated flax was physiologically and, in some cases, morphologically distinct from that on certain wild flax species.

2. Life Cycle. Allen (1) demonstrated the heterothallic nature of flax rust. She stated that the immature teliospores are dicaryotic. During germination the nuclei fuse to produce the diploid phase. Two successive divisions of the diploid nucleus, one of which is a reduction division, result in 4 haploid nuclei which migrate into the 4 sporidia in the promycelium. Sporidial infection of a susceptible host results in the formation of a pycnium. Each pycnium is incapable of further development unless pycniospores from a

pycnium of opposite mating type are transferred to it. Then it develops into an aecium bearing dicaryotic aeciospores. The aeciospores reinfect flax, producing the dicaryotic uredial stage which repeats itself.

3. Physiologic Specialization. Physiologic races of rusts are dicaryotic clones identified by the types of infection produced on selected varieties termed 'host testers' or 'differentials' (18).

Henry (23) suspected the occurrence of physiologic forms of Melampsora lini (Pers.) Lev. on cultivated flax but did not demonstrate their existence. Physiologic specialization in M. lini was first demonstrated by Flor (8). He differentiated 14 races by the reaction of 9 flax varieties. Using additional differential varieties, Flor (9) identified 10 additional races.

Physiologic specialization of flax rust has also been demonstrated in Argentine by Vallega (41), in Europe by Straib (40), in Australia by Waterhouse and Watson (44) and Kerr (27), in India by Prasada (34) and in New Zealand by Cruickshank (4).

III. Epidemiology

The varieties of flax grown in North America between 1931 and 1951 largely determined the prevalence of the different races. Flor (17) indicated that since this rust attacks only species of Linum, the survival of a particular race depends upon the continued production of varieties susceptible to it. Races unable to attack the current commercial varieties

disappear, while those attacking the predominant varieties tend to increase. Bison was released as a rust resistant variety in 1926 (17) but it has been susceptible to all collections of flax rust made in North America since 1931 when physiologic race studies were started. Dakota, carrying the Newland gene for rust resistance,was released in 1946. In 1948, however, it was attacked in Minnesota and North Dakota (17). Koto was immune from rust during several years of nursery tests, but was attacked while being increased for distribution.

IV. Differential Varieties

1. Origin of Differentials. The flax varieties that differentiate physiologic races of Melampsora lini (Pers.) Lev., were selected by the trial and error method. Flor (9) tested the reactions of 50 varieties of flax to 36 rust collections made in 1931 and 1932. He found the reaction of individual plants of varieties possessing some resistance to be extremely variable. It was, therefore, necessary to develop lines from these varieties which were pure for rust reaction. This was done by individual plant selection. Following this procedure Flor (9) isolated 14 physiologic races by the use of 7 differentials. These were Williston Brown, Akmolinsk, J.W.S.,Pale Blue Crimped, Kenya, Argentine selection (C.I. 705) and Abyssinian.

2. Isolation of Lines Bearing Unit Rust Conditioning Genes. Differential lines possessing single rust-conditioning

genes usually show less variation in infection type than do lines with 2 or more genes. Flor (18) stated that if each host tester possessed a single gene for rust resistance, race determinations would give a more nearly complete indication of pathogenicity, and the identification of rust resistant genes in varieties would be simplified.

Flor (18) in 1954 revised the flax rust differential varieties. Although two of the old differentials were dropped because they gave little information or had unsatisfactory reactions there was little loss in continuity of race identification as 16 of the 18 new differentials either were old differentials or were derived from those previously used. He developed these lines pure for each rust conditioning gene by backcrossing to Bison.

V. Inheritance of Pathogenicity

Flor (13) studied the reactions of sixteen rust differentiating varieties to races 6, 22 and 24, and to selfed cultures of these races. He also studied the F_1 and F_2 hybrid cultures of race 22 crossed with race 24 and race 6 crossed with race 22. He found that virulence was inherited as a recessive character, since F_1 cultures were unable to attack varieties resistant to either parent race. Varieties susceptible to both parent races were susceptible to the F_1 culture. Out of 133 F_2 cultures segregation ratios of 1:3, 1:15 and 1:63 of virulence : avirulence were obtained. The only exception was from the variety Williston Brown. In this instance a

ratio of 3 virulent : 1 avirulent was obtained. On the basis of these results Flor concluded that in the pathogen as a general rule, avirulence is inherited as a dominant character and virulence was inherited as a recessive character.

Pathogenicity to Ottawa 770 B, Bombay, Newland and Tammes Pale Blue was in each case conditioned by single genes different from one another. Pathogenicity to Bolley Golden and Italia Roma was in each case conditioned by pairs of duplicate factors, and pathogenicity to Morye was conditioned by 3 pairs of factors. Flor analysed these results on the assumption that for each rust conditioning factor in the host there is a specific factor for pathogenicity in the rust organism.

In the cross of race 22 by race 24 segregation for pathogenicity on the varieties Akmolinsk, Abyssinian and Leona was in a ratio of 23 virulent : 110 avirulent. Flor states that pathogenicity on these three varieties was inherited as a unit on a single factor basis. However, in the cross of race 6 with race 22 Flor found it necessary to assume a factor in Akmolinsk distinct from Abyssinian and Leona in order to explain the pathogenicity of 'hybrid A' derived from the above cross. Flor (13) states that since Abyssinian is susceptible to a number of North American races to which Leona is resistant, these two varieties possess different rust conditioning genes. Consequently, it is probable that separate but closely linked factors condition pathogenicity on Abyssinian and Leona.

In the pathogen Flor (13) identified 18 genes for pathogenicity occurring in 12 linkage groups. These linkage groups were identified on the basis of host reaction and are as follows:

Group I - Williston Golden and Williston Brown - inherited as a unit.

II - Akmolinsk, Abyssinian and Leona - inherited as a unit.

III - Pale Blue Crimped and Kenya - inherited as a unit.

IV - Ottawa 770 B
V - Bombay
VI - Newland
VII - Tammes Pale Blue

conditioned by single genes in each case different from one another.

VIII & IX - Bolley Golden and Italia Roma - conditioned by pairs of duplicate factors in each case.

X - Morye - conditioned by three pairs of factors.

XI - Buda - conditioned by two factors - one of these factors is linked with the factors for pathogenicity on Akmolinsk, Abyssinian and Leona.

XII - J.W.S. - immune from races 6, 22 and 24, was immune from 231 F_2 cultures of hybrids of these races.

Flor (15) claims that there is a parallelism in linkage between certain factors for rust reaction in the host and those for pathogenicity in the fungus. He states that pathogenicity to Akmolinsk, Abyssinian and Leona (host varieties having factors in the NN series) was also linked with that to Buda ($L^1L^1M^2M^2$) indicating that linkage between pathogenic factors 'transcends' the linkage groups conditioning

resistance in the host. Mayo (29) states that Flor apparently uses the term linkage to cover allelism. He states further that this would mean that members of a single linkage group in the pathogen correspond to genes belonging to more than one linkage group in the host, and this deals with correspondence between groups not yet established.

VI. Inheritance of Rust Reaction

Henry (24) in his studies on the inheritance of rust reaction in flax reported that the varieties Bombay, Ottawa 770 B and an Argentine selection were immune from several collections of flax rust from North America and one from Europe. He found that immunity was conditioned by single dominant factor-pairs in the varieties Ottawa 770 B and Bombay and by a pair of duplicate factors in the Argentine selection.

Myers (30) studied the inheritance of reaction to a field collection of flax rust and to a single physiologic race in 37 crosses involving 17 varieties or strains of flax. He found that immunity was dominant to resistance and susceptibility, and resistance was dominant to semi-resistance and susceptibility. The reaction to the collection and to the single physiologic race was similar. He explained his results by assuming duplicate factors in two different allelic series which he called L and M.

Flor (10) studied the inheritance of rust reaction in a cross between Buda and J.W.S. to several physiologic races.

He explained his results by assuming rust reaction to be conditioned by a single gene in J.W.S. and by 2 genes in Buda.

Flor (10) studied the interaction of genes for rust reaction in 16 varieties used as differentials and 4 additional varieties of flax. He identified 23 different genes, 19 of which conditioned rust reaction in the 16 differential varieties. He placed 16 genes in 3 series of multiple alleles or closely linked groups, designated L, M and N. He placed 7 in the L series, 4 in the M, and 5 in the N series. He observed crossing-over in some hybrids between varieties carrying genes designated by the symbol N.

Kerr (27) found that Koto, Bolley Golden and Italia Roma each carried a gene in the L series of allelomorphs conditioning immunity from the Australian race A. He identified 2 different genes at the M locus in Walsh, a variety mixed for rust reaction. Ottawa 770 B, Koto, Akmolinsk, Abyssinian, Tammes Pale Blue, Walsh and Argentine selection C.I. 462 carried allelic genes for rust reaction linked with the N gene conditioning rust reaction in Punjab. Argentine selection and Bolley Golden carried a gene for rust reaction at a locus other than that occupied by the L, M, or N series.

Flor (21) lists the genes for rust reaction in flax occurring as multiple alleles in at least 5 loci: one at the K locus, 11 at L, 6 at M, 3 at N, 4 at P and 7 unplaced genes.

Mayo (29) criticized Myers'(30) original evidence for allelism. He says that only 11 of the 37 crosses tested by Myers could contribute to his hypothesis and in these the data are often unsatisfactory. Furthermore, Myers used a field collection of the pathogen and a single physiologic race which he called 'stock form 4', which he claimed behaved identically for purposes of analysis but this is not shown in his results.

VII. Host-Parasite Interaction

Henry (24), Myers (30) and Flor (10) independently showed that resistance to rust in flax has been inherited as a dominant character, although with some genes dominance is incomplete. Avirulence in the flax rust fungus has been inherited as a dominant character on all varieties except Williston Brown (21).

Flor (21) states that the host-parasite interaction in flax rust may be explained by assuming a gene-for-gene relationship between rust reaction in the host and pathogenicity in the parasite. "Pustule type, the criterion of both reaction and pathogenicity is conditioned by specific pairs of genes, one in the host, and the other in the parasite."

Flor (21) used the symbols A and V to designate genes for avirulence and virulence respectively in the rust fungus with subscripts indicating the differential variety on which the gene conditioned pathogenicity. In 1955 Flor (20) used a new system of gene designation to show the specificity of

interaction of the genes in the host and parasite. The symbol of the gene in flax with which the gene in the rust fungus interacts is used as a subscript (A = avirulence dominant, and V = virulence dominant) to indicate this specific relationship.

According to Flor's complementary gene hypothesis, resistance occurs only when complementary genes in both host and parasite are dominant (except on the Williston Brown gene M^1 to which virulence is dominant). If either or both of each pair of complementary genes are recessive susceptibility results.

Flor (21) illustrated the parallelism of the inheritance of rust reaction and pathogenicity from his data of 1956 (20) and 1947 (16). In the cross of Ottawa 770 B x Bombay, plants possessing the Bombay gene (N) were resistant to race 22 and those possessing the Ottawa 770 B gene (L) were resistant to race 24. All host-parasite combinations other than N - A_N and L - A_L resulted in susceptibility.

In the cross of race 22 x race 24, cultures possessing the avirulent gene A_L of race 24 did not attack Ottawa 770 B and cultures possessing the avirulent gene A_N did not attack Bombay. All parasite-host combinations other than A_L - L and A_N - N resulted in susceptibility.

Mayo (29) states that Flor (1947-1954) developed the idea of specificity of reaction between host and parasite to the point where the effects of one dominant gene in the host

are exclusively specific to the effects of one dominant gene in the pathogen. For example the genes A_{ab}, A_{ak} and A_{le} must be separate in their specificities though they segregate together. Mayo indicates that the evidence is insufficient to support this extreme interpretation which is not necessary for the basic usefulness of the concept in breeding for disease resistance.

Mayo (29) also criticizes Flor's (1946) (15) conclusion that specificity between the effects of host and pathogen genes ensures that pathogen strains arising through hybridization will not attack host strains previously resistant to both parental strains of the pathogen. The fact, for example, that Bombay, immune from races 6 and 22,was immune from the 98 F_2 cultures of this cross could be due as Flor suggests (15) to races both 6 and 22 being homozygous for a dominant gene conferring avirulence when reacting with a dominant gene,for which Bombay is homozygous, conferring immunity. But Mayo (29) states that if the two races differed in their dominant genes for avirulence, then it could also be ascribed to the chance that 98 F_2 cultures failed to produce the necessary recombinant to permit virulence, either due to close linkage between the pathogen genes or to even more than 2 genes being concerned in the reaction.

MATERIALS AND METHODS

Source of Material

The materials used in this study came from the United States Department of Agriculture World Flax Collection. The following parental material was used in this study:

Variety	Origin	Probable rust reaction genotype
C.I. 1218	Argentine	-
C.I. 1219	"	-
C.I. 1223	"	-
C.I. 1225	"	-
Bison	North America	ll mm nn
Crystal	"	LL mm nn
Dakota	"	ll MM nn
Bombay	"	ll mm NN

Physiologic Races Used

Three physiologic races, namely D-8, D-10 and race 41 were used in this study. Race D-8 and D-10 were obtained from Dr. B. Peturson, of the Laboratory of Plant Pathology, Canada Department of Agriculture, Winnipeg. Race 41 was obtained from Dr. H. H. Flor, of the North Dakota Agricultural Experiment Station, U.S.A.

Seeding the Parental Material

The parental material was sown in the Dominion Cereal Breeding Laboratory, Winnipeg, in the winter of 1954. Six inch pots were used, and five seeds were sown per pot. Seedings were made at five dates at intervals of one week, commencing November 26, 1954.

Crosses Made

The following sixteen crosses were made in the greenhouse:

C.I. 1225 x Bison
" x Crystal
" x Dakota
" x Bombay

C.I. 1223 x Bison
" x Crystal
" x Dakota
" x Bombay

C.I. 1219 x Bison
" x Crystal
" x Dakota
" x Bombay

C.I. 1218 x Bison
" x Crystal
" x Dakota
" x Bombay

The varieties with the C.I. numbers were used as the female parents and the tester parents (Bison, Crystal, Dakota and Bombay) were used as the male parents. In order to overcome any heterogeneity that may exist between plants within the same variety it was originally planned to use one plant as a female parent in each of the varieties with C.I. numbers and one plant from each of the male tester parents as a source of pollen in each cross. However, this was found to be impracticable due to inadequate flowering by some plants, the inability to obtain flowering to coincide between male and female parents in every case, and the poor seed-set in some crosses. Therefore in each cross two plants were used as the female parents and on an average three plants were used as male parents. The identity of each cross was maintained by means of labelled tags.

Handling the F_1 Material

Twenty five F_1 plants were grown from each cross. The seeds were spaced 1 inch apart and seeded to a depth of 1/4 inch in a sterilized soil bed. The seeding was done in a greenhouse of the Cereal Breeding Laboratory, Canada Department of Agriculture, Winnipeg, in the summer of 1955. From the time the plants were about one foot tall they were supported upright by means of a wire mesh.

When the plants were ready for harvesting, seed was harvested from each plant separately. The F_2 seed derived from each F_1 plant was classified as an F_2 family. The identity of each family was maintained as follows:

e.g. Cr - 1218 - III - 5 - (1955)

where Cr = one parent in cross of Crystal x 1218

1218 = other parent in cross of Crystal x 1218

III = F_2 family number three

5 = F_2 plant number five within family III

1955 = year seed from F_1 plant was harvested

Handling the F_2 Material

In order to obtain a minimum of 200 F_2 plants and to account for losses due to poor germination, 250 F_2 seeds from each cross were seeded in $4\frac{1}{2}$ inch pots and grown in the greenhouse during the winter of 1955 to 1956 (Plate II). Five seeds were sown per pot. In addition twenty seeds from each parent, and 15 F_1 seeds, were seeded at this time. During the seedling stages the temperature in the greenhouse

was maintained between 60 and 70° F. Supplemental illumination was given the plants at this time.

The Excised Shoot Technique

The excised shoot technique that was developed by H. B. Kerr (26) was used. When the seedlings were 3 to 4 inches high, the shoots were excised about ½ inch above the cotyledons. The excised shoots were placed in specially constructed boxes containing water and were inoculated with the appropriate physiologic race immediately (Plate III).

The rooting boxes were made of plastic according to the following specifications: 2½ ft. x 1½ ft. x 6 in. The sides of the boxes were glued with ethylene dichloride. Fitting into each was a plastic lid. Holes 1/4 inch in diameter and ½ inch apart were made in the lid. Each box accommodated 1000 excised shoots.

Each excised shoot was placed in a hole and identified by means of a number marked beside each hole. Ten excised shoots from each parent were used as checks. In addition to this, ten excised shoots of Bison were used as a universal check in every test.

Inoculation Procedure

The shoots in each box were inoculated with the specific physiologic race as follows:

1. The leaves were moistened by means of a fine spray from an atomizer.
2. A small quantity of the inoculum was dusted on the leaves. The leaves were then rubbed gently between the fingers.

3. The leaves were moistened again by means of a fine spray and then dusted with a mixture of one part of inoculum to 50 parts of talc.
4. The leaves were moistened with a fine spray once again.

Incubation Procedure

The boxes containing the excised shoots were placed in a tray containing water, which was then covered with a lid. This provided the humidity conditions necessary during the incubation period. The boxes were left in this chamber for about 18 hours and then returned to the greenhouse bench. The temperature in the greenhouse was maintained between 60 and 70° F.

A portion of the F_2 data was gathered in the summer of 1956. The high temperatures in the greenhouse were circumvented by placing the boxes in a growth chamber after inoculation. The temperature in the chamber was controlled at 65° F.

Reading the Rust Reaction

Rust readings were taken as soon as pustules appeared on the leaves of the susceptible plants. This took place about 10 to 12 days after inoculation. The reading of the rust reaction was based on Flor's (18) system of classification with some modifications (Plate I), and was as follows:

Class of host reaction	Type	Description
Immune	0	No uredia developed; heavily inoculated leaves may be stunted and show variable necrosis; they may develop flecking with variable distortion or they may show no visible evidence of infection.
Resistant	1	Chlorotic fleck; chlorotic fleck surrounded by necrotic zone.
Resistant	2	Uredia minute to small, distinct and scattered; they may or may not be accompanied by chlorosis of surrounding tissue. Little necrosis or leaf distortion.
Resistant	3	Uredia small to medium, always associated with necrosis of adjacent leaf tissue and stunting and distortion of leaf, they may be rudimentary and distinct or may form crust-like aggregations in necrotic areas. Isolated pustules and those at margins of necrotic areas may be medium in size and produce an abundance of spores but are surrounded by a necrotic zone which becomes more distinct on ageing.
Susceptible	4	Uredia medium sized, vigorous but not compound. Development somewhat retarded in heavily infected leaves with areas adjacent to uredia becoming more or less chlorotic, especially as the pustules age; little necrosis or leaf distortion.
Highly susceptible	5	Uredia large and compound, if isolated, no necrosis and little chlorosis of adjacent tissue except under unfavorable growing conditions.

Each plant was examined carefully with a hand lens and classified as above. Where difficulties of classification were observed, notes were taken.

Handling the Second and Third Cuttings

Three physiologic races were used in this study. Consequently three cuttings were taken from each plant. The

second and third cuttings were taken about fifteen days after the first cutting. The cuttings were taken from the two shoots which develop from the axils of the cotyledons. The two shoots were placed in separate boxes. The boxes were filled with the excised shoots as described previously and each inoculated with a different race. The inoculation and incubation procedure was carried out in a manner similar to that described for the first cutting.

Increase and Storage of Inoculum

The original inoculum obtained was increased on the susceptible variety Bison. Ten seeds of Bison were sown per pot. When the plants were 3 to 4 inches high they were moistened with a fine spray and the specific physiologic race dusted on the plants. The leaves were rubbed between the fingers and sprayed again. The pots were then placed in a humid chamber for about 18 hours for incubation. The pots were removed from the humid chamber and placed in separate greenhouses to prevent contamination of the races. When the pots were placed in the same greenhouse, contamination of the races was prevented by means of a covering around the pots (Plate IV).

During the summer months the inoculum was collected into small vials and stored in the refrigerator.

Microtome Sectioning

Microtome sections of leaves were taken in order to classify a fleck-type reaction. The leaves were first fixed

in Formal-Acetic alcohol (FAA), which was made as follows:

Formaldehyde	90 cc
Glacial acetic acid	5 cc
50% Ethyl alcohol	5 cc

The material after fixing and dehydration was embedded in paraffin wax and stained in Carbol-thionine. Microtome sections 1/20 microns in thickness were taken. The sections were examined under the microscope. Photographs were taken of the sections showing infection. Photographs were also taken of the checks showing no infection.

Statistical Procedures Utilized

The Chi-square method of goodness of fit tests were applied to determine the probability of the proposed hypothesis being tenable.

Handling the F_3 Material

The F_3 material was grown in the greenhouse during the winter of 1956 to 1957. The F_3 lines were grown in 6 inch pots (Plate II). When the plants were 4 to 6 inches high cuttings were taken and placed in rooting boxes. The inoculation, incubation and reading of the rust reaction was carried out in a manner similar to that described for F_2.

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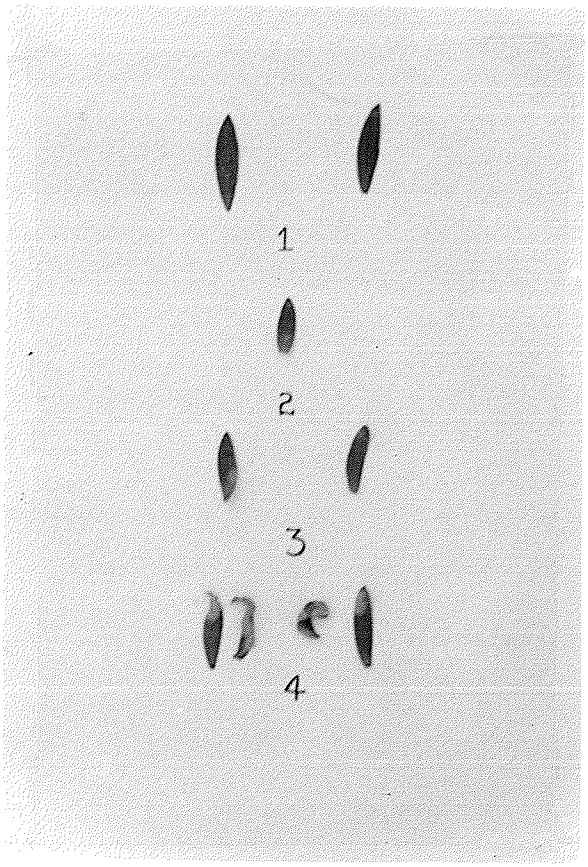


Plate I

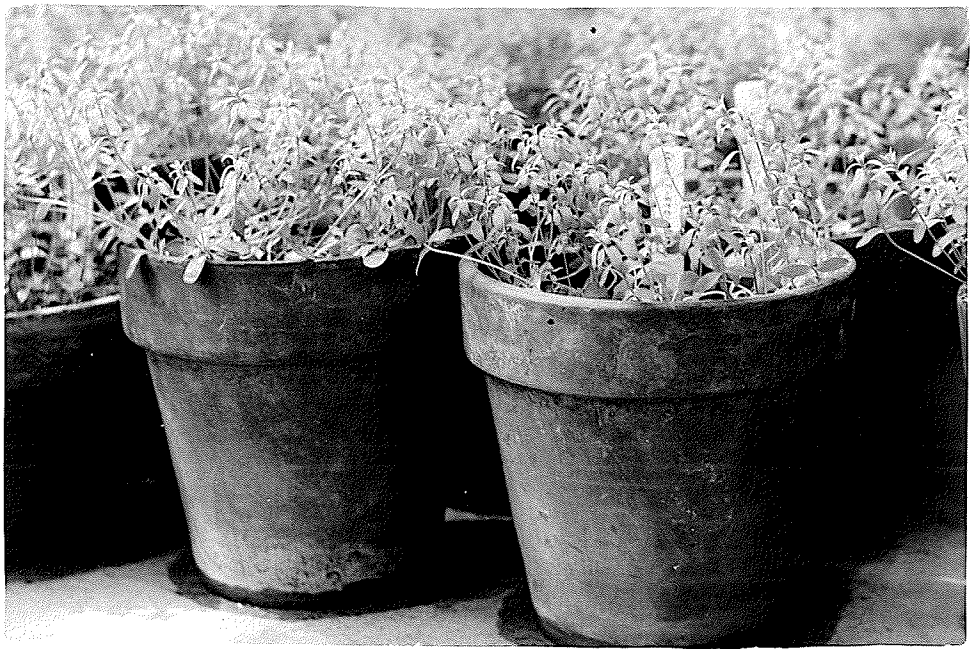


Plate II

Plate III

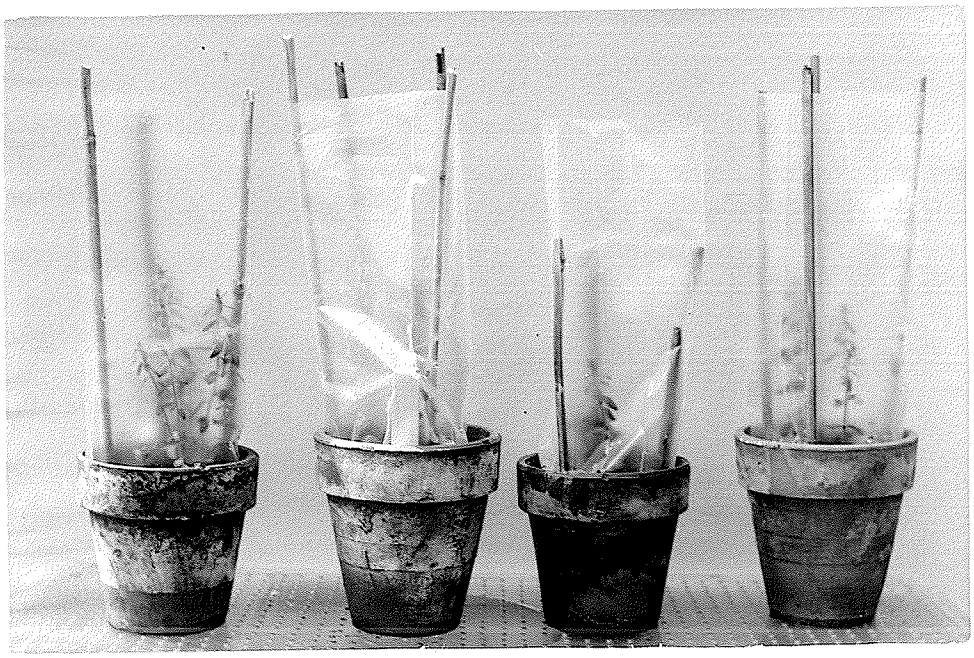
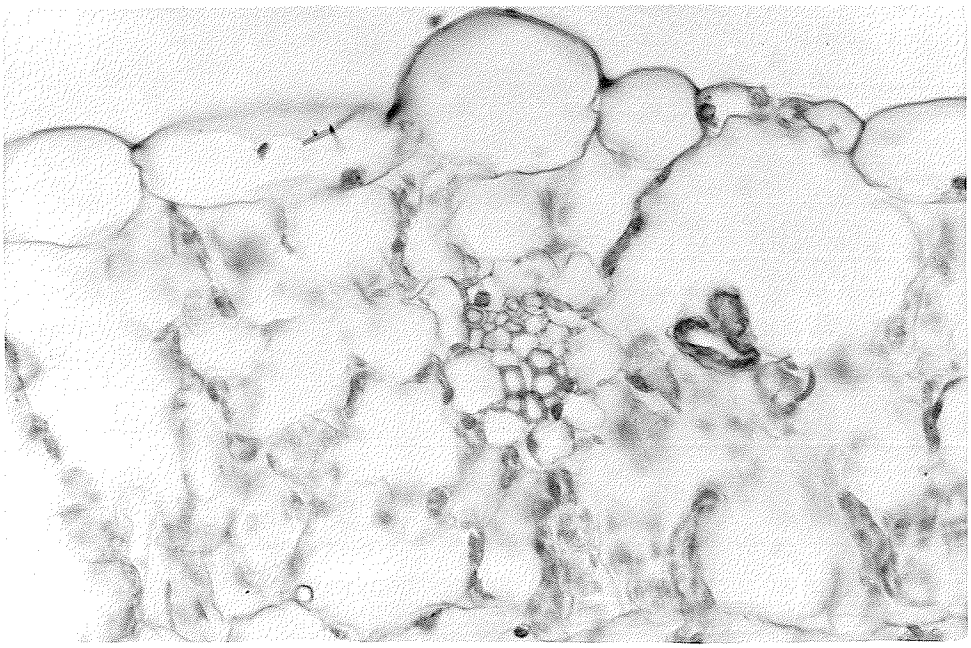


Plate IV



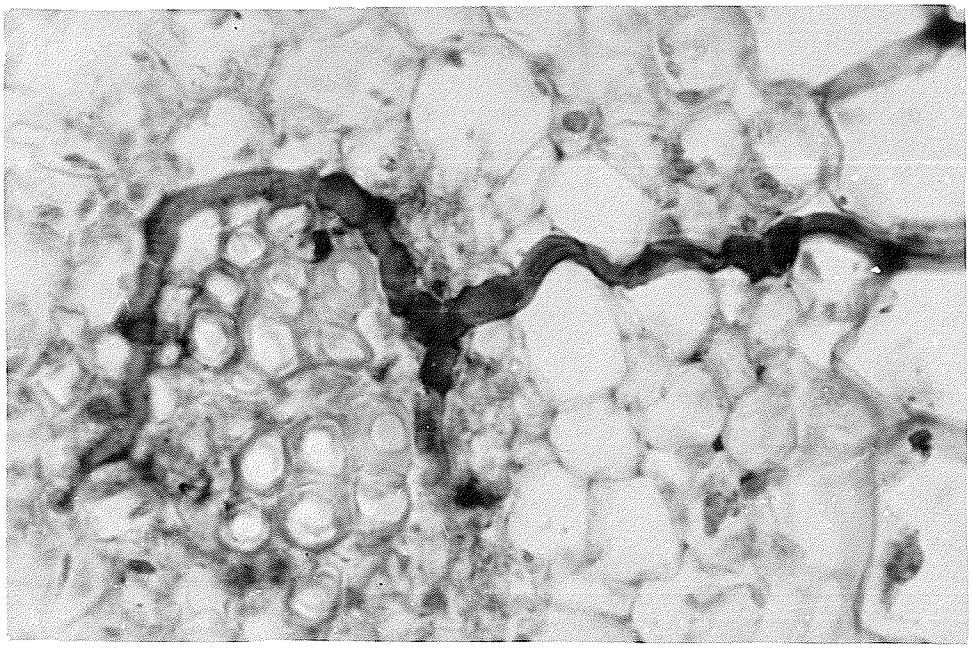


Plate V

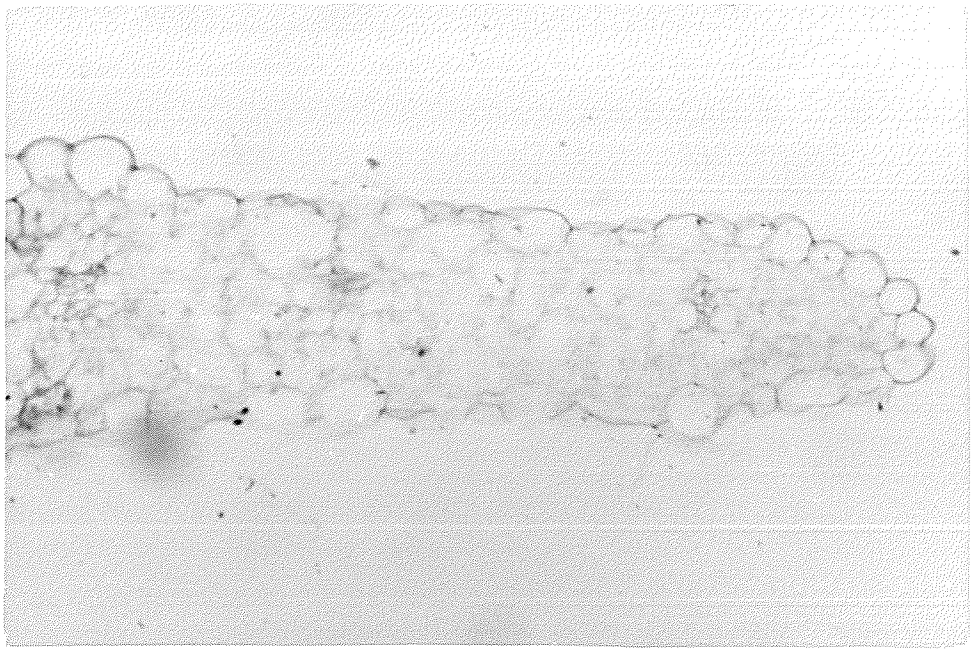


Plate VI

EXPERIMENTAL RESULTS AND DISCUSSION

The genetic analysis of the varieties used in this study is based on the F_2 and F_3 segregations of crosses involving these varieties with certain tester parents. The crosses involving each of the parents of unknown rust reaction genotype to the four testers are presented and discussed separately. The F_2 and F_3 data are presented in a tabulated form in the pages following. In the F_2 results, the F_2 plants in each cross are shown in groups for the purpose of indicating the difference or similarity in reaction of the individual plants to the three races. The F_3 lines are classified into three groups of immune or resistant, segregating, and susceptible.

Crosses Involving the Variety C.I. 1225

Table 1. F_2 segregation ratios in the cross Bison x C.I. 1225 to individual races.

Race	Parental reaction Bison	C.I. 1225		F_2 plants having indicated reaction I	R	S	Theoretical ratio
D-8	S	I	obs.	147.0	0	39.0	3:1
			exp.	139.5	0	46.5	
D-10	S	I	obs.	147.0	0	39.0	3:1
			exp.	139.5	0	46.5	
41	S	I	obs.	147.0	0	39.0	3:1
			exp.	139.5	0	46.5	

X^2 value for D-8 = 1.61 (P = between .20 and .30)
X^2 value for D-10 = 1.61 (P = between .20 and .30)
X^2 value for 41 = 1.61 (P = between .20 and .30)

Table 2. Reaction of parents, F_1 and F_2 in the cross Bison x C.I. 1225 to races D-8, D-10 and 41.

Race	Parents Bison	Parents C.I. 1225	F_1	F_2 plants showing indicated reaction	
				I	S
D-8	S	I	I	I	S
D-10	S	I	I	I	S
41	S	I	I	I	S
		plants observed		147.0	39.0
		plants expected		139.5	46.5

X^2 value for 3:1 ratio = 1.61 (P = between .20 and .30)

Table 3. F_2 segregation ratios in the cross Dakota x C.I. 1225 to individual races.

Race	Parental reaction Dakota	Parental reaction C.I. 1225		F_2 plants having indicated reaction I	R	S	Theoretical ratio
D-8	S	I	obs.	153	0	39	3:1
			exp.	144	0	48	
D-10	S	I	obs.	153	0	39	3:1
			exp.	144	0	48	
41	R	I	obs.	153	32	7	12:3:1*
			exp.	144	36	12	

X^2 value for D-8 = 2.25 (P = between .10 and .20)
X^2 value for D-10 = 2.25 (P = between .10 and .20)
X^2 value for 41 = 3.09 (P = between .20 and .30)
* X^2 value for 15:1 ratio for race 41 = 2.22 (P = between .10 and .20)

Table 4. Reaction of parents, F_1 and F_2 in the cross Dakota x C.I. 1225 to races D-8, D-10 and 41.

Race	Parents Dakota	Parents C.I. 1225	F_1	F_2 plants showing indicated reaction		
D-8	S	I	I	I	S	S
D-10	S	I	I	I	S	S
41	R	I	I	I	R	S
			plants observed	153	32	7
			plants expected	144	36	12

X^2 value for 12:3:1 ratio = 3.09 (P = between .20 and .30)

Table 5. F_2 segregation ratios in the cross Bombay x C.I. 1225 to individual races.

Race	Parental reaction Bombay	Parental reaction C.I. 1225		F_2 plants having indicated reaction I	R	S	Theoretical ratio
D-8	I	I	obs.	196.0	0	10.0	15:1
			exp.	193.1	0	12.9	
D-10	I	I	obs.	196.0	0	10.0	15:1
			exp.	193.1	0	12.9	
41	S	I	obs.	162.0	0	44.0	3:1
			exp.	154.5	0	51.5	

X^2 value for D-8 = .68 (P = between .30 and .50)
X^2 value for D-10 = .68 (P = between .30 and .50)
X^2 value for 41 = 1.46 (P = between .20 and .30)

Table 6. Reaction of parents, F_1 and F_2 in the cross Bombay x C.I. 1225 to races D-8, D-10 and 41.

Race	Parents Bombay	Parents C.I. 1225	F_1	F_2 plants showing indicated reaction		
D-8	I	I	I	I	I	S
D-10	I	I	I	I	I	S
41	S	I	I	I	S	S
			plants observed	162.0	34.0	10.0
			plants expected	154.5	38.6	12.9

X^2 value for 12:3:1 ratio = 1.56 (P = between .30 and .50)

Table 7. F_2 segregation ratios in the cross Crystal x C.I. 1225 to individual races.

Race	Parental reaction Crystal	Parental reaction C.I. 1225		F_2 plants having indicated reaction I	R	S	Theoretical ratio
D-8	I R^+	I	obs.	198	0	0	1:0
			exp.	198	0	0	
D-10	I R^+	I	obs.	198	0	0	1:0
			exp.	198	0	0	
41	I R^+	I	obs.	198	0	0	1:0
			exp.	198	0	0	

Table 8. Reaction of parents, F_1 and F_2 in the cross Crystal x C.I. 1225 to races D-8, D-10 and 41.

Race	Parents Crystal	C.I. 1225	F_1	F_2 plants showing indicated reaction	
D-8	I R^+	I	I	I	S
D-10	I R^+	I	I	I	S
41	I R^+	I	I	I	S
			plants observed	198	0
			plants expected 1:0	198	0

Table 9. Reaction of F_3 lines in the cross Bison x C.I. 1225 assuming segregation in a ratio of 1:2:1 to indicated races.

Reaction class	Race D-8 Observed	Expected	Race 41 Observed	Expected
Immune or resistant	12	10	15	10
Segregating	18	20	16	20
Susceptible	10	10	9	10
P value	.70-.80		.50-.70	

Table 10. Reaction of F_3 lines in the cross Dakota x C.I. 1225 assuming segregation in a ratio of 1:2:1 for race D-8 and in a ratio of 7:8:1 for race 41.

Reaction class	Race D-8		Race 41	
	Observed	Expected	Observed	Expected
Immune or resistant	15	10	21	17.5
Segregating	17	20	17	20.0
Susceptible	8	10	2	2.5
P value	.10-.20		.50-.70	

Table 11. Reaction of F_3 lines in the cross Crystal x C.I. 1225 assuming no segregation for the genes concerned.

Reaction class	Race D-8		Race D-10		Race 41	
	Observed	Expected	Observed	Expected	Observed	Expected
Immune or resistant	30	30	30	30	30	30
Segregating	-	-	-	-	-	-
Susceptible	-	-	-	-	-	-
P value	-	-	-	-	-	-

The variety C.I. 1225 was immune to all three races of M. lini used in this study. Bison was susceptible to all three races. Dakota was susceptible to races D-8 and D-10 but resistant to race 41. Bombay was immune to races D-8 and D-10 but susceptible to race 41. The Crystal parent showed a variation in reaction type. The range in reaction was from immune to pronounced flecks. The F_1 hybrids obtained from crosses involving C.I. 1225 with the four tester parents showed no departure from the immune reaction exhibited by the variety C.I. 1225.

The F_2 segregation behaviour of the variety C.I. 1225 crossed with Bison is shown in Table 1. All plants which were immune to race D-8 were also immune to races D-10 and 41. There were only four plants within this group which showed a variation from the immune reaction. These four plants showed distortion of the leaves but no visible evidence of flecking or pustule development. This could have been due to injury of the leaves due to inoculation. The susceptible plants ranged in reaction from moderately susceptible to fully susceptible to all three races. An acceptable fit for a 3:1 ratio was obtained for all three races with a P value between .20 and .30.

The F_2 data in the cross of Dakota with C.I. 1225 are shown in Tables 3 and 4. Since Dakota is susceptible to races D-8 and D-10, the resistant gene carried by Dakota could not be separated by these two races. Fortunately,

Dakota exhibits a chlorotic fleck to race 41 in contrast with the immune reaction of C.I. 1225. The Dakota reaction type appeared among 32 plants in the F_2 population when exposed to race 41 (Table 4). These 32 plants were therefore assumed to carry the dominant gene for resistance from Dakota and the recessive gene from C.I. 1225. The P value for a 12:3:1 ratio of immune : resistant : susceptible was acceptable and between .20 and .30.

The F_2 results of the cross of Bombay with C.I. 1225 are shown in Tables 5 and 6. The F_2 plants segregated into two classes; there was no variation within the classes and the plants exhibited either an immune or susceptible reaction. The higher proportion of plants that were immune to races D-8 and D-10 than to race 41 indicated the segregation of two factors when tested to the first two races. Since Bombay carries one gene, the other gene must have come from C.I. 1225. The P value for a ratio of 15 immune : 1 susceptible for the first two races was satisfactory. The F_2 results of the cross of Crystal with C.I. 1225 is shown in Tables 7 and 8. There were only 16 plants which exhibited a fleck type reaction. These plants were grouped into the immune class. There were no plants that were susceptible to any of the races.

The F_3 results from the cross of Bison with C.I. 1225 are shown in Table 9. A good fit for a single factor hypothesis was obtained for races D-8 and 41. It is evident from both the F_2 and F_3 results that the gene carried by C.I. 1225 is distinct from the genes carried by the tester parents Bombay

and Dakota. Table 10 shows the reaction of the F_3 lines in the cross of Dakota with C.I. 1225. Since Dakota is susceptible to race D-8, it is assumed that the 1:2:1 segregation with respect to race D-8 could have been obtained only through the operation of the C.I. 1225 gene. That this gene was operating independently of the Dakota gene is evident from the 7:8:1 segregation with respect to race 41.

The results from the F_2 and F_3 populations substantiate the conclusion that the variety C.I. 1225 carries a single dominant gene for immunity against the races tested. The absence of segregation in the cross of Crystal with C.I. 1225, Table 11, is taken to indicate that these two varieties have a gene in common. Since the variety C.I. 1225 gave an immune reaction in contrast to the immune to fleck reaction of Crystal, it is assumed that these two genes are allelic rather than identical.

Crosses Involving the Variety C.I. 1223

Table 12. F_2 segregation ratios in the cross Bison x C.I. 1223 to individual races.

Race	Parental reaction Bison	C.I. 1223		F_2 plants having indicated reaction I	R	S	Theoretical ratio
D-8	S	I	obs.	157.0	0	41.0	3:1
			exp.	148.5	0	49.5	
D-10	S	I	obs.	157.0	0	41.0	3:1
			exp.	148.5	0	49.5	
41	S	R	obs.	0	154.0	44.0	3:1
			exp.	0	148.5	49.5	

X^2 value for D-8 = 1.95 (P = between .10 and .20)
X^2 value for D-10 = 1.95 (P = between .10 and .20)
X^2 value for 41 = .81 (P = between .30 and .50)

Table 13. Reaction of parents, F_1 and F_2 in the cross Bison x C.I. 1223 to races D-8, D-10 and 41.

Race	Parents Bison	C.I. 1223	F_1	F_2 plants showing indicated reaction			
D-8	S	I	I	I	S	I	S
D-10	S	I	I	I	S	I	S
41	S	R	R	R	R	S	S
		plants observed		123.0	31.0	34.0	10.0
		plants expected		111.4	37.1	37.1	12.4

X^2 value for 9:3:3:1 ratio = 2.94 (P = between .30 and .50)

Table 14. F_2 segregation ratios in the cross Dakota x C.I. 1223 to individual races.

Race	Parental reaction Dakota	C.I. 1223		F_2 plants having indicated reaction I	R	S	Theoretical ratio
D-8	S	I	obs.	153	0	39	3:1
			exp.	144	0	48	
D-10	S	I	obs.	153	0	39	3:1
			exp.	144	0	48	
41	R	R	obs.	0	192	0	1:0
			exp.	0	192	0	

X^2 value for D-8 = 2.25 (P = between .10 and .20)
X^2 value for D-10 = 2.25 (P = between .10 and .20)

Table 15. Reaction of parents, F_1 and F_2 in the cross Dakota x C.I. 1223 to races D-8, D-10 and 41.

Race	Parents Dakota	C.I. 1223	F_1	F_2 plants showing indicated reaction		
D-8	S	I	I	I	S	S
D-10	S	I	I	I	S	S
41	R	R	R	R	R	S
			plants observed	153	39	0
			plants expected	144	48	0

X^2 value for 3:1 ratio = 2.25 (P = between .10 and .20)

Table 16. F_2 segregation ratios in the cross Bombay x C.I. 1223 to individual races.

Race	Parental reaction Bombay	C.I. 1223		F_2 plants having indicated reaction I	R	S	Theoretical ratio
D-8	I	I	obs.	196	0	0	1:0
			exp.	196	0	0	
D-10	I	I	obs.	196	0	0	1:0
			exp.	196	0	0	
41	S	R	obs.	0	158	38	3:1
			exp.	0	147	49	

X^2 value for 41 = 3.29 (P = between .05 and .10)

Table 17. Reaction of parents, F_1 and F_2 in the cross Bombay x C.I. 1223 to races D-8, D-10 and 41.

Race	Parents Bombay	C.I. 1223	F_1	F_2 plants showing indicated reaction		
D-8	I	I	I	I	I	S
D-10	I	I	I	I	I	S
41	S	R	R	R	S	S
			plants observed	158	38	0
			plants expected	147	49	0

X^2 value for 3:1 ratio = 3.29 (P = between .05 and .10)

Table 18. F_2 segregation ratios in the cross Crystal x C.I. 1223 to individual races.

Race	Parental reaction Crystal	C.I. 1223		F_2 plants having indicated reaction IR	S	Theoretical ratio
D-8	I R^+	I	obs.	178.0	7.0	15:1
			exp.	173.4	11.6	
D-10	I R^+	I	obs.	178.0	7.0	15:1
			exp.	173.4	11.6	
41	I R^+	R	obs.	177.0	8.0	15:1
			exp.	173.4	11.6	

X^2 value for D-8 = 3.01 (P = between .05 and .10)
X^2 value for D-10 = 3.01 (P = between .05 and .10)
X^2 value for 41 = 1.17 (P = between .20 and .30)

Table 19. Reaction of parents, F_1 and F_2 in the cross Crystal x C.I. 1223 to races D-8, D-10 and 41.

Race	Parents Crystal	C.I. 1223	F_1	F_2 plants showing indicated reaction					
D-8	I R^+	I	I	I	R^+	I	I	S	S
D-10	I R^+	I	I	I	R^+	I	I	S	S
41	I R+	R	I	I	R^+	R^+	S	R^+	S
		plants observed		127.0	21.0	23.0	7.0	6.0	1.0
		plants expected		112.7	26.0	26.0	8.7	8.7	2.0

X^2 value for 39:9:9:3:3:1 ratio = 5.40 (P = between .30 and .50)

Table 20. Reaction of F_3 lines in the cross Bison x C.I. 1223 assuming segregation in a ratio of 1 immune and resistant : 2 segregating : 1 susceptible to indicated races.

Reaction class	Race D-8		Race 41	
	Observed	Expected	Observed	Expected
Immune or resistant	13	10	17	10
Segregating	18	20	16	20
Susceptible	9	10	7	10
P value	.70-.80		.05-.10	

Table 21. Reaction of F_3 lines in the cross Dakota x C.I. 1223 assuming no segregation for race 41 and segregation in a ratio of 1 immune : 2 segregating : 1 susceptible for race D-8.

Reaction class	Race D-8		Race 41	
	Observed	Expected	Observed	Expected
Immune or resistant	12	8.25	33	33
Segregating	15	16.50	-	-
Susceptible	6	8.25	-	-
P value	.20-.30		-	

Table 22. Reaction of F_3 lines in the cross Bombay x C.I. 1223 assuming no segregation for race D-8 and segregation in a ratio of 1 immune : 2 segregating : 1 susceptible for race 41.

Reaction class	Race D-8		Race 41	
	Observed	Expected	Observed	Expected
Immune or resistant	40	40	15	10
Segregating	-	-	18	20
Susceptible	-	-	7	10
P value	-		.10-.20	

The variety C.I. 1223 was immune to races D-8 and D-10 but showed a variation in reaction type to race 41. The resistance shown by this variety to race 41 ranged from minute pustules surrounded by a necrotic zone to chlorotic flecks. The reactions of the other tester parents was the same as has been mentioned previously. The F_1 hybrids from crosses involving C.I. 1223 with the tester parents were immune to races D-8 and D-10 but showed a variation from immune to chlorotic flecks with respect to race 41. The predominant reaction type to race 41 was therefore a resistant reaction.

The F_2 results from the cross of Bison with C.I. 1223 are shown in Table 12. The F_2 plants gave a satisfactory fit to a 3 immune or resistant : 1 susceptible ratio for all three races indicating the segregation of a single gene. However, Table 13 shows that among the 157 plants which were immune to

races D-8 and D-10, there were 34 plants susceptible to race 41. Among the 41 plants that were susceptible to races D-8 and D-10, there were 31 plants resistant to race 41. On the basis of this segregation it was assumed that C.I. 1223 carried two genes at separate loci, one for immunity from D-8 and D-10 and one for resistance against race 41. A satisfactory fit for a 9:3:3:1 ratio with a P value between .30 and .50 was obtained.

The F_2 results from the cross of Dakota with C.I. 1223 are shown in Table 14. The F_2 plants segregated in a 3 immune : 1 susceptible ratio with respect to races D-8 and D-10 but gave no segregation for race 41. The absence of any susceptible segregates and the identical reactions of the parents, F_1 and F_2 to race 41 was assumed to be due to a gene in C.I. 1223 allelic to a gene for resistance in Dakota.

The F_2 results from the cross of Bombay with C.I. 1223 are shown in Table 16. The F_2 plants showed no segregation with respect to races D-8 and D-10 but segregated in a ratio of 3 resistant : 1 susceptible with respect to race 41. This was taken to indicate that the second gene postulated to be present in C.I. 1223 was allelic to a gene for immunity carried by Bombay.

The F_2 results from the cross of Crystal with C.I. 1223 are shown in Table 19. The typical resistant reaction, characteristic of the C.I. 1223 parent with respect to race 41 was not observed among the F_2 segregates. Instead, the fleck

reaction, typical of the Crystal parent was observed among some of the F_2 segregates. This seems to indicate that one of the two genes in C.I. 1223, which gives a typical resistant reaction to race 41 was reacting differently in the Crystal background. When the immune and fleck classes were grouped together (Table 18), a satisfactory fit to a 15 immune and resistant : 1 susceptible ratio was obtained for all three races. The classes of host reaction represented in Table 19 are explained on the basis of the interaction of the factor derived from Crystal with the two factors assumed to be present in C.I. 1223. The 21 plants showing the R^+ reaction to all three races are assumed to carry (L) gene derived from Crystal and the resistant gene from C.I. 1223. The 23 plants showing an immune reaction to races D-8 and D-10 and R^+ reaction to race 41 are assumed to carry the (L) gene derived from Crystal and the second gene assumed to be present in C.I. 1223.

The F_3 results from the cross of Bison with C.I. 1223 are shown in Table 20. It is assumed that the variety C.I. 1223 carries two genes for immunity or resistance against the races tested. One of these genes confers immunity against races D-8 and D-10 but gives a susceptible reaction to race 41. The second gene is ineffective against races D-8 and D-10 but gives a resistant to a hypersensitive fleck reaction to race 41. If this assumption is correct a segregation in a 1:2:1 ratio would be expected for all three races in the F_3.

Among the 9 lines which were susceptible to race D-8, there were 2 lines which were also susceptible to race 41. These two lines are assumed to be homozygous recessive for the two genes assumed to be carried by C.I. 1223. Among the remaining 7 lines, 5 lines were segregating in a ratio of 3 resistant : 1 susceptible while the other two lines were resistant to race 41. Out of the 7 lines which were susceptible to race 41, two lines were susceptible to race D-8, 3 lines were segregating in a ratio of 3 immune : 1 susceptible and two lines were immune to race D-8. Out of the 13 lines which did not segregate with respect to race D-8, there were 8 lines which were resistant, 3 lines segregating in a ratio of 3 resistant : 1 susceptible and two lines were fully susceptible to race 41. These 13 lines are assumed to carry a gene for immunity against race D-8 in a homozygous dominant condition. This gene, however, is ineffective against race 41. In order to explain the segregation within these 13 lines with respect to race 41, a second gene has to be postulated. This gene confers resistance only to race 41. Further evidence to this effect was obtained from 9 lines which were resistant to race 41 but segregating or susceptible to race D-8. A satisfactory fit to a 1:2:1 ratio was obtained, in the F_3 for races D-8 and D-10, Table 20), indicating agreement with the hypothesis under consideration.

The critical data from the F_2 and F_3 tests were obtained from crosses involving the variety C.I. 1223 with

Dakota and Bombay. The F_3 results from the cross of Dakota with C.I. 1223 are shown in Table 21. All 33 lines showed no segregation with respect to race 41 but segregated in a ratio of 1:2:1 with respect to race D-8. The results of the cross of Bombay with C.I. 1223 are shown in Table 22. All 40 lines gave no segregation with respect to race D-8 but segregated for race 41 in a ratio of 1:2:1.

The general conclusion that could be drawn from both F_2 and F_3 data is that the variety C.I. 1223 carries two dominant genes for immunity or resistance against the races. One of these genes is common to the (M) gene assumed to be carried by Dakota. The second gene is common to the (N) gene assumed to be carried by Bombay.

Crosses Involving the Variety C.I. 1219

Table 23. F_2 segregation ratios in the cross Bison x C.I. 1219 to individual races.

Race	Parental reaction Bison	C.I. 1219		F_2 plants having indicated reaction I	R	S	Theoretical ratio
D-8	S	I	obs.	163.00	0	48.00	3:1
			exp.	158.25	0	52.75	
D-10	S	I	obs.	163.00	0	48.00	3:1
			exp.	158.25	0	52.75	
41	S	I	obs.	163.00	0	48.00	3:1
			exp.	158.25	0	52.75	

X^2 value for D-8 = .57 (P = between .30 and .50)
X^2 value for D-10 = .57 (P = between .30 and .50)
X^2 value for 41 = .57 (P = between .30 and .50)

Table 24. Reaction of parents, F_1 and F_2 in the cross Bison x C.I. 1219 to races D-8, D-10 and 41.

Race	Parents Bison	C.I. 1219	F_1		F_2 plants showing indicated reaction	
D-8	S	I	I		I	S
D-10	S	I	I		I	S
41	S	I	I		I	S
				plants observed	163.00	48.00
				plants expected	158.25	52.75

X^2 value for 3:1 ratio = .57 (P = between .30 and .50)

Table 25. F_2 segregation ratios in the cross Dakota x C.I. 1219 to individual races.

Race	Parental reaction Dakota	C.I. 1219		F_2 plants having indicated reaction I	R	S	Theoretical ratio
D-8	S	I	obs.	153.0	0	47.0	3:1
			exp.	150.0	0	50.0	
D-10	S	I	obs.	153.0	0	47.0	3:1
			exp.	150.0	0	50.0	
41	R	I	obs.	153.0	36.0	11.0	12:3:1*
			exp.	150.0	37.5	12.5	

X^2 value for D-8 = .24 (P = between .50 and .70)
X^2 value for D-10 = .24 (P = between .50 and .70)
X^2 value for 41 = .24 (P = between .50 and .70)
* X^2 value for 15:1 for race 41 = .19 (P = between .50 and .70)

Table 26. Reaction of parents, F_1 and F_2 in the cross Dakota x C.I. 1219 to races D-8, D-10 and 41.

Race	Parents Dakota	C.I. 1219	F_1	F_2 plants showing indicated reaction		
D-8	S	I	I	I	S	S
D-10	S	I	I	I	S	S
41	R	I	I	I	R	S
			plants observed	153.0	36.0	11.0
			plants expected 12:3:1	150.0	37.5	12.5

X^2 value for 12:3:1 ratio = .30 (P = between .50 and .70)

Table 27. F_2 segregation ratios in the cross Dakota x C.I. 1219 to individual races.

Race	Parental reaction Bombay	C.I. 1219		F_2 plants having indicated reaction I	R	S	Theoretical ratio
D-8	I	I	obs.	151.00	0	5.00	15:1
			exp.	146.25	0	9.75	
D-10	I	I	obs.	151.00	0	5.00	15:1
			exp.	146.25	0	9.75	
41	S	I	obs.	124.00	0	32.00	3:1
			exp.	120.00	0	36.00	

X^2 value for D-8 = 2.47 (P = between .10 and .20)
X^2 value for D-10 = 2.47 (P = between .10 and .20)
X^2 value for 41 = .58 (P = between .50 and .70)

Table 28. Reaction of parents, F_1 and F_2 in the cross Bombay x C.I. 1219 to races D-8, D-10 and 41.

Race	Parents Bombay	C.I. 1219	F_1	F_2 plants showing indicated reaction		
D-8	I	I	I	I	I	S
D-10	I	I	I	I	I	S
41	S	I	I	I	S	S
			plants observed	124	27.00	5.00
			plants expected	117	29.25	9.75

X^2 value for 12:3:1 ratio = 2.91 (P = between .20 and .30)

Table 29. F_2 segregation ratios in the cross Crystal x C.I. 1219 to individual races.

Race	Parental reaction Crystal	C.I. 1219		F2 plants having indicated reaction I	R	S	Theoretical ratio
D-8	I R^+ R^-	I	obs.	101.0	38.0	7.0	12:3:1
			exp.	109.5	27.4	9.1	
D-10	I R^+ R^-	I	obs.	101.0	38.0	7.0	12:3:1
			exp.	109.5	27.4	9.1	
41	I R^+ R^-	I	obs.	101.0	38.0	7.0	12:3:1
			exp.	109.5	27.4	9.1	

X^2 value for D-8 = 5.28 (P = between .05 and .10)
X^2 value for D-10 = 5.28 (P = between .05 and .10)
X^2 value for 41 = 5.28 (P = between .05 and .10)
X^2 value for 15:1 = 0.53 (P = between .30 and .50)

Table 30. Reaction of parents, F_1 and F_2 in the cross Crystal x C.I. 1219 to races D-8, D-10 and 41.

Race	Parents Crystal	C.I. 1219	F_1	F_2 plants showing indicated reaction		
D-8	I R^+ R^-	I	I	I	R^+ R^-	S
D-10	I R^+ R^-	I	I	I	R^+ R^-	S
41	I R^+ R^-	I	I	I	R^+ R^-	S
			plants observed	101.0	38.0	7.0
			plants expected	109.5	27.4	9.1

X^2 value for 12:3:1 ratio = 5.28 (P = between .05 and .10)

Table 31. Reaction of F_3 lines in the cross Bison x C.I. 1219 assuming segregation in a ratio of 1 immune : 2 segregating : 1 susceptible for all races.

Reaction class	Race D-8		Race D-10		Race 41	
	Observed	Expected	Observed	Expected	Observed	Expected
Immune	13	10	13	10	14	10
Segregating	19	20	19	20	18	20
Susceptible	8	10	8	10	8	10
P value	.50-.70		.50-.70		.30-.50	

Table 32. Reaction of F_3 lines in the cross Dakota x C.I. 1219 assuming segregation in a ratio of 1 immune : 2 segregating : 1 susceptible for races D-8 and D-10 and in a ratio of 7 immune and resistant : 8 segregating : 1 susceptible for race 41.

Reaction class	Race D-8		Race D-10		Race 41	
	Observed	Expected	Observed	Expected	Observed	Expected
Immune or resistant	14	10	14	10	23	17.5
Segregating	17	20	17	20	15	20.0
Susceptible	9	10	9	10	2	2.5
P value	.50-.70		.50-.70		.30-.50	

Table 33. Reaction of F_3 lines in the cross Crystal x C.I. 1219 assuming segregation in a ratio of 7 immune : 8 segregating : 1 susceptible for race D-8.

Reaction class	Race D-8 Observed	Expected
Immune	20	17.5
Segregating	17	20.0
Susceptible	3	2.5
P value	.30-.50	

The variety C.I. 1219 was immune to all three races of M. lini. The reactions of the other tester parents have been mentioned previously. The F_1 hybrids from crosses involving C.I. 1219 with the other tester parents were all immune to the three races, indicating that immunity was dominant.

The segregation behaviour of the F_2 plants in the cross of Bison with C.I. 1219 is shown in Tables 23 and 24. All the F_2 plants were classified into two groups of immune and susceptible plants. There was no variation among any of the plants in the immune group. The susceptible plants ranged in reaction from moderately susceptible to fully susceptible. The segregation gave a good fit for a single factor hypothesis with a P value between .30 and .50.

The results of the cross of Dakota with C.I. 1219 are shown in Tables 25 and 26. On the assumption that the Dakota gene was distinct from the gene carried by C.I. 1219, 3/16 of

the F_2 population was theoretically expected to segregate with respect to race 41 for the resistant gene derived from Dakota. There were 23 plants which showed the typical chlorotic fleck reaction exhibited by Dakota. In addition to this there were 13 plants which showed a reaction intermediate between the immune and chlorotic fleck reaction. It is assumed that these 36 plants are homozygous or heterozygous for the resistant gene derived from Dakota and homozygous recessive for the gene for immunity derived from C.I. 1219. A good fit for a 12 immune : 3 resistant : 1 susceptible ratio was obtained for race 41 with a P value between .50 and .70.

The F_2 results of the cross of Bombay with C.I. 1219 are shown in Tables 27 and 28. The segregation behaviour suggests that Bombay and C.I. 1219 each contribute a single factor for immunity against races D-8 and D-10 and that the factor from C.I. 1219 also contributes immunity to race 41.

The F_2 results of the cross of Crystal with C.I. 1219 are shown in Tables 29 and 30. The segregation behaviour suggests that Crystal and C.I. 1219 each contribute a factor for immunity or resistance against the races tested.

The variety Crystal exhibited a range in reaction type from immunity to large and small flecks. The uniformity of the immune reaction among the F_1 hybrids indicates dominance of the C.I. 1219 gene over the Crystal fleck reaction.

The reaction of the F_3 lines in the cross of Bison with C.I. 1219 is shown in Table 31. The P value between .30 and .50 for race 41 and between .50 and .70 for races D-8 and D-10 indicate satisfactory agreement with the hypothesis that the variety C.I. 1219 carries a single dominant gene conferring immunity against all three races.

The reaction of the F_3 lines in the cross of Dakota with C.I. 1219 is shown in Table 32. It is evident that the resistant gene contributed by Dakota is not operative against races D-8 and D-10. If this gene were effective against races D-8 and D-10, it would have been theoretically expected to segregate in 4/16 of the population in a ratio of 12 immune : 3 resistant : 1 susceptible. Furthermore, there were 4 lines which were susceptible to races D-8 and D-10. Two of these lines were resistant and two segregating in a ratio of 3 resistant : 1 susceptible with respect to race 41. These two resistant lines are assumed to be homozygous recessive for the C.I. 1219 gene and homozygous dominant for the Dakota gene. The two segregating lines are assumed to be homozygous recessive for the C.I. 1219 gene and heterozygous for the Dakota gene. The Dakota gene confers resistance to race 41, and a two factor segregation was observed with respect to this race. The 23 immune and resistant : 15 segregating : 2 susceptible fits a 7:8:1 ratio with a P value between .20 and .30. Theoretically, 20 lines were expected to segregate. Of these 20 lines, 10 lines should segregate in a ratio of

12 immune : 3 resistant : 1 susceptible. Of the 15 lines that were observed to segregate, only 6 lines segregated in a 12:3:1 ratio. However, since there were only 20 plants per line, it is possible that this segregation was missed in some of the lines. Consequently, a correction was applied for the expected frequency of failure to detect segregation from heterozygous genotypes due to small numbers (46). When this correction was applied, the data gave an extremely good fit to an expected 9.20:5.80:1 ratio, with a P value of .90.

The reaction of the F_3 lines in the cross of Crystal with C.I. 1219 is shown in Table 33. On the assumption that two duplicate factor pairs are involved, a 7 immune : 8 segregating : 1 susceptible ratio would be expected in the F_3. The P value obtained between .30 and .50 indicated satisfactory agreement to a two-factor hypothesis. A notable feature was the absence of the fleck type reaction that was obtained in the F_2. The complete absence of this class of host reaction indicated that this reaction was probably influenced by environment. The F_2 population was tested in a growth chamber under conditions of artificial illumination, a controlled temperature of 65° F. and a relative humidity of 80 per cent. The F_3 lines were tested in the greenhouse under natural daylight conditions, an averaged temperature of 70° F. and a relative humidity of 66 per cent. The difference in the environmental conditions under which the two populations were tested could probably account for the

difference in reaction (Plates V and VI). Myers (30) states that if immunity in flax is dependent upon the limitation of development of the pathogen rather than its exclusion, it would perhaps not be surprising to find cases in which the balance between host and pathogen is so delicate that slight changes in the environment shift the reaction from one in which no macroscopic disease symptoms occur to one in which sufficient host cells are killed to produce a fleck-type reaction.

The F_2 and F_3 data substantiates the general conclusion that the variety C.I. 1219 carries a single dominant gene conferring immunity against all three races. This gene was not common to any of the genes assumed to be carried by the tester parents.

Crosses Involving the Variety C.I. 1218

Table 34. F_2 segregation ratios in the cross Bison x C.I. 1218 to individual races.

Race	Parental reaction Bison	C.I. 1218		F_2 plants having indicated reaction I	R	S	Theoretical ratio
D-8	S	I	obs.	146.0	0	50.0	3:1
			exp.	147.0	0	49.0	
D-10	S	I	obs.	143.0	0	53.0	3:1
			exp.	147.0	0	49.0	
41	S	I	obs.	181.0	0	15.0	15:1
			exp.	183.8	0	12.3	

X^2 value for D-8 = .03 (P = between .80 and .90)
X^2 value for D-10 = .44 (P = between .50 and .70)
X^2 value for 41 =1.19 (P = between .20 and .30)

Table 35. Reaction of parents, F_1 and F_2 in the cross Bison x C.I. 1218 to races D-8, D-10 and 41.

Race	Parents Bison	C.I. 1218	F_1	F_2 plants showing indicated reaction			
D-8	S	I	I	I	I	S	S
D-10	S	I	I	I	S	I	S
41	S	I	I	I	I	I	S
			plants observed	108.0	38.0	35.0	15.0
			plants expected	110.3	36.8	36.8	12.3

X^2 value for 9:3:3:1 ratio = 1.0 (P = between .30 and .50)

Table 36. F_2 segregation ratios in the cross Dakota x C.I. 1218 to individual races.

Race	Parental reaction Dakota	C.I. 1218		F_2 plants having indicated reaction I	R	S	Theoretical ratio
D-8	S	I	obs.	137.0	0	51.0	3:1
			exp.	141.0	0	47.0	
D-10	S	I	obs.	149.0	0	39.0	3:1
			exp.	141.0	0	47.0	
41	R	I	obs.	101.0	84.0	3.0	36:27:1*
			exp.	105.8	79.3	2.9	

X^2 value for D-8 = .45 (P = between .50 and .70)
X^2 value for D-10 = 1.82 (P = between .10 and .20)
X^2 value for 41 = .49 (P = between .30 and .50)
* X^2 value for 63:1 for race 41 = .0012 (P = .90)

Table 37. Reaction of parents, F_1 and F_2 in the cross Dakota x C.I. 1218 to races D-8, D-10 and 41.

Race	Parents Dakota	C.I. 1218	F_1	F_2 plants showing indicated reaction			
D-8	S	I	I	I	I	S	S
D-10	S	I	I	I	S	I	S
41	R	I	I	I	R	R	S
			plants observed	101.0	36.0	48.0	3.0
			plants expected	105.8	79.3		2.9

X^2 value for 36:27:1 ratio = .49 (P = .90)

Table 38. F_2 segregation ratios in the cross Bombay x C.I. 1218 to individual races.

Race	Parental reaction Bombay	C.I. 1218		F_2 plants having indicated reaction I	R	S	Theoretical ratio
D-8	I	I	obs.	184	0	8	15:1
			exp.	180	0	12	
D-10	I	I	obs.	187	0	5	15:1
			exp.	180	0	12	
41	S	I	obs.	185	0	7	15:1
			exp.	180	0	12	

X^2 value for D-8 = 2.4 (P = between .10 and .20)
X^2 value for D-10 = 4.4 (P = between .02 and .05)
X^2 value for 41 = 2.2 (P = between .20 and .30)

Table 39. Reaction of parents, F_1 and F_2 in the cross Bombay x C.I. 1218 to races D-8, D-10 and 41.

Race	Parents Bombay	C.I. 1218	F_1	F_2 plants showing indicated reaction				
D-8	I	I	I	I	I	S	I	S
D-10	I	I	I	I	S	I	I	S
41	S	I	I	I	I	I	S	S
			plants observed	174	4	7	6	1
			plants expected	162	9	9	9	3

X^2 value for a 54:3:3:3:1 ratio = 6.4 (P = between .10 and .20)

Table 40. F_2 segregation ratios in the cross Crystal x C.I. 1218 to individual races.

Race	Parental reaction Crystal	C.I. 1218		F_2 plants having indicated reaction I	R^+	R^-	Theoretical ratio
D-8	I R^+ R^-	I	obs.	129	53	40	1:0
			exp.				
D-10	I R^+ R^-	I	obs.	129	53	40	1:0
			exp.				
41	I R^+ R^-	I	obs.	129	53	40	1:0
			exp.				

Table 41. Reaction of parents, F_1 and F_2 in the cross Crystal x C.I. 1218 to races D-8, D-10 and 41.

Race	Parents Crystal	C.I. 1218	F_1	F_2 plants showing indicated reaction I	R^+	R^-	S
D-8	I R^+ R^-	I	I	I	R^+	R^-	S
D-10	I R^+ R^-	I	I	I	R^+	R^-	S
41	I R^+ R^-	I	I	I	R^+	R^-	S
			plants observed	129	53	40	0
			plants expected 1:0	129	53	40	0

The variety C.I. 1218 gave an immune reaction to all three races. The reactions of the tester parents have been mentioned previously. The F_1 hybrids obtained from crosses involving C.I. 1218 with the four tester parents were immune to all three races.

The F_2 segregation behaviour of the variety C.I. 1218 crossed with Bison is shown in Table 34. A good agreement to a 3 immune : 1 susceptible ratio for races D-8 and D-10 and a 15 immune : 1 susceptible ratio for race 41 was obtained. Table 35 shows the reactions of the identical plants to the three races. A hypothesis was derived on the basis of the segregation of 73 plants in the F_2 population. There were 38 plants which were immune to race D-8 but susceptible to race D-10. The remaining 35 plants were susceptible to race D-8 but immune to race D-10. All 73 plants, however, were immune to race 41. The reversal in reaction of the identical plants to the two races was explained by assuming that C.I. 1218 carried two factor-pairs for immunity. Table I of Appendix shows the genotypes and the phenotypic ratio obtained. The (A) factor confers immunity against race D-8. The (B) factor confers immunity against race D-10 and either one of them is effective against race 41. The P value for a 9:3:3:1 ratio, (Table 35) indicates satisfactory agreement with this hypothesis.

The F_2 results in the cross of Dakota with C.I. 1218 are shown in Table 36. The 3:1 segregation obtained with respect to races D-8 and D-10 substantiates the conclusions drawn

from the cross of C.I. 1218 with Bison. The 36:27:1 ratio obtained with respect to race 41 indicates that Dakota contributes a factor for resistance against race 41 distinct from the two factors for immunity contributed by C.I. 1218. Table II of the Appendix explains the reactions of the identical plants to the three races as is shown in Table 37. It is assumed that the (M) gene derived from Dakota, when present with either (A) or (B) alone confers resistance to race 41 instead of immunity. The only group that appears to be inconsistent with the hypothesis under consideration is the one carrying the genotypes aabbMM and aabbMm, giving susceptibility to races D-8 and D-10 and resistance to race 41. The absence of this class of host reaction (Table 37), indicates the possible interaction of MM with the recessives aa or bb thereby giving an immune reaction to races D-8 and D-10 respectively.

The F_2 results in the cross of C.I. 1218 with Bombay is shown in Table 38. The 15:1 ratio obtained for all three races is explained in Table III of the Appendix. It is assumed that Bombay carries a gene for immunity against races D-8 and D-10, different from the (A) or (B) genes assumed to be carried by C.I. 1218. The 15:1 ratio obtained for all races is explained by assuming the operation of the (A) or (N) genes for race D-8, the (B) or (N) genes for race D-10 and the (A) or (B) genes for race 41. The 54:3:3:3:1 ratio (Table 39), is also explained on the foregoing assumptions.

The F_2 results in the cross of Crystal with C.I. 1218 are shown in Tables 40 and 41. The absence of any susceptible segregates is taken to indicate that Crystal contributes two genes allelic to the genes assumed to be carried by C.I. 1218. However, there is some doubt as to whether the fleck reaction classes, namely (R^+) small fleck and (R^-) large fleck, are truly resistant classes. It is possible that optimum conditions were not obtained for susceptibility to result. However, the failure to obtain a susceptible plant in the large population tested makes this unlikely.

The F_3 results from crosses involving C.I. 1218 were inconclusive giving several irregular segregations. In addition, there were several fully susceptible lines which had a high percentage of escapes. These irregularities have been attributed to the following:

1. The small number of F_3 lines.
2. The small number of plants per line.
3. Non-viability of the inoculum used.
4. Optimum conditions for infection not being obtained.

However, it could be concluded that the variety C.I. 1218 carries two genes for immunity against the races tested. Further work, with larger populations is necessary to ascertain the genic relationships of this variety with the tester parents.

SUMMARY

The inheritance of rust reaction to races D-8, D-10 and 41 was studied in hybrid populations derived from crosses involving the varieties C.I. 1225, C.I. 1223, C.I. 1219 and C.I. 1218 with the tester parents Bison, Dakota, Bombay and Crystal.

F_2 and F_3 populations were grown in the greenhouse and inoculated with the three races utilizing the excised shoot technique (26). The F_2 and F_3 populations were analyzed for reaction to each of the three races of rust. Results of the analysis were as follows:

1. A gene for immunity against all three races carried by C.I. 1225. This gene is common to the (L) gene assumed to be carried by Crystal.
2. Two genes governing an immune or resistant reaction to all three races in C.I. 1223. One of these genes is common to the (M) gene assumed to be carried by Dakota. The second gene is common to the (N) gene assumed to be carried by Bombay.
3. A gene governing an immune reaction to all three races in C.I. 1219. This gene was not common to any of the genes assumed to be carried by the tester parents.
4. Two genes governing an immune reaction to all three races in C.I. 1218. These two genes are different from (M) and (N) genes assumed to be carried by Dakota and Bombay respectively.

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APPENDIX

Hypotheses suggested to explain segregations in crosses involving C.I. 1218.

AA - immunity factor of C.I. 1218, effective against race D-8.

BB - immunity factor of C.I. 1218, effective against race D-10.

AA or BB- either one effective against race 41.

MM - resistance factor of Dakota, effective against race 41 only.

NN - immunity factor of Bombay, effective against races D-8 and D-10 only.

MM - epistatic to AA or BB when AA or BB is present alone.

Table I. Table of genotypes and phenotypic ratios in F_2 for two segregating factor pairs in the cross C.I. 1218 x Bison.

Frequency	Genotypes	Phenotypic ratio in F_2 (AA) Race D-8	(BB) Race D-10	(AA or BB) Race 41
1	AABB	9 I	9 I	9 I
2	AaBB			
2	AABb			
4	AaBb			
1	AAbb	3 I	3 S	3 I
2	Aabb			
1	aaBB	3 S	3 I	3 I
2	aaBb			
1	aabb	1 S	1 S	1 S
16		12 I : 4 S or 3 I : 1 S	12 I : 4 S or 3 I : 1 S	15 I : 1 S

Table II. Table of genotype and phenotypic ratios in F_2 for three segregating factor pairs in the cross C.I. 1218 x Dakota.

Frequency	Genotypes	Phenotypic ratio in F_2 (A) Race D-8	(B) Race D-10	(A, B or M) Race 41
1	AABBMM	27 I	27 I	27 I
2	AABBMm			
2	AABbMM			
2	AaBBMM			
4	AaBbMM			
4	AABbMm			
4	AaBBMm			
8	AaBbMm			
1	AABBmm	9 I	9 I	9 I
2	AaBBmm			
2	AABbmm			
4	AaBbmm			
1	AAbbMM	9 I	9 S	9 R
2	AabbMM			
2	AAbbMm			
4	AabbMm			
1	aaBBMM	9 S	9 I	9 R
2	aaBbMM			
2	aaBBMm			
4	aaBbMm			
1	AAbbmm	3 I	3 S	3 R
2	Aabbmm			
1	aaBBmm	3 S	3 I	3 R
2	aaBbmm			
1	aabbMM	3 S	3 S	3 R
2	aabbMm			
1	aabbmm	1 S	1 S	1 S
64		48 I : 16 S or 3 I : 1 S	48 I : 16 S or 3 I : 1 S	36 I : 27 R : 1 S

Table III. Table of genotype and phenotypic ratios in F_2 for three segregating factor pairs in the cross C.I. 1218 x Bombay.

Frequency	Genotypes	Phenotypic ratio in F_2 (A or N) Race D-8	(B or N) Race D-10	(A or B) Race 41
1	AABBNN			
2	AaBBNN			
2	AABbNN			
2	AABBNn	27 I	27 I	27 I
4	AaBbNN			
4	AABbNn			
4	AaBBNn			
8	AaBbNn			
1	AABBnn			
2	AaBBnn	9 I	9 I	9 I
2	AABbnn			
4	AaBbnn			
1	AAbbNN			
2	AabbNN	9 I	9 I	9 I
2	AAbbNn			
4	AabbNn			
1	aaBBNN			
2	aaBbNN	9 I	9 I	9 I
2	aaBBNn			
4	aaBbNn			
1	AAbbnn	3 I	3 S	3 I
2	Aabbnn			
1	aaBBnn	3 S	3 I	3 I
2	aaBbnn			
1	aabbNN	3 I	3 I	3 S
2	aabbNn			
1	aabbnn	1 S	1 S	1 S
64		15 I : 1 S	15 I : 1 S	15 I : 1 S