A STUDY OF THE INHERITANCE OF REACTION TO FIVE RACES OF RUST IN CROSSES INVOLVING PREVENCHE FLAX

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ABSTRACT

The mode of inheritance of the reaction to rust (Melampsora lini (Pers.)Lev.) in Prevenche, a flax variety introduced from France, was studied by determining the reactions of the F_1 and the F_2 populations of six crosses involving Prevenche. The rust reactions of the F_3 lines from three of the six crosses were also studied.

By the use of selected races of rust it was demonstrated that Prevenche has a pair of complementary genes conditioning a variable reaction ranging from resistant to moderately susceptible with races 41, 166 and 210, and a moderately susceptible to susceptible reaction with races 8 and 178. These genes were subject to environmental influence and acted either as a pair of complementary recessive or as complementary dominant genes. This pair of complementary factors was inherited independently from factors in the L, M, or N series.

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INTRODUCTION

Rust, caused by Melampsora lini (Pers.) Lev., is a major disease of flax which occurs throughout the flax-producing areas of the world. The principal method of control has been the use of resistant varieties. Frequently, however, new virulent races of rust develop, by hybridization or mutation, which are pathogenic on varieties hitherto resistant.

Reports of an annual survey of the plant disease situation in Canada (3) illustrate how varieties can succumb to new races of rust shortly after their release. In 1942 and 1943, when the rust-susceptible variety, Bison, was the main variety grown in Manitoba, yields were greatly reduced by rust infection. Later a new rust-resistant variety called Dakota was released. However, new races of rust capable of attacking Dakota were soon prevalent, and by 1952 this variety was being rapidly replaced by Sheyenne, Redwood and Rocket. This situation emphasizes the necessity of a continuous search for new sources of rust resistance in flax.

The Central Experimental Farm, Ottawa, has introduced many varieties of flax in the hope that they might contribute desirable characteristics such as pasmo tolerance, rust resistance, etc., of value for incorporation into a general breeding program. One of these varieties, called Prevenche (C.D. 5954), came from the Plant Breeding Station, Versailles, France. In preliminary tests at Winnipeg it was resistant to a field collection of rust, while at Ottawa it appeared to be segregating for rust resistance. This variety was considered to be valuable as breeding stock because of its large seeds. The purpose of this study was to determine which genes for rust resistance are carried by Prevenche and the range of effectiveness of these genes.

LITERATURE REVIEW

THE HOST

Linnaeus (18) in 1753 named the cultivated species of flax *Linum usitatissimum*, meaning most useful, or most used, flax. It is the only economically important species of the genus *Linum*. The flax plant is an annual or, in mild climates, a winter annual ten to forty inches tall.

The origin of flax is uncertain. It is one of the oldest cultivated crops and was well developed in ancient Egyptian agriculture. Tammes (23) and Schilling (22) believe that common flax was developed by human selection from the wild species, *L. angustifolium* Huds. The wild species is prevalent throughout the Mediterranean region and is the only species with which the cultivated flax has been readily crossed. Vavilov (24) suggests that cultivated flax originated independently in two or three regions and that it was related to the wild *L. angustifolium*.

THE PARASITE

In 1801, Persoon (21) described the rust on flax and named it *Uredo miniata* var. *lini*. Leveille (17) transferred it to the genus *Melampsora* and named it *Melampsora lini*. Arthur (2) demonstrated that *M. lini* was an autoecious rust. He obtained pycnia and aecia on *L. usitatissimum* by inoculation with overwintering teliospores produced on this species. Allen (1) in 1933 proved that flax rust was heterothallic.

The occurrence of physiologic specialization in flax rust was first demonstrated by Flor (4) in 1935, when fourteen races were identified by their reaction on nine flax varieties. In 1940, Flor (5) identified ten more races, using a set of eleven differentials, which included eight of the nine differentials previously used and three new varieties. Later, five additional varieties were found that showed differential reaction distinct from those of the eleven then in use (8). In 1954, Flor (10) established a new set of eighteen differential lines each possessing single rust-conditioning genes.

INHERITANCE OF RUST REACTION

The first studies on the inheritance of rust reaction in flax were reported by Henry (12, 13) in 1926 and 1930. He crossed an Argentine selection and the varieties Bombay and Ottawa 770B with susceptible varieties. His work indicated that immunity to a North American collection of flax rust was determined by dominant factors in all crosses and was conditioned by single genes in Bombay and Ottawa 770B and by two genes in the Argentine selection.

In 1937, Myers (19) studied the inheritance of reaction to a field collection of flax rust and to a single physiologic race in thirty-seven crosses involving seventeen varieties or strains of flax. The reactions he obtained from both the collection of urediospores and the single physiologic race were similar, except for minor differences probably due to environment. The immunity, near immunity and resistance obtained from these crosses was explained by assuming that rust reaction was conditioned by genes in two different allelic series, which he designated by the symbols L and M. L and M were dominant duplicate factors conditioning immunity; l^n and m^n conditioned near immunity and were allelic with and recessive to L and M; and l^r and m^r conditioned resistance and were recessive to the more resistant L and M and l^n and m^n factors. Susceptibility was conditioned by the recessive factors ll and mm.

Flor (6) studied the inheritance of factors conditioning reactions to flax rust in a cross between J.W.S. and Buda. These varieties possessed differential reactions to a number of physiologic races of rust. J.W.S. was immune from race 4 and susceptible to race 7, while Buda gave the reverse reaction to these two races. The variety J.W.S. gave reactions of immunity from or susceptibility to each of the physiologic rust races used that could easily be distinguished in classification. On the other hand, the reaction of Buda to the races ranged from immunity, through the intermediate stages, to susceptibility. It was also found that these intermediate types of reaction were very sensitive to changes in environmental conditions. From his results, Flor assumed that rust reaction was conditioned by a single factor in J.W.S. and by two factors in Buda. The J.W.S. factor was allelic to one of the Buda factors, but not identical to it since the Buda factor conditioned immunity from some races, various degrees of resistance to others, and susceptibility to still others. The second Buda gene conditioned various degrees of resistance, semi-resistance and susceptibility to different races. The two Buda genes had an additive effect to some of the races, that is, plants carrying both genes were more resistant than plants carrying each separately.

In 1947, Flor (9) determined the rust-conditioning factors occurring in the sixteen flax rust differentials. He found that the factors for rust reaction lay in three allelomorphic series or linkage groups represented by the factors occurring in Ottawa 770B (LL), Newland (MM) and Bombay (NN). Rust reaction in each of these varieties is conditioned by a single pair of independently inherited factors. Crosses were therefore made between these three varieties and all other differentials. Results from these crosses showed that resistance was dominant and conditioned by single factors in twelve of the varieties studied, by two factors in six, and by three factors in two. The lack of segregates susceptible to races to which both parents were resistant was interpreted as indicating the presence of a rust-conditioning factor in the variety being studied which was allelomorphic to, or closely linked with, the factors for immunity in Ottawa 770B (LL), Newland (MM) and Bombay (NN) as the case might be. A number of susceptible segregates was taken to indicate lack of allelomorphism or linkage.

The results of this study showed that the sixteen differentials possessed collectively twenty-two pairs of rust-conditioning factors, of which at least nineteen were distinct. Seven differentials had factors in the LL series, four in the MM series, and five in a separate series which was named the NN group. The genes in the L group and the M group behaved as alleles, but crossing over was observed in some hybrids segregating for factors in the N group. This was considered to be a linkage group. In Australia, Kerr (14) obtained similar results using race K. However, with Australian race A he demonstrated resistance genes in Bison, Newland, and Ottawa 770B not discernible with race K.

Recently Kerr (16) identified three additional genes allelic to the L series and two allelic to the M series. He also determined a number of genes associated with the N linkage group. Studying this N linkage group in particular, he found that it consisted of two linked allelic series. The relationship of some of these N genes was determined. The symbol N was retained for those allelic to the rust-conditioning gene in Bombay, while the symbol P was assigned to the genes lying at the other locus. Kerr also found that the rust reaction in the host differential Clay was conditioned by an independently inherited gene to which the symbol K has been assigned.

Thus, there are five established loci for genes conditioning rust resistance in flax, with one allele for resistance at the K locus, eleven at the L, six at the M, three at the N and four at the P. The relationship of seven additional genes has not yet been established.

INHERITANCE OF PATHOGENICITY

In 1942, Flor (7) reported a study on the interaction of pathogenicity in various races of the flax rust fungus. This was done by selfing and hybridization of six physiologic races. Selfing revealed that three of the races were homozygous for pathogenicity, while the other three were heterozygous. In hybrids involving the six races virulence was found to be recessive. The segregation for pathogenicity of F_2 cultures from a cross of race 6 with race 24 on Akmolinsk, Bombay and Buda was explained by assuming that two pairs of incompletely dominant genes conditioned virulence on Buda, and that single independent pairs of genes conditioned virulence on Akmolinsk and Bombay. The results also indicated that one of the pairs of genes for pathogenicity on Buda was linked with the pair of genes conditioning pathogenicity on Akmolinsk.

The genetics of the inheritance of pathogenicity in crosses between several races was reported by Flor (8) in 1946. Race 22 of South American origin was crossed with races 6 and 24 of North American origin and the F_2 cultures were tested on the set of sixteen flax differentials. Of the differentials, only J.W.S. and Bombay were resistant to race 22. Williston Brown and Bombay were susceptible and Buda was moderately susceptible to race 24, while only Williston Golden and Williston Brown were susceptible to race 6. Virulence on all the differentials except Williston Golden and Williston Brown was inherited as a recessive character.

The segregation of the 133 F_2 cultures obtained from the cross of race 22 with race 24 indicated that pathogenicity was governed by one gene on each of Ottawa 770B, Newland, Bombay, Pale Blue Crimped, Kenya, Akmolinsk, Abyssinian, Leona, and Tammes Pale Blue; by two genes on Bolley Golden and Italia Roma; and by three genes on Morye (8). Of the remaining two varieties, Buda was moderately susceptible or susceptible to the parent races (22 and 24) and all the F_2 cultures, while J.W.S. was immune from them. The data also showed that the genes for pathogenicity on Pale Blue Crimped and Kenya, on Akmolinsk, Abyssinian and Leona, and on Williston Golden and Williston Brown were so closely linked as to be inherited as a unit. One of the pairs of genes for virulence on Bolley Golden was identical to or closely linked with one of the pairs of genes for virulence on Italia Roma. The pair of genes for virulence on Tammes Pale Blue was either the same as or closely linked with one of the three pairs of genes for virulence on Morye.

GENETICS OF HOST-PARASITE INTERACTION

Studies on the inheritance of pathogenicity in hybrids between races of flax rust (7, 8) and on the inheritance of rust reaction of the flax rust differentials (9) indicated to Flor that there is a relationship between factors for rust reaction in the host and those for pathogenicity in the pathogen. Flor (11) explained this host-parasite interaction by assuming a gene-for-gene relationship. Flor tested the pathogenicity of sixty-seven F_2 cultures from a cross of race 6 with race 22 on thirty-two varieties of flax that had been selected as carrying single rust-conditioning genes. Two of the varieties were resistant and six were susceptible to all the F_2 cultures used. These F_2 cultures segregated in a ratio of three avirulent to one virulent on twenty-three of the remaining twenty-four varieties. On Ottawa 770B, 2 genes conditioned pathogenicity.

The pathogenicity of the rust races identifies the genes for rust reaction in the host, while the reaction of the specific varieties of the host identifies the genes for pathogenicity in the parasite. Each gene that conditions rust reaction in flax apparently has a complementary gene in the rust that conditions pathogenicity. Flor (11) has set up a system of gene designation showing the specificity of the interaction between the genes in the host and the parasite. The symbol of the gene in the flax that interacts with the gene in the rust is used as a subscript to A and V. The symbols A and V designates genes for avirulence and virulence, respectively. For example, Bombay carries the N gene and, therefore, is resistant to all races of flax rust carrying the A_n gene and susceptible to all races homozygous for its alleles a_n a_n. Flor states that resistance occurs only when the complementary genes in both the host and the parasite are dominant except with the Williston Brown gene where virulence is dominant. Thus susceptibility will result when either or both the gene for reaction in the host and the gene for virulence in the parasite are homozygous recessive.

Mayo (20), in a recent publication, critically analysed Flor's work on the genetics of rust reaction in the host and the pathogen. Using a mathematical approach, Mayo claims that Flor's work does not give conclusive evidence for the existence of allelic series in the host and in the pathogen nor does it establish the presence of a gene-for-gene relationship. Flor bases his evidence for allelism on F_2 segregation from a repulsion phase heterozygote. This method, Mayo states, is very inefficient and therefore to get reliable results the population sizes would have to be much larger than the ones used by Flor. Further, Mayo suggests that a more critical test for allelism would be to cross an F_1 hybrid containing a pair of dominant genes to a universally susceptible variety. Using this method, some of the allelic series might be found to be cases of close linkage.

DIFFERENTIAL VARIETIES WITH SINGLE RUST-CONDITIONING GENES

The physiologic specialization in flax rust is of major importance in the development of rust resistant varieties. To identify the physiologic races, a set of host differentials must be inoculated with the rust. Some of the varieties in the series of host testers had more than one rust-conditioning gene, and some showed a reaction that was unstable in a fluctuating environment. In flax, according to Flor (7), there is a gene-for-gene relationship between resistance in the host and virulence in the pathogen. Resistance in flax is inherited as a dominant factor and virulence in the pathogen as a recessive factor. Varieties with two or more specific genes are resistant to all races not possessing the two or more corresponding genes for virulence. Consequently, such varieties do not differentiate between races effective against none, any one, or any combination of the genes present in the variety. From this it is apparent that the old host differentials (8) were of limited value in differentiating physiologic races of flax rust. Potentially pathogenic races could easily escape detection. A routine check with a series of host differentials each having a single rust-conditioning gene would detect any new races that might appear. The pathogenicity of these new races could easily be determined, and sources of resistance could readily be obtained, should resistance be present in one of the single factor differential lines or in other classified rust resistant material.

With this in mind, Flor (10) set up a project to isolate lines each apparently pure for a single pair of rust-conditioning genes. He crossed the sixteen old differentials with susceptible varieties, and using F_2 hybrid rust cultures from crosses between the highly virulent races 6 and 24, he selected F_2 plants that showed potential differentiation. These lines were increased and further tested to numerous rust races. Using this method, Flor isolated twenty-two lines each having only one rust-conditioning factor. Eight additional lines with single factors were secured from other varieties. From these thirty lines, Flor derived a new set of eighteen differentials each giving a sharp difference in reaction for easy identification of pustule type. The use of a series of eighteen differentials should not be too laborious since only three or four plants of each differential need be inoculated when checking the pathogenicity of a specific race of rust.

MATERIALS and METHODS

The varieties used as parents in these studies, together with their ancestry and rust reaction genotype are given in Table 1. All the crosses studied had Prevenche as one parent as follows: (The female parent of each cross is listed first) Prevenche x Bison, Prevenche x Dakota, Prevenche x Rocket, C.I. 1155 x Prevenche, Prevenche x Bombay and Crystal x Prevenche.

The crosses were made in the greenhouse during the winter of 1954-55. The F_1 plants were grown in a greenhouse bed during the summer of 1955. Due to extremely hot weather, the F_1 plants produced very little seed, and consequently, five to six families had to be used to obtain a large enough population for analysing each cross. The F_2 families were grown in the greenhouse during the winter of 1955-56, and were used in testing the reaction of the six crosses to five races of rust. During the summer of 1956 the F_3 lines from the six crosses were sown in the field in rows ten feet long and spaced twelve inches apart. Border rows of Bison, a susceptible variety, were sown along the front and back of each series.

The F_2 families to be tested with different races of rust were sown in five inch pots, with five or six seeds per pot. Kerr's excised shoot technique (15), which involves cutting the shoots above the cotyledons and growing them in tap water, was used in testing the seedlings with rust.

The equipment required for this technique consisted of a plastic rooting box, approximately two feet by one foot by three inches. The lid of this tray was perforated with one-eighth inch holes, which were spaced in a rectangular grid pattern with one-half inch intervals. When the seedlings were from four to six inches high the growing shoots were excised

TABLE 1 - ORIGIN OF PARENTS AND THEIR

KNOWN RUST REACTION GENOTYPE

Parents	Origin	Rust Genotype
Prevenche	A French Introduction	Unknown
Bison	A Selection	ll mm nn[1]
Dakota	Renew x Bison	ll MM nn
Bombay	An Indian Selection	ll mm NN
Crystal	Bison x Ottawa 770B	LL mm nn
C.I. 1155	Crystal x Redson (Minn.11-41-25)	LL mm nn
Rocket	Argentine 8C x Redwing	LL MM nn

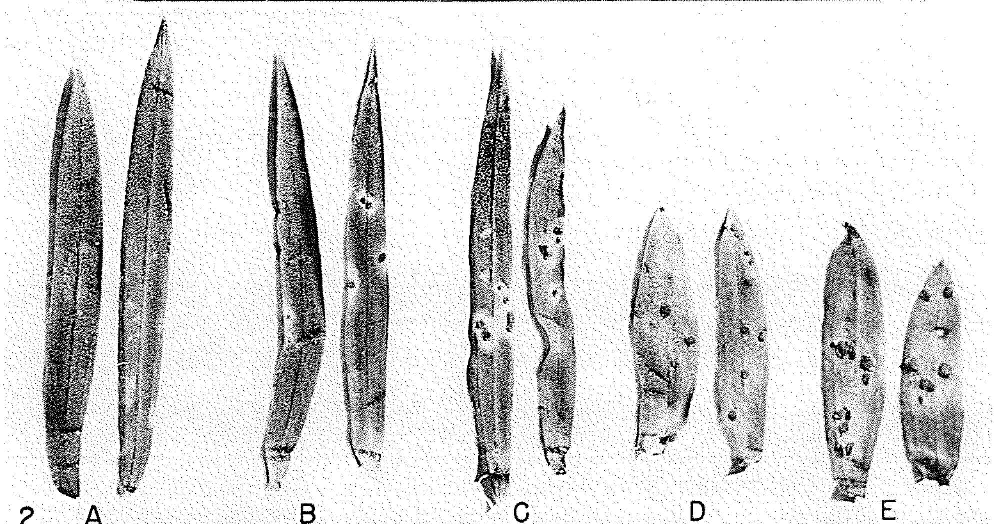
1. Kerr (14) recently found that Bison carries a single dominant factor for resistance to Australian race A, probably falling in the LL series.

just above the cotyledons. The stems of these excised shoots were then placed in holes in the grid (Figure 3, Plate 1). The tray was filled with water so that the stems were submerged. Each excised shoot was identified with a number corresponding to the seedling's pot number. The shoots in the one tray were immediately inoculated with a single race of rust. For inoculation the rust was mixed with talcum powder in a volumetric ratio of one part rust spores to about fifty parts talc. This mixture was applied directly to the leaves of the cuttings with a small puffer, and the plants were sprayed with water. The tray with the cuttings was then placed into a small metal glass-topped incubator for twenty-four hours. The temperature in the greenhouse was dropped 10 to 15 degrees F. to ensure high humidity. After incubation the tray with cuttings was kept in a greenhouse held at 65 to 70 degrees F. As soon as the rust had developed sufficiently to allow identification of the type of pustule produced (usually eight to ten days after inoculation) the type was recorded and the shoots were discarded, thus making the tray available for the next cuttings.

While the first shoots were being tested, the parent plants (the plants from which the cuttings had been made) were isolated from rust. These plants each produced at least two new shoots from just below the point of excision (Figure 1, Plate 1). In about two weeks these were again ready to be excised and were tested simultaneously with different races of rust. Using this method a single plant could be tested to an almost unlimited number of races. In this experiment the F_2 plants were tested to pure cultures of five different flax rust races (races 8, 41, 166, 178 and 210). After all these cuttings had been made and tested to rust, the parent plants were allowed to grow to maturity and the seed was harvested.

Cuttings made from the F_3 lines of the crosses Prevenche x Dakota

Plate 1



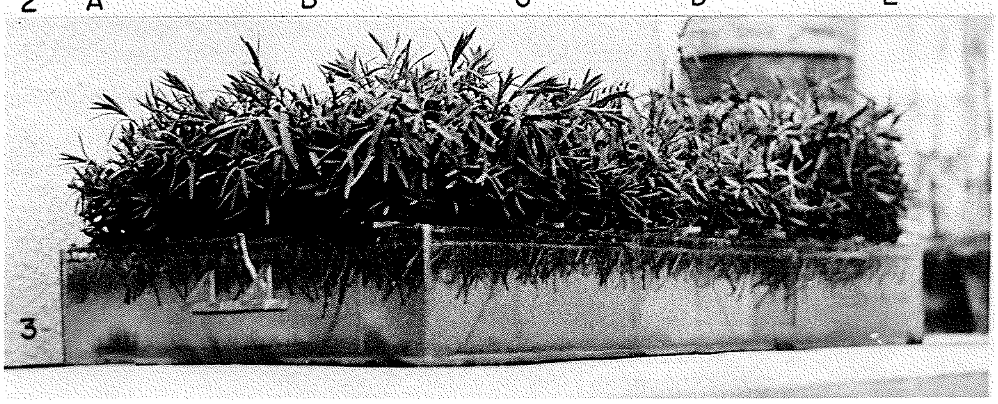


Figure 1 - An example of the prolific branching of flax seedlings when excised above the cotyledons.

Figure 2 - Types of rust infection used for classification of host reactions. Description in text.

Figure 3 - Plastic tray containing excised shoots of flax ready to be inoculated with rust.

and Prevenche x Bison were tested for reaction to three pure races of rust. The first twelve plants in each line from which cuttings were made were marked with wooden labels. The method of making the cuttings and inoculating them with rust was the same as that described for the F_2 population. This work was done during a hot summer period, and consequently, to promote normal rust development, the rust-inoculated excised shoots were placed in a growth cabinet. Here uniform temperature and light conditions were maintained. During this same period, flax rust in the greenhouse failed to develop or the flax plants were killed due to extremely high temperatures. Each of the twelve marked plants in the F_3 lines were tested to pure cultures of races 41, 166 and 178 of flax rust.

With the completion of individual race tests on the F_3 lines from the crosses Prevenche x Dakota and Prevenche x Bison, a rust epidemic was started in the field. A mixture of rust composed of the five races which were used on the F_2 population was applied to the border rows of Bison.

About one-hundred and fifty plants of the Prevenche parent and single plant progeny of Prevenche were tested to the same rust races used for the F_2 segregates. This bulk population was sown in five inch pots, with five or six seeds per pot. The same technique as described above was used to test each individual plant to the five rust races. The inoculated cuttings were also held under uniform conditions in a growth cabinet.

The infection classes, based on the type of pustule produced, was an adaptation from those described by Flor (10). The classes of host reaction used and a description of the corresponding types of rust infection follows. (See also Figure 2, Plate 1).

Class of host reaction		Description
A. Immune	-	No uredia produced; flecking may develop or no visible sign of infection.
B. Resistant	-	Few uredia, minute to small, usually accompanied by chlorosis or necrosis of surrounding tissue.
C. Moderately resistant	-	Uredia small to medium, surrounded by zone of heavy chlorosis.
D. Moderately susceptible	-	Uredia medium-sized, with little or no chlorosis.
E. Susceptible	-	Uredia large and confluent, with no chlorosis.

The immune, resistant and moderately resistant infection types were considered as resistant, while the moderately susceptible and susceptible were combined in the susceptible class.

The rust races used in this study were differentiated on the set of sixteen differentials established by Flor in 1946 (8). The numbers used to designate the five races were taken from Flor's key for the physiologic races of flax rust (10).

EXPERIMENTAL RESULTS

PARENTAL RUST REACTION

The six parental varieties used as testers in crosses with Prevenche were inoculated with the five races of rust used in this study. The rust reaction of these varieties, shown in Table 2, were uniform and consistent. Prevenche, on the other hand, proved to be very inconsistent in reaction. A bulk population of approximately one-hundred and fifty Prevenche plants were tested to each of the five races, and extremely variable results were obtained. The data in Table 3 illustrate how the tested plants varied in reaction from resistant to moderately susceptible to races 41, 166 and 210, and from moderately susceptible to susceptible to races 8 and 178. The reaction in these rust tests on Prevenche varied by almost imperceptible stages between the different pustule types, making their classification rather arbitrary.

The variable rust reaction exhibited by Prevenche was investigated by testing the progeny of a number of individual plants of the variety to the five races of rust used. The results obtained, as shown in Table 4, gave a range and frequency of reaction types similar to that obtained from the bulk population. This seemed to indicate that the Prevenche parent used in this study was not heterozygous for rust reaction.

In addition, the rust reaction of Prevenche was very unstable. The individual plants of the bulk population of Prevenche were reinoculated with race 41 and quite different results were obtained (Table 3). A few of the plants that were classified as immune in the first test were moderately resistant in the second, and one was even moderately susceptible. On the other hand, some of the plants were more resistant in the second test than in the first. Thus the type of reaction on individual plants varied between

TABLE 2 - PARENTAL AND F_1 REACTION TO VARIOUS RACES OF RUST[1]

Tester Parents and F1's	Races Tested 8	41	166	178	210
Bison	S	S	S	S	S
Dakota	I	I	S	S	S
Bombay	I	S	I	I	I
Crystal	I	I	I	I	I
C.I. 1155	I	I	I	I	I
Rocket	I	I	I	I	I
Prevenche x Bison	MS,S	R,MR,MS	MR,MS	MS,S	MR,MS
Prevenche x Dakota	I	I	MR,MS	MS,S	R,MR,MS
Prevenche x Bombay	I	MS	I	I	I
Prevenche x Crystal	I	I	I	I	I
Prevenche x C.I. 1155	I	I	I	I	I
Prevenche x Rocket	I	I	I	I	I

1 I, immune; R, resistant; MR, moderately resistant; MS, moderately susceptible; S, susceptible.

TABLE 3 - VARIATION OF RUST REACTION

BETWEEN INDIVIDUAL PREVENCHE PLANTS[1]

Races Used	Rust Reaction I	R	MR	MS	S
41 - first[2]	22	2	95	12	-
- second	19	4	98	10	-
166	37	1	95	3	-
210	31	-	62	3	-
8	-	-	3	124	32
178	-	1	9	131	11

1 I, immune; R, resistant; MR, moderately resistant; MS, moderately susceptible; S, susceptible.

2 The same plants were inoculated twice with race 41.

TABLE 4 - VARIATION IN RUST REACTION WITHIN

THE PROGENY OF INDIVIDUAL PREVENCHE PLANTS[1]

Races Used	Plant Numbers	Rust Reaction				
		I	R	MR	MS	S
Race 41						
	1	2	-	12	6	-
	2	2	-	11	9	-
	3	-	-	14	6	-
	4	1	1	13	5	-
	5	6	-	13	5	-
	6	3	1	12	6	-
Race 166						
	1	5	-	16	-	-
	2	4	1	16	1	-
	3	3	-	16	-	-
	4	1	1	19	1	-
	5	8	1	16	-	-
	6	1	3	17	-	-
Race 210						
	1	5	2	13	-	-
	2	2	5	14	-	-
	3	5	1	11	-	-
	4	6	1	13	-	-
	5	11	-	12	-	-
	6	12	-	10	-	-
Race 8						
	1	-	-	-	22	-
	2	-	-	1	22	-
	3	-	-	3	18	-
	4	-	-	2	20	-
	5	-	-	3	21	1
	6	-	1	2	18	-
Race 178						
	1	-	-	-	22	-
	2	-	-	1	19	3
	3	-	-	3	16	2
	4	-	-	-	21	2
	5	-	1	1	20	2
	6	-	-	-	21	1

[1] I, immune; R, resistant; MR, moderately resistant; MS, moderately susceptible; S, susceptible.

the two tests, but the percentage of plants falling into each reaction group was almost identical.

Prevenche's variable rust reaction suggested that the factors governing the resistance were very unstable and readily affected by changes in environment. Since these rust tests on the Prevenche population were conducted in the growth cabinet the environmental variation was held to a minimum. The only apparent variation was in the age or size of the plants from which the cuttings were made, since some of these plants would recover and send out new shoots much more quickly than others. The evidence obtained indicated that Prevenche's unstable and variable reaction to rust was not due to heterozygosity or heterogeneity, but that the expression of the degree of resistance was readily affected by any minor changes in external environment or by any variation in the condition or age of the plants.

RUST REACTION OF THE F_1 HYBRIDS

The reaction of the F_1 of the six crosses to the five races of rust is reported in Table 2. The F_1 plants from crosses where the tester parent was susceptible were generally less resistant than Prevenche, but the resistance exhibited by the F_1 tended to be more uniform than Prevenche in reaction. For example, the F_1 of Prevenche x Dakota gave a fairly uniform, moderately resistant to moderately susceptible reaction to race 166 (Table 2), while the reaction of Prevenche when tested to race 166 varied from immune to moderately susceptible (Table 3). The resistance of Prevenche is controlled, therefore, by a factor or factors incompletely dominant in reaction.

RESULTS FROM F_2 POPULATIONS

Results obtained from tests on F_2 populations to determine the number of factors for rust reaction possessed by the flax variety, Prevenche, are given in Table 5. Each cross was tested to five different races of rust. The fact that the rust reaction of Prevenche was unstable made the classification of the intermediate reactions difficult and consequently not as reliable as could be desired.

The data could best be explained by assuming that the rust reaction of Prevenche was controlled by two complementary factors, giving a moderately resistant reaction with races 41, 166 and 210, and a moderately susceptible reaction with races 8 and 178. The observed segregation ratios in the F_2 from crosses where Prevenche contributed all the resistance would fit either a 1 resistant :15 susceptible or a 9 resistant :7 susceptible ratio. A 1:15 ratio is obtained when a pair of complementary recessive genes are involved, while a 9:7 ratio is obtained when the pair of complementary genes behave as dominant factors. It would appear that environmental conditions may have been responsible for the differences in expression. In crosses where the tester variety was resistant to the races of rust used, a 1:15 or 9:7 ratio representing the resistance from Prevenche, was combined with the basic 3:1 or 15:1 ratio imposed by factors from the tester variety, depending on whether the tester carried one or two genes for resistance. Thus in Table 5 a 49:15 ratio represents the interaction of a single dominant factor from the tester, and a pair of complementary recessive genes from Prevenche; a 57:7 ratio is the interaction of a single dominant factor from the tester, and a pair of complementary dominant genes from Prevenche; and a 241:15 ratio is a combination of two dominant factors from the tester, and a pair of complementary recessive genes from Prevenche.

The tests on the F_2 populations from the six crosses with race 166, to which Prevenche was moderately resistant, supported the hypothesis that Prevenche carried two complementary genes for resistance, which acted as

TABLE 5 - ANALYSES OF F_2 DATA FROM SIX CROSSES

INVOLVING PREVENCHE TESTED TO FIVE RACES OF RUST

Cross and race tested	Rust Reaction[1] I	R	MR	MS	S	Observed ratio[2]	Calculated ratio	X^2 value	Probability between
Race 166									
Prevenche x Bison	29	11	38	21	21	78:42	9:7	3.7333	0.10 - 0.05
" x Dakota	53	10	32	30	38	95:68	9:7	0.2735	0.95 - 0.50
" x Bombay	67	-	31	27	2	98:29	49:15	0.0256	0.95 - 0.50
" x Crystal	112	5	6	7	9	123:16	57:7	0.0468	0.95 - 0.50
" x C.I. 1155	115	3	8	17	3	126:20	57:7	1.1418	0.30 - 0.20
" x Rocket	146	6	7	13	2	159:15	241:15	2.4081	0.20 - 0.10
Race 210									
Prevenche x Bison	13	-	-	22	82	13:104	1:15	4.4350	0.05 - 0.02
" x Dakota	69	5	14	20	56	88:76	9:7	0.4475	0.95 - 0.50
" x Bombay	68	-	13	40	12	81:52	(3)		
" x Crystal	98	-	1	5	25	99:30	49:15	0.0022	0.99 - 0.95
" x C.I. 1155	142	7	2	11	12	151:23	57:7	0.9298	0.50 - 0.30
" x Rocket	156	4	6	10	7	166:17	241:15	3.9402	0.05 - 0.02
Race 41									
Prevenche x Bison	13	-	-	35	68	13:103	1:15	4.8643	0.05 - 0.02
" x Dakota	116	9	3	14	24	128:38	49:15	0.0272	0.95 - 0.50
" x Bombay	5	2	8	39	109	15:148	1:15	2.4249	0.20 - 0.10
" x Crystal	104	11	1	8	19	116:27	49:15	1.6470	0.20 - 0.10
" x C.I. 1155	157	-	4	17	25	161:42	49:15	0.8541	0.50 - 0.30
" x Rocket	181	2	8	8	1	191:9	241:15	0.6701	0.50 - 0.30
Race 8									
Prevenche x Bison	1	-	-	3	116	1:119	(3)		
" x Dakota	103	2	3	2	47	108:49	3:1	3.2290	0.10 - 0.05
" x Bombay	74	7	14	16	9	95:25	49:15	0.4535	0.95 - 0.50
" x Crystal	112	-	-	-	32	112:32	49:15	0.1185	0.95 - 0.50
" x C.I. 1155	116	4	-	17	13	120:30	49:15	0.9891	0.50 - 0.30
" x Rocket	166	-	-	5	2	166:7	241:15	1.0311	0.50 - 0.30
Race 178									
Prevenche x Bison	1	-	3	13	109	4:122	1:15	2.0338	0.20 - 0.10
" x Dakota	6	-	8	30	119	14:149	1:15	1.5219	0.30 - 0.20
" x Bombay	74	8	15	11	40	97:51	(3)		
" x Crystal	109	-	-	1	32	109:33	49:15	0.0035	0.99 - 0.95
" x C.I. 1155	140	-	3	11	32	143:43	49:15	0.0834	0.95 - 0.50
" x Rocket	158	6	11	7	2	175:9	241:15	0.3122	0.95 - 0.50

1 I, immune; R, resistant; MR, moderately resistant; MS, moderately susceptible; S, susceptible.

2 Reactions I, R and MR were grouped into the number listed first, the resistant class; while reactions MS and S were grouped into the number listed second, the susceptible class.

3 No satisfactory ratio to fit the plants observed.

complementary dominant in some cases and as complementary recessive in others. The segregation to this race in the Prevenche x Bison and the Prevenche x Dakota crosses, where both Bison and Dakota were susceptible, gave good 9:7 ratios, thus indicating that Prevenche contributed a pair of complementary dominant genes. Similarly, in the Prevenche x Crystal and the Prevenche x C.I. 1155 crosses, where both tester parents were resistant to race 166, good fits to a 57:7 ratio were obtained. The results in the Prevenche x Bombay and the Prevenche x Rocket crosses suggested that the factors for resistance against race 166 in Prevenche were inherited as a pair of complementary recessive genes.

The rust reaction when the six crosses were inoculated with race 210 was in many respects similar to that obtained with race 166. Prevenche was also moderately resistant to this race. With race 210 the factors from Prevenche in the Prevenche x Bison and the Prevenche x Crystal crosses were complementary recessive, contrary to their behavior with race 166. A poor fit was obtained in the Prevenche x Bison cross, but a population size of 117 plants was too small to determine adequately a two factor segregation. This together with a few escapes could readily account for the high resistant class in this cross. The segregation in the Prevenche x Bombay F_2 population with race 210 did not fit the expected ratio, as the susceptible class was too large. In this cross a large number of plants fell into the moderately resistant and moderately susceptible classes. Since considerable difficulty was encountered in distinguishing between these intermediate reactions, it seemed possible that the susceptible class contained some plants that should have been placed in the resistant class.

When the rust reaction of race 41 on the six crosses was determined, it was found that the Prevenche resistance to race 41 was governed by a pair of complementary recessive genes in all cases; no complementary dominant reaction was observed. Prevenche itself was moderately resistant to race 41. A fairly good 1:15 ratio was obtained from the Prevenche x Bombay cross (Bombay susceptible). The segregation in the Prevenche x Bison cross was similar to that obtained using race 210. The other four crosses all gave satisfactory results when a 1:15 ratio was combined with the basic ratio derived from genes of the tester parents.

Segregation for rust reaction in the F_2 populations of the six crosses to races 8 and 178 could in most cases be explained by assuming that Prevenche possessed a pair of complementary recessive rust-conditioning factors. Prevenche gave a moderately susceptible reaction to these two races. The Prevenche x Bombay cross gave an excellent fit to a 49:15 ratio when race 8 was used, but with race 178 the results were similar to those with race 210 in that no logical ratio was obtained. The Prevenche x Dakota cross when tested to race 8 gave a segregating ratio with a large susceptible class. While this cross gave a fair fit to a 3:1 ratio, it did not fit the expected 49:15 ratio, indicating that the Prevenche contribution had little or no effect in this case. The Prevenche x Bison cross also was highly susceptible. These latter two crosses were tested at the same time and under the same environment. It is possible that this environment may have been conducive to the production of a greater susceptible reaction on the plants, thus accounting for the large susceptible classes.

The remaining crosses when tested to the various races supported the hypothesis that Prevenche carried a pair of complementary genes for rust reaction.

RESULTS FROM F_3 LINES

The rust reaction on cuttings made from the F_3 lines of the Prevenche x Dakota and Prevenche x Bison crosses, presented in Table 6, supported the

TABLE 6 - INHERITANCE OF REACTION TO RACES 41, 178 AND 166 OF F_3 LINES FROM TWO CROSSES, PREVENCHE X BISON AND PREVENCHE X DAKOTA

	Ratio	Rust[1] Class	Observed Ratio	Calculated Ratio	X^2	Probability Between
Race 41						
Prevenche	1	R	3	5.5	-	
x Bison	6	Seg	40	33.0	-	
	9	S	45	49.5	2.7030	0.30 and 0.20
Prevenche	19	R	44	33.34	-	
x Dakota	36	Seg	59	66.96	-	
	9	S	16	16.74	3.1008	0.30 and 0.20
Race 178						
Prevenche	1	R	4	5.5	-	
x Bison	6	Seg	34	33.0	-	
	9	S	50	49.5	0.4444	0.95 and 0.50
Prevenche	1	R	3	7.375	-	
x Dakota	6	Seg	51	44.250	-	
	9	S	64	66.375	3.7085	0.20 and 0.10
Race 166						
Prevenche	1	R	6	5.0	-	
x Bison	6	Seg	37	30.0	-	
	9	S	37	45.0	3.2312	0.20 and 0.10
Prevenche	1	R	3	7.43	-	
x Dakota	6	Seg	49	44.64	-	
	9	S	67	66.96	3.0672	0.30 and 0.20

[1] R, resistant; Seg, segregating; S, susceptible.

assumption that Prevenche possessed a pair of complementary genes conditioning a variable rust resistance. The segregation between the F_3 lines from Prevenche x Bison with races 41, 166 and 178 gave satisfactory fits to a 1 resistant: 6 segregating: 9 susceptible ratio. This ratio is obtained by a correction from the normal 1 resistant: 8 segregating: 7 susceptible, to make allowance for the fact that only twelve plants were tested in each line. Similarly, the F_3 lines from Prevenche x Dakota when tested with races 166 and 178, to which Dakota was susceptible, segregated in a 1:6:9 ratio. To race 41 the lines segregated 19 resistant: 36 segregating: 9 susceptible. Such a ratio would result in the F_3 of this cross tested to race 41 on the basis that Dakota carries a single dominant factor and Prevenche a pair of complementary factors for resistance to this race.

The rust epidemic on the F_3 lines in the field was not a success. The first rust was applied on the susceptible check rows quite late in the season. This delay was necessary so that the lines from the two crosses of Prevenche with Dakota and Bison could be tested to the three races of rust. By this time the plants were maturing rapidly and consequently the rust did not develop as well as expected. The results from the field were, therefore, discarded from all crosses except from Prevenche x Bombay. Race 41 was the only race in the collection of rust applied in the field that was pathogenic on Bombay. For some reason this race spread very rapidly on the Bombay check rows, killing the plants even before seed was set. The F_3 lines from this cross gave very good rust reactions and the results gave an excellent fit to a 1 resistant: 8 segregating: 7 susceptible ratio, as shown in Table 7. This also supports the hypothesis that Prevenche has two complementary rust reaction factors. In this case the ratio was not corrected for small population size, since each F_3 line grown in the field consisted of from thirty to sixty plants.

TABLE 7 - FIELD RUST REACTION OF F_3 LINES FROM THE PREVENCHE X BISON CROSS

	Rust Reaction[1]			X^2	Probability Between
	R	Seg.	S		
Ratio	1	8	7	-	
Observed ratio	8	54	56	-	
Calculated ratio	7.375	59	51.625	.7997	0.95 and 0.50

[1] R, resistant: Seg, segregating; S, susceptible.

DISCUSSION

The results obtained from rust tests on the F_2 and F_3 generations were explained by assuming that Prevenche possessed a pair of complementary genes for rust reaction. In the F_2 generation, under certain conditions, the Prevenche factors behaved as a pair of complementary dominant genes giving a 9:7 ratio; while more frequently they behaved as a pair of complementary recessive genes as expressed in a 1:15 ratio. The limited number of tests on the F_3 generation gave particularly good evidence of the presence of a pair of complementary genes in Prevenche. However, in working with F_3 lines, the only difference in the reaction of a complementary dominant or complementary recessive pair of genes would be in the ratios of reaction within the segregating lines. Since no such information was obtained it was impossible to determine whether or not the Prevenche factors behaved as dominant or recessive in the F_3 generation.

The rust reaction of Prevenche was shown to be quite unstable and readily affected by environmental changes. The various Prevenche crosses were inoculated with the five races of rust at different times during the winter, and consequently under somewhat different environmental conditions. It is possible that the variations in external environment, and in the stages of plant growth at which the cuttings were made could determine whether the Prevenche genes acted in a dominant or recessive manner. It is also probable that minor environmental changes could give ratios intermediate between 1:15 and 9:7. Some of the ratios obtained did indicate such a situation. The Prevenche x Bison cross gave a very poor fit to a 1:15 ratio with races 41 and 210. In both cases the resistant class was large, suggesting that the conditions under which these tests were made tended to shift the infection classes toward an intermediate reaction.

There were indications of a relationship between the races of rust used and the expression of Prevenche's complementary genes. Four of the six 9:7 ratios observed occurred when race 166 was used, the other two were found when race 210 was used, and none occurred against the other three races. Thus there appeared to be a tendency for the Prevenche resistant to act as complementary dominant factors against race 166, and to a lesser extent against race 210.

The behavior of the Prevenche genes is in some respects similar to that obtained by Flor (6), who found that the reactions of one of the factors conditioning rust resistance in Buda varied from highly resistant to moderately resistant. Flor also found that one of the factors for resistance in Buda was incompletely dominant, and plants heterozygous for this factor were distinctly less resistant than plants carrying the dominant factor in the homozygous condition. These quantitative factors were also subject to considerable influence by environment.

There was no evidence that the Prevenche factors were linked or allelic with the L, M or N factors represented in the tester plants.

The Prevenche genes tended to give a more uniform reaction in the F_1 plants than in Prevenche itself. This suggests that the genes, when transferred into another genetic background, might become more stable. Thus if these genes were transferred by a backcrossing program into another variety, they might eventually be of some value for flax improvement. It is possible that the Prevenche genes might give the variety a broader form of rust resistance. This is especially so in view of the fact that they are evidently not related to the genes in the L, M or N series as represented in the tester parents.

SUMMARY

The mode of inheritance of rust reaction in Prevenche was studied by determining the segregation to five races of rust (8, 41, 166, 178 and 210) in six crosses having Prevenche as one of the parents. The varieties with which Prevenche was crossed were chosen to represent various combinations of genes in the L, M and N series designated by previous investigators. Prevenche gave a variable reaction ranging from resistant to moderately susceptible with races 41, 166 and 210, and a moderately susceptible to susceptible reaction with races 8 and 178.

The results obtained were explained by assuming that the rust reaction in Prevenche was conditioned by a pair of complementary genes. Generally, these genes behaved as a pair of complementary recessives, while in a few cases the reaction was expressed as a pair of complementary dominant genes. The F_1 results indicated that the rust reaction was conditioned by incompletely dominant genes.

The factors conditioning rust reaction in Prevenche were found to be very unstable and readily affected by environmental changes. No evidence for allelism or linkage of these factors with the L, M and N factors was observed in the F_2 segregation.

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