

Interactions Between Genes Controlling Pathogenicity in the Flax Rust Fungus

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ABSTRACT

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Progeny obtained by self-fertilizing each of two strains of the flax rust fungus (*Melampsora lini*), and by intercrossing them, were individually tested for pathogenicity on 29 host-differential cultivars that possess 28 (or possibly 29) different resistance genes. The progeny segregated for pathogenicity on 19 of the differential cultivars. It was concluded that pathogenicity on 14 of these cultivars was determined, in each case, by a single pair of allelic genes, with avirulence dominant to virulence. For each of the remaining five cultivars possessing resistance genes M^1 , L^1 , L^7 , L^{10} , and L^x , it was concluded that an avirulence/virulence gene pair (A/a) and an inhibitor gene pair (I/i) interact to determine pathogenicity and that the

only avirulent strains are those with genotypes $ii Aa$ and $ii AA$. The data are consistent with the assumption that a common inhibitor gene (or group of closely-linked inhibitor genes) is involved in determining pathogenicity on the L^1 , L^7 , L^{10} , and L^x resistance genes and that this inhibitor gene (or genes) is closely linked to the inhibitor gene involved in determining pathogenicity on M^1 . The data also suggest that the avirulence genes A_{M^1} and A_{M^4} are linked (possibly very closely), that the differential cultivar Victory A possesses two genes conferring resistance (both of which belong to the "M" group [M^1 and M^4]) and that the differential cultivar Towner also possesses the M^4 resistance gene in addition to the L^8 gene.

Additional key words: gene-for-gene relationship.

In flax (*Linum usitatissimum* L.) 29 genes that confer resistance to flax rust caused by *Melampsora lini* (Ehrenb.) Lévy. have been identified. These occur in five series of closely linked or allelic genes, designated K , L , M , N , and P , which contain 1, 13, 7, 3, and 5 genes, respectively (6,11,19,20). In studies of the inheritance of pathogenicity in flax rust, single-gene segregation ratios indicating a dominance of avirulence have been obtained, on at least one occasion, on differential cultivars possessing 24 of the 29 known genes for resistance (2,3,5,7-9,17,18). Since these data are consistent with the assumption that the 24 pathogen genes segregating in these studies are different genes, it would seem, with the exception of five resistance genes that remain untested, that the pathogen possesses an avirulence gene specific and complementary to each of the resistance genes in the host. This one-for-one relationship, first elucidated by Flor (2), is called the "gene-for-gene" hypothesis.

However, the gene-for-gene hypothesis, as stated above, does not provide a complete description of the interaction between genes in the host and genes in the pathogen. It has been proposed (17) that data on the inheritance of pathogenicity on the host cultivar Williston Brown, which possesses the M^1 gene for resistance, can best be explained by postulating that two pairs of allelic genes in the flax rust fungus interact to determine pathogenicity on this cultivar.

This paper reports the results of three family studies on the inheritance of pathogenicity in flax rust. The results of these studies support the proposal that two allelic gene pairs in the flax rust fungus interact to determine pathogenicity on the M^1 resistance gene and also suggest that two allelic gene pairs similarly interact to determine pathogenicity on each of three, or possibly four, other genes conferring resistance to rust in flax.

MATERIALS AND METHODS

Three rust strains, designated C, H, and I, were used as primary parents in the family studies. Strain C was obtained by self-fertilizing New Zealand "race" 5 (see [1]). Strain H is "race" 228 of

North American origin and strain I is "race" 271 (North American series) of South American origin (H. H. Flor, *personal communication*). Three families of rust fungus progenies were obtained as follows: strain CH₅, a hybrid strain obtained from crossing strains C and H, was self-fertilized and yielded a family of 80 progeny; strain I was self-fertilized and yielded a family of 27 progeny; and strains CH₅ and I were intercrossed and yielded a family of 32 progeny. All rust progeny were individually tested for pathogenicity on a set of 29 host-differential cultivars. These cultivars collectively possess 28 of the 29 known genes for resistance and the gene L^x , a gene derived by rare recombination from resistance gene L^6 (K. W. Shepherd and G. M. E. Mayo, *unpublished*).

To produce the rust progeny, plants of cultivar Hoshangabad, which possesses no known resistance genes, were inoculated with haploid basidiospores by suspending germinating teliospores of a parent strain over the plants for several hours in a high-humidity chamber. The monokaryotic infections (pycnia) arising from that inoculation produced pycniospores in a liquid exudate (nectar) after 8-10 days. A day or two before the production of nectar, all leaves with more than a single focus of infection were removed from the plants. Crosses were made about 5 days after the first appearance of nectar by transferring pycniospores from one pycnium to another. Each recipient pycnium received nectar from only one donor pycnium, and a pycnium used as donor in one cross was not used in any other crosses. The dikaryotic aeciospores arising from successful crosses were inoculated onto Hoshangabad plants to obtain sufficient urediospores to test on the full set of differential cultivars: the urediospores derived from one pustule of aeciospores constituted a single rust progeny. Differential cultivars were inoculated as seedlings when they were 6-12 cm high. Inoculations with aeciospores and urediospores were done by suspending the spores in a small amount of tap water and applying the suspension to the leaves with a small brush. Plants were kept at high humidity overnight and then were moved to a greenhouse bench for the remainder of the incubation period.

Pathogenicity reactions were scored approximately 14 days after inoculation and were based on the amount of growth made by the rust fungus on the host plant. This ranged from free growth (+)

through restricted growth (+/- and -), to no growth (---). Full virulence (+) was characterized by large, often compound, urediospore pustules on leaves (both young and old) and on stems; avirulence (---) was characterized by necrotic flecks on inoculated leaves with no sign of pustule development. Semivirulent reactions showed restricted pustule development, the extent of which frequently, but not always, varied with the age of the leaf. Reactions showing small (0.5-mm diameter) pustules, generally on only the very young leaves, with no pustule development on older leaves, were scored as -, whereas reactions showing only slight restriction of pustule development on young leaves at the top of a stem, grading through smaller pustules to no pustule development on older leaves at bottom of stem, were scored +/- . Reactions intermediate between these categories were scored as -(+/-) or +/- (+) etc.

RESULTS

Segregation data. Segregation data are summarized in Table 1. The ratios of progeny showing less virulence (the "avirulent" progeny) to those showing greater virulence (the "virulent" progeny) are given for each family. Segregations showing a complete association of pathogenicity reactions in one or more of the families occurred on the following pairs, or groups, of cultivars (see Table 1): (i) Akmolinsk, Abyssinian, and Leona; (ii) Towner and Victory A; and either (iii) Wilden and Birio; (iv) Barnes and L^x; or (v) Wilden, Birio, Barnes, and L^x. Within each family, the segregation on any one cultivar was tested for independence from the segregations that occurred on each of the other cultivars. All segregations were independent except those in the CH₅-selfed and CH₅ × I families which are indicated in Table 2.

The reactions of individual progeny in both the CH₅-selfed and the CH₅ × I families on certain host differentials are summarized in Tables 3 and 4, respectively. Many of the segregations on these host

differentials were significantly associated; the relationships can be examined in detail in Tables 3 and 4.

Segregations indicating dominance of avirulence. The patterns of segregation for pathogenicity on the cultivars listed in Table 1 (part a) are consistent with the assumption that a single pair of allelic genes determines pathogenicity on each cultivar, with avirulence (or less virulence) dominant to virulence (or greater virulence). All segregations fit ($P=0.05$) the expected monohybrid ratios (3 avirulent:1 virulent) except on Koto, Akmolinsk, Abyssinian, and Leona in the CH₅ × I family in which a 1:1 ratio was expected. The data in Table 1 (part a) are, therefore, consistent with findings of previous studies (9) in which avirulence has been invariably inherited as a dominant character on all cultivars tested except Williston Brown.

Pathogenicity on the "P" gene differentials. The results of previous studies (3,5,8) show close linkage of the four pathogen avirulence genes A_{P^1} , A_{P^2} , A_{P^3} , and A_{P^4} that are complementary to host resistance genes P , P_1 , P_2 , and P_3 in Koto, Akmolinsk, Abyssinian, and Leona, respectively. Therefore, assuming linkage, the genotype of strain CH₅ is $A_{P^1}A_{P^2}A_{P^3}A_{P^4}/a_{P^1}a_{P^2}a_{P^3}a_{P^4}$ and that of strain I is $a_{P^1}a_{P^2}a_{P^3}a_{P^4}/a_{P^1}a_{P^2}a_{P^3}a_{P^4}$ (order arbitrary). In the CH₅ × I family approximately half the progeny (20 of 32) were virulent on Koto and avirulent on Akmolinsk, Abyssinian, and Leona, but the remainder had the reverse pathogenicity (Table 1). In the CH₅-selfed family there was a complete association between the reactions of rust progeny on Akmolinsk, Abyssinian, and Leona (Table 1) and the "unit" segregation on these three cultivars was not independent of the segregation on Koto (Table 2); in particular, no individual was virulent on all four cultivars. These are the expected observations assuming close linkage of the A_{P^1} , A_{P^2} , A_{P^3} , and A_{P^4} genes. The A_{P^4} gene is not associated with the closely-linked A_{P^1} , A_{P^2} , and A_{P^3} genes; segregation on the cultivar possessing the P^4 gene was independent of the segregation on the cultivars, possessing the other "P" genes.

TABLE 1. Segregation for pathogenicity on flax differential cultivars among *Melampsora lini* progeny obtained by self-fertilizing and intercrossing strains CH₅ and I

Cultivar ^x	Resistance gene(s) ^y	Pathogenicity of ^w									
		Parent strain		Strain CH ₅	CH ₅ -selfed progeny		Strain I	I-selfed progeny		CH ₅ × I progeny	
		C	H		Avir.	Vir.		Avir.	Vir.	Avir.	Vir.
(a)											
Stewart	L^2	+	---	---	55	25	---	27	0	32	0
Towner	L^8	+	---	-	66	14	---	18	9	23	9
L ¹¹	L^{11}	+	---	-	65	15	---	19	8	23	9
Dakota	M	+	---	---	63	17	---	18	9	21	11
Cass	M^3	+	---	---	62	18	---	27	0	32	0
Victory A	M^4	+	---	-(+/-)	66	14	---	18	9	23	9
Bombay	N	---	---	---	60	20	---	27	0	32	0
Koto	P	---	+	---	64	16	+	0	27	20	12
Akmolinsk	P^1	+	---	---	60	20	+	0	27	12	20
Abyssinian	P^2	+	---	---	60	20	+	0	27	12	20
Leona	P^3	+/-(-)	---	---	60	20	+	0	27	12	20
P ⁴	P^4	+	---	---	64	16	---	27	0	32	0
Wilden	L^5	+	---	-	59	21	---	24	3	20	12
Birio	L^6	+	---	---	59	21	---	24	3	20	12
(b)											
Williston Brown	M^1	+	---	+	18	62	+(+/-)	4	23	9	23
Barnes	L^7	+	-	+	22	58	-(+/-)	24	3	12	12+8 ^z
L ^x	L^x	+	---	+	22	58	---	24	3	12	12+8 ^z
B ¹⁴ × Burke	L^1	+	---	+	6	74	-(+/-)	18	9	11	21
Bolley Golden selection	L^{10}	+	-	+	0	80	---	27	0	18	14

^w Brackets in table join segregations showing complete association. + = full growth of rust; +/- = restricted growth; - = minute pustules, usually on youngest leaves only; --- = no growth.

^x Host differentials Clay (K), Ottawa 770B (L), Pale Blue Crimped (L³), Kenya (L⁴), Bison (L⁹), Ward (M²), M⁶, Polk (N¹), and Marshall (N²) have been omitted from this table since segregation for pathogenicity on these cultivars did not occur in any family. Cortland has also been omitted because, although segregation did occur in all three families, the reactions ranged in continuous spectrum from - to + and it was not possible to clearly distinguish a less-virulent group from a more-virulent group. Flor (10) states that the resistance conditioned by the Cortland gene (M⁵) is variable and difficult to study. Cultivars have been listed in groups a and b to facilitate discussion of the results, since the genetic control of pathogenicity on the cultivars in one group differs from that on the cultivars in the other group.

^y References (6, 11, 20) and K. W. Shepherd and G. M. E. Mayo (unpublished).

^z Twelve were fully virulent and eight were semi-virulent.

Pathogenicity on Williston Brown. The patterns of segregation for pathogenicity on the cultivars listed in Table 1 (part b) were exceptions to the usual findings in flax-rust inheritance studies because they indicated an apparent dominance of virulence in some families. The only previous report of a similar atypical segregation is by Flor (3), and that occurred on Williston Brown (M^1), one of the cultivars on which an atypical segregation occurred in this study. In Flor's study, F_2 progeny from a cross between his "race" 22 and "race" 24 segregate in the ratio 17 avirulent to 116 virulent on Williston Brown. Both parent strains and the F_1 were virulent on Williston Brown, as were nine selfed progeny of "race" 22 and 15 selfed progeny of "race" 24. An explanation that accounts for these observations (17) postulates that a dominant inhibitor gene, I_{M^1} , which is present in one of the parents, interacts with a dominant gene normally controlling avirulence on Williston Brown, A_{M^1} , that results in a virulent pathogen phenotype. Under this model the

TABLE 2. Chi-square values for independence of the segregations for pathogenicity on selected^w flax differential cultivars within progenies obtained by self-fertilizing *Melampsora lini* strain CH₅ and by intercrossing strains CH₅ and I

Cultivars, or cultivar groups, ^x on which the segregations for pathogenicity are tested for independence	Chi-square value ^y for:	
	CH ₅ progeny	CH ₅ × I progeny
Akmoilinsk-Abyssinian-Leona × Koto	5.10 **	27.88 ***
Wilden-Birio × Barnes-L ^x	9.01 **	9.10
Williston Brown × Barnes-L ^x	40.02 ***	6.44 *
Williston Brown × B ¹⁴ × Burke	10.25 **	13.30 ***
Williston Brown × Bolley Golden selection	...	7.42 **
Barnes-L ^x × B ¹⁴ × Burke	13.40 ***	3.33
Barnes-L ^x × Bolley Golden selection	...	12.22 ***
B ¹⁴ × Burke × Bolley Golden selection	...	10.47 ***

^w Cultivar combinations in which the segregations for pathogenicity were independent of each other have been excluded.

^x Cultivars on which completely-associated segregations occurred have been grouped and treated as a unit.

^y The 2 × 2 contingency table chi-square values were calculated by using Yates' continuity correction.

^z Asterisks indicate association between segregations that are: significant, $P = 0.05$ (*); highly significant, $P = 0.01$ (**); or very highly significant, $P = 0.001$ (***)

TABLE 3. Pathogenicity reactions on selected flax differential cultivars of progeny obtained by self-fertilization of *Melampsora lini* strain CH₅

Cultivar	Reaction pattern ^z								
Wilden - Birio	-	-	-	+	+	+	-	-	
B ¹⁴ × Burke	-	-	+	+	+	+	+	+	
Barnes - L ^x	-	-	-	+	+	+	+	+	
Williston Brown	-	+	-	+	-	+	+	+	
Towner - Victory A	-	+	-	+	-	+	-	+	
Progeny	5	1	11	5	2	4	15	4	33

^z + = full virulence or greater virulence; - = avirulence or lesser virulence.

TABLE 4. Pathogenicity reactions on selected flax differential cultivars of progeny obtained by inter-crossing *Melampsora lini* strains CH₅ and I

Cultivar	Reaction pattern ^z							
Wilden - Birio	+	-	-	-	+	+	+	+
B ¹⁴ × Burke	+	+	-	+	-	+	-	+
Barnes - L ^x	+	+	-	-	+	+	+	+
Williston Brown	+	+	-	+	+	-	-	+
Bolley Golden selection	+	+	-	-	-	-	-	-
Progeny	6	8	7	5	3	1	1	1

^z + = full virulence or greater virulence; - = avirulence or lesser virulence.

only avirulent strains are those with genotypes $ii Aa$ and $ii AA$. Flor's data can be accounted for if it is assumed that "races" 22 and 24 have genotypes $I_{M^1} I_{M^1} A_{M^1} A_{M^1}$ and $i_{M^1} i_{M^1} a_{M^1} a_{M^1}$, respectively; these "races," their selfed progeny, and the F_1 hybrid ($I_{M^1} i_{M^1} A_{M^1} a_{M^1}$) would be virulent on Williston Brown as was observed. Furthermore, a 3:13 ratio of avirulent to virulent F_2 individuals would be expected, and this also was observed ($\chi^2_1 = 3.11$; $P = 0.05-0.1$).

This model received support from a later study (9) in which progeny obtained from self-fertilizing a strain avirulent on Williston Brown segregated 61 avirulent to 29 virulent. This result fits a monohybrid ratio with avirulence dominant to virulence, thus indicating that an A_{M^1}/a_{M^1} gene pair does occur in the pathogen as predicted by the model: the genotype of the parent strain presumably is $i_{M^1} i_{M^1} A_{M^1} a_{M^1}$.

In this study the observed segregations for pathogenicity on Williston Brown in all three families (Table 1) were consistent with strains CH₅ and I both being heterozygous for the postulated inhibitor gene and either heterozygous or homozygous dominant for the A_{M^1}/a_{M^1} gene pair. If both parents are $I_{M^1} i_{M^1} A_{M^1} a_{M^1}$ a 3:13 (avirulent:virulent) ratio would be expected in all three families. If both are $I_{M^1} i_{M^1} A_{M^1} A_{M^1}$ a 1:3 ratio would be expected. If one is $I_{M^1} i_{M^1} A_{M^1} a_{M^1}$ and the other $I_{M^1} i_{M^1} A_{M^1} A_{M^1}$ a 1:3 ratio would be expected in the CH₅ × I family. Since the segregation ratios observed on Williston Brown in all three families are in agreement ($P = 0.05$) with both a 1:3 and a 3:13 ratio, it was not possible to infer from these segregation data alone the genotype of either CH₅ or I at the A_{M^1} locus. However, other evidence discussed below indicates that both CH₅ and I are heterozygous for A_{M^1} .

Pathogenicity on Barnes and L^x. In all three families unit segregation for pathogenicity occurred on cultivars Barnes (L^7) and L^x (Table 1). From such unit segregation it can be inferred that either L^7 and L^x are the same gene or, if different genes, that the complementary pathogenicity genes in the rust fungus are sufficiently closely linked to have always segregated as unit in these studies. Since it has not been established that L^7 and L^x are different genes (K. W. Shepherd and G. M. E. Mayo, unpublished), the inheritance of pathogenicity on these cultivars is discussed in relation to Barnes (L^7) alone.

Segregation on Barnes in the I-selfed family (Table 1) fits a classical 3:1 segregation ratio with avirulence dominant to virulence ($\chi^2_1 = 2.78$; $P = 0.05-0.1$), which is consistent with a single gene pair (A_{L^7}/a_{L^7}) controlling pathogenicity on Barnes, as reported by Flor (7,9). However, the pattern of segregation on Barnes in the CH₅-selfed and CH₅ × I families cannot be explained solely by the segregation of an A_{L^7}/a_{L^7} gene pair. To account for these atypical patterns it is proposed that CH₅ is heterozygous for a second inhibitor gene pair, I_{L^7}/i_{L^7} , in which I_{L^7} interacts with A_{L^7} to give a virulent reaction on Barnes (L^7) in the same way that it has been proposed that I_{M^1} interacts with A_{M^1} to give a virulent reaction on Williston Brown (M^1). The expected phenotypic ratio in the CH₅-selfed family is then, again, either 3:13 or 1:3 (avirulent:virulent), depending on whether CH₅ is heterozygous or homozygous for A_{L^7} .

There is evidence that CH₅ is heterozygous at the A_{L^7} locus. Previous inheritance studies (7,9,17) have shown that the genes for avirulence A_{L^5} , A_{L^6} , and A_{L^7} on Wilden (L^5), and Birio (L^6), and Barnes (L^7), respectively, are closely linked, for they have always segregated as a unit. In agreement with these earlier findings, unit segregation for pathogenicity occurred on Wilden, Birio, and Barnes in the I-selfed family, indicating that strain I is trihybrid $A_{L^5} A_{L^6} A_{L^7}/a_{L^5} a_{L^6} a_{L^7}$ and $i_{L^5} i_{L^6} i_{L^7}$. If strain CH₅ was also trihybrid in coupling phase, $A_{L^5} A_{L^6} A_{L^7}/a_{L^5} a_{L^6} a_{L^7}$, then unit segregation would be expected and it did occur on Wilden and Birio in the CH₅-selfed and CH₅ × I families (Tables 1,3, and 4). This unit segregation would not be expected to extend to Barnes because CH₅ is heterozygous $I_{L^7} i_{L^7}$. Some progeny of CH₅ possessing the $A_{L^5} A_{L^6} A_{L^7}$ group of genes also will possess I_{L^7} and thus will be virulent on Barnes, even though they are avirulent on Wilden and Birio. However, a relationship would still be expected between the segregation on Wilden-Birio with that on Barnes in the CH₅-selfed and CH₅ × I families since, if CH₅ is trihybrid $A_{L^5} A_{L^6} A_{L^7}/a_{L^5} a_{L^6} a_{L^7}$

a_{L^7} , progeny virulent on Wilden (L^5) and Birio (L^6) ($a_{L^5} a_{L^6} / a_{L^5} a_{L^6}$) also will be $a_{L^7} a_{L^7}$ (assuming no recombination) and therefore will be virulent on Barnes (L^7). Since this association was observed without exception in both the CH₅-selfed and CH₅ × I families (Tables 3 and 4) it is concluded that CH₅ is heterozygous for A_{L^7} .

If CH₅ is heterozygous at both the A_{L^7} and I_{L^7} loci, a 3:13 ratio of avirulent to virulent progeny on Barnes would be expected in the CH₅-selfed family, assuming that the genes at these two loci assort independently. The observed ratio, 22:58 (Table 1), differs significantly ($P=0.05$) from a 3:13 ratio ($\chi^2=4.02$, $P=0.02-0.05$). This may indicate linkage between the A_{L^7} and I_{L^7} loci. If there is linkage then (assuming the avirulent parent, H, contributed $i_{L^7} A_{L^7}$), CH₅ would be in repulsion phase, $i_{L^7} A_{L^7} / I_{L^7} a_{L^7}$, which would lead to an excess of avirulent progeny as was observed. However, because the putative linkage has only a small effect on the expected values in the CH₅-selfed family (3 of 16 progeny avirulent if independent assortment and 1 of 4 progeny avirulent if complete linkage in repulsion), and since the observed result is only marginally different from the expected based on independent assortment, for the purpose of calculating expected ratios in the remainder of this discussion, the I_{L^7} and A_{L^7} loci are conservatively assumed to assort independently.

Progeny virulent on Barnes in the CH₅ × I family were of two kinds (Table 1). The first of these, a group of 12, were fully virulent on Barnes; these were the same 12 that were virulent on Wilden and Birio. The other, a group of eight, although still scored as + in most cases, showed slightly less than full vigor. Individuals in this group were all avirulent on Wilden and Birio. (The same difference occurred on L^x , only more markedly. The 12 were fully virulent, but the eight were only semivirulent, giving a +/− or +/−(−) reaction.)

This observation suggests that in this family the interaction of A_{L^7} and I_{L^7} determines a lower degree of virulence on Barnes (L^7) than that determined by homozygosity for the recessive virulence allele, a_{L^7} . This conclusion follows because, given that the genotypes of strains CH₅ and I are $I_{L^7} i_{L^7}$; $A_{L^5} A_{L^6} A_{L^7} / a_{L^5} a_{L^6} a_{L^7}$ and $i_{L^7} i_{L^7}$; $A_{L^5} A_{L^6} A_{L^7} / a_{L^5} a_{L^6} a_{L^7}$, respectively, and assuming no recombination between $A_{L^5} A_{L^6} A_{L^7}$, then the 12 progeny fully virulent on Barnes (L^7) will all be $a_{L^7} a_{L^7}$, since they are virulent on Wilden (L^5) and Birio (L^6) and therefore must be $a_{L^5} a_{L^6} / a_{L^5} a_{L^6}$. In contrast, the eight progeny showing slightly less than full virulence on Barnes most likely will each possess an A_{L^7} gene with its expression inhibited by an I_{L^7} gene; since these eight progeny are all avirulent on Wilden and Birio, indicating that they possess $A_{L^5} A_{L^6}$, and therefore it is most likely that they possess A_{L^7} as well, given the close linkage of the $A_{L^5} A_{L^6} A_{L^7}$ genes.

In the CH₅-selfed family, occasional individuals were scored as having slightly less than full virulence on Barnes and L^x , and these were also avirulent on Wilden and Birio. Other individuals, however, although avirulent on Wilden and Birio, were scored as fully virulent on Barnes and L^x . Thus, the difference between virulence determined by $a_{L^7} a_{L^7}$ and that determined by the $I_{L^7} A_{L^7}$ interaction apparently was very minor in this family.

Pathogenicity on Bison¹⁴ × Burke. Strain CH₅ is virulent on B¹⁴ × Burke (L^1) and the progeny obtained from self-fertilization of CH₅ segregated six avirulent to 74 virulent (Table 1). This observation can be accounted for by postulating, first, that the genotype of CH₅ is $I_{L^1} i_{L^1} A_{L^1} a_{L^1}$, in which the dominant inhibitor gene I_{L^1} interacts with A_{L^1} to give a virulent phenotype and, second, that A_{L^1} is not expressed as a dominant gene in these progeny, so that the six progeny avirulent on B¹⁴ × Burke (like the H parent of CH₅) represent the 1 in 16 progeny ratio expected to be of genotype $i_{L^1} i_{L^1} A_{L^1} A_{L^1}$ and that the 2 in 16 progeny of genotype $i_{L^1} i_{L^1} A_{L^1} a_{L^1}$ were scored as virulent. This second assumption is supported by results of a study by Flor (2) in which he accounted for the segregation for pathogenicity on Buda ($L^1 M^2$) by postulating that heterozygosity at the A_{L^1} locus gives an intermediate reaction. In the present study, of the 74 progeny virulent on B¹⁴ × Burke, eight were scored as slightly less than fully virulent. These may be the individuals of genotype $i_{L^1} i_{L^1} A_{L^1} a_{L^1}$.

However, A_{L^1} apparently does not always display a lack of dominance; self-fertilization of strain I, which gives a − to +/− reaction on B¹⁴ × Burke, gave 18 “avirulent” progeny (range — to

+/−) to nine fully virulent progeny (Table 1). This fits a 3:1 ratio ($\chi^2=1.00$, $P=0.30-0.50$) suggesting that the genotype of strain I is $i_{L^1} i_{L^1} A_{L^1} a_{L^1}$ and that A_{L^1} is at least partially dominant in this family. Flor (5) and Statler and Zimmer (18) also obtained ratios of three avirulent to one virulent on L^1 hosts. Thus, background genes in the rust fungus may influence the expression of A_{L^1} on L^1 hosts, or, alternatively, there may be different forms of the A_{L^1} gene.

In the CH₅ × I family, 11 “avirulent” progeny (range from — to +/−(+)) were obtained on B¹⁴ × Burke to 21 fully virulent progeny (Table 1). This result fits the 3:5 ratio ($\chi^2=0.13$, $P=0.70-0.80$) which was expected on the assumption that the genotype of CH₅ is $I_{L^1} i_{L^1} A_{L^1} a_{L^1}$, the genotype of I is $i_{L^1} i_{L^1} A_{L^1} a_{L^1}$ and A_{L^1} is at least partially dominant.

Pathogenicity on the Bolley Golden selection. All selfed progeny of CH₅ were virulent on the Bolley Golden selection (L^{10}), whereas selfed progeny of strain I were all avirulent. However, progeny from intercrosses between CH₅ and I showed segregation of 18 avirulent to 14 virulent progeny (Table 1). Thus, the parent strains, even though showing no segregation when selfed, could not be homozygous for genes controlling pathogenicity on the Bolley Golden selection. These results can be explained by assigning genotypes $I_{L^{10}} i_{L^{10}} a_{L^{10}} a_{L^{10}}$ to strain CH₅ and $i_{L^{10}} i_{L^{10}} A_{L^{10}} A_{L^{10}}$ to strain I, where $A_{L^{10}}$ leads to an avirulent reaction on the Bolley Golden selection unless $I_{L^{10}}$ also is present. On this basis, no segregation for pathogenicity is expected within each of the selfed families, but, on inter-crossing strains CH₅ and I, half the progeny should be avirulent (genotype $i_{L^{10}} i_{L^{10}} A_{L^{10}} a_{L^{10}}$) and the other half virulent (genotype $I_{L^{10}} i_{L^{10}} A_{L^{10}} a_{L^{10}}$): the observed segregation of 18:14 fits this expectation ($\chi^2=0.50$, $P=0.30-0.50$).

If the genotype of CH₅ is $I_{L^{10}} i_{L^{10}} a_{L^{10}} a_{L^{10}}$, then the strain H parent of CH₅ must have contributed $a_{L^{10}}$ to CH₅. Therefore, since strain H is avirulent on Bolley Golden selection, it must be heterozygous $A_{L^{10}} a_{L^{10}}$: segregation among progeny obtained by self-fertilization of strain H has confirmed this.

In addition to strain CH₅, four other hybrid strains were produced by inter-crossing strains C and H, namely CH₄, CH₆, CH₇, and CH₈. Two of the five strains, CH₅ and CH₆, are fully virulent on the Bolley Golden selection. These strains presumably have the genotype $I_{L^{10}} i_{L^{10}} a_{L^{10}} a_{L^{10}}$. The remaining three hybrid strains however, showed less than full virulence and were scored as + to +/−. Presumably these received the $A_{L^{10}}$ gene from strain H and thus have the genotype $I_{L^{10}} i_{L^{10}} A_{L^{10}} a_{L^{10}}$. This is the same genotype proposed above for the “virulent” progeny in the CH₅ × I family, which also showed slightly less than full virulence on the Bolley Golden selection. These observations suggest that the interaction of $I_{L^{10}}$ and $A_{L^{10}}$ determined a lower degree of virulence on the Bolley Golden selection than that determined by homozygosity for the recessive virulence allele $a_{L^{10}}$.

Linkage of inhibitor genes. The postulated genotypes of strains CH₅ and I with respect to the genes determining pathogenicity on Williston Brown, Barnes, B¹⁴ × Burke, and the Bolley Golden selection are shown in Table 5. The segregations that occurred on Williston Brown, Barnes, and B¹⁴ × Burke in the CH₅-selfed family were not independent (Table 2). Also, the segregations that occurred on Williston Brown, Barnes, B¹⁴ × Burke, and the Bolley Golden selection in the CH₅ × I family were, with one exception, not mutually independent (Table 2). The exception, the association between the segregations on Barnes and B¹⁴ × Burke (Table 2), was however, close to the borderline of statistical significance ($\chi^2=$

TABLE 5. Postulated genotypes of *Melampsora lini* strains CH₅ and I with respect to the genes determining pathogenicity on flax cultivars Williston Brown, Barnes, B¹⁴ × Burke, and the Bolley Golden selection

Cultivar	Genotype of strain	
	CH ₅	I
Williston Brown (M^1)	$I_{M^1} i_{M^1} A_{M^1} a_{M^1}$	$I_{M^1} i_{M^1} A_{M^1} a_{M^1}$
Barnes (L^7)	$I_{L^7} i_{L^7} A_{L^7} a_{L^7}$	$i_{L^7} i_{L^7} A_{L^7} a_{L^7}$
B ¹⁴ × Burke (L^1)	$I_{L^1} i_{L^1} A_{L^1} a_{L^1}$	$i_{L^1} i_{L^1} A_{L^1} a_{L^1}$
Bolley Golden selection (L^{10})	$I_{L^{10}} i_{L^{10}} a_{L^{10}} a_{L^{10}}$	$i_{L^{10}} i_{L^{10}} A_{L^{10}} A_{L^{10}}$

3.33, $P = 0.05-0.1$). Data for the joint segregations on these cultivars can be extracted from Tables 3 and 4.

All the significant associations just referred to can be accounted for by postulating that the four inhibitor genes in CH₅ are linked in coupling phase, $I_{M^1}I_{L^1}I_{L^2}I_{L^3}/i_{M^1}i_{L^1}i_{L^2}i_{L^3}$ (order arbitrary). Because of the small family sizes, precise estimates of the recombination fractions between these genes cannot be obtained. However, the data are consistent with the assumption of complete linkage between all four inhibitor genes.

Thus, in the CH₅-selfed family, the observed numbers for the joint segregation classes on Williston Brown, Barnes, and B¹⁴ × Burke, considered in paired combinations, do not differ significantly from the expected numbers calculated on the assumption of complete linkage between I_{M^1} , I_{L^2} , and I_{L^1} (Tables 6 and 7). An estimate of the recombination fraction (r) between I_{M^1} and I_{L^2} does not differ significantly from zero, while leaving an upper limit at significance ($P=0.05$) of relatively loose linkage (see Table 6). Estimates of the recombination fractions between I_{M^1} and I_{L^1} and between I_{L^2} and I_{L^1} have not been made, because these would be expected to have similarly wide limits.

TABLE 6. Comparison of observed numbers for joint segregation classes on flax cultivars Williston Brown and Barnes in the CH₅-selfed family with expected numbers calculated on the assumption of no recombination between I_{M^1} and I_{L^2}

Postulated genotype of CH ₅ :	Joint segregation classes			
	$\frac{I_{M^1}I_{L^2}}{i_{M^1}i_{L^2}}$	$\frac{A_{M^1}}{a_{M^1}}$	$\frac{A_{L^1}}{a_{L^1}}$	
Williston Brown (M^1)	+	+	-	-
Barnes (L^2)	+	-	+	-
Expected proportions ²	$49-18r+9r^2$	$3+18r-9r^2$	$3+18r-9r^2$	$9-18r+9r^2$
	64	64	64	64
Expected numbers ($r=0$)	61.25	3.75	3.75	11.25
Observed numbers	56	6	2	16

$\chi^2 = 4.62$, $P = 0.05-0.10$ for two degrees of freedom (it is assumed that $r=0$ is the best-fit value of r as calculated from the observed data, therefore an additional degree of freedom is lost).

²The letter r = recombination fraction between I_{M^1} and I_{L^2} . An estimate of r obtained by applying maximum likelihood method to joint segregation data on Williston Brown and Barnes gives $r = -1.19\%$ with standard error, 5.40%. A negative recombination fraction is, of course, not possible: The two linked genes both interact with second, but different, genes to determine the phenotypes being scored, and, in such a circumstance, it is possible that if the true value of r is zero or a small positive value, that chance deviations will give observed numbers for the various phenotypic classes for which the best-fit value of r is a negative number. The present data do not exclude positive r values, as the upper limit of the r estimate is clearly greater than zero, even though the standard error estimate (5.40%) may not be meaningful since the maximum likelihood method assumes a normal distribution and distributions centered near zero are commonly skewed.

TABLE 8. Results of studies by Flor (5,8,9) in which *Melampsora lini* progeny obtained by self-fertilizing parent strains were tested for pathogenicity on flax differential cultivars Towner, Williston Brown, and Victory A

Cultivar	Proposed resistance gene(s)	Pathogenicity reported in:								
		1955 (5)			1960 (8)			1965 (9)		
		Parent ^w	Progeny		Parent ^x	Progeny		Parent ^y	Progeny	
		Avir.	Vir.		Avir.	Vir.		Avir.	Vir.	
Towner	$L^8 + M^4$	-	45	16	-	198	0	-	72	18
Williston Brown	M^1	+	0	67	+	0	198	-	61	29 ^z
Victory A	$M^4 + M^1$	+	0	67	+	154	44	-	61	29

^w"Race" 6 × "race" 22 hybrid: proposed genotype $A_{L^8}a_{L^8}a_{M^4}a_{M^4}a_{M^1}a_{M^1}$ (or if $A_{M^1}-$, then $I_{M^1}I_{M^1}$).

^x"Race" 1: proposed genotype $A_{L^8}A_{L^8}A_{M^4}a_{M^4}a_{M^1}a_{M^1}$ (or if $A_{M^1}-$, then $I_{M^1}I_{M^1}$).

^y"Race" 171 × "race" 228 hybrid: proposed genotype $A_{L^8}a_{L^8}a_{M^4}a_{M^4}A_{M^1}a_{M^1}i_{M^1}i_{M^1}$.

^zUnit segregation with that on Victory A.

Segregation for pathogenicity on Bolley Golden selection (L^{10}) did not occur in the CH₅-selfed family; thus, this family does not provide an opportunity to examine any possible linkage between $I_{L^{10}}$ and the other inhibitor genes. However, in the CH₅ × I family, segregation did occur on the Bolley Golden selection, and was significantly associated with the segregations on Williston Brown, Barnes, and B¹⁴ × Burke (Table 2). The assumption that $I_{L^{10}}$ is linked in coupling arrangement with I_{M^1} , I_{L^2} , and I_{L^1} in CH₅ accounts for these observations, and the data are consistent with the assumption of complete linkage between $I_{L^{10}}$ and I_{M^1} , I_{L^2} , and I_{L^1} . Thus, assuming no recombination, individuals in the CH₅ × I family that are virulent on the Bolley Golden selection because they received $I_{L^{10}}$ from the CH₅ parent, also should possess genes I_{M^1} , I_{L^2} , and I_{L^1} , and thus should be virulent on Williston Brown (M^1), Barnes (L^2), and B¹⁴ × Burke (L^1) as well. This result was obtained

TABLE 7. Comparison of observed numbers for joint segregation classes on Williston Brown and B¹⁴ × Burke and on Barnes and B¹⁴ × Burke in the CH₅-selfed family with expected numbers calculated on the assumptions that A_{L^1} is not expressed as a dominant and that no recombination occurs between I_{M^1} , I_{L^1} , and I_{L^2}

Postulated genotype of CH ₅ :	Joint segregation classes			
	$\frac{I_{M^1}I_{L^1}I_{L^2}}{i_{M^1}i_{L^1}i_{L^2}}$	$\frac{A_{M^1}}{a_{M^1}}$	$\frac{A_{L^1}}{a_{L^1}}$	$\frac{A_{L^2}}{a_{L^2}}$
W. Brown (M^1) or Barnes (L^2)	+	+	-	-
B ¹⁴ × Burke (L^1)	+	-	+	-
Expected proportions ^y	$51-6r+3r^2$	$1+6r-3r^2$	$9+6r-3r^2$	$3-6r+3r^2$
	64	64	64	64
Expected numbers ($r=0$)	63.75	1.25	11.25	3.75
Observed numbers for joint segregation on Williston Brown and B ¹⁴ × Burke	61	1	13	5
Observed numbers for joint segregation on Barnes and B ¹⁴ × Burke	58	0	16	6

Testing goodness-of-fit

(i) Joint segregation on Williston Brown and B¹⁴ × Burke

$$\chi^2 = 0.59 \text{ (two smallest classes combined)}$$

$$P = 0.30-0.50 \text{ for one degree of freedom}^2$$

(ii) Joint segregation on Barnes and B¹⁴ × Burke

$$\chi^2 = 2.72 \text{ (two smallest classes combined)}$$

$$P = 0.05-0.10 \text{ for one degree of freedom}^2$$

^yThe letter r = recombination fraction between I_{M^1} and I_{L^1} for joint segregation on Williston Brown and B¹⁴ × Burke and between I_{L^2} and I_{L^1} for joint segregation on Barnes and B¹⁴ × Burke.

^zIt is assumed that $r=0$ is the best-fit value of r as calculated from the observed values; thus, an additional degree of freedom is lost.

(Table 4).

However, some difficulty was experienced in classifying the reactions on the Bolley Golden selection in this family. The "avirulent" individuals were not fully avirulent, but generally gave a $-$ to $+/-$ reaction with small, pinhead-sized pustules forming on both the upper and lower leaves. The "virulent" individuals generally showed slightly less than full virulence, usually being classified as $+$ to $+/-$. The difference between these levels of virulence was sufficient for most progeny to be classified without difficulty, provided the final scoring was done 14–16 days after inoculation. Three progeny, however, gave intermediate reactions, being scored as $+/-$, and these have all been placed in the avirulent class. If any of these progeny properly belongs to the virulent class, then the conclusion of no recombination between $I_{L^{10}}$ and $I_{M^1} I_{L^7} I_{L^1}$ is in error. The observed numbers for the joint segregation classes on Williston Brown (M^1), Barnes (L^7), and $B^{14} \times$ Burke (L^1) in the $CH_5 \times I$ family, considered in paired combinations, also are consistent with the assumption of complete linkage between I_{M^1} , I_{L^7} , and I_{L^1} in CH_5 . However, these comparisons are not shown in detail, since detailed comparisons have been given earlier for the CH_5 -selfed family (Tables 6 and 7).

Pathogenicity on Towner and Victory A. In this section two associated segregations will be considered: the unit segregation for pathogenicity that occurred on Towner and Victory A in all three families (Table 1) and an association between the unit segregation on Towner-Victory A and the segregation on Williston Brown. These results, together with those obtained by Flor in studies of the inheritance of pathogenicity on Towner, Victory A, and Williston Brown (Table 8), can be accounted for if the following assumptions are made: that Victory A possesses the Williston Brown gene, M^1 , in addition to the M^4 gene; that Towner possesses the M^4 gene in addition to L^8 ; and that the A_{M^1} and A_{M^4} loci in the pathogen are linked.

The first assumption, that Victory A possesses both M^4 and M^1 , was proposed by Person (15) to explain the observation that no rust strains are known that are avirulent on Williston Brown and virulent on Victory A, although strains with reverse pathogenicity are known. Flor ([9]; see Table 8) observed unit segregation for pathogenicity on Victory A and Williston Brown among F_2 progeny from the cross, "race" 171 \times "race" 228. This observation supports Person's suggestion that Victory A also possesses the Williston Brown gene, M^1 , because a common gene can be a cause of unit segregation for pathogenicity.

If Victory A does possess both M^1 and M^4 , then it is expected that segregants avirulent on Williston Brown (M^1) also will be avirulent on Victory A. This was observed without exception in all three families (see Table 3 for CH_5 -selfed family). However, due to the small size of the families, absence of cultures avirulent on Williston Brown and virulent on Victory A did not result in a significant association between the segregations on these cultivars.

A second relationship also occurs between the segregations on Victory A and Williston Brown in the CH_5 -selfed family, which is revealed when the segregation on Barnes also is taken into account (Table 3). Progeny of particular interest are the six that are avirulent on Barnes and virulent on Williston Brown (see Table 3, columns 2 and 4). Being avirulent on Barnes (L^7), these individuals must have the genotype $i_{L^7} i_{L^7} A_{L^7} -$. Assuming no recombination between the I_{L^7} and I_{M^1} loci, then these individuals will be $i_{M^1} i_{M^1}$. Hence, to be virulent on Williston Brown (M^1) they must be $a_{M^1} a_{M^1}$. The point of interest is that these six individuals of presumed genotype $i_{M^1} i_{M^1} a_{M^1} a_{M^1}$ are, without exception, all virulent on Victory A and therefore must be $a_{M^4} a_{M^4}$. This suggests that the A_{M^1} and A_{M^4} loci are linked, because if they are not, the probability of all six being virulent on Victory A is very low ($P = (1/4)^6 = 0.000244$). The association cannot be due to linkage between the A_{M^4} and I_{M^1} loci because, on the model developed above, a_{M^4} comes from one parent of CH_5 (strain C) and i_{M^1} comes from the other parent (strain H).

Although these data indicate linkage between the A_{M^4} and A_{M^1} loci, they are inefficient for estimating the strength of this linkage. A simple estimate is that the six individuals of presumed genotype $i_{M^1} i_{M^1} a_{M^1} a_{M^1}$, which are also $a_{M^4} a_{M^4}$, represent a sample of 12

chromosomes all of which are nonrecombinant. This observed recombination fraction of 0% in a total of 12 has an upper limit of 26.5% at $P = 0.05$.

However, the assumption that the true value lies near 0% is supported in two ways. First, when the segregation on Barnes is also taken into account, the relationship in all three families between the segregations on Williston Brown and Victory A is consistent with the assumption of no recombination between A_{M^1} and A_{M^4} (significance tests not shown here). Second, the assumption of no recombination in these studies between A_{M^1} and A_{M^4} suggests an explanation for the unit segregation observed on Victory A and Towner in all three families (Table 1). If strains CH_5 and I are each $A_{M^1} A_{M^4} / a_{M^1} a_{M^4}$ and if no recombination occurs between A_{M^1} and A_{M^4} , the segregation for pathogenicity among the progeny of these strains that occurs on a cultivar possessing both M^4 and M^1 will be identical to that which occurs on a cultivar possessing M^4 alone; for strains possessing $a_{M^1} a_{M^4} / a_{M^1} a_{M^4}$ will be virulent on both cultivars and strains possessing $A_{M^1} A_{M^4} / -$ will be avirulent on both, irrespective of whether I_{M^1} is present or absent. Thus, an explanation to account for the unit segregation observed on Victory A and Towner is that Towner possesses, in addition to L^8 , the M^4 gene in Victory A, and that strains CH_5 and I both recognize M^4 , but not L^8 . The assumption that Towner possesses $L^8 + M^4$ is compatible with the results of Flor's studies as shown in Table 8. The possibility that Towner possesses both the Victory A genes ($M^4 + M^1$) in addition to L^8 , although consistent with the results of the present studies, is excluded by Flor's (9) study (see Table 8) in which unit segregation occurred on Williston Brown (M^1) and Victory A (presumably due to common gene M^1) and this segregation was independent of the segregation that occurred on Towner. The segregations would not be expected to be independent if Towner also possessed M^1 . Thus, the genotype of the F_1 strain in this study by Flor ([9]; see Table 8) presumably was $A_{L^8} a_{L^8}; A_{M^1} a_{M^1} / a_{M^4} a_{M^4}$. If this genotype is accepted, then the observation in Flor's study that the segregation on Towner was independent of that on Williston Brown-Victory A indicates that the A_{L^8} locus is not linked to the A_{M^1} locus and thus (assuming that A_{M^1} is closely linked to A_{M^4}) the possibility that the unit segregation observed on Towner and Victory A in the present studies is due to close linkage of the A_{L^8} and A_{M^4} loci can be excluded. The one remaining possibility that could account for the unit segregation on Towner and Victory A is that Victory A possesses the Towner gene, L^8 . This possibility, too, can be excluded, however, because rust fungus strains are known that are avirulent on Towner but virulent on Victory A (eg. [5]; see Table 8). Thus, the only explanation that accounts for the unit segregation on Towner and Victory A and is also consistent with Flor's observations, is that Towner possesses L^8 and M^4 .

In summary, therefore, the assumption that Victory A possesses $M^4 + M^1$ accounts for the observation that no rust strains are known that are avirulent on Williston Brown (M^1) and virulent on Victory A. The assumption that Towner possesses $L^8 + M^4$ accounts for the unit segregation on Towner and Victory A (due to common gene, M^4), and the assumption of close linkage between the A_{M^1} and A_{M^4} loci in the pathogen accounts for the association between the segregation on Williston Brown and that on Towner-Victory A observed in the present studies.

To test the assumption that both Towner and Victory A possess the M^4 gene, 199 F_2 progeny from the cross Towner \times Victory A were inoculated with strain CH_5 . All were resistant to CH_5 , which is the expected result if both parents possess M^4 and CH_5 recognizes M^4 as proposed above. In addition, 63 individuals from the cross Hoshangabad \times (Victory A \times Towner) were inoculated with CH_5 and again all were resistant as expected (Hoshangabad is susceptible to CH_5).

Further, that Towner possesses two genes for resistance is shown from the results obtained by inoculating Hoshangabad \times Towner F_2 progeny with strain K (a rust fungus strain not used in the family studies) to which Hoshangabad is susceptible and Towner is resistant. The F_2 progeny segregated in the ratio 143 resistant to four susceptible individuals which fits a dihybrid (15:1) ratio ($\chi^2 = 3.12$, $P = 0.05-0.1$). The same F_2 plants tested with CH_5 gave a

monohybrid ratio (110 resistant to 37 susceptible), suggesting that CH₅ recognizes only one gene in Towner. Strain K (collected from *Linum marginale*, a native Australian species) was selected for use in this test because it is avirulent on all flax differentials except Bombay and Akmolinsk, and therefore it was likely that if Towner did possess two resistance genes this strain would recognize both of them.

Strain K cannot similarly be used to demonstrate that Victory A possesses two genes, because the two genes proposed for Victory A, M^1 and M^4 , would be closely linked. Thus, a 3:1 F₂ ratio would be expected from the cross Hoshangabad × Victory A, irrespective of whether the tester strain recognized either both or only one of genes M^1 and M^4 . Tests of 147 Hoshangabad × Victory A F₂ progeny with strain K, and then with strain CH₅, both yielded monohybrid ratios: 109 individuals were resistant to both strains and 38 were susceptible to both.

Thus, the results of these host-segregation studies are consistent with the assumptions made to account for the results of the rust fungus inheritance studies. However, if these assumptions are correct, one additional assumption becomes necessary. This follows from the proposal above that the genotype of strain CH₅ with respect to the pathogenicity genes corresponding to the Towner genes for resistance (L^8 and M^4) is $a_{L^8}a_{L^8}A_{M^4}a_{M^4}$. Since the strain C parent of CH₅ is virulent, and the strain H parent is avirulent on Towner, the H parent (that is, Flor's "race" 228) must have contributed A_{M^4} and a_{L^8} to CH₅.

In Flor's (9) study of the segregation for pathogenicity among the F₂ progeny from the cross, "race" 171 × "race" 228 (see Table 8), the segregation on Towner was independent of the unit segregation that occurred on Williston Brown and Victory A. Therefore, it was postulated above that the genotype of the F₁ strain in this study by Flor was $A_{L^8}a_{L^8}A_{M^4}a_{M^4}$. Again, since "race" 228 was the avirulent parent of the F₁ strain, it is necessary to postulate that "race" 228 contributed A_{L^8} and a_{M^4} . Thus, "race" 228 apparently contributed a_{L^8} and A_{M^4} in one study and A_{L^8} and a_{M^4} in another. The possibility that "race" 228 is heterozygous at both loci is unlikely, because Flor (9) produced 58 progeny by self-fertilization of the "race" 228 used in the cross to "race" 171 and all were avirulent on Towner.

Thus, the assumption that the "race" 228 used in the present studies (strain H), while phenotypically identical to that used by Flor (9), is genotypically different seems most likely. This assumption is supported by Flor's (9) finding that progeny obtained by self-fertilization of "race" 228 segregated for pathogenicity on Wilden and Barnes, whereas those obtained by self-fertilization of the "race" 228 used as a parent of strain CH₅ (strain H) did not segregate on Wilden and Barnes, but did segregate on Bombay, Pale Blue Crimped, Kenya, and the Bolley Golden selection.

It was pointed out above that the segregation on Williston Brown (M^1) in the CH₅-selfed family, because it fitted both a 1:3 and a 3:1 ratio of avirulent to virulent, was consistent with CH₅ being homozygous dominant or heterozygous at the A_{M^1} locus. For the purposes of working out the recombination fraction between I_{M^1} and I_{L^7} however (see Table 6), it was assumed that CH₅ is heterozygous at the A_{M^1} locus: the observation in the CH₅-selfed family that cultures avirulent on Barnes (L^7) but virulent on Williston Brown are all virulent on Victory A and Towner (Table 3) supports this assumption because, as argued above, the association can be accounted for if CH₅ is heterozygous at both the A_{M^1} and A_{M^4} loci and these are linked. There is no ready explanation for this association if CH₅ is assumed to be homozygous dominant at the A_{M^1} locus.

If CH₅ is $A_{M^1}A_{M^4}/a_{M^1}a_{M^4}$, and assuming the linkage is close, then segregants virulent on Victory A and Towner ($a_{M^1}a_{M^4}$) will be $a_{M^1}a_{M^4}$ (assuming no recombination). Therefore, an opportunity exists to compare the virulence phenotype on Williston Brown (M^1) determined by $a_{M^1}a_{M^4}$ with that determined by the $I_{M^1}-A_{M^1}$ interaction. In the CH₅-selfed family little variation was expressed in virulence on Williston Brown. However, a few segregants were classified as having slightly restricted virulence on Williston Brown and these were all avirulent on Victory A and Towner, suggesting

that the $I_{M^1}-A_{M^1}$ interaction may not always give full virulence on Williston Brown in the CH₅-selfed family.

In the strain I-selfed family however, variation in the degree of virulence on Williston Brown was more evident; the nine segregants virulent on Victory A and Towner were scored as fully virulent on Williston Brown, while the 18 segregants avirulent on Victory A and Towner were, with three exceptions, all scored as having restricted virulence or avirulence on that host. These observations support the earlier assumption that strain I is heterozygous at the A_{M^1} locus, and suggest that in the I-selfed family $a_{M^1}a_{M^4}$ commonly gives a slightly more virulent phenotype than does the $I_{M^1}-A_{M^1}$ interaction. Three of the I-selfed progeny avirulent on Victory A and Towner (the exceptions noted above) were classified as fully virulent on Williston Brown. These may have resulted from environmental variability, differences in genetic background, errors in observation, or recombination between the two loci, A_{M^1} and A_{M^4} .

DISCUSSION

Results of the study reported here support those of studies by Flor (3,9) which indicate that two allelic gene pairs in the rust fungus interact to determine pathogenicity on the M^1 resistance gene and further suggest that two allelic gene pairs are similarly involved in determining pathogenicity on the L^1 , L^7 , and L^{10} resistance genes. In choosing to refer to these gene pairs as the avirulence/virulence gene pair (A/a) and the inhibitor gene pair (I/i), the nomenclature established by Shepherd (17) has been retained. Use of the term "inhibitor" gene, however, was not intended to imply any biochemical mode of action of this gene, but merely describes the phenotypic observation that A does not result in an avirulent reaction when I is present.

Our data are consistent with the assumption of complete linkage between all four inhibitor genes. The possibility that all four are the same gene was excluded because strain CH₅ is heterozygous for all four, $I_{M^1}I_{L^1}I_{L^7}I_{L^{10}}/i_{M^1}i_{L^1}i_{L^7}i_{L^{10}}$, whereas strain I is heterozygous for one and homozygous recessive for the other three, $I_{M^1}i_{L^1}i_{L^7}i_{L^{10}}/i_{M^1}i_{L^1}i_{L^7}i_{L^{10}}$. However, the possibility that I_{L^1} , I_{L^7} , and $I_{L^{10}}$ are the same gene was not excluded. If they are the same gene, then this is an exception to the general finding that genes determining pathogenicity in isolates of the flax rust fungus interact with only one resistance gene in the host. It is evident that further studies are needed to elucidate the relationships between the inhibitor genes. If I_{L^1} , I_{L^7} , and $I_{L^{10}}$ can be recombined, that would be evidence that they are not the same gene. Also, if it can be established that I_{M^1} is tightly linked to the other inhibitor genes, that would be an indication of a possible relationship between the reactions involving "M" genes and those involving "L" genes.

Virulence determined by the $I-A$ interaction was not always as vigorous as that determined by homozygosity for the recessive virulence allele. Summarizing the observations, the $I_{L^1}-A_{L^1}$ interaction gave full virulence on B¹⁴ × Burke (L^1) in both the CH₅-selfed and CH₅ × I families. The $I_{M^1}-A_{M^1}$ and $I_{L^7}-A_{L^7}$ interactions gave full, or virtually full, virulence on Williston Brown (M^1) and Barnes (L^7), respectively, in the CH₅-selfed family but gave less than full virulence in the CH₅ × I family. The $I_{L^{10}}-A_{L^{10}}$ interaction also gave less than full virulence on Bolley Golden selection (L^{10}) in the CH₅ × I family. The restricted virulence shown by three of the interactions in the CH₅ × I family could be due to temperature and daylength effects, because the CH₅ × I progeny were tested in early summer, whereas nearly all the CH₅-selfed progeny were tested in winter. Alternatively, background modifier genes contributed by the strain I parent may have reduced the effectiveness with which the $I-A$ interactions led to a virulent phenotype. However, as the level of virulence scored on Williston Brown, Barnes, and the Bolley Golden selection for the $I-A$ interaction phenotype was never less than + to +/-, it is likely that the $I-A$ interaction would confer effective virulence under field conditions on these host differentials. This is not true, however, of the host cultivar possessing L^x , in which the $I-A$ interaction gave a marked reduction in virulence (as low as +/- to -) in the CH₅ × I family, although in the CH₅-selfed family little or no reduction was observed.

Interactions between genes controlling pathogenicity have been reported in other rust fungus species besides those that cause flax rust. These interactions are of several different kinds. In wheat leaf rust (caused by *Puccinia recondita* f. sp. *tritici*), pathogenicity on the $Lr2^4$ gene, conferring resistance in wheat (*Triticum aestivum*) is determined by an A/a gene pair in which A_- confers avirulence unless a dominant inhibitor gene is present at a second locus (16); this interaction is identical to those proposed above for flax rust. Also, in wheat leaf rust, Haggag et al (13) propose that pathogenicity on the $Lr3$ gene in wheat is determined by an A/a gene pair complementary to $Lr3$ and aa gives a virulent phenotype unless the strain is homozygous recessive at a second locus in which case an avirulent phenotype results. In a study of stem rust of oats (*Puccinia graminis* f. sp. *avenae*) Green and McKenzie (12) reported that pathogenicity on the $Pg2$ (or A) gene conferring resistance in oats (*Avena sativa*) is determined by an A/a gene pair in which A_- confers avirulence unless the strain is homozygous recessive at a second locus, in which case semivirulence occurs. Finally, a fourth kind of interaction has been reported (14) in wheat stem rust (*Puccinia graminis* f. sp. *tritici*): two dominant complementary genes determine virulence on the $Sr1$ resistance gene in wheat; that is, a strain must possess dominant alleles at two loci to be virulent on $Sr1$. The existence of interactions such as these in rust fungus species suggests that caution should be exercised in inferring the genotype of a rust fungus strain from its phenotype. Furthermore, even obtaining progeny from a strain by self-fertilization may not reveal its genotype; this occurred in the present study with respect to the genes controlling the pathogenicity of strain CH₅ on the Bolley Golden selection; the full genotype of this strain was revealed only when it was intercrossed with strain I.

In all three families in this study L^x elicited reactions qualitatively the same as those elicited by L^7 . However, some quantitative differences were noted. Avirulent reactions on L^x always were fully avirulent (scored —) whereas avirulent reactions on L^7 were often, but not always, leaky (— or — to +/—). Also, in the CH₅ × I family, the $I-A$ interaction conferred a higher level of virulence on L^7 than on L^x . If these quantitative differences are a reflection of the different genetic backgrounds of L^7 and L^x then, from the results of this study, these two genes could be the same gene. Host genetic background can give rise to such quantitative differences. However, it remains a possibility that L^7 and L^x are different genes interacting with different pathogen genes that are closely linked. An additional possibility is that L^7 and L^x are not identical, but are sufficiently similar to interact with the same genes controlling pathogenicity in the rust.

The avirulence genes A_{L^5} , A_{L^6} , and A_{L^7} (A_{L^x}) segregated as a unit in the I-selfed family as they have in other studies (7,9,17). The finding that A_{L^x} in the pathogen is linked to A_{L^6} is of interest because L^x in the host was derived from L^6 by rare recombination (K. W. Shepherd and G. M. E. Mayo, unpublished). Thus, host genes that may possess some structural similarity, L^6 and L^x , interacted with closely associated genes in the rust fungus. This suggests that the closely linked genes in the rust fungus may also possess structural similarities. Thus, the $L^6-A_{L^6}$ interaction may be similar to the $L^x-A_{L^6}$ interaction. If this is the case, then the finding

that the inhibitor gene is not involved in the $L^6-A_{L^6}$ interaction, but is involved in the $L^x-A_{L^6}$ interaction, suggests that the nature of the recognition that occurs between the products of these genes is very specific.

A final observation of interest is that three of the four avirulence genes that interact with inhibitor genes are linked to other avirulence genes: A_{L^7} (A_{L^x}) is closely linked to A_{L^5} and A_{L^6} (7,9,17), $A_{L^{10}}$ is closely linked to A_{L^3} and A_{L^4} (5,9), and A_{M^1} is linked (possibly closely) to A_{M^2} . The significance of this observation may only be determined when the biochemical basis of the interaction is elucidated.

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