The Development of New Races of Flax Rust to Identify Multiple Gene Combinations in Flax

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New races of flax rust (incited by *Melampsora lini*) in 1973 left only three resistant flax cultivars: Linott, Foster, and Raja (6). Of these, only Linott was commercially acceptable to North Dakota flax producers.

A single mutation for virulence on the L^6 gene would have left growers without a commercially acceptable variety resistant to rust. New varieties with new genes for resistance to *M. lini* were urgently needed.

As early as 1956 Flor (2) established that the probability of a mutation for virulence would be much lower for a variety with two effective genes. Therefore, USDA and NDSU plant breeders and pathologists initiated a program to develop flax varieties with two effective genes, both of which are effective against prevalent races of *M. lini.*

There are two methods of determining whether the proposed genes are still present in a new variety. The first method is to cross the new variety with a susceptible variety, rust-test segregating generations, and determine the genetic composition of the parent by segregation ratios. This method is time consuming, and unless the correct rust cultures are used, genes other than those desired in the cultivar may be incorrectly identified.

The second method is the use of selective pathogenicity to identify resistance genes. Flor (1) proposed that resistance genes could be determined by successively inoculating plants with races that are virulent on all but one of the resistance genes involved in a cross. This method has been used by Flor and Comstock (3) and Zimmer (6) to identify plants carrying multiple genes for resistance. However, races of *M. lini* were not available in 1973 to detect effective two-gene combinations of rust resistance genes L^{11}, M^3, M^6 and P^3. Therefore, we developed a crossing program with the fungus to produce cultures of *M. lini* with selective pathogenicity to identify two or more combinations of resistance genes in flax.

This article reports manipulation of virulence of *M. lini* through hybridization and selfing to produce cultures necessary to identify combinations of effective host genes in flax.

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MATERIALS AND METHODS

Theoretical Planning

After new rust races were reported in 1973, the effective genes for resistance in commercially grown flax varieties were L^6 and genes in Raja and Foster.

The other effective genes available in 1973 were L^{11}, M^3, M^6, P^3, P^4 and Kugine. Because there was a limited number of genes available, the judicious use of combinations in new varieties was necessary. The release of varieties with only one of the above genes would expose this gene to the natural *M. lini* populations and perhaps negate its effectiveness in digenic combinations. This hypothesis is based on the reasoning that when a single-gene resistant cultivar is released it is usually only a matter of time until it is parasitized by a new virulent race.

Fungal Genetics

Virulence is recessive in *M. lini*. If a culture is virulent on a single-gene line, *L* for example, then both alleles must be homozygous recessive (a_La_L). If a culture is avirulent on a single-gene line, the alleles could be homozygous dominant (A_LA_L) or heterozygous (A_La_L). So, when planning crosses, if the desired combination is to be virulent on lines with L^{11}, M^3, and P^3, but avirulent on lines with M^6, then one or both cultures chosen for the cross must be virulent on L^{11}, M^3, and P^3, and at least one culture avirulent on M^6. The F_1 cultures from the crosses must be selfed to obtain cultures with the desired virulence. All crosses were planned in the above manner.

The crosses planned were as follows: 1 × 22, 22 × 97, 22 × 191, and 22 × 218. Races 1, 22, 97, 191, and 218 were also selfed to determine heterozygosity. If these races were heterozygous at many loci, it may be possible to obtain the desired virulence by selfing.

All cultures for crossing were purified by single pustule urediospore isolations and virulence was tested on single gene lines. 'Bison' flax plants, susceptible to all races, were separately inoculated when they were about 15 cm tall. Teliospores, formed when the inoculated plants began to mature, were conditioned to germinate by several alternate freeze-thaw, wet-dry cycles. After several cycles, the telia-laden straw was suspended over seedlings of Bison flax. When pycnia

formed, crosses were made by separately transferring honeydew and pycniospores from a pycnial infection of one race to a pycnial infection of another race (Table 1. X = cross) or to the same race for a self (S) (Fig. 1).

Aeciospores resulting from a cross or self were used to inoculate Bison plants. The resulting urediospore cultures were used to inoculate 29 isogenic flax lines, each with a different gene for resistance. Reactions were classified as resistant or susceptible.

RESULTS AND DISCUSSION

We made 75 crosses to obtain cultures with the desired selective pathogenicity. Several of the desired virulence combinations needed to test two-gene combinations of L^{11}, M^3, M^6 and P^3 were obtained from selfing race 218.

The cultures with selective pathogenicity to identify two-gene combinations in flax are listed in Table 1. For example, to identify host genes M^3P^3 in a flax variety, one culture would be virulent on lines with M^3 but avirulent on lines with P^3 and a second culture virulent on P^3 but avirulent on M^3. Hybrid X36 and the self 218 S48 have this selective pathogenicity. These two hybrids can be used to test for M^3 and P^3 either alone or together in a flax variety. These hybrids are virulent on many other host genes and, therefore, can be used to identify combinations of M^3P^3 even if other ineffective

Table 1. Virulence combinations obtained to test certain two-, three-, or four-gene combinations in flax for resistance to *Melampsora lini.*

	Identification of multi-gene combinations	
To identify combinations with:	Virulence combinations desired	Self or Cross
$L^{11}M^3$	Virulent on L^{11}; not M^3	X 36†
	Virulent on M^3, not L^{11}	X 59
$L^{11}P^3$	Virulent on L^{11}; not P^3	218 X 48
	Virulent on P^3; not L^{11}	X 57
$L^{11}M^6$	Virulent on L^{11}; not M^6	218 S 61
	Virulent on M^6, not L^{11}	X 15
M^3M^6	Virulent on M^3; not M^6	X 57
	Virulent on M^6, not M^3	X 36
M^3P^3	Virulent on M^3; not P^3	218 S 48
	Virulent on P^3, not M^3	X 36
M^6P^3	Virulent on M^6; not P^3	X 15
	Virulent on P^3, not M^6	X 57
$L^{11}M^3M^6$	Virulent on $L^{11}M^3$; not M^6	218 S 61
	Virulent on $L^{11}M^6$, not M^3	X 36
	Virulent on M^3M^6, not L^{11}	X 15
$L^{11}M^6P^3$	Virulent on $L^{11}M^6$; not P^3	218 S 48
	Virulent on $L^{11}P^3$, not M^6	218 S 5
	Virulent on P^3M^6, not L^{11}	X 64
$L^{11}M^3P^3$	Virulent on $L^{11}M^3$; not P^3	218 S 61
	Virulent on $L^{11}P^3$, not M^3	X 36
	Virulent on P^3M^3, not L^{11}	X 59
$M^3M^6P^3$	Virulent on M^3M^6; not P^3	X 15
	Virulent on M^3P^3, not M^6	X 57
	Virulent on M^6P^3, not M^3	218 S 63
$L^{11}M^3M^6P^3$	Virulent on $L^{11}M^3M^6$; not P^3	218 S 48
	Virulent on $L^{11}M^6P^3$, not M^3	X 36
	Virulent on $M^3M^6P^3$, not L^{11}	X 64
	Virulent on $L^{11}M^3P^3$, not M^6	Not available

† X15 (R1 X 22), X36 (R22 X 218), X57 (R22 X97) X59 (R22 X 191), and X64 (R22 X 97).
R = race, X = cross, S = self.

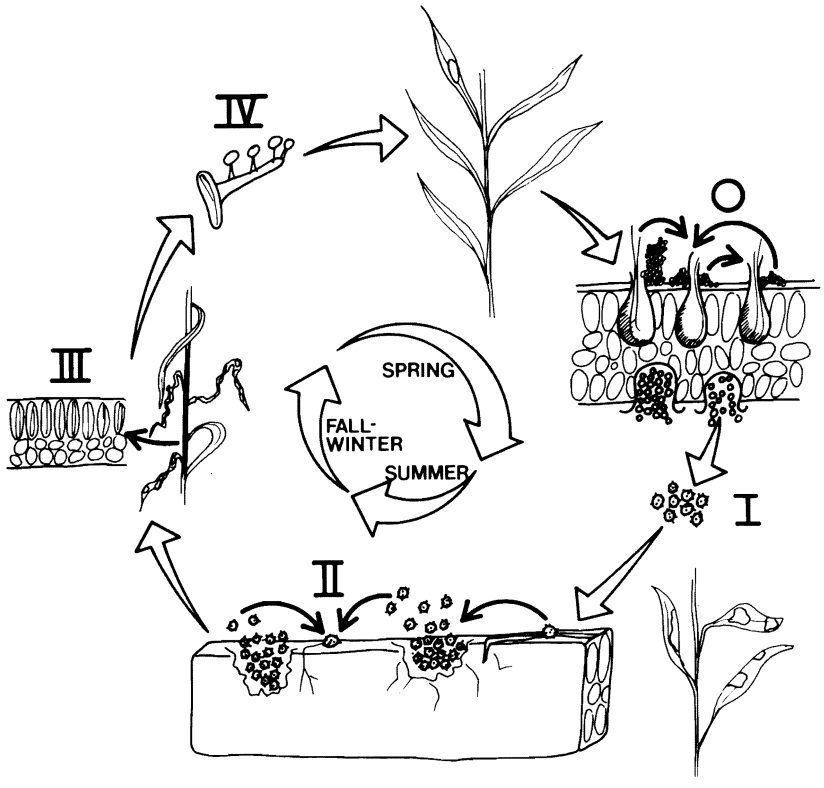

Figure 1. Disease cycle of flax rust.
O. Small light yellow pustules appear on the upper flax leaves. These are called pycnia and contain spores which allow sexual crossing.
I. Aeciospores arise from new pustules on the lower leaf surfaces soon after mating is completed.
II. Aeciospores infect flax. This results in pustules bearing yellow-orange urediospores. This is the summer-recycling stage which can increase rapidly.
III. In the fall urediospores are replaced by dark, thick walled over wintering spores called teliospores.
IV. The teliospores germinate in the spring to produce very delicate, light colored basidiospores which infect the new crop.

resistance genes are present in a flax variety. These cultures are currently being used in NDSU and USDA flax breeding programs. Flor, a variety with these two genes, was released in 1980. The two hybrids developed by this program were used to test for M^3P^3 in the new variety.

Cultures have been developed to identify all three-gene combinations of L^{11}, M^3, M^6 and P^3. All possible combinations of virulence on two of three genes are necessary to identify three-gene combinations of the above genes. The desired virulence combinations are listed in table 1.

Three of the four cultures necessary to test for four-gene combinations have been developed. Unfortunately, a culture virulent on L^{11}, M^3 and P^3 but not M^6 is not available from the crosses or selfs.

This system is effective for identifying genes when the breeder has a knowledge of the genes in his lines and wants to know whether certain gene combinations are still present in segregating populations.

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