Comparative Virulence of Basidiospore and Urediospore Cultures of Three Pathogenic Races of *Melampsora lini*

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Journal Series Paper 1032.
Accepted for publication 15 November 1979.

ABSTRACT

STATLER, G. D. and R. E. GOLD. 1980. Comparative virulence of basidiospore and urediospore cultures of three pathogenic races of *Melampsora lini*. Phytopathology 70:555-557.

The virulence of monokaryotic basidiospore cultures was compared to that of dikaryotic urediospore cultures in three races of *Melampsora lini* on 29 isogenic lines of flax (*Linum usitatissimum*), each with a single gene for resistance. The three races previously had been selfed to determine heterozygosity on the 29 isogenic lines. In most cases, basidiospores were virulent only on host isogenic lines for which they carried the gene for virulence. Exceptions to this general observation included comparisons of race 1 on lines with L^8, M^2, M^4, N, and P^4; race 370 on lines L, L^1, M^2, N^1, and P^4; and race 218 on P^4. Exceptions to the observation that basidiospores infect only host cultivars for which they carried the gene for virulence were of three types: (i) those with urediospore cultures homozygous avirulent and basidiospore cultures virulent on that host line; (ii) those with homozygous virulent urediospore cultures and basidiospores that produced only a few poorly developed pycnia; and (iii) those with urediospore cultures heterozygous for virulence but with avirulent basidiospore cultures. Cultures with urediospore cultures avirulent and basidiospores virulent on an isogenic line (case i) were selfed on that isogenic line; the resulting cultures resembled parental urediospore cultures which were avirulent on that isogenic line on which basidiospores were virulent.

Additional key words: flax rust, fungal genetics, pycnial reaction, urediospore virulence.

The gene-for-gene relationship implies that the pathogenicity genotype of a rust culture can be established by a study of the infection patterns expressed by its selfed cultures on isogenic host lines that contain single genes conditioning rust resistance (6). We have used this relationship in studies of host-parasite genetics of flax (*Linum usitatissimum*) and the flax rust pathogen *Melampsora lini* (Ehrenb.) Lev. (9,10). The pathogenicity of the haploid basidiospore stage, however, has not been studied in great detail (6).

An understanding of cytology of the different stages of *M. lini* was basic to our study which compared the virulence of monokaryotic basidiospore cultures with dikaryotic urediospore cultures. The cytology of *M. lini* has been studied by several workers (1,7) and discussed by Flor (5). The immature teliospores are binucleate or dikaryotic. Either prior to or during teliospore germination, the dikaryotic nuclei fuse and produce a diploid phase. Meiosis soon follows, resulting in four haploid nuclei. Each of these nuclei migrates into one of the four basidiospores (sporidia) that form on the basidium (promycelium). Infection of a susceptible host by a haploid basidiospore results in the formation of pycnia. Typically several pycnia are present in a small cluster, all resulting from infection by one basidiospore. Such pycnia produce hundreds of haploid pycniospores, each of which can function as a "male" gamete. Flor (5) reasoned that since all pycniospores from one pycnium are genetically identical, the fertilization of one pycnium by transfer of pycniospores from a pycnium of complementary mating type is comparable to the fertilization of one ovule by one pollen grain. The resulting dikaryophyte (ie, the aecia, uredia, and telia) is genetically the equivalent of a clone in higher plants, and behaves as a diploid in genetic studies.

Flor (3) reported that the haploid or monokaryotic basidiospores infect only host varieties for which they carried the gene for virulence, as might be expected. Our preliminary observations, however, suggested that this might not always be true. This study was conducted to investigate the possibility that basidiospores of the rusts might infect host cultivars for which the uredial culture of the same race did not have a gene for virulence. *M. lini* is an autoecious rust and therefore is good material for such studies.

0031-949X/80/06055503/$03.00/0

MATERIALS AND METHODS

Urediospore cultures of races 1, 370, and 218 of *M. lini* were purified by two successive single-pustule isolations. Purity was evaluated on near-isogenic lines of flax, each containing a single gene for resistance. Parental reactions are listed in Table 1.

Urediospore cultures of each race were used to inoculate Bison flax plants that were approximately 15 cm tall. Bison is susceptible to all known North American races of *M. lini*. Inoculated plants were held at approximately 100% relative humidity (RH) and 19 C for 18 hr and were incubated in greenhouses at 18 ± 4 C. Teliospores formed several weeks after inoculation and were collected while the plants were still green. They were conditioned to germinate by several alternate freeze-thaw, wet-dry cycles (9). For selfing studies, telia-laden straw was suspended over seedlings of Bison flax. Selfing was conducted by transferring honeydew and pycniospores from one pycnium to another of the same race in a different cluster.

The resulting aeciospores were used to inoculate Bison plants. The urediospore cultures thus produced were used to inoculate 29 isogenic flax lines, each containing a different gene for resistance (Table 1). The reaction type expressed on each line was classified 12–14 days after inoculation on the standard scale of 0–4 (2). Reaction types 0–2 were classified as resistant and types 3 and 4 were classified as susceptible for the genetic analysis. Chi-square tests were used to determine the probabilities of the segregating cultures fitting hypothetical ratios. The inheritance of virulence of races 370 and 218 was reported previously (9,10).

For studies of basidiospore pathogenicity, telia were suspended over plants of all 29 isogenic flax lines, with Bison plants included as a monitor of germination, in chambers held at 100% RH and 19 C for 48 hr prior to incubation in greenhouses at 18 ± 4 C. Teliospores were suspended over each isogenic line five times to insure teliospore germination. Isogenic lines were evaluated as immune (having no visible response), necrotic (producing necrotic flecks), or susceptible (producing well defined pycnia) 10–12 days after inoculation (Table 1). Plants with necrotic flecks and a few poorly developed pycnia were classified as resistant.

RESULTS AND DISCUSSION

Virulence is recessive in *M. lini* and therefore, basidiospores should infect only plants of host lines in which the uredial culture

from the same race is virulent or segregating for virulence (heterozygous). We observed this to be true in most cases when 29 isogenic flax lines were inoculated with three races of *M. lini* (Table 1). Exceptions to this general observation were of two kinds; either basidiospores were virulent on certain host lines even though selfed progeny indicated the original culture was homozygous for avirulence (race 1 on lines with N and P^4), or basidiospores failed to infect certain host lines even though selfed progeny indicated the original culture was homozygous, or heterozygous, for virulence (race 1 on lines with L^8, M^2, M^4; and race 370 on lines with L, L^1, M^2, and N^1). In addition to the above exceptions a few poorly developed pycnia were observed on plants of the line containing P^4 on which urediospores of 370 and 218 were avirulent.

Race 1 and all the urediospore cultures resulting from selfing our culture of race 1 were avirulent on the lines containing N and P^4. This indicated that race 1 was homozygous avirulent on lines with N and P^4. However, basidiospores of race 1 did infect lines with N or P^4. We separately selfed these basidiospore-derived cultures of race 1 on flax lines with N or P^4. The resulting cultures were comparable in virulence formula to other cultures resulting from selfing race 1 on the susceptible cultivar Bison, and they were not virulent on lines with N or P^4. The urediospore culture of race 1 displayed an immune reaction on lines with N or P^4 both before and after selfing on lines with N or P^4. In this study we tested the virulence of urediospore cultures derived from inoculating Bison with aeciospores since Flor (3) had reported that urediospores had the same pathogenicity as aeciospores from which they were derived.

Flor (3) fertilized pycnia of race 22 developed on Ottawa 770B (L) with pycniospores of race 6 (developed on Bison) to which Ottawa 770B was immune. Normal aecia developed, but their aeciospores were incapable of reinfecting Ottawa 770B on which they were borne or any of the other differential cutivars for which race 6 aecia were nourished by the monokaryotic hyphae of race 22 to which Ottawa 770B is a congenial host. This could not be an explanation for our cases in which the urediospore culture was homozygous avirulent, but the basidiospores were virulent on that host line (eg, race 1 on lines with N and P^4). Since all our selfed urediospore cultures of race 1 were avirulent on lines with N and P^4, race 1 was homozygous avirulent on these lines and did not contain a gene for virulence.

Urediospore cultures of races 370 and 218 as well as all the cultures resulting from selfing these two races were avirulent on the isogenic line containing P^4. Separate basidiospore inoculations of both race 370 and 218 on the line with P^4 resulted in resistant reactions with a few poorly developed pycnia. These depauperate pycnia from each race were separately spermatized in the manner previously described. The resulting urediospore cultures had the same virulence as other selfs of race 370 or 218 and did not attack the line with P^4. Both races had an immune reaction on the line with P^4 and after selfing still had an immune reaction on P^4. This is another exception to the concept that basidiospores infect only host cultivars for which they carry the gene for virulence.

Race 1 and all the uredial cultures resulting from selfing race 370 were virulent on the flax line containing M^2; however, basidiospores of race 1 were avirulent on the line with M^2 and only a few poorly developed pycnia were produced. We spermatized these pycnia, but aecia did not form. Thus, we concluded that basidiospores presumably carrying a gene for virulence do not necessarily express the virulence that would be expected of the virulence gene in question. Race 370 and all the uredial cultures resulting from selfing race 370 were virulent on lines containing M^2 and N^1, however, basidiospores of race 370 were avirulent on flax lines containing M^2 and N^1, and only a few poorly developed pycnia were produced. These were spermatized, but

TABLE 1. Parental urediospore reactions, segregation ratios of selfed urediospore cultures, and pycnial reactions for 29 isogenic flax lines inoculated with three races of *Melampsora lini*

	Race 1			Race 370[a]			Race 218[a]		
	Uredial			Uredial			Uredial		
Near isogenic lines with gene:	Parental reaction	Seg. ratio of S_1	Pycnial reaction	Parental reaction	Seg. ratio of S_1	Pycnial reaction	Parental reaction	Seg. ratio of S_1	Pycnial reaction
K	R[b]	HA[b]	I[b]	R	HA	I	I	3:1	S
L	I	HA	I	I	15:1	I	I	15:1	S
L^1	R	HA	N	R	3:1	I	I	3:1	S
L^2	I	HA	I	I	HA	I	I	HA	I
L^3	I	HA	I	I	HA	I	I	3:1	S
L^4	I	HA	I	R	3:1	S	I	3:1	S
L^5	I	HA	I	I	HA	I	S	HV	S
L^6	I	HA	I	I	HA	I	S	HV	S
L^7	I	HA	I	R	HA	N	S	HV	S
L^8	I	3:1	I	I	HA	I	I	3:1	S
L^9	S	HV	S	S	HV	S	S	HV	S
L^{10}	R	HA	I	R	3:1	S	S	3:1	S
L^{11}	I	HA	I	I	HA	I	S	HV	S
M	I	3:1	S	S	HV	S	I	3:1	S
M^1	S	HV	S	S	HV	S	S	HV	S
M^2	S	HV	R	S	HV	R	S	HV	S
M^3	I	HA	I	I	HA	I	I	3:1	S
M^4	R	3:1	R	S	HV	S	S	HV	S
M^5	R	HA	R	R	HA	I	I	3:1	S
M^6	I	HA	I	I	HA	R	I	3:1	S
N	I	HA	S	I	HA	R	I	HA	I
N^1	I	HA	I	S	HV	R	I	3:1	S
N^2	R	HA	I	I	HA	I	R	3:1	S
P	I	3:1	S	S	HV	S	I	3:1	S
P^1	I	3:1	S	I	HA	I	S	HV	S
P^2	I	HA	I	I	HA	I	R	3:1	S
P^3	I	HA	I	I	HA	I	R	3:1	S
P^4	I	HA	S	I	HA	R	I	HA	R
Kugine	I	HA	I	R	HA	I	I	HA	I

[a] Ratios derived from data in literature references 9 and 10.

[b] Abbreviations: R = resistant, S = susceptible, I = immune, R = Flecks plus a few poorly developed pycnia, N = necrotic flecks, HA = Homozygous avirulent, HV = homozygous virulent, 3:1 = three avirulent to one virulent, 15:1 = fifteen avirulent to one virulent. All 3:1 and 15:1 fit expected ratios with $P > 0.05$ or higher.

aecia did not develop. The fact that urediospore cultures of these races were virulent on M^2 and N^1, but basidiospores were avirulent is an additional exception to the assumption that basidiospores infect only host cultivars for which they carry the gene for virulence.

In 1941, Flor (3) reported the inability of aeciospores to reinfect the host upon which they were produced to be homologous with heteroecism although occurring in an autoecious rust. In our study, the inability of basidiospores to reinfect the host cultivar on which the urediospores were homozygous virulent also could be considered heteroecism in an autoecious rust. Heteroecism usually refers to obligatory parasitism of taxonomically unrelated hosts. The present instance of a pathogenic race that will attack a different spectrum of resistance genotypes of the same host species while in the monokaryotic vs. the dikaryotic stage may represent a type of heteroecism. As yet, no unrelated host species has been demonstrated for the races of *M. lini* incapable of completing their life cycles on a single cultivar of flax.

In most cases when the parental race was avirulent on an isogenic line and the cultures resulting from selfing that race were segregating for virulence, we observed normal pycnia. Selfs of race 218 segregated three avirulent to one virulent on 15 host genes and fifteen avirulent to one virulent on the line with *L*. In all 16 cases, normal pycnia were observed when the 16 lines were inoculated with basidiospores of race 218 (Table 1). This also was true for race one and the lines with *M, P,* or P^1, as well as race 370 and the lines with L^4 or L^{10}. However, selfed cultures of race 1 segregated three avirulent to one virulent on the lines containing L^8 or M^4, but only a few poorly developed pycnia developed on the line containing M^4 and none developed on those containing L^8 when inoculated with basidiospores of race 1 (Table 1). These poorly developed pycnia were spermatized, but aecia did not develop. In addition, selfed cultures of race 370 segregated 15 avirulent to one virulent on the line with the *L* gene, and three avirulent to one virulent on the line with L^1. No pycnia were formed when lines with *L* or L^1 were inoculated with basidiospores of race 370.

One explanation for no formation of pycnia on lines with L^1 by basidiospores from selfed cultures of race 370 which segregated 3:1 as a urediospore culture could be antagonism to virulent spores induced in host tissues by avirulent spores. In other words previous inoculation with an avirulent culture of *M. lini* might have provided induced resistance to inoculation with a virulent culture (8). However, in all of our studies with basidiospore inoculations some leaves were heavily infected and others had only a few pustules but all leaves had same reaction. So the theory of antagonism may not be a logical explanation. Also, normal pycnia were observed in most cases when the parental culture was segregating for virulence.

The fact that no, or at best a few, poorly developed pycnia were observed on host lines on which the same race was segregating for virulence was additional evidence for a different mechanism of virulence in haploid basidiospore isolates than in the dikaryotic urediospore culture. However, if some different, but regular, mechanism of conditioning virulence was present, the exceptions we found should be more common.

The exceptions we found to the concept that basidiospores infect only those host lines for which they carry the gene for virulence is also an exception to the generally accepted concept of Flor's (5) that the dikaryophytic aecia, uredia, and telia resulting from fertilization of a pycnium behave as a diploid. In our studies, the monocaryon did not always have the same virulence pattern as the dikaryon of the same race.

A final explanation may be that the exceptions we observed could have been due to a mutation, deletion, translocation, gene conversion, suppression or some other genetic change. However, if a genetic change caused the exceptions we observed, then the mutant should have been virulent on the line with *N* after selfing race 1 on this line, as well as other cases in which urediospores were immune but basidiospores were virulent on that line. A mutation could have caused the change when the urediospore culture was homozygous virulent or segregating and the basidiospores were avirulent. Since this resulted in an immune reaction, we could not further test the virulence of the culture.

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