

Comparative Virulence of Monokaryotic and Dikaryotic Stages of Five Isolates of *Uromyces appendiculatus*

J. A. Kolmer, B. J. Christ, and J. V. Groth

Graduate research assistants and associate professor, respectively, Department of Plant Pathology, University of Minnesota, St. Paul 55108. Present address of first author: Department of Plant Pathology, North Carolina State University, Raleigh 27650. Present address of second author: Department of Botany, University of British Columbia, Vancouver, Canada V6T 2B1.

Scientific Journal Series Paper 13,227, Minnesota Agricultural Experiment Station, St. Paul 55108.

Accepted for publication 25 July 1983.

ABSTRACT

Kolmer, J. A., Christ, B. J., and Groth, J. V. 1984. Comparative virulence of monokaryotic and dikaryotic stages of five isolates of *Uromyces appendiculatus*. *Phytopathology* 74:111-113.

The virulence of monokaryotic basidiospores and dikaryotic aeciospores and urediniospores of five isolates of *Uromyces appendiculatus* was compared on 11 lines and cultivars of beans (*Phaseolus vulgaris*). Virulence of monokaryotic basidiospores of each isolate was determined by placing germinating teliospores over plants of each line or cultivar. Segregating isolates produced both pycnia and flecks; homozygous isolates produced only flecks or pycnia. The fungal isolates were mass-selfed and the bean lines inoculated with bulked S₁ aeciospores to detect heterozygosity and

dominance in the dikaryotic stage. Segregating dikaryotic S₁ aecial collections gave rise to both flecks and uredinia; homozygous isolates produced only flecks or uredinia. With only two exceptions, in which avirulent basidiospores produced no reaction, isolates that segregated for pycnial infection types also segregated as dikaryotic S₁ aeciospores, and isolates that did not segregate as monokaryons did not segregate as S₁ aeciospores.

Additional key words: bean rust, genetics, necrotic fleck, telia, uredinia.

Flor (4,6) was the first to compare virulences of urediniospores and basidiospores of *Melampsora lini* on flax (*Linum usitatissimum*). Normal pycnia and aecia developed on six cultivars of flax following inoculation with basidiospores from an isolate of race 210. The dikaryotic urediniospores of the isolate were either homozygous or heterozygous virulent, on each of the six cultivars. All cultivars that were resistant to the urediniospores were also resistant to the basidiospores from race 210 teliospores. Normal pycnia developed on cultivar Bombay from race 210 basidiospores, but the aeciospores from these pycnia were incapable of infecting Bombay, as were the urediniospores of race 210 (6). Flor (6) thought Bombay was comparable to an alternate host in the life cycle of the appropriate isolate of *M. lini*.

In a recent paper, Statler and Gold (14) also compared the virulence of basidiospore and urediniospore cultures of three races of *M. lini*. In most cases, basidiospores were virulent on host lines for which the urediniospores carried the gene for virulence. Exceptions to this were of three types: homozygous avirulent urediniospore cultures whose teliospores produced basidiospores that were virulent on the same host line; homozygous virulent urediniospore cultures whose teliospores produced only poorly developed pycnia; or heterozygous urediniospore cultures whose telia produced only avirulent basidiospores. Statler and Gold (14) raised the possibility that the inability of basidiospores to infect hosts on which the urediniospores were homozygous virulent could be considered to be a form of heteroecism in an autoecious rust. In some cases, different genes may condition virulence in the dikaryotic and monokaryotic stages of the parasite. Isolates in which this occurs may be considered to be less evolutionarily advanced (14).

Uromyces appendiculatus (Pers.) Unger is an autoecious macrocyclic rust fungus. Andrus (1) was the first to observe and obtain all the stages in the life cycle of the fungus. The life cycle can be completed experimentally using the techniques of Groth and Mogen (8).

The objective of this research was to determine whether identical genes condition virulence and avirulence in monokaryotic and dikaryotic stages of *U. appendiculatus*.

MATERIALS AND METHODS

Five isolates of *U. appendiculatus* designated as P10-1, KW8-1, S1-5, P14-1, and 229 were used in this study. Each isolate was derived from a single uredinium and was increased and maintained separately on plants of susceptible bean cultivar Pinto 111. Plants were inoculated with either urediniospores or aeciospores one week after planting. The inoculation method was the same as that of Groth and Shrum (9). The inoculated plants were placed in chambers at 100% relative humidity (RH) for 18-24 hr then maintained on greenhouse benches at 20-28 C.

Leaves bearing telia of the five isolates were obtained after periods of up to 2 mo following inoculation of the plants with single-uredinial isolates of *U. appendiculatus*. Host differential cultivars Early Gallatin, US#3, and Roma were used to confirm the purity of the single-uredinial isolates used in telial production.

The 11 bean cultivars used in these studies were Montcalm, Aurora, US#3, Pinto 111, Early Gallatin, Bush Blue Lake, B1561, Roma, B2090, Golden Gate Wax, and 814. Infection types of the parental isolates and their S₁ progeny were rated on a scale of 1 to 9 (9). On this scale, types 1 and 2 are characterized by necrotic flecks, type 3 by minute uredinia, while types 4 to 9 are susceptible infection types with increasing uredinial size.

We used the methods of Groth and Mogen (8) to obtain the pycnial and aecial stages of *U. appendiculatus*. Teliospores were scraped from wetted leaves, concentrated by using a Millipore filter apparatus with Whatman 5.5 glass microfiber papers, resuspended in 2 ml of water at 2×10^6 per milliliter, and poured onto petri dishes containing 25 ml of solidified 2% water agar. Open dishes that contained germinating teliospores were inverted over young bean plants. Teliospores were kept over susceptible Pinto 111 plants for 1 wk at 20-28 C and 100% RH to ensure sufficient pycnia for mass-selfing. Plants with pycnia were kept in cages to avoid accidental fertilization by insects. Mass-selfing was done by putting a few drops of distilled water on leaves that bore pycnia and rubbing an alcohol-rinsed (95% EtOH), dry finger over the leaves.

Aeciospores collected 12 days after mass-selfing were used to inoculate the bean lines in the same manner as the urediniospores. Segregation ratios were determined from infection types recorded 12 days after inoculation.

Teliospores of each isolate were kept suspended over plants of the other 10 lines for an average of 5 days to obtain pycnial (monokaryotic) infection types. The pycnial infections were

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

recorded 12 days after the teliospores were removed from over the plants. In a follow-up experiment, teliospores of isolate P10-1 were germinated over cultivars Early Gallatin and Roma. P10-1 segregated as a monokaryon, producing both flecks and pycnia on Early Gallatin and Roma. The pycnia were mass-selfed as described earlier. Aeciospores from resultant aecia were used to inoculate plants of cultivars Early Gallatin, Roma, and Bush Blue Lake to determine whether segregation occurred in the dikaryons.

RESULTS

Segregating S_1 progeny produced either uredinia (infection types 4-9) and flecks (types 1-2) or, when the incompatible infection type was a minute uredinium (type 3), large uredinia and minute uredinia. Basidiospore inoculations from segregating isolates gave rise to non-necrotic pycnia and large necrotic flecks. Basidiospores from homozygous cultures produced only pycnia or flecks.

Ratios of uredinial infection types for S_1 progenies from isolates that segregated are shown in Table 1. The results of inoculations with monokaryotic basidiospores from segregating isolates are shown in Table 2. The remainder of the combinations of bean cultivars and isolates of *U. appendiculatus* did not segregate in either the S_1 dikaryotic stage or the monokaryotic basidiospore stage, being homozygous virulent or avirulent in both nuclear conditions. With only two exceptions, isolates that segregated in the S_1 with a dikaryotic nuclear condition (Table 1), also segregated as monokaryons (Table 2). In general, the segregation ratios were 3:1, or 1:3, for the dikaryotic stage (Table 1) and 1:1 for the monokaryotic stage (Table 2), indicating monogenic control of virulence to the bean cultivars tested. Virulence of aeciospores of isolate KW8-1 to line 814 appeared to be controlled by two recessive genes. With only two exceptions, isolates that segregated as S_1 dikaryons (Table 1), also segregated as monokaryons (Table 2). For the remainder of the combinations of bean cultivars and isolates of *U. appendiculatus* the isolates were either homozygous virulent or avirulent and did not segregate in either the S_1 dikaryotic stage or the monokaryotic basidiospore stage. These results indicate that in the five isolates that we tested the same genes condition infection types in both nuclear conditions. The only exceptions to this are the infection types of isolates KW8-1 and 229 on line 814.

Isolate KW8-1 produced infection type 3 (minute uredinium) on line 814, and the S_1 progeny segregated in a 15:1, minute:large uredinium ratio (Table 1). Basidiospores of KW8-1 gave rise to large fertile pycnia on line 814 (Table 2). Isolate 229 also produced avirulent, minute uredinia on line 814 and also segregated (3:1, minute uredinia:large uredinia) in the S_1 on line 814 (Table 1). Basidiospores of 229 produced large, fertile pycnia on 814 (Table 2). When these pycnia of 229 on line 814 were mass-selfed, and the

resultant aeciospores inoculated on 814, no segregation occurred; only large virulent uredinia were present. Apparently basidiospores of isolate 229 that carried the gene or genes conditioning type 3 uredinia were avirulent and never produced any pycnia or other observable symptoms. Thus, no segregation of pycnial infection types of 229 on line 814 would be apparent. The same phenomenon most likely occurred in the combination of KW8-1 and 814, in which the avirulent dikaryotic infection type also was the minute uredinium, and no observable avirulent monokaryotic infection types occurred.

In the follow-up experiment, isolate P10-1 was mass-selfed on cultivars Early Gallatin and Roma, on which flecks and pycnia were produced. The resultant aeciospores were inoculated onto plants of cultivars Early Gallatin, Roma, and Bush Blue Lake to see if further segregation occurred in the uredinial stage. If such segregation occurred, it could indicate that the genes conditioning virulence in monokaryotic infections differed from those conditioning virulence in the dikaryotic stage. The S_1 of P10-1 from Early Gallatin or Roma did not segregate for uredinial infection types on Early Gallatin, Bush Blue Lake, and Roma; only virulent uredinia were produced. The results indicate that the same gene(s) that condition avirulence to these cultivars in monokaryotic basidiospores also condition avirulence in dikaryotic aeciospores. If segregation for uredinial infection types had occurred in these selfed progeny, it would have indicated that some of the virulent basidiospores contained other genes conditioning a virulence in the dikaryotic stage.

The results of the follow-up experiment also suggest that Early Gallatin, Bush Blue Lake, and Roma share the same gene for resistance to isolate P10-1. If these cultivars had different genes for resistance to isolate P10-1, selfing P10-1 on Early Gallatin or Roma should not have eliminated genes for avirulence to the other two cultivars from all of the aeciospores of P10-1 S_1 produced on Early Gallatin or Roma. In that case some segregation of uredinial infection types would have been expected on the other two cultivars. This of course, assumes a gene-for-gene relationship between virulence and resistance.

Virulence in isolate KW8-1 to cultivars Early Gallatin, Roma, and Bush Blue Lake, is conditioned by a single dominant gene (Table 1). Virulence in isolate P14-1 to cultivar US#3 is also conditioned by a dominant gene (Table 1). Christ and Groth (2) previously found a dominant gene conditioning virulence in *U. appendiculatus*. Although dominant genes conditioning virulence are relatively rare, they have been noted in other rust fungi (7,10).

DISCUSSION

Various methods of selfing rust fungi have been used by several workers. Loegering (11), Flor (5), and Statler and Gold (14),

TABLE 1. Parental uredinial infection types and segregation ratios for infection types from S_1 aeciospore progeny from four isolates of *Uromyces appendiculatus* on five cultivars of *Phaseolus vulgaris*

Isolate	Cultivar	Parental infection type	Segregation of uredinial S_1 cultures (A:V) ^a			
			Observed	Expected	Ratio	P-value
P10-1	Early Gallatin	A	933:342	955:319	3:1	0.1
P10-1	Bush Blue Lake	A	279:144	317:106	3:1	0.001
P10-1	Roma	A	324:117	331:110	3:1	0.4
KW8-1	Early Gallatin	V	197:629	206:620	1:3	0.15
KW8-1	Bush Blue Lake	V	132:327	114:344	1:3	0.4
KW8-1	Roma	V	315:879	298:896	1:3	0.15
KW8-1	814	A	620:52	630:42	15:1	0.15
P14-1	US#3	V	135:412	189:417	1:3	0.7
229	Early Gallatin	A	396:160	417:139	3:1	0.01
229	Bush Blue Lake	A	321:129	337:112	3:1	0.01
229	Roma	A	202:83	213:71	3:1	0.01
229	814	A	222:58	210:70	3:1	0.3

^aA = avirulent and V = virulent.

TABLE 2. Parental uredinial infection types and segregation ratios for pycnial infection types from basidiospores from four isolates of *Uromyces appendiculatus* on five cultivars of *Phaseolus vulgaris*

Isolate	Cultivar	Parental infection type	Segregation of pycnial infection types (A:V) ^a			
			Observed	Expected	Ratio	P-value
P10-1	Early Gallatin	A	130:154	142:142	1:1	0.3
P10-1	Bush Blue Lake	A	84:92	88:88	1:1	0.7
P10-1	Roma	A	100:92	96:96	1:1	0.6
KW8-1	Early Gallatin	V	180:84	134:134	1:1	0.001
KW8-1	Bush Blue Lake	V	146:54	100:100	1:1	0.001
KW8-1	Roma	V	207:69	138:138	1:1	0.001
KW8-1	814	A	HV
P14-1	US#3	V	155:132	143:143	1:1	0.15
229	Early Gallatin	A	183:181	182:182	1:1	0.95
229	Bush Blue Lake	A	82:92	87:87	1:1	0.95
229	Roma	A	132:136	134:134	1:1	0.90
229	814	A	HV

^aA = avirulent, V = virulent, and HV = homozygous virulent.

fertilized individual pycnia with spermatia from only one pycnium. Others have mass fertilized the pycnia with a bulked collection of the spermatia (3,10,12,15). Miah (13) believed that errors arising from the pooled-spermatia approach could be caused by: a small number of pycnia used in intermixing the nectar, pooled spermatia that were not fully intermixed, a small number of aecial clusters, and use of the aeciospore rather than the aecial cup as the unit of segregation and fertilization. The problems in mass-selfing technique which Miah described may not apply to *U. appendiculatus* nearly as much as to the heteroecious cereal rust fungi. In our studies, a large number of pycnia were obtained, spermatia were fully intermixed, all pycnia produced aecia, and the aeciospore was used as the unit of fertilization and segregation.

Basing segregation ratios on aeciospores as the unit of segregation was confirmed by Christ and Groth for *U. appendiculatus* (2) and Dinoor et al for *Puccinia coronata* (3). The receptivity studies of Christ and Groth (2) with mixtures of virulent and avirulent *U. appendiculatus* urediniospores showed that counts of mass S₁ uredinia and flecks should represent an unbiased measure of virulent and avirulent aeciospores applied to the leaf. Dinoor (3) isolated single aecial cups after mass-selfing, and found that mixed genotypes can occur within a single cup. He concluded that single aeciospores may be a better unit of fertilization than single aecial cups. Green (7) selfed races 10 and 11 of *Puccinia graminis* sp. *tritici* by using both mass-selfing and individual selfs. In all cases, results from single and mass-selfing agreed. The mass S₁ ratios of Christ and Groth (2) agreed with individual crosses made between isolates of *U. appendiculatus*.

Our results in this paper support the hypothesis that identical genes condition virulence and avirulence in dikaryotic and monokaryotic stages of *U. appendiculatus*. We found no exceptions of the type described by Flor (6) and Statler and Gold (14) for *M. lini* for the five isolates of *U. appendiculatus* used in this study.

LITERATURE CITED

1. Andrus, C. J. 1931. Sex in *Uromyces appendiculatus* and *U. vignae*. J. Agric. Res. 42:559-587.
2. Christ, B. J., and Groth, J. V. 1982. Inheritance of virulence to three bean cultivars in three isolates of the bean rust pathogen. Phytopathology 72:767-770.
3. Dinoor, A., Khair, J., and Fleischmann, G. 1968. Pathogenic variability and the unit representing a single fertilization in *Puccinia coronata* var. *avenae*. Can. J. Bot. 46:501-508.
4. Flor, H. H. 1941. Pathogenicity of aeciospores obtained by selfing and crossing known physiologic races of *Melampsora lini*. Phytopathology 31:852-854.
5. Flor, H. H. 1942. Inheritance of pathogenicity of *Melampsora lini*. Phytopathology 32:653-669.
6. Flor, H. H. 1959. Differential host range of the monocaryons and the dikaryons of a eu-autoecious rust. Phytopathology 49:794-795.
7. Green, G. J. 1966. Selfing studies with races 10 and 11 of wheat stem rust. Can. J. Bot. 44:1255-1260.
8. Groth, J. V., and Mogen, B. D. 1978. A technique for completing the life cycle of bean rust. Phytopathology 68:1674-1677.
9. Groth, J. V., and Shrum, R. D. 1977. Virulence in Minnesota and Wisconsin bean rust collections. Plant Dis. Rep. 61:756-760.
10. Johnson, T. 1954. Selfing studies with physiologic races of wheat stem rust, *Puccinia graminis* var. *tritici*. Can. J. Bot. 32:505-522.
11. Loegering, W. Q., and Powers, H. R. 1962. Inheritance of pathogenicity in a cross of physiological races 111 and 36 of *Puccinia graminis* f. sp. *tritici*. Phytopathology 52:547-554.
12. Luig, N. N., and Watson, I. A. 1961. A study of inheritance of pathogenicity in *Puccinia graminis tritici*. Linn. Soc. N.S.W. Proc. 86:217-229.
13. Miah, M. A. 1968. Method of selfing F₁ cultures and interpreting genetic data in rust fungi. Can. J. Genet. Cytol. 10:613-619.
14. Statler, G. P., and Gold, R. E. 1980. Comparative virulence of basidiospores and urediospores of three races of *Melampsora lini*. Phytopathology 70:528-530.
15. Zimmer, D. E., and Shafer, J. 1965. Nature and inheritance of virulence in *Puccinia coronata*. Phytopathology 55:1320-1324.