Virulence Frequency in the Bean Rust Fungus: Comparison of Phenotypic vs. Genotypic Polymorphism

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ABSTRACT

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A collection of urediniospores of the bean rust fungus, *Uromyces appendiculatus*, made in 1982 from west central Minnesota on a highly susceptible cultivar, Pinto U. I. 111, was polymorphic for virulence (produced both uredinia and flecks) on numerous differential snap and dry bean lines. Four years later, another collection of the bean rust fungus from the same region was phenotypically monomorphic for virulence on many of the same differential lines. However, bulk-fertilization of the 1986 collection revealed genotypic polymorphism that was being maintained in the heterozygous condition. The temporal difference in phenotypic variability between the 1982 and 1986 populations may have originated from differences in founder populations or selection in favor of virulence hybrids (overdominance). Other populations of bean rust from Minnesota in 1986, collected 5, 145, and 180 km away from the first 1986 site, all differed from each other in frequency of virulence on several differential bean lines. This genetic divergence of *U. appendiculatus* in space and time within one state means that spore collections from different sites should not be pooled and single sites should not be used for testing new bean lines.

Additional keywords: heterozygosity, unnecessary virulence

Mass field collections of the bean rust fungus, *Uromyces appendiculatus* (Pers.:Pers.) Unger, frequently are mixtures of a number of virulence phenotypes (2,13,18), which are often called "races." When urediniospores from mass collections are inoculated on differential lines of dry or snap beans (*Phaseolus vulgaris* L.), polymorphic reactions will be produced (i.e., both uredinia and flecks) if not all of the races present are virulent on the differential host used (1,8,18). Deployment of new resistance genes may cause the frequency of virulence (percent uredinia produced, thus the percent of virulent individuals) in the population to change (7). There are few data, however, that help reveal the magnitude or speed of selection pressure that could change the frequency of virulence in a bean rust population. In addition, the geographic scale of diversity of the bean rust population is not well-known.

In 1982, two new cultivars of dry beans were introduced in commercial fields in Renville County in west central Minnesota. Between 600 and 800 of the more than 2,000 ha of Pinto beans in the area were planted with the Pinto-type cultivar, Olathe, which had been resistant to all races of the bean rust fungus in Colorado where it was bred (20). Nearly 80% of approximately 3,200 ha of navy-type beans (about 55% of the total dry bean hectarage in the region in 1982) (9) were planted with the new cultivar Fleetwood. Both cultivars have remained generally resistant to bean rust (18), but both became moderately to heavily rusted in that first year of appreciable hectarage in Minnesota (9). Fleetwood and Olathe differ in resistance. A sample of 12 single uredinial isolates of *U. appendiculatus*, selected on either Fleetwood or Olathe from Staples in north central Minnesota, contained one isolate that was virulent only on Olathe and two others virulent only on Fleetwood (9).

A collection of urediniospores from Renville County, originally made in 1976, showed no or only slight virulence when tested on Fleetwood and Olathe beans in 1982 (9). For the 1982 field population of the pathogen to be virulent on the two new cultivars, virulence genes had to have been introduced into the fungal population between 1976 and 1982, have been present in the pathogen in a nearby bean area, or have been recessive genes masked in a heterozygous population. A 1976 urediniospore collection from near Staples produced a low virulence frequency on Fleetwood and Olathe when tested in 1982 (9). Additionally, the genes introduced into (or hidden in) the Renville County rust fungus population would have had to be effective against these resistance genes to which the rust fungus probably had not been exposed previously. Data from trap plots of dry beans in north central Minnesota indicate that some "virulence to 'exotic' differentials is the rule" (i.e., differential lines containing genes not used locally [9]).

The populations of *U. appendiculatus* in Minnesota rapidly and simultaneously overcame two different resistances, which underscores the great adaptive ability of this pathogen. The details of how this came about are not known. If we are to understand and profit from this example, more information about phenotypic and genotypic polymorphism of the pathogen population is needed. The purpose of this study was to examine polymorphism for virulence in one of the important samples of the pathogen from 1982 and compare it to collections taken in 1986 from the same bean production area and from an adjacent region where beans are not grown commercially. The

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results document a change in phenotypic diversity in populations of the pathogen collected since (but not necessarily the result of) the introduction of race-specific resistance in the host.

MATERIALS AND METHODS

Origin of collections. Field collections of populations of the bean rust fungus were made on 26 August 1982 (collection P21) by H. M. Alexander (1) and 9 September 1986 by J. W. McCain (collections P24 and P25). Field collection P21 was made near Hector in Renville County on Pinto U. I. 111 beans, a cultivar with no observable resistance to rust collected from dry beans in Minnesota (12). P24 was collected 1 km south and P25 about 7 km southeast of Bird Island in Renville County, both within about 15 km of the P21 collection site. The host of P24 and P25, cultivar Agate Pinto, likewise showed no resistance to any part of the bean rust fungal populations that have been previously obtained from these regions of Minnesota (J. V. Groth, *unpublished*). A fifth collection (P26) was made approximately 145 km east of the Renville County bean production area from Pinto 111 beans in field plots at the University of Minnesota Agricultural Experiment Station, St. Paul, Ramsey County, on 16 September 1986 by J. W. McCain and B. A. Haile.

For each collection, 50–100 heavily rusted leaves were gathered from an area of the field 10–20 m in diameter. Within 24 hr of harvest, the urediniospores were removed from the leaves with a vacuum aspirator. Spores were collected from approximately 100 uredinia per leaf in order to obtain a large initial sample (the "field" collection). However, because collection P26 consisted mostly of teliospores, fewer urediniospores were obtained. All of the spore samples were then stored in reserve at −60 C in an ultra-low-temperature freezer.

About 5 mg of urediniospores from each sample was inoculated on young Pinto 111 plants in the greenhouse. Spores, suspended in Soltrol 170 light oil (Phillips Petroleum Co.), were lightly misted on half-expanded unifoliate leaves. After being placed in a 100% RH chamber overnight, the plants were grown in a 20–25 C greenhouse. New uredinia erupted after about 1 wk, and the spores were harvested 1 wk later by vacuum aspiration or by shaking the plants over a clean sheet of aluminum foil. The collected urediniospores were stored at −60 C until needed.

For each new 1986 field collection (P24, P25, and P26), 30–50 plants of Pinto 111 were inoculated for the previously mentioned increase. Each resulting "mass" collection, descended by one asexual generation from its respective field collection, was obtained from 6,000–10,000 uredinia. The P21 field collection had been increased in 1982 (1) and a sample of urediniospores from 1,000–1,500 pustules had been preserved. These were reincreased to obtain fresh urediniospores for this study in numbers comparable to the other collections. Thus, P21 was actually two generations removed from the field.

Bulk-fertilization technique. Plants of the increase inoculations of P21 and P24 were moved after urediniospore collection to a greenhouse at 15–20 C. By 4–6 wk after inoculation, most of the uredinia had converted to telia. Leaves were collected and washed overnight in running tap water to remove contaminant phylloplane organisms. Teliospores were scraped off as described previously (10) and stored on glass microfiber filters for at least 3 mo at 4 C to complete dormancy. The teliospores were then spread on 2% water agar in petri plates (25 mg of spores per plate) and incubated in a closed glass desiccator jar on a north-facing windowsill until the spores germinated (4). When one to two metabasidia were observed in a ×100 microscope field (500–1,000 spores per field), the plates were taken to the 15–20 C greenhouse. They were placed, inverted, for 2–3 days atop clear plastic cylinders over young Pinto 111 plants with half-expanded unifoliate leaves, two plants per pot, 10–15 pots per culture. Moisture condensed on the cylinders, producing a high-humidity environment. As the basidiospores matured, they were shot off the metabasidia and fell onto the susceptible leaf tissue.

After about 1 wk, pycnia appeared on the upper surface of the bean leaves. When the pycnia began exuding nectar, they were bulk-fertilized by the method of Christ and Groth (4). A few drops of distilled water were put on each leaf, and an alcohol-rinsed finger was used to spread the water and, hence, the nectar containing spermatia over all the pycnia on the leaf. Aecia (about 50 clusters per leaf) erupted 10–12 days later on the lower leaf surfaces. The aeciospores were collected by tapping them into a gelatin capsule before being inoculated onto fresh Pinto 111 plants to produce first-generation (F_1) urediniospores.

Virulence assays. Young plants of a wide range of snap and dry bean cultivars and experimental lines were grown from seed and inoculated with urediniospores when unifoliate leaves were about half-expanded. The bean lines tested included commercial cultivars as well as selected differential lines, such as a number of those developed by Ballantyne (2). While the identity or number of genes for resistance to bean rust in many of these cultivars is not known, previous inoculations with other bean rust collections suggested that there were many different resistance combinations included.

Urediniospores were inoculated on all available bean lines. Each rust collection was inoculated on the two unifoliate leaves of six to nine plants per culture. If possible, inoculations were done at the same time so that infections developed under similar environmental conditions. This eased comparison of the infection types produced.

Two wk after inoculation, the plants were scored for uredinial infection type, according to the scale of Groth and Shrum (12). All flecks and uredinia were counted, and the percentage of virulent events was calculated. For the purposes of this study, infection types 0–3 were considered avirulent (chlorotic fleck, necrotic fleck without or with small amount of sporulation, minute uredinia), and types 4–9 were grouped as virulent reactions (compatible, i.e., non-necrotic uredinia of increasing size). Usually, leaves bearing 50–200 uniformly distributed infection events (flecks or uredinia) were chosen for scoring—higher numbers caused overcrowding and coalescing and, therefore, unreliable measurements. Data were pooled from plants with lower numbers of infection events, with the goal of a sample size of over 1,000 events. For all possible cultivars, the inoculations were repeated on new sets of plants and the results, weighted for different sample sizes, were averaged.

Seventeen bean cultivars and lines were inoculated with urediniospores from the original P24 field collection and compared with plants inoculated with urediniospores from the first generation of asexual increase in the greenhouse. Although large changes in frequencies of virulence genes can occur over several generations of repeated greenhouse increase of spores (7), the change in one generation may be small. On these 17 cultivars, the greatest difference between the frequencies of virulence produced by the field collection and the mass generation was 11% (on bean line B1672). These changes were not expected to be qualitatively significant for this project. Therefore, the mass generation was used so that the field collections could be preserved in the ultra-low-temperature freezer as vouchers and for future studies.

Field data. Some disease ratings were also obtained by visual scoring of a few late-planted lines of dry beans in 1986 at the University of Minnesota Irrigation Center near Staples in Wadena County, approximately 180 km north of Renville County. The plants were infected by naturally occurring inoculum. Qualitative severity of rust infection was scored as trace, very light, light, moderate, or heavy. These ratings were not replicated.

RESULTS

Virulence comparisons of P21 and the 1986 collections. The mass collection P21 of urediniospores of *U. appendiculatus* produced polymorphic infection types (percent virulence $03<\%<97$) on 34% of the bean lines tested, but as few as 10% of the bean lines were found to bear both

uredinia and flecks when inoculated with one of the 1986 collections (Table 1). The differences between the two years 1982 and 1986 appeared either as increased or as decreased frequency of virulence, depending on the bean differential line tested. Frequency of virulence remained unchanged over time and space on some bean lines. All of the rust collections tested were 100% virulent on 33 bean lines and 100% avirulent on 20 other lines (Table 1). For all lines on which collection P21 was polymorphic for virulence, at least one of the 1986 collections differed noticeably in percent of virulence from P21 (Table 1).

The Cochran test for related observations (6) confirmed that the four populations were not all polymorphic on the same proportion of differential lines ($P = 0.005$). In addition, in pairwise Cochran tests, each mass collection differed in proportion of polymorphism from all three others ($P = 0.1$) except for P24 vs. P25 (significant only at $P = 0.25$) and P24 vs. P26 (no difference). There were significant differences ($P = 0.05$), however, between the proportions of virulent infection types produced by P24 vs. the other two 1986 collections

Table 1. Percent virulence (number of uredinia divided by number of uredinia + number of flecks) of mass collections of *Uromyces appendiculatus* on bean cultivars and experimental lines

	Bean rust mass collection number and year					Bean rust mass collection number and year			
	1982	1986				1982	1986		
Bean line	P21[a]	P24	P25	P26	Bean line	P21[a]	P24	P25	P26
Aurora	00[b]	00	00	00	Bush Blue Lake	21*	00	07*	00
B752	100	100	···	···	2C-120	100	100	100	100
B754	100	100	···	100	4C-109	100	100	100	100
B1049	00	00	00	00	Cascade	16*	00	···	···
B1257	00	00	00	00	Charlevoix Robinson	100	100	100	···
B1259	100	100	100	100	Checkmate	14*	02	···	···
B1263	100	100	···	100	Chief	29*	100	24*	66*
B1298	00	00	···	00	Columbia Pinto	72	···	100	···
B1349[c]	09*[d]	02	100*	00	Early Gallatin	18*	00	19*	00
B1400	00	00	···	00	Executive	17*	00	···	···
B1404	00	00	···	···	Fleetwood	20*	100	17*	70*
B1431	66*	24	92*	11*	Golden Gate Wax	100	100	100	100
B1464	54*	62	···	00*	Kentucky Wonder Pole	100	100	100	100
B1481	100*	42	···	···	Kinghorn Wax	27*	00	10*	00
B1484	00	00	···	···	Manitou Kidney	100	···	100	···
B1487	00	00	···	00	Michicran	100	···	100	···
B1494	100*	16	···	···	Montcalm	100	100	100	100
B1527	62*	00	94*	00	Olathe	36*	100	x[f]	58*
B1554 (#643)[e]	100	100	100	100	Pencil Pod Black	11*	02	07*	···
B1556	01*	100	100	100	Pinto 111	100	100	100	100
B1557	81*	100	100	100	Redlands Pioneer	100	100	100	···
B1558	100	100	72*	100	Roma	53*	00	03	00
B1562	89*	100	47*	100	Royal Red Kidney	100	100	100	100
B1597	65*	14	91*	00*	Seminole	100	100	100	100
B1600	00	00	00	00	Slenderette	11	00	07	00
B1606	100	100	···	···	Slimgreen	100	100	···	···
B1640 (Mexico 309)	58*	00	···	00	Stringless Green Pod	76*	01	···	···
B1654	00	00	00	00	Tenderette	09*	00	···	···
B1659	00	00	···	00	Tendergreen	19*	00	00	···
B1660 (Nep 2)	68*	14	89*	00*	Topcrop	100	100	100	100
B1666	00	00	···	00	Truegreen	100	100	···	···
B1667	00	00	100*	···	Upland	100	100	100	100
B1672 (UrH)	63*	70	84*	00*	Ur1[g]	00*	100	100	56*
B1754 (CNC)	00	···	00	···	Ur2	100	100	100	100
B1757	00	00	00	00	UrB	00	00	00	00
B1767	66*	91	···	···	UrC (Brown Beauty)	100	100	···	100
B1773	00	00	00	00	UrE	00	00	···	00
B1787	100	100	100	100	UrF	100	100	100	100
B1882	00	00	00	00	UrJ	100	100	100	100
B2027	100*	00	00	100*	UrK	00*	100	···	···
B7672	45	48	84*	00*	US #3	78*	99	100	100
Black Turtle	00	00	00	00	Y6-71	100	100	···	100
Bountiful	100	100	100	100	6Y-218	00	00	00	00
Brazil-1	73	71	42*	20*	#650	100	100	100	···
Brazil-2	100*	55	···	···	#765	02*	100	17*	66*
Brazil-3	81*	100	100	100	#814	100	100	100	100
Brazil-6	100	100	100	100					
Brazil-8	100	100	100	100					
Brazil-9	80*	100	100	100	Total lines polymorphic	33	11	18	7
Brazil-10	74*	100	100	100	Percent lines polymorphic	34	12	26	10

[a] P21 was collected on Pinto 111 beans in 1982; P24 and P25 on Agate Pinto in 1986. All collections were from Renville County, Minnesota. P26 was collected in 1986 in Ramsey County, Minnesota.
[b] Figures are the weighted average of two or more runs.
[c] Designations of bean lines beginning with the letter B indicate lines received from B. J. Ballantyne (2).
[d] * = percent significantly ($P = 0.05$) different from that of P24 by Li test (14).
[e] Names in parentheses indicates standard bean rust differential line that was source of this experimental line.
[f] A type of resistant reaction in which flecks and uredinia of various sizes are intermixed.
[g] B. J. Ballantyne (2) gene designation for rust resistance.

on several of the individual bean lines (Table 1) according to the Li test of the confidence interval about the difference of means (14). Groth and Alexander (9) reported that P21 differed "subtly" in virulence frequency on three bean differential lines from nearby collection P22, which was also from 1982 at Bird Island. Percent virulence for P21 was 64, 66, and 100%, and for P22 was 67, 40, and 96%, on bean lines US #3, Early Gallatin, and #814, respectively (9). P24 and P25, collected from fields of fully susceptible beans only about 5 km apart, differed significantly in virulence frequency on 16 of 65 bean lines (Table 1). Collection P26 from Ramsey County differed from collection P24 in proportion of the population virulent on 13 of 70 bean lines. Thus, although collection P24 was not overall more frequently virulent than the P25 or P26 collections, there were clear differences on several of the individual bean lines.

Although comparing the inoculations with the 1986 field severity data from Staples is inexact because of the different rating methods (exact counts of uredinia or flecks in the greenhouse vs. visual scoring of severity in the Staples fields), some differences were suggested. Rust infection at Staples was "heavy" or "moderate to heavy" on B1527 and UrB beans (Table 2), but no infection was obtained on these lines from the P24 and P26 collections. Two lines (Aurora and B1757) were scored "trace" at Staples, indicating some virulence in the rust population there, but P24, P25, and P26 were completely avirulent on these bean lines. In contrast, the Staples field infection was "very light" or "trace" (which could mean either low virulence frequency or lower inoculum potential) on B1556, Brazil-9, and Brazil-10 beans, on which the P24, P25, and P26 populations were 100% virulent, moderate to heavy infections.

Virulence of the bulk-selfed progeny. Segregation for virulence and avirulence was observed when P21 and P24 F_1 progeny were inoculated on a number of available differential lines (Table 3). In parental tests with P21, 41% of 68 bean lines showed polymorphic infections, compared to only 12% of 68 lines inoculated with P24 parental urediniospores. Not as many lines were inoculated with bulked F_1 progeny of P21 as for P24, because the phenotypic polymorphism already seen in the parental infection types for P21 must have arisen from genotypic polymorphism. Nevertheless, the many "fixed" phenotypes from P24 parental inoculations were found to mask almost as many heterozygous virulences (41% vs. 52.5%) as in P21. Of the virulences apparently fixed for avirulence in the parental generation, the F_1 generation segregated to reveal hidden (recessive) virulence genes on six of 15 bean lines inoculated with P21 and on 16 of 26 lines inoculated with P24. However, segregation for hidden avirulence (suggesting dominant virulence genes) was also noted, occurring in four of 21 lines on which parental P21 appeared fixed for virulence and in six of 34 lines for which P24 was apparently fixed phenotypically. On three of these lines with P21 and four lines with P24, the hidden avirulent reaction was very small uredinia; on the other lines, necrotic flecks appeared. Preliminary results indicate that although much of the P24 population was heterozygous for virulence, isolates from this collection are highly homozygous for five isozymes tested in polyacrylamide gel electrophoresis (J. W. McCain, *unpublished*).

DISCUSSION

The 1982 collection of bean rust urediniospores from Minnesota was polymorphic for virulence on numerous bean differential lines. Both dominant and recessive virulence genes previously have been shown to occur in *U. appendiculatus* (5). The frequency of the individual virulence genes in the population might be expected to change as a result of inmigration of new fungal individuals or by selection attributed to either the introduction of new resistance genes in the host or the relative fitness conferred by the virulence genes.

Significantly, of the bean lines that had both pustules and flecks in these inoculations, only a few have ever been grown on a large scale in the bean-production areas of Minnesota—Early Gallatin, Fleetwood, Olathe, Pinto 111, and Pinto 114. Thus, the P21 population of *U. appendiculatus* included numerous urediniospores carrying genes for virulence effective against host resistance genes not likely to have been encountered by the fungus in Minnesota. In other words, the fungus possessed genes for "unnecessary" or "excess" virulence (1,9). Excess virulence has been shown to occur in a number of plant pathogenic fungi (3,16,17). It has been suggested that the fitness cost exacted by possession of these excess genes would soon sort them out of the population (3,7). Although the 1986 phenotypes include more fixed virulence or avirulence and fewer polymorphic phenotypes (about one-third as many in P24 as in P21), this sampling technique cannot prove that selection against unnecessary virulence occurred.

Excess virulence could be maintained by heterosis. Many of the parental P21 infection types were polymorphic, so it could be directly inferred that the P21 population of urediniospores was a mixture of virulent and avirulent spores, and the F_1 ratios confirmed that many of these spores were heterozygotes. In contrast, parental P24 infection types were monomorphic (fixed) on many cultivars tested. Had there been no segregation in the F_1 progeny of P24, we would have concluded the P24 population was composed of homozygotes, either for virulence or for avirulence. However, the data from the selfed progeny show that for every differential line on which P21 was phenotypically polymorphic and P24 phenotypically fixed, P24 was still genotypically polymorphic. The simplest explanation for lack of fixation of virulence in the selfed progeny was the recombining of recessive virulence genes hidden in a heterozygous condition. The presence of hidden genes in the 1982 Renville County or nearby rust populations may account for the rapid appearance of virulence to the new cultivars, Olathe and Fleetwood. Moreover, some of the changes from parental fixation for phenotype to polymorphism in the selfed progeny were large, such as for B1400 and B2027 with P24. This indicated either that levels of heterozygosity were high, as is also suggested in a recent literature survey of heterozygosity of several rust fungi (11), or that strong selection occurred for virulent genotypes during the mass-fertilization process. Follow-up studies in progress (McCain et al, *unpublished*) confirm that frequency of heterozygotes in the P24 population for virulence to several differential bean lines is far in excess of expectations from the Hardy-Weinberg equilibrium theory. Other studies using various crossing methods suggest that selection sufficient to skew genetic ratios of allozymes does occur among progeny of *U. appendiculatus* during selfing (15) but not neces-

Table 2. Severity of bean rust in the field at Staples, Minnesota, 1986[a]

Bean line	Score[b]
Aurora	tr
B1259[c]	H
B1263	M
B1527	H
B1554	H
B1556	VL
B1562	tr
B1597	M-H
B1641	0
B1660	M
B1666	0
B1667	0
B1672	M-H
B1757	tr
B1773	0
B1787	H
Brazil-6	H
Brazil-9	VL
Brazil-10	L
Montcalm	L-M
Url	M
UrB	M-H
UrVer	M
643	tr
765	M
7C-119	H

[a] Bean rust from natural infection in the field.
[b] Rust severity score: H = heavy, M = moderate, L = light, VL = very light, tr = trace (1–5 uredinia on all plants), 0 = free of rust.
[c] B. J. Ballantyne (2) rust differential bean line.

Table 3. Frequency of virulence (%) in two mass collections of *Uromyces appendiculatus* and their progeny (from bulk-selfing on Pinto 111) on bean lines

Bean line	Bean rust collection			
	P21[a]		P24	
	Parental collection	Selfed progeny	Parental collection	Selfed progeny
Aurora	00	08[b]	00	03
B1049	00	00	00	00
B1257[c]	00	100	00	00
B1259	100	100	100	100
B1349	09	04	02	41
B1400	00	···	00	52
B1431	66	83	24	52
B1464	54	100	62	100
B1527	62	07	00	51
B1556	01	55	100	100
B1557	81	33	100	69
B1558	100	100	100	100
B1562	89	100	100	70
B1597	65	39	14	60
B1600	00	47	00	00
B1654	00	00	00	00
B1660	68	05	14	37
B1666	00	00	00	00
B1667	00	47	00	00
B1672	63	45	70	61
B1757	00	100	00	17
B1773	00	00	00	00
B1787	100	100	100	100
B1882	00	00	00	00
B2027	100	···	00	58
B7672	45	21	48	···
Brazil-1	73	24	71	85
Brazil-2	100	84	55	44
Brazil-3	81	71	100	64
Brazil-6	100	100	100	100
Brazil-8	100	100	100	100
Brazil-9	80	82	100	100
Brazil-10	74	74	100	63
Bush Blue Lake	21	04	00	14
4C-109	100	100	100	100
Cascade	16	···	00	25
Charlevoix	100	100	100	100
Chief	29	···	100	100
Early Gallatin	18	10	00	21
Executive	17	···	00	10
Fleetwood	20	x[d]	100	100
Golden Gate Wax	100	85	100	100
Kentucky Wonder	100	···	100	100
Kinghorn Wax	27	12	00	06
Montcalm	100	100	100	100
Olathe	36	16	100	100
Pencil Pod Black	11	···	02	07
Redlands Pioneer	100	···	100	87
Roma	53	17	00	13
Royal Red Kidney	100	100	100	100
Seminole	100	···	100	100
Slenderette	11	14	00	09
Slimgreen	100	100	100	100
Tenderette	09	15	00	···
Tendergreen	19	06	00	12
Topcrop	100	100	100	100
Ur-B	00	00	00	07
Ur-C	100	100	100	100
Ur-F	100	100	100	100
Ur-J	100	95	100	60
Ur-Ver	100	100	100	100
Ur-1	00	00	100	100
Ur-2	100	100	100	100
US #3	78	44	99	100
YC-71	100	100	100	100
6Y-218	00	00	00	11
#765	02	16	100	100
#814	100	86	100	100

[a] Collection P21 was made from Pinto 111 beans in 1982, P24 on Agate Pinto in 1986, from Renville County, Minnesota.
[b] Figures are percentage of uredinia, given as the weighted average of two or more inoculations.
[c] B. J. Ballantyne (2) rust differential bean line.
[d] A type of resistant reaction in which flecks and uredinia of various sizes are intermixed.

sarily to favor virulent phenotypes. Two avirulent parental collections yielded selfed progeny that were all virulent. This cannot be explained through simple modes of inheritance, except by invoking the action of selection. Finally, the several instances where 100% virulent collections yielded polymorphic selfed progeny would suggest dominance for virulence.

There were also differences in virulence frequency among the collections from the three 1986 locations. As each field epidemic likely arose from a different founder population of urediniospores or teliospores, it is unlikely the genetic makeup of all three populations would be identical. The 1982 collection differed from the 1986 P24 collection in virulence frequency on 39% (36/92) of the bean lines tested (Table 1). The intrayear variation between P24 vs. P25 and P26 of 29 and 19%, respectively, is less but nevertheless noticeable. (These data are not enough to show, however, that temporal variation is greater than geographic variation, or vice versa.) These differences in time and space suggest that the practice in some disease studies of bulking collections of spores from different parts of a region can cause underestimation of the diversity in the pathogen (19). For example, a pooled inoculation of spores from the collections P24, P25, and P26 would probably have produced a high amount of virulence on bean lines such as B1672 and Ur1, and this would have obscured the avirulence in the spores from the eastern collection site. From the opposite point of view, if bean line B1672 was tested at St. Paul in 1986, it would have appeared to be highly resistant to the races prevalent in the local population of the bean rust fungus. Yet, had this variety been planted in Renville County that same year, it would have become severely diseased. Thus, pooled collections and single-site testing should be avoided.

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