Virulence and Isozyme Differences for Establishing Racial Identity in Rusts of Maize

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ABSTRACT

A new race of *Puccinia sorghi* virulent to maize possessing resistant genes *Rp* ≤, *Rp* ≥, and *Rp* > was found in central Illinois in 1982. This race is designated race 2/*Rp* ≤<sub>2</sub>/<sub>10</sub> distinguish it from race 1, already known throughout the Corn Belt, that is avirulent on maize with these resistance genes. Resistance gene *Rp* ≤ conditioned a chlorotic fleck in seedlings to both races. Illinois races 1 and 2 were only distinguished on differential hosts and were indistinguishable in morphology, spore color, or reproductive growth rates on the plant. Illinois strains of races 1 and 2 were identical for two isozyme patterns, whereas a Wisconsin strain of race 1 was differentiated from these by both acid phosphatase and glucose-6-phosphate dehydrogenase banding patterns. *P. polysora* isozymes were readily distinguished from all *P. sorghi* biotypes.

Common rust of maize (*Puccinia sorghi* Schw.) is found wherever maize (*Zea mays* L.) is grown and generally thrives in the subtropics and highland tropics (I). Resistance is widespread among modern cultivars of maize and in *Tripsacum dactyloides* L. (2,9,10,12) and has been of two distinct forms, specific and general. Use of resistant maize hybrids is the most feasible means of control for common rust. Specific resistance is pathogen race-specific and is characterized by a hypersensitive, fleck-type reaction on the host. Inheritance is commonly monogenic with resistance dominant. At least six loci conditioning resistance have been identified by Hooker et al. (11). The gene *Rp* ≤ has also been transferred into major Hawaiian maize hybrids (5) and more recently into several Corn Belt inbreds (R. R. Bergquist, unpublished). The gene *Rp* ≥ has also been transferred into a Corn Belt inbred (3). General resistance of *P. sorghi* leads to a reduction in number and size of uredia and to reduced leaf chlorosis and necrosis (12,13). This resistance cannot be distinguished in seedlings and is often referred to as “mature plant resistance.” General resistance is believed to be racially nonspecific.

Under comparatively mild rust epiphytoties of the U.S. Corn Belt in past years, many inbreds have expressed good general resistance. Certain maize families, such as B14, have been notoriously susceptible to common rust. Common rust was prevalent throughout the entire Corn Belt, from Iowa and Nebraska in the west to the Carolinas of the eastern United States in epiphytotic proportions in 1982. Uredia began appearing on lower leaves of *Rp* ≥ and *Rp* > genetic stocks in the field near El Paso, IL, in late summer of 1982. Naturally occurring biotypes virulent to plants with genes *Rp* ≤, *Rp* ≥, *Rp* > have been observed in Hawaii (2).

The objective of this study was to examine the extent of virulence of this new biotype on a range of monogenic resistant maize differentials and also to determine whether simple isozyme analysis could resolve *P. sorghi* biotypes from each other and from the tropical rust *P. polysora*. The electrophoretic isozyme technique has been applied to wheat rusts (6).

MATERIALS AND METHODS
Techniques for obtaining and propagating collections of *P. sorghi* and classifying host reactions and types of infection were those conventionally employed for rusts of maize and other crops (7,14). Three collections of *P. sorghi*, designated 75-01, 82-01 (collected at El Paso, IL, in 1972 and 1982, respectively), and Wis-1 (collected at Madison, WI, in 1982) were used in these studies. A single collection of *P. polysora* Underw. (designated PP-1) was obtained from S. King (Mississippi State University) for comparison of isozymes and cultural growth patterns. The PP-1 collection was virulent to all host differentials used in this investigation and differed in time of uredial maturation, color of uredia, and lack of ability to be easily dispersed in growth chamber maintenance. Each collection was clonally propagated in the growth chamber and/or greenhouse at about weekly intervals. Rust collections 75-01 and Wis-1 were host-specific and avirulent to host plants with specific rust-resistant genes used in this study, whereas 82-01 consisted of a mixture of spores produced from an assortment of uredia collected from a deep corn hybrid with a high level of general resistance.

The host differentials used each had a single dominant gene for resistance, *Rp* ≤, *Rp* >, *Rp* >, and *Rp* > whereas a single hybrid with genes *Rp* >/*Rp* > was used for separating racial admixtures. Maize inbred Oh43, with little or no resistance, was used for maintenance of rust collections. Data were taken on number of days from inoculation to first appearance of uredia, color of uredia, and sporulation or lack of on all differentials. Data were recorded when the small white flecks produced 7-9 days after inoculation did not produce spores on resistant host differentials but erupted into uredia after about 7-9 days on susceptible hosts from which spores could be collected. Rust collection 82-01 was serially passed through digenic resistant host Oh43 *Rp* >/*Rp* > three times in 1 mo to remove avirulent forms and then used for comparison of virulence and isozyme differences with race 1 collections.

For isozyme analysis, freshly collected uredospores (10-30 mg) were germinated overnight in a monolayer on water. The germ tubes collected by gentle centrifugation were ground in liquid nitrogen and then extracted in 0.01 M Tris-HCl, pH 8.0, containing 1 mM β-mercaptoethanol (50 µl buffer/10 mg spores). The supernatant obtained after 5 min in an Eppendorf microcentrifuge was adsorbed onto filter paper wicks (10 µl on paper 6 X 3 X 3 mm) and inserted in the sample slot of a horizontal starch gel. A discontinuous borate, pH 8.6, Tris-citrate, pH 8.3, electrophoresis system was used. Further details for this and the isozyme-staining procedure have been described (6,8).

RESULTS
Serial passage of 82-01 through digenic Oh43 *Rp* >/*Rp* > host. A single collection
of *P. sorghi* rust spores (82-01) collected from a commercial hybrid in central Illinois was serially maintained at 10-day intervals on rust-susceptible inbred Oh43 in the growth chamber. Evidence for an admixture of virulent and avirulent rust strains in 82-01 was indicated by expression of both resistant flecks and uredia development when grown on Oh43 *Rp*[^4]/*Rp*[^14]. After three serial passages through host Oh43 *Rp*[^4]/*Rp*[^14], only the novel virulent reaction remained and this new biotype, designated 82-01.1, was used for further tests on monogenic resistant lines and for isozyme analysis.

**Identification of *P. sorghi* races.** Isolates 75-01 (Illinois) and Wis-1, virulent on Oh43 *Rp*[^4]/*Rp*[^14] and avirulent to Oh43 *Rp*[^4], *Rp*[^14], and Oh43 *Rp*[^14], were designated race 1. The new biotype 82-01.1, virulent to all hosts except *Rp*[^4], where it gave a resistant fleck reaction (Table 1), was designated race 2. The resistant fleck of races 1 and 2 on the *Rp*[^4] host differential appeared to be identical.

Susceptible uredia-producing reactions incited by race 2 were indistinguishable regardless of the resistant differential host (i.e. *Rp*[^4], *Rp*[^14], or *Rp*[^14]) in which they were produced and were similar in size, color, and rate of development to race 1. *P. sorghi* uredia typical of susceptible genotypes occurred on lower leaves of corn plants with *Rp*[^4] and *Rp*[^14] in late August 1983 in central Illinois, thus indicating the presence of race 2 in the field.

**Isozyme differentiation of *P. sorghi* rust races.** Two isozyme systems proved useful in differentiating races of *P. sorghi*. They were acid phosphatase (ACP) and glucose-6-phosphate dehydrogenase (G6PD). The two Illinois isolates (75-01 and 82-01.1) had identical isozyme banding patterns for both systems even though they could be clearly differentiated as races 1 and 2 on the basis of virulence on monogenic resistant lines. However, rust isolate Wis-1, which in terms of virulence on monogenic resistant differential, was a race 1 isolate and was distinguishable from either of the Illinois isolates by both isozyme systems (Fig. 1). The intensities of various ACP isozyme bands were markedly different, whereas the faster-migrating G6PD isozyme bands present in Wis-1 were absent from both Illinois isolates.

**Isozyme differentiation of *P. sorghi* and *P. polysora*.** Both isozyme systems differentiated the two species of maize rust (Fig. 1). Under identical conditions of preparation, no ACP isozyme bands were revealed from the *P. polysora* race. More significantly, the G6PD isozyme banding pattern was markedly different from those observed in either the Illinois or Wis-1 isolates of *P. sorghi*.

**DISCUSSION**

In this study, a single field collection from a hybrid with general resistance consisted of an admixture of strains virulent to *Rp*[^4] and avirulent to a host line with these two genes. General resistance apparently did not substantially favor selection of more virulent biotypes in nature over less virulent strains but supported a status quo balance of two biotypes. In three serial passages through digenic host *Rp*[^4]/*Rp*[^14], the 1982 field collection consisting previously of a racial admixture was reduced to a single more widely virulent strain. This more virulent strain of *P. sorghi* did not differ in uredium color or growth rate compared with a less virulent strain on a host with no specific genes conditioning resistance. If these features could be used as criteria and measurement of aggressiveness, that also was not changed as a result of host passage.

However, it is quite notable that the isozyme analysis revealed heterogeneity within the *P. sorghi* isolates. In the central Corn Belt, *P. sorghi* does not infect the intermediate host *Oxalis corniculata* L., thus providing no opportunity for the evolution of new races through genetic recombination. Spores are apparently windblown into the Corn Belt each summer, presumably from southern United States and Mexico, where the pathogen persists on living maize plants (15). The level of genetic heterogeneity in this population is unknown. However, our reproducible observations distinguished the Wis-1 isolate from the two rust isolates collected from El Paso, IL, by just the ACP and G6PD isozyme systems, indicating that this approach using a greater range of rust isolates and isozymes may provide a useful measure of genetic heterogeneity in the *P. sorghi* populations. The identical isozyme patterns for races 1 and 2 isolates collected from the same El Paso locality possibly show that the increased virulence of race 2 evolved from within the race 1 background; however, further analysis would be necessary to permit this conclusion.

**Table 1. Summary of disease reactions of rust isolates on differential maize hosts**

<table>
<thead>
<tr>
<th>Host line</th>
<th>Resistance genes</th>
<th><em>P. sorghi</em></th>
<th><em>P. polysora</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>75-01</td>
<td>82-01.1</td>
</tr>
<tr>
<td>P77</td>
<td><em>Rp</em>[^4]</td>
<td>-</td>
<td>+[^b]</td>
</tr>
<tr>
<td>P70</td>
<td><em>Rp</em>[^14]</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>P78</td>
<td><em>Rp</em>[^4]/<em>Rp</em>[^14]</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Oh43</td>
<td><em>Rp</em>[^4]/<em>Rp</em>[^14]</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Oh43</td>
<td><em>Rp</em>[^14]</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

[^4]: The - reaction is a resistant fleck.
[^14]: The + reaction is a susceptible pustule.

**Fig. 1.** Acid phosphatase and glucose-6-phosphate dehydrogenase isozyme banding patterns of (1) Maize coleoptile, *Puccinia sorghi* isolates (2) Wis-1, (3) 75-01, (4) 82-01.1, and *P. polysora* (5) PP-1.
In general, common rust *P. sorghii* has not been an economically serious problem in the central Corn Belt, and consequently, there has been little use of major dominant resistance genes. However, in some years and particularly in 1982, there was a significant early rust infection, thus causing losses in yield. The combined use of polygenic resistance and specific genes affecting resistance would obviously reduce the magnitude and rust infection (13). However, the use of such a genetic background would favor selection for more widely virulent rust races. Because common rust is initiated from subtropical maize with a general resistance background, a rather consistent and heterogeneous rust population could be expected each summer in the temperate Corn Belt. In the event of an early summer infection, there may be ample time for selection toward more widely virulent strains even though a late-season epiphytoty may not result in a marked shift toward increased virulence in the pathogen population because of insufficient time for racial evolution. In 1982, we observed the appearance of a new rust race that was virulent to hosts with genes *Rp*5 and *Rp*10, which were exposed in field trials. A similar picture has been reported from Hawaii, where sequential deployment of a number of resistance genes has led to the appearance of the corresponding new virulent rust races (2). Taken together, these observations indicate a high level of flexibility in *P. sorghii* for overcoming major gene resistance. Such a capability is well documented in other rust diseases, as for example, the yellow rust of wheat (4) and perhaps indicates that simple deployment of major resistance genes may not be a successful means for control of disease caused by *P. sorghii*.

Limited isozyme analysis clearly distinguished *P. sorghii* isolates from *P. polysora*. This result was not unexpected because these rusts are distinct species and differ in a number of readily observed physiological and morphological traits. For example, besides different spore color, they differed in rate of ureidium development (9 days for *P. sorghii* vs. 12 days for *P. polysora*). *P. sorghii* produced a much greater quantity of spores that were readily dispersed by slight leaf movement, unlike *P. polysora*, for which spores adhered tightly to the ureidium and required physical scraping for collection.

The results of this study have prompted us to question the concept of combining both specific and general rust resistance in population improvement for maize. Occurrence of rust biotypes virulent to *Rp*5/*Rp*10 indicates the fungus has the capacity to shift with changes in levels of host resistance and may indicate a wide range of potential variation in the fungal population.

**LITERATURE CITED**