Components Contributing to Partial Resistance in Maize to *Puccinia polysora*

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ABSTRACT

Zummo, N. 1988. Components contributing to partial resistance in maize to *Puccinia polysora*. Plant Disease 72:157-160.

Four uredinial characteristics (incidence, size, tumescence, and sporulation) of infection of corn (*Zea mays*) by *Puccinia polysora* were studied under greenhouse and field conditions to determine their usefulness in differentiating among corn genotypes for partial resistance to southern corn rust. Evaluation scales were used so that corn genotypes could be compared under divergent environments. Corn genotypes differed significantly in response to the fungus with regard to these characters. There was some variation in expression of these rust components among individual corn genotypes inoculated in the field, humidity chamber, and spore settling tower, but the ranking of genotypes for these components remained constant. Inoculated plants of a susceptible genotype (Pioneer Brand 3369A) consistently had greater numbers of uredinia, which were significantly larger and more tumid and sporulated more readily than those on corn genotypes with partial resistance. The consistent relationship of these characteristics on plants in the field and greenhouse indicates that greenhouse studies could be useful for identifying corn genotypes with partial resistance to *P. polysora*. This would allow screening for resistance to southern rust in areas where epidemics do not normally occur or in years when environmental conditions do not favor rust development in the field.

Puccinia polysora Underw., the incitant of southern corn rust in the United States, has the potential to cause severe crop losses if the disease occurs early in the growing season. King and Scott (5) reported that symptoms of southern rust first appeared at Starkville, MS, during the last 2 wk of July or the first week of August. Corn planted early enough to mature before the first week in August can generally escape injury. In the more humid areas of the Southeast, late-planted corn can be severely injured if environmental conditions become favorable for disease development. Rodriguez-Ardon et al (12) reported that three successive biweekly plantings of susceptible hybrids showed average yield reductions of 4, 23, and 45%. Although genotypes with high levels of resistance have been identified (2,16,19), races of *P. polysora* exist that limit their usefulness (1). Field tests at Starkville in 1983 and 1984 indicated that some corn genotypes infected with *P. polysora* produced fewer, smaller, less tumid uredinia containing fewer urediniospores. Also, uredinia on these partially resistant genotypes did not sporulate as readily as those produced on a susceptible genotype (Pioneer Brand 3369A). Although only a rust-resistant recovery of inbred B37 (B37R) was resistant to infection by *P. polysora*, partial resistance or slow rusting was present in other corn genotypes.

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Accepted for publication 2 September 1987.

Small-grain plants infected with rust have shown quantitative differences in rust development known as slow rusting (6,7,14,15,18). Shaner et al (15) reported that the latent period was longer on a resistant wheat cultivar and uredinia were smaller and less prevalent than those present on cultivars susceptible to *P. recondita* Rob. Johnson and Wilcoxson (4) found that *P. hordei* Fuckel had a shorter latent period and produced more urediniospores per square centimeter of leaf surface, more spores per uredium, more uredia per square centimeter of leaf surface, and larger uredia on fast-rusting than on slow-rusting barley cultivars. Luke et al (6) reported that slow rusting is a form of horizontal resistance to crown rust in oats and is controlled by a small number of genes showing slight partial dominance for susceptibility. They found a high heritability value (87%), indicating that selection for horizontal resistance should be effective. Subrahmanyam et al (17) studied incubation period, infection frequency, pustule diameter, percent ruptured pustules, and percent leaf area damaged in 30 peanut genotypes to determine components of resistance to *P. arachidis* Speg. They found that highly resistant and resistant genotypes had much smaller uredosori than did moderately resistant and susceptible peanut genotypes. Highly resistant and resistant peanut genotypes had very few uredosori ruptured at 30 days after inoculation. Ru-Hong et al (13) described slow-rusting resistance to *P. polysora* in corn based on longer latent period, fewer urediniospores produced per unit leaf area, lower infection frequency in terms of pustule number, and lower infection type.

Pataky (10) reported partial resistance in corn to *P. sorghi* Schw., the causal agent of common corn rust in sweet corn hybrid seedlings expressed as reductions in components of the infection cycle related to *P. sorghi* reproduction (number of lesions, number of sporulating uredinia, size of uredinia, and urediniospore production). Pataky found that time-related components (latent period, infectious period, and sporulating uredinia occurrence in time) did not differ among genotypes for *P. sorghi* infection. Bailey et al (1) reported the identification of slow-rusting resistance to *P. polysora* in maize inbreds and single crosses based on weekly assessments of pustule density to determine the area under the disease progress curve (AUDPC). They indicated that weekly pustule density assessments, if correctly timed, could be as effective for identifying slow rusting as AUDPC.

The objectives of this study were to evaluate four selected rust characters—pustule incidence, size, tumescence, and sporulation—to determine if they could be used to differentiate among corn genotypes for partial resistance to *P. polysora* in the field and greenhouse using numerical evaluation scales suitable for use with a preprogrammed DataMyte, which allows data to be transferred directly to a computer for analysis.

MATERIALS AND METHODS

The corn genotypes used in this study were selected because of their observed reactions to *P. polysora* in the field at Starkville over a number of growing seasons. Pioneer Brand 3369A is one of several commercial hybrids known to be susceptible to *P. polysora*. In naturally infected plants in the field, this genotype produced large uredinia with a high percentage of sporulation. Mp77:179, Mp77:378, and Mp78:25 are inbreds that were developed and released in Mississippi. When evaluated for southern corn rust resistance in the field, these inbreds were considered intermediate in resistance to

P. polysora because, when infected with *P. polysora*, they generally produced fewer smaller uredinia than those produced on 3369A. CI21 is an inbred, moderately susceptible to *P. polysora*, that grows well in the field but still appeared to produce fewer smaller uredinia when infected with southern corn rust than did Pioneer 3369A. B37R is a rust-resistant recovery of inbred B37 that showed a very high level of resistance to infection by *P. polysora* in the field.

Mp77:179, Mp77:378, and Mp78:25 were compared in separate field tests in 1985 and greenhouse tests in 1986 with CI21, hybrid 3369A, and B37R and with each other when inoculated in the spore settling tower in 1986 and 1987.

Inoculum for each trial was prepared from urediniospores collected from corn leaves in the field at Starkville the preceding year. Spores were air-dried at 21 C for 4 days on sheets of aluminum foil, sealed in glass vials, and stored in liquid nitrogen until used. The glass vials with urediniospores were immersed in 500 ml of H_2O at 40 C for 10 min immediately after removal from liquid nitrogen. Spore germination percentage was determined on 2% water agar. Spore concentration was adjusted so that about 0.125 g of viable urediospores per liter of water containing two drops of Tween 20 added as a wetting agent was used for the field and greenhouse tests.

In the field evaluation in 1985, Mp77:179, Mp77:378, and Mp78:25 were each grown with CI21 and hybrid 3369A in a field test in 20-plant rows replicated four times. Each plot consisted of three rows, each 6.1 m long by 1.02 m wide, randomly planted with CI21, hybrid 3369A, and a selected inbred line. A randomized complete block design was used. At the midsilk stage of growth, plants were misted (or irrigated) for 30 min with an overhead sprinkler system 1 hr before sunset. Plants were inoculated with a standardized urediniospore suspension using a Burgess (No. 7) 1-gal yard and garden tank sprayer. The spore suspension was sprayed into the air above the plants and allowed to settle on them. The plants remained moist until the following morning. Plants were evaluated for response to *P. polysora* on the leaf above the ear leaf 18 days after inoculation.

In the greenhouse evaluation in 1986, greenhouse-grown corn plants at the four- to five-leaf stage of growth in greenhouse flats with 15-plant rows were placed in a humidity chamber at dusk and inoculated (as described before) on 24 February. The experimental design was a randomized block with 12 replicates. The plants remained in the humidity chamber at 100% relative humidity until the next morning, when they were moved to the greenhouse bench, where the temperature ranged from 20 to 31 C. Using the fourth leaf from the apex, rust ratings were taken 11 days after inoculation.

In the spore tower evaluations in 1986 and 1987, two seeds of each of the six corn genotypes were planted in the greenhouse in randomized rows in 6×6 Ball Cell-Paks and thinned to one plant per cell 2 days after emergence. The experimental design was a complete randomized block with 12 replicates in the 1986 evaluations and seven replicates in the 1987 evaluations. Seedlings in four cell-packs were placed in the spore settling tower and inoculated by the method described by Melching (7) modified after Politowski and Browning (11) with an aliquot inoculator attached to the side of the settling tower so that 10 ml of suspension containing 2.8×10^6 urediniospores per milliliter was discharged into the air above 144 plants in four cell-packs within 1 min. After spore discharge, the seedlings remained in the settling tower for 10 min, were placed in a dew chamber at 28 C and 100% relative humidity for 24 hr, and then were returned to the greenhouse bench. The spore settling tower was sprayed with water between inoculations to eliminate possible residual urediniospores. Rust determinations were made 10 days after inoculation, using the fourth leaf from the apex.

The 1986 spore settling tower evaluations were made in January, when the greenhouse temperature ranged from 20 to 28 C. The 1987 evaluations were made in July, when the temperature in the greenhouse ranged from 24 to 35 C. Seed of Mp77:179 was lost in 1986; therefore, this genotype was not included in the 1987 spore settling tower test.

Individual rating scales were used to evaluate pustule incidence, size, tumescence, and sporulation so that data could be recorded directly into a DataMyte and transferred directly to a computer without further transcription. Component determinations for each character except incidence were made with a 30× hand lens to which a millimeter scale calibrated in 0.25-mm increments was affixed. Halos around pustules were disregarded in all evaluations.

Uredinial incidence was evaluated by the following scale: 1 = no uredinia; 2 = isolated individual uredinia; 3 = up to 1% of leaf area affected, some uredinia in small scattered clumps; 4 = 1.5–5% of leaf area affected, numerous uredinia, some in clumps; 5 = uredinia distributed over most of the leaf surface, 10–25% of leaf area affected; and 6 = uredinia distributed over entire leaf surface, >25% of leaf area affected.

Uredinial size was determined by measuring the long axis of at least five uredinia per leaf per plant on a minimum of six plants per replicate, and the average from each treatment was converted to the following scale: 1 = 0.2 mm, 2 = 0.3 mm, 3 = 0.5 mm, 4 = 0.8 mm, 5 = 1.0 mm, and 6 = 1.2 mm.

Uredinial plumpness was evaluated by determining the relative tumescence of all uredinia on a leaf and converting to the following scale: 1 = uredinia barely erumpent, basically flat; 2 = uredinia tumescent but height not exceeding 10% of uredinial length; 3 = uredinia tumid, height 10–20% of uredinial length; and 4 = uredinia very tumid, height in excess of 25% of uredinial length.

Uredinial sporulation was evaluated by determining the percentage of uredinia sporulating and converting to the following scale: 1 = no sporulation, 2 = >1–10%, 3 = >10–25%, 4 = >25–50%, 5 = >50–75%, 6 = >75–90%, and 7 = >90–100% of uredinia sporulating.

An analysis of variance was carried out for each test, and the means were separated using Duncan's multiple range test.

RESULTS AND DISCUSSION

Pustule incidence on individual corn genotypes inoculated in the field, spore settling tower, and humidity chamber was consistent relative to corn genotypes in all tests (Table 1). Hybrid 3369A had the highest pustule incidence, whereas B37R remained rustfree. Mp77:179, Mp77:378, and Mp78:25 had significantly lower pustule incidence than hybrid 3369A in all tests. Furthermore, uredinia on hybrid 3369A were larger than those produced on Mp77:378, Mp77:179, and Mp77:378. Uredinia on Mp77:378 were significantly less tumid than those produced on hybrid 3369A in all tests. Sporulation was consistently greater on hybrid 3369A than on Mp77:179, Mp77:378, and Mp78:25. CI21 was generally intermediate between hybrid 3369A and Mp77:179, Mp77:378, and Mp78:25 in pustule incidence, size, tumescence, and rupture. Mp77:378 had significantly fewer, smaller, and less tumid pustules than hybrid 3369A in all tests. There was some variation in expression of these rust components among individual corn genotypes whether inoculated in the field, humidity chamber, or spore settling tower, but corn genotypes could be separated from each other.

Pustule incidence was variable in these tests. This is not totally unexpected. Although the spore load applied as inoculum was adjusted so that uniform levels of urediospores were applied to the different tests, factors such as temperature, air movement, and relative humidity could not be completely controlled. In 1986, field tests inoculated with southern rust had to be abandoned when extremely low humidity resulted in failure to obtain infection despite large-scale attempts to raise the humidity. In spite of this variability, relative pustule incidence in Pioneer 3369A was always significantly higher than in all other genotypes tested. However, none of the corn genotypes tested in this study approached the level of resistance found in B37R.

Uredinial size was relatively consistent among the genotypes tested. Uredinia produced on hybrid 3369A were significantly larger than uredinia produced on all other genotypes except CI21 and Mp78:25. Uredinia produced in the greenhouse were larger than those produced in the field. Observations indicated that uredinial tumescence or plumpness played an important role in partial resistance because plumper uredinia produced and liberated more spores than flatter ones.

More of the uredinia produced in the greenhouse sporulated than did uredinia produced in the field; however, the relationship between slow-rusting and non-slow-rusting genotypes remained constant. This suggests that sporulation was influenced by the ambient environment.

Previous studies indicated that plant growth stage could affect the expression of components of slow rusting in wheat (8) and asparagus (3). Pustule size was limited on two wheat cultivars when plants were inoculated between flag leaf emergence and flowering, whereas pustule size was limited least when inoculations were made just after flowering. Observations over a 3-yr period indicated that partial rust resistance in corn was not affected by stage of plant growth. This is supported by the findings of King and Scott (5) who determined that rust severity was somewhat delayed on upper corn leaves because of environmental factors rather than resistance to the pathogen. Susceptibility of lower leaves to *P. polysora* did not vary significantly with maturity of the plant.

Data on incubation period were not included in these tests because incubation period varied more among tests than among genotypes. Pataky (10) also found that time-related components (latent period, infectious period, and sporulating uredinia in time) for *P. sorghi* did not express differences among five sweet corn hybrids. Parlevliet (9) reported that the latent period in four barley cultivars inoculated with *P. hordei* at various plant growth stages increased from the primary leaf to the young flag leaf stage for all cultivars, then decreased again. The differences in latent period among cultivars are small in the seedling stage and larger in the adult stage.

What often appeared to be flecks to the naked eye, when examined under 30×, actually were small flat uredinia that occasionally produced a few urediniospores (fewer than 100). Some corn genotypes produced very small (0.1 mm) uredinia which, when dissected in the laboratory, showed some fully developed urediniospores. These uredinia, which rated 1 on the tumescence scale, generally did not sporulate or release urediniospores until after collapse of the host tissue. Data on telia and teliospore production in the field are not presented because the role of teliospores in the disease cycle is not known.

The rating scales used in these studies to evaluate the pustule characteristics permitted separation of corn genotypes in regard to components of partial rust resistance under divergent environments. Corn genotypes differed significantly from each other with regard to these characters in response to infection by the fungus. The individual component rating scales also proved to be useful in recording data directly onto a preprogrammed DataMyte so that data could be transferred directly to a computer for analyses.

The consistent relationship of the resistance components evaluated in the field and greenhouse indicate that these

Table 1. Southern corn rust uredinial incidence, size, tumescence, and sporulation on corn genotypes in three experiments inoculated with *Puccinia polysora* in the field, greenhouse, and spore settling tower at Starkville, MS, in 1985, 1986, and 1987

Genotype	Incidence[v]	Size[w]	Tumescence[x]	Sporulation[y]
		Field evaluation (1985)		
Test 1				
Pioneer 3369A	3.66 a[z]	3.09 a	2.98 a	5.70 a
CI21	2.57 a	2.72 a	2.38 a	4.12 a
Mp78:25	0.44 b	1.41 b	1.13 b	1.22 b
B37R	1.00 c	1.00 c	1.00 c	1.00 c
Test 2				
Pioneer 3369A	3.71 a	3.11 a	2.91 a	4.48 a
CI21	1.41 b	2.21 ab	2.09 ab	2.05 b
Mp77:179	1.21 b	1.61 b	1.50 b	1.38 b
B37R	1.00 c	1.00 c	1.00 c	1.00 c
Test 3				
Pioneer 3369A	3.61 a	3.33 a	2.92 a	5.17 a
CI21	1.64 b	2.12 b	1.91 b	2.77 b
Mp77:378	0.48 b	1.37 b	1.37 b	1.36 b
B37R	1.00 c	1.00 c	1.00 c	1.00 c
		Greenhouse evaluation (1986)		
Test 1				
Pioneer 3369A	5.70 a	4.26 a	3.86 a	6.96 a
CI21	4.75 b	2.97 b	2.78 b	6.22 a
Mp77:378	4.31 b	1.66 c	1.64 c	6.43 a
B37R	1.00 c	1.00 d	1.00 d	1.00 b
Test 2				
Pioneer 3369A	5.58 a	5.07 a	3.36 a	6.86 a
CI21	5.00 b	4.08 a	3.37 a	6.21 b
Mp78:25	4.67 c	3.09 a	1.45 b	6.37 b
B37R	1.00 d	1.00 b	1.00 c	1.00 c
		Settling tower evaluation (1986)		
Pioneer 3369A	4.17 a	3.75 a	2.75 a	4.50 a
Mp78:25	3.08 b	3.42 ab	2.67 a	4.25 ab
CI21	3.17 b	2.83 bc	2.50 ab	3.50 bc
Mp77:378	2.92 b	2.75 bc	2.17 b	3.25 c
Mp77:179	3.33 b	2.33 c	2.58 ab	4.67 a
B37R	1.00 c	1.00 d	1.00 c	1.00 d
		Settling tower evaluation (1987)		
Pioneer 3369A	5.07 a	5.11 a	3.71 a	6.91 a
Mp78:25	3.53 b	2.63 c	2.93 b	4.71 b
CI21	3.89 b	3.46 b	3.00 b	5.77 b
Mp77:378	3.86 b	3.07 bc	2.50 b	5.23 b
B37R	1.00 c	1.00 d	1.00 c	1.00 c

[v] Scale of 1–6, where 1 = no uredinia; 2 = isolated individual uredinia; 3 = up to 1% of leaf area affected, some uredinia in small scattered clumps; 4 = 1.5–5% of leaf area affected, numerous uredinia, some in clumps; 5 = uredinia distributed over most of the leaf surface, 10–25% of leaf area affected; and 6 = uredinia distributed over entire leaf surface, >25% of leaf area affected.

[w] Determined by measuring long axis of at least five uredinia per leaf per plant on at least six plants per replicate and converting the average from each treatment to a scale of 1–6, where 1 = 0.2 mm, 2 = 0.3 mm, 3 = 0.5 mm, 4 = 0.8 mm, 5 = 1.0 mm, and 6 = 1.2 mm.

[x] Evaluated by determining relative tumescence of all uredinia on a leaf and converting to a scale of 1–4, where 1 = uredinia barely erumpent, basically flat; 2 = uredinia tumescent but height not exceeding 10% of uredinial length; 3 = uredinia tumid, height 10–20% of uredinial length; and 4 = uredinia very tumid, height >25% of uredinial length.

[y] Evaluated by determining the percentage of uredinia sporulating and converting to a scale of 1–7, where 1 = no sporulation, 2 = >1–10%, 3 = >10–25%, 4 = >25–50%, 5 = >50–75%, 6 = >75–90%, and 7 = >90–100%.

[z] Means within a test and within a column not followed by the same letter differ significantly at $P = 0.05$ according to Duncan's multiple range test.

components could be useful for identifying corn genotypes with partial resistance to *P. polysora* in the greenhouse. This would allow screening for resistance in areas where southern rust epidemics do not normally occur or in years when environmental conditions do not favor rust development in the field.

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