A Study of Race Populations of Puccinia recondita f. sp. tritici

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Portion of a thesis submitted by the junior author in partial fulfillment of the requirements for the M.S. degree, Oklahoma State University, Stillwater.

Journal Series Paper No. 3125, Agricultural Experiment Station Project 1224, Oklahoma State University.

Accepted for publication 9 November 1976.

ABSTRACT

YOUNG, H. C., JR., and J. M. PRESCOTT. 1977. A study of race populations of Puccinia recondita f. sp. tritici. Phytopathology 67: 528-532.

Uredial samples of the wheat leaf rust fungus were collected from a susceptible cultivar at 20 sites in Oklahoma. From 647 isolates of that uredial population eight races based on Unified Numeration (UN) differential cultivars, or 17 races based on North American Wheat Leaf Rust Research Workers Committee (NA65) differential cultivars were found. Telial samples from the same uredial population were used to produce aecia on *Thalictrum speciosissimum* or *T. dasycarpum*. Identification of races of the resulting uredia produced 24 races based on UN differentials and 25 races based on the NA65 differentials. Thus, in the original uredial population in the field, most of the genes conditioning avirulence must have been heterozygous since the uredia from the aecia either were F_1's or were the result of selfing.

Puccinia recondita Rob. ex. Desm. f. sp. *tritici* Eriks., the fungal pathogen that causes leaf rust of wheat, exists on wheat as numerous morphologically similar, but pathogenically distinct, races. Variation in pathogenicity may derive from mutation, hybridization, heterocaryosis, or parasexualism (9, 15, 16).

The gametophytic stage of this fungus occurs on species of *Thalictrum*, *Isopyrum*, and *Anemonella* in the Ranunculaceae, and on *Anchusa* in the Boraginaceae (6, 8, 14, 17, 20). Only species of *Thalictrum* and *Anemonella* grow in abundance in the U.S.A. and these are more resistant to *P. recondita tritici* than exotic ones. This may account for the rarity of naturally-occurring pycnial and aecial stages of the fungus in the U.S.A.

Brown and Johnson (5) collected teliospores on wheat straw from two locations in Canada and induced them to germinate by an alternate wetting and drying treatment. The sporidia were seeded on several exotic species of *Thalictrum* of known susceptibility. Aeciospores produced on these hosts gave rise to 36 uredial cultures; six were of races not found in fields from which the telial collections were made. On the other hand, certain races common in field collections of urediospores were not represented in the isolates from aecia. They concluded that sexual recombination occurred on the alternate host and that production of new or different races may arise from infections on *Thalictrum* sp.

In Portugal *Puccinia recondita tritici* commonly infects its alternate host in nature (17). In Spain, where infection of *Thalictrum* sp. is common, the race population identified on the Unified Numeration (UN) differential cultivars (2) for 1961-1965 consisted of 16 of the possible 32 races (22).

Natural infection of *Thalictrum* spp. by the wheat leaf rust fungus in the U.S.A. apparently is rare. The first report was by Levine and Hildreth (13) who found a naturally infected plant of *Thalictrum dioicum* L. in Minnesota; uredial isolates produced from the aecia behaved on differential cultivars (14) as a typical isolate of the prevalent race 2. Later, Young et al. (28) reported that inoculation of wheat with aecial collections from *Thalictrum dasycarpum* Fisch. & Lall. at Lyons, Colorado, produced a low percentage of uredial development. Cultures derived from those uredia possessed no new pathogenicity based on their differential cultivars (1).

Young (H. C. Young, Jr., *unpublished*) found that among uredial collections made in Oklahoma from 1950 to 1965, only eight races could be consistently identified on UN differential cultivars. This has been confirmed by results from other surveys made in the U.S.A. and Canada (10, 11, 12, 23). Such surveys, if based on a random sample of the population of the rust fungus, are useful indicators of the pathogenicity combinations prevalent in certain areas, and the genes required in wheat breeding programs.

The present study was designed to determine the degree of heterozygosity existing in the races that had evolved in Oklahoma where host selection for virulence was minimal. This was done by comparing combinations of pathogenicity (races) in the natural uredial population with those found in aecia derived from that population.

MATERIALS AND METHODS

Previous studies by Young (H. C. Young, Jr., *unpublished*) using UN differentials indicated that the first 100 random samples from a given uredial population

would reveal the principle races in that population, and the approximate percentage of each. However, that small a sample does not reveal the rare races, and so approximately 500 isolates were made from each population. We used the UN series of five cultivars and the North American Wheat Leaf Rust Research Workers Committee (NA65) series of five cultivars (27). We also used 14 additional wheat cultivars, the majority of which comprised the "Universally Resistant" and "Test Cultivar" classes established by the North American Wheat Leaf Rust Research Workers Committee (1, 27) to determine if new pathogenicity had occurred.

The methods devised for planting and inoculating the differential sets were described earlier (19). Leaf rust-infected leaves of the wheat cultivar Triumph (C. I. 12132), susceptible to all races of the pathogen in the field, were collected at many sites throughout Oklahoma. Each collection was kept separate in glassine packets and stored at 4 C. Upon removal from storage, leaves from each packet were used to brush-inoculate (4) leaves of wheat cultivar Cheyenne (C. I. 8885). Before and after inoculation, the plants were sprayed with a solution containing two-to-four drops/1,000 ml of the surfactant polyoxyethylene sorbitan monolaurate (Tween-20), placed in a moist chamber overnight, then moved to isolation chambers in a greenhouse at 20 ± 3 C. When leaves began to show flecking (3-5 days later), individual leaves were detached and placed in plastic petri dishes partitioned into two compartments. The basal ends of the leaves were submerged in a solution of benzimidazole (20-40 μg/ml) contained in one of the two compartments. The tip end of the leaf, supported by the petri dish divider, was suspended over the other compartment. This method was a modification of one devised by Browder (3).

Urediospores from a single, isolated uredium on an excised leaf were collected 7-10 days after detachment in a No. 00 gelatin capsule by means of a modified cyclone separator (25). Acetone was drawn through the separator after each collection to kill and remove spores adhering to it, then allowed to evaporate before the separator was used again. Urediospores collected in gelatin capsules were stored at about 4 C until used. Enough urediospores to inoculate 24 differential wheat cultivars were obtained in this way (19).

Plant material with telia was collected from four wheat cultivars (Bison, C. I. 12518; Triumph, C. I. 12132; Wichita, C. I. 11952; and Fulcaster 6121, C. I. 4862; each susceptible to all known races of the pathogen) from several locations in Oklahoma in June and stored at room temperature (25-30 C) until used in October. At that time, several samples of the telia-bearing plant material were soaked in water for 24 hr, then placed wet on several layers of cheese cloth and allowed to dry for 48 hr. The leaf-cheese cloth mat then was inserted between two layers of wide-mesh (~1 square per centimeter) screen wire and hung from the top of a 0.6 × 0.6 × 1.2-m plastic chamber suspended directly above young plants of *Thalictrum speciosissimum*, Loefl, an exotic species, or *T. dasycarpum*, a native species. The chamber was held in a greenhouse at approximately 19 C. Production of sporidia was stimulated by alternate wetting and drying of the telial mat; i.e., misted in the chamber with water plus a surfactant (Tween-20) until the mat appeared uniformly wet and small water droplets formed on the *Thalictrum* spp. leaves below, sealed in the chamber for at least 12 hr (usually overnight), then dried by opening the chamber for 10-20 hr before the next wetting. This was continued for 7-10 days, or until infection was observed on the plants of *T. speciosissimum* and/or *T. dasycarpum* which then were transferred to a bench in the same greenhouse. Within 1 wk, pycnial infections developed to the point that nectar could be intermixed.

A camel's-hair brush was used to thoroughly mix the pycnial nectar among all infections. Because the rate of pycnial development was variable, it was necessary to mix nectar on several successive days to insure fertilization of all pycnia. Aecia appeared 8-10 days after pycnial fertilization. The heaviest pycnial infection usually occurred on the leaves, but occasionally it occurred on stems, petioles, peduncles, and even floral parts as noted by Saari et al. (21).

Leaves bearing aecia were excised from the plant when

TABLE 1. The percentage of various "Unified Numeration" races of *Puccinia recondita tritici* identified from a sample of uredia from only sporophytic generations in the field compared with a sample derived after one gametophytic generation on *Thalictrum dasycarpum* and *T. speciosissimum* in the greenhouse using teliospores from the same field population

UN[a] race	Isolates from: Only sporophytic generations (%)	Isolates from: One gametophytic generation (%)	UN[a] race	Isolates from: Only sporophytic generations (%)	Isolates from: One gametophytic generation (%)
1	17	11	14	0	1
2	50	16	16	0	1
3	7	3	17	0	2
4	0	2	18	0	2
5	11	13	19	0	2
6	3	2	20	0	7
7	0	3	21	0	1
9	9	2	22	0	1
10	0	1	23	0	10
11	1	1	25	0	5
12	0	1	26	0	2
13	2	8	27	0	3

[a]A classification system devised by Basile. (2).

the peridium of the aecial cup began to rupture. Leaves then were floated on tap water in a 125-ml beaker with the aecia toward the water surface and the aeciospores were allowed to shower onto the surface of the water. Within 2 hr, enough spores had usually discharged to color the surface of the water yellow-orange. The leaves of *T. speciosissimum* or *T. dasycarpum* were then removed from the beaker and discarded. Pots with 20-25 seedling plants of wheat cultivar Cheyenne (C. I. 8885) were inoculated by dipping the wheat leaves into the beaker containing the aeciospores. The inoculated plants were misted with water and a surfactant (Tween-20), left overnight in a moist chamber, and then placed in isolation chambers in a greenhouse where the temperature was 20 ± 2 C. Infected leaves were detached and handled as described earlier.

The process and equipment used to inoculate the differential sets were those described by Prescott and Young (19). The inoculated differential sets were held in moist chambers overnight and then placed in growth chambers maintained at 20 ± 1 C. The photoperiod was 12 hr at 20,700 lux.

Infection types were recorded 10-12 days after inoculation. The information was recorded directly on computer data cards using a Wright Punch (Model 2600). Numbers designating infection types "1", "2", "3", and "4" of Stakman et al. (24) were retained, but "9" was used in place of "0;" to designate the "fleck" infection type; "5" was used in place of "X" for mesothetic infection type and "6" was used in place of "Y" to designate the infection type described by Johnston (10). A two-digit system was employed to accomodate the variation in infection that often occurred. Thus, a "fleck" infection type was recorded as "90", and a range of "0;" to "1" infection types was recorded as "91", etc.

RESULTS AND CONCLUSIONS

Sample of uredia from the field.—Eight of the 32 possible UN races were found among 647 single uredial pustule isolates (Table 1). This compares favorably with previous surveys in Oklahoma (H. C. Young, Jr., *unpublished*). Approximately 50% of the isolates were UN race 2, 18% were UN race 1, and 11% were UN race 5. Other races in order of prevalence were: UN races 9, 3, 6, 11, and 13.

Seventeen of the 32 possible NA65 races were found (Table 2); NA65 races 1 and 10 were by far the most common.

Sample of uredia derived from aecia developed on Thalictrum spp.—Aecial infections on *T. dasycarpum* and *T. speciosissimum* were used to provide uredial collections from this source. Races identified from isolates derived from these two species appeared similar and data from both sources therefore were combined. Race identification was completed for 766 isolates.

Pathogenicity in the sample derived from *T. speciosissimum* and *T. dasycarpum* differed from that in the field. The eight UN races found in the field also were obtained from *T. speciosissimum* and *T. dasycarpum* as well as 16 additional UN races. Of the 32 possible UN races, only 27 have been identified from uredial collections made in the field anywhere in the world (11). Of the five UN races still not found in nature, none was among isolates derived by hybridization and/or selfing on *T. speciosissimum* or *T. dasycarpum* in our study. Fifty percent of the entire race population in the field was UN race 2 and, if most isolates were homozygous, it would be expected to be among the most common races derived from the *Thalictrum* species if much selfing occurred. Only 15% of the isolates from the *Thalictrum* spp. were UN race 2 but, together with UN races 1 and 5, it was among the most commonly identified races. It would be expected that UN race 1 would be common among the isolates from the *Thalictrum* spp., since it has no pathogenicity on any of the differentials used and would result as F_1 from crosses of races homozygous at different loci. Ten percent of the isolates from the *Thalictrum* spp. were UN race 23, but that race was not found in the field in our study. This race, although originally identified in

TABLE 2. The percentage of various "NA65" races of *Puccinia recondita tritici* identified from a sample of uredia from sporophytic generations in the field compared with a sample derived after one gametophytic generation on *Thalictrum dasycarpum* and *T. speciosissimum* in the greenhouse using teliospores from the field sample

NA65[a] race	Isolates from: Only sporophytic generations (%)	Isolates from: One gametophytic generation (%)	NA65[a] race	Isolates from: Only sporophytic generations (%)	Isolates from: One gametophytic generation (%)
1	28	14	17	5	1
2	1	0	18	1	1
3	7	5	19	1	2
5	1	0	21	0	1
6	0	1	23	0	1
8	0	1	25	6	2
9	16	13	26	6	5
10	19	9	27	3	3
11	6	6	28	2	5
12	3	3	29	0	3
13	1	2	30	0	2
14	1	6	31	0	3
15	0	2	32	0	7
16	0	2			

[a]A classification system devised by the North American Wheat Leaf Rust Workers Committee (1, 27).

the United States, is rarely found in nature (11).

Pathogenicity in the sample derived from *Thalictrum* spp. as characterized by the NA65 differential cultivars, also differed from that found in the field. Twenty-five of the 32 possible races were identified compared with only 17 from the field. Field isolates produced NA65 races 2 and 5, but these races were not isolated from the *Thalictrum* spp. The *Thalictrum* spp. isolates produced NA65 races 6, 8, 15, 16, 21, 29, 30, 31, and 32, none of which was isolated from the field. Four NA65 races comprised 43% of all isolates identified from the *Thalictrum* spp. source, and three of these (races 1, 9, and 10) were also the most commonly identified races in the field. The fourth, NA65 race 32, comprised over 7% of the isolates from the *Thalictrum* spp., but was not identified from field material. Race 32 is the race of "universal virulence" (18) in this host-parasite system and would have genes for pathogenicity at least five loci, probably more since some of the differential cultivars are known to have more than one gene for reaction.

Among the 14 additional differential cultivars used in this study, five: Agatha, a selection of a complex 'Supressa' cross, Agrus, Agent, and Transfer, were not infected with any isolates regardless of the source. Two additional cultivars or selections, a selection of the cross Kanred by Hard Federation, and Wanken 2 were not infected by any of the isolates obtained from the field, but were infected with a few isolates obtained from the *Thalictrum* spp.

A few isolates derived from the *Thalictrum* spp. were not pathogenic on the cultivar Cheyenne, which has been used as a "universal suscept" (18) for increasing cultures of *P. recondita tritici*. The greatest differences between isolates from the field and from the *Thalictrum* spp. was on cultivar Waban 2; only 2% of the isolates from the field compared to 30% of the isolates from the *Thalictrum* spp. were pathogenic to Waban 2.

Our results indicate that eight UN races were originally present in the telial-bearing straw used in the studies on two *Thalictrum* spp. The fact that 24 UN races were identified after passing the organism through the alternate host, versus only eight races in the field collections indicates that sexual recombination occurred on the alternate host. If pathogenicity is generally recessive in this pathogen (7), then considerable heterozygosity must exist since we tested only F_1 or selfed isolates. Since over 90% of the wheat acreage in Oklahoma was seeded with cultivars susceptible to all races of *P. recondita* prevalent at the time of this study, then races present in the field must be selected on some basis other than specific pathogenicity. Under these conditions, which involve host, pathogen, and environment, heterozygosity may be useful to the organism in aggressiveness and, therefore, contribute to natural selection. However, stabilizing selection (26) must influence the population since UN races 1 and 2, and NA65 races 1 and 10 were the most prevalent in the field population and are among the races with the fewest genes for pathogenicity.

The study also indicates that the alternate host virtually is nonfunctional in nature, since none of the 16 additional races derived from infections on the *Thalictrum* spp. is normally encountered in the *P. recondita tritici* population of the Oklahoma area. In any population in which the alternate host functions only occasionally, as in this study, the number of races encountered should be higher than in a population where the alternate host does not function at all. One race having at least five genes for pathogenicity, namely UN race 13, was identified more often from the *Thalictrum* spp. sample than the field sample. If genes for pathogenicity in the pathogen are commonly heterozygous, as this study has indicated, then it would be expected that such races would occur as a result of recombination in the sexual cycle on *Thalictrum* spp. Similarly, NA65 race 32 constituted 7% of the *Thalictrum* spp. sample and was not found in the field.

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