A STUDY OF VARIATION IN RACE POPULATIONS OF *PUCCINIA RECONDITA* F. SP. *TRITICI*

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TABLE OF CONTENTS

LIST OF TABLES

Table | Page

LIST OF FIGURES

INTRODUCTION

Puccinia recondita Rob. ex. Desm. f. sp. tritici, the casual agent of the leaf rust disease of wheat, is an obligate parasitic fungus, characterized in its gramineous host relationship by a pronounced specialization in pathogenicity. The consequence is the existence of numerous morphologically similar but parasitically distinct biotypes. Various mechanisms are involved in production of these biotypes. It has been shown that mutation, hybridization, heterocaryosis, or parasexualism may contribute to variation in the cereal rusts (14, 21, 22). Having arisen by one of these means, natural selection pressure allows the pathogen to adapt to the host population, thus conditioning survival.

Puccinia recondita f. sp. tritici is a pleomorphic, heteroecious, long cycle rust with its sporophytic generation found on species of the genus Triticum in the family Graminese. The alternate host carrying the gametophytic stage of the wheat leaf rust fungus was shown to be in the Ranunculaceae family. Species of the genera Thalictrum, Isopyrum and Anemonella have all been shown to condition a susceptible response when inoculated with the leaf rust of wheat (6, 13, 30). Of these hosts, only species of the genus Thalictrum are found in any abundance in the United States.

Natural infections on Thalictrum by the wheat leaf rust fungus in the United States have been reported only rarely (19, 35). However, the finding of these infections has established that hybridization can

occur in nature and that there is a possibility that new combinations of virulence may arise in this manner. The study reported here was designed to investigate what role, if any, sexual recombination on the alternate host Thalictrum plays in the origin of new virulence or new combinations of virulence. At the same time the study was designed to reveal whether hybridization on the alternate host would produce combinations of virulence not ordinarily found in the natural uredial population.

LITERATURE REVIEW

Experiments involving hybridization in leaf rust of wheat were made possible by the discovery, in 1921, of the alternate host of this pathogen. Jackson and Mains (13), simulating as closely as possible natural conditions, screened many different genera of the family Ranunculaceae with massive sporidial inoculations. Only the genus Thalictrum proved to be susceptible, with the degree of susceptibility varying with the species. The exotic species proved to be more susceptible than did the native species tested, which may account for the apparent rarity of naturally occuring pycnial and aecial stages of P. recondita tritici in the United States.

Later, two other genera of the Ranunculaceae were reported to be susceptible to the leaf rust of wheat. Mains (20), in 1932, showed Anemonella thalictroides would condition a somewhat susceptible reaction to leaf rust of wheat. In 1937, Bryzagalova (6) reported that Isopyrum fumarioides was also susceptible to the gametophytic stage of wheat leaf rust in the Crimea.

When the alternate host was first found, however, the complete life cycle of the leaf rust of wheat was not fully understood. No one really knew what role the alternate host played in the life cycle of any of the cereal rusts. The function of the pycnia and the pycniospores was not known until 1927 when Craigie (8, 9) showed that the pycniospores function in fertilization; that is, in the establishment of the binucleate condition at the base of the aecium. Later he proved

(10) that when the pycniospore-containing exudate of a monosporidial infection is applied to many other such infections, aecia would develop in only about one-half of the infections receiving the exudate. From this fact he inferred the existence of two mating groups in the pycnia and in the uninucleate mycelia on which they are formed. He also found that of the four sporidia produced by a telispore, two belong to one mating group and two to another. His work formed the basis for the many genetic studies of the rust fungi which followed. Most of the early reports on hybridization were from investigations of Puccinia graminis Erikss. and Henn. on the alternate host barberry (Berberis vulgaris L.). Waterhouse (33), reported that two new physiologic forms of P. graminis tritici were obtained in Australia from crosses made on barberry. Newton, Johnson, and Brown (24) found that when certain physiologic forms were selfed on the barberry, segregation took place in the first generation with the production not only of some old forms but also certain new ones. They reported (14, 15, 23) that when race 9 and race 36 were used as parents, the resulting F_1 generation produced only race 17. When the F_1 generation was selfed the F_2 generation yielded the original parents, races 9 and 36, and also races 1, 11, 15, 17, 52, and 57. Genes governing virulence on different varieties of wheat appeared to be inherited independently. In crosses involving two different races, Johnson (14) stated that it was not uncommon to obtain races that had a broader range of virulence than either of the parent races. He also stated that hybrid races did not usually possess characteristics entirely foreign to the parent races, i.e. characters not shown either by the parent races themselves or by the progenies of their selfing.

Attempts to determine whether Thalictrum has an active function in the life cycle of the leaf rust of wheat under natural conditions have generally yielded negative results. However, Levine and Hildreth (19) reported finding a naturally infected specimen of Thalictrum dioicum in Minnesota. Aecial isolates produced an uredial infection on wheat and identification of this culture on the standard wheat leaf rust differential varieties (20) indicated a typical isolate of race 2. This was the first report of natural infection on Thalictrum in the United States. Young, et al. (35) reported that aecial collections from Thalictrum at Lyons, Colorado produced a low percentage of uredial cultures on wheat. These cultures did not possess any new virulence or any new virulence combinations detectable on differential varieties which were currently being used (1).

Brown and Johnson (5), using telial infected wheat straw, collected from two different locations in Canada, induced telial germination by alternate wetting and drying and then seeded the sporidia on several species of Thalictrum known to be susceptible. Aeciospores derived from these hosts gave rise to 36 uredial cultures. Identification of these cultures yielded six races not found in the field from which the telial collections were made. On the other hand, certain races common in the field collections were not represented in the isolates from aecia. They concluded that sexual recombination did occur on the alternate host and that production of new or different races may arise from infections on Thalictrum. They also found certain aecial isolates which produced red, yellow, and orange-yellow urediospores that were almost avirulent on the wheat variety Little Club (C.I. 4066) which they were using for uredial increase. These cultures were pathogenically

so weak on Little Club, and several other wheat varieties tested, that they were lost before any congenial host could be found.

Selfing of individual races also was studied by Brown and Johnson (5). They found that when race 3 was selfed on Thalictrum and the aecial derived urediospores were identified, they had obtained 14 races in the first generation, three of which had not been previously described from field-collected urediospores. Selfing of race 5A yielded only three races, while selfing of race 76 yielded five races. These data enabled them to postulate several possible homozygous and heterozygous relationships among the races of leaf rust of wheat studied.

It is reported that P. recondita tritici commonly infects its alternate host in nature in some parts of the world (25). Salazar (28) reports that in Spain where infection on Thalictrum is common, the "Unified Numeration" (3) race population for the period 1961-1965 was comprised of 16 of the possible 32 races. In the central plains area of the United States, however, where the alternate host is rarely infected (27), Young (Personal communication) has reported that for the past 15 years, only eight "UN" races have been consistently identified in Oklahoma. Johnston (16), reporting surveys conducted by the U. S. Department of Agriculture, and Samborski (29), reporting the results of surveys conducted in Canada, have obtained similar results. The data from leaf rust surveys (16, 18, 29), if based on a random sample of the rust population, can yield valuable information for the plant breeder and the pathologist. Interpretation of these data indicate which races or virulence combinations are prevalent in certain areas, and point out the genes for resistance required in breeding programs. Recently,

Persons (26) and Schafer (30) have proposed new methods of analyzing the same data which will indicate the association of genes for resistance in the varieties used as differentials in culture identification programs. Such analyses will assist in identifying usable combinations of genes for resistance, in utilizing the most efficient differential varieties, and in pointing out associations of virulence in the parasite.

MATERIALS AND METHODS

Planting of Differential Varities

Identification of races of leaf rust of wheat present in uredial collections from the field and uredial collections derived from aecial infections on Thalictrum requires the use of a minimum of ten varieties of wheat (3, 34). The possibility that new virulence types might be found in this study prompted the use of 14 additional wheat varieties, the majority of which comprise the "Universally Resistant" class of the North American Wheat Leaf Rust Research Workers Committee (34). The varieties used are listed in Table 1.

The most common method of handling wheat leaf rust differential sets (in pots) was inadequate for this study, due to the large amount of controlled environment space required, and the time and labor involved. A new method was devised that utilized 6 plant bands (Vita Band No. 10, $2\frac{1}{2}$" x $1\frac{1}{2}$" x $1\frac{1}{2}$") partitioned into 3/4 inch square cubicles and held together with a metal clip (Figure 1). The resulting plant container requires an area of only 13 1/2 sq. inches of controlled environment space and will accomodate 3 to 5 plants of each of the 24 wheat varieties used.

Hand planting such a container would have been impossible to accomplish satisfactorily, so a machine was built that would plant 3 to 5 seeds of each differential variety in the proper compartment of the plant container (Figure 2). The machine consists of 24 triangular seed

TABLE I

WHEAT VARIETIES AND SELECTIONS USED TO DIFFERENTIATE VIRULENCE IN PUCCINIA RECONDITA TRITICI

Variety or Selection	C. I.[a] or Selection No.	Abbreviation[b]
Webster	3780	Wst
Loros	3779	Ls
Mediterranean	332	Mi
Democrat	3384	Do
Dular	13373	Dlr
Sinvalocho	12096	Svl
Exchange	12635	Ech
Wesel	13090	Wsl
Lucero	14047	Lco
Agatha	14048	Aa
Kanred x Hard Federation	STW63R5003	Kfd*
Sps-Fz x Kv-Fz-Hg-Ill-Wbs-Tb^3-Hope-Hs	STW61R8533	Sft*
Cheyenne	8885	Cnn
Malakof	4898	Ma
Lee	12488	Lee
Waban 2	14018	Wbn 2
Westar	12110	Wtr
Agrus	13228	Ars
Agent	13523	Agt*
Transfer	13483	Tf
Aniversario	12578	Aiv
Wanken 2	14049	Wkn 2
Tst-Hry x Pn	STW60R7950	Tp*
Wardal 2	13628	Wd 2

[a] C. I. numbers are assigned by the Cereal Crops Research Branch, Crops Research Division, Agriculture Research Service, U. S. Department of Agriculture.

[b] Abbreviations used were made according to rules adopted by the National Wheat Improvement Committee. See Agron. J. 52:613, 1960, and U. S. Dept. of Agr. Tech. Bull. 1278, p. 131, 1963. The exceptions are those marked with an asterisk (*).

Figure 1. Plant container arrangement for 24 wheat leaf rust differential varieties

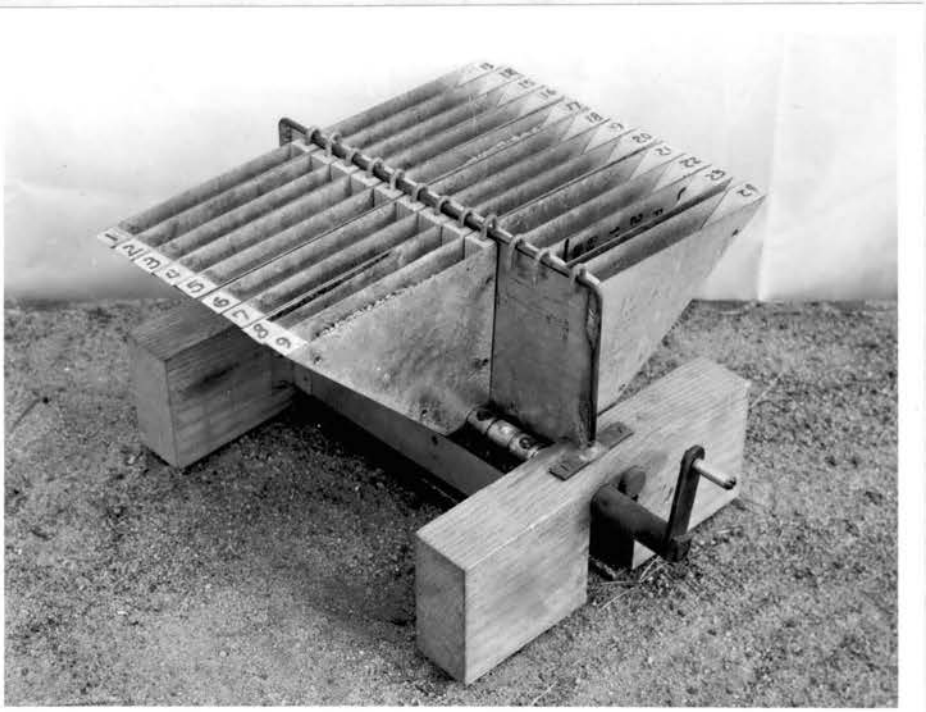

Figure 2. A mechanism designed to plant 3 to 5 seeds of each of 24 wheat varieties or selections into individual compartments of a plant container simultaneously

reservoirs mounted over two 5/8 inch metal shafts. Each shaft had twelve 3/8 inch diameter cup-shaped holes drilled on 3/4 inch centers. Depth of the hole was determined by the seed size of the variety used in a particular position so that equal numbers of seed of each variety would be discharged, regardless of seed size. Planting is accomplished by simultaneous rotation of both shafts, releasing the seed into the cup-shaped holes in the shaft into each compartment of the plant container inserted below. After planting was accomplished, the plant container was removed from the planter, the seed covered with soil, watered and placed in a rust-free controlled environment room.

Field Collections

Uredial infections on susceptible wheat varieties were collected at seven locations in the state of Oklahoma. At the time of collection the infected wheat leaves were placed in glycine packets for the return trip to Stillwater. It was not possible to identify the collections immediately so they were stored in a refrigerator at $\pm 4^{\circ}$ C. until used. Upon removal from storage the leaves from each packet were used to brush-inoculate plants of the wheat variety Cheyenne (C.I. 8885) to produce fresh urediospores. After inoculation, the plants were sprayed with water containing a surfactant (Tween 20, 2 to 4 drops per 1000 ml. of water) and placed in a moist chamber overnight. The next morning the inoculated plants were removed from the moist chambers and placed in isolation chambers in a controlled-temperature greenhouse room. Three to five days later, when the leaves began to show flecking, individual leaves were detached and placed in plastic petri dishes divided in to two compartments. The basal ends of the leaves were submerged in a

20-40 ppm solution of benzimidazole (Figure 3) contained in one of the two compartments. The tip end of the leaf, supported by the petri dish divider, was suspended over the remaining compartment. This method was a modification of Browder's detached leaf culture technique (4).

The urediospores from a single pustule were collected, 7 to 10 days after detachment, in a No. 00 gelatin capsule by means of a modified cyclone separator (37). After each collection, acetone was drawn through the separator to destroy and remove spores adhering to the separator. The acetone was then allowed to evaporate before the separator was used again. The urediospores collected in the gelatin capsules were then stored in a refrigerator at 4° C. until needed. Uredial lesions produced in this manner provided a quantity of urediospores sufficient to inoculate the entire compact set of differential wheat varieties used.

Thalictrum Collections

Plant material with telial lesions used to inoculate Thalictrum was collected from four varieties of wheat (Bison C. I. 12518, Triumph C. I. 12132, Wichita C. I. 11952, and Fulcaster 612 C. I. 4862) in June of 1965 and was stored at room temperature (25-30° C.) until used in October. At that time several bundles of the telial infected plant material were soaked in water for 24 hours. After this soaking period, while the material was still wet, the leaves bearing telial sori were removed and placed on several layers of cheese cloth and allowed to dry for 48 hours. The leaf-cheese cloth mat was then inserted between two layers of wide-mesh (2 squares per inch) screen wire. The mat was then hung from the top of a 2 x 2 x 4 ft. plastic inoculation chamber in

Figure 3. Petri dish culture of detached wheat leaves in a solution of benzimidazole

such a manner that it would be directly above Thalictrum plants placed in the chamber. The chamber was held in a greenhouse room cooled with refrigerated air to a temperature of approximately 60° F. A process of alternate wetting and drying of the telial mat was employed to stimulate the production of sporidia. The "wetting" phase was accomplished by misting the chamber with water plus a surfactant (Tween-20) until the mat appeared uniformly wet and small water droplets had formed on the Thalictrum leaves. The chamber was then sealed for at least 12 hours, usually overnight. The chamber was then opened and its contents allowed to dry for approximately 10-12 hours before the next wetting period. This alternate wetting and drying process usually continued for 7-10 days or until infection could be observed on the Thalictrum plants. The Thalictrum plants were then removed from the chamber and placed on a bench in the same greenhouse room. Within one week the pycnial infections developed to the point that crosses could be made.

Crosses were made at random using a camels hair brush to throughly mix the pycnial nectar of all infections. The rate of pycnial development was somewhat variable, necessitating the mixing of the pycnial nectar on several successive days to insure fertilization of all pycnia. Aecia began to appear about 8-10 days after fertilization of the pycnia. The heaviest pycnial infection usually occured on the leaves, but it should be noted that occassional pycnial infections were also observed on stems, petioles and peduncles.

The leaves bearing aecia were excised from the plant when the peridium of each aecial cup began to rupture. The leaves were then floated on tap water in a 125 ml. beaker with the aecia toward the water surface. This allowed the aeciospores to shower onto the surface

of the water. Usually within two hours sufficient spores had discharged so that the surface of the water was quite yellow-orange in color. The leaves were then removed from the beaker and discarded. Pots containing 20-25 plants of the wheat variety Cheyenne (C. I. 8885) 7 to 8 days old were then inoculated by dipping the wheat leaves into the beaker containing the aeciospores. When the leaves were slowly extracted from the beaker the aeciospores adhered to the wheat leaves. After inoculation the plants were misted with water and a surfactant (Tween-20), and left overnight in a moist chamber. The next morning the plants were placed in isolation chambers in a greenhouse room where the temperature was controlled with refrigerated air. The method of detaching the wheat leaves and allowing the pustules to develop in petri dishes before spore collection was the same as that used with the field collections.

Inoculation of Differential Varieties

Gelatin capsules containing urediospores were removed from the storage refrigerator and rehumidified in a moist chamber for approximately one hour prior to innoculation. After rehumidification the capsule was opened and 0.2 to 0.4 ml of an oil (Mobisol 100) was added to the spores in the bottom or inner half of the capsule. This portion of the capsule was then coupled to an aluminum atomizer and placed on a peg board until used.

Inoculation of 5 to 6 day old differential wheat varieties, grown in the plant containers previously described was accomplished in a machine designed for this purpose. It consisted of a conveyor belt which passed through an aluminum chamber (Figure 4). The conveyor belt was driven by a 1/2 horsepower 1725 rpm electric motor coupled to a

Figure 4. A machine used to inoculate differential wheat varieties for leaf rust race identification

300:1 gear reduction box which in turn was coupled by means of a V-belt to one of the conveyor belt pulleys. The resulting conveyor belt speed was approximately 1.25 inches per second. The movement of the conveyor belt, the air pressure (10 lbs. psi) used to disperse the spores from the capsule, and the water misting cycles were controlled by means of manual switches and electric solenoid valves.

The first step in the inoculation process was to attach an atomizer from the peg board to a special fitting on the air pressure line inside the chamber. The plant container of differential varieties was then placed on the input end of the conveyor belt and the belt was set in motion. As the plant container moved through the chamber the plants were inoculated by a two-second oilspore atomizing cycle. This was followed by a 2-4 second settling period which was in turn followed by a 25 second water misting cycle that wetted the inoculated plants and precipitated stray spores before the next plant container entered the chamber. Spring-loaded plastic doors at each end of the inoculation chamber prevented movement of spores into or out of the chambers. When the conveyor belt had carried the inoculated plants out of the inoculation chamber they were then placed in a moist chamber, misted lightly again with water and a surfactant (Tween-20), and left overnight. The next morning, the plants were removed from the moist chamber and placed in controlled environment chambers. The controlled environment chambers were maintained at a temperature of 20° C. with temperature fluctuation held to $\pm 1^{\circ}$ C. The photoperiod was set at 12 hours of 3000 f. c. and 12 hours of darkness. This temperature and light intensity produced excellent growth of both the wheat plants and the rust. Supplemental nitrogen was added every 48 hours since, in the

plant containers used, the soil-plant ratio was low and extreme yellowing would otherwise occur.

Reaction types were recorded 10-12 days after inoculation. In order that the data could later be assembled by machine, the reaction types were recorded directly on IBM data cards using a Wright Punch (model 2600). This machine is a portable key punching unit which could be used near the chambers where the plants were held. In order to accomplish this a slight revision had to be made in the symbols used to designate reaction types as originally described by Stakman, et al. (31). The numbers to designate the pustule types "1", "2", "3", and "4" were retained, but the figure "9" was used in place of "0;" to designate the "fleck" reaction, "5" was used in place of "X" for mesothetic type, and "6" was used in place of "Y" to designate the variable reaction described by Johnston (16). A two-digit system was employed to accomodate the variable reactions that often occured. Thus a "fleck" reaction was recorded as "90" and a "0;" to "1" reaction was recorded as "91" and so forth (Table II).

TABLE II

A TWO-DIGIT CODE DEVISED TO RECORD WHEAT LEAF RUST REACTION TYPES ON IBM DATA CARDS

Reaction Type[a]	Two-digit Code
0	00
0-0;	09
0;	90
0;-1	91
1	10
0;-2	92
2	20
1-2	12
0;-3	93
3[b]	30
1-3	13
2-3	23
0;-4	94
1-4	14
2-4	24
3-4	34
4	40
X	50
X-4	54
Y	60
4-Y	46
Segregating	88

[a] After Stakman, et al. (31).

[b] Reactions above the division line were placed in the "resistant" class and those below the line were placed in the "susceptible" class.

Data Analysis

The frequency of races from each source of uredial material is presented following the system of Basile (3) and also following a system which combines that of Basile and that proposed by the North American Wheat Leaf Rust Research Workers Committee (34). In addition, the percent of isolates from each source with virulence for each of the differentials used is presented. Finally, the data were analyzed following the method proposed by Schafer (30) to show associations among resistance genes contained in certain of the differential varieties used. In this method the frequencies of all of the isolates tested on the two varieties to be compared were fitted into a table as follows,

		VARIETY 1		
		R	S	
VARIETY 2	R	N_{11}	N_{12}	$N_{1\cdot}$
	S	N_{21}	N_{22}	$N_{2\cdot}$
		$N_{\cdot 1}$	$N_{\cdot 2}$	$N_{\cdot\cdot}$

where N_{11} equals the number of isolates developing a low infection type on both varieties, N_{21} equals the number of isolates developing a low infection type on variety 1 and a high infection type on variety 2, and so forth. If the genes for virulence in these isolates or the genes for resistance in these varieties are independent then the ratio of N_{11} to N_{12} will equal the ratio of N_{21} to N_{22} or the ratio of N_{11} to N_{21} will equal the ratio of N_{12} to N_{22}. On the basis of independence, a value M for the expected frequency of the observed class N_{22} was calculated from the formula:

$$M = \frac{N_{12} \times N_{21}}{N_{11}} .$$

A chi-square test for independence was then calculated from the formula:

$$\chi^2 = \frac{[(N_{12} \times N_{22}) - (N_{12} \times N_{21})]^2 \times N..}{N_1. \times N_2. \times N._1 \times N._2} .$$

RESULTS

Field Collections

The inoculation of differential variety sets with single uredial pustule collections from the field was initiated in May, 1966. A total of 1627 sets were inoculated. Of this munber, 980 had to be discarded due to a missing differential variety in the set, an ineffective inoculation, or for other causes. Identification of the other 647 single pustule isolates was made utilizing first the reaction of the five differential varieties suggested by Basile (3). Only eight of the 32 possible "UN" races were found (Table III), which compares favorably with previous surveys made in Oklahoma (Young, Personal Communication). Race UN2 constituted approximately 50 percent of all isolates identified, race UN1 made up 17.6 percent of the population, and race UN5, 11 percent. The other races found, in order of prevalence, were UN9, 3, 6, 11 and 13 respectively.

The same isolates were classified using the 5 differential varieties suggested by Young and Browder (34) for "NA65" races. In this case, 17 of the 32 possible races were found (Table IV). Races NA65-1, NA65-9 and NA65-10 were by far the most commonly identified.

The reactions on all 10 differential varieties were used to classify "UN-NA65" races. When this was done races UN2-NA65-1, UN2-NA65-9 and UN2-NA65-10 were by far the most abundant (Table V). It was also found that NA65-5 was associated only with UN3, NA65-13 and

TABLE III

NUMBER AND RELATIVE PROPORTION OF "UN" RACES OF *PUCCINIA RECONDITA TRITICI* ISOLATED FROM UREDIAL COLLECTIONS FROM THE FIELD IN OKLAHOMA IN 1966

"UN" Races	Number of Isolates	Percent of Total
1	114	17.6
2	325	50.3
3	46	7.1
5	71	11.0
6	16	2.5
9	61	9.4
11	4	0.6
13	10	1.5

TABLE IV

NUMBER AND RELATIVE PROPORTION OF "NA65" RACES OF PUCCINIA RECONDITA TRITICI ISOLATED FROM UREDIAL COLLECTIONS FROM THE FIELD IN OKLAHOMA IN 1966

"Na65" Race	Number of Isolates	Percent of Total
1	148	22.9
2	4	0.6
3	47	7.3
5	2	0.3
9	107	16.5
10	127	19.6
11	36	5.6
12	16	2.5
13	2	0.3
14	2	0.3
17	34	5.3
18	4	0.6
19	8	1.2
25	40	6.2
26	40	6.2
27	16	2.5
28	14	2.1

TABLE V

NUMBER OF "NA65" RACES OF PUCCINIA RECONDITA TRITICI FOUND IN CERTAIN "UN" RACES IN OKLAHOMA IN 1966

"NA65" Race Number	Number of Isolates of Each "UN" Race							
	1	2	3	5	6	9	11	13
1	45	68	12	15	2	16	-	-
2	4	-	-	2	-	-	-	-
3	8	23	4	8	2	2	-	-
5	-	-	2	-	-	-	-	-
9	22	61	8	9	2	11	-	-
10	15	67	10	21	2	14	2	2
11	6	26	-	4	2	2	-	-
12	6	6	2	4	-	2	-	-
13	-	-	-	-	-	-	-	2
14	-	-	-	-	-	-	-	2
17	-	18	2	-	2	4	-	-
19	2	2	-	-	-	-	-	-
20	-	-	-	-	-	2	-	-
25	4	16	4	-	2	4	2	-
26	-	24	2	4	2	2	-	-
27	2	4	-	-	-	2	-	2
28	-	6	-	4	-	-	-	-

NA65-14 were associated only with UN13, and NA65-20 was associated only with UN9. Only NA65-10 was found to be associated with all of the "UN" races isolated in this study. No "UN" race was found associated with all of the "NA65" races, although race UN2 was found to be associated with 12 of the 17 "NA65" races identified. Only four UN11 isolates were found, and these were associated with only two "NA65" races.

Thalictrum Collections

The inoculation of differential wheat variety sets with single uredial pustule collections derived from aecial infections on two species of Thalictrum was initiated at two different times. Isolates from T. dasycarpum were used to inoculate differential sets in the latter part of April, 1966. Differential sets were inoculated with isolates from T. speciosissimum in July, 1966. The races identified from these two sources appeared to be quite similar, and therefore the data from both sources were combined. A total of 1078 single pustule collections were used to inoculate wheat leaf rust differential sets. Complete race identifications could be made on only 766 sets.

The race population derived from Thalictrum was quite different from that found in the field. All eight of the "UN" races found in the field were also obtained from Thalictrum, as well as 16 additional "UN" races. Of the 32 possible "UN" races, only 27 have ever been identified from uredial collections made in the field anywhere in the world (3). Identification of the "UN" race population derived from isolates from Thalictrum in this study yielded 24 of the 27 known races (Table VI). It is worthy of note that none of the five "UN" races which have not been found in nature were found among isolates derived

TABLE VI

NUMBER AND RELATIVE PROPORTION OF "UN" RACES OF PUCCINIA RECONDITA TRITICI ISOLATED FROM UREDIAL COLLECTIONS DERIVED FROM AECIA FROM THALICTRUM DASYCARPUM AND T. SPECIOSISSIMUM UNDER CONTROLLED CONDITIONS

"UN" Race	Number of Isolates	Percent of Total
1	87	11.4
2	119	15.5
3	19	2.5
4	16	2.1
5	99	12.9
6	18	2.3
7	20	2.6
9	18	2.3
10	6	0.8
11	10	1.3
12	12	1.6
13	64	8.4
14	8	1.0
16	8	1.0
17	15	2.0
18	17	2.2
19	15	2.0
20	52	6.8
21	4	0.5
22	4	0.5
23	78	10.2
25	35	4.6
26	18	2.3
27	24	3.1

by hybridization or selfing on Thalictrum. Race UN2 comprised 50.2 percent of the entire race population in the field, while it comprised only approximately 15 percent of the isolates derived from Thalictrum. However, races UN1, UN2 and UN5 were the most commonly identified races both in the field and among isolates derived from Thalictrum. Race UN23 was found to comprise 10 percent of the isolates from Thalictrum, but was not even found in the field in this study. This race, although originally identified in the United States, is rarely found in nature (17).

The "NA65" race population derived from Thalictrum also differed considerably from that found in the field. Of the 32 possible races, 25 were identified from the Thalictrum material compared with only 17 from the field. The "NA65" races 2 and 5 were isolated from the field but not from the Thalictrum source. Races NA65-6, -8, -15, -16, -21, -23, -29, -30, -31, and -32 were isolated from Thalictrum sources but not from the field. Four "NA65" races comprised 42.8 percent of all isolates identified from the Thalictrum sources, and again, three of these races (NA65-1, NA65-9, and NA65-10) were also the most commonly identified races in the field (Table VII). The fourth, race NA65-32, comprised over seven percent of the isolates from Thalictrum, but it was not even identified from the field material.

The "UN-NA65" races most commonly isolated from the field collections, UN2-NA65-1, UN2-NA65-9, and UN2-NA65-10, were also among those races most commonly identified from the Thalictrum sources (Table VIII). However, UN1-NA65-1, UN1-NA65-9, and UN1-NA65-10 were also quite common among the Thalictrum isolates. No "NA65" race was found to be associated with all of the "UN" races isolated, although

TABLE VII

NUMBER AND RELATIVE PROPORTION OF "NA65" RACES OF PUCCINIA RECONDITA TRITICI ISOLATED FROM UREDIAL COLLECTIONS DERIVED FROM AECIA FROM THALICTRUM DASYCARPUM AND T. SPECIOSISSIMUM UNDER CONTROLLED CONDITIONS

"NA65" Race	Number of Isolates	Percent of Total
1	108	14.1
3	41	5.3
6	3	0.4
8	4	0.5
9	97	12.7
10	69	9.0
11	42	5.5
12	29	3.4
13	18	2.3
14	46	6.0
15	13	1.7
16	20	2.6
17	4	0.5
18	5	0.7
19	19	2.5
21	3	0.4
23	11	1.4
25	15	2.0
26	38	5.0
27	26	3.4
28	38	5.0
29	22	2.9
30	15	2.0
31	25	3.3
32	55	7.2

TABLE VIII

NUMBER OF "NA65" RACES OF PUCCINIA RECONDITA TRITICI FOUND ASSOCIATED WITH "UN" RACES IN ISOLATES DERIVED FROM THALICTRUM DASYCARPUM AND T. SPECIOSISSIMUM UNDER CONTROLLED CONDITIONS

"NA65" Races	"UN" Races																								
	01	02	03	04	05	06	07	09	10	11	12	13	14	16	17	18	19	20	21	22	23	25	26	27
1	23	19	-	4	-	-	3	2	2	-	-	-	-	-	-	-	-	12	-	4	19	12	4	-
3	3	-	3	-	12	-	-	-	-	8	-	-	8	-	4	-	-	-	-	-	3	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-
8	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	19	24	-	-	-	7	-	4	-	-	4	-	-	-	-	7	-	16	4	-	12	-	-	-
10	24	25	4	4	1	-	-	-	-	-	-	-	-	-	-	3	-	8	-	-	-	-	-	-
11	-	-	3	4	8	-	-	-	-	-	-	-	-	-	-	7	4	8	-	-	-	-	8	-
12	-	7	-	2	12	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	3	-	-
13	-	8	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	4
14	12	-	-	-	-	-	-	-	-	-	8	12	-	-	-	-	3	-	-	-	-	-	3	8
15	-	-	-	4	-	-	-	-	-	-	-	2	-	-	-	-	4	-	-	-	-	-	-	3
16	-	-	4	-	-	-	4	-	-	-	-	8	-	-	-	-	-	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-
18	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	3	-	-	-
19	2	2	-	-	8	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-
23	-	-	-	-	8	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	12	-	-	-
26	4	16	-	-	-	-	-	-	-	-	-	8	-	-	-	-	-	2	-	-	8	-	-	-
27	-	-	-	-	8	-	-	-	-	-	-	-	-	8	-	-	-	-	-	-	-	-	3	7
28	-	4	-	-	12	-	-	4	4	-	-	8	-	-	-	-	-	3	-	-	6	-	-	-
29	-	8	-	-	-	-	2	-	-	-	-	4	-	-	-	-	-	-	-	-	8	-	-	-
30	-	-	-	-	8	-	4	-	-	-	-	-	-	-	2	-	-	-	-	-	-	1	-	-
31	-	2	3	-	3	-	7	-	-	-	-	8	-	-	-	-	-	-	-	-	-	-	-	2
32	-	4	-	-	11	4	-	4	-	-	-	12	-	-	7	-	4	-	-	-	-	13	-	-

NA65-1 was associated with 12 of the "UN" races identified. Race NA65-6 was associated only with UN5, and NA65-17 and NA65-21 were associated only with UN23. Similarly, no "UN" race was associated with all of the "NA65" races isolated, although UN5 was associated with 13 of the 25 NA65 races identified. Race UN14 was associated only with NA65-3, UN16 was associated only with NA65-17, Un21 was associated only with NA65-9, and UN22 was associated only with NA65-1.

Varietal Reactions

The percentages of isolates from both the field and Thalictrum collections virulent on each of the 24 wheat varieties used in this study is given in Figure V. Only six of the 24 wheat varieties used in this study exhibited a reduction in susceptibility to isolates derived from the Thalictrum when compared to isolates from the field collection. The varieties Cnn, Mi, Do, Wtr, Wsl, and Tp (see Table I for explanation of varietal abbreviations) each exhibited some reduction in susceptibility. Increased susceptibility to the Thalictrum derived isolates compared to the field isolates was found on 12 of the 24 varieties used. Two of these 12 varieties (Kfd and Wkn 2) were resistant to all of the field cultures. There were 5 varieties which were resistant to all of the isolates used, regardless of the source. These were Aa, Sft, Ars, Agt, and Tf. It was interesting to note that a few isolates derived from Thalictrum were avirulent on the variety Cnn, which has been used as a universal suscept for increasing cultures of P. recondita tritici.

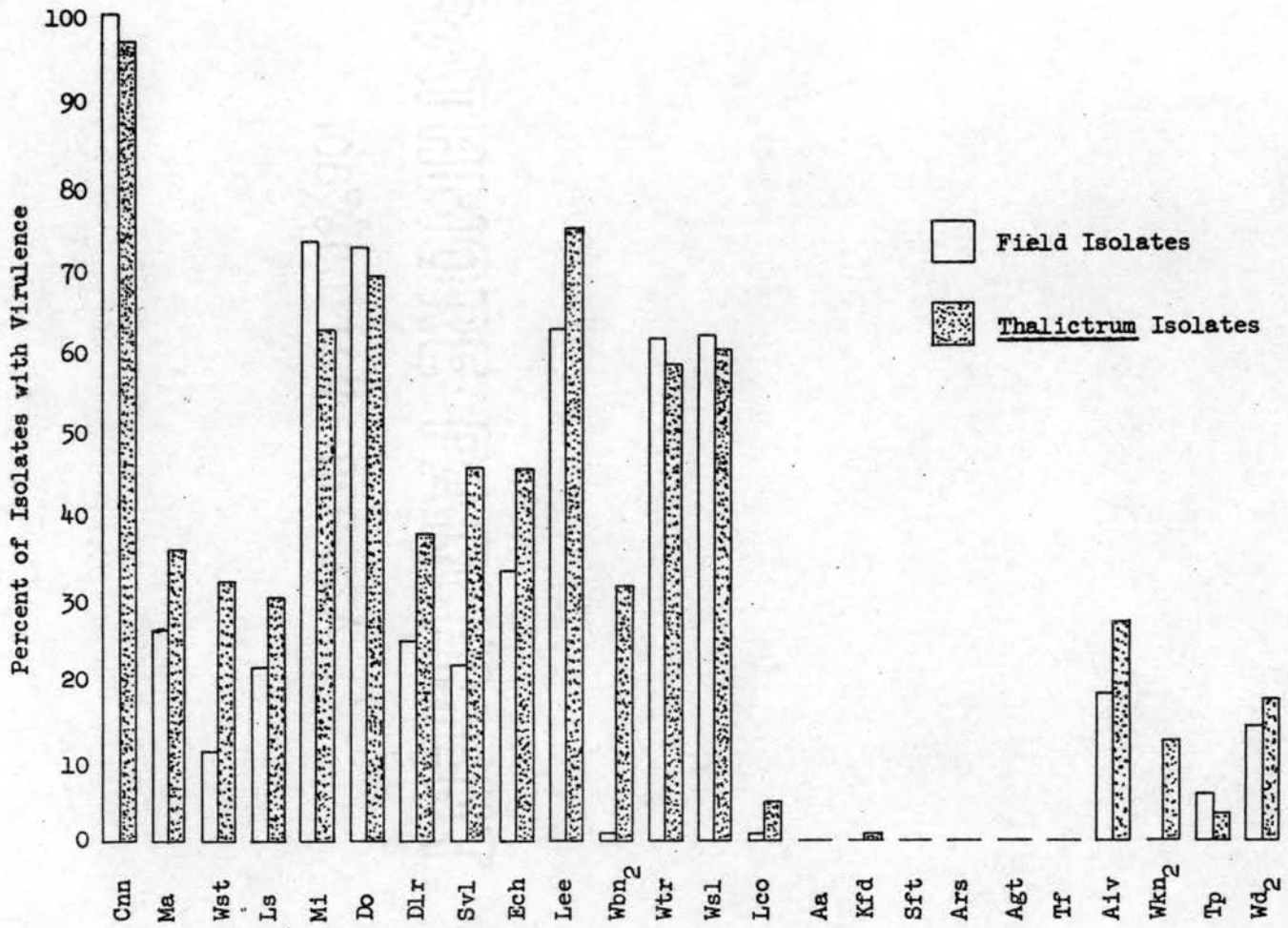

Figure 5. Percent of field and Thalictrum derived isolates of Puccinia recondita tritici virulent on each of 24 differential wheat varieties

Frequency Analysis

The analyses of rust reaction with isolates from the field on certain pairs of differential varieties by the frequency of virulence association method (30) are presented in Table IX. Three types of relationships were found: (a) random; (b) associated; and (c) inverse. A random relation was indicated whenever the ratio of the expected combined virulence class to the observed values of this class were approximately equal to one with a significant χ^2 value. An associated relationship between virulences existed whenever the expected to observed ratio was less than one and the χ^2 value was not significant. An inverse relationship was indicated whenever the expected to observed ratio was greater than one and the χ^2 value was not significant.

Specific conclusions (Table X) suggested by this method of analysis indicated that Lee and Ech possess a gene in common, since the class with virulence on both varieties was much higher than expected. Each of these varieties probably possesses a different additional gene since the association was not complete. This is shown in the following example.

		Lee		
		R	S	
Ech	R	239	197	436
	S	8	203	211
		247	400	647

Combined Susceptibility		Relationship	χ^2 for Independence
Expected	Observed		
7	203	Associated	156.8495

TABLE IX

PAIRED COMPARISONS OF THE FREQUENCY OF ASSOCIATION OF GENES FOR RESISTANCE AND SUSCEPTIBILITY IN THE 1965 NORTH AMERICAN WHEAT LEAF RUST SUPPLEMENTAL DIFFERENTIAL VARIETIES TO FIELD ISOLATES OF PUCCINIA RECONDITA TRITICI

Variety Comparisons	Combined Susceptibility		χ^2 for Independence	Relationship
	Expected	Observed		
1) Lee:Svl	96	80	26.2016	Inverse
2) Ech:Lee	7	203	156.8495	Associated
3) Ech:Svl	55	34	4.8048	Inverse
4) Dlr:Svl	30	38	4.2488	Associated
5) Dlr:Lee	66	110	6.5757	Associated
6) Wbn2:Svl	2	0	1.6269*	Random[a]
7) Wbn2:Lee	3	4	0.0602*	Random[a]
8) Ech:Dlr	46	56	0.1878*	Random
9) Ech:Wbn2	2	2	0.0014*	Random
10) Dlr:Wbn2	2	0	1.9242*	Random[a]

*Indicates a significant χ^2 value.

[a]The relationship indicated may be due to lack of virulent isolates on Wbn2.

TABLE X

HOST-PARASITE INTERACTIONS OF 1965 NORTH AMERICAN WHEAT LEAF RUST SUPPLEMENTAL DIFFERENTIAL VARIETIES AND RACES OF PUCCINIA RECONDITA TRITICI ISOLATED FROM FIELD COLLECTIONS FROM OKLAHOMA IN 1966 AND TABULATED ACCORDING TO THE SYSTEM OF PERSON

"NA65" Race	Lee[1]	Svl	Ech	Dlr	Wbn2	Number of Isolates
1						148
9	S[2]					107
3		S				47
2			S			4
17				S		34
5					S	2
11	S	S				36
10	S		S			127
25	S			S		40
13	S				S	2
19		S		S		8
18			S	S		4
12	S	S	S			16
27	S	S		S		16
26	S		S	S		40
14	S		S		S	2
28	S	S	S	S		14
					Total	647

[1]Abbreviations used were made according to rules adopted by the National Wheat Improvement Committee. See Agron. J. 52:613, 1960, and U. S. Dept. of Agr. Tech. Bull. 1278, p. 131, 1963.

[2]S designates susceptible reaction; blanks indicate resistance.

Svl and Lee also appear to have a gene in common, different from the common gene of Lee and Ech, as well as an additional gene each, also different from the common gene of Lee and Ech. Dlr appears to have a minimum of two resistance genes, one in common with Lee and another in common with Svl. The data suggest that Wbn2 has one independent gene and possibly one additional gene that is common with the Svl-Dlr gene. Definite conclusions concerning the additional Wbn2 can not be made with any certainty due to the lack of virulence to Wbn2 in the field isolates.

Analysis of the frequencies of association of genes for virulence suggests the following temporarily designated minimum genes for resistance in the differential varieties indicated.

Lee	ACD
Ech	AE
Svl	BC
Dlr	BD
Wbn2	F (and possibly B)

Data from isolates derived from Thalictrum were similarly analysed (Table XI). Only two types of relationships, random and associated, were found among the comparisons made.

Specific conclusions (Table XII) indicated by these data suggest that Lee, Ech, and Wbn2 possess one gene in common and two additional different genes in each variety. Lee, Svl, and Dlr possess one gene in common, different from the common gene in Lee, Ech and Wbn2, plus two additional genes each. Svl, Dlr, and Wbn2 possess one gene in common, different from the common gene in Lee, Svl, and Dlr, plus two additional genes each. Dlr and Ech possess one gene in common plus two additional

TABLE XI

PAIRED COMPARISONS OF THE FREQUENCY OF ASSOCIATION OF GENES FOR RESISTANCE AND SUSCEPTIBILITY IN THE 1965 NORTH AMERICAN WHEAT LEAF RUST SUPPLEMENTAL DIFFERENTIAL VARIETIES TO ISOLATES OF *PUCCINIA RECONDITA TRITICI* DERIVED FROM *THALICTRUM*

Variety Comparisons	Combined Susceptibility		χ^2 for Independence	Relationship
	Expected	Observed		
1) Lee:Svl	201	266	2.8501*	Random
2) Lee:Ech	27	324	13.0762	Associated
3) Svl:Ech	127	168	3.6387*	Random
4) Dlr:Svl	49	185	74.9512	Associated
5) Dlr:Lee	88	241	28.3724	Associated
6) Wbn2:Svl	62	139	26.4663	Associated
7) Wbn2:Lee	42	215	51.1283	Associated
8) Ech:Dlr	66	168	37.9063	Associated
9) Ech:Wbn2	60	140	28.6408	Associated
10) Dlr:Wbn2	39	135	60.0885	Associated

*Indicates a significant χ^2 value.

TABLE XII

HOST-PARASITE INTERACTIONS OF 1965 NORTH AMERICAN WHEAT LEAF RUST SUPPLEMENTAL DIFFERENTIAL VARIETIES AND RACES OF PUCCINIA RECONDITA TRITICI ISOLATED FROM UREDIAL COLLECTIONS DERIVED FROM AECIA FROM THALICTRUM DASYCARPUM AND T. SPECIOSISSIMUM UNDER CONTROLLED CONDITIONS AND TABULATED ACCORDING TO THE SYSTEM OF PERSON

"NA65" Race	Lee[1]	Svl	Ech	Dlr	Wbn2	Number of Isolates
1						108
9	S[2]					97
3		S				41
17				S		4
11	S	S				42
10	S		S			69
25	S			S		15
13	S				S	18
19		S		S		19
18			S	S		5
6			S		S	3
21				S	S	3
12	S	S	S			29
27	S	S		S		26
15	S	S			S	13
26	S		S	S		38
14	S		S		S	46
29	S			S	S	22
8		S	S		S	4
23		S		S	S	11
28	S	S	S	S		38
16	S	S	S			20
31	S	S		S	S	25
30	S		S	S	S	15
32	S	S	S	S	S	55
					Total	766

[1]Abbreviations used were made according to rules adopted by the National Wheat Improvement Committee. See Agron. J. 52:613, 1960, and U. S. Dept. of Agr. Tech. Bull. 1278, p. 131, 1963.

[2]S designates susceptible reaction; blanks indicate resistance.

different genes.

The following temporarily designated genes for minimum resistance are suggested from this method of analysis:

Lee	ACD
Ech	AEH
Svl	BCG
Dlr	BCE
Wbn2	ABF

DISCUSSION

Before initiation of this study, it was obvious that a major portion of time would be required to solve certain logistics problems. The large quantities of culture identifications to be made created problems with labor, time, and space. Reduction in the size of the container for each differential set, mechanization of planting and inoculation procedures, and the utilization of the increased spore accumulation of rust pustules grown in detached leaf culture provided means to overcome these problems. Otherwise it would have been almost impossible to analyze over 2500 single uredial pustule isolates within a few months time.

The success of the study was also threatened near the mid-way point due to failure of inoculation procedures on both the differential sets and the Thalictrum plants. After exhaustive examination of all steps of each inoculation procedure, the problem was found to be the temperature during the period that the plants, both wheat and Thalictrum, were held in the moist chambers. Chester (7) states that leaf rust urediospores will germinate over a wide range of temperatures (2° to 31° C.) with optimum germination at about 20° C. It was finally found that during the period when large numbers of inoculation failures occurred the temperature in the room where the moist chambers were located rose to near 30° C. When this situation was corrected and the temperature remained near 20° C. during the moist chamber period, inoculations were again successful. In the meantime, however, over

1200 differential sets were discarded due to inoculation failure.

Temperature has also been found to be a limiting factor during the infection of Thalictrum. Davis (11) reported that at temperatures above 24° C. infection of Thalictrum did not occur. A rise in temperature also occurred in the room where the Thalictrum plants were being held during the moist period. When the temperature was reduced to between 15° and 20° C., infections were again successful.

The survey of the leaf rust population in the field confirmed the results of previous survey reports, and also provided information on the races likely to have formed telial lesions so that the results could be used to compare with uredial populations derived from aecial infections on Thalictrum. It can be said with reasonable accuracy that a maximum of eight "UN" races were originally present in the telial infected straw used in hybridization studies on Thalictrum. The fact that 24 "UN" races were identified after passing the organism through the alternate host indicates that sexual recombination did occur, causing a reassortment of the genes for virulence which accounted for 16 "UN" races not found in the field. An obvious conclusion is that the races present in the field are selected on some basis other than virulence or virulence combinations. It is also apparent that the alternate host does not function to any appreciable extent in nature, since none of the 16 additional races derived from Thalictrum infections are normally encountered in the race population of the Central United States.

The "NA65" race populations identified in this study exhibited a pattern similar to the "UN" race populations. Following hybridization there was a definite increase in the number of races present.

Additional information can be gained from leaf rust surveys, if the reported data are analyzed in the manner described by Person (26) and by Schafer (30). Both types of analysis provide evidence of the genetic makeup of the differential varieties used in the absence of available genetic data. Analysis by the frequency of association of genes for virulence supports the analysis by Person's method whenever the relation is random or whenever the frequency of either or both of the "Resistant to one variety - Susceptible to the other variety" class is zero. When there is a positive association of the occurence of virulences and yet the frequency of neigher of the "Resistant to one variety - Susceptible to the other variety" class is zero, then the presence of a common host resistance gene is suggested in addition to the independent resistance gene in each variety indicated by Person's analysis. The occurence of a positive association of virulences in which all races which attack one variety also attack another indicates that both varieties possess the same genes for resistance and no independent resistance genes. Schafer (Personal communication) states that other degrees of association of virulences to the two varieties indicate a similar response of the varieties, yet mutual differences. This suggests that the two varieties concerned, rather than having only independent single genes as indicated by Person's (26) method of analysis, probably possess a gene or more in common plus a different additional gene each. Linked genes possibly offer an alternate hypothesis.

The inverse relation of virulences reported for several of the comparisons in this study is more difficult to explain. If virulence, per se, is deletrious to the pathogen, as suggested by Flor (12), then

there may be evolutionary pressure against virulence genes occuring together. Schafer (30) suggests that this is a plausible explanation if the alleles for avirulence of the different loci could to some degree substitute for each other in their presumed advantageous function.

Certain combinations of virulences may, by chance, be associated with other types of factors requisite to survival (Young, Personal communication). In which case, there would also be evolutionary pressure against the survival of certain virulence combinations.

The validity of conclusions drawn from gene combination frequency analysis is very dependent on the random sampling of the survey. Erroneous relationships could easily be concluded if the sampling was performed in a biased manner.

It should be pointed out that analyses by Person's method or by Schafer's method are not meant to take the place of precise genetical studies but merely to serve as an indicator of the possible location of leaf rust resistance genes and to act as a basis for future genetic studies on the nature and location of leaf rust resistance genes. However, if available genetic studies (2) are correct and all of the resistance genes in Lee are also present in Ech, then only 24 of the possible 32 "NA65" races should be found. The virulence combinations on races not possible would be those virulent on Ech and avirulent on Lee. Several such virulence combinations or races appeared in this study, however, indicating that there are additional resistance genes in Lee which are not found in Ech. Genetic studies with the appropriate cultures are needed to verify the conclusions drawn from the data reported here.

SUMMARY

1. The logistic problems of this study were solved by:
   (a) reduction in the size of differential variety plant containers; (b) construction of a 24-variety seed planter; (c) utilization of a detached leaf culture technique to aid in accumulation of urediospores from single pustules; and (d) construction of a rapid inoculating machine capable of utilizing urediospores from a single pustule.

2. The wheat leaf rust survey of races found in field isolates indicated only eight "UN" races present and 17 "NA65" races present in the uredial population.

3. Identification of uredial isolates derived from mass crossed pycnial infections on Thalictrum under controlled conditions exhibited a rust population consisting of 24 "UN" races and 25 "NA65" races.

4. A possibility of association may exist between "NA65" races 1, 9, and 10 with "UN" races 1, 2, and 20. Other than these associations, no definite pattern could be consistently observed.

5. A definite trend toward increased virulence of the pathogen was noted when a comparison of the virulence population of field isolates to the Thalictrum isolates was made. Several exceptions to this trend were evident, but the majority of the varieties showed

an increase in the total number of susceptible responses.

6. Analysis by frequency of virulence association of the field population indicated the following possible minimum resistance genes in the NA65 supplemental differential varieties: Lee (ACD), Ech (AE), Svl (BC), Dlr (BD), and Wbn2 (F and possibly B).

7. Analysis of frequency of virulence association of the Thalictrum isolates agreed with the indicated field results and, in addition, indicated the presence of several additional genes: Lee (ACD), Ech (AEH), Svl (BCG), Dlr (BCE), and Wbn2 (BAF).

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