THE USE OF HOST RESISTANCE GENES IN THE FIELD, RADIATION, AND A CHEMICAL MUTAGEN TO IDENTIFY OR CHANGE THE VIRULENCE SPECTRUM OF THE LEAF RUST PATHOGEN *PUCCINIA RECONDITA* ROB. EX. DESM. F. SP. *TRITICI*

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CHAPTER I

INTRODUCTION

The leaf rust disease of wheat, caused by Puccinia recondita Rob. ex. Desm. f. sp. triciti, is chronic and costly to winter wheat production almost every year in Oklahoma and the winter wheat growing area of the Great Plains. The fungus is an obligate parasite and consists of morphologically similar, but parasitically distinct races. The diverse races in the fungus population have been shown to originate from mutation, and possibly hybridization. In nature in the United States, new races of the leaf rust fungus develop only from mutation since the alternate host is nonfunctional and heterokaryosis or parasexualism have not been definitely shown to occur.

The strategy of utilizing single gene resistance has been widely used in wheat improvement programs, but this method of controlling the leaf rust disease is not based on an adequate consideration of potential genetic variability of pathogen (36). When this strategy is used, however, only one race avirulent on that specific resistance gene is capable of identifying the host genotype carrying that gene (5, 25). When two or more specific genes are incorporated into a single host variety, a longer-lasting resistance may

result (Young, H. C., Jr., Personal Communication), but two races of the pathogen, reciprocally virulent and avirulent on each gene, are necessary to identify the segregating lines carrying both resistance genes. Sometimes such specific races are not available in nature. Therefore, mutation to certain combinations of virulence and avirulence, either spontaneous or artificially induced, may serve as a means of obtaining the specific races desired for testing purposes.

Mutagenesis in fungi by the use of radiation and chemical mutagens has been widely reported. Flor (12) successfully used ultraviolet radiation with the flax rust fungus (Melampsora lini). Rowel et al., in 1963 (28), used X-rays to produce mutant cultures with added virulence in the stem rust fungus (Puccinia graminis tritici).

A chemical mutagen was used successfully to induce mutation by Mikhailova et al. (23). They used N-nitroso-N-methyl urea with leaf rust uredospores, and Higgins (16) reported the successful use of ethyl methane sulphonate with stem rust uredospores.

Crosses of wheat lines to combine leaf rust resistance genes LR-1, LR-3, and LR-24 have been made by wheat breeders in the Department of Agronomy, Oklahoma State University, Stillwater, Oklahoma. In order to identify plants or lines in the segregating generations that contain all three of these host genes for resistance, a culture of race virulent on LR-1 and LR-24, but avirulent on LR-3, was needed. Such

a culture was not available in the native pathogen population. This study was established in an attempt to develop such a culture. The following approaches were used:

1. Mutation induction using gamma radiation; and
2. Mutation induction using the chemical N-nitroso-N-methyl urea.

In both approaches, uredospores of a culture of a race with virulence on LR-1 and avirulence on both LR-3 and LR-24 were used.

3. A third aspect of this study involved a search for races virulent resistance genes LR-17 and LR-19 which were planted in an area (southeastern Texas) where the leaf rust disease normally is present and develops on wheat throughout its life. Cultures of races virulent on these two resistance genes have never been found in the native population of the leaf rust pathogen in Oklahoma.

CHAPTER II

LITERATURE REVIEW

The method of utilizing resistant plants against plant diseases is an old strategy which has been used almost since the beginning of the cultivation of crop plants. The development of resistant crops has been known since 1800 (3). The types of resistance which have been widely used could be divided into two types: specific or vertical resistance, which is highly resistant but unstable due to pathogen variability; and nonspecific or horizontal resistance, which may confer less resistance to the pathogen but is more stable and not affected by pathogen variability (36). The use of specific resistance is a highly successful method of controlling cereal rust (14), late blight of potato (2) and certain other diseases (27).

The number of resistance genes utilized in a crop variety has progressively increased from a single gene for resistance to multiple resistance genes due to the ability of the pathogen to produce new cultures or races which attack the resistance genes. Between host and pathogen there is an interrelationship involving a corresponding gene system (10, 11). The relationship is dynamic, and host and pathogen have evolved through the variability of their

respective genetic systems. Changes in host by the introduction of resistance genes leads to adaptive changes in the parasite and vice versa.

The success of using resistance genes is based primarily on the stabilizing selection hypothesis introduced by Vanderplank (36). He stated that the more virulence genes required by a pathogen to allow it to attack a host, the less likely it would be to survive. Eenink (8) stated that the stability of resistance utilized in a plant will increase if more numbers or more complicated virulence genetic systems are required in the parasite to overcome the genes responsible for resistance in the host plant. Stability of resistance may result from breeding strategies also, depending upon certain other factors such as types of genes, environmental factors affecting the expression of those genes, and the relationship of the host-parasite interaction. Most cultivars are bred to be resistant to the prevalent strains or races of a particular pathogen without much consideration being given to the presence of other strains or races in the pathogen population (3). Kilpatrick (19) made an intensive study of the stability of rust resistance in wheat cultivars released during the period 1970-1975. He found that the longevity of resistance of these cultivars varied within and between the countries where they were released, and by which species of rust fungi was involved in the original selection for resistance. The factors affecting the longevity of resistance of released

cultivars were changes in virulence of rust races in the population, insufficient screening of introduced cultivars, and unusual weather conditions favoring more severe disease epidemics. The longevity of crop plant resistance to rust diseases in general varied from 1-15 years: 2-12 years for stem rust, 1-15 years for leaf rust, and 1-10 years for stripe rust.

Generally, new races or biotypes of the pathogen are discovered only after cultivars with a new gene or gene combinations for resistance have been introduced and exposed to the pathogen population. However, races or biotypes could be mixed in the pathogen population which could attack the new resistance gene or gene combinations in the host and not be detected unless the specific corresponding resistance gene present in the host cultivars are used for identification (11). The common races of the cereal rust population were reviewed by Johnson and Newton in 1946 (17) using differential hosts available at that time. Since then, supplemental host cultivars that contain new resistance genes have been utilized to identify new races that have arisen in the rust population (Young, H. C., Jr., Personal Communication). One such new mutant found by Samborski (30) in 1963 was heterozygous for virulence to LR-9, a gene made available as a source of resistance in 1956 by Sears (32). By selfing this culture on Thalictrum sp. Samborski (30) was able to produce a race virulent on wheat having gene LR-9. A similar situation for stem rust was

reported from Australia (22). The cultivar Festiguay contained stem rust resistance genes Sr-9B and Sr-15, which conditioned resistance to all known races in Australia at the time. One year after Festiguay was released, a stem rust culture with a high infection type was found in the field, and within five years the variety Festiguay was forced from cultivation in Australia due to stem rust (22).

Variability in pathogenicity in rust fungi has been intensively studied by Waterhouse (37) beginning as early as 1919. The mechanisms of pathogen variability (other than hybridization during the sexual cycle) are somatic hybridization resulting from parasexualism or heterokaryosis, and mutation (9, 20, 24, 26, 38, 39). With the leaf rust fungus, heterokaryosis and parasexualism have not been demonstrated and the sexual cycle is rarely completed, leaving mutation as the principal means of variability in pathogenicity (26, 41).

Mutation rates in pathogens have been very difficult to measure, due to the many factors involved, and the frequencies of mutation rates obtained are not comparable between, nor even within, pathogens because of different kinds of evaluation methods and the loci involved (7, 27). Zimmer (42) measured the mutation rate in the oat leaf rust fungus Puccinia coronata var. avenae. He inoculated three races (202, 203 and 209) on a highly resistant host cultivar and counted the high infection types that occurred. The estimates of mutation rates in these experiments were

1:22,000 for race 202, and 1:7,900 for race 209. Flor (13) used four single gene differentials to study mutation rates in flax rust. He found two virulent mutants on the cultivar Birio from 200,000 uredospores used in screening, and one virulent mutant on the cultivar Cass from 600,000 spores used. The rates of occurrence of natural mutants was similar to rates observed with artificially induced mutants using X-ray radiation, which suggested that those loci most susceptible to the action of mutagens may be the least stable under natural conditions.

Naturally occurring mutations for pathogenicity have been found to occur gradually or in step-wise fashion. Watson and Luig in 1968 (40) found that race 80 of the wheat stem rust fungus which was avirulent on the cultivar Yalta containing Sr-11 (infection type 0;), had increased virulence after passing through a subculture on a susceptible host cultivar. After six cycles of reproduction on the susceptible host, a variant was found which gave infection types of 2^+, 3C, or 3 instead of the normal 0; found with the original culture of race 80 on the cultivar Yalta.

Mutation for uredospore color has been detected in several species of rust, including both P. recondita tritici (18) and P. graminis tritici (15). Watson and Luig (40) used a uredospore color mutant as a marker to study mutation in races NR-1, 11, 111, and GB-1 of the stem rust of wheat. A mutant occurred in an orange culture of race NR-1 on the

cultivar Lee, increasing from low infection type (0;-2) of the original culture to high infection type or virulence. The mutant culture was also virulent on the cultivars Goto, Gaza, and Kenya C6042. An orange culture of race 11 also produced a mutant with virulence on the cultivar Lee. The other races, 111 and GB-1, produced no mutants on Lee.

In 1980, Statler and Jones (34) studied the inheritance of uredospore color, uredial length and width, and virulence by crossing cultures 70-197 and 71-122 of P. recondita var. tritici on Thalictrum speciosissimum. They found that there was no indication of linkage between characters and that uredospore color was apparently controlled by a single dominant gene, independent of the genes which controlled virulence.

Many factors have been suggested to account for the occurrence of mutations in nature. Schwinghamer (13) suggested that spontaneous mutation may be caused by a combination of radiation levels that the fungus may be exposed to in nature, including ultraviolet radiation, terrestrial gamma rays, and emission from radioactive elements absorbed by the host plant. Potentially mutagenic chemicals, produced both by the host plant and the pathogen as a result of impaired metabolism, may be the cause of mutation. He further suggested that semi-resistance (intermediate infection types allowing a small amount of sporulation) of the host plant possibly may contribute to mutation by applying what he called "mutation pressure" as

well as selection pressure to the pathogen. These suggestions could explain the mechanism of "progressive increase in virulence or increase in pathogenicity" suggested by Watson and Luig (40). Schwinghamer (31) also suggested that higher rates of spore production of the pathogen could increase the mutability due to solar radiation or to inherent thermal instability of certain genes in the pathogen. Certainly then, mutation frequency in rust fungi depends upon the organism, the environment, and other factors (12).

In order to check segregating generations resulting from crosses in the host plants crossed to combine resistance genes, two races of the pathogen, one virulent and one avirulent, are required to identify plants with the combined resistance genes (25, 34). Such races may not be found in the natural populations; artificial acceleration of mutation by some means is then necessary. Physical mutagens, such as ultraviolet light, X-rays, gamma rays, and fast neutrons have been used, as well as chemical mutagens, such as ethyl methane sulphonate (EMS) and N-nitroso-N-methyl urea. Mutation to virulence at the specific loci can be identified by screening through a cultivar containing the appropriate resistance gene. Flor (12), in 1956, used ultraviolet light (UV) as a mutant to alter the virulence spectrum of the flax rust fungus, Melampsora lini. The dose used gave a 50 percent reduction in uredospore germination. He obtained seven mutants representing four different races

and four different virulences. The estimated mutation rate was very low, only about 0.3%. In 1958, Flor (13) used X-rays to induce mutation in M. lini. He applied X-rays to uredospores of F_1 culture from race 22 x race 1, which gave a heterozygous condition at 17 virulence/avirulence loci. His dosage rates were 55 and 88 kR (kilo rad.). From more than 100,000 uredospores, he obtained six mutants that differed from the original culture by a single virulence gene, and two mutants that differed by two genes. He suggested that mutant cultures which differed from the F_1 culture by virulence on one differential corresponded to a "single hit", and those that differed on two differentials corresponded to "two hits". He further suggested that mutation resulted in either a change in the gene itself or from destruction of a chromosome segment, or even destruction of the entire chromosome. He speculated that each locus in a pathogen may have greatly different mutability rates under these or natural conditions.

Schwinghamer, in work discussed previously (31), applied ultraviolet light, X-rays, gamma radiation and fast neutrons to flax rust uredospores. More than 4,000 pathogenic mutants were obtained that differed from the original cultures. Ultraviolet light, applied at the level of 10,000 ergs/mm^2, resulted in an LD_{70-80}, and gave the lowest rate of mutation. Ionizing radiation from X-rays, applied at a dosage that gave an LD_{90}, and gamma radiation at dosages of 32 and 46 kR produced higher mutation rates

than ultraviolet light, but gamma radiation at 46 kR reduced uredospore germination to the point that it was difficult to detect mutants. The results indicated an average maximum mutation frequency of 0.3% for ultraviolet light, 1.5% for X-rays and 0.2% for fast neutron radiation. He said that since ultraviolet light and fast neutrons resulted in proportional mutation frequency, it indicated that the mechanism was point mutations in the chromosome structure. However, the mutation frequency which occurred from ionizing radiation (X-rays and gamma rays) varied with the square of the dose indicating deletion of chromosome structure.

Rowell, Loegering and Powers (28, 29) applied X-rays to a culture of P. graminis tritici (race 111 x 36#5) at the rate of 121 kR. The cultivar Marquis was used to screen the mutants from the treated uredospores. They obtained cultures which produced a 2-3C infection type on Marquis. They postulated that a mutant change from P_1p_1 to p_1p_1 permitted the expression of P_2p_2 and certain other genes.

A number of chemical mutagens have been used to induce mutation in cereal rust fungi for research in the development of disease resistance cultivars. EMS (ethyl methane sulphonate) has been widely used in Australia to develop cultivars resistant to stem rust. More recently, Teo and Baker (35) treated oat stem rust (Puccinia graminis f. sp. avenae) uredospores and found mutation in traits such as uredospore color, and virulence were increased as mutagen concentration, duration of treatment, and temperature

increased. Recurrent mutagenic treatment increased mutation frequency and produced a wider spectrum of virulence among the mutants. Luig (22), in 1978, used EMS to treat uredospores of a culture of stem rust fungus to study the possible association of virulence on two wheat cultivars. He treated stem rust race 75-L-9 with 1.2% aqueous EMS. He found two virulent mutants on the cultivar W3592 which were double mutants. The cultures differed from the parent culture not only by their virulence on cultivar W3592, but also on Festiguay. Such a virulence had not been found before in Australia.

Mikailova et al. (23), worked with N-nitroso-N-methyl urea at pH 4.7-5.2. Uredospores of the leaf rust fungus P. recondita tritici race 10 were treated at concentrations of 0.1, 0.2, 0.4, and 0.6%. The percent of germination of the treated uredospores was reduced to 50% in the 0.1% treatment, 80% in the 0.2% treatment, 92% for the 0.4% treatment, and 96.8% for the 0.6% treatment. The mutation rates, measured from signs of aggressiveness (uredospore reproduction characteristics) was found to be 3×10^{-4}.

CHAPTER III

MATERIALS AND METHODS

A uredospore culture of race 9 of *Puccinia recondita* Rob. ex. Desm. f. sp. *tritici* Eriks used in this investigation, herein designated "9A" due to an additional virulence on LR-10, was obtained from a stock culture maintained in liquid nitrogen storage as suggested by Loegering (21) at Oklahoma State University, Stillwater, Oklahoma. It was increased on the susceptible cultivar Danne CI.13876 and checked for purity on the "standard" set of differential hosts (Table I). The reaction of race 9A, together with a culture of race 2, designated herein "2AAG" due to added virulence on LR-10 and LR-24, and a culture of race 5, herein designated "5A" due to additional virulence on LR-10, are shown in Table II along with the reaction sought in a mutant from the culture of race 9A.

Uredospores obtained from the stock culture were increased on the cultivar Danne twice each time spores were required. The first increase was made on 10 pots of approximately 30 eight-day-old plants. Inoculation was accomplished by the dipping method (5). These inoculated plants were held overnight in a previously surface sterilized moist chamber, and then placed in closed cages in

TABLE I

STANDARD DIFFERENTIAL SET OF WHEAT CULTIVARS USED IN IDENTIFICATION OF RACES OF LEAF RUST, *PUCCINIA RECONDITA TRITICI* IN THESE EXPERIMENTS

Cultivar Name	C.I. Number[a/]	Resistance Gene Contained
Malakof	4898	LR-1
Webster	15248	LR-2A
Loros	3779	LR-2C
Democrat	15246	LR-3
Transfer	13483	LR-9
Westar	12110	LR-10
Agent	13523	LR-24
Wesel	13090	LR-10&?

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

TABLE II

INFECTION TYPES OF SOME IMPORTANT RACES USED IN THESE EXPERIMENTS AND THE MUTANT SOUGHT

Races	Standard Differential Cultivars[a]							
	M	Web	L	D	T	West	A	Wes
	(LR-1)	(LR-2A)	(LR-2C)	(LR-3)	(LR-9)	(LR-10)	(LR-24)	(LR-10?)
2AAG	L[b]	L	L	H	L	H	H	L
5A	H	L	L	H	L	H	L	L
9A	H	H	H	L	L	H	L	L
M.[c]	H	-	-	L	-	-	H	-

M = Malakof; Web = Webster, L = Loros; D = Democrat; T = Transfer; West = Westar; A = Agent; Wes = Wesel.

[a] Symbols in parentheses are resistance gene symbols.

[b] L = Low infection type
H = High infection type

[c] Mutant race sought.

the greenhouse.

Fifteen days after inoculation, each of the 10 pots was used to inoculate 15 additional pots 30 eight-day-old plants of Danne. This innoculation was accomplished by the brushing technique (5), and the plants were then handled as previously described.

Fifteen days after this second increase, the uredospores were collected with a cyclone separator collector and stored in sealed glass vials, 0.05 gm per vial, in liquid nitrogen until required for the mutation experiments.

Gamma Radiation Experiments

Cobalt-60 (^{60}Co) contained in a "Gamma Cell 200" was used to develop the short wave length gamma rays for the radiation mutagenic experiments. This material and equipment was provided by the Department of Physics, Oklahoma State University. The "Gamma Cell 200" can deliver a maximum radiation dose of 300 R. (radiation dose) per minute. In these experiments five levels of radiation doses (72, 108, 144, 180, and 216 kR) were used with five replications of each dosage. The dosage levels were regulated by the time of exposure: 4, 6, 8, 10, and 12 hours, respectively.

Vials of uredospores of P. recondita tritici race 9A, stored in liquid nitrogen as described previously, were removed from storage and heat-shocked at 40 C in a water

bath for five minutes. Vials containing the heat-shocked uredospores were opened, and the spores remained in the vials. These vials were held at room temperature to re-hydrate for 12-15 hours before starting the radiation procedure.

Thirty of these opened glass vials of uredospores were placed in a 250 ml beaker in the chamber of the "Gamma Cell 200". At the end of each of 4, 6, 8, 10, and 12 hours, six vials were removed from the chamber, labeled with the dosage exposure, and stored in a refrigerator at approximately 4 C until used for germination tests and host inoculation.

Samples of uredospores from each replication of each radiation treatment were uniformly distributed on the surface of a water agar medium (1.5%) and incubated at 25 C for 12-15 hours. The percent of germinated uredospores was determined five times in each of four areas of the dish. In each area, approximately 150 to 250 spores were counted.

Since the mutant of culture 9A being sought would be virulent on LR-24, the irradiated spores were screened first on the cultivar Agent (CI 13523), which contains resistance gene LR-24. Seeds of cultivar Agent were planted as described for the cultivar Danne. Plants were grown in a rust-free greenhouse for 8-10 days.

Radiated vials of spores were held for 12-18 hours at room temperature, after which the uredospores were spread on the surface of water in a 15 cm plastic cup. The 8 to 10 day old seedlings of Agent were inoculated by the dipping

method (5). These inoculated seedlings were placed in moist chambers for 12-18 hours and transferred to cheesecloth-covered cages in a greenhouse. There were 300 seedlings of cultivar Agent inoculated in each replication of each treatment.

The inoculated seedlings were examined for disease reaction on the primary leaves after 16 days. The disease reactions that appeared on each leaf were counted and recorded. All the high infection type reactions were transferred by the brushing technique to leaves of 10-day-old seedlings of Agent to recheck the disease reaction. Only the high infection types appearing on this second group of plants were increased and tested on the standard differential host set (Table II). Pustules which were a high infection type on Agent were increased on that cultivar individually to provide spore production for the inoculation of differential hosts by the brushing technique. The entire experiment was repeated three times.

Chemical Mutagenesis Experiment

N-nitroso-N-methyl urea (NMU) was used in these experiments. It had been reported by Mikhailova (23) to be effective as a mutagen with the leaf rust fungus. The experiment was designed with 12 treatments composed of four concentrations of the NMU, 0.4, 0.6, 0.8, and 1.0 percent in 300 ppm "Tween 20" solution with three exposure periods: 6, 8, and 10 minutes.

Uredospores stored in glass vials in liquid nitrogen as described previously were used in this experiment also. The vials were removed from storage, heat-shocked in hot water at 40 C for five minutes, opened and held at room temperature for 12-18 hours. To form a stock solution N-nitroso-N-methyl urea was mixed with water containing a surfactant (300 ppm of "Tween 20", Polyoxyethelene 20 sorbitol monolaurate) in a magnetic swirl mixer for 30 seconds, and stabilized with HCl at pH6 to prepare the various concentrations used in the experiment. Three vials of uredospores were treated with the chemical in each of three replications.

After treatment, the spores were separated from the chemical by passing the spore suspension through a filter (Whatman #1 paper) followed by three washings with 150 ml each of the water-surfactant solution and three washings with sterile water. Uredospores then were vacuum dried at room temperature for 25-30 minutes. Samples of uredospores from each replication of treatment were distributed over a water agar prepared as previously described. Twelve to 16 hours later the level of germination was determined. The procedure for counting the percent germination of uredospores was done in the same manner as in the radiation experiment.

These chemically treated uredospores were used to inoculate 12 pots with 25 plants each of 10-day-old seedlings of the cultivar Agent by using the dip inoculation

technique (5). The inoculated seedlings were placed in moist chambers for a period of 12-18 hours and transferred to covered cages in a greenhouse. Disease reaction was examined after 15 days. The infection types that appeared on each primary leaf were recorded, and spores from the high infection types pustules were transferred to the primary leaf of 10-day-old seedlings of Agent to recheck the infection type. Isolates that produced a high infection type on both the original and the recheck plants were increased for further virulence identification on the standard differential hosts. The experiment was repeated three times.

Observation and Surveys of the Possibility of Natural Mutation

A survey of natural races pathogenic to resistance genes LR-17 and LR-19 was made in an area of southeastern Texas where the host and leaf rust pathogen are associated for an extended time period in environmental conditions optimum for disease development. The area chosen was at Beeville, Texas, where a substation of Texas A & M University is located. Two experimental wheat lines, Agatha/3* Comanche (OK75/R1A) containing the LR-19 host resistance to the leaf rust fungus, and Lucero/5* Comanche (OK77R6687) containing the LR-17 host resistance gene, were chosen for study. No culture of any race of the leaf rust

fungus has been found to be virulent on LR-19 in North America, and virulence on LR-17 has been found previously only in Canada (Young, H. C., Jr., personal communication). The two wheat lines were planted in the fall of 1980 in plots 1 x 20 m in cooperation with Mr. Lucas Reyes, Texas A & M University Agricultural Experiment Station, Corpus Christi, Texas. The experiment relied on natural infection. Examination for infection type was made in April 1981. High infection types were found on both wheat lines. Samples of these pustules were collected and returned to Stillwater where the uredospores were used to inoculate the cultivar Danne for increase. Individual pustules from this increase were used to verify virulence on wheat lines containing LR-19 and LR-17, respectively. High infection types on these lines were increased for identification of further virulence using the standard set of international differential wheat hosts.

CHAPTER IV

RESULTS

Radiation Experiment

This experiment was made to determine the possibility of using gamma radiation to induce a mutation to virulence on the corresponding wheat host resistance gene LR-24 in the leaf rust fungus Puccinia recondita f. sp. tritici race 9A. The effects of radiation at various dosages on uredospores were measured by percent germination, percent infection, variation in infection type from the original culture, and virulence spectrum on the eight standard differential hosts. The effect of radiation at various dosages (72, 108, 144, 180, and 216 kR) on uredospore germination is presented in Table III. The percent of uredospores that germinated decreased as the radiation dosage increased. At a level of 72 kR, the percent germination was 22 percent; at 108 kR, 15 percent, at 144 kR, 8.9 percent, at 180 kR, 5.8 percent and at 216 kR, 4.9 percent.

Uredospores treated with various dosages of gamma radiation were inoculated on the cultivar Agent to screen for mutants that would produce a high infection type on the corresponding host gene LR-24. The results of the

TABLE III

THE EFFECTS OF GAMMA RADIATION DOSAGE ON UREDOSPORE GERMINATION OF WHEAT LEAF RUST

Radiation Dosage in kR	Total Number of Spores Observed[a]	Number of Germinated Uredospores[b]	% Germination[c]	Reduction in Germination[d]
Control	3937	2259	57	-
72	3396	756	22	61
108	3906	621	16	71
144	3322	289	9	86
180	3965	230	6	90
216	3675	182	5	91

[a] Total number of spores observed from five replications of each treatment.

[b] Spores with germ tubes longer than the diameter of spore.

[c] Percent germination = $\frac{\text{Number of germinated uredospores in each treatment}}{\text{Total number of uredospores observed in each treatment}} \times 100$

[d] Percent reduction in germination = $\frac{\text{(Percent germination of non-treated) - (Percent germination in each treatment)}}{\text{Percent germination of non-treated}} \times 100$

observations of infection and infection types are given in Table IV. Infection types varied from a low infection type of 0; (corresponding to the original race 9A) to low infection types other than 0; and to high infection type 4. The total number of infections observed was gradually reduced as the radiation dosage increased. The number of infection type 0; lesions also decreased with increased dosage and in relative proportion to the total number of infections. Low infection types other than type 0;, and high infection types (3 and 4) were greatest at 108 kR and decreased with both increased and decreased radiation dosage. The percent reduction of infectivity was calculated on the basis of the non-treated control. As expected, infection was reduced as the dosage increased. The reduction was 80 percent at 72 kR and increased to 98 percent at 216 kR. The high infection type pustules recovered (Figure 1) were increased and identified on the standard set of differential hosts (Table V).

Of 39 mutant cultures obtained from radiation mutagenesis, three were lost during uredospore increase due to a lack of aggressiveness. Results of inoculating the differential hosts are given in Table V. All mutant cultures had high infection types on four cultivars: Malakof (LR-1), Democrat (LR-3), Westar (LR-10), and Agent (LR-24). Mutant culture R-9-39 showed a high infection type on Agent in the initial screening but an intermediate infection type when inoculated on the differentials. All

TABLE IV

NUMBER AND CLASSIFICATION OF INFECTION TYPES OBTAINED FROM GAMMA RADIATED WHEAT LEAF RUST UREDOSPORES SCREENED ON THE CULTIVAR AGENT

Radiation Dosage in kR	Total Number of Infections[a]	Infection Type 0;[b]	Other Low Infection Type	High Infection Type	% Reduction of Infectivity[c]
Control	2327	2325	2	0	-
72	447	420	24	3	80.7
108	475	406	47	22	79.5
144	178	157	12	9	92.4
180	72	61	10	1	96.9
216	49	39	10	1	97.9

[a] Total number of infections counted on 1,500 inoculated primary leaves of the cultivar Agent.

[b] Infection types classified according to Stakman et al. (33).

[c] Percent reduction in infectivity =

$$\frac{\text{Total number of infections of control} - \text{Number of infections in each treatment}}{\text{Total number of infections of control}} \times 100$$

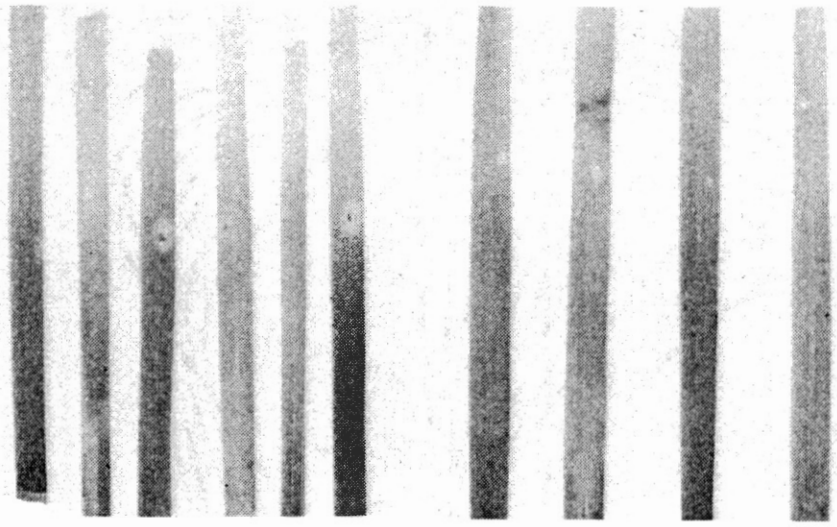

Figure 1. The Infection Type 0; of Original Leaf Rust Culture Race 9A on the Cultivar Agent Which Contains Leaf Rust Resistance Gene LR-24 (right), and High Infection Type Pustules Found on the Same Cultivar After Irradiation of Race 9A Uredospores (left)

TABLE V

THE INFECTION TYPES OF MUTANT ISOLATES OBTAINED FROM THE CULTIVAR AGENT AS A RESULT OF MUTAGENESIS WITH GAMMA RADIATION ON THE STANDARD SET OF WHEAT LEAF RUST DIFFERENTIAL HOSTS

Isolate Number	Host Differentials[a] Malakof (LR-1)	Webster (LR-2A)	Loros (LR-2C)	Democrat (LR-3)	Transfer (LR-9)	Westar (LR-10)	Agent (LR-24)	Wesel (LR-10+?)
9A[b]	88P[c]	88P	88P	04C	04C	88P	04C	15C
R-9-1	88P	04C	04C	88P	04C	88P	88P	78C
R-9-2	88P	04C	04C	88P	000	88P	88P	88P
R-9-3	88P	06C	04C	88P	04C	88P	88P	78C
R-9-4	88P	04C	04C	88P	04C	88P	88P	24C
R-9-5	88P	04C	04C	88P	04C	88P	88P	88P
R-9-6	88P	04C	04C	88P	04C	88P	88P	88P
R-9-7	88P	04C	06C	88P	000	88P	88P	26C
R-9-8	88P	04C	04C	88P	04C	88P	88P	88P
R-9-9	-[d]	-	-	-	-	-	-	-
R-9-10	88P	04C	24C	88P	000	88P	88P	88P
R-9-11	-[d]	-	-	-	-	-	-	-
R-9-12	88P	04C	26C	88P	000	88P	88P	88P
R-9-13	88P	02C	24C	88P	000	88P	88P	78P
R-9-14	88P	06C	26C	88P	88P	88P	88P	64C
R-9-15	88P	04C	06C	88P	000	88P	88P	64C
R-9-16	-[d]	-	-	-	-	-	-	-
R-9-17	88P	04C	78P	88P	04C	88P	88P	78P
R-9-18	88P	02C	06C	88P	000	88P	88P	26C
R-9-19	88P	04C	04C	88P	04C	88P	88P	24C
R-9-20	88P	06C	06C	88P	000	88P	88P	88P
R-9-21	88P	04C	04C	88P	000	88P	88P	78P

TABLE V (Continued)

Isolate Number	Host Differentials[a/]							
	Malakof (LR-1)	Webster (LR-2A)	Loros (LR-2C)	Democrat (LR-3)	Transfer (LR-9)	Westar (LR-10)	Agent (LR-24)	Wesel (LR-10+?)
R-9-22	88P	04C	06C	88P	88P	88P	88P	46C
R-9-23	88P	000	06C	88P	000	88P	88P	68P
R-9-24	88P	04C	06C	88P	000	88P	88P	88P
R-9-25	88P	04C	06C	88P	000	68P	88P	68P
R-9-26	88P	04C	04C	88P	000	88P	88P	88P
R-9-27	88P	04C	06C	88P	000	88P	88P	88P
R-9-28	88P	04C	04C	88P	000	88P	88P	88P
R-9-29	88P	000	06C	88P	000	88P	88P	88P
R-9-30	88P	04C	04C	88P	000	88P	88P	88P
R-9-31	88P	04C	04C	88P	000	88P	88P	88P
R-9-32	88P	04C	06C	88P	000	88P	88P	88P
R-9-33	88P	04C	04C	88P	000	88P	88P	67C
R-9-34	88P	04C	04C	88P	000	88P	88P	26C
R-9-35	88P	04C	88P	88P	24C	88P	88P	88P
R-9-36	88P	02C	26C	88P	000	88P	88P	88P
R-9-37	88P	04C	04C	88P	02C	88P	88P	88P
R-9-38	88P	04C	04C	88P	000	88P	88P	46C
R-9-39	88P	24C	78P	88P	000	88P	24C	88P

[a/] Symbols in parentheses are resistance gene symbols.

[b/] Parent culture.

[c/] Infection type according to Browder, and Browder and Young (4, 6).

[d/] Cultures lost during uredospore increase.

mutant cultures, in contrast to the parent culture, were avirulent on Webster (LR-2A), and all but three also were avirulent on Loros (LR-2C). There were two mutant cultures virulent on Transfer (LR-9) and most cultures were virulent or intermediate on Wesel (LR-10 plus an unknown gene). Therefore, there were mutant changes in virulence at the following loci: LR-2A from virulent (recessive) to avirulent (dominant or heterozygous); LR-2C from virulent (recessive) to avirulent (dominant or heterozygous); LR-3 from avirulent (dominant or heterozygous) to virulent (recessive); LR-9 from avirulent to virulent; Wesel unknown gene locus from avirulent to virulent or intermediate (heterozygous?); and of course LR-24 from avirulent to virulent. No changes occurred at the LR-1 or LR-10 loci. The culture sought, similar to the parent race 9A except on LR-24, was not obtained.

It was interesting to note that when the cultivar Newton (C.I. 17715), which is known to have LR-1 and LR-3, was used as a differential, all mutant cultures should have been virulent on it since all were virulent to both LR-1 and LR-3; however, four cultures (R-9-18, 34, 37, and 39) were avirulent on Newton.

A comparison was made of the virulence/avirulence (V/A) patterns found among the mutants derived from the different levels of radiation (Table VI). The dosage of 72 kR produced three mutants, each of which had a different V/A pattern. At 108 kR four patterns were found among 24

TABLE VI

PATTERNS OF INFECTION TYPES OF MUTANT CULTURES OBTAINED FROM DIFFERENT RADIATION TREATMENTS ON EIGHT STANDARD DIFFERENTIAL HOSTS

Dosage kR	No. of Isolates	Infection Type Patterns on the Differential Host (LR genes)								Group[a]
		1	3	24	2A	2C	9	10	Wesel	
72	1	H	H	H	L	L	L	H	L	A
	1	H	H	H	L	L	L	H	H	B
	1	H	H	H	L	L	H	H	L	C
108	6	H	H	H	L	L	L	H	L	C
	15	H	H	H	L	L	L	H	H	B
	2	H	H	H	L	H	L	H	H	D
	1	H	H	I	L	H	L	H	L	E
144	3	H	H	H	L	L	L	H	H	B
	3	H	H	H	L	L	L	H	L	A
	1	H	H	H	L	L	H	H	H	F
180	1	H	H	H	L	L	L	H	L	A
216	1	H	H	H	L	L	L	H	L	A
Parent Culture (9A)		H	L	L	H	H	L	H	L	
Mutant Sought		H	L	H	-	-	-	-	-	

[a] All isolates with the same letter had the same virulence/avirulence pattern.

cultures, two of those being the same as found among those at 72 kR. At 144 kR three patterns were found among seven cultures, two of which were the same as another pattern found with 72 kR and 108 kR. The other pattern was different. At 180 and 216 kR only one culture was found in each and the patterns of both were the same as found at 72 kR, 108 kR, and 144 kR. This pattern was the most common, and herein will be called group "A" (Figure 2). There were six mutant cultures in group "A", and at least one mutant with this pattern was found in each dosage treatment except 144 kR. The mutants in this group were virulent on LR-1, 3, 10, and 24 and avirulent on LR-2A, 2C, 9, and Wesel. The "B" group was the second most common, but found at dosages of 72 kR, 108 kR, and 144 kR only. This group was virulent on LR-1, 3, 10, and 24 and Wesel (LR-10+?) and avirulent on LR-2A, 2C, and 9, differing from group "A" only on Wesel (LR-10+?). There were only seven mutants in group "C" (Figure 3). They were found in dosages 72 and 108 kR, and were virulent on LR-1, 3, 9, 10, and 24 and avirulent on LR-2A, 2C, and Wesel (LR-10+?). It differed from group "A" by having virulence on LR-9. Group "D" had two mutants, both found at dosage 108 kR, and was one of only two groups virulent on LR-2C. These mutants were virulent on LR-1, 2C, 3, 10, 24, and Wesel (LR-10+?) and were avirulent on LR-2A and 9 only. Group "E" had only one mutant which occurred at dosage 108 kR. It was unique in that the original screening showed high infection type on Agent (LR-24) but after

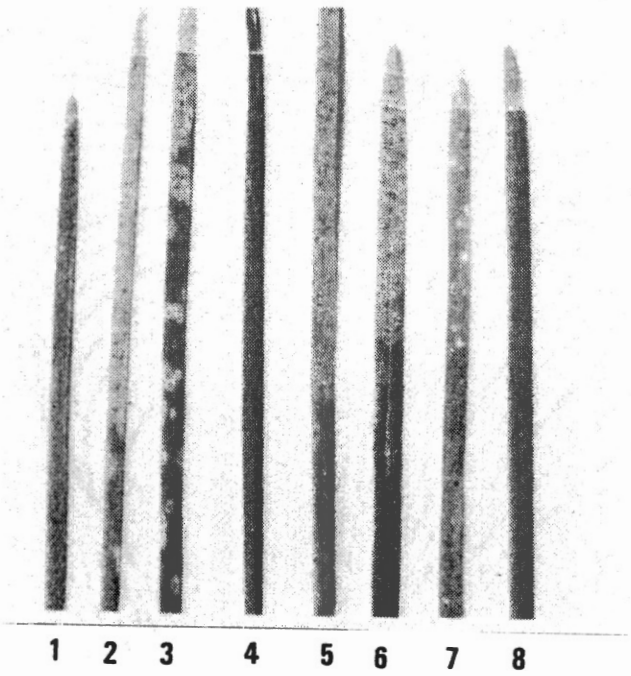

Figure 2. Mutuant Culture R-9-29, in Mutant Virulence/Avirulence Pattern Group "A" (the most common group), is Virulent on LR-1 (not shown), LR-3 (leaf 2), LR-10 (leaf 6), and LR-24 (leaf 5). It is Avirulent on LR-2A (leaf 1), LR-2C (leaf 7), LR-9 (leaf 4), Wesel (leaf 3), and Newton (leaf 8)

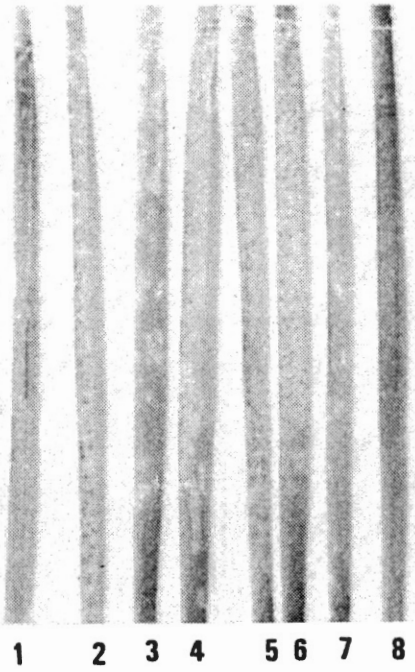

Figure 3. Mutant Culture R-9-22, in Mutant Virulence/Avirulence Group "C", is Virulent on LR-1 (not shown), LR-3 (leaf 2), LR-9 (leaf 4), LR-10 (leaf 6), and LR-24 (leaf 5). It is Avirulent on LR-2A (leaf 1), LR-2C (leaf 7), Wesel (leaf 3), and Newton (leaf 8)

increase, the reaction on Agent was intermediate (infection type 24C) (6). Otherwise it was similar to group "D", and was virulent on LR-1, 2C, 3, 10, and Wesel. There was only one mutant in group "F", which occurred at dosage 144 kR, and one of only two groups virulent on LR-9. It differed from group "C" only by virulence on Wesel (LR-10+?) and was virulent on LR-1, 3, 9, 10, and 24. It was avirulent on LR-2A and 2C.

An attempt was made to calculate the frequency of mutation from avirulence to virulence on resistance gene LR-24, by relating the number of mutant cultures obtained from each dosage treatment to the number of total infections obtained with untreated uredospores. Results indicate that the frequencies varied from 4×10^{-4} to 9.5×10^{-3} (Table VII). The highest mutation frequency obtained was with dosage 108 kR and the lowest mutation frequency in this experiment was with radiation dosage 216 kR.

Chemical Treatment

The chemical mutagen, N-nitroso-N-methyl urea, was used in this experiment and the results indicated it was effective in reducing uredospore germination. At the lowest concentration of 0.4 percent with six minutes exposure, the reduction in germination was 82 percent, and at the highest concentration of 1.0 percent with 10 minutes exposure germination was reduced 99 percent. The reduction of germination was calculated on the basis of the percent

TABLE VII

THE NUMBER OF INFECTIONS OBTAINED AND THE FREQUENCY OF MUTATION TO VIRULENCE ON HOST RESISTANCE GENE LR-24 FROM IRRADIATED UREDOSPORES OF *PUCCINIA RECONDITA* F. SP. *TRITICI* RACE 9A

Dosage in kR	Total Number of Infections Obtained[a]	Number of High Infection Types Recovered	Possible Mutation Frequency
72	447	3	1.3×10^{-3}
108	475	22	9.5×10^{-3}
144	178	9	3.8×10^{-3}
180	72	1	4.0×10^{-4}
216	49	1	4.0×10^{-4}
Control	2327	0	-

[a] Infection type 000 (6) may have easily been overlooked. Only infection types 01C and higher could readily be observed.

germination of the control. The procedure used with the "untreated" was to place spores in similar concentrations of "Tween 20" for 6, 8, and 10 minutes to indicate any effect the surfactant itself may have had. The percent germination obtained from the control treatments were 54 percent for six minutes, 53 percent for eight minutes, and 52 percent for 10 minutes exposure. These results are presented in Table VIII. The reduction in germination was quite high, perhaps too high, since the number of mutant high infection type infections on cultivar Agent (LR-24) was very low. Since there was little difference between lengths of exposure, possibly the chemical concentration should have been lower than 0.4 percent.

The total number of infections obtained when the primary leaves of cultivar Agent were inoculated with treated uredospores was counted and classified into low infection type (0;), low infection types other than 0; (1 and 2), and high infection types (3 and 4), as in the radiation experiment (Table IX). Although these are variable, possibly due to low recovery of uredospores from the dip inoculation methods, the total number of infections was found to decrease as the concentration and exposure increased. It was not determined if the occurrence of low infection types other than 0; represented mutations from a homozygous dominant avirulence state of the parent culture to heterozygosity, or if it represented a normal response to environmental factors as is sometimes encountered with the

TABLE VIII

OBSERVATION OF GERMINATION OF UREDOSPORES OF PUCCINIA RECONDITA F. SP. TRITICI RACE 9A FOLLOWING TREATMENT WITH THE CHEMICAL MUTAGEN N-NITROSO-N-METHYL UREA

Treatment Concentration	Exposure Time	Number of Spores Counted[a]	Number of Germinated Spores[b]	Percent Germination	Percent Reduction of Germination
0.4%	6	1991	188	9.4	82.6
	8	1630	121	7.4	86.0
	10	2475	107	4.3	91.7
0.6%	6	2382	103	4.3	92.9
	8	1917	55	2.9	94.9
	10	2014	68	3.3	93.7
0.8%	6	2051	41	1.9	96.3
	8	1976	39	1.9	96.4
	10	2179	15	0.7	98.7
1.0%	6	1889	10	0.5	99.1
	8	2113	11	0.5	99.1
	10	2256	13	0.5	99.0
Control	6	2043	1164	53.9	-
	8	2322	1232	52.9	-
	10	2129	1108	52.0	-

[a] A total of three replications of each treatment.
[b] Spores that had germ tubes longer than diameter of the spore.
[c] Control uredospores were exposed 6, 8, and 10 minutes in 300 ppm "Tween 20".

TABLE IX

THE TOTAL NUMBER OF INFECTIONS AND THOSE CLASSIFIED AS HIGH OR LOW INFECTION TYPES ON THE CULTIVAR AGENT INOCULATED WITH UREDOSPORES TREATED WITH THE CHEMICAL MUTAGEN N-NITROSO-N-METHYL UREA

Treatment Conc.	Exposure Time	Total No. Infections[a]	Infection Type		
			Low		High
			0;	Other Than 0;	
0.4	6	220	218	2	1
	8	196	191	4	0
	10	272	262	9	1
0.6	6	185	169	16	0
	8	156	143	10	3
	10	89	86	2	1
0.8	6	72	68	3	1
	8	78	71	6	1
	10	28	25	2	1
1.0	6	42	39	2	1
	8	30	23	5	2
	10	61	56	4	1
Control	6	1135	1135	0	0
	8	1079	1079	0	0
	10	1082	1082	0	0

[a] Total number infection types observed on 300 primary leaves of the cultivar Agent.

parent culture. In any case, there were 16 high infection types observed which were believed to result from mutation due to exposure to the mutagenic chemical. These cultures were evenly distributed over the range of both concentrations of the chemical used, and the exposure time at each concentration.

As indicated in the objective, this investigation was not designed to determine the frequency of mutation caused by the mutagenic chemical treatment; therefore, no attempt was made to calculate mutation frequency.

There were 16 mutant cultures obtained from the chemical treatment experiment (Table X). As with the parent cultures and with all mutants from the radiation experiment, all mutant cultures expressed virulence on the differential hosts, Malakof (LR-1) and Westar (LR-10). Also, as with the radiation experiment, all mutant isolates were induced to virulence on Democrat (LR-3) and Agent (LR-24), since the initial isolates were screened on that cultivar. On cultivar Webster (LR-2A), only the mutant culture C-9-7 had a high infection type, similar to the original culture; all remaining mutant cultures produced a low infection type on this differential. On the cultivar Loros (LR-2C), mutant cultures C-9-1, 2, 6, 7, 10, and 13 produced high infection types, as did the original culture; all other mutant cultures produced low infection types. All mutant cultures were avirulent on cultivar Tranfer (LR-9). Eleven of the 16 mutant cultures were virulent on the cultivar Wesel and five

TABLE X

THE INFECTION TYPES OF ISOLATES OBTAINED FROM TREATMENT OF UREDOSPORES OF *P. RECONDITA TRITICI* RACE 9A WITH N-NITROSO-N-METHYL UREA ON THE STANDARD SET OF DIFFERENTIAL HOSTS

Isolate No.	Host Differentials[a]							
	Malakof (LR-1)	Webster (LR-2A)	Loros (LR-2C)	Democrat (LR-3)	Transfer (LR-9)	Westar (LR-10)	Agent (LR-24)	Wesel (LR-10+?)
9A[b]	88P[c]	88P	88P	04C	04C	88P	04C	15C
C-9-1	88P	04C	88P	88P	000	88P	88P	46C
C-9-2	88P	04C	88P	88P	000	88P	88P	24C
C-9-3	88P	04C	04C	88P	000	88P	88P	88C
C-9-4	88P	06C	04C	88P	000	88P	88P	88C
C-9-5	88P	04C	06C	88P	000	88P	88P	88C
C-9-6	88P	04C	88P	88P	000	88P	88P	46C
C-9-7	88P	88P	88P	88P	000	88P	88P	88C
C-9-8	88P	04C	46C	88P	000	88P	88P	88C
C-9-9	88P	000	04C	88P	000	88P	88P	88C
C-9-10	88P	04C	88P	88P	000	88P	88P	67C
C-9-11	88P	04C	24C	88P	000	88P	88P	46C
C-9-12	88P	000	04C	88P	000	88P	88P	88C
C-9-13	88P	260	88P	88P	000	88P	88P	88C
C-9-14	88P	000	04C	88P	000	88P	88P	24C
C-9-15	88P	04C	04C	88P	000	88P	88P	78C
C-9-16	88P	04C	06C	88P	04C	88P	88P	88P

[a] Symbols within parentheses are resistant gene symbols.
[b] Original culture of race 9A, *P. recondita tritici*.
[c] Infection types classified according to Browder, and Browder and Young (4, 6).

produced an intermediate infection type, but none were as low as the original culture.

Therefore, it appears that no discernible mutations occurred at the LR-1 locus, as was true with the radiation experiment. At the LR-2A locus, all but one recovered isolates were different from the parent culture. At the LR-2C locus, 10 isolates differed from the parent, and the same association between these two loci occurred in the radiation experiment. In this case, no change occurred at the LR-9 locus, and all isolates were different from the parent culture on Wesel.

Later, all of these isolates were inoculated on the cultivar Newton and since all were virulent on both LR-1 and LR-3, all should have been virulent on Newton. However, only isolates C-9-3, 7, and 16 produced a high infection type on cultivar Newton. All other isolates produced a low infection type on that cultivar.

These isolates also were grouped according to their virulence/avirulence pattern (Table XI). The virulence spectrum of mutant cultures obtained from chemical treatment were classified into five different patterns according to their reaction on the eight standard differential hosts.

With the mutants produced by the chemical mutagen, the common pattern found was the one designated "B" in the radiation mutants. The most common group, "A", among the radiation mutants occurred only twice here, group "D" occurred once and two new groups, "G" (two isolates) and "H"

TABLE XI

INFECTION TYPE PATTERNS OF MUTANT CULTURES OBTAINED FROM N-NITROSO-N-METHYL UREA TREATED UREDOSPORES OF *P. RECONDITA TRITICI* RACE 9A

Treatment Conc. %	Exposed in min.	Number of Isolates	Infection Type Pattern on Differential Hosts (LR Genes) 1	3	24	2A	2C	9	10	W[b]	Group[a]
	6	1	H	H	H	L	L	L	H	H	B
0.4	8	0	-	-	-	-	-	-	-	-	-
	10	2	H	H	H	L	L	L	H	L	A
	6	0	-	-	-	-	-	-	-	-	-
0.6	8	2	H	H	H	L	L	L	H	H	B
	8	1	H	H	H	L	H	L	H	H	D
	10	1	H	H	H	H	H	L	H	H	H
	6	2	H	H	H	L	H	L	H	L	A
0.8	8	1	H	H	H	L	L	L	H	H	B
	10	1	H	H	H	L	L	L	H	H	B
	6	1	H	H	H	L	L	L	H	H	B
1.0	8	1	H	H	H	L	H	L	H	L	G
	8	1	H	H	H	L	L	L	H	H	B
	10	2	H	H	H	L	H	L	H	L	G
Parent Culture (9A)			H	L	L	H	H	L	H	L	
Mutant Sought			H	L	H	-	-	-	-	-	

[a] All isolates with the same letter had the same virulence/avirulence pattern.

[b] W = Wesel

(one isolate) also were found. Groups "C", "E", and "F", found among the radiation isolates, were not found here. The most common group "B" was found at all concentrations of the chemical, while the other groups were scattered among the other concentrations. The virulence spectrum of the mutant cultures both from radiation and chemical treatments was compared with races commonly found in the field in Oklahoma. Of the eight groups of mutant isolates, only one group, "A:, was similar to any common race, i.e., a variant of race 5 virulent on both LR-10 and LR-24. It is of interest that among radiation mutants, the group "A" isolates were the most common.

Survey of Possible Natural Mutation

High infection type pustules were found on both wheat lines planted in southern Texas, OK75/R1A and OK77R6687, which contained leaf rust resistance genes LR-19 and LR-17, respectively. Only a few pustules were found on OK75/R1A, 15 of which were collected and subsequently transferred to primary leaves of the same cultivar upon return to Stillwater. Two types of virulence patterns were found, the differences being virulence on LR-2A and LR-9 (Table XII and Figure 4). The isolate named BV-1 was virulent on LR-2A and avirulent on LR-9, and BV-2 was avirulent on LR-2A and virulent on LR-9. These cultures are the first found in the United States with the ability to attack LR-19.

The wheat line OK77R6687 apparently was segregating for

TABLE XII

THE INFECTION TYPE OF CULTURES OF LEAF RUST OBTAINED FROM THE WHEAT LINE OK/75R1A CONTAINING THE RESISTANCE GENE LR-19

Isolate[b]	Differential Hosts[a]							
	Malakof (LR-1)	Webster (LR-2A)	Loros (LR-2C)	Democrat (LR-3)	Transfer (LR-9)	Westar (LR-10)	Agent (LR-24)	Wesel (LR-10+?)
BV.-1	88P[c]	88P	88P	88P	46C	88P	04C	67C
BV.-2	88P	04C	88P	88P	88P	88P	04C	88P

[a] Symbols in parentheses are gene symbols.
[b] Abbreviation (BV.) according to the name of place where isolates obtained.
[c] Infection types classified according to Browder, and Browder and Young (4, 6).

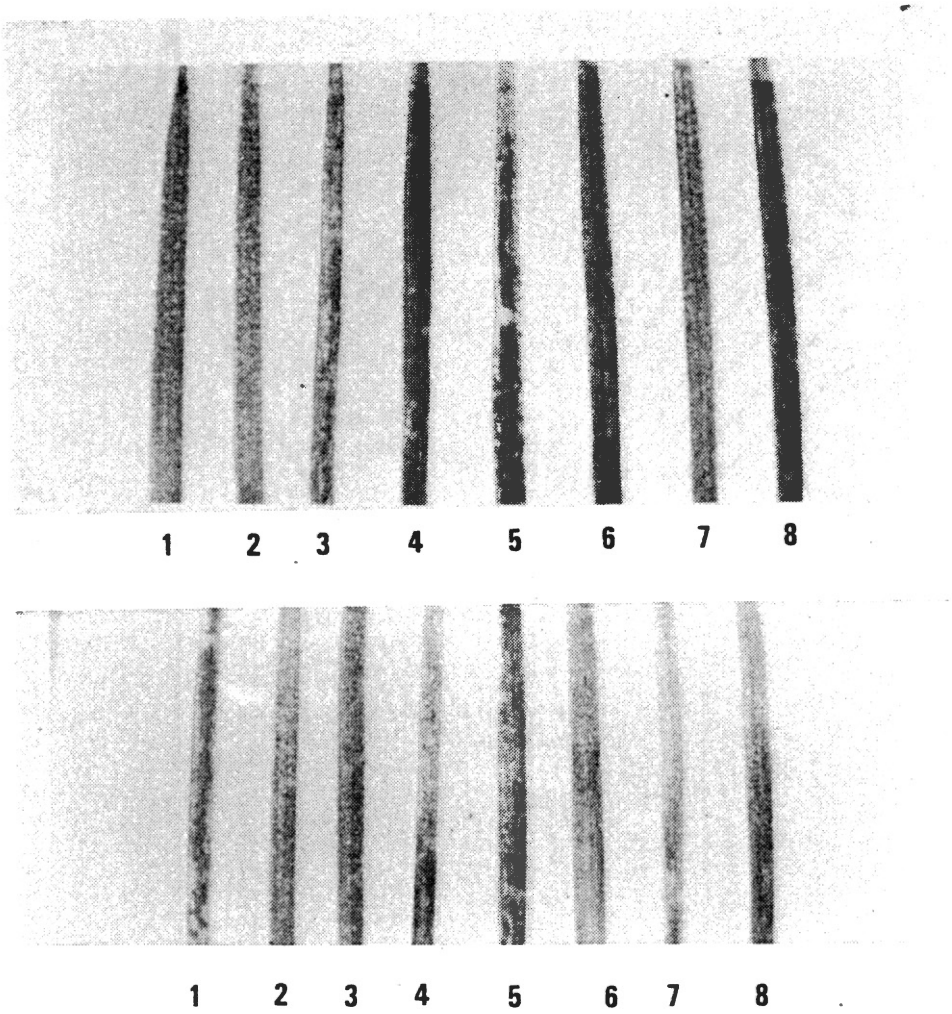

Figure 4. Infection Types of Isolates BV-1 (Top) and BV-2 (Bottom) on Eight Differential Hosts

Leaf Number	Differential
1	Webster (LR-2A)
2	Democrat (LR-3)
3	Wesel (LR-10+?)
4	Transfer (LR-9)
5	Agent (LR-24)
6	Westar (LR-10)
7	Loros (LR-2C)
8	Malakof (LR-1)

the resistance gene LR-17, and numerous plants had pustules. Many of these were collected and transferred to the same cultivar upon return to Stillwater. All such isolates failed to attack a pure line of wheat containing the LR-17 gene. It was apparent that the pustules were collected from the wrong segregate of the line OK77R6687. The other segregate was essentially free of leaf rust but there were a few plants with high infection type pustules. These were not collected, however.

CHAPTER V

DISCUSSION

The methods of determining the survival or germinability of the uredospores of this fungus *in vitro* were used to provide measures of lethality caused by mutagenic agents. In general, germination and infection, decreased as the dosage of mutagen increased. Such a response could be due to damage to both morphological structures and/or genetic components of the uredospores. Damage may be to membranes, enzyme inactivation, protein components or any number of altered structures or metabolic systems. Chromosome aberration, breakage and deletion also may result in non-functional uredospores (3, 31).

The dosage of mutagens which provided the maximum number of mutants in this study was similar to that reported by Rowell et al. (28, 29) for gamma radiation and by Mikhailova et al. (23) for N-nitroso-N-methyl urea. The radiation dosage treatment that provided the greatest number of viable mutants was between 108 and 144 kR and 0.4 to 0.8 percent concentration for six to eight minutes exposure for chemical treatment. As indicated by many investigators, the highest frequency of mutation usually occurred when the mutagen dosage produced lethality of 80 to 90 percent.

The experiments were not designed in such a way as to provide for calculation of mutation frequency. An attempt was made, however, to calculate mutation frequency from the results of the radiation experiment, but this calculation is speculative since the purpose of the study was to search for a specific mutant culture. No such attempt was made with the data from the chemical mutagen study, since the number of mutant cultures derived was so small. Many other mutants undoubtedly occurred with both mutagenic agents but were lost or not observed due to the screening on a single host genotype.

Mutagenic effects occur at random. In this investigation even when the exposed population was first screened for a single specific virulence locus, mutants occurred at all virulence loci investigated, except two (LR-1 and LR-10). It was obvious that certain loci mutated with a greater frequency than others; for example, many changes occurred at whatever locus or loci are involved with the differential Wesel, but few changes occurred at loci LR-2A, LR-2C, and LR-9.

Of special interest was the fact that each change which produced virulence at the LR-24 locus was accompanied by a change to virulence at the LR-3 locus. This suggests that these two loci are either closely associated, or that the changes that occurred were deletions (1, 31) of segments of chromatin material. If this is true then it may not be possible, or at least not simple, to produce the culture

that was sought at the onset of the study. It also may explain why such a culture has not been found in nature.

It was fortuitous that the mutant isolates were used to inoculate the cultivar Newton, which is known to contain both the resistance genes LR-1 and LR-3. The mutant cultures virulent on both LR-1 and LR-3 should have been virulent on the cultivar Newton. However, most of them were avirulent on Newton. It is certainly possible, then that cultivar Newton has one or more resistance genes that have not been identified and which expressed resistance to corresponding avirulence genes in the mutant cultures. Such information also suggests that there may be many genes for virulence present in the pathogen population in the avirulent condition (either homozygous dominant or heterozygous and therefore avirulent) that can not be identified due to unrecognized host resistance genes.

Such a theory is further substantiated by the study made of the presence of natural mutants in the pathogen population. Naturally occurring virulent mutants were recovered by using a host plant with the requisite resistance gene, in this case a wheat line containing resistance gene LR-19. While this same line has been planted in similar or greater quantities and distributed for several years (41), no high infection type pustules of leaf rust have ever been observed on it. However, in Oklahoma the host and pathogen are associated for a period of one to two months in the fall after planting and again in the

spring for about two months before harvest. In the area of southern Texas where this line was planted for the study, however, the host and pathogen are associated during favorable conditions for infection and disease development over the entire period from host emergence until ripening, a period of seven months or more. Under these conditions, a mutant in the pathogen population would have greater opportunity to infect a susceptible host than under conditions found in Oklahoma. It also is possible that the mutation rate could be higher in Texas than in Oklahoma. Schwinghamer (30) suggested that the cause of natural mutation may be a combination of radiation such as ultraviolet rays, terrestrial gamma rays and chemical mutagens at different levels to which the pathogen is exposed in nature. The chemical mutagens could be produced by the host plants or the pathogen as a result of impaired metabolism.

The unsuccessful attempt to find an LR-17 virulent mutant seems to have been due to a mixed or segregating host line and selection of uredia from the wrong segregate in the host population. The line should be purified and another attempt made so that LR-17 can be readily used in host resistance gene combinations.

CHAPTER VI

SUMMARY

1. Research was carried out to investigate the possibility of obtaining a specific mutant culture of Puccinia recondita f. sp. tritici race 9A that would be virulent on host resistance genes LR-1 and LR-24 and avirulent on host resistance gene LR-3 by using two types of mutagenesis treatments, radiation and chemical treatments.

2. In a radiation experiment, gamma radiation was used as the mutagen. Dosages of 72, 108, 144, 180, and 216 kR were applied to wheat leaf rust fungus uredospores of race 9A. The cultivar Agent, which contains the resistance gene LR-24, was used to screen the treated spores to detect virulence on that resistance gene.

3. Thirty-nine mutant cultures were obtained in the radiation experiment that were virulent on the cultivar Agent.

4. The highest number of mutant cultures was obtained from a radiation dosage of 108 kR.

5. Many of the mutant isolates obtained from the gamma radiation experiment had a virulence spectrum similar to race 5AAG on the differential hosts used.

6. In all, six virulence/avirulence patterns on the

eight differential hosts used in the study occurred. Some occurred more often than others, indicating variation in the rate of mutation at various loci.

7. All of the mutant isolates virulent on host resistance gene LR-24 were also virulent on LR-3. No mutants from the parent culture were found at the LR-1 and LR-10 loci. Mutants occurred at all other loci tested (LR-2A, LR-2C, LR-9, and the differential host Wesel which has LR-10 plus one or more unknown resistance genes).

8. In a chemical mutagen experiment, N-nitroso-N-methyl urea was used and uredospores of wheat leaf rust fungus race 9A were exposed at 0.4, 0.6, 0.8, and 1.0 percent concentrations for 6, 8, and 10 minutes at each concentration. The number of mutant isolates obtained by chemical mutagenesis were scattered in all treatments.

9. Sixteen mutant isolates were obtained from the chemical mutagen experiment that produced a high infection on the cultivar Agent (LR-24).

10. The virulence spectrum of mutant cultures fell into five groups, three of which were the same as those found in the radiation experiment. Again as in the radiation experiment, variation occurred at all loci except LR-1 and LR-10.

11. Although all mutant cultures from both the chemical and radiation experiments were virulent on host resistance gene LR-1 and LR-3, some mutant cultures did not attack the cultivar Newton which carried those two resistance genes,

indicating that there are possibly additional gene(s) in that cultivar.

12. A survey was made for races in nature that could produce high infection types uredia developed on wheat line OK75/R1A, planted in southern Texas, which carries the resistance gene LR-19. Two cultures virulent on LR-19 were recovered. Both were virulent on LR-1, LR-2C, LR-3, LR-10, and LR-24, and avirulent on LR-24. One culture was virulent on LR-2A and avirulent on LR-9, and the other was avirulent on LR-2A and virulent on LR-9.

13. No isolates virulent on LR-17 were obtained when uredospores were collected from a wheat line thought to carry LR-17 (OK77R6687) planted in southern Texas. That line was later found to be segregating for LR-17 and collection may have been taken from the wrong segregate.

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