# A STUDY OF MUTATION TO VIRULENCE IN *PUCCINIA RECONDITA* F. SP. *TRITICI*

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# INTRODUCTION

*Puccinia recondita* Rob. ex. Desm. f. sp. *tritici* is the causal agent of leaf rust of wheat. The population of this fungus consists of morphologically similar but parasitically distinct genetic biotypes. The biological mechanisms by which these biotypes have been shown to originate are mutation, hybridization, heterocaryosis, and parasexualism (10, 12, 26). Having arisen by one of these means, those biotypes, which by their inherent pathogenicity impart a more favorable chance for survival of the pathogen, have a reproductive advantage and are likely to become a dominant part of the rust population. As a result, newly developed disease-resistant cultivars commonly have succombed to new races of the pathogen not previously encountered. It is obvious that more needs to be learned about the function of these mechanisms in the derivation of new races of different virulence.

Heterocaryosis, parasexualism and hybridization are mechanisms by which new combinations of the virulence genes present in the gene pool are shuffled. It was found that hybridization could, but apparently does not, function to any appreciable extent in nature, since more virulent combinations were isolated from the alternate host on which sexual recombination occurs than were encountered in field collections (16).

Mutation is the only mechanism by which new virulence genes can occur. Naturally occurring mutations to greater virulence in the

cereal rusts have been reported to occur both in nature (11, 23, 25) and in the laboratory (7, 26, 28). The object of this investigation was to evaluate the stability of certain differential and resistant cultivars currently being used as sources of resistance in the breeding program by measuring the relative mutation rates of different cultures to virulence on these cultivars.

## LITERATURE REVIEW

The experience of observing a resistant cultivar succomb to a new biotype either before or soon after release for commercial production is common to most plant breeders. It is an experience as old as the science of plant breeding. However since these new biotypes do occur or arise in nature and must reach some proportion of the population before they are detected, it is often difficult to be sure where or how they arose.

Johnson and Newton (11) reviewed the occurrence of new biotypes of the cereal rusts in North America up to 1946. Following the stem rust epidemic of 1935, the spring wheat cultivar Thatcher was replaced by cultivars carrying the adult-plant type of resistance to stem rust derived from the cultivar Hope. By 1945 outbreaks of epidemic proportion on those cultivars were evidence that new sources of resistance were needed. The Hope type resistance was then replaced by other sources of resistance. Again in 1950, stem rust race 15B, a previously unimportant race, swept the wheat growing areas inciting widespread damage by 1953 and 1954 (22).

In Oklahoma where leaf rust is important, the stability of resistant cultivars is evaluated concurrently with their use in the breeding program by exposure to the natural population of the fungus in trap plots located over the state and in neighboring states. In 1967 a susceptable type reaction was noted for the first time on the cultivars Aniversario, Lucero, and a selection of the cross Wabash/

American Banner//Aniversario. Cultures isolated from these cultivars produced a virulent reaction type on these cultivars in the greenhouse and were found to be variants of race UN6. More recently, cultures have been collected from these plots that produce a type 2 reaction (22) on the cultivars Timpaw and Preska. These varieties previously exhibited only a fleck reaction to cultures isolated from the Oklahoma area (Young, personal communication). Similar histories have been recorded in the wheat growing areas of Australia-New Zealand where resistance breeding has been practiced (24, 25, 27).

Reports of naturally occurring mutations for urediospore color have been numerous since spore color changes are easily detected. Orange or grey-brown color mutants have been reported for many species of the rusts, such as; Puccinia graminis tritici (13, 24), Puccinia graminis secalis (4), Puccinia recondita (9), Puccinia glumarum (8), Puccinia anomala (5), and from a cross of Puccinia graminis tritici and Puccinia graminis agrostidis (23). A color mutant reported by Newton and Johnson (13) also differed in spore size and viability.

Johnston (9) reported an aberrant form of Puccinia triticina (recondita) that may have arisen through mutation. It was first sighted at the Denton, Texas, Agricultural Experiment Station in 1927. The length of the period from infection to spore production was 15 days instead of the usual 5 to 7 days. Flecks appeared at the site of the infection by the tenth day at which time other cultures had already developed uredia. Urediospore color was a light orange rather then the normal orange red. Individual sori of the aberrant form on susceptable cultivars were smaller in length and

width than those of other races and the urediospore size was smaller. Although not more virulent, the culture gave reactions on differential cultivars not previously described.

The occurrence of natural mutations for additional virulence during culture in the greenhouse has been rare. This is not surprising when the techniques used in the study of the parasite population are considered. Uredial cultures are often propagated on differential cultivars to lessen the chances of contamination. Mutations to avirulence are therefore lost due to inability of the pathogen to infect the differential cultivar. Mutations to virulence would be detected only if the culture was heterozygous at that locus or if simultaneous mutations occurred at the same loci in both dikaryotic nuclei. Even then the mutant would not be detected unless a differential for that particular gene were used.

Stakman et. al. (23) observed several aberrant forms that were considered to have arisen from form 1 of Puccinia graminis tritici which had been cultured for 13 years without change in virulence. One was more virulent on three cultivars, two were less virulent, and another was different from any previously tested culture in its range of virulence.

Newton and Johnson (14) were surprised to find that a culture of pure race 52 had changed virulence during storage in a refrigerator at 8C and appeared to be a mixture of races. The culture which had been in storage for 8 months was put back in the refrigerator for 4 months after which the entire culture had mutated to the new race. The new race, not previously described, was characterized by reduced virulence on four differentials and increased virulence on three differentials.

The resistance of Transfer (Aegilops umbellulata / Triticum aestivum var. Chinese Spring) was thought by some to be the ultimate in leaf rust resistance. However Samborski (19) observed a single sporulating type 1 pustule on Transfer growing in the field that had been routinely inoculated with bulk collections of urediospores. The culture was selfed on the alternate host and the aecial infections on the wheat host segregated 1:2:1 for virulence to the Transfer gene. The homozygous virulent segregate produced a 4 type pustule on Transfer and a 1 to 2 on Aegilops umbellulata indicating that modifier genes are present in that species. This was the only known North American culture capable of sporulation on Transfer.

Flor (7) used four single gene differentials to study natural mutation rates of flax rust. He found two mutants for the variety Birio from a minimum of 200,000 urediospores screened, one mutant for Cass from 600,000 spores screened and no mutants for Leona or Polk from a minimum of 300,000 and 900,000 spores screened, respectively. These natural mutations were the same as those which occurred in higher frequency following exposure to X-ray radiation suggesting that those loci most responsive to mutagens may be the least stable under natural conditions.

Watson (26) used a combination of spore color and pathogenicity to detect virulence mutants in stem rust of wheat. A mutation in the orange culture NR-1 was discovered on Lee. In addition to the virulent "X" or mesothetic reaction on Lee, the mutant culture possessed virulence on Gabo, Timstein, Gaza, and Kenya C6042 not possessed by the parent culture. From race 11, another orange culture, a mutant virulent on Lee produced the same reaction to Lee, Gabo, Timstein,

Gaza, and Kenya C6042 as the mutant of NR-1.

Zimmer et. al. (28) cultured three races of Puccinia coronata var. avenee on a susceptible cultivar for several generations and used some of the inoculum from each of the previous generations to inoculate selected resistant cultivars. The estimated rate of mutation of race 202 for virulence on the cultivar Ascencao was 1 in 2,200 infections; for virulence on Ukraine was 1 in 6,450 infections; and of race 290 for virulence on Ukraine was 1 in 7,900 infections. No mutants of race 203 for virulence on seven resistant cultivars were observed.

In order to study the mutation process, artificial acceleration of mutation rates is a necessary and valid prerequisite. Flor (6), the first to induce mutations for virulence, used ultraviolet light (UV) as a mutagen with urediospores of Melampora lini. Later he found a high frequency of induced mutants were obtained when X-rays were used (7). The culture irradiated was the F1 of a cross of race 1 and 22 which was heterozygous for avirulence on 17 single gene differentials. From the 100,000 to 200,000 viable spores screened on each of 13 single gene differential cultivars, a total of 154 mutants developed on 8 of the cultivars. The number of mutations per cultivar varied from 2 to 49. From the 94 mutants cultured, 92 differed from the F1 parent in virulence to one differential, corresponding to a single "hit", and two differed by virulence on two differentials corresponding to two "hits". In cases where virulence on 2 or more cultivars was known to be inherited as a unit, i.e. allelic or closely linked, mutants of these genes on all cultivars involved were identical.

The effect of radiation dose, spore water content, and type of ionizing radiations on the frequency of mutation for virulence at one locus was investigated by Schwinghamer (21). For UV and fast neutron radiation the frequency was proportional to dose, indicating "point" changes in chromosome structure. For X-ray and gamma-ray irradiation, the frequency varied with the square of the dose indicating deletion type mutations. The rate of mutation increased sharply as spore water content was varied from 45 to 70%. The average maximum frequency of mutations was 2.0% for fast neutrons, 1.5% for X-rays, and 0.3% for UV.

## MATERIALS AND METHODS

### Cultures used

The leaf rust culture, race UN1-NA65-1, expresses fewer genes for virulence than any other culture found in field populations. It is virulent only on the "Universally Susceptible" class of cultivars such as Bison and Cheyenne. Since it expresses no genes for virulence on the ten differentials of the Unified Numeration and North American 1965 sets of cultivars, it possesses the largest number of loci possibly heterozygous for virulence on these cultivars. The heterozygosity of this culture has been shown by Kucharek (unpublished data). For this reason this race was chosen to evaluate the rate of mutation to virulence on the 10 differentials and eight other supposedly resistant cultivars used in the present resistance breeding program. This culture was irradiated with 40 Kr. of X-rays and 40 Kr. of gamma-rays in separate experiments.

The other culture used was race UN13-NA65-15, which was virulent on eight of the ten differentials. It was irradiated with 55 Kr. of X-rays to evaluate the effect of a rather virulent culture on the origin of additional genes for virulence in that culture.

Both cultures were obtained from field collections. Single pustule isolates were propagated in isolation by inoculation to a large number of susceptible plants. Differential plants were inoculated simultaneously to check for contamination with each increase of urediospores.

Irradiation of urediospores

Bulked lots of urediospores were hydrated over a saturated solution of sodium bisulfate for 24 hours prior to irradiation to regulate spore water content (20). Spores were exposed to X-rays with a Picker X-ray machine, 70 Kvp (unfiltered), 10 ma, 65 R/ma X sec, 10 sec/min or to gamma-rays with a $Cs^{137}$ source at 990 r/min.

Planting of resistant cultivars

Thirty to sixty seeds of a resistant cultivar were planted in soil in 3-oz plastic-coated, paper, ice cream cups. In each cup eight seeds of the susceptible cultivar Cheyenne (C. I. 8885) were distributed among the seeds of the resistant cultivar. In the first experiment, four cups of each of 7 cultivars (See Tables I and II) were grown in isolation; three were inoculated with irradiated spores of race UN13-NA65-15 and one was inoculated with spores of UN13-NA65-15 not irradiated. In the second experiment, eight cups of each of 18 cultivars (See Tables I and III) were grown in isolation; seven were inoculated with irradiated spores of race UN1-NA65-1; one was inoculated with spores of UN1-NA65-1 not irradiated.

Inoculation of resistant cultivars

Irradiated spores were rehydrated in a saturated atmosphere for 24 hours prior to inoculation and germinability was checked each time on non-nutrient agar. Bulked lots of irradiated spores were divided into 10 mg samples and placed into No. 00 gelatin capsules. Six tenths ml of a light oil (Mobisol 100) was added and the oil and spores were mixed. A portion of the oil-spore mixture was transfered to a haemocytometer and the spores were counted to determine the number of

TABLE I

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

| Variety or Selection | C. I.[1] or Selection No. | Abbreviation[2] |
|---|---|---|
| Malakof | 4898 | MA |
| Webster | 3780 | WST |
| Loros | 3779 | LS |
| Mediterranean | 332 | MI |
| Democrat | 3384 | DO |
| Dular | 13373 | DLR |
| Lee | 12488 | LEE |
| Waban | 14018 | WBN |
| Sinvalocho | 12096 | SVL |
| Exchange | 12635 | ECH |
| Westar | 12110 | WTR |
| Wesel | 13090 | WSL |
| Aniversario | 12578 | AIV |
| Lucero | 14047 | LCO |
| Agent | 13523 | AG |
| Agrus | 13228 | ARS |
| Timpaw | 14154 | TPA |
| Preska | 14153 | PSK |
| 5*Wichita/Transfer | 13853 | 5*WI/TF |
| Marquillo/Oro//Pawnee/ 3/Frontana | S61R8538 | MQL/ORO// PN/3/FTN |
| Wanken 2 | 14049 | WK 2 |

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

[2] Abbreviations used were made according to rules adopted by the National Wheat Improvement Committee as amended by KONZAK. See Agronomy Journal. 52:613, 1960; U. S. Dept. of Agr. Tech. Bull. 1278, p. 131, 1963; and Wheat Newsletter 1965.

spores/ml of oil. The capsule was then fitted to an aluminum atomizing block. Resistant cultivars were inoculated by a variation of the method described by Prescott and Young (17). Cups of 5-6 day old resistant seedlings were placed on a 78 rpm record turntable inside an inoculating chamber and atomized with the spore-oil suspension for two turns of the turntable. Inoculated plants were placed in a moist chamber, misted lightly with water and a surfactant (Tween-20, 2 drops in 600 ml) and left overnight. The next morning, plants were removed from the moist chamber and placed in a controlled environment chamber. The controlled environment chamber was maintained at a temperature of 20C with fluctuations held to _1C. The photoperiod was set at 12 hours of light (3000 fc) and 12 hours of darkness. Plants were watered daily with a solution of "Hyponex" (1 tablespoon/gallon of water).

All rooms were misted with water and a surfactant and a plastic cage constructed aroung the controlled environment chamber was misted prior to exposure of the plants to settle the dust and minimize contamination by airborne spores.

Leaves with pustules varying from the normal type were detached and cultured by Prescott's modification (16) of Browder's detached leaf culture technique (2). Variants with sufficient vigor were increased on susceptible plants and returned to the cultivar on which they were first observed, together with the conplete set of ten differentials and the eight resistant cultivars.

## RESULTS

### Reduction in infectability

The reduction in the ability of the urediospores to infect the host due to radiation damage was measured by calculating the difference in the number of spores inoculated to produce an infection with the irradiated spores compared with the spores not irradiated. The following formula was used to find the percent reduction in infectability:

$$\frac{\dfrac{\text{number of spores per ml inoculated (irradiated)}}{\text{number of derived infections per plant}}}{\dfrac{\text{number of spores per ml inoculated (not irradiated)}}{\text{number of derived infections per plant}}}$$

The average reduction in infectability of race UN13-NA65-15 spores irradiated with 55 Kr. of X-rays was 67%.

### Number and frequency of mutants

The number of infections screened was derived from the average number of infections on the eight susceptible plants and the number of resistant plants in each cup. From the total number of infections screened and the total number of mutants for each cultivar the mutation frequency was expressed as a percent of the infections screened.

The number of mutants that developed on the six resistant cultivars inoculated with X-ray irradiated spores of UN13-NA65-15

varied from 0-12 (Table II). No mutants were observed from the estimated 1369 infections screened on the resistant cultivar 5*Wichita/Transfer. Several mutants developed on each of the other five cultivars with a mutation frequency of .38 to 2.03%. The inoculation on the cultivar Preska was not uniform enough to evaluate the number of infections screened.

Inoculations of 40 Kr., X-rayed UN1-NA65-1 to 7 cups each of the 18 resistant cultivars was unsuccessful on two different occasions. The germination of the urediospores on agar was 60% the first time and 84% the second, and similar plants inoculated at the same time with spores not irradiated developed normal uredia in expected numbers.

More infections were screened per plant in the experiment involving race UN1-NA65-1 treated with gamma-rays because the plants were misted with a finer mist following inoculation, which produced smaller droplets and therefore more individual infections. The number of mutants that developed on the eighteen resistant cultivars inoculated with irradiated spores of race UN1-NA65-1 varied from 0 to 116 (Table III). Again, no mutants were observed on the resistant cultivar 5*Wichita/Transfer from the more than 12,000 infections screened. In addition, no mutants were observed on the varieties Malakof, Webster, Waban and Exchange which were not tested in the previous experiment. A high rate of mutation to virulence on the cultivars Preska and Agent was noted, 1.28% and 0.93%, respectively. Intermediate rates were noted for mutation to virulence on Aniversario, Marquillo/Oro//Pawnee/3/Frontana, Loros and Mediterranean. Relatively low rates of mutation for virulence were observed on the cultivars

TABLE II

THE NUMBER OF UREDIAL INFECTIONS SCREENED AND THE FREQUENCY OF MUTATION TO VIRULENCE IN RACE UN13-NA65-15 ON SIX RESISTANT CULTIVARS

| Variety | Average number of infections on susceptible plants | Number of resistant plants inoculated | Total number of infections screened | Number of mutant uredia | Mutation frequency (percent of infections) |
|---|---|---|---|---|---|
| LCO[1/] | 6.3 | 94 | 595 | 12 | 2.03 |
| AIV | 20.0 | 80 | 1600 | 11 | 0.69 |
| TPA | 13.7 | 116 | 1589 | 6 | 0.38 |
| AG | 16.5 | 93 | 1534 | 12 | 0.78 |
| 5*WI/TF | 16.5 | 83 | 1369 | 0 | 0.00 |
| MQL/ORO// PN/3/FTN | 10.1 | 64 | 646 | 3 | 0.46 |
| PSK | [2/] | - | - | 4 | - |

[1/] Abbreviations used were made according to rules adopted by the National Wheat Improvement Committee as amended by KONZAK. See Agronomy Journal 52:613, 1960; U. S. Dept. of Agr. Tech. Bull. 1278, p. 131, 1963; and Wheat Newsletter 1965.

[2/] Unable to evaluate the number of infections screened.

TABLE III

THE NUMBER OF UREDIAL INFECTIONS SCREENED AND THE FREQUENCY OF MUTATION TO VIRULENCE IN RACE UN1-NA65-1 ON EIGHTEEN RESISTANT CULTIVARS

| Variety | Average number of infections on susceptible plants | Number of resistant plants inoculated | Total number of infections screened | Number of mutant uredia | Mutation frequency (percent of infections) |
|---|---|---|---|---|---|
| LCO[1/] | 41.1 | 302 | 12,399 | 19 | 0.15 |
| AIV | 11.5 | 332 | 3,809 | 21 | 0.55 |
| TPA | 15.7 | 335 | 5,255 | 6 | 0.11 |
| AG | 31.8 | 308 | 9,795 | 91 | 0.93 |
| 5*WI/TF | 40.3 | 303 | 12,222 | 0 | 0.00 |
| MQL/ORO// PN/3/FTN | 25.4 | 447 | 11,324 | 84 | 0.74 |
| ARS | 32.0 | 424 | 13,576 | 0 | 0.00 |
| PSK | 30.8 | 295 | 9,087 | 116 | 1.28 |
| MA | 40.2 | 146 | 5,868 | 0 | 0.00 |
| WST | 53.6 | 242 | 12,967 | 0 | 0.00 |
| LS | 28.4 | 206 | 5,841 | 39 | 0.67 |
| MI | 33.3 | 184 | 6,119 | 47 | 0.77 |
| DO | [2/] | - | - | - | - |
| DLR | 33.1 | 280 | 9,274 | 5 | 0.05 |
| LEE | 34.6 | 334 | 11,546 | 6 | 0.05 |
| WBN | 24.0 | 326 | 7,831 | 0 | 0.00 |
| SVL | 26.7 | 355 | 9,471 | 24 | 0.25 |
| ECH | 29.3 | 317 | 9,278 | 0 | 0.00 |

[1/] (See Table II)

[2/] Inoculation unsuccessful.

Lucero, Timpaw, Dular, Lee and Sinvalocho.

The growth of many mutants was not vigorous and they failed to sporulate sufficiently in detached leaf culture to continue propagation. Others were over-run with saprophytic fungi since the leaves were not surface sterilized prior to detached leaf culture. Mutant pustules were often associated with expanding necrotic circles with only limited sporulation. These necrotic circles turned dark brown rather than the bleached tan color normally observed for the resistant reaction and in the center a small black telial sorus occasionally developed. It was noted that while benzimidazol hastened the conversion to telial formation, these unusual sori were often seen on plants growing in the soil only 10 to 13 days after inoculation. This type of reaction was noted especially on the varieties Agent and Preska (Figure 1).

From the mutants isolated and cultured on detached leaves, 61 sporulated sufficiently to be increased on susceptible Cheyenne plants. Each was then inoculated to a pot of wheat seedlings of the cultivar from which the mutant pustules were originally isolated (Table IV). Most of the mutants maintained their virulence to the cultivars on which they were originally isolated. Marquillo/Oro//Pawnee/3/Frontana, resistant to the parent culture, produced a susceptible type reaction to the mutants isolated from this cultivar. The mutants to virulence on the other cultivars, though sporulating to a greater degree, did not produce a virulent reaction. A few of the cultures appeared to be environmental variants or perhaps tri-caryons and lacked the virulence which they had originally displayed when returned to the parent cultivar.

Many of the 61 cultures propagated on Cheyenne were also used to

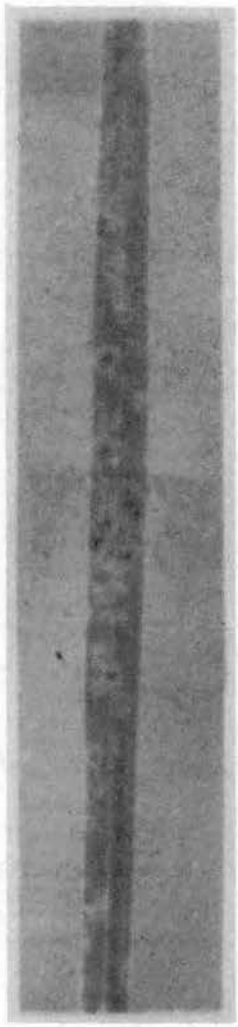

Figure 1. A mutant culture of race UN1-NA65-1 originally isolated on the cultivar Dular and grown on the cultivar Preska showing unusual pustules with black centers.

TABLE IV

THE VIRULENCE OF MUTANT CULTURES WHEN RETURNED TO THE CULTIVAR FROM WHICH THEY WERE ORIGINALLY ISOLATED

| Cultivar | Number of mutants tested | Number of mutants with more virulent reaction | Reaction of parent cultivars: to parent culture | Reaction of parent cultivars: to mutants |
|---|---|---|---|---|
| | | From race UN13-NA65-15 | | |
| MQL/ORO//PN/3/FTN[1/] | 11 | 11 | 0;-1 | 2-4 |
| TPA | 2 | 2 | 0; | 0; |
| AIV | 5 | 4 | 0;-1 | 1-2 |
| PSK | 4 | 4 | 0; | 0;-1 |
| LCO | 1 | 1 | 0; | 0; |
| AG | 5 | 5 | 0; | 0;-1 |
| | | From race UN1-NA65-1 | | |
| LS | 10 | 8 | 0; | 0;-1 |
| MI | 9 | 7 | 0; | 0;-2 |
| DLR | 4 | 1 | 0;-1 | 2 |
| LEE | 6 | 6 | 0; | 0; |
| SVL | 4 | 4 | 0; | 3-4 |

[1/] Abbreviations used were made according to rules adopted by the National Wheat Improvement Committee as amended by KONZAK. See Agronomy Journal. 52:613, 1960; U. S. Dept. of Agr. Tech. Bull. 1278, p. 131, 1963; and Wheat Newsletter 1965

inoculate the complete set of differentials in use for race identification and the nine cultivars used in resistance breeding. Infection types of some of the mutants of race UN1-NA65-1 cultured on the differential and resistant cultivars are listed in Table V and pictured in Figures 2 and 3. Most of the virulence changes in the pathogen resulted in changes from a 0; to a 0;-2 infection type. However one of the mutants isolated on Mediterranean produced a susceptible type reaction on Waban instead of the normal immune reaction (Figure 3) and an avirulent reaction on Wesel instead of the normal type 3 reaction. A mutant isolated on Sinvolocho also possessed additional virulence on Webster.

The reaction of two mutants isolated on Marquillo/Oro//Pawnee/3/Frontana is compared to the parent culture UN13-NA65-15 in Table VI. In addition to virulence on Marquillo/Oro//Pawnee/3/Frontana possessed additional virulence on Lucero, Agent, and Wanken 2 (Figures 4 and 5).

TABLE V

THE VIRULENCE OF THE PARENT CULTURE, RACE UN1-NA65-1 AND CERTAIN MUTANT CULTURES FROM IT TO CERTAIN DIFFERENTIAL AND RESISTANT CULTIVARS

| | Reaction to race UN1-NA65-1 | Reaction of mutants of race UN1-NA65-1 originally isolated from: | | | | | |
|---|---|---|---|---|---|---|---|
| | | MI | MI | DIR | LEE | LEE | SVL |
| Unified Numeration Differentials | | | | | | | |
| MA[1] | 0 | 0; | 0; | 0; | 0 | 0 | 0; |
| WST | 0 | 0 | 0; | 0 | 0 | 0 | 4 |
| LS | 0; | 0;-2 | 0; | 0; | 0; | 0;-1 | 0; |
| MI | 0; | 0;-2 | 0;-2 | 2 | 0;-1 | 0;-1 | 0; |
| DO | 0; | 0; | 0;-1 | 2 | 0;-2 | 0; | 0; |
| North American 1965 Differentials | | | | | | | |
| DIR | 1 | 0;-2 | 0; | 2 | 0;-2 | 0;-1 | 0; |
| LEE | 0; | 0; | 0; | 2 | 0;-1 | 0;-3 | 0; |
| WBN | 0; | 4 | 0; | 0; | 0 | 0 | 0; |
| SVL | 0; | 0; | 0; | 0; | 0 | 2/ | 1 |
| ECH | 0; | 0; | 0;-1 | 1 | 0; | 0;-1 | 0; |
| Other Differentials | | | | | | | |
| WIR | 0; | 2/ | 0; | 0; | 0; | 0; | 0; |
| WSL | 3 | 0; | 3 | 3 | 3 | 3 | 3 |
| Resistant Cultivars | | | | | | | |
| LCO | 0; | 0; | 0; | 0; | 0; | 0; | 0; |
| AIV | 0; | 0; | 0;-3 | 0; | 0; | 0;-1 | 0; |
| AG | 0; | 0; | 0; | 1 | 0; | 0; | 0; |
| ARS | 0 | 0 | 0; | 0; | 0 | 0 | 0 |
| TPA | 0; | 0; | 0; | 0; | 0; | 0; | 0; |
| PSK | 0; | 0; | 0;-1 | 1 | 0;-1 | 0; | 0; |
| WK 2 | 0 | 0 | 0; | 0 | 0 | 0 | 0 |
| 5*WI/TF | 0 | 2/ | 0 | 0 | 0 | 0 | 0 |
| MQL/ORO// PN/3/FTN | 0; | 0; | 0;-1 | 2 | 0;-1 | 0;-1 | 0; |

[1]/Abbreviations used were made according to rules adopted by the National Wheat Improvement Committee as amended by KONZAK. See Agronomy Journal. 52:613, 1960; U. S. Dept. of Agr. Tech. Bull. 1278, p. 131, 1963; and Wheat Newsletter 1965.

[2]/Cultivar not tested.

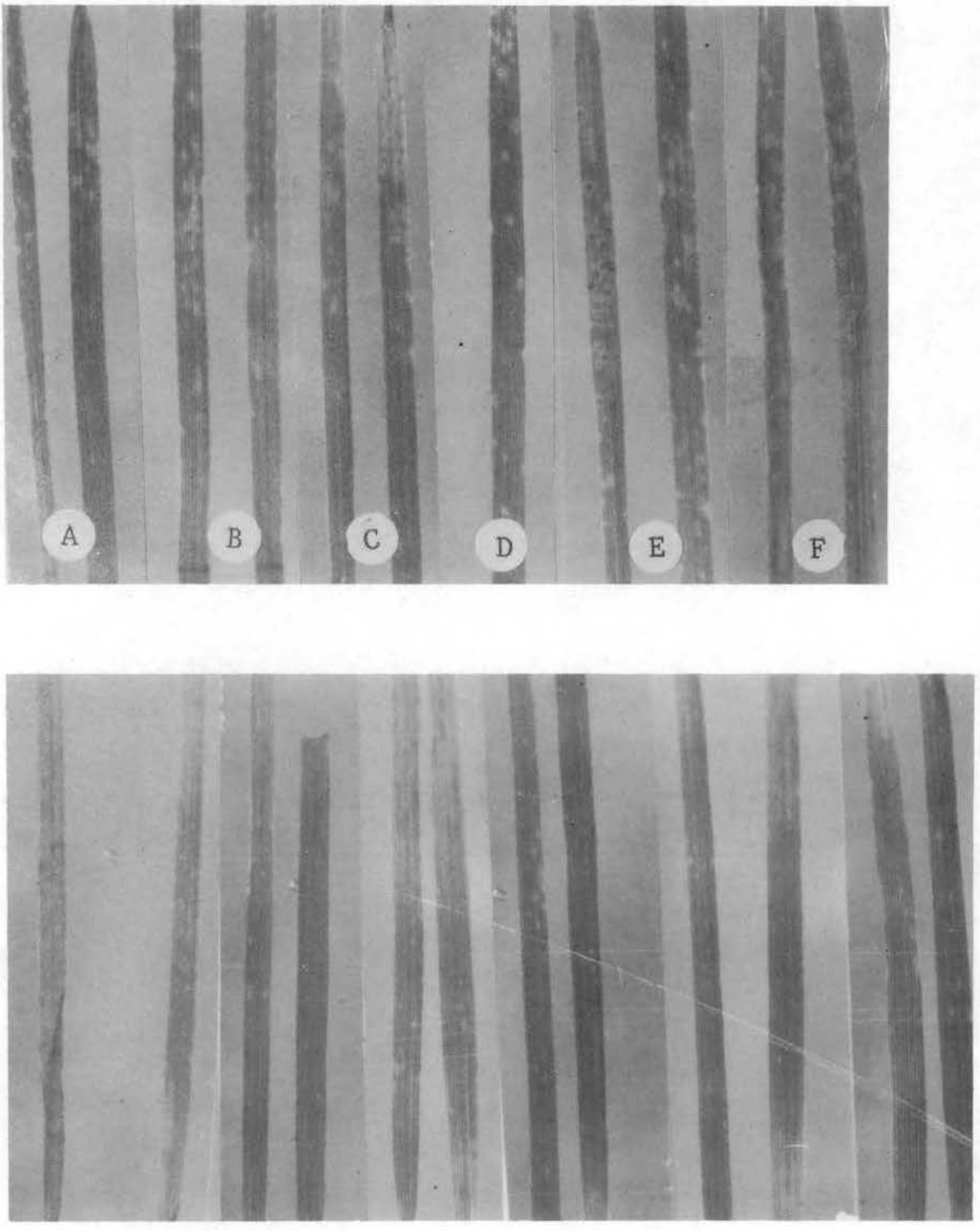


Figure 2. Reaction of a mutant of race UN1-NA65-1 originally isolated from Dular (A) on Lee (B), Exchange (C), Agent (D) Preska (E), and MQL/ORO//PN/3/FTN (F) in the upper row. In the lower row the reaction of the parent culture, race UN1-NA65-1, is shown on each of the cultivars.

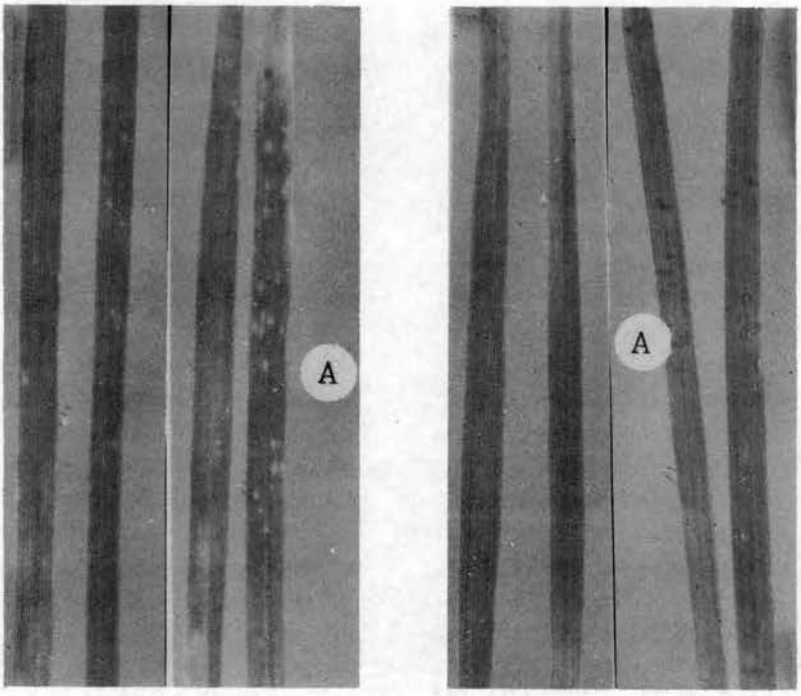


Figure 3. The reaction of Mediterranean (left) and Waban (right) to the parent culture, race UN1-NA65-1 and to a mutant culture (A) originally isolated on Mediterranean.

TABLE VI

THE VIRULENCE OF THE PARENT CULTURE, RACE UN13-NA65-15 AND CERTAIN MUTANT CULTURES FROM IT TO CERTAIN DIFFERENTIAL AND RESISTANT CULTIVARS

| | Reaction to race UN13-NA65-15 | Reaction of mutants of race UN13-NA65-15 originally isolated from MQL/ORO//PN/3/FTN | |
|---|---|---|---|
| | | A | B |
| **Unified Numeration Differentials** | | | |
| MA[1/] | 4 | 4 | 4 |
| WST | 4 | 4 | 4 |
| LS | 4 | 4 | 4 |
| MI | 4 | 4 | 4 |
| DO | 4 | 4 | 4 |
| **North American 1965 Differentials** | | | |
| DLR | 0; | 0; | 0;-1 |
| LEE | 4 | 4 | 4 |
| WBN | 4 | 4 | 4 |
| SVL | 4 | 4 | 4 |
| ECH | 2-4 | 3 | 3 |
| **Other Differentials** | | | |
| WTR | 4 | 4 | 4 |
| WSL | 2-3 | 3 | 3 |
| **Resistant Cultivars** | | | |
| LCO | 0; | 0;-2 | 0;-2 |
| AIV | 0;-1 | 2-4 | 0; |
| AC | 0; | [2/] | 0;-1 |
| ARS | 0 | 0 | 0 |
| TPA | 0; | 0; | 0; |
| PSK | 0; | 0; | 0; |
| WK 2 | 0; | 0; | 3-4 |
| 5*WI/TF | 0 | 0 | 0 |
| MQL/ORO// PN/3/FTN | 0;-2 | 2-4 | 0;-2 |

[1/] Abbreviations used were made according to rules adopted by the National Wheat Improvement Committee as amended by KONZAK. See Agronomy Journal. 52:613, 1960; U. S. Dept. of Agr. Tech. Bull. 1278, p. 131, 1963; and Wheat Newsletter 1965.

[2/] Cultivar not tested.

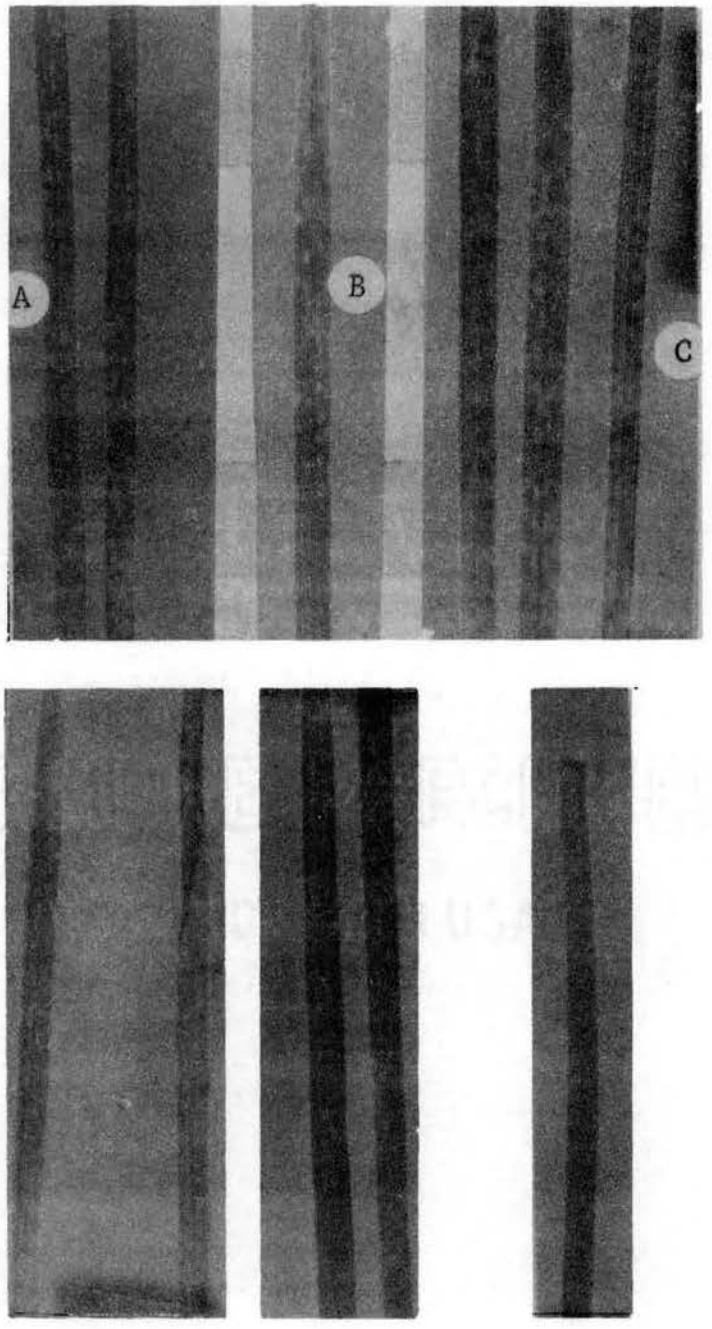


Figure 4. The reaction (upper row) of MQL/ORO//PN/3/FTN (left), Lucero (center), and Wanken 2 (right) to a mutant of race UN13-NA65-15 produced on MQL/ORO// PN/3/FTN. In the lower row the reaction of these cultivars to the parent culture is shown.

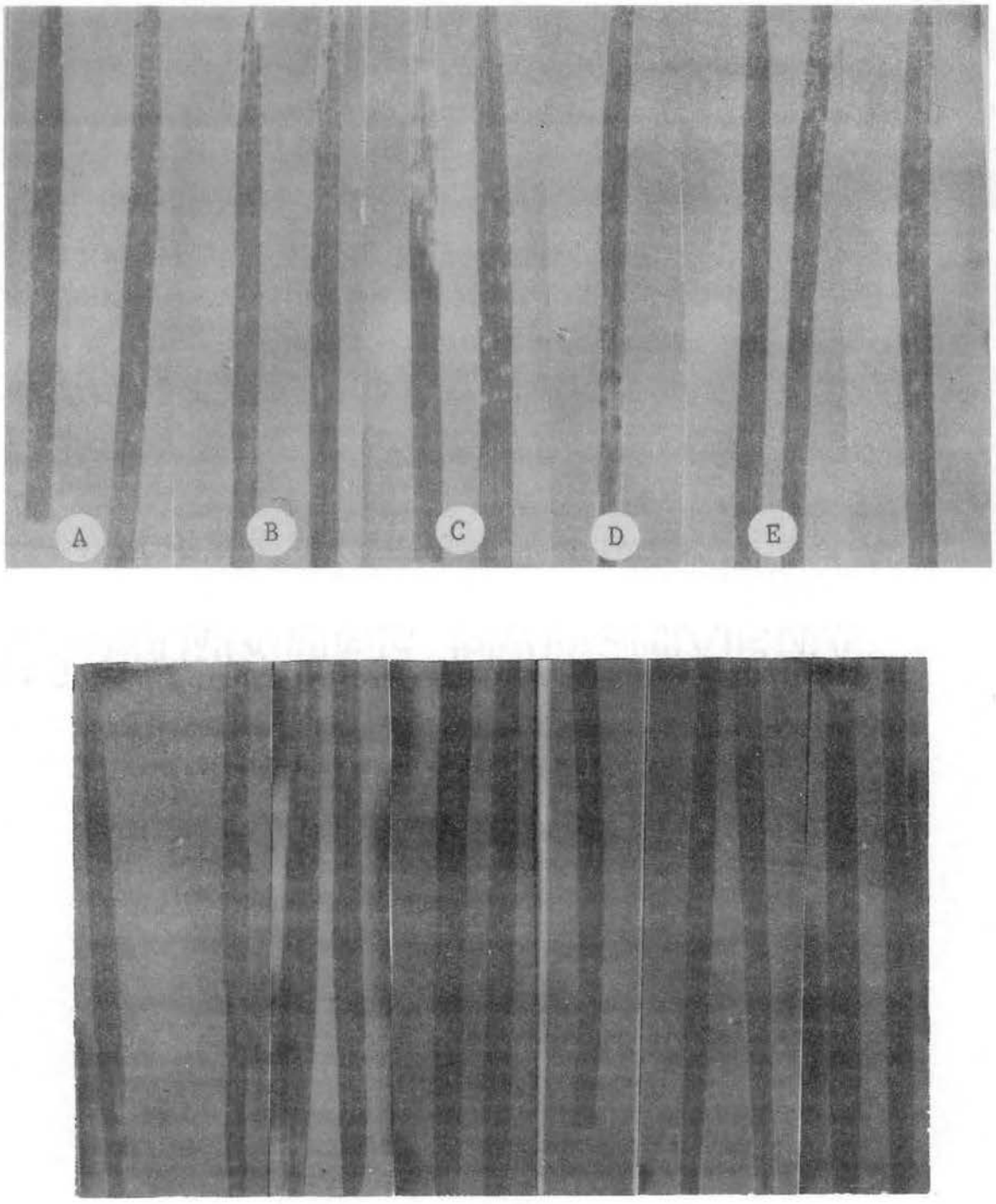


Figure 5. The reaction (upper row) of MQL/ORO//PN/3/FTN (A), Aniversario (B), Lucero (C), Wanken 2 (D), Timpaw (E), and Preska (F) to a mutant of race UN13-NA65-15 originally isolated on MQL/ORO//PN/3/FTN. In the lower row, the reaction of these cultivars to the parent culture is shown.

## DISCUSSION

Since Puccinia recondita f. sp. tritici is an obligate parasite, the standard method of determining the presence of mutants, colony counts on agar, could not be used. Infectability could not be determined or estimated by germination tests on agar media either. Schwinghaver (20) found that germination is not significantly reduced by doses of irradiation that completely inhibit infection. He suggested that delayed death is probably due to the fact that cell division and maybe nuclear division do not occur until the germ tube contacts or enters the stoma. However, since germination is a necessary prerequisite for infection, germinability was checked on non-nutrient agar. For maximum germination a longer period of rehydration was found to be necessary following irradiation than following storage in the refrigerator.

Explanation for the failure to urediospores of race UN1-NA65-1 to infect following X-ray irradiation is lacking. Spores taken from the vial prior to irradiation were viable and infective when inoculated as checks for infectability and host reaction. The process for irradiation and inoculation was the same as that used for race UN13-NA65-15 except the radiation dose was less. It was noted, however, that the vial was quite warm at the end of the irradiation period and the germination on agar was lower. Arthur (1) states that urediospores of P. glumarum will endure exposure at 45C for five minutes when moist and urediospores of Puccinia rubigo-vera bromi will germinate after

exposure at temperatures of 65-70C (moisture and length of exposure not stated). Provisions for a water bath to the surface of the vial did increase germination from 60% to 84% but infectability was not increased.

Several factors may account for the fact that observed mutations to virulence resulted in changes from immune (0-0;) to resistant (0;-2) rather than to susceptible (3-4) infection types. Avirulence is generally conditioned by dominant genes (3); therefore, a single mutation in a heterozygous clone or simultaneous mutations in a homozygous clone would result in a change from avirulence to virulence if the corresponding gene in the host were the only resistance gene present. This was borne out by the study of Rowell, Loegering, and Powers (18) who found that where more than one gene was present in the host and pathogen, mutation to virulence at one gene would allow the expression of other phenotypes, the interaction producing the lowest infection type being the one expressed. Analysis by Prescott (16) using Person's (15) and Schafer's (personal communication) methods suggest the presence of three genes in each of the North American 1965 differentials. Therefore, expression of a mutation at one gene locus would depend upon the genotype of the host and pathogen at other loci governing infection type.

Another factor may be radiation damage. Schwinghamer (21) reported that the prevalence of inhibited flecks and slowly developing pustules increased from 28 to 45 to 55% as radiation dose was increased from 30 to 60 to 90 Kr. Since inhibition was observed to occur in both mutant and nonmutant pustules, it was interpreted to be genetic damage.

It should be pointed out that mutants for virulence on Mediterranean, Dular, and Lee appear to be related in virulence on these

varieties as well as on Loros and Exchange. Prescott's analysis showed that some of the genes present in one variety may be common to one or more other varieties. Flor (7) states that in flax rust, where pathogenicity for several varieties appears to be inherited as a unit, i.e. allelic or closely linked, induced mutations to virulence to one variety also confers virulence on the other varieties in that group. It may be that pathogenicity for one gene of these varieties is inherited as a unit although Prescott's analysis of the five North American 1965 differentials did not show a gene common to Dular, Lee, and Sinvalocho. It did appear, however, that Webster and Sinvalocho may have a gene in common.

The appearance of type 4 pustules on Webster from a Sinvalocho mutant and on Waban from a Mediterranean mutant in interpreted as a "double hit", since more than one gene is probably involved in each variety (Young, personal communication) and no mutants were isolated from either of these varieties.

It appears that the mutation frequency for specific genes or combinations of genes varies widely from a low unknown rate to a rather high rate. The rate did not vary appreciably in this experiment from one race to the other except for mutants isolated on Lucero, Aniversario and possibly Marquillo/Oro//Pawnee/3/Frontana. The fact remains that a combination of genes for resistance is more stable since the mutation frequency for pathogenicity on this combination would be the product of the individual mutation frequencies.

Selection pressure has been suggested as the force responsible for the sudden occurrence of new virulences. Schwinghamer (21) suggests that in addition to this and the natural radiation of the

exposed fungus, semi-resistant varieties applying "mutation pressure" through the production of mutagenic chemicals during tissue senescence may contribute to the production of new virulences and should be further investigated.

## SUMMARY


1. A large number of irradiated spores of 2 races of the leaf rust fungus were screened on several resistant and differentially resistant wheat cultivars in order to determine the frequency of mutation to virulence.

2. Within a race, the frequency of mutation to virulence on the different cultivars tested varied greatly. From race UN1-NA65-1 no mutations to virulence were found for the varieties 5*Wichita/Transfer, Agrus, Malakof, Webster, Waban, and Exchange; whereas a high frequency was noted for Agent and Preska. From race UN13-NA65-15 no mutations for virulence on 5*Wichita/Transfer were found; whereas a high frequency of mutations for virulence on the cultivar Lucero was found.

3. The frequency of mutation to virulence on particular cultivars varied somewhat between the two races, but it is not known if the variation is statistically significant. For race UN13-NA65-15 the frequency of mutation to virulence on the cultivar Lucero was more than ten times that of race UN1-NA65-1 and on the cultivar Timpaw more than three times greater.

4. Some indication of the association of certain resistance genes among certain cultivars was shown by correlated changes in infection type.

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