A STUDY OF INHERITANCE OF PATHOGENICITY IN *PUCCINIA GRAMINIS* VAR. *TRITICI*.

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Synopsis.

The inheritance of pathogenicity in *P. graminis* var. *tritici* was studied by selfing strain 21 Anz-2 on *Berberis vulgaris* and determining the reactions of 285 isolates on 23 differential varieties. The three varieties Little Club, Marquis and Kota were susceptible to all progeny strains except one. Two varieties, Kenya 117A and Khapstein, were resistant to all strains. On the remaining varieties the segregation of the 37 strains showed the parent strain to be heterozygous for pathogenicity. On Kanred, Einkorn, Vernal Emmer and Mentana avirulence was inherited as a dominant character and conditioned by a single major factor in each instance. On Acme and Kubanka virulence was dominant and one of the factors for pathogenicity seemed to operate against both varieties. Virulence on Arnautka, Mindum and Spelmar was inherited as a unit with a single dominant factor involved. On Celebration which carries Marquillo-type resistance avirulence was recessive and due to a single factor. The same resistance operated in Thatcher which possesses also the immunity factor of Kanred. On Yalta virulence was dominant, while avirulence was dominant on Eureka, Bowie, Bokveld and W1656. There was evidence of association of certain of the genes for virulence and several strains showed abnormal characteristics. Abnormal uredosorus colour was associated with homozygous recessive genes for pathogenicity.

Introduction.

During the last fifteen years, new, virulent strains of wheat stem rust, *Puccinia graminis* var. *tritici* Eriks. and Henn., have appeared in the eastern Australian wheat belt and have rendered ineffective many sources of resistance. It was thought (Watson and Singh, 1952) that the main source of new strains in this country was mutation. These workers suggested the incorporation of two different genes for resistance into varieties to be released on the assumption that a combined resistance would remain effective for a longer period as it could be overcome only by a double mutation in the fungus. Later research, however, produced evidence of sexual hybridization of the pathogen on the alternate host, *Berberis vulgaris*, which is widespread in Tasmania, and also of somatic hybridization which was shown to occur readily between field strains (Watson and Luig, 1958*a*, 1958*b*). It could therefore be expected that two strains, both heterozygous for two recessive genes for virulence, by somatic hybridization would produce a strain pathogenic on a variety carrying combined resistances. The present study was undertaken to determine the genotype of the most prevalent Australian strain of wheat stem rust in order to evaluate individual sources of resistance. Those genes for resistance which remain effective to the progeny of existing strains of rust would be the most valuable for breeding.

Review of Literature.

In *Melampsora lini* (Pers.) Lév. extensive studies of a fundamental nature conducted by Flor have suggested a relationship between the genes for pathogenicity in the fungus and the genes for resistance in the host. In *Puccinia graminis*, apart from the Canadian work, little has been reported on the genetics of pathogenicity.

Waterhouse (1929) was the first to obtain experimental proof that new forms of stem rust had their origin on barberry. In 1930 Newton, Johnson and Brown reported that most races of wheat stem rust studied by them, including race 21, were heterozygous. By selfing race 21 six different races were isolated, viz., 11, 17, 21, 34, 49 and 56, indicating heterozygosity of genes for pathogenicity on Kanred, Einkorn,

Arnautka, Mindum, Spelmar and Kubanka. Five per cent. of the aecial cups gave rise to more than one physiologic form. In another study (1930*b*) the same investigators found the inheritance of normal red uredospore colour to have a Mendelian basis and to depend upon the interaction of two dominant complementary factors. Orange and greyish-brown were both conditioned by one or other of the complementary factors and white colour was explained by the presence of the two recessive alleles. Avirulence ("3-c" reaction) on Marquis and Kota was found to be due to maternal influences. In second generation hybrids there was evidence of an association of orange spore colour with avirulence on Marquis and Kota, and of an association of greyish-brown spore colour with the occurrence of races 36 and 85. These findings were confirmed in a later publication (Johnson, Newton and Brown, 1934). In a more recent study with *Puccinia graminis* var. *avenae* (Johnson, 1949), parallel results were obtained. Pathogenicity on the oat varieties Sevnothree and Joanette was found to be cytoplasmic with possible nuclear influences, and red spore colour was dominant over orange.

Mendelian inheritance of pathogenic characters in *P. graminis* var. *tritici* has been reported in two papers (Johnson and Newton, 1940; Johnson, 1954). In the first study, avirulence ("0" reaction) on the variety Kanred was found to be dominant and governed by a single factor pair. Virulence on Mindum ("4" type reaction) was dominant over avirulence ("1" type reaction) and was also due to a single factor. Virulence on Vernal Emmer, on the other hand, was governed by two recessive factors. Independence of all factors was indicated. Johnson and Newton concluded that despite the binucleate condition of the rust organism, genes for pathogenicity are inherited as if they were present in a single diploid nucleus. In the second study, the mode of inheritance of pathogenicity on the varieties Kanred, Mindum, Vernal Emmer, Einkorn, Marquis and Kota was studied by selfing 34 physiologic races of rust. The previous findings were confirmed and additional evidence indicated that avirulence on Einkorn was dominant. Johnson thought that apart from the "0" and "4" types of infection on the *durums* Arnautka, Mindum and Spelmar, which seemed to be inherited as a unit, there was no indication of linkage between genes for virulence. Fourteen additional differential hosts including Gabo, McMurachy, *Triticum timopheevi* and Kenya 117A were inoculated with mass cultures derived from 12 races (among them race 21), but only on the *durum* Carleton did segregation for susceptibility occur.

More recently Wilcoxson and Paharia (1958) isolated 15 different races by selfing race 111 to which the standard hosts, except Little Club, are resistant. It was suggested that race 111 arose as a hybrid of *P. graminis* var. *tritici* and *P. graminis* var. *secalis* and that the unusual behaviour of its progeny was due to the segregation of inhibitors suppressing pathogenicity in the parent race.

Materials and Methods.

The studies reported herein were carried out at the University of Sydney during the years 1957 and 1959. In 1957 teleutospore material from a summer crop of the variety Celebration was collected at Castle Hill. Uredospore samples from the same field were identified, the majority were of strain 21 Anz-2* and there were a few isolates of 21 Anz-1. At this time these two strains were the only Australian stem rusts attacking Celebration which carries Marquillo-type of resistance. Half the telia-bearing material was sent to Glen Innes Experimental Farm, N.S.W., and left in the open over the winter months to induce germination. The remainder was put into a deep-freeze chamber and at weekly intervals removed, wetted and allowed to thaw. Better infection was obtained with the material that had been allowed to overwinter at Glen Innes.

For the 1959 study teleutospore material was collected at Parkes, N.S.W., from a heavily rusted, self-sown crop of an unknown variety. This material had become infected in the autumn of 1959 and the teleutospores had overwintered under natural conditions. No artificial treatment was necessary to induce germination. From uredospores collected in the autumn strain 21 Anz-2 was found to make up almost all the inoculum. In addition, however, a few pustules of the resistant type were noticed

* Anz stands for the geographical area of Australia and New Zealand.

on the differential variety Yalta, and they subsequently proved to belong to a strain which resembled 34 Anz-1 except for the reaction types on Federation and C.I.12632. The mode of origin of this strain is not known, it has not been found previously in this country and, during the foregoing rust survey, all the samples collected in this part of New South Wales proved to be 21 Anz-2.

The following procedure was used in inoculating the barberry: Young shoots were sprayed with water and the leaves rubbed between the fingers to break down surface tension. An inoculating needle was then used to transfer the spores to the leaf surfaces. In addition, straw-bearing telia were suspended above the barberry plants which were set in an incubator for a period of two or more days.

Several attempts to obtain infection on barberry were entirely unsuccessful or produced only a few pycnia which were often sterile despite mixing of pycnial fluids. The complete sexual cycle finally developed mostly on young leaves, but also on older leaves, spines and stems. Rye stem rust inoculated by the same method onto barberry gave abundant infection.

After formation of the pycnia the nectar was intermixed by means of a blunt needle. When single aecial cups from different aecial clusters were inoculated onto seedlings of Little Club striking differences in the ability to infect were noted. Mass inoculation with subsequent random selection of pustules would have obscured this difference, and for this reason the mass-inoculation method was not employed. In each case a single aecial cup was carefully cut off by means of a sharpened needle, lifted onto seedlings of Little Club in a separate pot and smeared and crushed thereon. After infection occurred plastic covers were placed round the pots to prevent contamination. The cultures were built up and inoculated onto differential sets, and the reaction types recorded in the usual way.

Experimental Results.

(i) *Inheritance of Pathogenicity.*

Strain 21 Anz-2 was heterozygous for most of the genes for virulence under study. In 1957, 14 different strains were obtained from 180 isolates. In 1959, from the study of a second lot of material, 23 strains were obtained from 105 isolates (Table 1). Although different strains were found in the two studies, the results were not contradictory, as similar segregation patterns were observed for individual virulence genes. Most of these strains are new records for Australia.

Four of Stakman's twelve standard hosts, viz., Little Club, Marquis, Kota and Khapli, did not give a differentiating reaction to any of the isolates except strain 104 Anz-1. The first three varieties were susceptible and Khapli highly resistant.

The results of testing the isolates on Kanred agreed with those of Johnson and Newton (1940) and Johnson (1954), who reported a single recessive gene for pathogenicity in the fungus. Twenty-five strains were avirulent on Kanred and 12 were virulent (χ^2 for a 3:1 ratio = 1·090; P-value = 0·30–0·20).

The present studies also have shown that virulence on Einkorn is recessive and controlled by a single factor. Only 7 strains gave a susceptible reaction ("3" to "3$^+$" type), while 30 strains produced a variation from ";" to "2$^=$" (χ^2 for a 3:1 ratio = 0·730; P-value = 0·50–0·30).

Further it was observed that the majority of strains were avirulent on Vernal Emmer. Isolates from 28 strains produced a ";" to "2$^=$" resistant reaction, 4 strains gave a semi-susceptible reaction ("2" to "3^c") and to 5 strains Vernal Emmer was fully susceptible. Grouping the semi-virulent and virulent strains together would give a ratio of avirulent to virulent of approximately 3 to 1 (χ^2 for a 3:1 ratio = 0·009; P-value = 0·95–0·90). If the semi-virulent are grouped with the avirulent, however, a ratio in the vicinity of 15 to 1 is obtained (χ^2 for a 15:1 ratio = 3·332; P-value = 0·10–0·05). The latter ratio would be in agreement with the findings of Johnson and Newton (1940) and Johnson (1954). A two-factor segregation involving two genes unequal in their pathogenic effects could satisfactorily explain the present results and account for the semi-virulent strains.

TABLE 1.

Reactions of Strain 21 Anz-2 and of 35 Products of Selfing on 22 Wheat Varieties.

Strain.	Differentiating Variety.																					Uredosorus Colour.
	Little Club.	Marquis.	Kanred.	Kota.	Arn., Min.. Spm.*	Kubanka.	Acme.	Einkorn.	Emmer.	Khapli Emmer.	Eureka.†	Bowie.‡	Bokveld.‡	Yalta.	W1656.	Mentana.	Celebration.	Thatcher.	Khapstein.	Kenya 117A.	Federation.	Culture Number.	
21 Anz-2 ..	4	4	0	3	4	3^+	3^+	;	;	;	;	2^-	;	4	;1	2^-	3^+	0	$;1^+$	$;2^=$	4		Argus Brown
1957																							
NR 10§ ..	4	4	0	3	;	X	X^+	;	;	;	;	2^-	;	$;2^=$	;1	2^-	3^+	0	$;1^+$	$;2^=$	4	2P	Amber Brown
NR 11 ..	4	4	0	3	3^+	X	X	;	;	;	;	2^-	;	$;2^=$	;1	2^-	3^+	0	$;1^+$	$;2^=$	4	3C	Amber Brown
NR 12 ..	4	4	0	3	3^+	X	X^+	;	;	;	;	2^-	;	3	;1	2^-	3	0	$;1^+$	$;2^=$	4	3L	Amber Brown
NR 13 ..	4	4	0	3	3^+	X^+	X^+	;	;	;	;	2^-	;	3	;1	$2^=$	X	0	$;1^+$	$;2^=$	4	5X	Amber Brown
NR 14 ..	4	4	0	3	;	X	X	;	;	;	;	2^-	3^{-c}	$;2^=$	;1	2^-	3^+	0	$;1^+$	$;2^=$	4	2W	Amber Brown
21 Anz-1 ..	4	4	0	3	4	3^+	3^+	;	;	;	$;1^{++}$	2^-	;	$;2^=$	;1	2^-	3^+	0	$;1^+$	$;2^=$	4	2E	Amber Brown
21 Anz-2 ..	4	4	0	3	4	3^+	3^+	;	;	;	$;1^{++}$	2^-	;	4	;1	2^-	3^+	0	$;1^+$	$;2^=$	4	2X	Amber Brown
9 Anz-1 ..	4	4	0	3	3	X^{++}	3	3	3	;	;	2^-	;	$;2^=$	;1	3^+	3	0	$;1^+$	$;2^=$	4	6V	Orange Rufous
17 Anz-1 ..	4	4	0	3	3^+	3	3	3	;	;	;	2^-	;	$;2^=$	;1	3^+	3	0	$;1^+$	$;2^=$	4	3D	Sudan Brown
222 Anz-6 ..	4	4	3	3	X^+	;1	$X^=$	;	;	;	;	2^-	;	3^+	;1	3^+	$;1^+$	$;1^+$	$;1^+$	;1	4	5D	Amber Brown
222 Anz-7 ..	4	4	3	3	X^+	;1	$2^=$	;	;	;	;	2^-	2^c	3^+	;1	3^+	$;1^+$	$;1^+$	$;1^+$	$;1^-$	4	6L	Amber Brown
222 Anz-3 ..	3	3	3	3	X^+	$;1^-$	;1	;	;	;	3	3^-	3^c	3	;1	3	$;1^+$	$;1^+$	$;1^-$	$;1^-$	3	2ye	Mars Yellow
222 Anz-4 ..	3^+	3^+	3	3	X^+	$;1^=$	$;1^=$	;	;	;	3	3	3^c	3^+	3	3^+	$;1^+$	$;1^+$	$;1^-$	$;1^-$	3^+	2K	Orange Rufous
176 Anz-1 ..	4	4	0	3	;	3	3^+	;	;	;	;	2^-	;	$;2^=$	;1	2^-	3^+	0	$;1^+$	$;2^=$	4	2Y	Amber Brown

NR 15	..	4	4	3	3	3^+	X^-	3^+	;	;	;	;	X	3^{-c}	;$2^=$	X^{++}	3^+	X^-	1^+	;1^+	3^{-c}	4	B20	Sudan Brown
NR 16	..	4	4	0	3	3^+	X	X^+	;	;	;	;	2^-	;1	3^+	;1	2^-	3	0	;1^+	;$2^=$	4	E9	Sanfords Brown
NR 17	..	4	4	3	3	3^+	3^{-c}	3^+	;	;	;	;	3^{+c}	;	3^+	;1	3^+	3^-	X^{++}	;1	$3^{=c}$	4	E3	Amber Brown
NR 18	..	4	4	3	3	3^+	3^c	X^+	;	3	;	;1^{++}	3^{+c}	;	3^+	;1^+	3^+	3	3	;1^+	3^{-c}	4	D3	Bay
NR 19	..	4	4	3	3	3^+	X	X	;1	2	;	;	2^+	;1	3^+	;1^+	3^+	3	3	;$1^=$	$3^{=c}$	4	F2	Argus Brown
NR 20	..	4	4	0	3	3^+	3^+	X	;1^-	;1	;	;	2^-	;1	3^+	X^-	2	3^+	0	;	3^{-c}	4	Q3	Amber Brown
NR 21	..	4	4	0	3	3	X^+	X^+	;	;	;	;1^{++}	2	;	;2	;	2	3^+	0	;$2^=$	$3^{=c}$	4	R1	Amber Brown
NR 22	..	4	4	0	3	3^+	3^+	3^+	$2^=$	2^+	;	;	2^-	;	3^+	;	2^-	3^+	0	;$1^=$	$2\equiv$	4	A1	Amber Brown
NR 23	..	4	4	0	3	3	;1	;1	;	3^c	;	;	3^{-c}	;	3^+	;1^-	3^+	3	0	;1	;	4	C13	Bay
NR 24	..	4	4	0	3	;	3^+	1^{++}	3	;	;	;	$2^=$	;	3^+	;$1^=$	;$2^=$	3	0	;$1^=$	;$2^=$	4	O3	Amber Brown
NR 25	..	4	4	0	3	3^+	X^+	X^+	3	3	;	;	2^-	;	3^+	;1	2^-	3^+	0	;1	;1	4	D14	Amber Brown
NR 26	..	4	4	0	3	3	X	1^+	;	;	;	;	2^-	;	;1	;1	2^-	3	0	;1	;$2^=$	4	S3	Amber Brown
21 Anz-1	..	4	4	0	3	3^+	3^+	3^+	;	;1	;	;	2^-	;	;2	;1^+	2	3^+	0	;1	2^-	4	K2	Mars Yellow
21 Anz-2	..	4	4	0	3	3^+	3^+	3^+	;1^-	;1^-	;	;	2^-	;	3^+	;1	2^-	3^+	0	;	;$2^=$	4	Q2	Amber Brown
57 Anz-1	..	4	4	0	3	;1^-	X^{++}	3^+	3	3	;	;	$2^=$	;	3^+	;1^+	$2^=$	3^+	0	;1	$2^=$	4	F1	Argus Brown
100 Anz-1	..	4	4	3	3	3^+	3	;$X^=$	3	;	;	;	2^-	$2^=$	;$2^=$	;1	2	3^+	3^+	1^+	3^{-c}	4	H8	Orange Rufous
104 Anz-1	..	3	1	0	;	;	;	;	3	3^c	;	;	;	;	1^+	;	;	$X^=$	0	;1	;$1^=$	3	N2	Amber Brown
116 Anz-1	..	4	4	0	3	3	X^{++}	X^{++}	;	3	;	;	$3^{=c}$	;	3^+	;1	3^+	3	0	1^{++}	3^{-c}	4	C3	Tawny
213 Anz-1	..	4	4	3	3	3^+	X	X	;1^-	;	;	3^c	2	;	;2^-	X^{++}	2	3	3	;	$2^=$	4	B25	Sudan Brown
213 Anz-2	..	4	4	3	3	3^+	3^c	3^c	;	;	;	3	3^+	2	;1	;1^+	3^+	X	X	;	$2^=$	4	A5	Amber Brown
194 Anz-1	..	4	4	0	3	3^+	X	1^{++}	;1^-	;$1^=$	;	;	2^-	;1	3^+	$X^=$	2	3	0	;1^+	3^{-c}	4	P4	Amber Brown
176 Anz-2	..	4	4	0	3	;1	X^{++}	3	;1	;	;	;	$2^=$	;	3^+	;1	2^-	3^+	0	;1	2^-	4	S16	Amber Brown
213 Anz-3	..	4	4	3	3	3^+	X	X	;	;	;	;	1,$2^=$	;	3^+	;1	2^-	$X^=$	$X^=$	;1	$2^=$	4	S1	Sudan Brown

* Denotes Arnautka, Mindum and Spelmar.

† Reaction types in winter; Eureka gave a "3^+" reaction with all isolates in summer.

‡ Reaction types at temperatures of 81° to 85° F.

§ Designations NR1–NR9 were used in a previous publication (Watson and Luig, 1958*b*).

On Mentana W1124* a single recessive gene for virulence was indicated. Of the 37 strains 13 were fully virulent, while the remainder produced a semi-resistant reaction varying from a "2=" to a "3–c" (χ^2 for a 3:1 ratio = 2·027; P-value = 0·20–0·10). These degrees of semi-resistance are typical of Mentana when tested in the seedling stage with the 21 and 34 race complexes of Australian field strains. The ";" reaction on Mentana to strain 104 Anz-1 is highly correlated with the ";" reaction on Acme to strains such as 111, 196, NR-2 and NR-4 (Watson, 1957) and is almost certainly due to the segregation of a second gene (Luig, unpublished).

On Acme, susceptible and semi-susceptible reaction types ("4" and "x") were clearly predominant. Many strains gave an intermediate reaction and the data for resistant, intermediate and susceptible classes could fit a 1:2:1 ratio. As the parent strain 21 Anz-2 produces a fully susceptible reaction on Acme, however, these results cannot be explained on the basis of a single incompletely dominant gene. If it is assumed that the intermediate class is the result of segregation of a second minor gene for virulence, the presence of a major dominant gene is indicated (χ^2 for a 3:1 ratio = 0·081; P-value = 0·80–0·70). The ";" reaction of strain 104 Anz-1 is discussed above.

The segregation pattern on Kubanka resembled closely that on Acme. This might be due to the presence of a common factor for resistance in these two *durum* varieties, and segregation of the same major dominant gene in the pathogen would account for the similarity. Several of the strains, however, produced different reactions on Acme and Kubanka, indicating that each variety possesses at least one additional factor for resistance not present in the other.

At high temperatures (80°–90° F. during daylight) the inheritance of pathogenicity on the three *durums* Arnautka, Mindum and Spelmar was less complex than on Acme or Kubanka. Dominance of virulence was also evident: only 7 of the 37 strains were avirulent (";" to ";x=" reaction type) and statistically a single dominant gene for virulence was indicated (χ^2 for a 3:1 ratio = 0·730; P-value = 0·5–0·30).

The reactions of Celebration to the progeny suggested the segregation of a single dominant factor for virulence (χ^2 for a 3:1 ratio = 0·009; P-value = 0·95–0·90). Celebration carries the Marquillo-type of resistance derived from its Double Cross parent. This resistance is lower at high temperatures, but no difficulty was experienced in classifying virulent and avirulent strains.

Thatcher possesses at least two types of physiological resistance, one an immunity derived from Kanred, the other resistance as in Celebration derived from Marquillo. The immunity factor was epistatic to the Marquillo-type resistance, all strains showing a "0" reaction on Kanred showed a "0" reaction on Thatcher. Strains NR17, NR18, NR19, 100 Anz-1 and 213 Anz-1—virulent on both Kanred and Celebration—were the only strains capable of attacking Thatcher.

On the variety Yalta, which carries a resistance identical with that of Gabo against certain Australian field strains, the segregation of strains in the progeny could not be satisfactorily explained on the basis of a single gene, but dominance of virulence was indicated. The significance of this will be discussed later.

On the variety W1656 (C.I.12632) the progeny behaved as though virulence ("x++" and "4" type reaction) was due to the action of two independent, recessive, complementary genes (χ^2 for a 15:1 ratio = 0·218; P-value = 0·70–0·50).

Inheritance of pathogenicity on the varieties Eureka, McMurachy, Bowie and Bokveld was more complicated. Pathogenicity tests suggest that each of these varieties has the gene Sr6 on chromosome XX, a gene which is ineffective at temperatures over 75° F. McMurachy was used in the 1957 study and the reactions were similar to those of Eureka. At low temperatures (60–65° F.) Eureka was fully susceptible to only three strains: virulence in the pathogen was apparently conditioned by two independent, recessive factors (χ^2 for a 15:1 ratio = 0·218; P-value = 0·70–0·50). This result was not expected, as it is known that only a single factor for resistance, Sr6, is operative against certain Australian strains, this factor giving a very distinct necrotic reaction at low temperatures. If a gene-to-gene host-pathogen relationship exists with Sr6, then

* Refers to the University of Sydney Wheat Accession Register.

many more strains virulent on Eureka would have been expected (χ^2 for a 3:1 ratio = 5·630; P-value = 0·02–0·01). At temperatures of 65° to 75° F. Eureka gave a higher reaction with parent strain 21 Anz-2 than with some other strains obtained from selfing. Strain 213 Anz-1 was the only one to which Eureka gave an intermediate reaction at these temperatures; this could be due to strain 213 Anz-1 being recessive for a gene pair for virulence. Eureka was fully susceptible at high temperatures to all isolates.

Bowie gave a full range of reactions to the progeny at high temperatures. To most strains the reaction was "2–", "2", or "2+". Several strains produced necrotic reactions in the range of "1–" to "x+". In addition, Bowie was semi-susceptible or susceptible ("3+c" and "3" type reactions) to five strains, and produced a ";" reaction when tested with strain 104 Anz-1. Segregation of a single major factor could account for most of the reaction types observed on Bowie (χ^2 for a 3:1 ratio of resistant vs. semi-susceptible and susceptible = 2·604; P-value = 0·20–0·10).

A somewhat similar segregation pattern was apparent when Bokveld was used as a differential. In most instances, however, Bokveld was highly resistant (";" to "1" reaction) and in no case fully susceptible. It is evident that genes in Bowie and Bokveld differ. Again, a one-gene segregation could explain the behaviour of the progeny on Bokveld at high temperatures (χ^2 for a 3:1 ratio of resistant vs. semi-susceptible is 1·523; P-value = 0·30–0·20).

When tested with the 37 strains, Khapstein W1451, a *vulgare* derivative from Khapli, showed minor variations from a highly resistant ";" reaction to a semi-resistant "1++, 2–" reaction. These variations might have been due to one of the two independent factors, previously found in this variety (Athwal and Watson, 1956), being ineffective against some strains, or to the segregation of minor genes in the fungus.

Besides Khapli and Khapstein, another variety, Kenya 117A, remained resistant to all strains.

Thirty entries from the 1959 International Wheat Rust Nursery were tested with seven of the progeny strains. All these entries had been selected for their comprehensive resistance to Australian field strains. The aim was to determine whether such resistance could possibly be due to the operation of a single factor rather than to multiple factors. In addition two wheat accessions, W2538, W2539, carrying *Agropyron elongatum* resistance, kindly sent by Dr. D. R. Knott, were also tested with the seven strains. The results indicated that many of these "I.W.R.N." lines are resistant to all the strains and the accessions W2538 and W2539 also showed a high degree of resistance to all seven strains.

(ii) *The Occurrence of Abnormal Characters.*

During the separation and study of the strains obtained from the barberry many abnormalities were noted. Differences were shown between strains in their incubation periods which varied from 9 days in some to 15 days in others. Marked colour variations also occurred, some strains being very dark brown and others almost yellow, the differences being observed in the uredospores. No strains with grey-brown spores were obtained and spores from an aecium suspected of being white failed to give infection on Little Club. It was evident that strains having certain characters such as an inability to break the epidermis and a tendency to be associated with browning and necrotic areas of the host would have less chance of survival in the field.

(iii) *Studies of Association between Factors for Virulence.*

Possible association of factors for virulence was also studied in all combinations. In Table 2 the χ^2 and P-values are tabulated for independent assortment of avirulence or virulence on 13 varieties.

It will be seen from Table 2 that virulence on Yalta was not associated with virulence or avirulence on the other 12 varieties. On two other varieties, Vernal Emmer and Einkorn, factors for pathogenicity seemed to be loosely associated, but this was not apparent between the factors in these varieties and those on the remaining 11 varieties.

TABLE 2.

Linkage between Factors for Pathogenicity in P. graminis *var.* tritici.

χ^2-values and *P*-values for Independent Assortment of Factors for Pathogenicity.

Pathogenic on	Pathogenic on.												
Kanred.	Kanred.	Einkorn.	Vernal Emmer.	Mentana.	Celebration.	Mindum.	Kubanka.	Acme.	Yalta.	Eureka.	Bowie.	Bokveld.	W1656.
Kanred..	—												
Einkorn	1·297 0·30–0·20	—											
Vernal Emmer ..	0·566 0·50–0·30	5·051 0·05–0·02	—										
Mentana	12·384 <0·001	0·163 0·70–0·50	2·176 0·20–0·10	—									
Celebration	11·159 <0·001	0·473 0·50–0·30	1·128 0·30–0·20	5·188 0·05–0·02	—								
Mindum	4·144 0·05–0·02	3·225 0·10–0·05	0·085 0·80–0·70	4·676 0·05–0·02	0·473 0·50–0·30	—							
Kubanka	3·830 0·10–0·05	0·024 0·90–0·80	0·316 0·70–0·50	7·300 0·01–0·001	13·541 <0·001	0·024 0·90–0·80	—						
Acme	1·930 0·20–0·10	1·097 0·30–0·20	0·139 0·80–0·70	1·329 0·30–0·20	4·908 0·05–0·02	0·010 0·95–0·90	19·335 <0·001	—					
Yalta	0·765 0·50–0·30	0·987 0·50–0·30	1·656 0·20–0·10	0·794 0·50–0·30	0·253 0·70–0·50	0·987 0·50–0·30	1·693 0·20–0·10	0·632 0·50–0·30	—				
Eureka	6·801 0·01–0·001	0·741 0·50–0·30	1·049 0·50–0·30	6·027 0·02–0·01	10·157 0·01–0·001	0·741 0·50–0·30	6·116 0·02–0·01	2·601 0·20–0·10	0·001 0·98–0·95	—			
Bowie	12·044 0·001	1·349 0·30–0·20	0·059 0·90–0·80	10·673 0·01–0·001	3·997 0·05–0·02	1·349 0·30–0·20	2·407 0·20–0·10	0·493 0·50–0·30	1·012 0·50–0·30	20·894 <0·001	—		
Bokveld	8·467 0·01–0·001	1·671 0·20–0·10	2·302 0·20–0·10	7·300 0·01–0·001	13·541 <0·001	0·024 0·90–0·80	5·536 0·02–0·01	1·916 0·20–0·10	0·266 0·70–0·50	16·868 <0·001	8·360 0·01–0·001	—	
W1656	6·801 0·01–0·001	0·762 0·50–0·30	1·049 0·50–0·30	1·424 0·30–0·20	3·180 0·10–0·05	0·762 0·50–0·30	0·704 0·50–0·30	0·066 0·80–0·70	0·924 0·50–0·30	2·788 0·10–0·05	1·097 0·30–0·20	6·116 0·02–0·01	—

The factors for virulence on the varieties Kanred, Mentana, Celebration, Eureka, Bowie and Bokveld were associated. The genes for virulence on Kanred and Bokveld, however, also appeared associated with those for virulence on W1656.

Some of these associations could be explained by assuming that certain of these varieties carry one or more of the same genes for resistance in common. This would most likely be the situation with Bokveld, Bowie and Eureka on the one hand and with Kubanka and Acme on the other. Additional genes in these varieties, or alleles, must be assumed present to account for differences in the reaction to different strains.

The possibility of association of genes for abnormal uredosorus colour with genes for pathogenicity was also investigated. It was thought that, if abnormal uredosorus colour was due to homozygosity of recessive genes, a relationship might be established between abnormal uredosorus colour and homozygosity of genes for pathogenicity. The 37 strains obtained from selfing were classed according to their uredosorus colour as normal (group I), comprising "Amber Brown", "Sanford's Brown" and "Argus Brown", or abnormal (group II), comprising all other colours, viz., the very dark "Bay" and the light colours "Sudan Brown", "Tawny", "Orange Rufous" and "Mars Yellow". For each strain the number of homozygous recessive gene pairs for pathogenicity in respect of the 13 above-mentioned varieties was counted. In the case of a strain being fully virulent on Eureka or W1656 the count was increased by two. In all other instances only one gene pair was counted in order to avoid over-estimation of the number of recessive gene pairs. No single recessive gene pair for pathogenicity was present in all strains abnormal for colour. Mean numbers of 2·12 and 4·416 recessive gene pairs were calculated for group I and group II respectively. The significance of the difference between these two means was tested by the t test. The value of t was found to be 2·90, and for 35 degrees of freedom is highly significant. It is therefore concluded that the genes for abnormal uredosorus colour show association with genes for virulence in the cultures that were studied here.

It was also noticed that some strains (NR15 and 213 Anz-1) were more aggressive than others, and that this was expressed by a shorter incubation period, more abundant infection and sporulation and better survival under storage conditions. As these strains also have a number of recessive genes for virulence, it would appear that these latter would not necessarily place these strains at a disadvantage when in competition with strains having dominant genes for avirulence.

Discussion.

The methods employed in this study did not exclude the possibility of some experimental error. Contamination of cultures, environmental effects on rust reaction and possible impurity of the teleutospore material used, make it necessary to consider some results with caution. The linkage of certain genes for pathogenicity with genes causing the loss of ability to produce the complete sexual cycle on the barberry could have been a further source of error. Such abnormalities have been reported by Johnson and Newton (1938). Anomalies due to differential survival of strains were largely eliminated by the technique of initiating each culture from a single aecidial horn. Mass inoculations would probably have favoured the more aggressive cultures and so discriminated against those with virulence genes associated with genes for abnormal characters. Data from which genetic ratios and association of characters were calculated were obtained from the 37 different strains and no statistical use was made of the frequency of appearance of the same strain.

The selfing studies presented were mainly concerned with the inheritance of pathogenicity on 23 differential hosts, but any association of genes for virulence on 13 varieties was also noted.

On eight differential varieties, viz., Kanred, Einkorn, Emmer, Mentana, Celebration, Kubanka, Acme and Mindum, virulence seemed to be inherited according to Mendelian laws and to be governed by a single major factor in each instance. Four of these factors, viz., those concerned in the reactions of Kubanka, Acme, Celebration and Mindum, could, when heterozygous, enable the parasite to overcome the corresponding

resistance. The four remaining factors were recessive. The mode of inheritance of pathogenicity on the five varieties Yalta, W1656, Eureka, Bowie and Bokveld was more difficult to ascertain.

It would appear from the results of this study that in certain instances there operates a system of specific relationships between factors for pathogenicity in the fungus and factors for resistance in the host as established for *Melampsora lini* by Flor.

In Kanred, for example, we suggest that, provided the corresponding dominant genes are present both in the fungus and the host, an interaction results following infection, and this becomes evident in the immunity or hypersensitivity characteristic of this variety. The absence of the appropriate dominant gene either in the fungus or the host or both fails to produce an interaction following infection, and susceptibility is evident. Similar complementary relationships are suggested for Einkorn, Emmer and Mentana.

The relationships, however, are not always simple. It is known that Mentana possesses two or more major factors for resistance to strains such as 104 Anz-1 which produce a ";" reaction (Luig, unpublished), and from the present studies it has not been possible to determine the number of genes in the fungus responsible for this interaction.

There was good evidence that virulence on the five *durum* varieties Acme, Kubanka, Arnautka, Mindum and Spelmar, and on Celebration, was conditioned in each case by a major dominant factor in the pathogen. Virulence or avirulence on Arnautka, Mindum and Spelmar was inherited as a unit. This was probably due to the presence of the same gene for resistance in all three varieties, this gene being the only one conferring high resistance to the progeny from selfing.

Acme and Kubanka, although alike in their reactions to all Australian field strains, reacted dissimilarly when tested with some of the segregates from selfing. Apparently these two varieties share a major factor for resistance, but they differ in their other genes for resistance. The parent strain 21 Anz-2 was heterozygous for genes for virulence on both varieties, and the data suggest a single major factor segregation in each case. The occurrence of strains avirulent on both Acme and Kubanka precludes the possibility of two different alleles controlling pathogenicity on these two varieties.

Celebration, which derived the Marquillo-type resistance from its Double Cross parent, reacted to the progeny as if segregation of a single dominant factor in the parasite were involved. Resistance of Celebration to a non-virulent strain at low temperatures was a ";1" type, which developed into an "x" type at higher temperatures. The reactions on Celebration and Thatcher to strains capable of rendering ineffective the immunity factor in Thatcher were almost identical. It is concluded that the same Marquillo-type resistance is present in both Celebration and Thatcher.

The majority of segregates were fully virulent on Yalta, indicating dominance of virulence. The data, however, fitted not a three to one, but a nine to seven ratio. Yalta possesses the same resistance to many strains of stem rust as Gabo, Charter, Lee and Timstein. Although overseas work reported the presence of two linked, dominant, complementary factors in varieties carrying this resistance (Knott and Anderson, 1956), recent work by one of us (N.H.L.) has shown that probably only a single dominant factor is involved and that differential transmission of gametes could account for the results obtained (Luig, 1960). A gene-to-gene host-pathogen relationship would require in the fungus the segregation of a single factor for virulence on Yalta.*

The origin of the parental strain 21 Anz-2 may have some bearing on the genetical basis of the host parasite relationship. Strain 21 Anz-1, unlike previous Australian field strains in its behaviour on several differential varieties, was first recorded in 1954 from southern New South Wales, and increased rapidly during the following years. In spite of its excellent competitive ability, the extensive cultivation of varieties carrying the Gabo-type resistance limited its spread. In 1956 strain 21 Anz-2 was first isolated from Woodburn, in northern New South Wales. This strain was identical

* In the 1960 study 16 different strains were obtained from selfing strain 21 Anz-2. Thirteen were virulent on Yalta and three were avirulent.

with 21 Anz-1 on all standard differentials and on other hosts except for its virulence on Gabo. Since only one type of resistance differentiates strain 21 Anz-1 from 21 Anz-2, the latter could be considered as a likely mutation from 21 Anz-1. Studies of somatic hybridization in field strains, however, suggested that strain 21 Anz-2 could have arisen by somatic hybridization between strain 21 Anz-1 and a strain capable of attacking Gabo (222 Anz-2). No variants virulent on Gabo were obtained by mixing 21 Anz-1 and 126 Anz-1, nor from extensive selection experiments from pure cultures of 21 Anz-1 (Watson and Luig, 1958*b*; and unpublished data). These studies, as well as that reported herein, suggest that the gene for virulence on Gabo is not present in the heterozygous condition in strain 21 Anz-1, and that somatic hybridization could be an important mechanism for the origin of new strains where the appropriate cultures are mixed in the field.

Virulence on W1656 was apparently controlled by two independent, recessive factors in the fungus. Allard and Shands (1954) and Nyquist (1957) found that crosses between C.I.12633 (a sister selection of W1656 C.I.12632) and susceptible varieties gave more resistant F_2 plants than could be expected if only a single factor was involved. They advanced the hypothesis that resistance depended on the action of two dominant factors linked with a recombination value of approximately 15 per cent. However, Allard and Shands (1954) also found resistance to stem rust and resistance to powdery mildew (*Erysiphe graminis tritici* El. Marchal) to be so closely linked that no recombinants were obtained from 762 F_3 progeny. Nyquist (1957*b*), when studying the mode of inheritance of resistance to leaf rust—located on the same chromosome—obtained a two-factor segregation in a cross between C.I.12633 and Ramona, but a single-factor segregation in a cross with White Federation. These findings taken in relation to recent work (Luig, 1960) suggest that chromosome XIII, on which all these resistances are located, could be differentially transmitted. If this is so, resistance to stem rust is provided by a single factor, and it can be assumed that the two factors in the pathogen are needed to overcome this one factor in the host.

The inheritance of pathogenicity on Eureka, Bowie and Bokveld suggested the segregation of two or more factors. All three varieties appear to carry the Sr6 gene (or an allele of it). In Eureka this was the only gene which operated against the progeny. The behaviour of Bowie to field strain 126 Anz-1 at low and high temperatures could be due either to an allele of Sr6 which confers a resistance less sensitive to temperature, or to the presence of two closely linked genes, one of them being Sr6, the other not affected by higher temperatures (Luig, unpublished data). The resistance of Bokveld can be explained in similar terms, but it should be noted that in no case was this variety fully susceptible. It is not possible to interpret these results in terms of a gene-to-gene relationship, and further studies of the nature of the Sr6 locus are required.

In certain respects these findings may be compared with those of Johnson and Newton (1940) and Johnson (1954). The present conclusions concerning the inheritance of pathogenicity on Kanred and Mindum are in agreement with theirs. Johnson (1954) found that avirulence on Einkorn was dominant, but he did not state the number of factors involved. Upon selfing race 21 he obtained seven forms, two avirulent and five virulent, on this variety. The selfing of race 125 gave a ratio almost the reverse of this. The present work indicates that a single recessive factor is involved.*

Furthermore, Newton, Johnson and Brown (1930) and Johnson (1954), when selfing race 21, did not obtain progeny virulent on Vernal Emmer or avirulent on Acme. This is clear evidence that North American race 21 differs genotypically from the Australian race 21 in respect to genes for pathogenicity on Stakman's twelve standard varieties. It is a well-known fact that, owing to the heterozygous nature of the rust organism, races which are phenotypically similar may nevertheless be quite different in their genotypes. Again, Johnson (1954) did not obtain susceptible infection types on varieties carrying Sr6 and Sr11 Sr12 when inoculating with mass progeny cultures

* In the 1960 study 19 different strains were isolated from selfing strains 21 Anz-1 and 21 Anz-2, nine were avirulent and 10 virulent on Einkorn.

derived from selfing race 21. The progeny from selfing strain 21 Anz-2 segregated on varieties with one or other of these two resistances.

It appears from this work that there is association between certain of the genes for virulence on the differential varieties. Vakili (1959) has reported such association in his studies of the leaf rust organism. He found that nine of the twelve genes conditioning pathogenicity fell into two linkage groups. Four of the twelve genes were dominant for virulence and others were complementary recessives. The gene M2 appeared to operate against two completely different genes for resistance.

Regarding reports of linkage, it should be borne in mind that very little is known about the mechanism of sexual recombination in rusts, and that linkage of semi-lethal genes with genes for pathogenicity could mimic linkage between the latter.

Disturbed ratios and pseudo-linkage can result in cases where the teleutospore material did not consist of only one strain. As mentioned above, a new strain resembling race 34 on Stakman's differentials was found when the inoculum which provided the teleutospore material in 1959 was tested. If preferential germination of basidiospores on barberry had occurred, a high proportion of the pycnia might have been formed by the new strain. This strain differed from strain 21 Anz-2 by its reactions on Kanred and Yalta. If pycnia were formed by the new strain an association between reaction types on Kanred and Yalta in the resulting strains would be expected. No such association, however, was found.

The inbreeding of strain 21 Anz-2 gave rise to several strains manifesting abnormal characteristics. The fact that the same abnormality was observed with isolates from different aecial clusters suggests that the changes from the normal condition were not due to mutations occurring during the passage of the rust organism through the sexual cycle, but to recombinations of recessive genes. The majority of changes seemed to be detrimental to the fungus, but in some instances progeny from selfing showed greater vigour under glasshouse conditions.

The studies on inheritance of and association of genes for virulence was one aspect of this investigation. Equally important was that part which dealt with the potential virulence that could be released on selfing. Selections from the International Rust Nursery and two varieties which are used for breeding, Khapstein and Kenya 117A, remained resistant when tested with all progenies. In each variety at least one gene for resistance was effective against all strains. The gene Sr9 of Kenya 117A has been incorporated into several Australian commercial varieties, and it appears that the genes for rendering Sr9 ineffective are not present in strain 21 Anz-2. The variety Festival, carrying Sr9 and extensively grown in Queensland and in the northern wheat belt of New South Wales, has retained its resistance to stem rust for several years now. An advanced backcross breeding programme at the University of Sydney is aiming at incorporating the resistance of Khapstein into agronomically desirable types. The genes of Kenya 117A and Khapstein may prove to be valuable sources of resistance to present and future strains of stem rust in Australia.

Addenda.

Since this report has been written, further selfing studies with strain 21 Anz-2 and with strain 21 Anz-1 have been carried out. Sixteen different strains were isolated from selfing strain 21 Anz-2 and three from selfing strain 21 Anz-1. The latter strains resembled the parental 21 Anz-1 in their inability to attack Yalta. On Kanred, Vernal Emmer and Mentana avirulence was again inherited as a dominant character with ratios of 15:4, 17:2 and 14:5 respectively. On Arnautka, Yalta and Celebration virulence was dominant and here the ratios were 15:4, 13:3 and 14:5. Only one of the 19 progeny strains was virulent on W1656. On Einkorn nine strains were avirulent and ten virulent, a somewhat different result from that obtained in the 1957 and 1959 studies. The 19 strains showed variations from a "2,3–" to a "3" reaction when tested on Kota, and also segregated for pathogenicity on Renown, Glenwari and Spica which are believed to carry Hope-type resistance. Kenya 117A and Khapstein were again resistant to all isolates from selfing, while Little Club, Marquis and Federation were

susceptible. At temperatures over 75° F. Eureka was susceptible to all isolates. Acme and Kubanka for the most part reacted similarly, but to some isolates they were unlike in their reaction.

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