PATHOGENICITY OF THE HAPLOID PROGENY CULTURES FROM A CROSS OF *ERYSIPHE GRAMINIS* F. SP. *TRITICI* BY *ERYSIPHE GRAMINIS* F. SP. *AGROPYRI* ON FOUR WHEAT VARIETIES

Unji HIURA and Hideo HETA

INTRODUCTION

On the basis of host specialization, Em. Marchal (1902) distinguished the formae speciales of *Erysiphe graminis* DC. *tritici* on species of *Triticum*, and *agropyri* on species of *Agropyron*. Hardison (1944) determined the hosts for several cultures of *Erysiphe graminis*. A culture from *Triticum aestivum* infected *Triticum aestivum* and *Agropyron spicatum*, but not several other species of *Agropyron*. Hardison studied 2 cultures from *Agropyron repens*. One of the cultures infected *Hordeum vulgare*. Neither culture infected *Triticum aestivum*.

Flor's gene-for-gene hypothesis (1955) has been shown to be valid for wheat, *Triticum aestivum* L. and wheat mildew, *Erysiphe graminis* DC. f. sp. *tritici* (Powers and Sando, 1960). The hypothesis has also been valid for barley, *Hordeum vulgare* L. and barley mildew, *Erysiphe graminis* DC. f. sp. *hordei* (Moseman, 1959 ; Hiura, 1964a). Genetics of host-parasite interactions between formae speciales of *Erysiphe graminis* and the host species has not been studied. Hiura (1962, '64b) showed that different formae speciales of *Erysiphe graminis* can be hybridized. He found that some haploid progeny cultures from a cross between *E. graminis* f. sp. *tritici* × *E. graminis* f. sp. *agropyri* infected both wheat and *Agropyron sp.*

In this study the inheritance of genes conditioning pathogenicity on four wheat varieties will be determined from the pathogenicity of haploid progeny cultures from a cross of *E. graminis* f. sp. *tritici* by *E. graminis* f. sp. *agropyri* on those varieties. The relationships of genes conditioning the reactions of those varieties suggested by these data will also be discussed.

MATERIALS AND METHODS

The parent powdery mildew cultures were A_1 of *Erysiphe graminis* f. sp. *agropyri*, and t_2 of *Erysiphe graminis* f. sp. *tritici*. The cultures were collected from a field at the Ohara Institute for Agricultural Biology at Kurashiki in 1962, and were of the opposite mating types.

Four wheat varieties, *Little Club*, *Norin No. 4*, *Turkey Red*, *Champion White*, and one ecotype of *Agropyron tsukushiense* var. *transiens* Ohwi were used. These are all hexaploid species.

The cultures were crossed on *Agropyron* (*Agropyron* will be used in place of *Agropyron tsukushiense* var. *transiens* in this paper). The methods of crossing, monoconidial isolation, and inoculation for testing pathogenicity of progeny cultures were similar to those previously described by Hiura (1964a). Notes were taken according to the five classes of infection types used on barley mildew (Hiura, 1960). Finer infection type differences of *Erysiphe graminis* f. sp. *tritici* on wheat varieties are dependent upon the environmental conditions at the time of testing as has been pointed out by Wolfe (1965). Furthermore, even virulent progeny cultures from the cross between cultures A_1 and t_2 produced mostly intermediate infection types on wheat varieties. For this reason the pathogenicity of the progeny cultures was divided into those which were virulent and avirulent on the following basis:

Avirulent — included infection types 0 and 0-1
Virulent — all included infection types 1 through 4

E. graminis is an obligate parasite which can be cultured only on living plants. The pathogenicity of cultures of the powdery mildew fungus is selected by the genes conditioning the reaction of the plant on which those cultures are maintained. Progeny cultures from the cross of cultures A_1 by t_2 were isolated and maintained on three different host plants. The cultures were isolated by removing single conidia from individual pustules produced by ascospores discharged from cleistothecia of the cross of cultures A_1 and t_2. The cultures were maintained on the same types of host plant as the original pustule was formed. By this procedure 304 cultures were isolated and maintained on *Agropyron*, 46 cultures on *Norin No. 4* wheat, and 90 cultures on *Little Club* wheat.

The experiments were conducted in the greenhouse where the temperature was maintained at 15 to 18°C in the night (from 7 p. m. to 7 a. m.) and at 22 to 25°C in the day (from 7 a. m. to 7 p. m.).

RESULTS

1. *Parent Cultures*

The infection types produced by parent cultures A_1 and t_2 on the 4 wheat varieties, and on *Agropyron* are shown in Table 1. Culture A_1 is virulent on *Agropyron* producing infection type 4, but avirulent on the 4 wheat varieties producing infection type 0. Culture t_2 is virulent on the 4 wheat varieties producing infection type 4, but avirulent on *Agropyron* producing infection type 0.

2. *Progeny Cultures on Agropyron*

The 304 haploid progeny cultures from the cross of cultures A_1 by t_2 were obtained and maintained on *Agropyron*. The pathogenicity of the 304 progeny cultures on the 4 wheat varieties is shown in Table 2. The infection types produced by the progenies on the 4 varieties were different from those of the parent cultures. For example, on *Little Club* the parent cultures produced only infection

TABLE 1

Infection types of culture A_1 of *Erysiphe graminis* f sp. *agropyri* and culture t_2 of *Erysiphe graminis* f. sp. *tritici* on 4 wheat varieties and *Agropyron tsukushiense* var. *transiens*.

Wheat variety and *Agropyron*	Infection type of culture	
	A_1	t_2
Little Club	0	4
Norin No. 4	0	4
Turkey Red	0	4
Champion White	0	4
*Agropyron**	4	0

* *Agropyron tsukushiense* var. *transiens* OHWI

TABLE 2

Pathogenicity of progeny cultures from the cross of culture A_1 of *Erysiphe graminis* f. sp. *agropyri* × culture t_2 of *Erysiphe graminis* f sp. *tritici* on seedlings of 4 wheat varieties when progeny cultures were isolated and maintained on *Agropyron tsukushiense* var. *transiens*

Wheat variety	Observed number of progeny								Value of P for 3 : 1 ratio
	Avirulent			Virulent					
	0	0-1	total	1	2	3	4	total	
Little Club	196	39	235	32	30	5	2	69	.3—.5
Norin No. 4	217	9	226	14	42	18	4	78	.7—.8
Turkey Red	122	105	227	21	39	15	2	77	.8—.9
Champion White	102	21	123	18	18	1	0	37	.5—.7

types 0 and 4, respectively, but all except 2 of the progeny cultures produced avirulent or intermediate infection types. The 2 progeny cultures produced infection type 4 like parent culture t_2. Avirulent infection type 0 could easily be distinguished from virulent infection types 1, 2, 3, and 4, but infection types 0-1 (avirulent) and 1 (virulent) were difficult to distinguish. On *Norin No. 4* only 9 cultures gave infection type 0-1, but on the 3 varieties a considerable number of cultures gave infection type 0-1. Cultures producing infection types 0-1 and 1 were retested at least five times. Cultures producing infection type 1 in all those tests were classified as virulent type 1. Those cultures which produced infection type 0 in some tests, and 1 in other tests were classified as avirulent type 0-1. In this way the progeny cultures were divided into the two classes, avirulent and virulent. The observed number of haploid progeny cultures avirulent and virulent on each of the 4 wheat varieties fit the 3 : 1 ratio expected if the parent cultures differed by 2 genes conditioning pathogenicity on each variety.

TABLE 3

Relationship of genes conditioning pathogenicity of progeny cultures from the cross of culture A_1 of *E. graminis* f. sp. *agropyri* × culture t_2 of *E. graminis* f. sp. *tritici* on 4 wheat varieties when the progeny cultures were isolated and maintained on *Agropyron tsukushiense* var. *transiens*

Wheat variety		Number of progeny				Value of P for 1:1:1:5 ratio
x	y	V_xV_y*	V_xA_y	A_xV_y	A_xA_y	
Little Club	*Norin No. 4*	39	30	39	196	0.5—0.7
Little Club	*Turkey Red*	38	31	39	196	0.5—0.7
Little Club	*Champion White*	21	13	16	110	0.2—0.3
Norin No. 4	*Turkey Red*	43	35	34	192	0.7—0.8
Norin No. 4	*Champion White*	13	25	24	98	0.2—0.3
Turkey Red	*Champion White*	19	20	18	103	0.95—0.98

* V_x and A_x indicate the pathogenicity of cultures on var. x. V_y and A_y indicate pathogenicity of cultures on var. y. V=virulent, A=avirulent

3. *Relationship of Genes Conditioning Pathogenicity of the Progeny Cultures on the 4 Wheat Varieties*

The pathogenicity of progeny cultures from the cross of cultures A_1 by t_2 maintained on *Agropyron* indicates the two cultures differ by 2 genes for pathogenicity on each of the wheat varieties (Table 2). The relationships of the genes conditioning the pathogenicity of the progeny cultures maintained on *Agropyron* on the wheat varieties is shown in Table 3. The frequency of the 4 pathogenically different cultures on any 2 varieties fits the 1:1:1:5 ratio. A 1:1:1:5 ratio would be expected if one of the two genes conditioning the avirulence of parent culture A_1 on all the varieties was the same, but the other gene conditioning avirulence on each variety was different. The gene conditioning the avirulence of culture A_1 on all the varieties could be designated A_{uw} (universal gene to wheat). The genes conditioning avirulence of culture A_1 on *Little Club*, *Norin No. 4*, *Turkey Red*, and *Champion White* could be designated A_{1c}, A_{n4}, A_{tr}, and A_{cw}, respectively. The gene conditioning virulence of culture t_2 on *Little Club*, *Norin No. 4*, *Turkey Red*, and *Champion White* could be designated V_{1c}, V_{n4}, V_{tr}, and V_{cw}, respectively. The genotypes of cultures A_1 and t_2 for pathogenicity on *Little Club* and *Norin No. 4* would then be $A_{uw}A_{1c}A_{n4}$ and $V_{uw}V_{1c}V_{n4}$, respectively. The eight possible genotypes of haploid progeny from the cross of cultures A_1 by t_2 for pathogenicity on *Little Club* and *Norin No. 4* would be $V_{uw}V_{1c}V_{n4}$, $V_{uw}V_{1c}A_{n4}$, $V_{uw}A_{1c}V_{n4}$, $V_{uw}A_{1c}A_{n4}$, $A_{uw}V_{1c}V_{n4}$, $A_{uw}V_{1c}A_{n4}$, $A_{uw}A_{1c}V_{n4}$, and $A_{uw}A_{1c}A_{n4}$. Cultures $V_{uw}V_{1c}V_{n4}$ would be virulent on *Little Club* and *Norin No. 4*. Cultures $V_{uw}V_{1c}A_{,4}$ would be virulent on *Little Club* but avirulent on *Norin No. 4*, and cultures $V_{uw}A_{1c}V_{,4}$ would be avirulent on *Little Club* but virulent on *Norin No. 4*. Cultures $V_{uw}A_{1c}A_{n4}$ and those 4 genotypes having gene A_{uw}

would be avirulent on both *Little Club* and *Norin No. 4*. The expected ratio of the 4 pathogenically different progenies on the 2 varieties would be 1 : 1 : 1 : 5. The observed number of 39 progenies virulent on *Little Club* and *Norin No. 4*, 30 progenies virulent on *Little Club* but avirulent on *Norin No. 4*, 39 progenies avirulent on *Little Club* but virulent on *Norin No. 4*, and 196 progenies avirulent on both *Little Club* and *Norin No. 4* fits the 1 : 1 : 1 : 5 ratio expected if 3 genes conditioned the pathogenicity on *Little Club* and *Norin No. 4*.

4. *Progeny Culture on Wheat Varieties*

The pathogenicity on *Little Club* of the 46 progeny cultures isolated and maintained on *Norin No. 4* is shown in Table 4. The progeny cultures maintained on *Norin No. 4* would have the genotype $V_{uw}V_{n4}$. One-half of those cultures would have the gene A_{1c} and the other half the gene V_{1c}. Those progeny with gene A_{1c} would be avirulent on *Little Club*. Those progeny with gene V_{1c} would be virulent on *Little Club*. The frequency of avirulent and virulent cultures on *Little Club* would be 1 : 1. The frequency of 25 avirulent to 21 virulent cultures on *Little Club* fits the 1 : 1 ratio expected if the cultures maintained on *Norin No. 4* differed by one gene for pathogenicity on *Little Club*.

TABLE 4

Pathogenicity of 46 progeny cultures from the cross of culture A_1 of *Erysiphe graminis* f. sp. *agropyri* × culture t_2 of *E. graminis* f. sp. *tritici* on seedlings of *Little Club* wheat when progeny cultures were isolated and maintained on *Norin No. 4* wheat.

Wheat variety	Observed number of progeny								Value of P for 1:1 ratio
	Avirulent			Virulent					
	0	0-1	total	1	2	3	4	total	
Little Club	8	17	25	0	13	4	4	21	0.5—0.7

TABLE 5

Pathogenicity of 90 progeny cultures from the cross of culture A_1 of *Erysiphe graminis* f. sp. *agropyri* × culture t_2 of *Erysiphe graminis* f. sp. *tritici* on seedlings of *Norin No. 4* wheat when progeny cultures were isolated and maintained on *Little Club* wheat.

Wheat variety	Observed number of progeny								Value of P for 1:1 ratio
	Avirulent			Virulent					
	0	0-1	total	1	2	3	4	total	
Norin No. 4	42	8	50	1	9	24	6	40	0.2—0.3

The pathogenicity on *Norin No. 4* of the 90 progeny cultures isolated and maintained on *Little Club* is shown in Table 5. The progeny maintained on *Little Club* would have the genotype $V_{uw}V_{1c}$. One-half of those cultures would have the gene A_{n4} and the other half the gene V_{n4}. Those progeny with the gene A_{n4} would be avirulent on *Norin No. 4*. Those progeny with gene V_{n4} would be virulent on *Norin No. 4*. The frequency of avirulent and virulent cultures on *Norin No. 4* would be 1 : 1. The frequency of 50 avirulent to 40 virulent cultures on *Norin No. 4* fits the 1 : 1 ratio expected if the cultures maintained on *Little Club* differed by 1 gene for pathogenicity on *Norin No. 4*.

The frequency of avirulent and virulent cultures on each of *Little Club* and *Norin No. 4* of the progeny cultures maintained on *Agropyron* were 3 : 1 (Table 2). The frequency of avirulent and virulent cultures on each of *Little Club* and *Norin No. 4* of the progeny cultures maintained on *Norin No. 4* and *Little Club* were 1 : 1, respectively (Tables 4 and 5). These data would confirm that 2 genes condition the aviruleuce of culture A_1 on each of *Little Club* and *Norin No. 4*, and that one of the 2 genes is the same.

DISCUSSION

The pathogenicity of haploid progeny cultures from the inter-formae-speciales cross of cultures A_1 by t_2 on the four wheat varieties *Little Club*, *Norin No. 4*, *Turkey Red*, and *Champion White* showed that two genes conditioned the avirulence of culture A_1 on each variety. The same gene A_{uw} conditioned the avirulence of culture A_1 on all varieties. The second gene conditioning avirulence on each variety was different. If Flor's gene-for-gene hypothesis is valid, then the four wheat varieties each have two genes conditioning their resistant reaction to culture A_1. One gene is the same in all varieties. The other gene is different in each variety. The gene present in all varieties could be designated Ml_{uw} (universal gene in wheat). The other genes could be designated as Ml_{1c} in *Little Club*, Ml_{n4} in *Norin No. 4*, Ml_{tr} in *Turkey Red*, and Ml_{cw} in *Champion White*.

The 4 wheat varieties used are susceptible to wheat mildew. It is interesting that even those susceptible varieties each would have different gene for resistance to Agropyron mildew. It is also very interesting to verify that if in wheat varieties there is universally the gene Ml_{uw} for resistance corresponding to gene A_{uw} in the pathogen.

The data showed many haploid progeny cultures from the cross of cultures A_1 by t_2 were virulent on both wheat and *Agropyron*. In nature, however, a specialized form of *Erysiphe graminis* DC. is dominant (Cherewick, 1944). Those facts would be important in relation to the origen of host specialization in *Erysiphe graminis* DC.

SUMMARY

Pathogenicity of the haploid progenies from inter-formae-speciales cross of cultures A_1 of ***Erysiphe graminis*** DC. f. sp. ***agropyri*** Em. Marchal and t_2 of *E. graminis* DC. f. sp. ***tritici*** Em. Marchal on four wheat varieties was studied. Two genes were found to condition the avirulence of culture A_1 on each of four wheat varieties. The same gene, designated A_{uw} (universal gene to wheat), conditioned the avirulence on all varieties. The other gene conditioning avirulence on each variety was different. The second genes conditioning avirulence on each variety were designated as A_{1c} on ***Little Club***, A_{n4} on ***Norin No. 4***, A_{tr} on ***Turkey Red***, and A_{cw} on ***Champion White***. The five genes conditioning pathogenicity were inherited independently.

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LITERATURE

1. Cherewick, W. J. 1944. Studies on the biology of ***Erysiphe graminis*** DC. Canad. J. Res. C 22 : 52—86.
2. Flor, H. H. 1955. Host-parasite interaction in flax rust—its genetics and other implications. Phytopathology 45 : 680—685.
3. Hardison, J. R. 1944. Specialization of pathogenicity in ***Erysiphe graminis*** on wild and cultivated grasses. Phytopathology 34 : 1—20.
4. Hiura, U. 1960. Studies on the disease-resistance in barley IV. Genetics of the resistance to powdery mildew. Ber. Ohara Inst. landw. Biol. Okayama Univ. 11 : 235—300.
5. Hiura, U. 1962. Hybridization between varieties of ***Erysiphe graminis***. Phytopathology 52 : 664—666.
6. Hiura, U. 1964a. Genetics of host-parasite interaction in barley mildew. Ber. Ohara Inst. landw. Biol. Okayama Univ. 12 : 121—129.
7. Hiura, U. 1964b. Pathogenicity of the haploid progeny cultures from crosses between cultures of ***Erysiphe graminis*** f. sp. ***tritici*** and ***Erysiphe graminis*** f. sp. ***agropyri*** (Preliminary Note). Ber. Ohara Inst. landw. Biol. Okayama Univ. 12 : 131—132.
8. Marchal, E. 1902. De la spécialisation du parasitisme chez 1' ***Erysiphe graminis*** DC. Compt. Rend. Acad. Sci (Paris) 135 : 210—212.
9. Moseman, J. G. 1959. Host-pathogen interaction of the genes for resistance in ***Hordeum vulgare*** and for pathogenicity in ***Erysiphe graminis*** f. sp. ***hordei***. Phytopathology 49 : 469—472.
10. Powers, H. R. Jr.. and Sando, W. J. 1960. Genetic control of the host-parasite relationship in wheat powdery mildew. Phytopathology 50 : 454—457.
11. Wolfe, M. S. 1965. Physiologic specialization of ***Erysiphe graminis*** f. sp. ***tritici*** in the United Kingdom. Trans. Brit. mycol. Soc. 48 : 315—326.