PRODUÇÃO DE CONÍDIOS, PERÍODO DE ESPORULAÇÃO E EXTENSÃO DA LESÃO POR HELMINTHOSPORIUM SATIVUM NAS FOLHAS-BANDEIRA DE TRIGO[1]

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RESUMO - Foram realizados estudos sobre produção de conídios, período de esporulação e extensão da lesão de doze raças de *Helminthosporium sativum* nas folhas-bandeira de quatorze cultivares de trigo. O cumulativo total de conídios produzidos variou entre zero e 6.335 conídios e o período de esporulação variou entre zero e 30 dias, dependendo da raça e da cultivar em questão. O pico de esporulação variou entre 23 e 36 dias de inoculação, porém na maioria dos casos o pico foi atingido no 29º dia de inoculação. A quantidade máxima de conídios foi produzida na cultivar Paraguay 214 durante os 46 dias de inoculação. O máximo tamanho final da lesão foi de 4,4 cm^2 e a máxima percentagem de área foliar infectada por uma única lesão foi de 22,7. A extensão da lesão, na maioria dos casos, foi marcadamente alta durante o período de 22 a 32 dias de inoculação, o qual também correspondeu com o aumento drástico da produção de conídios durante o mesmo período, atingindo o pico de esporulação no dia 29. De modo geral, os componentes de resistência parcial, tais como: produção dos conídios, período de esporulação e taxa de extensão da lesão, agiram independentemente. A taxa de extensão da lesão e o tamanho final da lesão foram considerados como parâmetros desejáveis da resistência parcial. Algumas das melhores fontes de resistência parcial foram as cultivares BH 1146, LD 7831 e PAT 7219.

Termos para indexação: resistência parcial, *Helminthosporium sativum*, produção de conídios, extensão da lesão, trigo.

CONIDIAL PRODUCTION, SPORULATION PERIOD AND EXTENSION OF LESION OF HELMINTHOSPORIUM SATIVUM ON FLAG LEAVES OF WHEAT

ABSTRACT - Studies were carried on conidial production sporulation period and lesion extension of twelve races of *Helminthosporium sativum* on flag leaves of fourteen wheat cultivars. Cumulative total conidial production varied from zero to 6,335 conidia and the sporulation period varied between zero and 30 days depending on the race and the cultivar under study. The peak of sporulation varied between 23 and 36 days after inoculation but in most of the cases it was reached on the 29th day of inoculation. The maximum final lesion size was 4.4 cm^2 and the maximum percentage of leaf area infected by a single lesion was 22.7. The lesion extension in most of the cases was markedly enhanced during the period of 22 to 32 days of inoculation, which was correlated with the drastic increase in conidial production during the same period reaching the peak on the 29th day. In general, the components of partial resistance, like conidial production, sporulation period and the rate of lesion extension acted independently. The rate of lesion extension and final lesion size were considered to be the desirable paramaters of partial resistance. Some of the best sources of partial resistance were the cultivars BH 1146, LD 7831 and PAT 7219.

Index terms: partial resistance, *Helminthosporium sativum*, conidial production, lesion extension, wheat.

INTRODUCTION

Considering the importance of the leaf blight (Spot-blotch) of wheat caused by *Helminthosporium sativum* in Brazil, Mehta (printing) made some studies and identified 32 races of this fungus. Merely identifying the races does not help solve the problem, but it definitely serves as one of the tools towards the search for a higher degree of partial resistance. It also serves to identify and incorporate the resistance genes in agronomically desirable cultivars.

Relatively little information is available with respect to the sources of partial resistance against this disease. In part, it may be because the disease is not yet considered important in any part of the world as it is in Brazil (Weise 1977, Mehta 1978 and Mehta & Igarashi 1979). As already reported (Mehta & Igarashi 1979), varietal resistance is very much lacking in existing cultivars and there are no efficient fungicides to control the disease economically. Thus for the time being breeding for resistance is the only alternative left and should receive due priority.

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There are already some known sources of resistance against *H. sativum*, amongst which the most common ones widely used by the pathologists and breeders are 'Horizon', 'PF 72707' and 'BH 1146'. Yet, whether such cultivars in fact possessed resistance against a single race or a large number of locally existing races was not known, and consequently, the breeding for resistance was more a gamble than a guarantee, since what interests us is partial resistance and not resistance against a single race or a very few races.

It is for this reason, that the present investigation was undertaken. It comprises two main objectives:

1. To find out the sources of a high degree of partial resistance, and,

2. To study the correlation between the different components of resistance and to identify the most reliable and practical ones so as to simplify the work on detection and measuring the degree of resistance.

This paper presents detailed studies on the performance of fourteen wheat cultivars against twelve virulent races of *H. sativum* with respect to the conidial production, sporulation period and the rate of lesion extension. Mehta & Zadoks (1970) studied the uredospore production and sporulation period of *Puccinia recondita*, whereas Yarwood (1961) studied uredospore production by *Uromyces phaseoli*. Recently, Koizumi (1977) studied the process of lesion extension in *Xanthomonas citri*. However, the present investigation gives the first report of such studies with *H. sativum*.

MATERIAL AND METHODS

Plants. Twelve plants of each of the fourteen cultivars were grown (three plants per pot) in earthen pots of 20 cm of diameter and 25 cm of height, in sterilized soil. A total of four experiments were performed. Experiments 1 and 2 were the pilot experiments in which six cultivars were used, whereas in Experiment 3, thirteen cultivars were used and in Experiment 4 the number was reduced to six. Throughout the experiments, only the flag leaves were used, and the other leaves were cut away just before the inoculation in order to avoid their interference in observation.

Environmental conditions. All the experiments were carried out in uniform conditions in a greenhouse where the temperature and humidity were not controlled. The relative humidity and temperature were recorded throughout the experiments using a thermohygrograph. Average relative humidity and temperature were calculated by measuring the area under the curves of the weekly graphs and dividing it by the length of the graphs. The average temperature during the total length of the experiments was 23.6° C and the variation during the day was between 21° C and 33° C (mostly below 28° C), whereas the variation in the night was between 17° C and 20° C (mostly about 20° C). The average relative humidity was 82.1% with a variation during the day between 40 and 70 (mostly above 60) and variation during the night was between 70 and 100 (mostly over 90). Experiments 3 and 4 were considered the major experiments during the present investigation. Analysing the weekly average environmental conditions it was observed that in Experiment 3 the variation in the weekly average temperature was between 19° C 26° C and the variation between the weekly average relative humidity was between 79° C & 87,5%. In Experiment 4, the variation in weekly temperature was zero, remaining always 24° C, and the variation in weekly average relative humidity was between 71% and 89%. The optimum temperature for the disease development was reported to be 20° C (Clark & Dickson 1958).

Races. Only very virulent races were used. In Experiments 1 and 2, races 1 and 5 were used; in Experiment 3, race 5 was used, whereas in Experiment 4, ten races were used: i. e., races 2, 3, 4, 6, 7, 8, 9, 10, 11 and 17 (Mehta, printing).

Inoculum. Twelve flag leaves per cultivar were inoculated soon after their emergence with a conidial suspension in water. For this purpose, the inoculum was multiplied on autoclaved sorghum seeds in an "Erlenmeyer" flask which was shaken twice a day to avoid the formation of mycelium (Joshi et al. 1969). Three weeks later, 50 grams of seeds were suspended and shaken in distilled water. The conidial suspension thus obtained was filtered through a cheese cloth and the conidial concentration was adjusted to 24 x 10^2 conidia/ml by diluting with water. A drop of sticker (SANDOVIT) per 200 ml of suspension was added and the same was sprayed using a small atomizer and a pressure pump. The leaves were first washed with distilled water, gently rubbed between the fingers, and then each one held upright, was given a quick single round of spray, starting from the top of the leaf downwards and then from the bottom of the leaf to the top. The inoculated plants were then incubated for 16 hours in a completely dark moist chamber with a water-saturated atmosphere at about 20° C. Later, the plants were kept on the bench in the greenhouse. In general, the first symptoms of the disease were noticed after 48 hours of inoculation. Conidial concentration was always maintained very low so as to obtain only one to three well-separated lesions per leaf. Leaves showing overlapping of lesions or more than three lesions were discarded. Normally, leaves with only one lesion were preferred.

Lesion extension. The size of the lesion was traced carefully by a pencil on a transparent butter paper held over the lesion. Such traced areas were cut with a razor blade and the lesion area was determined periodically by means of an Automatic area meter Model AAC-400 (manufactured by Hayashi Denkoh. Co. Ltd. Tokyo, Japan), with an accuracy of ± 1%. Though the area meter was of very high precision, three readings were taken for each lesion and an average was determined. Always, only one lesion per leaf and per plant and a total of three to eight leaves per cultivar were studied throughout the experiment. There were some exceptions, like cultivar LD 7831, where only two leaves were studied, and cultivars IAS 58, Tobari and Sel. Londrina, where only three leaves were studied in Experiment 3. In Experiment 4, the exceptions were 'Horizon', 'BH 1146' and 'LD 7831', where only one leaf was studied against race 7, and 'Paraguay 214', where only two leaves were studied against race 11. The lesion extension measurements started six days after inoculation and continued till the leaves started showing senescense and natural drying. Considering the four experiments, a total of 247 lesions were periodically measured during a total period of over 46 days.

Leaf area. After the experiment was over, the leaves were removed from the plants, unrolled over a broad adhesive "scotch tape", and kept pressed within blotter papers for two days, and the area was determined in the same way as explained for the lesion area. The percentage of leaf area infected was determined by dividing the final lesion area by the leaf area and multiplying it by 100. For this purpose at least 18 leaves per cultivar were used.

Rate of lesion extension. Due to the nature of the experiments, the disease was considered as multiplying in the same way as a simple interest (Plank 1963), and hence, the lesion area in cm^2 was transformed into percentage of leaf area infected which was divided by 100 and was transformed into $\log_e (1/1\text{-}x)$. The rate of lesion extension was calculated through a regression analysis, using the formula $\text{Log}_e = a + bx$, and is expressed in terms of the regression coefficient (b). An overall average leaf area of 17.2 cm^2 was used for all the calculations both in Experiment 3 and 4.

Conidial production. The same leaves and the same lesions were used for the studies on conidial production and sporulation period as were used for the lesion extension studies. Conidia were harvested from the lesions, both from the superior and the inferior leaf portions, periodically (after every three to seven days and mostly after every four days) starting after eleven to 20 days from inoculation and continuing till no more conidia were formed. For this purpose, a piece of adhesive "Scotch tape", a little bigger than the lesion size, was fixed on both the surfaces of the lesion; it was then removed and affixed to a glass slide, and the number of conidia were counted with the help of a microscope. Sufficient care was taken for not to touch the lesions with the fingers and also to avoid the movement of the plants during the experiment. Lesion area was always measured soon after harvesting the conidia so as to avoid the loss of conidia, thereby reducing the experimental error. Including all the experiments, a total of 247 leaves were studied over a period of 46 days for the conidial production and a total of 57.725 conidia were counted.

Sporulation period. Sporulation period was considered the period during which the lesions continued to produce conidia. The number of conidia produced per lesion per day was calculated by dividing the cumulative total number of conidia by the period of sporulation in days.

Fungicides. Whenever necessary, one or two sprays with Butyl Triazol (0,4 l/300 l of water) + Ethirimol (1,0 l/300 l of water) were given to control the powdery mildew and leaf rust infections in the experimental plants. Application of such fungicides neither affeeted the sporulation nor the development of the lesions.

RESULTS

a. Conidial production and sporulation period

Experiment 1 and 2 were the pilot experiments. Experiment 1 was conducted to standardize the techniques for lesion extension studies. Experiment 2 was conducted to standardize the techniques for conidial production and sporulation period studies, and hence no results of this experiment are presented although the conclusions are mentioned in **Discussion**. In Experiment 3, conidial production, sporulation period and rate of lesion extension were studied, using race 5 of *H. sativum* on thirteen wheat cultivars (Table 1). The cultivars are placed in the order of the number of conidia produced per lesion per day. Both the conidial production and the sporulation period varied a lot from one cultivar to another. In this experiment, sporulation started 16 days after inoculation, with the exception of cultivar Sel. Londrina, in which it started only after 38 days of inoculation. In general, sporulation continued up to 42 days after inoculation, except for the cultivars PF 72707, IAC 5, IAS 54 and LD 7831, where it continued up to 46 days. The cultivars Sel. Londrina, LD 7831, BH 1146 and Tobari were considered highly resistant for race 5 since the sporulation period and the cumulative total conidial production were very low as compared to the rest of the cultivars. Sonora 64, on the other hand, was considered very susceptible in relation to the others, considering the conidial production. No correlation was observed between

TABLE 1. Conidial production, sporulation period and rate of lesion extension of race 5 of *Helminthosporium sativum* on flag leaves of some wheat cultivars.

Cultivar	Number of conidia produced per lesion days after inoculation[a]																Sporulation period in days	Total number of conidia produced	Average number of conidia per lesion/ day	Average lesion size in cm^2 [b]	% of leaf area infected[c]	Rate of lesion extension
	16		19		26		30		34		38		42		46							
	S	I	S	I	S	I	S	I	S	I	S	I	S	I	S	I						
Sonora 64	12	2	86	116	1673	1000	5	4	234	239	158	24	0	0	0	0	22	3553	162	2,17	14,8	0,004
PF 72707	2	2	9	2	247	67	5	0	1255	189	118	73	3	0	2	0	30	1977	67	2,31	11,3	0,004
Horizon	1	3	27	18	132	67	43	16	35	13	465	350	198	69	0	0	26	1427	55	1,65	9,6	0,003
IAS 62	8	2	10	5	192	7	0	0	680	170	11	4	6	3	0	0	26	1098	42	2,60	14,2	0,005
CNT 6	5	2	3	1	93	47	3	0	464	396	47	3	5	2	0	0	26	1071	41	2,20	11,8	0,004
IAC 5	7	1	14	1	251	60	9	31	316	244	33	22	3	1	0	2	30	1006	34	1,71	9,9	0,004
IAS 54	2	1	6	5	111	68	2	6	108	73	71	53	21	43	5	0	30	574	19	2,02	7,8	0,004
IAS 58	4	4	5	2	156	65	3	1	106	32	57	31	3	5	0	0	26	474	18	2,16		
Paraguay 214	3	0	7	6	128	6	3	6			158	102	20	7	0	0	26	448	17	0,79	3,7	0,003
Sel. Londrina	0	0	0	0	0	0	0	0	0	0	23	8	3	0	0	0	4	34	9	0,30	1,6	0,001
LD 7831	2	0	1	0	23	2	1	1	26	35	49	9	3	0	2	0	30	152	5	0,95	4,2	0,002
BH 1146	1	0	0	0	6	0	0	0	57	14	2	5	3	3	0	0	26	91	4	1,60	13,1	0,003
Tobari	3	1	7	5	21	3	0	0	0	0	0	0	0	0	0	0	10	40	4	0,93	5,5	0,003

a - Average of 4 - 6 leaves using only one lesion per leaf.
b - Final lesion size.
c - Based on 20 - 25 leaves.
S - Superior leaf portion; I - Inferior leaf portion.
OBS: Blank columns indicate that no reading was taken.

the sporulation period and the cumulative total conidial production. A higher sporulation period does not signify higher conidial production.

By and large, the superior leaf portions produced a greater number of conidia than the inferior leaf portions. This could be an artifact of experimental procedures, and further studies are needed to prove whether such a phenomenon really exists. Mehta & Zadoks (1970), while working with *Puccinia recondita,* did not find an appreciable difference either between the number of pustules or between the number of spores produced on the upper and lower leaf surfaces. In the present experiment, the sporulation peak was observed on the 34th day, whereas in 'Sonora 64', which is an early maturing cultivar, the peak was observed on the 26th day.

In Experiment 4, five cultivars which were supposed to be resistant, i.e., PF 72707, Horizon, BH 1146, LD 7831 and PAT 7219 were tested against ten virulent races along with a susceptible cultivar, Paraguay 214. The main purpose of this experiment was to determine whether such cultivars were really resistant against a number of races, so that these could be used in breeding programs to guarantee the success. The conidial production, sporulation period and the rate of lesion extension of these cultivars against ten races are demonstrated in Tables 2, 3, 4, 5, 6 and 7. As in the earlier experiment, the races are placed in the order of the number of conidia produced per lesion per day. In general, sporulation started eleven days after inoculation, except for the cultivars LD 7831 and PAT 7219, where the sporulation started 22 days and 20 days after inoculation respectively. Considering the six cultivars and the ten races, the sporulation period varied from zero to 29 days. Such differences are interpreted as differences between the genetic compositions of the cultivars and the races. A less pronounced difference in the sporulation period between the races was observed in 'Horizon', 'PAT 7219', and Paraguay 214', i.e., 11 to 25 days in 'Horizon' (Table 3), 18 to 22 days in 'Paraguay 214' (Table 4) and 10 to 20 days in 'PAT 7219' (Table 7). Considering the sporulation, all the cultivars do indicate that they have some dominant resistance genes operating against at least three to eight races. The cultivars PF 72707 and Horizon have been considered by many breeders and pathologists to be resistant in general against *H. sativum,* and have been widely used in breeding programs. On the contrary, the results presented herein demonstrate that these cultivars are susceptible to at least three or more races and possess a relatively smaller degree of resistance. 'BH 1146' and 'LD 7831' were moderately resistant to three races and highly resistant to other races (Tables 5, 6). With the exception of BH 1146', in this experiment all the cultivars produced over 1500 conidia at least against one race. In some cases, very few conidia were produced but at the same time the lesion size was expressed by "zero", indicating that the lesion size was very small and beyond the capacity of the area meter to measure it. (Table 2, 5 and 6). On the contrary, the lesion size of race 7 was as big as 1.18 cm^2 in 'LD 783', but no conidia were produced (Table 6).

The peak of the sporulation against all the races was observed on the 29th day in cultivars PF 72707, Horizon, Paraguay 214 and BH 1146. In LD 7831, the peak of sporulation was on the 36th day (with one exception which was on the 29th day) and in 'PAT 7219' it was reached on the 23rd day (with three exceptions). The difference of thirteen days in the peak of sporulation between some cultivars and races is not very well understood. In part it could be interpreted as the difference in the degree of virulence among the races and in part due to the underlying genetic mechanism of the cultivars. The difference in the total conidial production could also be interpreted in a similar way. The highest amount of total number of conidia produced was in 'Paraguay 214' and in subsequent order were the cultivars Horizon, PF 72707, PAT 7219, LD 7831 and BH 1146.

As stated under Experiment 3, no correlation was observed between the sporulation period and the total number of conidia produced. The latter gives a better understanding about the performance of cultivars against different races rather than the sporulation period and the average number of conidia produced per lesion per day.

b. Lesion extension

Studies on lesion extension were made in three experiments. Lesion extension of race 1 of *H. sativum* is presented in Fig. 1. The lesions did not

TABLE 2. Conidial production, sporulation period and rate of lesion extension of 10 races of *Helminthosporium sativum* on flag leaves of wheat cultivar PF 72707.

Races	Number of conidia produced per lesion days after inoculation[a]														Sporulation period in days	Total conidia produced	Number of conidia produced per lesion/day	Average lesion size cm^2 [b]	% of leaf area infected[c]	Rate of lesion extension
	11		18		22		29		33		36		40							
	S	I	S	I	S	I	S	I	S	I	S	I	S	I						
2	0	0	0	0	0	0	367	1383	13	184	0	0	0	0	4	1947	487	1,75	8,6	0,010
8	0	0	0	0	0	0	412	19	1	3	7	8	0	0	7	450	64	2,57	12,6	0,008
7	1	1	1	0	0	0	471	158	32	0	138	696	116	75	29	1689	58	2,06	10,1	0,006
9	0	0	1	1	0	0	659	103	21	19	0	0	0	0	15	804	54	1,45	7,1	0,006
3	0	0	0	0	0	0	18	27	11	3	0	0	0	0	4	59	15	0,95	4,7	0,004
4	0	1	0	0	0	0	45	7	4	1	0	0	0	0	4	7	14	0,92	4,5	0,004
11	0	0	0	2	0	0	98	38	19	12	0	0	0	0	15	169	11	1,11	5,4	0,004
6	0	0	0	0	0	0	2	3	0	0	0	0	0	0	1	5	5	0,00[d]	0,0	0,000
10	0	0	1	0	0	0	33	5	3	0	0	0	0	0	15	42	3	0,74	3,6	0,002
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,00	0,0	0,000

a - Average of 3 - 5 leaves using only one lesion per leaf.
b - Final lesion size.
c - Based on an average area of 25 leaves.
S - Superior leaf portion; I - Inferior leaf portion.

TABLE 3. Conidial production, sporulation period and rate of lesion extension of 10 races of *Helminthosporium sativum* on flag leaves of wheat cultivar Horizon.

Races	Number of conidia produced per lesion days after inoculation[a]												Sporulation period in days	Total conidia produced	Number of conidia produced per lesion/day	Average lesion size cm[2b]	% of leaf area infected[c]	Rate of lesion extension
	11		18		22		29		33		36							
	S	I	S	I	S	I	S	I	S	I	S	I						
3	0	0	4	0	0	0	926	1424	0	0	0	0	11	2354	214	2,12	15,1	0,007
7	0	0	1	1	0	0	1274	736	0	0	0	0	11	2012	183	1,74	12,4	0,008
11	0	3	0	0	0	0	1695	1283	31	183	0	0	22	3195	145	0,60	4,3	0,002
6	1	0	21	0	0	0	1704	999	18	13	0	0	22	2756	125	1,49	10,6	0,004
9	0	0	2	0	0	0	1324	463	17	12	0	0	15	1818	121	0,81	5,8	0,002
4	2	0	17	0	0	0	1262	20	0	0	5	11	25	1317	53	0,87	6,2	0,004
17	0	0	2	0	1	0	332	151	2	0	0	0	15	488	33	1,22	8,7	0,005
10	1	1	1	1	0	0	236	203	12	3	0	0	22	458	21	1,72	12,3	0,005
2	1	1	0	0	0	0	184	258	0	2	0	0	22	446	20	2,60	18,6	0,010
8	0	1	8	0	0	0	100	139	8	0	9	3	25	268	11	2,24	16,0	0,007

a - Average of 3 - 4 leaves using only one lesion per leaf.
b - Final lesion size.
c - Based on an average area of 25 leaves.
S - Superior leaf portion; I - Inferior leaf portion.

TABLE 4. Conidial production, sporulation period and rate of lesion extension of 10 races of *Helminthosporium sativum* on flag leaves of wheat cultivar Paraguay 214.

Races	Number of conidia per lesion days after inoculation[a]										Sporulation period in days	Total conidia produced	Number of conidia produced per lesion/day	Average lesion size in cm^2 [b]	% of leaf area infected[c]	Rate of lesion extension
	11		18		22		29		33							
	S	I	S	I	S	I	S	I	S	I						
2	1	1	28	2	1	0	3068	2768	86	131	22	6086	277	2,23	11,5	0,007
7	0	0	28	18	0	0	753	2362	8	4	22	3173	144	1,63	8,4	0,005
17	3	0	15	0	2	2	966	603	1	0	22	1590	72	4,41	22,7	0,011
9	0	0	21	2	0	0	621	256	46	38	15	988	66	2,21	11,4	0,006
3	1	1	6	0	1	1	683	597	62	93	22	1445	66	2,37	12,2	0,008
10	9	0	61	13	0	0	259	744	0	0	18	1086	60	1,79	9,2	0,007
4	0	1	0	0	0	0	848	417	3	18	22	1287	59	1,39	7,1	0,004
6	2	1	10	2	0	0	1	4	174	86	22	280	13	1,30	6,7	0,005
11	6	2	88	7	2	1	19	1	0	0	18	126	7	1,70	8,7	0,007
8	49	18	4	0	0	0	13	20	4	0	22	108	5	1,56	8,0	0,004

a - Average of 3 - 5 leaves using only one lesion per leaf.
b - Final lesion size.
c - Based on an average area of 25 leaves.
S - Superior leaf portion; I - Inferior leaf portion.

TABLE 5. Conidial production; sporulation period and rate of lesion extension of 10 races of *Helminthosporium sativum* on flag leaves of wheat cultivar BH 1146.

Races	Number of conidia produced per lesion days after inoculation[a]												Sporulation period in days	Total conidia produced	Number of conidia produced per lesion/day	Average lesion size in cm^2 [b]	% of leaf area infected[c]	Rate of lesion extension
	11		18		22		29		33		36							
	S	I	S	I	S	I	S	I	S	I	S	I						
2	0	0	0	0	0	0	25	11	5	1	0	0	4	42	11	0,97	6,4	0,004
8	0	1	0	0	0	0	62	71	0	0	0	0	18	133	7			
11	0	0	1	0	0	0	69	5	2	1	33	5	18	116	6	1,71	11,2	0,007
3	0	0	0	0	0	0	4	1	3	2	8	6	7	24	3	0,55	3,6	0,003
7	0	0	9	10	0	0	0	1	0	0	0	0	11	20	2	0,21	1,4	0,001
10	0	0	0	0	0	0	1	4	1	0	0	0	4	6	2	0,55	3,6	0,002
9	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0,00[d]	0,0	0,000
6	1	0	0	0	0	0	4	1	0	0	0	0	18	6	0,3	0,54	3,5	0,002
4	0	1	1	0	0	0	0	0	0	0	0	0	7	2	0,2	0,93	6,1	0,004
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,00	0,0	0,000

a - Average of 3 - 4 leaves using only one lesion per leaf.
b - Final lesion size.
c - Based on an average area of 20 leaves.
S - Superior leaf portion; I - Inferior leaf portion.

TABLE 6. Conidial production, sporulation period and rate of lesion extension of 10 races of *Helminthosporium sativum* on flag leaves of wheat cultivar LD 7831.

Races	Number of conidia produced per lesion days after inoculation[a]										Sporulation period in days	Total conidia produced	Number of conidia produced per lesion/day	Average lesion size in cm^2 [b]	% of leaf area infected[c]	Rate of lesion extension
	22		29		33		36		40							
	S	I	S	I	S	I	S	I	S	I						
11	0	0	50	56	13	5	923	360	76	41	11	1524	139	0,59	2,9	0,002
9	0	0	162	67	2	0	689	214	18	17	11	1169	106	1,95	9,6	0,005
8	0	0	168	20	5	7	0	0	0	0	4	200	50	1,39	6,8	0,005
3	0	0	8	3	0	0	0	0	0	0	1	11	11	0,00[d]	0,0	0,000
17	0	0	3	0	0	0	0	0	0	0	1	3	3	0,00[d]	0,0	0,000
7	0	0	0	0	0	0	0	0	0	0	0	0	0	1,18	5,8	0,005
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0,54	2,6	0,001
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0,00	0,0	0,000
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0,00	0,0	0,000
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0,00	0,0	0,000

a - Average of 4 - 6 leaves using only one lesion per leaf.
b - Final lesion size.
c - Based on an average area of 20 leaves.
S - Superior leaf portion; I - Inferior leaf portion.

TABLE 7. Conidial production, sporulation period and rate of lesion extension of 10 races of *Helminthosporium sativum* on flag leaves of wheat cultivar PAT 7219.

Races	Number of conidia produced per lesion days after inoculation[a]												Sporulation period in days	Total conidia produced	Number of conidia produced per lesion/day	Average lesion size in cm^2 [b]	% of leaf area infected[c]	Rate of lesion extension
	20		23		27		33		36		40							
	S	I	S	I	S	I	S	I	S	I	S	I						
9	13	8	768	447	29	54	1	0	1	0	0	0	16	1321	83	0,98	8,4	0,002
6	3	4	500	192	221	81	8	0	0	0	0	0	13	1009	78	1,34	11,6	0,006
8	4	1	484	255	12	7	3	0	0	1	0	0	16	767	48	0,75	6,5	0,002
7	4	5	179	76	206	72	47	18	0	0	0	0	13	607	47	2,06	17,8	0,008
10	4	2	198	86	144	16	80	0	0	0	0	0	13	530	41	0,79	6,8	0,006
17	17	3	85	27	103	85	223	8	10	1	0	0	16	562	35	1,13	9,7	0,004
3	5	2	155	27	204	9	1	3	0	0	0	0	13	406	31	1,70	14,7	0,006
4	4	1	262	132	36	4	2	0	2	0	0	0	16	443	28	1,22	10,5	0,003
2	13	4	149	98	65	16	30	1	2	1	1	1	20	381	19	1,31	11,3	0,004
11	0	0	6	0	34	1	1	0	0	0	0	0	10	42	4	0,94	8,1	0,004

a - Average of 3 - 7 leaves using only one lesion per leaf.
b - Final lesion size.
c - Based on an average area of 18 leaves.
S - Superior leaf portion; I - Inferior leaf portion.

grow in LD 7831 and PF 72707 since these cultivars were highly resistant to this race. In Horizon and PAT 7219 the lesions started developing twelve days after inoculation instead of nine days of inoculation, as shown for BH 1146 and Paraguay 214. Horizon being a resistant cultivar against race 1, the lesions stopped developing after 18 days of inoculation. On the other hand, the lesion extension was stopped after 33 days of inoculation in PAT 7219 and BH 1146. In the latter, the development was very slow, but it was very fast in Paraguay 214. In Experiment 3, race 5 was used, and the development of the lesions in thirteen cultivars is presented in Fig. 2.

In all cultivars the lesion extension initiated between six and eleven days, except for Sel. Londrina, in which it initiated as late as 30 days after inoculation and terminated 41 days after inoculation. As in the earlier experiment, the lesion extension in BH 1146 was reasonably slow. Plotting of the lesion area against time in most of the cases yielded almost a typical sigmoid curve with a slow and gradual slope. The sharp increase in lesion extension in LD 7831 cannot be explained. A sharp but less drastic increase was also noticed in three other cases with the same cultivar and during more or less the same period in Experiment 4 (Fig. 7).

Normally, the lesion extension continued only up to 41 days after inoculation except in the case of Horizon and CNT 6, where it continued up to 44 days. The maximum lesion size reached was as high as 2.60 cm^2 in the case of IAS 62, covering the second highest percentage of leaf area infected (14,2), the first being 14.8% in Sonora 64. No correlation was observed between the lesion size and the percentage of leaf area infected. This is mainly because the leaf area of each cultivar was different. Similarly, no correlation could be made between the total conidia produced and the percentage of leaf area infected.

The process of lesion extension in Experiment 4 is illustrated in Fig. 3, 4, 5, 6, 7 and 8. Races against which no lesion extension is shown indicate that the cultivars were highly resistant and the lesions did not develop at all. In such cases, only small necrotic spots appeared and could not be measured by the area meter.

The lesion extension in most of the cases was markedly enhanced during the period of 22 to 32

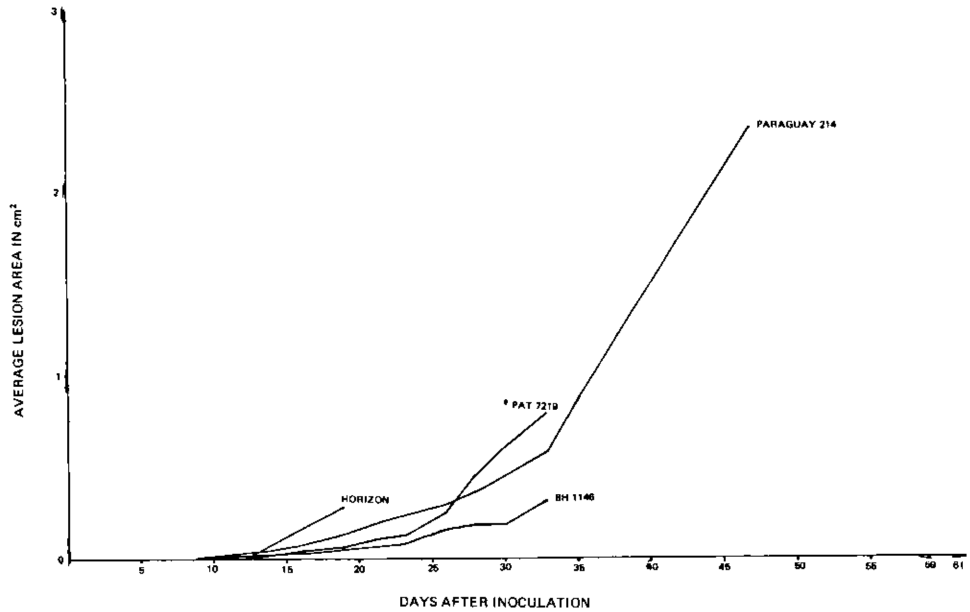

FIG. 1. Extension of lesions of race 1 of *Helminthosporium sativum* on flag leaves of four wheat cultivars.

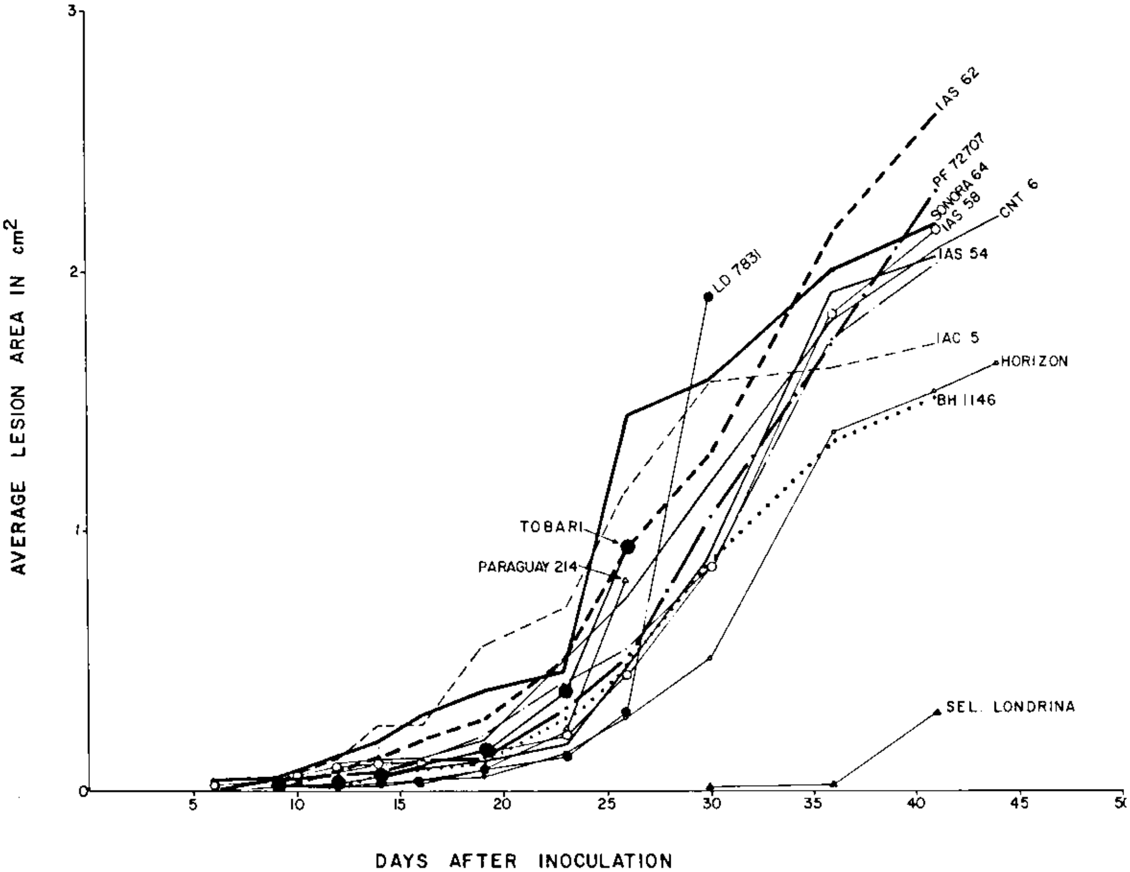

FIG. 2. Extension of lesions of race 5 of *Helminthosporium sativum* on flag leaves thirteen wheat cultivars.

days of inoculation. This was correlated with the drastic increase in conidial production during the same period, reaching its peak on the 29th day. In general, the process of lesion extension initiated eleven days after inoculation. Unfortunately, the early development of lesion in PAT 7219 was not recorded, due to practical difficulties. Nevertheless, in these two cultivars and in LD 7831 the process of lesion extension against all the races was considerably slow as compared to lesion extension against some of the races in other cultivars. Moreover, the final lesion size in BH 1146, LD 7831 and PAT 7219 never exceeded 2.06 cm^2, indicating their high degree of partial resistance. The longest lesion extension period of 42 days in PAT 7219 is ignored and considered unimportant as far as measuring the degree of partial resistance is concerned.

Considering the six cultivars, it was observed that races 4, 6 and 11 were in general less aggressive as compared to other races which were very variable and cultivar dependent.

The maximum final lesion size of 4.41 cm^2 was reached in Paraguay 214, covering a leaf area as high as 22.7% during a period of only 32 days. As in Experiment 3, no correlation was observed between the total conidial production and the final lesion size, or the percentage of leaf area infected. The final lesion size was proportional with the rate of lesion extension and linear correlation was obtained (correlation coefficient 0,85). Mehta & Igarashi (1979), while working with *Puccinia recondita* on wheat, also found some correlation between the components of partial resistance. Components of partial resistance, like

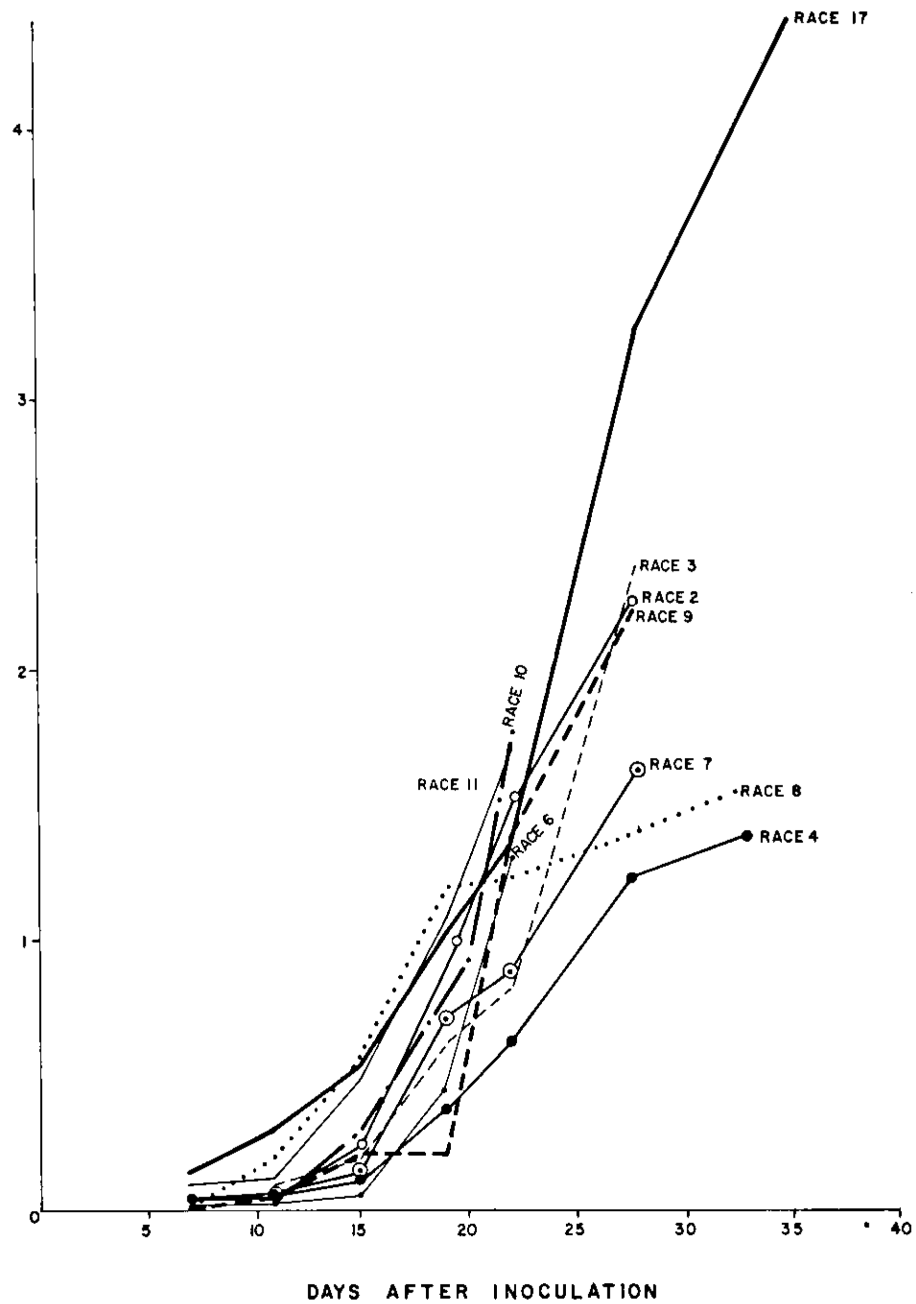

FIG. 3. Extension of lesions of ten races of *Helminthosporium sativum* on flag leaves of wheat cultivar Paraguay 214.

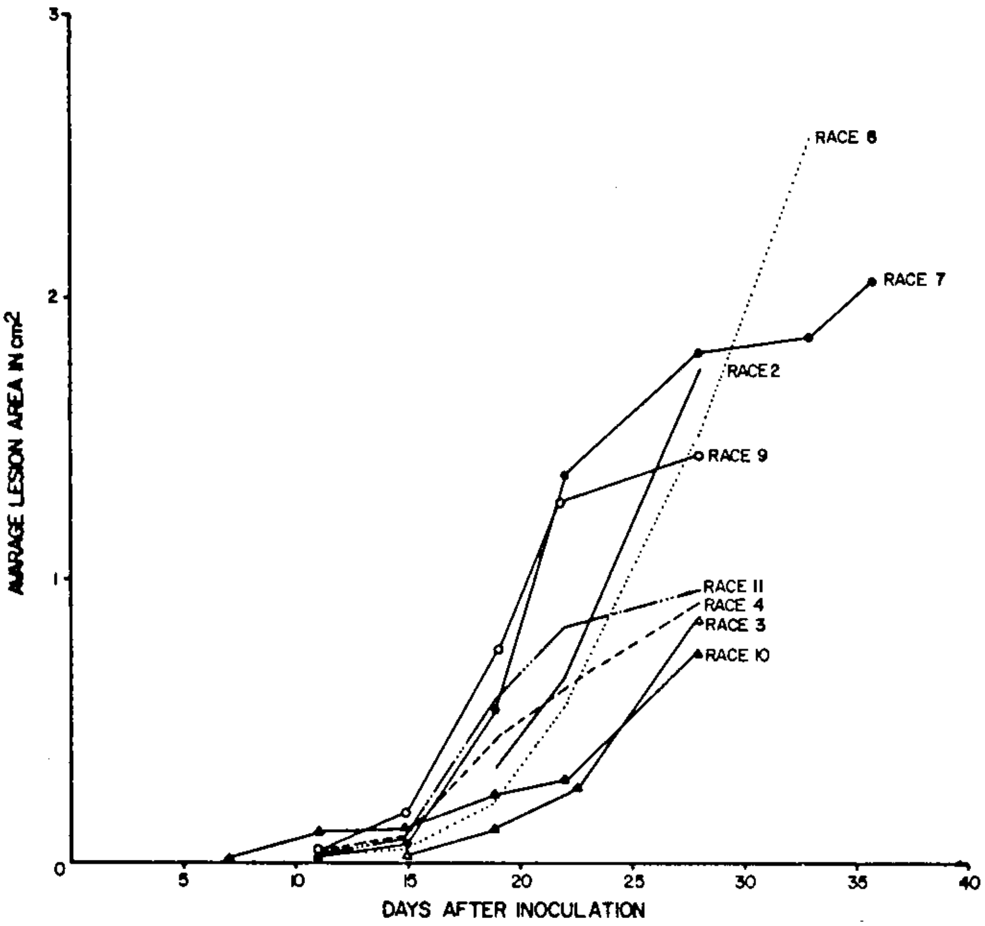

FIG. 4. Extension of lesions of eight races of *Helminthosporium sativum* on flag leaves of wheat cultivar PF 72707.

conidial production, sporulation period, and the rate of lesion extension, acted independently.

DISCUSSION

The major investigations were the spore production potential and the rate of lesion extension. In one of the experiments, sporulation was measured only 20 days after inoculation. Though small, some amount of conidia must have been lost due to the delay of seven to eight days in the first observation. Considerable variation was observed within the cultivar as regards the number of conidia produced between one lesion and another. Variation in lesion extension among the leaves of a cultivar and a particular race was not great. It is possible that the conidial production was underestimated by the fact that some conidia might have been lost in the air during the period between one reading and another. Moreover, due to the use of adhesive "Scotch tape" technique, the conidiophores - including the young ones - were also sometimes harvested along with the conidia, interfering thereby in their capacity of conidial production. In a pilot experiment (Experiment 2), a washing technique was used, which included washing the lesions after every 48 hours with the help of a pipette and distilled water with five drops of spreader (SANDOVIT) per litre, then collecting the conidial suspension in a beaker, and finally determining the number of conidia produced per lesion. Such a technique did not harvest the conidiophores, but it was not found suitable,

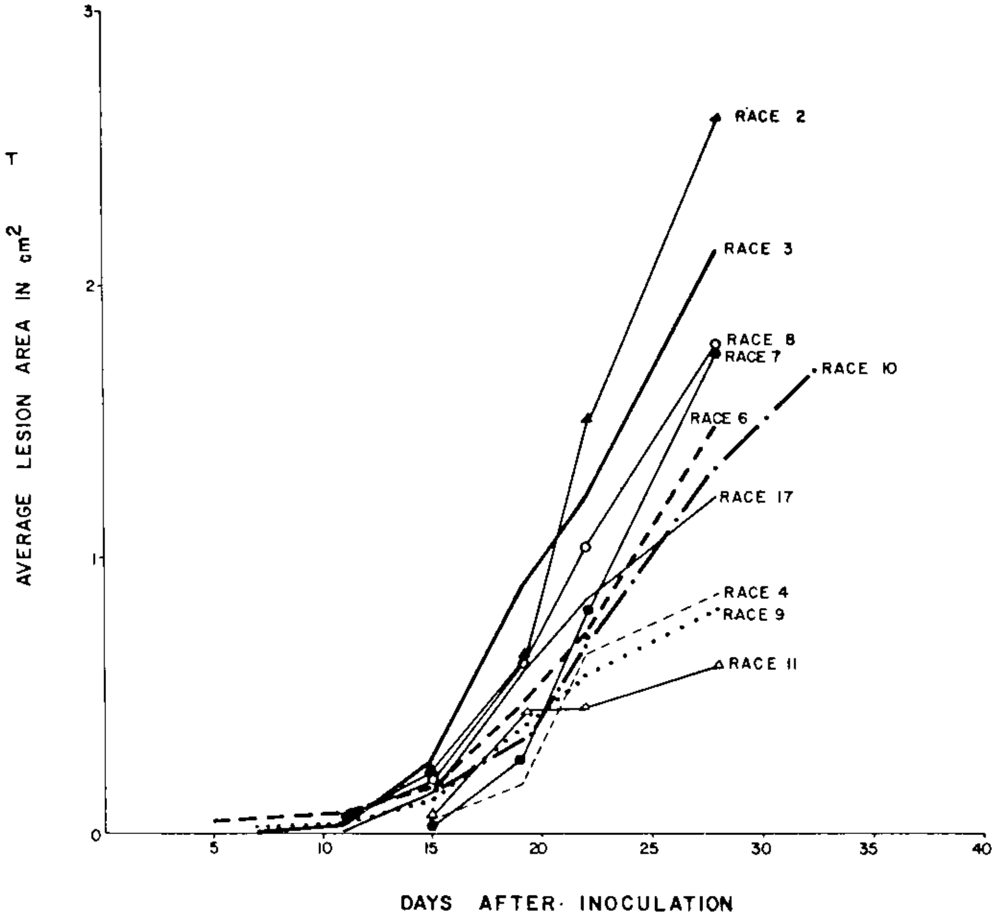

FIG. 5. Extension of lesions of ten races of *Helminthosporium sativum* on flag leaves of wheat cultivar Horizon.

since it was not possible to harvest all the conidia present in the lesion, and since the technique was too labourious and time consuming.

The maximum sporulation period was 30 days and the maximum conidial production per lesion per day was 487. These figures are a little low as compared to the sporulation period of up to 72 days and a spore production of about 767 per pustule per day in the case of *P. recondita* (Mehta & Zadoks 1970). Somewhat similar results were obtained by Yarwood (1961), while working with *U. phaseoli*. This indicates that the fungus *H. sativum* has a much lower production potential than *P. recondita* and *U. phaseoli*. Nevertheless, *H. sativum*, could be much more desastrous, due to the fact that the lesion extension of this fungus is very rapid and one single lesion could cover over 22.7% of the leaf area within 33 days of inoculation, especially in susceptible cultivars. The incubation period of *P. recondita* is about eight days, whereas in *H. sativum* it is only 48 hours, but the sporulation in both the pathogens initiates more or less at the same time.

The effect of the density of lesions per leaf on conidial production, sporulation period and lesion extension has not been studied. Mehta & Zadoks (1970) reported that, within limits, the higher the density of pustules of *P. recondita* per leaf, the lower will be the spore production and sporulation period. It is very likely that such a finding also holds good for *H. sativum*. Using fourteen cultivars and a total of twelve races, it was observed that the races also behave differently on adult plants. This finding confirms the earlier work on race identification (Mehta, printing).

As stated earlier, the environmental conditions in the greenhouse did not vary much, and hence it is hoped that the minor variations in temperature and humidity may not have interfered greatly in the present investigation.

In cultivars susceptible to one race or the other, the conidial production was markedly enhaced during the initial stages of lesion extension and was drastically reduced soon after the peak of conidial production was reached. In general, the peak

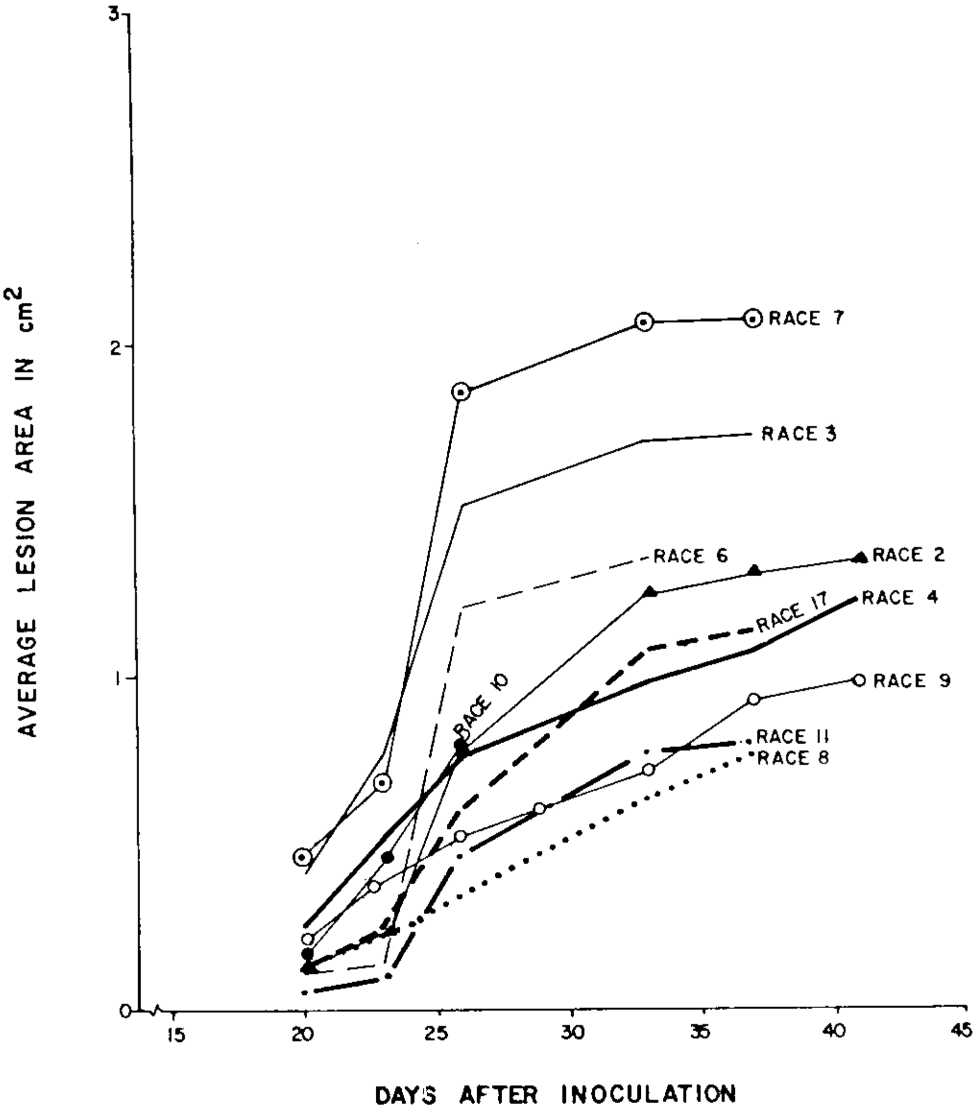

FIG. 6. Extension of lesions of ten races of *Helminthosporium sativum* on flag leaves of wheat cultivar PAT 7219.

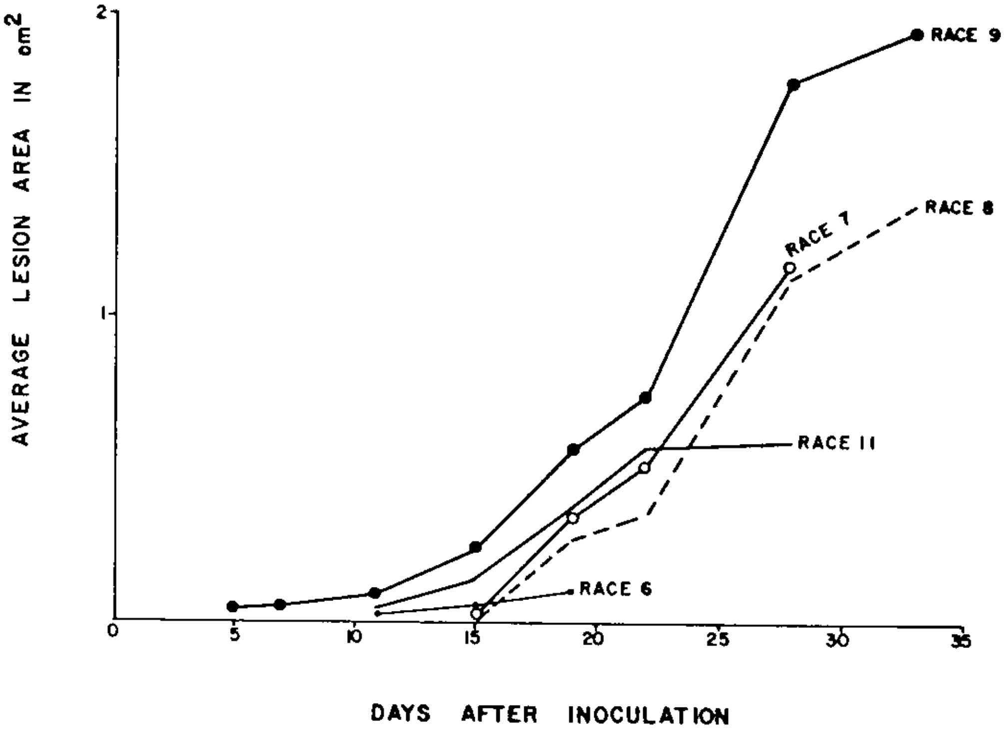

FIG. 7. Extension of lesions of five races of *Helminthosporium sativum* on flag leaves of wheat cultivar LD 7831.

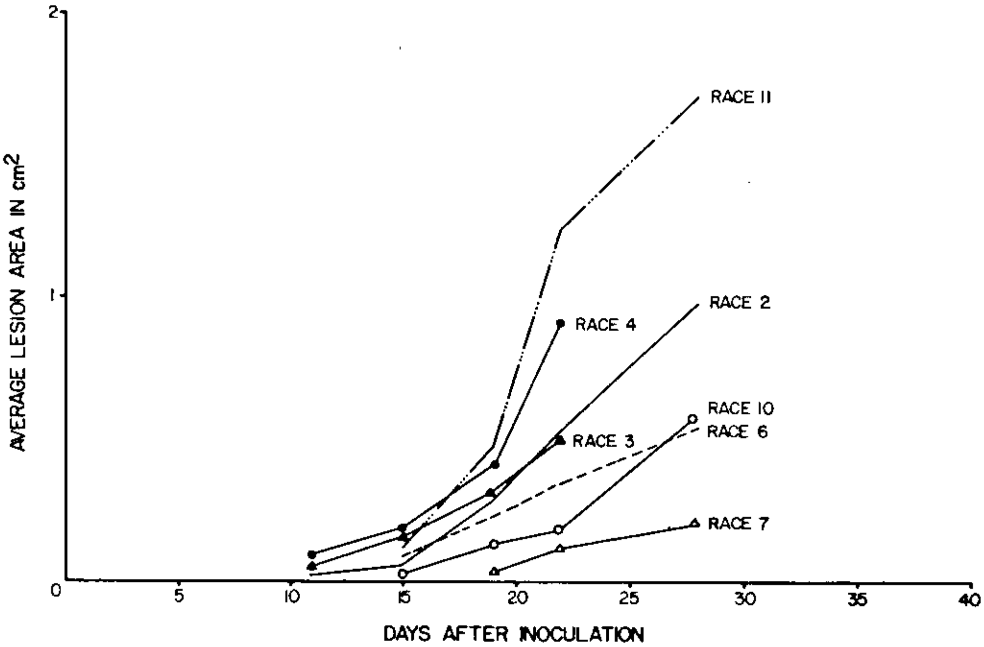

FIG. 8. Extension of lesions of seven races of *Helminthosporium sativum* on flag leaves of wheat cultivar BH 1146.

period lasted only one day as against a few days in *P. recondita* (Mehta & Zadoks 1970), *U. phaseoli* (Yarwood 1961), and *X. citri* (Koizumi 1977). Epidemiologically speaking, the conidial production after the peak is reached is of very little importance. It is apparent that chemical control measures should be used in the early stages of the disease development.

Undoubtedly, for the detection and measuring of the degree of partial resistance, the cultivars must be tested along with some of the highly susceptible cultivars, using virulent races. In other words, definition of a highly susceptible reaction is a prerequisite. In the present investigation, Paraguay 214 was considered to be highly susceptible, and the degree of resistance of six cultivars against eleven races was measured, using three parameters like conidial production, final lesion size and the rate of lesion extension. The resistance of 'Paraguay 214' against race 2 and 17 was considered to be zero in all the parameters and further calculations for the degree of resistance were made for all the races and cultivars. Considering Fig. 9, 10, and 11, it was observed that all the five cultivars possess partial resistance since none of the cultivars showed highly susceptible reaction (zero degree of resistance) against any of the eleven races when compared with Paraguay 214 . The degree of resistance varied considerably depending on the parameter used. The cultivars BH 1146 , LD 7831 and PAT 7219 showed a very high degree of partial resistance irrespective of the parameter.

When conidial production is considered as a parameter (Fig. 9), the cultivar 'BH 1146' could be taken as a cultivar with almost complete resistance against all the races. This does not hold true when final lesion size and rate of lesion extension are considered as parameters (Fig. 10 and 11). The degree of partial resistance can also be studied by grouping all the components of resistance into one index, the "relative resistance" as suggested by Zadoks (1971). The "relative resistance" is calculated by multiplying the degree of resistance of one component with another and then deducting the product from 1. In such a case, it is necessary that the highly susceptible cultivar expresses zero degree of resistance against the same race in all the parameters. In the present investigation, for example, Paraguay 214 showed zero degree of resistance against race 17 as far as final lesion size and the rate of lesion extension were concerned, but did not show zero degree of resistance when conidial production was concerned as a parameter. This indicates that a bigger lesion size and a higher rate of lesion extension does not also necessarily mean a higher conidial production. The degree of partial resistance should be determined after having studied more than one component of resistance, if not, misleading conclusions could be drawn. This is in contrast to what was reported earlier by Mehta & Igarashi (1979). In general, components of partial resistance are independent and hence a choice has to be made as to which component should be studied. Conidial production and rate of lesion extension are the epidemiologically important components and should receive considerable importance. Unfortunately, the former is relatively difficult and time-consuming.

The cultivars PF 72707 , Horizon BH 1146 , and PAT 7219 are widely used by breeders as the sources of resistance against *H. sativum*. The present studies indicate that some of the best sources of resistance are the cultivars BH 1146 , LD 7831 and PAT 7219 . Luz et al. (1976), in their preliminary studies, reported, that BH 1146 and PAT 7219 were highly resistant as far as ear and seed infections were concerned. Nonetheless, studies on inheritance of resistance in these cultivars still need to be done. Detection and measuring of partial resistance against a number of races does not explain its genetic background.

CONCLUSIONS

1. The identification of races of *H. sativum* on seedlings is valid, and some of the races identified in such a way also show great differences when tested on adult plants.

2. Conidial production, sporulation period and the rate of lesion extension vary to a great extent and are cultivar and race dependent.

3. No correlation was observed either between the conidial production and the sporulation period or between the conidial production and the final size of the lesions or the rate of lesion extension. There existed linear correlation between the final

lesion size and the rate of lesion extension, and hence these were considered to be the reliable and relatively easy parameters of partial resistance. Partial resistance should be detected and measured using at least two parameters.

4. Some of the best sources of a high degree of partial resistance are the cultivars BH 1146, LD 7831 and PAT 7219 instead of PF 72707 and Horizon. The latter two cultivars are commonly used breeders to incorporate their resistance in agronomically desirable cultivars and should be substituted by the former three cultivars.

5. Detection of more sources of partial resistance against *H. sativum* is very much needed. No information on inheritance of resistance against this pathogen is available in Brazil and hence due priority should be given for such investigation.

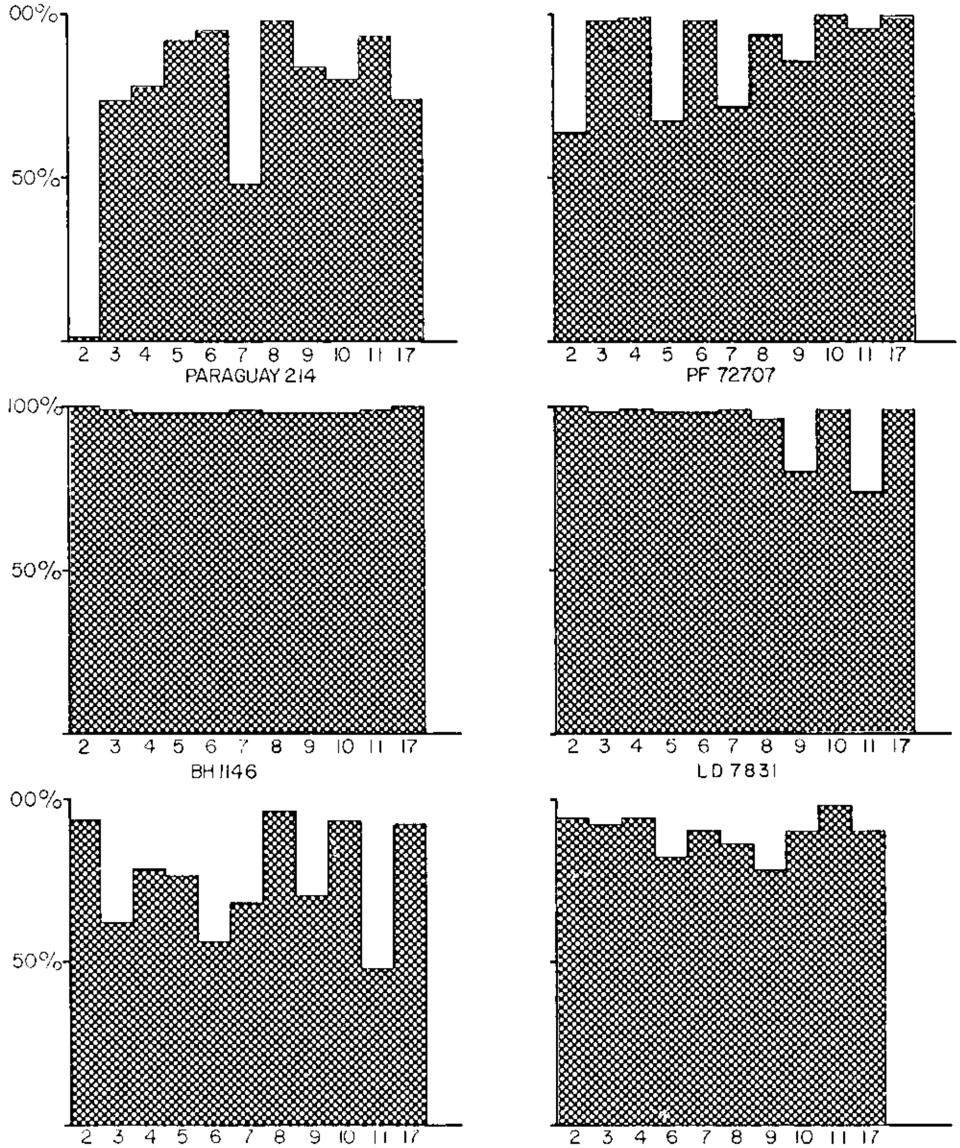

FIG. 9. Degree of resistance of six wheat cultivars against eleven races of *Helminthosporium sativum* using cumulative total conidial production as a parameter.

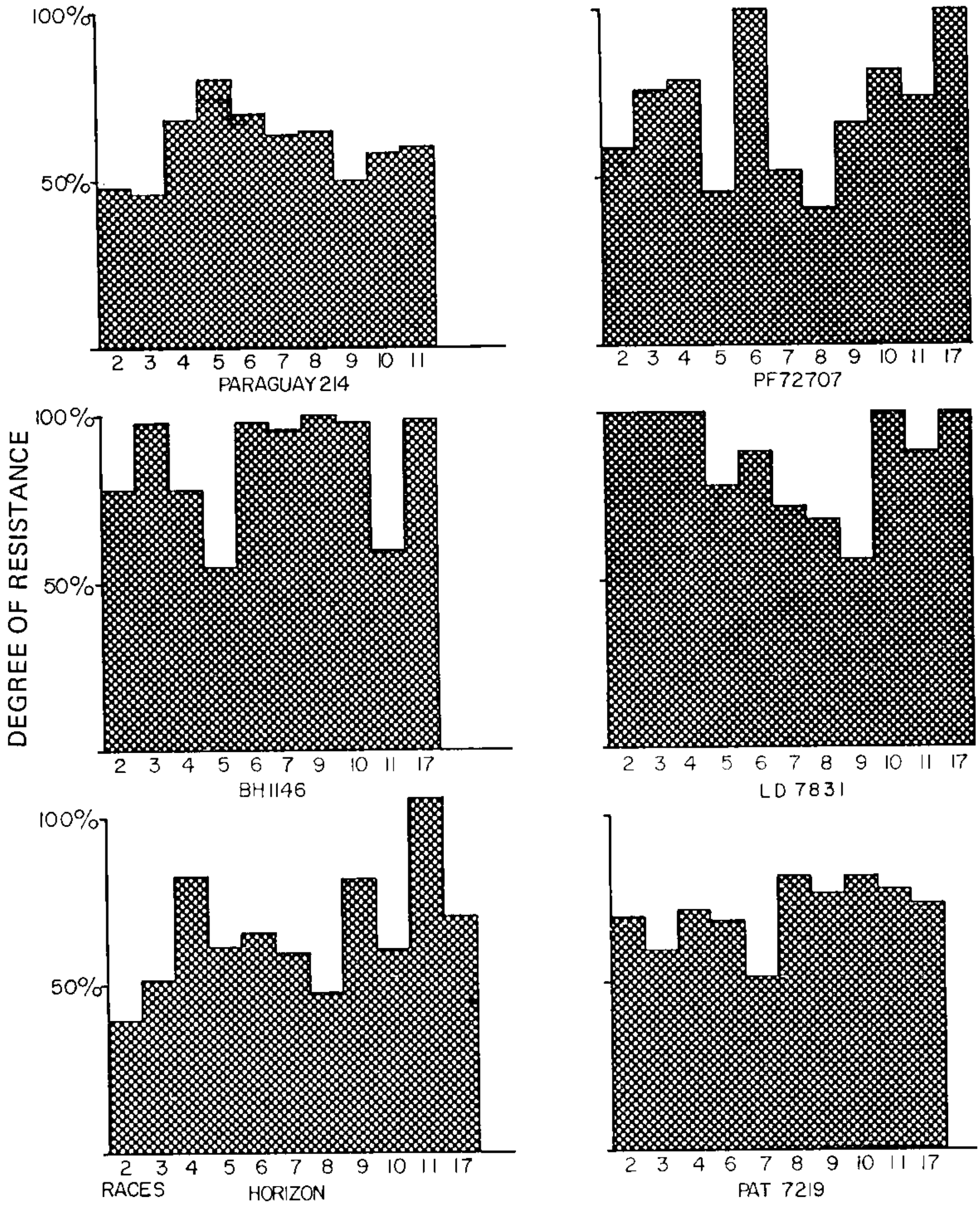

FIG. 10. Degree of resistance of six wheat cultivars against eleven races of *Helminthosporium sativum* using final lesion size as a parameter.

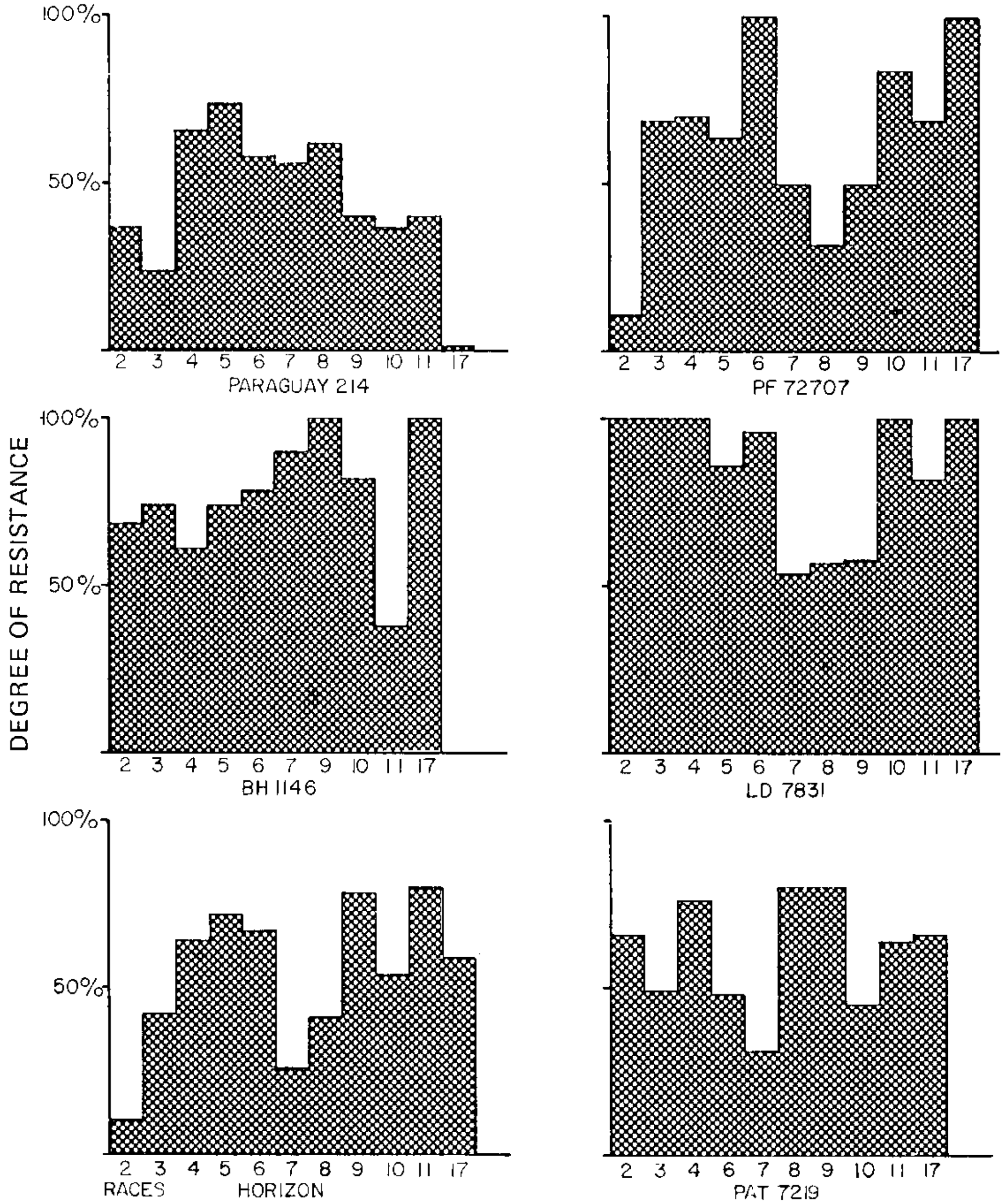

FIG. 11. Degree of resistance of six wheat cultivars against eleven races of *Helminthosporium sativum* using rate of lesion extension as a parameter.

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