STUDIES ON THE DISEASE-RESISTANCE IN BARLEY

II. PHYSIOLOGIC RACES OF Erysiphe graminis Hordei in Japan*

U. Hiura and H. Heta

Introduction

Honecker (9) estimated on the basis of experiments conducted in 1934 to 1935 the increase in yield of barley associated with mildew resistance to be roughly 10%. Schaller (20) determined the effect of various levels of mildew infection on yield of barley from differential performance of Atlas 46 and Atlas. In the absence of disease the performance of the two varieties was essentially alike. He estimated that when a barley crop was slightly mildewed in the early stages of development, the reduction in yield was 6.6 to 3.8%, and under severe infection the maximum reduction at any one location was 27.4% of the total yield. In Japan, through improvements in disease control measures and practices, it is seldom that a barley crop encounters a heavy loss in yield from the powdery mildew. In the central and western parts of Japan, where a majority of the barley varieties grown are highly susceptible to powdery mildew (16), most of the crops are infected to a certain extent, and even if the reduction in yield per unit area may be slight, a considerable damage can be estimated when the entire area is considered.

Although powdery mildew of barley can be controlled by the use of sulphur compounds, the use of fungicides in widespread areas is normally not economical, and the use of mildew-resistant varieties represents the most practical control method of this disease. For developing resistant varieties of barley informations on the occurrence and geographical distribution of physiologic races of the mildew are necessary.

This paper reports the results of experiments performed from 1950 to 1952 on the physiologic races of barley mildew and their geographic distribution in Japan.

Review of Literature

In 1902 Marchal (14) distinguished seven physiologic varieties of Erysipe-

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Erysiphe graminis DC. based on their host specialization: Tritici on species of Triticum, Hordei on species of Hordeum, Secalis on species of Secale, Avenae on species of Avena, Poae on species of Poa, Agropyri on species of Agropyron, and Bromi on species of Bromus. Salmon (18) and Reed (17) made studies on the powdery mildew occurring on various species of Hordeum. They found little evidence of differences in pathogenicity of the mildew from such different sources. Their results, therefore, would indicate that the Hordei race was rather uniform in its pathogenicity.

Physiologic races of Erysiphe graminis hordei were first demonstrated by Mains and Dietz (12). In 1930 they distinguished five races on barley in the United States. In this line of investigation Tidd (22) described two additional races in that country. Hardison (3) stated that a culture isolated from Agropyron repens collected at Yakima, Washington, could be classed as a new race of E. graminis hordei, because it produced a very ready infection on the barley variety, Arlington C.I. 702, which was resistant to all seven races of barley mildew in the United States. In Canada Cherewick (1, 15) reported four races of barley mildew in 1935, and five additional races, three of them in 1944 and the remaining two in 1947, making a total of nine physiologic races. Honecker (10) isolated nine races in Germany. In Argentine two races were described (2, 19). In Japan, Homma (7) made studies on the physiologic varieties of Erysiphe graminis DC. However, it was not until 1951 that Hiura and Heta (4, 5) first identified three physiologic races of barley mildew.

Materials and Methods

The Differential Varieties

In 1950 twelve varieties of barley, Akimaki-hokusëi, Nakaizumi-zairai, Yahazu, Kairyô-bôzu-mugi, Heiwa-hadaka, Hitokawa, Caucasian, Mamuth, Hordeum spontaneum nigrum (H. spont. var. transcaucasicum Vav.), Nudideficiens, Colsess la, and Kommopori were used to distinguish physiologic races of barley mildew. These varieties were selected on the basis of their reaction to mildew as distinguished by different types of infection as described in our previous work (16).

The differential varieties of barley used in 1951 were the same as those used by Tidd in the United States (22): Black Hull-less C.I. 666, Goldfoil C.I. 928, Heil's Hanna 3 C.I. 682, Nepal C.I. 595, and Peruvian C.I. 935. The seeds of these barley varieties were obtained through the kindness of Dr. Warren H. Leonard, Professor of Agronomy, Colorado A & M College, and Dr. G. A. Wiebe of the U. S. Department of Agriculture.

In 1952 four barley varieties, Chevron C.I. 111, and Colsess C.A. 772 for differentiating races and Duplex C.I. 2433, and Kairyô-bôzu-mugi for
differentiating biotypes were added to those used in 1951. The seeds of Chevron C. I. 1111 and Coless C. A. 772 were originally supplied by Dr. W. J. Cherewick of the University of Manitoba.

**Source of Cultures**

To survey the physiologic races of barley mildew present in Japan, inoculation experiments were made on the 18 cultures gathered from 16 prefectures in 1950, 119 cultures from 12 prefectures in 1951, and 106 cultures from 34 prefectures in 1952. These cultures were all established from conidia.

Owing to the short life of the conidia, very few cultures were established from conidia present on specimens received in envelope by mail from distance. Most cultures, however, established well from specimens when the samples were wrapped in moist material and packed in a bamboo-cylinder for shipment by mail. Such specimens, when received, were usually devoid of viable conidia, but fresh conidia developed within 2-3 days upon floating the infected leaves on a 5% sucrose solution held at 5-10°C. The conidia thus developed on the specimen were inoculated with a spatula tipped inoculating needle on the seedling of a susceptible barley variety, Kobinkatagi, grown in a test tube.

**Inoculation Methods**

Six seedlings, each representing different differential variety, were cultured in a pot 12 cm. in diameter. Plants in each pot were covered with a glass lamp chimney whose top was covered with a muslin-cloth and the base pressed down well into the soil in the pot. These glass covers were removed only at the time of the inoculation. The inoculations were made on seedlings when they have developed their second leaf. During the periods of May to June, plants covered with glass chimney were further encased in glass infection chambers covered at the top with galvanized iron sheets. But in September to November, it was found that the mildew hardly developed on a barley grown in glass infection chamber if it were not inoculated artificially, so only the chamber was used. The temperatures in the glass chambers never at any time reached above 25°C. In order to prevent dispersion of conidia into air, test tube cultures of conidia were applied to the surface of the leaf by stroking with the tip of the moistened Japanese writing brush. Numerous infection areas were produced after 7 to 10 days of inoculation. When a different infection types was noted on a host, the conidia from each pustule were isolated and cultivated again on a susceptible host. The pathogenicity of single pustule cultures were tested repeatedly for their purity.

Cherewick (1, 15) reported that the development of mildew in general was appreciably less at higher temperature (25°C.) than at lower temperature (15°C.) and it was completely arrested when a host plant was kept.
continuously at a temperature above 25°C. Tapke (21) reported that the resistance of barley to mildew was promoted by pre-inoculation conditions that "toughen" the plant, such as exposure to cold, heat, drought, and long intense light. However, the reaction of young seedling to pre-inoculation environment might differ from that of older plants. Honecker (8) stated that continuous high temperatures above 30°C. or low temperatures below 10°C., intense direct sunlight or deficient light inhibiting assimilation in the plant did affect the infection.

Several attempts have also been made by the writers to determine the effect of environmental conditions, such as temperature, humidity, light, and drought, on the development of barley mildew. It was confirmed that, as the informations of Tapke (21) showed, the environmental conditions to which plants were exposed before inoculation considerably influenced their reaction to mildew. It was found on some varieties in seedling stage, resistance was promoted by exposure to cold and drought. This was more striking in a moderately or slightly resistant variety. In a glass room at 10 to 25°C., however, as Cherewick (1) pointed out, the variation in infection types brought about by the environment was insufficient to cause undue difficulty in the identification of the races.

**Preservation and Purification of Cultures**

Difficulties were experienced in maintaining cultures of powdery mildew during summer months. Those races identified in the spring were, therefore, preserved till autumn in a refrigerator. Barley seedling grown to a height of about 2 cm. in the dark at 0 to 5°C. survived for more than two months under such conditions. Viable conidia of mildew developed on the pale yellowish host. In autumn the stored cultures were purified by means of single spore isolations. Conidia in test tube cultures were dusted over a sterilized slide glass and single spores were picked up on a dry needle manipulating under a low power microscope. The needle bearing single spore was then drawn over the leaf of susceptible host plant grown in a test tube. About 20 to 30 % of the single spores thus isolated established. The pathogenicity of these pure cultures were then retested.

**Experimental Results**

**Identification of Physiologic Races**

During the three years from 1950 to 1952, two biotypes (races 8B, and 8C) and three new races (races 13, 14, and 15) were isolated in Japan. The three new races resemble one another in their inability to infect Heil's Hanna 3 C I. 682, which apparently distinguish them from the twelve races identified in the United States and Canada (Table 1).
TABLE 1. Mean infection types produced on seedling leaves of nine varieties of barley by 15 physiologic races of Erysiphe graminis hordei

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<td>15*</td>
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*Races isolated by writers.

? Reactions of Duplex C.A. 1129

1—Highly resistant (immune). Plant is perfectly sound macroscopically.

0—Resistant. No mycelium but minute chlorotic or necrotic spots visible macroscopically.

The infection is evident.

1—Moderately resistant. Slight developments of mycelium and sporulation. Development of distinct chlorotic or necrotic spots.

2—Slightly resistant. Moderate development of mycelium, with a slight conidia formation.

Chlorotic or necrotic areas of various modifications.

3—Moderately susceptible. Moderate developments of mycelium and sporulation. Slight chlorotic or necrotic spots may or may not develop.

4—Susceptible. Abundant mycelium and conidia formation. Outline of pustule distinct. Infection areas do not show discoloration at first.

It was not possible to differentiate races 8B and 8C from race 8 isolated in Canada, by using the reactions of only six differential varieties of Cherewick (1). Duplex C.I. 2433 was very susceptible to races 8B and 8C while race 8 is known to cause no infection on Duplex C.A. 1129 (15). No attempt was made to confirm Duplex C.I. 2433 and Duplex C.A. 1129 to possess similar reaction to race 8. However, Duplex C.I. 2433 is highly resistant to seven races of mildew (races 1, 2, 3, 4, 5, 6, and 7) in the United States (12, 13, 22), and Duplex C.A. 1129 also highly resistant to eight races of mildew (races 3, 4, 6, 8, 9, 10, 11, and 12) in Canada (15). Therefore, it is presumed that both races 8B and 8C may be distinguished from race 8 by the reactions of Duplex C.I. 2433 or Duplex C.A.
1129. From the above reason, although it was impossible for us to differentiate the two cultures from race 8 by the use of Cherokee's differential varieties, they are designated races 8B and 8C as biotypes of race 8. Race 8C was first isolated from the conidia of moderately resistant type of pustule produced on J.5 barley which showed high resistance to race 8B. The race 8C was differentiated more distinctly from race 8B by the reactions of Kaiyô-bôzu-mugi.

Analytical key for the identification of physiologic races of *Erysiphe graminis hordei* on the basis of their pathogenicity on seven selected differential varieties of barley

<table>
<thead>
<tr>
<th>Goldfoil susceptible</th>
<th>Black Hull-less susceptible</th>
<th>Heil's Hanna susceptible</th>
<th>Heil's Hanna resistant</th>
<th>Black Hull-less resistant</th>
<th>Chevron susceptible</th>
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<tr>
<td>Goldfoil resistant</td>
<td>Chevron susceptible</td>
<td>Peruvian susceptible</td>
<td>Peruvian resistant</td>
<td>Nepal susceptible</td>
<td>Heil's Hanna susceptible</td>
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<td>Colless susceptible</td>
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<td>Nepal resistant</td>
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Race 14 was separated from the other races by its virulence to Goldfoil C.I.928 which is highly resistant to other races. The resistance of Goldfoil to race 14 was promoted when the seedling was grown under cold and drought conditions, producing slightly to moderately resistant types of infection. In greenhouse, however, the race 14 always developed considerably severe on the Goldfoil, representing a stable virulence.

Race 13 and race 15 could not be distinguished from each other without the use of Colless C.A.772 as a differential variety. The race 15 was isolated from Aizu No.2 collected at Iwate prefecture, Tôhoku district. It was
characterized by its high virulence to a majority of the varieties being grown in the Tôhoku district which are highly or moderately resistant to other Japanese races of mildew. These varieties that were susceptible to race 15 and resistant to other local races were as follows:

Ackermann's Isaria, Aizu No. 2, Aizu No. 4, Aizu No. 5, Aizu No. 6, Akimaki-hokusai, Closer ls, Closer lb, Closer C a, 772, Date No. 2, Hordeum spontaneum 5060, Hosogara No. 1, Hosomugi No. 3, Hosomugi C, Kachidoki, Kenkichi No. 1, Kenkichi No. 3, Maruchin No. 1, Mensury No. 2, Mukade-mugi, Murasaki-hadaka, Nakaizumi-zairai, Nihonsan, Omugi No. 2, Russia No. 12, Russia No. 35, Sangatsu, Sanjaku-honaga C, and Wase-hosogara.

Geographical Distribution of Physiologic Races

Geographic distribution of the races isolated during the three years (1950 to 1952) are represented in Table 2 and Figure 1. The races 8, 13 and 15 could have been distinguished by the reactions of the varieties used in 1950, but in this year only race 13 was isolated. As shown in Table 2 and Figure 1, race 13 was very widespread present having been almost over the whole of Japan except Hokkaido, comprising 87% of the isolates made in three years.

Race 8B was first isolated in 1951 from specimens received from the prefectures of Niigata (Hokuriku) and Nagano (Kanto-Tozan). In 1952, it was isolated from samples from Yamagata (Tôhoku), Ishikawa (Hokuriku), Guma (Kanto-Tozan), Aichi (Tôkai-Kinki), Yamaguchi (Chûgoku), Ehime and Kagawa (Shikoku) prefectures. The Hokuriku district and its neighboring prefectures and the prefectures of Ehime, Kagawa, and Yamaguchi comprised principally of race 8B. While in other districts it occurred mixed with race 13 in low proportions.

Race 8C was isolated only once from a field of barley at Ohara Institute (Chûgoku) in 1952.
Race 14 was first isolated in 1951 from a specimen sent from Tōkyō (Kantō-Tōzan). In 1952, it was again isolated mostly from Kantō-Tōzan district. It always occurred as a mixture to race 13. This race was never found on samples collected from western parts of Japan.

So far, race 15 was isolated only from materials received from Iwate prefecture, Tōhoku district in 1952. But the specimen received from Iwate prefecture in 1950 was a mildewed Iwate-Mensury which is moderately resistant to mildews other than race 15. Although a culture from this material was not established, it is presumed that it was infected by this race 15. This race may be widespread in Tōhoku district because there the varieties grown are resistant to all other prevailing races.
Discussion

Just as were found by Honecker (10) on German and Cherewick (1) on Canadian barley mildew, the six differential varieties of barley used by Cherewick were not adequate for differentiating physiologic races of barley mildew prevalent in Japan. Since Black Hull-less C.I. 666, Chevron C.I. Illl, Nepal C.I. 595 and Peruvian C.I. 935 could not be used for distinguishing the races prevalent in Japan, Colsess C.A. 772, Duplex C.I. 2433, and Kairyō-bōzu-mugi were added to the six differential varieties, and by so doing two biotypes (races 8B, and 8C) and three new races (races 13, 14, and 15) were identified.

Concerning 8B and 8C, they apparently belong to race 8 from the reaction shown on six differential varieties. However their further reaction to Duplex C.I. 2433 which we have on hand shows that they readily infect this variety. Assuming that Duplex C.I. 2433 and Duplex C.A. 1129 are the same variety with different numbers, 8B and 8C are distinct from race 8. It is unfortunate that we have only Duplex C.I. 2433 in possession to make such comparisons.

Race 15 was characterized by high virulence to many of the varieties that are moderately or slightly resistant to other races. A majority of these varieties susceptible only to race 15 are important agricultural varieties owing to their resistance to the snow mold of the northern Japan. Race 15 is very threatening in northern regions, and needs to be differentiated more distinctly from other races. It resembles race 13 in that it reacts identically to the six differential hosts. Further distinction is only possible by adding new differentiating varieties. Such a variety should be selected from those varieties of known reactions to certain known races as studied by previous investigators. For this purpose, Dr. W. J. Cherewick kindly sent us some seeds of varieties used in his investigation on the relative resistance of barley varieties to the races of mildew in Canada (15), and we selected Colsess C.A. 772.

In Argentine two races of barley mildew have been described. Honecker described nine races of mildew for Germany, but owing to the fact that he used none of the differential hosts used by Mains and Dietz and other workers, his races cannot be compared precisely with those isolated in the United States and Canada.

It is questionable that different selections of same agronomic variety of barley react quite equally to all races of mildew. An attempt was made here as shown in Table 3 to compare the pathogenicity of races on varieties whose names are mutually understood in Germany, Argentine and Japan (2, 11, 19). As shown in Table 3, nine races of Germany are characterized by high virulence to Ackermann’s Isaria, while in Japan only race 15 infected severely upon this variety. Race 15 also differed from the nine
TABLE 3. Infection types produced on the leaves of seedlings of four barley varieties by the physiologic races of Erysiphe graminis hordei of Germany, Argentine and Japan.

<table>
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<th>Variety</th>
<th>Races</th>
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<th>Argentina</th>
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<td>A  H  J</td>
<td>D  B  G  E  C  F</td>
<td>Arg.1</td>
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<tr>
<td>Ackermann's Isaria</td>
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<td>i  i</td>
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races of Germany in the pathogenicity to Goldfoil C. I. 928 and Hanna C. I. 906. On this subject Hoffmann and Kuckuck (6) stated that the naked barley varieties of Japan, being susceptible to mildew in Japan, were moderately resistant to the disease in Germany. It can be presumed, therefore, that the races of Japan differ from those of Germany. Two races of Argentine are differentiated from the races of Japan by the reactions of Kwan C. I. 1016. From the above, it can be concluded that the Japanese races described in the present investigation are different from all other known races of mildew in foreign countries.

Nishikado et al. (16) pointed out that a majority of barley varieties resistant to a race of mildew prevalent in the prefecture of Okayama (Chū-goku) were grown in the northern parts of Japan, and very rarely in western parts of Japan except in Ehime prefecture where resistant variety Kairyō-bōzu-mugi and moderately resistant Ehime-hadaka and Heiwa-hadaka existed. From the present investigation it was found that in the northern parts of Japan there exists race 15 which is characterized by a high virulence to the varieties grown in that region. Similarly, in Ehime prefecture there is race 8B which infects severely on Kairyō-bōzu-mugi, Ehime-hadaka and Heiwa-hadaka. This fact is very interesting when we refer to the studies on Erysiphe graminis of barley and wheat in Canada by Newton and Cherewick (15). It is concluded that a principal race of barley mildew in a region may be varied by the barley varieties grown in that region. From this point of view alone, it is difficult to explain why, whereas races 13 and 8B are both highly infectious to certain varieties, only race 13 is so widespread and prevalent over the whole of Japan.

Summary

During these three years (1950 to 1952) two biotypes (races 8B, and 8C) and three new races (races 13, 14, and 15) were isolated in Japan. Three of those (races 8B, 8C, and 15) could not be identified without including Colsess C. A. 772, Duplex C. I. 2433 and Kairyō-bōzu-mugi as differential varieties.

Race 13 was widespread almost over the whole of Japan. Hokkaido
was excepted on account of difficulties encountered in securing living samples. Race 8B was prevalent in the district Hokuriku, and in the prefectures of Ehime, Kagawa, and Yamaguchi. Race 14 was gathered mostly from the district of Kantō-Tozan, and none from the western parts of Japan. Only one isolate each was made on race 8C from Okayama prefecture (Chūgoku) and race 15 from Iwate prefecture (Tohoku).

It was concluded that the variety of barley contributes important influence upon the race of mildew that will prevail in a locality.

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