Use of Mobile Nurseries in Pathogenicity Studies of Erysiphe graminis hordei on Hordeum spontaneum

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ABSTRACT

"Mobile nurseries" consisting of small trays with seedlings of barley representing a diversified spectrum of resistance were used to determine the pathogenic variability of *Erysiphe graminis* f. sp. *hordei* in Israel. The trays were placed for several days at 69 sites across the country in stands of *Hordeum spontaneum* where powdery mildew incidence accompanied by abundant formation of cleistothecia has been observed for several years in succession. Subsequently, the trays were transferred to the greenhouse where infection types were scored at the end of the incubation period.

Wide variations in pathogenicity were noted, not only among cultures obtained by countrywide sampling, but also among cultures derived from small stands of *H. spontaneum* heavily attacked by powdery mildew. "Mobile nurseries" also aided in detecting rare but dangerous strains of the pathogen virulent on important sources of resistance to powdery mildew, such as 'Engledow India', 'Monte Cristo', 'Rupee', and 'Spiti'.

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Israel is one of the centers of origin and diversification of the wild barley species *Hordeum spontaneum* C. Koch, the progenitor of cultivated barley (4). Populations of this species constitute important components of natural vegetation in most parts of the country. They comprise a wide range of ecotypes or "races" (4), distinct in morphologic and biologic traits. Plants robust in all features are common in northern Israel as a part of lush, herbaceous vegetation, while xeric types penetrate into the steppes of the Negev Desert in the south. Various "races" hybridize freely in nature and produce intergraded forms (4). Likewise, plants of *H. spontaneum* introgress occasionally with barley cultivars. In addition to *H. spontaneum*, *H. bulbosum* L. [of tetraploid type, 2n = 28 (5)] and *H. murinum* L. also thrive abundantly and are represented by ubiquitous populations greatly varying in morphology and growth habits.

The mentioned *Hordeum* species and *H. marinum* Huds. in many regions annually support outbreaks of the powdery mildew disease caused by *Erysiphe graminis* (DC.) Merát *hordei* Em. Marchal. Cultures of the fungus isolated from each of the above *Hordeum* spp. were able to parasitize plants of all other species in reciprocal inoculation tests (Eyal & Wahl, *unpublished*). The concept of correlated host-parasite evolution implies that genetic differentiation in the populations of the indigenous *Hordeum* species is matched by corresponding variability in populations of *E. graminis hordei* (2, 3, 9, 17). The mechanism of evolution of such strains was discussed by Moseman (9). The prevalence of fertile cleistothecia of this fungus throughout Israel, liberating functional ascospores concomitantly with the development of congenial hosts in nature, greatly contributes to the genetic diversification of *E. graminis hordei* (6, 13). Results of preliminary studies (6, 9) revealed a wide range of pathogenic variability in this fungus, and the occurrence of rare and highly virulent strains.

The purpose of this study was to obtain a more accurate estimation of pathogenic variability in *E. graminis hordei* populations sampled in *H. spontaneum* on a countrywide basis and within small stands.

MATERIALS AND METHODS.—"Mobile nurseries" for assessing the pathogenic specialization and virulence of *E. graminis hordei* were used in the following manner. Seeds of nearly isogenic lines of barley with specific genes for resistance incorporated in the variety 'Manchuria' as the recurrent parent (8, 10) and of accessions with known resistance to the pathogen (11, 12, 14, 16, 17) were planted in garden soil in plastic trays. Each tray, 17-cm square and 10-cm deep, contained five to seven seedlings of each of 24 accessions, the majority of which were included in Moseman's International Evolution for Pathogenicity Nursery (10); these were 'Algerian'/4*'Manchuria (F-14) (Ml-a), CI 16137; Algerian (Ml-a and Ml-at), CI 1179; 'Goldfoil'/4*Manchuria (F-14) (Ml-g), CI 16139; Goldfoil (Ml-g), CI 928; 'Hanna'/4*Manchuria (F-12) (Ml-h), CI 16141; Hanna (Ml-h), CI 906; 'Kwan'/4*Manchuria (F-12) (Ml-k), CI 16143; Kwan (Ml-k), CI 1016; 'Psaknon'/4*Manchuria (F-14) (Ml-p) CI 16145; Psaknon (Ml-p), CI 6305; 'Multan'/4*Manchuria (F-15) (Ml-a?.A_1) CI 16147; Multan, CI 3401; 'Durani'/4*Manchuria (F-13) (Ml-a^{10}), CI 16149; 'Franger'/4*Manchuria (F-15) (Ml-a^6), CI 16151; 'Long Glumes'/4*Manchuria (F-15) (Ml-a?), CI 16153; Long Glumes (Ml-a?), CI 6168; 'Rupee', CI 4355; Rupee/4*Manchuria (F-13) CI 16155; Manchuria CI 2330; 'Monte Cristo' (Ml-a^9) CI 1017; 'Spiti' (Ml-ms), CI 4343-1; and 'Mianwali' (Ml-mw) CI 3400.

When seedlings were 8- to 10-cm tall, the trays were placed, one tray per site, at 69 sites situated in 34 locations over 11 geographic regions throughout the country. In 1970-71, detailed readings were made on seedlings exposed at 48 sites distributed over 25 locations (Table 1). Distances between sites within a location averaged 5-10 meters. At each site, the tray was placed in a stand of *H. spontaneum* severely infected with *E. graminis hordei*. At most sites,

TABLE 1. Infection types[1] produced by *Erysiphe graminis hordei* on seedlings of specified barley cultivars and isolines naturally infected when maintained at the indicated sites in 1970-71

Region[2]	Location	Site No.	Hosts[3]													
			a^1	a	b^1	b	c^1	c	d^1	d	e^1	e	f	g	h	i
A. Upper Galilee	I.	1.	1	1	1	0(3)[4]	3(1)	4	3(1)	3(1)	4(1)	3(1)	1	3(1)	3(1)	1
		2.	2	1	3	0	3(1)	3	3(1)	4(1)	3(1)	3(0)	1(3)	1(3)	3	0
	II.	1.	1	1(3)	3(1)	3(1)	3(1)	4(0)	0(3)	4	1(3)	3(1)	2	3(1)	4(1)	1
		2.	3	3(1)	3(1)	2	3(0)	3	3(1)	3(1)	4	1	1(3)	3(1)	3	0
	III.	1.	1(3)	3	0(3)	0(3)	1(3)	4	1(3)	1(3)	1(3)	1	1(3)	1(3)	3	0
		2.	1(3)	1(3)	1(3)	3(1)	1	3	1(3)	3(1)	1(3)	1(3)	3	1	1	0
	IV.	1.	0	1	0	0(3)	0	3	1(3)	1	0	0	0(3)	0	0	0
		2.	0	2	0(3)	0	0	3	1(3)	1(3)	3(0)	1	0	2	3	0
B. Western Galilee	I.	1.	2	1(3)	3(1)	3	3	3	3(1)	1(3)	3(1)	2	1	1	3	0
		2.	3(1)	3(0)	3(1)	0(3)	2	3(1)	3(1)	0	3(1)	3(0)	0	1	2	1
	II.	1.	3(1)	3(1)	3	1	2	4	3(0)	3(1)	4(1)	2	2	3(1)	3	2
C. Lower Galilee	I.	1.	2	3(1)	3(1)	2	3(1)	3(1)	1	1	2	3(1)	1	2	3(1)	0
		2.	1	3(1)	2	3(0)	3(1)	3(0)	3	0	2	3(0)	2	3(1)	3	3(1)
D. Jordan Valley	I.	1.	1(3)	1(3)	2	1	3(0)	4	(3(1)	3(1)	3(1)	3(1)	1	2	3	3(1)
		2.	1(3)	1(3)	2	3(0)	3(1)	3	3(1)	0(3)	2	3(1)	1	1	3	-
	II.	1.	1	1(3)	3(1)	0(3)	1(3)	4	0(3)	1(3)	1(3)	1	1	0(3)	3	1
		2.	1(3)	0	3	1	0	0(3)	0(3)	0	0	1	0	0	0	0
E. Bet Shean Valley	I.	1.	2	3(1)	1(3)	3	2	3	3(1)	3	2	3(1)	1(3)	3(1)	3(1)	0
	II.	1.	0	1	2	0	1	3	1	3(0)	1	0	1	3(1)	3(0)	2
F. Valley of Esdraelon	I.	1.	0	3(0)	3(1)	0(3)	0(3)	0	0(3)	3(0)	0(3)	1	0	1	3	1
		2.	0	1	2	0	1(3)	0(3)	0	0(3)	0(3)	0	1	1	0(3)	2
	II.	1.	3(1)	1(3)	3(1)	3(1)	3(1)	4	4(1)	3(1)	3	3(1)	3(1)	3(1)	3(1)	0
		2.	3	3	3	3(1)	4	4	4	4(1)	3	3(1)	4(1)	4	4(1)	0
		3.	3(1)	3(1)	3(1)	4	3	4	3(1)	0	0	0	3(1)	4	4(1)	3
G. Mt. Carmel	I.	1.	2	2	3(1)	2	3(1)	3	1	3(1)	3(1)	3(0)	2	3(0)	3	0
		2.	2	2	2	1(3)	4	1	3	4(1)	3(1)	3(1)	4	3	3	0
H. Central Coastal Plain	I.	1.	2	3(1)	3(1)	3(0)	3(1)	4	3(1)	2	3(1)	4(1)	2	3(1)	3	2
	II.	1.	3(1)	3(1)	2	3(0)	3(1)	4	1(3)	3(1)	2	3(1)	2	3	3(1)	0
		2.	3(1)	3(0)	3(0)	1(3)	3(0)	3	3(0)	3(0)	2	2	0	3(0)	3(0)	3(1)
	III.	1.	3(1)	2	2	0	3(0)	4	3(1)	3(0)	3	2	3(0)	3(0)	3(0)	3(1)
		2.	2	2	0	2	3(0)	3	2	3(0)	1	3(1)	3(0)	2	3(0)	3(1)
I. Southern Coastal Plain	I.	1.	3(1)	3	3	0	3(1)	4	3(1)	3	2	4(1)	3(1)	3(0)	3(1)	3(0)
		2.	2	2	0	0	4(1)	4	2	3	2	2	2	3(0)	3(0)	0
	II.	1.	0	0	2	0(3)	3(0)	3	3	0	3	0	0	2	0	1
	III.	1.	1	0	3(0)	2	3(0)	4	2	3(0)	2	3(0)	1	3(1)	3(0)	3
		2.	2	3	3(0)	3(1)	3(0)	3	2	3	1(3)	3(1)	2	2	2	3(1)
J. Judean Foothills	I.	1.	3(1)	1	4(1)	3(0)	4	4	3(1)	4	3	4(1)	3(1)	2	3(1)	0
		2.	3(1)	3	0	3(0)	3	-	3(1)	1(3)	1(3)	0	3(1)	3(1)	4(1)	2
		3.	3(1)	3(0)	0	4	3	4	3(1)	3(1)	1(3)	3(1)	4	3(0)	3	0
		4.	3(1)	3(0)	3(0)	4(0)	2	4	3(0)	0	3(1)	1(3)	2	3(1)	3(1)	0
	II.	1.	1(3)	3(1)	0(3)	0	0	4	0(3)	4(1)	0(3)	3	0(3)	3	3(1)	0
		2.	0	2	2	0(3)	3	4	2	2	3(0)	1	0	3(0)	3(1)	0
	III.	1.	1	2	3(1)	3(0)	4(1)	4	1(3)	3(1)	1(3)	3	2	2	3	3(1)
		2.	0	2	3(1)	0(3)	3	4	3(1)	4(1)	1	3(1)	0(3)	1(3)	1	3(1)
	IV.	1.	1	3(1)	3(1)	4(0)	3(0)	4	1(3)	3(1)	3(1)	3(1)	1	2	3(1)	4(0)
		2.	3	4	4(1)	4	4	4	3(1)	4(1)	3(1)	4	4	3(1)	3(1)	3(1)
K. Northern Negev Lowland	I,	1.	2	1(3)	3(0)	3	3	4	3	3	3	3(1)	2	3	3(1)	0
		2.	3	3	1(3)	3	3(1)	3	1(3)	1(3)	1	1(3)	2	1	1(3)	0

[1] Infection types as described by Moseman (7)

[2] See Fig. 1

[3] a^1 - Algerian/4 * Manchuria (F-14)(Ml-a) CI 16137
b^1 - Goldfoil/4 * Manchuria (F-14)(Ml-g) CI 16139
c^1 - Hanna/4 * Manchuria (F-12)(Ml-h) CI 16141
d^1 - Kwan/4 * Manchuria (F-12(Ml-k) CI 16143
e^1 - Psaknon/4 * Manchuria (F-14)(Ml-p) CI 16145
a - Algerian (Ml-a + Ml-at) CI 1179
b - Goldfoil (Ml-g) CI 928
c - Hanna (Ml-h) CI 906
d - Kwan (Ml-k) CI 1016
e - Psaknon (Ml-p) CI 6305
f - Durani/4 * Manchuria (F-13)(Ml-a^{10}) CI 16149
g - Franger/4 * Manchuria (F-15)(Ml-a^6) CI 16151
h - Manchuria CI 2330
i - Monte Cristo (Ml-a^9) CI 1017

[4] Numbers in parentheses indicate a second infection type, when present.

copious development of cleistothecia had been recorded on *H. spontaneum* in previous years. In studies of 1970-72, trays were maintained at each site for 48-72 hrs, while in tests of 1972-73 the duration of exposure was reduced to 30 min. Then the trays were returned to the greenhouse and kept at 20 ± 2 C. Each tray was maintained in a separate plastic chamber covered with five to six layers of cheesecloth. Readings of the infection types were made at least twice, usually after 7 and 10 days of incubation in the greenhouse. The designations of infection types used were those of Moseman (7). Infection types ranging from 0 to 2 signify a resistant reaction class, whereas infection types 3 and 4 denote susceptibility. Resistant and susceptible reactions were frequently observed on the same leaf.

Tests of 1970-71 were partially repeated in 1971-72 and supplemented by trials with the following barley accessions: 'Engledow India', CI 7555; 'Hispont', CI 8828; 'Lyallpur BS', CI 3395; 'Lyallpur 3647', CI 12115; 'Nigrate', CI 2444; and 'Union', CI 11807. Trays with seedlings of these entries and others were exposed for 48-72 hr in February and again in March 1972, in region F, location II, site 1; region H, location III, sites 1 and 2; and in region J, location III, site 1 (Fig. 1, Table 1). Previous years' tests revealed pronounced pathogenic variability of *E. graminis hordei* at these sites.

RESULTS.—Infection types formed by *E. graminis hordei* displayed distinct uniformity on some accessions and a wide range of variability on others (Table 1). Infection type 0 was observed at all sites on seedlings of Mianwali, CI 3400; Multan, CI 3401; Multan/4*Manchuria (F-15), CI 16147; Long Glumes, CI 6168; and Long Glumes/4*Manchuria (F-15), CI 16153; Rupee, CI 4355; Rupee/4*Manchuria (F-13), CI 16155; and Spiti, CI 4343-1 were similarly resistant except at several sites of regions F, I, and J where infection resulted in susceptible reactions on these plants.

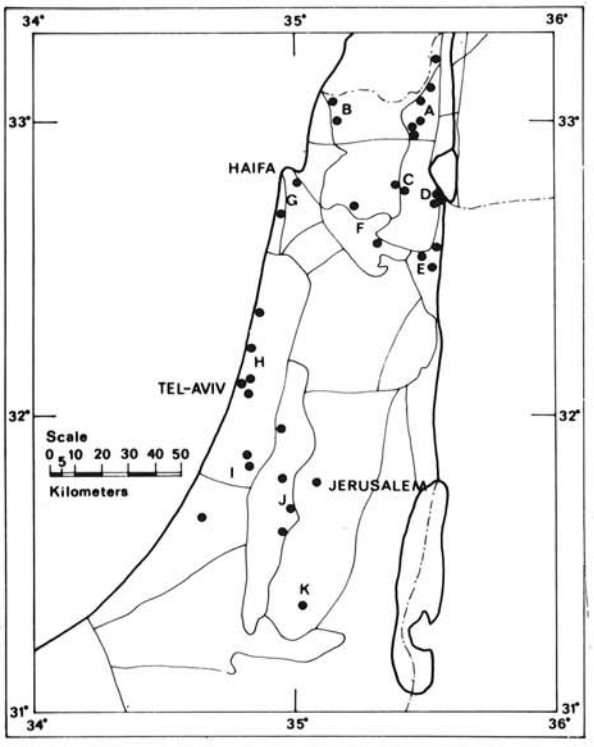

Fig. 1. Regions and locations where pathogenicity of *Erysiphe graminis hordei* was sampled. Designation of regions explained in Table 1.

Patterns of pathogenic variability.—The over-all pathogenic variability of *E. graminis hordei* was determined by comparing infection types shown in Table 1. The reactions recorded in vertical columns in Table 1 vary from resistance to susceptibility, and embrace a wide range of infection types. The magnitude of pathogenic variation of fungus cultures derived from neighboring sites was sometimes as great as between cultures originated in widely separated regions. We shall limit our discussion to variations in pathogenicity as reflected by the reactions of seedlings in a single tray. Deviations of disease reactions on the isoline from those of their respective donors fall into the following four classes:

(i) Donor variety susceptible, isoline and Manchuria resistant, as in accessions c and c^1 infected at sites A.III.2. and A.IV.1; and accessions e and e^1 at site J.III.2.

(ii) Donor variety and Manchuria susceptible, isoline resistant, as in accessions c and c^1 infected at site A.IV.2; and accessions b and b^1 infected at site J.I.3.

(iii) Donor variety resistant, isoline and Manchuria susceptible, as in accessions b and b^1 at sites A.I.2, B.II.1; accessions d and d^1 infected at site C.I.2; and accessions c and c^1 infected at site G.I.2.

(iv) Donor variety and Manchuria resistant, isoline susceptible, as in accessions b and b^1 at site D.II.2; accessions d and d^1 and e and e^1 at site I.II.1.

Studies were undertaken to explain the differences in the reaction of the donors and their isolines to *E. graminis hordei*. Powdery mildew cultures isolated from the donor in group (i) proved to be virulent in artificial inoculation tests on seedlings of the donor and avirulent on seedlings of the isoline and Manchuria. Evidently, resistance of the isoline was imparted by the recurrent parent. The reasons for deviations of reactions of the donors and the corresponding isolines in groups (ii), (iii), and (iv) are not known and are being further investigated.

The fact that seedlings within a small tray were attacked by more than one strain of *E. graminis hordei* attests to the pathogenic heterogeneity of powdery mildew populations which probably had originated within the small stands of *H. spontaneum*.

Virulence of E. graminis hordei.—Very virulent strains of the pathogen were identified on the "mobile nurseries". In tests of 1970-71 were detected strains of *E. graminis* virulent on Monte Cristo, Psaknon, Rupee, and Spiti which are considered to be highly resistant to powdery mildew in a number of

countries (3, 12). In tests of 1971-72, seedlings of Engledow India, Hispont, Union, Lyallpur 3647, Lyallpur BC, and Nigrate displayed susceptible reactions when naturally infected in the Central Coastal Plain, in the Valley of Esdraelon, and the Judean Foothills. Susceptible reactions on the seedlings of the latter two varieties in Israel have been recorded in previous trials (6). Susceptibility of Engledow India to this pathogen has not heretofore been reported.

DISCUSSION.—Vavilov (15) postulated that regions of maximum parasitic diversity in fungi or insects coincide with the regions of origin of the respective plant host species or their wild relatives. The findings of Bodenheimer's entomological studies in Israel were used by Vavilov to substantiate his theory. The parasitic specialization of *E. graminis hordei* in this country accords with Vavilov's hypothesis. Evolution of this specialization has presumably been influenced by the prevalence of functional cleistothecia on the ubiquitous *H. spontaneum*. Populations of this wild barley represent a heterogenic patchwork of genotypes that offer many selective ecological niches (1) favoring the survival of diverse fungus strains of ascospore origin.

The "mobile nurseries" used in this study have considerable merit in elucidating the variability and potential virulence of *E. graminis hordei*, and may be helpful in similar studies with other plant pathogens. Test seedlings assembled in a small tray are easily manageable and appropriate for sampling pathogen populations at chosen sites at suitable time intervals. The composition of the test seedling sets can be changed during the growing season and adjusted to the new needs which may emerge as research progresses. These nurseries make possible assaying populations of powdery mildew in wild barley stands in very arid regions, on rocky hills, and in woods. Cultivation of conventional nurseries in such locations is virtually impossible; nevertheless, wild barley thrives at such sites and harbors *E. graminis hordei*.

By periodic and countrywide distribution of "mobile nurseries" on a finer grid, rare but dangerous strains of the pathogen can be detected. For example, with the aid of these nurseries we discovered strains virulent on Engledow India, Rupee, and Spiti. Such strains, though rare at present, may become prominent if barley cultivars carrying genes of resistance derived from the mentioned accessions, are grown on a large scale (11). The pathways of evolution of the highly virulent strains were discussed by Moseman (9). It is noteworthy that some cultures trapped on the "mobile nurseries" proved to be avirulent on Manchuria (Table 1), thus invalidating the concept of a universally susceptible variety. Favret (3) arrived at similar conclusions.

Consistent resistance of Long Glumes in the present trials contrasts sharply with its susceptibility in the past (6) and indicates shifts in the pouplations of the pathogenic strains of the fungus. On the other hand, permanence of resistance of Mianwali and Multan over many years (6) proves their stability and makes incorporation of such resistance into commercial barleys desirable, despite the fact that strains of *E. graminis hordei* capable of attacking these two varieties are known to exist (16). According to Favret (3), Psaknon and Mutant C.20 possess nonspecific resistance to powdery mildew, because "these varieties have been tested in many different locations against many races and their resistance persists". Our results do not confirm this statement in respect to Psaknon, since infection types on this host varied from 0 to 4.

LITERATURE CITED

1. BAWDEN, F. C. 1957. The role of plant hosts in microbial ecology. p. 299-314. *In* R. E. O. Williams & C. C. Spicer [ed.]. Microbial Ecology. Seventh Sympos. Soc. General Microbiology, Cambridge Univ. Press.
2. ESHED, NAAVA, & I. WAHL. 1970. Host ranges and interrelations of Erysiphe graminis hordei, E. graminis tritici, and E. graminis avenae. Phytopathology 60:628-634.
3. FAVRET, E. A. 1971. The host-pathogen system and its genetic relationships. p. 457-471. *In* Barley Genetics II, Proc. 2nd Int. Barley Genetics Sympos., Pullman, Washington, 1970.
4. HARLAN, J. R., & D. ZOHARY. 1966. Distribution of wild wheats and barley. Science 153:1074-1080.
5. KATZNELSON, J., & D. ZOHARY. 1967. Diploid and tetraploid Hordeum bulbosum L. Israel J. Botany 16:57-62.
6. KOLTIN, Y., R. KENNETH, & I. WAHL. 1964. Powdery mildew disease of barley in Israel and physiologic specialization of the pathogen. p. 228-235. *In* Barley Genetics I, Proc. 1st Int. Barley Genetics Sympos., Wageningen 1963.
7. MOSEMAN, J. G. 1968. Reactions of barley to Erysiphe graminis f. sp. hordei from North America, England, Ireland, and Japan. Plant Dis. Reptr. 52:463-467.
8. MOSEMAN, J. G. 1971. Genes for specific resistance: powdery mildew of barley. Phytopathology 61:617-620.
9. MOSEMAN, J. G. 1971. Co-evolution of host resistance and pathogen virulence. p. 450-456. *In* Barley Genetics II. Proc. 2nd Int. Barley Genetics Sympos., Pullman, Washington, 1970.
10. MOSEMAN, J. G. 1972. Isogenic barley lines for reaction to Erysiphe graminis f. sp. hordei. Crop Sci. 12:681-682.
11. MOSEMAN, J. G., R. C. F. MACER, & L. W. GREELEY. 1965. Genetic studies with cultures of Erysiphe graminis f. sp. hordei virulent on Hordeum spontaneum. Trans. Brit. Mycol. Soc. 48:479-489.
12. NOVER, I., & CHR. O. LEHMANN. 1972. Resistenzeigenschaften im Gersten- und Weizensortiment Gatersleben. 14. Prüfung von Sommergersten auf ihr Verhalten gegen Mehltau (Erysiphe graminis DC. f. sp. hordei Marchal). Kulturpflanze 19:283-298.
13. SILBERSTEIN, ORA. 1972. Factors controlling formation and liberation of ascospores in Erysiphe graminis DC. f. sp. hordei Marchal. M.S. Thesis, Tel Aviv University (In Hebrew, with an English summary). 53 p.
14. SLOOTMAKER, L. A. J. 1970. The isolation of a further new race of Erysiphe graminis DC. f. sp. hordei Marchal and the genetical basis of the resistance of "Lyallpur 3645". Neth. J. Plant Pathol. 76:64-69.

15. VAVILOV, N. I. 1939. Selektsiýa ustoichivykh sortov kak osnovnoi metod bor'by a rzhavchinoi (Selection of resistant varieties as the basic method of rust control). p. 3-20. *In* Rzhavchina Zernovykh Kultur. Sel'khozgiz. Moscow (Russian).

16. WIBERG, A. 1970. Physiologic races of barley powdery mildew, Erysiphe graminis DC. f. sp. hordei Marchal in the Scandinavian countries. Phytopathol. Z. 69:344-365.

17. WOLFE, M. S. 1972. The genetics of barley mildew. Rev. Plant Pathol. 51:507-522.