ECOLOGICAL IMPACT ON THE VIRULENCE OF PHYTOPATHOGEN'S POPULATIONS.

A.V. Bogdanovich

Laboratory of Immunity of Agricultural Plants to Diseases,
Institute of Plant Protection,
33 Vasylkivska str, Kyiv, Ukraine, 03022
E-mail: a_bogdanovich@ukr.net

Abstract.
Since 1998 we are carrying out examination of virulence and race structure of populations of powdery mildew of wheat (*Erysiphe graminis f. sp. tritici*) from five regions of Ukraine: Kyiv district, Kherson district, Ternopol district, Zhitomir district and Ivano-Frankovsk district. These regions differ by levels of chemical pollution. The most polluted sown areas are nearby Kalush chemical plant (Ivano-Frankovsk district). It was found that in all investigated regions the most prevalent races had the virulence to resistance wheat genes *Pm5* and *Pm8*. At the same time, it was determined that fungus populations collected in the vicinity of Kalush was characterized by the most races diversity and had the greatest virulence as compared to populations from other regions. Our data suggest impact of chemical pollution of sown areas on the virulence and race structure of powdery mildew populations.

Introduction.
Powdery mildew, caused by *Erysiphe graminis f. sp. tritici* is an important disease of wheat throught wheat-growing areas of the world [1, 2] and significant yield losses have been reported in various countries [3, 4]. Cultivar resistance is considered the most practical, effective and economical means of managing powdery mildew [4, 5]. However, the deployment of single-gene resistance may be short-lived if variability of the pathogen is great.

Resistance genes in the host play an important role in determining powdery mildew virulence gene frequencies [6]. Seledction pressure applied by commonly grown cultivars carryng major resistance genes causes virulence shifts in the *Erysiphe graminis f. sp. tritici* population [7].

Any powdery mildew management program that includes the use of host resistance will require information on the virulence genes that exist, and the frequency at which they exist, in the pathogen population [3]. Surveys of *Erysiphe graminis f. sp. tritici* virulence frequencies are necessary to identify which resistance genes are ineffective as well as effective aids for resistance breeding [7].

The objective of this study was to characterize the powdery mildew population using conidia and to examine difference between populations from different regions of Ukraine.

Methods.
Collection of mass isolates. Isolates of *Erysiphe graminis f. sp. tritici* were collected from commercian cultivars of winter wheat in 5 districts of Ukraine (Kyiv district, Kherson district, Ternopol district, Zhitomir district and Ivano-Frankovsk district). A 5-cm leaf sections of the 8-day-old seedlings of the susceptible cultivar Erythrospermum 1516 were placed in petri plates on sterile filter paper moistened by 0,004% benzimidazole solution. Mass isolates were made by transferring conidia from each field isolate into a leaf sections of Erythrospermum 1516. Petri plates with diseased leaf sections were placed in a 18-20^{o}C growth chamber with 12h of fluorescent light. Mildew colonies sporulated profusely within 7 to 8 days. These conditions were used for all the following experiments [8].

Purification of single-colony isolates and inoculum production. Single-colony isolates were made from each mass isolate by transferring a few conidia from a single pustule to a noninfecting leaf sections in a petri plates using a sterile dissecting needle. This process was repeated three times in order to reduce the chance of isolate mixtures. Increase of inoculum were made by transferring conidia from single-colony isolates each 7-8 days in noninfecting leaf of Erythrospermum 1516. At 13-15 5-cm leaf sections with mildew colonies counted sufficient for identification of virulence genes [9].

Identification of virulence genes. At five 2-cm leaf sections of the eight-day-old seedlings of eight differential cultivars and Carsten (Tabl. 1) were placed in 15x20 cm plastic box on sterile filter paper moistened by benzimidazole solution. Differentials were inoculated with conidia from a single isolate using a sterile dissecting needle. The plastic box were covered by 15x20 cm glases and placed in a growth chamber. Eight days after inoculation, leafs were rated for their reactions to each isolate on an individual plant basis [9]. A 0 to 4 rating scale was used. A score of 0 to 2 indicated a resistant reaction of the host to the particular isolate, while a score 3 to 4 indicated a susceptible reaction [6]. A isolate that gave a rating of 3 to 4 on a particular differential line or cultivar was assesed as having the virulence gene that gave a compatible reaction with a corresponding resistance gene in the host. Frequency of virulence genes was calculated as percentage of isolates with corresponding gene from general number of isolates [3].

Table 1. *Pm* gene symbols for differential cultivars used in the assessment of *Erysiphe graminis f. sp. tritici* virulence.

Cultivar	*Pm* genes
Carsten	…*
Axminster	*Pm*1
Ulka	*Pm*2
Halle stamm 13471	*Pm*2+mld
Asosan	*Pm*3a
Chul	*Pm*3b
Weihenstephan	*Pm*4b
Hope	*Pm*5
Salzmunde stamm 14/44	*Pm*8

* No known gene for powdery mildew resistance.

Results.

A total of 175 isolates of *Erysiphe graminis f. sp. tritici* were recovered and analized. Isolates were collected from five different districts of Ukraine. Forty races of pathogen were detected, i. e forty different genotypes were represent the population of *Erysiphe graminis f. sp. tritici* (Tabl. 2).

Table 2. Races of *Erysiphe graminis f. sp. tritici* collected in different regions of Ukraine*.

Races**	Frequency (%)	District***	**Races****	Frequency (%)	District***
without v. genes	1.14	1, 4	1, 2, 5	0.57	4
8	19.43	1-5	1, 3b, 8	0.57	4
5	4.00	1, 2, 5	2, 2+mld, 8	0.57	3
1	1.17	4	2, 4b, 5	0.57	1
2	0.57	4	2+mld, 3a, 8	0.57	2
5, 8	28.00	1-5	1, 2, 5, 8	1.14	3, 5
1, 8	13.00	1-5	1, 2, 3b, 8	0.57	5
3b, 8	1.71	1, 3, 4	1, 4b, 5, 8	0.57	1
1, 5	1.14	1	2, 3b, 4b, 8	0.57	1
1, 4b	0.57	5	2+mld, 3b, 5, 8	0.57	2
2, 2+mld	0.57	1	3a, 3b, 4b, 8	0.57	3
2+mld, 8	0.57	1	3a, 3b, 5, 8	0.57	1
4b, 8	0.57	4	2, 2+mld, 3b, 5, 8	1.14	4
4b, 5, 8	6.29	1-3, 5	1, 3a, 4b, 5, 8	0.57	5
1, 5, 8	4.00	1, 2, 4	1, 3b, 4b, 5, 8	0.57	1
2, 5, 8	3.43	1-4	3a, 3b, 4b, 5, 8	0.57	5
2+mld, 5, 8	2.86	2-5	1, 2, 2+mld, 3b, 5, 8	0.57	4
3b, 5, 8	1.71	1, 3, 5	1, 2+mld, 3a, 4b, 5, 8	0.57	5
1, 4b, 8	1.14	1	2, 2+mld, 3a, 3b, 5, 8	0.57	3
2, 3b, 8	1.14	1	1, 2, 2+mld, 3a, 3b, 4b, 5, 8	0.57	4

* - sample size = 175;

** - races described in terms of ineffective host *Pm* genes in which isolates were tested against *Pm*1, 2, 2+mld, 3a, 3b, 4b, 5 and 8;

*** - districts: 1 - Kherson, 2 - Kyiv, 3 - Ternopol, 4 - Ivano-Frankovsk, 5 – Zhitomir.

Races differed in their frequency of occurrence, however virulence to *Pm5* and *Pm8* was most prevalent. A high percentage of isolates were virulent on differentials carryng *Pm5* and *Pm8* (60,57% and 89, 71% respectively). Virulence to *Pm1*, *Pm2* and *Pm4b* was found in 21,71%, 12,57% and 13, 71% of cases respectively. Virulence to three other resistance genes was more rare: to *Pm3b* – 12%, *Pm2+mld* – 9, 14% and *Pm3a* – 4, 57%.Most of the isolates had one, two or three genes for virulence (25, 14%, 40,57% and 23,43% respectively). Four or more genes for virulence had 9,71% of all the isolates. Two of all the isolates had not virulence genes.

Comparison of virulence of the isolates from different districts revealed following characteristic features:
1) fungus population collected in the Ivano-Frankovsk district was characterized by the most races diversity (Tabl. 3);

Table 3. Races diversity in the *Erysiphe graminis f. sp. tritici* populations from different regions of Ukraine.

District	Number of races (n)	Number of isolates (N)	n/N
Kherson	21	66	0.3182
Kyiv	10	31	0.3226
Ternopol	14	27	0.5185
Ivano-Frankovsk	18	29	0.6207
Zhitomir	13	22	0.5909

2)Nei's standart genetic distance is more considerable for the population from Ivano-Frankovsk region (Fig. 1);

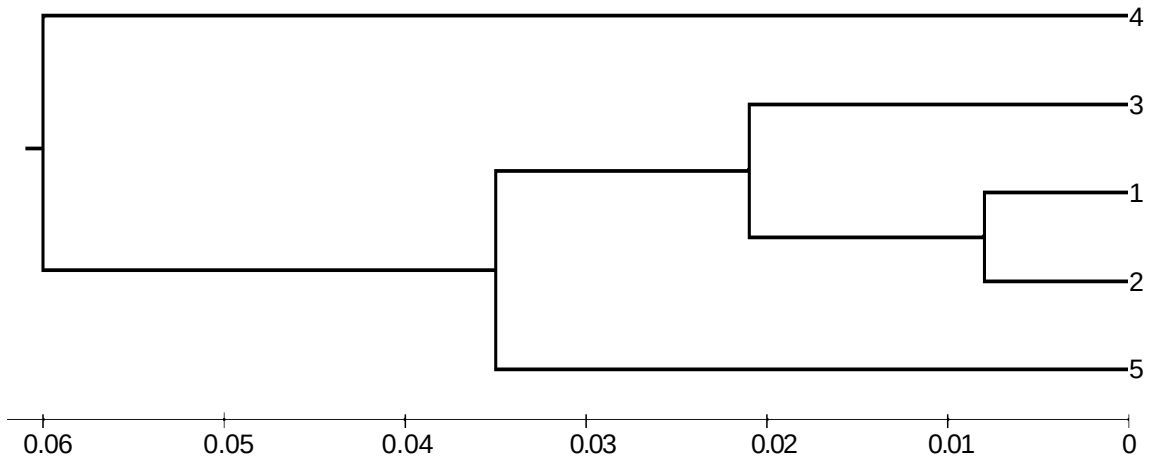

Fig. 1. Phenogram of similarities between regions based upon virulence frequencies in isolates of *Erysiphe graminis f. sp. tritici* (regions: 1 - Kherson, 2 - Kyiv, 3 - Ternopol, 4 - Ivano-Frankovsk, 5 - Zhitomir)

3) high virulence of isolates was one of properties of the population from Ivano-Frankovsk district (Fig. 2). Only the population from Zhitomir district had higher virulence;
4) amongst twenty three genotypes, that were detected only once, 7 were received from Kherson district, 6 – from Ivano-Frankovsk district, 5 – from Zhitomir district 3 – from Ternopol district and 2 – from Kyiv district. Therefore, population from Ivano-Frankovsk district was distinguished by high frequence of rare genotypes.

Discussion.
Resistance genes in the host play a most important role in determining of pathogen virulence. However, us were established, that cultivars from different regions have simillar resistance genes [10]. Suggest, that other factors are causes of differences between populations.
It is known, that sexual stage and migrations are causes of great variability of the pathogen. It is possible, that migrations of the pathogen from European countries is a cause of differences of Ivano-

Frankovsk population. At the same time, according to literature datas, great variability of *Erysiphe graminis f. sp. tritici* was found on radio-active polluted territory [11]. These regions differ by levels of chemical pollution. The most polluted sown areas are nearby Kalush chemical plant (Ivano-Frankovsk district).

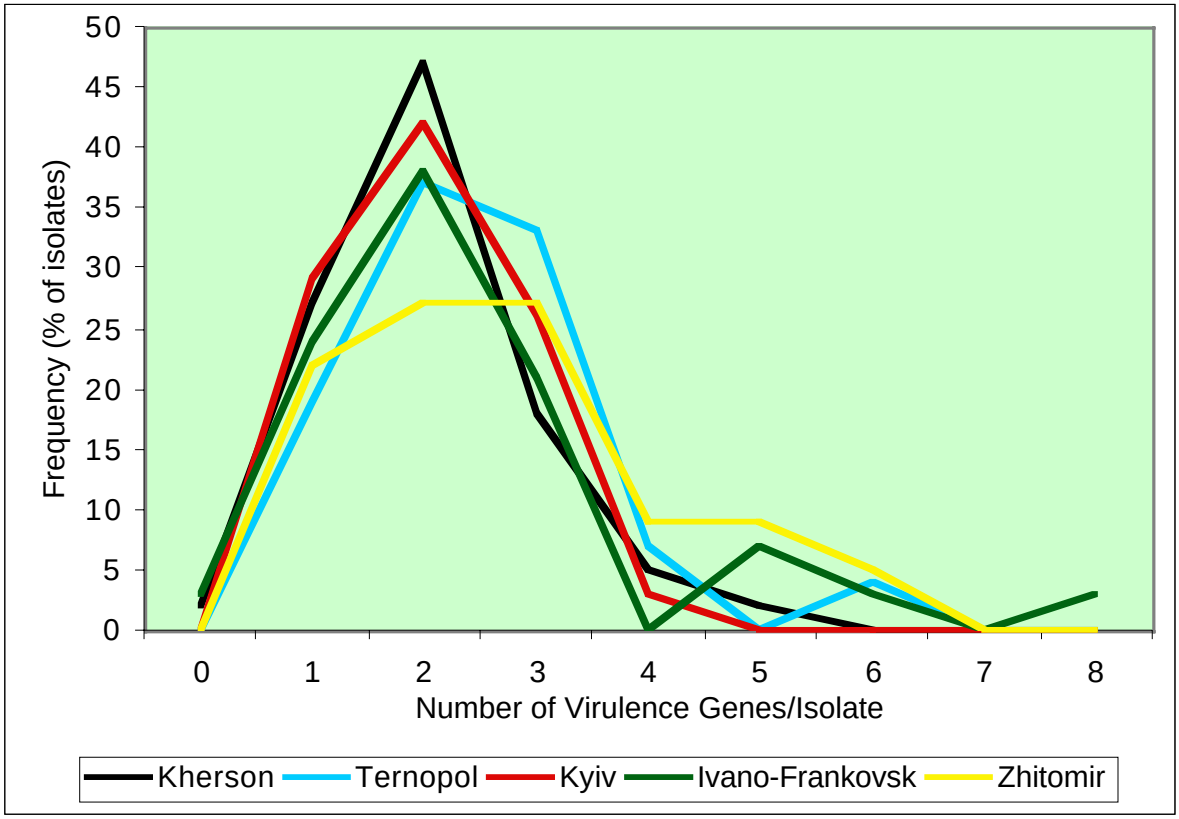

Fig. 2. Number of virulence genes in isolates of *Erysiphe graminis f. sp. tritici* in the different regions of Ukraine.

Conclusions.

A number of factors may be a cause of differences in virulence between populations of *Erysiphe graminis f. sp. tritici*.It is considered It is needed more detiled study of influence of environmental impact on phytopathogenic populations.

References.

1. M. HEUN, B. FRIEBE Introgression of powdery mildew resistance from rye into wheat, Phytopathology, **80**, 242-245 (1990)
2. P.E. LIPPS, L.V. MADDEN Effect of fungicide application timing on control of powdery mildew and grain yield of winter wheat, Plant Disease, **73**, 991-994 (1989)
3. R.R. PERSAUD, P.E. LIPPS Virulence genes and virulence gene frequencies of *Blumeria graminis f. sp. tritici* in Ohio, Plant Disease, **79**, 494-499 (1995)
4. L.T. BABAYANTS, L.K. SIMONENKO, V.K. SIMONENKO Introgressive resistance of wheat to powdery mildew pathogen derived from Agrotriticum and its genetic base, Tsytologia i genetika (Cytology and Genetics), **33** (2), 49-54 (1999)
5. L.O. NAMUCO, W.R. COFFMAN, G.C. BERGSTROM, M.E. SORRELLS Virulence spectrum of the *Erysiphe graminis f. sp. tritici* population in New York, Plant Disease, **71**, 539-541 (1987)
6. S. LEATH, M. HEUN Identification of powdery mildew resistance genes in cultivars of soft winter wheat, Plant Disease, **74**, 747-752 (1990)
7. A.S. NEIWOHNER, S. LEATH Virulence of *Blumeria graminis f. sp. tritici* on winter wheat in the eastern United States, Plant Disease, **82**, 64-68 (1998)
8. V.V. VOLKODAV **Method of state test of cultivars of agricultural plants**, Kyiv, 100 pp., (2000) [in Ukrainian]
9. V.I. KRIVCHENKO, E.H. SUHANBERDINA, V.A. Vershinina, T.V. LEBEDEVA **Studies of cereals resistance to powdery mildew**, Leningrad, 80 pp., (1980) [in Russian]

10. M.P. LISOVIY, A.V. BOGDANOVICH Genetic identity of virulence of populations of *Erysiphe graminis f. sp. tritici* from different soil-climatic zones of Ukraine, Visnyk agrarnoi nauky, **11**, 46-48 (2001) [in Ukrainian].
11. M.G. GARNAGA, O.S. SATSYUK Formation of new forms of pathogenes that causes diseases of winter wheat in CAES zone, Visnyk agrarnoi nauky, **1**, 21-22 (1998) [in Ukrainian]