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Selective Effect of Wheat Germplasm upon Isolates of *Mycosphaerella graminicola*

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The selection of *Septoria tritici* biotypes by Bobwhite'S' resistant wheat germplasm was studied. Seedlings of cultivars with Bobwhite'S' germplasm and with dwarf-mexican germplasm were inoculated in the greenhouse. Two *Septoria tritici* isolates (atypical yeast-like variant coming from Bobwhite'S' germplasm and stromatic from traditional argentine germplasm) were used. Isolates of *S. triciti* from each isolate × cultivar combination were analyzed, and the percentage of regular or variant colonies was registered. Significant pathogen population effect and cultivar effect were demonstrated. The regular isolate produced chlorotic and necrotic lesions with pycnidia in all the cultivar; the atypical variant had quite distinct reaction according to the cultivars, being predominant the non-pycnidial necrotic reaction. Pathogen population isolated from Bobwhite'S' germplasm produced higher levels of variant colonies, than did population isolated from dwarf mexican cultivar. Inoculation with the variant produced a high number of variant colonies in cultivars with both germplasm, indicating that Bobwhite'S' germplasm could induce the origin of less pathogenic variants from a heterogeneous pathogen population through a remarkable mechanism of selective effect and any genetic change.

The selection pressure exerted by diverse hosts upon their pathogens has been largely studied (Lincoln, 1940; Watson and Luig, 1968; Leonard, 1969; Brasier, 1987; Cordo et al., 1989; Rapilly et al., 1989).

The pathogens progressively adapt to their resistant hosts through a selective effect on such hosts. They produce a selective pressure (Krupinsky, 1982; Cunfer and Youmans, 1983) on the pathogen, showed throughout the increase or decrease of pathogenicity (Skajennikoff and Rapilly, 1983; Osburn et al., 1986). Moreover the lack of virulence in a biotype of the population is due to the mutants that have lost the genetic factors conditioning such feature (Stackman, 1947).

Spontaneous mutation, sexual recombination and somatic hybridisation are mechanisms of change is pathogenicity by which the new virulence combinations could be generated in a pathogen population. Additional variations occur from other pathogen populations or due to molecular and cytoplasmic changes (Burdón, 1992). Such examples as delay in chromosome separation in mitosis (Mc Clusky and Mills, 1990); suppressions producing polymorphism in nuclear DNA restriction fragment sequences (Mc Donald and Martinez, 1990); reduction in the pathogenicity of aggressive isolates because of the host cytoplasm or nucleus (Brasier, 1987; Rapilly et al., 1989), support such statement.

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Brasier (1977, 1982, 1983, 1987) studied aggressiveness changes in *Ophiostoma (Ceratocystis) ulmi* in elms populations. The reduction in pathogenicity would appear because of a combination of mutations that occurred in nuclear genes and for the influence of a cytoplasmic factor.

In Argentina, the population of *Septoria tritici* isolates, obtained from cultivars having a Bobwhite'S' resistant germplasm, showed new cultural types, with diminished pathogenicity, and a different physiological behaviour (Perelló et al., 1990). Besides, one of these isolates (P_3 isolate which produces microspores in cultures and pycnidia and microspores on leaves) could be selected from the pathogen local population (Sanderson, pers. comm. 1990).

This paper studies the evidence to support that Bobwhite'S' resistant germplasm selects *S. tritici* biotypes morphoculturally different and less pathogenic than the regular ones.

The spreading of wheat cultivars with resistant germplasm could cause alterations on the composition of *S. tritici* population with an increase of biotypes with diminished pathogenicity.

Materials and Methods

Cultural and morphometrical studies were carried out upon 10 pathogen isolates, obtained from cultivars with Bobwhite'S' (CM 33203) and Kavkaz, germplasm collected from naturally infected plants (Table 1).

Each fungal isolates was isolate from infected tissue samples or by spore transfer from a pycnidium. The cultures (6 replication for isolate) were grown on 2% PDA (Potato Dextrose Agar) under laboratory conditions (means temperature 20 °C, diffused

Table 1

Origin, cultural types and colony colour of ten *Septoria tritici* isolates

Isolate	Cultivar	Germplasm	Colour	Collected in
P_3	LPI/BW'S'	BW'S'	45 Buff	Pergamino
P_1	Bw/4/... Laj 3139	BW'S'	45 Buff	Pergamino
E_9	CST 169	BW'S'	45 Buff	Uruguay
35_B	CM 76751 × 0 (F_3)	BW'S'	61 Rossy Buff	Barrow
P_{3-4}, P_{3-6}	LPI/BW'S'	BW'S'	45 Buff	Pergamino
19_N	CM 61830	Kavkaz	45 Buff	Necochea
5_E	Millalew	Kavkaz	61 Rossy Buff	Uruguay
E_1	LI7	BW'S'	45 Buff	Uruguay
LH_{SM}	Los Hornos improved line	physiologic mutant	45 Buff	Los Hornos

Table 2

Size of budding cells and conidia of ten *Septoria tritici* variants developed "in vitro" on PDA media (Fitzgerald and Cook method) after seven days

Isolate	Cultivar type	Thallus type	In vitro length (µm)	In vitro width (µm)
P_3	mucous (yeast-like)	short and long cylindrical budding cells	(1.93) 2.15 3.66	(1.02) 1.25 (1.59) 1.50
P_1	albinic, mycelial plastery, "cordeé type"	elliptical budding cells	3.92	2.02
E_0	stromatic, albinic, dusty	elliptical conidia	6.02	3.75
35_B	albinic, mycelial, "cordeé type"	elliptical conidia	(4.3) 5.73 (6.0)	2.86
P_{14} P_{36}	mycelial, filamentous	unicellular and bicellular elliptical conidia	(4.5) 6.0 (7.5)	3.0
19_N	albinic, powdery, plastery, mycelial	secondary budding cells and conidia	(6.0) 7.5 (10.5) *	3.0 *
5_E	albinic, powdery plastery, stromatic	filiform conidia, secondary conidia chlamidospora	(7.15) 11.4 (12.) **	1.43 **
E_1	albinic, powdery plastery, stromatic	elliptical conidia	6.02	3.75
LH_{SM}	albinic, mycelial, "cordeé type"	prismatic budding cells	(5.78) 9.5 (13.5)	(1.8) 2.38 (2.6)

* These sizes correspond to budding cells only
** These sizes correspond to filiform conidia only

light) for 21 days. The aspect, colour (following Rayner, 1970), form, margin, internal structure, size and spore type were determined. Colonies were described after Garassini 1958 and Negroni 1938. Direct microscopic observations microculture techniques, micrometrical measurements and SEM spore observations were made. Fitzgerald and Cooke's method (1989, pers. comm.) was applied to determine the germination of each vegetative structure (Table 2).

Monospore colonies arising from resistant germplasm were studied and comparing with the phenotypes usually isolated from Argentine germplasm (pycnidial-stromatic).

Two isolates were assayed in order to test the selective effect of the Bobwhite'S' germplasm through artificial inoculation technique: P_3 atypical yeast-like and mucous isolate, as a variant and M89, regular and stromatic, as a control. Every isolate come from hosts with different genetic origin: P_3 from La Paz INTA/Bobwhite'S' line and M_{89} from Buck Poncho with argentine traditional germplasm.

Three wheat cultivars of different germplasm were chosen as hosts: Don Ernesto INTA (D. E. I.) and La Paz INTA/Bobwhite'S' line with CM 33203, AU/KAL/BB/

/3/WOP'S' ancestry, and Marcos Juarez INTA (M. J. I.) with Sonora 64/KLRE genealogy.

Artifical inoculations of the two isolates on the cultivars mentioned were perfomed on the 3rd leaf stage. The inoculum concentration for each isolate ranged between 1.8×10^7 for M_{89} and 2.2×10^7 for P_3. The inoculum was applied by pulverization until run off from the wheat leaves. The incubation period under wet chamber was of 96 hours. At the end all pots inoculated were maintained under greenhouse with controlled condition of temperature and humidity. The pycnidial coverage percentage (PCP) was registered on the 21st days and after the assessment of the disease all lesions of the middle part of the third leaf inoculated were cut out in 0,5 cm long pieces. Three pieces were randomly selected and, once desinfected (70% ethanol for 1/2 minute and 1‰ Cl_2Hg for 1 1/2 minute) were incubated on PDA. Within 7 to 10 days, the percentage of regular colonies (RCP) and variant colonies (VCP) for each selected piece were determined. The experimental design used was a random block with 10 replications. A variance analysis of PCP and VCP was carried out. The average differences were analyzed by Tukey test.

Results

The different isolates studied comprised two types according to thallus structure: yeast-like and albinic filamentous. The largest part of the material studied belonged to the latter (Table 2, Fig. 1).

Most isolates comprised cell types that germinated through terminal cell by budding or by fission. True conidia germinated by a tube.

A highly significant effect on cultivars and isolates ($P \leq 0.05$) was shown by the ANOVA for PCP (Table 3). The highest PCP was observed in all cultivars with the M_{89} isolate (Fig. 2).

The PCP has always been higher on LPI/Bbw'S', for the two isolates studied. No differences were found between other two cultivars (Fig. 3 and Table 4). Analyzing each isolate in particular, M_{89} caused chlorotic and necrotic lesions with pycnidia, when it was inoculated on 3 cultivars.

The P_3 isolate produced different reactions on inoculated cultivars. Very restricted or absent lesions on M. J. I. (Fig. 4), only small marginal lesions without pycnidia on D.E. I. (Fig. 5) and small lesions with or without pycnidia on LPI/Bbw'S' (Fig. 6).

A significant effect on cultivars and nonsignificant effect on isolates were showed by the ANOVA for VCP ($P \leq 0.05$) (Table 5). Among cultivars, M. J. I. did not produce variant colonies for M_{89} with respect the other two cultivars (Fig. 7). The cultivars behaviour with respect to each isolate was different (Fig. 8). The VCP produced by the inoculation of M_{89} isolate was significantly high in D. E. I. and LPI/Bbw'S' compared to M. J. I. With P_3 variant, VCP did not differ significantly among the three cultivars.

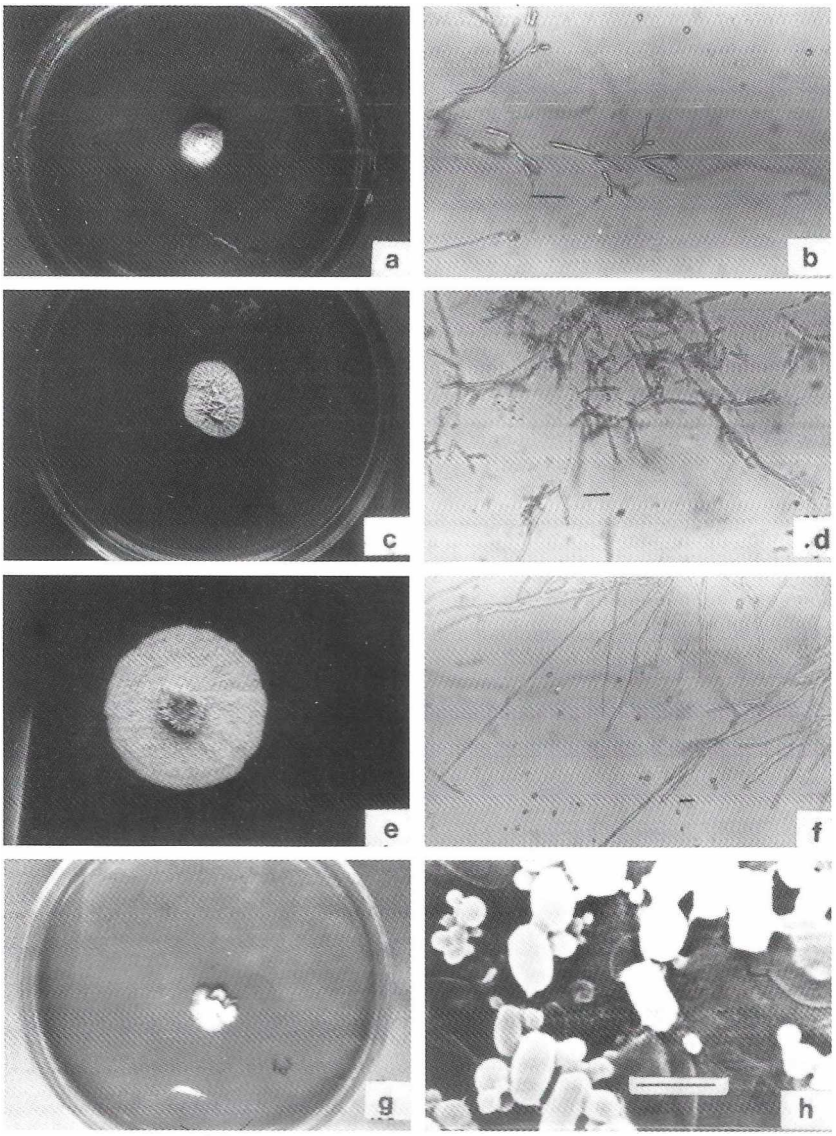

Fig. 1. Types of variants of *Septoria tritici* developed "in vitro" on PDA media after 21 days.
a) isolate LH_{5m}; monosporic colony upon a potato – dextrose agar. (PDA) (× 0.8)
b) isolate LH_{5m}; pseudomycelium hyphas with apex budding cells; bar = 20 μm
c) isolate P_1; monosporic colony upon PDA (× 0.8)
d) isolate P_1; pseudomycelium with end budding cells; bar = 1 μm
e) isolate 5_E; monosporic colony upon PDA (× 0.8)
f) isolate 5_E; elliptical hyaline conidia and secondary conidia; bar = 18 μm
g) isolate P_3; yeast-like monosporic colony upon PDA (× 0.8)
h) isolate P_3; cylindrical budding cells; bar = 2 μm

Table 3

ANOVA for percentage pycnidial coverage (PCP)

Source of variation	Sum of squares	d.f.	Mean square	F-ratio	Sig. level
MAIN EFFECTS	3869.0979	12	322.4248	16.374	0.0000
cultivar	286.5103	2	143.2551	7.275	0.0018
isolate	3297.3246	1	3297.3246	167.453	0.0000
block	285.2630	9	31.6959	1.610	0.1415
FACTOR INTERACTIONS	45.704698	2	22.852349	1.161	0.3225
cultivar isolate	45.704698	2	22.852349	1.161	0.3225
RESIDUAL	886.09738	45	19.691053		
TOTAL (CORR.)	4800.9000	59			

0 missing values have been excluded

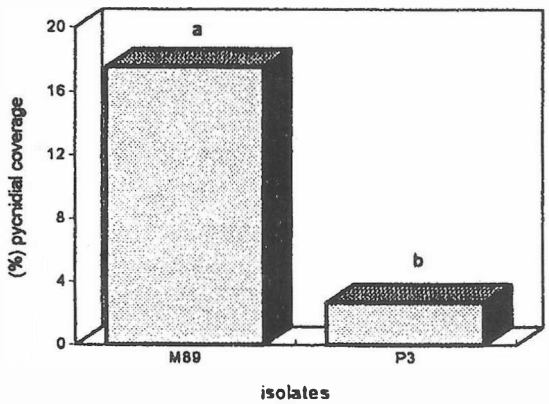

Fig. 2. Pycnidial coverage percentage of wheat cultivars inoculated with M_{89} and P_1 isolates. Different letters show significant differences by Tukey test

Table 4

Mean values (10 replications) for percetage pycnidial coverage (PC) and percentage variant colonies (VC)

Cultivar	Isolate	Pycnidial coverage %		Variant colonies %	
		M_{89}	P_1 (variant)	M_{89}	P_1 (variant)
MJI (Mexican Dwarf germplasm)		17.56	0.34	0	34
LPI/Bw'S' (Bbw'S' germplasm)		19.7	6.51	70	59
Don E. INTA (Bbw'S' germplasm)		15.1	1.12	55	58

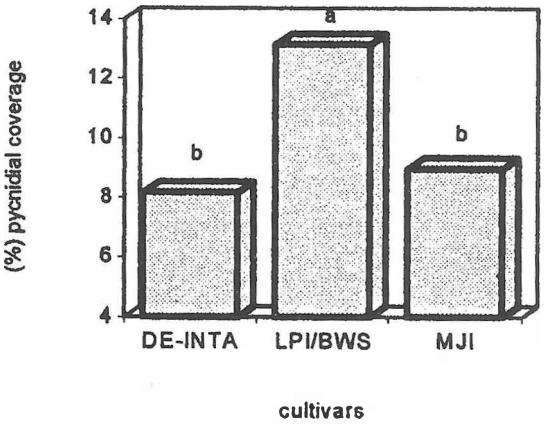

Fig. 3. Pycnidial coverage percentage on DE-INTA (Don Ernesto INTA); LPI/BWS (LPI/Bobwhite'S') and MJI (Marcos Juárez INTA) cultivars, inoculated with M_{80} and P_3 isolates. Different letters show significant diffrences by Tukey test

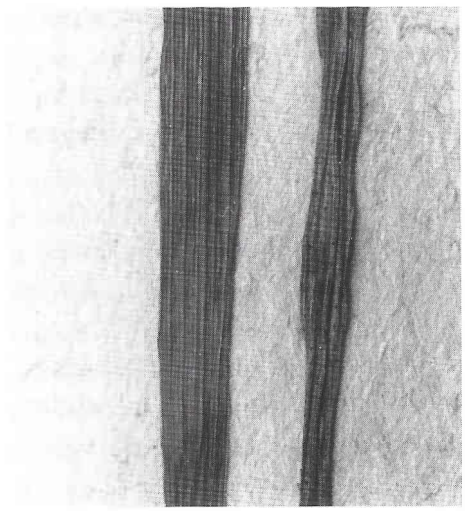

Fig. 4. Foliaceous lesions without pycnidia for P_3 × MJI (isolate × cultivar) interaction

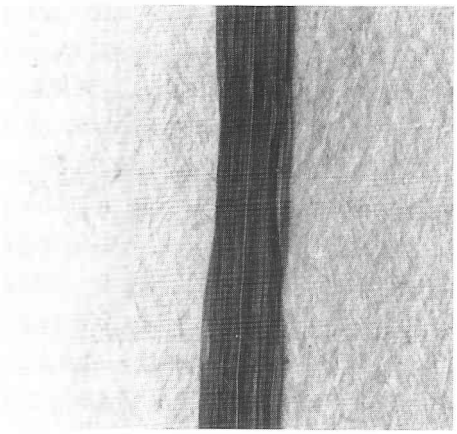

Fig. 5. Foliaceous lesions without pycnidia for P_1 × Don Ernesto INTA (isolate × cultivar) interaction

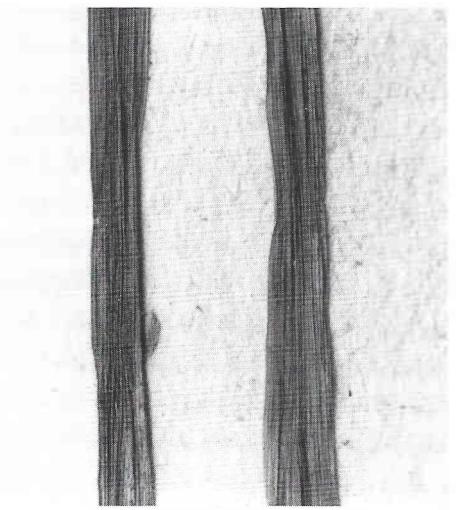

Fig. 6. Foliaceous lesions with or without pycnidia for P_1 × LPI/Bobwhite'S' (isolate × cultivar) interaction

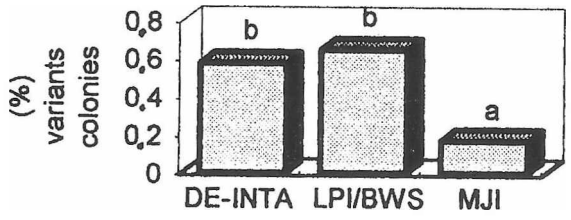

Fig. 7. Mean values of the variant colony percentage of isolates on DE-INTA (Don Ernesto INTA); LPI/BWS (LPI/Bobwhite'S') and MJI (Marcos Juárez INTA) cultivars. Different letters show significant differences by Tukey test

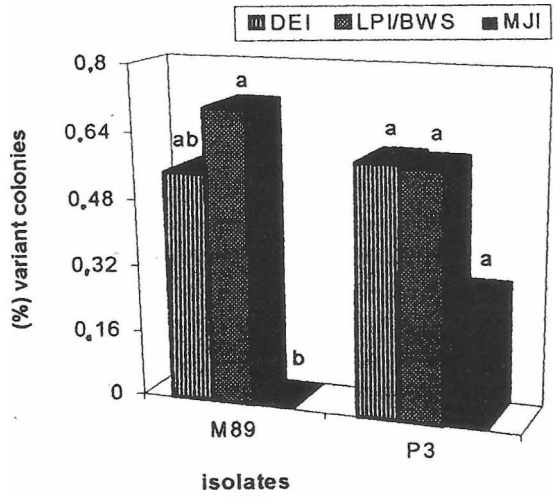

Fig. 8. Variant colonies percentage in DE-INTA (Don Ernesto INTA); LPI/Bobwhite'S' and MJI (Marcos Juárez INTA). Different letters show significant differences by Tukey test

Table 5

ANOVA for percentage variant colony (VCP)

Source of variation	Sum of squares	d.f.	Mean square	F-ratio	Sig. level
MAIN EFFECTS	4.8113400	12	0.4009450	3.141	0.0026
cultivar	2.3070400	2	1.1535200	9.036	0.0005
isolate	0.0299267	1	0.0299267	0.234	0.6357
block	2.4743733	9	0.2749304	2.154	0.0441
FACTOR INTERACTIONS	0.2465733	2	0.1232867	0.966	0.3884
cultivar isolate	0.2465733	2	0.1232867	0.966	0.3884
RESIDUAL	5.7443267	45	0.1276517		
TOTAL (CORR.)	10.802240	59			

0 missing values have been excluded

Discussion

The results obtained in this work showed the effect of wheat germplasm (specially Bobwhite'S'-CM 33203) in variants selection of *S. tritici* with lower pathogenicity. Rapilly et al. (1989) verified the selection pressure exerted by triticale isogenic lines upon *Septoria nodorum* cytoplasm.

Cordo et al. (1989) detected qualitative changes in the virulence of two *S. tritici* isolates with only one generation of subculture from a resistant wheat line with Bbw'S' germplasm. The virulence either increased or remained stable with regard to their original generation.

Current biochemical methods have allowed to study the genetic variability of *S. tritici* (Mc Donald and Martinez, 1990) suggested a great genetic variability among small isolates samples collected from small geographic areas. They found that different genotypes were present in the same lesion of the leaf.

The Bbw'S' germplasm had selected atypical yeast-like and albinic mycelial variants, from the total population of *S. tritici*. Besides, it has been demostrated that the P_3 isolate had lost pathogenicity. In order to show the selective effect of Bbw'S' wheat germplasm it must be marked that M89 isolate, did not produce variants colonies by passed throughout M. J. I. cultivar (control) but was affected when passed the first time throughout varieties with Bobwhite'S' germplasm. The evidence coincides with Stackman (1947) and Lincoln (1940) statements for different selection pressure examples. In both cases, the less pathogenic variants would have been caused by mutant selection during fungus multiplication in its host with loss of the pathogenicity factor.

In this work, as in Brasier's experiences (1977, 1982, 1983, 1987) polymorphic colonies (mycelial-albinic, stromatic-dusty-albinic and mycelial-filamentous) and less

pathogenic isolates have been described. They are morpho-cultural different from those traditionally stromatic (wild type in Argentina). This would suggest that these cultural types with low associated pathogenicity may be brought about the combination of a mutagenic effect and some cytoplasmic factor as Brasier mentioned for *Ceratocystis ulmi*.

The selective effect of Bbw'S' germplasm has been seen through various paths: 1) when inoculating a regular or a variant isolate on cultivars having such germplasm, variant isolates appeared in a high percentage, compared with the isolates obtained from the cultivar with dwarf-mexican germplasm. 2) the P_3 variant isolate was characterized by its weak pathogenicity on the 3 cultivars assayed.

The variants frequently isolated from cultivars with Bbw'S' germplasm could be originated by a serie of adaptative changes in the pathogen due to diverse mechanism. It could be thought that Bbw'S' germplasm act as a variant selector substratum. Also, certain character (pathogenicity, pigment production, spores feasibility) could been ruled by specific genes affected by the selection. These genes would be present and active in the regular isolate, but when infect the host could suffer an inactivation as a result of the adaptative change (Stackman and Harrar, 1957). If the combination of a mutagenic effect and cytoplasmatic factors lasted long, it could be expected that a more durable resistance would occur in cultivars containing this germplasm.

Acknowledgements

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Literature

Brasier, C. M. (1977): Dual origin of recent Dutch elm disease outbreaks in Europe. Nature 281, 78–79.

Brasier, C. M. (1982): Genetic of pathogenicity in *Ceratocystis ulmi* and its significance for elm breeding. In: Heybroek, H. M.,. Stephan, B. R and von Weissenberg, K. (eds): Resistance to Diseases and Pests in Forest Trees, Wageningen, The Netherlands, pp. 224–235.

Brasier, C. M. (1983): The future of Dutch elm disease in Europe. In: Burdekin, D. A. (ed.): Research on Dutch Elm Disease in Europe, London, pp. 96–104.

Brasier, C. M. (1987): Recent genetic changes in the *Ophiostoma ulmi* population: the threat to the future of the elm. In: Wolfe, M. S. and Caten, C. E. (eds): Population of Plant Pathogens: their Dynamics and Genetics, Oxford, pp. 213–226.

Burdón, J. J. (1993): Genetic variation in pathogen populations and its implications for adaptation to host resistance. In: Jacobs, Th. and Parlevliet, J. E. (eds): Symposium on Durability of Disease Resistance Wageningen, The Netherlands, 41–56.

Buxton, E. W. (1960): Heterocaryosis saltation and adaptation. In: Plant Pathology. New York, pp. 339–405.

Cordo, C. A., Perello, A. E., Arriaga, H. O. and Pascar, A. (1989): Study of the stability of *Mycosphaerella graminicola* (*Septoria tritici*) isolates. In: Fried, P. M. (ed.): Procceding of the Third International Workshop on Septoria Diseases. Zürich, Reckenholz, Switzerland, pp. 55–59.

Cordo, C. A., Perello A. E., Alippi, H. E. and Arriaga, H. O. (1997): Morphocultural variants of *Septoria tritici* isolates. Rev. Iber. Micol. (in press).

Cunfer, B. M. and Youmans, J. (1983): *Septoria nodorum* on barley relationship among isolates from several hosts. Phytopathology 73, 911–914.

Fitzgerald, W. and Cooke, B. M. (1989): Spore germination and pycnidial development in wheat and barley isolates of *Septoria nodorum* on cellulose film. Faculty of Agriculture, University Collage, Belfield, Dublin, Ireland (pers comm).

Garassini, L. A. (1958): Microbiologia. Sucre, Venezuela.

Krupinsky, J. M. (1982): Comparative pathogenicity of *Septoria nodorum* isolated from *Triticum aestivum* and *Agropyrom* sp. Phytopathology 72, 660–681.

Leonard, K. J. (1969): Selection in heterogeneous populations of *Puccinia graminis f.* sp. *avenae*. Phytopathology 59, 1851–1857.

Leonard, K. J. (1987): The host population as a selective factor. In: Populations of Plant Pathogens their Dynamics and Genetics (Blackwell Scientific eds), Oxford, pp. 163–179.

Lincoln, R. E. (1940): Bacterial with resistance and genetic host-parasite interaction in corn. Journal Agricultural Research 60, 217–240.

Mc Clusky, K. and Mills, D. (1990): Identification and characterization of chromosome length polymorphisms among strains representing fourteen races of *Ustilago hordei*. Molecular Plant Microbe Interaction 3, 366–373.

Mc Donald, B. A. and Martinez, J. P. (1990): Restriction fragment length polymorphism in *Septoria tritici* occur at a high frequency. Current Genetic 17, 133–138.

Negroni, P. (1938): Morfología y biología de los hongos. Técnicas micológicas. (El Ateneo ed), Buenos Aires, Argentina

Osburn, A. E., Scott, P. R. and Caten, C. E. (1986): The effect of host passaging on the adaptation *of Septoria nodorum* to wheat or barley. Plant Pathology 35, 135–145.

Perelló, A., Cordo, C. A. and Alippi, H. E. (1990a): Características morfológicas y patogénicas de aislamientos de *Septoria tritici* Rob ex Desm. Agronomie 10, 641–648.

Perelló, A., Cordo, C. A. and Alippi, H. E. (1990b): Influencia de las fuentes carbonadas y nitrogenades en el crecimiento de *Septoria tritici*. In: Resúmenes del II Congreso Nacional de Trigo, Pergamino, Buenos Aires, 22–29.

Rapilly, F., Richard, H., Skajennikoff, M., Cauderon, Y. and Roussel, J. (1989): Pression de Sélection exercé es par le noyau ou le cytoplasme del'hôte sur l'agressivité d'un parasite nécrotrophe du blé: *Septoria nodorum* Berk. Agronomie 9, 703–718.

Rayner, R. W. (1970): A mycological colour chart. The Commonwealth Mycological Institute, Key Surrey, England.

Row, K. V., Aist, J. R. and Crill, J. P. (1985): Mitosis in the rice blast fungus and its possible implications for pathogenic variablity. Canadian Journal of Botany 63, 1129–1134.

Skajennikoff, M. D. and Rapilly, F. (1983): Etudes sur l'agresivité de *Septoria nodorum* Berk. Effects des hotes (triticatc et blé) et des organes attaqués. Agronomie 3, 131–140.

Stackman, E. C. (1947): Plant diseases are shifty enemies. American Scientist 35, 321–350.

Stackman, E. C. and Harrar, G. J. (1957): The Genetics of Plant Pathology. In: Principles of Plant Pathology, (The Ronald Press Company), New York, pp. 121–177.

Watson, I. A. and Luig, H. (1968): Progressive increase of virulence in *Puccinia graminis f.* sp. *tritici*. Phytopathology 58, 70–73.