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A STUDY OF GENETIC SEGREGATION USING SINGLE ASCOSPORE ISOLATES OF MYCOSPHAERELLA GRAMINICOLA

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ABSTRACT

Traditionally, plant breeders have used major genes for resistance. However, because of the limited life expectancy of these genes, there has been a general swing towards the use of multi-genic resistance. Genetic studies allowing plant breeders to be fully conversant with the inheritance of the resistance they are working with have been few, because of the difficulties in detecting small step wise differences conferred by the different genes. Inheritance of aggressiveness in *Mycosphaerella graminicola* — the causal organism of septoria tritici blotch of wheat — is being studied using single ascospore isolates, in order to obtain a better understanding of the genetics of resistance in a system controlled by multi genes.

KEYWORDS

Septoria tritici blotch, speckled leaf blotch, multi-genic resistance, wheat.

INTRODUCTION

Selection pressures within wild plant populations favour disease resistance. Conversely, host susceptibility and hence pathogen aggressiveness is a trait inadvertently acquired during plant breeding programmes. As plant resistance is the most cost effective approach to combating disease, concurrent introduction of host resistance genes is an aim of most breeding programmes. The success of this depends wholly upon the pathogen. If the pathogen can match host resistance alleles with virulence alleles, and these become prevalent in the pathogen population, then the work of the plant breeder is largely undone. The decisive factor in breeding for resistance is therefore the population genetics of the pathogen, yet it is the genetics of host resistance, which is one step removed from the core of the problem, that has received attention in the literature. Host genes may influence the durability of resistance only indirectly, through their influence on the population genetics of the pathogen.

It was on the presence or absence of this host influence that Vanderplank (1978) classified resistance as vertical or horizontal. A selective influence of the host is present in the former whilst in the latter it is not. That is, with vertical resistance there is an observable interaction between host and pathogen. This has been wrongly interpreted as a single gene-for-gene relationship, whereas horizontal resistance has become synonymous with polygenic resistance. This was not Vanderplank's intention.

We would suggest that most resistance being considered in the context of plant breeding is vertical, there being a gene-for-gene relationship between host and pathogen: This is not always in the context implied by Vanderplank, who postulated a close biochemical relationship, but rather more in the context of the laws of physics: 'for every action there is an equal and opposite reaction'.

It is unfortunate that Vanderplank, in his treatise on plant resistance, centred his ideas on rusts, potato late blight and cereal mildew. These diseases occur occasionally, yet when they do, cause severe crop damage, an effect inherent in the use of single major genes. The majority of diseases are less dramatic and occur more frequently.

To understand the genetic relationship between pathogen and host, it is important to appreciate the life cycle of the pathogen and the significance of its sexual phase. Those diseases where the primary source of inoculum is the asexual spore, and where the sexual stage plays only a minor role in the life cycle (Fig. 1), are those which are likely to be associated with single major gene resistance e.g. *Puccinia striiformis* on wheat.

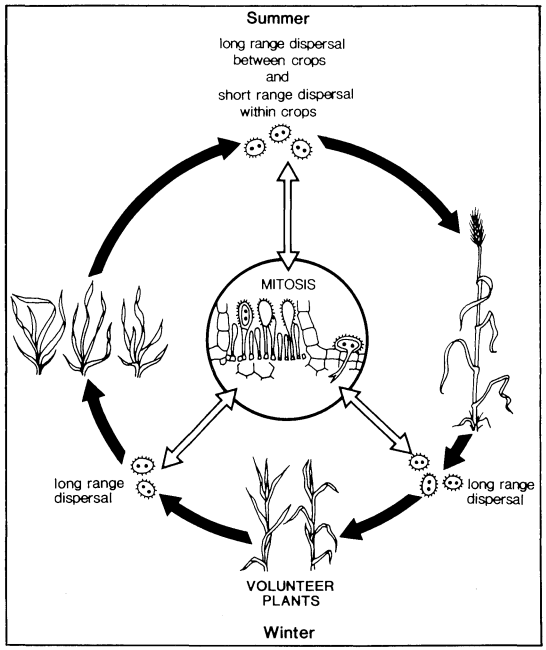

Figure 1. Life cycle of *Puccinia striiformis*. Asexual spores play a major role in the life cycle and are responsible for both long range and short range dispersal of the pathogen.

A population originating from asexual spores is largely haploid and homogeneous. A single mutation in the population produces an isoline, which, if virulent, results in a rapid swing in the pathogen population. In such instances, vertical gene-for-gene resistance is easily established.

With pathogenic ascomycetes, the perfect state may play a major role in the life cycle, (Fig. 2) with ramifications of outcrossing and screening by the host of a segreating pathogen population. This is a point missed by many plant breeders and pathologists.

In many instances, the primary inoculum is the sexual stage ascopore, being airborne and having the ability to travel long distances, thus disseminating genetic recombinants over a wide area. This segregating pathogen population is screened by the host each season. The pathogen, usually haploid at the time of infection, at some stage during its life cycle may become heterokaryotic, allowing the accumulation and maintenance of unexpressed alleles within the populations.

Current thinking by plant breeders and pathologists is that durable resistance will result from a cross containing a number of resistance genes, as it requires the accumulation of several mutations within the one pathogen 'race' before resistance is overcome. This is only true for two situations, the primary being where the sexual stage plays a minor role in the life cycle of the pathogen and infection is predominantly by asexual spores, for example, the rusts. The other is where the resistance mechanism is beyond the scope of adaptation of the pathogen.

For diseases where the sexual stage plays an important part in the fungal life cycle there is a strong possibility that resistance would not be durable. During a breeding programme, a new cultivar may only be exposed to low selection pressures. It is when the cultivar is grown extensively that it becomes exposed to a major selection pressure, allowing the pathogen to experiment with different gene combinations. The resultant accumulation of alleles for aggressiveness erodes the resistance of the new cultivar.

For the present study, it was assumed that for polygenic control there is a gene-for-gene relationship with genes for resistance/susceptibility being matched with genes for avirulence/virulence. Secondly, a study of the genetics of virulence will lead to an understanding of the inheritance of polygenic resistance.

METHODS

Isolates were obtained by discharging ascospores in culture. Pieces of senesced wheat leaves, collected at Hyslop Agronomy Farm, Oregon State Universtiy, Corvalis, were attached to the lids of petri dishes of water agar. Each dish was left standing overnight in a vertical position with the leaf piece at the top. Ascospores were sequentially discharged, each with less force, resulting in a line of ascospores down the agar. (Plate 1).

Following germination these were transferred to nutrient agar where growth rate, sporulation and colony morphology varied greatly between isolates (Figs. 3 & 4, Plate 2).

Experiments were conducted, comparing the pathogenicity of single ascospores of *M. graminicola* to a range of wheat cultivars: Bobwhite "S" = Aurora x Kalyansona-Bluebird/Woodpecker "S" (CM 33203-k-9M-2Y-1M-1Y-1M-0Y), Siete Cerros (7C) CI 14493, Bezostaya 1 (PI 345685), Fortuna (CI 13596), Colotana (CI 13556), Wampum, 81M UWWMN 2024 (Palmaress/(TF 1035) Fauereau/4/Martin/K3/Hohen 77/Oro/2/Capelle/

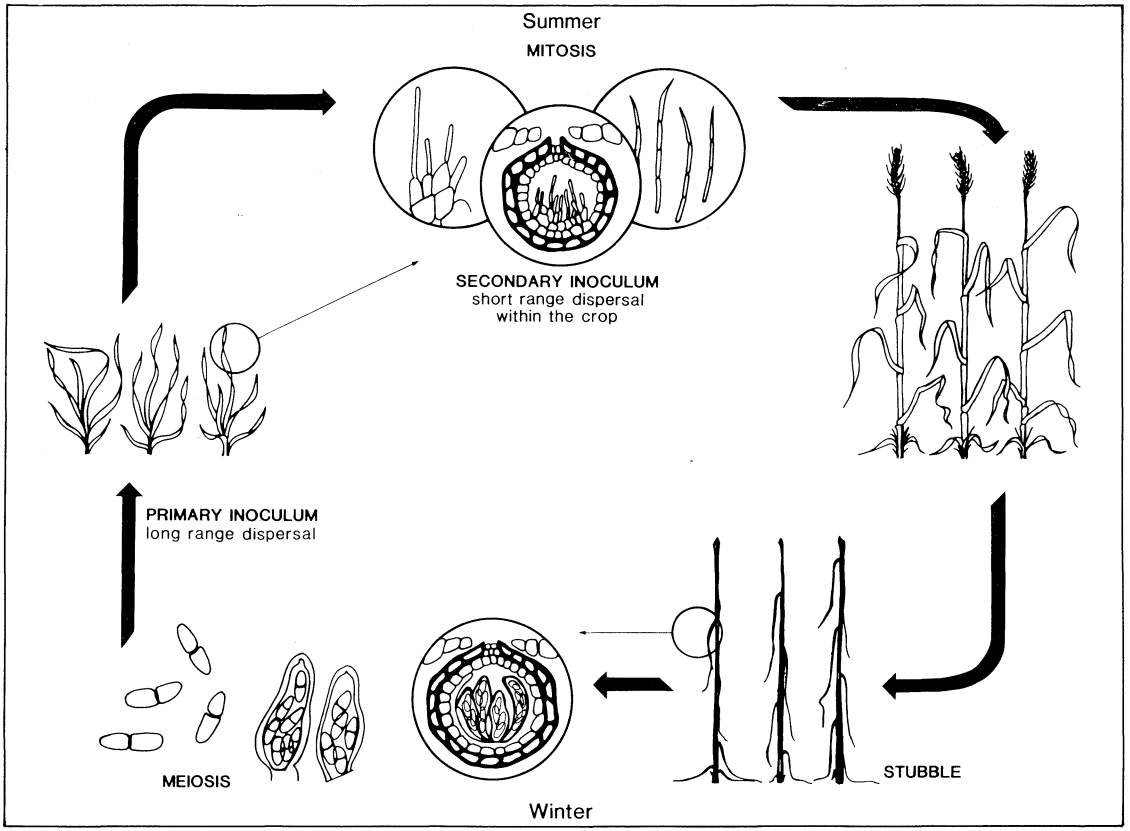

Figure 2. Life cycle of *M. graminicola*. The primary inoculum is the sexual stage spore which is capable of long range dispersal. The asexual spore is confined to short range dispersal within or between adjacent crops.

Magdalena) sel.1) and Weibull 7389.

Plants were inoculated with a conidial spore suspension (Eyal and Scharen, 1977) and scored for percent leaf area covered by pycnidia on a 0-9 scale. The response of the wheat cultivars was determined by using a cluster analysis where 6 response groups were arbitrarily established: very susceptible (VS), susceptible (S), moderately susceptible (M), moderately resistant (MR), resistant (R) and very resistant (VR). The distribution of the means of the 8 cultivars x 20 isolates is presented in Table 1. The 20 isolates included three check anamorphic isolates, one from the same location (ORG 82076), an isolate from Stillwater, Oklahoma (OK 83106) and an isolate from New Zealand (NZ1). Three sets of single ascus-derived isolates were used in the study: set 46 with 6 ascospore cultures; set 48 with 3 cultures, and set 53.1 with all 8 ascospore cultures.

The cutpoint between resistant and susceptible host responses was determined as 2.05 by adding the standard deviation of the moderately resistant response group to its mean.

Table 1. Cluster analysis of six cultivar response classes in a 20-isolate (*Mycosphaerella graminicola*) x 8-cultivar matrix.

Response class	Number of evaluations	Leaf area with pycnidia (scored on a 0-9 scale) ± standard deviation
Very susceptible	8	5.56 ± 0.49
Susceptible	14	4.00 ± 0.42
Moderately susceptible	18	2.74 ± 0.25
Moderately resistant	35	1.83 ± 0.22
Resistant	34	1.13 ± 0.20
Very resistant	51	0.34 ± 0.24

The analysis forms clusters of variables which are based on a measure of association (similarity)between the variables, or of distance (difference) between them.

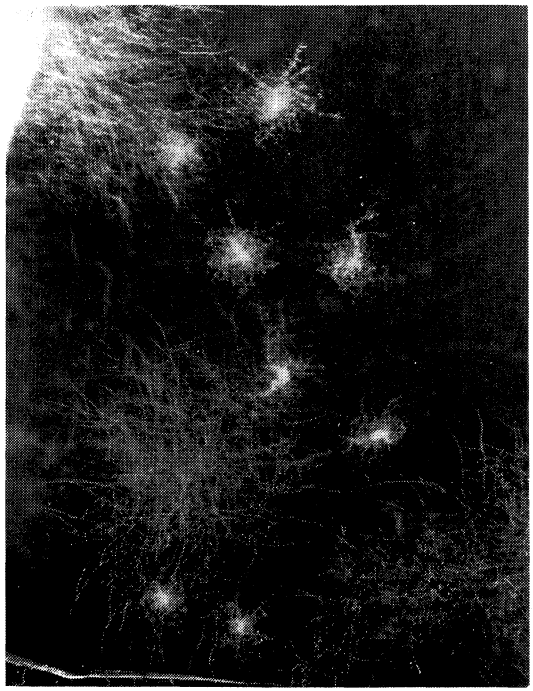

Plate 1. A line (down the agar) of 8 germinating ascospores of *M. graminicola* in 4 genotype pairs. The first ejected is at the top and the last at the bottom.

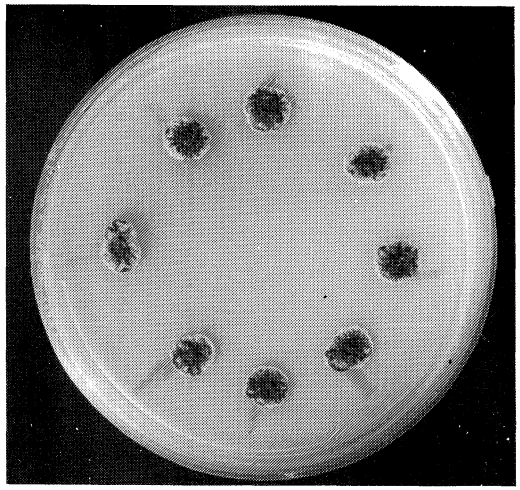

Plate 2. Eight colonies of *M. graminicola* from ascospores of one ascus, demonstrating the variation in growth rate and colony morphology, suggesting a possible segregation for colony morphology of 2:4:2.

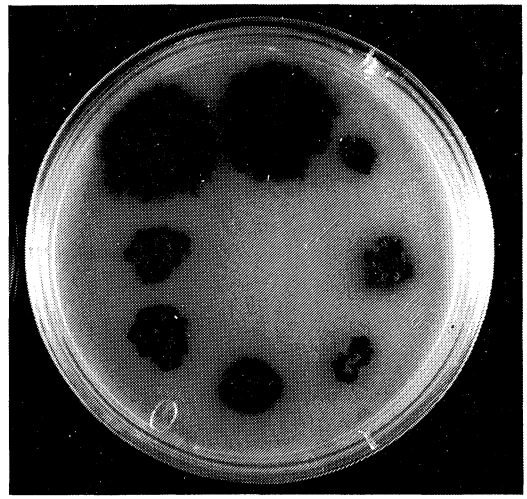

Plate 3. Eight colonies of *M. graminicola* from pycnospores derived from one pycnidium, demonstrating uniformity in colony morphology.

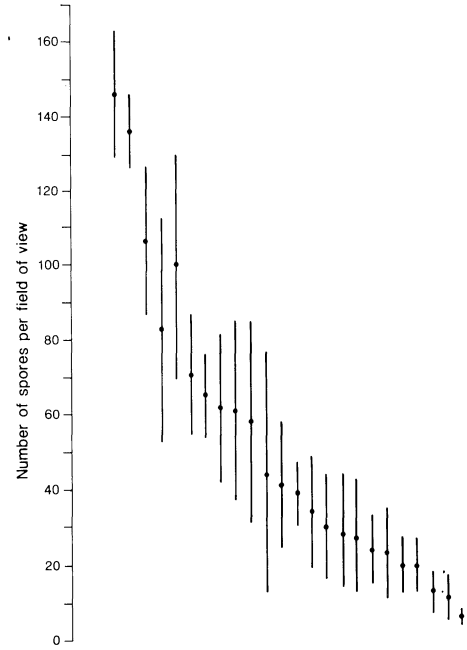

Figure 3. Variation in sporulation between 24 single ascospore isolates of *M. graminicola*. The vertical line for each isolate represents the variation in total number of spores between the five fields of view counted.

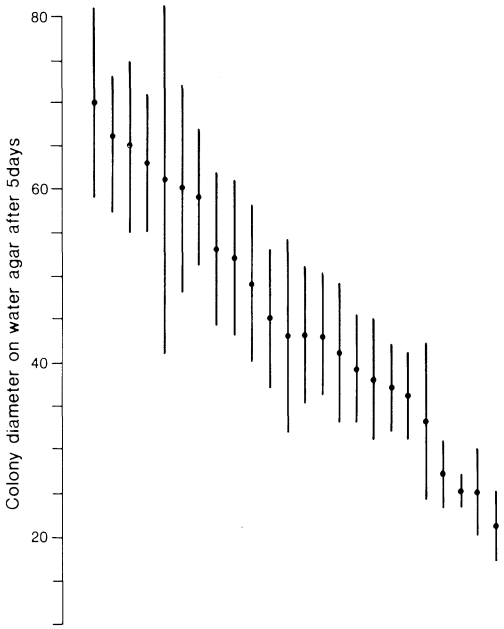

Figure 4. Diameter in μm of 24 single ascospore colonies of *M. graminicola*, after five days on water agar. Vertical lines represent the variation in growth rate between hyphae.

RESULTS & DISCUSSION

A cultivar (8) by isolate (20) matrix was formed using the host response of either resistance (R) or susceptibility (S) (Table 2). This matrix was analysed by the GENEALOGY computer analysis which estimates the minimum number of interacting genes assuming a gene-for-gene analysis (Eyal *et al.*, 1985, Kampmeijer, 1981, and Yechilevich-Auster *et al.* 1983). The gene-for-gene analysis identified 6 different, interacting, hypothetical genes in the 20-isolate x 8-cultivar matrix. The cultivar 8IM UWWMN 2024 was resistant to all 20 isolates of *M. graminicola* and thus it was not possible to assign it specific genes for resistance. The hypothetical genes assigned to the other 7 cultivars are present in Table 3. The cultivars Siete Cerros, Bezostaya 1, Fortuna and Colotana each possess one different gene for resistance. The cultivar Bobwhite "S" possesses 3 genes, with one common gene for resistance with Bezostaya 1. The cultivar Weibull 7389 possesses two genes for resistance, one common with Bobwhite "S". The hypothetical genes for virulence assigned to the 20 isolates are presented in Table 4. The isolate Oregon 82076 was assigned a gene virulent of Siete Cerros but not on Bezostaya 1 and Bobwhite "S" to which this isolate was virulent in a previous study (Eyal *et al.*, 1985).

Attempts to follow virulence patterns using ascospore arrangements should be possible according to host response types. However, in this instance — it would appear that differentiation between susceptibility and resistance was

Table 2. The distribution of susceptible host responses in the 20 isolate (*Mycospherella graminicola*) x 8-cultivar matrix.

Cultivar isolate	Siete Cerros	Bobwhite "S"	Bezostaya 1	Fortuna	Colotana	Wampum	81 M 2024	Weibull 7389
Oregon 82076		+	+		+	+		
Oklahoma 83106	+			+	+	+		
N.Z. 1	+		+	+	+	+		+
46A	+	+	+	+	+	+		
46B			+	+		+		
46C								
46E								
46F								
46G	+	+	+	+	+	+		+
48A	+		+		+	+		
48B								
48H								
53.1A		+	+		+	+		
53.1B	+			+	+	+		
53.1C								
53.1D					+	+		
53.1E						+		
53.1F	+					+		
53.1G	+		+			+		
53.1H	+		+	+		+		

+ denotes a susceptible host response

not consistent using the cut point as defined by the cluster analyses. The distribution of virulence genes within the two sets of ascospore isolates 46 and 53.1 did not follow the organised pattern expected of four genotype pairs of eight related ascospores.

Table 3. Hypothetical resistance genes assigned to the 7 cultivars from a gene-for-gene analysis using the GENEALOGY computer analysis on 20-isolates x 8-cultivar interacting matrix.

Cultivar	Genes for resistance 1	2	3	4	5	6	Hypothetical number of genes
Siete Cerros	+a						1
Bobwhite "S"			+	+		+	3
Bezostaya 1			+				1
Fortuna		+					1
Colotana				+			1
Wampum							0
81 M 2024	*	*	*	*	*	*	-
Weibull 7389					+	+	2

* resistant to all isolates

[a] presence of resistance genes based on P. Kampmeijer's (1981) DIFFER and GENEALOGY (3) analysis of incomplete person scheme of a variety x isolate reaction matrix.

Table 4. Hypothesized virulence genes derived from analysis of reaction matrix of 20 *Mycosphaerella graminicola* isolates tested on 8 cultivars.

Mycosphaerella graminicola isolate	Hypothesized virulence genes VST1	VST2	VST3	VST4	VST5	VST6
Oregon 82076	+[a]					
Oklahoma 83106		+	+	+		
N.Z. 1	+	+	+	+	+	
46A	+	+	+	+	+	+
46B		+	+			
46C						
46E						
46F						
46G				+	+	
48A	+		+	+		
48B						
48H						
53.1A			+	+		+
53.1B	+	+		+		
53.1C						
53.1D				+		
53.1E						
53.1F	+					
53.1G	+		+			
53.1H	+	+	+			

[a] presence of virulence genes based on P. Kampmeiher (3) analysis of incomplete Person scheme of a cultivar by isolate reaction matrix.

Although incomplete, the virulence arrangement for the two sets of isolates suggests a segregation pattern of 2 : 4 : 2 (Table 5) as demonstrated in plate 2. Such an arrangement (2 : 4 : 2) may indicate that during the second meiotic division a crossing-over occurred in the chromosome segment containing the virulence gene. If this assumption is correct, this leads to the conclusion that virulence of those cultures is based on two unlinked loci in these haploid ascospore-derived cultures.

The gene, VST3, virulent on Bezostaya 1 is an hypothesized gene and is a rather ambiguous marker for use in genetic analyses. This was the first attempt using *Mycosphaerella graminicola* to follow distribution of virulence as an outcome of meiosis.

The above suggestion should be carefully checked on a larger number of *M. graminicola* cultures with more refined techniques and genetical markers.

CONCLUSION

Stackman, in 1940, stated that a better understanding of the genetics of pathogenic micro-organisms is urgently needed for the advancement of knowledge of infectious diseases and their control. In 1941 Keitt and Langford published a paper entitled "*Venturia inaequalis* (Cke.) Wint. I A ground work for genetic studies", yet Stackman's views are as true today as they were in 1940. It is disturbing that there are still few published genetic studies of plant pathogenic ascomycetes. This may be due to a lack of appreciation of the importance of the work or an inability to duplicate this work with other pathogens.

In vitro attempts to produce the sexual state were not successful. Both this and the development of more refined techniques and improved genetical markers are currently being addressed.

ACKNOWLEDGEMENTS

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Table 5. Distribution of susceptible host responses for the isolate sets 46 and 53.1, suggesting a 2:4:2 ascospore segregation.

Cultivar isolate	Siete Cerros	Bobwhite "S"	Bezostaya 1	Fortuna	Colotana	Wampum	81 M 2024	Weibull 7389
46A	+	+	+	+	+	+		
B			+	+		+		
C								
-	-	-	-	-	-	-	-	-
E								
F								
G	+	+	+	+	+	+		+
-	-	-	-	-	-	-	-	-
53.1A		+	+		+	+		
B	+			+	+	+		
-	-	-	-	-	-	-	-	-
D					+	+		
E						+		
F	+					+		
G	+		+			+		
H	+		+	+		+		

+ denotes a susceptible host response

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SYMPOSIUM DISCUSSION

Dr K. Hammett, Division of Horticulture & Processing, DSIR

How did you select the specific ascus? Was it just any ascus, with this differentiation in resistance/virulance?

Sanderson

It's the luck of the draw which eight ascospores you are going to get. But, of course all the asci will be closely related although the segregation will be different.

Hammett

It seems remarkable that you got that amount of difference from a single ascus.

Sanderson

Yes, there can be very big differences in a single ascus.