

***Phoma macdonaldii* BOEREMA / *Helianthus annuus* L. INTERACTION**

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SUMMARY

Phoma macdonaldii, the causal agent of the sunflower black stem disease, is responsible for qualitative and quantitative damage which can result in up to 60% yield losses in France and worldwide. An early inoculation method has been developed in the laboratory to test sunflower genotype tolerance to *Phoma*. It was thus important to check whether the method was relevant by assessing the possible incidence of the growth stage of the plant on its reaction to *Phoma*. Six sunflower genotypes, which were known to be more or less susceptible to *Phoma*, were inoculated with an aggressive isolate at four different growth stages: cotyledon, unfolded leaves 3-6, budding and early flowering stage. Data analysis showed there is no interaction between the growth stage and the genotype: the test developed discriminates between genotypes early and the grading is representative of what occurs at a more advanced stage as it remains unchanged. Taking into account these data and the large genetic variability of *Phoma macdonaldii*, the strain-genotype interaction was investigated on isolates originating from France and other countries, in the search for possible pathotypes. For this purpose, ten isolates of *Phoma*, which were considered to be representative of the fungus variability, with differing aggressiveness, geographical origins and types of symptoms, were tested on ten different sunflower lines. Preliminary results suggest the existence of five pathotypes. Plant breeders could thus use these pathotypes for testing rapidly and reliably the tolerance of their genetic material to *Phoma*.

Key words: sunflower, black stem, early inoculation method, tolerance, variability, pathotype

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INTRODUCTION

The black stem disease of sunflower has become a matter of increasing concern recently, whether in France or worldwide. Yield losses may be quite high, particularly when the pathogenic agent induces early plant senescence (Figure 1). The inoculation method developed by our team allows sunflower genotype tolerance to *Phoma* to be tested. In a first step, it was thus important to check whether the method was relevant by assessing the possible incidence of the plant growth stage on its reaction to *Phoma*. In a second step, taking into account the wide genetic variability of *Phoma macdonaldii*, the strain-genotype interaction was investigated on isolates originating from France and two other countries so as to characterize the possible occurrence of pathotypes.



Figure 1: Disease symptoms on sunflower stem and collar

PART I – search for a possible incidence of sunflower phenological stage on plant reaction to *Phoma*

MATERIAL AND METHOD

The inoculation was achieved at the insertion of the cotyledon or leaf on the stem, depending on the phenological stage selected, using a pycniospore suspension of 10^6 spores/ml of an aggressive *Phoma* isolate. The plants were maintained under saturating humidity for 48 h. The severity of the symptoms was recorded 7 days after inoculation for the cotyledon stage (% necrotic area) and 6 weeks after inoculation for the other phenological stages (necrotic stem length).

RESULTS

Variance analysis showed the occurrence of significant differences between phenological stages and between genotypes ($p < 0.001$) and the absence of interaction between these two factors (Table 1). Independently of the genotypes, the attacks at the most advanced phenological stages were the most severe. Moreover, the grading of the varieties remained the same irrespective of the phenological stage considered. The absence of any phenological stage/genotype interaction clearly suggests that

genotype behavior is the same, irrespective of the phenological stage. The early inoculation test therefore allows a reliable discrimination between sunflower genotypes to be carried out.

Table 1: Susceptibility groups (VS : very susceptible, S: susceptible, MS: moderately susceptible and T: tolerant) of 6 sunflower varieties tested at 4 different phenological stages

Sunflower variety	4-6 leaf pairs, budding and early flowering stages	Cotyledon stage
EN11	VS	VS
EN22	MS	S
EN21	MS	MS
EN23	MS	MS
EN32	T	T
EN31	T	T

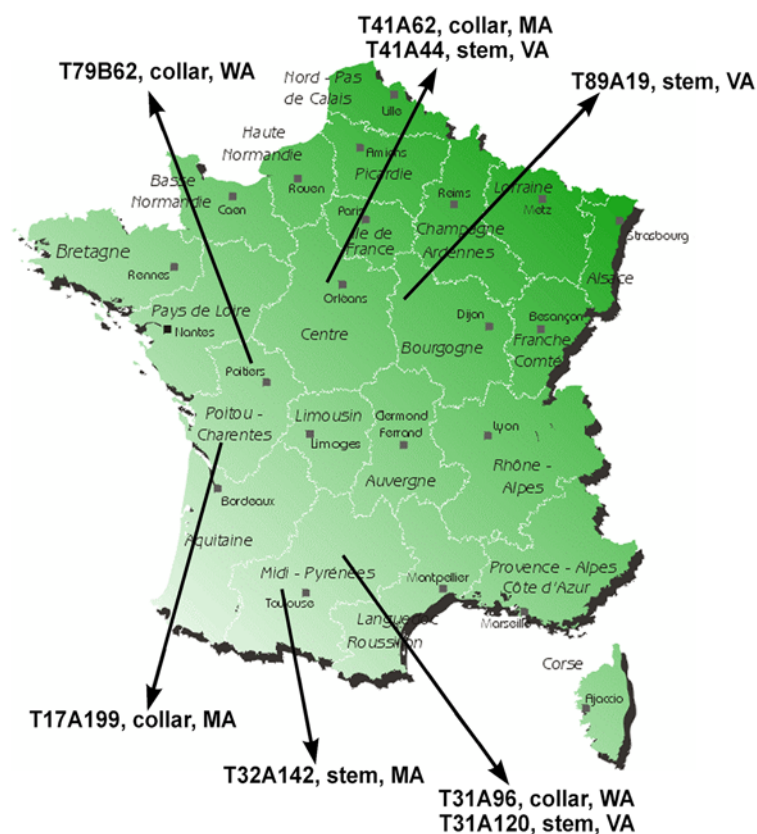


Figure 2: Geographical distribution of the French isolates issued from symptoms developed on stem or collar and characterized by differing aggressiveness (VA: very aggressive, MA: moderately aggressive, WA: weakly aggressive)

PART II – search for the possible occurrence of pathotypes

MATERIAL AND METHOD

Ten *Phoma* isolates, which were representative of the fungus variability (in terms of aggressiveness, geographical origin and symptom types) were selected: 8 isolates originated from France (Figure 2) and one isolate from each Argentina and Hungary. The confrontation between these isolates and ten sunflower lines was carried out using the early inoculation test described above and allowed the *Phoma*/sunflower interaction to be investigated.

RESULTS

Variance analysis showed the occurrence of significant differences ($p < 0.001$) between genotypes (Table 2) and isolates (Table 3) and the existence of a strain/genotype interaction (Table 4). Thus, the general behavior of a sunflower line vs *Phoma* may differ, depending on the isolate considered.

Table 2: Susceptibility groups (homogeneous groups obtained after variance analysis from symptom scoring) of the ten sunflower lines tested, all isolates included

Line	Susceptibility group (score)	
XRQ-B	A (7.36)	
PSC 8	B (7.02)	
PST 5	B (6.99)	
PSS 2	B (6.77)	
Rha 266	BC (6.65)	
PAC 2	BC (6.63)	
VAQ-B		D (6.13)
FU-B		E (5.66)
FN-B		E (5.42)
PAZ 2		F (5.07)

The genotypes can be distributed into 6 homogeneous groups. XRQ-B is highly susceptible, PSC 8, PST 5 and PSS 2 are susceptible, Rha 266 and PAC 2, are moderately susceptible, VAQ-B, FU-B and FN-B are moderately tolerant and PAZ 2 is tolerant.

The isolates can be distributed between 5 homogeneous groups. Isolates T89A19, T32A142, TArgC2 and THongC1 are aggressive. T79B62 is only very slightly aggressive, T41A62, T31A96 and T41A44 are slightly aggressive, and the other isolates are moderately aggressive.

The occurrence of pathotypes can be inferred from the existence of a strain/genotype interaction.

Table 3: Aggressiveness groups (homogeneous groups obtained after variance analysis from symptom scoring) of the 10 isolates tested, all lines included

Isolate	Aggressiveness group (score)				
T89A19	A (7.43)				
T32A142	A (7.34)				
THongC1	A (7.29)				
TArgC2	A (7.23)				
T31A120		B (6.77)			
T17A199			C (6.29)		
T41A62				D (5.57)	
T31A96				D (5.29)	
T41A44				D (5.29)	
T79B62					E (3.60)

Table 4: Strain/genotype interaction: tolerant (T) and moderately susceptible (MS) lines can turn into very susceptible (VS) lines in the presence of some isolates

Line		Isolate inducing changes in the general behavior of the line (for T to MS lines altered to VS)				
T	MS					
	Rha 266	T89A19	TArgC2		T32A142	T31A120
	PAC 2	T89A19	TArgC2	THongC2	T32A142	T31A120
	VAQ-B		TArgC2	THongC2		
FU-B			TArgC2	THongC2		T31A120
FN-B		T89A19	TArgC2			
PAZ 2		T89A19			T32A142	

CONCLUSION

The results presented above suggest the occurrence of at least 5 *Phoma* pathotypes. These results require further corroboration and developments through the investigation of a larger number of differential hosts and isolates. Plant breeders might then use the pathotypes thus characterized for the rapid and reliable testing of their genetic material vs *Phoma*.

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INTERACCION DE *Phoma macdonaldii* BOEREMA / *Helianthus annuus* L.

RESUMEN

Phoma macdonaldii, agente causal de la rona negra del tallo de girasol, responsable para los danos cualitativo y cuantitativo que pueden llevar a la perdida de 60% de rendimiento, en Francia y en el mundo. Fue creado un nuevo metodo de laboratorio para la infeccion artificial temprana, por el cual se investiga la tolerancia de genotipos del girasol a este patogeno. Era importante determinar la conveniencia del metodo por el control del efecto posible de la fase de crecimiento de la planta con respecto a su reaccion al patogeno. Seis genotipos del girasol, conocidos segun diversos niveles de sensibilidad a *Phoma*, fueron infectados artificialmente por un aislado agresivo en cuatro fases de crecimiento: fase de cotiledon, 3-6 pares de hojas, brotacion y fase temprana de florecimiento. El analisis de datos ha indicado que no existe la interaccion entre la fase de crecimiento y los genotipos: el metodo recién creado hace notar las diferencias entre los genotipos en las fases tempranas y las relaciones establecidas quedan invariables tambien en las fases posteriores. Considerando estos datos asi como la grande variabilidad genetica del patogeno *Phoma macdonaldii*, hemos investigado la interaccion de variedad-genotipo utilizando los aislados de Francia y otros paises con la intencion de encontrar patotipos posibles. A este efecto, diez aislados de *Phoma* considerados de representar la variabilidad del hongo con respecto a la agresividad, origen geografica, y a los tipos de sintomas, fueron investigados en diez diversas lineas del girasol. Los resultados preliminares indican la existencia de cinco patotipos. Los seleccionadores pueden utilizar esos patotipos para las pruebas rapidas y ciertas referentes a la tolerancia de su material genetico a *Phoma*.

INTERACTION *Phoma macdonaldii* BOEREMA/*Helianthus annuus* L.

RÉSUMÉ

Le *Phoma macdonaldii*, cause de la moisissure noire de la tige du tournesol est responsable de dégradations de qualité et de quantité qui peuvent conduire à une perte de rendement de 60% en France et dans le monde entier. Une méthode d'inoculation précoce a été mise au point en laboratoire pour tester la tolérance du génotype de tournesol envers ce pathogène. Il était important de vérifier si la méthode était adéquate en évaluant l'incidence possible du stade de croissance de la plante sur sa réaction au *Phoma*. Six génotypes de tournesol, connus pour leur plus ou moins grande susceptibilité au *Phoma*, ont été inoculés d'isolat agressif à quatre différents stades de croissance: phase du cotylédon, 3-6 paires de feuilles, bourgeonnement et phase précoce de floraison. L'analyse des données a montré qu'il n'y a pas d'interaction entre le stade de croissance et le génotype: la méthode nouvellement créée montre des

différences entre les génotypes dans les phases précoces et les liens établis ne changent pas dans les phases ultérieures. Tenant compte de ces données et de la grande variabilité génétique du *Phoma macdonaldii*, nous avons étudié l'interaction des génotypes au moyen d'isolats de France et d'autres pays dans le but de trouver de possibles pathotypes. Dans ce but, dix isolats de *Phoma*, considérés comme étant représentatifs de la variabilité du champignon du point de vue de l'agressivité, de l'origine géographique et des types de symptômes ont été testés sur dix différentes lignes de tournesol. Les résultats préliminaires suggèrent l'existence de cinq pathotypes. Les sélectionneurs devraient donc utiliser ces pathotypes pour tester rapidement et avec efficacité la tolérance de leur matériel génétique au *Phoma*.

