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PATHOTYPES OF SUNFLOWER DOWNY MILDEW IN SOUTHERN PARTS OF GERMANY

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SUMMARY

During the past six years the occurrence of *Plasmopara halstedii* and the regional distribution of its pathotypes in the southern parts of Germany was investigated. More than 50 isolates, representing fields of all regions relevant for sunflower production in the State of Baden-Württemberg, two areas in Bayern and one locality in Hessen were collected. Pathotype characterization, based on sunflower differential lines and evaluated according to a triplet code system, indicated the existence of at least five pathotypes. Among them, pathotype 730 (former race 4) dominated by far, frequently accompanied by 710 (former race 8), while pathotypes 330 (former race 7 or 9), 310 (former race 6) and 300 (former race 2) were only sporadically present. Studies on single spore isolates selected from certain field samples mostly documented the existence of more than one pathotype per field.

Key words: ***Helianthus annuus*, *Plasmopara halstedii*, pathotype spectrum in Germany, sunflower downy mildew**

INTRODUCTION

Within last 15 years the commercial production of sunflower in Germany, though still a relatively marginal crop in this country, increased significantly. Thus, the total area of sunflower cultivation expanded from 8,000 ha in the late 80's to approximately 40,000 ha towards the end of the millenium (FAO, 1998). About 80% of these areas are located in the States of Baden-Württemberg, Bayern and Brandenburg (Saatenunion, 1998).

P.halstedii, a worldwide major disease of sunflower, was first observed in Germany in 1986 (Spring *et al.*, 1991). Until the year 1994 the pathotypes 1, 4, and 5 were identified (Spring *et al.*, 1994). Since that time, intensified studies on the distribution and pathotype spectrum of *P.halstedii* in southern Germany were performed, the results of which will be reported here.

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MATERIALS AND METHODS

In order to collect field isolates of *P.halstedii*, areas of sunflower cultivation throughout Baden-Württemberg and Bayern were checked during the past six years. One additional sample derived from a field in Groß-Gerau, Hessen (sporangia provided by Dr. L. Brahm, University of Gießen). Leaves from systemically infected plants in the field were harvested and kept in darkness at 16°C in 100% humidity for 12 h for the induction of sporulation. Freshly developed sporangia were used to secure the isolates by transferring them to generally susceptible sunflower seedlings (HA-821) cultivated in a climate chamber as described earlier (Spring *et al.*, 1997).

Pathotype characterization based on sunflower differential lines with varying resistance. Classification was carried out according to the triplet code system proposed by Gulya (1995). The following nine sunflower differential lines were used:

set 1 HA-821 (universal susceptible), RHA-266, RHA-274;

set 2 DM-2, 799-2, 803-1;

set 3 HA-R4, HA-R5, HA-335.

In contrast to the lines proposed by Gulya (1995), RHA 265 was replaced by RHA-266, and PM-17 was replaced by 799-2, due to the unavailability of seed material. Both lines show the identical resistance type as the replaced lines.

Twenty-five pre-germinated seedlings per differential line were planted in soil and inoculated with 10,000 sporangia per seedling according to the soil drenching-method suggested by Goosen and Sackston (1968). Plants were grown in a climate chamber (16°C; 80% humidity; 14 h photoperiod). Evaluation of the tests was performed 14 days after inoculation. Plants with stunting symptoms and sporulation on cotyledons and true leaves were classified as susceptible. Plants with cotyledon-limited-infection (CLI) were interpreted as moderately resistant and were finally added to the symptomless resistant plants.

In order to examine pathotype homogeneity of field isolates, additional tests were performed using single-spore strains gained from certain samples. Single-spore strains were established through leaf disk infection with micromechanically selected zoospores according to a recently described method (Spring *et al.*, 1998).

RESULTS

Distribution of *P.halstedii* in Southern Germany

Commercial sunflower cultivation in Germany is climatically restricted to the southern parts of the country and certain areas in east Germany. Within the past six years, fields from all important sunflower regions in south Germany were checked with respect to the occurrence of sunflower downy mildew (Figure 1). In the State of Baden-Württemberg, this comprised in particular the areas of the upper

Rhein valley (A), the Bodensee region (B), the Neckar valley in the upper parts around Tübingen (C) and the lower parts north of Stuttgart (D). In the State of Bayern samples were collected from fields in the basin around München (E) and near Würzburg (F). One additional sample of *P.halstedii* derived from a locality at Groß-Gerau (G) in the State of Hessen.

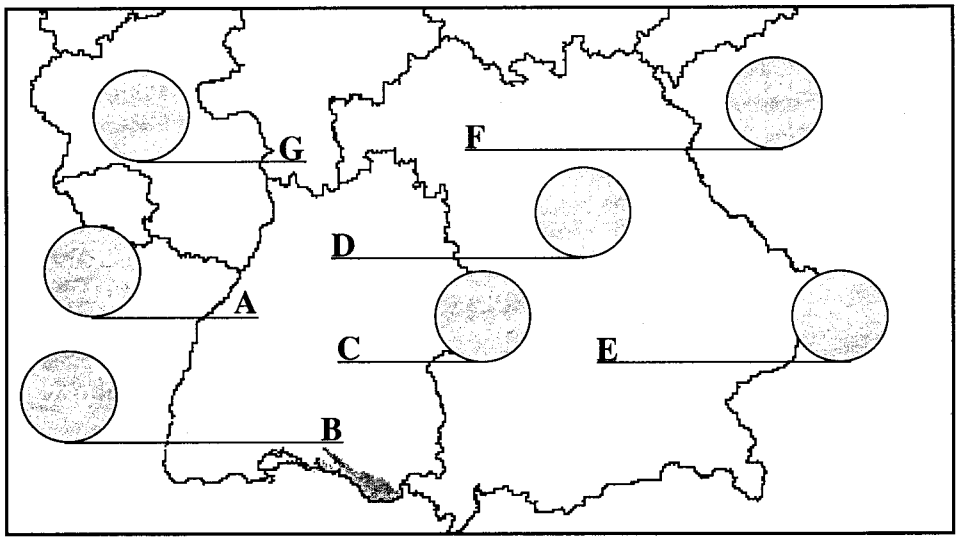

Figure 1: Distribution of P.halstedii and identified pathotypes in major areas of sunflower cultivation (A-G) of southern Germany: A=Upper Rhein valley, B=Bodensee region, C=Upper Neckar valley, D=Region north of Stuttgart, E=München, F=Würzburg, G=Groß-Gerau.

Out of 136 fields analyzed, 127 were commercial oilcrop fields, whereas the remaining nine were planted to sell ornamentals (Table 1). Infected plants were detected in 66 fields, a rate of almost 50%. Infection with downy mildew was found to be distributed in all investigated regions, and it appears noteworthy that the pathogen was present in all fields used for the cultivation of ornamentals. Overall, a total number of 54 isolates was collected, 27 of which were characterized for pathotype specificity.

Infection rates

The infection rates found were correlated to the mode of cultivation practiced in Germany (Table 2). Conventionally treated fields with metalaxyl-protected seeds showed generally low rates of infected plants (ranging from single affected plants up to 5%). However, significantly higher infection rates in the same fields (with up to 30% incidences) were occasionally found in those plants which grew up between the sown rows. Such individuals most likely derived from uncontrolled seeds, left over from a previous harvest and stored in the soil until plowing accidentally brought

them up again to the surface where they germinated. Such plants, since unprotected by fungicide, are readily susceptible for soil-borne downy mildew infection and appear to represent a major source of secondary infection for the surrounding plants. Generally high rates of infection (5-30%) were also found in fields of biologically controlled farming where fungicide treatment was prohibited. However, the highest rates of downy mildew infection were reached in fields of ornamental cultivation, where up to 70% of the sunflower plants were affected in one case. The latter appears to be due to the lack of sufficient crop rotation, to the use of low quality seeds as well as to the lack of fungicide treatment.

Table 1: Downy mildew incidences in major areas of sunflower production of southern Germany between 1994 and 1999

Sunflower-producing area		No. of investigated fields	No. of fields with system. infected plants	No. of collected field isolates	No. of field isol. characterized for pathotypes
State of Baden-Württemberg					
A	Upper Rhein valley	21	12	11	4
B	Bodensee region	8	4	4	1
C	Upper Neckar valley	18	12	5	2
D	Stuttgart	65	27	24	14
State of Bayern					
E	München	13	5	4	1
F	Würzburg	10	5	5	4
State of Hessen					
G	Groß-Gerau	1	1	1	1
Total		136	66	54	27

Spectrum of pathotypes and geographic distribution

A total of 27 field isolates from the areas A-G were characterized for pathotype by means of sunflower differential lines (Table 2). Employing the triplet code system suggested by Gulya (1995), 20 of the field isolates (75%) showed virulence pattern 730 (former race 4), while 5 (20%) were identified as pathotype 710 (former race 8) and the remaining two (5%) were characterized as pathotype 330 (former race 7 or 9). Consequently, the dominant pathotype was present in all investigated areas A-F, mostly accompanied by pathotype 710 (found in areas A, C-F). In contrast, pathotype 330 showed only sporadic distribution in two fields which are located about 150 km from each other. It appears noteworthy that both isolates derived from fields which were used for the cultivation of ornamentals. In one case, oilcrop fields only 100 m apart were infected with pathotype 730.

Since strains of weaker virulence in mixed samples of more than one pathotype may be overseen in such differential tests, additional experiments were carried out using single-spore isolates. Such tests, performed with the field isolates Ph3-95 (A), Ph1-94 (A), Ph5-96 (F) and Ph1-97(G) revealed the occurrence of more than one pathotype in three out of four cases. Thus, pathotype 710 was found to be inter-

Table 2: Virulence patterns of field isolates collected in southern Germany (1994 - 1999)

Field isolate	Region	Infection rates	Sunflower differential lines									Virulence pattern	Former race	Virulence patterns of single spore strains
			HA-821	RHA-266	RHA-274	DM-2	799-2	803-1	HA-R4	HA-R5	HA-335			
Ph3-95	A	+	S	S	S	S	S	R	R	R	R	730	4	730+710
Ph13-95	A	+	S	S	S	S	S	R	R	R	R	730	4	
Ph2-97	A	++	S	S	S	S	S	R	R	R	R	730	4	
Ph1-94	A	+	S	S	S	S	S	R	R	R	R	730	4	730
Ph21-95	B	++	S	S	S	S	S	R	R	R	R	730	4	
Ph5-95	C	+	S	S	S	S	R	R	R	R	R	710	8	
Ph8-97	C	+++ (*)	S	S	S	S	S	R	R	R	R	730	4	
Ph1-95	D	++	S	S	S	S	S	R	R	R	R	730	4	
Ph7-97	D	+++ (*)	S	S	S	S	R	R	R	R	R	710	8	
Ph1-98	D	+++ (#)	S	S	S	S	S	R	R	R	R	730	4	
Ph4-98	D	+	S	S	S	S	S	R	R	R	R	730	4	
Ph6-98	D	+	S	S	S	S	R	R	R	R	R	710	8	
Ph7-98	D	+	S	S	S	S	S	R	R	R	R	730	4	
Ph8-98	D	+	S	S	S	S	S	R	R	R	R	730	4	
Ph9-98	D	++++ (*)	S	S	R	S	S	R	R	R	R	330	7 or 9	
Ph10-98	D	+	S	S	S	S	S	R	R	R	R	730	4	
Ph13-98	D	+++ (*)	S	S	S	S	S	R	R	R	R	730	4	
Ph1-99	D	+++ (#)	S	S	S	S	S	R	R	R	R	730	4	
Ph2-99	D	+++ (#)	S	S	S	S	S	R	R	R	R	730	4	
Ph3-99	D	+++ (#)	S	S	S	S	R	R	R	R	R	710	8	
Ph4-99	D	+++ (#)	S	S	S	S	S	R	R	R	R	730	4	
Ph16-95	E	+	S	S	S	S	S	R	R	R	R	730	4	
Ph1-96	F	++	S	S	S	S	S	R	R	R	R	730	4	
Ph3-96	F	+	S	S	S	S	R	R	R	R	R	710	8	
Ph4-96	F	+	S	S	S	S	S	R	R	R	R	730	4	
Ph5-96	F	+	S	S	S	S	S	R	R	R	R	730	4	730+710
Ph1-97	G		S	S	R	S	S	R	R	R	R	330	7 or 9	330+310+300

(*) cultivation of ornamentals (without metalaxyl treatment); (#) fields of biologically controlled farming (without metalaxyl treatment)
Infection rates classified as: +<1%; ++ 1-5%; +++ 5-30%; ++++> 30%

mixed with 730 in two samples (Ph3-95 (A) and Ph5-96 (F)), and the field isolate Ph1-97 (G) revealed to be a mixture of the pathotypes 330, 300 (former race 2) and 310 (former race 6). In that way, the final number of different pathotypes detected in Southern Germany during this study raised up to five.

DISCUSSION

P.halstedii, since its first reported occurrence in 1986 (Spring *et al.*, 1991), has successfully invaded all major areas of sunflower cultivation in south Germany. Although no comparable data yet exist from the regions in east Germany, the presence of the pathogen in the remaining areas was observed in past years (I. Gröne, Südwestdeutsche Saatzucht; personal communication). In general, infection rates were still found to be very low, so that farmers are mostly not aware of a disease problem. Phytosanitary measures like crop rotation, the use of controlled seeds and particularly the treatment with metalaxyl kept economic impact still at a low level. Problems with fungicide resistance, as recently reported from France (Albourie *et al.*, 1998) could not be observed, although downy mildew incidences were found in about 50% of the metalaxyl-treated fields. Such infections could either be due to incomplete fungicide-coating of single seeds, or to secondary infections through zoosporangia of uncontrolled plants. The latter was formerly reported to be a very rare source for the systemic type of infection (Cohen and Sackston, 1973). However, our recent observations have shown numerous incidences of late systemic infections acquired by zoospores via local leaf infections under favorable weather conditions (O. Spring, personal communication).

A completely different situation is given in fields where fungicide treatment is either prohibited or considered to be unnecessary. Infection rates of up to 30% were found and will force farmers or gardeners to reconsider their mode of sunflower cultivation for the coming years.

In contrast to a former study (Spring *et al.*, 1994) which reported the pathotypes 1, 4 and 5 in Germany (equivalent to pathotype 100, 730 and 770 of the triplet code nomenclature), our current investigation resulted in the identification of pathotypes 730, 710, 330, 310 and 300, while 100 and 770 could not be observed again. A similar phenomenon has recently been reported from France, where the French pathotypes C and D (Lafon *et al.*, 1996) could not be redetected in a following investigation (Penaud, 1998). Such variation of the pathotype spectrum, on the one hand, could be due to the increased use of sunflower cultivars, resistant to low virulent pathotypes like pathotype 100 (former race 1). Hence, decreasing proportions of pathotype 100 were recently reported from other European countries as well (Kormany and Viranyi, 1997; Maširević, 1998). On the other hand, modifications introduced to the differential test (e.g., the use of new lines or differences in the evaluation of cotyledon limited infections) may be sources of apparent pathotype variation. Finally, experiments with single-spore isolates documented that pathotypes may easily be overseen in nonhomogeneous field samples, when the most virulent strain dominates and masks the infection pattern caused by the rest.

In comparison with the pathotype spectrum known from other European countries, the situation found in Germany was not surprising. All pathotypes detected in this study were formerly identified in other localities in Europe. So 730 is known from Bulgaria, Hungary, Spain and Yugoslavia, and pathotypes 710, 330, 310 and 300 occur in Hungary, Romania and Spain (Maširević, 1998; Molinero-Ruiz *et al.*, 1998; Viranyi and Gulya, 1995). Together with 100, 701, 732, 733 (Gulya *et al.*, 1996), the French types A to C (Mouzeyar *et al.*, 1994; Lafon *et al.*, 1996) and the new subtypes 3103, 7003, 7033 and 7133 (Maširević, 1998; Molinero-Ruiz *et al.*, 1998), not less than 16 varieties of *P.halstedii* currently endanger sunflower cultivation in Europe.

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PATOTIPOS DEL MILDIU DE GIRASOL EN LAS PARTES MERIDIONALES DE ALEMANIA

RESUMEN

Durante los últimos seis años fueron estudiadas la aparición del hongo *Plasmopara halstedii* y la distribución regional de sus patotipos en Alemania del Sur. Fueron coleccionados más de 50 aislados que representaban todas las regiones importantes para la producción en la provincia de Baden-Württemberg, dos áreas en la provincia de Bayern y un lugar en la provincia de Hessen. La caracterización de patotipos, hecha a base de lineas de girasol diferenciales y del triple sistema de claves, ha siñalado la existencia de cinco patotipos al minimo. El patotipo 730 (previamente raza 4) era mucho más dominante y muchas veces unido con el patotipo 710 (previamente raza 8), mientras los patotipos 330 (previamente raza 7 o 9), el patotipo 310 (previamente raza 6) y el patotipo 300 (previamente raza 2) eran solo en parte presentes. El estudio de esporas singulares aisladas de ciertas muestras del campo ha confirmado en general la existencia de más de unu patotipo por campo.

TYPES PATHOGÈNES DU MILDIOU DU TOURNESOL EN ALLEMAGNE MÉRIDIONALE

RÉSUMÉ

L'apparition de *Plasmopara halstedii* et la distribution régionale de ses types pathogènes a été observée en Allemagne méridionale au cours des six dernières années. Plus de 50 isolats ont été recueillis qui représentaient toutes les régions significatives pour la production du tournesol dans l'État de Baden-Württemberg, deux régions de l'État de Bavière et une localité dans l'État de Hessen. La caractérisation des types pathogènes, basée sur des lignes différentielles de tournesol et un système de code triple a montré qu'il existait au moins cinq types pathogènes. Le type 730 (anciennement race 4) dominait de loin les autres et était souvent accompagné du type 710 (anciennement race 8), alors que les types 330 (anciennement race 7 ou 9), le type 310 (anciennement race 6) et le type 300 (anciennement race 2) n'étaient que sporadiquement présents. L'observation de spores isolats individuels sélectionnés parmi certains échantillons de champ ont établi l'existence de plus d'un type pathogène par champ.