European Journal of Plant Pathology **108:** 593–596, 2002.

Short communication

Mating relationships between isolates of *Phaeosphaeria nodorum*, (anamorph *Stagonospora nodorum*) from geographical locations

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Accepted 8 May 2002

Key words: mating protocol, mating type, sexual reproduction, sterility, wheat glume blotch

Abstract

Mating types of *Phaeosphaeria nodorum* isolates (from North Africa, North America, Australia, Europe and Near East) were determined in laboratory conditions. Although both mating types were found, MAT1-1 and MAT1-2 were not evenly distributed among the isolates. Sexual compatibility pairings with standard mating type testers revealed that 56 of 101 isolates could be assigned to MAT1-1 and 5 to MAT1-2. Although the teleomorph occurred in different countries, the two mating types were observed only among isolates collected from France, Great Britain, Germany and Morocco. For Morocco, where no *P. nodorum* pseudothecia have been reported, this is the first report of the existence of the two opposite mating types. The remaining 40 isolates could not be designated to a specific mating type. However, in 17 crosses, pseudothecial initials or sterile pseudothecia were observed on wheat straw after two months. The implications of the predominance of one mating type are discussed.

Stagonospora nodorum (Berk.) Castellani and Germano (syn. *Septoria nodorum* Berk.), the anamorph of *Phaeosphaeria nodorum* (Müll.) Hedjar. (syn. *Leptosphaeria nodorum* E. Müll.), is an important leaf spot and glume blotch pathogen with a large geographical distribution. The sexual stage of the life-cycle takes place on wheat stubble during autumn and winter. The sexual stage has been induced in the laboratory and is controlled by a single mating type locus with two alleles similar to that in other heterothallic filamentous ascomycetes (Halama and Lacoste, 1991). The present work reports the results of attempts to induce mating between isolates of *P. nodorum* from different geographical locations and the mating testers.

The mating testers A/5 (MAT1-2) and 6/T (MAT1-1) were described previously (Halama and Lacoste, 1991; Rapilly et al., 1992). The isolates were provided from different culture collections maintained by scientists working on glume blotch of wheat. Among the French isolates, 14 were collected from the north of France. Of the 14 isolates from the USA, nine originated from Oregon and five from Texas. For Moroccan isolates, 12, 7 and 6 isolates came from Khenifra, Oued-Zem and Chefchouen, respectively. For the other countries, the precise origins are unknown. Conditions to obtain pseudothecia were described previously (Halama and Lacoste, 1992b). Sexual crosses were set up between the highly fertile reference mating type strains 6/T (MAT1-1), A/5 (MAT1-2) and a series of 101 isolates. The pseudothecia obtained after two months of incubation were distinguished from pycnidia by the absence of cirrhi and by their dark aspect. For each successful cross, pseudothecia were crushed and observed microscopically to confirm their fertility by observing the presence of mature asci containing ascospores. Isolates were crossed in replicate. When initial crosses with the two mating testers were sterile, experiments were repeated.

The mating ability of *S. nodorum* isolates originating from various countries was examined (Table 1). None of isolates produced fertile pseudothecia when grown alone. Mature pseudothecia with asci and ascospores developed in 61 pairings of the 202 crosses

Table 1. Origin and mating type of isolates after crosses with *Phaeosphaeria nodorum* mating tester isolates (6/T: MAT1-1; A/5: MAT1-2)

Country	MAT1-1		MAT1-1?[b] (No. of isolates)	MAT1-2		MAT1-2?[b] (No. of isolates)	Sterile isolates[c]	Total isolates
	No. of isolates	Pseudothecia production[a]		No. of isolates	Pseudothecia production[a]			
France	17	3.3	5	2	1.5	0	3	27
Switzerland	4	3.7	0	0	0	2	2	8
Germany	4	3.7	6	1	1	0	3	14
Great Britain	2	3.5	1	1	2	0	1	5
Sweden	1	4	0	0	0	0	0	1
Finland	1	1	0	0	0	0	0	1
Morocco	15	2.1	0	1	1	0	9	25
Australia	2	1.5	0	0	0	2	0	4
U.S.A.	9	3.6	0	0	0	1	4	14
Israël	1	1[*]	0	0	0	0	1	2

[a] Scale of pseudothecia production (from 1 (low production) to 5 (high production)); pseudothecia production of cross 6/T × A/5: 5.

[b] Formation of pseudothecial initials after incubation of two months.

[c] No pseudothecia formation after incubation of two months.

[*] Pseudothecia abnormal.

attempted. One-hundred and forty-one pairings failed to produce pseudothecia. None of the isolates formed pseudothecia with both testers, supporting the model of 2-allele heterothallism demonstrated previously with a smaller number of crosses (Halama and Lacoste, 1991). Seventeen of the 141 sterile pairings produced pseudothecial primordia comparable with a previous report (Halama et al., 1992). These 17 isolates were tentatively designated MAT1-1? or MAT1-2? The time required for pseudothecia to form did not vary among the crosses, but there were differences in the resulting densities of fertile pseudothecia. The number of pseudothecia formed varied from 1 to 20 per replicate and per cross. No correlation was observed between pseudothecial densities and conidiogenesis of isolates. All fertile isolates except one produced pseudothecia containing abundant asci with 8 ascospores. The isolate SAAD 88, originating from Israel, produced pseudothecia which differed from normal mature pseudothecia; their size was reduced and a few asci were observed with less than the 8 ascospores typically found in the asci. Isolates of both mating types were found in France, Germany, Great Britain and Morocco. Where both mating types of *P. nodorum* were found among isolates, one mating type (MAT1-1) occurred more frequently (92% of the mating types identified). Including the crosses producing pseudothecial initials, isolates designated as MAT1-2? occurred also in Switzerland, Australia and USA. For these last countries and for the others (Sweden, Finland and Israel), where no fertile crosses were obtained, a larger sampling would be necessary to confirm the absence or presence of the two mating types and their frequencies.

This study demonstrates that crossing protocols are valid for examining the mating ability of isolates from different locations. Where both mating types of *P. nodorum* were found, MAT1-1 occurred more frequently. The present report supports results of previous studies demonstrating unequal frequencies of mating types in other fungi including *Sphaerotheca fuliginea* (McGrath et al., 1996) and *Gibberella fujikuroï* (Elmer, 1995). The unequal distribution of *P. nodorum* mating types described above was unexpected. Indeed, this result suggests that sexual reproduction in nature would be affected by distribution and frequency of mating types. However, as previously stated, the sexual form *P. nodorum* occurred in many different countries. Our results reveal that both mating types are present among Moroccan isolates even though pseudothecia have yet to be reported in this country. In contrast, only MAT1-1 was found exclusively among the USA isolates although pseudothecia have been observed (Scharen and Sanderson, 1982).

It has been known for some time that the sexual stage of *P. nodorum* occurs in nature, but it has not been clear whether the sexual form makes substantial contributions to levels of primary inoculum in comparison with asexual inoculum. The occurence of *P. nodorum* pseudothecia in the field is limited to autumn and winter according to the optimal temperature condition (10 °C) of the crossing protocol (Halama and Lacoste, 1992b). Recent work on *P. nodorum* utilizing molecular biology technologies indicated a significant impact of sexual reproduction on the genetic structure of *P. nodorum* populations (McDonald et al., 1994; Keller et al., 1997; Caten and Newton, 2000).

Among the 101 isolates, 40 failed to produce pseudothecia after crosses with both mating testers. This result is in agreement with previous observations after pair-wise crosses between 8 single-ascospore strains from 3 tetrads (Halama, 1992). In that study, some of the intergroup mating type crosses did not form fertile pseudothecia as expected in usual bipolar heterothallism in spite of optimal conditions to produce pseudothecia (Halama, 1992). Unexpected sterile crosses were also reported in *Magnaporthe grisae* (Notteghem and Silué, 1992). There are several possible reasons for the failure to induce the sexual stage between certain isolates and the two mating testers. Isolates may have become infertile during culture. Indeed, a decrease in fertility and in conidiogenesis was sometimes observed among *P. nodorum* isolates after subculture (Halama and Lacoste, 1992a). Within the same species, certain mutant genotypes are characterized by impaired fertility (Perkins, 1994). This sterility might be attributed to mutations (Leslie and Raju, 1985) or to the absence of genes, other than the MAT genes, that are required for the sexual process (Sharon et al., 1996). Such factors might also explain the variation of fertility among compatible crosses, reflected in variations in the numbers of pseudothecia produced.

In certain intercrosses which have been called 'sterile', we observed pseudothecial initials comparable with previous observations on pseudothecial development (Halama et al., 1992) or sterile pseudothecia as they exist in cultures of single-ascospore strains cultivated on their own (Halama, 1992). No ascogenous development (ascogenous hyphae, asci and ascospores) was observed thus allowing the use of the term 'sterile' for these structures. In heterothallic species of the Pyrenomycetes, further development of the ascogonium into a mature fruiting body does not occur until fertilization. In contrast, in Loculascomycetes such as *P. nodorum,* the envelopment of the ascogonium by sterile hyphae can occur in the absence of fertilization (Glass and Nelson, 1994). For the cross with the SAAD 88 isolate in which pseudothecia producing few ascospores were formed, the mating ability between the mating type tester and this isolate is reduced.

The issue of specificity in the wheat-*S. nodorum* pathosystem has been discussed extensively in the literature. Unlike *S. tritici*, specificity in the wheat-*S. nodorum* pathosystem is much less distinct. Studies have demonstrated differential interactions (Allingham and Jackson, 1981), limited interactions (Fried and Meister, 1987) or non-significant interactions (Scharen et al., 1985). An earlier study on aggressiveness of *P. nodorum* after sexual reproduction showed more statistically significant isolate × cultivar interactions for MAT1-1 than for MAT1-2 (Rapilly et al., 1992). This result supports the evidence that the outcome of a study can be affected by the *P. nodorum* isolate selected and consequently more research is needed to estimate the natural frequency of the two mating types.

Because of the limited number of isolates in this study, the ratio of the two mating types is perhaps greatly influenced by sampling. More mating experiments are needed, in particular with a sampling scheme to collect isolates from different countries, to confirm or refute the unequal distribution of mating types in the field. A survey of mating abilities in *P. nodorum* populations from different countries and regions is needed as a basis for predicting the extent of sexual reproduction, to elucidate the role of ascospores in the epidemiology of the disease and the role of sexual recombination in the variation observed among *S. nodorum* isolates.

Acknowledgements

I thank C. Caten, G.M. Hoffman, T. Hunter, R. Loughman, B. Mc Donald, A. Obst, A. Ramdani, F. Rapilly and M. Skajennikoff for providing isolates. The author expresses thanks to Miss Céline Rauch and Miss Sylvie Bayart for comments on the English.

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