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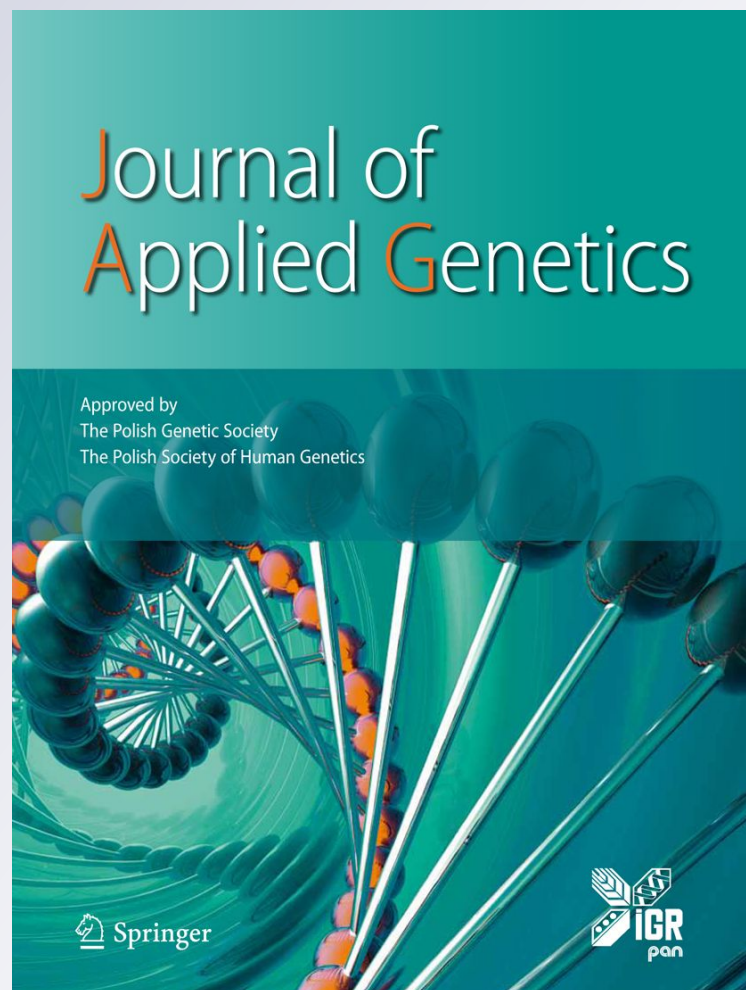
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## Mating behavior of a Northern Italian population of *Fusarium verticillioides* associated with maize

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**Abstract** *Fusarium verticillioides*, the most common causal organism of Fusarium stalk and ear rot of maize in Northern Italy, produces important mycotoxins such as fumonisins. Reproductive biology of *F. verticillioides* has been widely studied in numerous maize growing areas, but up to now no information is available on the mating behavior and genetic structure of this plant pathogen in Italy. Mating type and female fertility distribution and effective population number,  $N_e$ , were assessed for a population of 181 *F. verticillioides* strains isolated from three fields located in Lombardia region (Northern Italy) during 2007–2008 maize growing season. The ratio of *MAT-1*:*MAT-2* was significantly different from the theoretical 1:1 ratio expected in an idealized population in which individuals mate at random. The frequency of hermaphroditic strains was 20 % of the total population.  $N_e$  for mating type was 89 % of the count (total population) and the  $N_e$  for male or hermaphrodite status was 55 %. The number of isolates that can function as the female parent limited  $N_e$  in the examined population. Under equilibrium cycle, assuming that female fertility has been lost due to selection and mutation rate during asexual reproduction, sexual reproduction needed to occur only once per 40 to 118 asexual generations to maintain this level of sexual fertility.

**Keywords** Female fertility · *Gibberella moniliformis* · Mating type

*Fusarium verticillioides* (Sacc.) Nirenberg [synonym *Fusarium moniliforme* Sheldon, teleomorph *Gibberella moniliformis* Wineland synonym *Gibberella fujikuroi* (Sawada) Ito in Ito & Kimura mating population A], a heterothallic fungus belonging to the *Sordariomycetes* Erikss. & Winka, greatly affects maize production in temperate areas, such as Lombardia, the main corn producing region located in Northern Italy, and represents a potential threat to human and animal health due to its ability to produce fumonisins, a well-known group of harmful mycotoxins (Munkvold 2003).

Sexual reproduction in *F. verticillioides* requires the presence of two genetically distinct fungal isolates carrying alternate forms of mating types, referred to as mating types *MAT-1* and *MAT-2* (Kerényi et al. 1999). In addition, the male/female/hermaphroditic nature of the mating participants for successful sexual crosses is an important feature in *F. verticillioides*. The extent of sexual reproduction and its contribution to primary inoculum greatly influences both the evolutionary potential and epidemiology of numerous fungal plant pathogens. The frequencies of the two mating types and of male/hermaphrodite polymorphism in field population affect the amount of sexual crossing that can occur within a population. In fungal field populations, due to possible mutation or selection, the repeated asexual cycles reduce the frequency of hermaphrodites leading to a loss of female fertility. Reduction in female fertility can shift species to a mitosporic form which in turn has less adaptive advantages toward the selection pressure. The effect of genetic drift in the loss of female fertility during sexual reproduction can be estimated from the effective population number,  $N_e$  (Leslie and Klein 1996). Moreover,  $N_e$  is usually used to evaluate populations when mating is not randomly distributed and when individual members of the population do not contribute equally to the gene pool of

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the progeny (Caballero 1994). The mating behavior of *F. verticillioides* populations isolated mainly from cereals has been studied in several areas of the world (Chulze et al. 2000; Cumagun 2007; Danielsen et al. 1998; Leslie and Klein 1996; Mansuetus et al. 1997; Reynoso et al. 2006). To the best of our knowledge, no detailed information is currently available on the genetic structure of *F. verticillioides* populations in Italy as well as in other European maize growing areas, apart from the mating type assessment carried out by Moretti et al. (2004) on 24 Italian strains, isolated from maize plants showing ear and stalk rot. The objectives of this study were (1) to determine the mating types within a population of *F. verticillioides* isolated from maize in Lombardia, (2) to calculate the relative proportion of *F. verticillioides* female-sterile and hermaphroditic strains, and (3) to estimate the  $N_e$  for the examined *F. verticillioides* population.

The present investigation was carried out on 181 strains of *F. verticillioides*, arbitrarily selected among the fungal population isolated during the 2007/2008 maize cropping season from three fields located in Lombardia (Venturini et al. 2011). Mating type idiomorphs of *F. verticillioides* strains were identified by sexual crossing procedures as described by Leslie and Summerell (2006). Sexual crosses were carried out on carrot agar with standard tester strains for *G. moniliformis* FGSC7600 (*MAT-1*) and FGSC7603 (*MAT-2*) as female parents (Fungal Genetics Stock Center, Kansas City, MO, USA) and field *F. verticillioides* isolates as male parents. A set-up with tester strains alone served as control. A cross was considered fertile when a cirrhous of ascospores oozing from a mature perithecium was observed 2–6 weeks after fertilization. Female fertility of the field isolates was determined in crosses in which the field isolates were used as the female parents and the standard testers as the male parents. All crosses were repeated twice. Difference in frequencies was tested for significance using

the chi-square test with a single degree of freedom (PAST software ver. 1.95; Hammer et al. 2001). The equations proposed by Leslie and Klein (1996) were used in order to calculate the  $N_e$ , based on the mating type ratio  $[N_{e(mt)}]$  and the relative frequency of female-sterile and hermaphrodite strains  $[N_{e(f)}]$ , and also the average number of asexual generations per sexual generation.  $[N_{e(mt)}]$  was determined by the equation  $N_{e(mt)} = (4 N_{MAT-1} N_{MAT-2}) / (N_{MAT-1} + N_{MAT-2})$ , where  $N_{MAT-1}$  was the number of *F. verticillioides* strains with *MAT-1* idiomorph and  $N_{MAT-2}$  was the number of strains carrying the other idiomorph *MAT-2*. This equation, first derived by Wright (1931) for diploids with two discrete sexes, is used to reduce population size of ascomycetes if both mating types are not equally frequent.  $[N_{e(f)}]$  was calculated by the equation  $N_{e(f)} = (4N^2N_h) / (N + N_h)^2$ , where  $N$  was the total number of *F. verticillioides* strains and  $N_h$  was the number of hermaphrodites. This equation is based on the observation that in field populations many isolates are fertile as males but not as females, due to the mutations and selection against hermaphrodites during vegetative propagation: if asexual reproduction is an important part in the life cycle of the fungus, female-sterile strains could dominate the population. Moreover, when such population reproduces sexually, the relative lack of hermaphrodites reduces the effective population size.

The average number of female sterile mutations per strains ( $M$ ) was calculated assuming a random distribution of the mutations and using the frequency of the hermaphrodites as the zero term in the Poisson distribution,  $e^{-M}(M^i/i!)$  where  $M$  was the mean number of mutations per strains,  $i$  was the number of mutations in a given class of strains. The hermaphrodites were the only members in the class  $i=0$ .

*MAT-1* and *MAT-2*, the mating type idiomorphs of the *F. verticillioides* population consisting of 181 strains isolated from maize in Northern Italy, segregated in a ratio 120:61 (Table 1). According to the chi-square test ( $P<0.001$ ), this

**Table 1** Female fertility and inbreeding effective numbers for *F. verticillioides* populations

| Mating ratio | $N_{fs}^a : N_h^b$ | Effective population number, $N_e$ |   | Origin        | Reference               |
|--------------|--------------------|------------------------------------|---|---------------|-------------------------|
|              |                    | Mating type, $N_{e(mt)}^c$         | Male/hermaphrodite dimorphism, $N_{e(f)}^c$ |               |                         |
| 120:61       | 145:36             | 89                                 | 55  | Italy         | this study              |
| 27:23        | 35:15              | 81                                 | 42  | Philippines   | Cumagun (2007)          |
| 129:74       | 98:105             | 93                                 | 90  | Argentina     | Reynoso et al. (2006)   |
| 23:47        | 62:8               | 88                                 | 37  | Argentina     | Chulze et al. (2000)    |
| 16:23        | 10:29              | 97                                 | 98  | Costa Rica    | Danielsen et al. (1998) |
| 59:17        | 39:37              | 69                                 | 88  | Tanzania      | Mansuetus et al. (1997) |
| 237:446      | 342:341            | 91                                 | 89  | United States | Leslie and Klein (1996) |

<sup>a</sup>  $N_{fs}$  is the number of female-sterile male-fertile isolates;

<sup>b</sup>  $N_h$  is the number of hermaphroditic isolates;

<sup>c</sup>  $N_{e(mt)}$  and  $N_{e(f)}$  are expressed as a percentage of the actual count.

ratio was significantly different from the theoretical 1:1 ratio expected in an idealized random mating population (Leslie and Klein 1996). Since *MAT* idiomorphs were not present at equal frequencies in the *F. verticillioides* population, asexual reproduction was likely to occur in the field isolates. However, the occurrence of the two opposite mating types in the *F. verticillioides* population implied that the population members were capable of sexual reproduction. Among the 181 strains examined, 36 isolates, accounting for the 20 % of the total *F. verticillioides* population, were female-fertile (Table 1). Among the 36 hermaphrodites, *MAT-1* and *MAT-2* segregated in a 25:11 ratio. To determine the population size in relation to the size of population mating at random, the effective population number based on mating type ratios [ $N_{e(mt)}$ ] and on the relative frequency of female-sterile and hermaphrodites strains [ $N_{eff}$ ] was estimated (Table 1).

The mean number of female sterility mutations per strains ( $M=1.6$ ) showed a decrease in the number of hermaphrodites after each sexual reproduction cycle.

The mating type ratio (120:61), different from the theoretical 1:1 ratio, resulted in a decrease of the effective population number to 89 % of the count (Table 1). The number of female-sterile strains (145 strains), representing 80 % of the total *F. verticillioides* strains, caused a reduction of the effective population number in comparison with the population size expected if the entire population strains had been hermaphrodite, precisely 45 % reduction of the count (Table 1).

The relative frequencies of sexual and asexual reproduction of *F. verticillioides* could be also inferred in this study from the assessment of the effective population number,  $N_e$ .  $N_e$  is mainly affected by two factors: mating type and female sterility. In *F. verticillioides*, like in other *Fusarium* species belonging to the *G. fujikuroi* species complex, female fertility was far more important in determining  $N_e$  than the relative proportions of strains belonging to different mating types (Leslie and Klein 1996). Even if in the Italian population *MAT-1* frequency was twofold the *MAT-2* frequency (120:61),  $N_e$  was still near 90 % of the total count.

On the other hand, female-sterile:hermaphrodite ratio equal to 145:36 led to a significant decrease of  $N_e$  to 55 % of the total count. Such decrease of  $N_{eff}$  of the examined *F. verticillioides* population indicated the dominant role of asexual recombination, selection and drift rather than the sexual recombination in determining the genetic variation. Leslie and Klein (1996) argued for the absence of sexual reproduction in local populations of *G. fujikuroi* species complex by the presence of female sterility mutations resulting in an increased vegetative propagation capability.

If the population was at equilibrium, data could also be used to estimate the range in which the percentage of

hermaphrodites could fluctuate and the relative number of asexual generations per sexual generation (Leslie and Klein 1996). Depending on the assumptions concerning the combined effects of mutation rate on female sterility and the selection against hermaphrodites during the asexual portion of the life cycle, the average number of asexual cycles per sexual cycle, varied from 40 to 118 in the examined population, and the frequency of hermaphrodites in such an equilibrium population could range from 10 to 45 % (observed value, 20 %). The number of female sterility mutations per strains obtained by applying the model of Leslie and Klein (1996) to the *F. verticillioides* population ( $M=1.6$ ), was higher than those calculated by Leslie and Klein (1996); Mansuetus et al. (1997) ( $M=0.7$ ) and Reynoso et al. (2006) ( $M=0.6$ ). The *F. verticillioides* population associated with maize grown in Northern Italy therefore seemed to be characterized by a high tolerance to female sterility mutations, during asexual reproduction, at which female fertility was lost. The comparison between the  $N_{eff}$  values obtained in the present study with those calculated by other Authors pointed out that the structure of the examined Italian population more closely resembles the relatively infertile Argentinean (Chulze et al. 2000) and Philippine populations (Cumagun 2007). Agricultural practices, such as tillage, under which maize was grown in the examined fields, were not likely to provide opportunity for sexual recombination, as already pointed out by Reynoso et al. (2006). Moreover, a wider sampling region should allow to isolate *F. verticillioides* strains which could show a major level of female fertility (Reynoso et al. 2006). This is the first contribution on mating behavior of *F. verticillioides* population in Italy and results suggest the need to further investigate by genotyping studies other Italian *F. verticillioides* populations in order to clarify the pattern of genetic variation within and between populations.

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