

## Female Fertility and Mating Type Distribution in a South African Population of *Fusarium subglutinans* f. sp. *pini*†

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*Fusarium subglutinans* f. sp. *pini* is the causal agent of pitch canker disease of pines. The initial occurrence of *F. subglutinans* f. sp. *pini* in South Africa was limited to a single nursery, and isolates from this population are capable of reproducing sexually. We determined the effective population number,  $N_e$ , of this population by using mating type and male/hermaphrodite polymorphisms as indicators. The effective population number for mating type,  $N_{e(mt)}$ , is 99% of the count (total population), and that for male/hermaphrodite status,  $N_{e(♂)}$ , is 42 to 46% of the count (total population). The number of strains that can function as the female parent limits the effective population number of this population. If this population is stable, then, depending upon assumptions about mutation and selection, sexual reproduction need occur only once per 26 to 153 asexual generations to maintain this level of sexual fertility.

*Fusarium subglutinans* (Wollenweber & Reinking) P. E. Nelson, Toussoun & Marasas f. sp. *pini* Correll et al., is the causal agent of pitch canker, a destructive disease of pines (2, 11) with a global distribution (2, 13). Species of *Fusarium* in the section *Liseola*, including *F. subglutinans*, generally belong to different mating populations (biological species) of *Gibberella fujikuroi* (Sawada) Ito in Ito & K. Kimura (6, 9). Within each heterothallic mating population, two strains of opposite mating type may cross, leading to the formation of a fertile perithecium with viable ascospores (6). Some isolates of *F. subglutinans* have been previously assigned to the B or the E mating population of *G. fujikuroi* (6). The isolates of *F. subglutinans* f. sp. *pini* that we examined belong to neither the B nor the E mating population of *G. fujikuroi* and appear to be a unique mating population (1).

Comparing the sizes of fungal field populations can be difficult. The effective population number ( $N_e$ ) provides an estimate of a population's size relative to a randomly mating population (1a, 14). In a population genetics context, effective population number is usually used in the evaluation of field populations because mating is often not random and because not all members of the population leave an equal number of progeny. The equations used to generate an effective population number depend on the constraints placed on the population by the reproductive strategy operating within it (1a). For heterothallic ascomycetes, equations that can be used to estimate effective population size are available (7). The relative frequencies of the mating type alleles and hermaphrodites affect the ease with which the population can pass through the sexual cycle. Maximum effective population sizes in these fungi occur when the mating type alleles are present in a 1:1 ratio

and all of the strains are self-sterile hermaphrodites. The objectives of this study were (i) to determine the frequency of the two mating type alleles in the presumed founder population of *F. subglutinans* f. sp. *pini* in South Africa, (ii) to determine the relative proportion of female-sterile and hermaphroditic strains within the population, and (iii) to determine the mating type and inbreeding effective population numbers for this population.

### MATERIALS AND METHODS

Eighty *F. subglutinans* f. sp. *pini* isolates were obtained from diseased *Pinus patula* Schlecht. & Cham. seedlings growing in the Ngodwana nursery in South Africa (12). This commercial pine nursery is approximately 12 ha in size and grows only pine seedlings. Samples were taken at random from infected plants scattered throughout the nursery, but no more than one isolate was taken per seedling. Isolates were purified through single conidial subcultures and then frozen in 15% glycerol at  $-70^{\circ}\text{C}$ .

Two of these 80 strains, FR 442 (mating type “-”) and MRC 6213 (mating type “+”) were previously selected as tester strains for this population due to their high level of female fertility (1, 3). Crosses were made on carrot agar (4) and V8 juice agar (10) by using the protocol of Klittich and Leslie (4). Crosses were first made by using the testers as the female parent and the unidentified strains as the male parent. Once the mating type had been identified, the roles were reversed and the appropriate mating type tester was used as the male. All crosses were successful on at least two different occasions. Crosses were examined weekly and scored positive when ascospore-oozing perithecia were observed. The ascospores from these crosses were checked for viability by streaking a portion of the ascospore cirrus on 2% water agar and estimating the percent germination the next day.

The equations used to estimate effective population are those of Leslie and Klein (7). The effective population number for mating type [ $N_{e(mt)}$ ] is determined by the equation  $N_{e(mt)} = (4 N_+ N_-)/(N_+ + N_-)$ , where  $N_+$  is the number of strains of the “+” mating type and  $N_-$  is the number of strains of the “-” mating type. This relationship was first described by Wright (14) for diploids with two sexes and in ascomycetes is used to reduce population size if both mating types are not equally frequent.

The inbreeding effective population number [ $N_{e(i)}$ ] based on the relative frequency of female-sterile and hermaphroditic strains is determined by the equation  $N_{e(i)} = (4 N^2 N_h)/(N + N_h)^2$ , where  $N$  is the total number of individuals and  $N_h$  is the number of hermaphrodites. This relationship is based on the observation that in field populations many isolates are fertile as males but not as females. In populations in which sexual reproduction is important, female-sterile strains that can function only as males are rapidly lost because of their selective disadvantage relative to the self-sterile hermaphrodites who can contribute gametes to both the male and female gamete pool for the next generation. If the population reproduces asexually for much of its life cycle, however, then it is possible for the female-sterile strains not only to persist but even to dominate a population. When such a population reproduces sexually, the relative lack of female-fertile

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strains limits the effective population size. This equation corrects for this limitation. If the mutation rate ( $\mu$ ) to female sterility and the selection ( $\theta$ ) against the hermaphrodites during asexual reproduction can be estimated, then the relative frequencies of sexual and asexual reproduction can also be estimated.

## RESULTS AND DISCUSSION

Of the 80 strains examined, 64 were cross-fertile with one of the mating type testers and could produce fertile perithecia containing ascospores with 80 to 100% germinability. Among the 64 fertile isolates, "+" and "-" mating types segregated 35:29, giving an  $N_{eff}$  of 99% of the count (total population). Thus, the relative frequency of the different mating types does not appear to be reducing the effective size of the population. This result is similar to that found for other populations of *G. fujikuroi* (7, 8).

Among the 64 fertile isolates, 17 were hermaphrodites and 47 were female sterile, giving an  $N_{eff}$  of 42% of the count. This calculation ignores the 16 strains that belong morphologically to *F. subglutinans* f. sp. *pini*, which can cause disease, and that belong to the same vegetative compatibility group as some of the fertile strains (13). If the sterile strains are included in the calculation as female-sterile strains, then  $N_{eff} = 46%$  of the count. Among the 17 hermaphrodites, "+" and "-" mating types were distributed in a 11:6 ratio, so there is no interaction bias that could affect the availability of a suitable female-fertile parent. These values for  $N_{eff}$  are lower than those reported for the A and D mating populations of *G. fujikuroi* and slightly higher than those reported for the F mating population (7, 8) which is now called *Fusarium thapsinum* (5). Our report is the first of any effective population numbers for *F. subglutinans*.

If the population is at equilibrium, then these data can also be used to estimate the range in which the percentage of hermaphrodites can fluctuate and the relative number of sexual generations per asexual generation (9). If only the fertile strains are used for this calculation, then the range for hermaphrodite frequencies is from 7 to 52% (observed value, 27%) and the average number of asexual generations per sexual generation ranges from 26 to 133 depending on the combined effects of mutation rate on female sterility and the selection against hermaphrodites during the asexual portion of the life cycle, i.e.,  $0.98 < \mu(1 - \theta) < 0.99$ . If all strains are included, then the range for hermaphrodite frequencies is from 6 to 46% (observed value, 21%) and the average number of asexual generations per sexual generation ranges from 38 to 156, again depending on the values for  $\mu$  and  $\theta$ .

Sexual reproduction under field conditions has not been observed for this fungus, so we think that the number of asexual generations per sexual generation is towards the higher end

of our range rather than the lower. The number of strains that are poorly fertile, if they are fertile at all, is also consistent with this conclusion. If the population is relatively recent and has not yet reached equilibrium, then it is likely that the number of hermaphrodites would continue to fall and if eliminated completely from the population could lead to a totally asexual population and an evolutionary dead end. We are presently monitoring this population by sampling it on a yearly basis, but identifying trends could require the analysis of samples recovered over a period of 10 years or more.

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