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Genotypic diversity in a South African population of the pitch canker fungus *Fusarium subglutinans* f.sp. *pini*

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The genotypic diversity in a South African population of *Fusarium subglutinans* f.sp. *pini* (*F.s. pini*) was determined, based on the number of vegetative compatibility groups (VCGs). Isolates of *F.s. pini* from South Africa (69), California (five) and Florida (19) were included in the study. The *nit1* (or *nit3*) and NitM mutants were selected as chlorate resistant sectors and paired on minimal medium. The South African isolates of *F.s. pini* were assigned to 23 different VCGs. No heterokaryons formed between isolates from South Africa, California and Florida. The high degree of genotypic diversity in the South African population of *F.s. pini* is probably due to some level of sexual reproduction in the population.

INTRODUCTION

Fusarium spp. are ubiquitous fungi found in many different environments as saprophytes or pathogens (Nelson *et al*., 1983). The taxonomy of the genus is based on morphological characteristics, and has been a subject of controversy for many years (Nelson, 1990). Until recently, *Fusarium* species have not been widely used in genetic studies of variation among and within populations (Burnett, 1984). This situation has changed over the last 10 years, and many species have been subjected to genetic analysis (Van Etten & Kistler 1988; Kistler & Momol, 1990; Leslie, 1990, 1993; Correll *et al*., 1992). Characters such as heterokaryon formation between isolates of *Fusarium* are often used in studies of self/non-self recognition. Isolates able to form sexual and vegetative heterokaryons are referred to as sexually and vegetatively compatible, respectively (Leslie, 1993).

Vegetative compatibility systems have been reported in many different fungi, and serve as a natural means to subdivide a fungal population (Leslie, 1993). The number of vegetative compatibility groups (VCGs) in a population will be influenced by several factors (Leslie, 1993), including the number of loci that affect vegetative compatibility and the frequency with which sexual outcrossing occurs. Thus, where outcrossing is rare or nonexistent, as in strictly asexual fungi, populations will tend to have fewer VCGs, some of which may be associated with distinctive phenotypes (Puhalla, 1985).

Fusarium subglutinans (Wollenw. & Reinking) Nelson, Toussoun & Marasas is a pathogen of many plant species (Booth, 1971). The fungus is heterothallic, and has a teleomorph known as *Gibberella subglutinans* Nelson, Toussoun & Marasas (Nelson *et al*., 1983). Some isolates of *F. subglutinans* are responsible for pitch canker disease of pine trees (Dwinell *et al*., 1981). These isolates have been assigned to a *forma specialis*, referred to herein as *F. subglutinans* f.sp. *pini* (*F.s. pini*) (Correll *et al*., 1991). The teleomorph of *F.s. pini* has not been observed in nature and is rare in culture (Kuhlman *et al*., 1978; Correll *et al*., 1992). The genetic diversity of *F.s. pini* has been studied in Florida, where pitch canker is well established, and in California, where the disease was reported for the first time in 1986 (Correll *et al*., 1992; Gordon *et al*., 1996).

F.s. pini has recently been reported as a root pathogen of *Pinus patula* seedlings in a forest nursery in South Africa (Viljoen *et al*., 1994). In the present study we used vegetative compatibility tests to assess genotypic diversity within the South African *F.s. pini* population and to assess the relatedness of South African and North American isolates.

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Table 1 Source of isolation, geographical origin and number of vegetative compatibility groups (VCGs) of *Fusarium subglutinans* f.sp. *pini* used

Source of isolation	Geographic origin	Number of isolates	Number of VCGs
Pinus patula seedlings	Nelspruit, SA	69	23
Pitch canker	Florida, USA	19	19
Pitch canker	California, USA	5	5

SA, South Africa.

MATERIALS AND METHODS

Isolates

Ninety-three isolates of *F.s. pini* were used in this study (Table 1). These included single-conidium isolates from 69 *P. patula* seedlings in a forest nursery in South Africa collected during June 1990 (19 isolates), November 1990 (16 isolates), June 1991 (19 isolates) and August 1991 (15 isolates), and isolates recovered from pitch canker-infected trees in Florida (19) and California (five). The isolates from Florida and California were representative of the major VCGs that occur in those areas (Correll *et al*., 1992). All isolates are maintained in cryovials in 15% glycerol at −70°C (Department of Microbiology and Biochemistry, University of the Free State, Bloemfontein, South Africa). Pathogenicity of all the isolates was confirmed on 2-year-old pine seedlings (Viljoen, unpublished data).

Determination of vegetative compatibility groups

Fungal isolates were cultured on complete medium (Correll *et al*., 1987) for routine study. Vegetative compatibility tests were made using nitrate non-utilizing *nit* mutants generated and characterized according to standard protocols (Correll *et al*., 1987) with a slight increase (from 1·5% to 1·8%) in the amount of potassium chlorate included in the chlorate medium. The *nit* mutants were separated into *nit1*, *nit3* and NitM classes based on their ability to utilize nitrite and hypoxanthine as sole nitrogen sources (Correll *et al*., 1987). Mutants in different phenotypic classes were paired to determine vegetative compatibility. NitM and *nit1* mutants were used wherever possible. Pairings were made on 24-well plastic hybridoma plates (Klittich & Leslie, 1988) and incubated under lights for 7 days at 25°C. Pairings with a distinct line of prototrophic growth were considered to be vegetatively compatible and to belong to the same VCG. All pairings were repeated at least once.

RESULTS

Selection, characterization and pairing of *nit*-mutants

Chlorate-resistant sectors were produced by all the isolates used in this study. The number of sectors produced differed substantially among isolates. Most of these sectors were discarded when wild type growth was observed on minimal medium. Isolates showing sparse growth could readily be characterized as *nit1*, *nit3* or NitM mutants. The *nit1* and *nit3* mutants were able to produce heterokaryons when paired with NitM mutants of the same isolate, and no vegetatively (heterokaryon) self-incompatible isolates were found (Jacobson & Gordon, 1988; Correll *et al*., 1989).

VCG diversity in the SA population of *F.s. pini*

The 69 South African isolates of *F.s. pini* collected

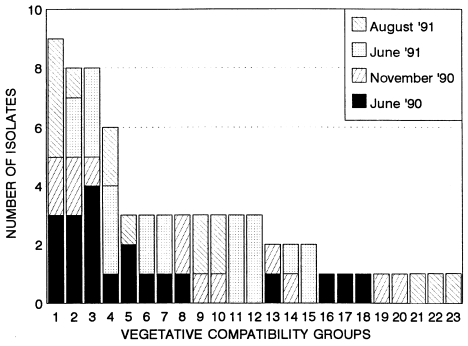

Fig. 1 The number of isolates in each of 23 vegetative compatibility groups representing the South African population of *Fusarium subglutinans* f.sp. *pini*. The isolates were obtained from diseased *Pinus patula* seedlings on four collection dates.

at the forest nursery could be assigned to 23 different VCGs (Fig. 1). Of these, VCGs SA-1, SA-2, SA-3 and SA-4 predominated and represented nine, eight, eight and six isolates respectively. The other groups consisted of mainly one, two or three isolates per VCG.

Isolates representing the same VCG were collected on several different dates (Fig. 1). VCG SA-2 was collected on all four dates, while representatives of VCGs SA-1, SA-3 and SA-4 were each obtained on three different dates of collection, and representatives of VCGs SA-5, SA-6, SA-7, SA-8, SA-9, SA-10, SA-13 and SA-14 were collected on two different dates.

Comparison of VCGs from SA, Florida and California

No isolates of *F.s. pini* from South Africa were vegetatively compatible with isolates from the United States. In addition, no isolates from Florida and California were vegetatively compatible (Table 1). These isolates were, however, few in number and each one represented a different compatibility group. A simple measure of diversity in a population is the number of species or phenotypes (*S*) in a sample from a population divided by the number (*N*) of individuals in the sample (Anagnostakis *et al*., 1986). The *S/N* ratio for the South African population was 0·33.

DISCUSSION

A high level of genotypic diversity, based on the number of VCGs found, is present in the population of *F.s. pini* in South Africa. This is despite the fact that the occurrence of the fungus has thus far been restricted to a single nursery in the country. VCG diversity may indicate a well-established fungal population, the introduction of numerous VCGs into a new area, and/or regular sexual reproduction (Puhalla & Spieth, 1985; Brasier, 1987, 1988; Correll *et al*., 1992). *F.s. pini* was found for the first time in South Africa in 1990 (Viljoen *et al*., 1994). Its presence in one nursery and its similarity to the pitch canker isolates in the United States (Viljoen *et al*., 1997) are consistent with the hypothesis that the fungus was recently introduced into South Africa.

The pitch canker pathogen has been present in the southern United States and Haiti for many years (Dwinell *et al*., 1981), whereas reports of its occurrence in California, Mexico, Japan and South Africa are more recent (McCain *et al*., 1987; Kobayashi & Muramoto, 1989; Santos & Tovar, 1991; Viljoen *et al*., 1994). The South African isolates of *F.s. pini* are phenotypically and genotypically similar to the population in the United States (Viljoen *et al*., 1997), and introduction of the pathogen from the southern United States, Haiti or Mexico, where the disease is apparently well established, seems likely. The present work, however, did not demonstrate that any South African and United States isolates were vegetatively compatible, but this may simply reflect a relatively small sampling of isolates from both areas.

Where a population is established by relatively few individuals, reduced genetic diversity is expected because of the founder effect (Nei *et al*., 1975). This is reflected by the *S/N* ratio of 0·02 reported for the Californian population of *F.s. pini* where a relatively small number of VCGs were apparently introduced and sexual reproduction does not occur (Correll *et al*., 1992). In contrast to the Californian population, the *S/N* ratio for the Florida population, where the pitch canker is well established, is 0·39. With the introduction of only a few genotypes, sexual reproduction could lead to a rapid increase in the number of VCGs within the new population (Brasier, 1988). The presence of both mating types of *F.s. pini* in South Africa has been confirmed by the production of fertile perithecia in culture (Viljoen *et al*., 1997). Thus, outcrossing could generate multiple VCGs within a short period of time and this may have contributed to the VCG diversity and high *S/N* ratio in the South African population of the pathogen.

The South African population of *F.s. pini* is homogeneous in virulence to pines but diverse in its genetic make-up. These characteristics may facilitate survival of the fungus in new environments. There are no native *Pinus* spp. in South Africa, so *F.s. pini* does not pose a threat to native forests. However, spread of *F.s. pini* from the forest nursery to pine plantations, consisting largely of susceptible pine species (Viljoen *et al*., 1995), could cause considerable economic damage.

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