Genetic Diversity in California and Florida Populations of the Pitch Canker Fungus *Fusarium subglutinans* f. sp. *pini*

J. C. Correll, T. R. Gordon, and A. H. McCain

First author, Department of Plant Pathology, University of Arkansas, Fayetteville 72701; second and third authors, Department of Plant Pathology, University of California, Berkeley 94720.

We thank George Blakeslee and Steve Tjosvold for their generous assistance in this study.
Accepted for publication 22 November 1991 (submitted for electronic processing).

ABSTRACT

Correll, J. C., Gordon, T. R., and McCain, A. H. 1992. Genetic diversity in California and Florida populations of the pitch canker fungus *Fusarium subglutinans* f. sp. *pini*. Phytopathology 82:415-420.

Isolates of *Fusarium subglutinans* that are pathogenic to pines, recently given the designation *F. subglutinans* f. sp. *pini*, were collected from slash and loblolly pines in Florida (116 isolates) and from various pine hosts (Monterey, bishop, aleppo, and Canary Island pine) in California (209 isolates). These isolates were characterized by vegetative compatibility, using nitrate nonutilizing mutants, and by mitochondrial DNA (mtDNA) restriction fragment length polymorphisms (RFLPs). Attempts also were made to characterize *F. s. pini* for sexual compatibility. Using four different restriction enzymes, no RFLPs were detected among isolates of *F. s. pini*. However, restriction patterns of mtDNAs of all nonpine isolates of *F. subglutinans* examined were different from those of *F. s. pini*. The characteristic restriction fragment patterns of mtDNAs of isolates of *F. s. pini* indicate that they are genetically related and may represent a distinct mating population within the morphological species *F. subglutinans*. However, we were unable to demonstrate the sexual fertility of any strains of *F. s. pini*. Vegetative compatibility group (VCG) diversity in the Florida population, where the disease had been established for at least 15 years, was high with 45 distinct VCGs identified among 117 isolates examined; one Florida isolate collected in 1977 was vegetatively self-incompatible and avirulent on pine. The predominate VCG at any one collection site in Florida made up 11–33% of the sampled population. In contrast, in the California population, only five VCGs were identified among 209 isolates examined. Furthermore, 70% of the entire California population sampled was composed of a single VCG (C1), indicating that the population is rather homogeneous with respect to VCG. This VCG structure would be indicative of an asexually reproducing pathogen and/or one that has recently been introduced.

Pitch canker disease of pine, caused by *Fusarium subglutinans* (Wollenweb. & Reinking) P. E. Nelson, T. A. Toussoun & Marasas (= *F. moniliforme* J. Sheld. var. *subglutinans* Wollenweb. & Reinking) f. sp. *pini* Correll et al (8), is a destructive disease of pine in the southeastern United States (13) and California (8,22). Pitch canker was first reported in North Carolina in 1946 (14) and is now widespread on pine throughout the southeastern United States, where it is considered endemic (13,20). Epidemics became quite severe in Florida in the mid to late 1970s when disease incidence in many slash pine plantations was estimated to be nearly 100%. In 1986, pitch canker was reported on Monterey pine (*Pinus radiata* D. Don) in California (22) and has since been identified in eight counties throughout the central and southern coastal areas of the state (8). A recent report indicated that pitch canker was present in Japan (23) and another (A. H. McCain, *unpublished*) indicated that it also was present in Mexico.

In California, pitch canker has caused considerable damage to Monterey pine planted along roadways, in landscape settings, and in tree nurseries (8). The dramatic increase in disease incidence and severity over the past 5 yr may reflect an increase in activity of an indigenous population of *F. s. pini* due to changes influencing host susceptibility, such as stand age, drought stress, unusual environmental conditions, or the activity of a new insect vector. Alternatively, the increase of pitch canker in California may be due to the recent introduction of the pathogen into the state. We hypothesized that if the pathogen were recently introduced, it would have a population structure in California very different from that in the southeastern United States where the pathogen has been present for many years.

Vegetative compatibility has been widely used to provide insight into the genetic structure of fungal populations. This, in turn, has provided insight into the epidemiology of several important fungal diseases (2,4,6,7,17,24,25,27). For example, Brasier (5) reported a high level of vegetative compatibility group (VCG) diversity in populations of *Ophiostoma ulmi* (Buisman) Nannf. (= *Ceratocystis ulmi* (Buisman) C. Moreau), the cause of Dutch elm disease, in areas where the disease is well established. This diversity apparently occurs because of the presence of both sexual mating types, leading to sexual recombination and the segregation of vegetative incompatibility loci (*vic* or *het* loci) that control vegetative compatibility. In contrast, in locations where Dutch

elm disease has only recently appeared, a single VCG, or clone, predominates in the epidemic front. Two to 3 yr after the onset of an epidemic, VCG diversity had returned to a high level more typical of the basal population (5).

Examination of vegetative compatibility in *Cryphonectria parasitica* (Murrill) Barr (= *Endothia parasitica* (Murrill) P. J. Anderson & H. W. Anderson), the cause of chestnut blight, has helped explain the dynamics of transmission and spread of a hypovirulence factor, presumably a virus, within populations of this pathogen (2). In Connecticut, movement of the hypovirulence factor is greatly impeded by a high level of VCG diversity in populations of *C. parasitica.* In Europe, pathogen populations are much less diverse (3). Vegetative compatibility also has been used to examine population diversity and to deduce the possible source of primary inoculum of *Leucostoma persoonii* Höhn. (1).

We were interested in determining if any differences in genetic diversity existed between populations of *F. s. pini* in California, where the disease has recently been found, and those in Florida, where the disease is well established. In this investigation, genetic diversity of *F. s. pini* was assessed by examining vegetative compatibility, sexual compatibility, and restriction fragment length polymorphisms (RFLPs) in mitochondrial DNA (mtDNA). A preliminary report of this work has been published (9).

MATERIALS AND METHODS

Isolates examined. A total of 326 isolates of *F. s. pini* recovered from diseased pine tissue (Monterey, *Pinus radiata* D. Don; slash, *P. elliottii* Engelm. var. *elliottii;* loblolly, *P. taeda* L.; bishop, *P. muricata* D. Don; aleppo, *P. halepensis* Mill.; and Canary Island, *P. canariensis* Chr. Sweet ex Spreng.) in California or Florida, and from insect and air samples in California, were examined for vegetative compatibility. The California isolates (209 total) were recovered between 1986 and 1989, predominately from Monterey pine. The collection sites included 20 locations in eight counties where the disease had previously been identified (Table 1) (8). All isolates recovered from insects and air samples were from Santa Cruz County. The majority of the Florida isolates (106 of 117 isolates) were recovered from four collection sites in May 1988 (Table 2). The four collection sites were in slash pine plantations in three different counties. Disease incidence in these plantations ranged from 5 to 30%. Eleven additional isolates from Florida were obtained from G. M. Blakeslee, who recovered them from infected pines or contaminated insects during the peak of a pitch canker epidemic in 1977. Almost all pine isolates of *F. s. pini* from California and Florida came from individual trees; nine isolates from the New Brighton site in California came from a single Monterey pine tree. A subsample of isolates from each location was previously tested for pathogenicity, and all were virulent on Monterey pine (8).

Vegetative compatibility. Isolates of *F. s. pini* were examined for vegetative compatibility using standard procedures (10,25). Nitrate nonutilizing (*nit*) mutants were generated by growing isolates on minimal medium containing either 1.5 or 3.0% potassium chlorate (MMC) (10). The phenotype of *nit* mutants generated on MMC was determined, and *nit*1 and NitM mutants were selected from all appropriate strains (10). The *nit* mutant testers from all isolates were paired in all combinations to determine whether isolates were vegetatively compatible. All isolates were assigned to a VCG based on results of repeated pairing reactions. The formation of dense aerial mycelium typical of prototrophic growth where two phenotypically distinct *nit* mutants came in contact indicated that isolates were vegetatively compatible.

Sexual compatibility. Thirty-eight arbitrarily selected isolates of *F. s. pini* (16 from California and 22 from Florida) were crossed with 19 isolates (Table 3) of either *F. s. pini,* nonpine isolates of *F. subglutinans,* or *F. moniliforme* in tests to determine sexual compatibility. Several of the 19 isolates previously had been reported to be sexually fertile and had been assigned mating types (18,19). In addition to crossing the 38 pine isolates with the 19 tester strains, over 200 sexual crosses between various California and Florida isolates of *F. s. pini* also were attempted. Sexual crosses were performed following the methods described by

TABLE 1. Vegetative compatibility groups (VCGs) identified among isolates of *Fusarium subglutinans* f. sp. *pini* from California

County	Site[a]	Pine host	Number of isolates examined	Number of isolates in each VCG				
				C1	C2	C3	C4	C5
Alameda	Holiday Inn	Monterey	4	4	...	...	...	...
	San Lorenzo	Monterey	18	17	1	...	...	...
	Lake Chabot	Monterey	2	2	...	...	...	...
	Hayward	Monterey	1	1	...	...	...	...
	Union City	Monterey	1	1	...	...	...	...
Los Angeles	Torrance	Monterey	6	...	...	5	...	1
Monterey	Salinas	Monterey	9	9	...	...	...	...
	Castroville	Monterey	1	1	...	...	...	...
	Aromas	Monterey	1	1	...	...	...	...
San Diego	Escondido	Monterey	8	8	...	...	...	...
San Francisco	Highway	Monterey	1	1	...	...	...	...
San Luis	Nipomo	Monterey	1	1	...	...	...	...
Santa Barbara	Highway	Monterey	14	...	...	14	...	...
	Highway	Canary Island	1	...	...	1	...	...
Santa Cruz	New Brighton	Bishop	4	3	1	...	...	...
	New Brighton	Monterey	22	22	...	...	...	...
	Capitola	Monterey	7	7	...	...	...	...
	Emeline	Aleppo	2	...	2	...	...	...
	Emeline	Monterey	4	2	2	...	...	...
	Soquel	Monterey	2	2	...	...	...	...
	Watsonville	Monterey	1	...	...	...	1	...
	Aptos	Bishop	1	...	1	...	...	...
	Aptos	Monterey	1	...	1	...	...	...
	Seacliff	Bishop	1	...	1	...	...	...
	Davenport	Monterey	1	1	...	...	...	...
	Scotts Valley	Monterey	1	...	1	...	...	...
Santa Cruz	All sites	Insects	32	16	15	...	1	...
Santa Cruz	All sites	Air samples	62	47	10	...	5	...
		(Totals)	209	146	35	20	7	1

[a] Locations where isolates of *F. s. pini* were collected. Torrance site was a Christmas tree nursery and Escondido a seedling nursery. Trees at all other sites were landscape plantings or volunteers in non-native locations.

TABLE 2. Vegetative compatibility group (VCG) diversity in California and Florida populations of *Fusarium subglutinans* f. sp. *pini*

State	Site	Number of VCGs identified (S)	Number of isolates examined (N)	S/N[a]
California	Statewide[b]	3	188	0.02
	Santa Barbara/Torrance	2	21	0.10
	(Totals)	5	209	0.02
Florida	Gainesville, Alachua County	14	31	0.45
	Maytown, Volusia County	13	28	0.46
	Osteen, Volusia County	13	21	0.62
	Carrabelle, Franklin County	16	26	0.62
	1977 collection,[c] Volusia County	7	11[d]	0.70
	(Totals)	45[e]	117	0.39

[a] S/N is a measure of genetic diversity (3) where S = number of VCGs identified and N = number of isolates examined.
[b] Statewide sites include all isolates throughout the state except isolates from Santa Barbara and Torrance.
[c] A miscellaneous collection of pine and insect isolates collected in 1977.
[d] Eleven isolates were examined, but one of these isolates was vegetatively self-incompatible. Thus, only the remaining 10 isolates could be assigned to a VCG.
[e] Some VCGs were found at more than one site. Thus, the total number of VCGs in the Florida collection is less than the sum of the number of VCGs found at each site.

Klittich and Leslie (18), except 400 ml of canned carrot juice per liter was used in place of fresh carrots. Isolates that functioned as the female parent were grown on carrot agar for 6–8 days at 25 C under a 12-h light/12-h dark cycle. The isolate to be used as the male parent was grown on a potato-dextrose agar slant under the same conditions for 6–8 days. Spores and mycelial fragments were removed from the male isolate with 2 ml of 0.01% water agar poured over the surface of the female isolate and mixed by rubbing the surface of the plate culture with a sterile glass rod. Fertilized cultures were returned to the incubation conditions previously described. Plates were observed for perithecia formation over the next 6 wk. Unfertilized female cultures were always run as controls. Also, known sexually compatible isolates of *F. subglutinans* (FK278 and FK281) and *F. moniliforme* (FK102 and FK149) were included in each group of crosses as positive controls.

Analysis of RFLP. Purified mtDNA was recovered from the virulent isolate FK863 of *F. s. pini* (from Monterey pine in California). This isolate belonged to the predominate VCG (C1) identified in California. Cultures for mtDNA isolation were obtained by inoculating 200 ml of complete medium broth (16) in a 0.5-L flask with a spore suspension. These cultures were incubated at 25 ± 3 C for 3–4 days on a rotary shaker. Mycelium was harvested on Miracloth and rinsed three times with sterile distilled water. The cells were disrupted with a rotary bead-beating apparatus (Biospec Products, Bartlesville, OK) to which extraction buffer was added (16). Intact mitochondria were isolated by sucrose gradient centrifugation, and mtDNA was recovered using published procedures (16,21). DNA extraction and purification also were accomplished by following published procedures (16,21).

Cultures for total DNA extraction were grown as previously described, and the mycelium was frozen at −80 C. Total DNA was extracted from mycelium by grinding with a mortar and pestle while continually adding liquid nitrogen and then following the "mini-prep" procedure described by Lee et al (21) as modified by Jacobson and Gordon (16).

Restriction digests and hybridization. Purified mtDNA from strain FK863 was digested with one of several restriction endonucleases according to the manufacturer's recommendations (Bethesda Research Laboratories, Gaithersburg, MD). Restriction fragments were separated electrophoretically in 0.7% agarose gels and visualized by staining with ethidium bromide and illumination with ultraviolet light.

Restriction fragments resulting from endonuclease digestions of total DNA were transferred from agarose gels to nylon membranes according to the manufacturer's recommendations (Nytran, Schleicher & Schuell, Keene, NH). These Southern blots then were probed with purified mtDNA from strain FK863 that was labeled with [α-^{32}P]dATP using a nick translation kit (Bethesda Research Laboratories). Southern hybridizations were conducted as described by Jacobson and Gordon (16). Kodak X-OMAT AR film was overlayed on the membrane and exposed in the dark at room temperature for 2–7 days depending on the intensity of the radioactive signal on the membrane.

TABLE 3. Isolates of *Fusarium subglutinans* f. sp. *pini*, *F. subglutinans*, and *F. moniliforme* used as testers in sexual compatibility tests

Species	Isolate	Mating type[a]	Host[b]	Geographic origin
F. s. pini	FK863	NR	Pine	California
F. s. pini	FK867	NR	Pine	California
F. s. pini	SK4	NR	Pine	California
F. s. pini	SK5	NR	Pine	California
F. s. pini	FL102	NR	Pine	Florida
F. s. pini	FL120	NR	Pine	Florida
F. s. pini	FL127	NR	Pine	Florida
F. s. pini	ATCC 38479	B−	Pine	North Carolina
F. subglutinans	ATCC 38480	B+	Ascospore	...
F. subglutinans	ATCC 38943	B+	Ascospore	...
F. subglutinans	ATCC 52135	B+/−	Corn	Iran
F. subglutinans	FK278	B+	Sugarcane	Taiwan
F. subglutinans	FK281	B−	Sugarcane	Taiwan
F. subglutinans	IG2	B−	Ascospore[c]	...
F. subglutinans	IG9	B+	Ascospore[c]	...
F. subglutinans	3693	E+	Corn	Illinois
F. subglutinans	3696	E−	Corn	Illinois
F. moniliforme	FK102	A+	Sorghum	California
F. moniliforme	FK149	A−	Corn	California

[a] Mating type of all ATCC cultures reported by Kuhlman (19), Klittich and Leslie (18), or J. F. Leslie, *personal communication*. The letter refers to the mating population, and the + or − designation refers to the mating type. NR = None reported.
[b] All pine isolates listed were virulent on Monterey pine (8). All nonpine isolates, including ATCC 38480 and 38943, were avirulent on Monterey pine.
[c] IG2 and IG9 were single ascospore progeny of a cross between FK278 and FK281.

mtDNA from three California and five Florida isolates was examined with four restriction enzymes (*Eco*RI, *Pst*I, *Bgl*II, and *Hae*III). A total of 25 pine isolates from California (including at least one isolate from each of the five VCGs) and Florida were examined with two restriction enzymes (*Eco*RI and *Pst*I). In addition, 12 nonpine isolates of *F. subglutinans* and two pine isolates of *F. proliferatum* were examined using a single restriction enzyme (*Eco*RI).

RESULTS

Recovery of *nit* mutants. *Nit* mutants were obtained from all isolates of *F. s. pini* examined, but there was considerable vari-

ability in the rates at which they were recovered. In general, California isolates were not restricted on 1.5% MMC; chlorate-resistant sectors were, however, readily recovered from these isolates on 3% MMC. In contrast, chlorate-resistant sectors were readily recovered from Florida isolates on 1.5% MMC.

California and Florida isolates differed in the percentage of chlorate-resistant sectors that were actually *nit* mutants. On the average, less than 10% of the chlorate-resistant sectors from any given California isolate proved to be *nit* mutants, whereas more than 60% of the chlorate-resistant sectors from the Florida isolates were identified as *nit* mutants. For several California isolates, only 1–2% of the chlorate-resistant sectors were *nit* mutants.

VCG diversity. Five distinct VCGs were identified among a collection of 209 California pine isolates of *F. s. pini* (Table 1). The majority of these isolates (70%) were found to belong to a single VCG designated C1. Isolates in VCG C1 were from numerous locations throughout California, from Lake Chabot in the north to Escondido in the south (800 km separation). The second California VCG, C2, was recovered much less frequently (17%) and predominately from sites in Santa Cruz County. A third VCG, C4, was recovered infrequently (3%) and only from Santa Cruz County. The majority of the isolates in VCG C4 were from air samples or insect isolations. A fourth VCG from California (C3) made up 9% of the total population sampled. C3 included isolates recovered from a Christmas tree nursery in Los Angeles County and all 15 isolates recovered from a 20- to 30-year-old pine stand of landscape trees in Santa Barbara County. One isolate from the Christmas tree nursery in Los Angeles County represented the only member of VCG C5.

A total of 45 VCGs were identified among a collection of 116 isolates of *F. s. pini* from Florida (Table 2). When all isolates were combined, the predominant VCG (F6) in Florida represented approximately 11% of the sampled population (Fig. 1). When the Florida isolates were separated by collection site, the predominant VCG for any given site represented 11–33% of the sampled population (Fig. 2). By pairing isolates from different collection sites, we determined that several VCGs were found at more than one site. For example, Florida VCG F12 was found at all four collection sites, whereas VCGs F2, F4, F6, F9, F12, and F25 were found at three of the four collection sites (Fig. 2). The Gainesville, Osteen, and Maytown collections had five to six VCGs in common (F2, F3, F4, F6, F7, F9, and F12), whereas the Carrabelle collection had only one VCG in common with the Osteen collection (F12), two VCGs in common with the Gainesville collection (F9 and F12), and three VCGs in common with the Maytown collection (F9, F12, and F25).

Seven VCGs were identified among the 11 isolates examined from the 1977 Florida collection (Table 2); one isolate (S24) was heterokaryon, or vegetatively, self-incompatible (11,15). This isolate also was somewhat unique in that it was the only pine isolate of *F. subglutinans* that was avirulent on Monterey pine.

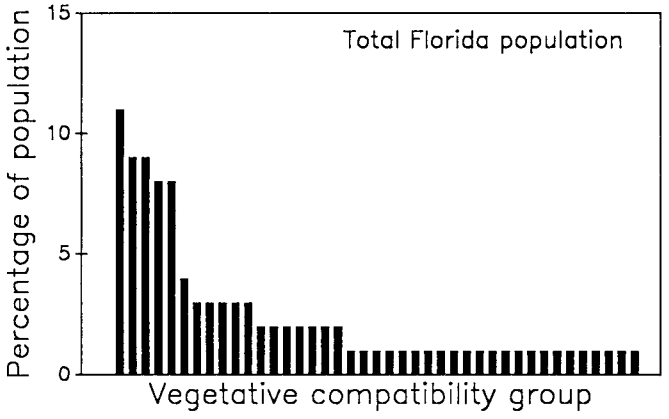

Fig. 1. The percentage of the total Florida population (106 isolates) of *Fusarium subglutinans* f. sp. *pini* made up by each of the 41 vegetative compatibility groups obtained in 1988.

Two isolates were in each of VCGs F2, F4, and F9; these three VCGs had been identified in three of the four collection sites made in 1988 (Fig. 2). Four of the isolates from the 1977 collection belonged to unique VCGs (F42, F43, F44, and F45). None of the VCGs identified in the Florida population was identified in the California population.

Interestingly, one Florida isolate (S18), which belonged to VCG F9, was collected from a naturally occurring slash pine in a native stand in Florida in 1977. This VCG was detected in the 1988 sample at all sites except the Osteen site (Fig. 2).

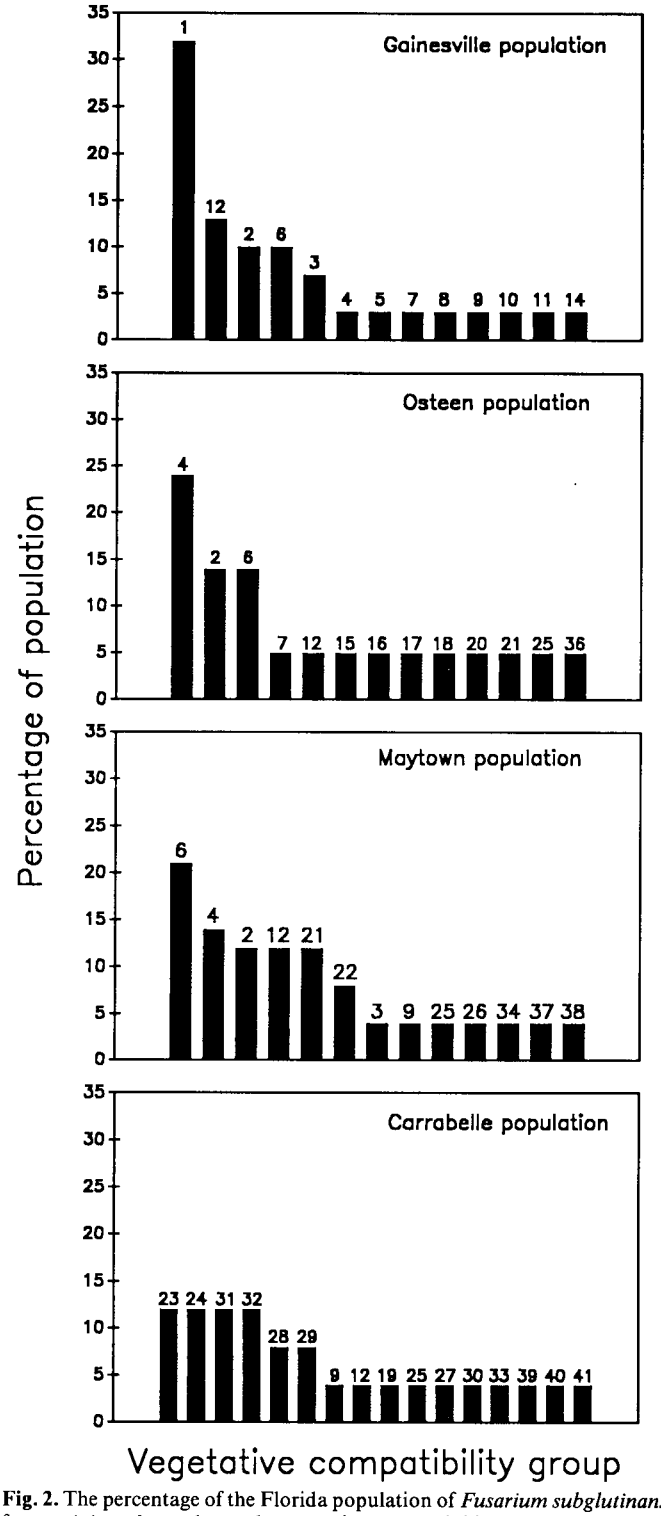

Fig. 2. The percentage of the Florida population of *Fusarium subglutinans* f. sp. *pini* made up by each vegetative compatibility group at each of four collection sites. The number at the top of each bar refers to the specific Florida vegetative compatibility group.

The S/N (number of VCGs identified/number of isolates examined) ratio, a measurement of genetic diversity (3), was very different between the California and Florida populations (Table 2). The S/N ratio for the total California and Florida populations was 0.02 and 0.39, respectively. The S/N ratio ranged from 0.45 to 0.62 for the individual collection sites (Table 2) in the Florida population.

Sexual compatibility. Isolates of *F. subglutinans* (FK278, FK281, IG2, IG9, 3693, and 3696) and *F. moniliforme* (FK102 and FK149), known to be sexually fertile (Table 3), consistently produced perithecia with viable ascospores when crossed with the appropriate tester strains (i.e., opposite mating types). Two of the ATCC cultures, 38480 and 38943 (both avirulent on pine), yielded fertile perithecia when crossed with IG9. No perithecia were produced in over 250 repeated crosses between pine isolates and fertile nonpine isolates of either *F. subglutinans* or *F. moniliforme* (Table 3). In addition, no perithecia were observed in over 100 arbitrary pine isolate by pine isolate crosses. On many occasions, however, what appeared to be perithecia initials (blue to blue-black amorphous to spherical structures) were observed in some of the pine isolate by pine isolate crosses, as well as the pine isolate by fertile nonpine isolate crosses. However, these structures never matured into full-sized perithecia, nor did they ever contain asci. We also were unable to produce perithecia when ATCC 38479 was crossed with any other strains. This pine isolate had previously been reported to be sexually fertile (19). Consequently, we were unable to demonstrate the sexual fertility of any isolates of *F. s. pini*.

Analysis of RFLP. Restriction digests of purified mtDNA from reference strain FK863 of *F. s. pini* indicated that it had a mitochondrial genome size of approximately 66 kb (Fig. 3, complete data not shown). No RFLPs were detected among any of eight isolates of *F. s. pini* (from California or Florida and from several different pine hosts) when examined with four different restriction digests (Fig. 3, only three isolates shown). Restriction patterns of mtDNAs of isolates of *F. s. pini* were, however, quite different from those of the nonpine isolates of *F. subglutinans* examined (Fig. 4), as well as from those of the pine isolates of *F. proliferatum* (data not shown). In addition, the nonpine isolates of *F. subglutinans* from a common host had similar restriction fragment patterns when cut with *Eco*RI. For example, all corn isolates examined had very similar restriction fragment patterns (Fig. 4). Each corn isolate belonged to a unique VCG (data not shown). One corn isolate from Mexico, however, had an additional fragment, approximately 3 kb in length, which was not found in other corn isolates (Fig. 4).

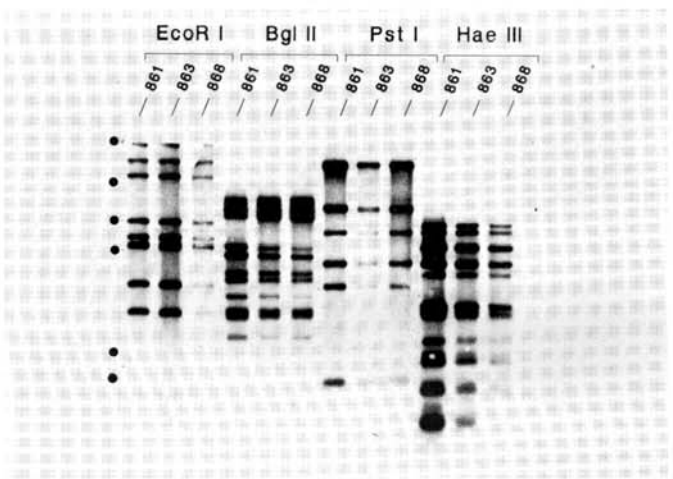

Fig. 3. Autoradiograph showing mitochondrial DNA of three isolates of *Fusarium subglutinans* f. sp. *pini* digested with four restriction enzymes. The Southern blot was hybridized with ^{32}P-labeled purified mitochondrial DNA from strain FK863 of *F. s. pini*. The three isolates are 861 from bishop pine, CA (VCG C1); 863 from Monterey pine, CA (VCG C1); and 868 from slash pine, FL (VCG F4). Circles indicate size references of lambda DNA digested with *Hin*dIII (23.1, 9.4, 6.6, 4.4, 2.3, and 2.0 kb).

DISCUSSION

F. subglutinans is a very diverse fungal species with a worldwide distribution and a broad host range (13). However, within this morphological species are isolates that are pathogenic to pine (8). Isolates that are pathogenic to pine have previously been designated *F. s. pini* (8). In this investigation, we found isolates of *F. s. pini* from different geographical locations, various pine hosts, and different VCGs to share identical mtDNA restriction fragment patterns (Fig. 3). The mtDNA restriction patterns of *F. s. pini* were, however, quite different from those of the nonpine isolates of *F. subglutinans* examined. Thus, on the basis of both virulence and mtDNA RFLP analysis, isolates of *F. s. pini* appear to represent a distinct subspecific group within the morphological species, *F. subglutinans*.

The VCG diversity in the Florida population of *F. s. pini* was very high relative to that of the California population. For example, 13–16 distinct VCGs were identified among a collection of 21–31 isolates from a single site in Florida where isolates were collected on trees in close proximity to one another. The high VCG diversity in the Florida population may be due to the introduction of multiple VCGs, or the introduction of both sexual mating types, followed by sexual reproduction and the segregation of multiple vegetative incompatibility loci (4,5,26). This would have led to a proliferation of VCGs in the population shortly after introduction of the pathogen. Such a shift in VCG diversity in fungal populations has been documented in populations of *O. ulmi* (5) in areas where it has been recently introduced.

In contrast to the Florida population, the VCG diversity of the California population was very low. Only five distinct VCGs

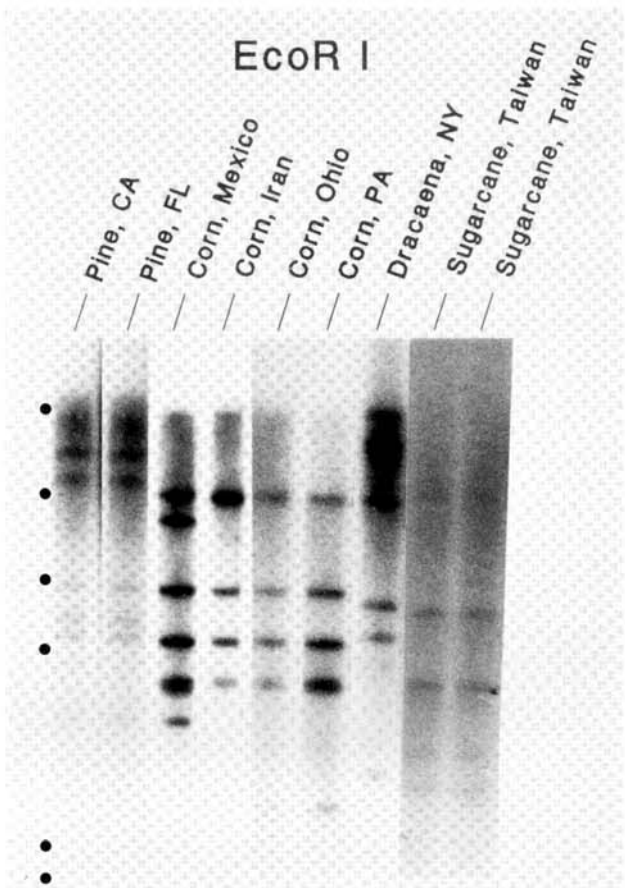

Fig. 4. Autoradiograph showing mitochondrial DNA of several pine and nonpine isolates of *Fusarium subglutinans* digested with *Eco*RI. The Southern blot was hybridized with ^{32}P-labeled purified mitochondrial DNA from strain FK863 of *F. subglutinans* f. sp. *pini*. The host and geographical origin of each isolate are listed at the top of each lane. Circles indicate size references of lambda DNA digested with *Hin*dIII (23.1, 9.4, 9.6, 4.4, 2.3, and 2.0 kb).

were identified among 209 isolates examined. Furthermore, the sampled population may be predominately clonal, with a single VCG (C1) constituting up to 70% of the sampled population. Two other VCGs, C2 and C3, were distributed among the collection sites where VCG C1 predominated, and they represented 17 and 3% of the population, respectively. Interestingly, at two locations, one near Santa Barbara and one in a Christmas tree plantation near Torrance, a different VCG (C4) predominated.

The chronology of this disease in California (22), its distribution (8), and the VCG structure favor the hypothesis that pitch canker was recently introduced into California. It is possible that a relatively small number of VCGs were introduced into the state and that different sexual mating types do not occur or conditions are not conducive for sexual reproduction. These data are consistent with VCG diversity observed in epidemic fronts of *O. ulmi* (5). Only examination of the California population in the future will reveal whether or not the VCG structure has shifted substantially. It is interesting to speculate at this time that VCG C1, which occurs throughout the state, and VCG C4, the predominant VCG in Santa Barbara and Torrance, represent separate introductions.

We were unable to demonstrate the sexual compatibility of any isolates of *F. s. pini.* Kuhlman (19) reported that certain isolates pathogenic to pine were sexually fertile and able to sexually cross with certain pine and nonpine isolates of *F. subglutinans.* However, no genetic markers were examined for segregation frequencies to confirm that the perithecia produced were the result of genetic complementation of distinct mating types. We were unable to repeat these results with several of the same tester strains. Although we could not demonstrate the sexual compatibility of any isolates of *F. s. pini,* this pathogen may indeed represent a distinct mating population or biological species. The fact that isolates of *F. s. pini* from different pine hosts and representing different VCGs have identical mtDNA restriction patterns implies that they have a common ancestry.

The nonpine isolates of *F. subglutinans* that we examined (each belonging to a different VCG) also had mtDNA restriction fragment patterns that correlated with host origin and not VCG. Possibly, the isolates from other hosts also represent, or were derived from, distinct mating populations. Within *F. subglutinans,* distinct subspecific groups may exist that are essentially biological species, some of which may have lost the ability to reproduce sexually. Isolates within these subspecific groups may be unified by a similar mtDNA genome yet have a high degree of VCG diversity. Preliminary evidence indicates that this pattern of VCG-mtDNA restriction fragment pattern relationship, where mtDNA restriction pattern is correlated with host, also is found in other fungi (12). In contrast, among pathogenic strains of the strictly asexually reproducing *F. oxysporum,* mtDNA restriction patterns are not correlated with host but generally are correlated with VCG, even when multiple VCGs are pathogenic on a given host.

LITERATURE CITED

1. Adams, G., Hammar, S., and Proffer, T. 1990. Vegetative compatibility in *Leucostoma persoonii.* Phytopathology 80:287-291.
2. Anagnostakis, S. L. 1987. Chestnut blight: The classical problem of an introduced pathogen. Mycologia 79:23-27.
3. Anagnostakis, S. L., Hau, B., and Kranz, J. 1986. Diversity of vegetative compatibility groups of *Cryphonectria parasitica* in Connecticut and Europe. Plant Dis. 70:536-538.
4. Brasier, C. M. 1987. The dynamics of fungal speciation. Pages 231-260 in: Evolutionary Biology of the Fungi. A. D. M. Rayner, C. M. Brasier, and D. Moore, eds. Cambridge University Press, Cambridge.
5. Brasier, C. M. 1988. Rapid changes in genetic structure of epidemic populations of *Ophiostoma ulmi.* Nature 332:538-541.
6. Correll, J. C. 1991. The relationship between formae speciales, races, and vegetative compatibility groups in *Fusarium oxysporum.* Phytopathology 81:1061-1064.
7. Correll, J. C., Gordon, T. R., and McCain, A. H. 1988. Vegetative compatibility and pathogenicity of *Verticillium albo-atrum.* Phytopathology 78:1017-1021.
8. Correll, J. C., Gordon, T. R., McCain, A. H., Fox, J. W., Koehler, C. S., Wood, D. L., and Schultz, M. E. 1991. Pitch canker disease in California: Pathogenicity, distribution, and canker development on Monterey pine (*Pinus radiata*). Plant Dis. 75:676-682.
9. Correll, J. C., Gordon, T. R., McCain, A. H., and Jacobson, D. J. 1988. Examination of genetic diversity in populations of the pitch canker pathogen, *Fusarium subglutinans.* (Abstr.) Phytopathology 78:1575.
10. Correll, J. C., Klittich, C. J. R., and Leslie, J. F. 1987. Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. Phytopathology 77:1640-1646.
11. Correll, J. C., Klittich, C. J. R., and Leslie, J. F. 1989. Heterokaryon self-incompatibility in *Gibberella fujikuroi* (*Fusarium moniliforme*). Mycol. Res. 93:21-27.
12. Correll, J. C., Weidemann, G. J., TeBeest, D. O., and Guerber, J. C. 1991. Genetic and molecular diversity in *Colletotrichum gloeosporioides.* (Abstr.) Phytopathology 81:1219.
13. Dwinell, L. D., Barrows-Broaddus, J. B., and Kuhlman, E. G. 1985. Pitch canker: A disease complex of southern pines. Plant Dis. 69:270-276.
14. Hepting, G. H., and Roth, E. R. 1946. Pitch canker, a new disease of southern pines. J. For. 44:742-744.
15. Jacobson, D. J., and Gordon, T. R. 1988. Vegetative compatibility and self-incompatibility within *Fusarium oxysporum* f. sp. *melonis.* Phytopathology 78:668-672.
16. Jacobson, D. J., and Gordon, T. R. 1990. The variability of mitochondrial DNA as an indicator of relationships between populations of *Fusarium oxysporum* f. sp. *melonis.* Mycol. Res. 94:734-744.
17. Joaquim, T. R., and Rowe, R. C. 1990. Reassessment of vegetative compatibility relationships among strains of *Verticillium dahliae* using nitrate-nonutilizing mutants. Phytopathology 80:1160-1166.
18. Klittich, C. J. R., and Leslie, J. F. 1988. Nitrate reduction mutants of *Fusarium moniliforme* (*Gibberella fujikuroi*). Genetics 118:417-423.
19. Kuhlman, E. G. 1982. Varieties of *Gibberella fujikuroi* with anamorphs in *Fusarium* section *Liseola.* Mycologia 74:759-768.
20. Kuhlman, E. G., Dianis, S. D., and Smith, T. K. 1982. Epidemiology of pitch canker disease in a loblolly pine seed orchard in North Carolina. Phytopathology 72:1212-1216.
21. Lee, S. B., Milgroom, M. G., and Taylor, J. W. 1988. A rapid, high yield mini-prep method for isolation of total genomic DNA from fungi. Fungal Gen. Newsl. 35:23-24.
22. McCain, A. H., Koehler, C. S., and Tjosvold, S. A. 1987. Pitch canker threatens California pines. Calif. Agric. 41:22-23.
23. Murамoto, M., and Dwinell, L. D. 1990. Pitch canker of *Pinus luchuensis* in Japan. Plant Dis. 74:530.
24. Ploetz, R. C., and Shokes, F. M. 1986. Evidence for homothallism and vegetative compatibility in southern *Diaporthe phaseolorum.* Can. J. Bot. 64:2197-2200.
25. Puhalla, J. E. 1985. Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. Can. J. Bot. 63:179-183.
26. Puhalla, J. E., and Spieth, P. T. 1985. A comparison of heterokaryosis and vegetative incompatibility among varieties of *Gibberella fujikuroi* (*Fusarium moniliforme*). Exp. Mycol. 9:39-47.
27. Zambino, P. J., and Harrington, T. C. 1990. Heterokaryosis and vegetative compatibility in *Leptographium wageneri.* Phytopathology 80:1460-1469.