Genetic Characterization by RAPD Analysis of Isolates of *Fusarium oxysporum* f. sp. *erythroxyli* Associated with an Emerging Epidemic in Peru

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ABSTRACT

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An epidemic of vascular wilt caused by *Fusarium oxysporum* f. sp. *erythroxyli* is currently occurring on *Erythroxylum coca* var. *coca* in the coca-growing regions of the Huallaga Valley in Peru. Random amplified polymorphic DNA (RAPD) analysis of isolates of the pathogen was undertaken to elucidate its genetic complexity, as well as to identify a specific DNA fingerprint for the pathogen. Two hundred isolates of *Fusarium* were collected from 10 coca-growing regions in Peru. Of these, 187 were confirmed to be *F. oxysporum*, and 143 of the *F. oxysporum* were shown to be pathogens of coca by a root-dip pathogenicity test. The pathogens could be grouped into two subpopulations based on RAPD analysis, and no polymorphism in RAPD pattern was observed among isolates of either subpopulation. Both subpopulations were present in the central Huallaga Valley, where earliest reports of the epidemic occurred. RAPD analysis could easily distinguish the isolates of *F. oxysporum* f. sp. *erythroxyli* from the nonpathogenic isolates of *F. oxysporum* from *E. coca* var. *coca*, indicating its utility in DNA fingerprinting.

Fusarium oxysporum Schlechtend.:Fr. (19) causes extensive disease problems as a vascular wilt pathogen on a variety of crops worldwide. In Peru, an epidemic of wilt disease has emerged on coca, *Erythroxylum coca* Lam. var. *coca* (2), the source of the narcotic cocaine. The forma specialis responsible for this disease has been designated f. sp. *erythroxyli*, and its host range includes several *Erythroxylum* species in addition to *E. coca* (17,18). Although definitive reports of the presence of the disease can be found as early as 1932, its incidence increased sharply in the 1980s, coinciding with the boom in coca production and the subsequent increased use of agricultural chemicals and introduction of a shortened cultivation time (1,20). The disease now occurs in epidemic proportions in most of the coca-producing areas of the Huallaga Valley, with the exception of the southernmost regions (3). Little is known about the genetic complexity of *F. oxysporum* f. sp. *erythroxyli* populations in Peru or about the origin and spread of the pathogen, i.e., whether it arose as a single lineage in one location or arose independently in several locations.

Random amplified polymorphic DNA (RAPD) analysis (24) has many advantages as a means of characterizing genetic variability such as speed, low cost, minimal requirement for DNA, and lack of radioactivity. Major polymorphisms in RAPD pattern indicate genetic distinctness and can be used to distinguish unrelated groups. Minor polymorphisms may indicate genetic distinctness within groups or may occur because of experimental variability and, therefore, must be verified by repetition. RAPD analysis has been used effectively to distinguish between species of *Fusarium* (23,27). RAPD analysis has also successfully delineated groups within *Fusarium* species including *F. avenaceum* (27), *F. graminearum* (13), and *F. moniliforme* (23), as well as within formae speciales of *F. oxysporum*. One group was delineated within *F. oxysporum* f. sp. *albedinis* (22), two groups within f. sp. *ciceris* (9), two groups within f. sp. *cubense* (5,14), one or two groups within f. sp. *dianthi* (11,26), two or three groups within f. sp. *pisi* (8,15), and three groups within f. sp. *vasinfectum* (4). For all of the formae speciales, except *albedinis* and *dianthi*, some minor polymorphisms in RAPD pattern were observed among isolates within each group.

The current study was undertaken to elucidate the genetic complexity of *F. oxysporum* f. sp. *erythroxyli* in Peru based on RAPD analysis and to provide insight into its geographic origin. A second objective was to develop a specific DNA fingerprint for *F. oxysporum* f. sp. *erythroxyli*.

MATERIALS AND METHODS

Fungal isolates. Field isolates were collected from an area in Peru where coca (*E. coca* var. *coca*) is cultivated on a large scale, covering much of the Huallaga Valley. The area was arbitrarily divided into 10 regions of near equal size, each region encompassing five field locations (Fig. 1). The field locations were surveyed for the presence of symptomatic coca plants. Plants that showed vascular wilt symptoms, or portions of those plants, were collected and brought to the laboratory at the Universidad Nacional Agraria de la Selva in Tingo María, Peru. Stem sections were taken from those plants and plated on slants of Komada's medium (10). In total, 200 isolates were collected. The isolates were brought to USDA-ARS facilities in Beltsville, MD, where each was single-spored, transferred to nährstoffarmer agar (0.1% KH_2PO_4, 0.1% KNO_3, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% KCl, 0.02% glucose, 0.02%

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saccharose, and 2% agar) (12), and identified microscopically. Those isolates that were confirmed to be *F. oxysporum* were preserved by allowing them to colonize toothpicks on water agar. The colonized toothpicks were dried and stored at 4°C.

Other formae speciales of *F. oxysporum* and other species of *Fusarium* that were isolated from their hosts either in neighboring locations in Peru or in the United States and confirmed to be pathogenic on their respective hosts were processed as above. *F. oxysporum* f. sp. *basilicum* was isolated from *Ocimum basilicum* (gift of R. Larkin, USDA, Beltsville, MD); *F. oxysporum* f. sp. *lycopersici* from *Lycopersicon esculentum* (gift of R. Larkin); *F. oxysporum* f. sp. *melonis* from *Cucumis melo* (gift of R. Larkin); *F. oxysporum* f. sp. *niveum* from *Citrullus vulgaris* (gift of A. Keinath, Clemson University, Clemson, SC); *F. acuminatum* from *Zea mays* (gift of W. Mao, USDA, Beltsville, MD); *F. culmorum* from *Zea mays* (gift of W. Mao); *F. graminearum* from *Zea mays* (gift of W. Mao); and *F. sambucinum* from *Oxalis tuberosa* (gift of T. Icochea, International Potato Center, Lima, Peru).

Coca seedlings. Fruits were harvested from plants of *E. coca* var. *coca* at the USDA-ARS Weed Science Laboratory field test site in Kauai, HI. Fruits were soaked in 10% bleach for 5 min, rinsed in water, and then soaked in water several days until the pulp fermented. The softened pulp was removed and the seeds were sterilized in 10% bleach for 5 min, rinsed with sterile water, and planted in Pro Mix potting soil (Premier Brands, Inc., Yonkers, NY) in seedling trays. The seedling trays were covered with plastic to retain moisture and maintained at 23°C with 12-h days/12-h nights. After germination (approximately 1 month), the plastic was removed and the seedling trays were transferred to a greenhouse, maintained at 26 to 30°C with 12-h days/12-h nights, and watered daily. Seedlings were grown to the six-leaf stage, which usually occurred 2 to 3 months after seeding.

Pathogenicity tests. Pathogenicity of all of the *Fusarium* isolates on *E. coca* var. *coca* was determined using a root-dip assay modified from that of Williams (25). Greater inoculum concentrations, inoculum incubation times, and overall pathogenicity test times were necessary, because *E. coca* var. *coca* is a woody plant. Pathogenicity tests were begun at weekly intervals, consisting of 20 to 30 isolates per test. In each test, a confirmed pathogenic isolate and a confirmed nonpathogenic isolate were included as controls. Each of the isolates was grown on potato dextrose agar (PDA), and plugs were used to initiate cultures in 100 ml of potato dextrose broth. Cultures were grown for 3 days on an orbital shaker (~120 rpm) at 26°C with 12-h days/12-h nights. The concentration of microconidia was determined for each culture, and it was diluted with water to a final concentration of 1×10^7 microconidia/ml in a total volume of 50 ml. Three dilutions were made for each isolate.

Coca seedlings at the six-leaf stage were removed from seedling trays, and their roots were trimmed to approximately half of their initial length and submerged in inoculum. Five seedlings were submerged in each 50 ml of inoculum, a total of 15 seedlings per isolate. After treatment for 3 h, the seedlings were planted in 4-inch-diameter pots (five seedlings per pot) in Pro Mix potting soil, and the soil was drenched with the inoculum. Plants were maintained in an environmentally controlled greenhouse cubicle with 12-h days at 28°C/12-h nights at 22°C.

Each week, plants were scored as healthy, chlorotic (many leaves dropped or yellowing leaves), or necrotic (all leaves dropped), and the stems of necrotic plants were cut at soil level. Three 1-cm-long sections were cut from each stem beginning at the base of the stem. The sections were surface-disinfected in 10% bleach for 2 min, rinsed twice in sterile water, and plated on Komada's medium. The culture dishes were incubated at 26°C in the dark, and after 2 to 3 days, the vascular tissue was checked for colonization by *F. oxysporum* f. sp. *erythroxyli*. In diseased plants, *F. oxysporum* f. sp. *erythroxyli* grew out of both ends of each stem section, indicating successful colonization of the vascular tissue. In plants that died as a result of pruning injury or transplant shock, no colonization by *F. oxysporum* f. sp. *erythroxyli* was observed, and death occurred within days rather than weeks. Also, in such cases, leaves generally remained attached to the stem after wilting, in contrast to diseased plants in which leaves dropped immediately after wilting. Pathogenicity tests were continued for a total of 12 weeks. At 12 weeks, all remaining plants (healthy, chlorotic, or necrotic) were removed from the pots and checked for colonization by *F. oxysporum* f. sp. *erythroxyli* as described above. The percentage of colonized plants was assessed for each isolate. The test allowed differentiation of the isolates pathogenic on coca from the nonpathogenic isolates. Isolates that caused symptoms of vascular wilt on any percentage of plants and that could be reisolated from the symptomatic vascular tissue were considered to be pathogens.

DNA isolation. Each isolate was transferred from PDA to 10 ml of Czapek-Dox broth with 1% casamino acids in a 50-ml conical centrifuge tube. Cultures were grown on an orbital shaker (~120 rpm) at 26°C for 3 days. Cultures were transferred to a 15-ml snap-cap tube and centrifuged at 4,000 × *g* for 10 min at 4°C. The supernatant was poured off, and cultures were frozen at –80°C for several hours and then lyophilized.

The lyophilized cultures were used for genomic DNA isolation according to the method of Rehner and Samuels (16) with minor modifications. Each lyophilized culture was ground directly in the snap-cap tube in liquid nitrogen with a sterile glass rod. The ground culture was transferred to a 1.5-ml tube and suspended in 600 µl of extraction buffer (2% sodium dodecyl sulfate, 100 mM Tris-HCl [pH 8.0], 100 mM NaCl, and 10 mM EDTA). Samples were incubated at 65°C for 30 min, extracted with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1), and centrifuged at 7,500 × *g* for 30 min. Approximately 500 µl of the upper aqueous phase was transferred to a clean 1.5-ml tube, and the DNA was precipitated with 300 µl of isopropanol at –20°C for 30 min. The tube was centrifuged at 7,500 × *g* for 2 min and dried briefly in a vacuum concentrator. The isolated DNA was dissolved in 50 to 100 µl of Tris-EDTA buffer (10 mM Tris-HCl [pH 7.4] and 1 mM EDTA [pH 8.0]) by heating for 5 to 10 min at 65°C. Isolated DNA was stored at 4°C until it was used for RAPD analysis.

Fig. 1. Map of Peru showing the 10 regions within the coca-growing area from which isolates of *Fusarium* were obtained.

RAPD analysis. Isolated *Fusarium* DNA was amplified by the RAPD method using the "A," "B," and "C" kits of random oligonucleotide (10-mer) primers from Operon Technologies, Inc. (Alameda, CA). All isolates were analyzed with the B-14 primer. Twenty isolates, representative of all of the coca-growing regions of Peru, were evaluated with all of the remaining primers from the A, B, and C kits. RAPD analysis was repeated at least three times per isolate. Approximately 50 ng of genomic DNA was combined with the following reaction mix to a final volume of 50 µl: 10 mM Tris-HCl [pH 8.0], 50 mM KCl, 3 mM $MgCl_2$, 0.001% gelatin, 100 µM dNTPs, and 0.5 µM primer. The DNA was denatured in the reaction mix for 5 min at 94°C, and then 2 units of *Taq* DNA polymerase (Promega Corp., Madison, WI) was added to each tube. The reactions were amplified in a thermocycler (M. J. Research, Inc., Watertown, MA) for 45 cycles of 1 min at 94°C, 1 min at 36°C, and 2 min at 72°C. The final extension was continued for an additional 5 min, and then reactions were held at 4°C until gel electrophoresis.

Amplified DNA was separated by electrophoresis on a 1% agarose gel in 1× Tris-borate-EDTA. The size of separated bands was determined by running in parallel a 1-kb DNA ladder (Gibco BRL, Gaithersburg, MD). Gels were stained with ethidium bromide to visualize the DNA, and photographs were taken using an MP4 Land Camera with 665 positive/negative black and white film (Polaroid Corp., Cambridge, MA).

RESULTS

Distribution of isolates. The area of the Huallaga Valley where coca is grown extensively was evaluated for the presence of coca plants exhibiting symptoms of vascular wilt disease. The disease was found to be present on coca plants in all of the coca-growing regions except region 10 (Fig. 1). A total of 200 fungal isolates was obtained from the nine regions where the disease was present. Few isolates were obtained from region 4, because of limited access to the region. However, at least 10 isolates were collected from all of the other coca-growing regions. Of the 200 isolates collected, only 13 were not *F. oxysporum* (Table 1).

Pathogenicity tests. Root-dip inoculation tests indicated that most of the *F. oxysporum* isolates were pathogenic. Of the 187 isolates confirmed to be *F. oxysporum*, 143 were found to be pathogenic on *E. coca* var. *coca* (Table 1). Pathogenic isolates caused wilt symptoms on at least some inoculated plants and could be reisolated from the vascular tissue of the symptomatic plants. When vascular wilt occurred, the percentage of plants exhibiting wilt symptoms ranged from 30 to 100%, with an average of 65%. Necrosis generally began at 3 to 4 weeks postinoculation and was near completion by 8 to 12 weeks.

RAPD analysis of pathogenic isolates of *F. oxysporum*. Genetic variability among the 143 pathogenic isolates from Peru was assessed by RAPD analysis. Using the random primer B-14, RAPD analysis of genomic DNA from the pathogenic isolates revealed the presence of two subpopulations. The subpopulations could be distinguished by the different banding patterns obtained after amplification of genomic DNA from the isolates using the primer (Fig. 2). No polymorphism was observed among isolates of the two subpopulations. Evaluation of all of the pathogenic isolates revealed that only these two subpopulations were present in the collection from Peru. Both subpopulations showed a wide geographic distribution across the coca-growing regions in Peru; however, subpopulation I was generally more prevalent than subpopulation II in all regions (Table 1). Subpopulation II was less prevalent in the north (regions 1 to 4) than in other regions, and subpopulation I was not detected in region 9 (Table 1).

Further characterization by RAPD analysis of 20 isolates representative of all regions of Peru indicated that the two subpopulations could be distinguished using every primer from the A, B, and C kits, a total of 60 primers. With all of the primers, each isolate always fell into the same subpopulation, and no polymorphism among isolates within each subpopulation was ever observed. Representative primers that could distinguish the two subpopulations and that showed distinctive banding patterns after amplification of genomic DNA are primer A-5 (Fig. 3) and primer A-17 (Fig. 4).

RAPD analysis of nonpathogens and other *Fusarium* species. To establish the utility of RAPD analysis as a DNA fingerprint for *F. oxysporum* f. sp. *erythroxyli*, isolates of the pathogen were compared with nonpathogenic isolates of *F. oxysporum* from *E. coca* var. *coca*. Amplification of genomic DNA from all of the nonpathogenic isolates with the B-14 primer gave banding patterns distinct from either of the two RAPD patterns characteristic of the pathogenic isolates (Fig. 5). In addition, the two characteristic patterns of isolates of *F. oxysporum* f. sp. *erythroxyli* obtained

TABLE 1. Number of *Fusarium* isolates collected from different regions in Peru

Region	Total	Not *F. oxysporum*	*F. oxysporum*	Non-pathogenic[a]	Pathogenic[a]	Subpopulation[b] I	Subpopulation[b] II
1	13	1	12	6	6	6	0
2	13	1	12	3	9	9	0
3	11	0	11	2	9	7	2
4	5	2	3	0	3	3	0
5	38	0	38	5	33	28	5
6	35	4	31	9	22	16	6
7	24	4	20	6	14	8	6
8	50	1	49	5	44	32	12
9	11	0	11	8	3	0	3
10	0	0	0	0	0	0	0
Total	200	13	187	44	143	109	34

[a] On coca.
[b] As defined by random amplified polymorphic DNA analysis (details in text).

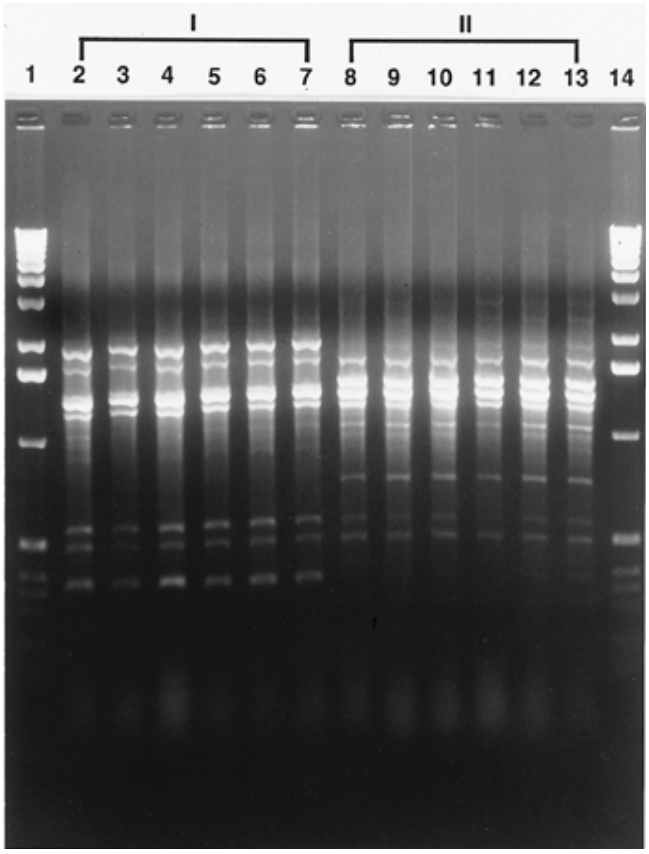

Fig. 2. Random amplified polymorphic DNA (RAPD) analysis of genomic DNA from isolates of *Fusarium oxysporum* f. sp. *erythroxyli* with the B-14 primer. The products were separated on a 1% agarose gel. Representative samples are shown. Lane 1, 1-kb DNA ladder; lanes 2 to 7, RAPD pattern of six isolates of subpopulation I; lanes 8 to 13, RAPD pattern of six isolates of subpopulation II; and lane 14, 1-kb DNA ladder.

after amplification of genomic DNA with the B-14 primer were distinct from the patterns obtained with the other formae speciales of *F. oxysporum* and the other species of *Fusarium* tested (Fig. 6).

DISCUSSION

In the root-dip assay, the variation in measured rate and extent of symptom development and fungal colonization was likely dependent on the success of initial entrance of the fungus into the root tissue as determined by the degree of injury to the roots during the pruning process. The significant heterogeneity among coca seedlings also contributed to the rate and extent of symptom development, as did the season of the year. Hence, the root-dip assay was used strictly as a qualitative test of pathogenicity and was not an effective test of aggressiveness.

Our results indicate that there is little genetic variability among isolates of *F. oxysporum* f. sp. *erythroxyli* in Peru as identified by RAPD analysis. The pathogens can be divided into two subpopulations, and no polymorphism in RAPD pattern occurred among isolates of the two subpopulations. The data suggest that the isolates of *F. oxysporum* f. sp. *erythroxyli* are derived from two genetically distinct clones. The limited genetic variability observed among isolates of *F. oxysporum* f. sp. *erythroxyli* as indicated by RAPD analysis would be expected for a pathogen that became widespread relatively quickly as a result of an increase in production of the host plant.

The limited variability among isolates of *F. oxysporum* f. sp. *erythroxyli* makes it difficult to speculate as to the center of origin or the direction of spread of the epidemic. However, both of the subpopulations are represented in region 5, where the earliest reports of the epidemic occurred (1,20). The complete homogeneity in RAPD pattern within the subpopulations contrasts with what has been observed for most other formae speciales of *F. oxysporum* (4,5,8,9,14,15). Interestingly, two other pathogens of woody tree species showed similar results. No polymorphism was observed in RAPD analysis of 42 isolates of *F. oxysporum* f. sp. *albedinis* from date palm (22) and near identical RAPD patterns were observed among 39 isolates of *F. oxysporum* from angsana (*Pterocarpus indicus*) (7).

Recent data indicate that *F. oxysporum* f. sp. *erythroxyli* is transmitted by seed (J. Gracia-Garza, *personal communication*), and hence may be closely associated with the host population. However, the limited genetic variability observed among isolates of *F. oxysporum* f. sp. *erythroxyli* based on RAPD analysis is not likely to be attributable to coevolution with a homogeneous host population. *E. coca* is highly outcrossed and shows considerable genetic variability (6), providing ample opportunity for genetic evolution of the pathogen population.

The two subpopulations are well dispersed across the coca-growing regions in Peru. Although subpopulation II is not well represented in regions 1 to 4 and subpopulation I has not been detected in region 9, the two subpopulations are not isolated geographically. The data indicate that the isolates exist in two subpopulations in Peru independent of geographic location. This is in contrast to what

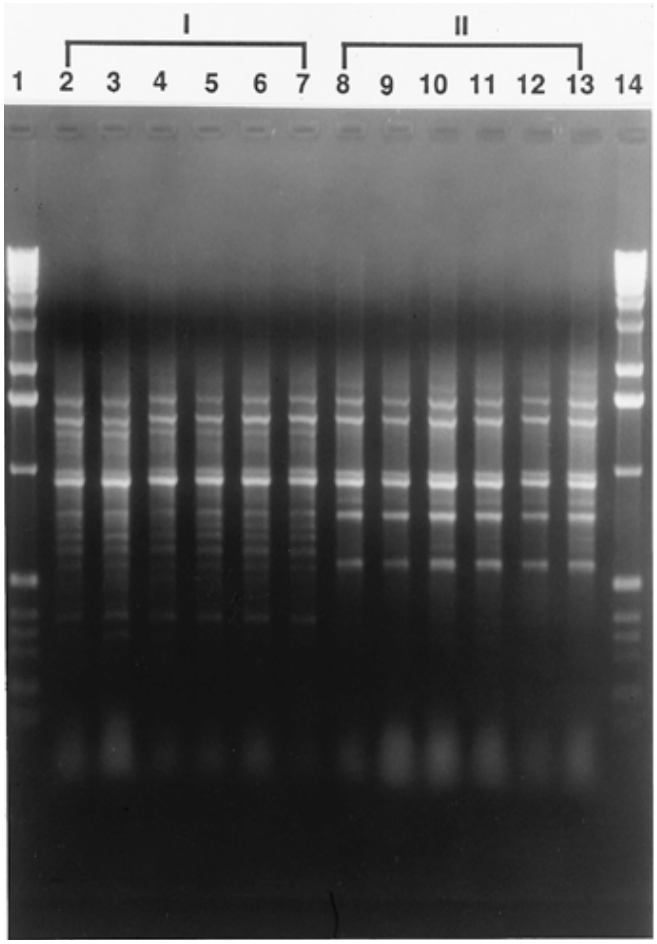

Fig. 3. Random amplified polymorphic DNA (RAPD) analysis of genomic DNA from isolates of *Fusarium oxysporum* f. sp. *erythroxyli* with the A-5 primer. The products were separated on a 1% agarose gel. Representative samples are shown. Lane 1, 1-kb DNA ladder; lanes 2 to 7, RAPD pattern of six isolates of subpopulation I; lanes 8 to 13, RAPD pattern of six isolates of subpopulation II; and lane 14, 1-kb DNA ladder.

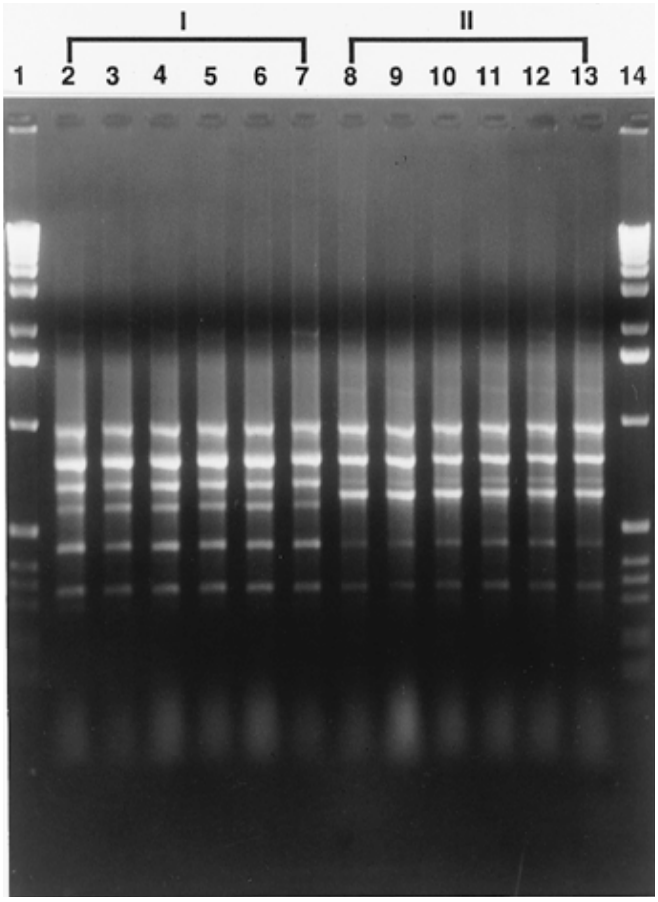

Fig. 4. Random amplified polymorphic DNA (RAPD) analysis of genomic DNA from pathogenic isolates of *Fusarium oxysporum* f. sp. *erythroxyli* with the A-17 primer. The products were separated on a 1% agarose gel. Representative samples are shown. Lane 1, 1-kb DNA ladder; lanes 2 to 7, RAPD pattern of six isolates of subpopulation I; lanes 8 to 13, RAPD pattern of six isolates of subpopulation II; and lane 14, 1-kb DNA ladder.

was reported for *F. oxysporum* f. sp. *vasinfectum*, in which a close correlation was observed between RAPD pattern and geographic location (4). However, a similar lack of correlation between RAPD pattern and geographic location was reported for *F. oxysporum* f. sp. *lycopersici* (21) and f. sp. *pisi* (15). Our recent results indicate that subpopulation II is also present on the Peruvian coast at Trujillo on *E. novogranatense* var. *truxillense* and in Hawaii on *E. coca* var. *coca*. Subpopulation II appears to be identical to the pathogenic isolate En4 previously characterized from Hawaii (18). Coca was brought to Hawaii from both Trujillo and Cuzco (region 9), and the disease was reportedly present on plants that were germinated from the newly transported seed (L. C. Darlington, *personal communication*), hence subpopulation II did not arise independently in the different geographic locations. Recent reports have indicated that *F. oxysporum* f. sp. *erythroxyli* may be present in the coca-growing regions in Colombia (1). It will be interesting to see if the same two subpopulations are present there.

Analysis of the same isolates from Peru by vegetative compatibility grouping (VCG) revealed an identical grouping of the isolates into two genetically distinct subpopulations (K. S. Elias, *personal communication*). Hence, the results from RAPD analysis and VCG analysis are corroborative and suggest a relative lack of genetic diversity among isolates of *F. oxysporum* f. sp. *erythroxyli*. Some correlation between RAPD data and VCG data was also found for *F. oxysporum* f. sp. *albedinis* (22), f. sp. *cubense* (5,14), f. sp. *dianthi* (26), and f. sp. *pisi* (15). For *F. oxysporum* f. sp. *cubense*, several VCGs were found within each RAPD group, indicating that although RAPD analysis and VCG analysis may both be good indicators of genetic variability within formae speciales of *F. oxysporum*, the same groupings of isolates may not always be achieved with the two methods of analysis (5,14).

Our results suggest that *F. oxysporum* f. sp. *erythroxyli* is distinguishable from other species of *Fusarium* by RAPD analysis, because no common RAPD bands were observed between *F. oxysporum* f. sp. *erythroxyli* and the other *Fusarium* species tested. This is in agreement with the results of Yli-Mattila et al. (27) and Voigt et al. (23). Also, *F. oxysporum* f. sp. *erythroxyli* appears to be distinguishable from other formae speciales by RAPD analysis, because distinct banding patterns were observed between *F. oxysporum* f. sp. *erythroxyli* and the other formae speciales tested. Testing of more isolates of other species of *Fusarium* and of other formae speciales of *F. oxysporum* will be necessary to confirm the uniqueness of f. sp. *erythroxyli* and to better understand its relationship to the other formae speciales within *F. oxysporum* and to other species of *Fusarium*.

That *F. oxysporum* f. sp. *erythroxyli* and nonpathogens of *F. oxysporum* from *E. coca* var. *coca* could be distinguished by RAPD analysis is consistent with reports for f. sp. *albedinis* (22) and f. sp. *dianthi* (11,26), but contrasts with results for *F. oxysporum*

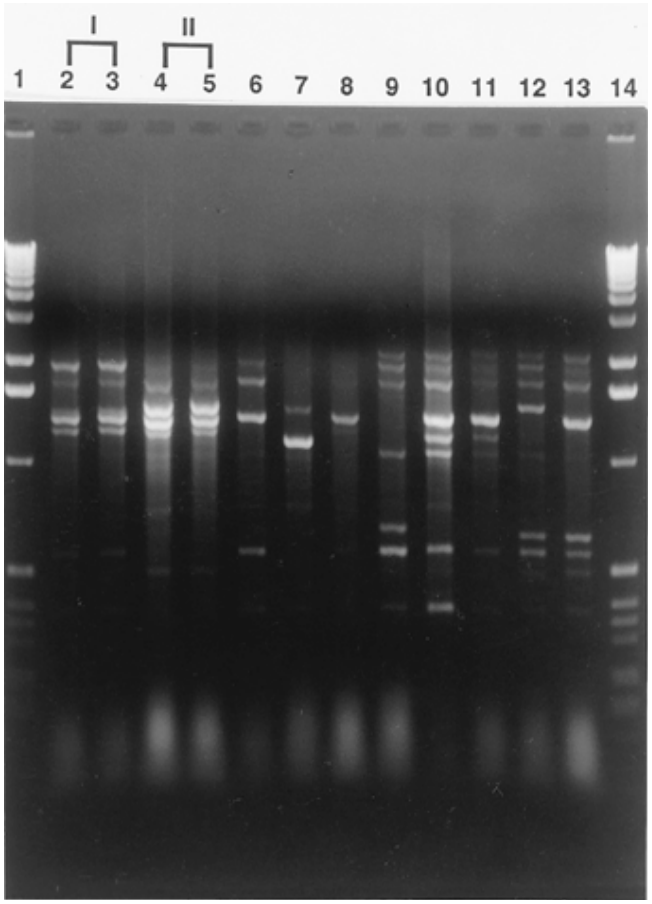

Fig. 5. Random amplified polymorphic DNA (RAPD) analysis of genomic DNA from nonpathogenic isolates of *Fusarium oxysporum* with the B-14 primer. The products were separated on a 1% agarose gel. Representative samples are shown. Amplified DNA from *F. oxysporum* f. sp. *erythroxyli* isolates is shown for comparison. Lane 1, 1-kb DNA ladder; lanes 2 to 3, RAPD pattern of two *F. oxysporum* f. sp. *erythroxyli* isolates of subpopulation I; lanes 4 to 5, RAPD pattern of two *F. oxysporum* f. sp. *erythroxyli* isolates of subpopulation II; lanes 6 to 13, RAPD pattern for eight different nonpathogenic isolates; and lane 14, 1-kb DNA ladder.

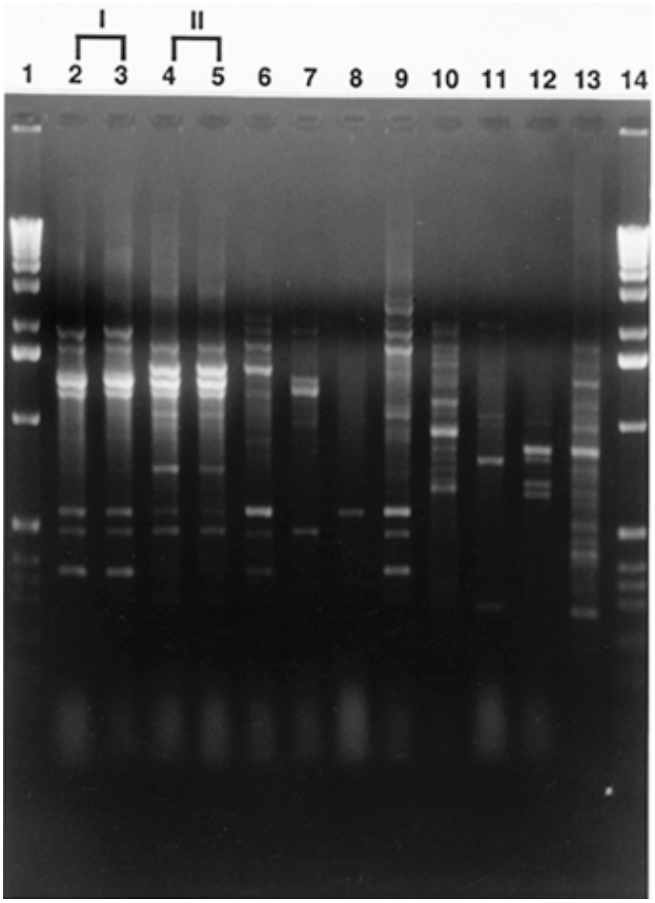

Fig. 6. Random amplified polymorphic DNA (RAPD) analysis of genomic DNA from various formae speciales of *Fusarium oxysporum* and various species of *Fusarium* with the B-14 primer. The products were separated on a 1% agarose gel. Representative samples are shown. Amplified DNA from *F. oxysporum* f. sp. *erythroxyli* isolates is shown for comparison. Lane 1, 1-kb DNA ladder; lanes 2 to 3, RAPD pattern of two *F. oxysporum* f. sp. *erythroxyli* isolates of subpopulation I; lanes 4 to 5, RAPD pattern of two *F. oxysporum* f. sp. *erythroxyli* isolates of subpopulation II; lane 6, *F. oxysporum* f. sp. *basilicum*; lane 7, *F. oxysporum* f. sp. *lycopersici*; lane 8, *F. oxysporum* f. sp. *melonis*; lane 9, *F. oxysporum* f. sp. *niveum*; lane 10, *F. acuminatum*; lane 11, *F. culmorum*; lane 12, *F. graminearum*; lane 13, *F. sambucinum*; and lane 14, 1-kb DNA ladder.

f. sp. *lycopersici* (21). Hence, it appears that the relationship between pathogens and nonpathogens of *F. oxysporum* varies with the host species. Some studies have indicated that RAPD patterns can be used to distinguish isolates with differing levels of virulence in *F. oxysporum* f. sp. *ciceris* (9), f. sp. *lycopersici* (21), and f. sp. *vasinfectum* (4). No such comparisons can be made for *F. oxysporum* f. sp. *erythroxyli*, because no virulence data are available for the different isolates given the limitations of the root-dip assay and the genetic variability among *E. coca* seedlings. In fact, the large amount of genetic variability among the host population has contributed to the problem in defining races of the pathogen. So, whereas RAPD analysis has been used to delineate at least some races of *F. oxysporum* f. sp. *cubense* (14), f. sp. *pisi* (8), and f. sp. *vasinfectum* (4), no such comparisons can be made at this time for *F. oxysporum* f. sp. *erythroxyli*.

RAPD analysis is an effective way to identify isolates of *F. oxysporum* f. sp. *erythroxyli* and distinguish them from nonpathogenic isolates of *F. oxysporum* from *E. coca* var. *coca*. Furthermore, RAPD analysis may also allow delineation of *F. oxysporum* f. sp. *erythroxyli* from other formae speciales of *F. oxysporum* and from other species of *Fusarium*. These results indicate that RAPD analysis can be effectively employed as a reliable DNA fingerprinting technique to study the spread of the pathogen.

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