

# Effect of Temperature, Soil Type, and Matric Potential on Proliferation and Survival of *Fusarium oxysporum* f. sp. *erythroxyli* from *Erythroxylum coca*

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## ABSTRACT

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Natural epidemics of Fusarium wilt on coca have stimulated interest in the causal agent *Fusarium oxysporum* f. sp. *erythroxyli*. Effects of constant and fluctuating temperatures, soil matric potential, and soil type on the proliferation of *F. oxysporum* f. sp. *erythroxyli* isolate EN4-FT from an alginate prill formulation were studied. Three or four soils were used: a Galestown gravelly loamy sand (GGLS), Hatboro loamy sand (HLS), and red clay subsoil (RC) were collected in Beltsville, MD. A clay loam soil from a planting of coca in Hawaii (HCL) was substituted for RC soil in some experiments. Prill containing *F. oxysporum* f. sp. *erythroxyli* were placed on the surface of GGLS, HLS, or HCL soils and maintained at -10, -100, or -500 kPa for 1 week. Matric potential and soil type significantly affected proliferation of the pathogen into the soils. Proliferation was greatest in HCL soil and least in HLS soil. The population density of *F. oxysporum* f. sp. *erythroxyli* at -10 and -100 kPa was approximately  $10^4$  to  $10^5$  CFU/g, whereas the population density at -500 kPa was approximately  $10^3$  CFU/g. During 17 weeks of sampling

GGLS, HLS, and RC soils maintained at -50 kPa, *F. oxysporum* f. sp. *erythroxyli* proliferated from prill into soil when constant temperatures ranged from 10 to 32°C, resulting in  $10^3$  to  $10^5$  CFU/g of soil after 1 wk of incubation. Optimum proliferation was at 25°C in all soils. Compared to other temperatures, lower numbers of propagules were recovered from soils stored at 40°C. Two regimes of fluctuating temperature (cycles of 12 h at 15°C followed by 12 h at 25°C or cycles of 12 h at 25°C followed by 12 h at 35°C) also were tested for their effect on proliferation of *F. oxysporum* f. sp. *erythroxyli* from prill into three soils maintained at -50 kPa. Population densities were initially  $10^3$  to  $10^6$  CFU/g of soil and declined over the 17-week test period. After 17 weeks, population densities in GGLS soil were greater than in HLS or RC soils in both temperature regimes. In HLS and RC soils, the number of CFU of *F. oxysporum* f. sp. *erythroxyli* per g was significantly higher in autoclaved than in nonautoclaved soil, indicating that these two soils were fungistatic. No differences were observed between autoclaved and nonautoclaved soils for HCL and GGLS soils, and these two soils were considered conducive to proliferation of *F. oxysporum* f. sp. *erythroxyli*.

*Additional keywords:* mycoherbicide.

*Erythroxylum coca* Lam. and *E. novogranatense* (Morris) Hieron. are grown in several South American countries as sources of cocaine (32). Natural epidemics of Fusarium wilt, caused by *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *erythroxyli*, occurring on coca in the Huallaga Valley of Peru and in Hawaii have stimulated interest in this pathogen, both from the viewpoint of controlling the disease in legal coca production and as a potential mycoherbicide to limit illicit production (4,5,21,32). Because of their host specificities and longevity in soil, *Fusarium* spp. have been used, or suggested, as mycoherbicides for several weeds, including Texas gourd, velvetleaf, prickly sida, congress weed, and the narcotic plant marijuana (6,7,12,20). A similar possibility may exist for coca.

For *F. oxysporum* f. sp. *erythroxyli* to be useful as a mycoherbicide, it must be able to proliferate from a formulated preparation and survive under a variety of environmental conditions. This research was undertaken to examine the effects of soil matric potential and temperature on proliferation of *F. oxysporum* f. sp. *erythroxyli* from an organic food base and its subsequent survival in different soils.

## MATERIALS AND METHODS

**Isolate and culture.** *F. oxysporum* f. sp. *erythroxyli* isolate EN4-FT was used for all studies. The pathogen was isolated originally from *E. coca* with wilt symptoms in Hawaii (21). The salmon color of this flat, pionnotal isolate aids in identification of the fungus on soil-dilution plates. Chlamydospores of *F. oxysporum* f. sp. *erythroxyli* were produced after 1 week of incubation with shaking in 1% nutrisoy (Lauhof Grain Co., Danville, IL) broth (P. Hebbbar, *personal communication*). These suspensions were mixed 4:6 (vol/vol) with glycerol (Sigma Chemical Company, St. Louis) and frozen until use. For experiments, chlamydospore suspensions of *F. oxysporum* f. sp. *erythroxyli* were formulated in alginate prill as follows: 29.4 g of nutrisoy plus 280 ml of distilled water (DW) were autoclaved together, and 8.4 g of sodium alginate (Sinport Corpco, New York) plus 420 ml of DW were autoclaved together. After cooling, the two autoclaved suspensions were mixed in a blender with 100 ml of chlamydospores in the broth in which they were grown. The suspension was adjusted to pH 7.5 with 1 N NaOH and added dropwise to 0.25 M CaCl<sub>2</sub>. Prill were collected, rinsed in tap water, and dried overnight under a chemical fume hood with the fan on (P. Hebbbar, *personal communication*). Prill contained approximately  $10^5$  CFU/g and were stored at 5°C for up to 6 months before use.

**Soils.** Three local soils and soil from a coca planting in Hawaii (21) were used. Local soils were collected the day before use and were passed through a 0.5-cm pore-size screen and stored over-

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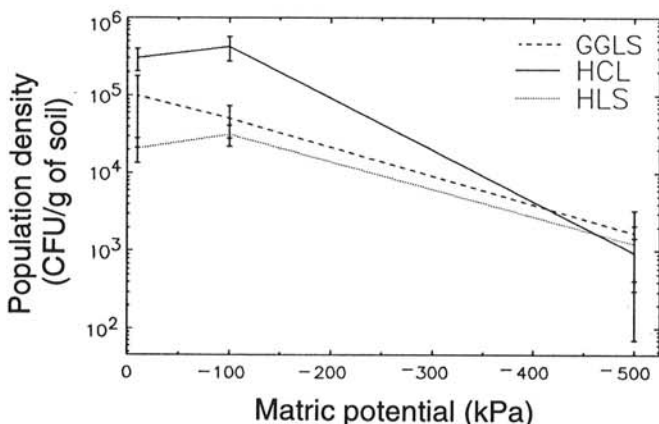
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night at 5°C before use. Soil from Hawaii was stored from 3 days to 4 months, as noted for each experiment, and was screened before use. Properties of the soils are as follows: Galestown gravelly loamy sand (GGLS), pH 5.8, sand 77.8%, silt 12.6%, clay 9.6%, organic matter (OM) 0.6%, and CEC 4.0; Hatboro loamy sand (HLS), pH 4.5, sand 59.4%, silt 28.8%, clay 11.8%, OM 3.2%, and CEC 4.5; red clay subsoil (RC), pH 4.4, sand 17.7%, silt 40.1%, clay 42.2%, OM 0.0%, and cation exchange capacity (CEC) 2.4; and Hawaiian clay (HCL), pH 5.0, sand 24.4%, silt 46.3%, and clay 28.8%.

**Matric potential.** Three ceramic plate extractors (Model 1500, SoilMoisture Equipment Co., Santa Barbara, CA) were used to control matric potential. Two extractors were fitted with valves and gauges for matric potentials down to -50 kPa, and the remaining extractor was fitted with valves and gauges for matric potentials down to -1,500 kPa. High-flow ceramic plates were used for matric potentials from -10 to -100 kPa. GGLS, HLS, and HCL soils were used in these tests. HCL soil was stored for 3 months prior to the first run of this experiment and for 4 months prior to the second run. Soil (25 g) was placed in a 5-cm-diameter rubber ring (one replicate) on ceramic plates in plastic containers with sufficient water to wet the plate. After soil equilibration in water for 6 to 15 h, plates were removed from the water. For treatments receiving prill, 10 prill containing chlamyospores of *F. oxysporum* f. sp. *erythroxyli* were placed on the soil surface of each ring. Control treatments did not receive prill.

Ceramic plates were placed in pressure chambers, and pressure was adjusted with a model 500F air compressor (SoilMoisture Equipment) to -10, -100, or -500 kPa. Soils were left undisturbed in the pressure chambers for 1 week. Prill were removed, and the soil for each replicate was mixed thoroughly. Serial dilutions of soil from each replicate were made on Komada's agar (KA) (13). Moisture content of the soil was determined by drying the soil overnight in an oven at 100°C so colony counts could be corrected for percent moisture. Dilution plates were incubated 40 cm from a fluorescent light (General Electric DLX cool white) for 1 week before recording population counts. Treatments were replicated three times, and the experiment was conducted three times.

**Constant temperature.** The GGLS, HLS, and RC soils were collected and screened as before. Before placing the soils at different temperatures, all soils were adjusted to -50 kPa with the ceramic plate extractor. Soil (25 g of each) was placed in each of 150 6-cm-diameter plastic petri dishes. Ten prill were placed on the soil surface of 75 of the petri dishes for each soil type. The remaining 75 plates served as control treatments and did not receive prill. Petri dishes were placed in a desiccator (valve not sealed) over water. Weekly throughout the experiment, water was added



**Fig. 1.** Effect of soil matric potential on proliferation of *Fusarium oxysporum* f. sp. *erythroxyli* from prill into three soils after 1 week. Soils tested included a Galestown gravelly loamy sand (GGLS) and Hatboro loamy sand (HLS) from Beltsville, MD, and a clay loam soil from Hawaii (HCL).

to soil in each plate, if needed, to bring it back to its original weight. Dishes were held at constant temperatures of 10, 18, 25, 32, or 40°C. Soils were sampled destructively after 1 week and at approximately 4-week intervals for 17 weeks. Prill were removed, and soils were mixed thoroughly before dilution plating on KA. Plates were incubated under light as before. Treatments were replicated three times, and the experiment was repeated once.

**Fluctuating temperature.** A setup similar to the constant temperature experiments was used to study proliferation of *F. oxysporum* f. sp. *erythroxyli* from prill into GGLS, HLS, and RC soils with fluctuating temperatures. Temperature regime 1 consisted of cycles of 12 h each at 15 and 25°C. Regime 2 consisted of cycles of 12 h each at 25 and 35°C. Plates were sampled destructively, as before, after 1 week and at approximately 4-week intervals for 17 weeks. Treatments were replicated three times, and the experiment was repeated once.

**Autoclaved and nonautoclaved soils.** In the above experiments, population densities of *F. oxysporum* f. sp. *erythroxyli* were significantly greater, or tended to be greater, in HCL and GGLS soils than in HLS and RC soils. To determine whether biotic or abiotic factors contributed to the differences in soil population densities, proliferation of *F. oxysporum* f. sp. *erythroxyli* in autoclaved and nonautoclaved soils was compared. GGLS, HLS, and RC soils were collected the day the experiment was initiated. In the first run of the experiment, HCL soil was collected 3 months before use and stored at 5°C until use. In the second run of the experiment, HCL soil was collected 3 days before use and stored under ambient conditions during shipping and at 5°C after receipt of the soil. Soils were screened as before to remove rocks and debris. Quantities (500 g each) of each soil were autoclaved for 1 h on each of two consecutive days, whereas the remaining nonautoclaved soil was stored at 5°C during this time. Ceramic plates for the pressure-plate apparatus were cleaned extensively with sterile distilled water (SDW) and placed under UV light for 14 h during

**TABLE 1.** Analysis of variance of the proliferation of *Fusarium oxysporum* f. sp. *erythroxyli* EN4-FT from prill into soils over time as affected by soil type and temperature when incubated under constant or fluctuating temperature regimes

Source	Constant temperature <sup>a</sup>		Fluctuating temperature <sup>a</sup>	
	df	Pr > F	df	Pr > F
Soil <sup>b</sup>	2	0.380	2	0.001**
RC vs. All	1	0.175	1	0.001**
GGLS vs. HLS	1	0.776	1	0.0035**
Temperature	4	<0.0001**	1	0.058
Linear	1	<0.0001**		
Quadratic	1	<0.0001**		
Cubic	1	0.006**		
Residual	1	0.75		
Soil × Temperature	8	0.009**	2	0.779
Error (soil)	75		30	
Time	4	<0.0001**	4	0.0001**
Linear	1	<0.0001**	1	0.0001**
Quadratic	1	0.0033**	1	0.071
Cubic	1	0.485	1	0.004**
Residual	1	0.427	1	0.626
Time × Soil	8	0.103	8	0.0015**
RC vs. All + Time	4	0.021*	4	0.031*
GGLS vs. HLS + Time	4	0.786	4	0.004**
Time × Temperature	16	0.012**	4	0.117
Time × Soil × Temperature	32	0.176	8	0.039*
Error (time)	300		120	

<sup>a</sup> Asterisks denote significant differences at  $P \leq 0.05$  (\*) and  $P \leq 0.01$  (\*\*).

<sup>b</sup> Soils included Galestown gravelly loamy sand (GGLS), Hatboro loamy sand (HLS), and a red clay subsoil (RC) from Beltsville, MD, and a clay loam soil from a field in Hawaii (HCL) infested with the pathogen.

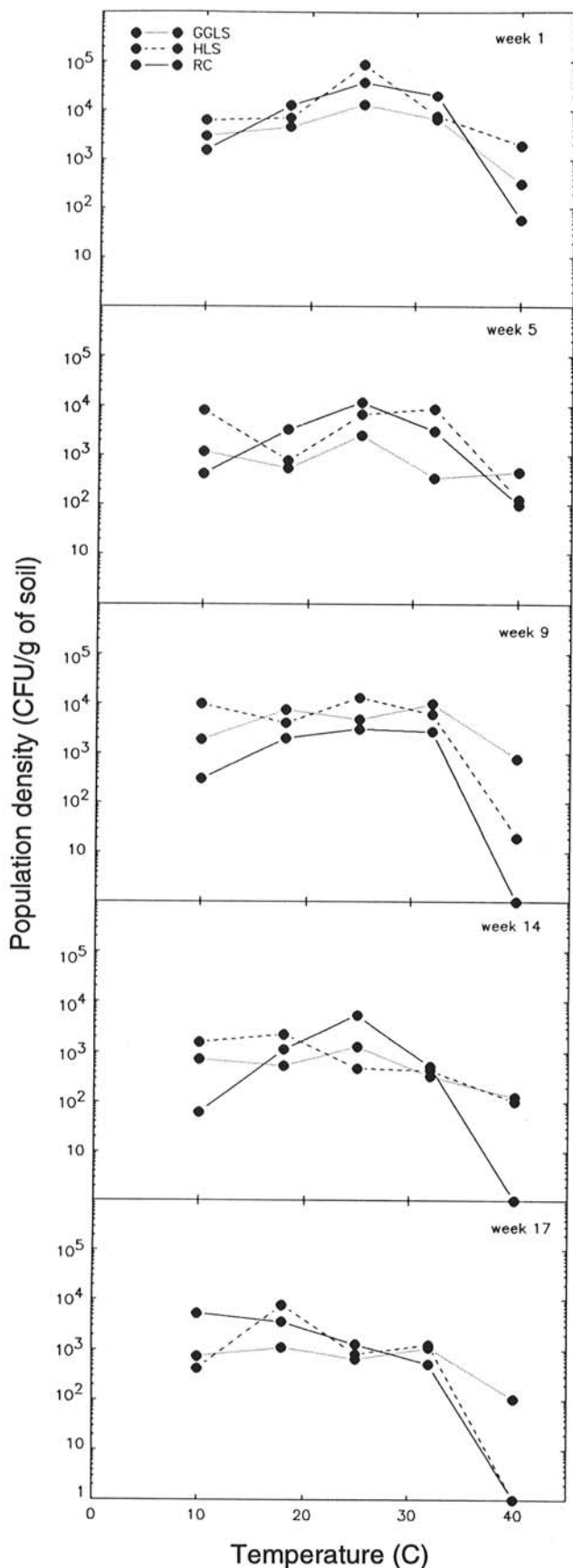


Fig. 2. Effect of constant temperature on survival and proliferation of *Fusarium oxysporum* f. sp. *erythroxyli* from prill into three soils. Soils tested included a Galestown gravelly loamy sand (GGLS), Hatboro loamy sand (HLS), and a red clay subsoil (RC) from Beltsville, MD.

each of two consecutive nights before use. Rubber rings for the ceramic plates were autoclaved.

Portions (25 g each) of autoclaved or nonautoclaved soil were placed in rubber rings as described above, and ceramic plates with rings and soil were equilibrated in pans of SDW for 8 h. Water was drained from the pans, and 10 nutrisoy prill with *F. oxysporum* f. sp. *erythroxyli* were placed on the surfaces of half of the soil rings. Control soils did not receive prill. Ceramic plates with soils were placed in the pressure-plate apparatus, and matric potential was adjusted to  $-50$  kPa. After 1 week of incubation, prill were removed, and soil population densities of *F. oxysporum* f. sp. *erythroxyli* were determined as described above.

**Data analysis.** Data were corrected for percent soil moisture and for background soil population densities of *F. oxysporum*, as well as expressed as  $\log_{10}$ , before analysis by the general linear models procedures of SAS, version 6.04 (SAS Institute, Cary, NC). Most experiments were designed as randomized complete blocks with a factorial treatment structure. Data collected over time were analyzed by repeated measures analysis of variance. Factor and interaction sources of squares were partitioned into planned orthogonal contrasts, as class contrasts for qualitative factors (soil type) and as polynomial trend contrasts for quantitative factors (temperature and time). Because data from the repetitions of each experiment were similar, data were combined for analysis.

## RESULTS

**Matric potential.** Matric potential and soil type affected proliferation of *F. oxysporum* f. sp. *erythroxyli* from prill ( $P \leq 0.0001$  and  $0.0074$ , respectively) (Fig. 1); however, an interaction between matric potential and soil type ( $P \leq 0.0154$ ) indicated significantly different responses of *F. oxysporum* f. sp. *erythroxyli* to matric potential among soils. In HCL soil, the highest population density was recovered from the  $-100$ -kPa treatment, whereas in GGLS soil the highest population density was recovered from the  $-10$ -kPa treatment. At all matric potentials, the populations of *F. oxysporum* f. sp. *erythroxyli* were most dense in HCL soil and least dense in HLS soil. Proliferation of *F. oxysporum* f. sp. *erythroxyli* in all soils at  $-500$  kPa was about  $10^3$  CFU/g, which was less ( $P \leq 0.05$ ) than proliferation at  $-100$  or  $-10$  kPa for all soil types.

**Constant temperature.** Populations of *F. oxysporum* f. sp. *erythroxyli* were affected significantly by incubation temperature (Table 1). *Fusarium oxysporum* f. sp. *erythroxyli* proliferated from prill into soils when temperatures ranged from  $10$  to  $32^\circ\text{C}$  (Fig. 2). After 1 week, population densities ranged from  $35$  to  $8.3 \times 10^4$  CFU/g of soil. Proliferation of *F. oxysporum* f. sp. *erythroxyli* from prill was greatest at  $25^\circ\text{C}$  on most sampling dates, whereas proliferation was less ( $P \leq 0.0079$ ) at  $40^\circ\text{C}$  than at all other temperatures. Overall, there were no significant differences in population densities of *F. oxysporum* f. sp. *erythroxyli* among the three soils tested, although the soil  $\times$  temperature interaction was significant, indicating that the response of population size to temperature differed among the soils. Population densities were fairly stable at  $10$  to  $32^\circ\text{C}$  but decreased slightly over time at all temperatures, with significant linear and quadratic trends in relation to time. A significant interaction between time and temperature indicated differential changes in population densities over time at different temperatures. This change was most apparent in the rapid decline of *F. oxysporum* f. sp. *erythroxyli* at  $40^\circ\text{C}$ . However, the time  $\times$  soil interaction was not significant (Table 1), indicating the changes in population densities over time were similar in all three soil types. Polynomial trend contrasts indicated significant linear, quadratic, and cubic trends with increasing temperatures.

**Fluctuating temperature.** There was no significant difference in the proliferation of *F. oxysporum* f. sp. *erythroxyli* between the two fluctuating temperature regimes; however, there were significant effects due to time and soil type (Table 1). Population densities of *F. oxysporum* f. sp. *erythroxyli* in all soils varied from  $10^4$

to nearly  $10^6$  CFU/g initially and declined somewhat in all soils over the 17-week experiment (Fig. 3). Population densities were greatest in RC soil initially but declined rapidly over time, whereas population densities were stable in GGLS soil. At 17 weeks, propagule numbers were higher in GGLS soil than in HLS and RC soils in both temperature regimes. Population densities of *F. oxysporum* f. sp. *erythroxyli* in RC and HLS soils under the 25 to 35°C regime were below the level of detection (<10 CFU/g) by 17 weeks. There was an interaction ( $P \leq 0.0015$ ) between time and soil type, primarily reflecting the more rapid decline in propagule numbers in HLS and RC soils compared to GGLS soil. This difference was especially evident in the 25 to 35°C regime. There was an interaction ( $P \leq 0.039$ ) among soil type, temperature regime, and time.

**Autoclaved and nonautoclaved soils.** Adding prill to autoclaved soil resulted in significantly greater population densities of *F. oxysporum* f. sp. *erythroxyli* than in nonautoclaved soil for HLS and RC soils (Fig. 4). In GGLS and HCL soils, propagules of *F. oxysporum* f. sp. *erythroxyli* recovered from autoclaved soil were not significantly different from those in the respective nonautoclaved soil. There were no significant differences among population densities of *F. oxysporum* f. sp. *erythroxyli* in the four autoclaved soils.

## DISCUSSION

*Fusarium oxysporum* f. sp. *erythroxyli* has the potential to be used as a mycoherbicide against illicit production of *E. coca*. Although death of plants may take as long as 2 to 3 years, *F. oxysporum* f. sp. *erythroxyli* stunts plants and limits production (5). *F. oxysporum* f. sp. *erythroxyli* is likely to persist in soil for several years and discourage replanting of the same crop. Natural epidemics of Fusarium wilt in Hawaii and Peru indicate that this pathogen has the ability to become established and spread. For *F. oxysporum* f. sp. *erythroxyli* to be useful in controlling *E. coca*, it must be able to function under a range of environmental conditions. Coca is produced on a variety of soils and under diverse environmental conditions, with air temperatures greater than 15°C (32).

Our study indicated that *F. oxysporum* f. sp. *erythroxyli* would proliferate at temperatures from 10 to 32°C and at matric potentials  $\geq -100$  kPa but that survival at 40°C would be poor. Optimum conditions for growth of other formae speciales of *F. oxysporum* have been reported as 28°C, with severe inhibition of growth below 10°C or above 35°C (9,18,19,33). Other formae speciales of *F. oxysporum* also are active over a wide range of moisture conditions, with optimal growth between -10 and -300 kPa and reduced growth at down to -1,000 kPa (8,10,19,24). Although *F. oxysporum* f. sp. *erythroxyli* isolate EN4-FT comes from a tropical climate, a response similar to that of other *F. oxysporum* was observed. Survival of isolate EN4-FT in the three soils also was similar to that observed for other pathogenic isolates of *F. oxysporum* in which population densities were fairly stable but that showed some decline in natural soils after 100 days or more (11,14).

One factor that may impede the general utility of *F. oxysporum* f. sp. *erythroxyli* as a mycoherbicide is the suppressiveness of some soils to this pathogen. Proliferation of *F. oxysporum* f. sp. *erythroxyli* from prill was observed in all soils tested. However, incubation with fluctuating temperature regimes of 15 to 25°C or 25 to 35°C accentuated the differences among soil types more than incubation at constant temperatures. Under the fluctuating temperature regime, and particularly at 25 to 35°C, populations declined more rapidly in RC and HLS soils than in GGLS. As indicated by comparison of proliferation of *F. oxysporum* f. sp. *erythroxyli* in autoclaved and nonautoclaved soils, two of the soils we tested (HLS and RC) were somewhat suppressive to proliferation of *F. oxysporum* f. sp. *erythroxyli* and two were not (HCL and GGLS). Our study did not determine subsequent incidence of Fusarium wilt on coca in these soils. However, natural infection of coca plants

in Hawaii, where soil was collected, indicates that HCL soil is conducive to wilt.

Soils that are naturally suppressive to *Fusarium* wilt diseases of numerous crops occur in many regions of the world (1,9,23,24, 31). As with the HLS and RC soils we tested, the cause of suppression in other systems is biological. However, the physical characteristics of the soil also are involved in suppressiveness. Generally, wilt-suppressive soils tend to have high clay and OM content, which support a large, diverse population of antagonistic bacteria and actinomycetes (1,3,9,30). Suppressiveness in these soils is

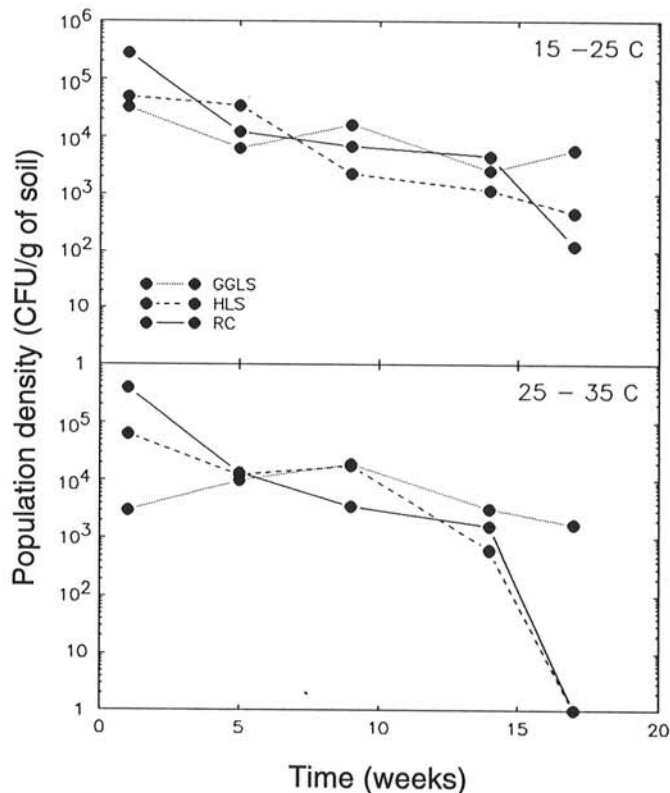


Fig. 3. Effect of fluctuating temperature on survival and proliferation of *Fusarium oxysporum* f. sp. *erythroxyli* from prill into three soils. Soils tested included a Galestown gravelly loamy sand (GGLS), Hatboro loamy sand (HLS), and a red clay subsoil (RC) from Beltsville, MD. The temperature regimes consisted of cycles of the lower temperature for 12 h followed by the higher temperature for 12 h.

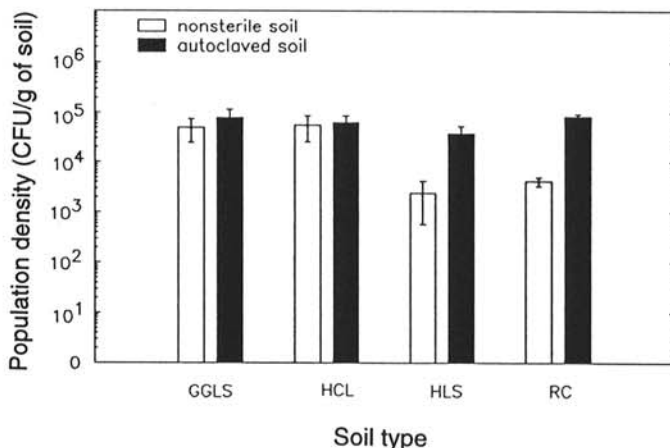


Fig. 4. Comparison of proliferation of *Fusarium oxysporum* f. sp. *erythroxyli* from prill into autoclaved and nonautoclaved soils after 1 week. Soils tested included a Galestown gravelly loamy sand (GGLS), a Hatboro loamy sand (HLS), and a red clay subsoil (RC) from Beltsville, MD, and a clay loam soil from Hawaii (HCL).

primarily a result of inhibition of chlamydospore germination and reduction in saprophytic growth of the pathogen (1,2,9,22,23,27). The two soils showing some suppressiveness in this study, RC and HLS, had the highest clay and OM content, respectively. These factors may be associated with the higher fungistatic activity of these soils due to higher populations of antagonists. The organisms primarily responsible for disease suppression in wilt-suppressive soils generally are considered nonpathogenic strains of *F. oxysporum* and fluorescent pseudomonads (1,2,9,14,22,23). However, numerous other organisms have been reported as causing or contributing to suppression, including species of *Trichoderma* (16, 17,25,26), *Penicillium* (15,18), *Arthrobacter* (27,28), *Alcaligenes* (34), *Bacillus* (35), *Hafnia* (28,29), and *Serratia* (29).

This study demonstrated that *F. oxysporum* f. sp. *erythroxyli* proliferated from prill and survived in soil under a wide range of temperature and moisture conditions and in a variety of soil types. However, suppression of this pathogen in some soils may limit its effectiveness and survival and must be taken into consideration. Additional experiments are planned to study the effect of additional edaphic parameters and relative humidity on proliferation of *F. oxysporum* f. sp. *erythroxyli* from prill into soil and on dissemination of the pathogen. The role of soil suppressiveness on the potential use of this pathogen as a mycoherbicide also is being investigated further.

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