Etiology

Wilt and Dieback of Mexican Lime Caused by *Fusarium oxysporum*

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This paper reports the results of research only. Mention of a pesticide or proprietary product does not constitute a recommendation by the U. S. Department of Agriculture to the exclusion of other suitable products, nor does it imply registration under the Federal Insecticide, Fungicide, and Rodenticide Act as amended.

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ABSTRACT

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Serious wilt and dieback of greenhouse-grown Mexican lime (*Citrus aurantifolia*) seedlings has occurred in Florida for several years. Early symptoms are reticulate chlorosis and epinasty of the young leaves followed by wilting, leaf abscission, shoot dieback, and gum exudation. Initially, symptoms are sectorial then the dieback progresses rapidly, and plants seldom survive more than 4–6 wk. *Fusarium oxysporum* was isolated consistently from the xylem of the main stem and from twigs of affected plants. The disease syndrome was reproduced in nearly 100% of the plants by dipping wounded and nonwounded root systems of Mexican lime seedlings in a suspension of spores and mycelial fragments of this fungus. The first symptoms appeared 4 wk after inoculation and all plants died within the following 2 wk. *Fusarium oxysporum* was reisolated from the main stem of all root-inoculated lime seedlings. Seedlings of *C. excelsa* inoculated by the root-dip method developed symptoms similar to those on Mexican lime. Rangpur lime (*C limonia*) seedlings exhibited chlorosis, mild wilt, and stunting but did not die when inoculated with *F. oxysporum*. Inoculated plants of nine other *Citrus* spp. and relatives showed no symptoms and the fungus could not be reisolated from stem tissue. The pathogen was designated as *F. oxysporum* emend. Snyd. et Hans. f. sp. *citri* form. nov. Most naturally infected Mexican limes with mild or moderate symptoms recovered following biweekly drench applications of benomyl at 1.3 g (a.i.)/L. Drench application of benomyl 2, 14, and 28 days after inoculation of plants with high spore concentrations delayed symptom expression, but all of the seedlings eventually died.

Additional key words: soil-borne pathogens.

A serious wilt and dieback of greenhouse-grown Mexican lime (*Citrus aurantifolia* [Christm.] Swingle) seedlings used for indexing citrus virus diseases has been observed in Florida for several years. Usually small seedlings were not affected. Seedlings began to collapse as they reached grafting size at 6–12 mo of age. Typically, plants developed chlorosis and epinasty which was

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followed by wilt, leaf abscission, and twig dieback; most of them eventually died. Incidence of the disease was variable, but a high percentage of the plants was lost before definitive results could be obtained in experiments in which Mexican lime was used as a host plant.

In India, Bhatmagar and Prasad (1) studied a twig dieback of Mexican lime, isolated *Fusarium solani* (Mart.) Appel. et Wr. emend. Snyd. et Hans. from affected tissues, and reproduced the disease by stem inoculation. In our attempts to isolate the pathogen from Mexican lime in Florida, *F. oxysporum* (Schlecht) emend. Snyd. et Hans. and not *F. solani* was recovered consistently. Takatsu and Dianese (4) in Brazil isolated *F. oxysporum* from declining field-grown Mexican lime trees. They demonstrated that this fungus caused stunting of Rangpur lime (*C. limonia* Osb.) seedlings, but did not reproduce the twig dieback on Mexican lime.

Fusarium oxysporum never has been proven to be the cause of a wilt disease in citrus. In the present study, we conclusively demonstrate the pathogenicity of *F. oxysporum* to citrus and determine the host range, symptomatology, and control measures for the disease. A preliminary report of the work has been published (5).

MATERIALS AND METHODS

Plant materials and growing conditions. Plants of the following *Citrus* spp. and relatives were grown from seed: Mexican lime, *C. excelsa* West., rough lemon (*C. jambhiri* Lush.), sour orange (*C. aurantium* L.), alemow (*C. macrophylla* West.), grapefruit (*C. paradisi* Macf.), sweet orange (*C. sinensis* [L.] Osb.), Cleopatra mandarin (*C. reticulata* Blanco), large-flowered trifoliate orange (*Poncirus trifoliata* [L.] Raf.), Carrizo citrange (*P. trifoliata* × *C. sinensis*), and Etrog citron (*C. medica* L. var. *ethrog* Engl.) Seeds were planted in flats containing a mixture of peat moss and vermiculite (1:1, v/v) which was sterilized with aerated steam at 80 C for at least 30 min. Rangpur lime seedlings were grown in a field seedbed which had been fumigated with methyl bromide at 50 g/m^2. When seedlings were 15–20 cm tall, they were potted singly in 1.5-L containers in a steam-sterilized mix of peat moss, vermiculite, and sand (7:2:1, v/v). All plants were grown in evaporatively cooled, partially shaded greenhouses. Temperatures usually ranged from 18 to 28 C, but higher temperatures occurred occasionally during the summer.

Natural infection occurred commonly in the greenhouses described above, especially in older seedlings. All observations and initial isolations of the pathogen were made from naturally infected seedlings grown there.

Completion of Koch's postulates. For isolation of the fungus, pieces of stem, leaf, or root were washed in detergent solution, rinsed with water, surface disinfested with 0.5% NaOCl for 0.5–1.0 min, rinsed with sterile distilled water, and plated on cornmeal agar (CMA). Plates were incubated at room temperature (22–25C).

For identification, several isolates of the fungus were grown on CMA for production of microconidia and on potato-dextrose agar (PDA) prepared from fresh potatoes for the production of sclerotia. Mycelial plugs from CMA were placed in sterile distilled water containing dried stem pieces of coastal bermuda hay (*Cynodon dactylon* Pers.) which had been sterilized with propylene oxide (2). These cultures were incubated under continuous fluorescent light (Westinghouse F15T8/cw) at an intensity of about 2,000 lux for about 2 wk for production of typical macroconidia and chlamydospores.

Inoculum for reproduction of the disease was prepared from colonies growing on CMA plates. Agar bearing the fungus was comminuted in distilled water for 1 min in a blender. Suspensions containing 2.3–9.0 × 10^5 microconidia per milliliter plus mycelial fragments and a few macroconidia were used for all inoculations. Root inoculations were made by dipping bare-rooted seedlings in the suspensions for 5 min. Wounds were made by cutting about 50 root tips per plant. After the dipped plants were repotted, about 50 ml of inoculum was added to the soil surface of each pot. Controls were treated similarly, except that sterile CMA plates were used to prepare the suspensions. Some plants were inoculated by adding a small amount of infested soil to the surface of the soil in the pot to determine whether the pathogen could be disseminated in contaminated soil in the greenhouse.

Stem inoculations were made by placing portions of a CMA culture bearing mycelium and microconidia of *F. oxysporum* on the cut ends of about 10 lateral branches per plant. Inoculated stem ends were covered for 48 hr with a small piece of moistened cotton enclosed in a plastic capsule, or the entire plant was covered with a plastic bag to maintain free moisture at the inoculation site.

Control with benomyl. To determine the efficacy of benomyl (methyl 1-[butylcarbamoyl]-2-benzimidazole-carbamate) for control of the disease, five apparently healthy Mexican lime seedlings and nine naturally infected seedlings in various stages of decline which were growing singly in 1.5-L containers were drenched at biweekly intervals with about 100 ml of benomyl at 1.3 g (a.i.)/L per plant. Two mildly affected plants were left as non-treated controls. Benomyl also was applied to root-dip inoculated plants growing in 1.5-L containers by drench at 2, 14, and 28 days after inoculation.

RESULTS

Symptomatology on naturally infected plants. The disease has been observed often on Mexican lime for the last several years and a similar syndrome was observed recently on *C. excelsa.* Symptoms have been especially severe and the incidence of the disease was high in a chamber in one greenhouse maintained at a constant 23 C. The disease has appeared at all times of the year. No symptoms were observed on many other *Citrus* spp. and relatives growing in the same greenhouses.

The general symptoms on naturally infected plants are shown in Fig. 1H. The first symptoms were usually a mild, reticulate chlorosis and epinasty of young leaves (Fig. 1B and 1C) followed by wilting, leaf abscission, and dieback of young twigs. Gum exudation usually occurred at various points along the dying twigs. Apical dieback, internal necrosis, and gum impregnation of the stem often were sectorial. The root system was not visibly affected even on

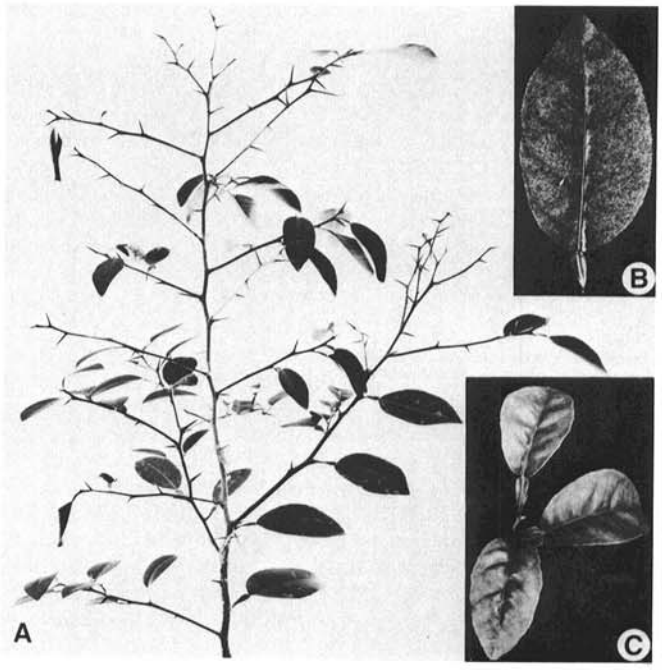

Fig. 1. Symptoms caused by *Fusarium oxysporum* f. sp. *citri* on naturally infected Mexican lime seedlings. **A,** wilt, leaf abscission, and general dieback of the entire plant; **B,** reticulate chlorosis of a mature leaf; **C,** epinasty and chlorosis of young leaves—an early symptom of infection.

plants with severe dieback. Affected plants normally died within 4–6 wk after appearance of the first symptoms.

Isolation and identification. A fungus, which was identified as *Fusarium oxysporum* Schlecht., was isolated from nearly 100% of the stem pieces from diseased Mexican lime and *C. excelsa* seedlings. The same fungus also was isolated frequently from the twigs, leaves, and roots of diseased plants. No other fungus was consistently isolated from diseased plants, and infected tissue pieces frequently yielded pure cultures of *F. oxysporum*.

The fungus produced abundant small, elliptical microconidia and later produced macroconidia on CMA. Abundant chlamydospores, borne singly and in pairs, and macroconidia were produced in sterile distilled water containing dried stem pieces of coastal bermuda hay (Fig. 2). Macroconidia were elongate, thin-walled, less than 4.5 μm in width, with an obvious foot cell and pointed apical cell. The fungus produced pinkish-purple pigment and blue-black sclerotia in older PDA cultures. Forty-five single-spored isolates, all resembling the parent mass culture morphologically, also were identified as *F. oxysporum*. Two single-spored isolates were deposited in the Florida Type Culture Collection, Department of Agriculture and Consumer Services, Gainesville, FL 32602, as FTCC #823 and 824 and the same cultures were deposited in the American Type Culture Collection as ATCC 38032 and 38033, respectively.

Inoculations. The entire disease syndrome was reproduced by root-dip inoculation of Mexican lime seedlings with a culture of *F. oxysporum* isolated from naturally affected Mexican lime (Fig. 3). Symptoms first appeared about 4 wk after inoculation. All inoculated plants usually died within 2–4 wk after symptoms appeared. Wounding did not accelerate symptom appearance in root-dip inoculated plants (Table 1). There was no visible root decay. Mexican lime seedlings inoculated by addition of a small amount of infested soil to the surface of the pot did not show symptoms during 4 mo of observation.

All Mexican lime plants inoculated with: the original mass isolate of *F. oxysporum* from a naturally infected Mexican lime; a single-spored isolate from that culture; and a reisolate from a diseased Mexican lime inoculated with mass isolate 1104 (Table 1) in a preliminary experiment, became infected and showed similar symptoms. An isolate of *F. oxysporum* from stems of naturally infected *C. excelsa* produced typical symptoms on Mexican lime. However, four isolates of *F. oxysporum* from stems of declining nursery trees of Valencia sweet orange on Carrizo citrange rootstock from a commercial nursery in Florida failed to produce symptoms on Mexican lime (Table 1).

Reisolations from xylem tissue of inoculated plants with symptoms consistently yielded *F. oxysporum*. Cultures reisolated from 38 inoculated Mexican lime plants (Table 1) were identified as *F. oxysporum*.

The disease consistently was not reproduced in the Mexican limes stem-inoculated with *F. oxysporum*. Two of six plants in one experiment and one of six in a second experiment developed symptoms of the disease. However, one of the six control plants in the first experiment also showed symptoms and all may have been naturally infected.

Host range. The host range was determined in a series of experiments in which root-dip inoculated Mexican lime seedlings served as the standard. Ten seedlings each of 12 citrus species and relatives were inoculated with *F. oxysporum*. All Mexican lime, *C. excelsa,* and Rangpur lime seedlings developed symptoms of the disease. The symptoms on *C. excelsa* were similar to those on Mexican lime. Inoculated Rangpur lime seedlings showed leaf chlorosis, mild wilt, and epinasty with occasional leaf abscission. The reaction of Rangpur lime differed from that of Mexican lime and *C. excelsa*; plants were severely stunted compared to noninoculated controls, but did not die (Fig. 3). *Fusarium oxysporum* was recovered from the stems of all diseased Mexican lime, *C. excelsa,* and Rangpur lime seedlings. Inoculated plants of rough lemon, sour orange, alemow, Duncan grapefruit, sweet orange, Cleopatra mandarin, Carrizo citrange, trifoliate orange, and Etrog citron grew vigorously and developed no disease symptoms. Isolations were attempted from five of the ten inoculated seedlings of each species and *F. oxysporum* could not be recovered from them. Since the pathogen is host specific, it is designated *F. oxysporum* (Schlecht) emend. Snyd. et Hans. f. sp. *citri* form. nov.

Control with benomyl. Of the naturally infected Mexican limes treated with benomyl as a soil drench at 2-wk intervals, those which were mildly affected recovered and produced new growth within

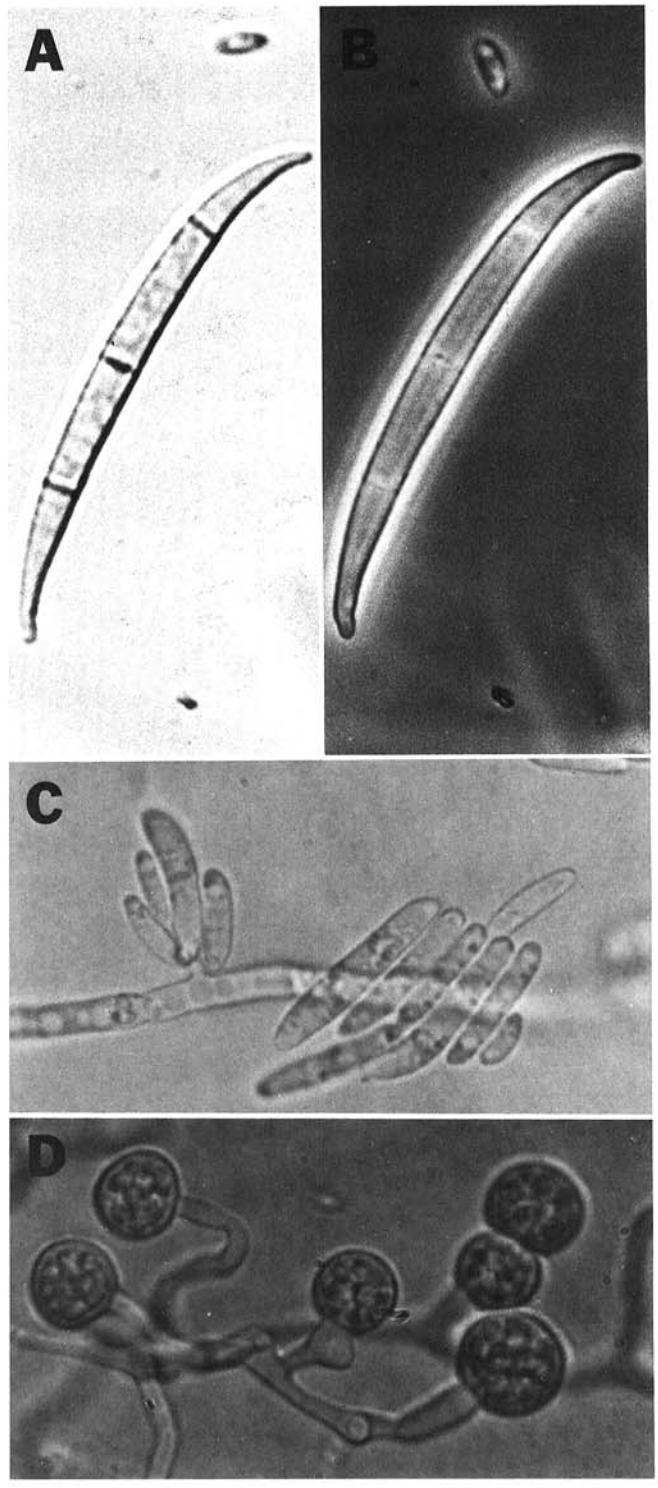

Fig. 2. Spores of the *Fusarium oxysporum* f. sp. *citri* pathogenic to Mexican lime: **A,B,** macroconidia, Nomarski differential interference contrast and phase contrast microscopy, respectively; **C,** microconidia; **D,** chlamydospores. Magnification of all spores about × 1,400.

TABLE 1. Effect of inoculation method and the source of the *Fusarium oxysporum* isolate on the incidence of wilt and dieback of Mexican lime

Isolate[a] no.	Source	Inoculation method	Ratio of no. diseased[b] to no. inoculated	*F. oxysporum* reisolated
1104	Mexican lime	root dip[c], wounded[d]	10/10	+
1104	Mexican lime	root dip, not wounded	10/10	+
1109	Mexican lime	root dip, wounded	10/10	+
FTCC 823	Mexican lime	root dip, wounded	5/5	+
1119	*Citrus excelsa*	root dip, wounded	5/5	n.t.[e]
...	Mexican lime	infested soil[f]	0/10	n.t.
1126A	Valencia orange	root dip, wounded	0/5	...
1126B	Valencia orange	root dip, wounded	0/5	...
1126C	Valencia orange	root dip, wounded	0/5	...
1126D	Valencia orange	root dip, wounded	0/5	...
...	...	control, wounded	0/10	...

[a] Isolate descriptions: 1104 = original mass isolate from a naturally infected Mexican lime; 1109 = reisolate from a diseased Mexican lime inoculated with 1104; FTCC 823 = a single-spored isolate of 1104; 1119 = mass isolate from a naturally infected *C. excelsa;* 1126A-D = four isolates from a declining Valencia orange on Carrizo citrange root stock from a commercial nursery in Florida.
[b] Number of plants with symptoms 5 wk postinoculation.
[c] Plants were bare-rooted, rinsed, dipped in a spore suspension of *F. oxysporum* ($2\text{-}9 \times 10^5$ spores/ml), repotted, and the spore suspension was added to the soil surface of the pot.
[d] Approximately 50 root tips were cut to provide wounds for entry of the fungus.
[e] n. t. = not tested.
[f] About 5 g of infested soil from a seedling killed by the disease added to the top of the pot.

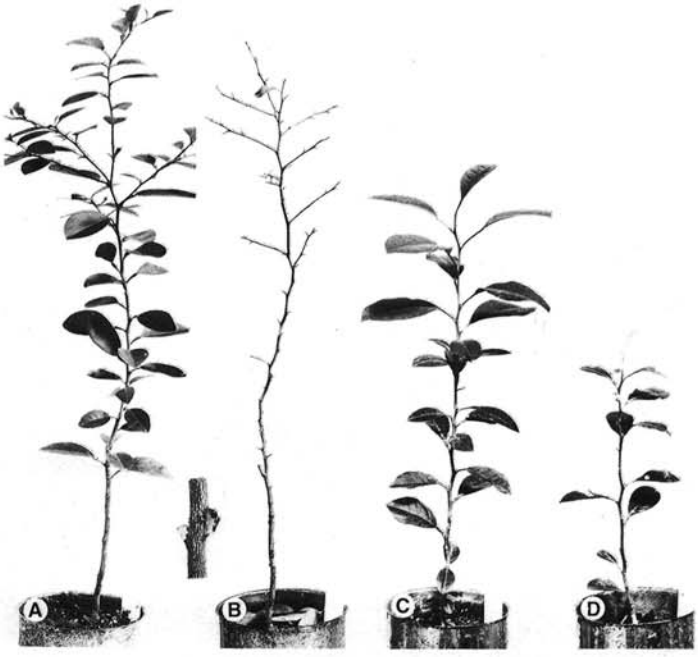

Fig. 3. Symptoms on Mexican lime and Rangpur lime seedlings inoculated by dipping roots in a suspension of *Fusarium oxysporum* f. sp. *citri* spores: **A,C,** healthy controls of Mexican lime and Rangpur lime, respectively; **B,** inoculated Mexican lime showing leaf abscission and twig dieback—inset shows gum exudation from the stem; **D,** inoculated Rangpur lime showing stunting and chlorosis. All plants photographed about 5 wk after inoculation.

2–4 wk. Affected plants partially recovered, produced new growth, and none of them died. Healthy plants treated with benomyl remained healthy. Mildly affected plants that were not treated continued to decline.

Treatment of root-dip inoculated plants with benomyl at 2, 14, and 28 days after inoculation delayed symptom expression in 7 of 10 plants for 5 wk postinoculation, but did not prevent infection. *Fusarium oxysporum* was reisolated from the treated plants, and all seedlings died within 10 wk after inoculation.

DISCUSSION

The wilt and dieback of Mexican lime in Florida greenhouses is caused by *F. oxysporum.* Koch's postulates have been fulfilled.

The host range of the fungus appears to be limited. *Citrus excelsa* was infected and exhibited symptoms similar to those on Mexican lime, but symptoms on Rangpur lime were less severe and no wilt developed on nine other *Citrus* spp. and relatives inoculated with the pathogen from Mexican lime. This is not surprising since isolates of *F. oxysporum* responsible for wilts of other plants are highly specialized pathogens.

The disease described here is probably the same as that described in Brazil on Mexican (Galego) lime on Rangpur lime rootstock (4). In Brazil, *F. oxysporum* was isolated from declining Mexican limes, and its pathogenicity to Rangpur lime seedlings was demonstrated by a root-dip inoculation method. Our data show that the stunting in Rangpur lime and the wilt and dieback of Mexican lime are caused by the same organism as previously suggested (4). However, in the case of the disease of Mexican lime described in India (1), the pathogen was identified as *F. solani* and stem inoculations were successful. That disease is probably distinct from the one reported here or the pathogen studied in India may have been misidentified.

The source of the pathogen remains unknown. Seedlings used in the greenhouses where the disease was found were grown in potting mixes treated with steam or methyl bromide. Airborne dispersal and reinvasion of steam-sterilized soil by *F. oxysporum* in greenhouse-grown tomatoes recently has been demonstrated (3). Recontamination of the potting mixture by airborne spores also may occur in Florida, but is probably slow, because seedlings usually do not become infected at an early age. Even the addition of a small amount of infested soil did not induce symptoms within 4 mo.

Application of a benomyl soil drench provided some control of the disease. Complete remission of symptoms occurred when mildly affected plants were treated. Benomyl applied after inoculation did not provide good control of the disease, probably because of the high spore concentrations used. Properly timed applications of benomyl at a sufficiently high rate will probably be effective in controlling natural infections.

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