Virulence of *Fusarium* Species Causing Fig Endosepsis in Cultivated and Wild Caprifigs

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ABSTRACT

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Fusarium spp. associated with endosepsis of figs were characterized for growth rate, sporulation, temperature optima, and virulence. *F. moniliforme* and *F. solani* were the dominant causal agents of fig endosepsis. A total of 62 *Fusarium* isolates were collected from cultivated and wild caprifigs with endosepsis symptoms from most fig-production areas of California. One isolate was *F. dimerum*, seven were *F. solani*, and 54 were *F. moniliforme*. In vitro growth rates of isolates within species differed significantly. Sporulation was determined after colony diameters were recorded by flooding the colonies with 10 ml of sterile deionized water and determining the number of spores using a hemacytometer. Sporulation correlated significantly ($r = 0.43$; $P = 0.0001$; and $n = 236$) with growth rate. The majority of *F. moniliforme* and *F. solani* isolates had a temperature optimum of 25 C; the *F. dimerum* isolate had an optimum temperature of 30 C for linear growth. The solitary *F. dimerum* isolate was moderately virulent; *F. solani* isolates were either virulent or highly virulent; and among *F. moniliforme* isolates, ~11% were avirulent, 67% were weakly to moderately virulent, and 22% were virulent to highly virulent. The teleomorph of most *F. moniliforme* isolates was *Gibberella fujikuroi* mating population A, and only a few were population F. *F. moniliforme* isolates from wild caprifigs were significantly more virulent than those from cultivated caprifigs, but no such differentiation was found with *F. solani*. Infusion of *F. moniliforme* from wild caprifigs into cultivated figs may cause significant, long-term problems for the fig industry in California.

Additional keywords: cultural practices, *Ficus carica*, pathogenicity.

California has been the leading producer of edible figs (*Ficus carica* L.) in the United States since commercial production first began in 1899. Four varieties have dominated commercial fig production and can be classified as persistent and caducous. Caducous figs require pollination (caprification) to produce mature fruits (9). Calimyrna (Smyrna figs grown in California), a highly valued variety that requires caprification, occupies nearly 50% of the total fig-production area in California and accounts for ~40% of the total revenue of the fig industry (2).

Calimyrna figs consist of botanically distinct male (caprifig) and female (edible) figs, which have different flowers in the syconium. Ecologically, the syconium simply represents a fruit. The caprifig syconium is lined internally with several hundred female florets and a few hundred male florets; the edible-fig syconium contains only female florets (32). Caprifigs provide pollen for the female crop and provide the hibernating site for the pollinator insect. Pollination is accomplished by the fig wasp (*Blastophaga psenes* L.), which resides in the ovaries of transformed pistillate flowers. Caprifigs produce three crops: a winter crop, called mamme, a spring crop, called profichi, and a summer crop, called mammoni. The profichi crop has the maximum number of staminate florets (13) and, consequently, the maximum amount of pollen; as a result, it is used to pollinate the Calimyrna figs.

Endosepsis (also called 'pink rot,' 'brown rot,' 'soft rot,' and 'eye-end rot'), named for the internal rot that can affect figs, has been a serious disease since 1920 (11) and causes significant losses yearly (9). The disease is caused primarily by *Fusarium moniliforme* J. Sheld. Two other species, *F. solani* (Mart.) Sacc. and *F. episphaeria* (Tode) W.C. Snyder & H.N. Hans. (=*F. dimerum* Penz. in Sacc.), also are capable of causing the disease (20). Endosepsis begins as brownish areas within the fig cavity when the syconium begins to ripen. When many florets are infected, the disease manifests externally as water-soaked lesions found primarily around the ostiole (eye) of the syconium. The disease progresses on the fruit toward the stalk, and a thick mycelial mat may appear on the surface of the syconium (Fig. 1). Caldis (3) described the pathogen-fig wasp relationship, outlined the disease and insect cycles, and showed that the fig wasp carries both pollen and fungal propagules to the receptive male and female figs. When this association was understood, control procedures for endosepsis were devised in the 1930s, involving fungicide treatment of mammes before caprifying profichis to obtain disease-free profichis. Caprification of the Calimyrnas was carried out with the presumed disease-free profichi caprifigs (26). The fungicides used in this approach have changed over the years, but the procedure has remained essentially the same. In recent years, however, growers have noticed a reduced vigor in the fig wasp population and an increase in the incidence of endosepsis and other diseases of figs that cause significant losses. The ecology of the different *Fusarium* spp. associated with this disease must be understood.

Little information is available on the identity and virulence of *Fusarium* spp. that cause fig endosepsis. During years when the supply of mamme figs from cultivated caprifigs is inadequate to caprify profichis, the growers commonly collect wild caprifigs to augment the supply. The short- and long-term effects of infusing cultivated figs with wild strains of *Fusarium* spp. on disease dynamics and the effects on the behavior of fig wasps are unknown. The objectives of this research were to characterize the *Fusarium* spp. associated with fig endosepsis, to develop a reliable procedure for testing the virulence of isolates, and to compare the relative virulence of *Fusarium* spp. collected from cultivated and wild caprifig trees. Preliminary results have been reported (28,30).

MATERIALS AND METHODS

Collection of isolates. Isolates of *Fusarium* spp. were collected during May–June from both cultivated and wild caprifigs from

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most of the fig-production areas in Butte, Fresno, and Tulare counties in California. Isolates from cultivated caprifigs originated from cultivars Stanford and Roeding. Caprifigs collected from different locations were brought to the laboratory, surface-disinfested separately with a 1% sodium hypochlorite solution for 3 min, and air-dried. The surface-disinfested caprifigs were sliced through the ostiole with a sterile knife. The inner scales surrounding the ostiole of the fig and scoops of flowers from different areas of the fig cavity were placed in petri dishes containing acidified (2.5 ml of 25% [v/v] lactic acid solution per liter of medium) potato-dextrose agar (APDA). Single-spore transfers were made from the developing colonies, and individual conidia were transferred to fresh APDA for species identification (21). Single-spore isolates were maintained on silica gel in a refrigerator at 4–6 C (31).

Effect of temperature on mycelial growth and sporulation of isolates. The growth of all isolates was evaluated at 15, 20, 25, 30, and 35 C. Five APDA dishes were seeded centrally with a 4-mm-diameter agar plug of each isolate. Cultures were incubated in the dark in a completely randomized design at each temperature. The diameter of each colony was measured when the leading edge of the fastest growing colony at any temperature had reached the edge of the dish. Culture dishes were flooded with 10 ml of sterile deionized water and brushed gently with a rubber spatula to dislodge conidia. Concentrations of conidia were determined with a hemacytometer. Five hemacytometer counts were made of each dish and averaged; the experiment was repeated once. Experiments were considered as blocks for analysis of variance.

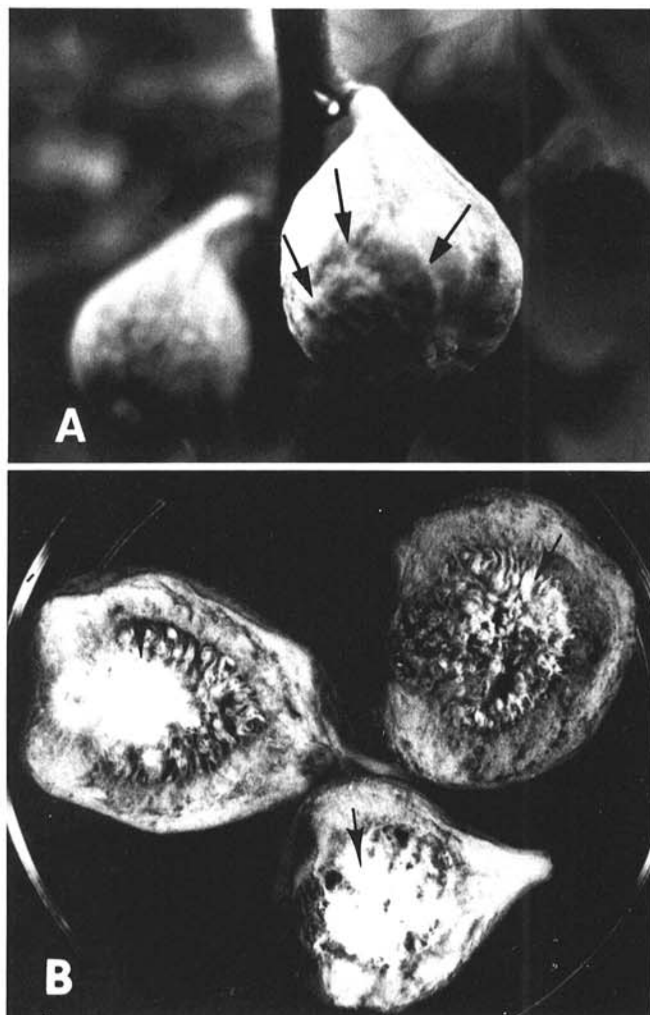

Fig. 1. Caprifig fruit showing endosepsis symptoms caused by *Fusarium* spp. **A,** Arrows indicate an external lesion on an infected caprifig. **B,** Caprifig halves showing various stages of colonization by *Fusarium* spp. Arrows indicate mycelia.

Linear regression analysis (27) was used to determine the effects of temperature on growth of *Fusarium* spp. Growth rates of all isolates at each temperature were calculated by dividing the colony diameter by the number of days of incubation. The Pearson correlation coefficients between growth rate and sporulation were estimated by PROC CORR in SAS (24).

Inoculation technique. Inocula used in the virulence test and in the inoculum-density experiments were produced by incubating isolate cultures of *Fusarium* spp. on APDA at 25 C in the dark for 9 days. Inocula consisting of conidial suspensions were prepared by flooding the culture of each isolate with sterile deionized water, dislodging spores with a rubber spatula, and adjusting the concentration to 10^6 conidia/ml.

To develop a suitable procedure for testing the virulence of the isolates and to quantify the disease, two methods of inoculation were tested. Figs surface-disinfested, as described above, were split in half longitudinally with a sterile knife, and 40 halves were placed over waxed wire screens (20 × 29.5 cm) with the cut surface facing down or up in separate, clear-plastic containers (23.5 × 32 × 10 cm). The fruits were inoculated by placing 5 μl of a conidial (10^6 conidia/ml) suspension on a wound, 2 mm wide and 2 mm deep, made either on the surface or in the cavity of each fruit half. Approximately 300 ml of deionized water was added to each container to increase the relative humidity (>97%), and the containers were incubated in the dark at 25 ±1 C. Lesion sizes were recorded on each fruit after incubation for 5 days.

Effect of inoculum density on lesion size. The effect of inoculum density of *F. moniliforme* on infection of figs was tested in two experiments; six inoculum densities and an uninoculated control treatment were evaluated in each experiment. Treatments were replicated five times (20 fig halves per replicate) in a randomized complete-block design. Inoculum densities of a highly virulent isolate (F50) were adjusted by dilution of a conidial suspension to 10^4, 10^5, 10^6, 10^7, and 10^8 conidia/ml. Fruits also were inoculated with a single germinating conidium transferred from water agar. Fig halves were placed face down over waxed wire screens and were inoculated on the exposed surface with a spore suspension of each inoculum density, as previously described. Fig halves inoculated with 5 μl of sterile distilled water served as a control treatment. The inoculated fruit halves were incubated, as described above, at 25 ±1 C, and lesion sizes were recorded after incubation in the dark for 5 days. The experiment was repeated once.

Isolate virulence tests. A total of 62 isolates of *Fusarium* spp. were obtained from cultivated and wild caprifigs. Virulence tests of all isolates were conducted on both profichi and mammoni caprifigs (experiments 1 and 2, respectively; Tables 1, 2, and 3). Fruits collected from the field were surface-disinfested, sliced through the ostiole, and placed with the cut surface touching the wire screens in clear-plastic containers, as previously described. Inoculum density of each isolate was standardized to 10^6 conidia/ml. Forty and 20 fruit halves of profichi and mammoni caprifigs, respectively, were wounded and inoculated with each isolate, as described previously. Inoculated caprifigs were incubated at 25 C in a completely randomized design. Each inoculated fig half was considered a replication. The interval between the time of inoculation and the time when at least 50% of the fruits exhibited the first visible lesion (incubation period) was recorded for each isolate. Lesion sizes (length × width) on all fruits were recorded after incubation for 5 or 7 days.

Data were tested for normality and homogeneity of variance before analysis of variance (24). Means were computed, and isolate means were compared with the least significant difference test (27). Frequency of isolates that caused lesions in size categories of 0 mm^2 (avirulent), 1–25 mm^2 (weakly virulent), 26–50 mm^2 (moderately virulent), 51–100 mm^2 (virulent), and >100 mm^2 (highly virulent) were calculated. Frequencies of isolates from cultivated and wild caprifigs in the lesion-size categories also were calculated. Groups of isolates of different *F. moniliforme* and *F. solani* from the cultivated and wild caprifigs were compared using linear contrasts (27).

Mating type determinations in *F. moniliforme*. The cause of fig endosepsis was recently reevaluated by comparing represen-

TABLE 1. Isolates of *Fusarium* spp. collected from cultivated (C) and wild (W) caprifigs and the results of mating-type determination and two virulence tests

Fusarium spp.	Isolate[a]	Year collected	Mating type	Female fertile	Original caprifig host	Lesion size (mm^2)[b] ± SE[c]	
						Experiment 1[d]	Experiment 2
F. moniliforme	F1	1989	A^+	No	C	0.0 ± 0.00	0.0 ± 0.00
	F2	1989	A^+	No	C	0.0 ± 0.00	37.6 ± 13.22
	F3	1989	A^+	No	C	12.7 ± 1.87	14.1 ± 3.21
	F4	1989	A^+	No	C	51.3 ± 9.63	80.6 ± 19.31
	F5	1989	A^+	No	C	27.9 ± 0.50	18.9 ± 4.14
	F6	1989	A^+	No	C	34.1 ± 1.66	43.6 ± 25.18
	F7	1989	A^+	No	C	40.4 ± 2.37	7.4 ± 2.74
	F8	1989	A^+	No	C	39.2 ± 4.57	17.9 ± 8.44
	F9	1990	A^+	No	C	10.2 ± 2.14	12.6 ± 6.49
	F11	1991	A^+	No	C	8.0 ± 2.61	94.1 ± 34.04
	F12	1991	A^+	No	C	14.2 ± 3.10	57.0 ± 14.81
	F13	1991	F^+	No	C	0.0 ± 0.00	0.0 ± 0.00
	F14	1991	A^+	No	C	20.5 ± 1.92	14.5 ± 2.08
	F15	1991	A^+	No	C	19.0 ± 2.03	6.4 ± 2.03
	F16	1990	A^+	No	C[e]	32.6 ± 10.68	10.9 ± 1.77
	F17	1991	A^+	No	C	31.8 ± 2.88	7.8 ± 2.40
	F18	1991	A^+	No	C	20.4 ± 4.70	7.0 ± 2.63
	F19	1991	A^+	No	C	25.7 ± 5.76	44.7 ± 23.06
	F20	1991	A^+	No	C	22.5 ± 10.31	38.4 ± 13.16
	F21	1991	A^+	No	C	22.5 ± 10.31	73.4 ± 22.82
	F22	1991	A^-	Yes	C	8.5 ± 4.20	35.9 ± 9.68
	F23	1991	A^-	No	C	32.1 ± 9.22	46.3 ± 12.53
	F24	1991	A^-	No	C	59.6 ± 19.18	118.2 ± 29.85
	F25	1991	A^-	No	C	28.7 ± 3.96	103.3 ± 18.24
	F26	1991	A^+	No	C	16.1 ± 2.28	7.5 ± 3.58
	F27	1991	A^+	No	C	20.1 ± 4.11	20.1 ± 10.95
	F28	1991	A^-	No	C	0.0 ± 0.00	4.9 ± 3.41
	F29	1991	F^+	No	C	4.1 ± 1.66	5.9 ± 3.85
	F30	1989	A^-	No	C	29.9 ± 3.39	6.9 ± 2.10
	F32	1989	A^+	No	C	34.1 ± 3.90	21.2 ± 8.91
	F33	1987	F^+	Yes	C	30.8 ± 3.72	23.3 ± 6.61
	F34	1987	A^+	No	C	17.1 ± 2.63	82.6 ± 19.59
	F35	1987	A^-	Yes	C	0.0 ± 0.00	0.0 ± 0.00
	F37	1987	A^+	No	C	12.5 ± 4.65	4.5 ± 1.84
	F38	1991	A^+	No	C	9.9 ± 2.93	10.7 ± 3.32
	F39	1991	A^+	No	C	7.4 ± 2.57	3.6 ± 1.64
	F40	1991	A^+	No	C	0.0 ± 0.00	0.0 ± 0.00
	F41	1991	A^+	No	C	3.2 ± 1.91	3.0 ± 1.64
	F42	1991	A^+	No	C	50.8 ± 7.69	26.2 ± 5.43
	F43	1991	A^+	No	W	25.8 ± 3.03	11.9 ± 2.94
	F44	1991	A^+	No	W	77.1 ± 24.30	88.8 ± 19.29
	F45	1991	A^+	No	W	25.6 ± 8.49	34.8 ± 12.05
	F48	1991	A^+	No	W	12.5 ± 5.00	16.4 ± 7.81
	F50	1991	A^+	No	W	139.1 ± 40.23	126.7 ± 16.37
	F52	1991	A^+	No	W	47.2 ± 14.70	88.0 ± 17.49
	F53	1991	A^+	No	W	0.0 ± 0.00	14.2 ± 7.82
	F54	1991	A^-	No	W	32.2 ± 2.91	39.3 ± 11.81
	F55	1991	A^+	No	W	35.7 ± 9.62	59.2 ± 10.42
	F57	1991	A^+	Yes	W	15.4 ± 5.20	13.6 ± 5.78
	F58	1991	A^-	No	W	55.7 ± 13.30	108.3 ± 22.06
	F59	1991	A^+	No	W	105.7 ± 15.50	119.5 ± 20.38
	F60	1991	A^+	No	W	33.8 ± 5.50	34.2 ± 11.12
	F61	1991	A^+	No	W	172.3 ± 30.92	118.0 ± 20.83
	F62	1991	A^+	No	W[f]	212.2 ± 29.52	131.5 ± 21.74
F. solani	F10	1991	...	...	C	246.5 ± 27.35	107.3 ± 17.87
	F36	1987	...	...	C	166.6 ± 39.30	71.2 ± 30.44
	F46	1991	...	...	W	448.4 ± 52.58	90.7 ± 13.83
	F47	1991	...	...	W	64.8 ± 11.59	64.2 ± 20.12
	F49	1991	...	...	W	353.8 ± 49.38	72.4 ± 18.25
	F51	1991	...	...	W	143.6 ± 17.30	119.9 ± 23.09
	F56	1991	...	...	W	76.8 ± 11.43	59.2 ± 10.42
F. dimerum	F31	1989	...	...	C	47.4 ± 2.61	24.9 ± 5.66

Contrast for lesion size	Experiment 1		Experiment 2	
	Sums of Squares	$P > F$	Sums of Squares	$P > F$
F. moniliforme (W) vs. *F. moniliforme* (C)	507,802.02	0.0001	38,133.00	0.0010
F. solani (W) vs. *F. solani* (C)	302,947.54	0.0001	186,379.20	0.0001
F. solani (W) vs. *F. moniliforme* (W)	21,027.84	0.1124	28,988.67	0.0042

[a] All isolates were from Fresno County, except F17–F25, F27–F29,F34, and F40 from Tulare County, and F62 from Butte County.
[b] Means represent the average of 40 and 20 inoculated caprifigs in experiments 1 and 2, respectively.
[c] Standard error of the mean.
[d] Experiments 1 and 2 tested profichi and mammoni caprifigs, respectively.
[e] Isolated from an infested fig wasp.
[f] Isolated from Black Mission, which is a persistent type of edible fig.

TABLE 2. Frequency of isolates from *Fusarium* spp. in different lesion-size classes in two virulence tests on caprifigs

	Number and percentage of isolates in lesion-size class (mm^2)[a]									
	0		1–25		26–50		51–100		>100	
Fusarium spp.	1[b]	2[b]	1	2	1	2	1	2	1	2
F. dimerum	0	0	1	0	0	1	0	0	0	0
	(0)	(0)	(100)	(0)	(0)	(100)	(0)	(0)	(0)	(0)
F. moniliforme	7	5	19	24	19	11	5	7	4	7
	(13)	(9)	(36)	(45)	(34)	(19)	(9)	(13)	(8)	(13)
F. solani	0	0	0	0	0	0	2	5	5	2
	(0)	(0)	(0)	(0)	(0)	(0)	(29)	(71)	(71)	(29)

[a] Numbers in parentheses are percents.
[b] Lesion sizes are the mean of 40 and 20 inoculated caprifigs in experiments 1 and 2, respectively. Isolates in the lesion-size classes 0, 1–25, 26–50, 51–100, and >100 are considered avirulent, weakly virulent, moderately virulent, virulent, and highly virulent, respectively. Experiments 1 and 2 tested profichi and mammoni caprifigs, respectively.

TABLE 3. Number and percentage (in parentheses) of isolates from *Fusarium* spp. collected from cultivated (C) and wild (W) caprifigs in different lesion-size classes in two virulence tests on caprifigs

Lesion-size class (mm^2)	Experiment	*F. moniliforme* C	*F. moniliforme* W	*F. solani* C	*F. solani* W
0	1[a]	6	1	0	0
		(16)	(7)	(0)	(0)
	2	5	0	0	0
		(13)	(0)	(0)	(0)
1–25	1	18	2	0	0
		(45)	(13)	(0)	(0)
	2	21	4	0	0
		(53)	(27)	(0)	(0)
26–50	1	12	6	0	0
		(32)	(40)	(0)	(0)
	2	7	3	0	0
		(18)	(20)	(0)	(0)
51–100	1	3	2	0	2
		(8)	(13)	(0)	(40)
	2	4	3	1	4
		(11)	(20)	(50)	(80)
>100	1	0	4	2	3
		(0)	(27)	(100)	(60)
	2	2	5	1	1
		(5)	(33)	(50)	(20)

[a] Experiments 1 and 2 tested profichi and mammoni caprifigs, respectively.

tative fig isolates with maize isolates of *F. moniliforme* (29). We wanted to determine if both the fig and maize isolates were members of the same mating population (or biologic species). Furthermore, we wanted to determine the toxigenic potential of the different isolates. We determined the mating population of all 54 fig isolates of *F. moniliforme* by crossing them with standard testers of the *Gibberella fujikuroi* ((Sawada) Ito in Ito & K. Kimura) A and F populations (16,17). Cultures of + and − mating types of both A and F populations were supplied by John Leslie (Kansas State University, Manhattan); mating type designations follow those of Klittich and Leslie (16).

Crosses were made on both V8-juice (21) and carrot agar (15). The media were dispensed into 90-mm-diameter petri dishes (15 ml per dish) left at room temperature (24 ±1 C) for 5 days to allow evaporation of any free moisture. In the first set of crosses, standard testers were used as female parents, and uncharacterized fig isolates were used as male parents. To initiate a cross, each standard tester was transferred to both V8-juice and carrot agar in a 4-mm-diameter plug from an actively growing culture. Simultaneously, each fig isolate also was grown on APDA slants. To minimize drying of media during the long incubation, cultures were placed in clear-plastic chambers (23.5 × 32 × 10 cm) and incubated on a laboratory bench at 25 ±1 C under a 12-h of dark/12-h of light cycle. After 8 days, 10 ml of sterile distilled water was added to each culture in slants to obtain a spore suspension. Two milliliters of this spore suspension was spread gently on the surface of the standard tester cultures to thoroughly wet the mycelium. Each cross was made on three replicate dishes of each medium, incubated as described above, and the crosses were repeated once. In a reciprocal set of crosses to test the female fertility of the fig isolates, each isolate was transferred to three replicate dishes of each medium, and simultaneously, the standard testers to be used as male parents also were transferred to APDA slants. Crosses were made, and cultures were incubated, as described above. All cultures were examined at 3-day intervals for the development of perithecia and exudation of ascospores.

RESULTS

***Fusarium* spp. and isolates.** A total of 62 isolates of *Fusarium* spp. were obtained from the caprifigs: 54 isolates were *F. moniliforme*, seven isolates were *F. solani*, and one isolate was *F. dimerum* (Table 1). Of the 54 *F. moniliforme* isolates, 39 were from cultivated caprifigs, and 15 were from wild caprifigs. Of the seven *F. solani* isolates, two were from cultivated caprifigs, and five were from wild caprifigs (Table 1). The solitary *F. dimerum* isolate was from a cultivated caprifig.

Effect of temperature on mycelial growth and sporulation of isolates. All isolates grew on APDA at temperatures between 10 and 35 C, but the rate of growth differed among isolates at a given temperature (*data not shown*). There was a significant isolate × temperature interaction. Approximately 6, 75, or 19% of the isolates of *F. moniliforme* had temperature optima of 20, 25, or 30 C, respectively. Five *F. solani* isolates grew best at 25 C, and the remaining two grew best at 30 C. *F. dimerum* grew best at 30 C.

Growth of *Fusarium* spp. increased up to 25 C and declined at higher temperatures (Fig. 2). Growth response to temperature was quadratic with a maximum at 25 C ($r^2 = 0.90$ and $P = 0.0001$). Sporulation by different isolates was highly variable and correlated significantly with the incubation temperature ($r = 0.43$ and $P = 0.0001$). Invariably, the isolates with higher growth rates produced a greater number of spores.

Inoculation technique. Inoculations both in the fig cavity and on the fig surface produced endosepsis symptoms. However, inoculations in the cavity resulted in irregular rotting symptoms in which lesion sizes were difficult to measure. Inoculations on the surface of the fig induced measurable, regular, circular or semicircular lesions that were well delimited, and as a result, this inoculation procedure was chosen for the virulence testing experiments.

Effects of inoculum density on lesion size. None of the control fruits developed external symptoms of endosepsis. Lesion sizes increased with inoculum densities between 10^4 and 10^8 spores per milliliter (Fig. 3). Inoculations with a single spore were also successful, although lesions were very small.

Virulence test. All isolates induced identical symptoms but took different lengths of time to do so. The incubation periods varied from 63 to 138 h among *F. moniliforme* isolates and from 63 to 79 h among *F. solani* isolates. Relative virulence of the isolates was not influenced by the choice of the caprifig crop. Varied degrees of endosepsis were induced by different isolates and species

of *Fusarium*. Although different lesion sizes were produced by a few isolates in the two experiments, the percentage of isolates of *F. moniliforme* in the lesion size-frequency classes were similar in the two experiments (Table 2).

The majority of the isolates of *F. moniliforme* from cultivated caprifigs were either avirulent, weakly virulent, or moderately virulent. Only ~9 or 3% (means of each of the two experiments) of the isolates were virulent or highly virulent, respectively. In contrast, ~15 or 30% of the isolates from wild caprifigs were virulent or highly virulent, respectively (Table 3). As a group, *F. moniliforme* isolates from the cultivated caprifigs were less virulent than were the isolates from wild caprifigs (Table 1).

Regardless of the origin host, all isolates of *F. solani* were either virulent or highly virulent (Table 3). Isolates of *F. solani* from cultivated caprifigs, however, were significantly more virulent than were those from wild caprifigs (Table 1).

The incubation period for the solitary isolate of *F. dimerum* was 79 h. The isolate was either weakly or moderately virulent in the two experiments.

Mating populations and fertility in *F. moniliforme*. Characteristic blue-black perithecia (4) resulted from all crosses after incubation for 9 days. The number of perithecia produced by different crosses varied from <10 to >100 in different crosses. Perithecia were solitary, clustered, or both in both A and F mating populations. Ascospore-oozing cirrhi appeared after incubation for 21 days. Production of perithecia was greater on carrot agar than on V8-juice agar. Mating population designations were not affected by the choice of crossing medium, however.

Of the 54 *F. moniliforme* isolates tested, 51 mated with population A and the remaining with population F (Table 1). Among the isolates in population A, 42 were designated as A^+, and nine were designated as A^-; all isolates in population F were of the + type. Only three isolates in A and one in F populations exhibited female fertility (Table 1). Characterization of the isolates from the two crossing experiments was identical. None of the isolates that crossed with population A also crossed with F, and all isolates crossed with only one mating type.

DISCUSSION

Three *Fusarium* spp. were isolated from caprifigs infected with endosepsis. In the laboratory, all three induced identical symptoms but varied in virulence. Of the 62 isolates collected, 54 were *F. moniliforme* and only seven were *F. solani*. Even though these frequencies indicate that *F. moniliforme* is the dominant cause of fig endosepsis, the greater virulence of *F. solani* isolates indicates that this species is also a significant cause. The one isolate of *F. dimerum* was only moderately virulent, so at this time, the species must be considered only a minor cause of fig endosepsis. The first report that these three *Fusarium* spp. caused endosepsis did not describe the relative virulence of the species (20). Both the findings of Michailides et al (20) and those of the present study contrast the previous conclusion that a morphologically distinct fungus, *F. moniliforme* var. *fici* Caldis (3), is the only causal agent of fig endosepsis. Morphology, pathogenicity, and host-specificity studies comparing fig isolates with maize isolates causing ear rot, however, showed the two pathogens were similar, and the variations within the two populations were as great or greater than the variations between them. The overlapping morphological characteristics and the ability of fig isolates to infect maize do not support the retention of variety *fici* for the fig endosepsis pathogen *F. moniliforme* (29). Theoretically, any of the three species can cause fig endosepsis and associated yield losses. Because of the very low isolation frequency, however, *F. dimerum* is probably not a major pathogen on figs.

Under optimal conditions, even a single spore can cause endosepsis, and the degree of infection depends on the number of spores available for infection. An infected caprifig with a large number of spores in the cavity also may lead to increased contamination of the wasps issued from them and in turn, may cause infection in a higher number of edible Calimyrna figs.

All three *Fusarium* spp. grew significantly at temperatures ranging from 10 to 35 C, which suggests high-survival and -pathogenic potential of each species under these conditions. The ability to grow over a wide temperature range may explain why the disease is prevalent in both male and female figs that bear fruit during spring and summer seasons, respectively (9). Variation in the optimal temperature requirements for linear growth occurred in both *F. moniliforme* and *F. solani* isolates. A majority of isolates within each species had a temperature optimum of 25 C. However, a few isolates of *F. moniliforme* grew best at 20 or 30 C, and a few isolates of *F. solani* grew best at 30 C. These results indicate that all three species may coexist in a fig at any given time. The genetic variability within the *F. moniliforme* populations from individual figs, based on vegetative compability grouping (15) or other appropriate molecular markers, may help determine whether a single strain or more than one strain can cause the observed symptoms.

Wasps contaminated with *Fusarium* spp. carry the fungus as well as pollen into healthy figs (3,9,26). Fruit rot symptoms, therefore, begin inside the fig cavity and manifest on the surface

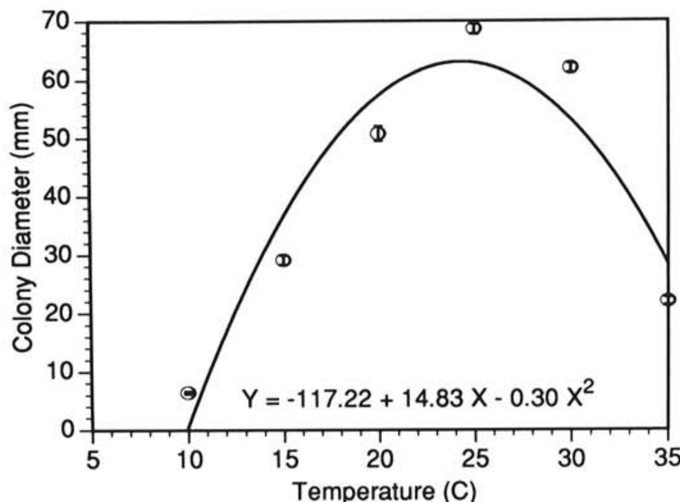

Fig. 2. Relationship between incubation temperature and mean colony diameter of all *Fusarium moniliforme* isolates after incubation for 9 days at different temperatures. Each point represents average colony diameters from five replicated acidified potato-dextrose agar cultures. Vertical bars associated with each point represent the standard error of the mean. Regression analysis was carried out on raw data.

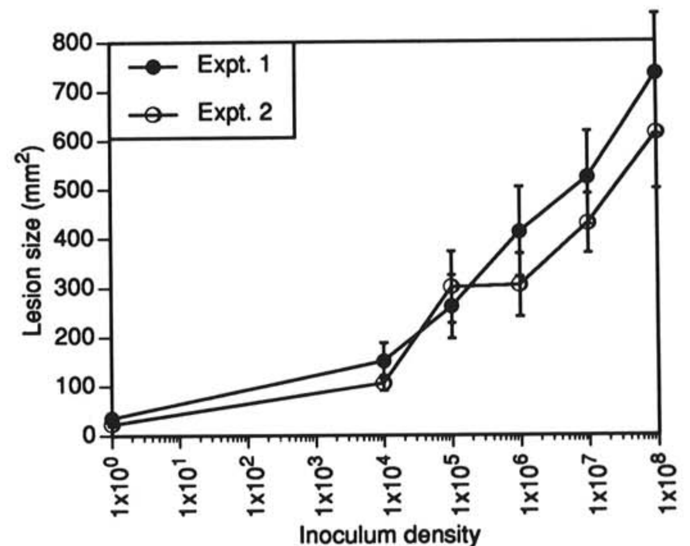

Fig. 3. Relationship between *Fusarium moniliforme* spore-inoculum density and lesion diameter on inoculated fruits after incubation for 5 days at 25 C. Virulent isolate F50 was used for inoculations in both experiments. Each point represents the average of five replicates (20 half-figs each). Vertical bars associated with each point represent the standard error of the mean.

later—hence the name endosepsis (3). Determination of the virulence of the different isolates on figs, however, requires a quantitative disease-assessment technique. Inoculating the fig cavity to mimic the natural process of infection results in irregular lesions difficult to quantify. Furthermore, background inoculum of *Fusarium* spp. that may already be present in the fig cavity can bias the results. Surface inoculation of figs usually results in circular lesions with well-defined margins and facilitates disease quantification and determination of virulence. This method of inoculation also is unaffected by the background inoculum in the cavity, is easily conducted, and, most importantly, provides repeatable results.

Isolates of *Fusarium* spp. differ in their ability to cause endosepsis. Whereas *F. moniliforme* isolates occurred in every category of virulence, *F. solani* isolates were only virulent or highly virulent. This predominance of virulent strains of *F. solani* may be attributable to the low number of *F. solani* isolates recovered in our study, but avirulent and moderately virulent isolates of *F. solani* that we did not detect may infect figs in the field. Although the *F. moniliforme* isolates showed differences in morphology, the differences were not specific to any one virulence group. Therefore, virulent and avirulent strains of the fungus were indistinguishable morphologically. Soilborne nonpathogenic strains of *F. oxysporum* can colonize and compete for the same ecological niche as pathogenic strains (1,7,25). This phenomenon has also been used to suppress disease incidence or severity in different host-pathosystems (1,6,7,8,22,25). Avirulent fig isolates of *F. moniliforme* might be exploited in a similar biocontrol program for endosepsis if they are competitive colonizers.

In this study, *F. moniliforme* isolates from wild caprifigs were significantly more virulent than were the isolates from cultivated caprifigs. Too few *F. solani* isolates were recovered to generalize a similar relationship for this species, although the two isolates from cultivated figs showed greater virulence than those from wild caprifigs. Figs were introduced into California only in the late 1700s (9), and essentially, there were no wild figs. The fig trees we termed "wild" resulted from dissemination of the seeds by birds to sites isolated from the cultivated areas. It is unclear why the isolates of *F. moniliforme* from the wild caprifigs were much more virulent than those from cultivated caprifigs. One possible explanation could be that the pathogen is genetically isolated. The average life-span of fig trees is estimated to be more than 100 yr (5), and wild caprifig trees are located in the Sierra Nevada Mountains far from commercial fig-production areas. In addition, fig wasp flights occur over distances of less than 100 m (T. J. Michailides and K. V. Subbarao, *unpublished data*).

In cultivated caprifigs, endosepsis has been managed by the use of fungicides since the early 1930s (26). Although different fungicides have been used over the years, the disease-management procedure has remained essentially the same. The isolates from cultivated caprifigs may have developed a certain degree of resistance to commonly used fungicides. In contrast, isolates from wild caprifigs, because of their physical and genetic isolation, may be expected to be sensitive to these fungicides. Preliminary evaluation of isolates from cultivated and wild caprifigs for benomyl resistance, however, did not show any difference between them (K. V. Subbarao and T. J. Michailides, *unpublished data*). Regardless of the operating mechanisms, the discovery of higher virulence in the isolates of *F. moniliforme* from wild caprifigs versus cultivated caprifigs has serious implications for disease management. During years with shortages of cultivated mamme caprifigs for caprifying the profichi pollinator crop, growers collect mamme caprifigs from wild fig trees to alleviate the shortage. As a result, some of the most virulent *F. moniliforme* isolates are introduced into the cultivated fig agroecosystem. Typically, this practice is used only after an extensive freeze of the mamme crop, but it can cause significant long-term problems for the fig industry in California and should be minimized.

F. moniliforme strains generally belong to *G. fujikuroi* mating populations A–F (12,16,17). Assignment of an isolate to a particular mating type also may indicate an ability to produce fumonisin B_1 (18). Strains in *G. fujikuroi* mating population A produce greater amounts of fumonisins than do those in the F population (18). The majority of *F. moniliforme* isolates included in our study belonged in *G. fujikuroi* mating population A, and only a few belonged in the F mating population. Fumonisin B_1 promotes cancer in rats (10) and causes equine leukoencephalomalacia (14,19) and human esophageal cancer (23). *F. moniliforme* isolates from maize and other cereals also produce large amounts of fumonisin B_1. The fig isolates of *F. moniliforme* that belong to *G. fujikuroi* mating population A (16) may produce fumonisin B_1 in amounts that can be correlated with fungal virulence. Maize isolates of *F. moniliforme* in comparison with fig isolates (29) were all from *G. fujikuroi* mating population A (K. V. Subbarao and T. J. Michailides, *unpublished data*), as were the majority of fig isolates.

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