

Fusarium Wilt and Crown Rot of Sweet Basil: Involvement of Soilborne and Airborne Inoculum

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ABSTRACT

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Fusarium oxysporum f. sp. *basilici* causes wilt and crown and root rot in sweet basil (*Ocimum basilicum*) in all basil-growing regions of Israel. Wilting and death of basil plants were recorded, and isolates of *F. oxysporum* were obtained at high frequency from roots and all above-ground parts of diseased plants. Inoculation tests with representative isolates verified that the causal agent is *F. oxysporum* f. sp. *basilici*. *F. oxysporum* f. sp. *basilici* causes both wilt and crown and root rot. The following symptoms were observed: wilt of plants at all stages of growth, brown and black discoloration of roots and lower stems, continuous and discontinuous black lesions on stems, blackening and drying

of vegetative apices, growth retardation, and xylem discoloration. The stems were frequently covered with a pink-orange layer consisting mainly of macroconidia, which could become airborne, as revealed by propagule trapping. Therefore, the pathogen has characteristics of both soilborne and airborne pathogens. *F. oxysporum* f. sp. *basilici* was readily recovered from structures inside the greenhouse, including concrete stumps, strings, pipes, and walls. Seeds collected from diseased plants were infested with the pathogen and produced diseased plants bearing infested seeds. Because the pathogen is easily disseminated, a holistic approach is necessary to control it. Because of the variety of symptoms associated with this disease, we suggest renaming it "Fusarium wilt and crown rot."

Additional keyword: herbs

Sweet basil (*Ocimum basilicum* L.) is grown for use as a spice in foods and for medicinal purposes. It is a relatively new crop in Israel, where it is grown commercially in monoculture in heated greenhouses and is the major herb crop produced for export. The types of sweet basil grown in Israel are local selections from imported European and American cultivars. In recent years, growth retardation, wilt, and occasionally plant death, resembling symptoms of Fusarium wilt of basil, have been observed. *Fusarium oxysporum* has been isolated at high frequency from symptomatic plants (18).

Fusarium wilt of basil has been reported from several countries, including southern Russia, Abkhazia, France, Italy, and the United States (2,4,5,9,11,15,20,21,23,30,31). The pathogen was identified in 1968 by Dzidzariya (7) as *F. oxysporum* f. sp. *basilicum* and was later renamed *F. oxysporum* f. sp. *basilici* (24). Reported symptoms of diseased plants include wilt (2,4,15,21,23,30), chlorosis and necrosis of leaves and apices (11,15,21,30), growth retardation (4,23,30), asymmetric growth (23,30), dark longitudinal streaks on stems and petioles and stem necrosis (2,4,11,21,30), vascular discoloration (2,11,15,23,30), root rot (11), and plant death (15,23,30). The disease may severely reduce crop productivity and yield. The pathogen may be transmitted via seeds (9,20,22).

In Israel, the disease has occurred in all basil-growing regions and progresses rapidly during the growing season. In addition to the symptoms of root rot and stem lesions, which are evident at all stages of growth and even on young plants, stems of diseased plants are often covered with a pale pink-orange layer. Microscopic examination has revealed that this layer consists mainly of macroconidia. A sporulating layer on the stem is an atypical sign of diseases caused by *F. oxysporum* and has been reported only in rare cases, such as in tomato crown and root rot (27,28) and for *F. oxysporum* f. sp. *perniciosum* on mimosa (26).

In this work, we studied (i) the etiology of the disease in Israel; (ii) the occurrence of the pathogen in plant parts, including seeds; (iii) the production of airborne inoculum on diseased plants; (iv) pathogen distribution in soil and greenhouse spaces; and (v) possible means of dissemination and survival of the pathogen.

MATERIALS AND METHODS

Media. Fungi were isolated from diseased plants on potato-dextrose agar (PDA) and modified peptone-pentachloronitrobenzene *Fusarium*-selective medium (FSM) (10). FSM was also used to enumerate *Fusarium* propagules. Inoculum for pathogenicity testing was grown on Czapek-Dox medium (CDM) (3).

Pathogen isolation. Symptomatic basil plants growing in soil or artificial growth substrates were collected from all basil-growing regions in Israel. We used these plants to document symptoms, isolate potential fungal pathogens, and determine the occurrence of the pathogen in various plant parts (Table 1).

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Segments (2 cm long) were taken from roots and along the stems of diseased plants, dipped in 70% ethanol, surface-sterilized with NaOCl (1%) for 1 min, and rinsed in sterile water. Each segment was then cut into three pieces, which were blotted and placed on PDA or FSM. Flowers were surface-disinfested, dissected into 3-mm segments, and plated as described above. Plates were incubated in the dark at 27°C for 3 to 7 days, and colonies of *F. oxysporum* and other fungi were identified microscopically. One monoconidial isolate was prepared from each of the colonies of *F. oxysporum* originating from plant parts (Table 1) and used for pathogenicity testing.

Pathogenicity tests. Isolates of *F. oxysporum* were transferred to CDM and incubated at 27°C for 7 to 9 days. The contents of each petri dish were macerated with 75 ml of water (19) to prepare an inoculum suspension (0.7 to 2.5×10^6 propagules, mostly microconidia, per milliliter).

Basil seedlings (4 to 5 cm high) were uprooted from soil 28 days after sowing. Their roots were washed free of soil and dipped in inoculum suspension for 2 min. Seedlings were then transplanted into 700-ml pots (five seedlings per pot, two pots per isolate) filled with Rehovot sandy soil (96.2% sand, 3.8% clay, 0.0% silt, and 0.4% organic matter; pH 6.9). The soil was free of *F. oxysporum* f. sp. *basilici* and other known pathogens before planting, as evidenced by planting it with noninoculated melon, tomato, and basil seedlings.

The inoculated seedlings were maintained in the greenhouse at 25 to 28°C for 20 days at natural daylight. Isolates were identified as *F. oxysporum* f. sp. *basilici* when 60% or more of the inoculated seedlings became diseased. Noninoculated basil seedlings served as control plants and were maintained under the same conditions. The noninoculated plants showed no disease symptoms. Inoculation tests were repeated with 50% of the isolates.

Enumeration of conidia from the stem surface. Stems with dry rot and covered with pale pink-orange masses of *Fusarium* macroconidia, as verified by microscopic examination, were cut into five 1-cm segments and placed in conical flasks containing 100 ml of sterile water agar (0.1%). Flasks were shaken for 20 min in a reciprocal shaker (200 strokes per minute), and conidia were counted directly in the suspension with a hemacytometer. Conidial suspensions were also serially diluted, and 0.2-ml samples were spread on five petri dishes containing FSM. Dishes were incubated in the dark at 27°C, and colonies were counted after 5 to 7 days. Results were expressed as number of conidia per centimeter of stem.

Twenty-eight germinating single conidia were transferred individually to CDM for pathogenicity testing (Table 1). Each of these monoconidial isolates originated from a different plant, and the plants represented seven sites nationwide.

Trapping of airborne propagules. The occurrence of airborne propagules of *F. oxysporum* was assessed in two commercial basil greenhouses. These greenhouses (0.2 ha each) were equipped with six fans (122 cm in diameter) that were used to aerate the greenhouse and were automatically operated along with window opening when the temperature was above 28°C. The plants were irrigated by drippers. Four to 6 weeks after planting, 60 to 80 FSM plates were placed in each greenhouse on stands 20 cm above the ground. The plates were exposed for 60 min and incubated at 27°C for 7 days. Colonies with *F. oxysporum* morphology were counted and, depending on the number of colonies, 15 to 100% of them were subcultured on CDM for pathogenicity testing (Table 2).

Propagules of *F. oxysporum* on the greenhouse structure. The presence of *F. oxysporum* propagules on the greenhouse structure was investigated in May 1994 in two basil greenhouses at two sites where a high incidence of disease had been recorded during crop growth (from September 1993 through March 1994). The greenhouse structures themselves (including concrete stumps, iron posts, and plastic walls) and fixtures inside the greenhouses (including heating pipes, plastic strings, plastic

boxes, and air-circulating fans) were tested for the presence of *F. oxysporum* propagules at heights ranging from 20 to 200 cm above the ground (Table 3). A 5-cm² patch of each tested object was wiped with a wet sterile cotton swab, which was then blotted onto five points on FSM plates. Plastic strings hanging in various locations in the greenhouse were cut into 1-cm segments and placed on FSM plates (five segments per plate). Plates were incubated at 27°C for 7 days. Segments or blots that yielded colonies of *F. oxysporum* were counted, and representative colonies were subcultured on CDM for pathogenicity testing. Results were expressed as percentage colonization by *F. oxysporum* or *F. oxysporum* f. sp. *basilici*. Temperature in the greenhouse at a height of 60 cm was recorded by T-type thermocouples connected to a data logger (21X; Campbell, Logan, UT).

Enumeration of *F. oxysporum* in nonrhizosphere and rhizosphere soil and a growth substrate. Enumeration was done as described previously (10). Samples were collected from two commercial greenhouses at each of two sites (Table 4). At one site (Ein Habor), basil was grown in sandy soil; at the other (Avigdor), it was grown in tuff (volcanic ash, an artificial growth substrate). At each site, one greenhouse contained diseased basil plants, whereas in the other, the basil crop was being grown for the first time, with no sign of diseased plants.

Nonrhizosphere soil or tuff samples were taken 50 cm or more away from plants, from three sampling points (replicates) in each greenhouse. Three 5-g subsamples of each nonrhizosphere replicate were added individually to 45 ml of sterile water agar (0.1%) supplemented with MgSO₄·7H₂O (0.1%).

Rhizosphere soil or tuff samples were obtained as described previously (10). Individual diseased or healthy plants, complete with roots and adhering soil or tuff substrate, were removed from three sampling points (replicates) in each greenhouse. The roots were shaken in sterile tubes to collect soil or tuff adhering to them. To collect the remaining particles, which adhered tightly to the roots (less than 5% of the total amount of rhizosphere soil or tuff), the roots were shaken in sterile water agar (0.1%) supplemented with 0.1% MgSO₄·7H₂O. The two fractions were combined to constitute the rhizosphere soil or tuff sample.

Nonrhizosphere and rhizosphere soil or tuff suspensions were shaken for 15 min on a reciprocal shaker and then serially diluted with the same solution. Samples (0.2 ml) were spread on five petri dishes containing FSM. Dishes were incubated in the dark at 27°C. After 5 days, colonies were counted from dilutions that provided 10 to 40 colonies per plate. Results were expressed as colony-forming units (CFU) per gram of soil or tuff (dried at 105°C for 48 h). Representative colonies were subcultured on CDM for pathogenicity testing (Table 4).

Seed infestation. Diseased plants with inflorescence stems carrying mature seeds were collected from commercial greenhouses at two sites. The presence of *F. oxysporum* f. sp. *basilici* in these plants was verified by isolation from the stems as described above. Seeds were shaken from inflorescences and divided into two batches. Seeds from one batch were placed on FSM plates and incubated in the dark at 27°C. *F. oxysporum* colonies on these seeds were counted after 5 to 7 days, and representative colonies were subcultured on CDM for pathogenicity testing (Table 5). Seeds of the other batch were sown singly in pots containing Rehovot sandy soil and were grown to maturity (about 10 to 15 weeks), when presence of disease was recorded. Seeds were collected from those plants that showed disease symptoms, and the presence of *F. oxysporum* f. sp. *basilici* was verified to determine infestation by *F. oxysporum* and *F. oxysporum* f. sp. *basilici* (Table 5).

Data analyses. *Fusarium* was enumerated in soil and tuff in triplicate samples. Tests were repeated. Because separate analysis of each trial showed that the variance of the experimental error of the two trials was homogeneous, data from both experiments were combined for analysis. Statistical analyses of the results included analysis of variance and *t* test. Percentages were trans-

TABLE 1. Pathogenicity of *Fusarium oxysporum* isolates originating from diseased basil plants, rhizosphere and nonrhizosphere soil or tuff, and basil greenhouses

Origin of isolates	Disease symptoms	Isolates tested (no.)	Pathogenic isolates (no.)	Pathogenic isolates ^a (%)
Plant parts				
Root	Dark root rot	21	10	48
Lower stem (crown)	Crown rot	57	34	60
Macroconidia on stem	Pale pink masses	28	28	100
Stem	Xylem discoloration, lesions	56	52	93
Apex	Necrosis, wilt	52	52	100
Flower	Necrosis	34	34	100
Immature seeds	None	30	30	100
Mature seeds	None	52	42	80
Soil, tuff^b				
Nonrhizosphere		30	23	77
Rhizosphere		30	29	95
Greenhouse				
Airborne propagules ^c		255	56	22
Greenhouse structures ^d		114	37	32

^a Pathogenicity was determined by the root dip method.

^b These data refer to isolates originating from soil or tuff only in greenhouses in which diseased plants were observed (details in Table 4).

^c Details in Table 2.

^d Details in Table 3.



Fig. 1. Sporulation of *Fusarium oxysporum* f. sp. *basilici* on the stem of a diseased basil plant.

formed to arcsine-square roots before analyses. All analyses were performed with the SAS program (SAS Institute Inc., Cary, NC; release 6.04 for personal computer) at $P \leq 0.05$.

RESULTS

Disease symptoms. An extensive survey of greenhouses at 12 sites in Israel where sweet basil is grown revealed instances of stunting and growth retardation, wilting, and death. Four to eight diseased plants were collected from each location. *F. oxysporum* was the fungus most frequently isolated from diseased plants.

The symptoms observed in diseased plants were as follows: typical wilt of plants at all stages of growth, including young plants; brown and black discoloration of roots and basal stems (crown); crown and root rot; continuous and discontinuous black lesions along the stems, including the inflorescence; black necrosis and desiccation of leaves and apices; leaf distortion; and vascular discoloration (Table 1). In many cases, plants eventually died. Thus, the disease syndrome included major symptoms incited by soilborne pathogens: wilt, vascular discoloration, stem lesions, and crown and root rot. Twenty to 40% of the diseased plants examined exhibited all or most of the symptoms. Appearance and severity of symptoms varied among individual plants and among greenhouses. Disease was especially severe in greenhouses with a long history of basil cropping.

The disease is widespread throughout the country; it was found in all regions in which the crop is grown, either in soil or in artificial growth substrates. Disease incidence and progress varied among greenhouses. In some cases, disease incidence was 50% or higher. In experimental plots with a history of seven or more years of continuous basil cropping, the percentage of dead plants reached 100%.

A pale pink-orange layer ranging in length from a few centimeters to 25 cm was often observed along stem lesions and on dead stems (Fig. 1). Microscopic examination revealed that this layer consisted mainly of masses of macroconidia (Fig. 2), with few microconidia of *F. oxysporum*. This sign resembles the sporulation layer of *F. oxysporum* f. sp. *radicis-lycopersici* observed on the basal stems of diseased tomato plants (27). Conidial production on stems was conspicuous when wilting occurred (from November through March), but decreased later in the season.

Isolation of the pathogen from plant parts and its identification. *F. oxysporum* was the fungus most frequently isolated from diseased plants, except those with advanced rotting. Of 330 monoconidial isolates of *F. oxysporum* obtained from various parts of diseased plants and tested for pathogenicity, 282 (85.5%) were pathogenic to basil seedlings (Table 1). The proportion of pathogenic isolates was very high among those originating from the upper stems, inflorescences, and seeds. In contrast, the proportion was relatively low among isolates from the roots and lower stems.

Basil seedlings inoculated with pathogenic isolates showed black root and stem discoloration, growth retardation, collapse, desiccation, and death. Pathogenic isolates originating from various plant parts did not differ in the symptoms they incited on inoculated seedlings. *F. oxysporum* was reisolated from inoculated diseased seedlings. Therefore, the pathogen was confirmed



Fig. 2. Macroconidia of *Fusarium oxysporum* f. sp. *basilici* collected from the stem surface of a diseased basil plant. Bar represents 50 μ m.

as *F. oxysporum* f. sp. *basilici* (syn. *F. oxysporum* Schlechtend.:Fr. f. sp. *basilicum* (Dzidzariya) Armstr. & Armstr. [30]).

F. oxysporum f. sp. *basilici* was found in all parts of diseased basil plants (Fig. 3) collected from commercial greenhouses at 12 sites in Israel. The pathogen colonized all stem parts, including vegetative apices and inflorescences of plants that showed either wilt or crown rot symptoms (Table 1). The pathogen could also be isolated from apices of both symptomatic and asymptomatic stems of the same plant, indicating the systemic nature of the disease. *Rhizoctonia* and *Pythium* spp. were seldom isolated, and then only from roots; these fungi were not further studied.

Conidia on stems. The number of conidia counted on the surface of stems ranged from 2.2×10^6 to 4.4×10^7 per centimeter. Colony counts confirmed that more than 95% of the conidia were viable. Conidial production occurred on one to five stems per diseased plant. The total length of stems bearing conidia ranged from 5 to 40 cm per plant, giving a calculated number of conidia per diseased plant with sporulation ranging from 1.1×10^7 to 1.8×10^9 . Twenty-eight monoconidial isolates, each derived from a different plant, were confirmed as pathogenic in inoculation tests (Table 1). The numerous conidia on diseased plants represent a potential source of inoculum that could infest the greenhouse during crop growth.

Trapping of airborne propagules. The conidial masses produced on the stem surfaces of diseased basil plants resembled sporulation of *F. oxysporum* f. sp. *radicis-lycopersici* on tomato basal stems, propagules of which have been trapped from the air in the greenhouse (13,28). We made similar attempts to detect airborne propagules of *F. oxysporum* f. sp. *basilici* in the fall of 1993 in two commercial basil greenhouses naturally infested with the pathogen. Trapping was started before or when disease symptoms began to show and macroconidia were first apparent on stems of diseased plants and was repeated three times over the following three months (Table 2). Of 255 isolates of *F. oxysporum* originating from airborne propagules in various trials, 56 were pathogenic to basil. The number of *F. oxysporum* propagules recovered from the air increased as the disease progressed. Moreover, the proportion of *F. oxysporum* f. sp. *basilici* in the trapped airborne propagules increased from 0 to 12% in mid-October to 27 to 30% three months later (Table 2). This increase paralleled the increase in the number of stems harboring conidial masses of *F. oxysporum* f. sp. *basilici*, which apparently were the source of the airborne propagules. During the following crop season (October 1994 to March 1995), trapping of airborne propagules was repeated in the same greenhouses plus an additional two greenhouses and again revealed the presence of airborne propagules of *F. oxysporum* f. sp. *basilici* (data not shown).

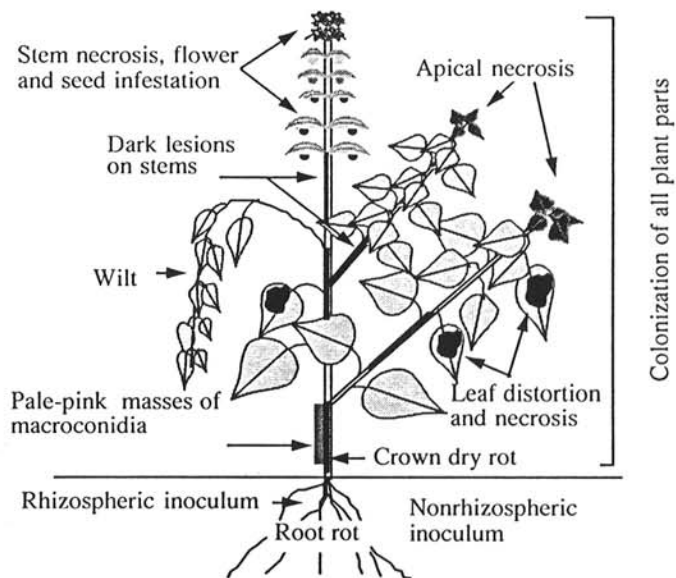


Fig. 3. Schematic diagram depicting the main symptoms of *Fusarium* disease in basil. Solid blocks represent necrosis and dark discoloration. Symptoms also include xylem discoloration.

TABLE 2. Airborne propagules of *Fusarium oxysporum* and *F. oxysporum* f. sp. *basilici* in two basil greenhouses

Greenhouse	Planting date ^a	Sampling date ^a	Diseased plants (%)	Colonies per plate ^b	Isolates tested (no.)	Pathogenic isolates (%) ^c
1	4 Sept.	18 Oct.	5	0.78	25	12
		7 Nov.	8	0.50	15	13
		19 Dec.	38	1.75	56	20
		13 Jan.	62	6.60	66	30
2	20 Sept.	18 Oct.	0	0.03	2	0
		7 Nov.	3	0.25	7	14
		19 Dec.	21	0.66	33	15
		13 Jan.	45	2.55	51	27

^a Dates refer to 1993 (September to December) and 1994 (January).

^b Plates of *Fusarium*-selective medium were exposed for 60 min.

^c Pathogenicity was determined by the root dip method.

Propagules of *F. oxysporum* on the greenhouse structure.

The greenhouse structures were tested for deposits of *F. oxysporum* propagules near the end of crop growth in May 1994. Isolates of *F. oxysporum* and *F. oxysporum* f. sp. *basilici* were readily recovered from various locations inside the greenhouse (Table 3). The pathogen was recovered at various frequencies from objects in the greenhouse up to 100 cm above ground level, including the concrete stumps supporting the greenhouse frame, plastic strings, plastic heating pipes, and plastic walls. Recovery of *F. oxysporum*, including *F. oxysporum* f. sp. *basilici*, from northern walls was about six times higher than that from southern walls, apparently because of the higher temperatures and direct solar radiation on the latter. The southern walls in May reached maximal temperatures between 38°C and 45°C, compared with 28 to 34°C for the northern walls. *F. oxysporum* propagules were not recovered from greenhouse components higher than 100 cm above ground level or from heat-conductive objects such as iron posts at any height. Maximal temperatures of iron posts ranged between 42°C and 51°C in May; these temperatures apparently are lethal to the propagules.

***Fusarium* propagules in soil and tuff.** Numbers of CFU of *F. oxysporum* f. sp. *basilici* (computed by multiplying number of *F. oxysporum* propagules by percentage of pathogenic isolates) recovered from nonrhizosphere soil or tuff from greenhouses with high disease incidence were 1,643 and 618 per gram, respectively

(Table 4). The respective numbers in the rhizosphere of basil plants were 12 to 33 times higher. *F. oxysporum* f. sp. *basilici* was also recovered from nonrhizosphere samples of new tuff substrate (but not from soil) in an adjacent greenhouse which had been cropped for the first time, as well as from the rhizosphere of apparently healthy plants growing there. *F. oxysporum* was also recovered from infested soil 12 months after the end of the crop season at Ein Habesor in numbers (1,300 CFU per g) not significantly different from those obtained at the end of the crop season.

TABLE 3. Recovery of *Fusarium oxysporum* and *F. oxysporum* f. sp. *basilici* from greenhouse structures^a

Source ^b	Ein Habesor			Avigdor		
	Colo-nization (%) ^c	Isolates tested (no.)	Patho-genic isolates ^d (%)	Colo-nization (%) ^c	Isolates tested (no.)	Patho-genic isolates ^d (%)
Concrete stumps	85	20	20	NA ^e	NA	NA
Iron posts	0	0	0	0	0	0
Plastic strings	28	17	47	23	14	50
Northern wall	47	21	38	85	15	20
Southern wall	8	3	33	16	4	25
Heating pipes	17	20	20	NT ^f	NT	NT

^a Experiments were conducted after crop removal in two greenhouses with high disease incidence.

^b Data refer to greenhouse structures and components tested at heights ranging from 20 to 100 cm. No isolates were recovered from iron posts, plastic strings, fans, plastic boxes, or walls tested at heights ranging from 100 to 200 cm.

^c A 5-cm² area of each tested object was wiped with a cotton swab and blotted on a plate of *Fusarium*-selective medium. Strings were cut into segments and plated. Results represent the percentage of plating points (out of 40 to 50 plates) that yielded colonies of *F. oxysporum*.

^d Pathogenicity was determined by the root dip method.

^e NA = not applicable (high tunnel).

^f NT = not tested.

TABLE 4. Inoculum density of *Fusarium oxysporum* and *F. oxysporum* f. sp. *basilici* in soil and tuff substrate at two sites

Site	Sub-strate	Green-house ^a	Nonrhizosphere ^b		Rhizosphere ^b	
			CFU/g × 100	Patho-genic isolates ^c (%)	CFU/g × 100	Patho-genic isolates ^c (%)
Ein Hab-esor	Sandy soil	I	17.3 a	95.0 a	208.0 a	95 a
		II	0.36 b	0.0 b	5.1 b	0 b
Avigdor	Tuff	III	10.3 a	60.0 a	216.0 a	95 a
		IV	3.85 b	40.0 b	50.0 b	33 b

^a Plants in greenhouses I and III showed *Fusarium* wilt disease (80 to 100%); all plants in greenhouses II and IV were apparently healthy.

^b Figures for each site within a column followed by different letters indicate a significant difference between greenhouses according to the *t* test ($P < 0.05$).

^c Ten to 15 isolates per treatment were tested for pathogenicity by the root dip method.

TABLE 5. Recovery of *Fusarium oxysporum* and *F. oxysporum* f. sp. *basilici* from seeds of diseased basil plants^a

Site	Plants tested (no.)	Plants with infested seeds (%) (A)	<i>F. oxysporum</i> -infested seeds ^b		<i>F. oxysporum</i> f. sp. <i>basilici</i>		Diseased plants ^e (%)
			Percentage (B)	Range (%)	Pathogenic ^c (%) (C)	Infested seeds ^d (%)	
Ein Habesor	20	70	37	10–90	91	34	62
Hazeva	16	44	61	10–100	68	41	45

^a Seeds were harvested separately from individual plants and tested for the presence of *F. oxysporum* on *Fusarium*-selective medium.

^b Seeds harvested from plants in (A).

^c Percentage of pathogenic isolates of *F. oxysporum* from (B), as tested by the root dip method.

^d Calculated as (B)×(C)/100.

^e Percentage of diseased plants obtained when seeds from (B) were sown in pathogen-free soil.

Seed infestation. Mature seeds were extracted from 36 diseased plants from two sites. A high proportion (44 to 70%) of the diseased plants carried seeds infested with *F. oxysporum*, and *F. oxysporum* was isolated from 37% and 61% of the seeds of these plants (Table 5). Pathogenicity tests showed that 34% and 41% of the seeds were infested with *F. oxysporum* f. sp. *basilici*.

We tested the potential of seeds originating from diseased plants to give rise to a new generation of diseased plants and infested seeds by planting the seeds in pathogen-free soil. Of the plants that emerged from such seeds, 62% and 45% showed disease symptoms after 5 to 9 weeks, and the presence of *F. oxysporum* f. sp. *basilici* was verified in these plants. Furthermore, when these new-generation plants were grown to maturity, 60% of the seeds extracted from diseased plants were infested with *F. oxysporum* f. sp. *basilici*. In one sample of infested basil seeds that was maintained at room temperature, the percentage infestation with *F. oxysporum* f. sp. *basilici* declined from an initial 90% to 55% at the end of 12 months (a significant difference at $P < 0.05$).

DISCUSSION

The syndrome incited in basil plants by *F. oxysporum* f. sp. *basilici* is manifested by a wide array of symptoms (Table 1 and Fig. 3). Some of these symptoms are typical of vascular wilt, while others are typical of foot, crown, and root rot. We obtained pathogenic isolates from all parts of diseased plants, including inflorescences and seeds. Isolates from different plant parts and from soil or artificial growth substrate and greenhouse structures produced similar disease symptoms on inoculated seedlings. Pathogenicity and vegetative compatibility tests have provided evidence that Italian and American isolates of *F. oxysporum* f. sp. *basilici* from roots, stems, and seeds belong to a single vegetative compatibility group (9).

Pathogenic forms of *F. oxysporum* cause vascular wilt and xylem discoloration via systemic infection in many crops (25) without any additional external symptoms on roots or stems. *F. oxysporum* f. sp. *radici-lupini* on lupine (32) and *F. oxysporum* f. sp. *radicis-lycopersici* on tomato (17) are notable exceptions because the major symptom they cause is crown and root rot, rather than systemic vascular infection. These two formae speciales are distinct from *F. oxysporum* f. sp. *lupini* and *F. oxysporum* f. sp. *lycopersici*, the vascular wilt pathogens of lupine and tomato. Another exception is *F. oxysporum* f. sp. *asparagi*, which causes both vascular wilt and crown and root rot diseases in asparagus (1,12). Similarly, *F. oxysporum* f. sp. *basilici* produces all the above symptoms and, in addition, foliar necrosis and sporulation on stems. In view of the diversity of symptoms manifested by infected basil plants, we suggest renaming the disease "Fusarium wilt and crown rot."

Sporulation on stems is considered very rare in *Fusarium* wilt diseases incited by pathogenic *F. oxysporum*. In mimosa trees, *F. oxysporum* f. sp. *perniciosum* emerges from lenticels in the bark and produces sporodochia bearing masses of macroconidia (26). Sporulation on stems is associated with the nonvascular tomato

pathogen *F. oxysporum* f. sp. *radicis-lycopersici* (27). Grasso (11) reported the appearance of a dense, whitish mycelium on stems of basil plants naturally infected with *F. oxysporum* f. sp. *basilicum*. It is possible that with changing agricultural practices, external sporulation may become more frequent in *Fusarium* vascular diseases. Whereas the sporulating layer on tomato plants was described as consisting of microconidia (27,28), in basil it consists almost exclusively of macroconidia.

Like the tomato pathogen *F. oxysporum* f. sp. *radicis-lycopersici*, *F. oxysporum* f. sp. *basilici* displays both soilborne and airborne behavior, a phenomenon that may reflect an epidemiological similarity. Airborne propagules of *F. oxysporum* f. sp. *basilici*, like those of *F. oxysporum* f. sp. *radicis-lycopersici* (13, 28), were readily trapped. The number of propagules trapped ranged from 5 to 1,164 per square meter per hour (calculated from Table 2). Hence, a significant number of airborne propagules can be deposited over several months. Such numbers are not surprising considering the heavy masses of conidia produced on diseased stems (up to 4.4×10^7 propagules per centimeter of stem), the apparent source of airborne inoculum. Rowe et al. (28) trapped 1.3 to 1.6 propagules of *F. oxysporum* f. sp. *radicis-lycopersici* per 2 h, a number which is within the range of our trapping (Table 2).

Most of the greenhouse structures had become contaminated with the pathogen by the end of the season (Table 3), apparently from airborne, soilborne, and plant debris sources. Because sprinkler irrigation is not used in basil greenhouses in Israel, and because the beds are mulched with plastic, pathogen spread via water splash from soil should not be significant. The large number of conidia produced and the frequent air circulation due to fan operation in the greenhouse greatly facilitate the dispersal of airborne propagules. Sprayer operation and other crop management practices might also enhance such dispersal. Control of air movement and measures that suppress conidial production should be helpful in reducing the fast spatial spread of this pathogen.

Seeds extracted from diseased plants carried the pathogen and gave rise to diseased plants, which in turn produced a new generation of infested seeds (Table 5). Contamination of commercial seed lots by *F. oxysporum* f. sp. *basilici* has been shown previously (9,20,22), and Elmer et al. (9) suggested that seeds are the logical vehicle for rapid dissemination of this pathogen to many countries within a relatively short period. Moreover, infested seeds may serve as a means of survival.

Soil pathogens that also have the characteristics of foliar pathogens are especially difficult to control. *F. oxysporum* f. sp. *basilici* was detected in a new tuff substrate in which basil was grown as a first crop. This finding demonstrates the strong contaminating potential of this pathogen, which can be attributed to the diversity of its means of dissemination and survival. Considerable potential for soil contamination and reinfestation has also been reported for *F. oxysporum* f. sp. *radicis-lycopersici* (13,28).

Such situations call for the development of a holistic, integrated approach to deal with the different inoculum sources at various sites before, during, and after planting. Primary inoculum, for example on seeds, greenhouse components, and potting mix in the nursery, must be prevented or eradicated. Because eradication of soilborne inoculum may not always be practical, for economic and technological reasons, efforts should be aimed at suppressing or reducing it to tolerable levels. Potential means of pathogen and disease management include regulation of production and transfer of propagation material, seed disinfestation, suppressive growth media (14), sanitation of the greenhouse by heating (space solarization) (29) or by formalin (27), crop rotation (31), soil and substrate disinfestation with methyl bromide (5–8) or by solarization, biocontrol agents (7,16,24), and fungicides (13,28). Avoiding or minimizing the use of pesticides is especially necessary with herb crops. A combination of appropriate control measures, emphasizing the eradication or avoidance of primary inoculum and the breeding of resistant cultivars, will achieve this goal. Three iso-

lates have been deposited with the *Fusarium* Research Center, Pennsylvania State University, College Park (FRC 0-1880, 0-1881, and 0-1882).

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