Effects of Inoculum Depth and Density on Fusarium Wilt in Carnations

Y. Ben-Yephet, M. Reuven, and A. Genizi

First and second authors: Department of Plant Pathology; and third author, Department of Statistics and Operations Research, Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel.
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ABSTRACT

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The effects of depth (0–30, 15–30, 30–45, 45–60, or 60–75 cm) and density (0, 6, 25, 120, 770, or 3,500 propagules per gram of soil) of *Fusarium oxysporum* f. sp. *dianthi* inoculum originating from naturally infested soil on wilt and flower yield in a highly susceptible cultivar of carnation (*Dianthus caryophyllus* 'Fantasia') were studied in concrete containers (100 × 50 cm) during three growing seasons. Depth of inoculum placement was related directly to time until symptom onset and inversely to the final percentage of plants that were infected. Rate of disease progress varied with depth of inoculum placement. At 15–30, 30–45, 45–60, or 60–75 cm, mean wilt incidence 185 days after planting was 50, 45, 33, or 22%, respectively. Flower yields were correlated with depth of inoculum. Mean yield losses were 15, 23, 31, and 39% when inoculum was placed at depths of 60, 45, 30, and 15 cm, respectively, relative to yield in pathogen-free soil. In experiments on the effect of inoculum density, time until symptom onset and final disease incidence were related inversely and directly, respectively, to propagule density. The rate of disease progress was the same at all propagule densities. At densities of 6, 25, 120, 770, and 3,500 propagules per gram of soil, mean percentages of infected plants 185 days after planting were 2, 5, 13, 34, and 57%, respectively. Flower yields were related inversely to inoculum density. The effects of inoculum depth and density on final disease incidence were fit by linear logistic models. At the end of the growing season, the vertical distribution of *F. o. dianthi* propagules through a 60-cm depth showed a similar trend whether the propagules had been placed at 0–30 cm or at 60–75 cm prior to planting. The number of propagules placed at various inoculum densities prior to planting had increased 10- to 100-fold by the end of the growing season.

Fusarium oxysporum Schlechtend.:Fr. f. sp. *dianthi* (Prill. & Delacr.) W. C. Snyder & H. N. Hans. causes severe wilting in carnation (*Dianthus caryophyllus* L.). In Israel, use of carnation cuttings free of *Fusarium* spp. (17) and fumigation of soil with methyl bromide prior to planting usually minimize carnation wilt caused by *F. o. dianthi.* However, these practices are not always successful in preventing *Fusarium*-induced losses, especially in greenhouses planted with susceptible cultivars. In a recent study (3), carnation wilt was observed even when soil fumigation prior to planting had reduced *F. o. dianthi* populations to below detection level down to a soil depth of 60 cm. The observed wilt may have been caused by *F. o. dianthi* propagules present in soil layers deeper than 60 cm or by rapid root colonization by the small number of propagules that had survived fumigation.

Effects of inoculum density on disease incidence caused by several formae speciales of *F. oxysporum* (4,7,9,15) and on root infections or disease incidence caused by *Verticillium dahliae* (1,6,11) have been described. Inoculum of *F. oxysporum* was distributed vertically through depths of 80 and 90 cm in experi-

ments conducted with *F. o. lycopersici* (5,8) and through 90 cm in an experiment with *F. o. apii* (4). The highest inoculum densities occurred at the soil surface (8) and in the upper 45 cm (5) for *F. o. lycopersici* and at 15–30 cm for *F. o. apii* (4). In a study of the vertical distribution of *F. oxysporum* among 10-cm soil layers to a depth of 40 cm in carnation greenhouses, the population of the fungus was greatest in the 0- to 10-cm layer, although it was still high at 30–40 cm (12,13). In a recent study in carnation greenhouses (14), *F. o. dianthi* populations were concentrated mainly in the 0- to 20-cm soil layer. No disease symptoms appeared in pea when inoculum of *F. solani* f. sp. *pisi* was placed deeper than 20 cm in 30-cm-deep containers (15). Little is known about the effects of depth or density of inoculum on Fusarium wilt in carnation.

The purpose of the present study was to determine the effects of inoculum depth and density on wilt symptoms and flower yields in a highly susceptible carnation cultivar planted in concrete containers filled with light, sandy soil. In addition, vertical distribution and amounts of *F. o. dianthi* inoculum in containers and in commercial carnation greenhouses at the end of a growing season were quantified.

MATERIALS AND METHODS

Soil infestation. Response of the carnation cultivar Fantasia, which is susceptible to *F. o. dianthi,* was evaluated in light, sandy soil in 100- × 50-cm (height × diameter) concrete containers. The soil was similar to that used in commercial carnation greenhouses in Israel. Standard agronomic practices for carnation production were followed.

The effect of inoculum depth on carnation wilt was studied over 3 yr in a series of four experiments with 4, 6, 5, and 4 replicates (experiments 1–4, respectively). Naturally infested soil was used as the source of *F. o. dianthi* inoculum and was mixed in a concrete mixer with pathogen-free soil of the same type to achieve ~2,000 propagules per gram (ppg) of soil. From this soil, five samples, each consisting of 10 subsamples, were taken for quantification of *F. o. dianthi* propagules, as described in the following section. A uniformly infested soil layer 15 cm thick was placed at a depth of 15–30, 30–45, 45–60, or 60–75 cm. Two additional treatments served as controls. In one, a double layer of the infested soil was placed at a depth of 0–30 cm; in the other, all soil in the container was free of *Fusarium.*

The effect of inoculum density on carnation wilt was studied over 2 yr in a series of three experiments (experiments 5–7) by placing propagules at different densities in the upper 20-cm soil layer. Four replicate containers were used for each propagule density tested. To achieve the different densities, naturally infested soil collected from areas with wilted carnations was diluted with *Fusarium*-free soil in a concrete mixer. The inoculum densities prepared were 0, 6, 25, 120, 770, and 3,500 ppg of soil for experiments 5 and 6 and 0, 100, 500, 1,500, and 4,500 ppg for experiment 7. At the end of the growing season, plants were removed, and *F. o. dianthi* propagules in the top 20 cm were quantified in composite soil samples, each prepared from five subsamples per container.

At the end of each growing season, soil in the containers was fumigated to eliminate viable *F. o. dianthi* propagules before starting a new growing cycle. To ensure maximal reduction of fungal propagules, soil was fumigated by the successive application of two highly concentrated fumigants, methyl bromide (1.25 g/kg of soil; i.e., ~15,000 kg/ha, which is 19 times the concentration used by growers) and metham-sodium (Vapam; 1.5 ml/kg of soil; i.e., ~15,000 L/ha, which is five times the concentration used by growers). Methyl bromide was injected into the soil by insertion of a metal tube with pores at 10-cm intervals to a depth of 60 cm into the center of each container under a polyethylene cover 0.1 mm thick. Four days later, metham-sodium was applied through the irrigation system with the water volume adjusted to ensure penetration of the fumigant throughout the soil column. Eight days later, the soil was irrigated with an amount of water equivalent to ~4,000 m^3/ha, i.e., ~100 L per container, to leach bromide toxic to carnation from the soil. After fumigation, the possibility that *F. o. dianthi* propagules were still present was examined by sampling soil from containers and quantifying viable propagules as described below.

In all experiments, 20 rooted stem cuttings were planted in each container and used as one replicate. Disease incidence was assessed by counting the number of wilted plants biweekly from mid-September to mid-December (85–185 days after planting). The number of flowers in each container was also determined.

Soil sampling in containers and greenhouses. In experiments 1 and 2, the vertical distribution of *F. o. dianthi* propagules toward the end of the growing season, 260 days after planting, was examined in pathogen-free soil and in soil containing propagules placed at 0–30 or 60–75 cm. An inflexible plastic tube with a longitudinal opening was used to collect four cylindrical soil samples (3 cm in diameter × 20 cm in height) from each container successively to a depth of 80 cm, and these were combined into a composite sample for each 20-cm depth. In addition, the vertical distribution of propagules down to 120 cm was examined toward the end of the growing season (~260 days after planting) in four greenhouses in which *F. o. dianthi*-induced wilt had occurred in susceptible carnation cultivars grown for commercial production. Carnations were planted to the end of June and terminated the next April. From each greenhouse, five paired sets of 7.5-cm-diameter cylindrical samples, at successive 20-cm depths down to 120 cm, were collected at random with the aid of an auger. Each pair was combined into a composite sample for each depth, and *F. o. dianthi* propagules were quantified. To minimize possible contamination from upper to lower layers, the following precautions were taken: the soil to be sampled was wetted to minimize its movement; any soil that slipped down was removed; and the auger or plastic tube was cleaned thoroughly after each 20-cm sample was obtained. In addition, a thin layer of soil (~5 mm) was sliced from the periphery of the sample, and the remainder was then used for the propagule count.

The number of *F. o. dianthi* propagules among the total number of *F. oxysporum* colonies counted in the soil samples was determined as described previously (2). Briefly, air-dried soil (5 g) was diluted in sterile dilute water-agar (0.1%), and 1 ml of this suspension was pipetted onto each of five plates containing pentachloronitrobenzene-agar medium (10). One hundred *Fusarium*-like colonies were transferred to PDS (potato-dextrose agar supplemented with 250 mg of dihydrostreptomycin per liter) for identification of *F. oxysporum.* For identification of *F. o. dianthi,* 30 colonies of *F. oxysporum* were tested for pathogenicity on the susceptible cultivar Fantasia.

Before planting, stem segments from 50 cuttings of cultivar Fantasia were plated in petri dishes on PDS and examined for possible contamination with *F. o. dianthi.* Cultures resembling *F. oxysporum* were evaluated for pathogenicity by inoculation of carnation plants (cultivar Fantasia).

Statistical design and analysis. All seven experiments were designed as randomized blocks, with each container representing an experimental unit. Disease incidence was analyzed by logistic regressions on inoculum depths or log inoculum densities for the final disease incidence and on log days for disease progress at different inoculum densities or depths. All log transformations were to base 10. The SAS (16) PROC CATMOD procedure was used, and maximum likelihood estimates were calculated and used to construct curves. Flower yields were regressed on inoculum depth and log(inoculum density + 10). Greenhouse-by-depth interaction was obtained by analysis of variance for propagule counts after log transformation.

RESULTS

Effect of inoculum depth on disease incidence and flower yield. Examination of plated stem segments revealed that prior to planting all cuttings were free of *F. o. dianthi.* Deep placement of inoculum resulted in low disease incidence (Fig. 1) and delay of symptom onset (Fig. 2). At depths of 0–30, 15–30, 30–45, 45–60, and 60–75 cm, mean wilt incidence in experiments 1–4, measured

185 days after planting, was 54, 50, 45, 33, and 22%, respectively. Regression of disease incidence on depth of inoculum placement (excluding the data for 0–30 cm) was fit by a logistic model ($P < 0.01$) with a common slope of −0.031 for all four experiments and with significantly different ($P < 0.01$) intercepts. Goodness of fit was indicated by a residual chi-square of 7.24 (10 df, $P = 0.70$). Disease progress associated with different depths of inoculum placement is shown in Figure 2, which presents results of experiment 3 as representative of experiments 1–4. Regression of logit-transformed disease incidence on log days yielded intercepts −14.2, −17.2, −26.7, −28.8, and −27.1 and slopes 6.9, 8.3, 12.3, 12.9, and 12.0 for depths 0–30, 15–30, 30–45, 45–60, and 60–75 cm, respectively (model $P < 0.01$; residual chi-square of 15.37, 20 df, $P = 0.75$). Disease incidence recorded at the end of the growing season did not differ significantly for inoculum placed at depths of 15–30 and 30–45 cm. However, disease symptoms took longer to appear when inoculum depth was 30–45 cm than when it was 15–30 cm. This was reflected by a significant time-by-depth interaction ($P < 0.01$). A similar pattern with respect to the effect of depth was found in experiments 1–4, although disease incidence differed significantly between experiments ($P < 0.01$).

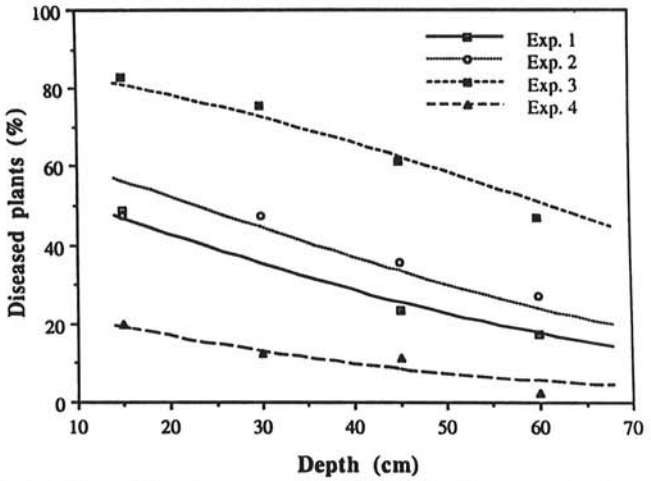

Fig. 1. Effect of *Fusarium oxysporum* f. sp. *dianthi* propagules placed at various depths on incidence of Fusarium wilt in carnation. The numbers of replicate containers used in experiments 1–4 for each soil depth tested were 4, 6, 5, and 4, respectively.

Relative to the flower yield from *Fusarium*-free soil, average yield losses for experiments 1–4 were 15, 23, 31, 39, and 60% when inoculum depths were 60–75, 45–60, 30–45, 15–30, and 0–30 cm, respectively (Fig. 3). A highly significant negative linear relationship was found between flower yield loss and inoculum depth ($P < 0.01$). In the regression model of the four experiments, R^2 was 0.97 and the residual standard deviation 4.26. Depth-by-experiment interaction was significant ($P < 0.01$).

Effect of inoculum density on disease incidence and flower yield. Disease incidence was related directly to inoculum density (Fig. 4). At densities of 3,500, 770, 120, 25, and 6 ppg of soil, average values of disease incidence (from experiments 5 and 6) 185 days after planting were 57, 34, 13, 5, and 2%, respectively. Regression of disease incidence on inoculum density was fit by a logistic model ($P < 0.01$) with a common slope of 1.52 and intercepts (not significantly different) of −5.4, −4.8, and −5.3 for experi-

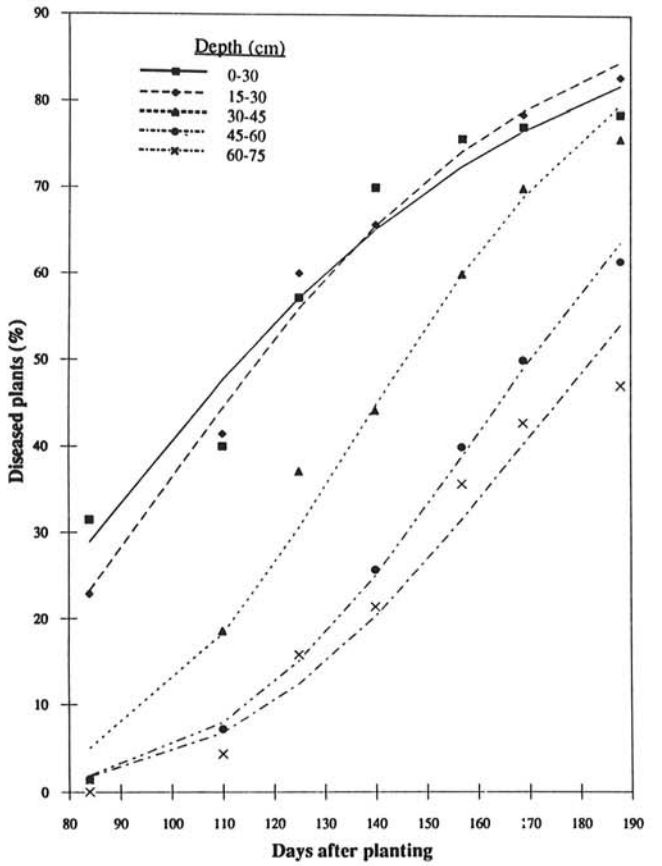

Fig. 2. Disease progress in carnation plants after placement of propagules of *Fusarium oxysporum* f. sp. *dianthi* at various depths (experiment 3). Five replicate containers were used for each soil depth tested. Logit regressions ($P < 0.01$) of disease incidence on log days for different depth treatments are presented. Residual chi-square = 15.37; df = 20; and $P = 0.75$.

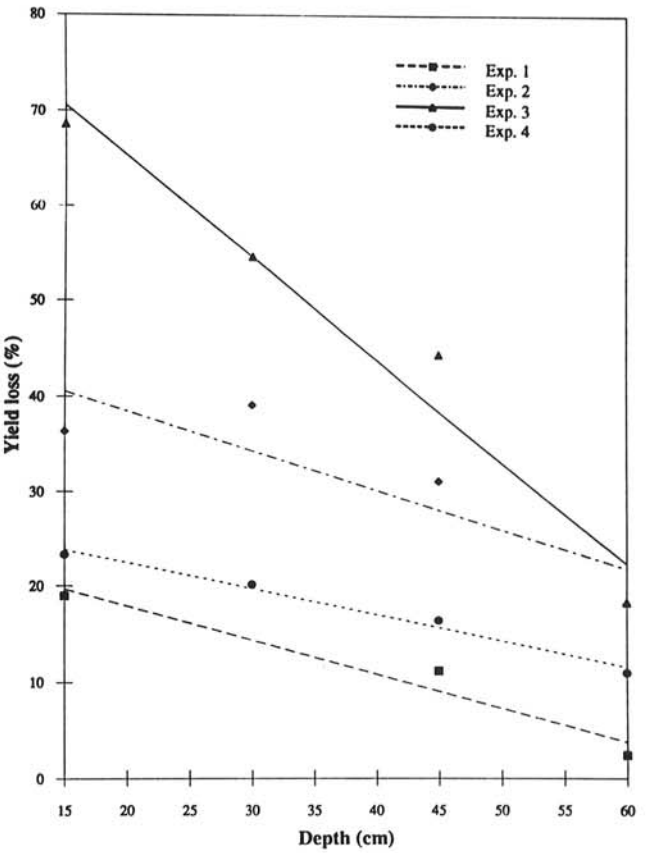

Fig. 3. Flower yield loss in relation to *Fusarium oxysporum* f. sp. *dianthi* propagules placed at various depths. The numbers of replicate containers used in experiments 1–4 for each soil depth tested were 4, 6, 5, and 4, respectively. Yield loss (%) = 25 − 0.35 (depth); 47 − 0.42 (depth); 87 − 1.07 (depth); and 28 − 0.27 (depth) for experiments 1–4, respectively. Standard deviation = 4.26, and R^2 for the regression model = 0.97.

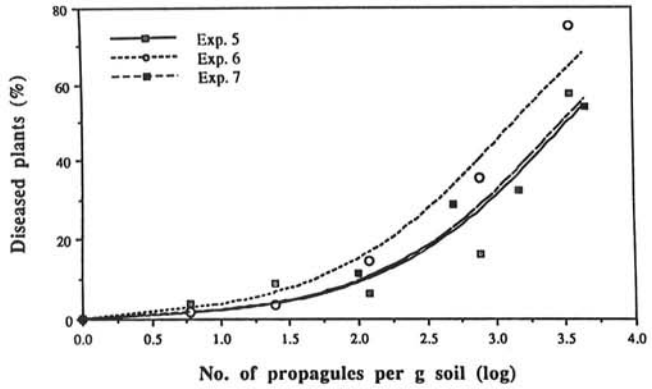

Fig. 4. Effect of propagule density of *Fusarium oxysporum* f. sp. *dianthi* on disease incidence in carnation plants. Four replicate containers were used for each propagule density tested in experiments 5–7.

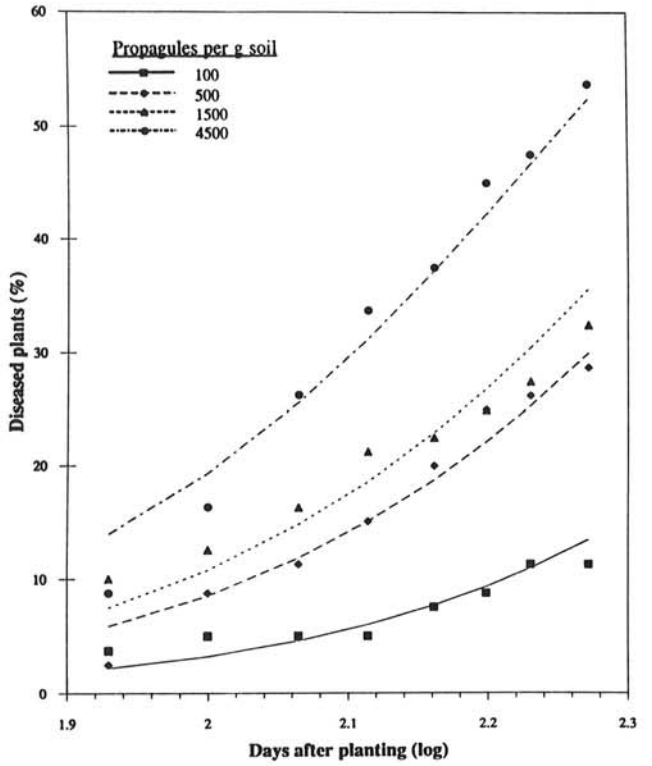

Fig. 5. Disease progress in carnation plants at several propagule densities of *Fusarium oxysporum* f. sp. *dianthi* (experiment 7). Four replicate containers were used for each propagule density tested.

TABLE 2. Analysis of variance for results of experiments 1 and 2

Experiment	Source of variation[a]	df	Mean square	*F*	Probability > *F*
1	Blocks	3	0.03	0.1	0.9723
	I	1	29.8	73.9	0.0001
	S	3	8.0	19.8	0.0001
	I × S	3	1.5	3.6	0.0301
	Error	21	0.4	...	...
2	Blocks	5	0.7	1.9	0.1270
	I	1	12.8	34.9	0.0001
	S	3	35.3	96.6	0.0001
	I × S	3	2.5	6.9	0.0009
	Error	35	0.4	...	...

[a] I = inoculum depth, and S = soil depth.

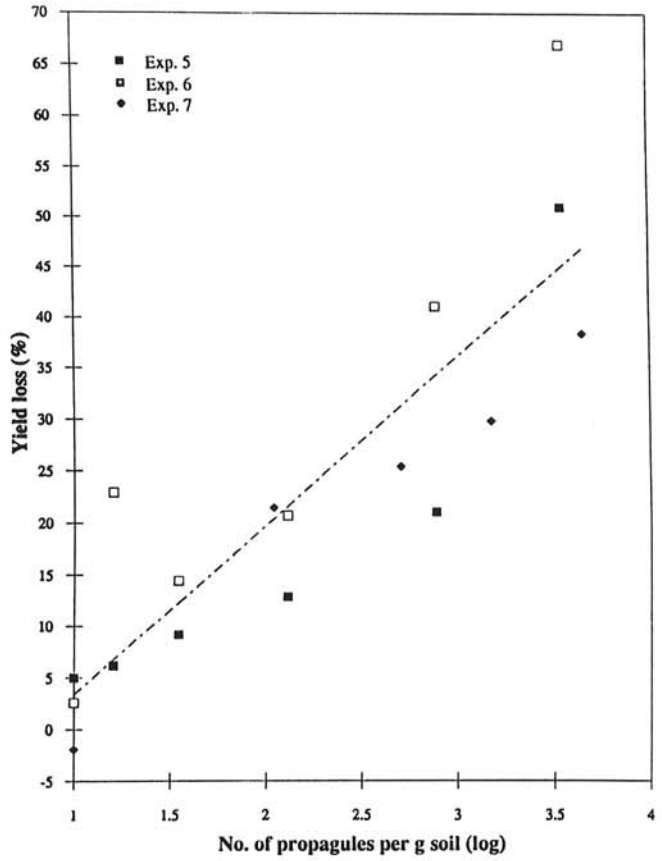

Fig. 6. Flower yield loss in relation to propagule density of *Fusarium oxysporum* f. sp. *dianthi*. Four replicate containers were used for each propagule density tested in experiments 5–7. Yield loss (%) = −13.01 + 16.37 log(number of propagules per gram of soil + 10). $R^2 = 0.76$, and $P < 0.01$.

TABLE 1. Effect of original placement of inoculum (0–30 or 60–75 cm deep) on distribution of *Fusarium oxysporum* f. sp. *dianthi* propagules after one growing season in carnations[a]

Experiment	Inoculum depth (cm)	Soil depth[b] (cm) 0–20	20–40	40–60	60–80	Slope[c]
1	0–30	14,743 (3,311)	2,253 (1,021)	1,173 (393)	916 (182)	−0.34
	60–75	868 (155)	346 (145)	108 (16)	422 (75)	−1.33
2	0–30	17,441 (3,573)	566 (250)	316 (82)	212 (43)	−0.8
	60–75	15,569 (1,517)	1,565 (618)	649 (145)	1,749 (278)	−1.1

[a] Containers were planted in mid-June 1992 with the highly susceptible carnation cultivar Fantasia. Soil was sampled in mid-March 1993.
[b] Values are numbers of propagules per gram of soil (means of four replicates for experiment 1 and six replicates for experiment 2). Values in parentheses are standard errors.
[c] Slope of linear log/log regression on four or three depths for inoculum originally placed at 0–30 or 60–75 cm, respectively.

TABLE 3. Distribution of *Fusarium oxysporum* f. sp. *dianthi* propagules in commercial greenhouses at the end of a growing season[a]

Greenhouse	Soil depth[b] (cm)					
	0–20	20–40	40–60	60–80	80–100	100–120
1	4,060 (661)	830 (188)	15 (6)	13 (2.4)	3 (0.5)	9 (5)
2	3,845 (502)	318 (64)	20 (3)	4 (3)	0.6 (1.3)	0 (0)
3	1,888 (929)	45 (20)	6 (3)	4 (3)	2.0 (2.0)	4 (4)
4	1,453 (382)	18 (9)	0 (0)	0 (0)	0 (0)	2.4 (3.3)

[a]After soil fumigation with methyl bromide (800 kg/ha), the four greenhouses were planted in the last week of June 1992 with carnation cultivars highly susceptible to *F. o. dianthi* (White Queen in two greenhouses and Smarty or Tomer in the other two). Soil samples were collected ~260 days after planting, and growth was terminated ~30 days later.
[b]Values are numbers of propagules per gram of soil (means of five replicates). Values in parentheses are standard errors.

TABLE 4. Analysis of variance of data from four greenhouses

Source of variation	df	Mean square	*F*	Probability > *F*
Greenhouse	3	19.5	7.22	0.0032
Depth	5	167.2	61.9	0.0001
Error	15	2.7	...	...

ments 5–7, respectively. Lack of fit was indicated by a residual chi-square of 22 (10 df, $P = 0.02$). For disease progress associated with different inoculum densities, regression of logit-transformed disease incidence on log days was highly significant ($P < 0.01$). The nonsignificant time-by-experiment interaction indicates a constant rate of progress in logit disease as a function of log density in the three experiments. The higher the inoculum density, the earlier the appearance of disease and the more quickly it progressed, as shown in Figure 5, which presents results of experiment 7 as representative of the three experiments. Logit regressions ($P < 0.001$) of disease incidence on log days revealed a common slope of 5.6 but significantly different ($P < 0.01$) intercepts of −14.7, −13.7, −13.4, and −12.8 for 100, 500, 1,500, and 4,500 ppg, respectively.

Relative to the flower yield from pathogen-free soil, average yield losses for experiments 5 and 6 (with similar propagule densities) were 11, 8, 15, 28, and 53% for 6, 25, 120, 770, and 3,500 ppg of soil, respectively. A highly significant positive linear relationship occurred between flower yield loss and inoculum density expressed as log(density + 10) ($P < 0.01$). The data for all three experiments (5–7) were fit by a simple regression model:

$$\text{Yield loss (\%)} = -13.01 + 16.37 \log(\text{number of propagules per gram soil} + 10)$$

in which $R^2 = 0.76$, $P < 0.01$, and residual standard deviation = 9.35 (Fig. 6).

Vertical distribution of *F. o. dianthi* propagule density after one growing season. *F. o. dianthi* propagules 260 days after planting in containers were distributed through 80 cm, whether the propagules were placed at a depth of 0–30 or 60–75 cm prior to planting (Table 1). For both treatments, the highest propagule densities (50–94% of the total amount of propagules retrieved from the entire profile) occurred at 0–20 cm. In experiment 1, final propagule densities were markedly lower, and the rate of their decline with depth was less when the original inoculum placement was at 60–75 cm than at 0–30 cm. Analysis of variance on log(number of propagules) indicated a significant treatment-by-depth interaction ($P < 0.01$) in both experiments (Table 2). The number of *F. o. dianthi* propagules decreased linearly with increasing soil depth on a log/log scale ($P < 0.01$). When the original inoculum placement was at 0–30 cm, linearity was evident over the entire 80-cm depth sampled. When the inoculum placement was at 60–75 cm, linearity was evident from 0 to 60 cm; in the 60- to 80-cm sample, inoculum density was high because of the survival of the inoculum originally placed at this depth (Table 1).

In the greenhouses, *F. o. dianthi* propagules toward the end of the growing season, i.e., ~260 days after planting, were found as deep as 120 cm (Table 3). The highest propagule densities occurred at 0–20 cm (82–98.6% of the total number of propagules), and the propagule densities found at 20–40 cm were 17, 7.6, 2.3, and 1.2% in greenhouses 1, 2, 3, and 4, respectively. At the other four depths (40–60, 60–80, 80–100, and 100–120 cm), there was no consistent trend of gradient for propagule distribution. Overall analysis of variance (Table 4) for log(number of propagules) of the four greenhouses shows the significance of differences between various depths.

Increase in *F. o. dianthi* propagule density after one growing season. The number of *F. o. dianthi* propagules recovered from the 0- to 20-cm layer at the end of the growing season was related directly to initial inoculum density. In experiments 5 and 6, inoculum densities of 3,500, 770, 120, 25, and 6 ppg of soil prior to planting resulted in 35,000, 10,210, 1,257, 2,179, and 534 ppg of soil, respectively, in the 0- to 20-cm layer after removal of plants from containers. A highly significant linear relationship ($P < 0.01$) occurred between the number of propagules at the end of the growing season and inoculum density prior to planting after a cube root transformation of both variables.

DISCUSSION

Fusarium wilt of the highly susceptible carnation cultivar Fantasia in containers was caused by inoculum placed as deep as 60–75 cm and by inoculum density as low as 6 ppg of soil. High inoculum density or shallow placement of inoculum was accompanied by greater final disease incidence, more rapid disease progress, and lower flower yields than low inoculum density or placement of inoculum deep in the soil. The linear correlation between inoculum density and Fusarium wilt may suggest that wilt incidence can be incited by an inoculum density lower than 6 ppg of soil.

Little information is available on the effect inoculum, placed at various depths, has on the host. No significant differences between plant stress measurements occurred when inoculum of *F. s. pisi* was distributed throughout containers or placed in the upper 10 cm of soil; furthermore, inoculum placed deeper than 20 cm had no detrimental effect on pea growth and development up to the time of flowering (15). In the present study, carnation wilt was caused by inoculum placed at all depths tested. The more deeply the inoculum was placed, the later the wilt symptoms appeared and the lower the final disease incidence. The appearance of disease symptoms when inoculum was placed in the deepest layer may suggest that wilt is induced in susceptible carnation cultivars by *F. o. dianthi* propagules, even at depths exceeding 60 cm. Build-up of inoculum during one growing cycle of carnations was indicated by the 10- to 100-fold increase in the number of propagules and by their distribution in the upper 60 cm when inoculum was placed at a depth of 60–75 cm prior to planting. These findings may explain the need to fumigate soil before new cuttings of susceptible cultivars are planted.

In many greenhouses in Israel, carnations have been grown as a monoculture for more than 10–15 yr, and growers fumigate the soil every year before planting. Since carnation has a high cash crop value, it is important that yield losses be kept to a minimum. Threshold levels of fungal propagules should be established chiefly on the basis of economic criteria, namely, the

yield losses that can be tolerated by growers. Also, in establishing such thresholds, one has to decide the depths at which soil samples should be taken. In crops grown under intensive monoculture, such as carnation, where disease can be incited by inoculum at a very low density and as deep as 60 cm, establishment of threshold levels seems impractical. In a recent study, varying degrees of wilt incidence were observed in susceptible carnation cultivars even though fumigation prior to planting had reduced the amount of propagules in the upper 60 cm to below the limits of detection (3). Failure to reduce wilt disease in these flowers might be caused by an accumulation of inoculum, via infected roots, at soil depths where fumigation is ineffective. This possibility is supported by the present finding of viable *F. o. dianthi* propagules to a depth of 120 cm after soil fumigation in commercial greenhouses for carnation monoculture.

Since fumigation alone does not adequately reduce *Fusarium*-induced yield losses in susceptible carnation cultivars, growers should be advised to use cultivars with greater degrees of resistance to the pathogen. These cultivars should be recommended, even though the resistant cultivars are less profitable when the pathogen is not present. Use of resistant cultivars results in smaller yield losses and less build-up of inoculum (3) and thus has the added advantage of allowing growers to reduce the amount of chemicals necessary for control of the pathogen prior to planting.

LITERATURE CITED

1. Ashworth, L. J., Jr., Huisman, O. C., Harper, D. M., and Stromberg, L. K. 1979. Verticillium wilt disease of tomato: Influence of inoculum density and root extension upon disease severity. Phytopathology 69:490-492.
2. Ben-Yephet, Y., Melero, J. M., and DeVay, D. E. 1988. Interaction of soil solarization and metham-sodium in the destruction of *Verticillium dahliae* and *Fusarium oxysporum* f. sp. *vasinfectum*. Crop Prot. 7:327-331.
3. Ben-Yephet, Y., Reuven, M., Szmulewich, Y., and Mor, Y. 1994. Effect of methyl bromide on control of *Fusarium oxysporum* f. sp. *dianthi* propagules in carnation greenhouse soil and on inoculum increase after growth cycle of carnation. Crop Prot. 13:357-361.
4. Elmer, W. H., and Lacy, M. L. 1987. Effects of inoculum densities of *Fusarium oxysporum* f. sp. *apii* in organic soil on disease expression in celery. Plant Dis. 71:1086-1089.
5. Farley, J. D., Hubbeing, N., and Jaberg, C. 1974. Vertical distribution of *Fusarium oxysporum* f. sp. *lycopersici* race 2 in greenhouse soil. Plant Dis. Rep. 58:320-321.
6. Grogan, R. G., Ioannou, N., Schneider, R. W., Sall, M. A., and Kimble, K. A. 1979. Verticillium wilt on resistant tomato cultivars in California: Virulence of isolates from plants and soil and relationship of inoculum density to disease incidence. Phytopathology 69:1176-1180.
7. Harris, A. R., and Ferris, H. 1991. Interactions between *Fusarium oxysporum* f. sp. *tracheiphilum* and *Meloidogyne* spp. in *Vigna unguiculata*. I. Effects of different inoculum densities on Fusarium wilt. Plant Pathol. 40:445-456.
8. Katan, J. 1968. Studies on the life cycle and multiplication in soil of the fungus *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder and Hansen, the incitant of the wilt disease in tomatoes. Ph.D. thesis. Hebrew University of Jerusalem, Rehovot.
9. Marois, J. J., and Mitchell, D. J. 1981. Effects of fumigation and fungal antagonists on the relationships of inoculum density to infection incidence and disease severity in Fusarium crown rot of tomato. Phytopathology 71:167-170.
10. Nash, S. M., and Snyder, W. C. 1962. Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. Phytopathology 52:567-572.
11. Nicot, P. C., and Rouse, D. I. 1987. Relationship between soil inoculum density of *Verticillium dahliae* and systemic colonization of potato stems in commercial fields over time. Phytopathology 77:1346-1355.
12. Orlikowski, L. 1978. Population density of *Fusarium oxysporum* f. sp. *dianthi* in relation to the growth of carnations. Pruce Inst. Sadownictwa Kwiaciarstwa Skierniewicach B3:143-150.
13. Orlikowski, L., and Dzieciol, R. 1977. Population of Fusarium wilt fungus at carnation growing sites. Acta Hortic. 71:123-125.
14. Reuven, M., Nitzani, Y., Mor, Y., Szmulewich, Y., and Ben-Yephet, Y. 1992. Distribution of *Fusarium oxysporum* and *Fusarium oxysporum* f. sp. *dianthi* inoculum in carnation greenhouses. Acta Hortic. 307:51-56.
15. Rush, C. M., and Kraft, J.M. 1986. Effects of inoculum density and placement on Fusarium root rot of peas. Phytopathology 76:1325-1329.
16. SAS Institute. 1989. SAS User's Guide: Statistics. Version 6. 4th ed. SAS Institute, Cary, NC.
17. Scovel, G. 1987. Improved agrotechnical and sanitation methods versus resistant cultivars as a means of avoiding Fusarium wilt. Acta Hortic. 216:55-61.