Neth. J. Pl. Path. 99 (1993) 3–12

Response of carnation cultivars to Fusarium oxysporum f.sp. dianthi in the field

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Accepted 23 September 1992

Abstract

The response of carnation (*Dianthus caryophyllus* L.) cultivars to *Fusarium oxysporum* f.sp. *dianthi (F.o.d.)* was evaluated in an artificially infested field from 1988/9 to 1991/2. Disease incidence was highly correlated with disease severity, indicating that disease incidence may be used to estimate the impact of *F.o.d.* on the host. Based on the results, the following stepwise procedure was developed for characterizing the response of carnation cultivars to *F.o.d.* First, the general response of the tested cultivar was classified as resistant or susceptible on the basis of disease incidence values recorded 180 days after planting. Empirical analysis of the data revealed that a disease incidence level of 75% may be taken as a reliable cut-off point for separation of cultivars into the two groups. Within each group, cultivars were then subjected to a more explicit classification: in the resistant group the records of actual disease incidence were used for classification, while in the susceptible group the linear rearrangement of the disease progress curve was calculated according to the Gompertz function, and the value of the intercept was used for classification.

Additional keywords: Dianthus caryophyllus, disease assessment, host resistance.

Introduction

Severe epidemics of wilt disease caused by *Fusarium oxysporum* f.sp. *dianthi* (Prill. and Del.) Snyd. and Hans. in carnation (*Dianthus caryophyllus* L.) in Israel prompted a search for development of cultivars resistant to the pathogen. Preliminary goals of that activity are the development of methods for inoculation of the tested cultivars and establishment of procedures for classifying their response to infection.

Several methods have been proposed for inoculation of carnation cultivars with *F. oxysporum* f.sp. *dianthi (F.o.d.)*. Root-dip inoculation in a spore suspension (Hood and Stewart, 1957; Garibaldi, 1978; Matthew and Arthur, 1978), stem inoculation with spore suspension (Baayen and Schrama, 1990), and inoculation of the substrate with spores before (Tramier et al., 1983) or after planting (Sparnaaij and Demmink, 1977) were all reported to result in adequate infections. In a previous study we observed that the response of carnation cultivars in greenhouse tests did not necessarily coincide with their response in the field (Ben-Yephet et al., 1992b).

The stage following inoculation is the establishment of procedures for classifying the

Contribution No. 3539-E series, from the Agricultural Research Organization

response to infection of the tested cultivars. The response of host plants to pathogens may be evaluated in several ways. Disease severity (i.e., the quantity of disease affected entities within a sampling unit) is often the measure of choice because it usually reflects the impact of infection on the host plant. On the other hand, disease incidence (i.e., the proportion of diseased entities within a sampling unit) can be more quickly and easily measured (James, 1974). Thus, any quantifiable relationship between these two measures would permit estimation of severity on the basis of incidence data. A simple correlation between disease incidence and disease severity is likely when the disease is systemic or affects the entire measured entity, as in the case of wilt diseases (Seem, 1984).

The purposes of this study were: (i) to examine the possibility of using disease incidence as an estimation of disease severity in the carnation–*F.o.d.* pathosystem and (ii) to develop a method for classifying the response of carnation cultivars to *F.o.d.*

Material and methods

Field infestation. The response of carnation cultivars to *F.o.d.* was evaluated in an artificially infested field. A 0.1-ha field with sandy soil was fumigated with methyl bromide (60 g/m^2) prior to infestation. Carnation plants infected with fusarium wilt were collected during March 1988 from commercial greenhouses in various parts of the country to ensure a wide-ranging collection of isolates and pathotypes existing in Israel. The *F.o.d.* pathotypes reported in Israel so far are the 2 and 4 (Ben-Yephet et al., 1992a). Isolates obtained from the wilted plants were of pathotype 2. The wilted plants were ground to a powder, which was spread on the soil furface in mid-May, 1988. The soil was disked to a depth of 15–20 cm, irrigated twice a week for one month and then planted with carnation cultivars to be tested. In subsequent growing seasons the soil in the experimental site was not subjected to further infestation, since examination of the soil each season before planting, according to Ben-Yephet et al. (1992b), revealed a high level of inoculum.

Experimental design. The infested field was divided into eight blocks (replicates), each containing about 40 experimental plots. Each plot consisted of a bed 1 m wide and 1 m long, with 32 rooted stem cuttings planted in four rows. About 40 carnation cultivars supplied by breeders in Israel and Europe were planted in the field in mid-June of each of the four growing seasons of the study (1988/9–1991/2). In addition to the new cultivars tested each year, seven reference cultivars with known levels of resistance were planted each season. These were the cultivars Eveline, Candy, Galit, Aviv, Lior, Raggio-di-Sole and Fantasia. Prior to planting, stem segments from 20 cuttings of each of the cultivars to be tested were examined for possible contamination with *F.o.d.* Segments were plated in Petri dishes on potato dextrose agar supplemented with 250 mg dihydrostreptomycin (PDS). Cultures resembling *F. oxysporum* were evaluated for pathogenicity by inoculation on carnation plants (cv. Fantasia). During the growing season, cultural practices were those recommended in Israel to commercial carnation growers.

Disease assessment. Disease incidence was recorded at biweekly intervals, from mid-September to mid-December (85–180 days after planting). Disease severity was recorded for all the tested cultivars on the last assessment date of the 1990/1 and 1991/2 seasons. In addition, disease severity was recorded in 14 representative cultivars four times through-

Fig. 1. Rating scale for evaluation of the response of carnation cultivars to *Fusarium oxysporum* f.sp. *dianthi*. 0 = healthy; 4 = completely wilted. Plants (right to left) have a rating of 0, 1, 2, 3, and 4, respectively.

out the 1991/2 growing season. Disease severity was determined as follows. First, the response of each of the plants to *F.o.d.* in the experimental plots was evaluated visually with the aid of a pictorial scale (Fig. 1), according to five categories (ranks) from 0 (healthy) to 4 (completely wilted). The number of plants in each category was then multiplied by the appropriate rank, the products were summed, and the outcome divided by the number of diseased plants in the sample. This procedure yielded a score from 0 to 4, reflecting the quantity of disease affecting entities within the sampling units (i.e., disease severity (Seem, 1984)).

Data analysis. Incidence–severity relationships were determined for data recorded in the 1990/1 and 1991/2 seasons. Scrutiny of plots of the incidence–severity data revealed that the variance was not stabilized across the entire data set, and a square root transformation was therefore used in order to linearize the relationship (Federer, 1955). A similar procedure was employed by Seem and Gilpatrik (1980) in the generation of incidence–severity relationship for powdery mildew on apple.

Data recorded in the field were used to evaluate the responses of the different carnation cultivars to *F.o.d.* A curve of disease progress over time was obtained by fitting of a Gompertz function to incidence values from each of the cultivars. This function was chosen because it provided the highest significant regression equations (as indicated by the P values), the highest values of the coefficient of determination (r^2) and the lowest values of error variance (MSE), compared to those achieved by the logistic or the monomolecular functions. The linear rearrangement of the Gompertz function was used for curve fitting. Disease incidence at the last assessment date was used as an estimation of the asymptotes for the Gompertz function because we did not have enough data points to estimate this parameter empirically. The intercept and the slope of the linear rearrangement of the Gompertz function were used as indicators of the response of diferent cultivars to *F.o.d.* These parameters will be referred to hereafter as the 'intercept' and the 'slope'. The units of the Gompertz function are Gompit. In the regression analysis, time (in terms of days after September 15, 85 days after planting) was the independent variable and disease incidence (in terms of Gompit values) was the dependent variable.

For some analyses, the relative area under the disease progress curve (RAUDPC) was calculated as described by Shtienberg et al. (1990). RAUDPC value integrates the response of a given carnation cultivar to *F.o.d.* because its calculation is based upon disease ratings throughout the entire growing season.

Results

During the four seasons of the study, all cultivars supplied by nurseries were free of *F.o.d.* The numbers of *F.o.d.* propagules counted in the field before planting were 3500, 5500, 6200 and 8800 per g air-dried soil in the 1988/9, 1989/90, 1990/1 and 1991/2 seasons, respectively.

Incidence–severity relationship. Disease incidence and disease severity recorded 180 days after planting were strongly correlated. Since the values of the regression coefficients calculated separately for the 1990/1 and 1991/2 seasons did not differ significantly ($P = 0.05$), data for the two seasons were pooled (Fig. 2). The correlation between disease incidence and disease severity estimates recorded at different times during the 1991/2 growing season was highly significant as well (Fig. 3). Consequently, for further analyses, disease incidence was used as an indicator of the response of carnation cultivars *F.o.d.*

Development of a method for classification of carnation response. In general, the response of the reference cultivars varied only slightly among the four growing seasons of the study. As an example, the disease incidence values 180 days after planting in the suc-

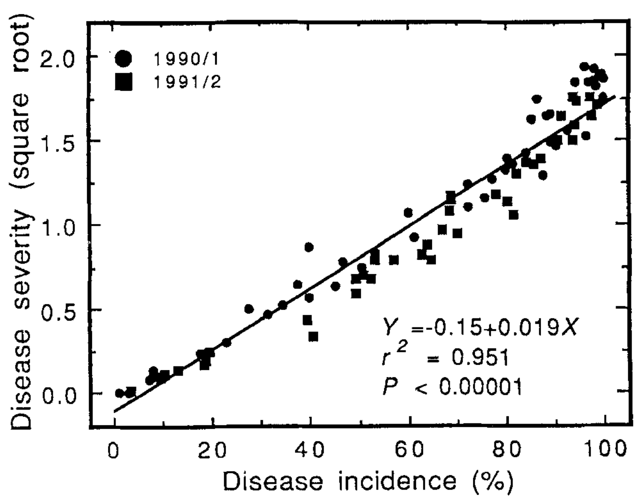

Fig. 2. Relationship between disease incidence and disease severity for the carnation – *Fusarium oxysporum* f.sp. *dianthi* pathosystem. Incidence and severity were recorded 180 days after planting.

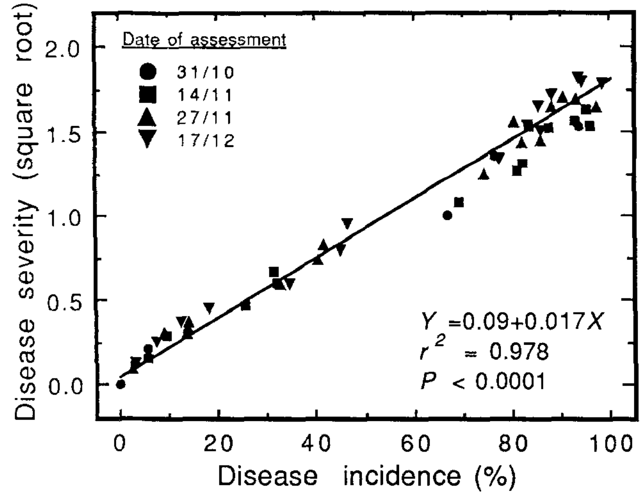

Fig. 3. Relationship between disease incidence and disease severity for the carnation – *Fusarium oxysporum* f.sp. *dianthi* pathosystem. Incidence and severity were recorded four times during the 1991/2 growing season.

cessive growing seasons were 6.6, 7.1, 7.8 and 3.5% for cv. Eveline, 41.1, 47.8, 39.8 and 49.6% for cv. Galit, and 99.2, 99.6, 96.5 and 97.3% for cv. Fantasia. The standard deviation values obtained for the disease incidence levels of the reference cultivars were relatively low, ranging from 1.5 to 4.8%

The different carnation cultivars showed considerable variation in their response to *F.o.d.* Disease progress for the reference cultivars in the 1991/2 season is presented in Fig. 4. Cultivar Eveline maintained relatively low incidence values throughout the entire growing season (Fig. 4A), corresponding to a low intercept and a modest slope (Fig. 4B). Disease incidence in cvs Candy, Galit and Aviv was initially low but gradually increased later, corresponding to a low intercept but progressively steeper slopes than that of cv. Eveline. Cultivars Raggio-di-Sole and Fantasia exhibited relatively high incidence levels even on the early disease assessment dates, corresponding to high intercept values. The shape of the curve obtained for cv. Lior was slightly different from that of the other cultivars: the disease incidence in this cultivar was low on the early assessment dates, but subsequent rate of disease progress was relatively high, corresponding to a low intercept but a relatively steep slope (Fig. 4).

In an attempt to develop criteria for classification of the response of carnation cultivars to *F.o.d.*, the relationships between RAUDPC and the coefficients of the linear rearrangement of the Gompertz function for the disease progress curves (i.e., the intercept and the slope) were explored. For cultivars in which the disease incidence 180 days after planting was lower than 75%, RAUDPC values were strongly correlated with the slope (Fig. 5). On the other hand, when disease incidence 180 days after planting exceeded 75%, the coincidence between these variables was poor. The relationship between RAUDPC values and the intercept was antonymous: the correlation was insignificant for a disease incidence of up to 75% and highly significant above 75% (Fig. 6). The value of 75% incidence was thus established empirically as the point at which the relationship between RAUDPC values and the regression coefficients changed. Analyses of the results of the other three

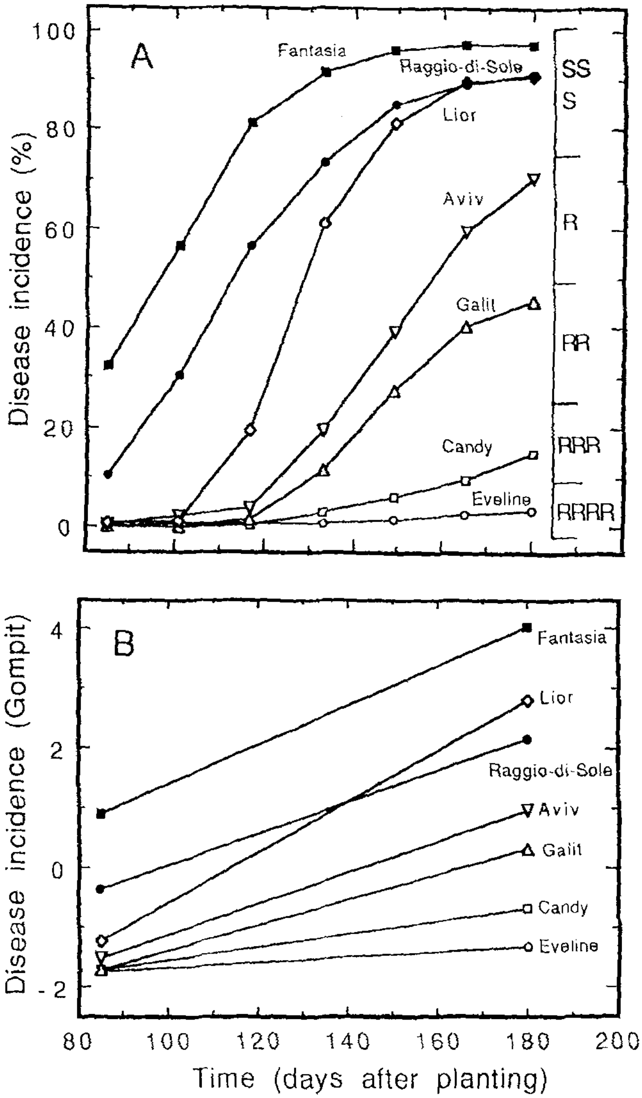

Fig. 4. Progress curves of *Fusarium oxysporum* f.sp. *dianthi* for seven carnation cultivars in the 1991/2 season. (A) Disease incidence; (B) linear rearrangement of the disease progress curve according to the Gompertz function. Letters (R-RRRR and S-SS) and ranges classify the response of the different cultivars to the disease. Host response was classified as highly resistant (RRRR); resistant (RRR); moderately resistant (RR); low resistant (R); susceptible (S) or highly susceptible (SS).

seasons showed trends similar to those presented in Figs 5 and 6.

Based on the above, the following stepwise procedure was developed for classifying the response of carnation cultivars to *F.o.d.* First, the general response of the tested cultivar was classified as resistant (if disease incidence 180 days after planting was lower than 75%) or susceptible (if disease incidence at that time exceeded 75%). A more explicit classification within each of these two groups was then determined. In the resistant group,

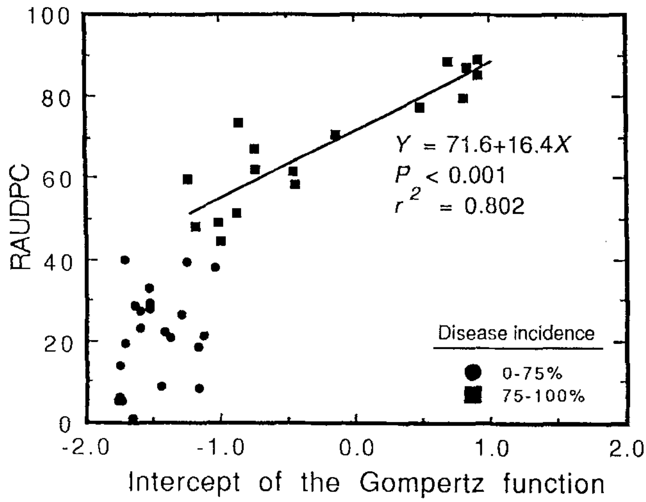

Fig. 5. Relationship between the relative area under the disease progress curve (RAUDPC) and the intercept of the linear rearrangement of the disease progress curve according to the Gompertz function. Disease data were recorded for different carnation cultivars in the 1991/2 growing season. The data are presented separately for cultivars in which disease incidence 180 days after planting was lower than 75% and cultivars in which it exceeded 75%.

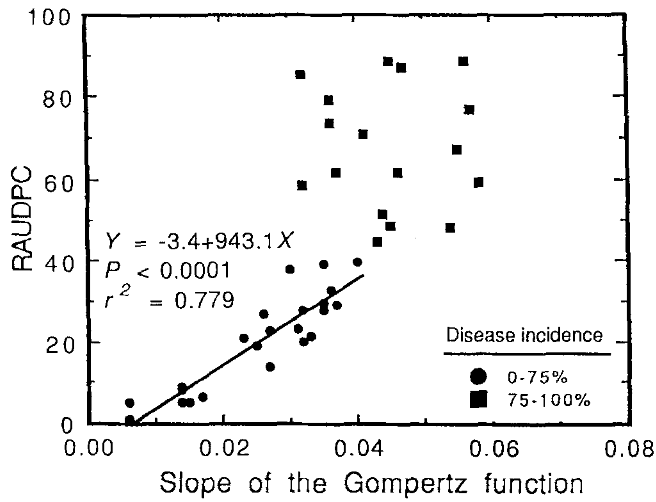

Fig. 6. Relationship between the relative area under the disease progress curve (RAUDPC) and the slope of the linear rearrangement of the disease progress curve according to the Gompertz function. Disease data were recorded for different carnation cultivars in the 1991/2 growing season. The data are presented separately for cultivars in which disease incidence 180 days after planting was lower than 75% and cultivars in which it exceeded 75%.

the records of actual disease incidence 180 days after planting were used for classification. In the susceptible group, classification was based upon the value of the intercept of the linear rearrangement of the disease progress curve. As a simple and useful identification sign, a symbol was designated for each of the classes, i.e., R-RRRR and S-SS (Table 1 and Fig. 4).

Table 1. Criteria for classification of the response of carnation cultivars to *Fusarium oxysporum* f.sp. *dianthi*.

Disease incidence (%)[a]	Intercept[b]	Classification	Classification symbol
0.0– 10.0		Highly resistant	RRRR
10.1– 25.0		Resistant	RRR
25.1– 50.0		Moderately resistant	RR
50.1– 75.0		Low resistant	R
75.1–100.0	< –1	Susceptible	S
75.1–100.0	–1 <	Highly susceptible	SS

[a] Recorded 180 days after planting.
[b] The intercept of the linear rearrangement of the disease progress curve according to the Gompertz function.

Discussion

The linear relationship between disease incidence and disease severity observed for the carnation–*F.o.d.* pathosystem (Figs 2 and 3) permitted the use of disease incidence records as a means of estimating the impact of the disease on the host. Disease incidence is not only quicker and easier to measure than disease severity, but its criteria are also more accurate and reproducible (Horsfall and Cowling, 1978).

A variety of methods have been used by a different authors for classifying the response of carnation cultivars to *F.o.d.* In principle, all methods are similar in that the response is ranked, according to certain criteria, into categories. The criteria for ranking vary slightly among the different studies, but all of them provide an estimation of disease intensity. The number of categories used for ranking varies in the different studies: some authors use four (Garibaldi, 1983; Blanc, 1983), while others use five (Vigodski-Hass et al., 1988) or six (Sparnaaij and Demmink, 1977).

The methods used for classification suffer from several drawbacks. In some cases, the distinction between two consecutive categories is determined on an arbitrary basis, without necessarily reflecting any biological meaning. For example, classifying the response of cultivars according to the proportion of plants that were completely wilted 130 days after planting in an infected substrate, Blanc (1983) defined four categories: 0–5%, very resistant; 5–15%, resistant; 15–30%, tolerant; and 30–50%, fairly tolerant. According to this scale, a cultivar with 14.9% wilted plants would be regarded as resistant, whereas one with 15.1% wilted plants would be considered tolerant. The various methods also have other drawbacks: in most studies host response was evaluated only once; artificial inoculation of young seedlings (if employed) may produce inaccurate results (Baayen and DeMaat, 1987; Tramier et al., 1987; Harling et al., 1988) and the response of a given cultivar in a particular test may not exclusively reflect its genetic characteristics. Additional factors such as the environment, the inoculum level, the type of growing substrate, etc. may also affect the results. This method for classification has, however, an important advantage in that it is simple, and allows the response of a given cultivar to be identified by means of an easily understood symbol (e.g., R = resistant; S = susceptible).

In an attempt to overcome the drawbacks mentioned above, while still retaining the advantage of simplicity, the following method of classification is proposed. The response of a given carnation cultivar to *F.o.d.* should be classified according to the simple identification symbol. In addition, the actual assessed value and its position in relation to the values of the nearest reference cultivars should be indicated. The following are examples of classification based on the criteria used in our study: In 1991/2, the response of cv. Splendid to *F.o.d.* was: 'RRR–18.4%–between Candy and Galit'. The response of cv. Snow-bird was: 'SS–94.1%–between Lior and Fantasia'.

During the four years of this study it was repeatedly observed that the response of certain carnation cultivars to *F.o.d.* was noticeably different from that of most of the others. A representative cultivar of this group is Lior. Cultivars of this group exhibited a low disease incidence initially, but supported a relatively high rate of disease progress (Fig. 4). Cultivars with this type of response were classified as a unique and separate group (S, Table 1). The physiological mechanisms and the practical consequences of this type of response are still unknown, and are currently under investigation.

Acknowledgements

The technical assistance of Y. Stzmulewich, M. Lampel and Y. Nitzani is gratefully acknowledged.

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