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# Inheritance of resistance in carnation against Fusarium oxysporum f.sp. dianthi races 1 and 2, in relation to resistance components

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**Abstract**

Four carnation cultivars, Novada (resistant to races 1 and 2 of *Fusarium oxysporum* f.sp. *dianthi*), Elsy (susceptible to race 1), Lena (susceptible to race 2) and Sam's Pride (susceptible to both races), were selfed and crossed. When three months old, the seedlings were inoculated via the roots or via the stems, after which wilting was recorded weekly according to a 5-point ordinal scale.

Analyses were carried out on the proportions of diseased plants. For race 1 variation between the progenies could be described by means of general combining abilities only; GCA values were not affected by the inoculation method used. Also for race 2 GCAs were most important but the GCA values appeared different for the two inoculation methods. It is concluded that resistance to both races is inherited in an additive way.

Indications for independently inherited root-specific resistance components (extravascular resistance) were only found with race 2. With both races, the ability to confine the pathogen at the infection site appeared the most important resistance component. Resistant progenies were also characterized by longer latent periods and lower wilting rates.

Both race 1 and race 2 induced the accumulation of the phytoalexins dianthalexin and methoxydianthramide S, but race 2 induced higher amounts than race 1. The accumulation of phytoalexins was positively correlated to the resistance level of the progenies against the respective races. The progenies of the double-resistant cultivar Novada appeared to produce particularly high levels of phytoalexins.

*Additional keywords*: *Dianthus caryophyllus*, additive resistance, localization ability, latent period, wilting rate, phytoalexins.

## Introduction

Vascular wilt diseases cause serious problems in carnation (*Dianthus caryophyllus* L.) growing areas all over the world. In particular Phialophora wilt and Fusarium wilt have caused major losses and are a constant threat to the culture of the world's major

flower crop. Breeding resistant varieties takes an important place in the control of these diseases. Resistance to both diseases is partial but in some cultivars of a very high level. The inheritance of resistance to *Phialophora cinerescens* (Wollenw.) Van Beyma is mainly additive; the resistance is probably polygenic and at least governed by two different gene pairs (Sparnaaij and Demmink, 1976). Resistance to the common race 2 of *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen f.sp. *dianthi* (Prill. & Del.) Snyder & Hansen is assumed to be polygenic and additive as well (Sparnaaij and Demmink, 1977 and unpublished), but this is not yet fully proved. In a recent survey of nine cultivars, resistance to race 1 appeared to be either complete or totally absent; intermediate resistance levels were not found (Demmink et al., 1989). Moreover, susceptibility to race 1 is less common than resistance (Garibaldi, 1983). It was therefore suggested that resistance to race 1 might be monogenic and dominant, which hypothesis is tested in the present study. Support for such a separate position is also found in the pathogenesis of cultivars susceptible to race 1, which proved quite different from that seen with race 2 (Baayen et al., 1988). Demmink et al. (1989) even raised the question whether race 1 could not be a distinct forma specialis instead of a physiologic race of *F. oxysporum* f.sp. *dianthi*.

Investigations on the inheritance of resistance in carnation to Fusarium wilt are a complex matter. Carnation is a highly heterozygous, naturally cross-pollinated but in commercial practice vegetatively propagated crop. Segregation analyses must therefore be carried out directly in crosses and selfings of commercial varieties, instead of in the F2 generations used in self-pollinated crops in which homozygous parental lines are investigated. However, the resistance level of individual carnation seedlings may only be known by clonal testing of each of the seedlings, because plants of a highly resistant genotype may develop a fully susceptible phenotype, and vice versa. Given the numbers of plants (50-100) needed for clonal testing of one single seedling, classical segregation analyses for determination of the number of genes involved in resistance are virtually impossible to perform. The mode of inheritance may nevertheless be studied in a diallel as presently carried out with four parents with large differences in resistance to races 1 and 2. Such an approach also allows for testing the hypothesis that resistance to race 1 is monogenic and dominant instead of additive as expected for resistance to race 2.

Resistance of carnation to Fusarium wilt may operate at various stages of the infection process. Inhibition or retardation of fungal growth may occur when the fungus attempts to penetrate the epidermis, cortex or endodermis of the roots (extravascular resistance), but also in the xylem of roots and stem (vascular resistance). In the xylem, resistance generally involves confinement of the invading fungus at the infection site by means of physical and chemical barriers such as the formation of vessel-occluding gums and the accumulation of fungistatic compounds (Baayen, 1988; Niemann and Baayen, 1988). The localization process in the xylem is similar in roots and stems and has been thoroughly studied (Baayen, 1988; Baayen et al., 1989). When inhibition of fungal growth fails, the plant may retard fungal growth and wilting may be slowed down. Tolerance to colonization appears not to be part of the resistance mechanism (Baayen, 1988).

The second aim of the present study was to analyze the contribution of the above components to the overall resistance level and its inheritance. The relative contribution

of extravascular resistance components to the overall resistance level of progenies may be investigated by comparing the effects of different inoculation procedures (stem or root inoculation) on the proportion of diseased plants. Progenies may be compared for localization ability by evaluation of the proportions of stem-inoculated plants that succeed in localizing the pathogen; such plants remain healthy until the end of the experiment when the number of visibly diseased plants does not increase any more. Contrary to root inoculation, stem inoculation is a one-time event leading either to colonization and death of the host, or to localization of the pathogen; the fate of the plants is determined in the first week after inoculation (Baayen, 1988 and unpublished) and the proportion of diseased plants remains constant thereafter. Possible differences among the progenies in the rate of wilting may be studied by monitoring the wilting process in those plants which fail to localize the pathogen.

Accumulation of phytoalexins may enhance the localization ability of plants as well as slow down the progress of colonization after a localization failure. The accumulation of two major phytoalexins of carnation, dianthalexin and methoxydianthramide S (Niemann and Baayen, 1988; Ponchet et al., 1988) was therefore also assayed.

## Materials and methods

*Plant material.* Four cultivars were selected with large differences in resistance to races 1 and 2 of *F. oxysporum* f.sp. *dianthi*: 'Novada', a spray carnation from the CPO breeding programme resistant to both races; 'Elsy', a mediterranean spray, susceptible to race 1 but moderately resistant to race 2; 'Lena', a standard (Sim) carnation resistant to race 1 but susceptible to race 2, and 'Sam's Pride', an American spray carnation susceptible to both races. The four parents were selfed and intercrossed at the CPO as shown in Table 1. Over 150 seeds from each cross were sown in sand trays in June, 1988. The seedlings were transplanted 5 weeks after sowing to single 8 cm diam pots with steamed soil and were further grown in a conditioned greenhouse at the WCS (temperature 20-25 (rarely 30) °C, additional light being provided during autumn and winter) until the end of the experiment (December, 1988).

*Preparation of inoculum and inoculation of plants.* Isolates WCS 827 (race 1, originally received from prof. A. Garibaldi, Turin, Italy) and WCS 816 (race 2, from the Netherlands) were cultured in Czapek Dox liquid medium (Oxoid) on a reciprocal shaker for 8 days at room temperature, after which the cultures were filtered through

Table 1. Ten crosses between carnation cultivars Elsy, Lena, Novada and Sam's Pride of which the progenies were evaluated for disease development and phytoalexin accumulation.

| ♂ | ♀ | | | |
|---|---|---|---|---|
| | 'Elsy' | 'Lena' | 'Novada' | 'Sam's Pride' |
| 'Elsy' | + | | | |
| 'Lena' | + | + | | |
| 'Novada' | + | + | + | |
| 'Sam's Pride' | + | + | + | + |

sterile glasswool. The resulting conidial suspensions were washed by centrifugation and adjusted to a concentration of $10^7$ conidia $ml^{-1}$. Two months after transplanting (i.e., September 1988), the seedlings were root- or stem-inoculated with either race 1 or race 2. Of each progeny of ca. 150 seedlings, 22 plants were left untreated while 128 plants were inoculated, of which 32 were stem-inoculated with race 1, 32 were stem-inoculated with race 2, 32 were root-inoculated with race 1 and 32 were root-inoculated with race 2. Of each set of 32 inoculated plants 20 were used for observation of disease development and 12 for extraction of phytoalexins. Root inoculation was accomplished by pouring 3 ml conidial suspension per plant on the soil. Stem inoculation ($1 \times 20$ $\mu$l per plant) was performed according to the method given by Baayen and Elgersma (1985) on plants which had been kept dry for three days. The experiments were carried out under quarantine conditions.

For the observations on disease development, 20 inoculated seedlings of each combination of progeny, inoculation method and race were placed on a greenhouse table in 10 replicates. Each replicate contained the four combinations of inoculation method and race, applied to two plants per progeny. Different treatments were separated by transparent plastic foil underneath and in between the plots (up to 10 cm above the soil level) in order to prevent cross-infections during watering. Watering was carried out by hand with care to avoid splashing. Also ten untreated controls per progeny were placed on the same greenhouse table.

For analysis of phytoalexin accumulation, the remaining 12 seedlings per progeny, inoculation method and race not used for the main experiment were placed in three replicates in an adjacent compartment of the greenhouse under the same conditions as in the parallel experiment described above. Each replicate comprised four main plots for the four treatments, each with ten subplots of four plants for the ten progenies, as well as a fifth main plot with untreated controls. The main (treatment) plots were again separated by foil and watered by hand. The stem-inoculated plants and the controls were harvested after seven days and extracted; the root-inoculated plants were used for comparison of the disease development with the parallel experiment.

*Observations on disease development.* The development of Fusarium wilt symptoms was recorded for all plants individually, using the following scale: 0. no disease symptoms; 1. very slight disease symptoms; 2. limited local symptoms; 3. well-developed symptoms on otherwise still healthy-looking plants; 4. severe wilt and 5. complete wilt and death. Wilting was recorded from the first appearance of diseased plants 23, 28 and 31 days, and 5, 6, 7, 8, 9, 10, 11 and 13 weeks after inoculation. The experiment was terminated when the number of stem-inoculated plants with disease symptoms hardly increased any more. A limited number of plants (15) succumbed to foot-rot diseases caused by other fungi and were omitted from the calculations.

*Statistical analysis.* Analyses were carried out on the proportions of diseased plants (indices 1 to 5)(healthy ones have index 0), recorded 7 and 13 weeks after inoculation. As pathogenesis is different for races 1 and 2, data of the two races were treated separately. The proportions of diseased plants were analyzed according to a generalized linear model for binary data (McCullagh and Nelder, 1989). The assumption is made that the proportions of diseased plants are distributed according to independent binomial distributions. For analysing the inheritance of resistance the logit of the

expected proportion of diseased plants $p_{ij}$ of the offspring of parent $i$ and parent $j$ is written as (Bulmer, 1985; p. 118)

$$\text{logit}\,(p_{ij}) = \mu + \tfrac{1}{2}g_i + \tfrac{1}{2}g_j + s_{ij} + \bar{d} + d_i + d_j\,(i = 1,2\ldots4) \qquad [1]$$

which may be written for selfings as

$$\text{logit}\,(p_{ii}) = \mu + q_i \qquad [2]$$

The logit is the inverse of the distribution function of the standard logistic distribution. It is used to remove non- additivity inherent to binary data and without genetical background. In [1] $\mu$ represents the grand mean, $g_i$ and $g_j$ are the general combining abilities (GCA's) of parents $i$ and $j$, $s_{ij}$ is the specific combining ability (SCA) of parents $i$ and $j$ and $\bar{d}$, $d_i$ and $d_j$ are called average and variety heterosis (summarized in Table 2 under Deviations). GCA's represent main (additive) effects of inheritance; SCA's represent the interactions (complementary effects). It should be noted that these parameters refer to the logit scale. In the analysis also the effect of the inoculation methods is considered. Results are summarized in analysis of deviance tables; for the testing of significance of effects deviances are compared with values from tables of the Chi-square distribution.

In order to study the overall effects of the four inoculation methods on the development of the disease, the time needed for plants to reach categories 1, 2, 3, 4 and 5 was analyzed. In this analysis no distinction was made between progenies, and only the results of those plants were used which developed any disease symptoms in the course of the experiment. Log-normal distributions were fitted to the data using the procedure 'cumdistribution' of Genstat (Payne et al., 1987).

Additionally, the mean lapse of time was determined for all progenies separately to the appearance of the first wilt symptoms (the latent period) and the mean period between the appearance of symptoms of index 1 and symptoms of index 3 (hereafter called the wilting period, a measure for the wilting rate of affected plants). In the latter calculations, the few diseased plants which had not yet reached index 3 at 13 weeks after inoculation were assumed to do so 2 weeks later. Correlation coefficients between the numbers of affected plants, latent period and wilting period were calculated for each method and race used, following standard procedures (Moroney, 1951).

*Accumulation of phytoalexins.* Of the stem-inoculated plants which had been selected for this purpose, fifty-millimeter-long stem parts directly above the inoculation site were cut out and processed, as were similarly sized and situated stem parts from the untreated controls. Per replicate and progeny, the segments from all four plants were pooled and extracted together, and extracts were analyzed by HPLC all according to the method described by Niemann and Baayen (1988). Chromatograms were analyzed with a Baseline 810 chromatography workstation. The peak areas in the chromatograms were expressed in (arbitrary) absorption units; for dianthalexin and methoxydianthramide S, 100 units correspond with ca. 3 $\mu$g compound (Baayen and Niemann, 1989). The accumulated amounts of phytoalexins were compared in an analysis of variance (ANOVA). The inheritance of phytoalexin accumulation was investigated as above but now by means of a linear model. The correlation between

the accumulated amounts of phytoalexins with a given race and the number of diseased plants with the same race and inoculation method in the parallel experiment was also determined.

## Results

*Disease development after infection with races 1 and 2.* The wilt symptoms of races 1 and 2 were different. Baayen et al. (1988) and Demmink et al. (1989) reported previously for a limited number of cultivars that race 1 induced pallescence of the leaves, whereas race 2 caused yellowing, of the midribs in particular. Both races caused withering of leaves. The xylem of plants affected by race 1 had a uniform brown colour, whereas the xylem of plants affected by race 2 was white with dark brown margins. Similar observations were made in the present study for the seedling progenies, irrespective of their genetic background.

Analysis of deviance of the proportions of diseased plants (Table 2) led to highly similar results both halfway the experiment and at the final observation. This is not at all self-evident, but may be taken as evidence that the obtained results were highly consistent throughout the experiment.

Highly significant main effects were found for root and stem inoculation, as well as for the progenies. Interactions between inoculation methods and progenies were not statistically significant, indicating that the ranking of the progenies was similar with root and stem inoculation.

A very strong interaction between inoculation methods and time was observed which appeared to be similar for both races (Fig. 1). Stem inoculation was followed by short latent periods, but after the first appearance of wilt symptoms wilting proceeded slowly. Root inoculation led to much longer latent periods (an expected main effect), but wilting proceeded much faster (an unexpected interaction). The faster wilting process is probably due to continuous inoculum pressure from the soil

Table 2. Analysis of deviance of the proportions of diseased carnation seedlings at 7 and at 13 weeks after root or stem inoculation with races 1 and 2 of *F. oxysporum* f.sp. *dianthi*. Df, degrees of freedom; GCA, general combining ability; SCA, specific combining ability. Significance levels: $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***).

| Source of variation | Df | Race 1 | | Race 2 | |
|---|---|---|---|---|---|
| | | 7 weeks | 13 weeks | 7 weeks | 13 weeks |
| Methods | 1 | 54.3*** | 15.2*** | 53.1*** | 23.9*** |
| Progenies | 9 | 168.0*** | 206.3*** | 90.4*** | 73.5*** |
| GCA | 3 | 166.3*** | 201.9*** | 77.6*** | 62.6*** |
| SCA | 2 | 0.8 | 0.8 | 1.2 | 0.4 |
| Deviations | 4 | 0.9 | 3.6 | 11.5* | 10.4* |
| Methods × Progenies | 9 | 6.8 | 10.1 | 14.3 | 14.6 |
| Methods × GCA | 3 | 2.6 | 4.9 | 9.5* | 9.7* |
| Methods × SCA | 2 | 0.8 | 2.2 | 1.0 | 0.7 |
| Methods × Deviations | 4 | 3.4 | 3.0 | 3.8 | 4.2 |

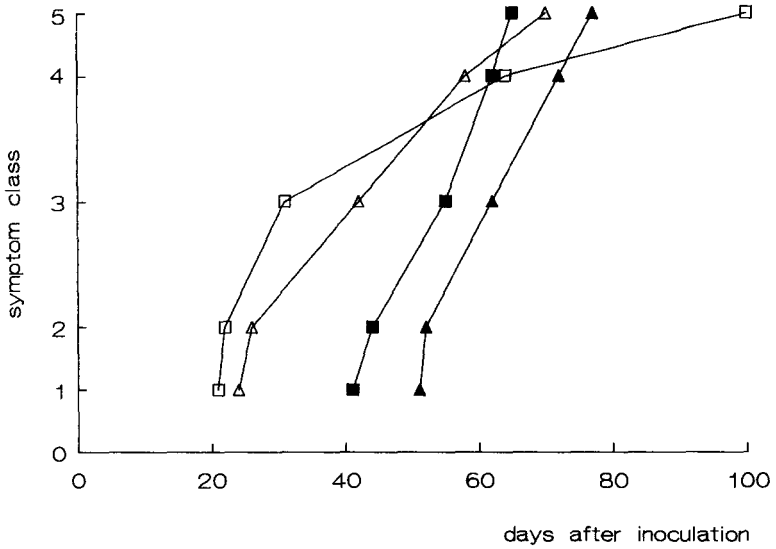


Fig. 1. Course of wilt development after stem (△, □) or root (▲, ■) inoculation with race 1 (△, ▲) or race 2 (□, ■) of *F. oxysporum* f.sp. *dianthi*, calculated as average for the combined nine carnation progenies. The ordinal disease scale on the Y-axis has been adapted in order to give a (subjective) indication of the distances between the classes.

on plants of decreasing health. In general, stem inoculation led to higher proportions of diseased plants than root inoculation.

*Inheritance of resistance.* The analysis of deviance of the proportions of diseased plants (Table 2) revealed that for race 1 the variation between progenies could be described by means of general combining abilities (main effects) only. Three different GCA values were found (Table 3) which were not affected by the inoculation method used. Resistance to race 1 thus appears to be inherited in an additive way. This does not exclude monogenic inheritance, however, if additivity is intragenic (incomplete dominance).

Analysis of the proportions of diseased plants with race 2 revealed that variation between progenies could be largely described by the general combining abilities of the parental cultivars (Table 2). However, the GCA values were different for the two inoculation methods (Table 3), which provides an indication for independent root-specific resistance components against race 2. At least two resistance mechanisms, and hence also two genes therefore seem to be involved. Further analysis indicated that with race 2 the crosses on average showed higher proportions of diseased plants than the selfings; this did not affect GCA values, however.

*Analysis of resistance components.* The absence of method-by- progeny interactions with race 1 (Table 2) indicates that extravascular resistance to race 1 was either too small to be detected, or was strongly correlated with the vascular resistance mechanisms. Indications for independent root-specific resistance components were only

Table 3. GCA values for resistance of the parental carnation cultivars against races 1 and 2, as calculated at 7 and 13 weeks after inoculation from the analysis of deviance of the proportions of diseased plants. GCA values for resistance to race 1 have been calculated from the combined data of stem (SI) and root (RI) inoculation. Values followed by different letters are significantly different at the 5% level (columnwise comparison only); l.s.d., least significant difference.

| Cultivar | Race 1 - SI/RI | | Race 2 - SI | | Race 2 - RI | |
|---|---|---|---|---|---|---|
| | 7 weeks | 13 weeks | 7 weeks | 13 weeks | 7 weeks | 13 weeks |
| Novada | 1.2 a | 1.1 a | 1.4 a | 1.1 a | 0.7 a | 0.8 a |
| Elsy | −0.7 b | −0.8 b | −0.1 b | 0.0 b | 0.5 a | 0.2 a |
| Lena | 1.3 a | 1.4 a | 0.0 b | 0.1 b | −0.4 b | −0.5 b |
| Sam's Pride | −1.8 c | −1.8 c | −1.3 c | −1.2 c | −0.8 b | −0.5 b |
| l.s.d. | 0.63 | 0.59 | 0.71 | 0.69 | 0.67 | 0.59 |

found with race 2. Although the total method-by-progeny interactions were not significantly different from zero, a significant method-by-GCA interaction was found. This indicates that the ranking of the parents for GCA was different for the two methods, although the magnitude of the effect was too small to be recognized in the overall method-by-progeny interaction, even at the 5% level. The presence of independent extravascular resistance components against race 2 therefore remains somewhat questionable. Moreover, ignoring the (questionable) interaction by pooling the two sets of data resulted in GCA values that better correspond to the known resistance levels of the parental cultivars: 'Novada' 1.2 (highly resistant), 'Elsy' 0.8 (moderately resistant), 'Lena' 0.5 (susceptible) and 'Sam's Pride' 0.0 (highly susceptible).

The percentages of stem-inoculated plants still healthy at 13 weeks after inoculation varied with both races from 0% to 95% (Table 4). The large variation among the progenies for this trait suggests that the ability to physically localize the invading fungus at the infection site is an important component of vascular resistance.

The latent periods and wilting periods of those plants of the various progenies which had developed wilt symptoms are given in Table 4. These parameters are of limited value for the resistant progenies because of the low numbers of diseased plants. Notwithstanding this shortcoming, significant correlations were found between latent period, wilting period, and the localization ability of the progenies. Correlations were higher if the time from inoculation to reach index 3 (LP + WP) was considered; for race 2 the correlation coefficients with stem and root inoculation thus were $r = -0.90$ and $r = -0.72$ respectively. The variation between the progenies in latent period (two- to threefold higher values in resistant progenies than in susceptible ones) and wilting rate was smaller than the variation in localization ability, which amounted to a factor 20. This may have been partially due, however, to the limited period of disease observation (13 weeks).

*Accumulation of phytoalexins.* The mean values for the accumulated amounts of phytoalexins are given in Table 5. The values for race 1 are based on a single replicate,

Table 4. Final numbers of diseased carnation seedlings ($n$) in the investigated progenies out of 20 inoculated ones, mean latent periods (LP, in days) and wilting periods (WP, in days) for the diseased plants, and correlation coefficients per race between $n$ (after stem inoculation) and LP or WP. Nov, 'Novada'; Els, 'Elsy'; Len, 'Lena'; Sam, 'Sam's Pride'.

| Progeny | Race 1 | | | | | | Progeny | Race 2 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | stem inoculation | | | root inoculation | | | | stem inoculation | | | root inoculation | | |
| | $n$ | LP | WP | $n$ | LP | WP | | $n$ | LP | WP | $n$ | LP | WP |
| Sam × Sam | 20 | 25 | 8 | 18 | 45 | 6 | Sam × Sam | 19 | 24 | 3 | 14 | 37 | 12 |
| Els × Sam | 20 | 28 | 18 | 15 | 60 | nc | Len × Sam | 19 | 26 | 5 | 16 | 40 | 9 |
| Els × Els | 16 | 32 | 17 | 14 | 64 | nc | Len × Len | 13 | 25 | 5 | 10 | 47 | 6 |
| Len × Sam | 12 | 30 | 17 | 4 | 47 | 4 | Els × Sam | 17[a] | 27 | 10 | 10 | 50 | 21 |
| Nov × Sam | 13[a] | 33 | 28 | 9 | 59 | 9 | Els × Len | 14 | 32 | 9 | 11 | 50 | 12 |
| Els × Len | 7 | 45 | nc | 2 | 53 | 18 | Nov × Sam | 15 | 30 | 11 | 5[a] | 42 | 20 |
| Els × Nov | 6 | 33 | nc | 5 | 67 | nc | Els × Els | 13 | 32 | 22 | 6 | 60 | 13 |
| Len × Nov | 2 | 63 | nc | 2 | 63 | 21 | Len × Nov | 9 | 39 | 16 | 12[a] | 52 | 32 |
| Len × Len | 1 | 49 | 7 | 1[a] | 91 | nc | Els × Nov | 12 | 31 | 10 | 5 | 59 | 10 |
| Nov × Nov | 1[a] | 31 | 60 | 0[c] | nc | nc | Nov × Nov | 3[b] | 61 | 14 | 1[d] | 49 | 28 |
| $r_1$ | | −0.68* | nc | | −0.60* | nc | $r_2$ | | −0.90*** | −0.55* | | −0.47[ns] | −0.57* |

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.

a, b, c and d, numbers out of 19, 18, 17 or 16, respectively, instead of 20 inoculated plants; nc, not calculated due to incomplete data.

$r_1$ and $r_2$, correlation coefficients calculated against the numbers of diseased plants after stem inoculation with race 1 and race 2, respectively.

Table 5. Mean accumulated amounts of dianthalexin and methoxydianthramide S in units absorption per gram fresh material weight of carnation stem segments. Extracts of plants from ten progenies, untreated or harvested 7 days after stem inoculation with races 1 and 2 of *F. oxysporum* f.sp. *dianthi*. Correlation coefficients ($r$) between the amounts of phytoalexins and the final numbers of diseased, stem-inoculated plants with the same race are given below the columns. Abbreviations and significance levels as in Table 4.

| Progeny | Dianthalexin | | | Methoxydianthramide S | | |
|---|---|---|---|---|---|---|
| | race 1 | race 2 | untreated | race 1 | race 2 | untreated |
| Sam × Sam | 120 | 201 | 4 | 11 | 55 | 4 |
| Len × Sam | 246 | 218 | 33 | 19 | 69 | 6 |
| Els × Sam | 193 | 329 | 3 | 19 | 79 | 2 |
| Nov × Sam | 188 | 483 | 4 | 19 | 128 | 2 |
| Len × Len | 205 | 289 | 8 | 28 | 65 | 5 |
| Els × Len | 180 | 305 | 6 | 26 | 63 | 3 |
| Len × Nov | 416 | 420 | 24 | 26 | 118 | 4 |
| Els × Els | 125 | 195 | 14 | 18 | 54 | 8 |
| Els × Nov | 275 | 368 | 4 | 34 | 90 | 6 |
| Nov × Nov | 300 | 933 | 4 | 36 | 347 | 3 |
| $r$ | −0.69* | −0.82** | | −0.86*** | −0.81** | |

as two replicates were lost by an accident during processing. The controls contained only small amounts of phytoalexins and were omitted from the analyses (given in Table 6). Dianthalexin accumulated in higher amounts than methoxydianthramide S. The progenies accumulated higher amounts of the phytoalexins after inoculation with race 2 than with race 1. Significant race-by-progeny interactions were not found.

With both races, the variation between the progenies could be described by general

Table 6. Analysis of variance of the accumulated amounts of dianthalexin (DX) and methoxydianthramide S (MDS) in the investigated carnation progenies, 7 days after inoculation with races 1 and 2 of *F. oxysporum* f.sp. *dianthi*. Abbreviations and significance limits as in Table 2.

| Source of variation | Df | Variance ratio (DX) | Variance ratio (MDS) |
|---|---|---|---|
| Races | 1 | 6.0* | 17.2*** |
| Progenies | 9 | 4.4** | 6.0*** |
| GCA | 3 | 11.6*** | 14.2*** |
| SCA | 2 | 0.3 | 1.1 |
| Deviations | 4 | 1.1 | 2.2 |
| Races × Progenies | 9 | 1.0 | 1.7 |
| Races × GCA | 3 | 1.7 | 4.0* |
| Races × SCA | 2 | 1.0 | 0.3 |
| Races × Deviations | 4 | 0.4 | 0.8 |
| Residual variance | 20 | 178.5 | 19.4 |

Table 7. GCA values for the parental carnation cultivars for accumulation of dianthalexin (DX) and methoxydianthramide S (MDS), calculated from the data on the accumulation of these compounds in the ten progenies at 7 days after inoculation with races 1 and 2 of *F. oxysporum* f.sp. *dianthi*. Values followed by different letters are significantly different at the 5% level (columnwise comparison only); l.s.d., least significant difference.

| Cultivar | Race 1 | | Race 2 | |
|---|---|---|---|---|
| | DX | MDS | DX | MDS |
| Novada | 4.74 a | 0.45 a | 16.90 b | 6.61 b |
| Elsy | −3.01 a | −0.05 a | − 6.40 a | −2.59 a |
| Lena | 1.72 a | 0.12 a | − 4.66 a | −2.03 a |
| Sam's Pride | −3.44 a | −0.52 a | − 5.85 a | −1.98 a |
| l.s.d. | 15.17 | 5.53 | 8.76 | 3.19 |

combining abilities only, indicating additive inheritance. The GCA values for accumulation of methoxydianthramide S were different for both races (Tables 6 and 7), showing that phytoalexin accumulation is not only dependent of the progenies' parents but also of the race of the pathogen used for inoculation. This was confirmed by the correlation between the phytoalexin accumulation and the number of diseased plants found for both races (Table 5).

In case of race 2, the GCA values for phytoalexin accumulation were significantly higher for 'Novada' than for the other progenies (Table 7). In the case of race 1 significant differences in the GCA values were not observed. This may be due to considerable overestimation of the variance for race 1 which had to be estimated from the variance for race 2. However, GCA values for phytoalexin accumulation with race 1 showed a similar tendency as the corresponding GCA values for resistance to this race (Table 3).

## Discussion

Resistance to the common race 2 of *F. oxysporum* f.sp. *dianthi* was assumed polygenic and mainly additive (Sparnaaij and Demmink, 1977 and unpublished; Baayen, 1988). The present data show that resistance is indeed inherited additively; significant SCA's were not found. The parental cultivars differed in extravascular and vascular resistance components. The two mechanisms seemed to inherit (partially) independently.

Contrary to previous expectations (Demmink et al., 1989), resistance to race 1 was not found to be monogenic and dominant. According to the evidence presented, resistance to race 1 is additive, but whether it is governed by one or several genes can not be judged from our data. Monogenic resistance to race 1 would fit the assumption of Demmink et al. (1989) that race 1 is a distinct forma specialis of *F. oxysporum* rather than a physiologic race of *F. oxysporum* f.sp. *dianthi*. Polygenic resistance to race 1 would fit the model presented by Parlevliet and Zadoks (1977) for polygenic resistance operating on a gene-for-gene basis. In their model, the expression of additive resistance genes is race-specific. Such an approach is in agreement with the occurrence of six other races not studied here, some of them being much more alike in virulence than

races 1 and 2 (Garibaldi, 1983; Baayen, 1988; Demmink et al., 1989). On the other hand, in a gene-for-gene system all races of the pathogen induce the same disease process, since the combination of (a)virulence genes and resistance genes only determines whether the disease process will occur or not, but not what the process will be like. This is evidently not the case in the presently investigated host-pathogen system (Baayen et al., 1988), which therefore does not wholly fit Parlevliet and Zadok's model. To elucidate the inheritance of resistance to race 1, further studies are needed in which the resistance level is quantified for all seedlings separately in order to analyse the segregation ratios. This may only be achieved by vegetative propagation and subsequent clonal testing of the individual seedlings, which renders such studies highly laborious and time-consuming.

Indications for the presence of extravascular resistance components were found for resistance to race 2 but seemed of minor importance. In histological studies, the resistance mechanism consisted largely of a localization response, both in roots and stem (Baayen, 1988). One of the main conclusions of the latter author: 'partial resistance to Fusarium wilt (race 2) appears to be mainly determined by the capacity of the various cultivars to localize the invading fungus, a parameter that can be measured as the percentage non-colonized plants in a clone at a given time after (stem) inoculation' (op. cit., page 156), is supported by the data presented here. The overall level of resistance appeared to be mainly determined by the localization ability, a component which is expressed early in the host-pathogen interaction. The contribution of latent period and wilting rate was correlated to the localization ability, probably because retardation of the invisible (LP) and visible (WP) phase of the colonization and wilting process is due to the same factors (such as phytoalexin and gum production) as determine the localization ability. The contribution of factors influencing the rate of the wilting process, but not the localization ability, appeared very small.

The progeny of the resistant cultivar Novada accumulated higher amounts of phytoalexins than the progeny of more susceptible cultivars. It could not be established whether this was due to a higher ability of 'Novada' and its progeny to produce phytoalexins (indicated by the strong GCA effects), or merely to the higher resistance of the progeny of 'Novada' to both races, of which phytoalexins could be a marker. The different GCA values found for both races with methoxydianthramide S indicate that the nature of the response to infection (resistant or susceptible) at least partially determined the accumulation of phytoalexins. Buiatti et al. (1987) were able to quantify the ability of carnation to accumulate phytoalexins, irrespective of resistance or susceptibility. These authors evaluated the level of fungitoxic activity in callus lines derived from F1 progenies from crosses between three susceptible and two resistant cultivars (including 'Elsy') after treatment with a probably aspecific cell wall-derived elicitor from *F. oxysporum* f.sp. *dianthi*, race 2. In their experiments, phytoalexin production appeared a dominant character, with 'Elsy' being homozygously dominant. This contradicts our observations on 'Elsy', as well as the apparently additive inheritance of phytoalexin accumulation.

It is remarkable that race 1 induced the accumulation of lower amounts of phytoalexins than did race 2. Similar results have recently been obtained in other experiments (Niemann et al., 1991). Race 1 appears to be less aggressive than race 2 (Baayen et al., 1988). Compared with race 2, race 1 causes little or no vascular degradation and

thus may have a lower eliciting activity on the host. The defence reactions of the host may therefore be milder as well.

The results of the present study offer opportunities for further development of screening techniques for resistance. Given the predominant role of the localization ability in the resistance mechanism, the proportion of non-colonized plants after stem inoculation should indicate the mean resistance levels in a quick and reasonably reliable manner. The important role of localization in the resistance mechanism, together with the apparent additivity of resistance, also suggests that the additive factor corresponds to the localization mechanism. A thorough knowledge of the localization process and its biochemical components is needed for future improvement of the efficiency of breeding and selection of Fusarium wilt-resistant carnations.

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**References**

Baayen, R.P., 1988. Fusarium wilt of carnation. Disease development, resistance mechanisms of the host and taxonomy of the pathogen. Ph.D. thesis, Utrecht University, The Netherlands, 165 pp.

Baayen, R.P. & Elgersma, D.M., 1985. Colonization and histopathology of susceptible and resistant carnation cultivars infected with *Fusarium oxysporum* f.sp. *dianthi*. Netherlands Journal of Plant Pathology 91: 119-135.

Baayen, R.P., Elgersma, D.M., Demmink, J.F. & Sparnaaij, L.D., 1988. Differences in pathogenesis observed among susceptible interactions of carnation with four races of *Fusarium oxysporum* f.sp. *dianthi*. Netherlands Journal of Plant Pathology 94: 81-94.

Baayen, R.P. & Niemann, G.J., 1989. Correlations between accumulation of dianthramides, dianthalexin and unknown compounds, and partial resistance to *Fusarium oxysporum* f.sp. *dianthi* in eleven carnation cultivars. Journal of Phytopathology 126: 281-292.

Baayen, R.P., Van Eijk, C. & Elgersma, D.M., 1989. Histology of resistant and susceptible carnation cultivars from soil infested with *Fusarium oxysporum* f.sp. *dianthi*. Netherlands Journal of Plant Pathology 95: 3-13.

Buiatti, M., Marcheschi, G., Venturo, R., Bettini, P., Bogani, P., Morpurgo, R., Nacmias, B. & Pellegrini, M.G., 1987. In vitro response to *Fusarium* elicitor and toxic substances in crosses between resistant and susceptible carnation cultivars. Plant Breeding 98: 346-348.

Bulmer, M.G., 1985. The mathematical theory of quantitative genetics. Clarendon Press, Oxford, England, 255 pp.

Demmink, J.F., Baayen, R.P. & Sparnaaij, L.D., 1989. Evaluation of the virulence of races 1, 2 and 4 of *Fusarium oxysporum* f.sp. *dianthi* in carnation. Euphytica 42: 55-63.

Garibaldi, A., 1983. Resistenza di cultivar di garofano nei confronti di otto patotipi di *Fusarium oxysporum* f.sp. *dianthi* (Prill. et Del.) Snyd. et Hans. Rivista della Ortoflorofrutticoltura Italiana 67: 261-270.

McCullagh, P. & Nelder, J.A., 1989. Generalized linear models (2nd ed.). Chapman and Hall, London, England, 511 pp.

Moroney, M.J., 1951. Facts from figures. 1986 Edition. Penguin Books Ltd, Harmondsworth, England, 472 pp.

Niemann, G.J. & Baayen, R.P., 1988. Involvement of phenol metabolism in resistance of *Dianthus caryophyllus* L. to *Fusarium oxysporum* f.sp. *dianthi*. Netherlands Journal of Plant Pathology 94: 289-301.

Niemann, G.J., Van der Bij, A., Brandt-de Boer, B., Boon, J.J. & Baayen, R.P., 1991. Differential response of four carnation cultivars to races 1 and 2 of *Fusarium oxysporum* f.sp. *dianthi* and to *Phialophora cinerescens*. Physiological and Molecular Plant Pathology 37, in press.

Parlevliet, J.E. & Zadoks, J.C., 1977. The integrated concept of disease resistance; a new view including horizontal and vertical resistance in plants. Euphytica 26: 5-21.

Payne, R.W., Lane, R.W. et al., 1987. Genstat 5 reference manual. Clarendon Press, Oxford, England, 749 pp.

Ponchet, M., Favre-Bonvin, J., Hauteville, M. & Ricci, P., 1988. Dianthramides (*N*-benzoyl and *N-para*coumarylanthranilic acid derivatives) from elicited tissues of *Dianthus caryophyllus*. Phytochemistry 27: 725-730.

Sparnaaij, L.D. & Demmink, J.F., 1976. Breeding for resistance to *Phialophora cinerescens* (Wr.) van Beyma in glasshouse carnations (*Dianthus caryophyllus* L.). Euphytica 25: 329-338.

Sparnaaij, L.D. & Demmink, J.F., 1977. Progress towards Fusarium resistance in carnations. Acta Horticulturae 71: 107-113.