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Involvement of phenol metabolism in resistance of Dianthus caryophyllus to Fusarium oxysporum f.sp. dianthi

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Abstract

Three carnation cultivars were investigated for the effect of stem inoculation with *Fusarium oxysporum* f.sp. *dianthi* on production of phenolic compounds and on fungistatic activity. Carnation stems were characterized by a complex mixture of cell wall-bound phenolics, several of which occurred in considerable amounts. Only very low amounts of phenolic compounds were present in the vacuoles.

Infection with *F. oxysporum* f.sp. *dianthi* induced the production and accumulation of a number of new compounds, both free in the cell sap and bound to the cell wall. In addition, the stem extracts acquired germination-inhibiting properties for conidia of the fungus. The accumulation of several phenolics and the fungistatic activity were roughly correlated to the degree of resistance of the three cultivars. Part of the differences in their resistance to *F. oxysporum* f.sp. *dianthi* might be due to an inhibition of the conversion of phenolic acid-type precursors into phytoalexins in the more susceptible cultivars.

Additional keywords: carnation, cell walls, dianthramides, hydroxyanthranilic acid, phenolic acid, phytoalexins.

Introduction

Resistance of carnation to *Fusarium oxysporum* f.sp. *dianthi* is governed by several gene pairs (Sparnaaij and Demmink, 1975; Blanc, 1983). Expression of the genes involved is strongly influenced by the environment, which renders the degree of resistance found for a cultivar (clone) largely variable. Under commercial conditions, and also for experiments, rooted cuttings are used for propagation. As shown by Sparnaaij et al. (1988), callus and phellem formation at the basal end of such cuttings remain insufficient to prevent passive entrance of the pathogen into the vessels for a long time. A major part of the mechanism of resistance, therefore, has to be found in the physiological response of the host after invasion of the fungus into the vascular system. From microscopic observation it appeared that the physiological response of the host includes the formation of gels in the affected vessels (Baayen and Elgersma, 1985). These gels seem to be formed in the xylem parenchyma cells adjacent to the infected vessels (Catesson et al., 1976; Baayen and Elgersma, 1985). Gel formation is a general phenomenon and probably is one of the factors in the plant response that provide for resistance to vascular invasion. The gels are composed of various amounts of polysac-

charides encrusted with (poly)phenolic compounds. Phenolics are probably released by the stimulus of infection in these same parenchyma cells. After infusion of the phenolics into gel and cell wall, oxidation and polymerization may occur, and thus a durable barrier may be provided at the interface of the zone of colonization and the zone of defense (Beckman and Talboys, 1981; Baayen, 1988).

In addition to their role in the formation of structural barriers, such products of phenolic metabolism may be fungistatic in their own right, whether in free form or bound to polysaccharides. Esterification of phenolic acids like ferulic and *p*-coumaric acid and their dimers to cell wall polysaccharides is found in the Poaceae and a number of other angiosperm families (Harris and Hartley, 1976; Hartley and Harris, 1981; Fry, 1984, 1987; Hartley et al., 1988). The fungistatic activity of this type of compounds is well documented (Kuc et al., 1956; Keen and Littlefield, 1979; Glazener, 1980; Haars et al., 1981; Ismail et al., 1987).

Ponchet et al. (1982) found a number of phytoalexins of phenolic nature in carnation after infection with *Phytophthora parasitica*. Preliminary investigations with carnations infected with *F. oxysporum* f.sp. *dianthi* indicated changes in free phenolic constituents as well, while a large array of phenolics was also present in esterified form in cell wall material (Niemann et al., 1987, 1988a, 1988b). This prompted us to evaluate the changes of phenolic nature after infection, to search for possible differences between susceptible and resistant reactions, and finally to examine whether a causal relationship exists between phenol metabolism and resistance. In the present investigation we restricted ourselves to free and esterified phenolics in three cultivars with different degrees of susceptibility to the fungus. Work on other cultivars and on more complex carbohydrate-phenolic entities is in progress.

Materials and methods

Inoculum. A virulent isolate of *Fusarium oxysporum* Schlecht. f.sp. *dianthi* (Prill. & Delacr.) Snyder & Hansen (IVT-AS1 or WCS 816) was cultured on potato dextrose agar culture slants. Conidial suspensions were obtained by shaking five of these cultures with demineralized water. For the germination tests the fungus was also cultured in Tchernoff's medium (Tchernoff, 1965) on a reciprocal shaker for 5 days at 23 °C. Mycelial fragments were removed by filtration through glasswool, and the suspension was adjusted to a concentration of 10^7 conidia ml^{-1}.

Plant material and inoculation of the plants. Rooted cuttings of the carnation cultivars Novada (highly resistant to fusarium wilt), Pallas (moderately resistant) and Lena (susceptible) were obtained by courtesy of Dr L.D. Sparnaaij and Ing. J.F. Demmink of the Institute for Horticultural Plant Breeding (IVT) at Wageningen. They were planted in steamed soil and allowed to grow in a greenhouse at 22 °C for 3 to 5 weeks prior to inoculation.

One week prior to inoculation the plants were moved to another compartment of the greenhouse, which was kept at a day-night schedule of 22 °C and 20 °C, respectively, and was provided with additional artificial light from 6.00 to 22.00 h (average of 6 to 7 Klux at soil level). The last three days before inoculation the plants remained unwatered. They were stem-inoculated as described previously (Baayen and Elgersma, 1985). Control plants of each cultivar remained untreated, or were treated with water

instead of the conidial suspension. For extraction, 5 cm stem segments taken just above the inoculation site were sampled at 1, 2, 3, 4, 5, 7 and 10 days after inoculation; at 2 and 5 days for the water controls and at 5 days for the untreated controls. For each sample (nine or) ten plants were used.

The experiment was repeated with samples obtained at 2, 5 and 10 days after inoculation. The collected stem segments were defoliated, weighed and kept in the deep-freeze until extraction.

Extraction of plant material. The stem segments were chopped up and then homogenized for 5 minutes in acetone with a Bühler homogenizer, after which the mixture was filtered. The residue was washed twice with acetone. The combined filtrates were evaporated to dryness and the residue was taken up in ethanol (0.25 ml g^{-1} fresh material) and analysed as such by high-performance liquid chromatography (HPLC), and in a number of cases by thin layer (TLC) and/or paper chromatography (PC). These extracts are further called 'acetone extracts'.

The residual cell wall material left on the filter was air-dried, weighed and shaken for 24 h in a closed vial in 20 ml 1 M NaOH. To prevent oxidation nitrogen was bubbled through the mixture for 5 min before closing the vials. Afterwards the solution was acidified with 11N HCl to pH 2, and filtered. The residue was washed twice with ethyl acetate and stored for further use. The washings were used to extract the acidified aqueous extract. The aqueous layer was discarded, while the ethyl acetate fraction was evaporated to dryness, taken up in ethanol, filtered over a cellulose acetate membrane filter, and again evaporated to dryness and taken up in ethanol (0.5 ml g^{-1} residual wall material). These 'hydrolysis extracts' were analysed by HPLC, TLC and/or PC.

Acid and alkaline hydrolysis of acetone extracts. One ml of the ethanolic solution of two of the acetone extracts of 'Novada' was hydrolysed in 2N HCl at 100 °C for 2 h. The solution was extracted twice with ethyl acetate, the ethyl acetate extract was evaporated to dryness and the residue taken up in methanol and subjected to chromatography. For alkaline hydrolysis of another ml of those acetone extracts the same procedure as that described above for the hydrolysis and extraction of cell wall material was used.

High-performance liquid chromatography. HPLC was performed with an LKB double-pump liquid chromatograph provided with a 2 × 10 cm Chromsep Lichrosorb RP18 (ID 3 mm) column, a Uvicord detector with 275 nm filter and a Spectraphysics SP 4270 integrator. Samples of 20 µl were injected and eluted with a gradient of increasing concentrations of MeOH in 0.05% aqueous phosphoric acid, starting with 25% MeOH from 0-2 min, followed by 25-75% from 2-15 min, 75-80% from 15-18 min, a linear part at 80% from 18-25 min and returning from 80-25% from 25-30 min with a constant flow of 0.5 ml min^{-1} (pressure around 60 bar).

Thin layer chromatography. Samples of the acetone and hydrolysis extracts were separated by TLC on 5 × 20 or 20 × 20 cm Merck silica gel 60 F_{254} plates, developed with cyclohexane – ethyl acetate 1 : 1 v/v (CE), hexane – ethyl acetate – methanol 60 : 40 : 1 v/v/v (HEM) and/or chloroform – acetic acid – methanol 75 : 5: 20 v/v/v (CAM 75). The compounds were detected under UV light (254 and/or 360 nm) either

viewing the plates as such or after fuming with ammonia. In addition, plates were sprayed with a 0.05% aqueous solution of *p*-nitrophenyldiazonium tetrafluoroborate (Merck-Schuchardt, NPD), followed by 10% Na_2CO_3. In other experiments plates were sprayed with a conidial suspension of *F. oxysporum* f.sp. *dianthi*.

Paper chromatography. Part of the extracts was also separated by one- or two-dimensional PC on Schleicher & Schüll no 2043a chromatography paper and developed with butanol – ethanol – water 4 : 1 : 2 v/v/v and 2% acetic acid. Detection was the same as for TLC.

Reference compounds. Most of the phenolic acids used as a reference were commercially available. Dianthalexin and methoxydianthramide S (= 'dianthramide A') were obtained by courtesy of Dr M. Ponchet, Antibes, France.

Evaluation of fungistatic activity. Fungistatic activity of the extracts was evaluated in two ways. In the first method TLC plates (developed with CE or HEM) were sprayed with a conidial suspension of *F. oxysporum* f.sp. *dianthi* or *Cladosporium herbarum* in Czapek-Dox medium or in Tchernoff's solution ($2 - 10 \times 10^6$ conidia ml^{-1}) and kept in a moist chamber for 48 to 72 h. Fungal development was evaluated as such or after treatment with iodine vapour.

In the second method 1 to 5 µl of the (diluted) ethanolic solution of the acetone extract was air-dried on a glass slide. The residue was thoroughly mixed with 20 µl of a conidial suspension ($1 - 5 \times 10^6$ conidia ml^{-1}) of *F. oxysporum* f.sp. *dianthi* in water. The slides were kept in a moist chamber for 5 to 24 h at room temperature or at 24 °C. Germination was then stopped with 2% formaldehyde and percentages of germination were determined by counting 100 conidia.

Results

Extracts of stem segments of untreated and water-treated controls. Comparison of the acetone and the hydrolysis extracts of stem segments of untreated controls by PC, TLC or HPLC gave surprisingly similar chromatograms for 'Novada', 'Pallas' and 'Lena' in spite of large differences between these cultivars in descent, morphology and resistance to fusarium wilt. The acetone extracts, mainly representing the vacuolar contents, contained very low amounts of UV-absorbing compounds (see Fig. 1a for HPLC). The acetone extracts from 'Novada' were also subjected to acid and alkaline hydrolysis. Acid hydrolysis yielded no recognizable aglycones. Flavonoids, common in the Caryophyllaceae (Hegnauer, 1964; Niemann et al., 1983), were not detected. Alkaline treatment released trace amounts of aromatic hydroxyacids tentatively identified (by R_f and colour only) as *p*-hydroxybenzoic, vanillic and ferulic acid.

Alkaline treatment of cell wall material yielded a large variety of compounds; some occurred in comparatively high concentration (Fig. 1b). Co-chromatography (PC, HPLC) and colour (UV, UV-NH_3, NPD, NPD-Na_2CO_3) tentatively revealed *p*-hydroxybenzoic, vanillic, salicylic, syringic, *p*-coumaric and ferulic acid. Some of the larger HPLC peaks which remained unnamed might be dimers (cf. Hartley et al., 1988). Identification of the cell wall-bound components of carnation is in progress and will be reported elsewhere.

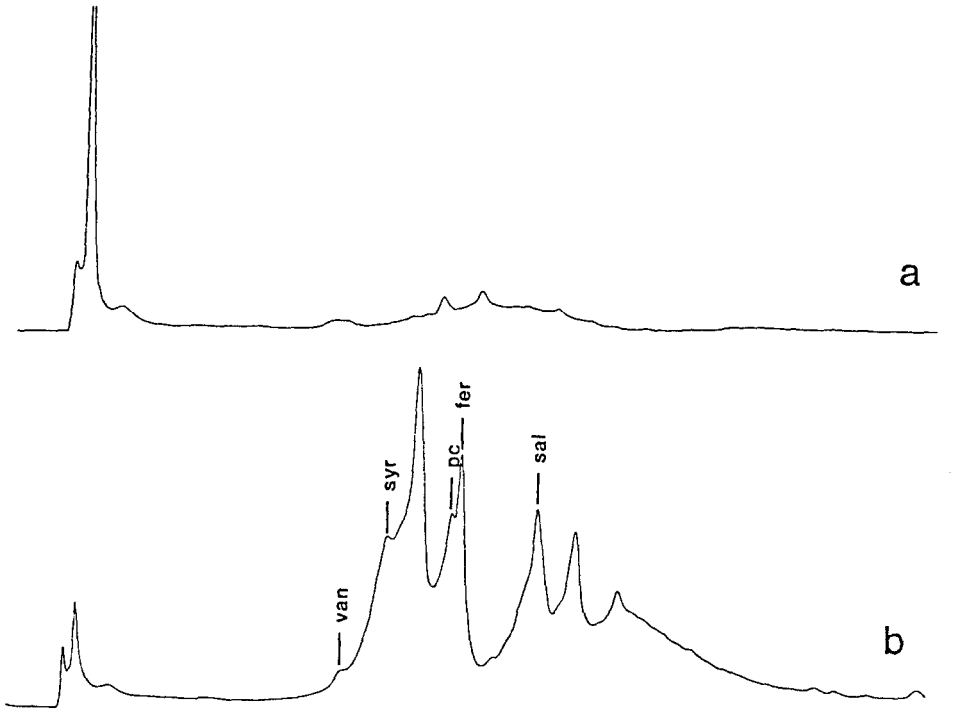

Fig. 1. HPLC-chromatograms of acetone (a) and hydrolysis (b) extracts of stem segments of water-treated 'Novada' carnations. Van: vanillic acid; syr: syringic acid; pc: *p*-coumaric acid; fer: ferulic acid; sal: salicylic acid.

Comparison of untreated and water-treated controls showed that mere wounding of the stem had little or no effect as far as compounds detectable in the chromatograms are concerned.

Effect of infection with F. oxysporum f.sp. dianthi; acetone extracts. Inoculation with *F. oxysporum* f.sp. *dianthi* directly influenced carnation metabolism and led to accumulation of a number of UV-absorbing compounds. Based on their change in UV absorption and fluorescence under alkaline conditions, these compounds were identified as phenolics. Fig. 2 illustrates the production within 24 hours of a number of new compounds in the cultivar Novada and the accumulation of several of them during the first 10 days after inoculation. Compound no. 1 practically disappeared after an initial accumulation. At least ten new metabolites were induced in this cultivar.

In the cultivars Pallas and Lena, infection induced the accumulation of the same substances, although with large differences in their relative concentrations. Accumulation of compound(s) 14, for instance, remained low in 'Pallas' and 'Lena' in comparison with 'Novada', whereas the opposite was seen for compound 5. The time-course diagram of two of the more eminent peaks, 11(+12) and 14, is given in Fig. 3. There were great differences in accumulation for compounds 11 + 12 and 14, with high values for 'Novada', low ones for 'Pallas' and still lower ones for 'Lena'. Compounds 6 and 8 accumulated to about the same extent in 'Lena' and 'Pallas', with higher values for

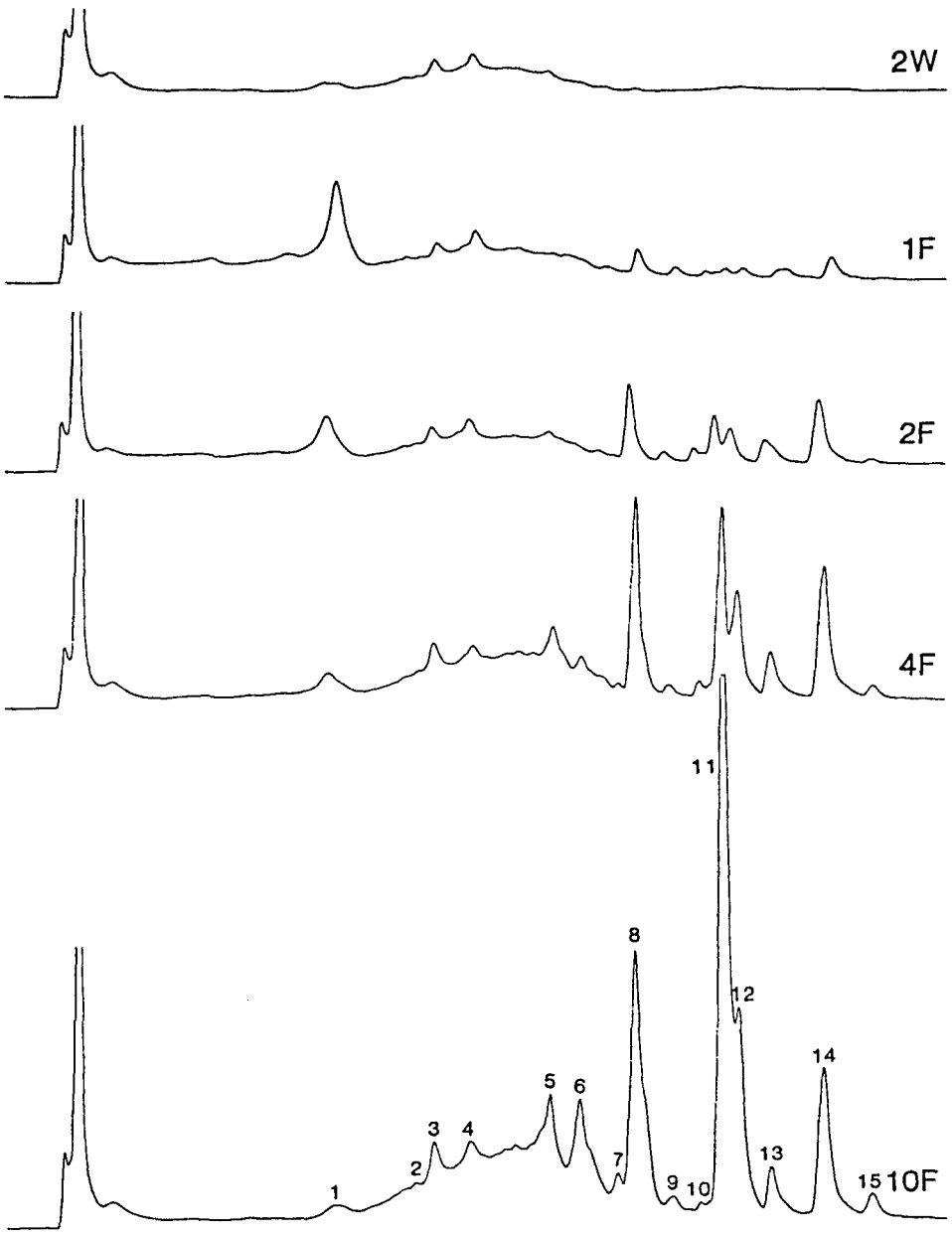

Fig. 2. HPLC-chromatograms of acetone extracts of stem segments of 'Novada', after treatment with water (2W, two days after treatment) and after inoculation with *F. oxysporum* f.sp. *dianthi* for 1 to 10 days (1F to 10F). The identity of the compounds present in peaks 7 to 15 is given in the text.

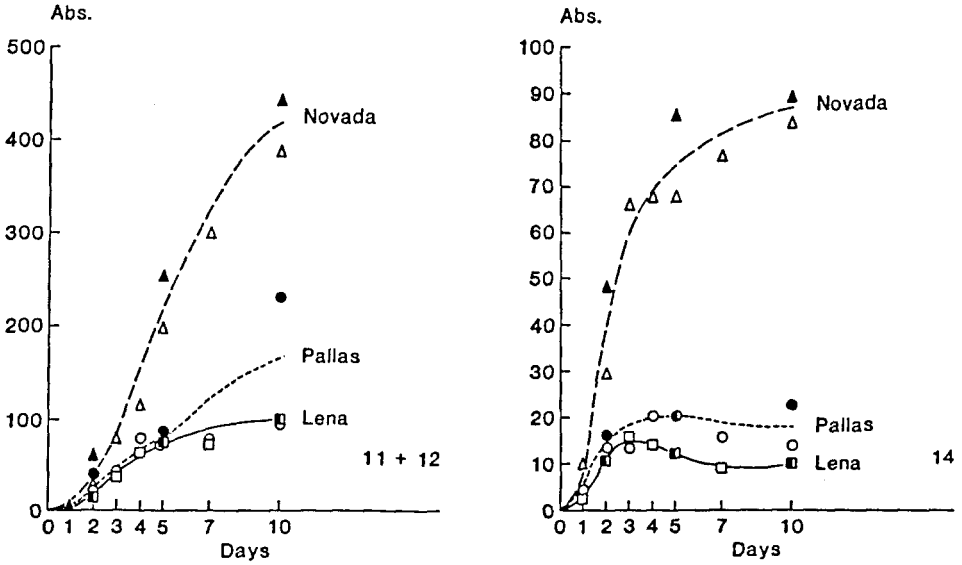

Fig. 3. Time-course diagrams of the accumulation of compounds 11 (DX) + 12 (MDR + MDB) and 14 (mainly MDS) in the acetone extracts of stem segments of 'Novada' (△ , ▲), 'Pallas' (○, •) and 'Lena' (□ , ■) after infection with *F. oxysporum* f.sp. *dianthi*. abs: absorbance in arbitrary units. Separate values from experiments 1 (△ , ○ and □) and 2 (▲ , • and ■).

'Novada' for compound 8. For compound 5 'Lena' and 'Pallas' surpassed 'Novada' in the course of the infection period.

Evaluation of the fungistatic properties of acetone extracts. Contrary to the water-treated control extracts, those of inoculated ones ('Novada', 4 or 5 days after inoculation) produced at least four inhibition zones in TLC-bioassays, among which one corresponding with HPLC peak 14. *C. herbarum* appeared to be more sensitive to these compounds than *F. oxysporum* f.sp. *dianthi*.

In the germination test, the acetone extracts of the infected cultivars inhibited germination of conidia of *F. oxysporum* f.sp. *dianthi* more than those of healthy plants (Table 1). Furthermore, extracts of infected 'Novada' proved much more active in this respect than extracts of infected 'Pallas', while these were slightly more active than those of infected 'Lena'.

Effect of infection with F. oxysporum f.sp. dianthi; hydrolysis extracts. Infection with *F. oxysporum* f.sp. *dianthi* also largely influenced the cell wall-associated compounds. Several novel compounds were observed within 24 hours which further accumulated during the first 10 days after infection (Fig. 4). These changes were restricted to compounds with relatively high retention times with HPLC; most aromatic hydroxyacids (compounds W1 to W7, Fig. 4) remained practically unaffected. Part of these new compounds appeared identical to some of those in the acetone extracts. For instance, W15 and W16 co-chromatographed with compounds 12 and 14, respectively, whereas W11 might either represent compound 8 or its shoulder. The latter compound, however, was

Table 1. Germination of conidia of *Fusarium oxysporum* f.sp. *dianthi* in water containing acetone extracts from healthy and infected stem segments (5 days after inoculation) of the carnation cultivars Novada, Pallas and Lena.

Concentration of extracts			
µl acetone extract used	1	5	5
dilution[1]	0	0	1
Period allowed for germination			
hours	24[2]	14	7
Cultivars and treatments	% germination		
Lena, control	49	100	98
Lena, inoculated	13	31	90
Pallas, control	20	96	90
Pallas, inoculated	6	31	83
Novada, control	30	97	90
Novada, inoculated	0	9	25
water control	41	95	97

[1] Dilution: 0 = undiluted, 1 = diluted 1 : 1 with ethanol before air-drying.
[2] Conidia from old culture slants, other tests with conidia from 5-day-old shake cultures.

present in much higher amounts in hydrolysis extracts than in acetone extracts of the same plants. Compound 11, on the other hand, was only represented in the acetone extracts and not in the hydrolysis extracts of the cell wall residue.

As for the acetone extracts, the three carnation cultivars differed in their pattern of accumulation of cell wall-bound components. 'Novada' in particular accumulated compounds W11, W15 and W16 (Fig. 5), whereas the situation was reversed for compound W8.

Identity of the new compounds. By co-chromatography the compounds 11 and 14 were tentatively identified as dianthalexin and methoxydianthramide S, respectively. This identity was confirmed by LC-MS (molecular ions only; co-operation with Prof. Dr J. van der Greef (Zeist) and Dr W.M.A. Niessen, (Leiden)). Four samples of acetone extracts were investigated by Dr M. Ponchet (Antibes). This led to the following tentative identification of peaks 7 to 15 (all of them dianthramides):

peak 7 – hydroxydianthramide R (HDR) + the dianthramide of *cis p*-coumaric acid (DPc),
peak 8 – hydroxydianthramide B (HDB) + the methoxydianthramide of *cis p*-coumaric acid (MDPc),
peak 9 – hydroxydianthramide S (HDS) + the dianthramide of *trans p*-coumaric acid (DPt),
peak 10 – dianthramide R (DR) + the methoxydianthramide of *trans p*-coumaric acid (MDPt),
peak 11 – dianthalexin (DX),
peak 12 – methoxydianthramide R (MDR) + methoxydianthramide B (MDB),
peak 13 – hydroxydianthramide S methyl ester (HDSM) + dianthramide S (DS) + unknown,

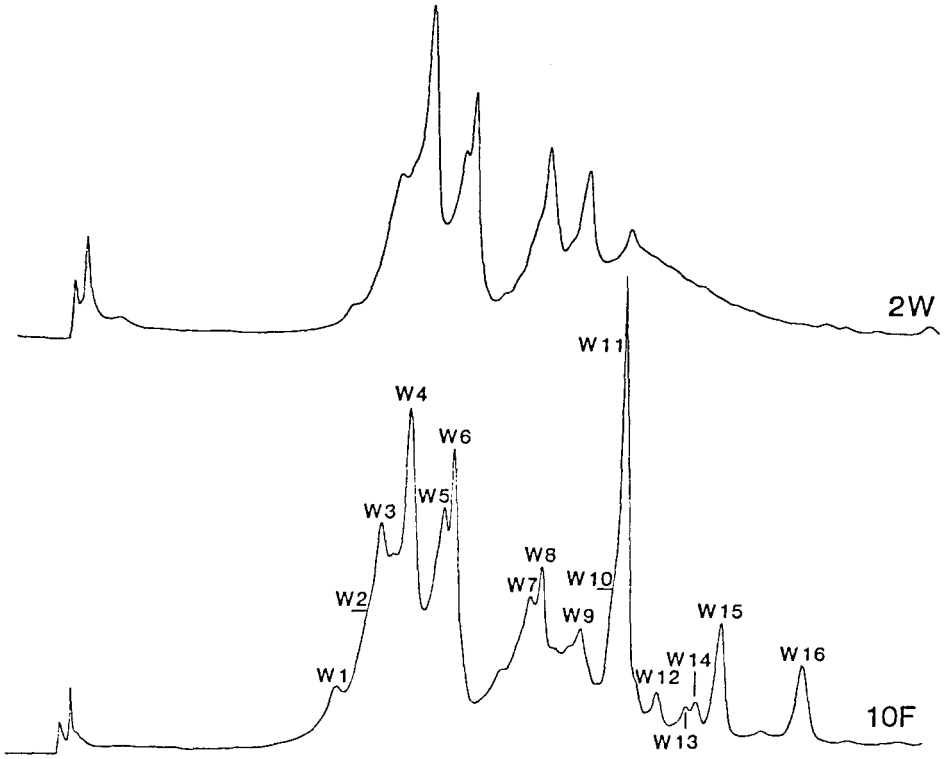

Fig. 4. HPLC-chromatograms of hydrolysis extracts of stem segments of 'Novada', after treatment with water (2W, 2 days after treatment) and 10 days after inoculation with *F. oxysporum* f.sp. *dianthi* (10F). Compound W1 co-chromatographed with vanillic acid, compound W3 with syringic acid, compound W5 with *p*-coumaric acid, compound W6 with ferulic acid, compound W8 with salicylic acid and compound W16 with methoxydianthramide S (MDS).

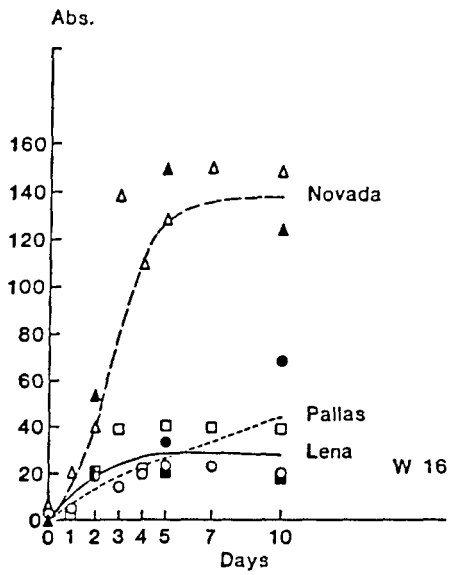

Fig. 5. Time-course diagram of the accumulation of compound W16 (MDS) in hydrolysis extracts of 'Novada' (△, ▲), 'Pallas' (○, •) and 'Lena' (□, ■) during infection with *F. oxysporum* f.sp. *dianthi*. abs: absorbance in arbitrary units. Separate values from experiments 1 (△, ○ and □) and 2 (▲, • and ■).

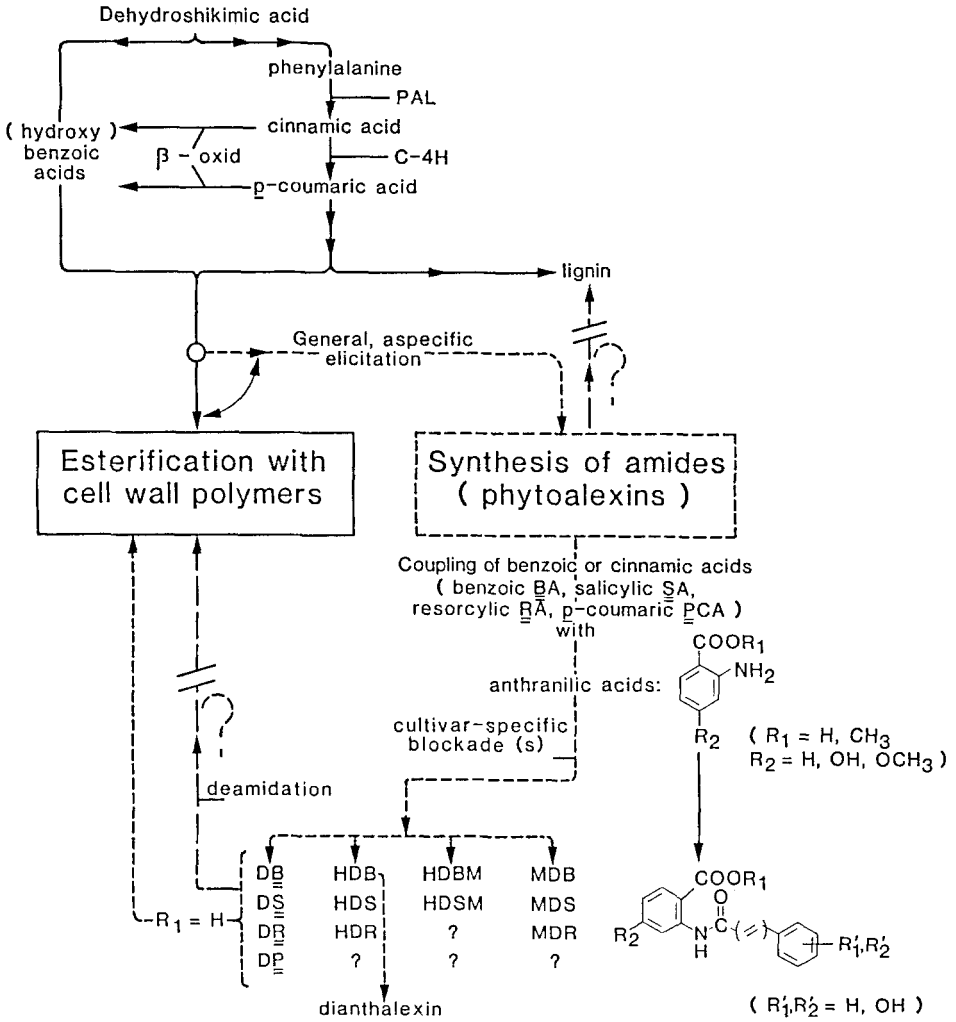

Fig. 6. Hypothetical metabolic pathways for integration of phenolic amides into wall components, and hypothetical regulation of phytoalexin synthesis by elicitation. The names of the dianthramides are given in the text.

-------- Pathway with accumulation of dianthramides, introduced by elicitation;

– – – hypothetical pathways which may be inhibited by elicitation.

peak 14 – methoxydianthramide S (MDS) + dianthramide M (DM), and peak 15 – methoxydianthramide M (MDM) + unknown.

B, S, R and M stand for benzoic, salicylic, resorcylic and 4-methoxysalicylic acid. Structures of these various dianthramides are given by Ponchet et al. (1988). A general structure of the dianthramides is given in Fig. 6.

Compounds 5 and 6 remained unidentified as yet.

Discussion

In healthy carnations phenolic acids accumulate to a large degree in the form of cell wall-bound esters. In this aspect *Dianthus caryophyllus* exemplifies the special position of the Caryophyllales among the dicotyledons (Hartley and Harris, 1981). The mixture of phenolic acids we found in this species, however, is much more complex than that reported for other Caryophyllaceae.

The accumulation of dianthramides found after infection with *F. oxysporum* f.sp. *dianthi* (in our experiments) or with *Phytophthora parasitica* (Ponchet et al., 1982, 1988) obviously is based on the metabolism of a number of the same phenolic acids as normally bind with cell wall-polysaccharides. It is tempting to suggest that elicitation in carnation merely induces a switch from the coupling of these acids (Fry, 1987) with polysaccharides, to the formation of amides with (hydroxy)anthranilic acid (HAA). A still simpler explanation of the large number of novel products found after elicitation could be that HAA is an intermediate in the integration of phenolic acids into wall material. A blockade of such an integration as a consequence of elicitation would directly cause the formation of the numerous new compounds. Fig. 6 shows some of the possible pathways. The proposed role of HAA might be similar to that of phenylethylamines or tyramine in other plants. The latter compounds have been suggested as intermediates in the formation of lignin-like compounds via their cinnamic acid amides (Negrel and Jeandet, 1987). The formation of avenalumin I after elicitation in *Avena sativa* (Mayama et al., 1981) indicates that HAA can have such a role in widely different plant families. Like many other Gramineae *Avena sativa* is rich in cell wall-bound phenolic acids (Harris and Hartley, 1976); avenalumin I is the product obtained after ring closure in the *p*-coumaric acid amide of 3-hydroxyanthranilic acid.

Esterification of the new compounds to the cell wall polymers of carnation depends on the availability of a free carboxyl group. Part of the differences found between accumulation in acetone and hydrolysis extracts can thus be explained. Dianthalexin (compound 11) is formed by ring closure of the benzoic acid amide of 4-hydroxyanthranilic acid (HDB) and lacks a free carboxyl group. Therefore, its occurrence is restricted to the acetone extracts. In general, the pattern found in the hydrolysis extracts probably reflects that of the free carboxylic acids in the acetone extracts.

The occurrence of free, cell wall- (or gel-)bound fungistatic compounds might contribute to the mechanism of resistance. The differences in accumulation of several vacuolar (8, 11+12, 14) and cell wall-bound (W11, W15 and W16) phenolic constituents between the three cultivars were considerable, and roughly reflected the known differences in resistance. The differential accumulation of phytoalexins in the cultivars studied was also reflected in the germination-inhibiting capacity of the total extracts. However, the present results only point to a slightly higher resistance of 'Pallas' than that of 'Lena', compared to the high resistance of 'Novada', while 'Pallas' in practice has a more intermediate position between 'Novada' and 'Lena'.

The accumulation of compounds 5 and 6 and that of the phytoalexins 11 to 15 appeared to be inversely related among the three cultivars studied. This suggests that, in addition to a general 'elicitation switch' (Fig. 6) as present in all three cultivars, there may be 'cultivar-specific switches' as well. Such switches could possibly direct the phenolic metabolic pool either towards (precursor-type?) compounds such as 5 and 6, which may well represent lower molecular phenolic acids, or towards the formation

of their (fungitoxic) amides. This might determine (part of) the level of resistance to fusarium wilt.

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Samenvatting

De betrokkenheid van de fenol-stofwisseling bij resistentie van Dianthus caryophyllus tegen Fusarium oxysporum f.sp. dianthi

Drie anjercultivars werden onderzocht op de effecten van stengelinoculatie met *Fusarium oxysporum* f.sp. *dianthi* op de produktie van fenolische verbindingen en op de fungistatische activiteit van stengelextracten. Anjerstengels bleken een complex mengsel van celwand-gebonden fenolzuren te bevatten, waarvan er verschillende in vrij grote hoeveelheden voorkwamen. Daartegenover waren de hoeveelheden fenolische verbindingen in de vacuole bij gezonde anjerstengels erg laag.

Infectie met *F. oxysporum* f.sp. *dianthi* induceerde de produktie en accumulatie van een aantal nieuwe verbindingen in het celsap, alsook gebonden aan de celwand. Tevens bleek de remming van de kieming van conidiën van *F. oxysporum* f.sp. *dianthi* onder invloed van deze stengelextracten sterk verhoogd, in vergelijking met de remming veroorzaakt door extracten van gezonde stengels. De accumulatie van een aantal fenolen en de verhoging van de fungistatische activiteit van de extracten waren in grote lijnen gecorreleerd met de uit de praktijk bekende resistentie van de drie cultivars. De verschillen in resistentie tegen *F. oxysporum* f.sp. *dianthi* zouden deels kunnen berusten op remming van de omzetting van precursors (fenolzuren) in fytoalexinen in de meer vatbare cultivars.

References

Baayen, R.P., 1988. Responses related to lignification and intravascular periderm formation in carnations resistant to Fusarium wilt. Canadian Journal of Botany 66: 784-792.

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.

Beckman, C.H. & Talboys, P.W., 1981. Anatomy of resistance. In: Mace, M.E., Bell, A.A. & Beckman, C.H. (Eds), Fungal wilt diseases of plants. Academic Press, London, p. 487-521.

Blanc, H., 1983. Carnation breeding for resistance to *Fusarium oxysporum* f.sp. *dianthi*: Practical achievement of resistant cultivars. Acta Horticulturae 141: 43-47.

Catesson, A.M., Czaninski, Y., Péresse, M. & Moreau, M., 1976. Sécrétions intravasculaires de substances 'gommeuses' par les cellules associées aux vaisseaux en réaction à une attaque parasitaire. Bulletin de la Societé Botanique de France 123: 93-107.

Fry, S.C., 1984. Incorporation of [^{14}C]cinnamate into hydrolase-resistant components of the primary cell wall of spinach. Phytochemistry 23: 59-64.
Fry, S.C., 1987. Intracellular feruloylation of pectic polysaccharides. Planta 171: 205-211.
Glazener, J.A., 1980. Defense mechanisms in tomato fruit after infection with *Botrytis cinerea*. Thesis, Utrecht University, 81 pp.
Haars, A., Chet, I. & Hüttermann, A., 1981. Effect of phenolic compounds and tannin on growth and laccase activity of *Fomes annosus*. European Journal of Forest Pathology 11: 67-76.
Harris, P.J. & Hartley, R.D., 1976. Detection of bound ferulic acid in cell walls of the Gramineae by ultra-violet fluorescence microscopy. Nature 259: 508-510.
Hartley, R.D. & Harris, P.J., 1981. Phenolic constituents of the cell walls of dicotyledons. Biochemical Systematics and Ecology 9: 189-203.
Hartley, R.D., Whatley, F.R. & Harris, P.J., 1988. 4,4'-Dihydroxytruxillic acid as a component of cell walls of *Lolium multiflorum*. Phytochemistry 27: 349-351.
Hegnauer, R., 1964. Chemotaxonomie der Pflanzen. Band 3. Dicotyledoneae: Acanthaceae – Cyrillaceae. Birkhäuser Verlag, Basel und Stuttgart. p. 389-390.
Ismail, M.K., Salama, A.-A.M., Ali, M.I.A. & Ouf, S.A.-E., 1987. Effect of some phenolic compounds on spore germination and germ-tube length of *Aspergillus fumigatus* and *Fusarium oxysporum* f.sp. *lycopersici*. Cryptogamie – Mycologie 8: 51-60.
Keen, N.T. & Littlefield, L.J., 1979. The possible association of phytoalexins with resistance gene expression in flax to *Melampsora lini*. Physiological Plant Pathology 14: 265-280.
Kuc, J., Henze, R.E., Ullstrup, A.J. & Quackenbush, F.W., 1956. Chlorogenic and caffeic acids as fungistatic agents produced by potatoes in response to inoculation with *Helminthosporium carbonum*. Journal of the American Chemical Society 78: 3128-3125.
Mayama, S., Tani, T., Matsuura, Y., Ueno, T. & Fukami, H., 1981. The production of phytoalexins by oat in response to crown rust, *Puccinia coronata* f.sp. *avenae*. Physiological Plant Pathology 19: 217-226.
Negrel, J. & Jeandet, P., 1987. Metabolism of tyramine and feruloyltyramine in TMV inoculated leaves of *Nicotiana tabacum*. Phytochemistry 26: 2185-2190.
Niemann, G.J. & Baayen, R.P., 1988a. Chemische verschillen tussen vatbare en resistente anjercultivars. Vakblad voor de Bloemisterij 43 (15): 39.
Niemann, G.J. & Baayen, R.P., 1988b. The biochemical response of different cultivars of *Dianthus caryophyllus* L. to infection by *Fusarium oxysporum* f.sp. *dianthi*. Acta Botanica Neerlandica 37: in press.
Niemann, G.J., Baayen, R.P. & Elgersma, D.M., 1987. Phenol metabolism in higher plants: relation with resistance against vascular wilt in carnation. Acta Botanica Neerlandica 36: 206.
Niemann, G.J., Koerselman-Kooy, J.W., Steijns, J.M.J.M. & Brederode, J. van, 1983. Flavone distribution in leaves of different genotypes of *Silene pratensis* (Rafn.) Godron & Gren. (Caryophyllaceae). Zeitschrift für Pflanzenphysiologie 109: 105-112.
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.
Ponchet, M., Martin-Tanguy, J., Andréoli, C. & Martin, C., 1982. Apparition de substances de type phénolamide lors de l'interaction *Dianthus caryophyllus* L. var. 'Scania' – *Phytophthora parasitica* Dastur. Agronomie 2: 37-44.
Sparnaaij, L.D. & Demmink, J.F., 1975. 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., Rattink, H. & Baayen, R.P., 1988. Fusarium dringt langs vele wegen in anjerplant binnen. Vakblad voor de Bloemisterij 43: 32-33.
Tchernoff, V., 1965. Methods for screening and for the rapid selection of elms for resistance to Dutch elm disease. Acta Botanica Neerlandica 14: 409-452.