Induced Resistance and Phytoalexin Accumulation in Biological Control of Fusarium Wilt of Carnation by *Pseudomonas* sp. Strain WCS417r

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ABSTRACT

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The involvement of mechanisms other than competition for iron in biological control of *Fusarium oxysporum* f. sp. *dianthi* of carnation by *Pseudomonas* sp. strain WCS417r was investigated. Pathogen and antagonist were spatially separated by bacterizing the roots while inoculating the stem with the pathogen. When the roots were bacterized with strain WCS417r 1 wk prior to stem inoculation with *F. o. dianthi*, the number of diseased plants in all experiments with cultivar Pallas was reduced from about 50 to 20%, and in one experiment with cultivar Lena from 69 to 38%. Strain WCS417r could not be isolated from stem tissue and control of Fusarium wilt by strain WCS417r, therefore, is not due to competition between *F. o. dianthi* and strain WCS417r, but has to be ascribed to induced resistance. Along with induced resistance, there was an increased accumulation of phytoalexins in stems of bacterized and inoculated plants compared with nonbacterized, fungus-infected plants. No accumulation of these compounds was found when the plants were bacterized but were noninfected. We concluded that signals, provided by strain WCS417r at the root system, induce in the stem sensitization of defense responses against *F. o. dianthi*, such as synthesis and accumulation of phytoalexins.

Additional keywords: *Dianthus caryophyllus*, dianthramides.

Biological control of Fusarium wilt by antagonistic pseudomonads has been often attributed to competition for certain nutrients such as iron (8,24,28,29) or carbohydrates (8,15). Mechanisms of biological control are complex, however, and often cannot simply be explained by a single factor. Results of previous studies on biological control of Fusarium wilt in carnation indicated involvement of mechanisms other than merely competition for iron (27). Severity of Fusarium wilt in the susceptible cultivar Lena and the moderately resistant cultivar Pallas was reduced when Fe-ethylenediamine di(*o*-hydroxyphenylacetic acid) (EDDHA) was used as an iron source instead of Fe-diethylenetriamine pentaacetic acid (DTPA). DTPA has a lower affinity for $Fe3^{+}$ than EDDHA. This suggests that competition for iron may be involved. With the moderately resistant cultivar Pallas, control of the disease could further be intensified by bacterization with *Pseudomonas* sp. strain WCS417r in combination with Fe-EDDHA as the sole iron source. Such an enhancement was not found with susceptible cultivar Lena. The positive effect of bacterization on disease reduction may depend also on the level of Fusarium wilt resistance of the cultivar.

This enhancement in resistance can no longer be explained by competition for iron alone. It rather suggests that the level of resistance can be increased as a consequence of bacterization in the moderately resistant cultivar.

Induced resistance of plants against fungal diseases often has been observed after preinoculation with weakly aggressive strains or avirulent or incompatible forms of the disease-causing fungus (11,13,25). A few reports also have mentioned the possible involvement of induced resistance in the biological control of soilborne pathogens with plant growth-promoting rhizosphere pseudomonads (1,30). In the latter cases, however, sufficient experimental evidence for the occurrence of induced resistance was not obtained because the competition for nutrients could not be entirely excluded.

To test experimentally whether induced resistance indeed plays a role in the biological control of Fusarium wilt of carnations by *Pseudomonas* sp. strain WCS417r, the pathogen and antagonist have to be separated spatially. This can be achieved by bacterizing

the roots while inoculating the stem with the pathogen.

Upon fungal infection, carnations accumulate a large number of new compounds, benzoic or cinnamic acid amides of (hydroxy)-anthranilic acid or derivatives thereof, known as dianthramides (16,19,21). About 25 of such compounds have been identified (19,21), and with a number of others, the structure still needs to be elucidated (19; G. J. Niemann, *unpublished data*). Several of these compounds are fungitoxic (6,23) and thus are phytoalexins. Production of these phenolic amides is correlated with resistance expression (4,16) and may even be manipulated within a cultivar by pretreatment with salicylic acid or phenylserine (17,20). Accumulation of the dianthramide-type phytoalexins and their possible involvement in induced resistance, therefore, also were examined.

Evidence for induced resistance to Fusarium wilt by bacterization with *Pseudomonas* sp. strain WCS417r sp. is presented with data on phytoalexin accumulation.

MATERIALS AND METHODS

Plant cultivation. Carnation (*Dianthus caryophyllus* L.) cuttings of the Fusarium wilt susceptible cultivar Lena and the moderately resistant cultivar Pallas, both of which had been rooted on rock wool granulate, were obtained from B. V. van Staaveren, Aalsmeer, Netherlands. Plants were placed on rock wool cubes (365 cm^3) (Grodan, Roermond, Netherlands) and grown in a glasshouse at 22 C in summertime under natural daylight conditions (cv. Lena; experiment I), but starting in September artificial light was used from 6:00 a.m. to 10:00 p.m. A nutrient solution (7), modified for iron (15 μM Fe-EDDHA), was manually applied. Depending on the season, evaporation, and growth stage, plants received 25–50 ml of the nutrient solution three times a week.

Bacterization and inoculation of plants. *Pseudomonas* sp. strain WCS417 was isolated from wheat rhizosphere and demonstrated to promote growth of several crops (28) and to be effective in suppression of *Gaeumannomyces graminis* var. *tritici* in wheat (14) and of *Fusarium oxysporum* Schlecht. emend. Snyder & Hans. f. sp. *dianthi* Prill. & Delacr. W. C. Snyder & H. N. Hans. in carnation (27,29). A rifampicin-resistant mutant of strain WCS417 (WCS417r) was used for bacterization of plant roots. Strain WCS417r was grown at 27 C for 24 h on King's medium B (KB) (12). Cells were suspended in sterile distilled water. Plants were bacterized by pouring 25 ml of a 10^7 cells ml^{-1} suspension on the rock wool cube of each plant, 1 wk after the cuttings had been placed on those cubes. Starting 3 days before inoculation with *F. o. dianthi*, the plants did not receive nutrient solution, but were fertilized an hour after inoculation.

A microconidial suspension of race 2 (isolate WCS816) of *F. o. dianthi* was obtained from cultures grown for 7 days on potato-dextrose agar at 23 C. Sterile distilled water was poured on agar plates and was collected after carefully abrading the agar. The microconidial suspension was filtered through sterile glass wool and adjusted to a concentration of 10^6 conidia ml^{-1}. At the time of bacterization or 1 wk after bacterization, plants were stem-inoculated with *F. o. dianthi* between the first and the second pair of leaves as described previously (4). Thirty microliters of the microconidial suspension was deposited in the axil of an artificial (Parafilm) pair of leaves tied to the stem. The stems were incised horizontally through the droplet just into the xylem. Control plants were mock-inoculated with sterile distilled water or bacterized with strain WCS417r.

To exclude competition between *F. o. dianthi* and strain WCS417r, the presence of strain WCS417r in the stems was checked both at the time of inoculation with *F. o. dianthi* and at the end of the experiment. Stem segments, 2 cm in length, were taken just above the stem base, surface-sterilized, cut into 2-mm-long segments, and incubated on KB supplemented with rifampicin (KBrif) (150 mg l^{-1}) for 2–4 days. Thereafter, growth of strain WCS417r on the medium was determined. Stem segments of nonbacterized plants were plated also.

Root surface colonization by strain WCS417r was determined at 0, 1, 2, 5, and 9 wk after inoculation with *F. o. dianthi* for Lena and at 0, 1, 2, 5, and 15 wk after inoculation for Pallas. Random samples of root pieces (0.3 g) were shaken in glass tubes containing 5 ml of 0.1 M $MgSO_4$ and 2.5 g of glass beads (0.17 mm diameter) for 1 min. Serial dilutions were plated on KBrif medium and incubated at 27 C for 48 h. Root pieces from non-bacterized plants were similarly plated. The number of colony-forming units (cfu) per gram of root tissue was determined.

Disease development. The number of diseased plants was scored weekly. A plant was considered to be diseased when the first local symptoms (chlorotic leaves, crook-neck shoots [3]) had developed. Disease was evaluated at 9 wk after inoculation.

Statistical differences in disease development among the treatments were analyzed by an analysis of variance at $P = 0.05$ followed by Fisher's least significant difference test for mean separations.

Experimental design. Five separate experiments were carried out, three for Pallas and two for Lena. In each experiment a total of 450 plants were used. Treatments were arranged in randomized block design and replicated six times. A total of 32 plants per cultivar and treatment were kept for evaluation of disease development.

Extraction of plant material. For extraction of phytoalexins, 5-cm-long stem segments were used (from 0 to 5 cm above the

TABLE 1. Major components in high-performance liquid chromatography chromatograms of acetone extracts after treatment of carnation with *Fusarium oxysporum* f. sp. *dianthi*[y]

Peak numbers	Components
5	Dianthramine (MW 289)
8	Hydroxydianthramide B[z] or HDB (MW 257)
11	Hydroxydianthalexin B or HDxB (MW 239)
12	Methoxydianthramide A or MDA (MW 301) + (low amounts of) methoxydianthramide R or MDR (MW 303) and methoxydianthramide B or MDB (MW 271)
13	Hydroxydianthalexin S or HDxS (MW 255) + hydroxydianthramide S methyl ester or HDSM (MW 287)
14	Methoxydianthramide S or MDS (MW 287) + unknown (MW 285)
15a	Methoxydianthramide A methylester or MDAM (MW 315)

[y] Only major peaks and major components given. Identification of the components is partly based on co-chromatography, mainly on pyrolysis-16 eV EI-MS and HR-40 eV EI-MS. Molecular weights given correspond with the molecular ions found. HDxS, MDA, and MDAM are new natural products (G. J. Niemann, *unpublished data*).

[z] A, B, S, R, and M stand for: *p*-anisic, benzoic, salicylic, resorcylic, and 4-methoxysalicylic acid, respectively.

TABLE 2. Incidence (%) of Fusarium wilt in carnation cultivars treated with *Pseudomonas* sp. strain WCS417r (Ps) and/or *Fusarium oxysporum* f. sp. *dianthi* (FOD)[x]

	Pallas			Lena	
Treatment[y]	I	II	III	I	II
Ps + FOD	22 a[z]	25 a	19 a	38 a	69 a
FOD	53 b	47 b	56 b	69 b	72 a
Ps/FOD	41 b	38 ab	53 b	59 b	75 a
LSD	18	11	17	12	...

[x] Results of three experiments for cultivar Palla and of two experiments for cultivar Lena.

[y] Plants were stem-inoculated with a spore suspension of *F.o. dianthi* either simultaneously with (Ps/FOD) or 1 wk after (Ps + FOD) bacterization with strain WCS417r. As controls, plants were inoculated with a cell suspension of WCS417r or water. Plants treated with water or WCS417r alone developed no disease symptoms.

[z] Numbers followed by the same letter within columns do not differ significantly, LSD ($P = 0.05$).

inoculation site), harvested at the time of inoculation and at 1, 2, 5, and 9 or 15 wk after inoculation with *F. o. dianthi* or after mock-inoculation. In one experiment, the roots were harvested as well, using the whole-root system, and were cleaned as far as possible from rock wool material. Phytoalexin accumulation was determined for 10 plants in a duplicate series of five plants each. Leaves were removed from the stems, and the stem segments were weighed, frozen, chopped while frozen, and homogenized for 5 min in acetone (25 ml) with a Bühler homogenizer (Edmund Bühler, Tübingen, Germany). The mixture was filtered, and the residue was washed twice with acetone. The combined filtrates were evaporated to dryness, and the residue was taken up in ethanol and analyzed by high-performance liquid chromatography (HPLC). In a number of cases, thin-layer chromatography (TLC) was applied to evaluate the fungitoxic activity of components in the acetone extracts (see TLC section).

HPLC. HPLC was performed with an LKB 2152 HPLC controller with two LKB 2150 HPLC pumps (LKB Produktor AB, Gromma, Sweden) with a 2- × 10-cm Chromsep Lichrosorp RP18 (ID, 3 mm) column (Chrompack, Middelburg, Netherlands), a Uvicord detector with a 275-nm filter, and a Spectraphysics SP4270 integrator (Spectra-Physics, Inc., San Jose, CA) or a Baseline 810 chromatography workstation (Base-Line, Inc., Kent, WA) . For some analyses, a Hewlett-Packard 1040A photodiode array detector (Hewlett-Packard Co., Avondale, PA) was used to obtain a complete UV spectrum to control possible deviations from peak identities. Samples (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 to 2 min, 25–75% from 2 to 15 min, 75–80% from 15 to 18 min, a linear part at 80% from 18 to 25 min, and returning from 80–25% from 25 to 30 min with a constant flow of 0.5 ml/min (pressure about 60 bar). For collection of HPLC peak fractions for pyrolysis mass spectrometry (PyMS), phosphoric acid in the solvent was replaced by formic acid (0.01%).

The large number of dianthramides that accumulate after fungal elicitation (16,18,19,21) allow no complete separation in one HPLC run, and most HPLC peaks contain a mixture of compounds. A number of the dianthramides (and dianthalexin) had previously been localized in the HPLC peaks (16). Identification of the major constituents was, among others, based on HPLC-MS (G. J. Niemann, *unpublished data*). For Pallas, HPLC peak fractions also were collected, rechromatographed, and analyzed by PyMS (19; G. J. Niemann, *unpublished data*) and, where necessary, by high resolution mass spectrometry (G. J. Niemann, *unpublished data*). Compounds present in the different HPLC peaks are listed in Table 1, with their molecular ions found by Py-low voltage EI-MS. PyMS was described previously (18,19). For the compound names, the system of Ponchet et al (21) was used, which is based on the type of anthranilic acid substitution (4-hydroxy (H)-, 4-methoxy (M)-dianthramide) and the type of coupled benzoic acid (B: benzoic, S: salicylic, R: resorcylic, and M: 4-methoxysalicylic; an A has to be added for the recently found *p*-anisic acid derivative) (G. J. Niemann, *unpublished data*). Analogous to the dianthramide nomenclature (21), dianthalexin was renamed as hydroxydianthalexin B or HDxB because other dianthalexins, such as hydroxydianthalexin S (MW 255), were found as well (G. J. Niemann, *unpublished data*).

Differences in phytoalexin accumulation between the treatment with strain WCS417r 1 wk before inoculation of the pathogen and inoculation with the pathogen alone were analyzed by a polynomial regression analysis and Student's *t* test (26). Correlations between peak accumulation and disease indices were tested for their significance by a rank correlation test (26).

TLC. To test for fungitoxic activity of the extracted samples, acetone extracts were separated by TLC on 20- × 20-cm Merck silica gel 60 F_{254} plates (E. Merck, Darmstadt, Germany), developed with cyclohexane-ethyl acetate (1:1, v/v). Compounds were detected under UV light (254 and 360 nm). Thereafter, TLC plates were sprayed with a 10^6 microconidia ml^{-1} suspension of *F. o. dianthi* or *Phialophora cinerescens*, another vessel parasite of carnations. Both were cultured in Czapek-Dox liquid medium. Plates were kept in a moist chamber for 48–72 h. Fungal growth was evaluated after treatment with iodine vapor.

Effects of methoxydianthramide S (MDS) and HDxB on germination of *F. o. dianthi*. Serum flasks, containing 2.5 ml of 40% strength Hoagland's solution (10), pH 7.0, buffered with 200 g of $CaCO_3$ l^{-1}, were supplemented with different concentrations of MDS or HDxB. Microconidia of *F. o. dianthi* were added to achieve a final concentration of 10^4 conidia ml^{-1}. Flasks were incubated for 15 h at 27 C. Thereafter, percentages of germination and germ-tube length were recorded.

RESULTS

Disease development. The incidence of Fusarium wilting of the two cultivars was significantly reduced when the plants had been bacterized 1 wk before inoculation with *F. o. dianthi* compared with plants that were inoculated with *F. o. dianthi* alone (Table 2). When *F. o. dianthi* and strain WCS417r were co-inoculated, no significant disease reduction was found in comparison with *F. o. dianthi* alone. Populations of strain WCS417r on roots decreased with time and ranged from 4.2×10^4 to 6.5×10^6 cfu g^{-1} root (Fig. 1). No significant differences in root colonization by strain WCS417r were found between the two cultivars (data not shown) or between *Fusarium*-inoculated or noninoculated plants. Strain WCS417r was not detected in the sliced stem segments.

Accumulation and identification of phytoalexins. Several UV-absorbing compounds (peaks 7–15), in particular components of peaks 11–15 (Table 1), accumulated in stem segments of *F. o. dianthi*-treated plants of Pallas (Fig. 2A,B). In the water-treated plants these peaks were present only at low levels. There was no effect of bacterization on accumulation of these compounds in the absence of *F. o. dianthi*. A significantly higher accumulation of peaks 11–15 was found in plants treated with strain WCS417r 1 wk before inoculation compared to nonbacterized *F. o. dianthi*-treated plants. For Lena, similar effects, although somewhat

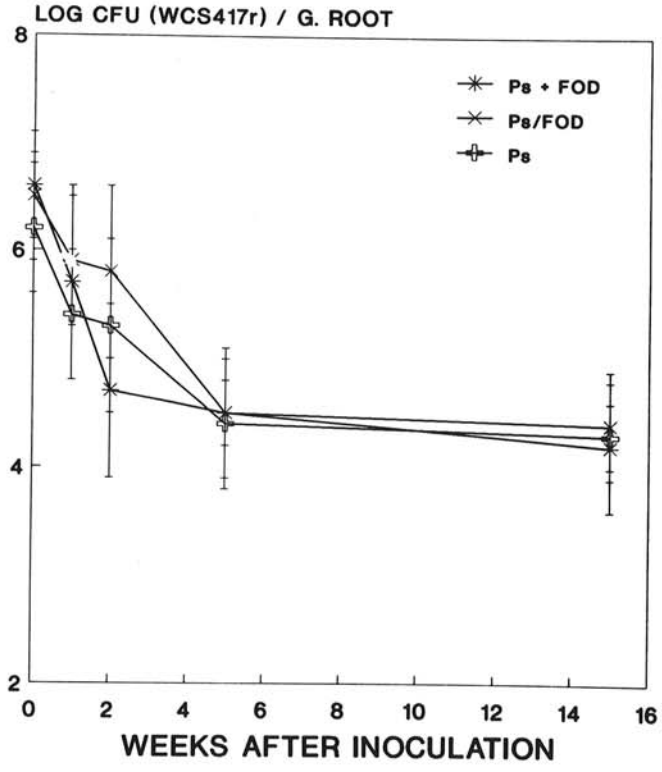

Fig. 1. Population size of *Pseudomonas* sp. (Ps) strain WCS417r on root surfaces of carnation cultivar Pallas. Plants were stem-inoculated with *Fusarium oxysporum* f. sp. *dianthi* (FOD) either simultaneously (Ps/FOD) or 1 wk after bacterization (Ps + FOD). The bars represent standard error.

retarded, were found in experiment I (Fig. 2C), but not in experiment II (Fig. 2D). In experiment II, there also was no effect of bacterization on disease incidence. Peaks 11–15 were sometimes slightly, but never significantly, increased in plants that were simultaneously bacterized with strain WCS417r and inoculated with *F. o. dianthi* compared with plants that had been inoculated with the fungus alone. Accumulation of the compounds of peaks 11–15 was inversely correlated with the number of diseased plants ($r = -0.90$; $P = 0.01$).

The identity of the compounds in the HPLC peaks was checked by comparison of the UV spectra in peak tops and shoulders obtained during an HPLC run with a diode array detector, with those of previous extracts (16; G. J. Niemann, *unpublished data*) and by peak isolation followed by PyMS and/or high resolution

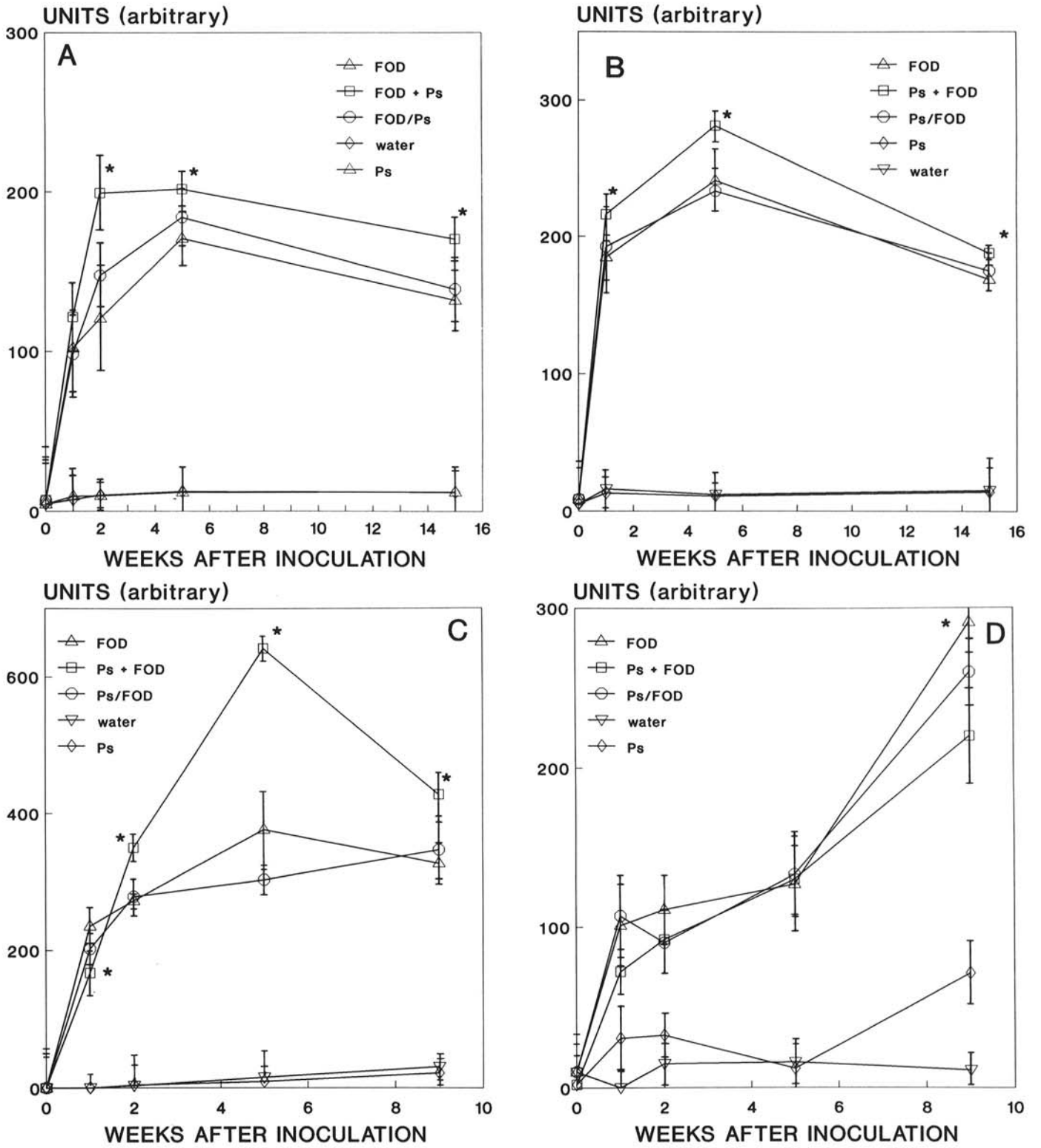

Fig. 2. Accumulation of phenolic compounds (present in peaks 11–15) in acetone extracts of stem segments of the carnation cultivars Pallas (**A, B**) and Lena (**C, D**) in two successive experiments. Plants were inoculated with *Fusarium oxysporum* f. sp. *dianthi* (FOD) and bacterized with *Pseudomonas* sp. strain WCS417r (Ps) either 1 wk prior to inoculation with *F. o. dianthi* (Ps + FOD) or simultaneously (Ps/FOD). As controls, the plants were bacterized with strain WCS417r alone or treated with water instead of *F. o. dianthi* (water). Amount (g^{-1} fresh weight of extracted segments) is given in arbitrary units. The bars represent the standard error.

MS. Although UV spectra are not very specific, the λ max of 255 nm of peak 5 (dianthramine), 252 nm of peak 8 (hydroxydianthramide B [HDB]), 260 nm of peak 11 (HDxB; 6), or 253 nm of peak 14 (MDS), with their respective secondary maxima and/or shoulders, proved rather indicative. Mass spectra of isolated HPLC peaks are, of course, more confirmative. A complete analysis of the extract from Pallas is outside the scope of the present article and will be published elsewhere. Molecular ions, found in the respective peaks, have been tabulated with the respective compounds in Table 1, however. Practically all UV absorption found for peaks 8–15 could be ascribed to dianthramide-type constituents. Dianthalexin appeared not the only benzoxazinone-type compound, some other substituted derivatives were present as well (G. J. Niemann, *unpublished data*). Dianthalexin, therefore, is further called hydroxydianthalexin B. The results confirm our earlier concept that all carnation cultivars are capable of synthesizing and accumulating all dianthramides and dianthalexins, although the amounts and proportions depend both on elicitor type and cultivar (G. J. Niemann, *unpublished data*).

Evaluation of the different HPLC peaks showed that especially the compounds responsible for peak 14 (which include, among others, MDS) (Fig. 3B) and to some lesser extent peaks 11–12 (whose major constituents are HDxB in peak 11 and methoxydianthramide A or MDA in peak 12, with many minor ones) (Table 1; Fig. 3A) accumulated to significantly higher levels in bacterized and *F. o. dianthi*-inoculated plants compared to plants inoculated with *F .o. dianthi* alone.

In roots, UV-absorbing compounds were found after all treatments (data not shown). In contrast to the accumulation of phytoalexins in the stem, no differences were found between the various treatments, and therefore this aspect was not investigated in detail.

Fungitoxic activity of acetone extracts. Acetone extracts of treated and control plants were chromatographed on TLC and sprayed with a microconidial suspension of *F. o. dianthi* or *P. cinerescens*. In contrast to the water-treated and bacterized plants, only treatments with *F. o. dianthi* induced three UV-absorbent spots that were not overgrown by *F. o. dianthi* and one or two more after spraying *P. cinerescens*. Two of these spots had R_f values similar to those of MDS and HDxB.

Effect of MDS and HDxB on germination of *Fusarium* conidia. Both MDS and HDxB significantly reduced the percentage of germination of microconidia (Fig. 4) as well as germ-tube length (data not shown) with concentrations of 5 nmol and greater of MDS and HDxB. At equal concentrations of both phytoalexins, inhibition on germination by MDS was slightly higher than that by HDxB. Nevertheless, the LD_{50} of both phytoalexins was of the same magnitude, approximately 100 nmol.

DISCUSSION

Fusarium wilt of stem-inoculated carnations of the moderately resistant cultivar Pallas was significantly reduced by root bacterization with *Pseudomonas* sp. strain WCS417r. This reduction was found only when the plants were bacterized 1 wk before stem inoculation with *F. o. dianthi* and not when they received both treatments simultaneously (Table 2). Strain WCS417r was not detected in the stem, which indicated that the bacterium and the fungus remained spatially separated during the experiment. Because strain WCS417r and the pathogen were spatially separated, we conclude that control of the disease is not due to competition. Strain WCS417r induced an enhanced resistance to *F. o. dianthi* in carnation. A lag period appeared to be necessary. This is in agreement with results obtained with fungi that induce systemic resistance (13).

Inoculation of carnation with *F. o. dianthi* resulted in an increase of dianthramide-type phenolic compounds in the stem. This increase could be manipulated by bacterization with strain WCS417r (Figs. 2 and 3). Along with the induced resistance, we observed a higher accumulation of the compounds in HPLC peaks 11–15. Accumulation of dianthramides was not found when plants had been bacterized only. Accumulation of compounds in HPLC peaks 11–15 was inversely correlated with the number of diseased plants. This is in agreement with previously reported results (4) obtained with 11 carnation cultivars that differed in

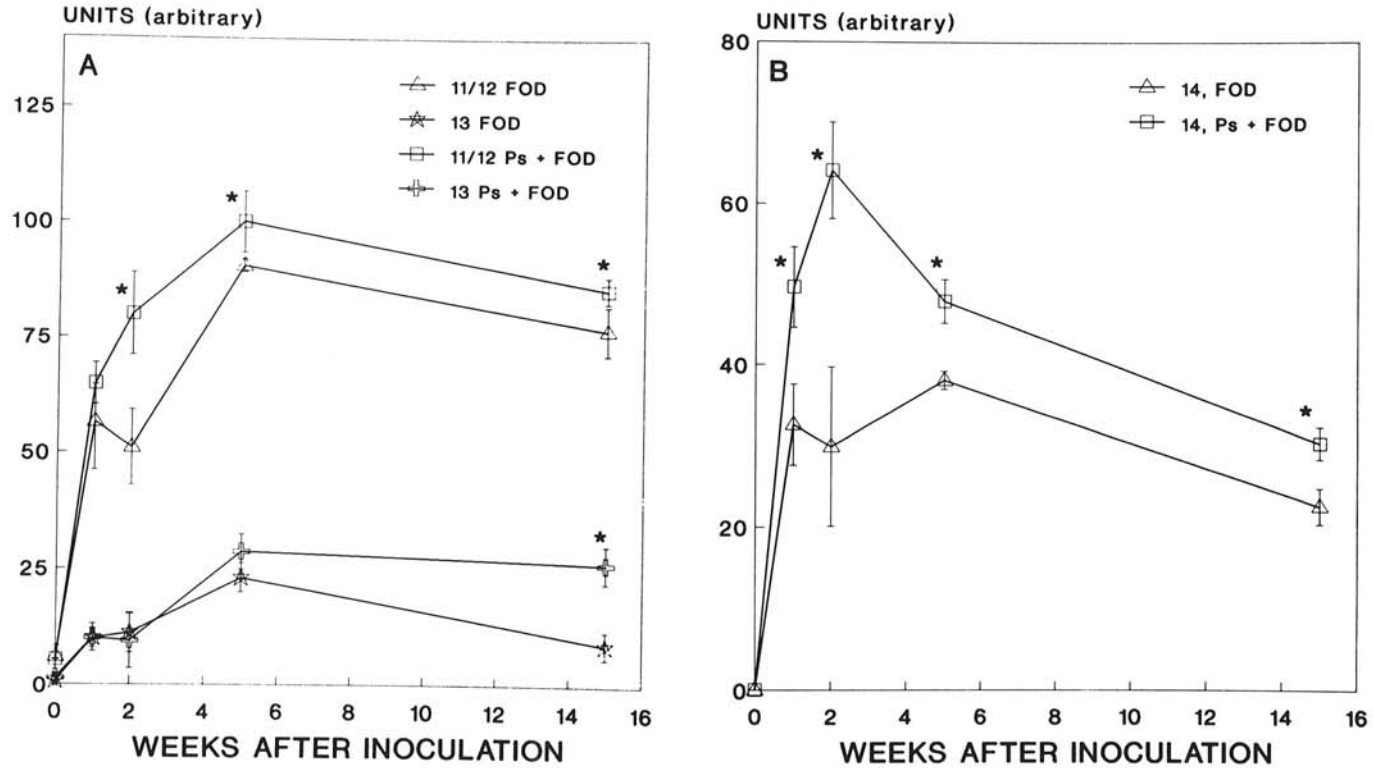

Fig. 3. Accumulation of the compounds of the individual peaks 11 + 12, 13 (**A**) and 14 (**B**) in acetone extracts of stem segments of the carnation cultivar Pallas. Plants were inoculated with *Fusarium oxysporum* f. sp. *dianthi* (FOD) or bacterized with *Pseudomonas* sp. WCS417r (Ps) 1 wk prior to inoculation with *F. o. dianthi*. Amount (g^{-1} fresh weight of extracted segments) is given in arbitrary units. Asterisks indicate significant differences.

their resistance level to *F. o. dianthi*.

Considering the efforts of the Antibes-Lyon research group (6,9,20–23) in isolation, identification, and synthesis of dianthalexins and dianthramides, their reports on the in vitro fungitoxicity of those compounds are comparatively meagre. This may be due to testing of variously substituted dianthalexin and dianthramide analogues (9) suggesting fungitoxicity for all hydroxy- and methoxy-substituted dianthramide analogues, more or less independent of the number and/or location of the substituent. An in vitro fungitoxic effect has been reported for HDxB (6,9), for HDB and hydroxydianthramide S (HDS) (9), and for hydroxydianthramide S methyl ester (HDSM) (or dianthramide B) and MDS (or dianthramide A) (22,23). The fungitoxic effect of HDxB and MDS, main components of peaks 11 and 14, also was demonstrated in the present study (Fig. 4). In addition, three other components were found to be fungitoxic, two of which could probably be ascribed to HDB (peak 8) and HDSM + HDxS (peak 13). Thus, all major HPLC peaks in the series 11–15 represent phytoalexins.

Whether these phytoalexins and resistance are causually related is not known. Accumulation of phytoalexins at the site of infection (18) may retard fungal growth and allow the plant to occlude the infected vessel and consequently encage the pathogen (2,3,5). Accumulation of phytoalexins in carnation stems also seems to mark an induced resistance response. The observation that phytoalexins did not accumulate to a higher extent when induced resistance could not be demonstrated is another example that phytoalexins are indicators of resistance level (Fig. 2D). Possibly, phytoalexin accumulation is more variable in susceptible than in more resistant cultivars. This variability may be due to unknown environmental factors. It was already noted by Ponchet et al (20) that phytoalexin production fluctuated depending on the season of the year.

Bacterization of roots with strain WCS417r affected both the synthesis and accumulation of phytoalexins in the stem, but only when the stem was inoculated with *F. o. dianthi*. Obviously signals provided by strain WCS417r at the root surface induced sensitization of the stem. The identity of these signals and their translocation from the root to the site of infection in the stem is currently being investigated.

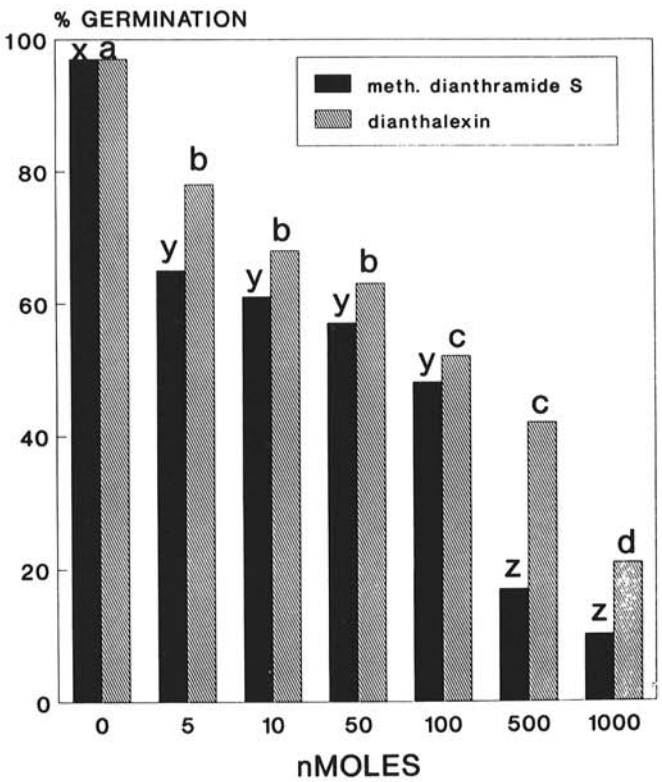

Fig. 4. Effect of various concentrations of methoxydianthramide S and dianthalexin or hydroxydianthalexin B on germination of microconidia of *Fusarium oxysporum* f. sp. *dianthi*. Percentage of germination was determined 15 h after incubation. Bars topped with corresponding letters are not significantly different ($P \leq 0.05$).

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