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Identification of a polyacetylenic phytoalexin in tomato plants after inoculation with Verticillium albo-atrum

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Up till now rishitin has been the only identified phytoalexin accumulating in stem and root tissues of tomato plants after infection with *Verticillium albo-atrum* Reinke & Berth. Although several other phytoalexins have been detected with bioassays on thin-layer plates, these compounds have never been isolated and characterized (Tjamos and Smith, 1974; Elgersma, 1980).

In an attempt to identify these compounds, we inoculated stems of six-week-old plants of the susceptible tomato cultivar Maascross with a suspension of 10^7 conidia.ml^{-1} as previously described by Elgersma (1980). One week after inoculation stem pieces of about 30 cm length, cut 0.5 cm above the site of inoculation were sampled and stored at -20 °C. After storage the cortex was removed and the vascular tissue excised and ground in liquid nitrogen in a mortar.

The vascular tissue was extracted as previously described by Elgersma (1980). The frozen tissue powder (300 g) was suspended in methanol (5 ml per g tissue) and kept at 4 °C in the dark for 24 h. After filtration (Whatman No.1) the residue was resuspended in fresh methanol and stirred at room temperature for 1 h. After filtration the methanol fractions were pooled and evaporated to dryness. The residue was dissolved in 60% methanol (1 ml per g fresh weight of vascular tissue) and partitioned three times with an equal volume of chloroform. The combined chloroform fractions were evaporated and chromatographed on a silica gel (Fluka, 100 mesh) column (12×5 cm). The column was eluted with cyclohexane : ethyl acetate (1 : 1, v/v). By this procedure a crude separation of the various phytoalexins and other compounds was obtained.

The fractions containing the antifungal compound with R_f 0.42 on silica gel thin-layer plates developed in cyclohexane : ethyl acetate (1 : 1, v/v). were pooled, dried and thin layer chromatographed (silica gel, 0.5 mm thickness) in the solvent system mentioned above. The band of silica gel containing the antifungal compound was eluted with chloroform. The eluate was further purified by t.l.c. (silica gel, 0.5 mm thickness) in chloroform : methanol (96 : 4, v/v) and the band of silica gel containing the antifungal compound (R_f 0.28) was eluted with chloroform. By this procedure 1.2 mg of a sufficiently pure compound was obtained for further characterization.

The spectral data (u.v., n.m.r. and m.s.) were completely in accordance with those recently recorded for the polyacetylene cis-tetradeca-6-ene-1,3 - diyne-5,8-diol by De Wit and Kodde (in press) (Fig. 1), found in tomato fruits or leaves after inocula-

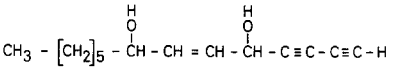

Fig. 1. Structural formula of cis-tetradeca-6-ene-1,3-diyne-5,8-diol.

Fig. 1. Structuur formule van cis-tetradeca-6-een-1,3-diyn-5,8 diol.

tion with *Cladosporium fulvum*. We concluded, therefore, that our isolated phytoalexin was identical with that compound and that this compound can also be produced in stems of tomato plants after inoculation with *V. albo-atrum*. Isolation and identification of other phytoalexins induced by *V. albo-atrum* in tomato stems are in progress.

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Samenvatting

Identificatie van een polyacetyleen-fytoalexine in tomateplanten na inoculatie met Verticillium albo-atrum

Een week na inoculatie van vatbare tomateplanten met *Verticillium albo-atrum* werd uit het vaatweefsel een fytoalexine geïsoleerd, dat idententiek bleek te zijn aan het polyacetyleen cis-tetradeca-6-een-1,3-diyn-5,8-diol.

References

Elgersma, D. M., 1980. Accumulation of rishitin in susceptible and resistant tomato plants after inoculation with *Verticillium albo-atrum*. Physol. Pl. Path. 16: 149-153.

Tjamos, E. C. & Smith, I. M., 1974. The rôle of phytoalexins in resistance of tomato to *Verticillium* wilt. Physiol. Pl. Path. 4: 249-259.

Wit, P. J. G. M. de & Kodde, E., 1981. Induction of polyacetylenic phytoalexins in *Lycopersicon esculentum* after inoculation with *Cladosporium fulvum* (syn. *Fulvia fulva)*. Physiol. Pl. Path. (in press).

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