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Lack of phytoalexin involvement in the antagonism of Alternaria tenuissima against Alternaria zinniae on dwarf bean leaves

J. VAN DEN HEUVEL, A. H. VERHEUS and C. J. KRUYSWIJK

Phytopathological Laboratory 'Willie Commelin Scholten', Baarn

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In studies on the antagonistic effects of epiphytic microorganisms on infection of dwarf bean (*Phaseolus vulgaris* L.) leaves by *Alternaria zinniae* Pape, it has been shown that *Alternaria tenuissima* (Fr.) Wiltsh. reduced numbers of *A. zinniae* lesions by inhibiting spore germination (Van den Heuvel, 1970). It was suggested that this antagonistic effect was mainly due to the action of inhibitors whose formation by the leaf was induced by *A. tenuissima*. Since then, however, we have obtained evidence indicating that no known bean phytoalexins are involved in this antagonism.

Primary leaves of 10- to 13-day-old dwarf bean (cv. Corene) plants were sprayed with suspensions of *A. tenuissima* and/or *A. zinniae* conidia (5×10^6 conidia/ml and 6.25×10^4 conidia/ml, respectively). The inoculated plants were incubated in humidity chambers in the greenhouse at about 23 °C. At various times after inoculation, leaves were collected, frozen in liquid nitrogen, ground with a pestle in a mortar and lyophilized. Two-hundred-mg (dry weight) samples were analysed for the presence of phytoalexins by methods of extraction and separation as described elsewhere (Van den Heuvel and Grootveld, 1978), except that sometimes chloroform instead of 60% methanol was used in the extraction. Quantitative analysis of phytoalexins was carried out by UV spectrometry of ethanolic eluates of compounds scraped from developed silica gel thin-layer chromatography (TLC) plates.

In leaves inoculated with *A. zinniae* alone, an average of 98 μg phaseollin/g dry weight of tissue was found 3 days after inoculation (Fig. 1). Less phaseollin accumulated in leaves pre-inoculated with *A. tenuissima* 1 day before inoculation with *A. zinniae*. The same was true in other mixed inoculations, where *A. tenuissima* was applied to leaves simultaneously, or 2 days prior to inoculation with *A. zinniae*. Mixed inoculations gave rise to fewer lesions than did inoculation with *A. zinniae* alone. No phytoalexins could be detected in leaves inoculated with only *A. tenuissima*. Besides phaseollin, no other known bean phytoalexins were found in leaves infected by *A. zinniae*, except that sometimes low concentrations of phaseollinisoflavan were detected.

In bioassays carried out by spraying suspensions of *A. zinniae* spores on developed TLC plates, no other consistent inhibitory compounds could be detected in extracts from bean leaves inoculated with *A. tenuissima* alone or in combination with *A. zinniae*.

The results indicate that none of the known bean phytoalexins or any other inhibi-

Fig. 1. Concentration of phaseollin in bean leaves inoculated with *A. zinniae* alone (■), with *A. zinniae* one day after a pre-inoculation with *A. tenuissima* (●), or with *A. tenuissima* alone (▲). Vertical lines denote standard deviations from means of three to seven replicate values.

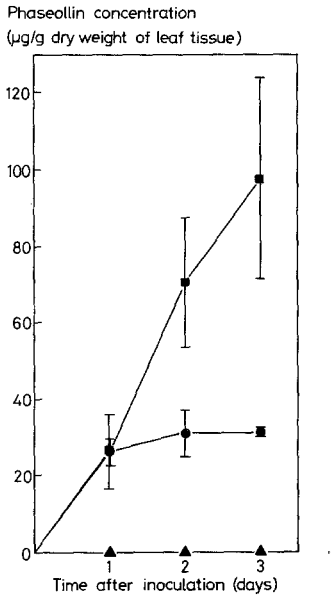

Fig. 1. Concentratie van phaseolline in bonebladeren geïnoculeerd met alleen A. zinniae (■), met A. zinniae één dag na een voor-inoculatie met A. tenuissima (●), of met alleen A. tenuissima (▲). De vertikale lijnen geven de standaardafwijkingen aan van het gemiddelde van drie tot zeven waarnemingen.

tory compound of similar polarity is involved in the antagonism of *A. tenuissima* against *A. zinniae* on dwarf bean leaves. The concentration of phaseollin that accumulated in leaves infected by *A. zinniae* seemed rather to be controlled by the number of lesions, since higher concentrations of phaseollin were found as leaves contained more lesions three days after inoculation.

Samenvatting

Geen fytoalexinen betrokken bij het antagonisme van Alternaria tenuissima tegen Alternaria zinniae op bonebladeren

In bonebladeren geïnoculeerd met alleen *Alternaria zinniae* werd altijd meer phaseolline aangetroffen dan in bladeren geïnoculeerd met een combinatie van *A. zinniae* en de antagonist *A. tenuissima* (Fig. 1). Soms was ook wat phaseollineïsoflavaan aanwezig. In bladeren geïnoculeerd met de antagonist alleen werden geen fytoalexinen gevonden. Ook konden geen andere remstoffen worden aangetoond.

Geconcludeerd wordt dat geen van de bekende fytoalexinen of een remstof van gelijke polariteit betrokken is bij het antagonisme van *A. tenuissima* tegen *A. zinniae* op bonebladeren.

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References

Heuvel, J. van den, 1970. Antagonistic effects of epiphytic microorganisms on infection of dwarf bean leaves by *Alternaria zinniae*. Thesis, Utrecht. Meded. phytopath. Lab. Willie Commelin Scholten No. 84, 84 pp.

Heuvel, J. van den & Grootveld, Dineke, 1978. Phytoalexin production in French bean leaves infected by *Botrytis cinerea*. Neth. J. Pl. Path. 84: 37–46.

Address

Phytopathologisch Laboratorium 'Willie Commelin Scholten', Javalaan 20, 3742 CP Baarn, the Netherlands.