Neth. J. Pl. Path. 74 (1968) 44–47

SHORT COMMUNICATIONS

Breakdown of pisatin by some fungi pathogenic to Pisum sativum

A. de Wit-Elshove

Laboratory of Phytopathology, Wageningen

Accepted 20 November, 1967

Peas *(Pisum sativum)* are known to synthesize an antifungal compound, pisatin (Cruickshank and Perrin, 1960). In healthy plants no pisatin or only small amounts are present, but after inoculation with a fungal spore suspension (or after certain chemical treatments) pisatin is formed. This compound is supposed to play a major role in the resistance of pea plants to non-pathogenic fungi. Pathogens of peas are less sensitive to pisatin than non-pathogens (Cruickshank, 1962). An answer to the question, why pea pathogens are less sensitive, has not yet been given. To study this, the following investigations were carried out.

For a better understanding of the experimental results, some physical and chemical properties of pisatin will first be mentioned. Pisatin shows U.V. absorption maxima in alcohol at 309 mμ (log ε 3.86) and 286 mμ (log ε 3.68) (Perrin and Bottomley, 1961). In aqueous solutions these maxima shift to lower wavelengths: 306 and 284 mμ. At lower pH pisatin is transformed to anhydropisatin and this causes a bathochromic shift and intensification of the U.V. absorption spectrum with maxima at 339 mμ (log ε 4.58) and 358 mμ (log ε 4.60). The quantitative formation of anhydropisatin from pisatin has been used to demonstrate low levels of pisatin.

Fifty-ml flasks, containing 10 ml nutrient medium (Jermyn, 1959), were inoculated with 1 ml each of a spore suspension of 100,000 spores/ml of the following fungi: *Fusarium oxysporum* f. sp. *pisi race 1* Sny. et Hans. and *Mycosphaerella pinodes* (Berk. et Blox.) Stone (pathogenic to pea plants); and also with *Cladosporium cucumerinum* Ellis et Arth., *Colletotrichum lindemuthianum* (Sacc. et Magn.) Bri. et Cav. and *Monilinia fructigena* (Aderh. et Ruhl) Honey (non-pathogenic to pea plants). The flasks were incubated as shake cultures at 24 °C for 1 week. Thereafter to half of the flasks (A) 5 ml of an aqueous solution of pisatin were added and to the other half (B) 5 ml distilled water. One flask (C), containing the nutrient medium but not inoculated, served as a control to test for stability of pisatin; pisatin was added after 1 week, and the flask was incubated with the others. Two weeks after the beginning of the experiment, the contents of the flasks were centrifuged at 18,000 × g for 30 min. U.V. absorption spectra of the supernatants were measured in a Beckman DB-G spectrophotometer, and the quantities of pisatin were determined. The pellets, mainly containing the mycelia, were washed with distilled water, extracted in 4 ml distilled

alcohol and U.V. absorption spectra of these extracts were recorded. Differences in the absorption spectra at 309 mμ between the extracts of cultures A and B of each fungus could provide an indication of the presence of pisatin in cultures A. To confirm these results, all extracts of cultures A were acidified and kept in the dark at 4 °C for 24 h; under these circumstances pisatin is converted to anhydropisatin. The presence of anhydropisatin could be demonstrated by measuring the U.V. absorption spectra.

The supernatants of the media to which pisatin had been added and which had been inoculated with *C. cucumerinum*, *C. lindemuthianum* and *M. fructigena* still contained pisatin, as could be deduced from the U.V. absorption spectra; the U.V. absorption spectra of supernatants from media inoculated with *F. oxysporum* f. sp. *pisi* and *M. pinodes* did not show the presence of pisatin. The results of some of these experiments are given in Fig. 1. A comparison of the ultraviolet absorption spectra of the alcohol extracts of the pellets before and after acidification proved that only the extracts originating from *C. cucumerinum* (A), *C. lindemuthianum* (A) and *M. fructigena* (A) contained pisatin. Fig. 2 shows the absorption spectra obtained in some of these experiments.

The quantities of pisatin present in the supernatants and the alcohol extracts of the pellets, as calculated from the absorption maxima of the spectra, are given in Table 1. The results of the experiments, which have been repeated several times, show that pisatin could not be recovered in the supernatants or the mycelia of the pathogenic fungi *F. oxysporum* f. sp. *pisi* and *M. pinodes*; on the other hand, nearly all the pisatin added could be recovered in the supernatants or in the mycelia of the three non-pathogenic fungi. The results suggest that *F. oxysporum* f. sp. *pisi* and *M. pinodes* possess a mechanism to metabolize pisatin, and that the other three fungi do not have such a mechanism. This may explain why *F. oxysporum* f. sp. *pisi* and *M. pinodes* are pathogens of peas, while the other three fungi are not pathogenic to peas. These results seem to be contradictory to those of Cruickshank and Perrin (1965), who found that the mycelium of *Ascochyta pisi* Lib. did not break down pisatin. An explanation for this apparent discrepancy cannot yet be given.

The experiments are being extended now to some other pea pathogens and non-pathogens, using labeled pisatin.

Table 1. The quantities of pisatin present in the supernatants and in the alcohol extracts of the pellets, as calculated from the absorption maxima of the spectra

	μg pisatin, recovered in the supernatants A	*μg pisatin, present in the mycelium extracts A, measured from the anhydropisatin peaks*
Fusarium oxysporum f. sp. *pisi*	0	0
Mycosphaerella pinodes	0	0
Cladosporium cucumerinum	±285	±140
Colletotrichum lindemuthianum	±500	± 30
Monilinia fructigena	±500	± 20
Control C	±520	–

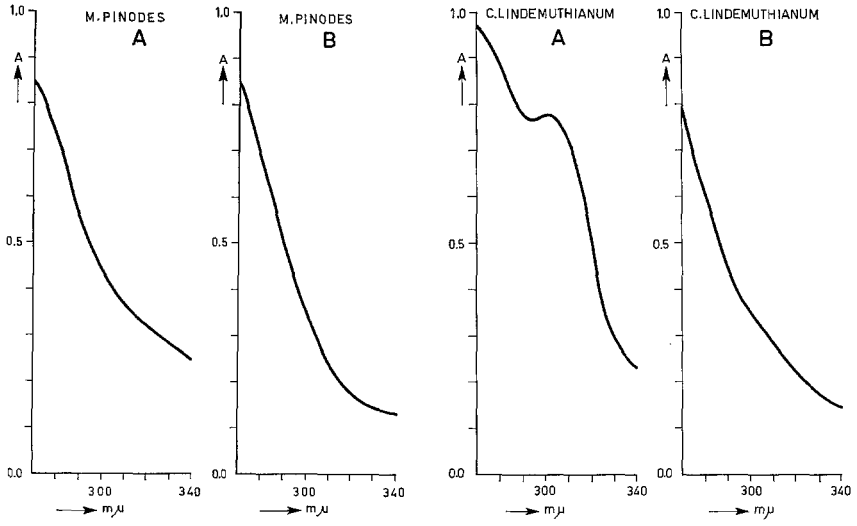

Fig. 1. U.V. absorption spectra of the supernatants from cultures of *M. pinodes*, *C. lindemuthianum* and *C. cucumerinum*, diluted 1:2.
A: pisatin added to the culture medium.
B: water added to the culture medium.
C: control, containing nutrient medium and pisatin.
(For further details, see text)

Samenvatting

Afbraak van pisatine door enige pathogene schimmels van Pisum sativum

Aan een voedingsmedium, geïnoculeerd met elk der schimmels *Fusarium oxysporum* f. sp. *pisi* ras 1, *Mycosphaerella pinodes* (pathogenen van de erwt), *Cladosporium cucumerinum*, *Colletotrichum lindemuthianum* en *Monilinia fructigena* (niet-pathogenen van de erwt) werd pisatine toegevoegd. Na incubatie gedurende een week werd het medium gecentrifugeerd. Zowel de bovenstaande vloeistof als het mycelium werd onderzocht op de aanwezigheid van pisatine. Uit de U.V.-absorptiespectra van de vloeistof en alcoholextracten van het mycelium blijkt, dat beide pathogenen het pisatine kunnen afbreken, maar de drie niet-pathogenen niet.

References

Cruickshank, I. A. M., 1962. Studies on phytoalexins. IV. The anti-microbial spectrum of pisatin. Aust. J. Biol. Sci. 15:147–159.

Cruickshank, I. A. M. and Perrin, D. R., 1960. Isolation of a phytoalexin from Pisum sativum L. Nature, Lond. 187:799–800.

Cruickshank, I. A. M. and Perrin, D. R., 1965. Studies on phytoalexins. VIII. The effect of some further factors on the formation, stability and localization of pisatin in vivo. Aust. J. biol. Sci. 18: 817–828.

Jermyn, M. A., 1959. Some comparative properties of β-glucosidases secreted by fungi. Aust. J. biol. Sci. 12:213–222.

Perrin, D. R. and Bottomley, W., 1961. Pisatin: an antifungal substance from Pisum sativum L. Nature, Lond. 191:76–77.

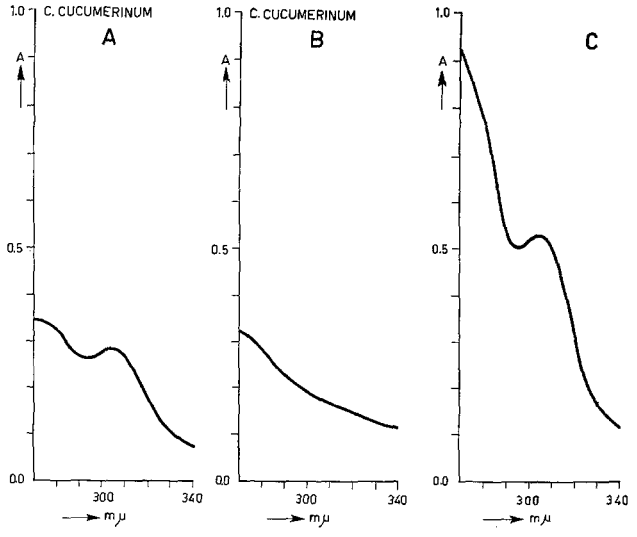

Fig. 1. *Continued*

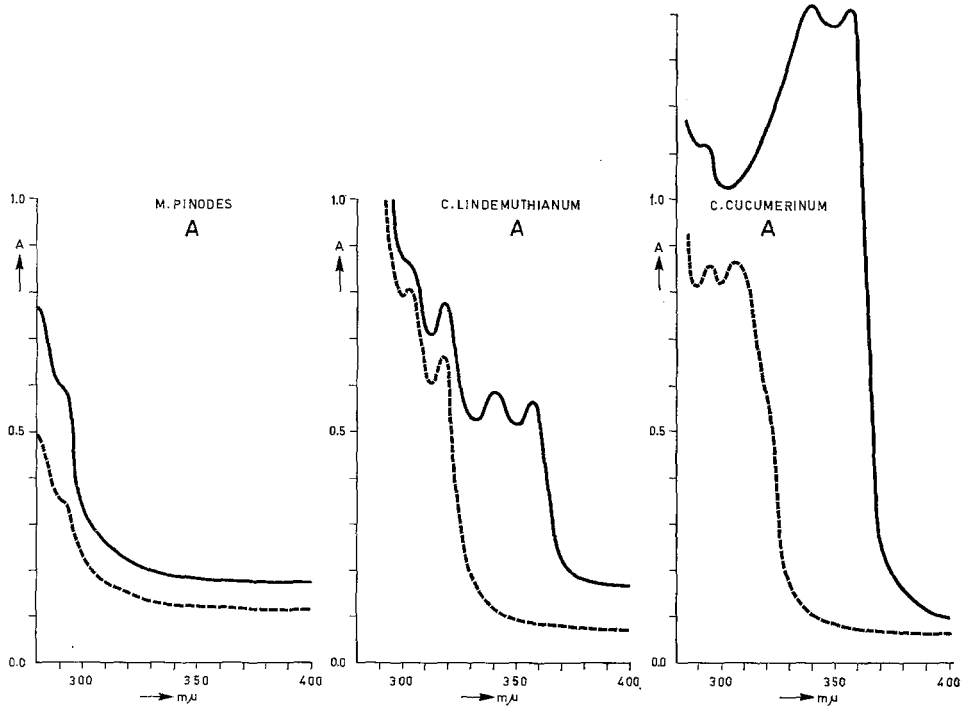

Fig. 2. U.V. absorption spectra of the alcohol extracts of the pellets of *M. pinodes*, *C. lindemuthianum* and *C. cucumerinum*, before (dotted line) and after acidification (solid line), diluted 1:2.
A: pisatin added to the culture medium. (For further details, see text)