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Indirect effect of 6-azauracil on Pythium debaryanum in cucumber

E. STĂNKOVÁ-OPOČENSKÁ[1] and J. DEKKER

Laboratorium voor Fytopathologie, Wageningen

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Abstract

Protection of cucumber seedlings against damping off, caused by *Pythium debaryanum*, was obtained by soaking the seeds in a solution of 1 ppm 6-azauracil (AzU), but not by treatment with a 10 or 100 ppm AzU solution. After the 1 ppm AzU treatment of the seeds, the rhizosphere microflora of the developing seedlings was changed; an increase of the bacterial population and a decrease of the fungal population was observed. Since *P. debaryanum* is rather insensitive to AzU in vitro, it is suggested that the control of damping off is obtained in an indirect way, possibly via the microflora of the roots.

Introduction

6-Azauracil (AzU) provides systemic control of various powdery mildew diseases and cucumber scab. Using *Cladosporium cucumerinum* as a test organism, Dekker (1968) showed that this compound is converted by the fungus into 6-azauridine-5'-phosphate, which interferes with pyrimidine biosynthesis. Various other fungi, among which *Pythium debaryanum*, are rather insensitive to AzU. In spite of this, control of damping off in cucumber seedlings was obtained after treatment of the seeds by this chemical at extremely low concentrations. Control of fungal plant diseases by systemic compounds, which do not inhibit the pathogen concerned in vitro, has been reported in the literature. In most cases it is not clear in which way the systemic does enhance the resistance of the host plant against the pathogen. Vraný et al. (1962) observed that application of urea to the above-ground parts of wheat plants increased the number of bacteria on the roots, but decreased the number of fungi. He noticed qualitative and quantitative changes in the root exudates of treated plants (Vraný, 1965). This led us to investigate whether 6-azauracil, when applied to the seed or to the leaves of cucumber plants, did provide protection against *P. debaryanum* by influencing the composition of the rhizosphere microflora.

Materials

6-Azauracil was obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio, U.S.A. The experiments were carried out with cucumber cv. 'Groene'. The nutrient media used, were composed as follows: Thornton medium (modified) for the assessment of bacteria and actinomycetes: 1000 ml H_2O, 1 g K_2HPO_4, 0.2 g

[1] Present address: Research Institute of Ornamental Gardening, Průhonice, Czechoslovakia.

$MgSO_4.7H_2O$, 0.1 g $CaCl_2$, 0.1 g NaCl, 0.01 g $FeCl_3$, 1 g $(NH_4)_2SO_4$, 1 g peptone, 1 g glucose, 20 g agar-agar; pH 7.

Medium containing rose Bengal with streptomycin for the assessment of fungi: 1000 ml H_2O, 1 g $NaNO_3$, 1 g K_2HPO_4, 10 g glucose, 15 g agar-agar; after sterilization 0.07 g rose Bengal and 0.003 g streptomycin were added; pH 6.8.

Taylor medium I for the assessment of bacteria, using nitrogen in the mineral form: 1000 ml H_2O, 0.5 g $(NH_4)_2HPO_4$, 0.4 g K_2HPO_4, 0.05 g $MgSO_4$, 0.1 g NaCl, 0.01 g $FeCl_3$, 1 g glucose, 15 g agar-agar.

Taylor medium II for the assessment of bacteria, using nitrogen in the form of amino acids; 1000 ml H_2O, 0.4 g K_2HPO_4, 0.05 g $MgSO_4$, 0.1 g NaCl, 0.01 g $FeCl_3$, 1 g glucose, 4 g casamino acids free of vitamins, 15 g agar-agar, pH 7.

Results

Influence of AzU on germination of seeds

Seeds of cucumbers were soaked for 6, 10 and 24 hours in aqueous solutions containing, 1, 10, 100 and 1000 ppm of AzU, and plated out on wet filter paper in petridishes of 15 cm diameter. The number of germinated seeds was counted after 16, 24 and 40 h (Table 1). Results obtained when using 1000 ppm have not been included because of serious phytotoxicity.

It appears that the germination of AzU-treated seeds proceeds faster than that of water soaked seeds. After germination a retardation of root and shoot growth was observed at 100 ppm AzU.

Control of damping off by AzU

Cucumber seeds were disinfected with 0.1 % sublimate and subsequently soaked in aqueous solutions of 1, 10 and 100 ppm AzU for 10 h. One hundred seeds were then plated out on wet filter paper in petridishes, and after 48 h covered with a 1.5 cm thick layer of sterilized sand, infested with *P. debaryanum*. The number of seedlings which, after emergence, were killed by the fungus, was recorded after 7, 8 and 9 days (Table 2). In a similar series without the fungus no diseased seedlings were observed.

It appears that the emerging seedlings are protected against attack by *P. debaryanum*, when the seeds have been soaked in 1 ppm AzU, but not when treated at a much higher AzU-concentration of 10 and 100 ppm (Fig. 1).

Table 1. Influence of 6-azauracil on germination of cucumber seeds; 100 seeds were soaked during 6, 10 and 24 h in aqueous solutions of AzU 1, 10 and 100 ppm.

Germinated after	6 hours				10 hours				24 hours			
ppm	0	1	10	100	0	1	10	100	0	1	10	100
16 hours	39	79	79	80	80	93	94	91	20	58	47	63
24 hours	87	96	95	92	98	96	96	96	99	100	99	100
40 hours	98	100	100	100	99	99	100	99	99	100	100	100

Tabel 1. Invloed van 6-azauracil op kieming van komkommerzaden; 100 zaden werden gedurende 6, 10 en 24 uur gekweekt in oplossingen van 1, 10 en 100 ppm AzU in water.

Table 2. Effect of AzU-treatment of cucumber seeds on damping off.

ppm	0	1	10	100
Germinated after 36 hours	91	96	94	90
Killed by *P. debaryanum* after: 7 days	16	0	6	8
8 days	37	0	26	27
9 days	47	0	41	35

Tabel 2. Het effect van een AzU-behandeling van komkommerzaden op aantasting der kiemplanten.

Fig. 1. Effect of AzU treatment of cucumber seeds on damping off: protection is obtained by 1 ppm but not by 10 or 100 ppm AzU. Upper row: seeds sown in *Pythium*-infested sand. Lower row: control, not infested. From left to right: water, 1, 10 and 100 ppm AzU.

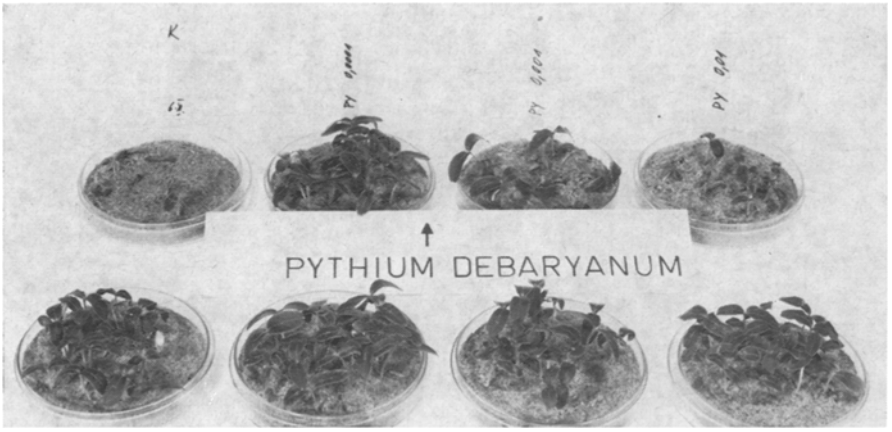

Fig. 1. Het effect van AzU-behandeling van komkommerzaden op aantasting der kiemplanten: bescherming wordt verkregen met 1 ppm, echter niet met 10 of 100 ppm AzU. Bovenste rij: zaden gezaaid in zand geïnfecteerd met Pythium. Onderste rij: contrôle, niet met Pythium geïnfecteerd zand. Van links naar rechts: water, 1, 10 en 100 ppm AzU.

Roots of these seedlings were washed with sterile water, plated out on malt agar, and incubated at 24 °C for 24 h. No mycelial growth occurred in the 1 ppm treatment, moderate growth in the 10 ppm treatment and normal growth of *P. debaryanum* in the 100 ppm treatment (Fig. 2). These results agree with the observed control of damping off obtained by treatment of the seed.

In vitro *P. debaryanum* is rather insensitive to AzU. Growth of the mycelium was scarcely retarded on potato dextrose agar, containing 1000 ppm of this chemical. It is therefore suggested that the effect of AzU against damping off at 1 ppm is due to an indirect action against the fungus.

Influence of AzU on the rhizosphere microflora

Ten days old seedlings were sprayed with a 100 ppm solution of AzU. After 2 days root samples were analysed for the occurrence of bacteria and fungi. One gram of

Fig. 2. *Pythium debaryunum*, developing from the roots of cucumber seedlings; seeds soaked in water, 1, 10 and 100 ppm AzU (from left to right). Inhibition of mycelial growth in the 1 ppm AzU treatment, but little or not in the 10 or 100 ppm treatments.

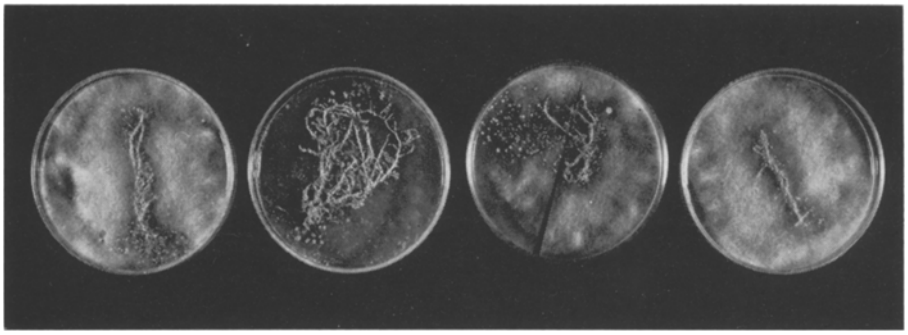

Fig. 2. Pythium debaryanum groeiend van de wortels van komkommerzaailingen;zaden geweekt in water, in 1, 10 en 100 ppm AzU (van links naar rechts). Myceliumgroei wordt geremd na de 1 ppm AzU-behandeling, doch weinig of niet na behandeling met 10 of 100 ppm.

roots was shaken in sterile water. Aliquots of the suspension obtained were plated out on different media (see materials). From the number of colonies which developed an estimate was made of the number of micro-organisms present on the root surface. It should be emphasized that the figures obtained have only a comparative, and not an absolute value (Table 3).

The AzU treatment of cucumber seedlings does increase the bacterial flora on the roots of the developing seedlings, as appears from the results obtained with the Thornton medium, but it seems to decrease the fungal microflora. Bacteria which prefer nitrogen in the form of amino acids seem to be favoured especially.

Analogous results were obtained with seedlings from seed, soaked in a 1 ppm solution of AzU. The number of bacteria found on the roots of seedlings 10 days after treatment of the seed increased 4–6 times, while the fungal population showed a sharp decrease.

Table 3. Effect of a 100 ppm AzU spray treatment on the rhizosphere microflora of cucumber seedlings. Bacteria in millions per gram of fresh root weight, fungi in thousands; assessment 2 days after treatment.

Organisms	Medium	No AzU	AzU
bacteria	Thornton	100	211
bacteria	Taylor I (with $(NH_4)_2HPO_4$)	63	108
bacteria	Taylor II (with amino acids)	112	227
fungi		56	10

Tabel 3. Het effect van een bespuiting met een 100 ppm oplossing van AzU op de rhizosfeer microflora van komkommerzaailingen. Bacteriën in millioenen per gram vers wortelgewicht, schimmels in duizendtallen; vastgesteld 2 dagen na behandeling.

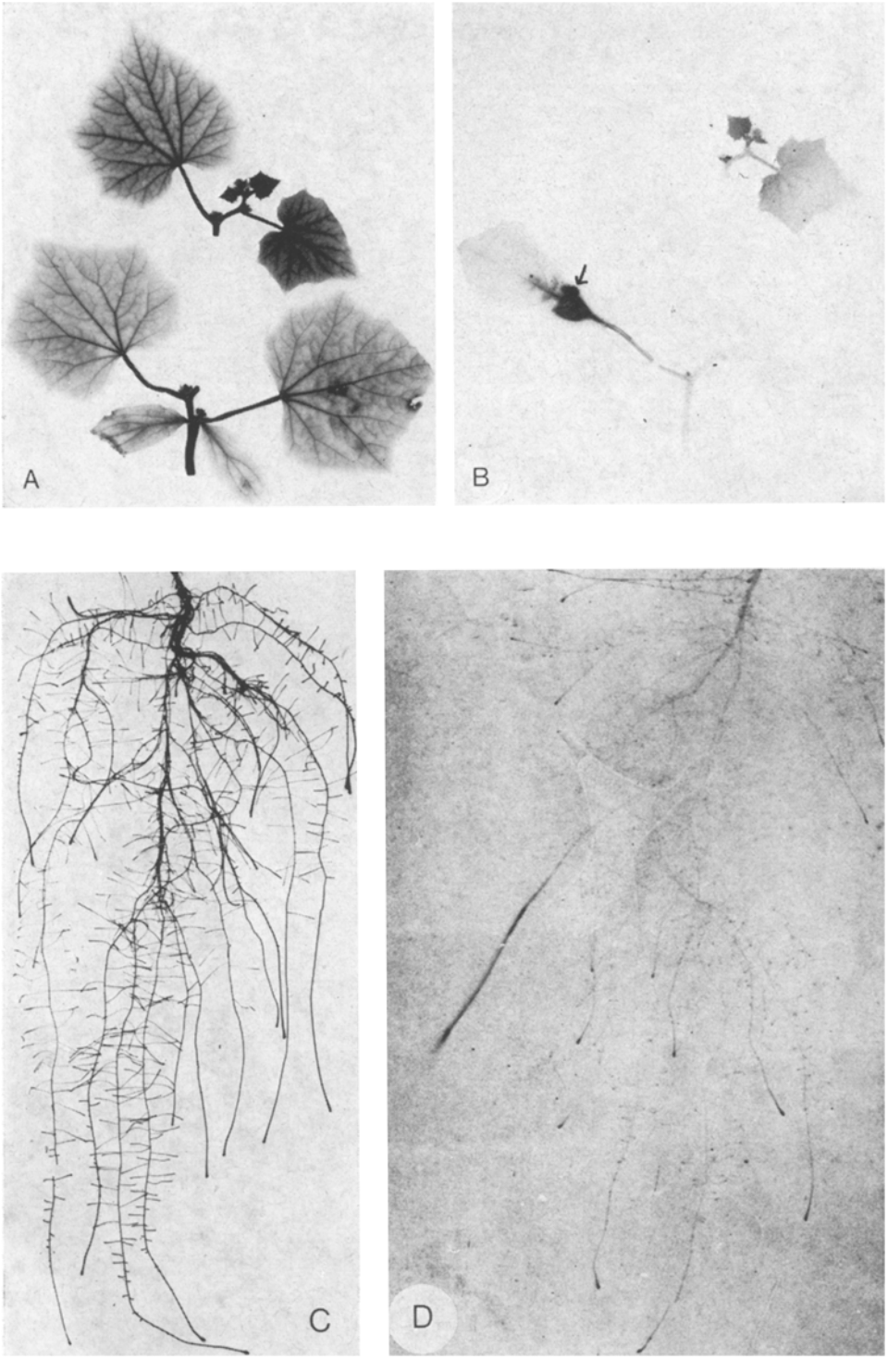
A
B
C
D

Fig. 3. Transport of AzU-2-^{14}C in cucumber plants. Left: from the roots to the leaves; roots immersed in a AzU-2-^{14}C solution. Right: from a leaf to the roots; drop containing AzU-2-^{14}C placed on leaf (arrow).

Fig. 3. Transport van AzU-2-^{14}C in komkommerplanten. Links: van de wortels naar de bladeren; wortels gedompeld in een AzU-2-^{14}C oplossing. Rechts: van een blad naar de wortels; druppel met AzU-2-^{14}C op blad (zie pijl).

By the use of ^{14}C-labelled AzU it was shown that this compound, when applied to the leaves, is translocated to the roots. Young cucumber plants, with one full grown leaf, were transferred from steamed soil to a nutrient solution, following a technique described by de Stigter (1969). After 12 days one drop of water, confined by a vaseline ring and containing 1 μ C of AzU-2-^{14}C, was placed on the second leaf. After 24 h the plants were lyophilized for 2 weeks, and placed under Kodirex X-ray film for one week. The result is shown in Fig. 3 (right); for comparison the autoradiogram after uptake of AzU by the roots has been included (left).

It appears that the label is transported especially to the growing points.

Conclusion and discussion

Protection of cucumber seedlings against damping off is obtained by soaking the seeds in a 1 ppm AzU solution, but not or little by treatment in 10 or 100 ppm AzU. In accordance herewith no mycelium of *P. debaryanum* developed on agar medium from roots of plants grown from seed treated with 1 ppm AzU. Notable changes were observed in the rhizosphere flora after AzU treatment of the seed or of the above ground parts of the seedlings. The number of bacteria increased, especially of those bacteria, which prefer N in the organic form, and the fungal flora decreased after AzU treatment. Since *P. debaryanum* is rather insensitive to AzU in vitro, it is suggested that control of damping off might be due to an indirect action of AzU. AzU is known to interfere with the pyrimidine biosynthesis; it is conceivable that an inhibition of this pathway leads to a change in the composition of the root exudates, favouring the development of certain bacteria. Growth of fungi, including *P. debaryanum* might be antagonized by these bacteria.

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Samenvatting

Indirect effect van 6-azauracil op de aantasting van komkommer door Pythium debaryanum

Bescherming van komkommerzaailingen tegen aantasting door *Pythium debaryanum* werd verkregen na weken der zaden in een oplossing van 1 ppm 6-azauracil (AzU);

10 en 100 ppm AzU waren niet werkzaam. Na behandeling der zaden met 1 ppm AzU traden veranderingen in de rhizosfeermicroflora der kiemplanten op; de bacteriepopulatie nam toe en de schimmelpopulatie nam af. Aangezien *P. debaryanum* in vitro vrijwel ongevoelig is voor AzU, wordt verondersteld dat de bescherming tegen aantasting door deze schimmel op indirecte wijze verkregen wordt, mogelijk via de microflora van de wortels.

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