

Effects of Nitrogen Compounds on Resistance of *Gossypium arboreum* Seedlings to *Colletotrichum gossypii*

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ABSTRACT

Gossypium arboreum 'Nanking' seedlings, which are resistant to *Colletotrichum gossypii*, were grown in a medium supplemented singly with 23 amino acids, KNO_3 , $\text{NH}_4\text{H}_2\text{PO}_4$, $(\text{NH}_4)_2\text{SO}_4$, and NH_4NO_3 . Plants were inoculated by placing a spore suspension of *C. gossypii* on the hypocotyls. Asparagine-amended medium induced complete susceptibility, and was not toxic to noninoculated plants. Other amino acids either induced less disease

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or were phytotoxic. Except for those which were phytotoxic, inorganic amendments did not affect resistance. Susceptibility was induced by growing the seedlings in asparagine-amended medium that had no contact with the inoculum. Evidence indicates that the supplements predispose the seedlings rather than increase virulence of the fungus. *Phytopathology* 61: 1394-1395.

Some investigators believe that crop decomposition products increase the incidence of certain diseases by enhancing root exudation, thereby increasing the inoculum potential of pathogens (5).

We reported (1) that seedlings of most USA cotton cultivars (*Gossypium hirsutum* L.) are very susceptible to *Colletotrichum gossypii* South., but that an Asian cultivar, *G. arboreum* L. 'Nanking', is highly resistant in the seedling stage. To determine the nature of anthracnose resistance in Nanking, we attempted to induce susceptibility in the seedlings. Since amino acids are produced during degradation of crop residues, we added them to the growth medium of Nanking seedlings to determine if amino acids might break down resistance. We also supplemented the medium with inorganic nitrogen compounds (2) and with glucose.

Seeds of Nanking were acid-delinted and immersed in water at 80 C for 2 min to break dormancy. They were surface-sterilized by soaking in 0.2% HgCl_2 for 10 min, then rinsing with sterile water. The seeds were germinated by incubating them 65 hr at 28 C between sterile, moistened filter papers. The seedlings were transferred to acid-washed, sterile sand in test tubes having 10 ml of a basal medium that consisted of distilled water containing 0.02% $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, at ca. pH 6. This medium was supplemented singly with the L-isomers of 23 amino acids and with four inorganic nitrogen compounds at a rate that furnished 0.5 g of nitrogen/liter. We also amended the medium with glucose in the amount that supplied 0.8556 g carbon/liter. When the addition of a supplement altered the pH of the medium, it was adjusted to pH 6 with NaOH or HCl. The hypocotyl of each seedling was inoculated with 0.5 ml of an aqueous suspension containing ca. 30,000 spores/ml. The spores were produced by the growing of *C. gossypii* for 7 days at 24 C on potato-dextrose agar containing 100 ml of V-8 juice/liter of medium. The cultures were illuminated with fluorescent light for 12 hr daily. Noninoculated plants were included in all tests to determine

whether the supplements alone were toxic. We grew the seedlings at ca. 27 C for 10 days in the greenhouse. The inoculated plants were rated for disease symptoms, and disease indexes were calculated by the following method: the number of seedlings with slight hypocotyl lesions was multiplied by 10, the number with moderate lesions by 20, the number with severe lesions by 30, and the number of dead plants by 100, and the sum was divided by the total number of seedlings.

The disease index for inoculated seedlings grown in unamended medium was 13. All inoculated seedlings died in medium supplemented with asparagine, with no visible symptoms of toxicity in noninoculated plants. Other nontoxic amendments induced the following seedling disease indexes: aspartic acid, 66; cysteic acid, 37; and histidine, 39. Cystine and cysteine were slightly toxic, and inoculated plants in media supplemented with these amino acids had disease indexes of 71 and 73, respectively. The moderately phytotoxic amendments produced disease indexes in inoculated plants as follows: arginine, 81; β -alanine, 19; citrulline, 29; proline, 29; serine, 61; and threonine, 31. The other 11 amino acids all caused severe toxicity in noninoculated plants. Inoculated seedlings grown in media amended with these 11 supplements had the following disease indexes: alanine, 85; glutamine, 100; glutamic acid, 23; glycine, 59; isoleucine, 81; leucine, 37; lysine, 70; methionine, 100; phenylalanine, 100; tyrosine, 19; and valine, 74. All inoculated seedlings died in medium containing 2.5 g/liter of peptone, but noninoculated plants were not affected.

The medium was amended singly with three inorganic ammonium-nitrogen sources, $\text{NH}_4\text{H}_2\text{PO}_4$, NH_4NO_3 , and $(\text{NH}_4)_2\text{SO}_4$. Only $(\text{NH}_4)_2\text{SO}_4$ predisposed the plants to disease, and it caused severe toxicity in noninoculated plants. These results indicate that release of ammonia probably is not directly responsible for susceptibility induced by amino acids.

We supplemented the medium with KNO_3 and

glucose, both singly and in combination, but none of these treatments predisposed the seedlings to disease. Since the KNO_3 -glucose combination supplied nitrogen and carbon in amounts equal to that in asparagine at the concentration that induced complete susceptibility, the results suggest that asparagine did not cause disease by a direct nutritional effect on the pathogen.

To gain further insight into the nature of induced susceptibility, we used a technique described by Borum & Sinclair (3) that kept the inoculum separate from seedling roots and their medium. Two compartments were made by filling Erlenmeyer flasks with vermiculite and basal medium, and covering the mouths with plastic wrap in which a cuplike depression was made. Seedlings were inserted in holes in the wrap, and sterile, moistened vermiculite was placed in the depression. This scheme provided separate chambers for the hypocotyls and roots. The plants were inoculated by adding 1 ml spore suspension to the vermiculite around the hypocotyls. For one treatment, the medium in the lower chamber was amended with asparagine at the rate of 0.5 g of nitrogen/liter. In another treatment, only the vermiculite around the hypocotyls in the upper chamber was moistened with the asparagine-amended medium. For controls, we grew plants with unamended inoculum in flasks containing basal medium. All seedlings died in the flasks with the asparagine-supplemented medium and unamended inoculum. Plants that were in contact with asparagine-amended inoculum but with the roots in the unamended medium had only very slight hypocotyl lesions similar to those in the inoculated controls. These results supply additional evidence that asparagine did not induce disease by directly increasing the nutrient supply of the pathogen. Since the inoculum had no contact with the roots, the results also suggest that asparagine did not predispose the plants to disease by enhancing root exudation. This hypothesis differs from the explanation given by some other investigators (5) of the

method by which organic amendments may increase the incidence of certain diseases.

Our findings, and those of other workers, suggest that asparagine is taken up by Nanking seedlings whose metabolism is then altered in a manner that renders them susceptible. Ting et al. (6) found that asparagine is the dominant amino acid in seedling roots of *G. hirsutum* 'Acala SJ-1', and that it is an active metabolite in the roots. We found that Acala SJ-1 seedlings are susceptible to anthracnose. Guinn & Brinkerhoff reported (4) that the asparagine content of cotton seedling roots is increased by reduction of the temperature. We tested Nanking with *C. gossypii* at temperatures (18 and 21 C) below optimum for seedling growth, and the plants became highly susceptible.

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