some spores did survive after 4 days of exposure to 500,000 ppm of 2,4-D.

Concentrations of 300 and 475 ppm of 2,4-D caused an initial lag in the decomposition of gelatin to amino acids or to ammonia; 500 ppm inhibited these changes. The inhibition of ammonia production from a selected amino-acid medium was achieved with 900 ppm of the herbicide.

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CULLIN, DOROTHY WILSON 1946 The effect of 2,4-dichlorophenoxyacetate upon the morphological and physiological characteristics of certain microorganisms associated with food spoilage. M. S. thesis, Ohio State University.


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The Effect of Herbicides on Soil Microorganisms

II. The Effect of 2,4-Dichlorophenoxyacetic Acid on Some Phases of the Nitrogen Metabolism of Pseudomonas fluorescens and the Microorganisms of a Soil Suspension

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In a previous report (Johnson and Colmer, 1955), the importance of acquiring some knowledge of the effect of 2,4-dichlorophenoxyacetic acid (2,4-D) on certain microbiologically induced processes essential to the soil's fertility was emphasized. When the common soil sporeformer Bacillus cereus was grown either in a gelatin or an amino acid medium, there was an initial lag in its decomposition of the substrates with concentrations of 2,4-D up to 500 ppm. At this level, growth of the organism was inhibited.

This report concerns the effect of 2,4-D on the decomposition of the same substrates when Pseudomonas fluorescens, a common soil inhabitant, and the mixed microflora of a cane field were the test agents.

EXPERIMENTAL PROCEDURES AND RESULTS

The procedures used were the same as described in the previous paper. Pseudomonas fluorescens and the mixed microflora were from Mhoon sugar cane soil.

Effects of 2,4-D when gelatin was the substrate. No appreciable amounts of amino acids or ammonia were produced from gelatin by P. fluorescens.

Figure 1 shows that the microorganisms of the soil suspension rapidly produced amino nitrogen from gelatin in the absence of 2,4-D. In the presence of 5,000 ppm and 10,000 ppm of 2,4-D, there was little change in the concentration of the amino acids up to the 9th day of incubation. By the end of the test period, however, the organisms of the 5,000 ppm test flask had recovered from the inhibitory effects of the 2,4-D and had hydrolyzed the gelatin to yield amino acids comparable in amount to that of the control. Although at the 10,000 ppm level there was some recovery after continued incubation, the 20,000 ppm concentration inhibited the formation of amino acids.

The course of ammonia formation during decomposition of gelatin by the microorganisms in the soil inoculum was similar to that for the production of amino nitrogen (figure 2).
Fig. 1. Effect of 2,4-D on amino acid formation from gelatin by microorganisms of a soil suspension.

Fig. 2. Effect of 2,4-D on ammonia production from gelatin by microorganisms of a soil suspension.

Fig. 3. Effect of 2,4-D on utilization of amino acids by *Pseudomonas fluorescens*.

**Effects of 2,4-D when amino acids were the substrate.** Figures 3 and 4 show that 5,000 ppm of 2,4-D inhibited the action of *P. fluorescens* on the amino acid substrate when either the utilization of it or the production of ammonia from it served as a gauge of activity. Concentrations of 2,4-D less than 5,000 ppm had but little effect on the organism.

Fig. 4. Effect of 2,4-D on ammonia production from amino acids by *Pseudomonas fluorescens*.

**Discussion**

*P. fluorescens*, a representative organism of the group of gram-negative nonsporeforming rods common to the soil, has been reported by Conn (1948) as one of the most strongly proteolytic types in soil. Results presented here and in the earlier report (Johnson and Colmer, 1955) emphasize the differences between *P. fluorescens* and *B. cereus* as “proteolytic” organisms. The latter organism could hydrolyze gelatin with production of appreciable quantities of free amino acids and, eventually, ammonia. *P. fluorescens*, however, was markedly less active in its action on gelatin. It may then be quite true, as pointed out by Berman and Rettger (1918), that the ability of an organism to liquefy gelatin is no sure indication of its proteolytic properties.

Although the pattern of activity of *P. fluorescens* was the same as that of *B. cereus* on the amino acid medium, the gram negative rod was less active than the spore former. The resistance of *P. fluorescens* to the harmful action of the 2,4-D, however, was much greater than that of *B. cereus* on this substrate. Whereas *B. cereus* was inhibited by 900 ppm of the chemical, it was the 5,000 ppm level which inhibited *P. fluorescens*.

Bright and Conn (1919) and Waksman and Lomanitz (1925) have reported that different organisms may take an active part in different stages of the process of protein decomposition in the soil, bacteria like *B. cereus* being active in the first stages of hydrolysis, and bacteria like *P. fluorescens* in those later stages leading to the formation of ammonia. The results of these studies, made with solutions of gelatin and amino acids, indicate that *B. cereus* is quite active in both stages and was more active than *P. fluorescens* in ammonia formation whether gelatin or amino acids served as the substrate.

Figures 1 and 2 show that the effect of 2,4-D on the decomposition of gelatin by the mixed microflora was primarily a matter of degree rather than a marked change from that characterized by the two pure cultures. More 2,4-D was required to gain the initial
inhibition, and more 2,4-D was required to produce complete inhibition of the amino acid production or ammonia production. It is interesting to note the rise both in amino acid and ammonia production after the 12th day of the test. The ammonia production was quite marked after this period. From microscopic examinations of the contents of the flasks, together with a qualitative sampling by plating out on appropriate media, it appeared that these changes were a reflection of the activity of the rod-shaped bacteria rather than of the actinomycetes and fungi.

**SUMMARY**

A 5,000 ppm concentration of 2,4-dichlorophenoxyacetic acid (2,4-D) inhibited the action of *P. fluorescens* on the amino acid substrate when either the utilization of it or the production of ammonia from it served as a gauge of activity. Concentrations of 2,4-D less than 5,000 ppm had but little effect on the organism.

The production of amino acids and ammonia from gelatin by the microorganisms of a soil suspension was inhibited by 20,000 ppm of 2,4-D. Concentrations of 5,000 and 10,000 ppm inhibited these activities during the first period of incubation; with 5,000 ppm, however, there was a marked recovery of activity in the later periods.

**REFERENCES**


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**ERRATUM**

In the article, "Metabolic Behavior and Chlortetracycline Production by *Streptomyces aureofaciens* in Liquid Culture," by G. Biffi, G. Boretti, A. Di Marco and P. Pennella, which appears in the September issue of *Applied Microbiology*, the following corrections should be made.

Page 290, section *Effect of Changes in Medium Composition During Fermentation*, the first line should read, "By the addition of KH₂PO₄ (0.03 per cent or more) to ..." The media in tables 4, 5, and 6 should read, "4N + 0.03 per cent K₂HPO₄" and "4N + 0.05 per cent KH₂PO₄." The readings concerning figures 6, 7, and 8 should read "0.03 per cent," and figures 10 and 11 should read "0.05 per cent."