The Action of 2,4-D Upon the *Azotobacter* of Some Sugarcane Soils

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The value of 2,4-dichlorophenoxyacetic acid (2,4-D) and its various formulations as phytotoxic agents has been predicated upon their toxicity to the unwanted weeds in relation to their toxicity to the wanted crop plants; but this evaluation is based on only a part of the farmers' plants. No one questions the beneficial roles played by many of the bacteria, actinomycetes, and fungi, the microscopic plants, in maintaining the fertility of our soils; thus it follows that any deleterious effect upon them by the indiscriminate use of the herbicide might be of serious consequence in the continued productivity of the soil.

There have been reports (Newman, 1947; Newman, Thomas, and Walker, 1952; Smith, Dawson, and Wenzel, 1945) on the effects of various herbicides upon the diverse organisms which make up the soil's microflora. Because of the role of *Azotobacter* in the nitrogen economy of the soil, it was thought to be worthwhile to study the effect of 2,4-D, the herbicide commonly used to control weeds in sugarcane, upon these beneficial bacteria.

**Materials and Methods**

Three soils of the sugarcane area, Mhoon, a sandy loam; Commerce, a very fine sand; and Sharkey, a clay, served as test materials after being air-dried and sifted through a 2 mm screen. The Mhoon and Commerce soils had so many *Azotobacter* that a portion of the soil had to be autoclaved and then mixed with approximately 10 per cent of the unheated soil to get a sample which would ensure discrete colony formation on the plaque surface.

The triethanolamine salt of 2,4-D (Dow 25W-110, 40 per cent acid eq.) served as the test herbicide. The soil plaque method of Winogradsky and Ziemiecka (1928) was used to test the response of *Azotobacter* of the soils to the herbicide. Fifty g portions of a soil, 0.5 g of mannitol, 5 ml of 3 per cent aqueous solution of K₂HPO₄, and distilled water were worked with a spatula on a glass plate into a homogeneous pasty mass. This material, after being placed in an ointment box and with the surface of the plaque smoothed, served as the control. The amount of distilled water needed to get the proper consistency to the plaque varied with the type of soil used; with the Mhoon soil the quantity added was 10 ml. The ointment box had a 47 mm diameter and a 15 mm depth.

To prepare the test plaques, chosen volumes from a freshly prepared aqueous solution of the herbicide (10,000 ppm concentration) were added to the portion of soil in lieu of a part of the distilled water. The concentrations of the agent present in the plaques, so calculated that they were equivalent to that of the 2,4-D acid, were increased in strength by increments of 200 ppm. To assure uniformity in the consistency and homogeneity of the control and test plaques, the total volume of liquid in each plaque was kept the same, and each soil portion, in its agitation to get the pasty mass, was kneaded the same length of time.

The plaques were incubated at room temperatures for 2 to 3 days in a moist chamber. The colonies of *Azotobacter* which had developed upon the surfaces of the plaques by that time were counted with the aid of a binocular dissecting microscope at a 15-fold magnification.

Pure cultures of *Azotobacter* were secured from the colonies on the control plaques by repeated purification on the N-free agar medium of Fred and Waksman (1928). They were identified as *A. chroococcum* by means of *Bergey's Manual* (Breed, et al., 1948). These isolates together with a representative culture of *A. agile*, were tested for their response to 2,4-D. The medium used was the N-free broth of Fred and Waksman (1928). Fifty ml of the broth were placed in a 250 ml Erlenmeyer flask, and 2,4-D was added from the freshly prepared stock solution to secure the desired concentration of the agent. For some of the higher concentrations the 2,4-D was used directly from the original material. The inoculum for all the flasks was 0.1 ml of a 24- or 48-hour culture grown in the N-free broth. All flasks were agitated at the lowest speed of a variable speed Brunswick shaker. The determination of the numbers of *Azotobacter* present at the beginning and end of a test was made by plating with the N-free agar.

To test for the possibility of increased resistance of *Azotobacter* to 2,4-D, serial subcultures were made from the flask of N-free medium containing the highest concentration of 2,4-D which had barely permitted growth into new media with the same concentration of the agent.

1 Dow chemical Co., Midland, Mich.
ACTION OF 2,4-D ON AZOTOBACTER

RESULTS

Figure 1 shows the appearance of the soil plaques after incubation when either the Mhoon or Commerce type of soil was used. Repeated tests with the Sharkey clay indicated that this soil normally had such small numbers of Azotobacter that it would not serve for this type of study. Table 1 gives the results of eight typical tests made by this plaque mode of study on the Mhoon soil. Both figure 1 and table 1 show that when the concentrations of the 2,4-D had reached 200 ppm, the numbers of Azotobacter developing were much less than in the control plaque, and they decreased in number as the concentration of the agent increased.

The gas production in the plaques, as shown in figure 1, was inversely correlated with the concentration of the 2,4-D. As the concentration of the agent increased, there was a marked decrease in gas formation. A concomitant finding was made when the plaques were removed from the ointment boxes. The solvent odor associated with the growth of some anaerobes was strong in the control plaque and in the plaques where gas had been produced, but was absent in the plaques where no gas was evident.

A different correlation was found with the fungi; their growth was directly correlated with the presence of the 2,4-D; the higher the concentration of the herbicide, the more profuse the fungal growth on the surface of the plaques.

A response quite characteristic of the pure cultures of Azotobacter to 2,4-D is shown in tables 2 and 3. The apparent stimulation of growth shown by the 100 ppm level on A. chroococcum as shown in table 2 and the 1000 ppm level on A. agile as shown in table 3 was not demonstrated in every test series, but its frequency of occurrence indicates a borderline concentration which might effect stimulation of growth or inhibition of growth.

The culture of A. agile was less affected by the 2,4-D than were the cultures of A. chroococcum. Table 3 indi-

![Figure 1: Mhoon soil plaques. Top row left to right: Control, no 2,4-D; 200 ppm; 400 ppm. Bottom row left to right: 600 ppm; 800 ppm; 1000 ppm.](http://aem.asm.org/)

Table 1. Numbers of Azotobacter chroococcum developing on soil plaques in presence of 2,4-D triethanolamine salt

<table>
<thead>
<tr>
<th>AMOUNT OF 2,4-D</th>
<th>TEST</th>
<th>AVERAGE DECREASE</th>
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<tbody>
<tr>
<td>ppm</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>none</td>
<td>211</td>
<td>230</td>
</tr>
<tr>
<td>200</td>
<td>61</td>
<td>63</td>
</tr>
<tr>
<td>400</td>
<td>37</td>
<td>30</td>
</tr>
<tr>
<td>600</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>800</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>1000</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

* Calculated as acid equivalent.
5,000 ppm concentration of the herbicide. By this technique A. chroococcum was also able to grow in a concentration of the herbicide which, at first, had limited its growth.

Table 2. Effect of 2,4-D triethanolamine salt on Azotobacter chroococcum in N-free medium

<table>
<thead>
<tr>
<th>2,4-D* ppm</th>
<th>NO./ML thousands</th>
</tr>
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<tbody>
<tr>
<td>None</td>
<td>1,615</td>
</tr>
<tr>
<td>100</td>
<td>19,650</td>
</tr>
<tr>
<td>200</td>
<td>126</td>
</tr>
<tr>
<td>300</td>
<td>89</td>
</tr>
<tr>
<td>400</td>
<td>82</td>
</tr>
<tr>
<td>500</td>
<td>62</td>
</tr>
</tbody>
</table>

Time of incubation—48 hrs.
Original inoculum—21.3.
* Calculated as acid equivalent.

Table 3. Effect of 2,4-D triethanolamine salt on Azotobacter agile in N-free medium

<table>
<thead>
<tr>
<th>INOCULATION TIME</th>
<th>2,4-D (PPM)*</th>
<th>NO./ML (X 10⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>none</td>
<td>1000</td>
</tr>
<tr>
<td>24 hr</td>
<td>31,500</td>
<td>44,500</td>
</tr>
<tr>
<td>48 hr</td>
<td>500,000</td>
<td>715,000</td>
</tr>
</tbody>
</table>

Original inoculum—141.5.
* Calculated as acid equivalent.

Fig. 2. Azotobacter agile and 2,4-D. Material removed from incubation flasks for photographing.
Left tube: Freshly inoculated medium.
Center tube: Amount of growth by unadapted culture in 5,000 ppm after 24 hours.
Right tube: Amount of growth by adapted culture in 5,000 ppm after 24 hours.

Discussion

The increasing use of 2,4-D to destroy weeds in sugarcane plantations has raised the question of the possible concomitant injury to the microorganisms of the soil. Hoover (1952) found that the bacteria, actinomycetes, and fungi were not deleteriously affected by the 1 to 2 ppm (2 to 4 lb/acre) concentrations commonly used. The results of the experiments made with Azotobacter indicate that there is a wide margin of safety between the rates of field application and the experimental rates capable of being withstood by these N-fixing bacteria.

The ability of the Azotobacter, upon serial subculturing, to grow readily in concentrations of 2,4-D which at first had prevented good growth, is of double significance. One thought concerns the ability of the organism to adjust to higher concentrations of the herbicide should some circumstances of field application rates demand it, and the other thought concerns the ability of the organism to use the herbicide as a nutrient. Should this latter be the case, the lasting phytotoxic effect of the herbicide would be diminished—a circumstance not desired when pre-emergence treatments are used. Unfortunately the attempts at assaying the residual 2,4-D in the flasks after the growth of the Azotobacter were not of the desired accuracy to gauge whether or not 2,4-D was being used by the organisms.

Even though no A. agile representatives were found in the soils tested, it is of interest to note its marked resistance to the harmful action of the 2,4-D. A wide examination of representatives of this organism might indicate that this species can be separated from A. chroococcum by its response to 2,4-D.

Sackett and Stewart (1931) have used successfully soil plaques and Azotobacter to gauge mineral deficiencies in soil. The soil plaque mode of study, comprising as it does the total microflora in an environment somewhat comparable to natural soil conditions, certainly permits an interplay of the herbicide on components of this microflora, such as the Azotobacter, and simultaneously affords the possibility of an interplay by the microorganisms on the herbicide. There is the concomitant possibility that the inhibition of a component or components of the microflora might afford, by such an imbalance, the marked growth of another group or groups. One such interplay was shown when the increasing 2,4-D concentrations caused a simultaneous decrease in bacterial activity and an increase in fungal growth.

Acknowledgments

The author would like to express his thanks to the Dow Chemical Company for the 2,4-D and to Mr. Victor Monsour for the culture of A. agile.

Summary

The commonly used field application rates of 2,4-D to control weeds in sugarcane did not harm the Azotobacter population of the soils tested. It was only when rates were used which would be prohibitive for use in sugarcane that the Azotobacter growth was harmed.
The *A. agile* tested was far more tolerant of 2,4-D than the *A. chroococcum* isolates.

It was possible to increase the tolerance of both species of *Azotobacter* to the herbicide by serial subculturing in the presence of the agent.

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**Studies on Disinfection of Clinical Thermometers**

I. Oral Thermometers

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A nation-wide survey of procedures currently employed for disinfecting thermometers in hospitals and public health agencies has revealed the use of a wide variety of substances and methods. For many of these procedures there exists no known experimental basis; their effectiveness is unknown and in some instances seems dubious. In an attempt to evaluate these procedures neither condemnation nor recommendation of any was possible because of lack of exact experimental data. In view of this situation, and because of a wide demand for a method of disinfecting clinical thermometers which could be recommended, on the basis of experimental evidence, as reasonably safe from the standpoint of transmission of communicable diseases, the present study was undertaken. The objectives of the study were (a) to obtain data on which to base evaluations of one or more currently used methods of disinfecting clinical thermometers, and (b) to develop, if necessary, one or more new methods which could be recommended in place of existing procedures.

As a first step, the literature on the subject was carefully reviewed. It became evident that, while in-vitro tests of the action of numerous disinfectants against pure cultures of organisms suspended in broth media, saline solutions, or distilled water have been carried out, the action of disinfectants against infectious organisms protected in body secretions or excretions, such as often contaminate clinical thermometers, has been neglected. Cognizance is taken, of course, of the work of Chick and Martin (1908); Klarmann and Wright (1944), Horton and Kitchin (1933), McCulloch and Fuller (1941), and Rahn (1945) on the effect of serum and other organic materials on the disinfectant value of bichloride of mercury, phenol, and related compounds. However, these substances are not widely used in the disinfection of clinical thermometers. Therefore, it was decided to undertake an investigation of the efficacy of various bactericidal agents for disinfecting clinical thermometers artificially contaminated with sputum containing *Mycobacterium tuberculosis* var. *hominis*, *Corynebacterium diphtheriae*, streptococci, and staphylococci. It was felt that these organisms would be sufficiently representative of the pathogenic bacteria common in the mouth. Because of limitations of time, equipment, space, and personnel, no cultural tests were made for *Borrelia*, *Treponema*, *Hemophilus*, *Neisseria*, or *Diplococcus*. No attempt was made in this laboratory to determine the susceptibility of any

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