The Effect of Herbicides on Soil Microorganisms

III. The Effect of Some Herbicides on the Respiration of Azotobacter

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The effects of chemical agents upon microorganisms have been extensively studied, and many experimental approaches have been used to gauge their action. Some workers have used manometric methods in the evaluation of the effect of certain germicides (Bronfenbrenner, Hershey, and Doubly, 1939, Ely, 1939, and Greig and Hoogerheide, 1941) while others have used the versatility of these methods in exploring the metabolic activities of the bacteria concerned with nitrogen fixation (Wilson and Burris, 1947).

With the increasing use of chemical agents in agriculture, an increasing interest has grown concerning the action of them upon the microflora of the soil. In one study of herbicides and this microflora, Gamble, Mayhew, and Chappell (1951) made use of a manometric approach. The following report is concerned with the effects of some herbicides upon the respiration of Azotobacter as gauged by the manometric method.

MATERIALS AND METHODS

The bacteria used were stock cultures of Azotobacter agile and Azotobacter vinelandii strain Original and a culture of Azotobacter chroococcum isolated from Mhoon type sugarcane soil. The cells were grown at 30 °C on bottle slants of nitrogen-free medium (Fred and Waksman, 1928). The cells were harvested by washing from the slants with 0.126 M phosphate buffer at pH 7.2, and the resulting suspensions were adjusted to a desired turbidity by use of the Klett-Summerson colorimeter.

The oxygen uptake was determined in the Warburg respirometer, with 1 ml each of cell suspension and nitrogen-free medium in the main compartment, 0.1 ml of 20 per cent KOH in the center well, and 0.5 ml of the herbicide in the side-arm. The concentrations of the herbicides used are expressed on the basis of their final concentrations in the contents of the main flask. The herbicide was tipped into the main compartment after 1 hr of active respiration, and the measurement of oxygen uptake was continued for 2 hr after the addition of the herbicides.

The herbicides used in the study were: the triethanolamine salt of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T); the triethanolamine salt, the polypropylene glycol butyl ether, and the isopropyl ester of 2,4-dichlorophenoxyacetic acid (2,4-D); the sodium salt of trichloroacetic acid (TCA); sodium chlorate (NaClO3); the sodium salt of 2,2-dichloropropionic acid (Dalapon); and dinitro-orthosubstituted butyl phenol (DNOSBP). The concentrations of the chemicals are expressed in terms of the acid rather than in terms of the derivatives used.

RESULTS

The results of the respiration studies are expressed by plotting the total oxygen uptake in microliters against the time in minutes. On each figure, the point at which the herbicide was added is indicated with the letter T, and the turbidity of the cell suspension used
is indicated by the designation K-S. Because the results were somewhat similar for each of the three organisms, only the figures for *Azotobacter agile* are given here. Only the graph of the results with the ester 2,4-D is presented, since it indicates the type of response of the test organisms to both it and the ether formulation.

A summary of the effects of the herbicides on the respiration of the three species is presented in table 1.

**DISCUSSION**

The inhibition of oxygen uptake of the *Azotobacter* by the formulations of 2,4-D varied markedly. The lack of activity by the ester and ether formulations of 2,4-D in comparison with that demonstrated by the amine salt is probably explained by the lack of solubility of the ester and ether in the menstruum of the flask. With the amine salt of 2,4,5-T, there was a more toxic reaction on the test organisms than when the 2-chlorine hydrocarbon compound was used, a condition that holds, in general, with comparative field uses of these herbicides.

The dinitro-orthosecondarybutyl phenol was not only the most toxic of the agents tested but it also presented technical difficulties because of its volatility. The graph of the results with this compound indicates that at the higher levels the DNOSBP reacted with the menstruum in such a fashion that gas was liberated. This is reflected by the lower curves of the graph.

The chlorinated acetic acid (TCA) and the chlorinated propionic acid (Dalapon) differed in their inhibition of the respiration of the *Azotobacter*; *A. agile* was more sensitive to the Dalapon, and *A. chroococcum* was more sensitive to TCA. *A. vinelandii* was less sensitive to both agents than its companion species.

There is a tendency in the field use of herbicides toward a combined application of these agents, and frequently there appears to be a desirable synergistic relation to their use. The test reported here with TCA and 2,4-D indicated that the mixture of herbicides produced somewhat more inhibition than would be expected if the effects were additive. However, the existence of a definite synergistic relation was not established.
Fig. 3. The effect of the triethanolamine salt of 2,4,5-T upon the respiration of Azotobacter agile.

Fig. 4. The effect of the sodium salt of trichloroacetic acid upon the respiration of Azotobacter agile.

Fig. 5. The effect of a mixture of sodium trichloroacetate and triethanolamine 2,4-D upon Azotobacter agile as compared with the effects of the two herbicides acting separately.

Fig. 6. The effect of sodium chlorate upon the respiration of Azotobacter agile.
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The inhibitory levels of NaClO₃ were similar to those of 2,4,5-T in their toxicity to the Azotobacter; yet in field uses of these compounds the NaClO₃ is applied at much higher levels.

Although these studies were made with pure cultures and not under conditions which would be found in the soil, it is felt that those levels of these herbicides which are commonly used in the field are so small in comparison with the levels found here to inhibit respiration, there is little danger in the use of the herbicides in relation to the Azotobacter.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Perry W. Wilson for the culture of Azotobacter vinelandii strain Original and the Dow Chemical Company for aid in the support of this research.

SUMMARY

Manometric methods served to demonstrate the levels of some representative herbicides which are required to inhibit the oxygen uptake of some members of the genus Azotobacter.

No marked difference in susceptibility to the herbicides was found in the species tested.

2,4,5-trichlorophenoxyacetic acid was more toxic than the 2,4-dichlorophenoxyacetic acid formulations.

Dinitro-orthosecondarybutyl phenol and sodium chlorate were more toxic than trichloroacetic acid or 2,2-dichloropropionic acid.

The levels of any of the herbicides used in these respiration studies which proved toxic to the Azotobacter

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>Azotobacter agil</th>
<th>Azotobacter vinelandii</th>
<th>Azotobacter chroococum</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4-D amine</td>
<td>10,000</td>
<td>10,000</td>
<td>10,000</td>
</tr>
<tr>
<td>2,4-D ether</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,4-D ester</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,4,5-T</td>
<td>2,000</td>
<td>1,500-2,000</td>
<td>2,000</td>
</tr>
<tr>
<td>TCA</td>
<td>15,000</td>
<td>15,000-20,000</td>
<td>3,000</td>
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<tr>
<td>NaClO₃</td>
<td>2,500</td>
<td>2,500</td>
<td>2,000</td>
</tr>
<tr>
<td>Dalapon</td>
<td>7,000</td>
<td>5,000-10,000</td>
<td>7,000</td>
</tr>
<tr>
<td>DNOSBP</td>
<td>1,500</td>
<td>1,500</td>
<td>700</td>
</tr>
</tbody>
</table>

Fig. 7. The effect of the sodium salt of 2,2-dichloropropionic acid upon the respiration of Azotobacter agile.

Fig. 8. The effect of dinitro-orthosecondarybutyl phenol upon the respiration of Azotobacter agile.
are exceedingly higher than the levels used in field applications.

REFERENCES


The Sterilization of Carbohydrates with Liquid Ethylene Oxide for Microbiological Fermentation Tests

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The insecticidal properties of gaseous ethylene oxide were noted by Cotton and Roark in 1928. Subsequent investigation by numerous workers revealed this compound to be an effective bactericidal agent. In a series of publications from the Chemical Corps Biological Laboratories (Camp Detrick, Md.), Phillips and Kaye (1949), Phillips (1949), Kaye (1949), and Kaye and Phillips (1949) reported on several facets of this subject. Wilson and Bruno (1950) successfully used liquid ethylene for the sterilization of liquid culture media. Since these reports, liquid ethylene oxide has been employed in our laboratory for the sterilization of the following substances used as substrates in microbiological studies: a-conidendrin (Konetzka, Pelczar, and Gottlieb, 1952), sodium lignosulfonate (Day, Gottlieb, and Pelczar, 1952); lignin (Konetzka, 1952), and decalcified dentin (Konetzka, Burnett, and Pelczar, 1955).

The study reported here was undertaken for the purpose of determining the practicability of using liquid ethylene oxide for the sterilization of the carbohydrates commonly employed in bacteriological fermentation tests.

EXPERIMENTAL METHODS

Procedure for Sterilization with Ethylene Oxide

Preliminary trials with liquid ethylene oxide revealed that the following procedure would effectively sterilize solutions containing a heavy inoculum of bacterial spores.

The liquid to be sterilized was cooled in an ice bath to 3 to 5° C and 1 volume per cent liquid ethylene oxide was added by means of a chilled pipette or syringe. The solution was agitated in order to insure mixture and allowed to remain in the ice bath for 1 hr. After this time, the solution was removed to a warm water bath circa 45° C (located under a hood) in order to allow complete volatilization of ethylene oxide. During this period, the interior of the bottle as well as cap or cotton plug is sterilized by the gaseous vapor. Sterility tests after this treatment have shown the material to be sterile and ready for incorporation into a previously sterilized fermentation base medium.

Carbohydrates Employed

Five per cent solutions of each of the carbohydrates listed below were prepared in distilled water, divided into 3 aliquots of 100 ml each, which in turn were sterilized by (a) liquid ethylene oxide, (b) autoclaving (10 lb, 15 min), and (c) Seitz filtration.

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Carbohydrate</th>
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<tbody>
<tr>
<td>Adonitol</td>
<td>Lactose</td>
</tr>
<tr>
<td>Aesculin</td>
<td>Maltose</td>
</tr>
<tr>
<td>Arabinose</td>
<td>Mannitol</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>Raffinose</td>
</tr>
<tr>
<td>Dextrin</td>
<td>Rhamnose</td>
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<tr>
<td>Dulcitol</td>
<td>Salicin</td>
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<tr>
<td>Fructose</td>
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<tr>
<td>Galactose</td>
<td>Starch</td>
</tr>
<tr>
<td>Glucose</td>
<td>Sucrose</td>
</tr>
<tr>
<td>Inositol</td>
<td>Trehalose</td>
</tr>
<tr>
<td>Inulin</td>
<td>Xylose</td>
</tr>
</tbody>
</table>

1 Heating was required to effect solution of these compounds. In the aliquot cooled for ethylene oxide sterilization, the carbohydrate precipitated out of solution. After sterilization and just prior to use in the fermentation base medium, solubilization was effected by heating.