INTERACTIONS BETWEEN 2,4-DB * AND THE ROOT-NODULE BACTERIA OF LOTUS CORNICULATUS

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INTRODUCTION

Microbial response to the presence of foreign compounds in the environment is varied and often difficult to predict. It has been noted that in a microbial community one or more species become dominant, either by genetic modification or by physiological adaptation, in response to alterations in the micro-environment such as brought about by the introduction and subsequent degradation of new compounds (Alexander 1). Nevertheless, some compounds, by nature of their molecular structures, persist and accumulate in the immediate microbial habitat. Studies have shown, for instance, that although 2,4-(dichlorophenoxy) acetic acid is easily broken down by soil micro-organisms a closely related herbicide, 2,4,5-(trichlorophenoxy) acetic acid (2,4,5-T) has prolonged durability under the same conditions 2 3 4 24.

Current practices in the production of legumes emphasize the use of herbicides for weed control, thus raising questions regarding interactions between the applied chemicals and the root-nodule bacteria. Indeed, several workers have tried to approach this problem by determination of the Minimum Inhibitory Concentration (M.I.C.) of different substituted phenoxy herbicides on some species of Rhizobium 6 7 8 9 12 19 26. Such in vitro studies of herbicide effects on rhizobia show that different species are adversely affected

* 4-(2,4-dichlorophenoxy) butyric acid.
by different concentrations of the same herbicide. *Rhizobium lupini* and *R. trifolii* were found to be the most sensitive to 2,4-D and MCPA (4-chloro-2-methylphenoxyacetic acid) whereas *R. meliloti* was the least sensitive. With different workers the M.I.C. for *R. trifolii* ranged from 300 µg/ml (Carlyle and Thorpe 6) to 2,000 µg/ml (Jensen and Petersen 12). Differences in sensitivity among strains within the same species also were observed, the growth of *R. leguminosarum* 317 being inhibited by 570 µg/ml while that of *R. leguminosarum* 308 being inhibited by 1,150 µg/ml of the same herbicide (Nickell and English 18). Fletcher and Raymond 9 have shown that *R. trifolii*, when examined after 4 days of incubation, was inhibited by 50–100 µg/ml of 2,4-D, MCPA, 2,4,5-T, γ-(4-chloro-2-methylphenoxy) butyric acid (MCPB) or 2,4-DB but recovered after 10 days of incubation except in the case of 2,4,5-T. These results might explain in part the discrepant results obtained by different workers since the degree of bacterial inhibition observed depends upon the length of incubation prior to examination. Stimulation of growth, on the other hand, has been reported for *R. trifolii* and *R. lupini* grown in the presence of 10 µg/ml 2,4-D (Wroebel 26) although Nilsson 19 found that concentrations of 10 µg/ml 2,4-D and 0.01 to 10 µg/ml MCPA had neither a stimulatory nor a depressing effect on a mixed culture of *R. meliloti*.

M.I.C. determination is only a one-sided attempt to study the herbicide-rhizobia relationship and, obviously, becomes meaningless except for comparisons of those concentrations actually used for weed control. Another approach would be to study the effect of herbicides on certain of the physiological properties of rhizobia. Such studies become particularly important in view of the finding that 2,4-DB application causes substantial decreases in nodulation and nitrogen fixation in birdsfoot trefoil 10.

**EXPERIMENTAL**

In the cellular growth experiments *Lotus corniculatus* rhizobia (Strain LC 296)* were incubated at 30°C in modified medium 79 (Wilson 25) containing 0.2% yeast extract, unless otherwise specified.

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