# Inhibition of Nitrogen-Fixing and Nitrifying Bacteria by Seed Plants III. Comparison of Three Species of Euphorbia[1]

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In a previous study of 20 species of seed plants which are of some importance in succession in abandoned fields in Oklahoma, *Euphorbia corollata* L. was included among 16 species which were found to exert inhibitory activity against selected nitrogen-fixing and nitrifying bacteria (Rice 1964). The goals of the present project were to determine if two other common species of *Euphorbia* (*E. marginata* Pursh and *E. supina* Raf.) produce inhibitors of the same bacteria and, if so, to determine the relative inhibitory activities of the three species.

## Materials and Methods

Two American Type Culture (ATC) strains of *Azotobacter* and one each of *Rhizobium* and *Nitrobacter* were used as test bacteria (Table I). The same media, growing conditions, and test procedures as previously reported (Rice 1964) were used.

The test extracts were made by adding 10g of leaves to 100 ml of distilled water, grinding in a Waring Blendor for 5 min., and filtering. Sensitivity discs were saturated with the extracts and placed on plates inoculated with the test organisms. Two discs of each extract were tested against each bacterial strain. Diameters of zones of inhibition were measured after 3 days in all cases except with *Nitrobacter*. Because of its slow growth rate, zones were measured after 6 days.

## Results and Discussion

Extracts of all three species of *Euphorbia* tested were found to be inhibitory to the test bacteria with one exception, the extract of *E. marginata* was not inhibitory to *Nitrobacter* (Table I). *E. marginata* was consistently the least inhibitory to all test bacteria. *E. corollata* was most inhibitory to ATC strain 9043 of *Azotobacter* and to *Nitrobacter*, but *E. supina* was most inhibitory to ATC strain 9104 of *Azotobacter* and to the test strain of *Rhizobium*.

[1]This project was done under Grant No. G-21909 with the National Science Foundation. Typing aid was furnished by the Faculty Research Committee. Thanks are extended to Mr. Robert Parenti for his excellent work as a research assistant.

The large inhibitory zones resulting from extracts of *E. corollata* and *E. supina* are remarkable when one considers that a $10^{-3}$M solution of the very common food preservative, methyl-p-hydroxybenzoate, has little inhibitory effect on the test organisms. Many strains of *Rhizobium* have been found to be very resistant to many commonly used antibiotics also (Kecskes and Manninger 1962).

By use of bioautography, hydrolysis of inhibitory substances, and chromatographic procedures, the bacterial inhibitors in *E. corollata* were previously found to be gallotannins (Rice 1965). The most prominent appeared to be the pentadigalloyl ester of glucose.

When ferric chloride was added to the extracts of *E. marginata* and

TABLE I. Inhibition of nitrogen-fixing and nitrifying bacteria by three species of *Euphorbia*.

| Source of Test Extract | Avg. Diameter of Inhibited Zone (mm)* | | | |
|---|---|---|---|---|
| | A9043 | A9104 | R10314 | N14123 |
| Euphorbia corollata | 18 com.** 25.5 par.† | 21 par. | 15 par. | 30.5 com. |
| Euphorbia marginata | 18.5 par. | 15.5 par. | slight†† | 0 |
| Euphorbia supina | 19.5 par. | 16 com. | 20 par. | 22 par. |

*A, *Azotobacter*, R, *Rhizobium*, N, *Nitrobacter*. The numbers are American Type Culture strains.

** com. = zone of complete inhibition. If this designation is not given, no zone of complete inhibition resulted.

† par. = zone of partial inhibition

†† No definite zone present but an obvious inhibition apparent.

*E. supina* a blue-black precipitate formed just as in the case of *E. corollata*. Such a precipitate is characteristic of gallic acid and the gallotannins on the addition of ferric chloride. Probably the bacterial inhibitors in *E. marginata* and *E. supina* are gallotannins, therefore, just as in *E. corollata*.

## LITERATURE CITED

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