STUDIES ON THE RHIZOSPHERE MYCOFLORA OF BROAD BEAN AND COTTON

II. SEED AND ROOT EXUDATES AND THEIR EFFECTS ON SPORE GERMINATION AND GROWTH OF THE PREVALENT FUNGI ISOLATED FROM THE RHIZOSPHERE

by

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INTRODUCTION

Although there are many investigations on the excretion of organic substances by plant seed and root (SCHROTH & COOK, 1964; SCHROTH, TOUSSOUN & SNYDER, 1963; TURNER, 1963; COOK & SNYDER, 1965; ROVIRA, 1956; KATZNELSON et al., 1954—55) little has been done on the effects of these exudates on fungi isolated from the rhizosphere. It is generally accepted that the main factors for the stimulation of microorganisms in the rhizosphere are the exudation of organic substances from the underground parts and sloughing off of root hairs and epidermal cells.

Seed exudation was reported by SCHROTH & COOK (1964) who tested the effect of seed exudation from three bean varieties varying in susceptibility to pre-emergence damping-off on Rhizoctonia solani and Pythium spp. FRIES & FORSMAN (1951) recorded that germinating pea seeds excrete lysine, arginine, methionine, uridine (or cystidine or both), adenine and guanine (or the corresponding nucleosides). Amino-acid excretion by excised roots of maize growing aseptically has been demonstrated by KANDLER (1951), and by pea root tips suspended in distilled water by FORSMAN (1955).

Working with excised root tips, LUNDEGARDH & STENLID (1944) showed that glucose, flavanones and nucleotides were excreted by wheat while peas excreted glucose and nucleotides but not flavanones. In a study of the growth factor requirements of mycorrhizol fungi of forest trees, MELIN (1954) reported that cultures of excised pine and tomato roots produced an unidentified substance, factor, “M”, which promoted the growth of mycorrhizol fungi in a medium containing salts, sugars, B-vitamins and amino-acids. Later work by MELIN & RAMA DAS (1954) showed that this “M” factor was produced by a wide range of leguminous and nonleguminous plants.
Pea roots, however, also produced an inhibitory factor for the mycorrhizal fungi and the authors suggest that such mechanisms of stimulating and inhibiting excretions are responsible for the specificity of the fungi. The object of the present investigation was to study the nature of seed and root exudates of different varieties of broad bean and cotton, and their effects on the predominating fungi previously isolated from the rhizosphere.

**Materials and Methods**

**Extraction and identification of amino-acid and sugar constituents of seed and root exudates**

Seeds of broad bean (*Vicia faba* Linn.) of the varieties, “Giza 1”, “Giza 2”, and “Rebaia 40”; and of cotton (*Gossypium barbadense* Linn.) of the varieties, “Monofy”, “Giza 47”, and “Giza 66”, were used.

Seed exudates were obtained by placing mercuric chloride treated seeds with intact seed coats in large sterile Petridishes, containing moistened purified sand that had been acid washed and ignited, at the rate of 25 seeds per dish. After 24 h. incubation at room temperature, the seeds and portions of the sand were tested for contamination by culturing on Czapek’s agar. The contents of 6 dishes then were washed five times with distilled water, filtered, evaporated in vacuo to 10 ml at 35°C and stored at 0°C.

Root exudates were collected by placing sterile, pregerminated seeds in sterile, widemouth, 500-ml flasks at the rate of 10 seeds/flask. Each flask contained 300 g purified sand, that had been acid washed and ignited. The sand was moistened with 70 ml of modified Crone’s solution (ROVIRA, 1956). Fine-mesh glass fiber cloth placed over the silica prevented the seeds, but not the root, from touching the sand. Autoclaved filter paper confetti was moistened with sterile distilled water and added with the seeds to keep them moist and enhance germination. The plants were grown aseptically for 7 days at which time the contents of each flask were cultured and checked for contaminations. The exudates were collected and concentrated in the manner already described.

Seed and root exudates, at the rates of 10 and 20/spot were placed on Whatman No. 1 paper and descending runs were made. For amino acid determinations two-phase solvent of butanol-glacial acetic acid and water in a ratio (4 : 1 : 5) by volume was used. Two way chromatograms were also made with the first run in this solvent followed by a second in 80 % phenol adjusted to pH 5.0 with NaOH. Ninhydrin reactive spots were recorded. For the study of sugars the solvent pyridine-ethyl acetate-water in a ratio (20 : 80 : 10) was used. The chromatograms were then developed with aniline hydrogen phthalate reagent. Identification of amino acids and sugars was made by Rf values, patterns on the chromatograms, colours of the