Enhancing growth and nitrogen uptake by soybeans using pesticides

A. K. MAQBUL HOSSAIN* and MARTIN ALEXANDER
Department of Agronomy, Cornell University, Ithaca, NY 14853, USA

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Summary Benomyl applied to the seeds of soybean (Glycine max L. Merrill) inoculated with a benomyl resistant strain of Rhizobium japonicum increased the relative abundance of nodules formed by the inoculum strain and the numbers of the added rhizobium on the roots, the total N content, the percentage N, the yield at one plant density and, in one of four soils, the pod weight of soybeans grown in the greenhouse. Oxamyl applied to the seeds, foliage or both of soybeans inoculated with an oxamyl resistant strain of R. japonicum increased the yield, N content, percentage N, and weight of nodules, pods and grain and enhanced the relative frequency of nodules formed by the inoculum strain. It is suggested that pesticides or other antimicrobial agents and rhizobia resistant to these inhibitors may provide a new means for increasing nitrogen fixation by soybeans.

Introduction

Inoculation of legumes does not always result in extensive nodulation, and nodulation by an effective Rhizobium does not always result in increased plant yields. The absence of nodulation even after inoculation is especially common in tropical regions, where no more than 5% of the nodules may sometimes be formed by the inoculated strains. In countries of the temperate regions, such as in the United States, recoveries of inoculated R. japonicum strains have been low in many areas where soybeans are grown. Nodulation failures following inoculation of legumes have also occurred in Australia and New Zealand.

Legume seeds are often treated with pesticides to protect them from soil-borne pathogens, but many investigators have shown that pesticides, and in particular fungicides, are toxic to rhizobia and may affect the functioning of the Rhizobium-legume symbiosis. One way of overcoming the toxicity of pesticides to Rhizobium is to use pesticide resistant mutants for inoculation.

The present study was designed to establish the consequences of joint use of such resistant mutants of R. japonicum together with pesticides of agricultural importance.

* Present address: Soil Microbiology Laboratory, Bangladesh Agricultural Research Institute, Joydebpur, Dhaka, Bangladesh.
Materials and methods

Samples of Howard (loamy-skeletal, mixed, mesic Glossoboric Hapludalf; pH 7.5), Honeoye-Lima (fine-loamy, mixed, mesic Glossoboric Hapludalf; pH 7.8), Eel (fine-loamy, mixed, non-acid, mesic Aquic Udifluent; pH 7.6), and Palmyra (fine-loamy over sandy or sandy-skeletal, mixed, mesic Glossoboric Hapludalf; pH 7.4) from the top 15 cm were dried in air and passed through a 2 cm sieve before use.

From *R. japonicum* 311b138, which was provided by Dr. D. F. Weber, a mutant (designated SEB-5) resistant to 100 μg of benomyl [methyl-1-(butylcarbamoyl)-2-benzimidazole-carbamate], 1.0 mg of streptomycin, and 50 μg of erythromycin/ml of agar medium was isolated by the method of Odeyemi and Alexander. A second mutant (designated SEO-3) was obtained that was resistant to 500 μg of oxamyl (methyl N',N'-dimethyl-N-[(methylcarbamoyl)-oxy]-1-thiooxaminidate), 1.0 mg of streptomycin and 50 μg of erythromycin per ml of agar. Strains SEB-5 and SEO-3, which were effective on soybeans, were used to study the effects of benomyl and oxamyl, respectively. The cultures were grown at 30°C in 100 ml of YEM broth contained in 250 ml Erlenmeyer flasks incubated on a rotary shaker operating at 120 rpm. After 7 to 10 days, the cells were collected by centrifugation, washed 4 to 5 times with the sterile salts solution and used to inoculate seeds at a rate of 1.0 × 10^5 cells per g of seed.

*R. japonicum* was counted on either yeast extract-mannitol (YEM) agar or YEM agar containing 1.0 mg of streptomycin, 50 μg of erythromycin and either 50 μg of benomyl or 100 μg of oxamyl per ml. Streptomycin, erythromycin and benomyl or oxamyl were added to the molten agar cooled to 46°C. The inorganic ingredients of YEM broth were used as a buffer and for making serial dilutions. Streptomycin sulfate and oxamyl in distilled water, benomyl in N,N-dimethylformamide and erythromycin in 95% ethanol were sterilized by passage through 0.45 μm filters before their addition to autoclaved media.

Soybean (*Glycine max* var. Evans) seeds were treated with a 40% aqueous solution of gum arabic and 2.0 ml of the sterile salts solution containing (i) rhizobia, (ii) oxamyl or benomyl, (iii) rhizobia and the pesticide or (iv) nothing. Benomyl or oxamyl was added at a rate of 0.20 g of active ingredient per 100 g of seed. The seeds treated with gum arabic (2.0 ml of solution per 100 g of seed) were dried thoroughly to prevent uneven coating with the pesticide. The population of *R. japonicum* on the seeds was counted immediately before planting using 60 seeds for each dilution series. For counts on the roots, the plant material below the soil surface was removed and shaken gently to remove part of the adhering soil, and about 10 g was used to prepare dilutions. The amounts of plant and soil material contained in the bottles were determined by drying the samples at 105°C for 20 to 24 hours.

To determine the strain of Rhizobium in nodules, nodules excised from the roots were immersed in 95% ethanol for 5 min, soaked in a 5% Na hypochlorite solution for 3 to 5 min and washed 5 to 6 times in sterile distilled water. Each nodule was separately crushed in 2 to 3 ml of sterile distilled water contained in sterile petri dishes, and the resulting liquid was added to YEM agar with and without the inhibitors. Growth was noted after an incubation period of 7 to 10 days at 30°C.

Ten seeds were planted in soil contained in plastic pots, 21 cm top diameter, 21 cm high. The inoculum was *R. japonicum* SEB-5. After 10 days, the plants were thinned to 3 or 1 per container. Each treatment was replicated 4 or 6 times, and the plants were grown in the greenhouse under lights (300 μmol m⁻² s⁻¹) for 14 h at 28°C and in the dark at 20°C for 10 h each day.

In studies of the effect of benomyl on growth, nodulation and N₂ fixation, plants grown in Howard soil were harvested at 60 d, and all nodules were collected for counting, dry weight determination and nodule typing. The aboveground plant material and the root system were dried at 70°C for 2 to 3 days for dry weight determinations, and portions were ground for the determination of total nitrogen by the Kjeldahl method.

For assessment of the influence of benomyl on rhizobial populations, soybeans in Howard soil that had been inoculated with strain SEB-5 were harvested at 70 d, and the entire root system was removed. Individual roots were cut with a sterile scalpel at depths of 0–5, 5–10