# Comparison of Axenic and Microbially Contaminated Soybean Plants

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Accepted for publication 15 May 1973.

ABSTRACT

Axenic and microbially contaminated soybean plants 'Lee 68' were grown within Plexiglas isolators for 10 weeks. Both types of plants grew vigorously in isolators and were similar in appearance. Axenic plants flowered earlier, reached senescence first, weighed more (both fresh and dry weight), and contained 28% more protein than contaminated soybeans. Axenic soybean tissue, analyzed by optical emission spectrography and atomic absorption spectrophotometry, contained 17 elements in greater abundance than contaminated soybean tissue.

Phytopathology 63:1501-1504

*Additional key words:* elemental abundance, gnotobiology, tissue analysis, *Glycine soja.*

To date, most of the literature on nutritional studies comparing contaminated to axenic plants indicates that certain soil microorganisms are beneficial to plant growth (1, 3, 6, 7, and 9). Phosphorus (3) and several organic nutrients (1, 9) appear to be absorbed more rapidly by plants grown in nonsterile soil. Lindsey (6), found that inoculation of axenic plants by the addition of contaminated soil to their substrate stimulated the growth of most plants tested. Addition of *Rhizopus nigricans* or *Fusarium roseum* to roots of axenic dwarf tomato plants resulted in increased height or dry weight (7). In other studies, Rovira (8) showed that axenic wheat responded favorably to inoculation with *Azobacter* spp. and *Clostridium* spp. while axenic maize and tomato were unaffected.

Except for plant pathogens, evidence that microorganisms in the rhizosphere exert a deleterious effect on plants is meager. Bowen & Rovira (2) found that in most instances total root length was significantly shorter in the presence of microorganisms. During the past 5 years, we have grown and maintained a large number of species within sterile isolators (12). Many species grew normally, appeared exceptionally green, produced large numbers of viable axenic seed, and appeared to benefit from the absence of microorganisms. Our study attempted to further explore these phenomena, and in this paper we describe the effect of soil microorganisms on soybean growth and development.

MATERIALS AND METHODS.–Soybean seeds 'Lee 68' were surface disinfested for 5 min in a 0.5% sodium hypochlorite solution buffered with 0.1 M $KH_2PO_4$, at pH 7.0, plus Triton X-100 (Rohm & Haas). These seeds were rinsed three times with sterile distilled water and then germinated on nutrient agar in half pint jars. Five ml of tryptic soy broth was added to the top of the nutrient agar and the jars were sealed. Seeds were incubated at 23 C for 5 days and those free of microbial growth and qualifying as axenic (13) were chosen for the tests in the chambers.

Seedling jars, heat sterilized sand and perlite (1:1, v/v), Hoagland's solution (4), plastic pots, sterile soil suspension, microbiological detection media (thioglycollate, tryptic soy, Bristol's, potato dextrose, and nutrient), cotton applicators, and instruments were placed in a Plexiglas isolator. The suspension intended to contain rhizosphere organisms was prepared by collecting fresh soil samples and making a 5% (w/v) soil suspension in distilled water.

The isolator and its contents were sterilized using formaldehyde gas generated from the addition of potassium permanganate to 37% formalin (10). Following a 24-hr contact period the isolator was air-washed for 48 hr. The isolator and contents were checked for microbial contamination by submerging cotton applicators in a phosphate buffer solution (0.1 M) and swabbing the walls of the isolator and its contents. The treated applicators were placed in the microbial detection media and allowed to incubate for 48 hr. If microbial growth was not apparent at this time, soybean seedlings were transplanted to plastic pots containing moistened sand and perlite.

TABLE 1. Wet and dry weights of soybean plants grown under axenic and nonaxenic conditions

| Tissue | Wet weight (g) | | Dry weight (g) | |
|---|---|---|---|---|
| | Axenic | Nonaxenic | Axenic | Nonaxenic |
| Root | 8.0549*[a] | 4.5654 | 1.0313*[a] | 0.5279 |
| Stem | 5.2803 | 5.5908 | 1.6416 | 1.8618 |
| Leaves | 3.5577 | 4.7226 | 0.9531 | 1.3120 |
| Pods | 8.8909*[a] | 5.0983 | 2.2743*[a] | 1.1356 |
| Total | 25.7838 | 19.9771 | 5.9003 | 4.8373 |

[a] *The differences between the axenic and nonaxenic plants were statistically significant ($P < 0.05$).

Five ml of soil suspension was added to each pot. The isolator and contents were checked subsequently for microbial contamination at 5-day intervals. Leaf, stem, and root sections from plants were placed into the detection media and incubated at 37 C for 2 weeks.

Nonsterile soybean control plants were grown under identical conditions except the soil suspension added to the pots was not sterile. Following 70 days growth the plants were removed, weighed, dried, and analyzed for protein and mineral elements (11).

RESULTS.–Microbiological samplings and portions of axenic plants incubated in contamination detection media gave negative growth. Internal fungal and bacterial growth was absent in light and electron microscopic examination of randomly sampled root, leaf and stem tissues from the axenic plants.

In all experiments, axenic and contaminated plants grew vigorously and appeared healthy. The only visible difference was an earlier initiation of flowering and senescence of axenic soybeans. Root and pod weights were greater from axenic plants than from contaminated ones; leaf weights were less under

TABLE 2. Average number of leaves and pods per soybean plant grown within Plexiglas isolators

| Tissue | Number of leaves | Number of pods |
|---|---|---|
| Axenic | 29.3[a] | 10 |
| Nonaxenic | 43.3 | 11.3 |

[a] Plant had senesced prior to harvest and some leaves had fallen. When fallen leaves were added to intact leaves the axenic and contaminated plants had equal numbers.

TABLE 3. Total protein in axenic and nonaxenic soybean plants

| Tissue | Total protein (mg) | | | |
|---|---|---|---|---|
| | Roots | Stems | Leaves | Pods |
| Axenic | 433*[a] | 207 | 248 | 591 |
| Nonaxenic | 285 | 237 | 294 | 340 |

[a] *Differences between the axenic and nonaxenic plants were statistically significant ($P < 0.05$).

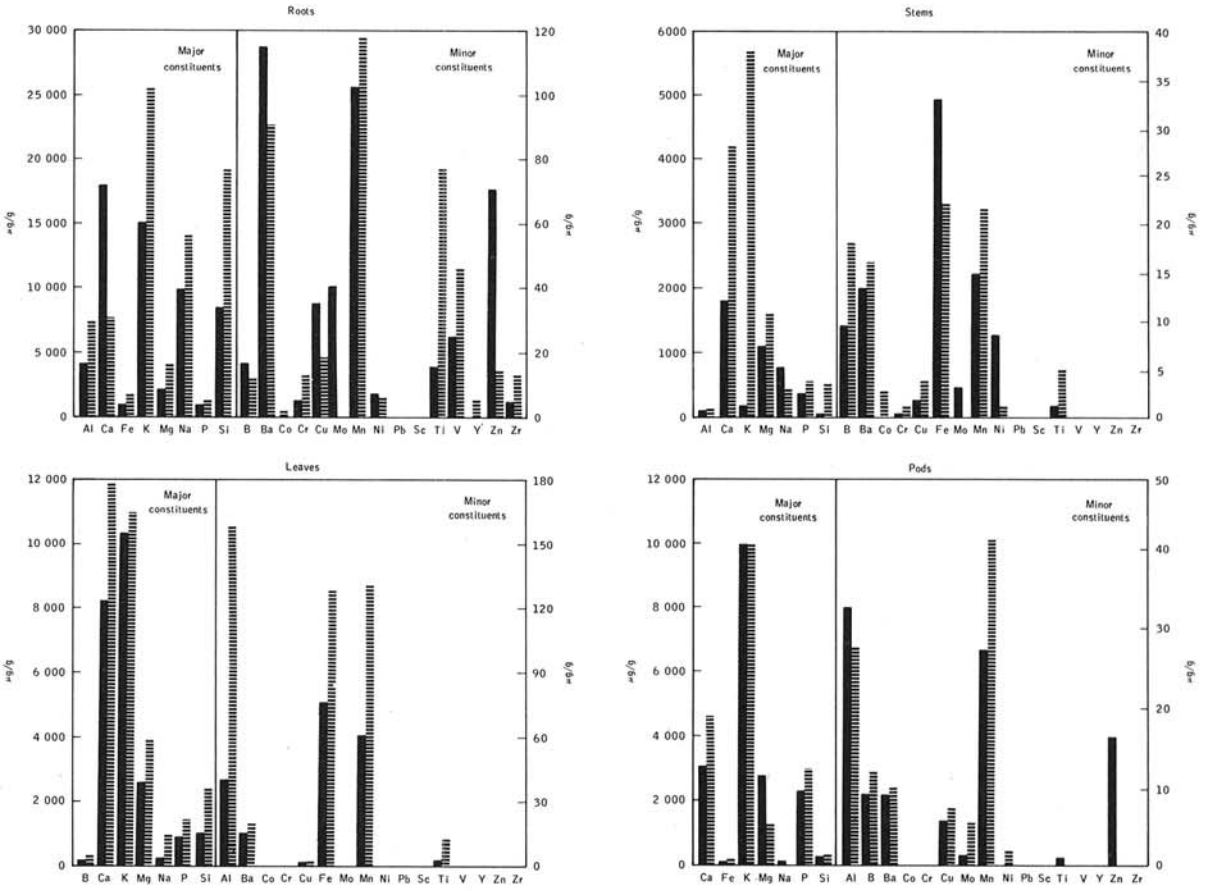


**Fig. 1.** Comparisons of elemental abundances in dried tissues harvested from conventionally grown, microbially contaminated (solid bar) and axenically grown (broken bar) soybean plants.

TABLE 4. Elemental abundances of soybean plants grown within axenic and microbially contaminated isolators

| | µg/plant | | µg/g | |
|---|---|---|---|---|
| Elements | Axenic | Contaminated | Axenic | Contaminated |
| Al | 8,040 | 1,610 | 1,360 | 334 |
| B | 242 | 169 | 41 | 35 |
| Ba | 162 | 114 | 27 | 24 |
| Ca | 36,800 | 27,900 | 6,250 | 5,770 |
| Co | 9 | ND[a] | 2 | ND[a] |
| Cr | 17 | 4 | 3 | 1 |
| Cu | 49 | 40 | 8 | 8 |
| Fe | 2,110 | 630 | 357 | 130 |
| K | 69,400 | 42,000 | 11,800 | 8,690 |
| Mg | 13,200 | 8,330 | 2,240 | 1,720 |
| Mo | 14 | 53 | 2 | 11 |
| Mn | 377 | 182 | 64 | 38 |
| Na | 15,800 | 5,760 | 2,670 | 1,190 |
| Ni | 13 | 38 | 2 | 8 |
| P | 10,400 | 5,150 | 1,770 | 1,060 |
| Pb | ND[a] | ND[a] | ND[a] | ND[a] |
| Sc | ND[a] | ND[a] | ND[a] | ND[a] |
| Si | 22,500 | 5,300 | 3,810 | 1,100 |
| Ti | 94 | 22 | 16 | 5 |
| V | 46 | 14 | 8 | 3 |
| Y | 4 | 1 | 1 | ND[a] |
| Zn | 14 | 93 | 2 | 19 |
| Zr | 13 | 3 | 2 | 1 |

[a] ND = not detected by either analytical technique.

axenic conditions at harvest (Table 1). Dropped leaves which were larger in number for axenic plants due to accelerated senescence were not considered in the leaf weights. A count of the dropped leaves and the intact ones gave a similar total leaf number for the two plant types (Table 2).

Differences between protein content per gram of tissue from axenic and nonaxenic plants were small. However, total protein in roots and pods was higher in axenic plants (Table 3).

Analysis of dried soybean tissues demonstrated that 70% of the elements measured were more concentrated in the axenic plants (Table 4). Seven elements were consistently higher (Al, Ca, Fe, Mg, Na, P, and Si). The total milligram weight of these elements in axenic plants was 280% higher than in contaminated soybeans. The comparative distribution of elements in stem, leaf, root, and pod tissue are given in Fig. 1. Differences in the elemental abundances between the two soybean root types was large.

DISCUSSION.–This paper shows that axenic soybean plants can have a higher fresh and dry weight, a higher total protein content, a faster rate of maturing and a higher concentration of many

elements than those grown in contaminated environments.

Of special importance was the observation that axenic soybeans developed a more extensive root system than contaminated plants. One possible explanation for this is that metabolites formed in the rhizosphere by microorganisms inhibit root growth (5). In the absence of microorganisms, roots might continue to develop and absorb water and nutrients. Such assimilation would explain the increased fresh weight, dry weight, and elemental abundance measured for the axenic soybeans. Another cause for the increased root growth of axenic plants might be due to rhizosphere microorganisms increasing the availability and uptake of nutrients, thus, making it unnecessary for roots of contaminated plants to develop as extensively as those of axenic soybeans. This would explain differences in root size for the two plant types, but it would not explain differences in elemental composition.

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