# Suppression of Anthracnose of Soybeans by Calcium

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**ABSTRACT**

MUCHOVEJ, J. J., R. M. C. MUCHOVEJ, O. D. DHINGRA, and L. A. MAFFIA. 1980. Suppression of anthracnose of soybeans by calcium. Plant Disease 64:1088-1089.

Application of $CaSO_4$, $CaCO_3$, and $CA(OH)_2$ significantly reduced seedling anthracnose of soybeans in the greenhouse. As little as 200 μg/g of Ca (1 meq of Ca/100 g soil) was effective. Reduction in disease was similar in seedlings grown in calcium-amended hydroponic solutions. In both hydroponic and greenhouse studies, tissue calcium increased with increased calcium applied.

Additional key words: cerrado soil, fertilizer, nutrition

Soybean production is being increased in Brazil by introducing this crop in the cerrado areas, which represent 25% of the country's total area. The soils of the cerrado are characterized by high levels of exchangeable aluminum and virtual absence of mineral nutrients such as calcium, magnesium, phosphorus, and zinc (8). In a survey of soybean diseases in the cerrado area in 1976-1977, more than 90% seedling blight, caused by *Colletotrichum dematium* (Pers. ex Fr.) Grove var. *truncata* (Schuv.) v. Arx, was observed in some fields; in adjacent fields the disease was virtually absent or stems and leaf veins had only a few necrotic specks (O. D. Dhingra, *unpublished*). Farmers or extension agents later indicated that fields with low disease levels had been limed but severely affected fields had not. This indicated that calcium might play an important role in reducing the severity of anthracnose. This study was done to verify the field observations.

## MATERIALS AND METHODS

A preliminary study was done with Hoagland's solution containing 0, 50, 100, 200, 400, 600, 800, or 1,000 μg/g of $Ca^{+2}$ supplied as $Ca(NO_3)_2$. Sodium nitrate was added to maintain the nitrate level in each solution at a level equal to that in the 1,000 g/g of $Ca^{+2}$ solution. Soybean seeds (cv. UFV-1) were germinated in moistened vermiculite, and two seeds were transferred after 5 days to each 130-ml glass bottle filled with Hoagland's solution. Plants were held in place with foam plugs, and the bottles were wrapped in black paper to minimize algal growth. Seedlings were grown under 18 hr of light and 6 hr of darkness with the solutions aerated for 15 min/hr. Each treatment included 30 plants and was repeated three times.

The soil was a calcium-deficient sandy loam oxisol (Ca+Mg, 0.05 meq/100 g; exchangeable $Al^{+3}$, 0.4 meq/100 g; CEC 3.2 meq/100 g; pH, 4.8) collected from a cerrado area destined for soybean cultivation. Calcium as $Ca(OH)_2$, $CaCO_3$, or $CaSO_4$ was added to supply 0, 200, 400, 600, 800, or 1,000 μg/g of $Ca^{+2}$. The dry soil and the amendment were mixed in a motorized twin arm mixer for 1 hr, moistened to field capacity with deionized water, and incubated in polyethylene bags for 4 mo. The soil was then distributed into 200-ml plastic cups, one seed was planted per cup, and the soil moisture was maintained at 80% of field capacity. There were 10 cups in each of 10 repetitions per treatment, and the experiment was repeated twice.

Seedlings grown in solutions or soil were spray-inoculated with a conidial suspension ($10^6$ conidia per seedling) of *C. dematium* var. *truncata* when the seedlings had reached the primary leaf stage. Seedlings sprayed with sterile water served as controls. All seedlings were placed in a lighted moisture chamber for 48 hr, transferred to the greenhouse for an additional 24 hr, and then rated for disease. Each seedling was rated for disease on a 1–5 scale where 1 = asymptomatic to occasional minute necrotic flecks, 2 = isolated small lesions, 3 = 20–50% necrosis of stem or leaf veins, 4 = 50–90% necrosis, and 5 = dead plants.

Immediately after disease was rated, the aerial part of the inoculated and uninoculated plants were bulk-harvested in the repetitions, dried for 48 hr at 80 C, ground, digested with $HNO_3$ and $HClO_4$ and analyzed for $Ca^{+2}$ by the EDTA method (11).

## RESULTS AND DISCUSSION

The disease index on seedlings grown in Hoagland's solution decreased signifi-

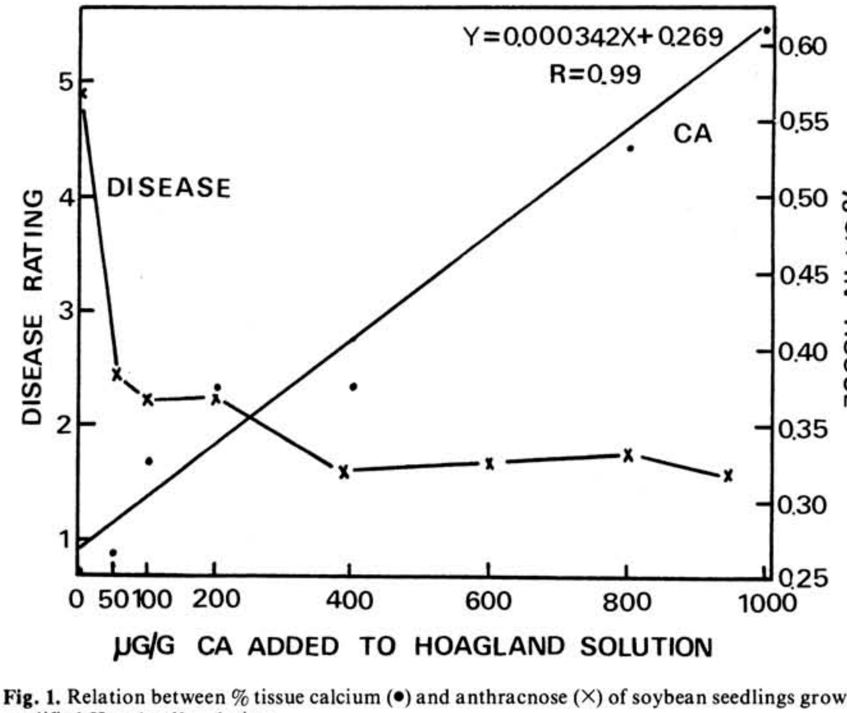


**Fig. 1.** Relation between % tissue calcium (•) and anthracnose (×) of soybean seedlings grown in modified Hoagland's solution.

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Supported by a UFV-FINEP contract.

0191-2917/80/12108802/$03.00/0

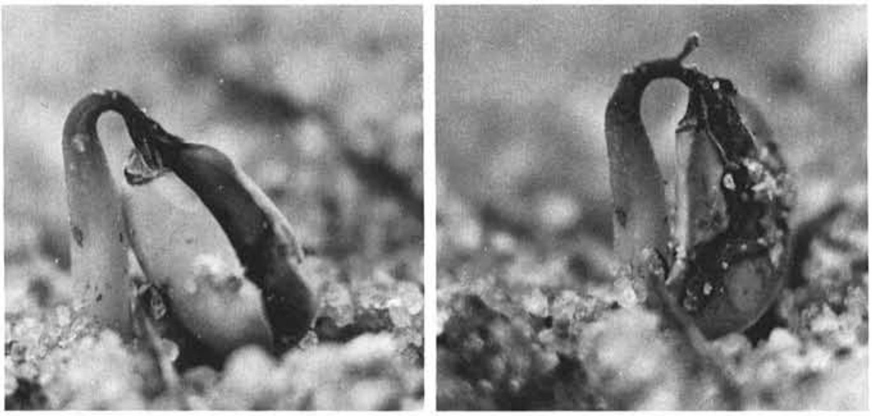

**Fig. 2.** Calcium-deficient soybean seedlings with anthracnose caused by seedborne *Colletotrichum dematium* var. *truncata*: **(Right)** Seedling with cotyledon removed shows internal damage.

cantly with additional calcium (Fig. 1). Inoculated seedlings grown in calcium-deficient solutions died within 3 days of inoculation. Addition of 50–200 $\mu g/g$ of calcium reduced the disease index by almost one-half. The disease index declined significantly when the calcium content of the solution was raised to 400 $\mu g/g$, and no further change occurred. The calcium content of the tissue increased in linear relation to the calcium content of the solution (Fig. 1). The disease index of inoculated seedlings grown in soil amended with $CaCO_3$, $Ca(OH)_2$, or $CaSO_4$ followed the same trend as that of seedlings grown in nutrient solution. The disease index declined from 5 for seedlings grown in unamended soil to 1.3 for seedlings grown in soil amended with 200 $\mu g/g$ of Ca. Uninoculated controls showed no disease.

Inoculated seedlings grown in soil containing 400 $\mu g/g$ of Ca or more were asymptomatic or showed only occasional minute necrotic flecks on the stems or major leaf veins. There was no significant difference among sources of calcium on disease. Tissue calcium of inoculated plants increased from 0.3% in seedlings grown in unamended soil to 0.6% in plants grown in soil amended with 200 $\mu g/g$ of Ca. There was no further significant increase in tissue calcium with increased soil calcium and no significant difference among calcium sources.

Calcium nutrition of the plant suppresses certain plant diseases (1,3,5,7,12), and the effect is often attributed to poor activity on Ca-pectates by pathogens' pectinolytic enzymes (1,3,5,7,10). Tissues of calcium-deficient plants are poorly organized, have thin cell walls, large intercellular spaces, and underdeveloped middle lamellae (6,7); all of these may facilitate fungal colonization of such tissues.

Symptoms of soybean seedling anthracnose caused by *C. dematium* var. *truncata* are similar to those of soft rot, and the fungus is known to produce pectinolytic enzymes (2). Anthracnose is an important disease of soybeans in the cerrado regions of Brazil. Its seedborne nature frequently allows seedling blight to occur (4,9) (Fig. 2). The disease is relatively less severe in areas of Brazil other than the cerrado regions. Soil application of calcium appears to be an effective way to reduce disease intensity in calcium-deficient soils.

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