

## Effect of *Endogone* Mycorrhiza on Soybean Yields

J. P. Ross and J. A. Harper

Plant Pathologist and Agricultural Research Technician, respectively, Crops Research Division, ARS, USDA, P.O. Box 5397, Raleigh, North Carolina 27607.

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### ABSTRACT

Growth and yield of soybean plants growing in previously fumigated small isolated plots were increased 34-40% in the presence of a chlamydosporic species of the vesicular-arbuscular mycorrhizal fungus *Endogone*. Inoculum for these plots was produced by culturing *Endogone* monoxenically on soybean roots to assure the absence of other soil

microorganisms. Mycorrhizal plants accumulated greater amounts of P, N, Ca, Cu, and Mn in their foliage than nonmycorrhizal plants. Infesting previously fumigated field plots with *Endogone* resulted in yield increases of 29% over fumigated-noninfested plots. This effect did not occur in nonfumigated soil. *Phytopathology* 60:1552-1556.

*Additional key words:* *Glycine max.*

Gerdemann (5) recently called attention to vesicular-arbuscular mycorrhizae and emphasized the need for additional work to evaluate their economic significance in relation to plant growth. Since previous work in this area was carried out mainly under controlled greenhouse conditions, information on yield from field-grown plants is lacking.

Nematode experiments carried out in methyl bromide-fumigated microplots (11) have required the addition of a small amount of nonfumigated soil from the previous season's control plots to assure good soybean (*Glycine max* L. Merr.) growth and yield. Heat sterilization of the added soil destroyed the stimulating effect and indicated that a soil-borne biological factor(s) was stimulating soybean growth and yield. This phenomenon was examined further with soil from a field in Mississippi (courtesy of E. E. Hartwig) with a history of high soybean yields. This soil was used in experiments to determine whether a stimulatory effect on soybean yield could be obtained, and whether this stimulation was related to an *Endogone* sp. isolated from the Mississippi soil.

**MATERIALS AND METHODS.—Plots.**—Experiments were conducted in small plots (1968 and 1969) and in field plots (1969). The small plots consisted of rectangular bins (1 × 1.5 m) constructed of fiberglass sheets (2 mm thick) cemented together at the corners to provide an impregnable barrier. Bins were placed in the ground to a depth of 0.9 m; the fiberglass sides rested on the clay subsoil and projected about 15 cm above the surrounding soil surface. Plots were filled with sandy loam to about the same level as the outside soil.

In 1968, nine plots were installed and soil was fumigated 1 month before planting with 6.1 ml of methyl bromide and 4.8 ml of chloropicrin/m<sup>2</sup>. In 1969, nine additional small plots were installed; the soil was treated with 6.1 ml/m<sup>2</sup> of methyl bromide, and 3.6 ml/m<sup>2</sup> of a mixture of 68% D-D, 17% methyl isothiocyanate, and 15% chloropicrin was injected to a depth of 23 cm with a fumigun. After treatment, the plots

were covered with polyethylene plastic for 2 days. In 1968, commercial inoculant of *Rhizobium japonicum* was added to surface-sterilized seed, and in 1969 a suspension of bacteria from a pure culture was applied to the soil at planting. Seed was surface-sterilized with 1.5% NaClO and planted in two rows, 51 cm apart, on 22 May of both seasons.

Field plots were established at two locations in North Carolina: Central Crops Research Station, Clayton, on a sandy loam; and Tidewater Research Station, Plymouth, on a Portsmouth fine sandy loam. Plots were treated with the same fumigants in the same manner and at the same rates as the small plots in 1969. Each plot contained three rows 3.0 m long and 0.9 m apart. Commercial *Rhizobium* inoculant was applied to seed planted on 23 and 27 May, respectively, at Clayton and Plymouth. At Plymouth, seedling emergence in fumigated plots was poor and these plots were replanted on 10 June. Yield comparisons were made between middle rows.

Both the small plots and field plots were fertilized with 0-10-20 at the rate of 444 kg/hectare at planting. Mycorrhizal inoculum was distributed in 15-cm-deep furrows; the furrows were filled and seed of cultivar Lee planted directly above the inoculum. Treatments consisted of various mycorrhizal inocula and were replicated 3 times in all experiments.

**Estimation of mycorrhizal development.**—Mycorrhizal development was estimated from the number of *Endogone* chlamydospores recovered per unit wt of root at the end of the 1969 growing season. Soil samples were collected with a tube 2.54 cm in diam to a depth of 20 cm. The sample from each plot was divided into three 500-ml aliquots, and root fragments were recovered by flotation and sieving. Roots were blotted, weighed, and blended in water. Chlamydospores were separated from root debris by sieving and decanting, suspended in 100 ml of water, and counted in three 10-ml aliquots.

During and after the growing season, root fragments recovered from soil samples were stained with acid-

fuchsin and examined for *Endogone* vesicles and mycelium.

*Leaf and seed analyses.*—Leaf samples for elemental analyses were taken on 4 September 1969 from plants in each small plot. These analyses were conducted by the Department of Soil Science, North Carolina State University. The percentage of protein and oil in seed harvested from the small plots in 1969 was obtained through the courtesy of the U.S. Regional Soybean Laboratory, Urbana, Illinois. The following plant responses were measured: (i) dry wt of aboveground growth of mature plants; (ii) wt of seed yield; and (iii) wt of 100 seed.

*Inoculum preparation.*—Plots were infested in 1968 with either of two types of inoculum, 1,800 g of field soil from Mississippi or 32 g of washed and denodulated soybean roots greenhouse-grown in Mississippi soil for 7 weeks. The same amounts of soil and roots used in the treatments were autoclaved twice for 1 hr on successive days and added to the control plots.

Inoculum for the 1969 small plots consisted of soil and roots containing *Endogone* isolated from soybean roots grown in the 1968 plots. To eliminate other soil-borne organisms from the inoculum, the following procedure was followed.

In January 1969, dead roots containing *Endogone* chlamydospores were dug from plots, washed, and blended for 30 sec. The suspension was passed through a sieve with 250- $\mu$  openings to remove coarse debris, and the spores were retained on a second sieve with 53- $\mu$  openings. The spores were concd by settling, layered on top of a sucrose density gradient column (40, 50, 60, 70%), and centrifuged for 3 min at 1,500 rpm (10). The spores formed a broad band and were drawn off and washed to remove the sucrose. At this stage very little extraneous material remained, and the spores were individually picked up under the dissecting microscope by a siphon-energized suction apparatus (3). The spores were disinfested in 0.05% NaClO for about 1 min, rinsed 30 times in sterile distilled water, and plated out on potato-dextrose agar (PDA) in petri plates. After a 2-day incubation period at 25-30 C, spores free of contaminating microorganisms were aseptically picked up by suction and used to inoculate sterile soybean root systems.

The method of culturing soybean with a sterile root system is illustrated in Fig. 1. Wide-top glass jars (475 ml) were filled with screened soil and autoclaved for 1 hr on each of 4 successive days. Sterile soil was added to make the soil surface level with the top of each jar. The soil was moistened with sterile water before the jar was covered with two thicknesses of a thin laboratory film (Parafilm M-6). Soybean seed were disinfested with NaClO and germinated on PDA slants to assure freedom from microorganisms. A probe was inserted through the film covering each jar and 3-5 cm into the underlying soil. Thirty to 100 sterilized chlamydospores were injected through the perforation to various depths in the soil with a sterile, 14-gauge, 10-cm laboratory cannula and syringe. The radicle of a germinated seed was then inserted through the film



Fig. 1. Soybean plant supporting monoxenic culture of *Endogone* on its root system.

into the soil. The elastic properties of the film provided a tight union around the hypocotyl. In an area of the film removed from the point of seedling insertion, a sterile bent glass tube (5 mm inside diam) containing a cotton wad was inserted for root aeration. The young seedlings were covered with moist cheesecloth until the roots became functional. Plants were

placed in a growth chamber with 30-C days and 24-C nights. Only one irrigation was needed during the 5-week growth period. Sterile water was injected with a cannula and syringe through the film after it had been wiped with 80% ethyl alcohol. Root growth penetrated the soil to the bottom of the jar.

After the growth period, soil samples were taken aseptically from each jar with a cork borer, and the soil was plated on PDA and placed in nutrient broth to test for contaminating microorganisms. The plastic coverings and plant tops were removed, and the jars replanted with surface-disinfested soybean seed; plants were grown for 14 more weeks in the greenhouse. Root samples were then taken, stained with acid-fuchsin, and observed for the presence of vesicles within and outside of the roots. *Endogone* developed in plants in 5 of 13 culture jars and were free of detectable contamination. Soil and chopped roots from these five cultures were composited and used as inoculum (200 or 1,990 g/plot) for the small plots initiated in 1969.

Field-plot inoculum consisted of chlamydo spores extracted from roots from the 1968 plots plus a mixture of soil and *Endogone*-infested soybean roots from 11-week-old greenhouse cultures established with chlamydo spores. Control plots received soil and soybean roots from greenhouse cultures free of *Endogone*.

The *Endogone* isolate obtained from the Mississippi soil appears to be a chlamydo sporic species closely related to *Endogone macrocarpa* (Tul.) Tul. var. *geospora* (9). It differs most from this variety by forming chlamydo spores with walls having two distinct layers.

**RESULTS.—Small plots.**—In 1968, no differences in plant growth were recognized between the treatments. Rainfall was virtually nil and the plots were irrigated. Harvest data showed that addition of the Mississippi soil to plots increased plant dry wt and seed size and yield, whereas addition of the roots from plants grown in the Mississippi soil increased only plant dry wt (Table 1).

In 1969, plant growth in control plots was noticeably less and the leaves were lighter green and smaller than those in *Endogone*-infested plots (Fig. 2-A, B, C). Terminal racemes of plants in the latter were noticeably larger and bore more flowers (Fig. 2-D). Plant growth did not respond differently to the two inoculum levels.

Observation of root fragments separated from soil samples taken on 17 July 1969 and stained with acid-fuchsin revealed *Endogone* vesicles only in roots from infested plots (Fig. 2-E). After plant maturity, the average number of chlamydo spores (Fig. 2-F) per g

TABLE 1. Effect on soybean growth and yield of adding Mississippi field soil or soybean roots grown in Mississippi field soil to previously fumigated small plots in 1968

Inoculum	Total dry plant wt <sup>a</sup>	Seed yield	Wt of 100 seed
	g	g	g
Roots (32 g)	2,056**	364	16.6
Soil (1800 g)	2,162**	506*	17.2*
Control	1,708	333	16.3

<sup>a</sup> Wt of above-ground plant at harvest. \*, \*\* = Statistically different from control at 5 and 1%, respectively.

TABLE 2. Effect of *Endogone* on the elemental composition of leaves from soybeans grown in previously fumigated soil in small plots in 1969

Inoculum level <sup>a</sup>	Per cent <sup>b</sup>					ppm <sup>b</sup>		
	N	P	K	Ca	Mg	Fe	Cu	Mn
g								
1,990	4.22 a	0.25 a	1.2 a	1.20 a	0.23 a	120 a	12.1 a	260 a
200	4.04 b	0.25 a	1.3 a	1.21 a	0.22 a	116 a	11.7 a	257 a
0	3.18 c	0.13 b	1.3 a	0.89 b	0.22 a	108 a	8.4 b	221 b

<sup>a</sup> Inoculum consisted of soil and soybean roots containing *Endogone*.

<sup>b</sup> Numbers with different letters are statistically different (5% level).

of roots from the low and high inoculum plots were 4,770 and 6,270, respectively. No *Endogone* chlamydo spores were found in roots grown in control plots.

The mycorrhiza affected the foliar content of certain elements (Table 2). P was increased almost 100% by the fungus, and N content was influenced not only by the fungus but also by its inoculum level. Ca, Cu, and Mn were also greater in leaves of mycorrhizal plants than in those of the control.

Soybean yields from plots infested with low and high inoculum levels were increased 37 and 43%, respectively, over yields from control plots (Table 3). The wt per 100 seed and dry wt of mature plants from *Endogone*-infested plots were also greater than those from control plots; percentages protein and oil of the seed were not altered by the fungus. Although none of the differences between the data from the two inoculum levels was significant, the average values from plots infested with the high inoculum level were consistently greater than these values for plots infested with the low inoculum level.

**Field plots.**—Initial plant growth in fumigated plots at both locations was stunted. By July, plants in certain fumigated plots infested with *Endogone* were noticeably larger than those in adjacent noninfested, fumigated plots (Fig. 2-G). In the nonfumigated plots, plant growth in infested and noninfested plots was similar.

At Clayton, yields from fumigated plots infested with *Endogone* were 29% greater than yields from noninfested plots; other differences were not significant (Table 4). Weight of 100 seed was not affected.

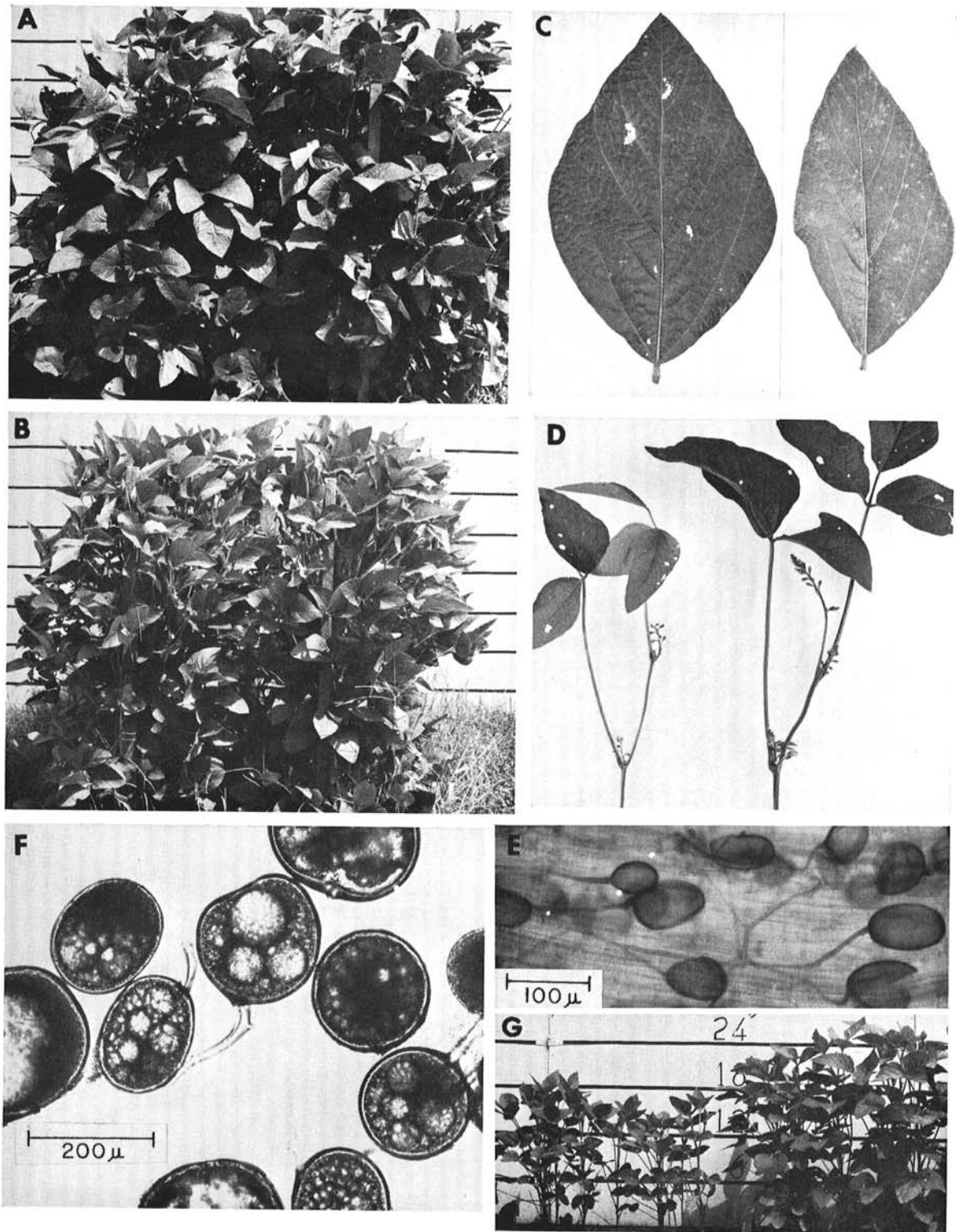
TABLE 3. Effect of infesting fumigated soil in small plots with *Endogone* on soybean growth and yield in 1969

Inoculum level <sup>a</sup>	Seed				Total plant wt <sup>b</sup>
	Yield	Size	Protein	Oil	
g	g	g/100 seed	%	%	g
1,990	1,161***	14.4**	39.7	23.1	3,450**
200	1,108**	14.3**	39.4	22.7	3,273**
0	812	12.9	39.3	23.1	2,567

<sup>a</sup> Inoculum consisted of soil and soybean roots containing *Endogone*.

<sup>b</sup> Plant top wt at maturity.

<sup>c</sup> \*\* = Statistically different from 0 inoculum level at 1%.



**Fig. 2.** A) Soybeans growing in soil infested with *Endogone*. B) Soybeans growing in soil not infested with *Endogone*. C) Comparable leaflets from soybean plants growing in *Endogone*-infested (left) and noninfested soil (right). D) Terminal portion of soybean stems showing development of racemes from plants grown in *Endogone*-infested soil (right) and noninfested soil (left). E) Vesicles of *Endogone* developed in racemes from soybean roots. F) Chlamydospores of *Endogone* extracted from roots of mature soybean plants. G) Growth of soybeans in previously fumigated field plots infested with *Endogone* (right) and noninfested (left).

TABLE 4. Effect of infesting fumigated and nonfumigated field soils with *Endogone* on soybean yield

Treatments	Yields <sup>a</sup>		Seed size	
	Clay-ton	Ply-mouth	Clay-ton	Ply-mouth
	g	g	g/100 seed	
Fumigated + <i>Endogone</i>	696*	483	14.4	14.3
Fumigated	538	450	13.8	13.8
Nonfumigated + <i>Endogone</i>	821	744		
Nonfumigated	757	755		

<sup>a</sup> Yield from 10 ft. of row. \* = statistically different from noninfested-fumigated control (5%).

At the end of the growing season, endogenous chlamydospores of the fungus were found only in roots from fumigated plots infested with *Endogone*. The average number of spores per g of root tissue was 1,914.

DISCUSSION.—Results of the 1968 experiment demonstrated that certain soil-inhabiting organisms, besides nodulating bacteria, contribute to increasing soybean yields. The use of soil inoculum derived from monoxenic cultures established from isolated *Endogone* chlamydospores that stimulated soybean growth and yields supports the hypothesis that *Endogone* may enhance soybean productivity.

Since response to the fungus occurred only in previously fumigated soil with reduced populations of soil microorganisms, including any *Endogone* (if present), it is impossible to conclude from these experiments whether other soil-inhabiting microorganisms, besides *Endogone*, might not also stimulate soybean growth. The failure of soybean yields to respond to the addition of *Endogone* to nonfumigated field plots and the absence of the fungus in soybean roots from these plots in July indicate that soil microorganisms inhibit or compete with this mycorrhizal fungus.

The striking increase of P in the foliage of plants growing in the *Endogone*-infested small plots verifies results of previous investigations carried out under controlled greenhouse conditions (1, 2, 4, 6), and supports the contention that *Endogone* was playing a major role in increasing soybean growth and yield. The increased foliar content of P, N, Ca, Cu, and Mn indicates that the *Endogone* mycorrhiza may be involved in the uptake of several essential elements. The positive correlation between the foliar N content and initial inoculum levels may indicate either (i) a close relationship between the mycorrhiza and the N fixation process; or (ii) that mycorrhizal plant roots are more efficient in N uptake. The increase of plant dry wt coupled with the increases in percentage composition of certain essential elements in the foliage indicate that the increased absorption of these elements by the mycorrhizal plants was even greater than that indicated by the foliage analyses. Although a tenfold difference in the inoculum level did not significantly alter yields, the trend for greater yields, plant wt, and up-

take of certain elements to be associated with the higher inoculum level indicate that a greater inoculum differential may give a significant dosage response.

The relevance of the vesicular-arbuscular mycorrhizal fungi to plant pathology may be considerable. The direct effect of these fungi on pathogens and/or their indirect effect by influencing the response of a plant to disease could play important roles. Ectotrophic mycorrhizae are believed to have considerable effects on root diseases of trees (7, 12). In plant disease studies, soils are commonly treated to control soil-inhabiting pathogens, and populations of mycorrhizal fungi such as *Endogone* are probably reduced (8). If differences between mycorrhizal and nonmycorrhizal plants of other species are as great as those of the soybeans in the small plot experiment, interpretation and extrapolation of results from plant disease experiments could be erroneous.

Occasional soybean yields of over 100 bu/acre (6,740 kg/hectare) in the USA have led agricultural scientists to search for underlying causes of these super yields. Association of high *Endogone* densities with high soybean yields would support the evidence presented here that vesicular-arbuscular mycorrhizae increase soybean yields.

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