# Effect of Chemical Soil Treatment on Plant Growth, Nitrogen Fixation, and Fungal Colonization of *Rhizobium* Nodules of Soybeans

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## ABSTRACT

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Hodgson soybeans, inoculated with *Rhizobium japonicum*, were grown in Vapam-fumigated and nonfumigated field soil for two growing seasons. Plants in nonfumigated soil showed significantly lower acetylene reduction and plant dry weight than did plants in fumigated soil during the period of pod formation in 1978. Nodule fresh weight was significantly inhibited more often than nodule number on plants grown in nonfumigated soil. Significantly more nodules and roots were colonized by fungi in nonfumigated than in fumigated soil at all but one sampling in 1979. In a one-season fungicide trial, application of captan as a soil drench did not result in significantly more plant growth and nitrogen fixation than did propamocarb drench or water alone. *Gliocladium, Myrothecium, Corynespora, Trichoderma, Fusarium,* and *Phoma* were the fungal genera most frequently isolated from soybean nodules and roots in these studies.

*Additional key words*: root rot, soil fumigation, soil fungi, soil fungicides.

Recently, attention has been focused on biological factors that affect the essential process of symbiotic nitrogen fixation. Viruses, bacteria, actinomycetes, and nematodes have been associated with *Rhizobium* spp. or with *Rhizobium* nodules of legumes (9,19,20,22,27), and some of these organisms reportedly have detrimental effects on nodule initiation, development, or function (1,2,18,26).

Many soilborne fungi colonize root nodules and may also inhibit nodulation and/or nitrogen fixation (3,6,23). Orellana (16) found that *Rhizoctonia solani* (Kuhn) significantly reduced plant top and nodule weights of Lee and Kent soybeans inoculated with *Rhizobium japonicum* (Kirchn.) Buchanan, compared to those from plants grown with the bacterium alone. Soybean root nodules also showed evidence of internal cell breakdown when grown in the presence of *R. solani* (17). *Rhizobium* spp. may interact with root rot fungi, in some cases intensifying (4) and in other cases decreasing (11,24,25) the severity of legume root diseases. Chhonkar and Subba-Rao (3) isolated numerous soil fungi from the surface of root nodules of many leguminous hosts and demonstrated that the fresh weight and nitrogen content of *Trifolium alexandrinum* L. was decreased significantly due to colonization by a species of *Cephalosporium*. Chemical treatment of soil with fumigants or fungicides has been shown to decrease severity of root rots and increase plant yield (5).

Although fungi apparently can colonize nodule tissue, and pathogenic soilborne fungi can have an adverse effect on nodule production and nitrogen fixation, the effects of fungal colonization on nodules have not been studied in the field. Because pods maintain priority over roots for photosynthate as plants mature, the contribution of soil mycoflora to decline of roots and nodules during this stage of stress is of particular importance. Objectives of the study were to identify fungal colonizers of soybean nodules and roots, to attempt to relate these fungi to nitrogen fixation potential under field conditions throughout the normal growing season, and to evaluate possible beneficial effects of soil fumigation and fungicidal treatment on plant growth and nodule function.

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## MATERIALS AND METHODS

Two similar soil fumigation experiments were made in 1978 and 1979; a third experiment was undertaken in 1979 to look at the influence of two soil fungicides on plant growth and colonization of roots and nodules by soil fungi.

**Fumigation experiment—1978.** Field plots were laid out in a randomized complete block design with four blocks (replicates) each containing two plots (treatments) on a silt loam that had been cropped to soybeans each season for the last 35 yr. Before fumigation, plots were sprayed with Treflan (trifluralin, Elanco Products Co., a division of Eli Lilly Co., Indianapolis, IN) which was incorporated into the soil. One plot (7.5 $m^2$) in each block was fumigated with sodium methyldithiocarbamate (Vapam) in early May at a rate of 125 L/ha by roto-tilling the fumigant solution to a depth of approximately 10 cm, watering, and covering plots with 4-mil polyethylene sheets. Plots were uncovered after 7 days and roto-tilled several times, and four rows of Hodgson soybeans per plot were planted on 3 June at a row width of 46 cm. A commercial granular mixture (Nitragin Co., Milwaukee, WI 53209) of *R. japonicum* was sown with the seed.

All pods were removed from plants in two of four rows of each plot at 6- to 10-day intervals from pod formation until plant senescence to determine the effect of allowing more photosynthate to go to the root system. Soil dilutions and isolations from plant roots and nodules were made to relate fungal genera in soil to genera isolated from plant tissue. Four times during the growing season, two root samples were dug up, removed from each plot, and subjected to several measurements. Acetylene reduction, an assay of nitrogenase activity, was calculated on two 10-cm root sections (including nodules) per sample. Samples were washed in running tap water and placed in 0.98-L jars with serum stoppers fitted into the lids. Roots and nodules were incubated in a 6% acetylene atmosphere for 30 min, after which a 0.5-cc gas sample was

removed for analysis in a Varian model 3700 gas chromatograph (Varian Instrument Division, Palo Alto, CA) with a Poropak N 90C column and a flame ionization detector. Results were expressed as μmoles of ethylene evolved per gram of nodule fresh weight per hour (7). Nodules were removed from each set of roots used in the acetylene reduction assay, counted, and weighed. Aboveground parts of plants were dried with moving air at 95–100 F for 72 hr, and dry weights were determined for all samples. Fungi were isolated from 10 g of soil taken from within the rows of plots of each treatment. The sample was added to 100 ml of 0.2% water agar (WA) and agitated for 30 sec. Serial dilutions were made in 0.2% Difco potato-dextrose agar (PDA) (1.8% WA) (pH 4.5–5.0), and fungal colonies were counted after 3 days of incubation at room temperature. Nodules and roots were washed in running tap water for 20 min, surface sterilized in 0.05% sodium hypochlorite for 2 min, rinsed twice with sterile distilled water, and plated separately on 0.2% Difco PDA (1.8% WA). Colonies arising from nodules and roots were counted and fungi identified after 7–10 days of incubation at room temperature.

**Fumigation experiment—1979.** Plots were set up similarly to those of 1978 at an adjacent location on silt loam soil that had been continuously cropped to soybeans for 6 yr. Plants were not depodded in 1979, and three samples of plant material and plot soil were taken from each plot six times during the growing season. Acetylene reduction, nodule number, nodule weight, plant dry weight, soil dilutions, and fungal isolations from nodules and roots were determined as in 1978.

Nodules were collected throughout the growing season from plants grown in fumigated and nonfumigated field soil, fixed in 2.5% glutaraldehyde (0.01 M $NaPO_4$ buffer, pH 7.0), dehydrated in an acetone series that included infiltration with osmium tetroxide, dried in an Omar SPC-50/EX critical point dryer, and coated with platinum/palladium. Prepared nodule surfaces were viewed with a scanning electron microscope.

Yield and quality of seed from five randomly selected mature plants in each plot were determined (29). Percent total nitrogen was determined by the Kjeldahl method (13) at the Analytical Services Laboratory, Department of Soil Science, University of Minnesota.

**Fungicide experiment.** Hodgson soybeans were planted with *R. japonicum* inoculum in one 4.6-m row per plot with four plots per treatment. One of three treatments was applied as a soil drench before sowing: captan at 1.1 kg a.i./ha (1 lb a.i./a), propamocarb (experimental fungicide $C_9H_{20}N_2O_2$, Nor-Am Agricultural Products, Chicago, IL) at 3.4 kg a.i./ha (3 lb a.i./a), or water control.

Two samples of plant material and soil from each plot were collected during the flowering and the pod-filling stages, and seeds from five plants per plot were analyzed for yield and quality. All samples were subjected to the same analyses as samples taken from the 1979 fumigation trial.

## RESULTS

**Fumigation 1978.** Acetylene reduction by nodules from plants in fumigated plots was significantly greater at the pod-formation and pod-filling stages (Table 1) than at preflowering or flowering. Depodding plants apparently had little effect on the amount of acetylene reduced, and the differences between normal and depodded plants were significant only in two cases (nonfumigated plants at flowering and fumigated plants at pod-fill). Numbers and weights of nodules from plants in fumigated soil were not consistently different than those from plants grown in nonfumigated soil (Table 1). Plant dry weights were significantly greater from fumigated plots than from nonfumigated plots during the preflowering and pod-formation stages (Table 1).

During the growing season, no consistently significant differences were found in the number of nodules and roots from plants in fumigated and nonfumigated soil colonized by fungi; however, nodules and roots from plants in the fumigated-depodded treatment were colonized with significantly fewer fungi than most other treatments at the last two harvest dates (Table 2).

In most treatments, percentage of tissue pieces colonized by fungi increased and more fungal species were isolated as the season progressed. The number of nodules and root pieces colonized by fungi increased for most treatments, particularly during the period of plant flowering and pod formation, and the fungal genera isolated were similar for different treatments and tissue types. Although fungal colonization was affected somewhat, depodding plants did not appear to have an effect on the variety of fungal genera that could be isolated from plant tissue. The predominant fungal genera isolated from nodules and roots were *Gliocladium, Corynespora, Fusarium, Stysanus,* and *Phoma.* Of the fungi isolated from nodules and roots, all but *Corynespora* and *Chaetomium* spp. were also observed on soil dilution plates.

**Fumigation 1979.** Plants matured late because of unseasonably cool and wet weather in September. Plants grown in fumigated soil tended to reduce more acetylene than plants grown in nonfumigated soil (Table 3); however, this difference was significant only for samples collected before flowering.

The number of nodules on plants of both treatments was greatest when pods were filling (Table 3). Plants from fumigated plots had

TABLE 1. Measurements during four growth stages of depodded and nondepodded (control) Hodgson soybeans grown in fumigated and nonfumigated soil in 1978[a]

| Growth stage | Treatment | Acetylene reduction[b] | Plant dry weight (g) | Nodule number | Nodule fresh weight (g) |
|---|---|---|---|---|---|
| Preflowering | Fumigated control (FC) | 5.09 a | 3.55 a | 81 a | 0.53 a |
| | Fumigated depodded (FD) | 4.68 a | 3.29 a | 105 ab | 0.56 a |
| | Nonfumigated control (NFC) | 3.78 a | 2.32 b | 103 ab | 0.72 a |
| | Nonfumigated depodded (NFD) | 3.78 a | 2.49 b | 107 b | 0.63 a |
| Flowering | FC | 3.72 ab | 9.00 ab | 165 a | 1.39 ab |
| | FD | 4.58 a | 10.4 a | 163 a | 1.20 a |
| | NFC | 4.49 a | 6.33 b | 170 a | 1.84 b |
| | NFD | 2.84 b | 8.56 ab | 151 a | 1.30 ab |
| Pod-forming | FC | 9.35 a | 19.5 a | 127 a | 1.37 a |
| | FD | 10.7 a | 21.7 a | 178 b | 2.18 b |
| | NFC | 6.14 b | 14.1 b | 152 ab | 2.01 b |
| | NFD | 5.12 b | 13.1 b | 129 a | 1.46 a |
| Pod-filling | FC | 3.05 a | 31.7 a | 147 a | 1.99 a |
| | FD | 4.56 b | 25.4 ab | 156 a | 2.47 a |
| | NFC | 1.64 c | 28.2 ab | 155 a | 2.45 a |
| | NFD | 1.58 c | 21.7 b | 157 a | 1.99 a |

[a] All values are means of eight samples. Means followed by the same letter within a column and growth stage are not significantly different as determined by Duncan's new multiple range test ($P = 0.05$).

[b] Micromoles of ethylene evolved per gram (fresh weight) of nodule per hour.

TABLE 2. Colonization of soybean nodule and root tissue by fungi in fumigated and nonfumigated field soil, 1978

| | Growth stage[a] and treatment[b] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Flowering | | | | Pod-forming | | | | Pod-filling | | | |
| | F | | NF | | F | | NF | | F | | NF | |
| | C | D | C | D | C | D | C | D | C | D | C | D |
| Nodules (n = 20) | | | | | | | | | | | | |
| Percent colonized[c] | 15 a | 32 ab | 25 ab | 52 b | 45 a | 10 b | 75 a | 65 a | 45 a | 15 b | 75 a | 70 a |
| Fungus | | | | | | | | | | | | |
| *Gliocladium* | | X | | X | | X | X | X | | | | |
| *Myrothecium* | | X | | | | | X | | | | | |
| *Corynespora* | | X | X | X | | | X | X | X | | X | X |
| *Cylindrocarpon* | | | | | | | | | X | | X | X |
| *Fusarium* | | X | X | X | X | | X | X | | | X | |
| *Phoma* | | X | | | X | | | | | | | |
| *Stysanus* | | X | X | | X | X | | | | | | X |
| *Trichoderma* | | | X | X | | | | X | | | | |
| *Penicillium* | | | | | | | | | | | | |
| *Aspergillus* | | | | | X | | | | | | | |
| *Cladosporium* | | | | | | | | | | | | |
| *Alternaria* | | X | | | | | | | X | | | |
| *Phytophthora* | | | | | | | X | X | | | | |
| *Chaetomium* | | X | X | X | | | | | | | | |
| Other | X | | | | | | | | | X | | |
| Roots (n = 10) | | | | | | | | | | | | |
| Percent colonized[c] | 40 | 0 | 35 | 35 | 60 ab | 20 a | 80 b | 90 b | 40 ab | 30 a | 80 bc | 90 c |
| Fungus | | | | | | | | | | | | |
| *Gliocladium* | | | | | | X | | | | | | |
| *Myrothecium* | | | | | | | | | | | | |
| *Corynespora* | | | X | X | | | | | | | | |
| *Cylindrocarpon* | | | | | | | | | | | | |
| *Fusarium* | | | X | X | | | X | X | | | X | |
| *Phoma* | X | | X | X | X | | | | | | | |
| *Stysanus* | | | X | | | X | | | | | | |
| *Trichoderma* | | | | X | | | | | | | | |
| *Penicillium* | | | | | | | | | | | | |
| *Aspergillus* | | | X | | | | | | | | | |
| *Cladosporium* | | | | | | | | | | | | X |
| *Alternaria* | X | | | | X | | | | | | | |
| *Phytophthora* | | | | | | | | | | | | |
| *Chaetomium* | | | | | | | | | | | | |
| Other | | | X | | | | | X | X | X | | |

[a] At the preflowering stage, colonization was slight: 5% of nodules with the F-D treatment were colonized by *Fusarium* and 10% of roots with the NF-D treatment were colonized by minor (other) species.
[b] Treatments: fumigated (F), nonfumigated (NF), control (C), depodded (D).
[c] Values within a growth stage followed by the same letter are not significantly different as determined by chi-square analyses.

TABLE 3. Effects of soil fumigation on plant growth and nodule characteristics of field-grown Hodgson soybeans from flowering to senescence[a]

| Growth stage | Treatment[b] | Acetylene reduction[c] | Plant dry weight (g) | Nodule number | Nodule fresh weight (g) |
|---|---|---|---|---|---|
| Preflowering | F | 5.34 a | 0.54 a | 50.5 a | 0.11 a |
| | NF | 2.28 b | 0.49 a | 48.2 a | 0.14 b |
| Flowering | F | 4.17 a | 2.40 a | 64.3 a | 0.17 a |
| | NF | 4.45 a | 1.87 a | 44.8 b | 0.18 a |
| Pod-forming | F | 3.93 a | 12.8 a | 81.5 a | 0.26 a |
| | NF | 2.74 a | 10.0 a | 77.4 a | 0.33 b |
| Pod-filling | F | 7.95 a | 29.5 a | 167 a | 0.79 a |
| | NF | 7.45 a | 24.1 a | 163 a | 1.04 a |
| Late pod-filling | F | 4.94 a | 38.9 a | 157 a | 1.18 a |
| | NF | 4.88 a | 32.1 b | 176 a | 1.64 b |
| Senescence | F | 3.25 a | 51.2 a | 113 a | 1.44 a |
| | NF | 2.67 a | 48.0 a | 109 a | 2.13 a |

[a] All values are means of 24 samples. Means followed by the same letter within a column and growth stage are not significantly different as determined by an F-test ($P = 0.10$).
[b] Treatments: fumigated (F) with sodium methyldithiocarbamate (Vapam) at 125 L/ha, and nonfumigated (NF).
[c] Micromoles of ethylene evolved per gram (fresh weight) of nodule per hour.

significantly more nodules than those from nonfumigated soil only at the flowering stage. At preflowering, pod formation, and late pod-fill growth stages, nodule weights were significantly greater for plants grown in nonfumigated plots (Table 3).

Dry weight of plants grown in fumigated plots was signficantly greater at the late pod-fill stage than that of plants grown in nonfumigated soil (Table 3). Although the trend was toward greater seed size and yield in fumigated plots, no significant differences were found. Percent total nitrogen of seeds from plants in fumigated soil was significantly greater than that of seed from plants grown in nonfumigated soil (Table 4).

TABLE 4. Seed yield and quality for plants grown in fumigated, fungicide-treated, and untreated field soil, 1979[a]

| Treatment | Yield[b] (g) | Size[c] (g/150 seeds) | Total nitrogen[c] (%) |
|---|---|---|---|
| Fumigated | 114 a | 17.3 a | 6.7 a |
| Nonfumigated | 108 a | 17.0 a | 6.5 b |
| Fungicide | | | |
| Control (water) | 95 a | 17.0 a | 6.7 a |
| Captan | 124 a | 17.0 a | 6.7 a |
| Propamocarb | 115 a | 18.0 a | 6.8 a |

[a] For the fumigation experiment, means within a column followed by the same letter are not significantly different as determined by a *t*-test for paired samples ($P = 0.05$); for the fungicide experiment, means within a column followed by the same letter are not significantly different as determined by a Duncan's new multiple range test ($P = 0.05$).
[b] Values for the fumigation experiment represent means of eight samples and those for the fungicide experiment, means of four samples. Each sample was the combined seed of five plants per plot.
[c] Values for the fumigation experiment represent means of 16 samples and those for the fungicide experiment, means of eight samples.

In all but the late pod-fill stage, significantly more nodules from plants grown in nonfumigated soil were colonized by fungi than nodules from plants grown in fumigated plots (Table 5). The same types of fungi occurred on nodules and roots of both treatments, but some fungi, such as *Corynespora* sp., were isolated more often from nodules and roots of plants grown in nonfumigated than from those in fumigated soil. The predominant genera isolated from soybean nodules and roots were *Gliocladium, Fusarium, Trichoderma*, and *Phoma* (all of which occurred on nodules and roots with a similar frequency). Members of the genera *Corynespora* and *Myrothecium* were isolated more frequently from nodules than roots (Table 5). Fungi colonizing nodules and roots showed no obvious succession during the growing season.

Species of *Fusarium, Phoma, Trichoderma,* and *Gliocladium* were found on soil dilution plates, but *Corynespora* sp. was never recovered by this method. Observations with the scanning electron microscope showed fungal mycelium to be present on nodule surfaces from fumigated and nonfumigated soil throughout the growing season. The mycelium often appeared to penetrate the outer layer of cortical cells.

**Fungicide soil treatment.** Nodules collected from roots of flowering plants grown in soil treated with captan exhibited higher

TABLE 5. Colonization of soybean nodule and root tissue by fungi in fumigated and nonfumigated field soil, 1979

| | Growth stage and treatment[a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Preflowering | | Flowering | | Pod-form | | Pod-filling | | Late pod-filling | | Senescence | |
| | F | NF | F | NF | F | NF | F | NF | F | NF | F | NF |
| Nodules (n = 100) | | | | | | | | | | | | |
| Percent colonized[b] | 8 a | 36 b | 28 a | 47 b | 53 a | 88 b | 83 a | 96 b | 54 a | 58 a | 68 a | 86 b |
| Fungus | | | | | | | | | | | | |
| *Gliocladium* | | X | X | X | X | X | X | X | X | X | X | X |
| *Myrothecium* | | | | X | X | X | X | X | X | X | X | |
| *Corynespora* | | X | X | X | | X | | X | X | X | X | X |
| *Cylindrocarpon* | | X | X | | | | | | X | | | |
| *Fusarium* | X | X | X | X | X | X | X | X | X | X | X | X |
| *Phoma* | | | X | X | | X | X | | X | X | | X |
| *Stysanus* | | | X | X | X | | X | | X | | | |
| *Trichoderma* | | | | X | | X | | X | X | X | X | X |
| *Penicillium* | | | X | X | X | | | | X | X | | |
| *Aspergillus* | X | | | | X | X | X | X | | | | |
| *Cladosporium* | | | | | | | | | X | | | |
| *Alternaria* | | | | | | | | | | | | |
| *Phytophthora* | | X | X | X | | | X | | X | X | | X |
| *Chaetomium* | | | X | | | | | | X | | | |
| Other | X | X | X | | X | | | X | X | X | X | X |
| Roots (n = 50) | | | | | | | | | | | | |
| Percent colonized[b] | 14 a | 84 b | 42 a | 68 b | 84 a | 80 a | 70 a | 84 a | 58 a | 82 b | 74 a | 100 b |
| Fungus | | | | | | | | | | | | |
| Gliocladium | X | X | X | X | X | X | X | | X | X | X | X |
| Myrothecium | | | | | | X | | | X | | | |
| Corynespora | | X | | X | | | | X | X | X | | X |
| *Cylindrocarpon* | | X | | | | | | | | X | | |
| Fusarium | X | X | X | X | X | X | X | X | X | X | X | X |
| *Phoma* | | | X | X | X | | | | X | X | X | X |
| *Stysanus* | X | | X | | | | | | | | X | X |
| *Trichoderma* | | X | | X | | X | | X | X | X | X | X |
| *Penicillium* | X | | X | | X | | | | X | | | |
| *Aspergillus* | X | | | | | | X | X | | | | |
| *Cladosporium* | | X | | | | | | | | | X | |
| *Alternaria* | | X | | | | | | | X | X | X | |
| *Phytophthora* | | X | X | | | | | | X | | | |
| *Chaetomium* | | X | X | | | | | | | | | |
| Other | X | X | | X | | | | | | | | |

[a] Treatments: F = fumigated, NF = nonfumigated.
[b] Values within a growth stage followed by the same letter are not significantly different as determined by chi-square analyses.

acetylene reduction values than those of the control or propamocarb treatment, but differences were not significant (Table 6). At pod-fill stage, nodule numbers from plants grown in captan-treated soil were significantly greater than those from plants grown in untreated soil, and nodule weights from plants in both fungicide treatments were significantly greater than those in untreated soil (Table 6). Plant dry weights were not significantly different (Table 6). Differences in total nitrogen, size, and weight (yield) of seeds were not significant (Table 4). At flowering but not at the pod-filling stage, plants grown in propamocarb-treated soil showed significantly greater fungal colonization of nodules than did plants in other treatments (Table 7).

Fungal genera isolated from nodules and roots and from soil dilutions were the same as in fumigation experiments. Frequency of occurrence of these genera was also similar. Of the predominant genera isolated, *Stysanus* and *Myrothecium* were isolated only from nodules (Table 7).

## DISCUSSION

The seasonal pattern of nitrogen fixation, as shown by acetylene reduction by nodules of Hodgson soybeans in 1978 and 1979, was similar for plants in both treated and untreated soils. Few values were significantly higher in fumigated and fungicide-treated soils, most of the significant differences occurring between fumigated and nonfumigated treatments in 1978. A trend toward higher acetylene reduction values in treated soils was observed, but the relatively low number of samples harvested may have affected tests for significance.

Soil treatment with fungicides or fumigants reduced the number of soil fungi and fungal colonization of nodules and roots. This reduction, combined with some apparent trends in greater acetylene reduction and plant growth values, may indicate that soil fungi are normally associated with inhibition of growth and nitrogen fixation of soybeans. The apparent beneficial effect of soil fumigation in 1978 may reflect the presence of more specialized soybean pathogens and/or depletion of nutrients caused by the prolonged soybean cultivation (35 yr) of the 1978 plots versus the 6-yr soybean history of the 1979 field plots.

Depodding plants had little effect on nitrogen fixation, plant growth, or colonization of nodules by fungi. Even though nodules compete with pods for photosynthate, Mondal et al (12) found that once the pods are removed, photosynthesis is reduced, resulting in no increase in nitrogen fixation.

Captan, a broad-spectrum soil fungicide that reportedly has no harmful effects on soil rhizobia (14), showed no significant superiority to propamocarb, an experimental fungicide mainly active against phycomycetous fungi, in increasing plant growth. Nodule number and fresh weight were significantly increased by the fungicide treatments at the pod-fill stage; the fungicide treatments may act to decrease fungal growth. Horton and Gibson (*personal communication*) observed an increase in nodule weight and number on primary roots of greenhouse-grown soybeans treated with Terraclor. From field and greenhouse studies, they concluded that fungicides exert a direct effect on root microflora and indirectly increase plant growth by inhibition of fungi. Even though the effect of less fungal parasitism on roots and nodules could allow the plant to increase growth of nodules, increased nodule size is not necessarily linked to increased nitrogen fixation.

Soil mycoflora were initially reduced by fumigation, but recolonization of soil and early and sustained colonization of nodules and roots was evident in fumigated and nonfumigated soil. In all experiments, few differences were seen in the genera of fungi isolated from soybean nodules and roots. In some instances a "preference" for roots or nodules was noted for fungi such as *Myrothecium* sp. Studies on the influence of crop rotation on soil fungi indicate a host selectivity for certain fungal genera and species (30,31). A separate study by the authors (28) also suggests a stimulation of mycoflora by the legume host.

A marked increase in the percentage of roots and nodules colonized by fungi was often observed after flowering, when

TABLE 6. Effects of fungicide soil treatment on plant growth and nodule characteristics of field-grown Hodgson soybeans during flowering and pod-filling in 1979[a]

| Growth stage | Treatment | Acetylene reduction[b] | Plant dry weight (g) | Nodule number | Nodule fresh weight (g) |
|---|---|---|---|---|---|
| Flowering | No fungicide | 4.04 a | 4.03 a | 52.0 a | 0.17 a |
| | Captan | 5.43 a | 5.52 a | 59.5 a | 0.18 a |
| | Propamocarb | 3.33 a | 4.7 a | 60.6 a | 0.16 a |
| Pod-filling | No fungicide | 3.09 a | 37.3 a | 104 a | 0.78 a |
| | Captan | 5.18 a | 50.3 a | 169 b | 1.40 b |
| | Propamocarb | 5.09 a | 32.8 a | 134 ab | 1.23 b |

[a] All values are means of 12 samples. Means followed by the same letter within a column and growth stage are not significantly different as determined by a Duncan's new multiple range test ($P = 0.05$).
[b] Micromoles of ethylene evolved per gram (fresh weight) of nodule per hour.

TABLE 7. Colonization of soybean nodule and root tissue by fungi in fungicide-treated and untreated field soil

| | Nodules (n = 100) Growth stage and treatment[a] | | | | | | Roots (n = 50) Growth stage and treatment[a] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Flowering | | | Late pod-filling | | | Flowering | | | Late pod-filling | | |
| | N | C | P | N | C | P | N | C | P | N | C | P |
| Percent colonized[b] | 35 a | 42 a | 57 b | 72 a | 69 a | 64 a | 58 a | 76 b | 78 b | 84 a | 52 b | 72 a |
| Fungus | | | | | | | | | | | | |
| *Gliocladium* | X | X | X | X | X | X | X | X | X | X | X | X |
| *Myrothecium* | X | | X | | X | X | | | | | | |
| *Corynespora* | X | X | X | X | X | X | X | X | X | X | X | X |
| *Cylindrocarpon* | X | | X | X | | | X | | X | X | | |
| *Fusarium* | X | X | X | X | X | X | X | X | X | X | X | X |
| *Phoma* | | | X | X | X | X | X | X | X | X | X | X |
| *Stysanus* | X | X | | X | X | X | | | | | | |
| *Trichoderma* | X | X | X | X | X | X | X | X | X | X | X | X |
| *Penicillium* | X | | | | | X | | | X | X | | |
| *Aspergillus* | X | | | X | | | X | | | | | |
| *Cladosporium* | | X | | | | | | | | | | |
| *Alternaria* | | | | | | | | X | | | X | X |
| *Phytophthora* | X | X | | | X | | X | X | X | | | |
| *Chaetomium* | | | | | | | | | | | | |
| Other | X | X | | X | X | | | | | X | | X |

[a] N = no fungicide, C = Captan, P = propamocarb.
[b] Values within a growth stage followed by the same letter are not significantly different as determined by chi-square analyses.

nitrogen fixation is at its peak. An apparent relationship also existed between physiological stress of the host during pod-fill and an overall increase in fungi colonizing roots and nodules in untreated soil. Physiological status of the plant is important in nodule function and senescence, and physiological effects that contribute to nodule deterioration must be clearly separated from premature senescence due to microbial parasitism.

The major fungal genera isolated from nodules and roots included representative species (eg, *Corynespora cassiicola, Gliocladium roseum,* and *Myrothecium verrucaria*) reported elsewhere as plant pathogens of varying virulence on legume hosts (8,15,21). Soil fungi may exert an indirect effect on nodule function by their effects on plant roots or may, as in the case of *Rhizoctonia solani* (17), induce a breakdown of internal cellular components of the nodule due to toxic metabolites that are translocated from the outer cortical cells to the internal nodular tissues. Fungal colonization of intact, healthy nodules normally does not proceed beyond a layer of thick-walled sclerenchyma cells in the nodule cortex; however, fungal colonization of entire senescent or damaged nodules can occur (17; *unpublished*).

In a study by Kittle and Gray (10), combined soil fumigation and foliar fungicide sprays controlled several major soybean pathogens and increased plant yield. Gray (5) also found that, after soil fumigation, average seed yield of Amsoy-71 soybeans was significantly higher and severity of root rot was significantly lower than for plants in nonfumigated plots. Although our data do not always show that common soil- and root-inhabiting fungi inhibit soybean growth and acetylene reduction by nodules, these fungi may have some influence on nodule size, nitrogen fixation, and consequently plant yield. Some genera of soil fungi are consistently associated with soybean roots and nodules, and some of these seem primarily attracted to nodules. The effects of these fungi are more obvious under long-term continuous soybean cropping and may be of most importance under suboptimal growing conditions. The soil fungi associated with root and nodule tissue could interact with other soil microflora and fauna and soil environmental conditions during the growing season to cause nodule deterioration.

More research is needed, particularly on the selectivity of nodules for soil fungi, the effects of economically important soybean pathogens on nodule structure and function, and the toxic and disruptive effects that may be exerted on the nodule by common fungal colonizers.

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