Effect of Soilborne Plant-Pathogenic Fungi on the Herbicidal Action of Glyphosate on Bean Seedlings

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ABSTRACT


The apparent herbicidal action of glyphosate can be separated from its growth-inhibitory action by the use of vermiculite or sterilized soil as a growth medium. The results suggest that glyphosate-linked death of bean plants in unsterilized soil was actually due to their parasitization by fungal root-rot pathogens in the growth medium. This effect occurred at treatment levels several-fold lower than those required to cause death in the absence of fungal root-rot pathogens. Although bean seedlings grown in the absence of soil-associated root pathogens did not die, they showed markedly inhibited growth when treated with doses of glyphosate that were lethal in unsterilized soil. However, when Pythium sp. or Fusarium sp. was added to sterilized soil or vermiculite, glyphosate-treated plants died within 6 or 11-12 days, respectively. The herbicidal, but not the growth-inhibitory, action of glyphosate on bean seedlings grown in sterilized medium reinvested with Pythium was blocked by metalaxyl, a phycotoxic-specific, systemic fungicide. However, metalaxyl did not block the herbicidal action of glyphosate on plants growing in untreated field soil, probably because such soils contain root-rot pathogens other than Pythium that are not suppressed by metalaxyl.

Additional key words: herbicide, Phaseolus vulgaris.

Glyphosate (N-phosphonomethyl glycine) is a broad-spectrum herbicide that is absorbed passively (13) by foliage and rapidly translocated in the phloem to areas of meristematic activity (e.g., roots) in plants (26). In addition to its herbicidal properties glyphosate is inhibitory to many microorganisms. Jaworski (16) proposed that the herbicidal activity of glyphosate was due to inhibition of aromatic amino acid biosynthesis because growth inhibition of Lemna gibba and Rhizobium japonicum by glyphosate was overcome by the addition of aromatic amino acids.

Amrhein et al (4) showed that glyphosate is a specific and potent inhibitor of the higher plant conversion of shikimate to chorismate and that it therefore lead to decreased phenylalanine biosynthesis. These workers (3,5,27) also provided evidence indicating that glyphosate inhibits the enzyme enolpyruvyl shikimate-3-phosphate (EPSP) synthase of both bacterial and higher plant origin. Studies using recombinant DNA technology (11,22) have provided, at least in enteric bacteria, direct biological evidence for this hypothesis.

In beans, Phaseolus vulgaris L., resistance to Colletotrichum lindemuthianum (Sacc. & Magn.) Scriber, the casual agent of bean anthracnose, is correlated with the accumulation of the phytotoxins phaseollin, phaseollinosiflavon, phaseollin, and kievitone (7,9,20,21,25,28). Phenylalanine is a precursor of these isoflavonoid phytotoxins (12,18,28). We proposed to exploit the inhibitory action of glyphosate on EPSP synthase and its likely blockage of phytotoxin synthesis in bean plants to more critically test the possible association of phytotoxin production with the resistance of bean plants to C. lindemuthianum.

In the course of the study (still ongoing) we observed that the herbicide applied at concentrations lethal to bean seedlings growing in soil did not kill seedlings growing in vermiculite. This observation led to a study of the effect of growth medium and associated root-invading fungi on the herbicidal action of glyphosate on bean plants which is described below.
MATERIALS AND METHODS

Four experiments were conducted to evaluate the effect of organisms associated with various growth media on the herbicidal action of glyphosate on seedlings of *P. vulgaris*. All plants were grown from surface sterilized seeds of the bean cultivar Topcrop. Seeds were planted aseptically (unless otherwise indicated) about 3 cm deep in sterilized or unsterilized muck field soil or unsterilized vermiculite in sterilized plastic pots and subsequently maintained under fluorescent light (14:10 hr; day:night photoperiod) at 22–24 C. Plants growing in vermiculite were given Hoagland-Krom 1 nutrient solution every fifth day; plants growing in soil were given distilled water once weekly.

In all experiments, 14-day-old seedlings were treated on the upper surface of one primary leaf with glyphosate or distilled water (control) by applying 10 droplets (each 1 µl) of test solution at 10 approximately equidistant locations. Two sources and two levels of glyphosate were used in the first experiment. These consisted of aqueous dilutions of a commercial formulation of glyphosate (Roundup®) or a surfactant-free preparation of glyphosate (MON-0139), containing 10 or 1 µg a.i./µl. The same materials were tested in the second experiment but at concentrations of 10, 10, and 0.5 µg a.i./µl. In the third and fourth experiments only the commercial formulation of glyphosate at a concentration of 1 µg a.i./µl was used. Each experiment was done at least twice.

**First experiment.** Visual observations of plant growth and health were made every 2 days, and 25 days after treatment all plants, whether dead or living, were harvested. Three 1-cm segments were taken from each of the root, crown, and hypocotyl portions of each plant, surface sterilized for 2 min in 1% NaOCl and placed on potato-dextrose agar (PDA) to isolate the organism(s) present within the plant tissues. Pure cultures, for identification, were made from hyphal tips of every fungus that grew from the tissue segments on PDA.

**Second experiment.** In this experiment, plant infection was studied as a function of time after glyphosate treatment. From each medium-dose treatment including controls, at 0, 1, 3, 5, 8, and 12 days after treatment, two plants were removed from the pots. Fungi present within the root, crown, and hypocotyl portions of these plants were isolated on PDA and identified, as in the first experiment.

**Third experiment.** Plants grown in sterilized soil and vermiculite were inoculated with spore suspensions of either *Pythium* sp. or *Fusarium* sp. from subcultures of these fungi isolated during the second experiment, before treatment with glyphosate.

Spore suspensions (~10⁶ oospores per milliliter) of *Pythium* sp. were prepared from 2-wk-old culture mats grown in V-8 cholesterol broth (6). Cultures were inoculated in 12 ml of medium in sterile petri dishes in the dark at 24 C. Oospore suspensions were prepared from freshly harvested mycelial mats by first homogenizing the cultures and then filtering the suspension through four layers of cheesecloth.

Spore suspensions (macroconidia, microconidia, and chlamydospores) of *Fusarium* sp. were prepared from 2-wk-old cultures grown on PDA at 24 C in the dark. Plate cultures were flooded with sterile water, their surfaces were rubbed with a rubber policeman, and the resulting suspensions were homogenized and filtered through four layers of cheesecloth and adjusted to ~10⁷ spores per milliliter. Three methods were used to inoculate the plants or growth media: Seed treatment—After surface sterilization, seeds were soaked for 10 min in the spore suspension of either fungus and then planted. Root-zone injection—Just before glyphosate treatment, 1 ml of spore suspension of either fungus was injected into the root zone of aseptically grown plants. Wounding—A wound on the hypocotyl was made by peeling downward, but not removing, an epidermal strip ~2.5 mm wide and 10 mm long. A drop of spore suspension was placed in (or on) the wound.

Plants inoculated with each separate organism, but not treated with glyphosate, served as controls. Visual observations of plant health (dead or living) were made at 6, 9, and 12 days after treatment with glyphosate and the plants were harvested at 12 days.

Harvested plants were checked for the presence of fungi within their tissues by using the PDA-petri plate culture procedure mentioned earlier.

**Fourth experiment.** To further substantiate the evidence that glyphosate-linked death of treated bean plants was due to parasitization by microorganisms associated with growth medium, the sterilized soil and vermiculite media were first infested by injecting inoculum of *Pythium* into the root zone, and the plants were treated with metalaxyl (a systemic fungicide specific for phycomycetes fungi) before and after glyphosate treatment. Metalaxyl was prepared from Ridomil 2.4E as an aqueous solution containing 10 µg a.i./ml and applied as a combined foliar spray and soil drench at 20 ml per pot per application when bean plants were 10, 12, and 16 days old. Plants treated with metalaxyl, but not with glyphosate, served as controls. All data were obtained as in the third experiment.

RESULTS

All bean plants growing in sterilized muck soil died within 7–8 days following treatment with the high dose (100 µg a.i. per plant) of glyphosate from either formulation (Roundup or MON-0139) and within 10–12 days after the low dose (10 µg a.i. per plant) treatment. Plants growing in sterilized soil or vermiculite were killed only by the high dose treatment (Table 1); death occurred 11–20 days after treatment.

Symptoms characteristic of damping-off or root rot occurred on plants killed by glyphosate in unsterilized soil. Fungi isolated from surface sterilized tissues of bean plants after harvest are listed in Table 2. *Pythium* and *Fusarium* were consistently isolated and *Acremonium* and *Trichoderma* were occasionally isolated from the root, crown, and hypocotyl tissues of plants killed by glyphosate in unsterilized soil. Control plants were healthy and free of any detectable fungus.

*Fusarium* was frequently isolated from plants killed by glyphosate in sterilized soil. Although none of the control or low-dose treated plants growing in sterilized soil died, some of them yielded *Rhizopus* and *Penicillium*; *Alternaria*, *Botrytis*, and *Fusarium* were isolated from plants killed by glyphosate while growing in vermiculite. Plants growing in vermiculite and subjected to control, low dose, and (in some cases) high dose treatments were not killed and their surface sterilized tissues yielded no fungi.

Studies of plant infection as a function of time after glyphosate treatment (second experiment) further revealed the importance of growth medium-associated microorganisms on the herbicidal action of glyphosate (Fig. 1). A yellow-pigmented fungus (unidentified) was always the first to infect glyphosate-treated plants growing in sterilized muck soil, and this predispation of bean plants by glyphosate (characterized by the first appearance of the yellow fungus) was formulation- and dose-dependent. Compared to surfactant-free glyphosate (MON-0139), the herbicidal effect of glyphosate plus the surfactant (Roundup) was more rapid. The effectiveness in each case was directly related to the concentration of the herbicide applied to the plant.

The yellow fungus was detected as early as 1 day after treatment with doses of 100 or 10 µg a.i. per plant of glyphosate plus surfactant (Roundup). In all the later samples, although the yellow fungus was also sometimes detected, *Pythium* and *Fusarium* were predominant and were consistently isolated. *Pythium* and

| TABLE 1. Effect of growth medium on the herbicidal effect of glyphosate on Phaseolus vulgaris |
|---------------------------------|-----------------|-----------------|-----------------|
| **Dose and treatment**          | **Growth Medium** | **MON 0139 Roundup®** | **MON 0139 Roundup®** |
| **100 µg/plant**                | **10 µg/plant**  | **0 µg/plant**    |
| **Unsterilized Soil**           | 8/8              | 8/8              | 8/8             |
| **Sterilized Soil**             | 7/8              | 0/8              | 0/8             |
| **Vermiculite**                 | 6/8              | 7/8              | 0/8             |

* No. of plants killed/ no. of plants treated, rated 25 days after treatment.
Fusarium were first recovered only from the root segments (3 days post-treatment), in the next sample (5 days post-treatment) they were present in the crown segments as well, and in the last sample (7 and later days post-treatment) the hypocotyl was also infected. Whenever detected, the yellow fungus was only recovered from the roots. By 12 days after treatment, all of the remaining plants treated with glyphosate at 10 μg a.i. per plant or more were dead; 60% of the plants treated with glyphosate plus surfactant at 5 μg a.i. per plant also died, whereas none of the plants treated with glyphosate alone at 5 μg a.i. per plant died. Some fungi (Penicillium and Rhizopus) were also isolated from the glyphosate-treated plants grown in sterilized soil and vermiculite (Fig. 1), but no regular pattern of fungal infection was evident. None of the plants remaining at the end of the experiment died although plants treated with ≥10 μg a.i. per plant showed marked growth inhibition, chlorotic leaves, and death of apical meristem. Some of these plants appeared to be drying out from the top downward. None of the controls (plants not treated with glyphosate) died or yielded any fungus.

Since the effect of glyphosate with surfactant was more rapid but otherwise similar to the effect of glyphosate without surfactant, the formulation with surfactant (Roundup®) was used at 10 μg a.i. per plant to evaluate the effects of *Pythium* and *Fusarium* on the herbicidal action of glyphosate on bean plants grown in a sterilized

| TABLE 2. Predominant genera of fungi isolated from surface-sterilized root, crown, and hypocotyl tissue of *Phaseolus vulgaris* grown in various media, 25 days after treatment of primary leaves with glyphosate or water |
|---------------------------------|-----------------|-----------------|-----------------|
| Growth Medium                  | MON 0139        | Roundup®        | MON 0139        | Roundup®        | 0 μg/plant      |
| Unsterilized Soil              | Acrocnema       | Acrocnema       | Acrocnema       | Acrocnema       | None            |
| Sterilized Soil                | Fusarium        | Fusarium        | Fusarium        | Fusarium        | Fusarium        |
| Vermiculite                    | Penicillium     | Penicillium     | Penicillium     | Penicillium     | Penicillium     |
|                                | Rhizopus        | Rhizopus        | Rhizopus        | Rhizopus        | Rhizopus        |
|                                | Botrytis        | Alternaria      | Botrytis        | Alternaria      | None            |
|                                | Fusarium        | Botrytis        | Fusarium        | Botrytis        | None            |

**Fig. 1.** Predominant genera of fungi isolated from surface-sterilized root, crown, and hypocotyl tissue of *Phaseolus vulgaris* grown in various media at various times after treatment of primary leaves with two formulations of glyphosate. * Dose of glyphosate (micrograms a.i. per plant). ** No fungus recovered (), fungus recovered (■); letter indicates genus: P = *Pythium*, F = *Fusarium*, R = *Rhizopus*, Y = Yellow fungus, T = *Trichoderma*. Pn = *Penicillium*, C = *Chaetomium*.**
medium. All plants died within 6 days when the sterilized growth medium was infested with *Pythium* (Fig. 2). In the presence of *Fusarium* the herbicidal effect of glyphosate occurred later than in the presence of *Pythium*, and not all plants were killed. The fungus isolated from treated plants always coincided with the type of fungus used to infest the sterilized growth medium. Plants grown in unsterilized soil gave both *Pythium* and *Fusarium* and occasionally *Trichoderma*. Both root-zone injection and seed treatment methods of fungal infestation of *Pythium* in the growth medium resulted in equal kill of glyphosate-treated plants; the wounding method of fungal inoculation by *Pythium* was less effective. For *Fusarium*, root-zone injection was the least effective mode of application. None of the control plants (glyphosate-treated plants without fungi added, or untreated plants in soil infested with fungi) died (Fig. 2).

Metalaxyl effectively protected the bean plants from the herbicidal action of glyphosate when the plants were grown in sterilized medium reinvested with *Pythium* (Fig. 3). No fungus was recovered from the metalaxyl-protected glyphosate-treated plants. Metalaxyl was ineffective in blocking the herbicidal action of glyphosate on plants growing in unsterilized medium. Only *Fusarium* and *Trichoderma* were isolated from these plants. Metalaxyl alone, at the concentrations applied, had no adverse effects on bean plants.

**DISCUSSION**

The apparent herbicidal action of glyphosate on bean plants can be separated from its growth-inhibitory action by the use of vermiculite or sterilized soil as a growth medium. We hypothesize that what has been considered to be the herbicidal action of glyphosate on bean seedlings is due at least in part to attack by root pathogenic soil fungi on treated plants. This conclusion is based on the following experimental observations:

(i) Bean plants growing in unsterilized field soil died within 10–12 days after receiving a 10 μg a.i. per plant dose of glyphosate. In contrast, plants treated with the same dose growing in vermiculite or sterilized soil were not killed. However, their growth was inhibited. Glyphosate at 100 μg a.i. per plant caused death of plants growing in vermiculite or sterilized soil, apparently directly (Table 1). Dead plants were found to be colonized by various fungi (Table 2). The types of fungi isolated varied with the medium in which plants were grown. Plants grown in unsterilized field soil yielded mostly *Pythium* and *Fusarium* (soil-associated root pathogenic fungi) following treatment with glyphosate whereas those grown in sterilized soil or vermiculite yielded mostly *Botrytis, Rhizopus, Penicillium*, and *Alternaria* (predominantly air-borne seed-disseminated saprophytes and facultative parasites). *Fusarium* was also isolated from glyphosate-treated plants grown in sterilized growth media, although infrequently. Its occurrence was possibly due to incomplete sterilization and survival of chlamydompores; alternatively, it may have arisen via contamination from the adjacent pots containing unsterilized soil. Healthy plants never yielded pathogenic fungi.

(ii) Time-course studies of fungal infection (Fig. 1) clearly demonstrated that infection and colonization by pathogen(s)
preceded the growth inhibition, wilting, and death of treated plants. Uninfected plants did not wilt and die. It may be that practically any soilborne pathogen which first happens to come in contact with the treated plants may be able to parasitize them; the pathogens most frequently isolated are all comparatively fast-growing (the yellow fungus appears to be an exception, it is comparatively slow-growing on FDA. This organism does not match the description of any known plant pathogen).

(iii) When *Pythium* or *Fusarium* was added to sterilized soil or vermiculite media, glyphosate-treated plants died within 5–6 or 11–12 days, respectively (Fig. 2). *Pythium* caused more rapid death of plants than did *Fusarium*, perhaps because its inherent growth rate is faster than that of *Fusarium*.

(iv) The herbicidal action of glyphosate on plants grown in sterilized medium reinfested with *Pythium* was blocked by metalaxyl (Fig. 3). However, metalaxyl was ineffective in reversing the herbicidal action of glyphosate on plants growing in unsterilized soil, probably because such soils contain root-rot pathogens other than *Pythium* that are not suppressed by metalaxyl.

Glyphosate at the concentrations used in this study either inhibited or did not affect the growth of the fungal root-rot pathogens isolated from glyphosate-treated plants (unpublished). Its apparent herbicidal effect, therefore, is concluded to be entirely on plant metabolism.

The higher dose of glyphosate (ie. 100 μg a.i. per plant) used in these experiments is comparable to the recommended field rate of application for Roundup. At this concentration, glyphosate killed most of the bean plants even in vermiculite or sterilized soil; when not parasitized by pathogens, plants died (dried out from top downward) after 20 or more days following treatment, but they died (rotted or wilted) within 8–10 days of treatment when parasitized. In unsterilized medium, plants died within 8 days when treated with the higher dose. While this implies that the field efficacy of the commercial formulation of glyphosate can be attributed substantially to direct metabolic effects of glyphosate on plant metabolism, the symptoms (rotting, wilting, necrosis, and chlorosis) of herbicidal damage usually observed in the field suggest that soil phytopathogens may augment the direct effect of glyphosate and thus contribute to its herbicidal action in the field.

Certain herbicides predispose plants to disease (2, 14) and this has been attributed to suppression of defense mechanisms in some cases (23). Glyphosate inhibited both accumulation of the phytalexin glyceolin and resistance expression in soybean plants inoculated with an incompatible race of *Phytophthora megasperma f. sp. glycinea* and *Pseudomonas syringae pv. glycinea* (15, 17). Glyphosate may increase the leakage of electrolytes from treated plants by changing plasma membrane permeability (8, 10), through possible hydrolysis of storage carbohydrate (1, 8, 29), and by affecting the composition of cell walls. Susceptibility of bean plants to damping-off during germination has been correlated with the amount of exudation, this being higher in susceptible cultivars (24). Thus, glyphosate-induced susceptibility of bean plants to root-rot pathogens may be attributed to suppression of defense mechanism(s) and/or to increased leakage of nutrients from the treated plants.

Lynch and Penn (19) observed that soil fungi were associated with the decaying rhizomes of couch grass (*Agropyron repens*) killed by glyphosate. They isolated various fungi, with *Fusarium culmorum* being the most common. However, they interpreted their observations to mean that these fungi were acting as saprophytes on glyphosate-killed tissues, rather than the cause of death of treated plants. They showed, however, that stimulated growth of *Fusarium culmorum* in soil after herbicidal death of plants by glyphosate caused damage to the succeeding cereal crop. This report and our work showing that the herbicidal action of glyphosate on bean plants is due to the parasitization of treated plants by root-rot pathogens associated with the growth medium suggest that, if the mechanism we have shown is universal, there is some risk in the use of glyphosate as an herbicide, at least with crops susceptible to root-rot pathogens.

It is apparent that further investigations will be required to sort out the specific site(s) and type of interaction of glyphosate with defense mechanism(s) in bean plants. It should also be emphasized that before this phenomenon of “glyphosate-linked death of plants by pathogenic fungi” can be generalized, there is need to extend these studies to other plant taxa. Variations in response to glyphosate by various plant taxa may well be associated with differential uptake, translocation, and metabolism of the herbicide, differences in the regulatory controls of the system(s) with which glyphosate interferes, and differences in the sensitivity of different plants to fungal root-rot pathogens.

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**Fig. 3.** Effect of metalaxyl (R) on the herbicidal action of glyphosate on *Phaeois�� vulgaris* seedlings grown in unsterilized soil, sterilized soil, vermiculite, *Pythium*-amended sterilized soil, and *Pythium*-amended vermiculite. **P** R’ = control plants grown in unamended growth medium without metalaxyl application; **P** R = control plants grown in *Pythium*-amended sterilized soil and *Pythium*-amended vermiculite without metalaxyl treatment; **P** R’ = metalaxyl-treated plants grown in *Pythium*-amended sterilized medium; **P** R’ = metalaxyl-treated plants grown in unsterilized soil. *** Glyphosate, 10 μg a.i. per plant.**
LITERATURE CITED


