HERBICIDE INTERACTIONS WITH FUNGAL ROOT PATHOGENS, WITH SPECIAL REFERENCE TO GLYPHOSATE

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KEY WORDS: plant disease, microorganism, microflora, predisposition, phytotoxicity

INTRODUCTION

Weeds are the most economically important of all pests with respect to sales of pesticides and limitations to crop yields (62). Herbicide sales represent more than two thirds of the $4.36 × 10^6$ kg of pesticides used annually in United States (113), and almost one half of the $21$ billion worldwide pesticide market (8). Numerous cases where an herbicide treatment influences disease outcome have been studied by plant pathologists as well as weed scientists. The concepts and the literature on interactions between herbicides, microorganisms, and plant diseases have been extensively reviewed (2-4, 40, 51, 59, 78, 129, 150). Most of the literature on herbicides deals with their direct effects on weeds and the effects of weed control on crops (Figure 1A). The effects of herbicides coming in direct contact with crops are considered secondarily in the weed-management literature (Figure 1B). The protection or predisposition of crops to disease that is sometimes observed following the use of herbicides is of particular interest to plant pathologists.

As we document in this review, herbicides can alter soil ecosystems by having a direct effect on various components of the soil microflora, such as
plant pathogens, antagonists, or mycorrhizae (Figure 1C). These effects can result in increased or decreased incidence of plant disease, for example through promotion or suppression of the activities of beneficial microorganisms (Figure 1D). Nonspecialized pathogens can increase their inoculum potential on weeds and subsequently affect crops (Figure 1E). As still another effect, herbicides can predispose pathogens to fungicides or act as synergists (Figure 1F).

Natural fluctuations in the physical environment and changes in microenvironment that occur as a result of weed control can also influence the interactions described above, but these effects are not discussed here. This review covers the recent reports on the types of herbicide-disease interactions that are depicted in Figure 1. The characteristics and interactions of glyphosate with soil fungi are emphasized because of: (a) the extensive applied and fundamental literature on its properties and effects summarized below, which provides good examples of the key information essential to define principles in herbicide-disease interactions; (b) the widespread use and economic importance of this herbicide; (c) the increased attention to development of glyphosate-tolerant crops (65, 125) and its use as a crop desiccant to accelerate ripening (35, 149); and (d) the fact that its herbicidal action is strongly influenced by the presence of certain soil fungi—a new concept in herbicidal activity (70, 91a, 117). Finally, some of the new or potential uses of herbicides in phytopathology are discussed.

CHARACTERISTICS OF GLYPHOSATE

Glyphosate (N-[phosphonomethyl]glycine) is a broad-spectrum, water-soluble herbicide sold under the trade names of Kleenup, Roundup, Rodeo, and Vision. It can effectively control 76 of the world’s 78 worst weeds (43). Total sales of Roundup recently topped $1 billion, and its share of the $9 billion a year world herbicide market (8) is expected to increase (75). In 1988, 4 years after its registration for use in forestry in Canada, glyphosate was used on 176,536 ha, representing 81% of the overall Canadian forest area treated with herbicides that year (20).
Herbicide-disease interactions must be kept in perspective; they are only one component of the many potential side effects of an herbicide. The effects of glyphosate on litter decomposition (42), insects (63), water fauna (83), aquatic bacteria (27), mineralization of organic matter (11), and general microbial activities in soil (23) have been reviewed.

The detection of herbicide residues in various parts of the plant, in the rhizosphere, and in the soil is essential to study its role in herbicide-disease interactions. Persistence and availability are key properties in the development and expression of any side-effect of a pesticide (104). The fate and behavior of several herbicides in soils was recently reviewed (147). One major difficulty in studying herbicide-disease interactions is to separate direct effects that an herbicide might have on pathogens or beneficial microorganisms in the rhizosphere from indirect effects such as root colonization owing to predisposition of the plants by the herbicide. Reliable routine assays of herbicide residue are essential to distinguish between direct and indirect effects. For most herbicides, and for glyphosate in particular, detection of herbicide residues in plants and soil is difficult. The usual method of detection of glyphosate is by gas chromatography following extraction, fractionation, and derivatization steps; and research efforts to develop improved techniques for routine assay are ongoing (119, 137, 140).

Despite these technical difficulties, there is a growing body of literature on the fate of glyphosate in agricultural and forest ecosystems. Once in contact with the soil, glyphosate is readily adsorbed to soil particles by electrostatic force and hydrogen bonding (45, 120, 130, 131), and it also forms insoluble metal complexes (135). Adsorption of glyphosate to soil particles is positively correlated with clay content (45) and generally decreases as pH decreases (80, 100). Glyphosate can be degraded by various microorganisms (58, 114, 116) and by ultraviolet light (93). In forests of northern Sweden, 8% of applied glyphosate was still present in the soil after two years, whereas only 1% of applied glyphosate could still be recovered from soils of southern forests after one year (138). In western Canada, glyphosate residues in soil decreased by 50% within 45–60 days after being aerially applied on a forest canopy by plane at a rate of 2 kg/ha (41). After one year, the residue concentration was 6–18% of that seen immediately after spraying. Almost all the glyphosate residues were in the top 15 cm of soil.

One reason for the popularity of glyphosate is its effect on root or rhizome systems following foliar application. Glyphosate is readily translocated throughout the plant within a few days after treatment (123, 132). Both glyphosate and its major metabolite, aminomethylphosphonic acid, were recovered from roots after foliar application of Roundup (99). Roots of hydroponically grown soybean seedlings showed arrested cell division three days after application of glyphosate to the hydroponic solution or to the
cotyledons (144). As plants mature toward flowering or dormancy, their ability to translocate glyphosate to the roots decreases (55, 151). Very little is know about the extent of exudation of glyphosate from roots of treated plants. Residue leakage from the leaves of yellow nutsedge treated with glyphosate was not detected (22).

The mode of action of an herbicide, both on plants and the plant- and soil-associated microorganisms, must be known to understand its effect on plant disease. There is convincing evidence that the primary target of glyphosate in plants and microorganisms is an enzyme of the shikimic acid pathway, namely, 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase (13, 29, 69, 134). It is worth noting that in the same year in which the mode of action of glyphosate was described, Baillie et al (6) reported their unsuccessful attempt to develop an herbicide that would block the shikimic acid pathway. They reasoned that such an herbicide would be desirable because the absence of the shikimic acid pathway in animals would render it nontoxic to animals. Glyphosate was one of the synthesized putative metabolites of phosphonic acid, a product that had shown weak herbicidal activity in perennials (152).

NATURE OF HERBICIDE EFFECTS ON ROOT DISEASES

Predisposition of Crops to Disease

One of the most commonly reported problems of herbicide use on crops is direct damage due to phytotoxicity, including cases where the enhancement of disease is obvious. A higher incidence of rotted potato tubers after desiccation of foliage with paraquat has been reported (54). Soil-incorporated trifluralin produced swelling and cracking of soybeans through which Fusarium oxysporum infected the plants (24). Inoculation of rice plants at the 4–5 leaf stage with both an isolate of Pythium arrhenomanes (from plants with "Dead Tiller Syndrome" (86)) and the herbicide fenoxapop caused 48% plant mortality, whereas P. arrhenomanes or fenoxapop alone caused 4% and 10% mortality of the plants, respectively (F.N. Lee & J. Hoy, personal communications). In Chehalis sandy loam soil, injury and death of Italian ryegrass (Lolium multiflorum) after glyphosate treatment were attributed to Pythium spp., whereas in Crooked sandy loam the damage appeared to be caused solely by the chemical (80). The mechanisms through which a plant is predisposed are unknown for most of the reported cases.

It is generally accepted that herbicide-induced weakening of the plant can predispose the plant to infection by facultative-type pathogens. Current knowledge on mechanisms of disease resistance in plants can be integrated with information on the mode of action of herbicides to better understand the mechanisms of predisposition (49). By blocking the synthesis of phenylalanine-derived phenols via the inhibition of the EPSP synthase, glyphosate
inhibits the production of phenolics, including lignin precursors and some classes of phytoalexins involved in resistance of plants to diseases. Glyphosate blocked the production of phaseollin, phaseollinosflavan, phaseollidin, and kievitone in bean (71, 72), as well as glyceollin in soybean (64). Sublethal doses of glyphosate inhibited the expression of resistance in soybean to *Phytophthora megasperma* f.sp. *glycinae* (81), in bean to *Colletotrichum lindemuthianum* (71) and in tomato to *Fusarium* spp. (15). The production of lignin in asparagus (122) and flax (127), as well as phenolic compounds in roots of tomato seedlings (15) and bulbs of *Cyperus esculentus* (21) were reduced by glyphosate. The implications for pest management strategies of using glyphosate, or any other pesticide that alters production of secondary metabolites such as phytoalexins, were reviewed by Lydon & Duke (95).

**Sublethal Doses of Herbicides on Crops**

Several registered uses of herbicides on crops result in intentional or accidental exposure to sublethal amounts of the herbicide. For example, low doses of glyphosate are applied to sugarcane to hasten ripening, enhance sugar content, and permit earlier harvest (38). Sublethal doses of herbicides also reach crops that are intentionally sprayed during a safe window, i.e. when absorption or translocation in those crops is minimal compared with the target weed species. Glyphosate application to conifers follows the latter principle and can provide a rapid increase in growth of desired tree species (94, 151). With tools and techniques for directed application, small amounts of herbicides unavoidably reach some of the crop plants, particularly when the weeds are within the rows (148). Our research program on glyphosate was started as a result of empirical observations on young apple trees that accidentally received a few drops of 1% Roundup spray on their bark—trees that the next year showed poor vigor, which appeared to be associated with root rot.

Low doses of glyphosate can be applied to certain crop species to control parasitic plants. Foliar applied glyphosate selectively killed broomrape (*Orobanchus* spp.) parasitic in the roots of beans (5, 124) or sunflowers (25), and controlled dodder (*Cuscuta* spp.) in alfalfa (33). Methods for controlling parasitic plants with herbicides, including glyphosate, were reviewed recently (112).

**Protection of Plants from Disease**

Obviously not all herbicides shift the host-pathogen interaction toward increased disease. Bruckart et al (17) found that bentazon, which they applied at low concentrations to improve the efficacy of the rust fungus *Puccinia canaliculata* for biological control of yellow nutsedge, actually reduced
disease by 50%. This result is not surprising since obligate parasites such as rust fungi do best when growth rate and vigor of their plant host is high.

Dinitroaniline herbicides applied in soil or on leaves caused a 40–98% reduction in infection of tomato seedlings by the vascular wilt pathogen *Fusarium oxysporum* f. sp. *lycopersici* compared to control plants (52). Two months after harvest, potato tubers from plants in plots treated with the postemergence herbicide metribuzin showed a 25% decrease in tuber rot caused by *Erwinia carotovora* ssp. *atroseptica* compared to tubers from plots where weeds were controlled by mechanical cultivation (92).

Herbicides might be able to stimulate a disease-resistance response through a mechanism similar to what has been described for the phosphates that induce resistance in plants against pathogens (34, 48). This mechanism may explain why it took 1000 times more of the systemic herbicide dicamba than the systemic fungicide cyproconazole to inhibit 90% of the radial growth of *Rhizoctonia cerealis* in vitro, whereas when applied to wheat leaves, it took only three times more dicamba than cyproconazole to achieve 90% control of infection (79).

**Direct Effect of Herbicides on Soil Microorganisms**

Unlike the effect of herbicides on plants, very little is known on the mode of action of herbicides on fungi. Glyphosate blocks the shikimic acid pathway in *Neurospora crassa* (14) and, presumably, could have the same effect in other fungal species. The addition of phenylalanine to agar medium reversed the inhibitory effect of glyphosate on radial growth and germination of *Colletotrichum lindemuthianum* (72). Certain combinations of phenylalanine, tyrosine, and tryptophan increased growth of *Calonectria crotalariae* in a medium amended with glyphosate but did not support growth equal to that on the medium without glyphosate (10). On the other hand, Wardle & Parkinson (146) found a strong negative correlation between growth of seven fungal species on water agar and their occurrence in soil with glyphosate at 200 μg g⁻¹; the fungal species most strongly inhibited by glyphosate on water agar plates were among those least suppressed and even stimulated by glyphosate in soil. A better correlation occurred between growth on glyphosate-amended soil extract agar and populations in amended soil, but there were still some important discrepancies. For example, glyphosate at 200 μg g⁻¹ reduced the radial growth of *Fusarium oxysporum* by 70% on soil extract agar, whereas the same concentration in soil had no effect on the population of this fungus (146). Dicamba was by far the least toxic out of four herbicides tested for inhibiting in vitro growth of *Rhizoctonia cerealis*, whereas it was one order of magnitude better than the closest other herbicide when tested for control of *Rhizoctonia* infection on wheat seedlings (79).
In vivo studies are complicated because of the heterogeneity of the soil environment and the diversity of fungal populations. The effect of triazine herbicides on soilborne conidia of *Cochliobolus* spp. was investigated for different species of *Cochliobolus* and different isolates of *C. sativus* (66). These herbicides induced germination of conidia under fungistasis in certain isolates of *C. sativus* but not in other isolates of that species or in other species of this genus. The effect was also dependent on soil type. Further testing with atrazine showed that the deleterious effect of this triazine on populations of *C. sativus* in soil could not be explained by the effect of this herbicide on microflora components that induce fungistasis (68). The negative effect of atrazine on *C. sativus* was increased by lowering soil pH or increasing humic acid content (67).

Little is known about the effects of herbicides on antagonists of plant pathogens. In vitro growth tests showed that bacterial antagonism to *Phytophthora cactorum* was not affected by a range of herbicides (142). Trifluralin did not affect in vitro growth of *Trichoderma viride*, whereas paraquat resulted in retarded growth and a decrease in total fatty acids present in cell lipids (44). The addition of glyphosate to raw soil increased the conducive-ness of the soil to take-all of wheat caused by *Gaeumannomyces graminis* var. *tritici* (101). It appeared that glyphosate selectively suppressed the activity of microorganisms antagonistic to *G. g. var. tritici* and increased the incidence of take-all via this indirect effect on some component of the soil microflora (101, 102). If the relevant antagonistic component of the soil microflora were known, this hypothesis might be testable with glyphosate-resistant antagonists.

Herbicides can also affect plant symbioses in the rhizosphere. Effects of pesticides on mycorrhizal fungi were reviewed by Trappe et al (139). Following application of glyphosate to a single pine needle (*Pinus banksiana* or *P. taeda*), translocation occurred to roots and to two mycorrhizal species, but glyphosate was not detected in roots of untreated pine seedlings growing in the same container (76). A sublethal dose of glyphosate increased the incidence of infection by vesicular arbuscular mycorrhizal Glomus spp. (106). In contrast to this positive effect, glyphosate, diclofop-methyl, and amitrole each reduced nodulation of *Rhizobium trifolii* in *Trifolium subterraneum* (39). Analogous to the effect of herbicides on the incidence of disease in specialized host-fungi pathosystems, it is possible that a detrimental effect on the health of a plant caused by an herbicide has a negative effect on the symbiotic associations. The effect of propazine and chlorthal dimethyl on root morphology and physiology of *Pinus radiata* appeared to have been the reason for the reduction in root infection by mycorrhizae (97). Glyphosate at 0.54 and 3.23 kg/ha did not affect plant growth or root infection by *Paxillus involutus* in seedlings of *Pinus resinosa* (26).
Figure 2  Diagrammatic illustration of direct and indirect effects of herbicides propazine and chlorthal dimethyl on incidence of Phytophthora root rot in seedlings of Pinus radiata in soil infested with Phytophthora cinnamomi. (Adapted from Marks & Cerra (98) with permission from R. Cerra and the widow of G. C. Marks.)

One example from Australia of an herbicide-induced root disease problem includes several of the effects discussed above. When radiata pine was established in the mid-1960s in a nursery at Benalla, Victoria, root diseases were not a significant problem (98). By the mid-1970s, the incidence of damping off and root rot caused by Pythium spp. and Phytophthora cinnamomi, respectively, increased to the point where they could not be controlled with metalaxyl (77). However, these diseases ceased to be problems soon after the herbicide propazine was replaced by chlorthal dimethyl. A model was constructed by evaluating the effects of these two herbicides on incidence of the Phytophthora root rot in steamed, naturally conducive and naturally suppressive soils and their effects on growth of several components of the soil microflora in vitro and in vivo (Figure 2). The empirical observations that chlorthal dimethyl reduced and propazine increased Phytophthora root rot were verified. In the rhizoplane, chlorthal dimethyl suppressed populations of Gram-positive and -negative as well as spore-forming bacteria by about 90%, whereas propazine increased them (98). The spore-forming bacteria stimulated by propazine increased sporangial production in P. cinnamomi. More work is needed to verify the proposed mechanisms, but this example illustrates the potential complexity of herbicide-disease interactions in soils.

**Herbicide-Fungicide Interactions**

Some herbicides can increase the efficacy of fungicides via direct synergism. On wheat seedlings inoculated with Rhizoctonia cerealis, the herbicides DNOC, dicamba, ioxynil, and bromoxynil, when sprayed onto leaves in combination with the fungicide cyproconazole, controlled infection up to 1.9,
2.4, 3.3, and 4.6 times more effectively, respectively, than the expected additive effects of these fungicide-herbicide combinations (79). This effect could be the result of either direct herbicide-fungicide synergism or herbicide-induced resistance. In vitro, the same herbicides were up to 3.1, 4.8, 2.3, and 2.4 times more effective, respectively, in inhibiting radial growth than the expected additive effects of individual cyproconazole-herbicide combinations. Another example was reported with the herbicide metribuzin and the fungicide chlorothalonil, which are both commercially used on potatoes. In vitro tests on Alternaria solani, the causal agent of early blight, showed that the presence of metribuzin in the agar medium reduced the efficacy of chlorothalonil (60). There are few studies on such interactions, which is surprising considering the common use of herbicide-fungicide tank mixes or sequential applications of herbicides and fungicides in the field.

Some herbicides apparently have the potential to predispose fungi to effects of a fungicide. Radial growth of Pseudocercosporella herpotrichoides sub-cultured on unamended PDA after exposure to dicamba, ioxynil, bromoxynil, or no herbicide was the same regardless of herbicide composition or dose (79), but if 5-mm disks from the edge of colonies growing on an herbicide-amended medium were transferred to PDA containing various concentrations of the fungicide cyproconazole, the concentrations resulting in 90% inhibition of radial growth (EC<sub>90</sub>) were drastically reduced compared to colonies established from herbicide-free PDA. Cyproconazole was 5, 2.6, and 1.4 times more effective as an inhibitor of colony growth if the fungus had been previously exposed to dicamba, bromoxynil, and ioxynil, respectively, regardless of the herbicide concentration. These herbicides did not predispose Rhizoctonia cerealis to greater sensitivity to cyproconazole. If one assumes a negligible concentration of herbicide in the inoculum disk, a somewhat stable herbicide-induced physiological change must have taken place to account for the predisposition of P. herpotrichoides.

Certain herbicides might also predispose plant pathogenic fungi to the effect of antagonists. Just as it is important to understand the effects of herbicides on mechanisms of plant disease resistance, a better understanding of the effects of herbicides on susceptibility of pathogens to antagonists is needed to clarify pathogen-antagonist interactions in the presence of herbicides.

**Inoculum Density and Damage to Crops**

The first microorganism to occupy the tissues of dying weeds after herbicide treatment has an advantage over other would-be colonists of this substratum. If an herbicide applied to control weeds selectively or nonselectively causes an increase in certain groups of unspecialized, facultative-type pathogens in the root systems of treated plants, the effect of this herbicide might be to
Soil/Site Factors and Variation in the Efficacy of Glyphosate

Glyphosate applied at recommended rates is slow acting and rarely kills plants within one week following treatment. Glyphosate treatment of field bindweed required at least three weeks (37) and volunteer potato plants up to one month (85) to be lethal. Poor control was obtained during the first year but excellent control was obtained during the two subsequent years when reduced doses (0.45–1.23 kg/ha) of glyphosate were used on quackgrass (9). In pin cherry and trembling aspen, percent mortality, leaf and stem length, as well as visual rating of injury were still affected two years after low doses (0.04–0.5 kg/ha) of glyphosate had been applied (133).

Glyphosate still appears to be one of the best herbicides for chemical control of weeds in forestry. It provided better control of willows (Salix spp.) than 2,4-D or hexazinone (115). Yet Canadian forest managers find that the efficacy of herbicides in forestry varies considerably for reasons that still have to be elucidated (20). No long-term studies have been conducted in Canada on the effect of herbicides on forest vegetation (20). Depending on locations, site preparation with glyphosate had either a positive or no effect on the survival of conifer seedlings compared to unsprayed controls (111). In three out of the five tree species studied in the southeastern United States, the mean efficacy
of aerially applied glyphosate, expressed as the percent decrease in wood volume, was lower than the corresponding standard deviation (32). Among several weather and physiological parameters measured, only sugar content in white oak at the time of glyphosate application was positively correlated with efficacy of glyphosate. For the other three hardwood species, none of the parameters could be correlated to efficacy.

There are also inconsistencies in the efficacy of glyphosate used in agriculture. Better consistency was the reason why paraquat and HOE 3986 were preferred over glyphosate as preplant/postemergence herbicides in sorghum or soybeans for the control of three different species of legume cover crop (50). In an evaluation of the efficacy of glyphosate in controlling regrowth of oatgrass (*Arrhenatherum elatius* var. *bulbosum*) from corms, the best control was obtained at the growth stage where translocation to corms was the lowest (136). Complete control of regrowth was obtained when glyphosate was applied at the 4–5 or 6–7 leaf stage, compared to 45% regrowth if applied at the 2–3 leaf stage, whereas the proportion of absorbed 14C from 14C-glyphosate translocated to corms was 3–7 times higher in plants that had been treated at 2–3 leaf stage compared to the stages that had been completely controlled.

Reported site variations in efficacy of glyphosate led us to compare the efficacy of glyphosate on bean grown under controlled conditions with five different autoclaved and raw soils. Three of the soils were from an arid interior region (southern Okanagan Valley of British Columbia) under commercial apple production, and two were from a humid coastal region (Fraser Valley of British Columbia) under commercial vegetable and nursery crop production. The interior soils varied in cropping and apple root disease histories and were designated on this basis as “virgin”, “crown-rot”, and “replant” soils. A tenfold range in the LD50 values for beans was observed among the five raw soils under otherwise experimentally identical conditions (Figure 4). The virgin soil required the highest dose of glyphosate to kill the bean seedlings, and the muck soil the lowest. These differences between soil types were less apparent in autoclaved soils, where 15–20 times more glyphosate was needed to kill bean seedlings than was required in the corresponding raw soils. We hypothesize that the range in sensitivity of beans to glyphosate in these different soils is due to differences in the fungal populations in these soils, and that higher doses of glyphosate were required in the virgin soil because fewer fungi capable of root colonization were present in this soil. Two unpublished observations support this hypothesis: (a) fewer fungal colonies were detected on dilution plates of the virgin soil than of the other two interior soils, and (b) there was virtually no colonization, and thus no chance for a difference in colonization, of the roots of untreated and glyphosate-treated apple seedlings grown in the raw virgin soil, nor did we find any difference in LD50 values for apple seedlings
grown in autoclaved and raw virgin soil (117). Soil-associated factors other than differences in the resident fungal microflora could also affect the sensitivity of bean seedlings to glyphosate, because significant differences in LD$_{50}$ values were detected in soils even after autoclaving. Such differences might reflect variations in available plant nutrients and hence plant vigor among the soils, or quantitative and qualitative differences in the microflora that colonize these soils after autoclaving.

**Fungal Colonization of Glyphosate-Treated Plants**

Fungal colonization of the roots occurs very rapidly after glyphosate is applied on plants. One day after glyphosate treatment of seedlings of a tomato cultivar (CR6) resistant to the crown and root rot agent, *Fusarium oxysporum* f. sp. *radicis-lycopersici*, the pathogen entered the root epidermis. A day later, it had penetrated the suberized hypodermis, and after that, the hyphae had penetrated the endodermis (15). Hyphae grew inter- and intracellularly similar to growth in the fully susceptible tomato cultivar MR13, yet roots of untreated resistant CR6 seedlings were not colonized by the pathogen. In the field, rapid colonization of several weed species by *Fusarium* spp. occurred after glyphosate treatment (91). In bean and wheat seedlings grown in pots,
colonization by *Pythium* spp., measured as the number of colony-forming units present in the intact root system (88), was detected at an increasing frequency starting 24 hr after glyphosate treatment at two different temperatures and soil moistures (87, 89), whereas *Pythium* spp. could not be isolated from the roots of the untreated seedlings. *P. sylvaticum* was the predominant colonizer of glyphosate-treated bean and wheat seedlings grown in a loam soil, whereas *P. ultimum* was prevalent in treated seedlings grown in a muck soil (87, 90). Because of this rapid colonization of the roots and potential buildup of inoculum, herbicides should be used with some caution to control vegetation around small experimental plots (109) or to simulate plant death in yield loss assessment studies (73).

Pathogenicity and Host Specificity of Glyphosate-Associated Colonizing Fungi

Knowledge of specialization and pathogenicity of fungal colonists of plant roots is an important aspect in studying the effect of glyphosate on inoculum buildup in soil after killing weed cover. The *Pythium* spp. that colonized roots of glyphosate-treated wheat and bean seedlings caused pre- and/or postemergence blight of these plants in the absence of glyphosate (90). There was no indication among the isolates tested of host specialization, i.e. isolates of *Pythium* obtained from wheat did not cause a higher incidence of seedling blight on wheat than on beans and vice versa.

Three fungal genera, namely, *Pythium*, *Fusarium* and *Cylindrocarpon*, were the predominant fungal root colonizers of glyphosate-treated apple seedlings grown in the crown rot and replant soils (C. A. Lévesque & J. E. Rahe, unpublished data). A representative isolate from each genus, and one representative isolate of *P. ultimum* obtained from glyphosate-treated bean seedlings grown in organic soil, were established in autoclaved loam soil. On McIntosh apple seedlings, *Pythium* sp. and *Fusarium* sp. isolated originally from apple resulted in lower LD_{50} values for glyphosate (91a). In contrast, *P. ultimum* from beans and *Cylindrocarpon* sp. from apple did not affect the LD_{50} values of glyphosate on apple seedlings compared with those values recorded in autoclaved soil alone, demonstrating that the synergistic effect does not apply to all host-colonist combinations. Much more remains to be learned about the host specificity of glyphosate synergistic fungi.

NEW WAYS OF EXPLOITING HERBICIDE-MICROORGANISM INTERACTIONS

*Melanconis alni* is a weak pathogen that produces cankers on alder, a species presently considered an important weed in forests of British Columbia (36). This pathogen does not kill the trees directly, nor does glyphosate cause
detectable damage to the trees when used at one-twentieth of the recommended dose to control alder. However, when this sublethal rate of glyphosate is applied in combination with *M. alni*, the trees die because of the canker (C. E. Dorworth, personal communication).

Gotlieb & Watson (46) tested up to 12 different herbicides for synergism with *Alternaria cassiae*, *Colletotrichum coccodes*, *C. truncatum*, and *Fusarium lateritium* for biological weed control. The most effective synergistic effect under greenhouse conditions was observed using a combination of sethoxydim (Poast) and *A. cassiae* to control sickle pod (*Cassia obtusifolia*). Neither the herbicide nor the microbial agent had any effect on the weed species when tested alone, but 100% control was achieved when they were used in combination.

*Cyindrocarpon destructans* is a natural colonizer of roots of thimbleberry (*Rubus parviflorus*), considered a weed in some forests of British Columbia (145). It caused little more than leaf spots or foliar browning of the upper portion of thimbleberry when added to soil, but plants either wilted, lost 60–90% of their leaves, or died when *C. destructans* was used in combination with a low dose of glyphosate (145a). Glyphosate alone caused about 50% leaf damage. The use of soilborne fungi for biocontrol of weeds has received very little attention (74).

The combination of a weak pathogen and sublethal doses of herbicide offers the advantage of restricting the spread of the biocontrol agent in both time and space. This is particularly appropriate when the weed to be controlled is a desirable plant in other situations. Because herbicides can also increase efficacy of biocontrol agents, microorganisms shown to be ineffective in killing weeds, such as *Exserohilum turcicum*, *Colletotrichum graminicola*, *Bipolaris halepense*, and *Gloeocercospora sorghi* tested by Chiang et al (28) to control johnsongrass, should be investigated further in combination with low doses of herbicides.

Combining microorganisms with herbicides can also expand host range of a microbial weed-control agent, a desirable characteristic for marketing. For example, bacterial plant pathogens with a narrow host range can be effectively combined with low doses of herbicides (31). It appears that stress on plants caused by the herbicide can increase the host range of some of the bacteria being tested (A. L. Christy, personal communication). The details are still classified at this time.

Herbicide-microorganism combinations could also be used to increase the selectivity of an herbicide and thereby reduce predisposition of crops to disease caused by pathogens in soils that have residues of persistent herbicides. Seeds of soybean and peas inoculated with dicamba-degrading strains of *Pseudomonas* sp. or *Moraxella* sp. grew much better than uninoculated seeds in soil treated with dicamba in both growth chambers and the field (84). The concentration of dicamba in soil treated with the inoculated seeds was
rapidly reduced. The effect of inoculation in the absence of the herbicide was minimal.

Paraquat can be used as a research tool to detect infection of lupines (*Lupinus angustifolius*) by *Phomopsis leptostromiformis* earlier than is otherwise possible (30). Lack of symptoms in the early stage of this disease makes epidemiological studies difficult. Likewise, it might be possible to use glyphosate-treated plants to assess the presence and activity of certain soil-borne fungi since fungal colonization occurs very rapidly after treatment (89).

**CONCLUSION**

General rules regarding the management of weeds without increasing and preferably while minimizing disease are difficult to develop, given the many variables that can affect, positively or negatively, herbicide-plant disease interactions. At present, it is difficult to predict the circumstances under which disease incidence will increase or will be reduced after an herbicide application. It is likely that the result of herbicide-disease interactions will be specific to certain geographical areas or crop production systems and will depend on fungal populations and herbicide residues. Monoclonal antibodies used in Enzyme Linked Immunosorbent Assay (ELISA) and DNA probes with nonradioactive labeling systems should make detection and quantification of key fungal pathogens involved in herbicide-disease interactions easier to perform on a routine basis and provide essential information to generate reliable recommendations. ELISA can even be used to detect herbicide residues (82). These technologies will lead to an ever-increasing quantity of data and known parameters for making management decisions with respect to herbicide-disease interactions. Conspicuous variables such as weed composition, density, size, crop cultivar, cultivation method, herbicide choices, costs and methods of application, and information about yield are already numerous enough to justify the use of computers and expert systems to provide “intelligent” herbicide recommendations adjusted to local conditions (1, 47, 107). It will be necessary to have herbicide-disease prediction models included in such expert systems with as many relevant variables as possible to be able to recommend herbicides that optimize disease management.

Better standardization of experimental protocols and cooperation between working groups will be necessary to accumulate a data base in the area of herbicide-disease interactions applicable for routine recommendations. Heitefuss (59) outlined five basic points that should have priority in studying herbicide-disease interactions. These summarized points are: (a) practical relevance of the side effects; (b) significance of crop rotation or alternate hosts; (c) relationships with weed density; (d) use of proper weed-free controls; and (e) distinction between direct and indirect effects. To this we would add the importance of reporting accurately what was used as potting soil for
any type of controlled herbicide experiments, and whether the soil was pasteurized, fumigated, or sterilized, information that all too often is missing. An impressive amount of data on side effects of pesticides on beneficial organisms has resulted from the joint testing program of the entomologically oriented Pesticides and Beneficial Organisms working group of the International Organization for Biological Control (57). This group has developed a standard sequential approach where the side effects are studied in laboratory, semifield, and field conditions. Such a team approach might be applicable to the study of herbicide-disease interactions. Collaborations between weed scientists and plant pathologists are increasing due to growing interests in microbial herbicides, and there are good reasons for such collaborations to expand into various areas of herbicide-disease interactions.

SUMMARY

Sublethal doses of herbicides may either protect or predispose crops to disease. Herbicides can directly alter the nature of soil ecosystems through promotion or suppression of activities of plant pathogens or beneficial microorganisms. Recently, it has been shown that herbicides can predispose pathogens or act as synergists to fungicides. Fungal colonization of roots rapidly follows the application of certain herbicides. Nonspecialized facultative-type pathogens can increase their inoculum potential on weeds or volunteers treated with herbicide and subsequently cause more disease on crops grown on that site. Soilborne fungi can also act as synergists in the herbicidal action of glyphosate, possibly because glyphosate blocks the production of phenolics involved in disease resistance of plants to these pathogens. Combinations of weakly pathogenic fungi and low doses of herbicides may have potential for weed control. Fundamental information on mode of action and effects of herbicides on crops and microorganisms is needed to better understand and predict the interactions between herbicides and fungal root pathogens. New tools for rapid detection of fungi and herbicide residues, as well as expert systems to make recommendations based on many variables, should help plant pathologists and weed scientists in optimizing herbicide efficacy and minimizing damage caused by diseases.

ACKNOWLEDGMENTS

We thank F. N. Lee, J. Hoy, R. Prasad, S. F. Shamoun, C. E. Dorworth, and A. L. Christy for sharing unpublished information. We are grateful to R. J. Cook, Z. K. Punja, T. C. Vrain, M. McCann, L. Ward, H. S. Pepin, and R. Stace-Smith who provided critical reviews for this manuscript. Our work reported in this review has been supported by the Natural Sciences and Engineering Research Council of Canada and by Agriculture Canada.
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