Fungicidal Effects of Glyphosate and Glyphosate Formulations on Four Species of Entomopathogenic Fungi

W. E. MORJAN,[1] L. P. PEDIGO,[2] AND L. C. LEWIS[3]

Environ. Entomol. 31(6): 1206–1212 (2002)

ABSTRACT Fungicidal effects of glyphosate and glyphosate formulations on the entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metchnikoff) Sorokin, *Nomuraea rileyi* (Farlow) Samson, and *Neozygites floridana* Weiser & Muma were evaluated under laboratory conditions. Media previously inoculated with entomopathogenic fungi were exposed to distilled water, glyphosate (active ingredient), seven glyphosate formulations, and five blank formulations (carrier only). The fungicidal activity was determined by measuring inhibition in mycelial growth in solid media (*B. bassiana*, *M. anisopliae*, and *N. rileyi*), and spore concentration in liquid medium (*N. floridana*). Glyphosate did not have fungicidal activity against any of the fungi tested. Fungicidal properties of glyphosate formulations varied among fungal species. *Neozygites floridana* and *M. anisopliae* were susceptible to all glyphosate formulations. RoundUp Ready-To-Use was consistently the glyphosate formulation with one of the strongest fungicidal properties. Fungicidal activity of some formulations had a synergistic effect with glyphosate. RoundUp Original was the only formulation that did not show any interaction on fungicidal activity between glyphosate and the formulation. The results showed that the four fungi tested are susceptible to various glyphosate formulations when exposed to field concentrations.

RESUMEN Los effectos fungicidas de glyphosato y formulaciones de glyphosato fueron evaluados bajo condiciones de laboratorio en los hongos entomopatógenos *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metchnikoff) Sorokin, *Nomuraea rileyi* (Farlow) Samson, y *Neozygites floridana* Weiser & Muma. Medios de crecimiento previamente inoculados con los hongos entomopatógenos fueron expuestos a agua destilada, glyphosato (ingrediente activo), siete formulaciones de glyphosato, y cinco formulaciones sin ingrediente activo (solamente inertes). La actividad fungicida fue determinada en medios de crecimiento sólidos midiendo el área de crecimiento miceliar inhibido (*B. bassiana*, *M. anisopliae*, y *N. rileyi*), y en el medio de crecimiento líquido determinando la densidad de esporas (*N. floridana*). Glyphosato no tuvo ninguna actividad fungicida para ninguno de los hongos. Las propiedades fungicidas de las formulaciones de glyphosato diferieron entre las especies de hongos. *Neozygites floridana* y *M. anisopliae* fueron susceptibles a todas las formulaciones de glyphosato. RoundUp Ready-To-Use fue consistentemente una de las formulaciones de glyphosato con propiedades fungicidas mas fuertes. La actividad fungicida de varias formulaciones tuvieron un effecto sinérgico con glyphosato. RoundUp Original fue la única formulación que no mostró ninguna interacción en la actividad fungicida entre glyphosato y la formulacion. Los resultados muestran que los cuatro hongos en este estudio son susceptibles a varias de las formulaciones de glyphosato cuando expuestos a concentraciones de campo.

KEY WORDS entomopathogens, nontarget effects, fungicidal effects, roundup, herbicide, biological control

LITTLE IS KNOWN about the interaction among herbicides and microorganisms compared with the knowledge of the effects of herbicides on plants. Some direct effects of herbicides, besides killing weeds, may include predisposition of fungi to fungicides, acting as synergistics or having fungicidal properties (Levesque and Rahe 1992). Herbicides also may have indirect effects caused by weed control. These may include changes in the normal interaction between fungi, crop, and weeds, and changes in microenvironment (Levesque and Rahe 1992).

One of the herbicides that ranks among the top 10 herbicides used in the United States in agricultural and nonagricultural situations is glyphosate (Cox 1995). Glyphosate (N-[phosphonomethyl]glycine) is a

[1] Monsanto Company, Northern Corn Entomology, Waterman, IL 60556 (e-mail: wilmar.e.morjan@monsanto.com).
[2] Department of Entomology, Iowa State University, Ames, IA 50011–3140.
[3] USDA–ARS, Iowa State University, Ames, IA 50011–3140.

0046-225X/02/1206–1212$02.00/0

broad-spectrum herbicide sold under the trade names of Roundup, Rodeo, Accord, Kleenup, and Vision. The total sales of Roundup were approximately $1 billion in 1991 (Levesque and Rahe 1992). In Canada in 1988, 81% of the forests treated with herbicides were treated with Roundup (Levesque and Rahe 1992).

Glyphosate inhibits the biosynthesis of aromatic amino acids in shoots and roots tips in plants and some microorganisms by inhibiting the enzyme EPSP (5-enolpyruvylshikimate-phosphate) of the shikimic acid pathway (Jaworski 1972, Steinrücken and Amrhein 1980). The absence of the shikimic acid pathway in animals means that glyphosate has very low toxicity for animals (Levesque and Rahe 1992).

Because glyphosate is a nonselective herbicide, its use in agriculture crops was limited to preplanting sprays, spot sprays, or screen sprays. Thus, sprays rarely covered 100% of the crop area once the crops emerged. However, with genetically engineered crops resistant to glyphosate, such as Roundup Ready Soybean and Roundup Ready Corn, glyphosate is now sprayed on nearly 100% of the planted area after emergence.

Some formulated glyphosate have shown some fungicidal properties. These properties vary among pathogenic and saprophytic fungal species (Grossbard and Atkinson 1985, Chakravarty and Chatarpaul 1990a, Wardle and Parkinson 1992). For example, fungicidal effects from formulated glyphosate have been reported in species such as *Rhizoctonia solani* Kühn (Black et al. 1996), *Fusarium solani* (Martius) Saccardo (Abdulsalam et al. 1990, Kawate et al. 1992), *Glomus etunicatum* Becker & Gerdemann (Paula and Zambolim 1994), *Nectria galligena* Bresadola (Burgiel and Grabowski 1996), *Septoria nodorum* Berkeley (Harris and Grossbard 1979), and *Phytophthora* spp. (Utkhede 1982, Kassaby 1985, Kassaby and Hepworth 1987); but no detrimental effects from these formulations have been reported against species such as *Drechslera teres* (Saccardo) Shoemaker (Toubia-Rahme et al. 1992) and *Pythium ultimum* Trow (Kawate et al. 1992).

Although glyphosate may negatively affect microorganisms, it has been suggested such effects are not likely under field conditions (Cox 1995). The reasoning is that glyphosate binds to clay and many soil types, reducing its movement in the soil (Cox 1995). Therefore, glyphosate formulations will be present in the surface of the soil of the areas treated. Knowing this, and that the half-life of glyphosate can be up to 141 d in Iowa soils (Cox 1995), negative consequences may occur to entomopathogenic fungi that generally inhabit the soil surface.

In Iowa and other midwestern states, entomopathogenic fungi play a key role in causing diseases in arthropod populations. With adequate moisture and temperature, entomopathogenic fungi are considered the most important mortality factor of several arthropod pests, maintaining populations below economic thresholds. The twospotted spider mite (*Tetranychus urticae* Koch) in soybeans and corn is maintained at subeconomic levels by *Neozygites floridana* Weiser and Muma in fields with high relative humidity (Klubertanz 1989, Klubertanz et al. 1991). Another pest is the green cloverworm [*Hypena scabra* (F.)] on soybeans, which is usually suppressed by *Nomuraea rileyi* (Farlow) Samson (Rice 1997). Other pests suppressed by fungi include the alfalfa weevil [*Hypera postica* (Gyllenhal)] by *Zoophthora phytonomi* (Arthur) Batko in alfalfa fields (Giles et al. 1994), and grasshopper (Acrididae) populations by *Entomophaga grylli* (Fresenius) Batko (Pickford and Riegert 1963).

In crops where environmental conditions or chemicals negatively affect entomopathogens, spider mites usually become a serious pest very rapidly (Campbell 1978, Brandenburg and Kennedy 1983, Bower et al. 1995). This is caused by their extremely short generation time (14 d to complete a generation) and female fecundity (≈300 eggs per female) (Huffaker et al. 1969). In Iowa in 1988, 2.4 million acres of soybeans were treated with insecticide because of a spider mite outbreak (Higley and Boethel 1994), and many fields that were not treated on time had yield losses of 40–60% (Higley and Wintersteen 1989). In 1997, small outbreaks of spider mites in fields planted with transgenic soybeans were reported in Story County, IA (L. P. Pedigo, personal communication). If entomopathogenic fungi are susceptible to glyphosate formulations, spraying this herbicide with 100% coverage in soybean fields may be detrimental to the entomopathogenic fungi. Thus, this could enhance pest outbreaks requiring the use of insecticides.

Therefore, the objective of this study was to evaluate the effects of glyphosate and glyphosate formulations on the entomopathogenic fungi *Beauveria bassiana*, *Nomuraea rileyi*, *Metarhizium anisopliae* (Metchnikoff) Sorokin, and *Neozygites floridana.*

Materials and Methods

Isolates. Four species of entomopathogenic fungi were obtained from USDA-ARS collection of entomopathogenic fungi (ARSEF) Ithaca, NY. The species included: (1) *Beauveria bassiana* (Deuteromycetes: Moniliales) culture No. 501 (R. L. Jones) isolated from European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), (2) *Nomuraea rileyi* (Deuteromycetes: Moniliales) culture No. 762 (T. A. Coudron) isolated from green cloverworm, *Hypena scabra* (Lepidoptera: Noctuidae), (3) *Metarhizium anisopliae* (Deuteromycetes: Moniliales) culture No. 324 (Commonwealth Scientific and Industrial Research Organization FI48) isolated from *Austracris guttulosa* (Orthoptera: Acrididae), (4) *Neozygites floridana* (Zygomycetes: Entomophthorales) culture No. 662 (R. A. Humber) isolated from twospotted spider mite *Tetranychus urticae* (Acari: Tetranychidae).

Growth Media. *B. bassiana* and *M. anisopliae* were grown on Sabouraud dextrose agar plus 1% yeast extract (SDAY) (4% dextrose, 1% peptone, 1.5% agar, and 1% yeast extract). *N. rileyi* was grown on Sabouraud maltose agar plus 1% yeast extract (SMAY) (4% maltose, 1% peptone, 1.5% agar, and 1% yeast

Table 1. Treatments used in this study (treatments at 0.96% [AI])

Treatment with glyphosate	Treatment without glyphosate
RoundUp Ready-To-Use[a]	
RoundUp Ultra 2[b]	
RoundUp Original[b]	RoundUp Original (blank)[b]
RoundUp Pro[b]	RoundUp Pro (blank)[b]
RoundUp Ultra[b]	RoundUp Ultra (blank)[b]
RoundUp RT[b]	RoundUp RT (blank)[b]
RoundUp Ultra RT[b]	RoundUp Ultra RT (blank)[b]
Glyphosate (99% purity)[c]	Distilled water (control)

Blank formulations were diluted to the equivalent of the dilutions of glyphosate formulations (0.96% [AI]).

[a] Formulation obtained from commercial retailer (WallMart, Ames, IA)

[b] Formulation obtained from Monsanto Company (St. Louis, MO)

[c] Active ingredient obtained from Chem Service (West Chester, PA)

extract). Each petri dish had 13 ml of media. *N. floridana* was grown in liquid tissue culture medium (Grace's medium, and 5% fetal bovine serum).

Treatments. The treatments consisted of seven glyphosate formulations, five blank formulations (formulations without the active ingredient, isopropylamine salt), glyphosate (pure active ingredient as isopropylamine salt) as a control for formulations, and distilled water as a control (Table 1). The concentration of glyphosate, glyphosate formulations, and blank formulations were standardized to 0.96% active ingredient, the lowest recommended dose for field applications.

In Vitro Tests. Experiments were conducted at 21 ± 2°C, and fungi were grown for 21 d. Fungal inocula were cultured from the original isolates in petri dishes (solid media) for *B. bassiana*, *M. anisopliae*, and *N. rileyi*, and in tissue culture flasks (liquid medium) for *N. floridana*. Conidia grown in petri dishes were harvested in 100-ml vials containing sterilized distilled water. This spore-suspension was used for inoculation in the experiment with fungi grown in solid media. The *N. floridana*, inoculum consisted of the liquid medium with fungus without dilutions. The concentration of spores was 13.7×10^6 spores/ml for *B. bassiana*, 6.2×10^6 spores/ml for *M. anisopliae*, 5.5×10^6 spores/ml for *N. rileyi*, and 180×10^6 spores/ml for *N. floridana*.

Petri dishes containing media specific for each fungus were inoculated by pouring 1 ml of spore suspension on the media and distributed with a sterilized cell spreader (6 cm long). Treatments were applied by individual blank paper discs of 6.36 mm diameter (BBL, Fisher, Pittsburgh, PA). The blank paper discs were submerged individually for 5 min in beakers containing designated treatments (Table 1). Then the discs were placed on a sterile petri dish to let them dry, and after dried, they were placed in the inoculated petri dish (Fig. 1). Each treatment had 10 replicates.

For *N. floridana*, 8 ml of medium was poured into tissue culture flasks (50 ml capacity) and inoculated with 1 ml of spore suspension. Treatments (Table 1) were applied to individual culture flasks with 1 ml of treatment solution. Each treatment had six replicates.

Growth Inhibition Measurements. The fungicidal effects of the treatments were evaluated after 21 d. Fungicidal effects in *B. bassiana*, *M. anisopliae*, and *N. rileyi* were quantified as the area (cm^2) around the blank paper discs with no and poor mycelial development. Measurements were taken by capturing digital pictures of the petri dishes using an Apple Computer (Cupertino, CA) G3 computer equipped with a Scion Corporation (Federick, MD) CG-7 frame-grabber and a Sony (Itasca, IL) DXC-3000A color video camera. This apparatus was set to capture images at a resolution of 18.8 pixels/mm^2. The photographs were analyzed using Scanalytic, Incorporated (Fairfax, VA) IPLab image analysis software, measuring the area around paper discs showing fungal inhibition. For *N. floridana*, fungicidal properties of the treatments were measured as the spore concentration (primary conidia/ml) by using a hemacytometer.

Data were analyzed as a completely randomized design and separation of means was done using least significant comparison analysis (Fisher protected least significant difference [LSD]) (PROC analysis of variance, SAS Institute 1985).

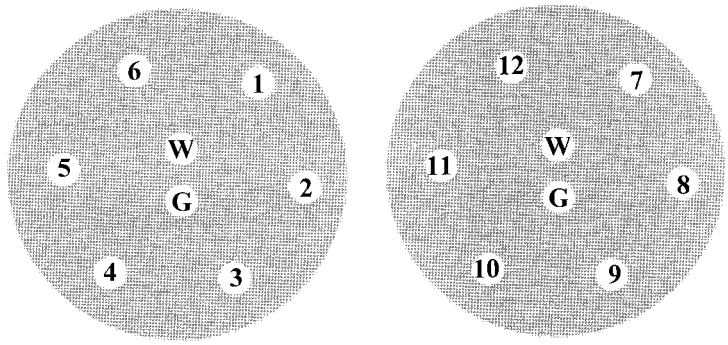

Fig. 1. Graphic representation of petri dishes inoculated with entomopathogens and distribution of treatments applied with blank paper discs. Numbers and letter represent treatments. W, distilled water; G, glyphosate; 1, RoundUp Ready-To-Use; 2, RoundUp Ultra2; 3, RoundUp Original; 4, RoundUp Original blank; 5, RoundUp Pro; 6, RoundUp Pro blank; 7, RoundUp Ultra; 8, RoundUp Ultra blank; 9, RoundUp RT; 10, RoundUp RT blank; 11, RoundUp UltraRT; 12, RoundUp UltraRT blank

Table 2. Effect of glyphosate, glyphosate formulations, and blank formulations on the in vitro growth of three entomopathogenic fungal species

Treatment	Entomopathogenic fungi		
	Beauveria bassiana	*Metarhizium anisopliae*	*Nomuraea rileyi*
RoundUp Ready-To-Use	2.93 ± 0.84a	6.84 ± 1.70a	7.55 ± 1.63a
RoundUp Ultra 2	0.56 ± 0.80cde	4.25 ± 1.73b	3.11 ± 1.65c
RoundUp Original	0.23 ± 0.26e	2.80 ± 1.38bcd	0.43 ± 0.60d
RoundUp Original blank	0.33 ± 0.42de	1.91 ± 1.45d	0.08 ± 0.21d
RoundUp Pro	2.67 ± 1.09a	4.17 ± 1.56b	0.85 ± 1.01d
RoundUp Pro blank	0.99 ± 0.60c	2.15 ± 0.99cd	0.27 ± 0.41d
RoundUp Ultra	3.13 ± 1.01a	3.10 ± 2.47bcd	3.30 ± 2.58c
RoundUp Ultra blank	1.89 ± 0.9b	1.96 ± 1.96d	2.84 ± 1.82c
RoundUp RT	0.91 ± 0.68cd	1.64 ± 1.48d	1.38 ± 1.65d
RoundUp RT blank	0.44 ± 0.49cde	2.47 ± 2.18cd	5.22 ± 2.64b
RoundUp Ultra RT	2.92 ± 0.50a	3.55 ± 1.54bc	3.53 ± 1.79c
RoundUp Ultra RT blank	0.99 ± 0.54c	2.97 ± 2.65bcd	3.56 ± 1.74c
Distilled water	0e	0.09 ± 0.20e	0.19 ± 0.37d
Glyphosate	0e	0 ± 0e	0.23 ± 0.26d

Data are average area of fungal growth inhibited (cm^2) ± SD.

Results and Discussion

Effect of Glyphosate. No statistically significant differences in fungal inhibition were detected between glyphosate (N-[phosphonomethyl]glycine) and distilled water on the four entomopathogenic fungi tested ($P > 0.05$) (Table 2; Fig. 2). These results indicate that spore germination and mycelial growth of *B. bassiana*, *M. anisopliae*, *N. rileyi*, and *N. floridana* are not affected by glyphosate active ingredient when exposed to a field concentration of 0.96% (AI). In contrast, studies with the plant pathogenic fungi *Fusarium solani* and *Pythium ultimum* have shown that mycelial growth and spore production are stimulated or inhibited by glyphosate (pure active ingredient), depending on concentrations of the active ingredient (Kawate et al. 1992).

Even though studies on the fungicidal properties of glyphosate against several species of plant pathogenic, saprophytic, and entomopathogenic fungi have reported high variability, we were not able to compare these data with our results. Those studies used herbicide formulations containing glyphosate, but glyphosate (active ingredient) alone was not tested (Harris and Grossbard 1979; Utkhede 1982; Johal and Rahe 1984; Kassaby 1985; Chakravarty and Sidhu 1987; Kassaby and Hepworth 1987; Mekwatanakarn and Siv-

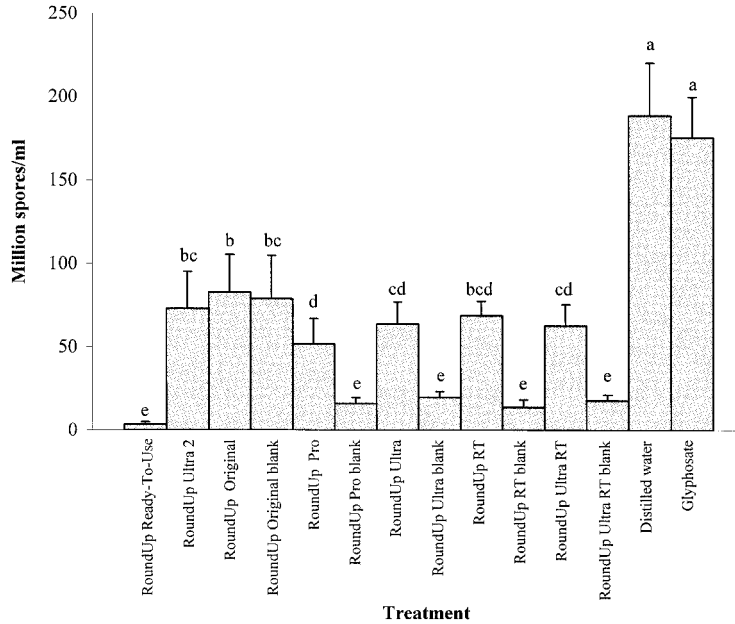

Fig. 2. Effect of different glyphosate, glyphosate formulations, and blank formulations on the in vitro growth of *Neozygites floridana*. Data are average spore concentration (spores $\times 10^6$/ml; ± SD). Bars with same letters are not statistically different ($P \leq 0.050$, Fisher protected LSD test)

asithamparam 1987; Estok et al. 1989; Sharma et al. 1989; Abdulsalam et al. 1990; Chakravarty and Chatarpaul 1990a, 1990b, Mietkiewski et al. 1990; Martensson 1992; Abdel-Mallek et al. 1994; Paula and Zambolim 1994; Burgiel and Grabowski 1996). Fungicidal activity of commercial herbicide formulations may be caused by the active ingredient, the inert ingredients, or an interaction between these. Therefore, differences in fungicidal activity observed among studies may vary because of differences in the formulations tested, rather than glyphosate alone.

Effect of Glyphosate Formulations. The fungicidal property of glyphosate formulations varied among species of the fungi tested (Table 2; Fig. 2). *N. floridana* and *M. anisopliae* were susceptible to all glyphosate formulations, but not to glyphosate (active ingredient) (Table 2; Fig. 2). Among the entomopathogenic fungi tested in solid media, *B. bassiana* showed less susceptibility to glyphosate formulations, with a growth inhibition of <3.13 cm^2, compared with *M. anisopliae* (6.84 cm^2) and *N. rileyi* (7.55 cm^2) ($P \leq 0.05$) (Table 2). RoundUp Ready-To-Use was consistently the glyphosate formulation with one of the strongest fungicidal properties on the four entomopathogenic fungi ($P \leq 0.05$) (Table 2; Fig. 2).

Beauveria bassiana growth was inhibited by five of the seven glyphosate formulations (Table 2). RoundUp Ready-To-Use, RoundUp Pro, RoundUp Ultra, and RoundUp Ultra RT had the strongest fungicidal properties, inhibiting spore germination and mycelial growth in an area ranging from 2.67 to 3.13 cm^2 (Table 2). The formulations RoundUp Original and RoundUp Ultra two did not significantly inhibit mycelial growth and spore germination of *B. bassiana* ($P > 0.05$) (Table 2).

Metarhizium anisopliae growth was inhibited by all glyphosate formulations ($P \leq 0.05$) (Table 2). The formulation with the highest fungicidal properties was RoundUp Ready-To-Use, which inhibited an area of 6.84 cm^2 (Table 2). The formulation with the lowest fungicidal properties on *M. anisopliae* was RoundUp RT, inhibiting an area of 1.64 cm^2 (Table 2).

Nomuraea rileyi mycelial growth and spore germination was inhibited by three of the seven glyphosate formulations ($P \leq 0.05$) (Table 2). RoundUp Ready-To-Use had the highest fungicidal activity, inhibiting 7.55 cm^2 (Table 2). There were no statistically significant differences among three glyphosate formulations (RoundUp Original, RoundUp Pro, and RoundUp RT) and the controls (glyphosate and water) which suggests that fungicidal activity against *N. rileyi* is very low or nonexistent with these formulations (Table 2).

Neozygites floridana spore concentration (primary conidia/ml) was reduced by >50% by all glyphosate formulations ($P \leq 0.05$) (Fig. 2). RoundUp Ready-To-Use was the formulation with the strongest fungicidal activity, having less than 4×10^6 spores/ml compared with 189×10^6 spores/ml with distilled water ($P \leq 0.05$) (Fig. 2). The glyphosate formulations with the lowest fungicidal activity were RoundUp Original (83×10^6 spores/ml), RoundUp Ultra two (73×10^6 spores/ml), and RoundUp RT (69×10^6 spores/ml) (Fig. 2).

Inhibition in colony growth by a glyphosate formulation (not identified in the study) has already been documented on four entomopathogenic fungi, *B. bassiana*, *M. anisopliae*, *Paecilomyces farinosus* (Holmskiold) Brown and Smith, and *Paecilomyces fumosoroseus* (Wize) Brown and Smith (Mietkiewski et al. 1997). Similarly, inhibition of mycelial growth in *B. bassiana* has been shown with RoundUp 4E (Gardner and Storey 1985). These studies did not test technical glyphosate alone. Thus, our finding that glyphosate does not have fungicidal activity against *B. bassiana*, *M. anisopliae*, *N. rileyi*, and *N. floridana*, but several formulations did, lead us to believe that the fungicidal activity observed in those studies was caused by the formulations (inert ingredients alone or interactions among inert ingredients and glyphosate).

Interactions of Glyphosate and Formulations. Interactions in fungicidal properties between formulations and active ingredient were observed in four of the five formulations tested. Every fungus had at least one blank formulation (formulation without active ingredient) that had significantly higher or lower fungicidal activity when compared with its corresponding glyphosate formulation ($P \leq 0.05$) (Table 2; Fig. 2). RoundUp Original was the only formulation that did not show any interaction in fungicidal activity between formulation and glyphosate ($P > 0.05$) (Table 2; Fig. 2). Thus, its fungicidal activity observed on *M. anisopliae* and *N. floridana* was from the inert ingredients alone.

Greater fungicidal activity was observed against *B. bassiana* by three of the five formulations containing glyphosate (RoundUp Pro, RoundUp Ultra, and RoundUp Ultra RT) compared with their corresponding blank formulations ($P \leq 0.05$) (Table 2). Similarly, one formulation containing glyphosate (RoundUp Pro) showed higher fungicidal activity against *M. anisopliae* than its corresponding blank formulation ($P \leq 0.05$) (Table 2). Thus, inert ingredients in these formulations seem to have a synergistic effect with glyphosate, perhaps inhibiting mycelial growth and spore germination. Lack of statistically significant differences ($P \leq 0.05$) between the glyphosate formulations and their equivalent blank formulations suggests that glyphosate does not interact with the inert ingredients on their fungicidal activity on *B. bassiana* (RoundUp Original and RoundUp RT) and *M. anisopliae* (RoundUp Original, RoundUp Ultra, RoundUp RT, RoundUp UltraRT) (Table 2).

The glyphosate formulation RoundUp RT had significantly less fungicidal activity against *N. rileyi* than did its blank formulation ($P \leq 0.05$) (Table 2). This indicates that glyphosate interferes with the inert ingredients present in that formulation in their fungicidal activity on *N. rileyi.* No statistically significant differences between the other four glyphosate formulations and their respective blank formulations suggest that there are no interactions on *N. rileyi* spore germination and mycelial growth ($P > 0.05$) (Table 2).

Neozygites floridana spore concentration was significantly higher in four of the five glyphosate formulations (RoundUp Pro, RoundUp Ultra, RoundUp RT, and RoundUp Ultra RT) than their equivalent blank formulations ($P \leq 0.05$) (Fig. 2). This indicates an antagonism or interference by glyphosate in the fungicidal activity of these formulations. These results may be caused by differences in the pH of the glyphosate formulations versus their corresponding blank formulations as has been shown in studies with tadpoles of four Australian frogs (Mann and Bidwell 1999).

Differences in fungicidal activity between glyphosate and different formulations are not surprising since some of the inert ingredients in formulations may increase or reduce the toxicity of the pesticide to specific organisms (Ware 1994). Though some inert ingredients may increase the toxicity of the pesticide, they are not toxic in themselves (Levesque and Rahe 1992, Ware 1994, Pedigo 2002). Glyphosate and glyphosate formulations (processed active ingredient plus addition of compounds that improve its properties) have been shown to have different toxicity to four species of frogs (Mann and Bidwell 1999). In the frog study, RoundUp R was the most toxic to the immature frogs, compared with Touchdown R, RoundUp R Bioactive, and glyphosate (pure active ingredient) (Mann and Bidwell 1999). Also, differences in species sensitivity to the different glyphosate formulations were observed (Mann and Bidwell 1999). Similarly, our study found that fungicidal effects of glyphosate formulations varied among species of entomopathogenic fungi, with *B. bassiana* being less susceptible than *M. anisopliae* and *N. rileyi* (Table 2). Of the fungi grown in the solid media, only RoundUp RT with *N. rileyi* showed a negative interaction in the fungicidal activity, when glyphosate was present (Table 2). In contrast, all the interactions detected between glyphosate and the inert ingredients with respect to fungicidal activity against fungi grown in solid media, were positive synergistic effects (Table 2). However, not all formulations were tested for interactions of inert ingredients with the active ingredient because some blank formulations were unavailable (RoundUp Ready-To-Use and RoundUp Ultra 2).

The results of our in vitro study indicate that the four entomopathogenic fungi tested (*B. bassiana*, *M. anisopliae*, *N. rileyi*, *N. floridana*) are susceptible to various glyphosate formulations when exposed to field concentrations. Determining which inert ingredients are interacting with glyphosate or causing the fungicidal activity to the entomopathogens should be evaluated. Even though formulations usually are classified information of most companies, cooperative studies between them and public researchers would be beneficial. In such studies, the companies could provide information about the formulation's inert ingredients to be tested for their fungicidal activity on entomopathogens. However, if information about the inert ingredients is not available, analytical studies should be conducted to establish what the inert ingredients are, so they could be tested both separately and in combination. Determining the fungicidal activity or interaction of individual inert ingredients could produce valuable information necessary to better understand the nontarget effects of herbicide formulations.

Application of herbicides with fungicidal properties detrimental to entomopathogenic fungi may have a direct impact on the natural epizootics necessary for adequate pest regulation in treated fields. Therefore, field responses of entomopathogenic fungi and other microorganisms important in agroecosystems to glyphosate formulations and other agents with fungicidal activity should be known and considered in integrated pest management (IPM) decision making.

Acknowledgments

We thank R. Gunnarson (USDA-ARS, Iowa), L. Tiffany (Iowa State University), and M. Owen (Iowa State University) for their assistance, and R. Humber (USDA-ARS Plant Protection Research Unit) for providing entomopathogenic cultures. This research was supported by Leopold Center for Sustainable Agriculture Grant No. 2000–41. This is a joint contribution: USDA, Agricultural Research Service and journal paper No. J-19576 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA, Project No. 3527, and supported by Hatch Act and State of Iowa Funds.

References Cited

Abdel-Mallek, A. Y., M.I.A. Abdel-Kader, and A.M.A. Shonkeir. 1994. Effect of glyphosate on fungal population, respiration and the decay of some organic matters in Egyptian soil. Microbiol. Res. 149: 69–73.

Abdulsalam, K. S., M. A. Rezk, M. I. Abdel-Megeed, and A. E. Mussa. 1990. Non target activity of certain pesticides against soil born fungi. Ann. Agric. Sci. 35: 459–467.

Black, B. D., J. S. Russin, J. L. Griffin, and J. P. Snow. 1996. Herbicide effects on *Rhizoctonia solani* in Vitro and Rhizoctonia Foliar Blight of soybean (*Glysine max*). Weed Sci. 44: 711–716.

Bower, K. N., L. P. Berkett, and J. F. Costante. 1995. Nontarget effect of a fungicide spray program on phytophagous and predacious mite populations in scab-resistant apple orchard. Environ. Entomol. 24: 423–430.

Brandenburg, R. L. and G. G. Kennedy. 1983. Interactive effects of selected pesticides on the twospotted spider mite and its fungal pathogen *Neozygites floridana.* Entomol. Exp. Appl. 34: 240–244.

Burgiel, Z., and M. Grabowski. 1996. The effect of selected herbicides on pathogenicity and growth of *Nectria galligena* Bres. Folia Horticult. 8: 13–19.

Campbell, W. V. 1978. Effect of pesticide interactions on the twospotted spider mite on peanuts. Peanut Sci. 5: 83–86.

Chakravarty, P., and S. S. Sidhu. 1987. Effect of glyphosate, hexazine and triclopyr on in vitro growth of five species of ectomycorrhyzal fungi. Eur. J. For. Pathol. 17: 204–210.

Chakravarty, P., and L. Chatarpaul. 1990a. Non-target effect of herbicides: I. Effects of glyphosate and hexazinone on soil microbial activity. Microbial population, and in-vitro growth of ectomycorrhizal fungi. Pestic. Sci. 28: 233–241.

Chakravarty, P. and L. Chatarpaul. 1990b. Non-target effect of herbicides: II. The influence of glyphosate on ectomycorrhizal symbiosis of red pine (*Pinus resinosa*) under greenhouse and field conditions. Pestic. Sci. 28: 243–247.

Cox, C. 1995. Glyphosate, part 2: human exposure and ecological effects. J. Pestic. Reform 15: 14–20.

Estok, D., B. Freedman, and D. Boyle. 1989. Effects of the herbicides 2,4-D, glyphosate, hexazinone, and triclopyr on the growth of three species of ectomycorrhyzal fungi. Bull. Environ. Contami. Toxicol. 42: 835–839.

Gardner, W. A., and G. K. Storey. 1985. Sensitivity of *Beauveria bassiana* to selected herbicides. J. Econ. Entomol. 78: 1275–1279.

Giles, K. L., J. J. Obrycki, T. A. Degooyer, and C. J. Orr. 1994. Seasonal Occurrence and impact of natural enemies of *Hypera postica* (Coleoptera: Curculionidae) larvae in Iowa. Environ. Entomol. 23: 167–176.

Grossbard, E., and D. Atkinson. 1985. Effects of glyphosate on the microflora: with reference to the decomposition of treated vegetation and interaction with some plant pathogens, pp. 159–185. *In* E. Grossbard and D. Atkinson (eds.), the herbicide glyphosate. Butterworth, Boston, MA.

Harris, D., and E. Grossbard. 1979. Effects of the herbicides gramoxone W and RoundUp on *Septoria nodorum.* Trans. Br. Mycol. Soc. 73: 27–33.

Higley, L. G. and D. J. Boethel. 1994. Handbook of soybean insect pests. Entomological Society of America, Lanham, MD.

Higley, L. G., and Wintersteen. 1989. Consequences of the 1988 spider mite outbreak, p. 40. *In* Crops, soils, and pests newsletter. Iowa Coop. Serv. Bull. IC 458(5).

Huffaker, C. B., M.V.D. Vrie, and J. A. McMurtry. 1969. The ecology of tetranychid mites and their natural control. Annu. Rev. Entomol. 14: 125–174.

Jaworski, E. G. 1972. Mode of action of N-phosphonomethylglycine: Inhibition of aromatic amino acid biosynthesis. J. Agr. Food Chem. 20: 1195–1198.

Johal, G. S., and E. Rahe. 1984. Effect of soilborne plant-pathogenic fungi on the herbicidal action of glyphosate on bean seedlings. Phytopathology 74: 950–955.

Kassaby, F. Y. 1985. Interaction of four herbicides with *Phytophthora cinnamomi.* Australasian Plant Pathology 14: 21–22.

Kassaby, F. Y., and G. Hepworth. 1987. Phytophthora cinnamomi: Effects of herbicides on radial growth, sporangial production, inoculum potential and root disease in *Pinus radiata.* Soil Biol. Biochem. 19: 437–441.

Kawate, M. K., S. C. Kawate, A. G. Ogg, Jr., and J. M. Kraft. 1992. Response of *Fusarium solani* f. sp. *pisi* and *Pythium ultimum* to Glyphosate. Weed Science 40: 497–502.

Klubertanz, T. H. 1989. Environmental and host plant effects on the twospotted spider mite in soybean. Ph.D. dissertation, Iowa State University, Ames, IA.

Klubertanz, T. H., L. P. Pedigo, and R. E. Carlson. 1991. Impact of fungal epizootics on the biology and management of the twospotted spider mite (Acari: Tetranychidae) in soybean. Environ. Entomol. 20: 731–735.

Levesque C. A., and J. E. Rahe. 1992. Herbicide interactions with fungal root pathogens, with special reference to glyphosate. Annu. Rev. Phytopathol. 30: 579–602.

Mann, R. M., and J. R. Bidwell. 1999. The toxicity of glyphosate and several glyphosate formulations to four species of southwestern Australian frogs. Arch. Environ. Contam. Toxicol. 36: 193–199.

Martensson, A. M. 1992. Effects of agrochemicals and heavy metals on fast-growing rhizobia and their symbiosis with small-seeded legumes. Soil Biol. Biochem. 24: 435–445.

Mekwatanakarn, P.. and K. Sivasithamparam. 1987. Effect of certain herbicides on saprophytic survival and biological suppression of the take-all fungus. New Phytol. 106: 153–159.

Mietkiewski, R., A. Sapiecha, and Z. Mietkiewska. 1990. Growth of the entomopathogenic fungi on the medium containing herbicide used in orcharding. Acta Mycolog. 25: 35–50.

Mietkiewski, R. T., J. K. Pell, and S. J. Clark. 1997. Influence of pesticide use on the natural occurrence of entomopathogenic fungi in arable soils in the UK: Field and laboratory comparisons. Biocontrol Sci. Tech. 7: 565–575.

Paula, Jr., T. J.. and L. Zambolim. 1994. Efeito de fungicidas e de herbicidas sobre a micorrizacao de *Eucalyptus grandis* por *Glumus etunicatum.* Fitopatol. Bras. 19: 173–177.

Pedigo, L. P. 2002. Entomology and pest management, 4th ed. Prentice Hall, Upper Saddle River, NJ.

Pickford R., and P. W. Riegert. 1963. The fungus disease caused by *Entomophthora grylli* Fres. And its effect on grasshopper populations in Saskatchewan in 1963. Can. Entomol. 96: 1158–1166.

Rice, M. 1997. Green cloverworms: a soybean surprise. Integrated Crop Manage. September 2.

SAS Institute. 1985. Sas user's guide: statistic, version 5 ed. SAS Institute, Cary, NC.

Sharma, U., E. A. Adee, and W. F. Pfender. 1989. Effect of glyphosate herbicide on pseudothecia formation by *Pyrenophora tritici-repentis* in infested wheat straw. Plant Dis. 73: 647–650.

Steinrücken, H. C., and N. Amrhein. 1980. The herbicide glyphosate is a potent inhibitor of 5-enolpyruvyl-shikimic acid-3-phosphate synthase. Biochem. Biophys. Res. Commun. 94: 1207–1212.

Toubia-Rahme, H., G. Barrault, L. Albertini, and B. Bendahmane. 1992. Etude in vitro de L'action de certains herbicides sur le developpement de *Drechslera teres* Sacc., parasite de L'orge. Phytopathol. Medit. 31: 88–95.

Utkhede, R. S. 1982. Effect of six herbicides on the growth of *Phytophthora cactorum* and a bacterial antagonist. Pestic. Sci. 13: 693–695.

Wardle, D. A., and D. Parkinson. 1992. The influence of the herbicide glyphosate on interspecific interactions between four soil fungal species. Mycol. Res. 96: 180–186.

Ware, G. W. 1994. The pesticide book, 4th ed. Thomson Publications, Fresno, CA.

Received for publication 4 October 2001; accepted 30 April 2002.