Use of Dicamba-Degrading Microorganisms To Protect Dicamba Susceptible Plant Species

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Three strains of dicamba-degrading microorganisms were able to reduce the herbicidal activity of dicamba in the rhizosphere quickly enough to allow dicamba susceptible crop species to grow. Pea seedlings planted immediately after inoculation had higher weights over the uninoculated controls at the 0.5 and 4.0 lb/acre rates in growth chamber studies. Peas seedlings planted 2 or 5 days after inoculation had higher mass over the uninoculated controls at all treatment rates. The concentration of dicamba in the soil was reduced dramatically at all treatment rates as compared to uninoculated controls. Dicamba-degrading bacteria also showed activity in field test plots, where soybeans were protected from dicamba damage even at the 8 lb/acre application rate.

INTRODUCTION

Dicamba (3,6-dichloro-2-methoxybenzoic acid) is used as a pre- and postemergent herbicide for the control of broad-leafed weeds and several grassy weeds infesting corn, small grains, sugarcane, and turf. Dicamba has the properties of an auxin-like growth regulator; however, the mechanism of action is still speculative (Ashton and Crafts, 1981). The symptoms of dicamba damage include abnormalities in flowering and in leaf and stem formation.

Dicamba is readily absorbed by leaves and roots and readily translocated via the symplast and apoplast systems (Augenstein and Thompson, 1972). Resistant species that absorb and translocate dicamba are able to metabolize it, while susceptible species cannot easily do so (Chang and Vanden Born, 1971). The dissipation of dicamba from treated plants can occur by exudation through the roots, by metabolism within the plant, and by loss from the leaf surface (*Herbicide Handbook*, 1989). The major plant metabolite of dicamba appears to be 5-hydroxydicamba (Broadhurst et al., 1966). Dicamba susceptible crop species include dicots such as peas and soybean.

The use of microbial inoculants such as *Rhizobium* to improve crop yields has been a common practice for some time (Brown, 1974). Additionally, rhizosphere-colonizing strains of *Pseudomonas fluorescens* and *Pseudomonas putida* have been used as inoculants to promote growth and increase yields (Moores et al., 1984; Schroth and Hancock, 1982). The commercialization of plant inoculums has received much attention over the past decade (Okon, 1985; Schank and Smith, 1984; Smith et al., 1984). However, there are no reports in the literature demonstrating the use of defined herbicide degrading microbes as an inoculum to protect susceptible plant species.

Several strains of bacteria have been isolated that can rapidly metabolize dicamba as a sole carbon source (Krueger et al., 1989). This study describes the use of previously isolated dicamba-degrading microbes to protect dicamba susceptible crops (peas and soybeans) in a growth chamber and in field test plots.

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MATERIALS AND METHODS

Chemicals and Microbial Medium. An authentic reference standard of dicamba (99% purity) was used for inoculum cultivation and for soil treatment. [^{14}C]Dicamba (U-*phenyl*-^{14}C, 11.5 mCi/mmol, radiochemical purity >98%) was synthesized by Pathfinder Labs. To increase solubility, the dicamba stock solution was prepared by titration with NaOH to pH 7.0. The dicamba stock solution used for cultivation of inoculums was filter sterilized through a 0.2-μm Teflon filter before being added to sterile media. Inoculums were cultured in reduced chlorine medium as described by Krueger et al. (1989). All other chemicals were of reagent grade or better, and all solvents were of glass-distilled quality.

Organisms and Inoculum Preparation. Three strains of dicamba-degrading organisms isolated and described by Krueger et al. (1989) were used for all experiments. The three strains were as follows: strain DI-6, *Pseudomonas* sp.; strain DI-7, *Moraxella* sp.; and strain DI-8, *Pseudomonas* sp.

Inoculums for the growth chamber study were prepared by growing each dicamba-degrading strain for 2 days at 30 °C in 1 L of reduced chlorine medium containing 1000 μg/mL dicamba. Cells were harvested by centrifugation at 4500*g* for 10 min. Pellets were washed in sterile medium, centrifuged, and resuspended in 60 mL of sterile medium. The number of viable cells present in each concentrated inoculum was determined by plate count on nutrient agar.

Inoculums for the field study were prepared by growing each dicamba-degrading strain for 2 days at 30 °C in 15 L of reduced chlorine medium containing 1000 μg/mL dicamba. Cells were harvested by centrifugation at 4500*g* for 10 min. Pellets were washed in sterile medium, centrifuged, and resuspended in 100 mL of sterile medium. Each cell concentrate was frozen quickly in a thin layer in an acetone–dry ice bath. Frozen cells were stored at −70 °C until use. Inoculation solutions were prepared in the field by thawing cells at room temperature and diluting to a volume of 200 mL with sterile medium. The viability of frozen dicamba-degrading strains has been described by Krueger et al. (1989).

Growth Chamber Study. Wisconsin clay loam soil was sieved through 2 mm diameter openings and moistened to field level (19.1% moisture). The percentage of sand, silt, clay, organic carbon, and organic matter and pH and moisture capacity of the Wisconsin clay loam soil were determined according to the methods of Weber (1977).

Field moist soil (150 g) was weighed into 4-oz cups (6.5-cm diameter) and incubated at 15 °C for 24 h. [^{14}C]Dicamba (in deionized water) was pipetted onto and mixed with the soil to yield the following application rates: 0.5 lb/acre (1.2 μg of dicamba/g); 4.0 lb/acre (9.58 μg of dicamba/g); 8.0 lb/acre (19.18

0021-8561/91/1439-1000$02.50/0

Table I. Weights of Pea Seedlings Planted in Dicamba-Treated Soil Inoculated with Dicamba-Degrading Organisms under Controlled Growth Chamber Conditions

concn of dicamba in the soil, lb/acre	strain no.	mass of pea seedlings, g (av of four plants)								
		planted immediately after inoculation			planted 2 days after inoculation			planted 5 days after inoculation		
		stems	roots	total	stems	roots	total	stems	roots	total
0.0	DI-6	2.22	3.18	5.40	1.54	2.55	4.09	2.07	2.71	4.78
	DI-7	2.22	2.69	4.91	1.73	2.39	4.12	1.28	1.66	2.94
	DI-8	2.78	2.59	5.37	2.69	3.02	5.71	1.37	2.08	3.45
	uninoculated	1.84	2.36	4.20	1.60	2.16	3.76	2.06	2.71	4.77
0.5	DI-6	1.74	1.81	3.55	1.35	1.70	3.05	2.44	3.18	5.62
	DI-7	2.35	2.45	4.80	2.08	2.70	4.78	2.36	2.36	4.72
	DI-8	2.18	2.56	4.74	1.92	2.24	4.16	1.38	1.79	3.17
	uninoculated	0.00	1.08	1.08	0.11	1.20	1.31	0.83	2.03	2.86
4.0	DI-6	0.00	1.17	1.17	1.15	2.70	3.85	1.38	2.51	3.89
	DI-7	0.00	1.17	1.17	1.76	2.46	4.22	1.59	1.99	3.58
	DI-8	0.43	1.98	2.41	1.98	2.51	4.49	0.00	1.90	1.90
	uninoculated	0.00	1.18	1.18	0.00	1.61	1.61	0.00	1.34	1.34
8.0	DI-6	0.00	1.07	1.07	0.99	2.22	3.21	1.04	1.95	2.99
	DI-7	0.00	0.64	0.64	1.67	1.48	3.15	1.80	2.08	3.88
	DI-8	0.00	0.82	0.82	1.61	2.11	3.72	2.02	2.62	4.64
	uninoculated	0.00	0.62	0.62	0.00	0.85	0.85	0.00	1.13	1.13

μg of dicamba/g). Water was used for the 0.0 lb/acre control. All treated cups were weighed and randomly distributed in a growth chamber at 15 °C. Lights were set to be on for 12 h and off for 12 h. One cup from each treatment rate was removed and frozen as a 0 time sample. Cups were incubated for 2 weeks at 15 °C to simulate a preemergence dicamba application. All moistures were maintained by the periodic addition of water to maintain 0 time weights.

After 2 weeks, two cups from each treatment rate were removed and frozen. The remaining cups received 2.0 mL of concentrated inoculum or 2.0 mL of water (control). Four pea seeds (Ferry Morse, Alaska Pea), which were soaked for 2 h in deionized water, were planted in each of two cups of each treatment group.

At 1, 2, and 5 days after inoculation, one cup from each treatment group was removed and frozen. These samples were used to determine the soil dissipation of dicamba. Four pea seeds were also planted in one container of each treatment group at 2 and 5 days after inoculation.

At 21 days after inoculation, stems and roots were separated from the soil. Roots were washed to remove soil, and excess water was removed by drying with tissue. Stems were separated from roots, and the fresh weights of both were determined.

Dicamba remaining in soil samples was extracted into ether according to Sandoz Crop Protection GC Residue Method AM-0766. Recovery (spiked soil samples) and check samples (untreated soil) were also analyzed. To determine unextractable residues, duplicate 0.5-g samples of soil were mixed with cellulose powder and combusted to CO_2 by using a Packard 306 sample oxidizer. Standards consisting of 0.5 g of Wisconsin clay loam mixed with cellulose powder and a known amount of [^{14}C]dicamba were combusted to determine recovery.

Ether extracts were evaporated under N_2 to near dryness and taken up in water. Samples were analyzed by using a Waters high-pressure liquid chromatographic system with a 300 × 3.9 mm C_{18} 10-μm column. The detector wavelength was set at 235 nm and 1 AUF. A mobile phase of 60% methanol, 39% water, and 1% acetic acid at a flow rate of 1 mL/min was used for 10 min, followed by a mobile phase of 100% methanol at a flow rate of 1.5 mL/min for 5 min. Duplicate 30-μL aliquots from each extract were analyzed. Dicamba standards were prepared in water and used for quantitation.

Field Study. The study was conducted at a typical midwestern site located in northern Illinois. Soil samples were removed, and the percentage of sand, silt, clay, organic carbon, and organic matter and pH and moisture capacity of the soil were determined according to the methods of Weber (1977). The study was started early in May so that conditions under which dicamba persistence is sometimes a problem could be simulated.

The fenced site was divided into 48 8-ft^2 plots (2 × 4 ft each). Each plot was separated from other plots by a 3-ft border of land on each side. Each plot represented one replicate. Air and soil temperatures and rainfall were monitored daily throughout the experiment.

Each of the following rates of dicamba was sprayed onto the entire surface of 12 plots: 0, 0.5, 2.0, and 8.0 lb/acre. Plots were randomly distributed throughout the field test site. All test plots were allowed to incubate for 2 weeks to simulate a preemergent treatment.

At 2 weeks after initial dicamba treatment, three plots from each treatment rate were sprayed with water or one of the inoculation solutions from one of the three dicamba-degrading strains (prepared as described). Organisms were incorporated into the soil by tilling the plots to a 5-in. depth. One row of soybeans (dicamba susceptible species) was planted in each plot at 0, 7, and 32 days after inoculation.

Stand counts of soybeans were made at 12, 16, 18, and 24 days after 0-day plantings; 6, 10, 12, and 18 days after 7-day plantings; and 8 days after the 32-day planting. At 30 days after planting, alternate plants from soybeans planted 7 days after inoculation were harvested. The number of plants, their weights, and lengths were recorded. The remaining plants were grown to maturity (late September). Plants were harvested, and the number of plants, their weights, and lengths were recorded.

RESULTS AND DISCUSSION

Protection of Pea Seedlings from Dicamba Injury in a Growth Chamber. All three dicamba-degrading strains were able to decrease the herbicidal activity of dicamba at all concentrations to a level at which pea seedlings could survive. Inoculation of dicamba-treated soil with dicamba-degrading organisms resulted in increased pea seedling weight for all treatment groups (Table I). Pea seedlings planted immediately after inoculation had higher weights over the uninoculated controls at the 0.5 and 4.0 lb/acre rates. Additionally, pea seedlings planted 2 or 5 days after inoculation had higher weights over the uninoculated controls at all rates (Table I). No differences between strains of inoculums were evident.

HPLC analysis of soil confirms that dicamba is rapidly removed in inoculated soil at all concentrations (Table II). The concentration of dicamba in soil was reduced dramatically at all treatment rates as compared to uninoculated controls. The rate of dicamba removal in inoculated soils was also much higher than in uninoculated controls (Table II). The half-life of dicamba in other aerobic soil studies in which no inoculation was used has been reported to vary from 17 to 45 days depending on the soil tested (Altom and Stritzke, 1973; Smith, 1973, 1974, 1984).

Table II. Concentration of Dicamba in Soil Inoculated with Dicamba-Degrading Organisms in a Growth Chamber

strain no.	concn of dicamba in soil, μg/g: 0 time	at inoculation	2 days after inoculation
uninoculated	1.20	0.87	0.65
DI-6	1.20	0.87	ND[a]
DI-7	1.20	0.87	ND[a]
DI-8	1.20	0.87	ND[a]
uninoculated	9.58	5.80	3.30
DI-6	9.58	5.80	ND[a]
DI-7	9.58	5.80	ND[a]
DI-8	9.58	5.80	ND[a]
uninoculated	19.18	12.00	6.20
DI-6	19.18	12.00	ND[a]
DI-7	19.18	12.00	ND[a]
DI-8	19.18	12.00	ND[a]

[a] Limit of detection 0.1 μg/g dicamba.

Table III. Properties of Wisconsin Clay Loam Soil

% organic C	4.6	% sand	67.0
% organic matter (calcd from % organic C)	8.0	% silt	0.0
pH (deionized water)	6.8	% clay	33.0
pH (0.01 M $CaCl_2$)	6.5	textural class	clay loam
75% of 0.33-bar level (g of H_2O/100 g of dry soil)	20.7		

Dicamba-degrading organisms were able to survive and have activity in the presence of naturally occurring microfauna in a soil and temperature for which dicamba persistence is sometimes a problem. Soil characteristics of Wisconsin clay loam are presented in Table III. Initial inoculum sizes (in cells/gram of field moist soil) for each strain were as follows: DI-6, 1.28×10^7; DI-7, 1.03×10^8; DI-8, 1.22×10^8. Smaller inoculum sizes may be equally efficacious and would result in reduced inoculum costs.

Dicamba-degrading organisms may be useful for the rapid removal of dicamba from soil and the protection of dicamba susceptible crops. To protect a dicamba susceptible crop species, organisms must degrade dicamba to less than phytotoxic levels by the time the seed germinates. Any alteration in the structure of dicamba will decrease its herbicidal activity; therefore, extensive metabolism is not necessary. However, other authors indicate that dicamba-degrading organism rapidly and completely mineralized dicamba to CO_2 in soil (Krueger et al., 1989; Krueger, 1989).

This study simulates a treatment strategy in which an unseeded field is treated with dicamba. Dicamba susceptible weeds are killed, the field is inoculated with dicamba-degrading organisms, and the crop is planted. Other treatment strategies utilizing dicamba-degrading organisms, such as seed coating, may be possible.

Protection of Soybeans from Dicamba Injury in Field Plots. Dicamba-degrading organisms were able to protect soybeans (a dicamba susceptible species) planted in dicamba-treated soil from dicamba injury in field test plots. Differences between inoculated and uninoculated plots were most dramatic at the 8.0 lb/acre rate in which the uninoculated plots did not show any germination or subsequent stand of soybeans but inoculated plots did (Table IV). Soybean plants that grew in inoculated plots showed no evidence of dicamba damage. Inoculated and uninoculated plots at the lower rates, including the 0.00 lb/acre control, did not differ. Weights and stem heights of plants from all the lower treatment rates were similar to those in the plots treated with 8.0 lb/acre dicamba and planted 0 days after inoculation with DI-6 (Table IV).

Table IV. Heights and Weights of Mature Soybeans Planted in Soil Treated with 8 lb/Acre Dicamba and Inoculated with Dicamba-Degrading Organisms in Field Test Plots

planting time, days after inoculation	strain no.	av[a] no. of stems	total mass, lb	av stem height, in.
0	uninoculated	0	0	0
	DI-6	3	2.1	32.58
	DI-7	0	0	0
	DI-8	0	0	0
7	uninoculated	0	0	0
	DI-6	5	3.4	27.70
	DI-7	2	0.1	3.22
	DI-8	0	0	0
32	uninoculated	0	0	0
	DI-6	8	1.6	16.38
	DI-7	3	0.1	5.27
	DI-8	2	0.1	5.50

[a] Average of three plots.

Table V. Rainfall and Temperature during the Field Study

month	av air temp, °F	normal air temp, °F	av soil temp, °F	av rainfall, in.	normal rainfall, in.
May	63	60	62	5.19	3.52
June	72	70	73	4.18	4.55
July	76	73	75	5.91	4.62
Aug	68	72	73	14.32	3.69
Sept	62	64	64	2.71	3.54

Table VI. Properties of Kanesville Loam Soil

% organic C	2.6	% sand	24.0
% organic matter (calcd from % organic C)	4.4	% silt	50.0
pH (deionized water)	6.6	% clay	26.0
pH (0.01 M $CaCl_2$)	6.5	textural class	loam
75% of 0.33-bar level (g of H_2O/100 g of dry soil)	24.0		

Higher than average rainfall (Table V) after dicamba treatment may have leached most of the dicamba out of the root zone, resulting in a treatment effect only at the highest rate. Dicamba-degrading strain DI-6 had the best activity in field test plots (Table IV).

Dicamba degradative activity in the field represents a significant step toward the practical application of dicamba-degrading organisms. Degradation occurred in a typical midwestern agricultural soil (Kanesville loam) exposed to natural air and soil temperatures in the presence of naturally occurring soil microfauna. Rainfall amounts and temperatures during the field study are shown in Table V, and the properties of the Kanesville loam soil are presented in Table VI. Results of this field study indicate that dicamba-degrading organisms have potential for removal of dicamba from soil and for use as a crop inoculum. Further optimization of inoculum growth conditions and of the required inoculum size would make the use of dicamba-degrading organisms more feasible. In addition, the use of dicamba-degrading organisms as a seed coating may represent a more practical application method.

CONCLUSIONS

All three dicamba-degrading strains were able to protect dicamba susceptible crop species at the application rates tested. A typical dicamba application rate is 0.5 lb/acre; therefore, results indicating protective activity at the 8.0 lb/acre rate represent a 16-fold safety margin. Typically, peas and soybeans are very susceptible to dicamba damage

and will show symptoms of damage even at very low concentrations of dicamba.

A problem with many microbial inoculants is their inability to survive and colonize the plant root zone. Soil usually acts as a biological buffer, and hence any change in the microbial population is only temporary (Alexander, 1977). Inoculated microbes must compete with natural microbe populations for available nutrients. Dicamba-degrading organisms have their own carbon source available to them. Therefore, competition with natural microbes for an available carbon source is not a concern. Furthermore, the dicamba-degrading organisms need only be present to degrade the dicamba, so a long survival rate in the soil is not necessary. Results demonstrate that the dicamba-degrading strains quickly degrade the dicamba in the soil (Table II).

Inoculation techniques must be practical to the farmer and simple to apply. Previous studies indicate that dicamba-degrading strains survive desiccation (Krueger et al., 1989). Therefore, application of an inoculum as a seed coating may be a practical application method. Additionally, all three dicamba-degrading strains can be grown quickly in liquid culture to provide a readily available source of inoculum.

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Received for review July 27, 1990. Revised manuscript received November 28, 1990. Accepted December 19, 1990.