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Foliar Dicamba Application Has No Lasting Effects on Microbial Activities in the Soybean Rhizosphere

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Abstract

The proliferation of glyphosate-resistant weeds has resulted in significant losses in the productivity of crops such as corn, soybean, and cotton. As a result, new crop varieties with resistance genes from other herbicides, such as 2,4-D and dicamba, have been developed as part of alternative weed control cropping systems. However, little is known about how the application of these herbicides impacts the microorganisms that carry out nutrient cycling in the soil of these cropping systems, particularly in the rhizosphere, the soil compartment immediately adjacent to the root system which is pivotal to plant nutrient uptake. The purpose of the current study was to assess the effects of dicamba on soil enzyme activities linked to C, N, and P cycling in the rhizosphere of resistant soybean plants. While dicamba had no significant effects on the activities of enzymes linked to C or P cycling in the rhizosphere, N-acetylglucosaminidase activity was temporarily inhibited, but recovered by three days after application. These results suggest there are no long-lasting negative effects of dicamba in the rhizosphere of treated plants when applied at field rates.

Keywords

Dicamba, Rhizosphere, Soybean, Soil, Microbial Activities

1. Introduction

The herbicide, glyphosate, has been heavily replied upon by farmers for the control of weeds since the introduction of glyphosate-resistant crops (such as soybean, corn, and cotton) in the 1990s, and the overreliance on it for weed control has resulted in the development and spread of weeds resistant to this herbicide

[1]. Uncontrolled proliferation of weeds in the field can cause significant losses in crop yield, leading to billions of dollars in lost revenue to farmers [2]. As such, much effort has gone into the development of additional weed control options, including the creation of new crop varieties stacked with genes conferring resistance to multiple herbicides, thereby providing farmers with more herbicide options to combat resistant weeds during the growing season.

Some of these new cropping systems include those engineered with resistance genes to auxin herbicides (such as 2,4-dichlorophenoxyacetic acid (2,4-D) or 3,6-dichloro-2-methoxybenzoic acid (dicamba)) in addition to glyphosate. In dicamba resistant systems, crops have been engineered to express the dicamba monooxygenase (dmo) gene from *Pseudomonas maltophilia* (strain DI-6), which encodes an enzyme that catalyzes the conversion of dicamba to 3-6-dichlorosalicylic acid [3]. Dicamba has a long history of use for early season weed control in corn, but its use later in the growing season has been limited before the introduction of dicamba resistant soybean and cotton, owing to its tendency for drift and volatilization, which can damage sensitive crops in neighboring fields [4]. As such, the introduction of dicamba resistant crops has been accompanied by new formulations designed to minimize drift, including XTendiMax® (which contains a diglycolamine (DGA) salt of dicamba) and Engenia (containing an N,N-Bis-(aminopropyl) methylamine salt of dicamba) [4].

Exposure of resistant crops to herbicides may still induce changes in root exudation. In the case of glyphosate, this herbicide is released through root exudates from treated plants [5] and induces increased carbohydrate and amino acid concentrations in treated plants [6]. Such changes in root exudation can impact soil microorganisms, altering substrate utilization patterns in the rhizosphere and potentially altering nutrient availability [7]. The presence of herbicides in root exudates could potentially have negative impacts on nutrient cycling potential in the rhizosphere, as some herbicides have been found to inhibit activities linked to P and C cycling, such as glyphosate and 2,4-D on phosphatase [8] [9] and 2,4-D on beta-glucosidase [9].

Less is known about how dicamba influences nutrient cycling potential, as most studies on dicamba in soil have focused on microbial degradation of this herbicide [10], with little to no research on its effects in the rhizosphere. However, while dicamba can be utilized as a carbon source by some bacteria [11], it is known to have toxic and inhibitory effects on others [12], and has been observed to inhibit phosphatase activity [13] and nitrification [14] [15] in soils. While these effects tended to be transient in nature and more pronounced at high application rates, herbicide exposure combined with changes in nutrient composition in root exudates may interact to produce differing effects.

Given that many of the previously mentioned studies were carried out using bulk soils or with levels of herbicide above the rate applied in the field, it is important to investigate how dicamba may affect the activity of soil microbial communities under agriculturally relevant conditions. Since such herbicides are

typically applied from above as foliar sprays, the bulk of the herbicide applied will end up on the leaves or the soil surface, with decreasing concentrations in greater depths of bulk soil. As such, herbicide effects in soil may be more prevalent in the rhizosphere, the soil compartment most likely to be impacted by herbicide induced changes in root exudation. As roots are the site for nutrient uptake by the plant, shifts in nutrient cycling activities in the rhizosphere could potentially impact plant nutrition. The effects of new 2,4-D formulations on microbial activities in the soybean rhizosphere have already been evaluated [16]. Dicamba resistant soybeans were unavailable at the time that study was conducted, but they have since become available. Therefore, the purpose of the current study was to conduct a rapid evaluation of the potential impacts of two new dicamba formulations on the rhizosphere of resistant soybean (*Glycine max* L. Merr.) at label rates.

2. Materials and Methods

2.1. Soybean Growth and Greenhouse Conditions

Dicamba resistant soybean seeds (Progeny 4816) were germinated using a ragdoll method to ensure even germination and developmental stage. Briefly, sheets of pre-wetted Anchor germination paper were placed over a sheet of aluminum foil. Approximately 50 soybean seeds were spread across each piece of germination paper, and the layers of foil, paper, and seeds were rolled together and secured with rubber bands. Rolls with seeds were placed in a 1 L beaker with water in the bottom, covered in aluminum foil, and allowed to incubate at room temperature in the dark for 2 days. Seeds that had germinated were transferred to round pots (15.2 cm diameter and depth) containing a Boskett sandy loam soil collected from a field site in Stoneville, MS at the rate of 4 germinated seeds per pot and covered with soil to a depth of 2.5 cm. Pots were placed in rectangular trays in the greenhouse and watered from below every two to three days as needed. Greenhouse conditions were maintained at 28˚C in the day and 22˚C at night with a 13-hr photoperiod of natural light supplemented with high pressure sodium lights providing 400 $mmol \cdot m^{-2} \cdot s^{-1}$ light intensity. A total of 65 pots were set up, allowing for 20 pots per treatment, with five extra pots. With the extra pots, any plants displaying delayed development could be discarded while still leaving enough so that all plants included the experiment were at the same developmental stage.

2.2. Herbicide Application

Soybean plants were treated 20 days after planting when they were in the second trifoliate developmental stage. Treatments included dicamba formulation 1 (N,N-Bis(3-aminopropyl) methylamine (BAPMA) salt of 3,6-dichloro-o-anisic acid (dicamba)), Engenia®, BASF, Research Triangle Park, NC), dicamba formulation 2 (diglycolamine (DGA) salt of dicamba, XtendiMax®, Monsanto, St. Louis, MO), and no herbicide control. Dicamba formulations were applied at the

maximum label rate for a single application of 0.56 kg acid equivalent (ae) per hectare. A total of 20 pots were sprayed per treatment, allowing of four replicate pots per timepoint within each treatment.

2.3. Rhizosphere Collection

Rhizosphere soil was collected at 1, 3, 7, 14, and 30 days post application (DPA) of herbicide treatment. For each time point, the soil-root mass from four pots for each treatment was removed, gently crumbled to break up bulk soil, and then shaken to remove all but the soil tightly adhered to roots. Approximately 5 g of root+rhizosphere were transferred to a 50 mL centrifuge tube. 50 mM acetate buffer (pH 5) was added to each tube until the root sample was submerged. The exact volume varied according to the size of the root mass, which varied by plant age, but was approximately 20 mL at the 1 DPA sampling, and 30 mL by 30 DPA due to the larger root systems compared to younger plants. Rhizosphere soil was dislodged from roots by vortexing each tube for 1 min followed by 1 min in a sonicating water bath. Roots were removed from the tubes using sterile forceps. The resulting rhizosphere soil slurry mixture was used for enzyme assays.

2.4. Enzyme Assays

Rhizosphere slurries were assayed for the activities of β-glucosidase, cellobiohydrolase, N-acetylglucosaminidase (NAGase), and phosphatase in 96-well plate format using p-nitrophenol (pNP)-linked assays as described in [17], with some modifications described in [16]. Briefly, for each enzyme and sample, 150 µL of rhizosphere slurry was mixed with 150 µL of a substrate in four wells on a 96-well deep well block, plus two non-substrate control wells containing rhizosphere slurry and blank buffer. Deep well blocks were incubated at 25˚C with shaking, and centrifuged. Supernatant from each well (150 µL) was transferred to clear 96-well plates, mixed with 150 µL of 0.067 M NaOH stop reagent, and absorbance at 410 nm was recorded. Reagents were prepared as previously described [17]. Incubation times, slurry dry weight determination, and calculation of the amount of substrate consumed per reaction were performed as described in [16]. Enzyme activities were calculated as the µmole of substrate consumed per g dry weight per hour.

2.5. Statistics

Two-way analysis of variance (ANOVA) was used to compare the effects of herbicide treatment across time point. Differences between rhizosphere activities from each herbicide treatment compared to no herbicide controls for each time point were determined by pair-wise T-tests. All analyses were performed in JMP version 11.2.0 (SAS Institute Inc., Cary, NC).

3. Results and Discussion

The role of root exudates in regulating a plant's interaction with the soil micro-

bial community is complex [18]. As such, external factors with the potential to influence their chemical composition, such as herbicide application, have the potential to alter the activities of soil microbes that play a pivotal role in nutrient cycling and availability to the plant. The current study found the activities of microbially secreted exoenzymes in the rhizosphere more affected by sampling time point than herbicide treatment with dicamba according to 2-way ANOVA ($p < 0.006$). Meanwhile, the effects of dicamba application at each individual timepoint varied by the enzyme.

In regard to enzyme activities associated with organic matter turn over, neither β-glucosidase (**Figure 1(A)**) nor cellobiohydrolase (**Figure 1(B)**) were significantly altered by dicamba formulation compared to no herbicide control at any time point. Phosphatase activities from the dicamba treatments appeared to be depressed relative to controls at 7 days post application (**Figure 2(B)**). Owing to the higher variability in rhizosphere activities, this difference in phosphatase was not statistically significant, and no other timepoints displayed differential levels of phosphatase activities between treatments. However, this observation is still notable as it is consistent with those of [13], who reported dicamba had an inhibitory effect on phosphatase in bulk soil. While they looked at a substantially lower application rate compared to the current study (0.15 $kg{\cdot}ha^{-1}$ versus 0.5 $kg{\cdot}ha^{-1}$), it is likely that only a small amount of dicamba would have made it into the rhizosphere from foliar application. In addition, [13] reported biochar application attenuated the inhibitory effect of dicamba on phosphatase activity. As such, it is possible nutrient inputs from root exudation also lessened any dicamba inhibition on this enzyme in a similar way.

In contrast to the other enzymes assayed, NAGase activity was inhibited in

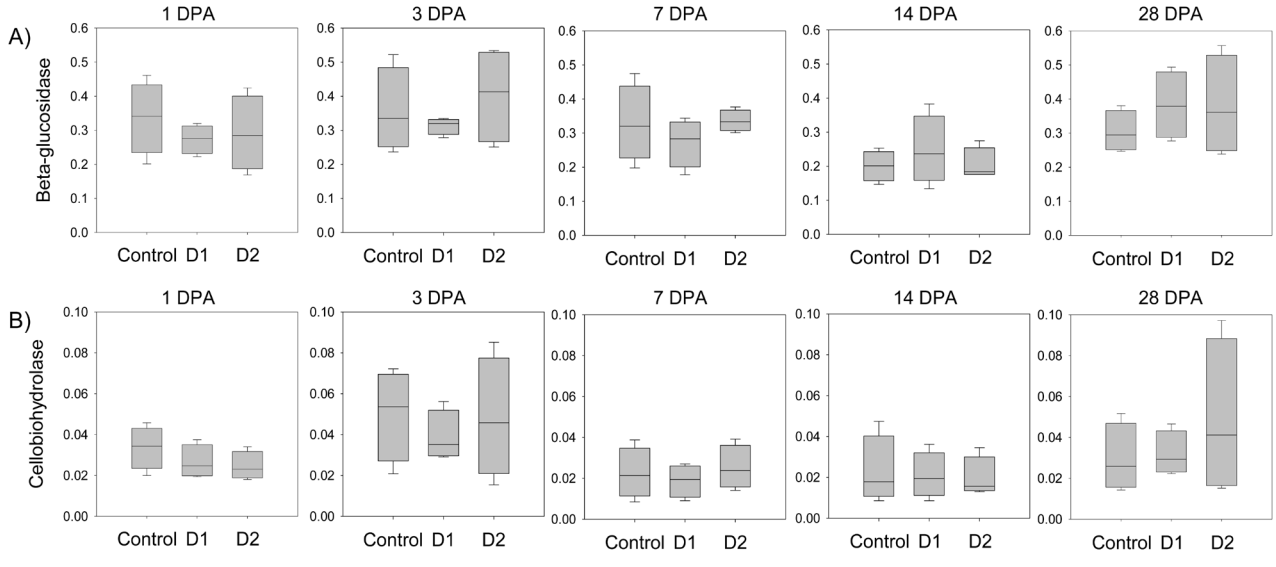

Figure 1. Box plots of beta-glucosidase (A) and cellobiohydrolase (B) activities in the rhizospheres of resistant soybean plants at 1, 3, 7, 14, and 28 days post application (DPA)of dicamba formulation 1 (D1), formulation 2 (D2), or no herbicide treatment (Control). Value from each treatment and timepointrepresents four replicates, each composed of the pooled rhizosphere soil from four plants, for a total of 16 soybean rhizospheres. Data on the y-axis is reported in units ofμmole of substrate consumed pergram dry weight of soil per hour.

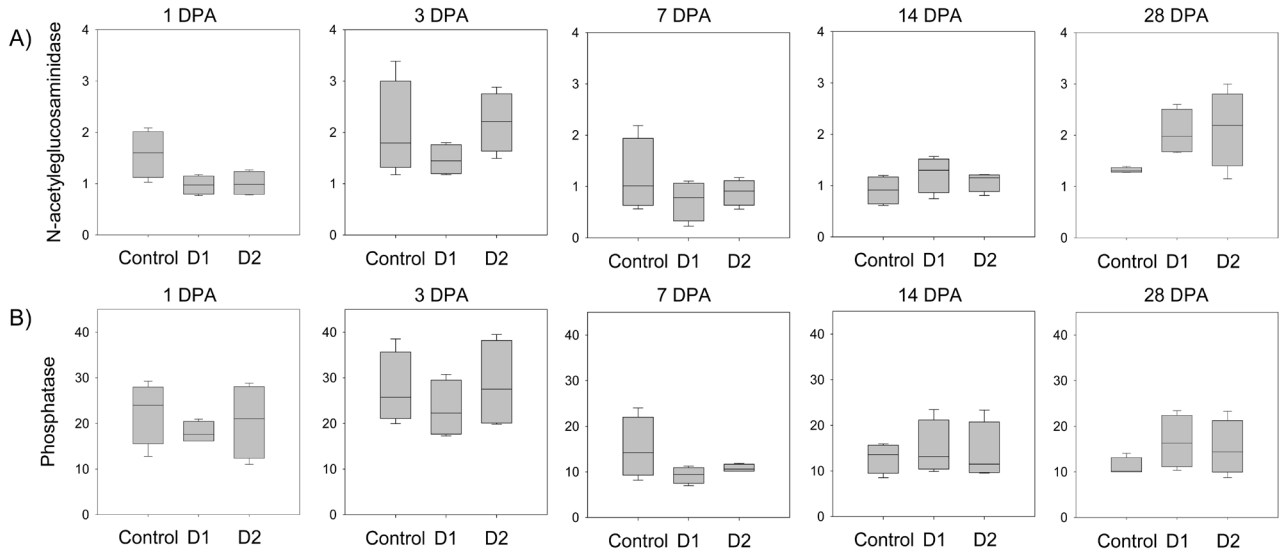

Figure 2. Box plots of N-acetylglucosaminidase (A) and phosphatase (B) activitiesin the rhizospheres of resistant soybean plants at 1, 3, 7, 14, and 28 days post application (DPA)of dicamba formulation 1 (D1), formulation 2 (D2), or no herbicide treatment (Control). Value from each treatment and timepointrepresents four replicates, each composed of the pooled rhizosphere soil from four plants, for a total of 16 soybean rhizospheres. Data on the y-axis is reported in units ofμmole of substrate consumed pergram dry weight of soil per hour.

response to the application of both dicamba formulation 1 ($p = 0.0254$) and formulation 2 ($p = 0.0305$) on the first day after application, but by the third, these activities were indistinguishable from the no herbicide control (**Figure 2(A)**). As NAGase activity is correlated with N mineralization [19], its inhibition in response to dicamba application observed here in the rhizosphere is consistent with observations made by [14] and [15], who both found dicamba inhibited nitrification in bulk soil. However, the effects on bulk soil were longer lasting than those observed in the rhizosphere during the current study, with inhibition detected 1 - 2 weeks after application by [14] and 2 - 3 weeks after application by [15]. In comparison, the initial inhibition of NAGase within only the first day after dicamba application is unlikely to have any detrimental effects on nitrogen dynamics in the soil. Additionally, as a legume, soybeans receive most of their nitrogen from symbiotic *Bradyrhizobium* in root nodules, and such minor and transient shifts in N-cycling potential in the rhizosphere are unlikely to have any negative effects on crop growth and nutrition.

Taken together, it can be concluded that the application of dicamba has no long-term detrimental effects on the extracellular activities of soil microbes in the rhizosphere of resistant soybean plants. Coapplication with other herbicides could have an additive effect, although prior work on glyphosate and 2,4-D has found differences between treatments containing one-versus two-herbicides to be inconsistent across experiments and not statistically significant [16]. While these specific dicamba formulations will be discontinued after the 2020 field season, these results may prove to be useful if new dicamba formulations are introduced or reapproved for midseason use on resistant crops at a later date, since

the problems of glyphosate-resistant weeds are still an ongoing issue requiring alternative herbicide options for control. In addition, these results provide insight into the concept of herbicide applications indirectly altering the rhizosphere activities of the plants they are applied to. The lack of detrimental effects seen in response to dicamba is similar to prior observations of soybean rhizospheres in response to 2,4-D application [16], suggesting that herbicide application, in general, may be unlikely to elicit long-lasting impacts on extracellular enzyme activities in the soybean rhizosphere.

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Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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