THESIS

INFLUENCE OF SOIL PROPERTIES AND SOIL MOISTURE ON THE EFFICACY OF INDAZIFLAM AND FLUMIOXAZIN ON KOCHIA SCOPARIA

Submitted by

Derek James Sebastian

Department of Bioagricultural Sciences and Pest Management

In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Spring 2015

Master's Committee:

Advisor: Phil Westra
Co-Advisor: Scott Nissen

Dale Shaner
Ken Barbarick

ABSTRACT

INFLUENCE OF SOIL PROPERTIES AND SOIL MOISTURE ON THE EFFICACY OF INDAZIFLAM AND FLUMIOXAZIN ON KOCHIA SCOPARIA

Indaziflam and flumioxazin are two broad spectrum preemergence herbicides both labeled for control of kochia (*Kochia scoparia* L.). There is currently limited understanding of the significant effect of soil properties and soil moisture on the efficacy of these herbicides. Soil water retention curves were generated for soils with a wide range of soil physicochemical properties. The direct effect of soil moisture was then evaluated in a greenhouse bioassay. The dose required for 80 percent growth reduction (GR_{80}) for both herbicides showed correlations with percent organic matter and cation exchange capacity. Results from the linear regression analysis show the single best parameter explaining the highest proportion of variability in the GR_{80} rates was soil organic matter (R^2 = 0.792 and 0.721) and CEC (R^2 = 0.599 and 0.354). There were two significant multiple regression models for indaziflam (R^2 = 0.914 and 0.901) and one for flumioxazin (R^2 = 0.814). As soil matric potential increased there was a significant effect of soil moisture on kochia percent dry weight reduction. Indaziflam and flumioxazin phytotoxicity was shown to be greatly reduced at -2 and -4 bars, and previous research has shown that kochia can germinate at moisture potentials greater than six times these values. The driving factors that were found to be correlated with this moisture effect was percent organic matter, CEC, percent sand, and percent clay. In these studies, kochia was found to germinate at moisture potentials below the moisture required for herbicide activation, and is likely why this weed is difficult to control with preemergence herbicides. There is a complex interaction between soil properties and soil moisture that influences kochia herbicide efficacy.

ACKNOWLEDGEMENTS

I would like to first thank my advisor Dr. Phil Westra for recruiting me to be a member of his weed research team at Colorado State University. Dr. Phil Westra and my co-advisor Dr. Scott Nissen have built a very successful program over their careers, and I am grateful to have been a part of it these past two years. For the countless hours of guidance, support, and assistance I cannot thank them enough. A special thanks to my other committee members Dr. Dale Shaner and Dr. Ken Barbarick; and Dr. Greg Butters who graciously assisted in this work. It was a pleasure collaborating with them all.

The time I was able to work alongside Galen Brunk I will forever cherish. I will never forget the times that he taught me fundamental lab techniques that I will surely use well into the future. Galen was an influential role model to all of the students at CSU and he is greatly missed.

I would also like to thank Bayer CropScience for funding these projects including David Spak, Chris Olsen, and Suresh Mislanker. I appreciate their guidance and freedom they gave us on these projects, ultimately allowing me to answer real world questions using many different techniques.

It has been great having the supportive team to collaborate with in the weed science program at CSU, including my fellow graduate students (Craig Beil, Tom Getts, Darci Giacomini, Curtis Hildebrandt, Kallie Kessler, Anita Kuepper, Eric Patterson, Dean Pettinga, Christopher Van Horn, and Eric Westra). I thank them for always being there to answer questions, helping with projects, and for the many memories outside of work.

I cannot thank my family enough for always being a constant support no matter what paths I have chosen in life. It has been a great opportunity these past two years to work alongside my dad Jim Sebastian, a great scientist that I am lucky enough to have been able to learn from. A final thank you to my beautiful wife Dana Sebastian and two sons, Miles and Pierce. This has been a challenging point in our lives, and I can say that I wouldn't be where I am without the constant support they have provided.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Introduced to the United States from Europe as an ornamental, kochia (*Kochia scoparia* L.), has escaped cultivation and spread across cultivated fields, along roadsides, and in waste areas throughout North America[1]. Resistance has been documented in three countries, against four different sites of action[1, 2]. Kochia germinates very early in the spring and is tolerant to heat, cold, drought, pH, and salt[1, 3-5]. A single plant can produce up to 15,000 seeds[6]. Kochia can germinate at soil moisture potentials between -13.2 and -16 bars, and will often favor dry soil moisture conditions[3, 4, 7]. Efficacy of many preemergence (PRE) herbicides for control of kochia differs among soils based on soil physicochemical properties and soil moisture. Without adequate soil moisture for herbicide activation, PRE herbicide efficacy decreases dramatically[8-11].

Previous studies showed the efficacy of many PRE herbicides differs among soils based on soil properties[12-20]. Better understanding the interaction between herbicide efficacy and soil physical properties on kochia control, may provide additional information to make more accurate PRE use recommendations, while decreasing negative environmental impacts such as off-target movement. Soil organic matter is often directly correlated with herbicide availability, and may be the only soil parameter found to be correlated with herbicide efficacy[18]. Among 12 herbicides previously investigated, percent organic matter and cation exchange capacity was highly correlated with herbicide phytotoxicity[15]. Adsorption of herbicides onto organic matter reduces concentrations in soil solution, decreasing phytotoxicity. Herbicides chemical properties are additional factors that help predict herbicide response to different soil properties. Basic compounds, such as indaziflam, tend to be in the non-ionized form at soil pH close to the pK_a (3.5), whereas nonionic herbicides like flumioxazin are not affected by pH. Previous studies with indaziflam have shown a significant positive correlation between the Freundlich distribution coefficient (K_f) and percent organic carbon (r = 0.990), and percent clay (r = 0.880) in Brazilian soils[21]. In United States mollisols, there were significant correlations between K_f and percent organic carbon (r = 0.970), pH (r = -0.870), and percent clay (r = -0.998)[21]. These correlations suggest that sorption of indaziflam increases as percent organic carbon increases, and pH decreases. Previous studies with flumioxazin have demonstrated similar results, indicating a significant correlation between K_f and percent organic carbon (r = 0.950), cation exchange capacity (r = 0.860), percent sand (r = -0.950), percent silt (r = 0.920), and percent clay (r = 0.700)[22]. In these studies, sorption varied for flumioxazin by approximately 22-fold, and indaziflam approximately 4-fold, across soils with different physical properties. Hysteresis of these two compounds also suggest that a portion of the applied herbicide is very strongly/irreversibly bound to soil and does not readily desorb without adequate soil moisture[21, 22].

The efficacy of most soil applied PRE herbicides is highly influenced by soil moisture, yet this effect is not well understood[8-11, 23, 24]. Using low rates of chloramben, PRE applications with adequate moisture resulted in increased weed control[9]. There have been similar findings with dichlobenil, atrazine, EPTC, simazine, atrazine, terbutryn, isoproturon, and diuron[8, 11, 25-29]. Soil moisture is a critical factor in better understanding the efficacy of PRE herbicides because as moisture levels decrease, herbicides are not transported to plants by mass flow or diffusion, decreasing phytotoxicity (Fig. 1.14)[23]. This rainfall activation is necessary for (1) bringing the herbicide into solution, (2) redistributing the herbicide into the surface soil layers, and (3) herbicide availability to the weed seedlings[30].

Learning how herbicide adsorption changes in response to different soil and moisture regimes will provide a better estimate for the quantity of a pesticide in soil solution, and thus determine its persistence, leaching, mobility, and bioavailability[19]. Soils contain different hydraulic and retention properties, so in order to determine moisture contents of soils with different soil properties at specific matric potentials, soil water retention curves are generated[31]. To minimize confounding influence of dosage on indaziflam and flumioxazin performance when evaluating the effect of soil moisture, plant responses are normalized based on soil properties using 80 percent growth reduction (GR_{80} values)[17].

Data describing the PRE efficacy of indaziflam and flumioxazin on kochia is limited. Our first objective of these studies was to determine the amount of herbicide required to produce GR_{80} in kochia for a range of soils with different properties. Our second objective was to assess the effect of soil moisture on herbicide phytotoxicity of kochia, using soil water retention curves and the GR_{80} standardized herbicide rates. This work expands on past research of PRE herbicides by showing the direct influence of soil properties and soil moisture on the efficacy of indaziflam and flumioxazin on kochia.

MATERIALS AND METHODS

Chemicals. Indaziflam [N-[(1R,2S)-2,3-dihydro-2,6-dimethyl-1H-inden-1-yl]-6-[(1RS)-1-fluoroethyl]-1,3,5-triazine-2,4-diamine] is a selective alkylazine herbicide used for broad spectrum PRE control of over 75 grass, broadleaf, and annual sedge species. Indaziflam is a cellulose-biosynthesis inhibitor and is used in industrial vegetative management, turf, and established citrus, grape, and tree nut crops[32]. Application rates range between 51 and 102 $g{\cdot}ai{\cdot}ha^{-1}$ with a yearly maximum of 146 $g{\cdot}ai{\cdot}ha^{-1}$. Long soil persistence ($t_{1/2}$ >150 days) and broad spectrum control makes this herbicide a viable option in many management systems. Indaziflam has a water solubility of 2.80 $mg{\cdot}L^{-1}$ and a Log K_{ow} ranging from 2.0 to 2.8; requiring adequate moisture for activation[33, 34]. Previous work has shown that sorption of indaziflam and its metabolites is affected by soil properties. Further work is necessary to better understand the main effect of soil moisture on the activation and phytotoxicity of indaziflam[21, 35].

Flumioxazin [N-(7-fluoro-3,4-dihydro-3-oxo-4-prop-2-ynyl-2H-1,4-benzoxazin-6-yl)cyclohex-1-ene-1,2-dicarboxamide] is a dicarboximide herbicide used PRE for broadleaf control in soybean (*Glycine max*), cotton (*Gossypium hirsutum*), peanuts (*Arachis hypogaea*), and several other crops. It is also used as a selective herbicide in industrial vegetative management and non-cropland areas. The mechanism of action is the inhibition of the enzyme protoporphyrinogen oxidase and is used at rates between 214 and 429 $g{\cdot}ai{\cdot}ha^{-1}$ with an annual maximum application of 857 $g{\cdot}ai{\cdot}ha^{-1}$. Flumioxazin has a short half-life ($t_{1/2}$ <18 days) unlike indaziflam, but has similar lipophilic chemical properties with a water solubility of 1.79 $mg{\cdot}L^{-1}$ and log K_{ow} of 2.6[36]. Indaziflam and flumioxazin chemical properties are summarized in Table 1.1.

Soil Preparation. Seven soils were selected for this study based on a wide range of physicochemical properties. Soils were collected from the 0 to 10 cm depth, air-dried, and passed through a 2 mm brass sieve**.** Soils were stored at 10°C until use. Soil physicochemical properties were analyzed at the Colorado State University Soil Testing Laboratory using the methods from Sparks *et al*.[37]. The physicochemical properties of the soils are summarized in Table 1.2.

Soil Water Retention Curves. Soil-water retention curves were generated for each soil using a pressure plate apparatus. Pressure plate extractor methods from Jury *et al*[31] were used. Individual soils were packed into 2.5 cm tall by 4.95 cm diameter aluminum columns; bottoms were each covered with a single filter paper and square cheese cloth, and secured with a rubber band**.** Four replications were constructed for each soil and randomly assigned to one of three pressure plates through completion of the experiment. Using an air compressor and pressure regulation system, soils were subject to increasing air pressure resulting in decreasing matric potentials. Soils were allowed to equilibrate for 3 days at each pressure of -0.05, -0.10, -0.20, -0.33, and -0.50 bars. For pressures -1, -2, -4, -6, and -10 bars a total of 5 days was allowed for equilibration, due to increased flow resistance at these higher pressures[31]. Once equilibrium was reached at each specific matric potential, samples were removed from the chamber and immediately weighed. The gravimetric water content (θ_g), bulk density (ρ_b), and volumetric water (θ_v) content of the soil samples were determined. Using the HYDRUS-1D software package, van Genuchten soil water retention parameters were estimated, and resulting curves were generated for each soil with $R^2 > 0.96$ (Fig. 1.1). These curves were used for calculation of volumetric water content values for each soil, at specific matric potentials.

Kochia Dose Response for GR_{80} Determination. Preliminary greenhouse bioassays were performed for indaziflam and flumioxazin to determine a range of concentrations above and below estimated visual GR_{80} values. Herbicide rates ($mg \cdot kg^{-1}$) were calculated by assuming the herbicide was equilibrated within the top (6 cm) of soil. These rates ranged from 0.050 to 0.003 $mg \cdot kg^{-1}$ for indaziflam, and 0.200 to 0.013 $mg \cdot kg^{-1}$ for flumioxazin. Herbicide solutions were prepared in a volumetric flask (10 mL), and then transferred to a glass air brush spray vial (10 ml) for application. Herbicide rates in $mg \cdot kg^{-1}$ are given in Table 1.3.

Prior to spraying, soils were oven dried and individually weighed (1 kg). For each desired herbicide rate, soils were flattened on butcher block paper for increased contact, and sprayed using an airbrush, air compressor apparatus. After approximately every third of the herbicide solution was applied (3-4 mL), soils were mixed by hand using a quartering method to ensure equilibration. Each sprayed soil (1 kg) was then transferred into a soil V-blender and allowed to equilibrate for 30 minutes. To reduce microbial degradation following equilibration, soils were transferred to glass mason jars and placed in a freezer (-20 °C) until planting. This airbrush method was used to insure the entirety of the herbicide was equilibrated in the soils, and each kochia seed was accurately coming into direct contact with the same herbicide concentration. The method for treatment applications was a Paasche double action airbrush, sprayed with a constant air pressure (138 kPa), with a 0.73 mm tip. The air brush technique is a common method used to apply a precise amount of pathogen inoculum in plant pathology research, in addition to use in insecticide research[38-41].

For planting, the treated soil (1 kg) was subdivided into four square flats (12x12x6 cm), constituting the four replications for each rate. The study was set up as a randomized complete block design. A total of five herbicide rates and an untreated treatment was used for the indaziflam and flumioxazin dose response. Pure kochia seed was individually weighed for each replication of soil (0.10 g), to ensure uniform germination throughout the experiment. Kochia seed was planted 1 cm below the soil surface and covered with soil equilibrated with the desired herbicide treatments. Flats were transferred to a greenhouse with a 25/20°C day/night temperature regime at an approximate relative humidity of 60%. Natural light was supplemented with high-intensity discharge lamps when light level was below 25 mW/cm^2, to give a 15 hour photoperiod. Soils were sub-irrigated daily in the greenhouse to simulate optimum moisture conditions. In addition, soils were misted several times a day to decrease crusting on the soil surface and increase germination rates. Experiment was repeated and data combined for statistical analysis.

Soil Moisture Effect on Herbicide Efficacy

Moisture Content Determination. Using the previously generated soil water retention curves (Fig. 1.1), the volumetric water content was determined for each soil at each matric potential of interest (-0.10, -0.33, -1, -2, and -4 bars). This volumetric water content (θ_v) was then converted to gravimetric water content (θ_g) using the bulk density (ρ_b) of each soil. The gravimetric water content was used as the basis for obtaining the desired water content for each soil, at each matric potential of interest. Experiment was setup as a randomized complete block design with 5 moisture potentials and 3 replications for each soil. An untreated treatment was planted for each potential and soil combination. Herbicide phytotoxicity was standardized based on soil properties and was represented as the GR_{80} rate. This rate was held constant within soil types, and used across moisture levels at specific matric potentials. Herbicide treatments were applied as before, using the airbrush apparatus and V-blender for equilibration. Soils were transferred to glass mason jars and placed in a freezer (-20 °C) until planting.

Planting. Gravimetric water contents (θ_g) were calculated from oven dry soils with a known weight. These specific water contents were equilibrated with each soil and matric potential combination. To each glass petri dish (95.43 cm^3), moisture amended soil was flattened into the bottom of the petri dish (0.75 cm), and was the base for which the kochia seed was planted. Pure kochia seed (0.10 g) was spread evenly over the soil in the petri dish. Seeds were then covered with the same moisture amended soil (0.50 cm). This procedure was performed for each soil-moisture potential combination, for the check treatment and treated soils (GR_{80} rates). This resulted in a total of 30 petri dishes per soil. To reduce moisture loss, directly after planting, each dish was immediately placed in plastic germination boxes (11x11x4 cm). Wet paper towel was placed around each dish in the germination box to reduce water loss to the head space. Boxes were then placed under a shade-cloth in the greenhouse to reduce moisture loss, under the same greenhouse conditions as the dose response. Boxes were opened once a day to allow gas exchange, until completion of the 10 day bioassay. Upon completion, individual moisture boxes were visually evaluated for percent control of check, harvested for dry weight biomass, and counted number of living plants per area (111 cm^2). Experiment was repeated and data combined for statistical analysis. An average moisture loss of 19.7% was recorded for the 10 day bioassay.

RESULTS AND DISCUSSION

Effect of Soil Properties on PRE Herbicide Efficacy

Dose Reponse (GR_{80} values). Visual evaluations were taken as percent control of the untreated at the six different rates. Study was also harvested for dry weight biomass, and number of surviving plants per square flat area (144 cm^2). The SAS statistical program, Proc Probit, was used to calculate GR_{80} vales[42]. The GR_{80} values (Fig. 1.2) represent the herbicide rate in mg kg^{-1} required for 80 percent control of kochia for each of the seven soils. The GR_{80} values were determined for each soil from a sigmoidal curve with rate on the X axis and percent control on the Y axis. Untreated treatments were omitted for calculation of GR_{80} values. Calculation of GR_{80} values were performed using visual percent control data in addition to percent of check dry weight biomass. There was no statistical difference for both methods after performing an F-test for differences in variance ($p = 0.116$), so visual percent data was used for analysis (Fig. 1.2). GR_{80} values for each soil and herbicide combination were calculated using an lsmeans statement in Proc Mixed (Fig. 1.2).

Using Proc Probit and the Output statement, fitted GR_{80} probabilities were calculated based on the model for each herbicide, rate, and soil; averaging across replications. Linear regression was calculated from the observed v. predicted probability values to determine goodness of fit for the GR_{80} estimates ($R^2 = 0.879$). The calculated GR_{80} herbicide rates showed increased variation across herbicides and soils. GR_{80} values for indaziflam ranged from 0.0046 to 0.0385 mg kg^{-1} and flumioxazin values ranged from 0.0479 to 0.1593 mg kg^{-1} (Fig. 1.2).

Correlation and Regression of Soil Properties with GR_{80} Values. Pearson correlation coefficients (Table 1.5) and linear regression of GR_{80} (Table 1.6) with soil properties were calculated. A $\log_{10}(GR_{80})$ transformation was required to meet assumptions of normality for analysis. Results showed a significant positive correlation of $\log_{10}(GR_{80})$ with percent soil organic matter (%SOM) and cation exchange capacity (CEC), for both indaziflam and flumioxazin. The CEC would not be expected to be significantly correlated to the efficacy of nonionic herbicides like flumioxazin, so this relationship was likely due to the confounding effect of SOM and CEC[16]. After combining data for both herbicides, organic matter was the only parameter that was found to be correlated. Similar findings have shown that from 10 to 100 times more herbicide was required for growth reduction of several PRE herbicides for a soil with 16.8% SOM, as compared to a soil with 0.4% SOM[15]. For flumioxazin, a majority of the SOM adsorption may be largely due to hydrogen bonding[18]. The transformed GR_{80} values for both herbicides, averaging across soils, was found to be correlated with each other ($r = 0.867$). These results demonstrate a similar trend in herbicide adsorption across two different PRE herbicide modes of action; cellulose biosynthesis inhibitor (CBI) and inhibition of the enzyme protoporphyrinogen oxidase (PPO). It is important to remember that these r values are a measure of the extent to which X and Y are linearly related. Legitimate correlation does not imply causation, and further analysis must be done to look further at the cause and effect relationship between soil properties and herbicide phytotoxicity. This work provides additional evidence for the overall effect of SOM on the efficacy of PRE herbicides. Herbicides with a low water solubility like indaziflam and flumioxazin, are able to partition into the hydrophobic organic phase more easily[22]. This may explain the decrease in kochia phytotoxicity with an increase in SOM.

Using Proc Reg, linear regression analysis results suggest the two best single term models describing the influence of soil properties on the efficacy of both indaziflam and flumioxazin was %SOM and CEC (Table 1.6). Multiple regression was also performed to try and best predict kochia response using these two herbicides, across soils with different physicochemical properties (Table 1.7). Multiple regression was used to account for additional variation in the model for predicting herbicide efficacy, that one predictor cannot account for by itself. Mallow's Cp model selection (best subsets) generated the top 5 models after trying every possible model. These regression parameters were re-run to determine which models were statistically significant.

Results from the regression analysis show the single best parameter explaining the highest proportion of variability in the GR_{80} values across soils was SOM, for both indaziflam and flumioxazin ($r^2 = 0.792$ and 0.721). The regression of SOM using this model says that a unit increase in percent organic matter is associated with a 1.375 mg kg^{-1} increase in GR_{80} starting with an initial rate of 0.004 mg kg^{-1}, for a soil with no soil organic matter. These linear equations describe herbicide efficacy (GR_{80}) as a function of individual soil properties. Although SOM and CEC are significant predictors of phytotoxicity, there is the possibility that multiple predictors together in a regression model explain more of the variability than one soil property alone. In the multiple regression we found that both OM and CEC were not needed in the model because both are highly confounded ($r = 0.850$ and $p = 0.0001$). Using a wider range of pH in future work would help further evaluate this main effect, and provide greater confidence in use recommendations based on soil pH. The multiple regression equations (Table 1.7) give an estimation of rate adjustment based on knowledge of soil properties prior to application. The best three term models explaining the influence of soil properties on PRE efficacy for indaziflam were:

1. $y = 0.0163 + 1.3722(\%OM) + 0.7473(pH) + 1.0121(\%Sand)$ ($r^2 = 0.914$)
2. $y = 0.0459 + 1.3562(\%OM) + 0.7588(pH) + 0.9827(\%Silt)$ ($r^2 = 0.901$).

For flumioxazin, the only significant two term model was:

1. $y = 0.0521 + 1.2365(\%OM) + 0.9865(\%Silt)$ ($r^2 = 0.814$)

These linear and multiple regression equations help to better explain the variability of the model for predicting the efficacy of these two PRE herbicides (GR_{80}), for kochia control.

Two-way ANOVA of GR_{80} Values. Separate logistic regression was run for each treatment and replication combination in order to run a two-way ANOVA. This provides the variability in the data so that herbicide, run, replication, and soil main effects can be analyzed (Table 1.4). Proc Probit was used again to calculate GR_{80} values but this time by herbicide, soil, and replication. Once these GR_{80} values were calculated for each replication, Proc Mixed was used to generate p-values for type 3 tests of fixed effects, to evaluate main effects and significant interactions.

Using the transformed GR_{80} values, results showed a significant main effect of soil and herbicide, as well as a significant interaction between soil and herbicide (Table 1.4). It is important to analyze this interaction in further depth to attempt to explain herbicide phytotoxicity of kochia in different soils. Using a Slice statement in Proc Mixed, we were able to determine differences in soils and herbicides with pairwise comparisons. A Tukey adjustment was made to control MEER separately for each group of comparisons. Using the Slice statement by herbicide, results showed a difference between indaziflam and flumioxazin, averaging over soils ($p<.0001$). This suggests a higher rate of flumioxazin is required for equal efficacy as compared to indaziflam, averaging over soils. Using a Slice statement by herbicide and soil, there was a significant difference between soils for indaziflam ($p<.0001$). There was a significant difference between the 6.2% SOM soil and all other soils, the 0.9% SOM soil was significantly different from all soils but 1.5% SOM and 3.3% SOM, and the 2.8% SOM, 2.5% SOM, and 4.0%SOM soils were not significantly different. We also found a significant difference between soils for flumioxazin ($p<.0001$). Looking at pairwise comparisons of flumioxazin for each soil we found a significant difference between the 6.2% SOM soil and every other soil, the 0.9% SOM soil was significantly different from all soils but 2.8% SOM, and the 3.3% SOM, 2.5% SOM, and 4.0% SOM soils were not statistically different from each other. The IL soil (6.2% SOM) was statistically different from all other soils for both herbicides, which provides additional evidence that soil properties such as SOM directly influence kochia phytotoxicity. Soils with increased SOM are particularly variable in terms of PRE control of kochia, often requiring an increased rate for similar control to soils with decreased SOM.

These data provide evidence for the variability in the indaziflam and flumioxazin rate required for kochia control, applied in soils with different soil properties. The soil property most highly correlated with an increased GR_{80} value was organic matter ($r=0.890$ for indaziflam and $r=0.849$ for flumioxazin). These studies provided similar results to other PRE herbicide studies evaluating the influence of soil properties on herbicide efficacy. Khan (1978) states that the adsorption of pesticides by organic matter may exert the most profound influence of the several processes operating to determine the fate of a pesticide in soil, which directly supports our findings. Although there are other factors to consider such as an herbicides water solubility and pKa (Table 1.1), this work better explains the direct effect of soil properties on the efficacy of these two chemically similar molecules.

Effect of Soil Moisture on PRE Herbicide Efficacy

The effect of soil moisture on PRE herbicides has previously been well documented. Evaluating soil moisture requires standardizing herbicide rates in order to look at the main effect of soil moisture. Soil water retention curves were used for determining specific matric potentials of interest, and herbicide rates were standardized based on soil physicochemical properties (GR_{80} rates). This allowed for direct evaluation of the influence of soil moisture on kochia phytotoxicity.

Significant Main Effects and Interactions. Performing an ANOVA using Proc Mixed, we evaluated the main effects and interactions of herbicide, soil, and matric potential. A $\log_{10}$ transformation of matric potential was made for analysis. Using this same model in Proc Glm resulted in a highly significant model ($p<.0001$) with an R^2 of 0.911. Averaging across all other means there were significant herbicide ($p=0.0414$), $\log_{10}$(matric potential) ($p < .0001$), and soil ($p < .0001$) main effects. These main effects can be explained by understanding the effect of soil properties and soil moisture on PRE herbicide efficacy. Both herbicides performed differently across soils, and efficacy was directly influenced by the soil water potential and soil physicochemical properties. The significant interactions in the model included $\log_{10}$(matric potential)*herbicide ($p = 0.0094$), $\log_{10}$(matric potential)*soil ($p<.0001$), herbicide*soil ($p<.0001$), and $\log_{10}$(matric potential)*herbicide*soil ($p<.0001$). These interactions illustrate the complexity of the effect of soil physicochemical properties and matric potentials on herbicide phytotoxicity. It is important to recognize these herbicide, soil, and moisture factors involved in controlling highly competitive, tolerant, and adaptive weeds such as kochia, using PRE herbicides.

To analyze the $\log_{10}$(matric potential)*herbicide interaction, Proc Mixed least squares means were calculated for each herbicide and matric potential combination, averaging over soils (Fig. 1.4). The only significant difference between herbicides came at matric potentials of -2 and -4 bars ($p = 0.0026$ and $p = 0.0344$). For each herbicide separately, the only non-significant lsmean across matric potentials for indaziflam was between matric potentials of -0.11 and -0.33

bars, whereas for flumioxazin each mean was statistically different for each matric potential ($p < .0001$). To evaluate the $\log_{10}$(matric potential)*herbicide*soil interaction, means were calculated for each combination of matric potential, soil, and herbicide (Fig. 1.3). Kochia can germinate at moisture potentials less than -13 bars, and these results show decreased herbicide activation beginning at -1 bar. This trend is consistent across both herbicides and can help explain why kochia is such a problematic weed across the United States. For PRE herbicides such as indaziflam and flumioxazin, results demonstrate that kochia is difficult to control because of its competitiveness to germinate with very little moisture. The implications of these results are that indaziflam and flumioxazin phytotoxicity was shown to be greatly reduced at -2 and -4 bars, and we know that kochia can germinate at moisture potentials less than 6 times these values. When evaluating herbicide efficacy, both soil properties and soil moisture are key factors driving phytotoxicity[23].

Individual Regression for Each Soil (A_{50}). An important question in understanding the efficacy of indaziflam and flumioxazin is how soil properties and moisture combined, effect kochia phytotoxicity. It is important to further analyze the highly significant interaction of matric potential and soil properties. Logistic regression was performed for each soil and herbicide using Proc Probit. Parameter estimates and standard errors were recorded in table 1.8. The logistic regression curves for the 5 soils (Fig. 1.5) show the variation in dry weight reduction in relation to matric potential changes, for each soil and herbicide combination. Looking at the logistic regression estimates comparing indaziflam and flumioxazin, it is important to recognize that the slope is greater for flumioxazin for three of the five soils. This suggests that soil moisture has a greater effect on kochia phytotoxicity for flumioxazin as compared to indaziflam. From these individual regression curves, we were able to determine the exact matric potential that resulted in 50% dry weight reduction of kochia, for each soil. We termed this value the A_{50}, or matric potential required for 50% activation of the herbicide treatments. The same herbicide rate was standardized for each soil for the moisture bioassay (GR_{80}), therefore the matric potential that corresponded to 50% reduction in dry weight biomass was directly related to herbicide activation. Proc Corr was used to analyze the relationship between the A_{50} values for each soil, and individual soil properties. No soil properties were found to be directly correlated with the A_{50} matric potentials for this analysis.

To better understand the direct relationship of soil properties and soil moisture on herbicide efficacy, we standardized the A_{50} values. Using the soil water retention curves generated for each soil, we were able to determine the volumetric water content (θ_v) that corresponded directly to the A_{50} matric potential. For an herbicide to show equal activation (A_{50}) across soils, soils with different soil properties require a different matric potential, and resulting volumetric water content (θ_v). We determined the Pearson Correlation coefficients for the water content (θ_v) of each soil that resulted in A_{50}, with the soil physicochemical properties, to evalaute how herbicide efficacy changes in response to soil properties and moisture. When correlating these water content values with soil properties we found significant correlations with %SOM, CEC, percent sand, and percent clay (Table 1.9). These correlations suggest that as %SOM, CEC, and percent clay are increased, there is a strong linear relationship suggesting an increased water content required for A_{50}. The significant percent sand correlation suggests that there is a negative linear relationship between water content and percent sand. This work suggests that there are multiple soil properties together, that influence the interaction between herbicides, soils, and soil matric potentials.

By plotting significantly correlated soil properties with the θ_v required for A_{50}, the interaction between these two variables can be evalueated. A linear and power regression were analyzed for each soil, describing the relationship between soil properties and kochia phytotoxicity of each herbicide (Fig. 1.6-1.13). The regression equations help explain the relationship between moisture activation of indaziflam and flumioxazin as a function of soil properties. Across soil properties we can see a trend toward increasing or decreasing phytotoxicity with changing moisture contents. These results showed a relationship between percent organic matter and phytotoxicity. As organic matter increases, an increased water content was needed for kochia phytotoxicity (Fig. 1.6 and 1.7). These results are consistent with other results on the adsorption of PRE herbicides and the importance of soil moisture for activation. Without adequate moisture for soils with a higher percent organic matter, the percent reduction in dry weight biomass would decrease. Similar relationships were found with CEC (Fig. 1.8 and 1.9), percent sand (Fig. 1.10 and 1.11), and percent clay (Fig. 1.12 and 1.13). For each herbicide, the equation describing the influence of soil water potential required for 50% reduction in dry weight biomass, as a function of soil properties, are included in the graphs with R^2 fit values.

CONCLUSION

In comparing herbicides, both indaziflam and flumioxazin showed similar relationships between soil properties and soil moisture on kochia phytotoxicity. The relationship between volumetric water content and soil physical properties can be partially explained by the difference in phytotoxicity of the herbicides in different soils with different soil properties (GR_{80}). These results demonstrate how soil properties, herbicide properties, and moisture contents all play a significant role in kochia phytotoxicity. Understanding these relationships and their interactions, will ultimately allow for greater herbicide efficacy for controlling kochia, and provide better predictability of how specific PRE herbicides perform with a variation in soil properties and moisture. Kochia is a highly problematic weed that has many physiological attributes that allow it to survive in a wide range of climates and soils. In addition, these studies suggest that kochia can germinate at moisture levels below herbicide activation. This is important to understand because indaziflam and flumioxazin are both labeled for kochia control, but we saw that efficacy greatly decreased with decreasing soil matric potentials. Further work should be done in analyzing the effect of multiple moisture events on herbicide reactivation, and the importance of initial moisture directly following indaziflam and flumioxazin applications. PRE herbicides including the ones used in this study, were shown to be greatly affected by soil physicochemical properties and moisture conditions. Further work is necessary, due to the variability in soils and climactic factors throughout the United States, where these PRE herbicides are being used for kochia control.

Table 1.1 Herbicide chemical properties					
Herbicides	Log K_{ow}	pKa	Water Solubility (mg/L)	Kochia Rate (oz/A)	Kochia Rate (g ai ha^{-1})
Indaziflam	pH 2: 2 pH 4,7, and 9: 2.8	3.5	4.4 (pH 4, 20 C) 2.8 (pH 9, 20 C)	3.5 - 7	51 – 102
Flumioxazin	2.55 (20 C)	None	1.79 (25 C)	6 - 12	214 – 429

Table 1.2 Physicochemical properties of soils[a]

Soil	Soil Series	pH	OM (%)	CEC (meq/100g)	Sand (%)	Silt (%)	Clay (%)
QLS	Quincy	8.0	0.9	8.3	88.8	10.0	1.2
NFR	Ascalon	7.6	1.5	11.5	62.0	16.0	22.0
IC	Haplustolls	6.3	2.5	8.7	56.0	26.0	18.0
KS	Farnum	5.9	2.8	18.5	42.0	34.0	24.0
DBS1	Otero	7.6	3.3	14.3	50.0	26.0	24.0
RM	Connerton	7.7	4.0	14.6	48.0	33.0	19.0
IL	Swygert	6.8	6.2	23.8	66.0	16.0	18.0

[a] Soil characterization provided by Colorado State University Soil Testing Laboratory, Fort Collins, CO.

Table 1.3 Application rates for GR_{80} dose response

Indaziflam (mg kg^{-1})	Flumioxazin (mg kg^{-1})
0.050	0.200
0.025	0.100
0.013	0.050
0.006	0.025
0.003	0.013
0.000	0.000

Table 1.4 ANOVA of $Log_{10}(GR_{80})$ for significant main effects and interactions	
Effect	P-value
Soil	<.0001
Herbicide	<.0001
Rep	0.5387
Study	0.3980
Herbicide*Soil	<.0001

Table 1.5 Pearson correlation coefficients of $\log_{10}(GR_{80})$ and soil physicochemical properties								
Indaziflam			**Flumioxazin**			**Pooled**		
Soil Property	**r**	**p-value**	**Soil Property**	**r**	**p-value**	**Soil Property**	**r**	**p-value**
OM	0.890*	<.0001	OM	0.849*	.0001	OM	0.387*	0.0422
CEC	0.774*	0.0012	CEC	0.595*	0.0248	CEC	0.311	0.1068
pH	-0.472	0.0887	pH	-0.157	0.5913	pH	-0.154	0.4336
Sand	-0.184	0.5297	Sand	-0.046	0.8755	Sand	-0.057	0.7717
Silt	0.115	0.6944	Silt	-0.097	0.7415	Silt	0.014	0.9246
Clay	0.228	0.4335	Clay	0.204	0.4835	Clay	0.097	0.6246
* Significant at P < 0.05								

Table 1.6 Linear Regression of GR_{80} to explain variability in the model with predictors. Predictors converted back from transformed to original scale.

Herbicide	Soil Property	Regression Coefficients Intercept	Slope	R^2	P value
Indaziflam	OM	0.0041	1.3747	0.792	<.0001*
	CEC	0.0032	1.0905	0.599	0.0012*
	pH	0.1374	0.6995	0.222	0.0887
	% Sand	0.0166	0.9927	0.034	0.5297
	% Silt	0.0090	1.0078	0.013	0.6944
	% Clay	0.0078	1.0181	0.052	0.4335
Flumioxazin	OM	0.0401	1.2157	0.721	0.0001*
	CEC	0.0392	1.0438	0.354	0.0248*
	pH	0.1246	0.9262	0.025	0.5913
	% Sand	0.0774	0.9988	0.002	0.8755
	% Silt	0.0795	0.9958	0.009	0.7415
	% Clay	0.0599	1.0104	0.042	0.4835

Table 1.7 Multiple regression to better explain variability in the model for predicting GR_{80}. Predictors converted back from transformed to original scale.

		Multiple Regression Coefficients		
Herbicide	**Soil Variable**	**Parameter Estimates**	**R^2**	**P value**
Indaziflam	Intercept	0.0163	0.914	<.0001
	OM	1.3722		0.0054
	pH	0.7473		<.0001
	Sand	1.0121		0.0214
	Intercept	0.0459	0.901	0.0020
	OM	1.3562		0.0103
	pH	0.7588		<.0001
	% Silt	0.9827		0.0474
Flumioxazin	Intercept	0.0521	0.814	<.0001
	OM	1.2365		<.0001
	% Silt	0.9865		0.0390

Table 1.8 Logistic regression curves for each individual soil

Soil Series	Parameter	Indaziflam		Flumioxazin	
		Estimate	**Standard Error**	**Estimate**	**Standard Error**
DBS1	Intercept	-1.430	0.333	-2.106	0.183
	Potential	0.693	0.167	1.998	0.167
IC	Intercept	-0.891	0.217	-1.124	0.293
	Potential	1.068	0.170	0.821	0.176
IL	Intercept	-2.473	0.237	-3.534	0.328
	Potential	1.634	0.163	2.999	0.287
QLS	Intercept	-0.711	0.211	-1.084	0.210
	Potential	0.582	0.119	1.109	0.159
RM	Intercept	-1.945	0.328	-1.409	0.157
	Potential	1.321	0.221	0.964	0.097

Table 1.9 Pearson correlation coefficients of volumetric water content required for 50% herbicide activation (A_{50}) for each soil, with soil properties

Soil Property	r	p-value
OM	0.910*	0.0003
pH	-0.425	0.2208
CEC	0.760*	0.0108
Sand	-0.692*	0.0266
Silt	0.570	0.0856
Clay	0.731*	0.0162

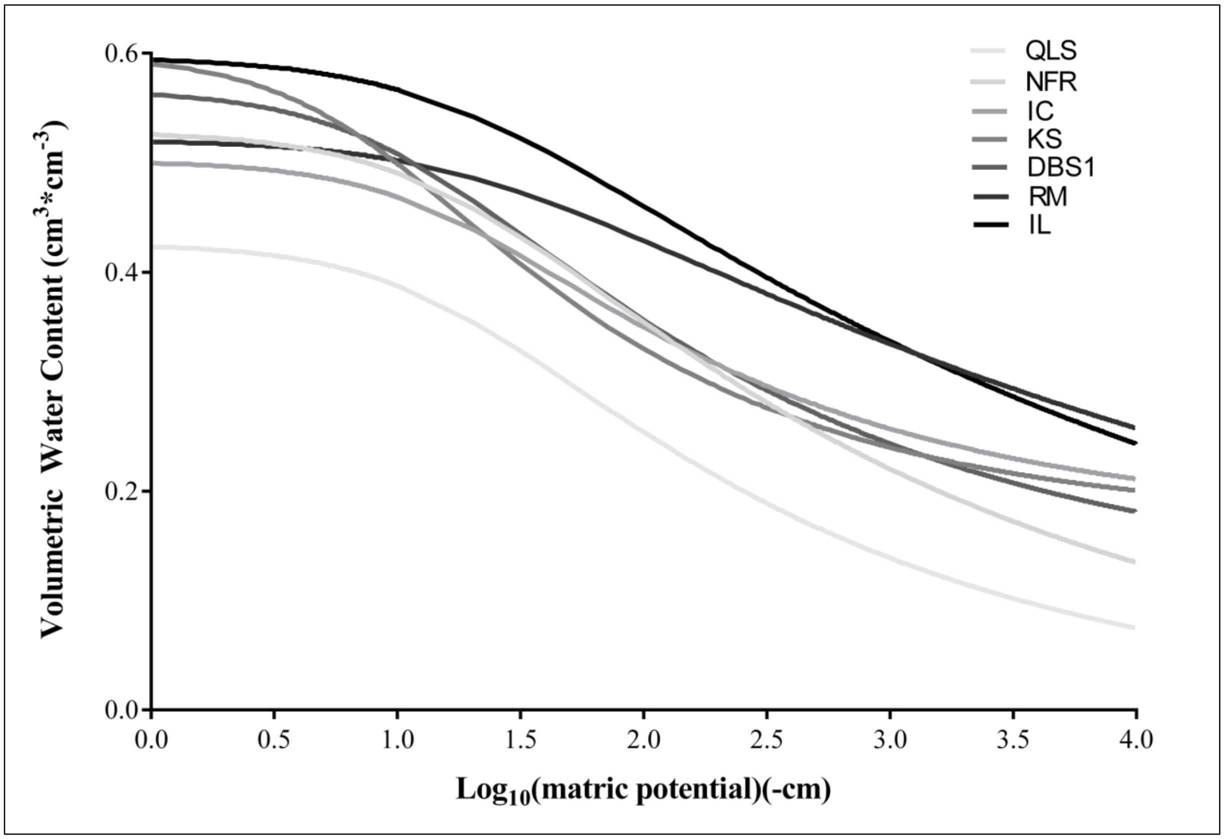


Figure 1.1: Soil water retention curves of seven soils generated using pressure plate apparatus and HYDRUS-1D software.

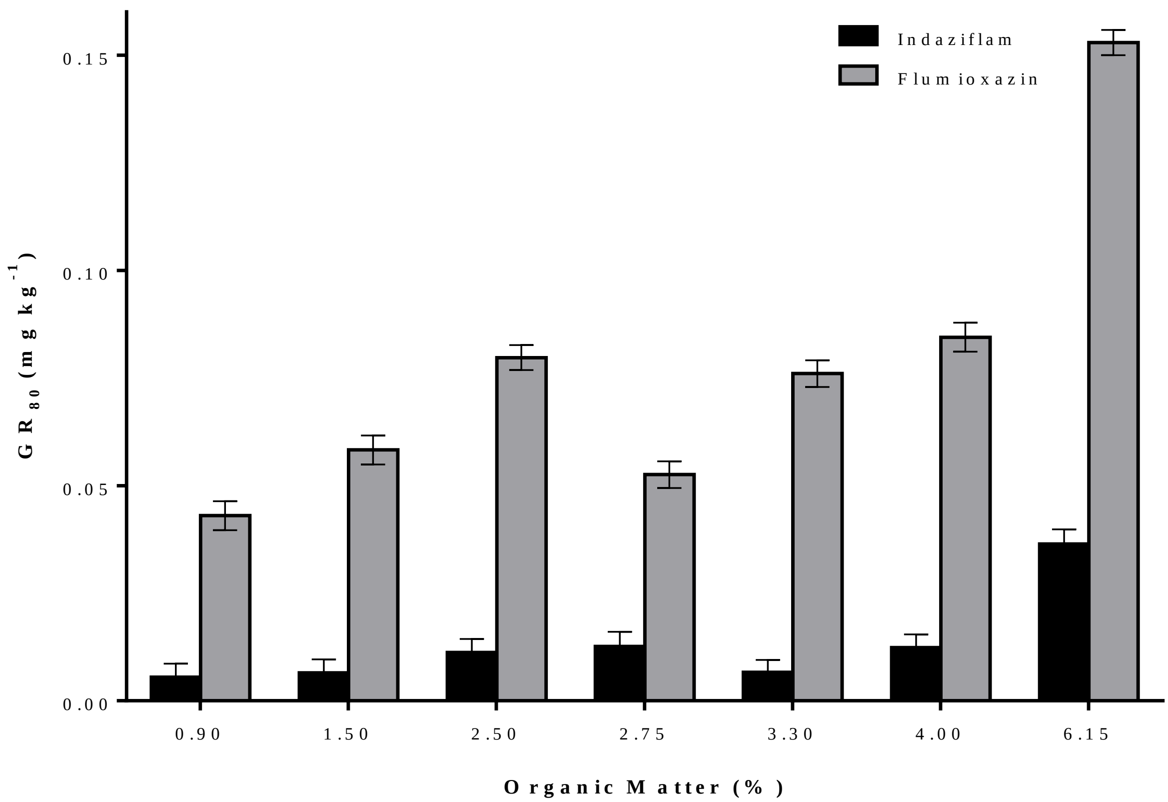
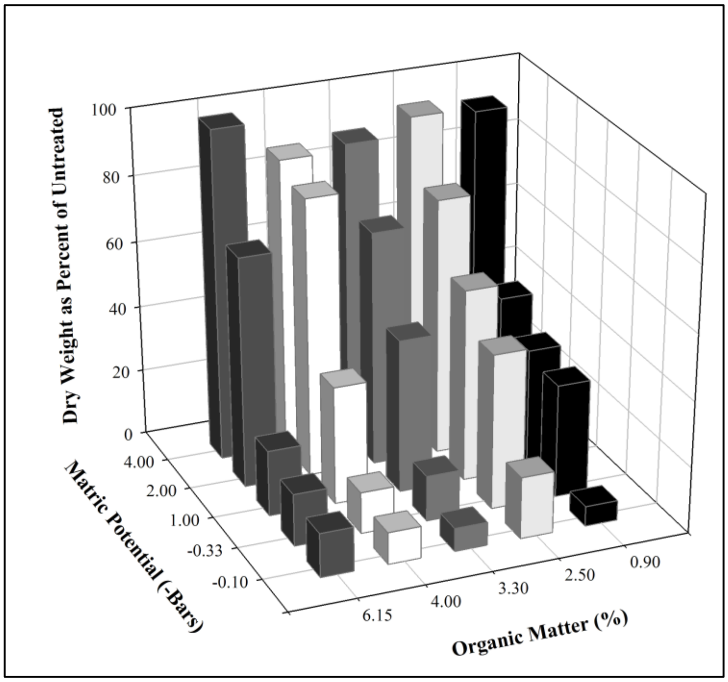


Figure 1.2: Least squares means of Indaziflam and flumioxazin rate required for 80 percent growth reduction of kochia.

a.



b.

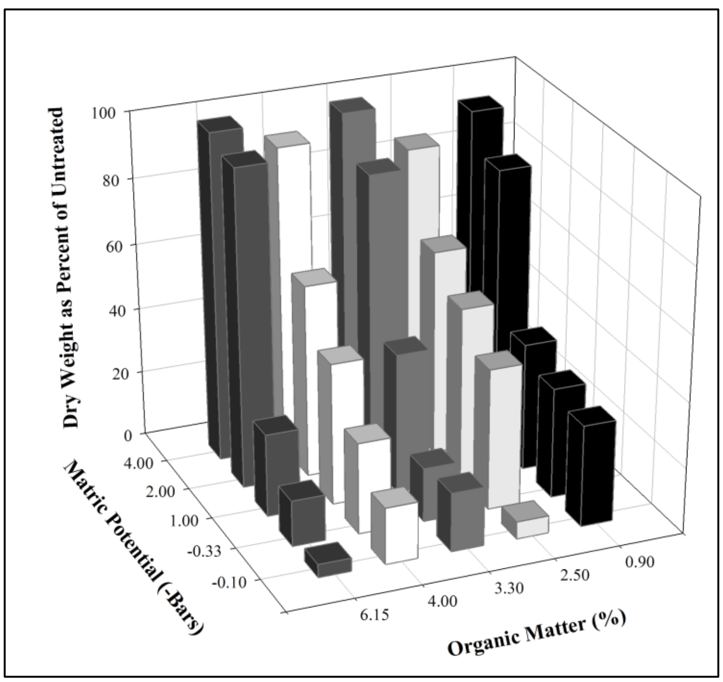
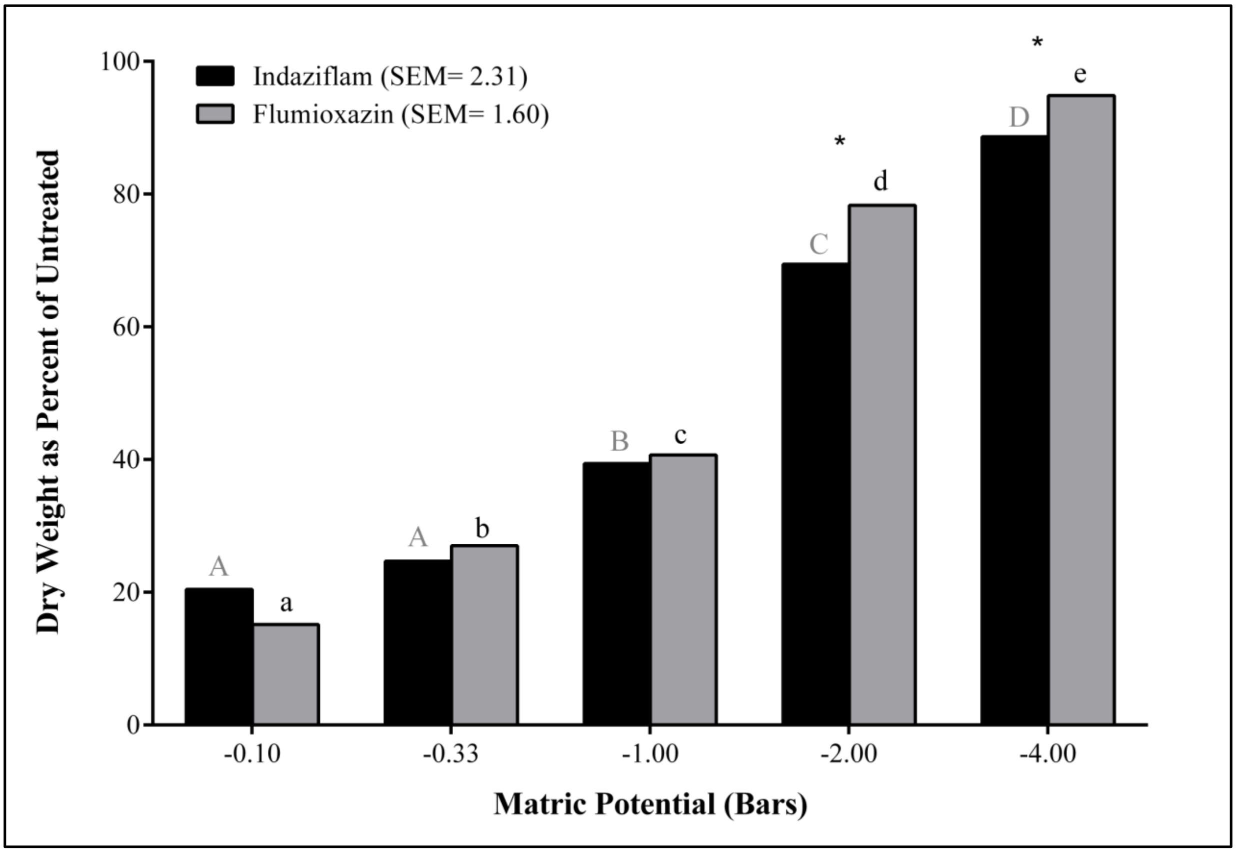


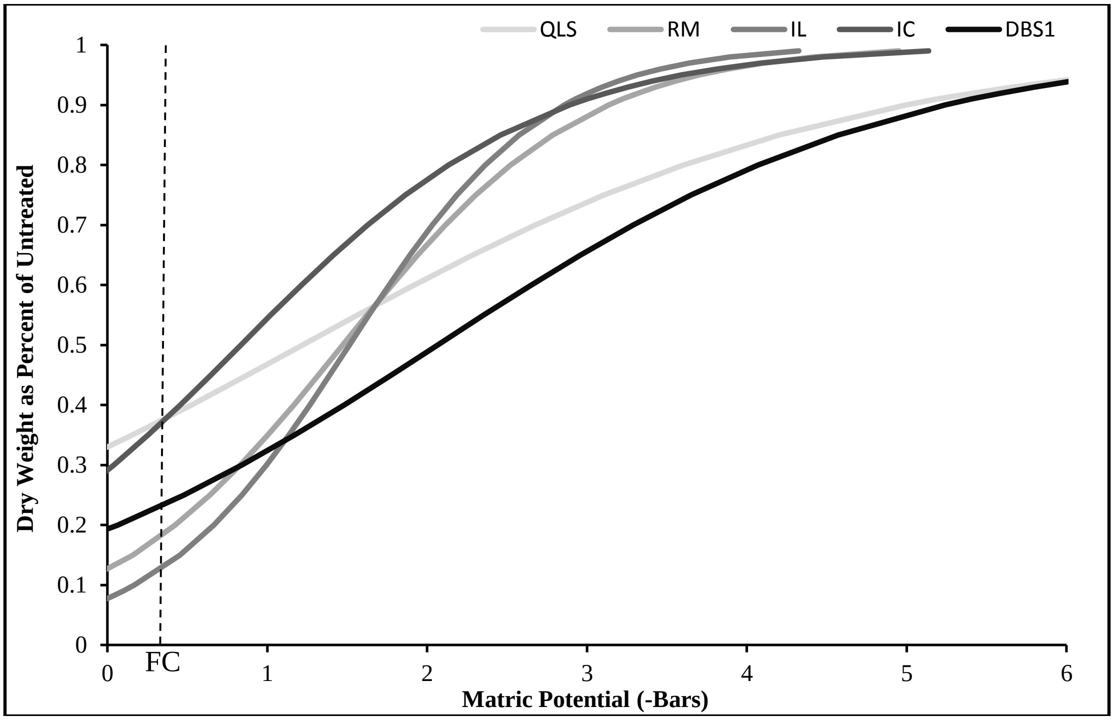
Figure 1.3: Three-way interaction of kochia phytotoxicity represented by dry weight as percent of untreated, matric potential, and soils with different percent organic matter for indaziflam (a.) and flumioxazin (b.).



* = significant difference in herbicides at a specific matric potential (water content)
Letters signify significant differences between potentials for each herbicide

Figure 1.4: Kochia dry weight as percent of untreated at five matric potentials showing the effect of soil matric potentials of seven soils on the efficacy of indaziflam and flumioxazin.

a.



b.

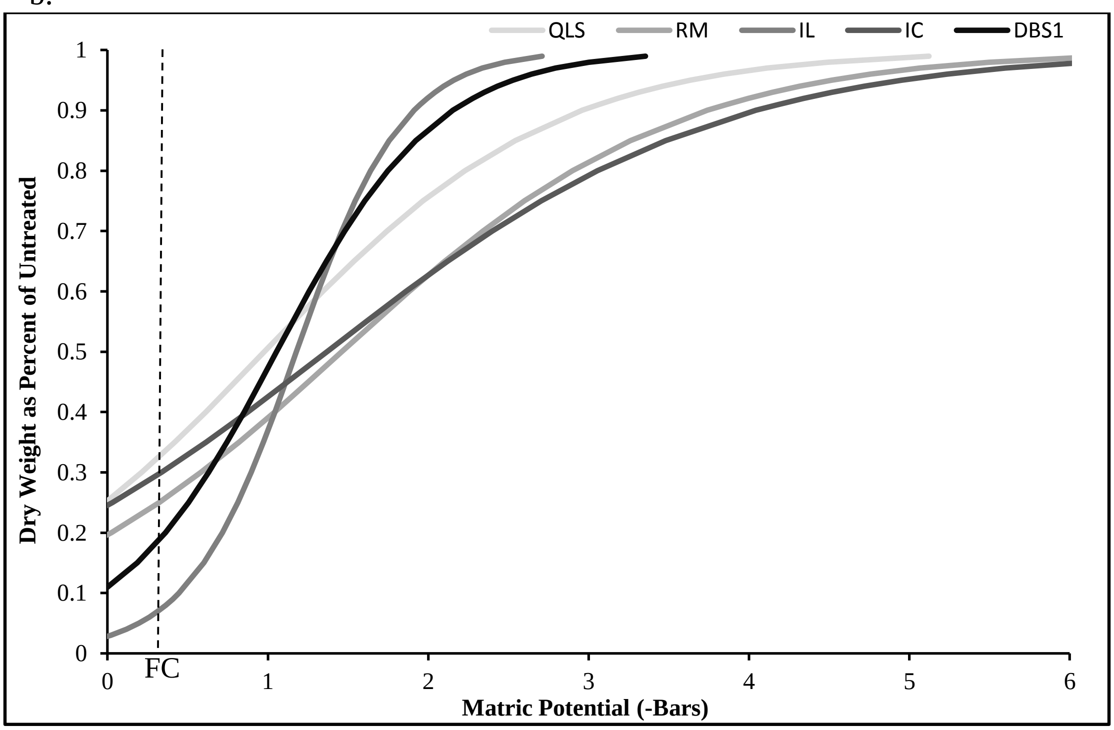


Figure 1.5: Logistic regression curves of five soils, evaluating the effect of matric potential on percent dry weight of kochia for indaziflam (a.) and flumioxazin (b.).

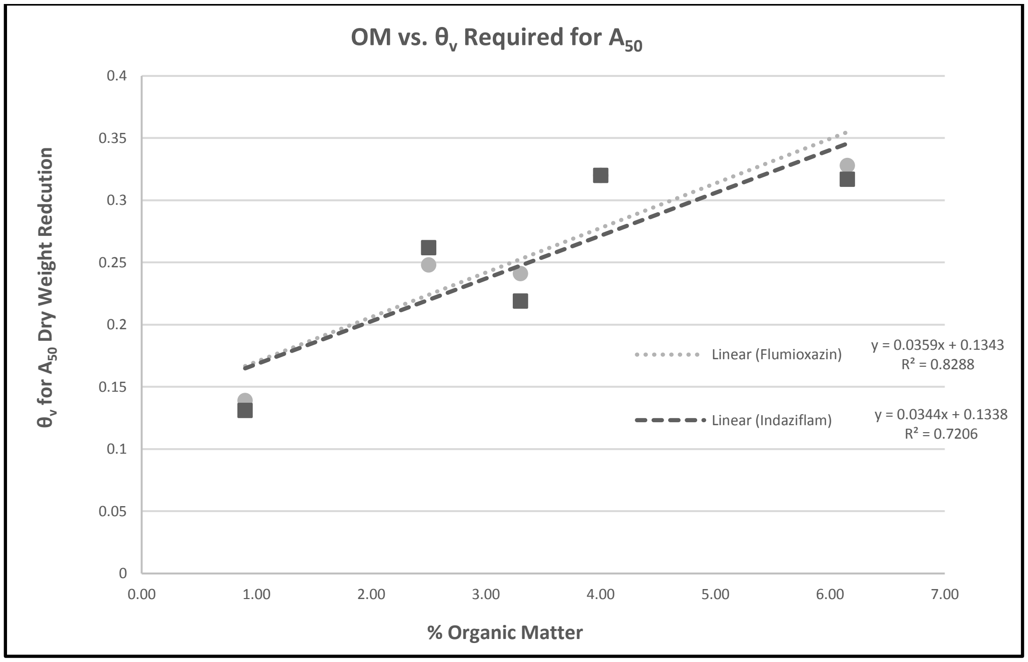


Figure 1.6: Linear regression of percent organic matter and volumetric water content required for 50% dry weight reducton of kochia for each soil. This represents the relationship between percent organic matter and volumetric water content on kochia phytotoxicity.

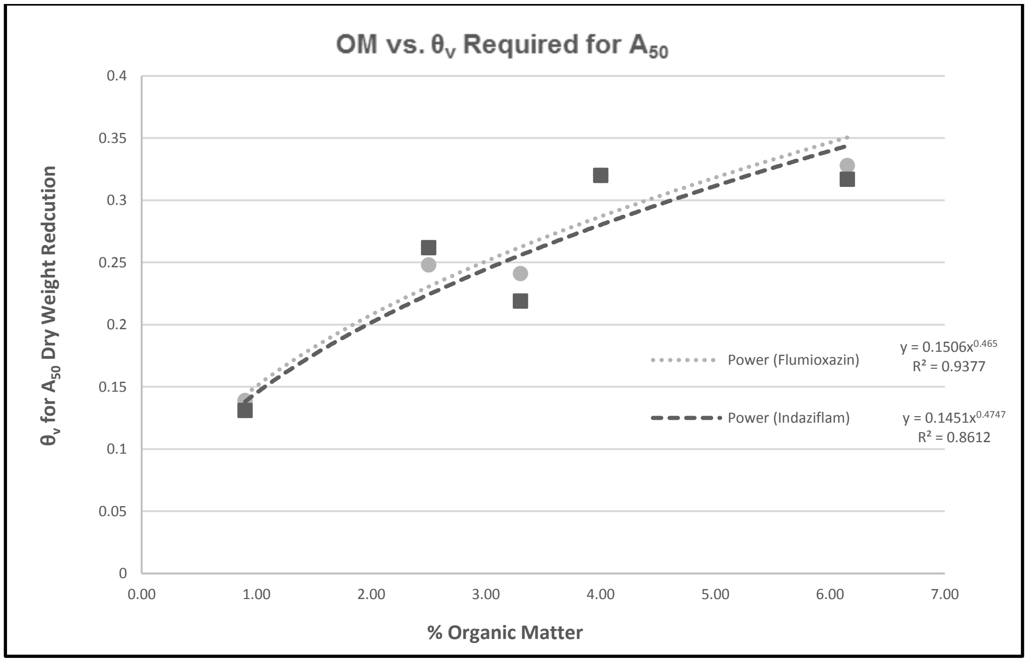


Figure 1.7: Power regression of percent organic matter and volumetric water content required for 50% dry weight reducton of kochia for each soil. This represents the relationship between percent organic matter and volumetric water content on kochia phytotoxicity.

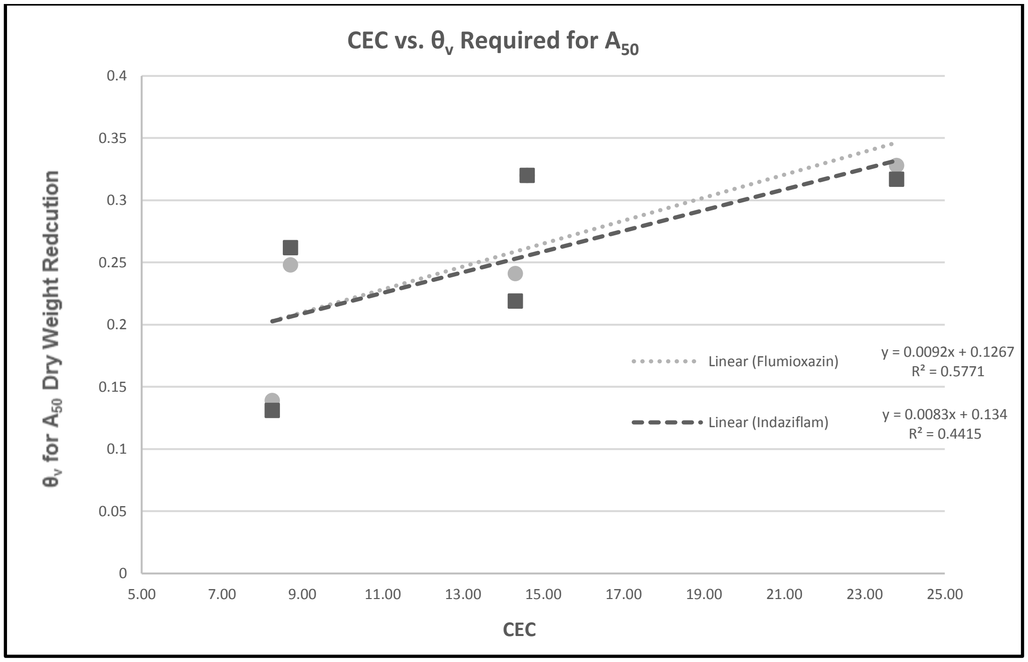


Figure 1.8: Linear regression of cation exchange capacity and volumetric water content required for 50% dry weight reducton of kochia for each soil. This represents the relationship between CEC and volumetric water content on kochia phytotoxicity.

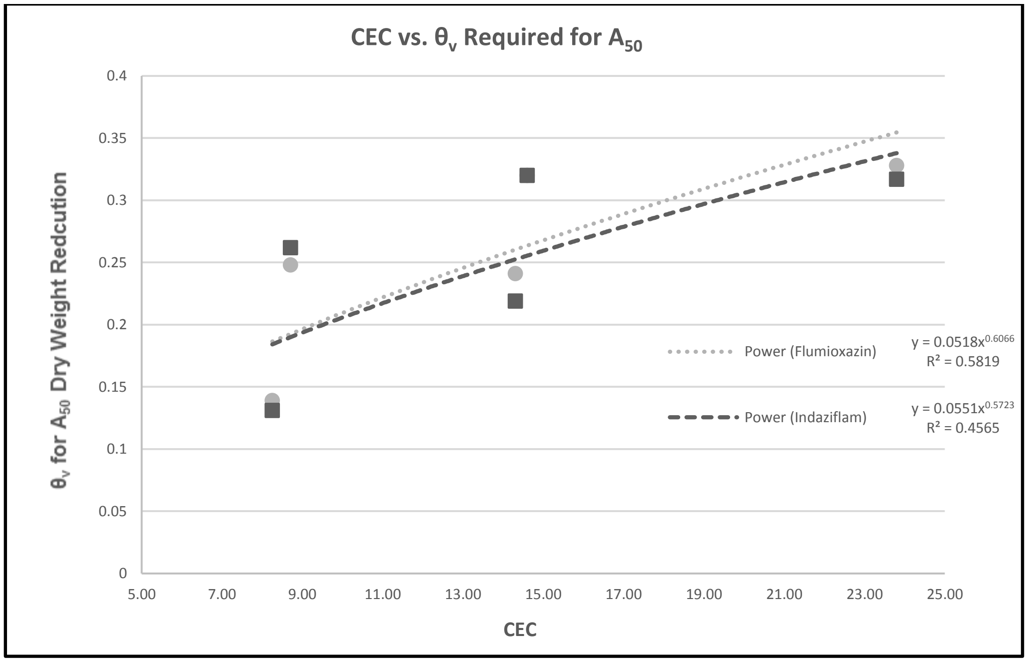


Figure 1.9: Power regression of cation exchange capacity and volumetric water content required for 50% dry weight reducton of kochia for each soil. This represents the relationshup between CEC and volumetric water content on kochia phytotoxicity.

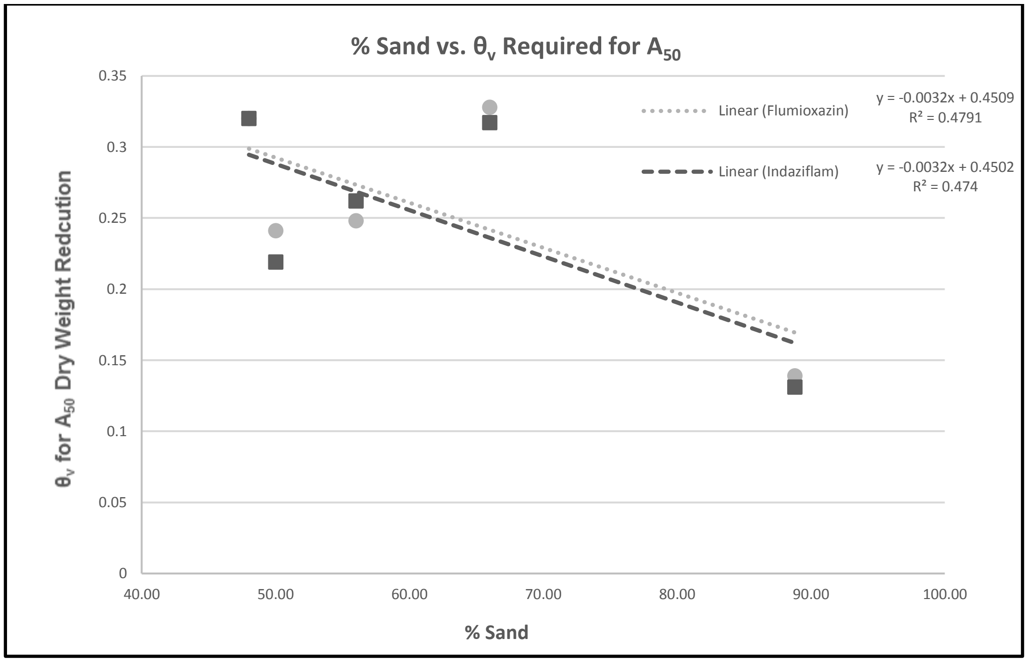


Figure 1.10: Linear regression of percent sand and volumetric water content required for 50% dry weight reducton of kochia for each soil. This represents the relationshup between percent sand and volumetric water content on kochia phytotoxicity.

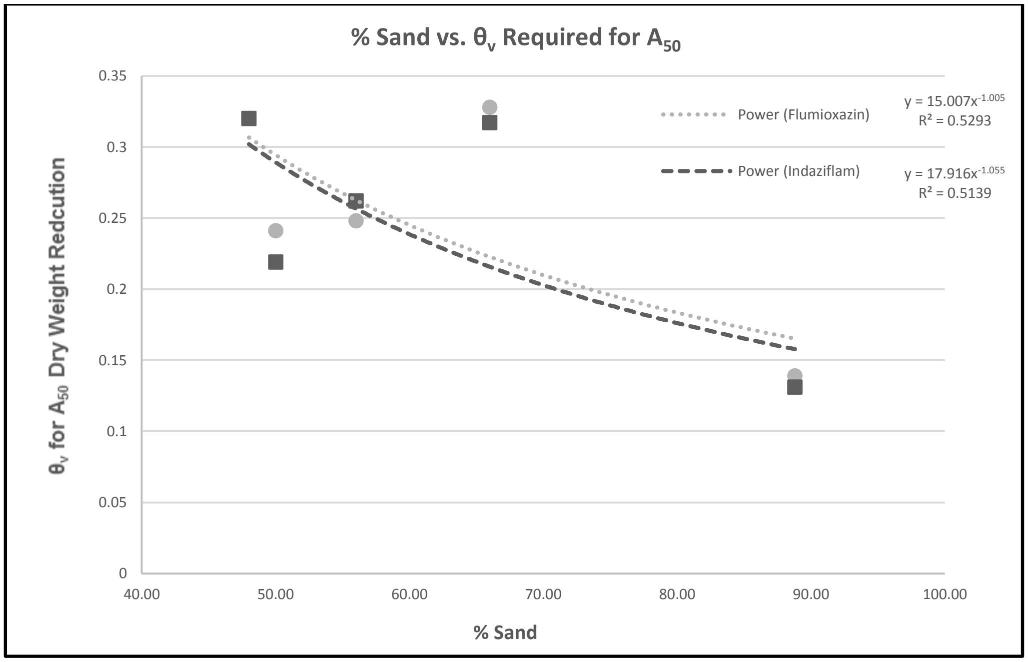


Figure 1.11: Power regression of percent sand and volumetric water content required for 50% dry weight reducton of kochia for each soil. This represents the relationshup between percent sand and volumetric water content on kochia phytotoxicity.

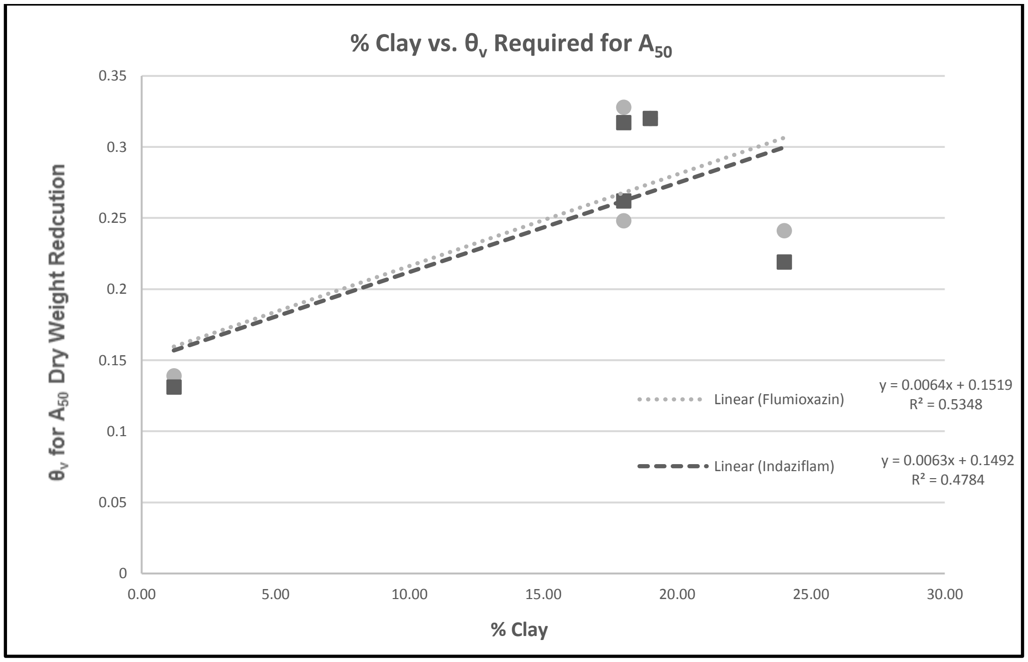


Figure 1.12: Linear regression of percent clay and volumetric water content required for 50% dry weight reducton of kochia for each soil. This represents the relationshup between percent clay and volumetric water content on kochia phytotoxicity.

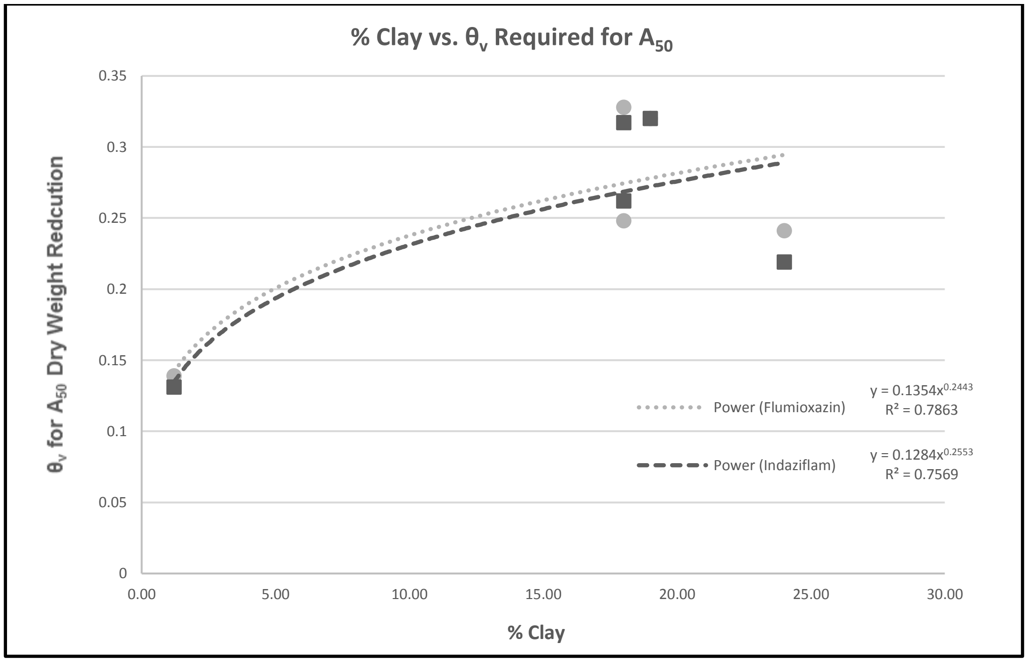


Figure 1.13: Power regression of percent clay and volumetric water content required for 50% dry weight reduction of kochia for each soil. This represents the relationshup between percent clay and volumetric water content on kochia phytotoxicity.

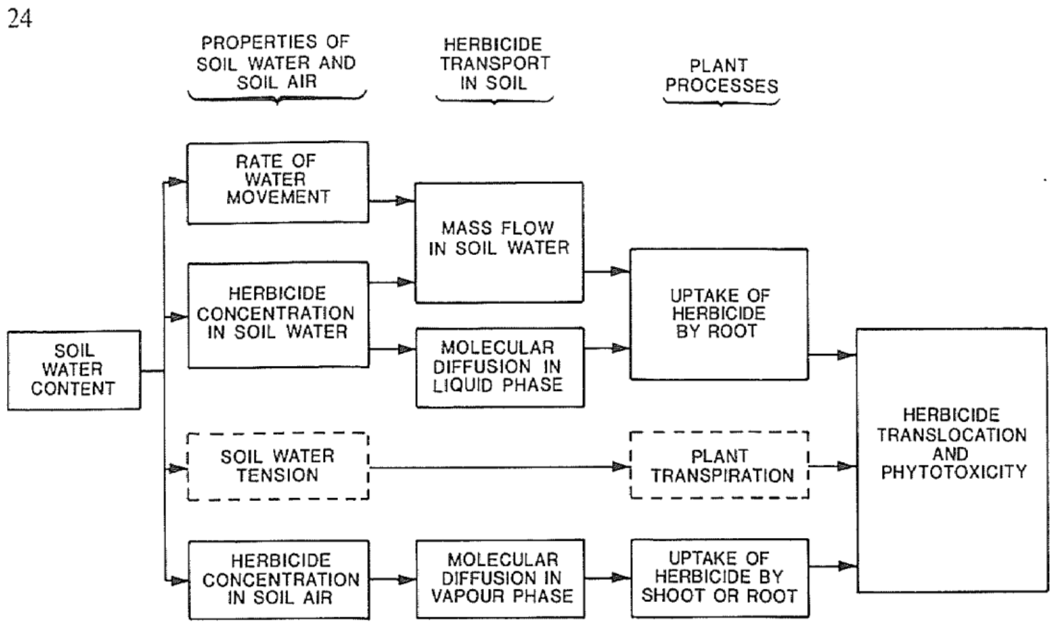


Figure 1. Schematic diagram showing probable mechanisms by which soil water content influences the phytotoxicity of a soil-applied herbicide at water contents above the wilting point (23).

Figure 1.14: Diagram from Moyer 1987 in Reviews of Weed Science showing the importance of soil water content on herbicide translocation and phytotoxicity.

REFERENCES

1. Eberlein C and Fore Z, Kochia biology. *Weeds today (USA)* 1984).
2. Heap I, Global perspective of herbicide-resistant weeds. *Pest Management Science*; **70**(9): 1306-1315 DOI Electronic Resource Number (2014).
3. Everitt JH, Alaniz MA and Lee JB, Seed germination characteristics of Kochia scoparia. *Journal of Range Management*; **36**(5): 646-648 DOI Electronic Resource Number (1983).
4. Evetts L and Burnside O, Germination and seedling development of common milkweed and other species. *Weed Science* 1972).
5. Friesen LF, Beckie HJ, Warwick SI and Van Acker RC, The biology of Canadian weeds. 138. Kochia scoparia (L.) Schrad. *Canadian Journal of Plant Science*; **89**(1): 141-167 DOI Electronic Resource Number (2009).
6. Stevens OA, The number and weight of seeds produced by weeds. *American Journal of Botany*; **19**(9): 784-794 DOI Electronic Resource Number (1932).
7. Wiese AF and Vandiver CW, Soil moisture effects on competitive ability of weeds. *Weed Science*; **18**(4): 518-519 DOI Electronic Resource Number (1970).
8. Barnsley GE and Rosher PH, The relationship between the herbicidal effect of 2,6-dichlorobenzonitrile and its persistence in the soil. *Weed Research*; **1**(2): 147-158 DOI Electronic Resource Number (1961).
9. Burnside OC and Lipke WG, The effect of applied water on preemergence applications of amiben. *Weeds*; **10**(2): 100-103 DOI Electronic Resource Number (1962).

10. Knake EL, Appleby AP and Furtick WR, Soil incorporation and site of uptake of preemergence herbicides. *Weeds*; **15**(3): 228-232 DOI Electronic Resource Number (1967).

11. Splittstoesser WE and Derscheid LA, Effects of environment upon herbicides applied preemergence. *Weeds*; **10**(4): 304-307 DOI Electronic Resource Number (1962).

12. Pedersen HJ, Kudsk P and Helweg A, Adsorption and ED50 values of five soil-applied herbicides. *Pesticide science*; **44**(2): 131-136 DOI Electronic Resource Number (1995).

13. Nishimoto RK and Rahman A, Effect of incorporation depth and soil organic matter content on herbicidal activity. *N Z J Agric Res*; **28**(4): 531-535 DOI Electronic Resource Number (1985).

14. Sheets TJ, Crafts AS and Drever HR, Soil effects on herbicides, influence of soil properties on the phytotoxicities of the s-triazine herbicides. *J Agric Food Chem*; **10**(6): 458-462 DOI Electronic Resource Number (1962).

15. Upchurch RP and Mason DD, The influence of soil organic matter on the phytotoxicity of herbicides. *Weeds*; **10**(1): 9-14 DOI Electronic Resource Number (1962).

16. Blumhorst MR, Weber JB and Swain LR, Efficacy of selected herbicides as influenced by soil properties. *Weed Technol*; **4**(2): 279-283 DOI Electronic Resource Number (1990).

17. Blackshaw RE, Moyer JR and Kozub GC, Efficacy of downy brome herbicides as influenced by soil properties. *Canadian Journal of Plant Science*; **74**(1): 177-183 DOI Electronic Resource Number (1994).

18. Weed SB and Weber JB. Pesticide-organic matter interactions. In *Pesticides in Soil and Water*, ed. by Guenzi WD. Soil Science Society of America, pp. 39-66 (1974).

19. Khan S, The interaction of organic matter with pesticides. *Soil organic matter*; **137**(1978).

20. Upchurch R, Selman F, Mason D and Kamprath E, The correlation of herbicidal activity with soil and climatic factors. *Weeds*: 42-49 DOI Electronic Resource Number (1966).

21. Alonso DG, Koskinen WC, Oliveira RS, Constantin J and Mislankar S, Sorption-desorption of indaziflam in selected agricultural soils. *J Agric Food Chem*; **59**(24): 13096-13101 DOI Electronic Resource Number (2011).

22. Ferrell JA, Vencill WK, Xia K and Grey TL, Sorption and desorption of flumioxazin to soil, clay minerals and ion-exchange resin. *Pest Management Science*; **61**(1): 40-46 DOI Electronic Resource Number (2005).

23. Moyer J, Effect of soil moisture on the efficacy and selectivity of soil-applied herbicides. *Reviews of weed science (USA)* 1987).

24. Ochsner TE, Stephens BM, Koskinen WC and Kookana RS, Sorption of a hydrophilic pesticide: effects of soil water content. *Soil Science Society of America Journal*; **70**(6): 1991-1997 DOI Electronic Resource Number (2006).

25. Stickler RL, Knake EL and Hinesly TD, Soil moisture and effectiveness of preemergence herbicides. *Weed Science*; **17**(2): 257-259 DOI Electronic Resource Number (1969).

26. Upchurch RP, The influence of soil-moisture content on the response of cotton to herbicides. *Weeds*; **5**(2): 112-120 DOI Electronic Resource Number (1957).

27. Wu CH, Santelma.Pw and Davidson JM, Influence of soil temperature and moisture on terbutryn activity and persistence. *Weed Science*; **22**(6): 571-574 DOI Electronic Resource Number (1974).

28. Gaillardon P and Dur JC, Influence of soil moisture on short-term adsorption of diuron and isoproturon by soil. *Pesticide science*; **45**(4): 297-303 DOI Electronic Resource Number (1995).

29. Gaillardon P, Influence of soil moisture on long-term sorption of diuron and isoproturon by soil. *Pesticide science*; **47**(4): 347-354 DOI Electronic Resource Number (1996).

30. Walker A and Roberts H, Effects of incorporation and rainfall on the activity of some soil-applied herbicides. *Weed Research*; **15**(4): 263-269 DOI Electronic Resource Number (1975).

31. Jury WA. *Soil physics*. John Wiley, Hoboken, NJ, (2004).

32. Brabham C, Lei L, Gu Y, Stork J, Barrett M and DeBolt S, Indaziflam herbicidal action: a potent cellulose biosynthesis inhibitor. *Plant physiology*; **166**(3): 1177-1185 DOI Electronic Resource Number (2014).

33. Kaapro J, Indaziflam - a new herbicide for pre-emergent control of weeds in turf, forestry, industrial vegetation, and ornamentals 2012).

34. Tompkins J. Environmental Protection Agency Pesticide Fact Sheet: Indaziflam (2010).

35. Trigo C, Koskinen WC and Kookana RS, Sorption-desorption of indaziflam and its three metabolites in sandy soils. *J Environ Sci Health Part B-Pestic Contam Agric Wastes*; **49**(11): 836-843 DOI Electronic Resource Number (2014).

36. WSSA. *Herbicide handbook*. Weed Science Society of America, Lawrence, KS, (2014).

37. Sparks DL, Page A, Helmke P, Loeppert R, Soltanpour P, Tabatabai M, Johnston C and Sumner M. *Methods of soil analysis. Part 3-Chemical methods*. Soil Science Society of America Inc., (1996).

38. Peng G, Wolf TM, Byer KN and Caldwell B, Spray retention on green foxtail (Setaria viridis) and its effect on weed control efficacy by Pyricularia setariae. *Weed Technol*; **19**(1): 86-93 DOI Electronic Resource Number (2005).

39. Zhang WM, Sulz M and Bailey KL, Evaluation of Plectosporium tabacinum for control of herbicide-resistant and herbicide-susceptible false cleavers. *Weed Science*; **50**(1): 79-85 DOI Electronic Resource Number (2002).

40. Mensah G, Accumulation of malathion residues in grain stored on malathion-treated wood surfaces. *Pesticide science*; **20**(3): 161-166 DOI Electronic Resource Number (1987).

41. Abdel-Kader M, Webster G, Loschiavo S and Watters F, Low-temperature degradation of malathion in stored wheat. *Journal of Economic Entomology*; **73**(5): 654-656 DOI Electronic Resource Number (1980).

42. SAS Institute. SAS/STAT 9.3 user's guide Cary, NC, USA: SAS Institute, Inc. (2010).

Appendix 1: Using a Pressure Plate Apparatus for Generating Soil Water Retention Curves

A pressure plate apparatus is one device used for constructing soil water retention curves for soils. It consists of an airtight chamber enclosing a porous ceramic plate, connected on its underside to a tube that passes through the chamber to the open air[1] (Fig. A1.1). Soil samples are fully saturated, packed into aluminum columns, and placed in contact with the ceramic on the top side. The chamber is then pressurized, which causes water to flow from the soil pores through the ceramic, and out the exit tube. Soil water retention curves from saturation to permanent wilting point can be generated by adjusting the matric potential energy of the water in the system. Soils with different physical properties were analyzed using a pressure plate apparatus at decreasing matric potentials in order to generate soil water retention curves.

Seven untreated soils were selected for this study based on a wide range of physicochemical properties. Soils were collected from the 0-10 cm depth, air-dried, and passed through a 2 mm brass sieve**.** Soil physicochemical properties were analyzed at the Colorado State University Soil Testing Laboratory using the methods from Sparks *et al*. 1996[2]. Individual soils were packed into 2.5 cm tall by 4.95 cm diameter aluminum columns with the bottom each covered with a single filter paper and square cheese cloth, and secured with a rubber band**.** Four replications were constructed for each soil and randomly assigned to one of three pressure plates through completion of the experiment.

When a soil column is placed on a wetted porous ceramic plate in a pressure chamber and the gas pressure is raised to above atmospheric pressure, the soil dries as the matric potential decreases and becomes more negative. Inside the plate, assuming gravitational potential $\Psi_z = 0$ and neglecting solutes, we determined specific matric potentials using the following equations[1];

$$\Psi_T = \Psi_m + \Psi_a$$

where Ψ_T is the total soil water potential, Ψ_m is the matric potential, and Ψ_a is the air pressure potential. The air pressure potential can also be written as $P_{soil} – P_0 = \Delta P$, and the point where the water exits the tube we reach equilibrium resulting in the $\Psi_T = 0$. The equation can therefore be rewritten as;

$$\Psi_m = -\Delta P$$

where the $-\Delta P$ (gauge pressure) is the matric potential of interest. With increased gas pressure the water moves out of the soil, through the ceramic plate, and through the plastic tube outflow system into a beaker of water. Once equilibrium is reached water flow through the tube will cease, and all points of the water in the soil columns have the same matric potential energy.

Pressure plate extraction methods from Dane *et* al. 2002 were used throughout the entirety of the experiment[3]. The individual soil columns were sub-irrigated overnight in a 0.005 M $CaSO_4$ solution to reach saturation. Once saturated, the columns were placed on porous ceramic plates in the pressure chamber. When two points of a porous medium at different potentials are brought into contact, water will flow from high to low potential until the two points are at equilibrium[1].

Using an air compressor and pressure regulation system, soils were subject to increasing gas pressure and decreasing matric potentials. Soils were allowed to equilibrate for 3 days at each pressure of -0.05, -0.10, -0.20, -0.33, and -0.50 bars. For pressures -1, -2, -4, -6, and -10 bars a total of 5 days was allowed for equilibration because there is much higher flow resistance at these high pressures. Once equilibrium was reached at each specific matric potential the samples were removed from the chamber and immediately weighed. The gravimetric water content (θ_g), bulk density (ρ_b), and volumetric water (θ_v) content of the soil samples were determined from equations;

$$\theta_g = \frac{\text{mass of water}}{\text{mass of oven dry soil}}$$

$$\rho_b = \frac{mass\ of\ oven\ dry\ soil}{volume\ of\ soil\ sample}$$

$$\theta_v = \frac{\rho_b * \theta g}{\rho_w}$$

Several different hydraulic functions with different parameters are used to represent most accurately the wide range of water retention curves. Parameters must be determined by curve fitting specific soil-hydraulic functions to measured data[1]. HYDRUS-1D implements the soil-hydraulic functions of van Genuchten (1980) who used the statistical pore-size distribution model of Mualem (1976) to obtain a predictive equation for the unsaturated hydraulic conductivity in terms of soil water retention parameters[4]. Using this software package, volumetric water contents of each soil at each potential from the pressure plates were entered into a custom application. The software provided soil hydraulic parameter estimates to use in the single porosity Van Genuchten model. For parameter estimation we assumed a constant pressure head, no hysteresis, and weighting by standard deviation was performed with a maximum of 10 iterations. The van Genuchten equation is as follows;

$$\theta_v = \theta_r + \frac{(\theta_s - \theta_r)}{[1 + |\alpha h|^n]^m}$$

The equation contains four parameters θ_r, θ_s, α, and n. θ_r and θ_s are residual and saturated water contents, α (L^{-1}) is the inverse of the air-entry value (or bubbling pressure) to scale the matric head, and n and m are dimensionless parameters[2, 4]. The n value is generally restricted to values greater than one, so the slope of the soil water retention curve is zero as the water content approaches the saturated water content[2]. The air-entry value (α) and pore-size index (n) in HYDRUS-1D are considered to be empirical coefficients affecting the shape of the hydraulic functions. The *m* (1-1/n) value is not constant in the van Genuchten function, which allows for an inflection point in the soil water retention curve. This allows the model to perform better than the Brooks and Corey (1964) type model for soils with S-shaped retention curves[2]. The Brooks and Corey model however, does not account for an inflection point but does have a distinct air-entry value. This type of model performs well with soils that have a distinct air-entry value and J-shaped retention curves[2]. Pressure plate data using the van Genuchten function to generate parameters resulted in water retention curves with $R^2 > 0.96$ (Figure 1).

From the soil water retention curves it is important to relate the soil properties to the overall curves generated by the HYDRUS-1D software using the van Genuchten function. Typically, fine textured soils have a larger saturated water content due to their greater total porosity. The residual water content of fine textured soils is also larger because it takes more energy to remove water from the soil films at higher matric potentials. Fine textured soils have a larger specific surface (surface area/mass) so can hold on to water molecules more tightly. The final factor influencing the S-shaped retention curves is the overall slope. Slope is affected by each soils pore size distribution. Coarse soils have many large pores that release water molecules at lower matric potentials all at once. Fine textured soils have a wider pore size distribution so have a much more gradual slope. From these curves we are able to determine the volumetric water content for any of these soils at matric potentials of interest. After determining the herbicide rates of indaziflam and flumioxazin that result in 80% growth reduction (GR_{80}) as a means of eliminating the herbicide rate variable, as well as the volumetric water contents of these soils at specific matric potentials, then we were able to evaluate the influence of soil moisture on the efficacy of these two herbicides.

Figure A1.1: Pressure plate apparatus

REFERENCES

1. Jury WA. *Soil physics*. John Wiley, Hoboken, NJ, (2004).
2. Sparks DL, Page A, Helmke P, Loeppert R, Soltanpour P, Tabatabai M, Johnston C and Sumner M. *Methods of soil analysis. Part 3-Chemical methods*. Soil Science Society of America Inc., (1996).
3. Dane JH, Topp G, Campbell GS, Horton R, Jury WA, Nielsen DR, van Es HM, Wierenga PJ and Topp GC, Part 4, Physical Methods. *Methods of Soil Analysis* 2002).
4. Simunek J, Van Genuchten MT and Sejna M, The HYDRUS-1D software package for simulating the one-dimensional movement of water, heat, and multiple solutes in variably-saturated media. *University of California-Riverside Research Reports*; **3**(1-240 DOI Electronic Resource Number (2005).