METRIBUZIN TOLERANCE OF EARLY-MATURING SOYBEAN GENOTYPES

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

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ABSTRACT

Metribuzin is an alternative herbicide to control glyphosate-resistant common ragweed (*Ambrosia artemisiifolia* L.) in soybean (*Glycine max L.* Merr). Metribuzin has potential to injure soybean. A screening technique to grow soybean genotypes in hydroponic solution was developed to determine differences in tolerance and sensitivity. Twenty-two named and experimental genotypes from the North Dakota State University breeding program were screened for visual injury rating, root and shoot weight, and plant height. Metribuzin × genotype interaction was significant for visual injury rating and shoot weight in both greenhouse experiments. Two tolerant and two sensitive genotypes were screened in the field for tolerance at three rates of metribuzin on soils with pH greater than 7.5. Although some genotypes were more sensitive to metribuzin in the greenhouse, none of the genotypes were sensitive to metribuzin in the field.

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INTRODUCTION AND LITERATURE REVIEW

Weeds that are resistant to herbicides have become increasingly more common in recent years. Weeds are continuously developing resistance to different herbicide modes of action. Glyphosate-resistant common ragweed (*Ambrosia artemisiifolia* L.) is becoming a widespread problem in the state of North Dakota. Every year as soybean (*Glycine max L.* Merr) hectares in North Dakota increase, the potential for glyphosate-resistant common ragweed increases. Metribuzin (4-amino-6-(1,1,-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4*H*)-one) herbicide is a control option for common ragweed in soybean. Past research has demonstrated metribuzin has the potential to injure soybean crops, especially with high pH soils. There are genetic differences among soybean for tolerance to metribuzin. Research to evaluate soybean tolerance of metribuzin has been conducted for decades across the United States and Canada, but no research has been conducted with the high pH soils of cultivars grown in North Dakota. Screening genotypes in the field on high pH soils and in greenhouse experiments will give growers information on the level of tolerance of early-maturing soybean cultivars to metribuzin.

Soybean is one of the most economically important and widely grown crops in North Dakota. In 2016, approximately 2.4 million ha^{-1} were planted (USDA/NASS). Soybean is a dicotyledonous, annual plant in the legume family and is categorized by its determinate and indeterminate growth types. Determinate cultivars stop vegetative growth before reproductive growth, or flowering, occurs. In contrast, indeterminate cultivars continue vegetative growth throughout flowering. Indeterminate cultivars are traditionally grown in northern regions of the United States, such as North Dakota. Genotype selection is an important consideration for soybean production, as genotypes can differ in yield, resistance to pests or herbicides, maturity, etc. Proper selection for a specific geographical area will result in the best yield.

Soybean Plant Density

Researchers have suggested different soybean planting densities for optimal yield, ranging from 26 plants m^{-1} to 36 plants m^{-1} (Weber and Staniforth, 1957; Lueschen and Hicks, 1977). Generally, as the population per unit of land area of soybean increases, pod and seed number produced per plant, which are important considerations for yield, decrease (Lueschen and Hicks, 1977). Increasing population density increases the height of the lowest pod-bearing node on the main stem. In indeterminate cultivars, half of the total yield comes from the bottom third of the plant.

Plant density is important for weed competition. Soybean is not a species that competes well with weeds, particularly in the early portion of the season. Soybean plant density has a role in weed control and yield (Friesen and Wall, 1986). Weber and Staniforth (1957) conducted a study with natural and planted weed infestations. Natural weed populations reduced soybean yield at a higher rate than planted weed populations. Soybean yield reductions from natural weed populations were between 17 to 23%, compared to planted populations, which reduced yield 9 to 18%. Furthermore, greater yield reductions occurred at lower soybean planting densities when weeds were present. Soybean was best able to compete with natural weed populations when weeds were of short height. If weed species were tall enough to shade soybean canopies, however, large yield reductions were more likely to occur. While weed competition decreased yield, it did not affect plant maturity, height, lodging, or seed weight of soybean. Weed competition decreases when more moisture is present in the seedbed. Determining an adequate soybean population to compete against weeds can pose a challenge. Lower soybean stands, in general, have a larger proportion of weeds throughout the season, which can delay harvest and create storage and soybean seed cleaning problems. Higher soybean stands reduce the impact of weed infestations, but have an increased potential for soybean lodging. Other problems can arise with higher soybean stands at harvest, such as delaying drying down or plugging the combine.

Soybean Row Spacing

Between-row width spacing is also important in soybean production. Hanson and Lukach (1988) found a between-row width spacing of 0.15 m had a 13%, or 215 kg ha^{-1} yield increase over a between-row width spacing of 0.3 m. In addition, both 0.15 and 0.3 m between-row spacing had yield advantages over wider between-row spacing of 0.6 and 0.9 m. These researchers indicated, between-row spacing is especially important for yield in North Dakota, as there is a shorter growing season. A quicker canopy cover in narrower rows may lead to increased yield compared with the late canopy cover in wider rows. Earlier canopy cover can help shade out weed species.

Soybean row width has an effect on yield, but does not influence plant height or test weight. Later-maturing soybean cultivars use extra space between rows more efficiently since they grow taller and produce more nodes and branches per plant (Dominguez and Hume, 1978). Wider between-row widths are also important for increased branching. Wider between-row spacings have more light available, which allows soybean plants to produce more branches. Even with extra space from wider plant spacings, extra branching per plant is finite as natural pod abortion in soybean is high (Hinson and Hanson, 1962). Branching only occurs below the first flowering node and its position is determined by a photoperiod response. Soybean genotypes respond differently to row spacing, and as a result of pod abortion, cultivars differ in maturity date, fruiting period, and average seed weight.

Metribuzin

Many factors affect the level of injury or tolerance of metribuzin on soybean. Soil factors, environmental factors, and genetics are all important considerations for metribuzin application in soybean. Metribuzin is used as either an early pre-plant, pre-plant incorporated (PPI), pre-emergence (PRE), or post-applied (POST) product (Zollinger et al., 2017). In North Dakota, metribuzin is labeled for PRE application in potato (*Solanum tuberosum*) and soybean. Weed control ratings vary for common weed species found in North Dakota soybean production systems. For many grass species, metribuzin has a control rating of poor to fair. This rating includes both barnyardgrass (*Echinochloa crus-galli*) and yellow foxtail (*Setaria pumila*). Weed control ratings vary from poor to excellent for broadleaf species. Metribuzin provides poor to fair weed control for common lambsquarters (*Chenopodium album*) and common ragweed. Control ranges from fair to excellent for redroot pigweed (*Amaranthus retroflexus*) and waterhemp (*Amaranthus tuberculatus*). Metribuzin provides medium residual weed control, which ranges from two to six weeks after application.

Metribuzin is an asymmetric triazinone photosystem (PS) II inhibitor herbicide. Photosystem II herbicides were developed in the 1950s and were among the first herbicides to be developed (Devine et al., 1993). The photosynthetic inhibiting properties, which are how these herbicides were classified, were discovered after their release. For PS II herbicides to be effective as PREs, the herbicide needs optimal water solubility, high plant uptake mobility, partitioning of thylakoid membranes, and strong binding to the herbicide binding niche. Metribuzin is an excellent example of an herbicide that meets many of these requirements.

The PS II class of herbicides inhibits electron transport, which in turn stops photosynthesis, but not energy harvest from photons. Photosystem II herbicides bind to the D1 protein on the plastiquinone binding site in the thylakoid membranes in chloroplasts on the reducing side of photosystem II (Devine et al., 1993; Shaner et al., 2014). The DI protein binding-site where the herbicide binds has been identified as large (Devine et al., 1993). The herbicide binding that occurs at the binding site stops electron transport from Quinone A (Q_A) to Quinone B (Q_B). Many effects occur as a result of electron transport inhibition. First, a buildup of electrons causes excessive radiation, which blocks pigment production. When the plastiquinone is not reduced, heat and fluorescence rise and triplet chlorophyll accumulates. As a result, maximum excess florescence is emitted, and energy spills over to oxygen and other molecules. The pigment β-carotene works to quench triplet chlorophyll after heat and florescence are produced and re-emitted. Excess triplet chlorophyll can overload the β-carotene forming singlet oxygen (Devine et al., 1993; Shaner et al., 2014). Beta-carotene production is important as it quenches singlet oxygen and provides protection for the chlorophyll from photooxidation (Devine et al., 1993). Triplet chlorophyll and singlet oxygen formed can extract hydrogen from unsaturated lipids (Shaner et al., 2014). A lipid radical is produced as a result of singlet oxygen, which causes a chain reaction resulting in lipid peroxidation. This peroxidation is not limited to lipids, and may also extend to proteins, nucleic acids, and pigments (Devine et al., 1993). Peroxidation causes membranes to lose semipermeable properties and fluidity. As a result, proteins and lipids are attacked and oxidized, which decreases chlorophyll and carotenoid production.

Other effects of metribuzin application include decreased carbon dioxide (CO_2) fixation and decreased ATP and NADPH production (Shaner et al., 2014). CO_2 fixation starts to slow within hours of electron transport inhibition. Photooxidation and phytotoxicty at the organelle, cellular, and tissue level of the plant also occur (Devine et al., 1993). After herbicide binding, damaged protein was not removed, and irreversible blocking of the electron transport system occurred. Due to the inhibition of election transport, the D1 protein is not continuously replaced. Depending on the irradiance level, plant tissue will start to show symptomology of metribuzin injury within 5 to 10 hr of photosynthesis ending. Higher irradiance levels will first show chloroplast swelling and membrane rupture.

Devine et al. (1993) noted the amount of herbicide that reaches plant tissue depends on the amount of water moving through the plant. Therefore, a high level of plant transpiration is also important for metribuzin to work efficiently. While metribuzin is able to move into the phloem, or symplasm, it fails to stay in this tissue as it diffuses into the xylem, or apoplasm. Metribuzin movement from the phloem to the xylem is due to water movement. Metribuzin is an herbicide that is readily translocated through the xylem of the plant. Once in the xylem, metribuzin will accumulate in metabolically active leaves, specifically at the leaf tip and margins. Both leaf tips and margins are considered endpoints of water flow through leaves. Chlorosis is the initial symptom of metribuzin application, which first appears on older leaf margins. Interveinal chlorosis follows chlorosis of the leaf margins in susceptible weed species. Even distribution of the herbicide in interveinal areas can lead to pronounced chlorotic effects. For hydrophilic herbicides such as metribuzin, chlorosis is more uniform in its coloration of yellow or light green before plant tissue turns white. Chlorosis appears four to six days after application, followed by leaf curling and eventual necrosis (Devine et al., 1993). The herbicide is in its most concentrated form in mature tissue such as roots and shoots and is less concentrated in fruits and seeds (Hargroder et al., 1974; Shaner et al., 2014).

Metribuzin is soil and foliar applied, with better plant uptake occurring when soil applied. For foliar applications to be effective, an adjuvant or surfactant needs to be added. A surfactant is used to increase contact between the leaf surface and herbicide (Devine et al., 1993). It also decreases the contact angle and surface tension, and increases leaf wetting properties. Reducing the contact angle allows the herbicide to better stay on the leaf surface by spreading the size of the herbicide droplet and covering a larger area of the cuticle. A surfactant is able to penetrate the cuticle and underlying tissue because it is able to break down part of the epicuticular wax on the leaf surface. Surfactants are also important for foliar application of herbicides, as residue from the droplet is left behind on the leaf surface. As a residue is left on the plant, this can result in symptomology appearing sooner. For metribuzin and other PS II herbicides, in foliar applications chlorosis can appear as early as two to five d when exposed to sunlight (Shaner et al., 2014), as opposed to four to six d when soil applied (Devine et al., 1993).

Metribuzin Metabolism

Metabolism is integral for tolerance of metribuzin in soybean. In general, there are three mechanisms used for herbicide metabolism in plants. The methods include conversion, conjugation, and deposition (Devine et al., 1993). Each mechanism in the metabolic process can also be broken down into further steps. Conversion includes hydrolysis, oxygenation, and oxidation and reduction. Conjugation typically involves sugar, amino acids, or peptides as conjugation partners of metabolites. Conjugation with glutathione is common for many plants and herbicides, including PS II inhibitors. Homoglutathione conjugation is common in soybean. The third mechanism of metabolism is deposition. Deposition occurs in vacuoles or cell walls, depending on reactions that occur during conjugation. Plants do not utilize all steps in the metabolic process to breakdown herbicides.

Metribuzin specifically depends on oxidation and reduction reactions in the first step of metabolism, which is conversion. Devine et al. (1993) noted reductive metabolism is rare in plants, but has been observed as reductive deamination in metribuzin. Deamination is the removal of an amino group from an amino acid or other compound. Following deamination, deaminated metribuzin undergoes conjugation with glutathione or homoglutathione. A glutathione anion acts as a nucleophile, while another portion of the herbicide molecule acts as the leaving group. These leaving groups include chlorine, *p*-nitrophenol, or alkyl-sulphoxide. The glutathione reaction is catalyzed with glutathione-*S*-transferases. There are both constitutive and inducible glutathione-*S*-transferases, which have a role in herbicide detoxification.

Anomalies exist with the conjugation of glutathione, in which it is replaced with homoglutathione. Homoglutathione, as opposed to glutathione, can be further metabolized in plants by peptide hydrolysis, sulfur oxygenation, or *N*-/O-malonylation (Devine et al., 1993). Soybean specifically uses homoglutathione instead of glutathione as the portion of the molecule that is transferred to the herbicide. The conjugation method in soybean after metribuzin application occurs after oxidation of a sulfur bridge to sulfoxide by S(O) methyl-displacement. Glutathione and homoglutathione conjugation can occur with amino acids or sugars. Conjugation with glucose involves nucleophilic displacement as opposed to carboxylic acid formation with amino acids. The amino acid conjugates that form as a result of metribuzin conjugation include alanine, leucine, glutamine, α-aminobutyrate, phenylanline, asparagine, and proline.

Devine et al. (1993) noted β-D-glucopyranoside is commonly formed as a conjugate in plants, along with *N*–/*O*-glycosides. Glucose can form or be added to a glycoside linkage. Plants use glucose in *N*-glucoside formation in the form of metribuzin-β-D-(*N*-glucoside). The glucoside is malonylated to metribuzin-6-*O*-malonyl-β-D-(*N*-glucoside). *N*-malonylation of metribuzin is the formation of *N*-malonyl conjugate via an exocyclic amino group. Other sugar conjugates that form in metribuzin reactions include galactose, mannose, arabinose, and rhamnose. Malonylation often is the final step of herbicide metabolism, as plants may use this step to compartmentalize and/or terminate metabolism. Following conjugation, deposition of conjugates occurs. As metribuzin forms glucosides, these conjugates are eventually deposited into the vacuole (Devine et al.,1993). Falb and Smith (1987) found conjugates that form in soybean following metribuzin metabolism are not phytotoxic. The rate at which a soybean cultivar degrades metribuzin is more important than the conjugates found within the plant.

Abusteit et al. (1985) evaluated metribuzin injury in two soybean species and determined tetraploid soybean plants were better able to metabolize metribuzin than diploid soybean plants. Diploid plants contained more metribuzin in plant tissue four and eight d after metribuzin application than tetraploid plants. Diploid plants contained 97 and 86% metribuzin in their shoots, while tetraploid plant shoots contained 34 and 21% metribuzin at the same time after application. Diploid roots contained 98 and 93% metribuzin, while tetraploid species contained 8 and 4% metribuzin after the same application time. As a result, metribuzin phytotoxicity may occur in soybean as plants are unable to inactivate absorbed metribuzin soon enough after application. Metribuzin absorption rate was relatively constant for diploid soybean plants at 6 to 24 hr after metribuzin application.

Metribuzin Sensitivity of Soybean

Greater concentrations of metribuzin herbicide accumulate in sensitive soybean cultivars compared to tolerant cultivars (Wax et al., 1976; Friesen and Wall, 1986). Many metribuzin tolerance studies have evaluated genotypes ‘Tracy’ and ‘Semmes’, which have continuously been identified as sensitive genotypes (Barrentine et al., 1976; Wax et al., 1976; Littlejohns et al., 1977; Hardcastle, 1979; Eastin et al., 1980). One recessive gene, *hm*, has been identified as the gene responsible for sensitivity to metribuzin in soybean (Kilen and Barrentine, 1983; Hanson and Nickell, 1986). The *hm* gene for metribuzin sensitivity is found in soybean of all maturity groups (Hanson and Nickell, 1986). Edwards et al. (1976) evaluated soybean cultivars Semmes and 'Hood' and their reciprocal crosses. The F_1 progeny were moderately sensitive and showed injury, but no death. The F_2 and backcross generations fit a 3:1 segregation ratio for tolerance: sensitivity to metribuzin. Kilen and Barrentine (1983) performed an experiment evaluating sensitivity of four soybean cultivars, one breeding line, and their respective crosses. Tracy and Semmes were used as parents, and F_2 populations comprised of 400 individuals from these crosses resulted in all metribuzin sensitive plants. This study again confirmed *hm* is responsible for sensitivity to metribuzin in soybean crops. Eastin and his colleagues (1980) also evaluated crosses derived from Tracy and Semmes. Two crosses where Semmes was used as a parent were considered moderately tolerant to metribuzin at the recommended use rate of 0.6 kg ha^{-1}.

While the results of sensitivity in Tracy and Semmes were consistent among experiments, both are determinate cultivars, which are not grown in North Dakota. Field and greenhouse experiments have also evaluated indeterminate cultivars. 'Harosoy 63' and 'Clay' have been identified as moderately tolerant cultivars to metribuzin (Barrentine et al., 1976; Wax et al, 1976). Both cultivars have maturities adapted to North Dakota. Wax et al. (1976) found Harosoy 63 and Clay showed similar response when subjected to three rates of metribuzin. At a rate of 0.56 kg ha^{-1}, neither cultivars showed signs of injury. Plant injury was 20 to 25% of all plants evaluated when a rate of 0.84 kg ha^{-1} metribuzin was applied. Plant injury was 50 to 55% when metribuzin was applied at the highest rate of 1.17 kg ha^{-1}.

Metribuzin Sensitivity in Other Crop Species

Sensitivity to metribuzin has also been identified in other cultivated species such as durum (*Triticum durum*), chickpea (*Cicer arientinum*), and field pea (*Pisum sativum*). Durum and other wheat species vary in their tolerance to metribuzin. Villarroya et al. (2000) evaluated one tolerant and one sensitive durum wheat cultivar and their reciprocal crosses for metribuzin sensitivity. Cytoplasm was proposed to have a role in metribuzin sensitivity, but was not confirmed at the conclusion of the study. More genetic variability for tolerance to metribuzin was found within the sensitive cultivars than tolerant cultivars. Results from the reciprocal crosses did not show a significant difference in tolerance to metribuzin inherited from the two durum wheat cultivars and their F_1 progeny. Tolerance was controlled by dominant alleles in this cross, although it was not complete dominance. These results confirmed metribuzin tolerance was not due to cytoplasmic inheritance. Metribuzin tolerance in durum was complex and controlled by many alleles.

Chickpea is also sensitive to metribuzin. Due to the growth rate and establishment of chickpea, few herbicides are labeled for POST use (Gaur et al., 2013). Gaur et al. (2013) evaluated 300 chickpea genotypes for tolerance to metribuzin, using a 1 to 5 scale. On the rating scale, one indicated highly tolerant to metribuzin and five was highly sensitive. Injury scores for metribuzin tolerant lines ranged from 1.5 to 2.88, and the most sensitive lines ranged from 4.5 to 4.75. The scale was a simplified version of a 0 to 9 scale and was considered a reliable estimate of metribuzin injury. Genotypes that were most tolerant to metribuzin were included in crosses with high yielding cultivars to establish tolerant chickpea breeding lines.

Field pea, much like chickpea, has a limited number of herbicide options apart from metribuzin. As a result, metribuzin is one of the only control options, and some genotypes express sensitivity to metribuzin (Al-Khatib et al., 1997). Greater herbicide injury in field pea occurs when metribuzin is POST applied or on sandy soils (Al-Khatib et al., 1997). Javid et al. (2017) evaluated 185 recombinant inbred lines (RILs) derived from a cross of a tolerant and sensitive parent to metribuzin. A preliminary assay was conducted to validate a metribuzin rate most injurious to plants. Following the preliminary assay, three separate assays were conducted to differentiate injury between RILs and parents. A metribuzin rate of 10 ppm was most effective in differentiating tolerant and sensitive RILs. The assay studies evaluated visual injury symptoms on a 0 to 6 scale, as well as in addition to percent necrosis, reduction of shoot dry weight, plant height, and number of nodes per plant two wk after application. All germplasm screened in the preliminary study showed some level of sensitivity to metribuzin at 10 ppm. A tolerant parent, tolerant RILs, and a moderately tolerant check showed significantly less necrosis and visual injury score than a sensitive parent, sensitive RILs, and a sensitive check. Plant dry weight was significantly reduced for all RILs in the assay studies. The dry weight of the tolerant parental line was reduced 46 to 54%, while the dry weight of the sensitive parent was reduced 84 to 88%. Plant height was significantly reduced for all RILs, and the height of the parent lines were not significant when compared to each other. Nodes per plant were significantly reduced for all RILs. The metribuzin tolerant parental line produced more nodes per plant compared to the metribuzin sensitive parent. Metribuzin inheritance was determined to be simple, indicated by heritability estimates of 0.79 to 0.96 for visual injury score and percent necrosis.

Soil Factors Affecting Sensitivity to Metribuzin

Wax et al. (1976) and Hardcastle (1979) indicated many soil factors contribute to metribuzin injury of soybean. These include mobility, adsorption, (Wax et al., 1976) microbial degradation, soil texture and organic matter (OM), and soil moisture/rainfall (Hardcastle, 1979).

Risk of crop injury from metribuzin increases as soil pH increases (Ladlie et al., 1976a). Ladlie et al. (1976a) observed a clear dividing line between soil pH of 5.0 and 5.4. Slight injury occurred at the lower pH compared to more severe injury at a pH of 5.4. In a separate study, Ladlie et al. (1976b) found more residual metribuzin was extracted from the top 5 cm of soil solution at the soil pH of 4.6 compared to soil pH of 6.7. Less soil adsorption occurred at the pH of 6.7 and more metribuzin was bound to soil particles at the pH of 4.6. Metribuzin phytotoxicty is more likely to occur in high pH soils because more metribuzin is in the soil solution and less is adsorbed to soil particles. Soybean phytotoxicity decreases with lower pH soils since metribuzin is more tightly held to soil particles and has a higher affinity for soil OM. Metribuzin that is adsorbed by soil particles is later broken down by soil organisms. It is a moderately water-soluble herbicide with a Ksp value of 1100 mg L^{-1}, which can also result in a potential carryover issue the following growing season (Shaner et al., 2014). Leaching potential decreases with higher soil OM, but leaching potential increases when the pH of the soil increases (Shaner et al., 2014). Moshier and Russ (1981) discovered sandy loam soils with high pH and low OM can reduce injury if metribuzin applications occur three wk before planting.

Coble and Schrader (1973) found metribuzin tolerance of soybean increased as the soil OM content increased. They evaluated three rates of metribuzin and five soils with varying OM percentages. On a soil with 1.1% OM, the lowest percentage evaluated, injury ranged from 32 to 95% at use rates of 0.56, 1.12, and 1.68 kg ha^{-1}. Soil OM percentages of 3.3 and 7.8 had less than 25% plant injury at a 1.12 kg ha^{-1} metribuzin rate. At the same OM matter percentages of 3.3 and 7.8, injury ranged from 35 to 44% at the 1.68 kg ha^{-1} rate. As the soil OM increased to 16.8%, 1 to 2% injury was expressed at 1.12 and 1.68 kg ha^{-1} rates, respectively. When soil OM was approximately 40%, there was no observed injury at any of the metribuzin rates evaluated.

Coble and Schrader (1973) concluded as soil OM percentages rose above 20.2, soybean has a high tolerance to metribuzin up to rates of 2.24 kg ha^{-1}.

Moomaw and Martin (1978) found metribuzin phytotoxicity was influenced by rainfall on a soil with a pH of 7.9. Years with more rainfall and cooler temperatures resulted in more injury compared to warmer, drier years. Rainfall after application provides incorporation. Injury can differ between cultivars depending on whether it was or was soil incorporated into soil after application (Wax et al., 1976). Using recommended rates of metribuzin on soils with a pH less than 7.0 caused minor injury to soybean while using a rate of 0.6 or 0.8 kg ha^{-1} on a soil with a pH of 7.9 resulted in greater than 60% injury (Moomaw and Martin, 1978). Reductions in plant stand were not significant as the rate increased. Coble and Schrader (1973) also found rainfall influenced metribuzin injury in soybean. Using simulated rainfall, they found metribuzin injury was less severe when no water was applied in the first ten d after application. Injury increased as the herbicide rate and OM increased, regardless of the amount of rainfall the soybean plants received.

Belfry et al. (2015) applied metribuzin and S-metalachlor + metribuzin at rates of 2. 240 kg a.i. ha^{-1} and 3.2 kg + 1.3 kg a.i. ha^{-1} to different soils at different locations with pH ranging between 6.4 and 7.8 and OM between 3.0 and 7.1%. Soybean height was reduced 41 and 30%, respectively, on sensitive cultivars compared to untreated control cultivars where soil pH was 7.7 to 7.8 and OM was 3.0 to 3.8%. The second location, where pH was between 6.4 and 7.0 and OM was 5.9 to 7.1%, height was reduced 5% or less relative to the untreated control cultivars. Yield was reduced between 21 and 28% on the higher pH soil on sensitive cultivars, compared to reductions of 10% or less at the location where soil pH was 6.4 to 7.0. Relative to the control, three sensitive cultivars experienced a 62 to 69% reduction in yield from metribuzin applications depending on soil pH and OM at the location. A major difference between the two locations was the OM percentage. Some cultivars were less sensitive to injury than other cultivars. However, due to variable soil conditions and weather, location-specific factors and cultivar specific tolerances shared a role in injury differences between the two locations.

Belfry et al. (2016) conducted a similar study, which evaluated PRE and POST herbicide options in conventional soybean. The experiment was conducted on soil with a pH ranging from 7.4 to 7.8 with OM contents ranging from 2.4 to 4.8%. Six treatments with metribuzin applied in combination with other herbicides were applied as PREs. Herbicide injury was low for all treatments two wk after soybean emergence. All metribuzin treatments provided greater than 90% control of common ragweed. At four wk after emergence, three metribuzin combinations with imazethapyr, S-metolachlor, or S-metolachlor + cloransulam + methyl, controlled common ragweed 79 to 88% four wk after emergence. Some metribuzin combinations reduced soybean yield, but reductions were not significant between treatments.

Greenhouse Screening

Researchers have screened for tolerance of several soybean cultivars to metribuzin in greenhouse experiments. Barrentine et al. (1976) screened 23 genotypes in a hydroponic solution and observed injury of cultivars evaluated at a metribuzin rate of 0.125 ppm. Hardcastle (1979) conducted a similar experiment and quantified injury using dry weight percentages. Soybean cultivars with a dry weight threshold of 87% or higher as a percent of the untreated control were considered tolerant to metribuzin and those with a dry weight percentage below 54% were deemed sensitive (Hardcastle, 1979). Eastin et al. (1980) screened 16 soybean genotypes in soil and applied four rates of metribuzin PPI. They used 0, 0.33, 0.66, and 1.1 kg ha^{-1} rates of metribuzin. The rate of 1.1 kg ha^{-1} resulted in injury of all genotypes. Injury was more easily differentiated between application rates when 0.66 kg ha^{-1} was used compared to rates of 0 and 0.33 ha^{-1}. The sensitive genotypes were injured at a rate of 0.66 kg ha^{-1}, but injury did not significantly increase when the rate was increased to the 1.1 kg ha^{-1} rate.

Objective and Rationale

Plant scientists have studied soybean injury caused by metribuzin for decades. Soil properties are important factors to determine the level of injury caused by metribuzin in soybean, but there is also evidence genetics play a role in the level of injury (Ladlie et al., 1976a; Wax et al., 1976). There are genetic differences among soybean cultivars for tolerance to metribuzin. Soil factors may have a greater influence on soybean injury than whether or not the genotype is sensitive to metribuzin injury. Metribuzin applied on high pH soils may cause injury to soybean depending on the rate of application and other factors. This is important in North Dakota and Minnesota where the soil pH is often greater than 7.5. The objective of this study was to determine whether an interaction was present between metribuzin rate and soybean genotype. Soybean genotypes were screened in the greenhouse and in field trials where soil pH was greater than 7.5. Both experiments were conducted to determine which genotypes of soybean were sensitive or tolerant to metribuzin.

MATERIALS AND METHODS

Experiments were conducted in the greenhouse and field. Genotype and metribuzin rate were considered fixed effects for the greenhouse experiments. Experiment runs were considered as random effects. Locations were considered random effects, and cultivar and metribuzin rates were fixed effects in the field experiment. Data from both the field and greenhouse experiments were subjected to analysis of variance through SAS (SAS Institute, 2011). A $P < 0.05$ value was used to determine significance of metribuzin injury between cultivars. An LSD with a Type I error rate of 0.05 was used to determine significance of injury rating, plant height, and root and shoot weights among cultivars (Carmer et al., 1989).

Greenhouse

Experimental Design

Soybean genotypes were screened using a hydroponic method. The treatment design was a split-plot with 0 ppm metribuzin and 0.125 ppm metribuzin treatments assigned to whole plots. Experimental lines or released cultivars were assigned to the sub-plots of the experiment, and the experiment was repeated twice. There were three replications of these randomized block experiments. The experimental unit consisted of four soybean plants that were grown side-by-side and spaced 3.8 cm apart. A preliminary study was conducted before experimental genotypes from the North Dakota State University soybean breeding program were screened.

Light and Temperature Control

Light and temperature settings were controlled to simulate natural growing conditions of indeterminate soybean cultivars grown in North Dakota, set at 16 h light per d, and 8 h of dark per night. The timer started the light cycle at 6:00 AM and ended at 10:00 PM. The soybean plants were grown at a temperature of 21°C. Due to constraints of greenhouse environmental conditions, the optimal time to perform greenhouse experiments was November through March. The preliminary experiment started November 2016 and concluded January 2017. The breeding genotype experiments started January 2017 and concluded April 2017.

Genotype Selection

The preliminary experiment evaluated four check cultivars, two which were sensitive and two which were tolerant to metribuzin. The breeding genotype experiments utilized one sensitive and one tolerant cultivar from the preliminary study, two proprietary releases, and 22 genotypes from the NDSU breeding program. Fourteen genotypes were screened in each of two different experiments. Each genotype was screened twice, once in each of the separate greenhouse trials. 'Altona' and Clay were the cultivars used as the sensitive and tolerant check, respectively, in both the preliminary and breeding genotype experiments (Wax et al., 1976). Altona, Clay, and a proprietary release were included in each run of the breeding genotype experiments, along with 11 experimental breeding genotypes.

Seed of each genotype was planted in vermiculite in plastic starter trays 13 cm deep and watered daily for a week to promote soybean germination. The seedlings were rinsed with distilled water to remove excess vermiculite before transplanting. After seedling germination, but before the VE stage, seeds were transplanted to plastic containers and grown hydroponically in a Hoagland's nutrient solution (Hardcastle, 1979). Plants were placed in 1.3 cm thick foam insulation boards cut to the size of each tub, with holes cut large enough for the seedlings to sit on and the roots to fit through. Each hole was 0.6 cm in diameter, spaced 2.5 cm apart, so the plants did not fall through, but had sufficient space to grow (Hardcastle, 1979). After transplanting, seedlings were left in the hydroponic solution for a wk before the metribuzin was added. All genotypes were grown in the same polyethylene tub. Seedlings were grown in black, spray painted 20 L containers in the experimental genotype experiments, and 5.7 L containers for preliminary experiments. Painting the containers black decreased available light on the perimeter, and decreased algae growth during the duration of the experiment.

Hydroponic Solutions

Each genotype was grown in a hydroponic solution. The hydroponic solution was composed of six nutrient solutions mixed together. Solutions were mixed in the following order to reduce potential of precipitates forming. Formulas of the six nutrient solutions, and their rates of addition to the final hydroponic solution are shown below (Table 1).

Figure 1. Polyethylene tubs used for hydroponic experiments.

Table 1. Formulas for six nutrient solutions needed for hydroponic solutions.

Solution	Compound	Formula	Concentration	Molecular wt (g)	mL made	g	mL L^{-1}
1	Potassium phosphate	KH_2PO_4	1 M	136.10	500	68.00	1
2	Potassium nitrate	KNO_3	1 M	101.10	1000	101.10	5
3	Calcium nitrate	$Ca(NO_3) \times 4H_2O$	1 M	236.20	1000	236.20	5
4	Magnesium sulfate	$MgSO_4 \times 7H_2O$	1 M	246.50	1000	246.50	2
5	Boric acid	H_3BO_3	-	2.86	1000	2.86	1
	Magnesium chloride	$MnCl_2 \times 4H_2O$	-	1.81		1.81	
	Zinc sulfate	$ZnSO_4 \times 7H_2O$	-	0.22		0.22	
	Copper sulfate	$CuSO_4$	-	0.05		0.05	
	Ammonium molybdate		-	0.02		0.02	
6	Fe-EDTA		-	4.10	500	4.10	1

The seedling growth containers were filled with 5 L or 15 L of hydroponic solution, depending on the experiment. Preliminary experiments utilized 5 L of hydroponic solution when genotypes were screened. The experiments where experimental breeding genotypes were screened utilized 15 L of hydroponic solution. Fifteen liters of hydroponic solution were mixed at a time, and transferred to each tub. Planting to harvest required three weeks. The Hoagland's solution was added to the hydroponic solution once for the duration of the experiment. The transplanted seedlings were left for a week to equilibrate in the hydroponic solution before metribuzin was added. As plant uptake and heating of the greenhouse occurred, distilled water was added to bring solutions to volume. The containers of hydroponic solution did not require aeration, but received some aeration from extra holes drilled in the foam insulation. Barrentine et al. (1979) indicated aeration, or lack thereof, does not affect plant injury from metribuzin. The pH of the hydroponic solution was kept at 5.6 to 5.7. The pH was monitored daily using a traceable pH/ORP meter after metribuzin was applied, as the pH of containers with metribuzin had a tendency to rise rapidly following the application. As the pH rose, 0.01 or 0.1 M of hydrochloric acid, as required, was added to bring the pH back down to 5.6 or 5.7. Increases in the solution pH above 5.7 results in iron deficiency chlorosis, which is undesirable. Symptoms characteristic of iron deficiency chlorosis and metribuzin injury are differentiated by the location injury first appears. Iron deficiency initially appears on new leaf tissue, whereas metribuzin injury initially affects older leaf tissue (Devine et al., 1993).

Metribuzin Application

Metribuzin was applied to whole plots which received this treatment after the first trifoliolate leaf expanded, which was seven to ten d after the soybean was transplanted. The metribuzin that was added was a technical grade form. The powder form of metribuzin was added to 98 ml of warm, deionized water and 2 ml of 95% ethanol was added to help dissolve the herbicide. The distilled water was warmed utilizing a hot plate before metribuzin was added. After the distilled water was warm, a stir bar was added to the 100 ml solution for 15 minutes to fully dissolve the metribuzin. The herbicide was immediately added to the preliminary and breeding genotype experiments at a rate of 0.00063 g and 0.0019 g. These calculations follow the 0.125 ppm rate provided by numerous researchers (Barrentine et al., 1976; Edwards et al., 1976; Hardcastle, 1979).

Visual Injury Scale

Visual herbicide injury ratings were taken once, at the time of harvest, using a 1 to 5 subjective scale, at increments of 0.5. In this scale, a rating of 1 represents no metribuzin injury, 2 represents 25% metribuzin injury, 3 represents 50% metribuzin injury, 4 represents 75% metribuzin injury, and 5 is total death. The soybean plants were grown for a week following metribuzin application before they were harvested. The height of each soybean plant was collected at the time of harvest to determine whether metribuzin reduced plant height between treatments. Shoots and roots were harvested separately. The shoots (stems and leaves) and roots of the soybean plants were oven-dried to measure total dry weight (TDW) at a temperature of 66°C for 72 h. Shoot and root dry weight measurements were collected once, at the time of harvest. Plant height and shoot and root reductions were quantified percent reducation. Percent reduction was defined as 1- (response of metribuzin/no metribuzin). Hardcastle (1979) used a threshold of TDW greater than 87% as tolerant and TDW less than 54% as susceptible, relative to the untreated control. Following the drying period, the shoot and root harvested from each genotype were weighed and recorded. The results of the weight measurements were analyzed using SAS to determine any statistical differences between the control (no metribuzin applied) compared to the metribuzin applied containers.

Field

Experimental Design

Field experiments in 2016 and 2017 were randomized complete block designs and the treatment design was a split-plot. Metribuzin rates of 0, 0.33, and 0.67 kg ha^{-1} were assigned to whole plots and four soybean genotypes were assigned to the subplots. Field experiments were conducted at four locations and included three replicates at each test site. Selected soil characteristics for the field sites are shown below (Table 2). Soils tests for phosphorus and potassium were in the very high category (data not shown).

Table 2. 2016 and 2017 soil factor data across the four locations.

	Location			
Soil factor	Arthur	Casselton	Fairmount	Prosper
2016				
pH	7.8	7.2	7.9	7.1
Soil mapping unit	Lankin-Gilby loam	Kindred-Bearden silty clay loam	Wheatville silt loam	Kindred-Bearden silty clay loam
Organic matter (%)	3.5	4.0	3.6	4.9
Soluble salts (EC mmhos cm^{-1})	0.31	1.6	0.78	0.45
2017				
pH	7.4	7.2	7.8	6.8
Soil mapping unit	Lankin-Gilby loam	Kindred-Bearden silty clay loam	Antler clay loam	Bearden-Lindaas silty clay loam
Organic matter (%)	3.4	3.9	4.5	3.8
Soluble salts (EC mmhos cm^{-1})	0.30	0.58	0.60	0.42

Genotype Selection

Genotypes with tolerance and sensitivity to metribuzin were evaluated at two rates of metribuzin, and compared to a control without metribuzin. Four genotypes were planted at locations in eastern North Dakota in 2016 and 2017: Prosper, Casselton, Fairmount, and Arthur. In both 2016 and 2017, locations were planted in early to mid-May. In 2016, Casselton was planted on 4 May, Prosper on 7 May, Arthur on 9 May, and Fairmount was planted on 17 May. In 2017, Casselton was planted earliest on 5 May, followed by Fairmount on 11 May, and both Arthur and Prosper were planted on 12 May. The genotypes planted in 2016 and 2017 had similar maturities. They were 0 or 00 maturity groups. Released cultivars Clay, ‘Wilkin’, Altona, and ‘Norman’ were planted in 2016. In 2016, Clay and Wilkin were the tolerant cultivars, and Norman and Altona were the sensitive cultivars to metribuzin. Three experimental genotypes, ‘ND12- 24081’, ‘ND14-6120’, and ‘ND14-5732’, and one released cultivar, ‘ND17009GT’, from the NDSU soybean breeding program were planted in 2017. The tolerant genotypes in 2017 were ND14-5732 and ND14-6120, and the sensitive genotypes were ND12-

24081 and ND17009GT. The level of tolerance for each genotype was determined from greenhouse experiments. An early-maturing soybean cultivar was planted as a border to minimize any border effects in 2016 and 2017. The cultivar planted in 2016 was conventional, and the cultivar planted in 2017 was glyphosate-tolerant.

Seed for the 2016 field season was grown in a winter nursery in Chile fall 2015. Only a limited amount of seed was available for field experiments in 2016. Seed for the 2017 field season was grown at Casselton and Prosper, ND in 2016. Each experimental unit was planted as a two-row plot, 6.4 meters long with a 0.76 meter between-row spacing using a Max Emerge 2 planter modified for plot research. Plots were end trimmed to a harvest length of 4.3 m. The plots were seeded at a rate of 489,300 live seeds ha^{-1} in 2016, and a seeded at a rate of 244,650 seeds ha^{-1} in 2017. The seed planted in 2016 was from cultivars released in the 1960s and 1970s. As a result, the germination percentage was lower, and therefore, soybean was planted at a higher seeding rate than 2017. After emergence, a one-meter row length in each plot was counted to determine percentage of emergence of each genotype.

Herbicide Applications

Metribuzin was applied at 0, 0.33, and 0.67 kg ha^{-1} rates on high pH soils. The recommended rate of metribuzin application for soil types with a pH above 7.5 is 0.33 kg ha^{-1} formulated as a dry flowable. Metribuzin was applied once pre-emergence. To reduce or eliminate metribuzin spray drift, each whole plot had a 1.5 m wide border. In addition to metribuzin, sulfentrazone (N-[2,4-dichloro-5-[4-(difluorpmethyl)-4,5-dihydro-3-methyl-5-oxo-1*H*-1,2,4-triazol-1-yl]phenyl] methanesulfonamide) was applied to all whole plots to provide general weed control. In the plot-area, herbicide treatments were applied with a custom-built four-wheeler mounted sprayer designed by technicians in the soybean project. The sprayer held a 37.9 L spray capacity and utilized High Flo Gold Series spray booms at a rate of 14.4 L per minute at a pressure of 310 kPa. The total boom length was 3.2 m with seven XR Tee Jet 80015V3 nozzles applied delivering a rate of 95 L ha^{-1}. After emergence and spray applications, field sites were checked weekly for weeds and weeded when necessary. The weeds were removed by hand pulling and hoeing. Glyphosate was applied post-emergence as needed in 2017 as the genotypes selected in field experiments were glyphosate-tolerant.

Visual Injury Rating

The first injury rating was taken approximately one mo after planting at each of the four locations. Ratings were taken on a weekly basis over the course of four wk to evaluate metribuzin injury on the four genotypes planted. Injury ratings were taken roughly one, two, three, and four wk after soybean emergence. A fifth injury rating was taken approximately one month after the fourth injury rating. Visible injury was measured using the same subjective, 1 to 5 scale utilized for greenhouse injury ratings. Taking injury measurements over the course of multiple weeks provided data on herbicide damage as the season progressed.

Soil Sampling

Soil samples were collected at Casselton, Arthur, Prosper, and Fairmount at a depth of 0 to 0.15 m in 2016 and 2017. The soil pH and OM were the desired factors to characterize response. Nine soil cores were obtained from each field location, one from each experimental unit in a replicate. The Arthur location had a soil pH of 7.4 and OM of 3.4%. The Casselton location had a soil pH of 7.2 and OM of 3.9%. The pH at the Prosper location was lower than expected, with a pH of 6.8, and OM of 3.8%. The Fairmount location had the highest soil pH at 7.8 and OM of 4.5%. Soil samples confirmed each of the four locations had a high pH soil. A soil pH greater than 7.5 is the threshold at which metribuzin injury is more likely to occur in soybean. For this reason, the high soil pH was desirable for our field experiments. Soils samples were also collected in 2017 to determine whether any soybean cyst nematodes were present at any location. No soybean cyst nematode eggs were detected at any of the four sites.

Harvest

Each two-row soybean plot was harvested with a research combine. The maturity date was the date when 95% of the pods had reached mature pod color. Yield and moisture data of each experimental unit was collected with the combine at the time of harvest. After harvest, yield was adjusted to 130 g kg^{-1} moisture.

RESULTS AND DISCUSSION

Preliminary Greenhouse Experiments

A preliminary study was conducted to validate a hydroponic technique utilized by other researchers (Barrentine et al., 1976; Hardcastle, 1979). Clay and Wilkin were used as tolerant genotypes. Altona and Norman were used as sensitive genotypes.

Table 3. Analysis of variance of four soybean genotypes with and without metribuzin, evaluated in the greenhouse.

		Mean Square			
Source of Variation	df	Injury rating	Shoot weight	Root weight	Plant height
Experiment (E)	1	0.26**	0.96**	0.0225	280.82**
Rep (E)	4	0.08	0.02	0.0006	18.55
Metribuzin (M)	1	26.26	1.92*	0.0547	46.61
Genotype (G)	3	2.69	0.01	0.0117	106.29
M × G	3	2.69	0.06*	0.0001	20.39*
M × E	1	0.26	0.01	0.0005	15.08
G × E	3	0.69	0.03	0.0040	20.98
G × M × E	3	0.69	0.01	0.0006	0.77
Rep × M × E†	4	0.08	0.01	0.0005	9.70
Error b	24	0.06	0.01	0.0009	3.27

*, **Significant at 0.05 and 0.01 levels, respectively.
†Error a.

Metribuzin rate influenced shoot weight (Table 3). The genotype source of variation was not significant for any of the variables evaluated. A metribuzin rate × genotype interaction was significant for shoot weight and plant height. No significance was detected for metribuzin × experiment interaction, genotype × experiment interaction, genotype × metribuzin × experiment interaction, and rep × metribuzin × experiment interaction. The lack of significance for genotype × metribuzin × experiment interaction shows that the results obtained in this experiment were repeatable. Injury rating was non-significant in the combined ANOVA, but it was significant when experiments were analyzed individually.

Ratings for the metribuzin visible injury were consistent across experiments for three of the four cultivars evaluated (Table 4). The visual injury rating for Wilkin was greater in the first experiment than in the second experiment by more than 1.5 units. The visual injury ratings of the other three cultivars were similar between the two experiments.

Table 4. Injury ratings of four soybean genotypes from individual and combined greenhouse experiment, at 0.125 ppm level of metribuzin application.

	Rating†		
Genotypes	Run 1	Run 2	Combined runs
	----------Score----------		
Altona	3.50	3.33	3.42
Clay	1.50	1.67	1.58
Norman	1.50	2.00	1.75
Wilkin	4.00	2.33	3.17
LSD (0.05)‡	0.28		

†A metribuzin injury rating of 1 represents no metribuzin injury, 2 represents less than 50% metribuzin injury, 3 represents 50% metribuzin injury, 4 represents greater than 50% metribuzin injury, and 5 is total death.
‡LSD compares means of genotypes, averaged across metribuzin rates.

Altona and Wilkin were consistently the more sensitive genotypes in greenhouse experiments. Cultivars with an injury rating of 2 or less were considered highly tolerant in the context of my greenhouse study. Wax et al. (1976) reported Norman was sensitive and Wilkin was tolerant, which was not consistent with my results. A possible explanation for the inconsistency between Norman and Wilkin injury ratings may have been seed source. The seed used for the greenhouse experiment came from a germplasm bank, and was increased in a winter nursery. The seed sources were not uniform and were a mixture of unknown genotypes. The seed sources were identified as a mixture of genotypes, based on maturity data in the field. Seed for other greenhouse experiments came from seed increases at Prosper and Casselton in 2016, which were considered pure.

Hanson and Nickell (1986) noted symptoms of plant injury appeared within three days of metribuzin application. They evaluated Altona since previous research concluded it was sensitive to metribuzin. As shown in Table 4, Altona had an average injury rating of 3.42 in the combined runs of the preliminary study. Wilkin had an average injury rating of 3.17. In one of the individual runs, Wilkin was rated as high as 4. Four was the highest rating of any experiment, and no plant death was expressed. Any plants that were killed were classified as sensitive in a study conducted by Hanson and Nickell (1986). They did not mention a scale, i.e. one to five, to differentiate moderately or highly sensitive. They also discovered that placing tolerant plants next to each other in the container delayed injury symptoms due to an unnamed environmental effect. This could account for the large difference in injury rating of Wilkin from the first run of the experiment, a 4, to the second run where it was identified as 2.33. Other reasons for the difference may be due to heating of the greenhouse or type of water used in the experiment.

Table 5. Means of shoot weight of four soybean genotypes grown in greenhouse experiment, with and without metribuzin.

Genotypes	No metribuzin	Metribuzin	Weight reduction
	---------------------------g	--------------------------	-------------%-------------
Altona	1.17	0.62	47
Clay	1.10	0.83	25
Norman	1.05	0.77	27
Wilkin	1.17	0.67	43
LSD (0.05)†			17
LSD (0.05)‡	0.73		

†LSD compares means of same level of metribuzin across different level of genotype.
‡LSD compares means of different levels of metribuzin across same level of genotype.

Averaged across four cultivars, the mean shoot weight when no metribuzin was applied was 1.12 g and the mean shoot weight when metribuzin was applied was 0.72 g. When no metribuzin was applied, Altona and Wilkin had the greatest shoot weights. Metribuzin reduced the shoot weights by almost half for both genotypes (Table 5). Shoot weight was reduced for Clay and Norman when metribuzin was added, but the reductions were not as great compared to Altona and Wilkin.

Metribuzin did not have an effect on root weight for any of the four genotypes evaluated. While root weight was unaffected, metribuzin had an effect on plant height. Plant height was reduced 5% for Norman when the metribuzin treatment was compared to the no metribuzin treatment (Table 6). Clay and Altona were identified as tolerant and sensitive, respectively. The increase in plant height of Clay when metribuzin was added showed metribuzin had no effect on the height of Clay. Plant height of Altona and Wilkin was reduced 16% when metribuzin was applied. Altona and Wilkin were sensitive cultivars. Plant height reduction was consistent with injury rating, as height reduction occurred with metribuzin application for sensitive genotypes. My results were consistent with Littlejohns et al. (1977).

Table 6. Plant height means of four soybean genotypes grown in greenhouse experiment, with and without metribuzin.

Genotypes	No metribuzin	Metribuzin	Height reduction
	-------------------------cm-------------------------		-------------%------------
Altona	26.32	22.18	16
Clay	21.38	22.55	-5
Norman	18.00	17.18	5
Wilkin	25.53	21.43	16
LSD (0.05)†			9
LSD (0.05)‡	14.15		

†LSD compares means of same level of metribuzin across different level of genotype.
‡LSD compares means of different levels of metribuzin across same level of genotype.

Experimental Genotypes Experiments

First Set of Experimental Genotypes

After the preliminary experiment was conducted, and visual injury rating was deemed repeatable, experimental breeding genotypes from the NDSU soybean breeding program were evaluated. Two sets of 14 genotypes were evaluated. Clay was used as a tolerant control, and Altona as a sensitive control in both experiments. The ANOVA from the first set of 14 genotypes was provided (Table 7).

Table 7. Analysis of variance of the first set of fourteen soybean genotypes with and without metribuzin, evaluated in the greenhouse.

		Mean Square			
Source of Variation	df	Injury rating	Shoot weight	Root weight	Plant height
Experiment (E)	1	0.00	0.59*	0.0115	460.66*
Rep (E)	4	0.12	0.06	0.0044**	30.47**
Metribuzin (M)	1	94.50**	3.23	0.0546	234.86
Genotype (G)	13	1.49**	0.06**	0.0073**	18.61
M × G	13	1.49**	0.12**	0.0019	16.48*
M × E	1	0.00	0.13	0.0004	19.07**
G × E	13	0.11	0.03	0.0007	5.15
G × M × E	13	0.11	0.02	0.0014	2.73
Rep × M × E†	4	0.12	0.04	0.0004	34.60
Error b	104	0.05	0.03	0.0008	3.20

*, **Significant at 0.05 and 0.01 levels, respectively.
†Error a.

The rate of metribuzin was significant for visual injury rating. Genotype was significant for visual injury rating, shoot weight, and root weight. A metribuzin × genotype interaction was significant for visual injury rating, shoot weight, and plant height. The metribuzin rate × genotype interaction is evidence the influence of metribuzin was not the same for genotypes, relative to other genotypes. No significance was detected for genotype × experiment interaction, genotype × metribuzin × experiment interaction, or rep × metribuzin × experiment interaction. The non-significant genotype × metribuzin × experiment interaction is evidence the results were repeatable across both experiments.

An ANOVA was also provided to determine percent reduction for shoot and root weight, and plant height (Table 8). Metribuzin rate was no longer one of the factors because percent reduction is one minus the response to metribuzin divided by the response to zero metribuzin. Genotype was significant for shoot weight and plant height. Genotype was non-significant for root weight. Genotype x experiment was non-significant for shoot and root weight, and plant height when percent reduction was evaluated.

Table 8. Analysis of variance of first set of genotypes, for percent reduction.

		Mean Square		
Source of Variation	df	Shoot weight	Root weight	Plant height
Experiment (E)	1	0.25	0.01	0.05*
Rep(E)	4	0.12	0.01	0.14**
Genotype (G)	13	0.22**	0.11	0.05**
G x E	13	0.03	0.06	0.01
Error b	52	0.07	0.04	0.01

*, **Significant at 0.05 and 0.01 levels, respectively.

Averaged across 14 genotypes, mean rating when no metribuzin was applied was 1, and the mean rating when metribuzin was applied was 2.50 (Table 9). Altona was rated 3.58 in the metribuzin treatment and considered sensitive, which is consistent with preliminary results. This result was consistent with the results of Wax et al. (1976). Clay was visibly less injured in the preliminary study, but was rated moderately tolerant in this experiment at 2.42. Wax et al. (1976) reported that Clay was tolerant to metribuzin injury while my results indicated that Clay was moderately tolerant. The most tolerant and sensitive genotype from the soybean breeding program were ND12-15628 and ND10-2763, respectively. ND Stutsman and Asgrow 00932 were also identified as tolerant, with visual injury ratings of 1.67 and 1.83, respectively. The newly released cultivar ND17009GT was considered moderately sensitive to metribuzin. Most genotypes evaluated were highly or moderately tolerant, with an injury rating of 2.5 or less. These results are promising as some experimental genotypes will continue through the program for potential cultivar release in upcoming years.

Table 9. Mean herbicide injury ratings of first set of 14 soybean genotypes grown in a greenhouse experiment, with and without metribuzin.

Genotypes	No metribuzin	Metribuzin†
Altona	1	3.58
Asgrow 00932	1	1.83
Ashtabula	1	2.58
Clay	1	2.42
ND Bison	1	2.08
ND Stutsman	1	1.67
ND10-2763	1	3.83
ND12-15628	1	1.58
ND12-24081 (GLY)	1	2.83
ND13-8892	1	2.25
ND13-8894	1	2.08
ND1700GT	1	3.33
ProSoy	1	2.83
Sheyenne	1	2.08
LSD (0.05)‡	0.52	
LSD (0.05)§	0.47	

†Metribuzin rate was 0.125 ppm.
‡LSD compares means of same level of metribuzin across different level of genotype.
§LSD compares means of different levels of metribuzin across same level of genotype.

Barrentine et al. (1976) and Hardcastle (1979) evaluated various genotypes at the same rate of metribuzin used in this experiment. Hardcastle (1979) only evaluated determinate cultivars, but conducted research on some of the same determinate cultivars as Barrentine (1976). Hardcastle (1979) noted the results obtained in his study were consistent with those of Barrentine et al. (1976). Another important consideration of Hardcastle's greenhouse research, was pH does not have an effect on metribuzin injury when planted in a nutrient-sand culture. Barrentine et al. (1976) evaluated both determinate and indeterminate cultivars. Two determinate soybean cultivars were completely killed by metribuzin, while none of the indeterminate cultivars were killed by metribuzin. The overall results of Hanson and Nickell (1986) were also consistent with my results, aside from death of the Altona plants. Altona plants died in their study, but no plants of this genotype died in my experiment. In my experiments, Altona was one of the most severely injured genotypes from metribuzin application.

Table 10. First set shoot weight means of 14 soybean genotypes grown in greenhouse experiment, with and without metribuzin.

Genotypes	No metribuzin	Metribuzin	Weight reduction
	------------------------g--------------------------		-------------%-------------
Altona	1.30	0.55	58
Asgrow 00932	1.13	1.05	7
Ashtabula	0.98	0.73	26
Clay	1.13	0.82	27
ND Bison	0.93	0.75	19
ND Stutsman	0.85	0.68	20
ND10-2763	0.88	0.40	55
ND12-15628	0.83	0.90	-8
ND12-24081 (GLY)	0.83	0.67	19
ND13-8892	0.95	0.63	34
ND13-8894	0.90	0.77	14
ND1700GT	0.97	0.57	41
ProSoy	1.25	0.77	38
Sheyenne	1.05	0.83	21
LSD (0.05)†			23
LSD (0.05)‡	0.21		

†LSD compares percent shoot weight reduction across cultivars, across columns.
‡LSD compares means of different levels of metribuzin across same level of genotype, across rows.

When averaged across 14 genotypes, the mean shoot weight when no metribuzin was applied was 1.0 g and the mean shoot weight when metribuzin was applied was 0.72 g. Metribuzin had minimal effect on the shoot weight of ND12-15628 (Table 10). The shoot weight of ND12-15628 increased 8% when metribuzin was added. For all other genotypes evaluated, shoot weight decreased as metribuzin was applied. ‘Asgrow 00932’ had a minimal reduction in shoot weight when metribuzin was added, decreasing from a weight of 1.13 g to a weight of 1.05 g, or 7%. Shoot weight decreased over 50% for the two sensitive cultivars, Altona and ND10-2763. The shoot weight of ND10-2763 in the no metribuzin container was 0.88 g, but decreased by 55% to a weight of 0.4 g when metribuzin was added. ND1700GT also had a large reduction in shoot weight. The shoot weight of this genotype was reduced 41% between the metribuzin and no metribuzin treatments.

Three genotypes had a greater plant height when treated with metribuzin. They were Ashtabula, ND12-15628, and Sheyenne (Table 11). This change was attributed to effects such as water type, heating of greenhouse, or number of plants grown for each genotype. Four seedlings were planted in the hydroponic solution, but often one or two genotypes had a plant that grew less than 10 cm and needed to be discarded. The height of the each plant was averaged across the number of plants of each genotype in the tub. Some genotypes grew faster than others, so adequate space was sometimes an issue since all genotypes grew in the same container. Environmental effects were likely the major reason for increases in height of the three genotypes between the two treatments.

Table 11. Plant height means of first set of 14 soybean genotypes grown in a greenhouse experiment, with and without metribuzin.

Genotypes	No metribuzin	Metribuzin	Height reduction
	------------------------cm-----------------------		-------------%-------------
Altona	27.83	21.31	23
Asgrow 00932	25.54	24.41	4
Ashtabula	21.96	22.31	-2
Clay	26.92	24.19	10
ND Bison	23.50	22.58	4
ND Stutsman	23.48	22.09	6
ND10-2763	24.75	17.15	31
ND12-15628	22.03	22.75	-3
ND12-24081 (GLY)	24.69	22.87	7
ND13-8892	23.78	20.49	14
ND13-8894	24.34	22.72	7
ND1700GT	24.38	20.82	15
ProSoy	24.50	21.89	11
Sheyenne	24.50	25.53	-4
LSD (0.05)†			13
LSD (0.05)‡	14.42		

†LSD compares percent plant height reduction across cultivars, across columns.
‡LSD compares means of different levels of metribuzin across same level of genotype.

When tolerance was based on visual ratings, tolerant genotypes like ND Bison, ND Stutsman, and Asgrow 00932 had small differences in plant height between the no metribuzin and the metribuzin treatments. Change in plant height between treatments for ND Bison and

Asgrow 00932 were both 4%. The change in plant height for ND Stutsman was 6% between the two treatments. Moderately tolerant or sensitive genotypes, identified from visual injury ratings, had similar height reductions compared to each other. Tolerant ND13-8892 had height reduction of 14% between treatments, and this genotype had a similar height reduction compared to the moderately sensitive ND17009GT of 15% between treatments. Generally, height reductions for any genotype considered tolerant were not as large as the highly sensitive genotypes. Highly sensitive genotypes Altona and ND10-2763 had height reductions between treatments of 23% and 31%, respectively.

Second Set of Experimental Genotypes

The results from the following ANOVA were from the second set of experimental genotypes evaluated for metribuzin injury.

Table 12. Analysis of variance of a second set of fourteen soybean genotypes with and without metribuzin, evaluated in the greenhouse.

		Mean Square			
Source of Variation	df	Injury rating	Shoot weight	Root weight	Plant height
Experiment (E)	1	0.43*	12.11*	0.2281*	422.88*
Rep (E)	4	0.05	0.99*	0.0128	44.31
Metribuzin (M)	1	86.43*	7.76	0.1755	46.45
Genotype (G)	13	11.42**	0.13*	0.0067**	16.22
M × G	13	11.42**	0.16*	0.0028*	4.41
M × E	1	0.43*	0.45	0.0061	35.13
G × E	13	0.92	0.04	0.0013	8.01
G × M × E	13	0.92	0.04	0.0009	3.15
Rep × M × E†	4	0.18	0.12	0.0021	5.39
Error b	104	0.08	0.05	0.0012	3.97

*, **Significant at 0.05 and 0.01 levels, respectively.
†Error a.

Injury rating was significant for rate of metribuzin (Table 12). Injury rating, shoot weight, and root weight were significant for genotype. A metribuzin × genotype interaction was present for injury rating, shoot weight, and root weight. Injury rating was significant for the metribuzin × experiment interaction. Genotype × experiment interaction, genotype × metribuzin × environment interaction, and rep × metribuzin × experiment interaction were non-significant for all parameters evaluated. The non-significant genotype × metribuzin × experiment interaction was evidence that the genotype × metribuzin rate interaction was repeatable across both experiments.

Table 13. Analysis of variance of second set of genotypes, for percent reduction.

		Mean Square		
Source of Variation	df	Shoot weight	Root weight	Plant height
Experiment (E)	1	0.03	0.002	0.10**
Rep(E)	4	0.05	0.02	0.01
Genotype (G)	13	0.01	0.07**	0.01
G x E	13	0.05	0.02	0.01
Error b	52	0.05	0.03	0.01

*, **Significant at 0.05 and 0.01 levels, respectively.

The following ANOVA provides results from the second set of genotypes when percent reduction of shoot and root weight, and plant height were evaluated (Table 13). Genotype was significant for root weight. Genotype was non-significant for shoot weight and plant height. Genotype x experiment interaction was non-significant for shoot and root weight, and plant height.

Averaged across 14 genotypes, the mean visual injury rating when no metribuzin was applied was 1 and the mean rating when metribuzin was applied was 2.43. The mean visual injury rating of the first set of 14 genotypes was similar, with an injury rating of 2.5 when metribuzin was added. Four genotypes were considered highly tolerant, based on the visual injury rating. An injury rating of 2 or less was considered highly tolerant.

Table 14. Mean herbicide visual rating of a second set of 14 soybean genotypes grown in greenhouse experiment, with and without metribuzin.

Genotypes	No metribuzin	Metribuzin†
Altona	1	3.83
Asgrow 0832	1	2.25
Clay	1	2.83
ND Benson	1	2.58
ND Henson	1	2.75
ND13-4508	1	2.00
ND13-7564	1	2.83
ND13-8129 (GLY)	1	2.67
ND13-8691	1	2.33
ND13-9073	1	2.00
ND14-5732 (GLY)	1	1.75
ND14-5895 (GLY)	1	2.17
ND14-6120 (GLY)	1	1.83
ND14-6238 (GLY)	1	2.25
LSD (0.05)‡	1.33	
LSD (0.05)§	1.38	

†Metribuzin rate was 0.125 ppm.
‡LSD compares means of same level of metribuzin across different level of genotype
§LSD compares means of different levels of metribuzin across same level of genotype.

When metribuzin was applied, ND14-5732 had a visual injury rating of 1.75 and ND14-6120 had a visual injury rating of 1.83 (Table 14). Both of these genotypes are known to be glyphosate-tolerant. ND13-4508 and ND13-9073 had visual injury ratings of 2.0. Four genotypes were visually rated for injury between 2.0 and 2.5, and were considered moderately tolerant. ND14-5895 had a visual injury score of 2.17. Asgrow 0832 and ND14-6238 had a visual injury rating of 2.25. ND13-8691 had a visual injury rating of 2.33. ND Benson had a visual injury rating of 2.58 and was considered moderately sensitive. All other genotypes evaluated were considered sensitive or moderately sensitive to metribuzin. Altona was highly sensitive with an injury rating of 3.83, which is consistent with my results and results from a hydroponic study conducted by Barrentine et al. (1976). Clay was identified as moderately sensitive with an injury rating of 2.83. The injury rating of Clay was higher than ratings received in previous greenhouse experiments I conducted, using this same cultivar. The sensitivity rating of Clay is consistent with two studies, one conducted in the greenhouse and one in the field. Barrentine et al. (1976) evaluated Clay in their greenhouse screenings and also found this genotype to be moderately tolerant to metribuzin. Environmental effects of the greenhouse such as water source, available light, thrip damage, etc. are most likely the cause for the difference in results between experiment runs. A field experiment that was reported by Wax et al. (1976) also found the tolerance of Clay to metribuzin ranged from tolerant to moderately tolerant. They found that as the rate of metribuzin applied in the field increased, tolerance become more moderate, but this was at a high application rate of 1.7 kg ha^{-1}. At metribuzin rates of 0.56 or 0.84 kg ha^{-1}, less than 25% injury occurred on the Clay cultivar. Overall, the results obtained from these two experiments are somewhat consistent with my current greenhouse results for Clay.

Altona and Clay had the greatest shoot weights when no metribuzin was applied. The shoot weight of Altona was reduced by over half from the non-metribuzin treatment to the metribuzin treatment (Table 15). The shoot weight of Clay was reduced 39% when metribuzin was applied, compared to the treatment without metribuzin. Shoot weights of nine of the genotypes evaluated were only reduced by 25% or less when metribuzin was added. ND13-4508 and ND13-7564 had the smallest difference in shoot weight between metribuzin treatments. ND13-7564 had a reduced shoot weight of 8% from the no metribuzin to the metribuzin treatment. The shoot weight of ND13-4508 was reduced 16% between metribuzin treatments. While these two genotypes had the smallest reduction in shoot weight, one was considered sensitive based on visual injury and one was a tolerant genotype. ND13-7564 had the smallest shoot weight reduction, but was a moderately sensitive genotype with a visual injury rating of

2.83. ND13-4508 was considered tolerant with a visual injury rating of 2.0. Visual injury rating was not a good measure of whether or not metribuzin would reduce the shoot weight of a given genotype.

Table 15. Second set shoot weight means of 14 soybean genotypes grown in a greenhouse experiment, with and without metribuzin.

Genotypes	No metribuzin	Metribuzin	Weight reduction
	---------------------------g---------------------------		-------------%------------
Altona	2.00	0.97	52
Asgrow 0832	1.75	1.28	27
Clay	1.92	1.17	39
ND Benson	1.57	1.18	25
ND Henson	1.88	1.40	26
ND13-4508	1.45	1.22	16
ND13-7564	1.33	1.22	8
ND13-8129 (GLY)	1.53	1.28	16
ND13-8691	1.62	1.23	24
ND13-9073	1.67	1.40	16
ND14-5732 (GLY)	1.70	1.30	24
ND14-5895 (GLY)	1.77	1.18	33
ND14-6120 (GLY)	1.55	1.20	23
ND14-6238 (GLY)	1.45	1.13	22
LSD (0.05)†			10
LSD (0.05)‡	1.93		

†LSD compares percent shoot weight reduction across cultivars, across columns.
‡LSD compares means of different levels of metribuzin across same level of genotype, across rows.

Four genotypes varying in tolerance to metribuzin had a difference in root weight between metribuzin treatments of 0.08 g (Table 16). ND14-5895 and Asgrow 0832 were the most tolerant cultivars, based on visual injury ratings of 2.17 and 2.25. ND Benson was moderately tolerant with a visual injury rating of 2.58. Altona was sensitive with a visual injury rating of 3.83. The root weights of ND Henson and Clay were reduced 30 and 41%, respectively, between the two levels of metribuzin treatments, which is consistent with the visual injury rating of these two cultivars. Based on visible injury, both of these genotypes were considered moderately sensitive. Three genotypes, ND13-7564, ND13-8691, and ND14-6238, had the lowest reductions in root weights, ranging from 14 to 18%. Their tolerances to metribuzin based on visual injury ratings ranged from 2.25 to 2.83. Due to similar weight changes between the metribuzin and no metribuzin treatments for both tolerant and sensitive genotypes, root weight was not the most reliable indicator of tolerance.

Table 16. Second set root weight means of 14 soybean genotypes grown in greenhouse experiment, with and without metribuzin.

Genotypes	No metribuzin	Metribuzin	Weight reduction
	---------------------g---------------------		------------%-----------
Altona	0.23	0.11	52
Asgrow 0832	0.26	0.18	31
Clay	0.27	0.16	41
ND Benson	0.24	0.16	33
ND Henson	0.27	0.19	30
ND13-4508	0.19	0.15	21
ND13-7564	0.19	0.16	16
ND13-8129 (GLY)	0.21	0.17	19
ND13-8691	0.21	0.18	14
ND13-9073	0.24	0.20	17
ND14-5732 (GLY)	0.19	0.14	26
ND14-5895 (GLY)	0.24	0.16	33
ND14-6120 (GLY)	0.23	0.17	26
ND14-6238 (GLY)	0.17	0.14	18
LSD (0.05)†			17
LSD (0.05)‡	0.27		

†LSD compares percent root weight reductions across cultivars, across columns.
‡LSD compares means of different levels of metribuzin across same level of genotype, across rows.

In the combined analysis, plant height was not significant for any sources of variation, except experiment (Table 12). In the individual analyses of the experiment, there was significance (data not shown). In the second run, genotype, metribuzin, and metribuzin × genotype interaction were all significant. None of the parameters evaluated were significant in the first run. Lack of significance in average plant height in the first run resulted in no effect of metribuzin and no metribuzin × genotype interaction in the combined ANOVA.

Table 17. Summary of visual injury ratings for tolerance to metribuzin for individual genotypes, in greenhouse experiments.

Genotype	Injury rating[†]	Level of tolerance
Altona[‡]	3.71	Sensitive
Asgrow 00932	1.83	Tolerant
Asgrow 0832	2.25	Moderately tolerant
Ashtabula	2.58	Moderately sensitive
Clay[‡]	2.46	Moderately tolerant
ND Benson	2.58	Moderately sensitive
ND Bison	2.08	Tolerant
ND Henson	2.75	Moderately sensitive
ND Stutsman	1.67	Tolerant
ND10-2763	3.83	Sensitive
ND12-15628	1.58	Tolerant
ND12-24081 (GLY)	2.83	Sensitive
ND13-4508	2.00	Tolerant
ND13-7564	2.83	Sensitive
ND13-8129 (GLY)	2.67	Moderately sensitive
ND13-8691	2.33	Moderately tolerant
ND13-8892	2.25	Moderately tolerant
ND13-8894	2.08	Tolerant
ND13-9073	2.00	Tolerant
ND14-5732 (GLY)	1.75	Tolerant
ND14-5895 (GLY)	2.17	Tolerant
ND14-6120 (GLY)	1.83	Tolerant
ND14-6238 (GLY)	2.25	Moderately tolerant
ND17009GT	3.33	Sensitive
ProSoy	2.83	Sensitive
Sheyenne	2.08	Tolerant

[†]A rating of 1 represents no injury, a rating of 5 represents death.
[‡]Averaged across two experiments, or 4 experimental runs. All other genotypes averaged across one experiment, or two experimental runs.

Field Experiments

2016 Site Year

Weather is reported because rainfall was needed to incorporate the PRE metribuzin into the soil (Table 18). Incorporation was essential, as without it, the metribuzin would not have been available to plant roots. In 2016, three of the locations received over 8 cm in May, after the soybean genotypes had been planted. Fairmount, ND, using the closest weather station in

Campbell, MN, received less rainfall at 3.3 cm (NDAWN). Soil pH ranged from 7.1 to 7.9 for each location. Soil OM also varied between locations, from 3.5 to 4.9%. Both pH and OM are important for metribuzin activity. The soil at each location was a loam type (USDA/NRCS)

Table 18. 2016 soil factor and May rainfall data across the four field locations.

	Location			
Soil factor	Arthur†	Casselton†	Fairmount‡	Prosper
pH	7.8	7.2	7.9	7.1
Soil Type	Lankin-Gilby loam	Kindred-Bearden silty clay loam	Wheatville silt loam	Kindred-Bearden silty clay loam
Organic Matter (%)	3.5	4.0	3.6	4.9
Soluble salts (EC mmhos cm^{-1})	0.31	1.60	0.78	0.45
Rainfall (cm)	8.2	8.2	3.3	8.2

†Prosper was closest weather station for each location.
‡Campbell, MN was the closest weather station.

Four soybean genotypes with tolerance and sensitivity to metribuzin on high pH soil were evaluated at four field locations. The same genotypes evaluated in the preliminary greenhouse experiment were evaluated in the field. Three rates of metribuzin were evaluated for each genotype. The metribuzin rates were 0, 0.33, and 0.67 kg ha^{-1}.

Table 19. Analysis of variance of four soybean genotypes on high pH soils in eastern North Dakota in 2016.

		Mean Square					
Source of Variation	df	Stand	Injury 1	Injury 2	Injury 3	Injury 4	Injury 5
Experiment (E)	3	51585026100**	0	0.10**	0.52**	0.19**	1.75**
Rep(E)	8	16469783100	0	0.05**	0.05	0.08**	0.39**
Metribuzin Rate (M)	2	3412863800	0	0.06	0.13	0.08	0.23
M × E	6	7182067900	0	0.04	0.04	0.09	0.27
Rep × M × E	16	8167005900	0	0.08	0.08	0.07**	0.12
Genotype (G)	3	40410803300	0	0.01	2.06*	0.07	0.74
G × E	9	22854213900	0	0.02*	0.35	0.04	0.50**
M × G	6	15341689700	0	0.002	0.04	0.01	0.13
G × M × E†	18	5914233100	0	0.004	0.06	0.01	0.09
Error b	72	10439151800	0	0.01	0.06	0.02	0.09

*, **Significant at 0.05 and 0.01 levels, respectively.
†Error a.

Genotype was significant for the third injury rating across locations (Table 19). Genotype × experiment interaction was significant for the second and fifth injury ratings. Metribuzin rate, metribuzin × experiment interaction, metribuzin × genotype interaction, and genotype × metribuzin × experiment interaction were not significant for any injury rating. Each genotype, regardless of metribuzin rate, was rated 1 at each of the four field sites when the first injury rating was collected. Differences in injury did not appear until the second injury rating. The soybean stand ha^{-1} was not significantly different amongst the four genotypes used in the field, regardless of location, which was the expected result. We can conclude, based on these results, metribuzin rates that I applied had minimal effect on the emergence of soybean when applied pre-emergence. The lack of stand reduction is consistent with results by Littlejohns et al. (1977) when lower rates of metribuzin were applied pre-emergence. They reported that at a rate of 0.56 kg ha^{-1} plant stand was 18 plants $plot^{-1}$, compared to a stand of 20 plants $plot^{-1}$ when no metribuzin was applied. They found increased metribuzin rates of 0.84 and 1.12 kg ha^{-1} significantly reduced plant stand ha^{-1}.

In 2016, two soils had a pH of 7.5 or greater, which was the desired pH for the objective of the experiment (Table 18). Soil pH was below 7.5 in two locations, but did not seem to affect level of injury. Visual injury ranged from 1 to 2 at all locations during the season. After rains in late June and July, some plots were more severely injured at the Casselton and Prosper locations. The soil pH for these two locations was 7.2 and 7.8, respectively. Using weather data from Prosper, both sites received 23.4 cm of rain through the season. The rainfall resulted in greater injury to the cultivar Clay. This cultivar was developed in 1927, (Hymowitz et al., 1977), and is not tolerant to phytophthora root rot. Injury ratings of 3 or 3.5 for Clay were likely due to phytophthora root rot injury, which could not be distinguished from metribuzin injury.

Otherwise, metribuzin injury for all four genotypes at Casselton and Arthur was minimal. Prosper, with a pH of 7.2, and Fairmount, with a pH of 7.9, had minimal injury throughout the season, with ratings of 1 or 1.5 for sensitive and tolerant genotypes at all weeks of injury ratings and all metribuzin rates. Rainfall in Prosper was 23.4 cm, and rainfall in Fairmount was 29 cm. Prosper received comparable rainfall to Casselton and Arthur, but phytophthora rot root of Clay at Prosper, Arthur, and Fairmount was not severe enough to increase late season injury ratings. In this field experiment, pH did not affect metribuzin injury in soybean. Other factors, such as disease, had a larger impact on higher injury ratings throughout the season.

After injury notes were collected, harvest maturity notes were collected before harvest. When maturity notes were collected, it was discovered the cultivars Altona and Clay were segregating for maturity. This posed a problem as all the cultivars evaluated during the field season were assumed to be pure seed at the time of planting. The seed of all the cultivars came from a germplasm bank, and were increased the previous winter. Due to the mixture of unknown genotypes of Altona and Clay, none of the plots were harvested. For this same reason, the genotype effects for stand and injury are suspect.

2017 Site Year

Total rainfall in May was lower for the Arthur, Casselton, and Prosper, ND, locations in 2017 compared to 2016 (Table 20). Rainfall amounts at Fairmount were consistent between both years (NDAWN). While rainfall totals were lower at Casselton, Arthur, and Prosper locations in 2017, enough rain fell to incorporate metribuzin into the soil and activate the herbicide. Soil pH ranged from 6.8 to 7.8 among the four locations in 2017 (Table 20). These pH levels are slightly lower than those of sites used in the 2016 field season. Like the 2016 locations, all soils were some type of loam (USDA/NRCS). Four soybean genotypes developed in the NDSU soybean breeding program were evaluated for tolerance to metribuzin on high pH soil at all four field locations. The four genotypes evaluated in the field in 2017 had previously been evaluated in the greenhouse. Their tolerance and sensitivity in a hydroponic solution was known prior to planting. Three rates of metribuzin were evaluated for each genotype. The rates were 0, 0.33, and 0.67 kg ha^{-1}.

Table 20. Soil factor and May rainfall data across four 2017 field locations.

	Location			
Soil factor	Arthur†	Casselton†	Fairmount‡	Prosper
pH	7.4	7.2	7.8	6.8
Soil Type	Lankin-Gilby loam	Kindred-Bearden silty clay loam	Antler clay loam	Bearden-Lindaas silty clay loam
Organic Matter (%)	3.4	3.9	4.5	3.8
Soluble salts (EC mmhos cm^{-1})	0.30	0.58	0.60	0.42
Rainfall (cm)	1.7	1.7	3.3	1.7

†Prosper was closest weather station for each location.
‡Campbell, MN was the closest weather station.

Table 21. Analysis of variance of four soybean genotypes on high pH soils in eastern North Dakota in 2017.

		Mean Square						
Source of Variation	df	Stand	Injury 1	Injury 2	Injury 3	Injury 4	Injury 5	Yield
Experiment (E)	3	1708337500**	2.21**	8.31**	7.85**	4.88**	0	1109800
Rep(E)	8	228667600**	0.04*	0.14**	0.07	0.35**	0	571200
Metribuzin Rate (M)	2	80033700	0.01	0.19	0.06	0.04	0	229300
M × E	6	10480600	0.01	0.05	0.02	0.15	0	102600
Rep × M × E	16	157209000	0.01	0.05	0.03	0.09*	0	148300
Genotype (G)	3	42875200	0.03	0.07	0.11	0.13	0	5250000**
G × E	9	62883600	0.05**	0.04	0.05	0.09	0	397100
M × G	6	8575000	0.01	0.01	0.03	0.03	0	108400
G × M × E†	18	134342200	0.01	0.04	0.04	0.05	0	92200
Error b	72	574527300	0.01	0.04	0.04	0.04	0	141000

*, **Significant at 0.05 and 0.01 levels, respectively.
†Error a.

Genotype × experiment interaction was significant for the first injury rating averaged across the four locations (Table 21). Metribuzin rate, genotype, metribuzin × experiment interaction, metribuzin × genotype interaction, and genotype × metribuzin × experiment interaction were not significant for any variable evaluated.

Some visual injury appeared in 2017 in the early weeks of injury rating. Differences among treatments were not significant (Table 20). A rating of 1 was considered an absence of injury from metribuzin. Herbicide carryover from the previous field season could have played a role. After the initial injury ratings were conducted during vegetative growth, one last rating was conducted after flowering. At this fifth date of visual injury rating, each genotype was rated 1. These ratings showed all genotypes, independent of metribuzin rate, had outgrown all early season injury. Much like 2016, soil pH had minimal effect on metribuzin injury in 2017. Yield data was collected for each genotype to determine whether there were yield reductions due to metribuzin rate. The metribuzin × genotype interaction was non-significant for soybean yield. Therefore, yields of the genotypes that were applied with a 0.33 or 0.67 kg ha^{-1} rate were not different from the genotypes that did not have metribuzin applied. This data shows metribuzin should not reduce soybean yields regardless of the rate applied.

Multiple researchers have evaluated effects of metribuzin rates. Griffen and Habbetz (1989) found when metribuzin was applied at the recommended rate soybean height was not significantly reduced. The metribuzin was applied at a rate of 0.42 kg ha^{-1}. They stated yield reductions seldom occur when metribuzin was applied PRE at twice the recommended rate. This result is consistent with my results that the 0.67 kg ha^{-1} rate did not visibly reduce plant height or yield in 2017. In contrast, Belfry et al. (2015) found metribuzin injured and reduced plant height of soybean on soils with a pH of 7.7 to 7.8. They applied a metribuzin rate of 2.24 kg ha^{-1}, and plant injury was around 20% two wk after application. Using the same application rate, plant dry weight was reduced 41% relative to the control. Yield was also reduced 62 to 69% for three cultivars, relative to the control.

Littlejohns et al. (1977) found all cultivars evaluated expressed leaf injury at 0.56 kg ha^{-1}, and injury increased as the rate increased when applied pre-emergence. Yield and plant stand were not significantly reduced at this rate, but both were reduced as the rate increased to 1.12 kg ha^{-1}. Wax et al. (1976) also concluded minimal injury occurs at a rate of 0.56 kg ha^{-1}. Aside from research conducted by Wax et al. (1976) all experiments evaluated metribuzin applied pre-emergence.

Researchers have reported results that both agreed and disagreed with those obtained from my field experiments of 2016 and 2017. The metribuzin rate used by each of the researchers played a large role. The results of Griffen and Habetz (1989) are most consistent with my results, and they used a rate closest to the recommended rate of 0.33 kg ha^{-1}. The studies conducted by Belfry et al. (2015) and Littlejohns et al. (1977) used much higher rates than those recommended in North Dakota. The rate used by Belfry et al. (2015) is almost a 7X rate of the 0.33 kg ha^{-1} rate. A rate of 1.12 kg ha^{-1}, used by Littlejohns et al. (1977) is a 3X rate of the North Dakota recommended rate. Each researcher's results are valuable, but must be taken within context. Rates of metribuzin this high are unlikely to be used outside of a research setting. The information on injury, dry matter reduction, and yield loss are important, but the experiments from which these results were obtained are not rates used by farmers in North Dakota. Each researcher provided evidence soybean is sensitive to metribuzin, but more field research should be conducted with metribuzin at rates applicable to farmers.

Previous researchers noted the importance of soil factors, but focused more on metribuzin rates. Other researchers focused on metribuzin rate, but emphasized the importance of soil factors when determining injury from metribuzin. Soil factors such as OM, pH, and soil type are extremely important for metribuzin activity. Ladlie et al. (1976a) researched both metribuzin rate and soil pH effects on soybean injury. They found an increase of soil pH resulted in height reduction, some dead plants per plot, and yield reduction. Effects of soil pH on soybean was averaged over rate and years. They noted each rate itself was not significant when analyzed separately. Injury that did occur in the field was more severe at a pH of 5.0 to 5.4. The rates of metribuzin were comparable to those used by other researchers (Littlejohn et al., 1976; Wax et al., 1976). Additionally, they conducted a greenhouse study that also evaluated the effect of metribuzin rate as pH varied. At an application rate of 0.28 kg ha^{-1}, metribuzin reduced soybean dry weight when soybean was grown in soil. Furthermore, metribuzin did not reduce the dry weight of soybean compared to the control when grown in a sand culture. Since this research was conducted in the greenhouse, yield was not measured. However, reporting information on metribuzin tolerance at a rate of 0.28 kg ha^{-1} is more pragmatic to farmers. This rate is closer to the rate that a farmer would use in a field setting. Ladlie et al. (1976a) also indicated while pH did not significantly affect tolerance to metribuzin, pH was important as it affected the availability in the soil. Less metribuzin was absorbed by the roots at lower soil pH.

Soil OM is important for metribuzin adsorption to the soil particles, and therefore effective control of weed species. Organic matter percentages of the field locations in 2016 ranged from 3.5 to 4.9%, and 3.4 to 4.5% in 2017. These OM percentages were comparable to those used by Coble and Schrader (1973). At three use rates, they reported metribuzin injury on soybean cultivars ranged from 4 to 35%. The least amount of injury, 4%, occurred at the 0.56 kg ha^{-1} rate on a soil with an OM of 3.3%. Coble and Schrader (1973) noted visual injury increased as the use rate increased. This study was conducted in a greenhouse, but had results applicable to my 2016 and 2017 field experiments. Minimal injury occurred at the rate used by Coble and Schrader (1973) and was comparable to injury ratings of my field experiments in 2016 and 2017. Overall, very little visual injury was expressed in 2016 and 2017 trials at a rate of 0.67 kg ha^{-1}, which was similar to the 0.56 kg ha^{-1} rate used by Coble and Schrader (1973).

Sharom and Stephenson (1976) evaluated the effect of OM and as well as soil pH at three rates of metribuzin on eight soil types. They found soil adsorption was significantly correlated with OM, but not clay content. A silt loam and loam type soils had significantly higher metribuzin adsorption to soil particles, compared to a sand or sandy loam type soil. The loam and silt loam had OM ranging from 5.7 to 7%, and 6.4 to 7.2 for soil pH. Sharom and Stephenson (1976) also noted the reason less metribuzin was adsorbed to soil particles was the low surface area and adsorptive capacity of the elements that comprise these soils. Soils with greater OM content, such as the loam and silt loam, were better able to adsorb more metribuzin than soils with a lower OM content, like a sand or sandy loam soil. Increased OM resulted in greater metribuzin adsorption to soil particles, making the herbicide more effective. Both the OM and pH evaluated by Sharom and Stephenson (1976) were higher than soils evaluated in both of my field experiments, but the overall efficacy was comparable.

In 2016, more metribuzin injury appeared in my experiments later in the season. The metribuzin may not have been as tightly bound to the soil particles due to OM percentage. As a result, 2016 weed control with metribuzin was marginal and required weekly weeding. Common ragweed and red root pigweed were often found within plots at each location. Weed control was much better in 2017, as fields were cleaner throughout the season as a result of glyphosate applications and metribuzin activity. Early season common ragweed control was excellent in 2017.

Metribuzin rate and soil factors have been important considerations for metribuzin activity, but rain also has a role on activity and injury. Sharom and Stephenson (1976) noted rainfall on sandy loams may cause metribuzin leaching through the soil. This leaching would result in metribuzin ending up in deeper soil than weed seeds, thus decreasing the effectiveness. Sharom and Stephenson (1976) evaluated rates of 0.28, 0.56, and 1.12 kg ha^{-1} on cucumber (*Cucumis sativus*) to determine effect on dry weight. Significant metribuzin leaching occurred in their experiment due to 26.5 cm of rain that was recorded at the locations in a three month period. At the highest rate of 1.12 kg ha^{-1}, there was a significant reduction in cucumber dry weight, compared to the dry weights of plants where 0.28 or 0.56 kg ha^{-1} metribuzin was applied. The rate of 1.12 kg ha^{-1} was enough to reduce the dry weight of cucumber, although most of the metribuzin had been leached to a lower depth of soil. Only at this high rate of metribuzin was there a reduction in cucumber dry weight, as leaching that occurred from the rainfall did not injure the plants at lower application rates or closer to soil surface.

Moshier and Ross (1981) also found rainfall had an effect on metribuzin activity and soybean injury in a two year study they conducted. Over the course of their study, each of the two years had contrasting results in comparison to each other. Injury was more severe when metribuzin was applied three wk before planting, but injury was less severe the following yr when applied immediately following planting. In 1978, the first year the study was conducted, 10.5 cm of rain fell in the three wk before planting, causing metribuzin to leach through the roots. In 1979, no rainfall occurred in the three wk after metribuzin application but before planting, followed by 17.5 cm of rainfall recorded in the later portion of the season. In this instance, visible metribuzin injury in soybean was less severe in 1979 compared to 1978 than when metribuzin was applied immediately before planting.

The rainfall totals for my experiments in 2016 and 2017 were greater than amounts recorded in the study by Moshier and Ross (1981). In my 2016 experiments, the rainfall totals ranged from 23.4 to 29 cm in Arthur, Casselton, Fairmount, and Prosper. In my 2017 experiments, the rainfall totals ranged from 20.7 to 26.3 cm at the same four locations. These rainfall totals were recorded over the course of four mo. While the timing and amount of rainfall was important for injury in 2016 and 2017 experiments, metribuzin rate was the most important consideration.

The rainfall amounts and level of injury from the study conducted by Moshier and Ross (1981) are important as the lower metribuzin rate they evaluated was similar to the high rate evaluated in my field experiments. At the 0.6 kg ha^{-1} rate used by Moshier and Ross (1981), no yield or plant height reductions occurred when metribuzin was applied pre-emergence. When metribuzin was applied at a rate of 1.1 kg ha^{-1} pre-emergence, height and yield reductions occurred. This is important as rain that fell before planting and pre-emergence metribuzin application caused metribuzin that was in the soil to leach beyond the root zone and possibly degrade. The results of the 1.1 kg ha^{-1} rate used by Moshier and Ross are important, but this rate is close to a 3X recommended rate of metribuzin in North Dakota. It is unlikely that a rate this high would be used by soybean growers in North Dakota.

My results at the 0.67 kg ha^{-1} rate of metribuzin were consistent with the Moshier and Ross (1981) results when they applied metribuzin at the 0.6 kg ha^{-1} rate. Both studies showed no reduction in yield or plant height at those rates. The 0.67 kg ha^{-1} metribuzin rate is the 2X rate in North Dakota for soils with a pH greater than 7.5. With the lack of yield and plant height reductions at the 2X rate, growers should not be concerned about yield or plant height reductions when applying metribuzin at 0.33 kg ha^{-1}, the recommended rate for North Dakota.

CONCLUSIONS

Metribuzin injury on soybean has been evaluated by multiple researchers in both the greenhouse and field, with varying results. The most relevant results to North Dakota soybean farmers are from data collected in greenhouse and field trials in North Dakota. In the greenhouse, metribuzin sensitive and tolerant genotypes were identified in a hydroponic screening. The information has already proved beneficial in determining whether or not to release genotypes as named cultivars. A metribuzin × genotype interaction in the greenhouse has shown there are genetic differences in tolerance to metribuzin within the NDSU soybean breeding germplasm. The results were consistent, for repeated experiments, making this procedure useful to determine the tolerance of other genotypes in the breeding program. In the greenhouse, visible injury was considered the most reliable and pragmatic estimate of metribuzin injury on the genotypes evaluated. Generally, genotypes considered sensitive from visual injury ratings were identified as sensitive for at least one other trait. Plant height reductions for each genotype varied as metribuzin was applied. Height reductions occurred for most genotypes, and those reductions that did occur were greater for sensitive genotypes, consistent with the visual injury rating. Visible plant injury was not always consistent with a reduction in shoot weight of genotypes. Root weight reductions were similar for both tolerant and sensitive genotypes. As a result, both shoot and root weight reductions were not considered the most reliable variables to measure tolerance to metribuzin. Both visual injury rating and plant height reductions were better estimates for identifying metribuzin injury. Greenhouse injury ratings were expected to over-estimate metribuzin injury in the field because hydroponic solution did not have soil particles to adsorb the herbicide.

Although sensitive and tolerant genotypes were identified from greenhouse screenings, the same result was not true in the field. The genotypes used as tolerant and sensitive controls did not express meaningful injury in the field during 2016 and 2017. Issues arose with seed source in 2016. After the problems of seed mixtures were solved for the 2017 field season, visible injury in the field was still minimal. While the soybean genotypes failed to show metribuzin injury over both the 2016 and 2017 field seasons, the results are not in vain. This information is positive for soybean growers who grow a genotype that research has considered sensitive. The field rates evaluated were those recommended for growers in North Dakota. Metribuzin injury observed by researchers in the past was typically using rates 3 to 7X greater than the 0.33 or 0.67 kg ha^{-1} rates used in my field experiments. High rates are unlikely to be used outside of a research setting. In other experiments where use rates were closer to the recommended rates of North Dakota, little injury to soybean occurred. On high soil pH, ranging from 6.8 to 7.9 in the 2016 and 2017 field experiments, minimal visible injury was found; therefore an economic impact is not expected at the 0.33 or 0.67 kg ha^{-1} rates of metribuzin. With high input costs of herbicide and seed and potential problems of glyphosate-resistant weeds, metribuzin injury on high pH soils may not be a concern.

Metribuzin control of common ragweed varied from adequate to excellent, depending on the field location, and could be a good alternative herbicide to glyphosate application in upcoming years. This is promising as glyphosate-resistant weeds are on the rise within the state. Future research should still be conducted to determine whether metribuzin can injure at the recommended use rate on soybean crops planted on high pH soils, especially as soil pH and soil types in this experiment are not representative of all soils present in North Dakota and Minnesota.

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APPENDIX

Table A1. 2017 yield data for individual genotypes, at three rates of metribuzin.

Genotype	Metribuzin rate† 0	0.33	0.67
	Yield		
	Kg ha^{-1}		
ND12-24081 (GLY)‡	2464	2642	2657
ND14-5732 (GLY)§	2877	2845	2758
ND14-6120 (GLY)§	3488	3724	3449
ND17009GT‡	3021	3250	3044
LSD (0.05)¶	NS		

†Reported as kg ha^{-1}.
‡Sensitive genotypes to metribuzin.
§Tolerant genotypes.
¶LSD compares means of different levels of metribuzin across same level of genotype, across rows.