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Characterization of the Relationships between Soybean Yield, Trifoliolate Leaf Chloride Concentration, and Cultivar Chloride Inclusion/Exclusion Rating

Dillon Cox
University of Arkansas, Fayetteville

Characterization of the Relationships between Soybean Yield, Trifoliolate Leaf Chloride Concentration, and Cultivar Chloride Inclusion/Exclusion Rating

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Dillon Douglas Cox
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University of Arkansas

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Nathan A. Slaton, Ph.D.
Thesis Director

Trent L. Roberts, Ph.D.
Committee Member

Edward E. Gbur, Jr, Ph.D.
Committee Member

Jeremy Ross, Ph.D.
Committee Member

ABSTRACT

Chloride toxicity is recognized as yield limiting problem in soybean [*Glycine max* (L.) Merr.] production. Limited information is available to accurately diagnose and manage Cl toxicity. The only recommendation for Cl toxicity management is to plant an excluder cultivar, however the cultivar Cl sensitivity rating system (excluder, includer, and mixed) does not appear to capture the variability in cultivar Cl tolerance. The objectives of this research were to i) develop critical tissue-Cl concentrations in which yield loss occurs for excluder and includer cultivars and ii) investigate the variability in cultivar Cl ratings. A study was conducted across five site-years using six soybean cultivars including three Cl-includer and three Cl-excluder cultivars. Solution containing Cl was applied to the soil beginning at late vegetative growth with final rates ranging from 0 to 1010 kg Cl ha^{-1}. Critical trifoliolate leaflet-Cl concentrations at the R3 stage were developed by regressing relative soybean yield across leaf-Cl concentration for each cultivar Cl rating. For the second objective, composite trifoliolate leaflet and individual trifoliate leaf samples were collected during reproductive growth from variety trials and analyzed for Cl concentration. The research verified that the yield of Cl-includer cultivars is reduced more (4-20%) than Cl-excluder cultivars (0-8%) in high Cl environments. Relative grain yield declined linearly for cultivars within each Cl rating group with 5% yield loss expected when Cl concentrations at the R3 stage averaged 3923 mg Cl kg^{-1} for Cl includers and 1885 mg Cl kg^{-1} for Cl excluders. Across more than 100 cultivars sampled in three Arkansas Soybean Performance Tests, tissue-Cl concentration ranged from <100 to >5000 mg Cl kg^{-1} and showed no clear groupings of the three cultivar Cl-traits suggesting that many cultivars labeled as includers are a mixture of includer and excluder plants. Chloride concentrations of 528 individual plants from eleven cultivars showed 34% and 31% of the plants had Cl concentrations

≤500 or 1000-2000 mg Cl kg^{-1} with only one cultivar having a pure population of Cl excluder plants. A new rating system is warranted to more accurately characterize the proportion of Cl include and excluder plants of each cultivar.

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CHAPTER 1

Literature Review

INTRODUCTION

Irrigated land produces one-third of the world's food, but crop production on an estimated 20% of irrigated hectares is negatively affected by salinity (Xu et al., 2000). High salt concentration adversely affects essential plant functions such as seed germination, seedling growth, flowering, and fruit set (Sairam and Tyagi, 2004). Crops are classified based on their ability to tolerate saline soils while maintaining regular growth. Soybean [*Glycine max* (L.) Merr.] is considered moderately tolerant to salinity (Maas and Hoffman, 1977). Plants considered low and moderately tolerant to salinity are termed glycophytes (Sairam and Tyagi, 2004). Salinity may be caused by different salts or by specific ions found in the soil solution, such as chloride (Cl).

Chloride toxicity has been recognized as a problem in soybean fields of the Mississippi River Delta in Arkansas and is usually associated with irrigation water that contains high amounts of soluble Cl salts, greater than 2-3 mmol Cl L^{-1} (Rupe et al., 2000; Slaton et al., 2013b). Studies and surveys conducted on water quality offer ample evidence that soluble salts in Arkansas soils are largely supplied by irrigation water (Gilmour et al., 1977; 1985; 1983). As of 2014, 82% of the Arkansas soybean hectarage was irrigated making Cl toxicity a relevant and potentially widespread problem for the production of salt sensitive crops like soybean in eastern Arkansas (USDA, 2014). The detrimental effect of excess Cl on the productivity of irrigated soybean is a topic of interest primarily in the mid-South USA, but published information on Cl salinity is scant. Recent research has focused on developing or comparing screening methods for detecting cultivar sensitivity to excess Cl (Lee et al., 2008; Valencia et al., 2008). Research has yet to define critical soil- or tissue-Cl concentrations to predict or diagnose Cl toxicity during the growing season. The lack of diagnostic information is problematic in that growers are unable to determine whether soybean yield will be adversely affected by Cl toxicity before plant symptoms

such as chlorosis become visible. There is also very little information on field and crop management once Cl toxicity is identified as a threat to soybean yield. Performance and yield of modern soybean cultivars categorized as Cl-excluders or Cl-includers has not been examined in the field as the bulk of this research is often conducted in a greenhouse setting. This literature review will summarize the research on Cl toxicity, screening methods to categorize cultivars as 'excluders' or 'includers', and speculate on problems that require additional research.

Chloride Function in Plants

Broyer et al. (1954) conducted research officially recognizing Cl as an essential micronutrient for plant growth. However, Warburg and Luttgen (1946) proved Cl to be necessary for plants to carry out the water splitting reaction in photosystem II. Chloride also functions in opening and closing stomata and stimulation of proton pumping by adenosine triphosphatase at the tonoplast (Marschner, 1995). Chloride is taken up by plants from the soil solution as the anion Cl^-. Typically Cl concentrations in plants range from 2 to 20 g Cl kg^{-1} greatly exceeding the normal requirement of 200 to 400 mg Cl kg^{-1} to achieve healthy plant growth (Marschner, 1995).

Chloride deficiency, although not a widespread problem, has been documented on small grain crops in the Great Plains area of the United States where annual rainfall is low, Cl deposition from ocean salts is non-existent, soils are naturally high in K, and there is no or little history of KCl fertilization (Lamond and Leikman, 2002). Deficiency symptoms often include reduced leaf growth and wilting, followed by chlorosis, bronzing, and necrosis. Roots become stunted and the development of lateral roots is reduced. The number and size of reproductive structures (e.g., fruit or grain) are decreased by Cl deficiency (Johnson et al., 1957; Xu et al., 2000). Chloride deficiency of plants is poorly understood because it is relatively uncommon.

Critical tissue-Cl concentrations that can be used to diagnose Cl deficiency via plant analysis are, as a general rule, not available for most crops. Chloride toxicity is thought to be more common than Cl deficiency, but, like Cl deficiency, the literature lacks information regarding specific plant tissue-Cl concentrations where plant growth and yield are limited by too much Cl. A better understanding of plant Cl nutrition requires research on both deficiency and toxicity of this essential element.

Measuring Chloride in Soil

Soil testing for available nutrients is a common practice for production agriculture to identify potential for deficiencies and toxicities and predict crop response to fertilization. Many macronutrients and some micronutrients have soil-test-based fertilizer recommendations, however recommendations for Cl are uncommon. Soil thresholds regarding salinity are more commonly used to quantify potential Cl problems rather than deficiencies. Saline soils are defined as soil that contains enough soluble salt to interfere with the growth of most crop species. The amount of soluble salts damaging to plants varies among plant species, soil texture and water holding capacity, and the composition of the salts (Marschner, 1995).

Salinity is usually quantified by the measurement of electrical conductivity (EC) using a meter and conductivity cell that indicates the amount of soluble salts in an extract or soil:water mixture. The more dissolved salts a solution contains, the higher the EC value (Rhoades, 1996). A saturated soil-paste or the solution extracted from a saturated paste (EC_{SPE}) is considered to be the best representation of the soluble salt composition of soil and most closely related to soil:water conditions in the field. A saline soil is defined as having an EC_{SPE} of ≥ 4 dS m^{-1} (U.S. Salinity Laboratory Staff, 1954), which converts to an $EC_{1:2}$ of about 0.9 dS m^{-1} (Norman et al., 2003). The standard definition of a saline soil is based on a saturated paste extract, which is a

very tedious and specific method described by Rhoades (1996). Because the preparation (e.g., amounts of soil and water) of a saturated paste differs among soils it is too time consuming for many laboratories to perform on large numbers of soil samples. Currently, most soil-test laboratories use standardized recipes or ratios of soil and water such as 1:1, 1:2, or 1:5. The interpretation of EC values based on a 1:1 or 1:2 soil:water ratio requires that one can estimate with reasonable accuracy what these EC values would equal if measured as a saturated paste extract. Hogg and Henry (1984) formulated relationships between the commonly used soil:water mixtures and the saturation extract method. Multiple comparisons were made, but the one of primary interest is the relationship between the 1:2 suspension and the saturated paste extract, since the 1:2 suspension is typically used to report soil EC in Arkansas (Espinoza et al., 2012). Hogg and Henry (1984) reported that Eq .1 could be used to convert an $EC_{1:2}$ to EC_{SPE}.

[**Eq. 1**] $EC_{SPE} = 3.17 (EC_{1:2}) - 0.47$ (where units are dS m^{-1})

Recommendations for soil testing and their interpretation in regards to salinity are provided by some universities. Kansas State University provides Cl fertilization recommendations for Cl-deficient soils. Mengel et al. (2009) reported winter wheat (*Triticum aestivum* L) yields were increased 7-9% by application of 11 kg Cl ha^{-1} as KCl. Overall, 11 to 22 kg Cl ha^{-1} is recommended for corn (*Zea mays* L.), grain sorghum (*Sorghum bicolor* L.) and winter wheat when soil-Cl concentrations are ≤6 mg Cl kg^{-1} in a 60 cm deep soil sample. While Cl deficiency has not been identified as a problem in Arkansas, the 60-cm deep soil sampling depth recommended in Kansas to identify Cl deficiencies could be used to identify toxic levels of resident Cl in soils (Lamond and Leikman, 2002). A collaborative effort by Oregon State University, University of Idaho and Washington State University created guidelines for soil testing and interpretation of soil salinity in the northwestern USA. Soils with an EC_{SPE} ≥4 dS m^{-}

[1] are defined as high risk for soil salinity related problems. No protocol regarding sampling depth or area is given, but it is recommended to test the EC of the soil using the saturated paste method if salinity problems are expected (Horneck et al., 2007). University of Georgia recommendations provide instruction for soil sampling potentially saline soils, as well as threshold values for interpreting soil EC. Soil samples should be taken from the 0-15 cm depth and each composite sample should include a total of ten soil cores with the interpretation of $EC_{1:2}$ as Very High (damaging to most plants) when $EC_{1:2}$ is 1.76-2.00 dS m^{-1} (Sonon et al., 2015). As an alternative to soil analysis, Slaton et al. (2013b) suggested guidelines for troubleshooting possible Cl toxicity problems in soybean by collecting 15-20 mature trifoliolate leaves at the R2 growth stage for Cl analysis. Preliminary recommendations suggest that leaf-Cl concentrations of 2000-4000 mg Cl kg^{-1} are considered normal for healthy soybean (e.g., no yield loss expected), but this likely depends on whether the cultivar is a Cl excluder or includer. The Cl concentrations in the plant may be a better diagnostic measure than soil EC since the vertical and lateral mobility of Cl makes proper sampling depth and timing difficult in temperate environments, areas that are irrigated, or areas with seasonal rainfall. Mass and Hoffman (1977) defined the minimum soil EC_{SPE} threshold for the initiation of yield loss in soybean as 5.0 dS m^{-1}. Soybean yield is expected to decline linearly by 20% for each additional 1.0 dS m^{-1} increase in EC_{SPE} (EC_{SPE} = 6 dS m^{-1} would result in 80% yield). Use of soil-EC to diagnose potential salinity requires knowledge of the soil and water ratio, recent rain or irrigation, and a proper interpretation of the EC values. Additional information regarding soil tests as well as tissue-Cl concentrations will allow growers multiple methods to detect salinity problems. More research is needed to assess the accuracy of these threshold values as well as to compare includer and excluder soybean cultivar response to soil EC.

Symptoms of Cl Toxicity

The high solubility of Cl-containing salts and the common presence of Cl in the landscape make Cl toxicity a greater concern than Cl deficiency, especially in the Arkansas Delta. Physical signs of Cl toxicity of field-grown plants are difficult to identify during the early vegetative growth of soybean (Parker et al., 1983). Early signs of Cl toxicity observed under experimental conditions include reduced plant height and development of small, dark green leaves (Abel and MacKenzie, 1964). The leaves of Cl-affected soybean plants begin to turn chlorotic (yellow) as Cl accumulates to a toxic level, but these symptoms may not be evident until pod development (Parker et al., 1983). Necrosis, or the death of plant tissues and cells, eventually develops from Cl toxicity. At this point of toxicity the plant's ability to carry out photosynthesis is diminished and large yield reductions are expected.

Chloride toxicity symptoms of field-grown soybean tend to be different than those expressed in greenhouse or laboratory settings. Parker et al. (1983) studied the physical signs of Cl toxicity exhibited by soybean grown in production fields from the use of KCl fertilizer. Symptoms of Cl toxicity were not noted during the first month of vegetative growth. However, the first signs of leaf scorch were observed during reproductive growth around the R3 growth stage (early pod development) and the R6 stage (Fehr et al., 1971). The leaf scorch symptoms begin on the tips of the mature leaves at the bottom of the plant, and move upward into the middle and upper canopy as stress continues. Drought stress intensifies these symptoms and can cause premature plant death. Similar symptoms and timing of appearance were described by Slaton et al. (2013b) for soybean affected by Cl toxicity in Arkansas fields. Parker et al. (1983) suggested that soybean producers are often unable to visually identify signs of Cl toxicity until

reproductive growth, which highlights the need for diagnostic methods to identify soil or plant stress before it is expressed visually by plants.

Diagnostic nutrient concentrations for soybean are usually listed for the early bloom growth stage (R1-R3; Small and Ohlrogge, 1973; Mills and Jones, 1996; Sabbe et al., 2000). The most recent and fully mature trifoliolate leaves should be taken from plants and submitted for analysis. Thresholds of soybean tissue Cl concentrations that define deficient, normal, and toxic concentrations are not listed by Small and Ohlrogge (1973), Mills and Jones (1996), or Sabbe et al. (2000). The lack of information for interpreting soybean tissue Cl concentrations prevents one from knowing what normal Cl concentrations are and from diagnosing Cl nutritional problems from plant tissue analysis.

Chloride toxicity of soybean is a relatively common problem for soybean production in Georgia and Arkansas USA and has been shown to be detrimental to yield (Parker et al., 1983; Rupe et al., 2000). Soybean plants do not have an established Cl concentration that is considered toxic, although Cl concentrations in the soybean tissues have been measured in some of the published research. Parker et al. (1983) showed that leaf scorch ratings and leaf-Cl concentration were correlated and that Cl-sensitive cultivars had greater leaf-Cl concentrations than Cl-tolerant cultivars with an average leaf-Cl concentration ratio of 18.6. Abel (1969) showed that Cl concentrations were 10 to 36% higher in soybean leaflets than in petioles. Trifoliate leaf (leaflet and petiole) Cl concentrations ranged from <1000 mg Cl kg^{-1} for cultivars exposed to salinity but showed no symptoms (Cl-excluder cultivars) to 7000-9000 mg Cl kg^{-1} for cultivars that showed leaf scorch (Cl includer cultivars). Abel and MacKenzie (1964) reported that soybean plants that died prematurely from Cl toxicity had 15,000 to 30,000 mg Cl kg^{-1} in leaves and stems. Slaton et al. (2013a) provided a preliminary estimate suggesting that a normal (e.g., healthy) Cl

concentration is <3500 mg Cl kg^{-1} for includer cultivars and < 1500 mg Cl kg^{-1} for Cl excluder cultivars. Plant-Cl concentrations exceeding these tentative values suggest that yield loss will occur. The toxic concentration of Cl and the effects of increasing leaf-Cl concentrations on soybean yield are currently unknown. The ability to monitor plant Cl concentrations would enable growers to determine whether Cl toxicity is a yield-limiting factor present in their fields, adjust production practices, and perhaps monitor plants in individual fields to make in-season crop management decisions.

Soybean Responses to Salinity and Chloride

Soil salinity does not imply Cl toxicity, although soils with high amounts of soluble salts are likely to have high concentrations of Cl or other anions. Sodium (Na), an element that is not essential for plant growth, is commonly found with Cl and can also be toxic to plant growth (Bernstein, 1975). Sodic soils are defined as a soil with Na ions occupying 15% or more of the cation exchange sites. Symptoms of Na toxicity are similar to that of Cl toxicity such as leaf tip burn and stunted plant growth, but sodic soils will have a black powdery residue on the soil surface, as well as poor drainage (Waskom, 2012). Sodium toxicity is not widespread in Arkansas (Gilmour et al., 1983), but field observations (N.A. Slaton, personal communication) suggest that soils with high Na concentrations also tend to have high EC and retain high Cl concentrations that may cause or contribute to poor soybean growth. Chloride toxicity problems are more prevalent than Na toxicity in Arkansas.

The first physiological reaction of plants exposed to saline conditions is reduced entry of water into roots (Abel and MacKenzie, 1964). Plants exposed to a saline soil solution must overcome both the soil water potential as well as the osmotic potential due to salts. Increased osmotic pressure of the soil solution resulting from increased salt content impairs a plant's ability

to absorb water (Norman et al., 2003). These two processes can be thought of as additive making less of the soil water supply available for crop growth and requiring more frequent irrigation (Ayers and Westcot, 1985). The relationship between osmotic pressure of the soil solution and salinity were published by Reeve and Fireman (1967). There is growing evidence that salt stress affects the uptake, transport, and use of mineral nutrients such as N, P, K, and Ca in nonhalophytes (Essa, 2002; Jouyban, 2012). Salinity may cause nutrient deficiencies and imbalances due to Na, Cl, or both competing with other nutrients for plant uptake or plant uptake of excessive amounts of Na and Cl (Jouyban, 2012).

Soybean seed germination can be negatively affected when planted in saline soil. Abel and MacKenzie (1964) showed that soybean exhibited salt tolerance during germination and early growth regardless of whether the cultivar was rated as a Cl excluder or includer. They reported that seed germination and emergence were not affected when soybean was grown in a solution with 0.0% NaCl (soil EC_{SPE} = 3.1 dS m^{-1}), but as the NaCl levels increased to 0.05% (soil EC_{SPE} = 6.0 dS m^{-1}), 0.10% (soil EC_{SPE} = 8.1 dS m^{-1}), 0.15% (soil EC_{SPE} = 10.3 dS m^{-1}), and 0.20% (soil EC_{SPE} = 11.8 dS m^{-1}) germination decreased at an increasing rate. The decrease in germination and seedling emergence was noticeable when the solution exceeded 0.10% NaCl. Shao and Wan (1994) determined the early developmental stages of germination exhibited higher salt tolerance than later stages, with imbibition of water being the most tolerant and growth of lateral roots as the least tolerant. While soybean may have some degree of salt tolerance during germination, the seedling stage is considered sensitive to salt stress, especially if the seedling is exposed to dry conditions (Hosseini et al., 2002).

Parker et al. (1983) reported Cl toxicity was associated with the application of a K-fertilizer that contained Cl on the poorly drained, flatwood soils of the Atlantic Coast in Georgia.

Averaged across two Cl-sensitive soybean cultivars, non-irrigated soybean fertilized preplant with 169 kg Cl ha^{-1} as KCl fertilizer showed severe chlorosis late in the growing season, had elevated leaf- and seed-Cl concentrations, and produced 42% lower grain yield than soybean that received no KCl fertilizer at the Berrien County research site. Parker et al. (1983) also observed that leaf scorch symptoms appeared prominently at the R6 growth stage at the Tift County site but yield was not affected by Cl addition. At the Berrien County site, leaf scorch occurred on soybean regardless of the Cl rate. The leaf-scorch susceptible cultivars yielded 37% less than tolerant cultivars, seed weighed 25% less, and contained significantly greater average leaf concentrations of P (2.8 vs 2.6 g P kg^{-1}), K (14.8 vs 13.8 g K kg^{-1}), Ca (10.7 vs 8.3 g Ca kg^{-1}), Mg (5.6 vs 4.1 g Mg kg^{-1}), and Cl (16.7 vs 0.9 g Cl kg^{-1}) than leaf-scorch tolerant cultivars. The observations reported by Parker et al. (1983) suggest that Cl can accumulate in poorly drained soil during drought years and carryover from one year to the next. Their research also suggests that leaf-Cl concentration may be a suitable means of differentiating among Cl excluder and includer cultivars but that seed-Cl concentration was not a suitable tissue for analysis in low Cl environments.

Identification of Chloride Including and Excluding Traits

Abel and MacKenzie (1964) and Abel (1969) provided evidence that certain soybean cultivars were able to exclude Cl ions from the plant shoots, while other cultivars transport high concentrations of Cl ions to the aboveground plant structures. Large differences among glycophytes regarding where Cl accumulates in the plant also exist. Chloride tends to accumulate in the older leaves at the bottom of the plant due to rapid growth and low transpiration of new expanding leaves while older leaves continually take up Cl with minimal recycling. The field experiment conducted by Abel (1969) used a saline soil whose conductivity

(EC_e) measured 5 to 7 dS m^{-1} and produced Cl-includer cultivars with leaf Cl-concentrations of 7000-9000 mg Cl kg^{-1} compared to 600-1000 mg Cl kg^{-1} for Cl-excluder cultivars. The leaf-Cl concentration ratio (excluder/includer cultivars) ranged from 7.6 to 14.6:1 suggesting that leaf-Cl concentration can be used to classify a cultivar's ability to include or exclude Cl. Abel (1969) crossed parent plants similar in Cl accumulation, and found no significant differences in the F2 generation regarding Cl accumulation based on necrosis and tissue Cl-concentrations. However, crossing a Cl includer cultivar with an excluder cultivar resulted in an F2 population ratio of 3:1 (non-necrotic:necrotic or excluder:includer). After extensive crossing of known includer and excluder cultivars, Abel (1969) concluded that Cl accumulation in soybean was controlled by a single gene, and Cl exclusion was dominate over Cl inclusion.

Valencia et al. (2008) also examined the Cl concentration in the leaves and roots of Cl-excluder and includer cultivars to develop a quick screening method for classifying cultivars as excluders or includers. Soybean seedlings were grown in 0, 40, 80, 120, and 160 mmol L^{-1} NaCl solutions for 14 d before Cl concentrations were determined in the leaves and roots. The Cl concentrations of excluder and includer cultivars were relatively low (~1000 mg Cl kg^{-1}) in young plants exposed to a solution having 0 mmol L^{-1} NaCl, but differences were apparent in 40-160 mmol L^{-1} NaCl solutions. At 80 mmol L^{-1} NaCl, includer cultivars contained a mean leaf concentration of 37,090 mg Cl kg^{-1} compared to 13,497 mg Cl kg^{-1} in the leaves of the excluders. The leaf-Cl concentration ratio between excluder:includer cultivars ranged from only 1:1 to 2:1, which is much lower than that from field research published by Abel (1969) and Parker et al. (1983). Valencia et al. (2008) also showed that root-Cl concentrations were not always different between Cl excluder and includer cultivars. This result along with the results of Abel and MacKenzie (1964) question whether the mechanism of Cl tolerance in excluders is from plants

retaining Cl in the root system or multiple mechanisms that include root exclusion of Cl uptake from the soil solution and a second mechanism that reduces Cl transport from root to shoots. Valencia et al. (2008) reported the Cl excluder cultivars had significantly lower leaf Na concentrations when grown in solutions containing 40 to 80 mmol L^{-1} NaCl indicating that these cultivars 'exclude' both Cl^- and Na^+ ions. Chloride includer cultivars had a leaf to root Cl ratio of 0.42 to 1.06:1 compared to 0.18 to 0.53:1 for Cl excluders.

Lee et al. (2008) also sought to develop a quick method for screening soybean salt tolerance. Their method used small plastic containers (e.g., cone-tainers or PC method) filled with a sandy soil as a growing medium and compared the results directly to a hydroponic-screening method [similar to Valencia et al. (2008)]. Soybean plants were placed into a nutrient solution after emergence and exposed to either a 0 or 100 mmol L^{-1} NaCl solution at the V2-V3 growth stage. Leaf scorch occurred 8 to 10 d after salt exposure and trifoliolate leaf samples (no petiole) were analyzed for Cl concentration. The results of the PC method were closely correlated with the hydroponics method. Chloride includer cultivars exposed to the 100 mmol L^{-1} salt solution had leaf-Cl concentrations between 3690 and 5230 mg Cl kg^{-1}. Excluder cultivars in the same solution had leaf-Cl concentrations ranging from 2830 to 3370 mg Cl kg^{-1}.

The ratio of Cl concentration between Cl excluders and includers grown under the same conditions is quite different between field and greenhouse conditions. Lee et al. (2008) and Valencia et al. (2008) both showed the includer:excluder Cl concentration ratio between aboveground plant tissue was generally less than 2:1 in greenhouse trials, regardless of Cl addition rate. In contrast, the includer:excluder Cl-concentration ratio is much wider in field trials usually exceeding 6:1. The extremely high (20,000-60,000 mg Cl kg^{-1}) leaf-Cl concentrations commonly measured in greenhouse trials are seldom measured in field trials,

where leaf-Cl concentrations in field trials are usually less than 20,000 mg Cl kg^{-1} (Abel and Mackenzie, 1964; Parker et al., 1983; Rupe et al., 2000). The great differences in leaf-Cl concentration, uptake of high Na concentrations and the very narrow ratio between the includer:excluder leaf-Cl concentration ratio questions whether the greenhouse screening techniques are actually measuring Cl sensitivity or tolerance since the high concentrations of Cl and Na used for screening are not representative of what soybean experiences in commercial field conditions.

Sources of Chloride in Soils

Irrigation water contains dissolved salts (e.g., Cl and Na) and other trace elements which can impact water quality. Eastern Arkansas (especially southeastern AR) uses large amounts of ground water from the alluvial aquifer for crop irrigation that can contain relatively high concentrations of dissolved salts (Gilmour, 1989; Kresse et al., 2000). The dissolved salts that pose potential threats to crop growth and yield have been the focus of research in Arkansas include chloride, calcium bicarbonate, and sulfur (Gilmour et al., 1976; 1983; 1989).

Water Cl concentration is determined in a similar manner to soil Cl, but is much simpler since soil is not involved and the soil to solution ratio is not a factor for interpreting the EC (Rhoades, 1996). Chloride in water has been found to account for 85% of the water EC (EC_w) reading, making it a good indicator for potential Cl toxicity problems sourced from irrigation water (Gilmour et al., 1983). University of Arkansas recommendations classify poor quality irrigation water as containing 2 to 3 mmol Cl L^{-1} (Slaton et al., 2013b). Irrigation water Cl concentrations above 3.0 mmol Cl L^{-1} (100 mg Cl L^{-1}) have potential to cause salinity problems with long-term use (Ayers and Westcot, 1985; Henry et al., 2014). Irrigation water used in rice (*Oryza sativa* L.) production exceeding 100 mg Cl L^{-1} can cause toxicity for soybean in a rice-

soybean rotation (Henry et al., 2014). Other EC_w thresholds include ranges specified by Ayers and Westcot (1985) suggest that <0.75 dS m^{-1} indicates no problem, 0.75-3.0 dS m^{-1} indicates an increasing problem, >3.0 dS m^{-1} indicates a severe problem. Guidelines regarding Cl concentration in irrigation water from Mississippi State University show low, medium, and high hazard for the Cl ranges of 0-142, 143-355, and >356 mg Cl L^{-1}, respectively (Thomas, 2001). Irrigation practices such as the use of a center pivot for overhead sprinkler irrigation may call for even lower Cl concentrations in the water. Thomas (2001) recommends water used for sprinkler irrigation should contain <107 mg Cl L^{-1}. Foliar absorption of Cl leads to greater chances of plant injury (leaf burn) and requires higher quality water (Mass et al., 1982; Thomas, 2001). Additional factors determining plant susceptibility to salt damage include the leaching fraction (LF) and the permeability of the soil (Ayers and Westcot, 1985). The LF can be calculated by dividing the depth of water leached below the root zone by the depth of water infiltrating the soil. The LF can be also be calculated as the percentage of water not used by the crop or lost through evaporation (e.g., If 85% of the applied water is used by the crop or evaporates, then the LF = 0.15). Once estimated, the LF along with EC_{DW} (electrical conductivity of water draining below the root zone) and EC_W (electrical conductivity of the irrigation water) can be used as part of an equation to estimate salt accumulation in the upper soil profile.

[Eq.2] $EC_{DW}\,(dS\ m^{-1}) = EC_W\,(dS\ m^{-1}) \div LF$

Small leaching fractions (< 0.1) indicate the salt in the irrigation water is not completely removed through leaching and can build up in the upper portion of the soil profile.

Arkansas irrigation water is supplied mostly from five aquifers including the Alluvial, Cockfield, Sparta/Memphis Sand, Wilcox, and Nacatoch Sand (Scott et al., 1998). The Alluvial Aquifer is the largest source and provides 94% of Arkansas' irrigation water. Irrigation water

salinity problems were documented as early as 1955 in Chicot County, AR (Onellion and Criner, 1955). More recent studies conducted by Kresse et al. (2000) surveyed the water quality in regards to salinity (e.g., Cl concentration) by collecting 24 water samples across a portion of Chicot County. The mean Cl concentration was 527 mg Cl L^{-1} (14.9 mmol Cl L^{-1}), with a maximum of 1460 mg Cl L^{-1} (41.2 mmol Cl L^{-1}). Slaton et al. (2000) assessed the dissolved salt content of 16 irrigation sources (13 well sources and 3 relifts) in Monroe County, AR at multiple times during the growing season. Wells contained between 35 and 319 mg Cl L^{-1} (1-9 mmol Cl L^{-1}) with the Cl concentration being relatively stable during the growing season. Using the average irrigation water requirement for soybean of 25 to 38 ha^{-1} cm $year^{-1}$ (10-15 ac in^{-1}) reported by Tacker and Vories (2000) irrigation water containing 69 mg Cl L^{-1} (2 mmol of Cl L^{-1}) will add 178 to 267 kg Cl ha^{-1} (160 to 240 lb Cl ac^{-1}) in a single growing season (7 kg Cl ha^{-1} cm). Additionally, Slaton et al. (2000) speculated that re-lift water from drainage ditches may be of poor quality due to the accumulation of salts and an increase in Cl concentration resulting from evaporation before use. Studies by Wilson et al. (2000) in Desha County, AR assessed the water quality from 1496 irrigation sources. Results from show 50.7% of the wells tested (624) had Cl concentrations > 2 mmol Cl L^{-1} and are considered potentially harmful to irrigated crops. Similarly, 62% of surface water sources (86) contained >2 mmol Cl L^{-1} (Wilson et al., 2000). Moore et al. (1993) took water samples from 151 sources (108 well and 20 surface) across Ashley, Chicot, Desha, Drew and Monroe counties and found that, on average, surface water contained significantly lower EC_w concentrations than well water, 0.6 dS m^{-1} and 1.6 dS m^{-1}, as well as lower Cl concentrations 164 mg Cl kg^{-1} to 360 mg Cl kg^{-1} respectively (Moore et al., 1993), but the average value of each source was potentially harmful. The high Cl concentrations

in the irrigation water used in eastern Arkansas highlight the potential for Cl toxicity or salinity to reduce the yields of irrigated crops.

Application of Cl-containing fertilizers can also contribute substantial amounts of Cl to fields used for irrigated crop production. Muriate of potash or KCl is the most common fertilizer applied to fields with K-deficient soil. Muriate of potash is 48% Cl and supplies near equal amounts of K and Cl. Soils that have a very low soil-test K level may receive recommendations for 150 kg K ha^{-1} or more when cropped to soybean, which supplies similar amounts of Cl (Slaton et al., 2013b). Research by Parker et al. (1983) in Georgia, showed non-irrigated Cl excluder soybean cultivars fertilized with KCl produced yields that were 37% greater than the yield of include cultivars and application of 169 kg Cl ha^{-1} reduced the yields of two include cultivars by 65% compared to the yield of soybean receiving 0 kg Cl ha^{-1}.

Behavior of Cl in Soils

The Cl anion is not adsorbed onto soil particles, making it highly leachable (e.g., mobile) in the soil profile (Xu et al., 2000). Chloride, because it is an anion, tends to be repelled by negatively charged soil colloids (Bohn et al., 1979). Chloride is often used as a tracer element for soil-water movement research because it is not adsorbed onto colloid surfaces, is highly soluble (seldom precipitates as secondary minerals), and is not chemically altered by soil organisms (White and Broadley, 2001). Burns (1974) tracked the movement of $CaCl_2$ salt placed 15 cm deep in a sandy loam soil. Soil samples collected to a depth of 45 cm showed that Cl readily moves with water. The mobile nature of anion make testing for potential Cl problems in fields difficult because evaporation results in water and dissolved salt movement towards the soil surface, while additions of water via irrigation and rainfall leach the soluble salts deeper into the soil profile. Shannon et al. (1998) reported that salinity problems in California rice fields usually

occur in paddies having the lowest elevation because the floodwater salt concentration increases as the water flows with gravity across the field and accumulates at the lowest elevation. Bernstein and Fireman (1957) investigated the movement of soluble salts in a furrow-irrigated system and showed that the wetting front (~1.25 cm of soil behind wetting front) typically contained 80 to 90% of the total added salinity, and when evaporation occurs, the dissolved salts accumulate on the top, middle portion of the bed (e.g., soil ridge), which is where the crop is typically planted. Based on the aforementioned research, furrow-irrigated soybean appear especially vulnerable to Cl toxicity, especially on the lowest elevation points in the field.

Research regarding Cl movement in soil has also been conducted in Arkansas. Gilmour et al. (1985) investigated soluble salt movement in a Sharkey clay (very-fine, smectitic, thermic Chromic Epiaquerts), which is a common soil series found in the lower Mississippi Delta. Salinity sensors were buried at five depths ranging from 4 to 91 cm below the soil surface of a Sharkey soil amended with 1052 kg Cl ha^{-1} and cropped to rice. Soil cores were taken for analysis 36 (before flooding rice), 140 (5 d after rice harvest) and 339 d (following spring) after salt addition. The 140 d samples showed that $EC_{1:2}$ peaked at 15 and 90 cm but the following spring $EC_{1:2}$ was relatively constant from 0 to 60 cm and then increased with increasing soil depth to 90 cm. In contrast, 140 d after application, soil-Cl concentration was greatest at a single depth of about 15 cm (no peak at lower soil depth like $EC_{1:2}$). The following spring, soil-Cl concentrations were relatively uniform and greatest in the 0-40 cm depths compared to 40 to 90 cm. Their results support that Cl is highly mobile in the profile of a clayey soil and shows evidence of high Cl concentrations remaining in the top 15 cm.

High Cl concentrations in the upper part of the root zone have shown to be more detrimental to plant growth than high salinity concentrations at lower depths because roots

located in the upper root zone are generally more active in water and nutrient uptake (Bernstein and Francois, 1973). Arkansas soybean growers who rotate rice in the same fields typically encounter fragipans and plow pans. Fragipans are impermeable layers underlain at some depth but generally found less than 120 cm deep. Plow pans are present much closer to the soil surface about 7 cm deep and extending to the 15 cm depth (Norman et al., 2003). Although both of these characteristics improve the soils water holding capacity for rice production, this same feature can prevent soluble salts from leaching downward in the profile salinizing the soil surface and inhibiting soybean growth. Thus, management of salinity in the upper root zone is paramount for minimizing salinity damage and achieving maximum crop yield.

Preliminary numbers by Ayers and Westcot (1985) show that soil Cl concentrations in the rooting zone tend to be three times greater than the Cl concentrations found in the irrigation water. The large irrigation requirement of rice (187 cm ha^{-1} $year^{-1}$; 30 ac in^{-1}), assuming a water Cl content of 3 mmol Cl L^{-1}, can supply a field with 800 kg Cl ha^{-1} (720 lb Cl ac^{-1}; Henry et al., 2014). This large amount of Cl introduced into the soil via irrigation water is not taken up by the rice plant and must be removed by leaching, run-off, or both (Gilmour et al., 1976; Ayers and Westcot, 1985. Arkansas growers rely mostly on rainfall to supply adequate run-off (approximately 94.7 ha cm^{-1}) during the winter months to remove excess salts from their fields (Gilmour et al., 1976). Winter seasons with below average rainfall may result in inadequate run-off (~5 acre inches), which fails to remove excess salts and can result in accumulation to a toxic level for the subsequent crop. About one-third (0.4 to 0.5 million ha $year^{-1}$) of the soybean hectares grown in Arkansas follow rice in the rotation, which is of concern since rice irrigation averages about 188 ha cm^{-1} yr^{-1} (30 ac in^{-1}) and may add large amounts of Cl that could accumulate if winter precipitation and run-off are low.

SUMMARY

Chloride toxicity is a problem that has adverse effects on soybean growth and yield and is believed to be a common problem for irrigated-soybean production in eastern Arkansas. While soybeans have been classified as moderately tolerant to salinity, research has categorized soybean cultivars into two categories known as Cl includer and Cl excluder cultivars to aid growers in selecting cultivars that will be least affected by Cl-specific salinity. The most economical and beneficial option for managing Cl toxicity is to plant a Cl-excluding cultivar. Methods of identifying Cl toxicity during the season would be helpful for crop management and establishing what percentage of soybean hectares are affected by Cl toxicity. Soil or plant tissue samples collected during the season would allow a producer to perhaps alter management to avoid further yield loss or to select a production system that is least vulnerable to Cl accumulation.

Chloride excluder cultivars will have substantially lower tissue Cl concentrations, but thresholds that define yield-damaging concentrations for each cultivar category (e.g., Cl excluders and includers) are not available. Preliminary research by Slaton et al. (2013a) suggests critical leaf-Cl concentrations that define when yield loss will begin should be different for Cl-exlcuder and includer cultivars. Additional research is needed to determine whether Cl excluder cultivars are able to retain greater concentrations of Cl in their roots or whether they take up less Cl from the soil solution and to establish more concrete tissue Cl thresholds for identifying Cl toxicity and predicting yield loss.

Accurately defining soybean cultivars as a Cl includer or excluder is of great importance to growers and is needed for breeding commercially acceptable Cl excluder cultivars. Valencia et al. (2008) estimated that only 20% of the southern soybean cultivars are Cl excluders, making

the development of effective screening methods to identify Cl excluder cultivars and monitor Cl accumulation during the season an important research objective. The number of Cl includer to excluder cultivars available to growers is limited in the late maturity group IV cultivars and almost non-existent in maturity group III and early IV cultivars. Several researchers have suggested that leaf tissue analysis could be used to categorize cultivars as includers and excluders but no one has sought to validate tissue analysis from cultivar yield trials as a viable screening method. The advantage of leaf analysis is that small and large seed companies could easily collect tissue samples for analysis eliminating the need for greenhouse screening trials. An additional advantage is that no extra labor or greenhouse space and expense are needed if the field trials can be used to identify this trait. The current screening method appears to be somewhat inconsistent as a cultivar may be listed as a 'mixed' population in one year and an includer or excluder in another separate screening (Table 1). For example, 'Hutcheson' soybean is considered to be a Cl includer cultivar and was used as a standard Cl includer in research conducted by Lee et al. (2008). Cultivar screening results raise the question as to whether Hutcheson is a Cl includer, a mixed population of includers and excluders, or a Cl excluder. Incorrectly categorizing the Cl includer/excluder trait could be a costly mistake for growers.

Irrigation water is believed to be the primary Cl source that contributes to the problem on the large majority of Arkansas soybean hectares. Soybean producers rely on adequate rainfall each year to remove excess Cl and other soluble salts via leaching or runoff (e.g., lateral movement across fields) between crops and to reduce their dependence on irrigation water that may be high in Cl during the growing season. A gradual buildup of Cl in the beds of soybean fields is believed to be the major Cl problem for Arkansas soybean production. Minimal research has been conducted in the field to develop threshold soil EC values that could be used to

determine the threat of Cl toxicity during the growing season. Soil testing alone may not be adequate for detecting Cl toxicity due to the highly mobile nature of Cl ions, however current recommendations in Kansas of sampling to a depth of 60 cm (24 inches) seem to be appropriate for detecting Cl deficiencies and toxicities in the root zone (Lamond and Leikman, 2002). Development of such information would allow the Arkansas soybean industry (seed companies, and breeders specifically) to quantify how widespread the Cl toxicity problem is and how much yield is lost to this problem annually. Assuming that Cl toxicity is relatively widespread and represents a significant yield loss, additional research to remove Cl from water, ameliorate Cl problems with other soil amendments and fertilizers would be warranted.

A summary of the potential importance of Cl toxicity's negative effect on soybean production in Arkansas can be put into perspective using common statistics that describe Arkansas soybean production systems and irrigation water. In Arkansas, 82% of the soybean crop is irrigated (USDA, 2014) and 38% of the irrigation water contains potentially damaging levels of salinity ($\geq$1.2 dS m^{-1}) (Gilmour et al., 1983). The greatest water requirement for soybean occurs from flowering to pod fill, which typically occurs during the months of July and August (Tacker and Vories, 2000). These same months have the lowest and most variable rainfall amounts, as well as the highest evaporation rates (Scott et al., 1998). Information on the behavior of Cl shows that it accumulates at or near the top middle portion of the bed of furrow-irrigated crops (Bernstein and Firemen 1957; Burns, 1974) where the Cl can cause the greatest damage to soybean growth and yield (Bernstein and Francois, 1973). All of these factors considered, Cl toxicity is a very relevant problem for soybean production in Arkansas and additional research to understand and ameliorate Cl toxicity of soybean is warranted.

The immediate research needs for managing Cl toxicity of irrigated soybean in Arkansas include:

1. Quantifying the importance of the selection of the proper cultivar trait in regards to Cl inclusion or exclusion for soybean production.
2. Examining the feasibility and accuracy (compared to existing screening methods) of categorizing the Cl inclusion/exclusion trait from tissue analysis in field trials.
3. Defining leaf-Cl concentrations that are considered normal and toxic for Cl includer and Cl excluder cultivars.
4. Correlating leaf-Cl concentration with relative yield or yield loss from Cl toxicity.
5. Defining soil EC or Cl concentrations that can be used to quickly assess accumulation of damaging Cl levels during the growing season.
6. Assessing how widespread the Cl toxicity problems are across the soybean-producing area within Arkansas to develop an estimate of annual yield loss from Cl toxicity.
7. Assessing how Cl movement through the furrow with irrigation water influences yield loss spatially within individual fields.
8. Assess how production system (furrow irrigation on beds vs flat planted and flood irrigated vs sprinkler irrigation) influences Cl toxicity.

This thesis research will focus on the first four objectives in the above list. Based on the information presented in the literature review, the hypotheses for these objectives are i) Cl excluder cultivars will produce similar yields as Cl includers in the absence of damaging Cl concentrations and yield loss from Cl toxicity will be less for Cl excluder cultivars, ii) leaf tissue analysis for Cl concentration will be more accurate and consistent for categorizing the Cl inclusion/exclusion trait than current screening methods, iii) critical leaf-Cl concentrations will be different for Cl includer and Cl excluder cultivars and will be significantly, and negatively correlated with soybean relative yield.

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Table 1.1. Soybean relative maturity group (RMG), and Cl rating category for selected cultivars showing inconsistencies among cultivar ratings generated in 2013, 2014, and 2015 (Ross et al., 2014, 2015).

Cultivar	RMG	2013	2014	2015
		Chloride rating category		
Eagle Seed ES 5400RR	5.4	Mixed	Excluder	Mixed
Halo 4:95	4.8	Includer	Mixed	Includer
NK S-45 V8 Brand	4.5	Includer	Mixed	Includer
Pioneer 50T64	5.0	Mixed	Excluder	Excluder
Progeny 4930LL	4.9	Mixed	Mixed	Includer
Progeny 5160 LL	5.1	Includer	Includer	Mixed
Progeny 5460 LL	5.4	Includer	Mixed	Includer
Rev 55R63	5.7	Excluder	Mixed	Excluder
Rev 47R53	4.7	Includer	Mixed	Includer

CHAPTER 2: Characterizing Soybean Yield Loss from Chloride Toxicity

ABSTRACT

Chloride toxicity is recognized as yield limiting problem for soybean [*Glycine max* (L.) Merr.] production throughout the mid-south USA. However, limited information is available to accurately diagnose and manage Cl toxicity. A study was conducted to determine how incremental additions of Cl effect the yield of Cl-excluder and –includer cultivars and develop critical leaf-Cl concentrations that indicate when yield loss from Cl toxicity begins. Six soybean cultivars, three Cl-includer and three Cl-excluder cultivars, were planted at five site-years. Chloride solution containing $CaCl_2 \cdot 2H_2O$ and $MgCl_2 \cdot 6H_2O$ salts was applied to the shoulder of 76-cm wide beds beginning at late vegetative growth with final rates ranging from 0 to 1010 kg Cl ha^{-1}. Leaf-Cl concentration was measured by sampling trifoliolate leaflets during reproductive growth. Critical leaflet-Cl concentrations at the R3 stage were developed by regressing relative soybean yield across leaf-Cl concentration for each cultivar Cl rating. Chloride addition reduced grain yield, on average, by 17% for Cl includer and 5% for excluder cultivars. Leaflet-Cl concentration explained 56% of the variability in relative grain yield for Cl excluders and includers. Relative grain yield declined linearly at 2.6% $(1000 \text{ mg Cl kg})^{-1}$ for cultivars within each Cl rating group with 5% yield loss expected when concentrations exceeded 3923 mg Cl kg^{-1} for includers and 1885 mg Cl kg^{-1} for excluders. The ability to predict when potentially toxic amounts of Cl are taken up using tissue analysis is the first step in developing management strategies that may help mitigate yield loss.

INTRODUCTION

Chloride toxicity is a yield-limiting problem for irrigated soybean [*Glycine max* (L.) Merr.] produced in Arkansas and other soybean-producing states in the mid-South USA (Parker et al., 1983; Rupe et al., 2000). Poor soil drainage, irrigation water and application of Cl-containing nutrient sources (e.g., muriate of potash fertilizer and poultry litter) contribute to salinity problems (Gilmour et al., 1976; Parker et al., 1983). In most years and in many fields, application of fertilizer and irrigation water are inputs critical for the production of high soybean yields, yet the Cl added in these inputs can contribute to yield loss. Irrigation water with Cl concentrations ≥3.0 mmol Cl L^{-1} (117 mg Cl L^{-1}) has the potential to cause soil salinity problems (Ayers and Westcot, 1985; Henry et al., 2014). Surface (e.g., reservoir) and subsurface (e.g., aquifer) water used for irrigation in eastern Arkansas often has ≥3 mmol Cl L^{-1} and is typically the single greatest source of Cl addition (Gilmour et al., 1983; Kresse et al., 2000; Slaton et al., 2000). For example, 0.123 ha m^{-1} of irrigation water with 3 mmol Cl L^{-1} contributes approximately 300 kg Cl ha^{-1} during the growing season. Chloride toxicity of soybean tends to occur more frequently and severely during summers with below average rainfall when the reliance on irrigation water is greatest and run-off producing rainfall events may not occur to aid in Cl removal.

Soybeans are classified as moderately tolerant to saline conditions (Mass and Hoffman, 1977). The moderately tolerant classification does not account for genetic variation in Cl accumulation among soybean cultivars. Commercial soybean cultivars are screened and most often categorized as Cl includers, Cl excluders, or a ‘mixed’ or segregating population (Green and Conaster, 2017; Ledesma et al., 2016). Although a cultivar may receive a rating of ‘mixed’ in university screening trials nearly all commercially available cultivars are listed as being either

Cl excluders or includers. The excluder cultivars tend to be more tolerant of saline conditions and accumulate less Cl in the aboveground portion of the plant than includer cultivars (Abel and MacKenzie, 1964; Parker et al., 1983). Abel (1969) reported that Cl exclusion was the dominant trait over Cl inclusion and was controlled by a single gene. Despite Cl exclusion being a dominant trait, only about 20% of late maturity group IV and 30% of early maturity group V cultivars are classified as excluders (Green and Conaster, 2017). The percentage of excluders available in lower maturity groups is largely unknown since Cl toxicity does not appear to be a problem in the Midwest USA soybean-growing states. Yang and Blanchar (1993) noted that none of the maturity group II, III and IV cultivars tested were Cl excluders.

Options for managing Cl toxicity of soybean other than cultivar selection based on Cl rating have not been researched. Limited field research is available that confirms cultivar Cl classification accurately describes cultivar response to high Cl field environments. Abel and MacKenzie (1964) summarized that soybean plant death occurred when the Cl concentration of leaves was >30,000 mg Cl kg^{-1} and stems >15,000 mg Cl kg^{-1} with leaves being the tissue that best indicated Cl uptake. The Cl concentration of includers is several times greater than the Cl concentration of excluders when grown under field conditions. Because excluder cultivars have substantially lower tissue-Cl concentrations than Cl includer cultivars (Abel and MacKenzie, 1964; Abel, 1969; Parker et al., 1983; Rupe et al., 2000), thresholds that define yield-damaging tissue-Cl concentrations at a critical growth stage or across growth stages for each cultivar category are needed but not available. Diagnostic tissue-Cl concentrations that describe sufficient and toxic Cl concentrations and enable one to identify soybeans that are at risk for accumulating a toxic level of Cl resulting in yield loss before the onset of Cl toxicity symptoms have not been developed.

Information describing Cl accumulation or leaf-Cl concentration over time is limited. Yang and Blanchar (1993) recorded the leaf-Cl concentration of known excluder and includer cultivars every 2 wk. The results showed leaf-Cl concentrations were greatest and most variable during vegetative growth compared to the lower and more consistent leaf-Cl concentrations measured during reproductive growth. Understanding how tissue-Cl concentrations change over time would be useful for developing a sampling protocol to accurately identify toxicity problems. Diagnostic Cl concentrations would be useful for surveying the incidence and severity of Cl toxicity in a geographic region, and be of value for examining other cultural practices that might be used to mitigate damage or reduce the risk of Cl toxicity.

Our research objectives were to i) characterize trifoliolate leaflet-Cl (tissue-Cl) concentrations of three includer and three excluder cultivars during soybean reproductive growth; ii) compare the seed yields of three includer and three excluder cultivars as affected by four levels of soil-applied Cl; and iii) develop a critical leaf-Cl concentration for Cl-includer and -excluder cultivars. Based on the aforementioned research, we hypothesized that the tissue-Cl concentrations will increase as Cl rate increases, the seed yield of includer cultivars will be reduced with the addition of less Cl than excluder cultivars, and that critical tissue-Cl concentrations that indicate yield loss will be lower for excluders than includers. We also expect that in high Cl field environments tissue-Cl concentration will increase during reproductive growth but in low Cl environments the rapid rate of dry matter accumulation during reproductive growth will be greater than the rate of Cl uptake and cause tissue-Cl concentration to decline with time.

MATERIALS AND METHODS

Site Description

Field trials were established at the Pine Tree Research Station (PTRS) during 2014, 2015, 2016 and Rohwer Research Station (RRS) during 2014 and 2016. Locations will be referred to by the site abbreviation and year (e.g., PTRS-2016). Soil at the PTRS was mapped as a Calloway silt loam (fine-silty, mixed, active, thermic Aquic Fraglossudalfs) that followed soybean in 2014, corn *(Zea mays* L.) in 2015, and grain sorghum (*Sorghum bicolor* L.) in 2016. Soil at the RRS was mapped as a mixture of Sharkey (very-fine, smectitic, thermic Chromic Epiaquerts) and Desha (very-fine, smectitic, thermic Vertic Hapludolls) clays and followed soybean in the rotation in both 2014 and 2016. Each of the soils is described as being very poorly to somewhat poorly drained and very slowly permeable (0.5-1.6 cm h^{-1}) (USDA-NRCS, 2016). Selected agronomic and research management information for each site is listed in Table 2.1 and soil chemical property means (n = 4, composite soil samples from 0- to 10-cm depth) for each field are listed in Table 2.2. Seeding rate (370,500 seed ha^{-1}), irrigation, and pest control closely followed recommendations from the University of Arkansas System Division of Agriculture (University of Arkansas, 2000). Soybean was grown on beds and furrow irrigated as needed with well water containing 24 mg Cl kg^{-1} at PTRS and 122 mg Cl kg^{-1} at RRS. Muriate of potash (500 g K kg^{-1}) and triple superphosphate (200 g P kg^{-1}) were applied preplant to each site to supply 60 to 80 kg K ha^{-1} and 20 to 30 kg P ha^{-1}, respectively.

Treatments

Six cultivars were seeded in 16, 55-m long strips and received one of four different Cl rates. Some cultivars changed from one year to the next (Table 2.3). Plots for each individual cultivar contained four, 9.15-m long rows. Cultivars were selected to represent the mid to late

IV maturity group and included three Cl includer and three excluder cultivars in 2014, two Cl includer, three Cl excluder and one Cl mixed cultivar in 2015, and two cultivars from each of the three categories in 2016. Note that each of the selected cultivars were initially rated as either a Cl excluder or includer. The mixed Cl designation was assigned to some cultivars based on information gathered during our research. Each Cl rate strip was separated by four border rows to ensure Cl from one strip did not influence soybean growth in adjacent strips.

Chloride treatments were made using a combination of $CaCl_2 \cdot 2H_2O$ and $MgCl_2 \cdot 6H_2O$ salts (Bulk Reef Supply Co., Golden Valley, Minn.) applied in a 3:1 cation molar ratio, which approximates the exchangeable Ca and Mg molar ratio in the soil common to each research site. The total amount of Cl was applied in five applications to achieve rates of 0, 280, 560, and 840 kg Cl ha^{-1} at all site years except PTRS-2016 where an additional application increased rates to 340, 670, 1010 kg Cl ha^{-1} (Table 2.1). The Ca and Mg salts for each Cl rate were preweighed for each replicate, dissolved in deionized water, and applied at spray volumes of 533 L ha^{-1} at PTRS and 683 L ha^{-1} at RRS to the plots on the dates listed in Table 2.1. Salt solutions were delivered using a 4-nozzle boom with drop nozzles (Teejet XR8004VS at the PTRS and the Teejet XR8006VS at the RRS; Teejet Technologies, Wheaton, Ill.) that applied solution to the bed shoulders of two rows simultaneously. Late in the season when the soybean canopy closed, a single-nozzle boom was used to apply the salt solution on the shoulder of each bed.

Plant Collection and Harvest

Fully expanded trifoliolate leaflets from the third node from the top of 15 plants in the middle two rows were collected at four to six different growth stages to monitor leaf-Cl concentration at all site-years except RRS-2016 where one sample was taken (Table 2.1). Leaf samples were dried at 65 °C to a constant moisture, ground to pass a 1-mm sieve, a 0.1 or 0.2 g

subsample was extracted with 15 mL deionized water to determine leaf-Cl concentration (Liu, 1998), and the Cl concentration in the extract was analyzed by inductively coupled plasma atomic emission spectroscopy (Arcos-130 SOP, SPECTRO Analytical Instruments, Kleve, Germany).

Two composite soil samples, one representing the includers and one representing the excluders, were collected from each Cl rate replicate in August of each year during the R5 growth stage. Each composite sample contained six, 2.5 cm o.d. by 10 cm deep cores collected from the top of the bed from each main plot. Two soil cores from each cultivar having the same Cl rating were composited in each main plot replicate (6 cores main plot^{-1}). Note that at the time of sampling, cultivars eventually labeled as 'mixed' were included in the Cl rating category given originally by the seed company. Soil samples were oven dried for 3 d at 65°C, ground to pass a sieve with 2-mm openings, and electrical conductivity ($EC_{1:2}$) was measured in 20 g soil and 40 mL deionized water mixture (Wang et al., 2014).

The two middle rows of each plot were harvested using a small-plot combine equipped with a moisture meter and scale. Soybean-seed moisture content at harvest was adjusted to 130 g H_2O kg^{-1} for the calculation of grain yield. A subsample of seed from each plot was collected and stored in an air-conditioned laboratory for 7 to 8 wk until seed reached an equilibrium moisture of 70 g H_2O kg^{-1}. Stems, pods and other foreign matter present in the harvested seed sample were removed before analysis. The moisture content of each seed subsample was recorded using a grain moisture meter (model GAC 2100, Dickey-John Corp., Auburn, IL) and 1000 seed were counted and weighed.

For determining the relationship between trifoliolate-leaflet Cl concentrations and grain yield, actual yield was converted to percent relative yield by dividing the overall actual mean

yield from each Cl rate and cultivar combination (n = 24 site-year^{-1}) by the highest mean yield for each cultivar and multiplying by 100. Based on this calculation each site-year had six relative yields that were 100% (one per cultivar) and 18 relative yields that were ≤100%. The calculation of relative yield for each cultivar assumes that salinity or Cl-specific ion toxicity was not yield limiting for soybean at the lowest applied Cl rate and there was no yield benefit from the applied Ca and Mg.

Statistical Analyses

Each experiment was a randomized complete block with split-plot treatment structure. The whole plot consisted of four Cl rates across four blocks and the split plot was the six cultivars nested within Cl rating. The Cl rate and cultivar Cl rating were treated as fixed effects and the block and cultivar nested within Cl rating were treated as random effects. For all measured variables (grain yield, trifoliolate leaf Cl, 1000-seed weight, and soil $EC_{1:2}$), ANOVA was conducted by site-year using the GLIMMIXED procedure in SAS v. 9.4 (SAS Institute, Inc., Cary, N.C.). For each experiment, the fixed effects of Cl rate and Cl rating were examined along with their interaction using ANOVA. When appropriate, mean separations were performed using Fisher's protected least significant difference (LSD) method at a significance level of 0.10.

The relationship between relative soybean yield and trifoliolate-leaflet Cl concentration was determined by regressing the mean relative soybean yield against the mean tissue-Cl concentration for samples collected at the R3 growth stages. Linear or quadratic models using the MIXED procedure or the linear plateau model using the NLIN procedure of SAS v9.4 were fit to the data by cultivar Cl rating trait (e.g., Cl includer or excluder). The model with the best fit was determined by examination of the coefficients of determination and studentized residual plots to explain the relationship.

The behavior of leaf-Cl concentration across time was examined by regressing replicate tissue-Cl concentration data from the R3 growth stage across days after the R1 stage (DAR1) as estimated by the SoyMap program and verified in the field (Popp et al., 2016). The regression was done by cultivar-Cl rating due to the large difference in overall magnitude of leaf-Cl concentrations. The regression model included the linear and quadratic functions of time (DAR1) and allowed regression coefficients to depend on Cl rate and its interaction with DAR1 (analysis of covariance). The regression analysis was performed by site-year for each site-year except RRS-2016 using the MIXED procedure of SAS v9.4 (SAS Institute, Cary, NC). The full model was simplified by sequentially removing the most complex nonsignificant model term ($P > 0.15$) until the simplest significant model was obtained. Regression coefficients in the final model were interpreted as significantly different from 0 when $P \leq 0.10$.

RESULTS AND DISCUSSION

Soil $EC_{1:2}$

Soil $EC_{1:2}$ was affected by the interaction between Cl rate and cultivar Cl rating at PTRS-2014, but only Cl rate, averaged across cultivar Cl rating, was significant at the other four site-years (Table 2.4). The lack of a significant cultivar Cl rating effect for four of the five site-years suggests that plant uptake of Cl has little influence on the EC of the bulk soil. Soil samples were collected based on the original Cl-rating that was associated with the cultivar for trials conducted in 2015 and 2016, which resulted in one cultivar with a mixed rating being sampled with the includers in 2015 and two cultivars with a mixed rating, one in each respective Cl rating, in 2016. Cultivar-Cl rating influenced soil $EC_{1:2}$ only at the PTRS-2014 when the cultivars in each group were all includers or excluders, but the response was not consistent across the four Cl

rates. In general, soil $EC_{1:2}$ increased with each incremental Cl rate increase, with the soil $EC_{1:2}$ being similar between cultivar Cl ratings within each Cl application ≤560 kg Cl ha^{-1}.

Chloride rate influenced soil $EC_{1:2}$ at each of the five site-years with the $EC_{1:2}$ increasing numerically and sometimes statistically as Cl rate increased (Table 2.4). Soil receiving the highest Cl rate had greater $EC_{1:2}$ than soil receiving 0 to 340 kg Cl ha^{-1}. The $EC_{1:2}$ results show that the added Cl solution had the desired effect of increasing soil electrical conductivity. Although soil $EC_{1:2}$ among the five site-years was not compared, the numerical range of $EC_{1:2}$ in soil that received 0 kg Cl ha^{-1} ranged from 0.153 to 0.281 dS m^{-1}. When converted to a saturated paste EC (EC_{SPE} of 2 dS m^{-1} = $EC_{1:2}$ of 0.78 dS m^{-1}) using the equation described by Hogg and Henry (1984), these values would not be considered saline (EC_{SPE} of 4 dS m^{-1}) or moderately saline (EC_{SPE} of 2 dS m^{-1}). Soil receiving the greatest Cl rate had $EC_{1:2}$ values that ranged from 0.254 to 1.047 dS m^{-1}. Soils having $EC_{1:2}$ values > 1.41 dS m^{-1} would be considered saline (US Salinity Laboratory Staff, 1954) when converted using the equation by Hogg and Henry (1984).

The cumulative rainfall from 1 June through 31 August totaled 389 mm at PTRS-2014, 378 mm at RRS-2014, 252 mm at PTRS-2015, 172 mm at PTRS-2016, and 390 mm at RRS-2016 with 3, 6, 4, 1, and 4 daily rainfall events > 25 mm d^{-1}, respectively. The relationship between $EC_{1:2}$ in the zero Cl rate and total rainfall 2 wk before soil samples were collected produced a negative linear response with an R^2 of 0.73 (P = <0.0001) suggesting soil $EC_{1:2}$ decreased as rainfall total increased (Appendix 2.1). Rainfall events producing substantial rainfall or an intensity that exceeds infiltration rate may result in runoff that effectively flushes Cl that has accumulated near the soil surface from the field. In turn, high temperatures coupled with low amounts of rainfall can encourage the movement of soluble salts to the soil surface which might promote Cl toxicity (White and Broadley, 2000). Thus, the amount and intensity of

rainfall events for each site-year might partially explain the soil $EC_{1:2}$ and numerical differences in yield response to Cl among the sites.

Trifoliolate Leaflet-Cl Concentration across Time

Trifoliolate leaflet Cl-concentrations were regressed across time (DAR1) to assess how tissue-Cl concentration responded during reproductive growth at four of the five site-years (Tables 2.5 and 2.6; Figs. 2.1 and 2.2). Knowledge of how tissue-Cl concentration changes across time is useful for interpreting tissue-Cl concentration relative to critical tissue-Cl concentrations at a particular growth stage. Tissue-Cl concentration was a quadratic function of time that depended on Cl addition rate for each of the four site-years. The coefficients of determination were numerically higher for the includer cultivars for each site-year with time (DAR1) explaining 14 to 47% of the variation in tissue-Cl concentration for excluders and 35 to 76% of the variation for includers (Table 2.6).

Tissue-Cl concentrations of excluders at PTRS-2014 were a quadratic function of time with the intercept and linear coefficients depending on Cl rate with a common quadratic coefficient (Fig. 2.1A and Table 2.6). All coefficients were statistically different than zero ($P \leq 0.10$) except the linear terms in the 0 and 280 kg Cl ha^{-1} rate (Table 2.6). The predicted tissue-Cl concentrations between 10 and 50 DAR1 for soybean receiving 560 and 840 kg Cl ha^{-1} increased by 440 to 540 mg Cl kg^{-1}, respectively. In contrast, tissue-Cl concentrations of soybean receiving 0 and 280 kg Cl ha^{-1} remained relatively constant or decreased (Δ -73 to -243 mg Cl kg^{-1}) between 10 and 50 DAR1 (Fig. 2.1A). Tissue-Cl concentrations of includers were 5 to 17 times greater than those of excluders but showed similar trends across time for each Cl rate (Fig. 2.2A). The change in includer tissue-Cl concentrations between 10 and 50 DAR1 ranged from -1502 mg Cl kg^{-1} for soybean receiving 0 kg Cl ha^{-1} to 4361 mg Cl kg^{-1} for soybean receiving 840

kg Cl ha^{-1}. The trends suggest that when soil Cl concentrations are low soybean dry matter increases at a rate greater than that of Cl accumulation, but the opposite occurs when Cl availability is high.

The quadratic equation coefficients (Table 2.6) predicting tissue-Cl concentrations changed among site-years, Cl rates, and cultivar Cl ratings, but the general trends across time described for PTRS-2014 showed many similarities with the trends exhibited by RRS-2014, PTRS-2015- and PTRS-2016 (Fig. 2.1A-D and Fig. 2.2A-D). First, within each sample time, tissue-Cl concentration generally increased as Cl addition rate increased. The second similarity was that includers had tissue-Cl concentrations that were, on average, 10 times greater (range 5 to 29:1) than excluders with the ratio numerically declining as Cl rate increased. Finally, regardless of cultivar-Cl rating, tissue-Cl concentrations tended to be relatively constant from early reproductive growth until soybean seed fill was nearly complete. With the exception of includers and excluders at the PTRS-2016 (Figs. 2.1D and 2.2D), tissue-Cl concentrations increase substantially, especially for includers, as soybeans approach the R6 stage. One possible reason why Cl concentration increases in late reproductive growth is soybean dry matter accumulation peaks near the R6 growth stage (Bender et al., 2015) but Cl continues to be taken up with soil water resulting in an increase in Cl concentration due to limited dilution by dry matter. Parker et al. (1983) showed that seed is not a major Cl sink and even in high Cl environments only a small percentage is translocated from the leaves to the seed. The spike in leaflet-Cl concentration was most notable at PTRS-2015 where predicted tissue-Cl concentrations of includers exceeded 15,000 mg Cl kg^{-1} (Fig. 2.2C). The observed increase in tissue-Cl concentrations as soybeans approach maturity coincided with the appearance of leaf

scorch symptoms caused by Cl toxicity of soybean receiving moderate to high rates of Cl in our study.

The literature contains limited information regarding soybean tissue-Cl concentration changes or accumulation across time. Research has examined nutrient uptake and partitioning by soybean but has not reported information for Cl (Harper, 1971; Bender et al., 2015). Yang and Blanchar (1993) collected leaf samples every 2 wk after planting from known Cl sensitive and tolerant cultivars and plotted the concentrations across time. Leaf-Cl concentration was greatest for Cl sensitive and tolerant cultivars during early vegetative growth (V2-V3) with concentrations being the lowest at flowering. During reproductive growth, tissue-Cl concentration of the includers increased gradually while the Cl concentration of the excluders remained fairly constant. The trend exhibited in our results suggests that a critical toxic tissue-Cl concentration established for soybean at one growth stage during early or mid-reproductive growth might be a good estimate of potential toxicity for other reproductive growth stages before the R5-6 stages.

Grain Yield

Either the treatment main effects or their interaction influenced grain yield at four of the five site-years (Table 2.7). No differences in grain yield were measured among treatments at RRS-2016 where seed yield averaged 3316 kg ha^{-1}. Grain yield was significantly affected by the Cl rate by cultivar rating interaction at PTRS-2014, PTRS-2015 and PTRS-2016 and Cl application rate, averaged across cultivar rating, at RRS-2014. At the RRS-2014, seed yield decreased numerically as Cl addition rate increased with yield losses of 4 to 8% from the addition of 280 to 840 kg Cl ha^{-1} compared to soybean that received no Cl. Reasons for the lack of a cultivar Cl rating effect at the two RRS sites are unclear but perhaps are related to

differences in varietal performance at this site. The variation in actual yield and relative ranking among soybean cultivars in variety yield trials is well documented, although the reasons are not always clear (Chen and Wiatrak, 2010).

Seed yield was significantly affected by the Cl rate by Cl rating interaction at each of the three PTRS site-years (Table 2.7). A significant interaction would be expected provided the selected cultivars have comparable yield potential, Cl is present in amounts that range from normal to excessive, and Cl-excluding cultivars are more tolerant of excessive amounts of Cl. At PTRS-2014, excluders and includers produced statistically similar yields when no Cl or 280 kg Cl ha^{-1} was applied. The yield of includers decreased incrementally as Cl rate increased with yield losses of 5 to 20% for soybean receiving 280 to 840 kg Cl ha^{-1}. The yield loss of excluders attributed to Cl toxicity was only 5 to 8% for the 560 to 840 kg Cl ha^{-1} rates.

At PTRS-2015, includers produced statistically similar yields as excluders only when no Cl or 280 kg Cl ha^{-1} was added (Table 2.7). The seed yield of includers declined significantly with each Cl rate increase beyond 280 kg Cl ha^{-1} while the yield of excluders did not decline significantly, regardless of Cl rate. The greatest yield loss for includers was 17% for soybean receiving 840 kg Cl ha^{-1} compared to a nonsignificant numerical difference of 5% for excluders receiving 840 kg Cl ha^{-1}.

At the PTRS-2016, the greatest numerical seed yields were produced by soybean receiving 340 kg Cl ha^{-1} rate, regardless of Cl rating (Table 2.7). The seed yield of excluders was statistically the same across Cl application rates and greater than the yields produced by includers except for the includer yield in the 340 kg Cl ha^{-1} rate. The seed yield of includers was greatest when 0 and 340 kg Cl ha^{-1} were applied and declined 11 to 13% for soybeans receiving 670 and 1010 kg Cl ha^{-1}. The yields of excluders varied by less than 2% among the four Cl rates.

We attempted to induce greater yield loss from Cl toxicity at this site by applying more Cl compared to previous trials, but our attempt to intensify Cl toxicity was not successful. Soil $EC_{1:2}$ values (Table 2.4) were numerically lower for PTRS-2016 in the high Cl rates (i.e., not statistically compared) than in previous years, which could explain the limited differences in grain yield. The cultivars having a mixed rating in 2016 produced yields that were numerically (i.e., not statistically different at $P = 0.10$) greater than Cl includers and less than Cl excluders when treatment rates exceeded 280 kg Cl ha^{-1} (data not shown). Trifoliolate leaflet-Cl concentration of mixed cultivars showed a similar trend, suggesting the mixed cultivars have an intermediate tolerance to Cl toxicity. Additional research is needed to confirm the response of cultivars with a mixed rating and better categorize soybean Cl tolerance.

Yield reduction of includers attributed to Cl toxicity ranged from 4 to 20% compared to 0 to 8% for excluders which is comparable with results reported by Yang and Blanchar (1993; 16 and 0% yield loss for Cl-includer and –excluder cultivars, respectively). Symptoms of Cl toxicity were visible at all site years, but leaf symptoms consistent with Cl toxicity were present only after the R5.5 stage. The variation in yield loss could be attributed to the mobile nature of Cl in the soil and different environmental stressors such as temperature and rainfall across locations (White and Broadley, 2000). Parker et al. (1983) also noted Cl-sensitive cultivars developed leaf scorch during pod development and yields were 37% less compared to tolerant cultivars in soil with naturally high Cl concentrations. The observations made in our trials suggest yield loss can occur from Cl toxicity without visible symptoms until very late in the growing season, presumably due to chronic accumulation of Cl from applied irrigation water. The late appearance of symptoms is an important aspect of Cl toxicity and highlights the need for diagnostic information that would aid in early detection of Cl toxicity. Although effective Cl management

strategies are not currently available, the ability to detect potentially toxic Cl concentrations will lead to a better understanding of this malady and perhaps facilitate research on how to manage Cl toxicity.

Seed weight

Seed weight was significantly affected by either the main effects or their interaction at three of five site-years (Table 2.8). Neither the main effects nor their interaction were significant for PTRS-2016 or RRS-2016. At the PTRS-2014 and PTRS-2015, the Cl rate by Cl rating interaction significantly influenced 1000-seed weight and at the RRS-2014 only the main effect of Cl rate was significant where 1000-seed weight declined, on average, by 4.0% between the no Cl and 840 kg Cl ha^{-1} rate. In general, the seed weight of the includers was lower than that of the excluders. The most important components of the interaction are the relative seed weight response to Cl rate within each Cl rating and how seed weight responded to increasing Cl within each category. At the PTRS-2014 site, the seed weight of excluders was greatest when 0 and 280 kg Cl ha^{-1} were applied and declined significantly by 2.6% for soybean receiving 580 kg Cl ha^{-1} and 4.7% for soybean receiving 840 kg Cl ha^{-1}. An incremental and significant decrease in seed weight with each addition of Cl occurred for the includer cultivars. As compared to soybean that received 0 kg Cl ha^{-1}, the seed weight decrease attributed to Cl toxicity ranged from 3.9 (280 kg Cl ha^{-1}) to 12.9% (840 kg Cl ha^{-1}).

Seed weight at the PTRS-2015 was also affected by the Cl rate by Cl rating interaction (Table 2.8). The excluders, regardless of Cl rate, had significantly greater seed weight than includers. The 1000-seed weight of excluders receiving 0 and 840 kg Cl ha^{-1} were similar and significantly greater than the 1000-seed weight of soybean receiving 280 kg Cl ha^{-1}. For the

includers, seed weight was similar for soybean receiving 0 and 280 kg Cl ha^{-1} and then declined by 2.2 to 4.4% when Cl rate increased to 560 and 840 kg Cl ha^{-1}.

The results from three of five site-years indicate that seed weight is frequently reduced by high rates of Cl and the seed weight of includers is often reduced more than that of excluders making seed weight one potential means of yield loss. Parker et al. (1983) also found that the seed weights of Cl-accumulator cultivars were significantly lower than tolerant cultivars and accounted for 50% of the yield reduction. In contrast, Yang and Blanchar (1993) reported theaddition of Cl had no significant influence on seed weight. The effect of chronic Cl toxicity on soybean yield components warrants further investigation.

Critical Tissue-Cl Concentration

Tissue-Cl concentration predictions at which soybean seed yield begins to decline were developed using linear and linear plateau models where relative yield was regressed across tissue-Cl concentration at the R3 growth stage from all site-years (Table 2.9; Fig. 3A-C). These relationships were developed initially using only data from cultivars that were believed (e.g., based on trifoliolate leaflet-Cl concentration) to be true Cl excluders and includers. The relationship between soybean relative yield and tissue-Cl concentration at the R3 growth stage showed a negative linear response to tissue-Cl concentration that was dependent on cultivar-Cl rating (Fig. 3A). The rate of yield loss (e.g., linear coefficient) was the same for both cultivar-Cl ratings but the different intercepts resulted in the prediction of lower critical tissue-Cl concentrations for excluders. Since the intercept values of the derived regression equations were not exactly 100%, maximal soybean yields were considered as relative yields within 5% of the maximal predicted relative yield (98.1% for excluders and 100% for includers). Relative yields 5% less than maximal were produced when tissue Cl exceeded 1885 mg Cl kg^{-1} for excluders

and 3923 mg Cl kg^{-1} for includers. The predicted relative yield at the R3 growth stage declined by increments of 5% as tissue Cl increased by 1884 mg Cl kg^{-1} for excluders and 1923 mg Cl kg^{-1} for includers (Table 2.9). The limited range of relative yield data prevents yield loss predictions lower than 85% of maximum yield for excluders.

The same data were analyzed using a linear plateau model with the analysis done by Cl-rating (Fig. 3B and C). The relationship between relative yield and tissue-Cl concentration was stronger for Cl-includers (n = 48; R^2 = 0.58; Fig. 3B) than Cl-excluders (n = 52; R^2 = 0.22; Fig. 3C). For excluders, relative seed yield declined at a rate of 0.0102% mg^{-1} Cl kg^{-1} when leaf-Cl was >1113 mg Cl kg^{-1} (Fig. 3B). Chloride concentrations > 2148 mg Cl kg^{-1} caused yield to decline by 0.0029% mg^{-1} Cl kg^{-1} for includers (Fig. 3C). Yang and Blanchar (1993) reported no significant relationship between tissue-Cl concentration and grain yield for individual cultivars.

The yield and tissue-Cl concentration means from cultivars that were eventually rated as being a mixed population of includer and excluder plants were omitted from the initial model development but later added to see what effect their inclusion would have on the predictions. When observations of mixed cultivars (n = 20) were included in the regression procedure the relationship remained linear but the R^2 decreased from 0.56 to 0.50 when mixed data were included in the Cl-excluder category or 0.49 when mixed cultivar data were grouped with Cl includer data (not shown). The limited data available for mixed cultivars and the possibility of having a wide range of includer to excluder plant ratios prohibits us from making a recommendation regarding which category (e.g., includer or excluder) cultivars with a mixed population should be included for assessing the critical tissue-Cl concentration. Perhaps the first step in this process would be developing a cultivar-Cl rating system that estimates with reasonable accuracy the ratio of includer to excluder plants in the mixed population and the

resultant rating might then be used to determine which regression equation is most appropriate. For example, we speculate that cultivars with a mixed population comprised of >50% includer plants might best be associated with Cl-includer prediction equation and vice versa.

Research regarding critical tissue-Cl concentrations is scant, however, data collected by Rupe et al. (2000) regarding yield and tissue-Cl concentration in includer and excluder cultivars was analyzed by Slaton et al. (2013) to develop preliminary critical Cl concentrations. While the data is limited (n = 12 excluder; n = 12 includers), the critical concentrations developed suggested includers suffered 5% relative yield loss when tissue-Cl concentrations exceeded approximately 3500 mg Cl kg^{-1}. These results are within 10% of the predictions of our research (Table 2.9). Since both data sets produced a negative linear response, the Rupe et al. (2000) data was combined with our own to create a more robust data set. The additional observations increased the amount of variability explained in relative yield by tissue-Cl concentration from 56 to 66% but changed the critical concentrations very little. These results suggest that our critical concentrations are reasonably accurate and can provide a baseline value for identifying toxic tissue-Cl concentrations before the development of visual symptoms.

CONCLUSIONS

Our research confirmed that the addition of Cl reduces the yield of soybean more for Cl includer cultivars compared to Cl excluder cultivars. Additionally, our research suggests tissue-Cl concentrations remain fairly constant from early to late reproductive growth stages at which point leaflet-Cl concentrations increase especially in high Cl environments. The relationship between tissue-Cl concentration and relative soybean yield was used to develop critical tissue-Cl concentrations for Cl includer and excluder cultivars. The results were consistent with our

original hypothesis in that critical concentrations in which yield loss occur are dependent on cultivar Cl rating due to differing affinities for Cl uptake.

The novel aspects and contributions of our research pertain to the development of critical tissue-Cl concentrations in which yield loss occurs for includer and excluder cultivars from Cl toxicity. While it is documented that excluders produce greater yields than includers in high Cl environments, threshold tissue-Cl concentrations at which yield loss begins are not available to diagnose the problem before visual symptoms appear and significant yield loss is probable. Leaf samples analyzed for Cl concentration at the R3 growth stage were good indicators of potential Cl toxicity problems. Early detection of Cl toxicity is the first step to implementing management practices to mitigate yield loss and determine the extent of yield reduction from Cl toxicity in which visual symptoms may not be present.

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TABLES AND FIGURES

Table 2.1. Selected agronomic information and dates for experiments conducted at the Pine Tree Research Station (PTRS) and Rohwer Research Station (RRS) in 2014, 2015, and 2016.

Information or event	Pine Tree Research Station			Rohwer Research Station	
Soil series	Calloway Silt Loam	Calloway Silt Loam	Calloway Silt Loam	Sharkey and Desha Clay	Sharkey and Desha Clay
Year	2014	2015	2016	2014	2016
Previous crop	Soybean	Corn	Grain Sorghum	Soybean	Soybean
Bed width	76 cm	76 cm	76 cm	96.5	96.5
Seed rate†	383,000	383,000	383,000	371,000	371,000
Seeding Date	23 May	10 June	5 May	21 May	12 May
SoyMap R1	2 July	15 July	12 June	23 June	15 June
Chloride application	25 June (V7)‡	7 July (V6)	14 June (V7)	25 June (R1)	22 June (R1)
	3 July (R1)	15 July (R1)	21 June (R1)	2 July (R2)	28 June (R2)
	9 July (R2)	29 July (R2)	29 June (R2)	9 July (R2)	6 July (R2)
	24 July (R3)	4 Aug (R3)	5 July (R2)	23 July (R3)	13 July (R2)
	9 Aug (R5)	11 Aug (R4-R5)	12 July (R3)	5 Aug (R5)	21 July (R4)
	-	-	19 July (R4)	-	-
Tissue sample	9 July (R2)	22 July (R1)	29 June (R2)	9 July (R2)	13 July (R3)
	17 July (R3)	29 July (R2)	5 July (R2)	15 July (R3)	-
	6 Aug (R5)	4 Aug (R3)	12 July (R3)	5 Aug (R5)	-
	21 Aug (R5.5)	11 Aug (R4-5)	19 July (R4)	22 Aug (R6)	-
	-	19 Aug (R5-5.5)	3 Aug (R5)	-	-
	-	14 Sept (R6.5)	16 Aug (R6)	-	-
Soil sample	14 Aug (R5)	20 Aug (R5-5.5)	3 Aug (R5)	22 Aug (R6)	2 Aug (R5)
Harvest date	7 Oct	14 Oct	6 Oct	14 Sept	21 Sept

† Seeding rates in seed ha^{-1}

‡ Date and (growth stage) of Cl solution application, tissue sample collection, soil sample collection, or harvest.

Table 2.2. Selected soil chemical property means for experiments conducted at the Pine Tree Research Station (PTRS) and Rohwer Research Station (RRS) in 2014, 2015, and 2016.

			Mehlich-3 nutrients§					
Site-year	pH†	SOM‡	P	K	Ca	Mg	S	Na
		g kg^{-1}	----------mg kg^{-1}----------					
PTRS-2014	7.1	26.0	101	139	1844	323	16	46
PTRS-2015	7.1	22.0	24	61	1556	250	9	41
PTRS-2016	7.1	22.0	43	102	1592	271	10	31
RRS-2014	7.3	24.0	82	208	2542	537	6	54
RRS-2016	7.5	32.0	62	190	3211	647	6	55

† pH and electrical conductivity ($EC_{1:2}$) measured in 1:2 soil:water mixture (Sikora and Kissel, 2014)

‡ SOM, soil organic matter determined by weight loss on ignition (Zhang and Wang, 2014).

§ Mehlich-3 extractable nutrients determined by inductively coupled plasma atomic emission spectroscopy (Zhang et al., 2014).

Table 2.3. Chloride rating and name of soybean cultivars used in experiments conducted at the Pine Tree Research Station and Rohwer Research Station in 2014, 2015, and 2016.

	Cultivar Cl rating	
Site-year	Excluder	Includer
PTRS-2014	Armor 49-R56 Northrup King S46-L2 Pioneer 49T80R	Armor 48-R66 Northrup King S45-V8 Pioneer 94Y82
PTRS-2015	Armor 49-R56 Northrup King S46-L2 Pioneer 47T36R	Armor 48-R66 Northrup King S45-V8 Pioneer 48T53R†
PTRS-2016	Armor 49-R56 Pioneer 47T36R Northrup King S48-D9†	Armor 48-R66 Northrup King S47-K5 Pioneer 48T53R†
RRS-2014	Armor 49-R56 Northrup King S46-L2 Pioneer 49T80R	Armor 48-R66 Northrup King S45-V8 Pioneer 94Y82
RRS-2016	Armor 49-R56 Pioneer 47T36R Northrup King S48-D9†	Armor 48-R66 Northrup King S47-K5 Pioneer 48T53R†

† Denoted cultivars did not behave consistently with Cl rating in regards to tissue-Cl concentration and are believed to be a mixed population of include and excluder plants.

Table 2.4. Soil electrical conductivity measured in a 1:2 soil water mixture ($EC_{1:2}$) at the soybean R5 stage as affected by the Cl rating by Cl rate interaction or Cl rate main effect (Mean), averaged across cultivar Cl rating, for experiments conducted at the Pine Tree Research Station (PTRS) in 2014, 2015, and 2016 as well as the Rohwer Research Station (RRS) in 2014 and 2016.

	Site-year					
	PTRS-2014		RRS-2014	PTRS-2015	PTRS-2016	RRS-2016
Cl Rate	Includer	Excluder	Cultivar mean†	Cultivar mean	Cultivar mean	Cultivar mean
kg Cl ha^{-1}	---------- $EC_{1:2}$ (dS m^{-1})----------					
0	0.281 a‡	0.239 a	0.153 a	0.190 a	0.184 a	0.228 a
280 (340)§	0.421 b	0.450 b	0.179 b	0.468 b	0.229 a	0.289 b
560 (670)	0.557 c	0.501 bc	0.229 c	0.618 b	0.305 ab	0.384 c
840 (1010)	0.775 e	0.658 d	0.254 d	1.047 c	0.429 b	0.424 c
P-values						
Cl rate	<0.0001		<0.0001	0.0007	0.0325	0.0003
Cl rating	0.0223		0.5928	0.9528	0.9280	0.1439
Interaction	0.0824		0.3683	0.9894	0.2659	0.2345

† Mean indicates the listed values are the average soil $EC_{1:2}$ from Cl-includer and -excluder cultivars in each Cl rate.

‡ Means within the same site-year followed by the same letter are not statistically different by Fisher's protected LSD ($P = 0.10$).

§ Chloride application rates at PTRS-2016 totaled 340, 670, and 1010 kg Cl ha^{-1}.

Table 2.5 Analysis of covariance *P* values for soybean leaflet-Cl concentration (mg Cl kg^{-1}) regressed across time expressed as days after the R1 stage (DAR1) as affected by Cl rate (CR) for two cultivar Cl ratings at four site-years of research conducted at the Pine Tree Research Station (PTRS) or Rohwer Research Station (RRS) in 2014, 2015, or 2016.

Source of variation	df†	PTRS-2014	RRS-2014	PTRS-2015	PTRS-2016
Cl-Excluder		----------*P* value----------			
CR	3	<0.0001	0.0003	<0.0001	-§
DAR1‡	1	-	-	<0.0001	-
CR x DAR1	3	<0.0001	<0.0001	-	<0.0001
$DAR1^2$	1	0.0836	-	-	-
CR x $DAR1^2$	3	-	<0.0001	<0.0001	0.0003
Cl-Includer					
CR	3	<0.0001	<0.0001	-	0.0791
DAR1	1	<0.0001	-	-	-
CR x DAR1	3	-	<0.0001	<0.0001	<0.0001
$DAR1^2$	1	-	-	-	-
CR x $DAR1^2$	3	<0.0001	<0.0001	<0.0001	0.0013

† The df for the final model is the sum of the df for each model term (intercept, linear, and quadratic) listed as a source of variation. For example, the df for R is 3 and the df for DAR1 is 1.

‡ DAR1 as predicted using SoyMap (Popp et al., 2016). Note that $DAR1^2$ represents the square of the regression model term of DAR1.

§ The model term or interaction was not significant ($P > 0.15$) in the final model or is accounted for in the interaction term using the Mixed procedure of SAS v9.4.

Table 2.6 Regression coefficients for leaflet-Cl concentration regressed across time expressed as days after R1 stage as affected by Cl rate measured during 2014, 2015, and 2016 at the Pine Tree Research Station (PTRS) and 2014 at the Rohwer Research Station (RRS).

Trial	Cultivar Cl-rating	Cl-rate	Parameter estimate†			Model R^2
			Intercept	Linear	Quadratic	
		kg ha^{-1}	----------Coefficients (SE‡) ----------			
2014-PTRS	excluder	0	353 (157)	9.7 (9.3)§	-0.266 (0.153)	0.35
		280	617	13.0§		
		560	866	25.4		
		840	1043	27.8		
2014-PTRS	includer	0	5655 (485)	-207.7 (35.4)	3.031 (0.643)	0.55
		280	6827		3.634	
		560	7776		4.508	
		840	9235		5.423	
2014-RRS	excluder	0	456 (506)	-21.0 (43.5)	0.335 (0.708)	0.47
		280	747	-33.1	0.513	
		560	775	-40.0	0.704	
		840	1227	-66.3	1.120	
2014-RRS	includer	0	3849 (704)	-225.3 (60)	4.776 (0.978)	0.76
		280	6376	-371.5	7.695	
		560	7619	-472.1	10.116	
		840	8116	-509.6	11.468	
2015-PTRS	excluder	0	576 (93)	-25.8 (5.6)	0.316 (0.085)	0.25
		340	683		0.408	
		670	879		0.433	
		1010	964		0.472	
2015-PTRS	includer	0	6547 (500)	-364.2 (32.4)	5.566 (0.449)	0.64
		340		-270.9		
		670		-213.3		
		1010		-176.5		
2016-PTRS	excluder	0	485 (158)	-7.3 (8.6)§	0.038 (0.268)§	0.14
		340		11.9§	-0.215§	
		670		10.7§	-0.155§	
		1010		26.9	-0.355	
2016-PTRS	includer	0	4088 (1106)	-40.2 (54.4)§	0.241 (0.585)§	0.35
		280	2683	72.6§	-0.606§	
		560	1669	173.4	-1.252	
		840	256	274.5	-2.099	

† Quadratic regression model ($y = a + bx + cx^2$) where y = tissue-Cl concentration (mg Cl kg^{-1}), x = d after R1 growth stage, a = intercept, b = linear slope, and c = quadratic slope.

‡ Coefficient standard error.

§ The coefficient was not significantly different than 0 ($P > 0.10$).

Table 2.7 Soybean seed yield as affected by the cultivar Cl rating (includer or excluder) by Cl rate interaction or Cl rate main effect, averaged across cultivar Cl rating, for experiments conducted at the Pine Tree Research Station (PTRS) in 2014, 2015, and 2016 and the Rohwer Research Station (RRS) in 2014 and 2016.

	PTRS-2014		RRS-2014	PTRS-2015		PTRS-2016		RRS-2016
Cl Rate	Includer	Excluder	Cultivar mean†	Includer	Excluder	Includer	Excluder	Cultivar mean
kg ha^{-1}	----------	----------	----------	kg ha^{-1}	----------	----------	----------	----------
0	4358 abc†	4361 ab	3307 a	3295 a	3081 bc	3364 a	3581 a	3308
280 (340)§	4149 abcd	4390 a	3180 b	3196 ab	3159 abc	3392 a	3603 a	3417
3560 (670)	3932 d	4133 bcd	3143 bc	2965 c	3080 bc	3011 b	3549 a	3305
840 (1010)	3468 e	4024 cd	3057 c	2750 d	3002 bc	2969 b	3536 a	3235
P-values								
Cl rate	0.0052		0.0275	0.0247		0.0801		0.1811
Cl rating	0.2748		0.8817	0.7392		0.2027		0.1004
Interaction	0.0034		0.5174	0.0007		0.0876		0.1663

† Mean indicates the listed values are the average soil $EC_{1:2}$ from Cl-includer and -excluder cultivars in each Cl rate.

‡ Means within the same site-year followed by the same letter are not statistically different by Fisher's protected LSD (P = 0.10).

§ Chloride application rates at PTRS-2016 totaled 340, 670, and 1010 kg Cl ha^{-1}.

Table 2.8 Soybean 1000-seed weight as affected by the cultivar Cl rating by Cl rate interaction or the Cl rate main effect, averaged across cultivar Cl rating (mean), for experiments conducted at the Pine Tree Research Station (PTRS) in 2014, 2015, and 2016 and the Rohwer Research Station (RRS) in 2014 and 2016.

	PTRS-2014		RRS-2014	PTRS-2015		PTRS-2016	RRS-2016
Cl Rate	Includer	Excluder	Cultivar mean†	Includer	Excluder	Cultivar mean	Cultivar mean
kg ha^{-1}	----------1000 seed weight (g)----------						
0	140.4 c‡	146.6 ab	139.4 a	138.4 c	150.7 a	131.9	125.5
280 (340)§	135.0 d	149.7 a	137.5 ab	137.5 cd	146.9 b	130.9	123.5
560 (670)	127.9 e	142.6 c	136.8 b	134.4 de	149.6 ab	128.8	124.0
840 (1010)	122.3 f	145.8 b	133.8 c	132.3 e	150.2 a	126.1	121.1
P-values							
Cl rate	0.0003		0.0142	0.1922		0.1417	0.2315
Cl rating	0.1485		0.3970	0.0040		0.5686	0.7120
Interaction	<0.0001		0.4040	0.0085		0.6054	0.8393

† Mean indicates the listed values are the average soil $EC_{1:2}$ from Cl-includer and -excluder cultivars in each Cl rate.

‡ Means within the same site-year followed by the same letter are not statistically different by Fisher's protected LSD (P = 0.10).

§ Chloride application rates at PTRS-2016 totaled 340, 670, and 1010 kg Cl ha^{-1}.

Table 2.9 The predicted relative yield and associated leaflet-Cl concentrations at the R3 growth stage for Cl-excluder and –includer soybean cultivar categories as predicted using linear and linear plateau (LP) regression models. The model and coefficient p-values were <0.0001.

	Cl includer		Cl excluder	
Relative yield	Linear	LP	Linear	LP
%	----------trifoliolate leaflet-Cl concentration (mg Cl kg^{-1})---------			
95	3,923	3,862	1,885	1,593
90	5,846	5,586	3,769	2,069
85	7,769	7,310	5,654	2,544
80	9,692	9,034	-†	-
75	11,615	10,759	-	-
Intercept‡	105.2 (1.35)	106.2 (2.18)	98.1(0.51)	108.4(10.2)
Slope	-0.0026 (0.0003)	-0.0029 (0.0004)	-0.0026 (0.0003)	-0.0102 (0.0006)
Join & plateau§	-	2148 (838), 100	-	1113 (314), 97

†Yield loss range beyond limits of collected data.

‡ Linear equation ($y = mx + b$), where y is relative yield, m is the slope value [% relative yield change (mg Cl $kg^{-1})^{-1}$], and b is the intercept value. The values in () are the standard error of the coefficient.

§ Join & plateau, the two listed values indicate the x-axis join point (mg Cl kg^{-1}) and the relative yield (%) plateau at which increasing Cl concentration causes relative yield to decline.

Figure 2.1. Chloride excluder soybean leaflet-Cl concentration regressed across days after predicted R1 growth stage at the (A) Pine Tree Research Station (PTRS) in 2014, (B) Rohwer Research Station (RRS) in 2014, (C) PTRS-2015, and (D) and PTRS-2016. Regression coefficients that define each curve are listed in Table 2.6.

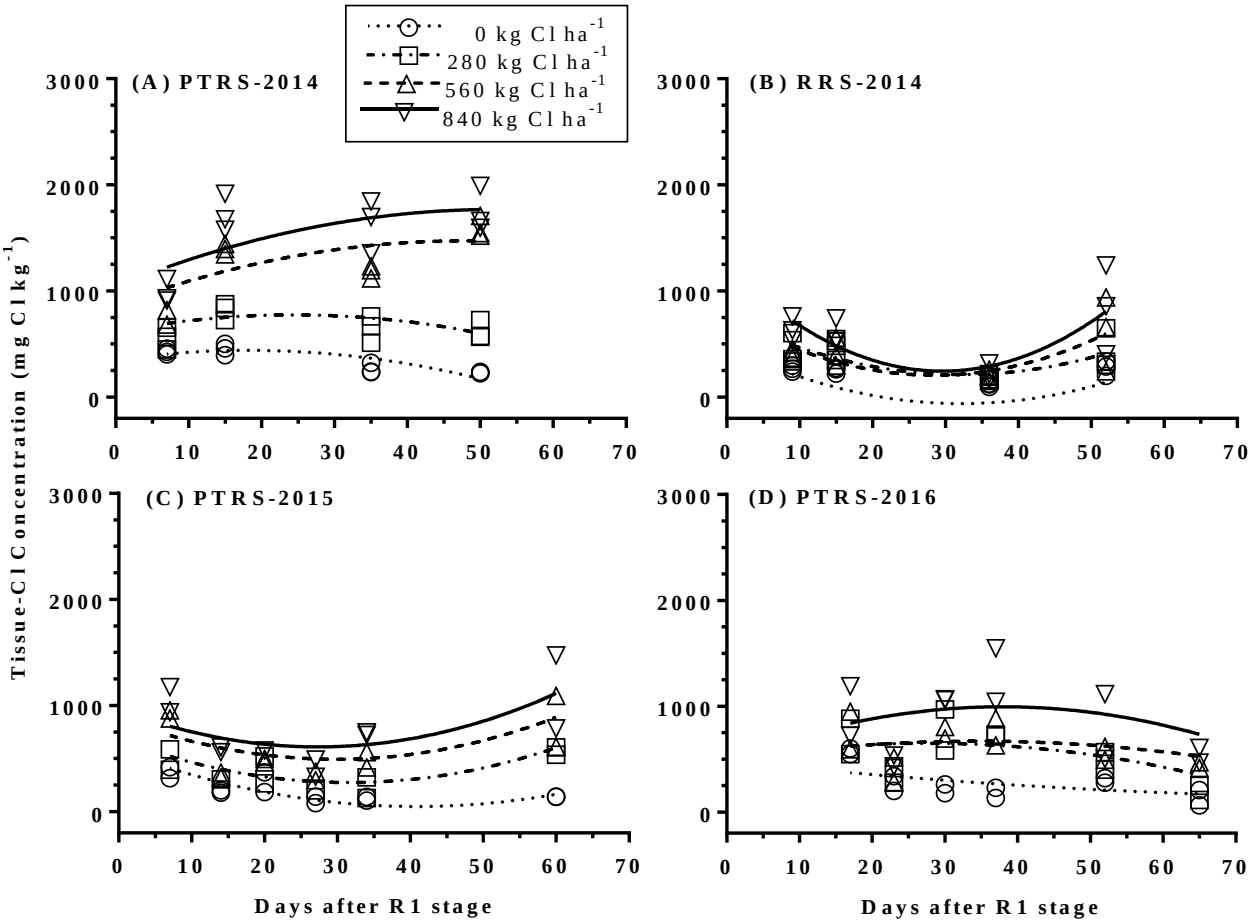


Figure 2.2. Chloride include soybean leaflet-Cl concentration regressed across days after predicted R1 growth stage at the (A) Pine Tree Research Station (PTRS) in 2014, (B) Rohwer Research Station (RRS) in 2014, (C) PTRS-2015, and (D) PTRS-2016. Regression coefficients that define each curve are listed in Table 2.6.

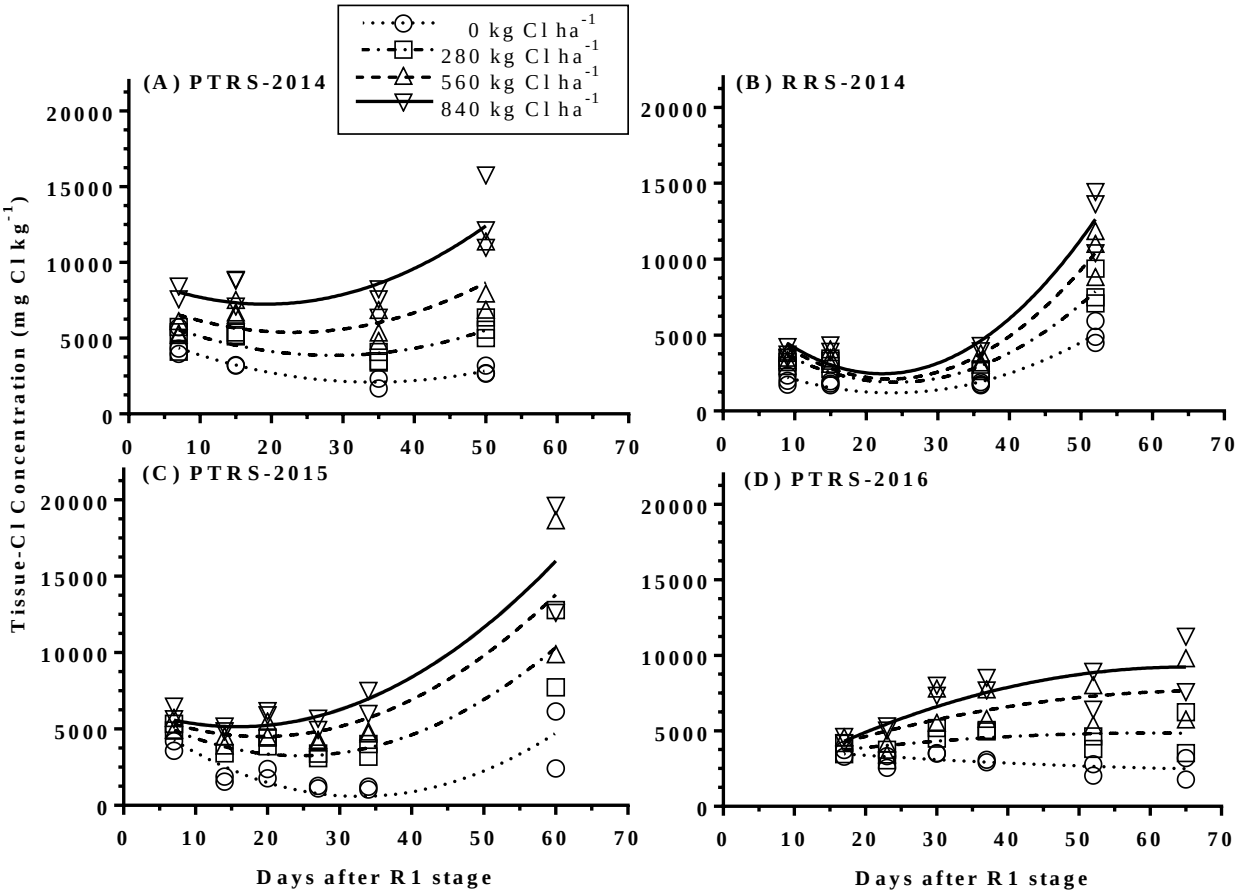
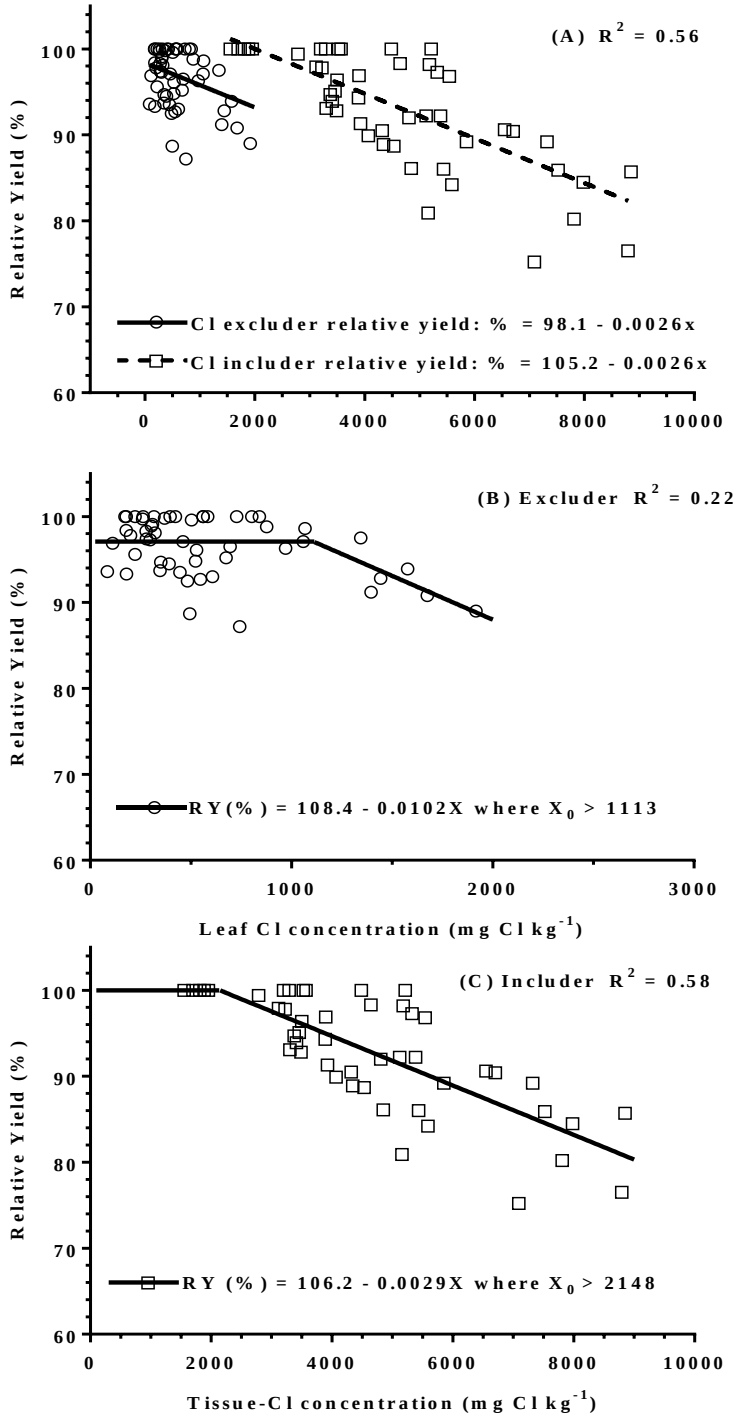
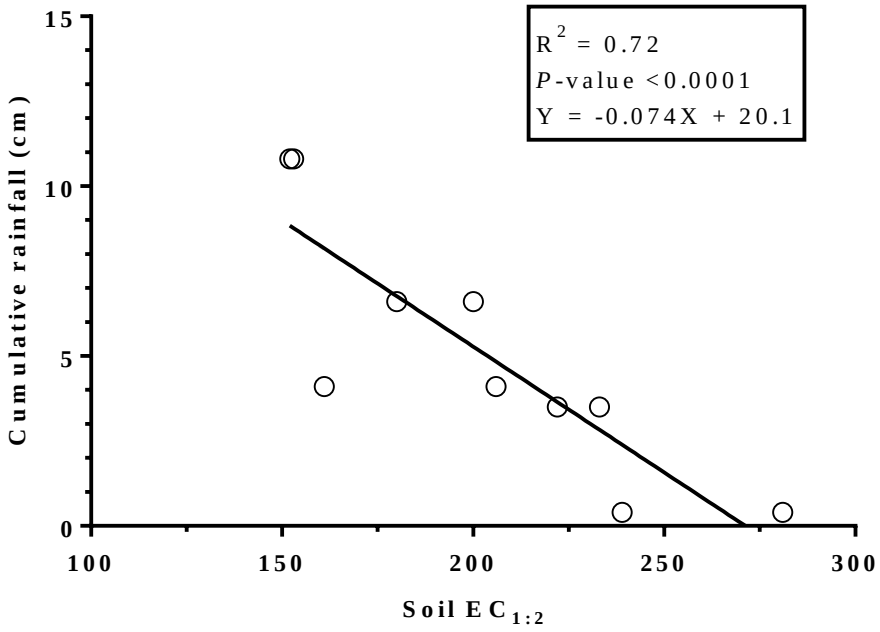


Figure 2.3. The relationship between soybean relative yield and trifoliolate leaflet-Cl concentration at the R3 growth stage as predicted by a (A) linear model for Cl-excluder and -includer cultivars and (B) linear plateau model for excluder and (C) includer cultivars. The symbols represent means for each cultivar and Cl rate from five site-years of research. Note that the X-axis scale is not consistent among all figures and X_0 indicates the join point (Table 2.9).



Appendix 2.1. The relationship (n = 10) between soil electrical conductivity as measured in 1:2 soil water mixture ($EC_{1:2)}$ from soil collected at the R5 growth stage in the 0 kg Cl ha^{-1} rate and the cumulative rainfall 2 wk prior to soil sampling. Note that each symbol represents the mean $EC_{1:2}$ for the Cl includer or excluder cultivars for five site-years. Note the model and coefficient p-values were <0.01.



CHAPTER 3

Identification of Chloride Trait in Soybean through Trifoliolate Leaflet-Chloride Concentration

ABSTRACT

Soybean (*Glycine max* (L) Merr.) cultivars are classified for Cl tolerance based on a three-rating system (excluder, includer, and mixed). The ratings sometimes change from one year to the next which questions the precision and accuracy of the rating method. Our research investigated possible reasons why cultivar Cl ratings are inconsistent by examining individual plant and multiple plant (composite) sample tissue-Cl concentrations. Tissue samples were collected from individual plants in a preliminary greenhouse study and an eleven-cultivar field trial and composite tissue samples were collected from three site-years of the Arkansas Soybean Performance Tests (ASPT). The tissue-Cl concentrations of 112 cultivars from the 2015 ASPT and 47 cultivars from two site-years in the 2016 ASPT show a gradual increase in leaflet-Cl concentrations with a clear set of cultivars having low Cl concentrations classified as Cl excluders but no clear distinction between mixed and includer cultivars. Many cultivars may be a mixed population of includer and excluder plants. Chloride concentrations from individual plants of eleven cultivars showed that all plants of only one cultivar had low ($\leq$500 mg Cl kg^{-1}) and uniform Cl concentrations as would be expected of a Cl excluder. When all 525 samples were analyzed, 34% and 31% of the plants had Cl concentrations $\leq$500 or 1001-2000 mg Cl kg^{-1}, respectively. Tissue-Cl concentration results showed that many cultivars contain both includer and excluder plants. A new rating system to better categorize the soybean Cl tolerance is warranted to explain the variability in Cl concentration in a single cultivar.

INTRODUCTION

Soybean grown in the southeastern United States are at risk for yield limitations from chloride (Cl) toxicity. The source of Cl can be from Cl-containing fertilizers, irrigation water, or poorly drained soils with large concentrations of resident Cl. In Arkansas, it is common for irrigation water to contain electrical conductivities (EC) greater than 1.2 dS m^{-1} in which 85% of the variability in EC can be explained by water Cl concentration (Gilmour et al., 1983). Marginal quality irrigation water, coupled with frequent irrigation during periods of high temperatures and limited rainfall create conditions favorable for Cl toxicity.

Soybean cultivars have been classified as sensitive (Lauchli and Wieneke, 1979) to moderately tolerant (Maas and Hoffman, 1977) to salinity. The different classifications may be attributed to genetic differences in salt tolerance among soybean cultivars related to their ability to take up Cl. Cultivar differences in Cl tolerance were first documented by Abel and MacKenzie (1964) who summarized that excluders had much lower leaf-Cl concentrations than Cl-includer cultivars. Abel (1969) later reported that Cl exclusion was controlled by a single dominant gene (*Ncl*). More recently, additional genetic mapping studies involving Cl tolerance have reported quantitative trait locus markers associated with the salt tolerant allele S-100 (Lee et al., 2004; Zeng et al., 2014). Field studies performed by Parker et al. (1983), Rupe et al. (2000), and Yang and Blanchar (1993) have reported that excluder cultivars yield 16 to 37% greater in high Cl soils compared to includer cultivars. These potential yield differences highlight the importance of accurately categorizing commercial soybean cultivars as includers or -excluders.

Essa et al. (2002) summarized the majority of research regarding Cl tolerance in soybean had been performed in the greenhouse and information regarding field-grown soybean response to Cl and environmental stress factors was limited. The majority of Cl tolerance screening

studies are performed in the greenhouse, where soybean in early vegetative growth (V2-V4) stages are exposed to high concentrations of Cl salts (60-120 mmol Cl L^{-1}). These high Cl concentrations result in rapid injury to leaf tissue or "leaf scorch" by which ratings are then assigned based on the premise that includers show symptoms before excluder cultivars (Lee et al., 2008; Valencia et al., 2008). The method of detecting Cl tolerance via greenhouse screening relies on cultivar response to acute Cl toxicity. Acute Cl toxicity is seldom observed in Arkansas production fields, as Cl toxicity typically manifests itself chronically where symptoms are not observed until late reproductive growth when Cl has accumulated to a damaging concentration from season-long Cl inputs (Parker et al., 1983). It is unknown whether Cl screenings done in the field under natural environmental stress could more accurately model soybean cultivar tolerance to Cl.

A greenhouse screening program has been used in Arkansas to assign one of three Cl ratings (i.e., includer, excluder or mixed) to soybean cultivars based on the leaf-Cl concentrations from five plants as compared to known Cl-includer and -excluder cultivar standards (Green and Conatser, 2017). A rating of excluder or includer is only given to a cultivar when the Cl concentration of all five plants being tested is consistent with the check cultivars. If one or more plants of a single cultivar does not produce a consistent concentration, then the cultivar is rated as 'mixed'. This testing procedure has produced inconsistencies for the same cultivar across multiple testing years where the Cl rating fluctuates between the includer and mixed ratings or excluder and mixed ratings. To our knowledge no single cultivar has been rated as an includer and an excluder in separate screenings. The inconsistent Cl ratings would suggest that the current three-category, Cl-rating system is not sufficient for capturing the variability expressed by soybean regarding Cl tolerance and a more robust rating system may be

needed. As a general observation, commercially available cultivars are seldom categorized as a mixed or segregating plant population. The lack of cultivars with a mixed rating may be an artifact of the screening process as Green and Conatser (2017) categorized less than 15% of the screened cultivars as mixed. The inconsistent ratings also suggest that many cultivars may be a mixed population of includer and excluder plants rather than pure populations. Research by Ledesma et al. (2016) supports the notion that the population of plants within many soybean cultivars is probably a combination of includer or excluder plants or plants.

Alternative Cl rating methods that would increase rating accuracy and better characterize the proportion of plants that are Cl includers and excluders in a mixed population are warranted. We could find no information in the literature suggesting how many individual plants need to be examined to accurately characterize a cultivar's Cl rating. Parker et al. (1983) and Yang and Blanchar (1993) both suggested that seed-Cl concentration would not be an effective means to categorize includers and excluders as seed-Cl concentrations were highly variable across studies and the progeny did not always behave consistently with regard to Cl accumulation. Yang and Blanchar (1993) did report large differences in leaf-Cl concentrations between includer and excluder cultivars grown on low Cl soils. They reported that leaf-Cl concentration of field-grown soybean could be an effective means of discerning excluder and -includer cultivars, although they did not specify how well this approach might perform for cultivars with a mixed population.

The overall goal of our research was to establish a more robust cultivar Cl rating system based on leaf-Cl concentrations of field-grown soybean. The specific objectives were to i) determine the number of soybean plants needed to accurately categorize a cultivar's Cl rating, ii) examine the consistency of leaf-Cl concentration among individual soybean plants of the same cultivar, and iii) examine the consistency of leaf-Cl concentration ranking in a population of

soybean cultivars grown at two sites. Use of leaf-Cl concentrations of field-grown plants would allow both public and private breeding programs to assign Cl ratings. Based on the previous research and the inconsistencies seen in current cultivar Cl tolerance ratings, we hypothesize that more than five individual plants of a single cultivar need to be analyzed for leaf-Cl concentration to accurately assign cultivar-Cl ratings. Additionally, we hypothesize that many soybean cultivars contain a mixed population of includer and excluder plants in varying ratios. If this hypothesis is true, a more extensive rating system for Cl tolerance (i.e., 1-5 or 1-10 scale) will be more effective at accurately representing the plant ratios, allowing growers to plant the best available cultivar in fields with a history of Cl problems.

MATERIALS AND METHODS

Preliminary Greenhouse Trial

A greenhouse trial examined the variability in tissue-Cl concentration among individual plants within 12 selected cultivars (Table 3.1). Soybeans were grown in the greenhouse using cone-tainers [model SC10 (3.8 × 21 cm, 164 mL volume); Stuewe and Sons, Inc., Tangent, OR] and racks as described by Lee et al. (2008). Each cone-tainer was filled with 165 g of Dewitt (fine, smectitic, thermic Typic Albaqualfs) silt loam with 5.4 pH (1:2 soil water mixture), 0.73 dS m^{-1} soil $EC_{1:2}$ and Mehlich-3 extractable nutrients averaging 18 mg P, 179 mg K, 914 mg Ca, 150 mg Mg kg^{-1}. Electrical conductivity and soil pH were measured in a 20 g soil mixed with 40 mL deionized water (Wang et al., 2014). Thirty-five seeds of each cultivar were treated with 0.17 g of Bradyrhizobium inoculant before planting (Becker Underwood Inc., Ames, Iowa). The planted cone-tainers (one seed cone-$tainer^{-1}$) and racks were placed into a 39 L container filled with deionized water allowing the soil to moisten from the bottom up. Soybean plants were arranged in four separate racks with each rack holding 98 cone-tainers. Each rack contained five

blocks containing each of twelve soybean cultivars. The perimeter holes in each rack contained a planted cone-tainer (non-treatment) to help reduce the border effect and equalize competition among soybean plants.

Overhead UV lighting was turned on 7 d after planting and set to a 12 h photoperiod. The temperature in the greenhouse was maintained at 22°C. At 16 d after planting, when soybean was at the V1-V2 stage, the water in each container was replaced with 8 L Hoagland nutrient solution without N (Hoagland and Arnon, 1938). At the V3 stage, 25 d after planting, the deionized water in each container was replaced with 10 L of Hoagland's solution. Once the Hoagland's solution was depleted, two of the plastic containers received 10 L of a 200 mg Cl L^{-1} solution from KCl (Low Cl, 5.64 mmol Cl L^{-1}). The other two containers received 10 L of a 2000 mg Cl L^{-1} solution consisting of 200 mg Cl L^{-1} from KCl, 900 mg Cl L^{-1} from $MgCl_2 \cdot 6H_2O$, and 900 mg Cl L^{-1} from $CaCl_2 \cdot 2H_2O$ (High Cl, 56.4 mmol Cl L^{-1}). At 42 d after planting (V5 stage), the soybean plant in each cone-tainer was cut at the cotyledonary node, placed in a labeled paper bag, dried in an oven at 65°C for 7 d, ground to pass through a sieve with 2 mm openings, extracted with deionized water (Liu, 1998), and analyzed for whole plant-Cl concentration by inductively coupled plasma spectroscopy (Arcos-130 SOP, SPECTRO Analytical Instruments, Kleve, Germany).

Individual soybean whole plant-Cl concentration data were subjected to ANOVA using the GLIMMIXED procedure in SAS v. 9.4 (SAS Institute, Inc., Cary, N.C.). The experiment was a randomized complete block with a 2 (Cl rates) × 12 (cultivars) factorial treatment structure and 10 replications. Cultivar and Cl rate were fixed effects and block was a random effect. When appropriate, Cl concentration means were separated using Fisher's protected least significant difference (LSD) method at a significance level of 0.10.

Arkansas Soybean Performance Trial Trifoliolate-Leaflet Sampling

The ASPT planted at the Rice Research and Extension Center (RREC), near Stuttgart, AR in 2015 and 2016 and Rohwer Research Station (RRS) near Rohwer, AR in 2016 were used to evaluate the range of trifoliolate leaflet-Cl concentrations among cultivars having a maturity group rating of 4.8 to 5.3 (Bond et al., 2014, 2015, 2016). At the RREC-2015 and RREC-2016, soybeans were planted at 321,000 seeds ha^{-1} on beds spaced 76 cm apart in soil mapped as a Dewitt silt loam on 8 June 2015 and 13 May 2016. Each year 29 kg P and 60 kg K ha^{-1} were applied preplant as muriate of potash (500 g K kg^{-1}) and triple superphosphate (200 g P kg^{-1}). The field was furrow irrigated with reservoir water. At the RRS-16, soybeans were planted on beds spaced 96 cm apart in soil mapped as a mixture of Sharkey (very-fine, smectitic, thermic Chromic Epiaquerts) and Desha (very-fine, smectitic, thermic Vertic Hapludolls) clays on 17 May 2016. The field was furrow irrigated with well water from the Alluvial aquifer. Additional information regarding pest control, monthly rainfall, and irrigation for each site-year was summarized by Bond et al. (2015; 2016). Each ASPT plot contained four, 7.5-m long rows of soybean with 112 cultivars (late IV and early V maturity groups) examined at RREC-2015 and 47 (early V maturity group) cultivars examined at RREC-2016 and RRS-2016 (Appendix 3.1). Each cultivar was represented in each of three blocks in a randomized complete block design.

Twelve fully-expanded trifoliolate leaflets from one of the top three nodes were collected on 19 August 2015 at RREC-15, 12 July at RREC-2016, and 21 July RRS-2016 when the average soybean growth stage was R4-5, R2-3, and R2-3, respectively (Fehr and Caviness, 1971). Leaflet samples were collected from the first two blocks of late IV MG cultivars at

RREC-15 and all three blocks for early V MG cultivars at RREC-2015, RREC-2016 and RRS-2016. The sampled tissue was processed and analyzed as previously described.

The mean tissue-Cl concentration was calculated for each cultivar and the cultivar Cl rating (includer, excluder, or mixed) provided by Ross et al. (2014, 2015) was assigned. The cultivars were ranked in increasing order of tissue-Cl concentration to examine the relationship between cultivar Cl rating and leaf-Cl concentration. Linear regression was used to compare the trifoliolate leaflet-Cl concentrations and the ranking of these concentrations in ascending order at each location. A similar regression process was used to examine the year to year (e.g., different seed sources) relationships for 24 cultivars that were planted in both the 2015 and 2016 ASPT.

2016 Field Trial

A field trail was established at the Pine Tree Research Station during 2016 on a Calloway silt loam (fine-silty, mixed, active, thermic Aquic Fraglossudalfs). Selected mean soil chemical properties from two composite soil samples (0- to 10-cm depth) included 6.3 pH, 88 μmhos cm^{-1} for soil $EC_{1:2}$, 22 mg kg^{-1} Mehlich-3 P, 106 mg kg^{-1} Mehlich-3 K, 256 mg kg^{-1} Mehlich-3 Mg, 1161 mg kg^{-1} Mehlich-3 Ca, and 16 mg kg^{-1} water-soluble Cl (Wang et al., 2014). No fertilizers or soil amendments were added to the field prior to or during the growing season. The field had been fallow for at least two years.

Eleven cultivars representing the 4.7 to 5.3 maturity groups were selected using previous greenhouse and field trial information (Table 3.2). Cultivars were planted (approximately 321,000 seed ha^{-1}) on 76.2 cm wide beds, in eight row wide strips that were 180 m long into a conventionally tilled seedbed. The trial was established 45 m inside the west border of the field where three, 15-m long blocks spaced 15 m apart were established in each cultivar. During the V6 growth stage, 16 individual plants in each block were selected from the fourth and fifth row

and flagged for identification throughout the season. Soybean management in regards to pest control and irrigation closely followed the University of Arkansas System Division of Agriculture production guidelines (University of Arkansas, 2000). Soybean was furrow irrigated with surface-water from a nearby pond (61 mg Cl L^{-1}).

Once plants reached the R2-R3 growth stage, trifoliate leaf samples (leaf and petiole) were collected by removing the top four mature leaves and petioles from each plant. The trifoliate leaves from each plant were placed in a labeled paper bag, oven dried at 65°C and processed for tissue-Cl concentration as described previously. Upon reaching maturity, each plant was hand-harvested for seed to further evaluate the progeny generation of cultivars to assess the consistency of Cl accumulation within a cultivar.

Statistical Analyses

The experiment consisted of eleven cultivars planted across three blocks in a strip trial design. The studentized residual of each block was plotted in order to assess the variability in tissue-Cl concentration associated with location in the field (Appendix 3.1). An ANOVA was performed on mean tissue-Cl concentration using the GLIMMIXED procedure in SAS v9.4 in which block and cultivar were treated as fixed effects. Frequency tables were developed from the tissue-Cl concentrations using all observations and then by each cultivar separately using the FREQ procedure in SAS v9.4. The range of Cl concentrations (0-500, 501-1000, 1001-2000, 2001-3000, 3001-4000, >4000 mg Cl kg^{-1}) were developed from previous observations from field studies that showed Cl-excluder cultivars rarely contain tissue-Cl concentrations >1000 mg Cl kg^{-1} in fields having low to moderate Cl while Cl includers nearly always had concentrations greater than this threshold when no additional Cl was added (Slaton et al., 2016). Linear regression was performed using the REG procedure in SAS v9.4 to evaluate selected

relationships between mean tissue-Cl concentration and the percentage of individual plants within defined tissue-Cl concentration ranges. Studentized residuals (±2.5) and Cooks D statistic were used to identify and remove outlying and influential data points, respectively. The CORR procedure in SAS v9.4 was used to evaluate the relationship between tissue-Cl concentrations (Pearson Correlation) and tissue-Cl concentration rankings (Spearman rank correlation) between cultivars and site-years.

One objective of the tissue-Cl concentration experiment was to determine the minimum number of plants needed to make a consistent and accurate cultivar-Cl rating. Tissue-Cl concentration data from 48 individual plants from each of eleven cultivars previously classified as excluders (4), includers (5) or mixed (2) populations were used to evaluate the accuracy of the initial cultivar-Cl rating. For each cultivar, 1000 subsamples of 5, 10, 15, 20, 25, and 30 individual plants were selected with replacement. The data were manipulated by utilizing the MEANS, FREQ, and TABULATE procedures in SAS v9.4 to produce descriptive tables to categorize the results of each cultivar. For each subsample, the cultivar was classified as an excluder if 90% of the plants had tissue-Cl concentrations ≤1000 mg Cl kg^{-1}. If the tissue-Cl concentration of ≥90% of the plants was >1000 mg Cl kg^{-1} the subsample was classified as an includer. If the subsample did not meet the definition of excluder or includer, the subsample was classified as a mixed population. The generated result for each subsample was categorized based on the percentage of plants having ≤1000 mg Cl kg^{-1} (excluder) or >1000 mg Cl kg^{-1} (includer). Three levels of precision, 80, 85, or 90% of subsample results in agreement with a single Cl rating, were used to classify each cultivar. If these percentage thresholds of 80, 85, or 90% were not met then the cultivar was classified as mixed.

The sampling number equation [Eq. 1] described by Moore et al. (2014) was also used to determine the minimum number of individual plant samples needed to accurately represent the Cl concentration variability of a cultivar. In Eq. 1, Z represents the z-statistic for desired confidence level at 80% (1.30), S is the standard deviation of the mean, and D represents the 95% confidence interval width.

$$\textbf{[Eq. 1]} \quad n = [(Z^2 \times S^2) / D^2]$$

RESULTS AND DISCUSSION

Greenhouse trial

The greenhouse trial resulted in mean cultivar whole plant-Cl concentrations ranging from 1100 to 25,059 mg Cl kg^{-1} in the low Cl treatment and 8547 to 45,651 mg Cl kg^{-1} in the high Cl treatment, but the interaction between cultivar and Cl solution concentration was not significant (Table 3.1). The main effect of solution Cl concentration, averaged cross cultivars, was significant with soybean grown in the low Cl solution having tissue Cl concentrations 14,370 mg Cl kg^{-1} lower than plants grown in the high Cl solution (Fig. 3.1A). Averaged across Cl solution treatments, three of the four cultivars (NK S48-D9 was the exception) rated as a Cl excluder had the lowest mean tissue-Cl concentrations, cultivars with a mixed rating had intermediate Cl concentrations and includer cultivars had the greatest Cl concentrations.

To examine how cultivars responded to different Cl environments, the tissue-Cl concentrations of the twelve cultivars within the two solution Cl treatments were ranked 1 to 12 and the rankings regressed (Fig. 3.1B). This linear relationship suggests that relative Cl uptake by cultivars is proportional between low and high Cl environments. Knowing that the relative difference among cultivars in different Cl environments is consistent is significant if the tissue of

field-grown plants will be used to determine the cultivar Cl rating as the soil and irrigation water Cl concentrations may differ among site-years, fields or years.

Greenhouse methods that use leaf scorch scores and tissue-Cl concentration have been used to screen cultivars for Cl-tolerance and recent research has focused on developing these methods (Lee et al., 2008; Valencia et al., 2008). These greenhouse methods expose young soybean plants to high Cl solutions (40-160 mmol Cl L^{-1}) that result in very high tissue-Cl concentrations (>50,000 mg Cl kg^{-1}) and a narrow ratio (e.g., includer/excluder) of tissue-Cl concentrations compared to field-grown plants or plants that are exposed to much lower Cl concentrations. Ledesma et al. (2016) reported greenhouse Cl screening results for 111 cultivars with the results suggesting that a significant portion of the cultivars had segregating or mixed plant populations. The mixed rating was given to cultivars in which approximately 50% of plants ($n = 4$) became necrotic when exposed to a 120 mmol L^{-1} NaCl solution, and tissue-Cl concentration values were intermediate compared to excluders and includers. These results are consistent with our greenhouse study in which multiple cultivars rated as mixed had intermediate average Cl concentrations.

Greenhouse screening methods that use high solution Cl concentrations enable one to base ratings on visual leaf scorch symptoms, which may reduce labor and analytical costs associated with tissue analysis, but the high Cl concentration is not representative of the chronic Cl accumulation that usually occurs in the field. Field studies regarding Cl toxicity in soybean rarely report tissue-Cl concentrations that exceed 20,000 mg Cl kg^{-1} even under high Cl environments (Parker et al., 1983; Yang and Blanchar 1993; Rupe et al., 2000). Examining field-grown soybean for tissue-Cl concentration to determine Cl tolerance was proposed by Yang and Blanchar (1993) as an effective method to distinguish Cl includers from exlcuders.

Assessment of cultivar Cl ratings using field trials has the advantages of requiring no greenhouse space or additional labor to prepare and maintain a greenhouse study, tissue-Cl concentrations may be of value for explaining yield differences among cultivars, and a greater number of plants are available for sampling.

Arkansas Soybean Performance Trial Trifoliolate-Leaf Sampling

The RREC-2015 ASPT was used to assess the range of trifoliolate leaflet-Cl in recently released soybean cultivars and examine the trends among the three different cultivar Cl ratings (Fig. 3.2). Assuming that cultivars rated as includers and excluders are pure populations and cultivars within each category have similar abilities to take up Cl, we expected to see three clusters of data points with clear separation between each cluster. For example, Cl excluders would have low Cl concentrations, Cl includers would have high Cl concentrations and the few mixed cultivars would have intermediate Cl concentrations. Among the 112 cultivars in the RREC-2015, 40 were rated as excluders, 63 were rated as includers, and 9 were rated as mixed (Ross et al., 2015). The results in Fig. 3.2A-C clearly show a distinct cluster of cultivars with low Cl concentrations that represent Cl-excluders, but, regardless of maturity group, Cl concentrations of all remaining cultivars slowly increase from a point near the mean Cl concentration of excluders (< 200 mg Cl kg^{-1}) to concentrations that approach or exceed 3000 mg Cl kg^{-1}. The mean Cl-concentration ratio (includer/excluder) averaged 12.0:1 for the late maturity group IV cultivars, 10.9:1 for early maturity group V cultivars and 10.9:1 for all cultivars.

The RREC-2015 results also showed that the Cl concentration of cultivars rated as mixed were intermingled with the includer cultivars. This same trend was evident at the RRS-2016 and RREC-2016 (Fig. 3.3A-B). Yang and Blanchar (1993) reported similar Cl concentration trends

for 60 cultivars. The variability measured in tissue-Cl concentration across soybean cultivars can be explained by two possibilities. First, soybean cultivars have different affinities to accumulate Cl regardless of the presence of the Ncl gene (or Cl rating) that may be influenced by other genetic traits (e.g., root growth and water uptake). Second, few cultivars are pure populations of plants with a single Cl trait but are a population of plants containing both includer and excluder plants present in a specific ratio. Research assessing a large number of field-grown cultivars should provide valuable insight to explain the variability seen in tissue-Cl concentration.

The ranking of the 47 cultivars in the RRS-2016 and RREC-2016 ASPT show the average tissue-Cl concentrations were 62 and 58% greater at RRS-2016 compared to RREC-2016 for excluder (Fig. 3.3A) and includer (Fig 3.3B) cultivars, respectively. The greater tissue Cl at RRS-2016 compared to RREC-2016 is consistent with the southeast Arkansas region having groundwater that contains high concentrations of dissolved salts (Gilmour et al., 1983). Despite the greater tissue-Cl concentration at RRS-2016 than RREC-2016, the ratio of the average tissue-Cl concentration between includers and excluders was 18:1 and 17:1 at the RREC and the RRS, respectively. Regressing the leaf-Cl rankings at one location across the rankings at the other produced a relatively strong linear relationship for the 47 cultivars with an $R^2 = 0.76$ (Fig 3.3C). Thus, both greenhouse and field experiments suggest that relative cultivar Cl concentrations are consistent across locations. The similar Cl concentration ranking and ratio suggest that cultivars respond proportionally to field environments with different amounts of Cl.

Twenty-four early V maturity group cultivars were included in both the 2015 and 2016 ASPT and allowed us to assess year-to-year Cl concentration variability between different seed lots of the same cultivar. The relationship between RREC-2015 and RREC-2016 rankings was not as strong as anticipated ($R^2 = 0.59$, Fig. 3.3D) and a similar R^2 of 0.64 was found between Cl

concentration ranking for RREC-2015 and RRS-2016 (data not shown). Unlike cultivar Cl ranking, the relationship between cultivar tissue-Cl concentrations at the RRS-2016 and RREC-2016 (e.g., same seed source) produced a strong linear relationship (Fig. 3.3E). Likewise, the tissue-Cl concentration of the 24 cultivars present at RRS-2016 and RREC-2015 produced a strong linear relationship (Fig 3.3F). These data suggest leaf-Cl concentration behaves consistently for the same cultivars across time and location. Additional research investigating the uniformity of Cl concentration and the ratio of includer to excluder plants in the plant population of a single cultivar from different seed sources is warranted.

Yang and Blanchar (1993) collected and analyzed recently mature trifoliate leaves for Cl concentration from 60 cultivars belonging to maturity groups II through VI at 15 (V2-V3) and 80 d (R1) after planting. When no Cl was added, the average tissue-Cl concentration of excluders and includers was approximately 900 and 1800 mg Cl kg^{-1} 15 d after planting and 290 and 500 mg Cl kg^{-1} 80 d after planting, respectively. When tissue-Cl concentration was plotted against cultivars ranked in ascending order of tissue-Cl concentration, notable amounts of variability around the mean of tissue-Cl concentration for both excluder and includer cultivars existed, regardless of sampling time or Cl treatment. The trend of tissue Cl among soybean cultivars from RREC-2015 (Fig 3.2), RREC-2016 (Fig. 3.3A), and RRS-2016 (Fig. 3.3B) mirror the trend reported by Yang and Blanchar (1993) and suggest only a few cultivars have relatively "pure" populations of excluder and includer plants.

Soybean Population Dynamics of Tissue-Cl Concentration

This experiment aimed to answer two questions raised by Yang and Blanchar (1993) and our results (Figs. 3.2 and 3.3) including do individual, field-grown plants of a single cultivar have similar trifoliate leaf-Cl concentrations, and, more comprehensively, why are cultivar-Cl

ratings inconsistent among years or screening times? The annual Cl ratings from the 2013 to 2016 Cl-screening trials are listed in Table 3.2 for eleven cultivars selected for field study. The average (n = 48) tissue-Cl concentrations for the eleven cultivars ranged from 221 to 3309 mg Cl kg^{-1}. Spatial variability did not cause large fluctuations in the studentized residuals of the average tissue-Cl concentration in each block (Appendix 3.2). The lack of a spatial effect suggests the variability in tissue-Cl concentration was attributed to cultivars having differing affinities for Cl uptake and translocation, and not to the variability of Cl concentration in the soil, movement of Cl by irrigation water, or native soil properties.

Analysis of variance of the mean tissue Cl concentrations for the eleven cultivars showed significant differences among cultivar mean Cl concentrations but no clear grouping of cultivars to differentiate the three Cl ratings (Table 3.2). Numerically, the range of values shows cultivars that have low, intermediate and high tissue Cl concentrations as was observed for a larger number of cultivars in Fig. 3.2 and 3.3. The distribution of Cl concentration in individual plants provides some insight as to the numerical ranking and statistical differences identified in the ANOVA. As the mean tissue-Cl concentration increased, in general, the percentage of individual plants with Cl concentrations ≤1000 mg Cl kg^{-1} decreased and individual plants with Cl concentrations >1000 mg Cl kg^{-1} increased (Table 3.2). The distribution of individual plant Cl concentrations provide clear evidence that the population of plants of most cultivars is a blend of includer and excluders plants.

The distribution of tissue-Cl concentrations among the 528 individual plants showed that 34% of the individual plants had ≤500 mg Cl kg^{-1} and 31% of the plants had 1001 to 2000 mg Cl kg^{-1}, representing the two most common tissue-Cl concentration ranges (Table 3.3). The tissue Cl concentration distribution represented by these eleven cultivars may not be representative of

all commercial cultivars as these eleven cultivars were selected for specific behavior concerning Cl uptake in order to produce tissue-Cl concentrations, which encompass a wide range of results.

The most common tissue-Cl concentration ranges were numerically different among the eleven cultivars (Table 3.3) and showed that some cultivars are comprised of a population of plants that exhibit relatively consistent tissue-Cl concentrations (e.g., Pioneer P49T80R, Progeny 4900RY, and Armor 47-R70) while other cultivars are comprised of plants that exhibit a diverse range of tissue-Cl concentrations (e.g., Dynagro S52RY75 and Asgrow AG5233). The results in Tables 3.2 and 3.3 suggest that many cultivars should likely be rated as mixed populations rather than Cl includers or excluders. The diversity of plants in the population of some cultivars coupled with the cultivar-Cl rating decisions based on only five plants as outlined by Green and Conaster (2017) is likely responsible for the inconsistent annual cultivar-Cl ratings. The results for some cultivars (e.g., Dynagro S52RY75 and Armor 47-R13) also suggest that plants may have different propensities to regulate Cl uptake, but additional research is needed to clarify whether plants with and without the *Ncl* gene take up different amounts of Cl or this is simply due to the ratio of include and excluder plants.

The results presented in Tables 3.1 through 3.3 and Figs 3.1 and 3.3 provide ample evidence supporting the need for a more robust cultivar-Cl-trait rating system that accounts for the ratio of Cl includer and excluder plants in a population. The basis for a new rating system was examined by regressing mean tissue-Cl concentration across either the percentage of plants with $\leq$1000 mg Cl kg^{-1} or >1000 mg Cl kg^{-1} (Table 3.2). Both regressions produced strong linear relationships ($R^2 = 0.82$; Fig. 3.4) and suggest that mean tissue-Cl concentrations, rather than individual plants, could possibly be used to assign cultivar-Cl ratings.

The relationships depicted in Fig. 3.4 can be used as the basis of a more robust cultivar-Cl rating system that estimate the makeup of the plant population of each cultivar. For example, using the relationship of percentage of plants >1000 mg Cl kg^{-1}, a rating scale of 1-5, with one being a true excluder and 5 being a true includer, cultivars would be assigned ratings based on 20% increments (Table 3.2). It should be noted that for the field environment where the eleven cultivars were planted, 1000 mg Cl kg^{-1} appeared to be a good threshold for distinguishing between Cl includers and excluders, but a different threshold may be required in fields with more or less available Cl. For this reason, a field-screening protocol for cultivar-Cl ratings should include the use of standard Cl excluder, includer, and mixed cultivars or individual plants of selected cultivars could be sampled to calibrate the relationship.

Besides the greenhouse and hydroponic screening methods proposed by Green and Conatser (2017) and Ledesma et al. (2016), we could find no literature utilizing individual-plant tissue-Cl concentrations. Newsome et al. (2017) reported strong evidence that the soybean root stock plays a major role in the uptake and translocation of Na and Cl into the foliage of the plant. Excluder cultivars showed no significant difference in the uptake of these ions when grown in deionized water or 100 mmol NaCl solution while includers showed significantly higher concentrations under the NaCl treatment. Salt tolerant cultivars were genotyped at the GmSALT3 locus. Future research involving genetic mapping as well as tissue-Cl concentration could provide more detailed information from which to classify cultivars for salt tolerance.

Determination of Plant Sample Size to Represent Cultivar Cl-trait

The number of plants needed to accurately represent the diversity of plants in a population is an important aspect regardless of whether tissue from multiple plants is composited or tissue from individual plants is analyzed. Using the mean and standard deviations listed in

Table 3.2, Eq. 1 calculated that 20 individual plants would need to be sampled for each cultivar to represent the mean tissue Cl concentration at an 80% confidence level (data not shown). The second method, based on 1000 simulations using different random subsamples of the 48 individual plants within each cultivar, showed 5 to 48 individual plants were required to provide an accurate (90% accuracy) assessment of the plant population (Table 3.4). As the percentage of plants matching the evaluation rule increased from 80 to 90%, the number of plants required to identify the cultivars Cl rating remained constant for seven cultivars, increased for three cultivars (Armor 47-R13, Armor 47-R70, and Progeny 4900RY) and decreased for one cultivar (Progeny 5333R). Based on the simulation data, at the lowest percentage of plants meeting the rule (80%), only five cultivars (1 excluder, 2 includer, and 2 mixed) could be assigned Cl-ratings with 90% accuracy using five individual plants. Four cultivars required 10-20 plants to arrive at a rating and two cultivars (GoSoy 4914GTS and Progeny 5333R) required all 48 plants to satisfy the classification parameters (Table 3.4).

The screening process performed by Green and Conatser (2017) generates cultivar Cl ratings based on the response of five individual plants, which according to results in Table 3.4, would provide an accurate Cl rating for seven of the eleven cultivars in our experiment. It is important to note that our results pertain only to the information collected regarding tissue-Cl concentration and do not directly verify the presence or absence of the S-100 allele or any other gene associated with Cl tolerance (Lee et al., 2004). Our results were based entirely on tissue-Cl concentration of individual plants and mean multi-plant Cl concentrations, which served as indicators of whether the plants included or excluded Cl. Verification of the presence of absence of the Cl excluder/includer gene in the individual plants of a cultivar could provide invaluable insight on Cl uptake and tissue-Cl concentrations differences.

CONCLUSIONS

Our research showed that the inconsistent cultivar Cl ratings from a greenhouse screening method that uses five plants are likely because many cultivars are a mixture of plants that contain a range of Cl concentrations. Although we did not evaluate plants for the presence or absence of the *Ncl* gene, our tissue Cl concentration results provide strong evidence that the plant population of many cultivars is a mixture of excluder and includer plants and the cultivar Cl rating of mixed is appropriate and should be more common. The novel aspect of our research was the collection and analysis of tissue Cl concentration from individual soybean plants and demonstrating that mean tissue Cl concentrations from composite samples representing multiple plants can be used to predict the percentage of includer or excluder plants in a population. The percentage of excluder (low tissue Cl concentration) plants or includer (high tissue Cl concentration) plants is highly correlated with mean tissue-Cl concentration and the relationship may provide an alternative method for assigning Cl tolerance ratings to new soybean cultivars. Chloride analysis of tissue taken from field-grown soybean plants is a feasible method for assigning soybean cultivars a numerical rating that provides a reasonably accurate assessment of the percentage of plants that would be considered a Cl includer or excluder. A new rating system that accurately identifies the ratio of excluder to includer plants in the population would benefit soybean producers with fields having a history of Cl toxicity by allowing the selection of the proper cultivar with the largest percentage of Cl excluder plants.

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TABLES AND FIGURES

Table 3.1. Cultivar, relative maturity group (RMG), Cl-rating category and tissue Cl concentration means and standard errors for 12 soybean cultivars exposed to two Cl levels in a greenhouse trial conducted in 2015.

		Tissue-Cl Concentration							
		Annual Cl Rating§			200 mg Cl kg^{-1}		2000 mg Cl kg^{-1}		Overall
Cultivar†	RMG‡	2013	2014	2015	Mean	SE¶	Mean	SE	mean#
					----------mg Cl kg^{-1}----------				
Progeny 4900 RY	4.9	Excluder	Excluder	Mixed	1,100	378	8,547	832	4,824 a
UA 5213C	5.2	Excluder	Excluder	Excluder	2,873	810	15,577	2573	9,225 a
Pioneer 49T80R	4.9	NA	NA	Excluder	2,927	611	10,659	1455	6,789 a
Hutcheson	5.5	Mixed	Mixed	Mixed	8,942	3625	25,004	5885	16,973 b
Pioneer 48T53R	4.8	Mixed	Mixed	Mixed	12,615	2725	21,039	3786	16,827 b
NK S48-D9	4.8	NA	NA	Excluder	12,713	3339	27,390	4841	20,051 bc
Asgrow AG5233	5.2	Mixed	Mixed	Mixed	14,912	3934	23,758	5505	19,335 b
Armor 53L55	5.3	NA	NA	Mixed	17,025	2690	33,008	3956	25,016 cd
Armor 48-C5	4.8	NA	NA	Includer	17,966	2643	43,803	1910	30,918 ef
Halo 5:26	5.2	Includer	Includer	Includer	18,800	1004	35,248	3463	27,024 de
Asgrow AG4934	4.9	Includer	Includer	Includer	23,332	3175	38,861	1326	31,096 ef
ES4840RY	4.8	NA	Includer	Includer	25,059	1759	45,651	3525	35,356 f
								P-values	
								Cultivar	<0.0001
								Cl rate	<0.0001
								Cult× Cl rate	0.1158

† Abbreviation definitions: UA, University of Arkansas; NK, Northrup King; ES, Eagle Seed.

‡ RMG, Relative Maturity Group.

§ Cl ratings are published annually in the Arkansas Soybean Update (Ross et al., 2014, 2015, 2016) NA, not available. 2016 Cl ratings: Progeny 4900 RY, excluder; UA 5213C, excluder; Hutcheson, includer; NK S48-D9, mixed.

¶ SE, standard error of mean

Tissue-Cl concentration averaged across the 200 and 2000 mg Cl kg^{-1} treatments. Means followed by the same letter are not statistically different by Fisher's protected LSD (P = 0.10).

Table 3.2. Cultivar, Cl-rating category, tissue-Cl mean and standard error, and the percentage of plants in two leaf-Cl concentration categories for eleven cultivars from the field trial conducted at Pine Tree Research Station in 2016.

	Cl Rating Category‡			Leaf-Cl Concentration		Percentage of Plants		
Cultivar†	2013	2014	2015	Mean	SE§	≤1000 mg Cl kg^{-1}	>1000 mg Cl kg^{-1}	New rating¶
				mg Cl kg^{-1}		---------%-------		
Pioneer 49T80R	Excluder	Mixed	Excluder	221 a#	8	100	0	1
Progeny 4900RY	NA	Excluder	Mixed	400 ab	97	92	8	1
Progeny 5333RY	Excluder	Excluder	Mixed	437 ab	75	83	17	1
GoSoy4914GTS	Mixed	Excluder	Excluder	759 abc	37	85	15	2
NK S48-D9	NA	NA	Excluder	875 abc	121	56	44	2
Asgrow AG5233	Mixed	Mixed	Mixed	1045 bcd	132	53	47	3
Asgrow AG4934	Inlcuder	Includer	Includer	1319 cd	66	34	66	3
Armor 47-R70	NA	NA	Includer	1693 de	74	4	96	4
Armor 47-R13	Includer	Includer	NA	2225 e	162	6	94	5
Pioneer 49T09BR	NA	NA	Includer	2350 e	204	0	100	5
Dynagro S52RY75	NA	Mixed	Includer	3309 f	302	0	100	5
			P-values					
			Cultivar	<0.0001				
			Block	0.3270				

† Abbreviation definitions; NK, Northrup King

‡ Cl ratings are published annually in the Arkansas Soybean Update (Ross et al., 2014, 2015, 2016). NA, not available. 2016 Cl rating: Progeny 4900RY, excluder; NK S48-D9, mixed; Armor 47-R70, includer; Dynagro S52RY75, includer.

§ SE, Standard error.

¶ New rating based on equation y = 0.051X – 11.46 where x is the mean leaf Cl concentration, y is the percentage of plants with leaf-Cl concentrations >1000 mg Cl kg^{-1}. Ratings were assigned on a 1-5 scale in increments of 20% (e.g., 1 = 20% or less of plants with leaf-Cl concentrations ≤1000 mg Cl kg^{-1}). The model p-value was <0.0001 with intercept and linear slope coefficient p-values of 0.32 and <0.0001, respectively.

Means followed by the same letter are not statistically different by Fisher's protected LSD (*P* = 0.10).

Table 3.3. The percentage of individual plants from eleven cultivars having tissue-Cl concentrations within six Cl concentration ranges from a field trial conducted at Pine Tree Research Station in 2016.

	Leaf-Cl Concentration Range (mg Cl kg^{-1})					
Cultivar	0-500	501-1000	1001-2000	2001-3000	3001-4000	>4000
	----------% of plants----------					
Pioneer 49T80R	100	0	0	0	0	0
Progeny 4900 RY	85	7	0	6	2	0
Progeny 5333RY	79	4	15	2	0	0
GoSoy4914GTS	13	72	15	0	0	0
NK S48-D9†	50	6	33	11	0	0
Asgrow AG5233	43	11	32	13	2	0
Asgrow AG4934	0	34	62	4	0	0
Armor 47-R70	0	4	71	23	2	0
Armor 47-R13	0	6	50	27	8	8
Pioneer 49T09BR	0	0	44	48	4	4
Dynagro S52RY75	0	0	21	44	17	18
All cultivars	34	13	31	16	3	3

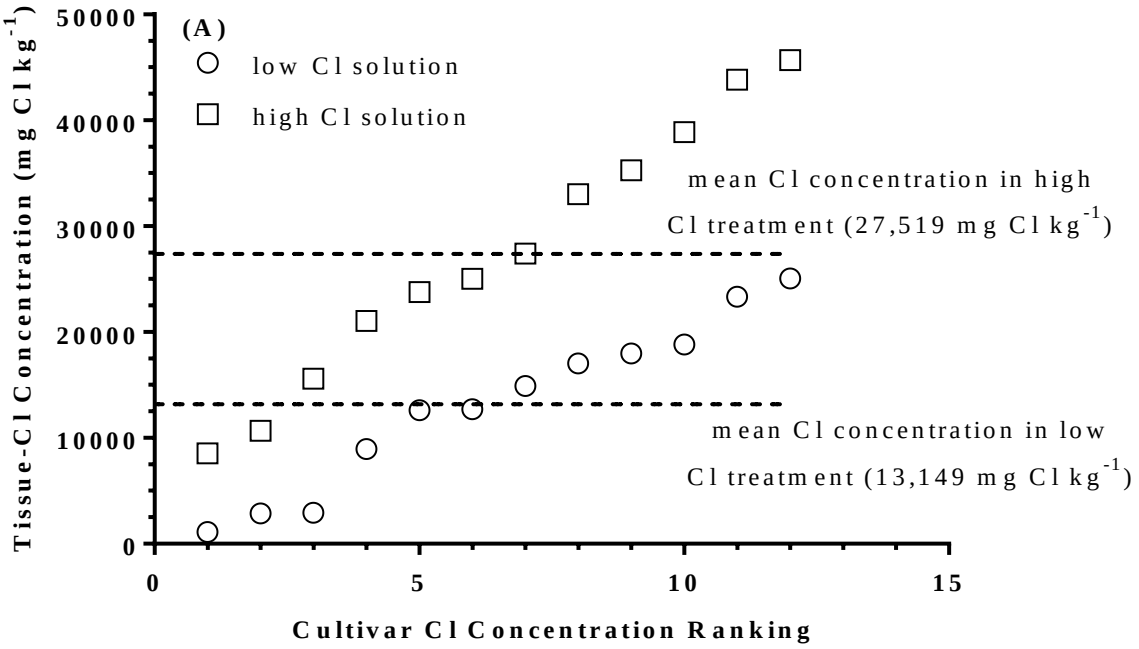
† Abbreviation definitions; NK, Northrup King

Table 3.4. Results of a simulation program in which cultivar-Cl rating classification was assigned based on the percentage of plants having tissue-Cl concentrations ≤1000 mg Cl kg^{-1} or >1000 mg Cl kg^{-1}.

		Percentage of plants for classification§			New classification¶		
Cultivar Cl-rating†	Cultivar‡	80%	85%	90%	80%	85%	90%
		------Number of samples------					
Excluder	GoSoy4914GTS	5	5	5	Mixed	Mixed	Mixed
	NKS48-D9	5	5	5	Mixed	Mixed	Mixed
	Pioneer 49T80R	5	5	5	Excluder	Excluder	Excluder
	Progeny 4900RY	48#	48	48	Excluder	Excluder	Mixed
Includer	Armor 47-R13	15	25	48	Includer	Includer	Includer
	Armor 47-R70	10	10	25	Includer	Includer	Includer
	Asgrow 4934	10	10	10	Includer	Includer	Includer
	Dynagro S52RY7	5	5	5	Includer	Includer	Includer
	Pioneer 49T09BR	5	5	5	Includer	Includer	Includer
Mixed	Asgrow 5233	5	5	5	Mixed	Mixed	Mixed
	Progeny 5333R	48	48	20	Mixed	Mixed	Mixed

† Chloride ratings taken from screenings as described by Green and Conatser (2017) published in Ross et al. (2014, 2015, 2016).
‡ Abbreviation definitions: NK, Northrup King.
§ The smallest number of individual plants needed to accurately establish a cultivar Cl rating. The three columns labeled 80, 85 or 90% represent the percentage of plants that satisfied the rules established for defining a excluder or includer plant (≤1000 mg Cl kg^{-1} or >1000 mg Cl kg^{-1}, respectively) and ≥90% of the 1000 simulations met the definition of includer, excluder or mixed cultivar rating.
¶ Classification assigned when ≥90% of the simulations produced the same Cl-rating.
# The variability was too large among subsamples for the cultivar to be classified without using all observations.

Figure 3.1. The tissue-Cl concentration (A) and Spearman correlation ranking (B) of twelve soybean cultivars grown in the greenhouse and exposed to either low (200 mg Cl kg^{-1}) or high (2000 mg Cl kg^{-1}) solution Cl concentrations.



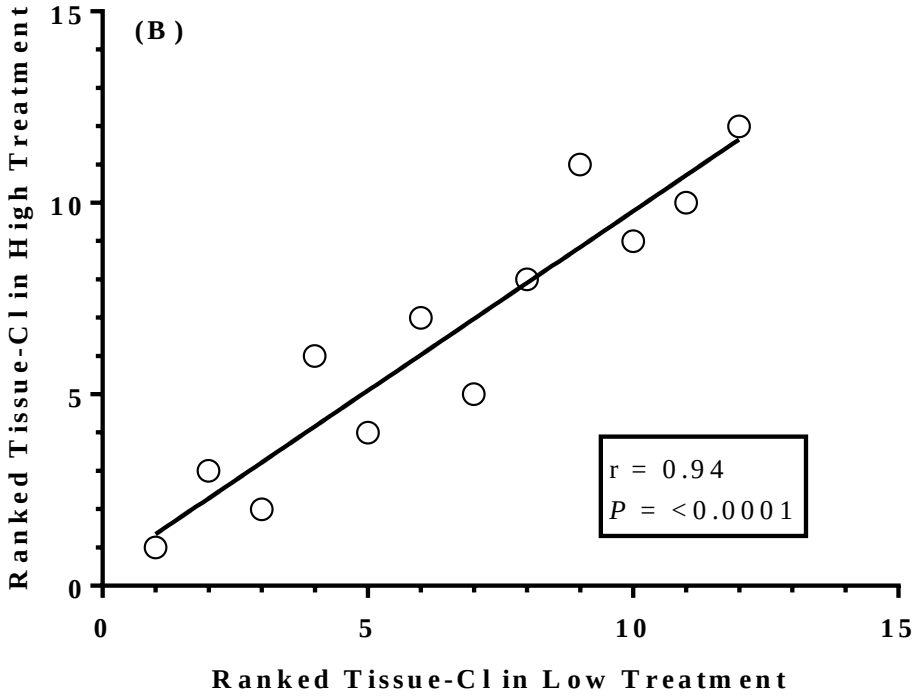


Figure 3.2. The distribution of tissue-Cl concentrations ranked in order of ascending sequence for cultivars in the (A) the late IV maturity group (MG), (B) early V MG, and (C) both MG combined for 112 cultivars planted in the 2015 Arkansas Soybean Performance Test located near Stuttgart, AR. Cultivar names are listed in Appendix 3.1.

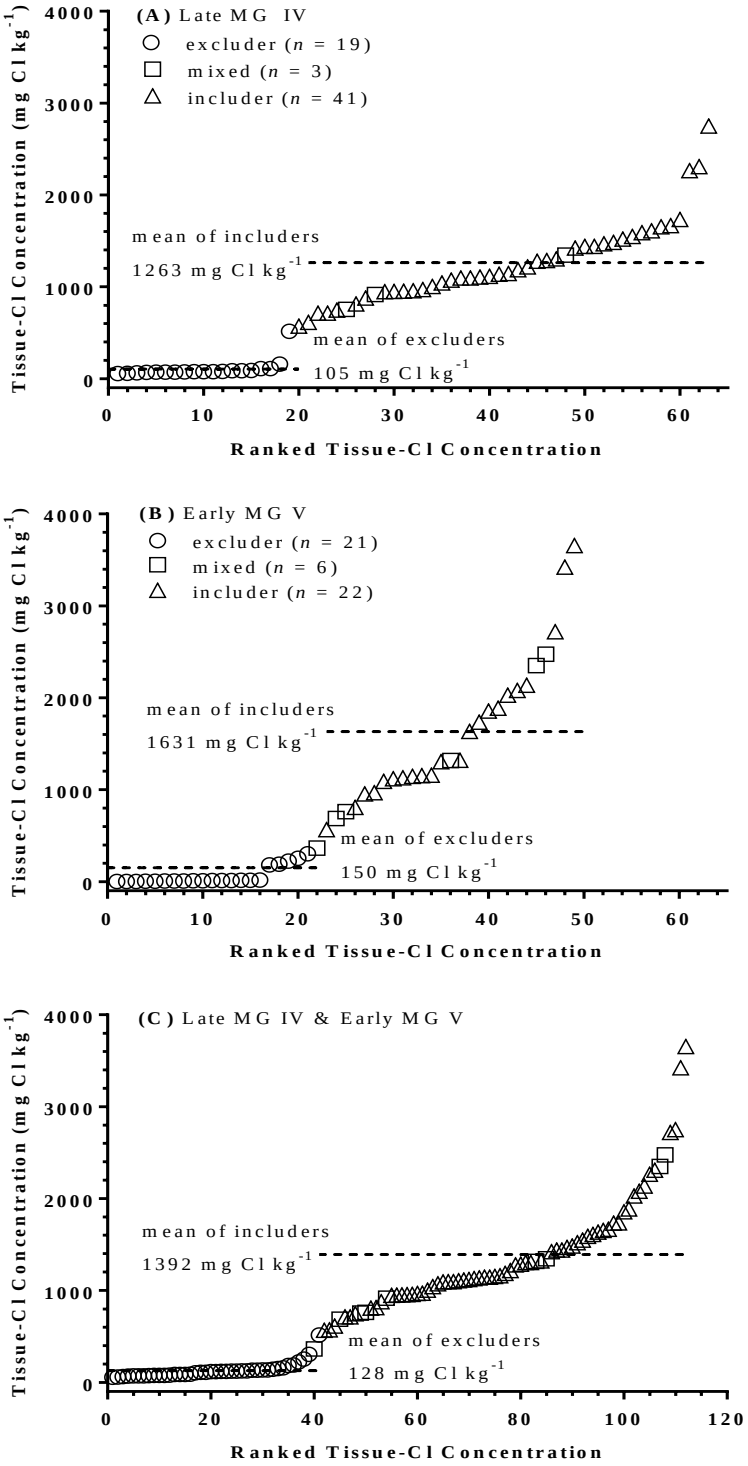


Fig. 3.3. Leaflet-Cl concentration and concentration rank in ascending order by cultivar-Cl rating from samples taken from the Arkansas Soybean Performance Tests at the (A) Rohwer Research Station (RRS) and (B) Rice Research and Extension Center (RREC) in 2016. Correlation analysis was performed using Spearman ranked correlation for the same cultivars at each location (C) and ranking the 24 cultivars present at the RREC tests in both 2015 and 2016 (D). Pearson correlation analysis was performed using the Leaflet-Cl concentrations at RREC-2015 and RREC-2016 (E) and RREC-2015 and RRS-2016 (F). Cultivar names are listed in Appendix 3.1.

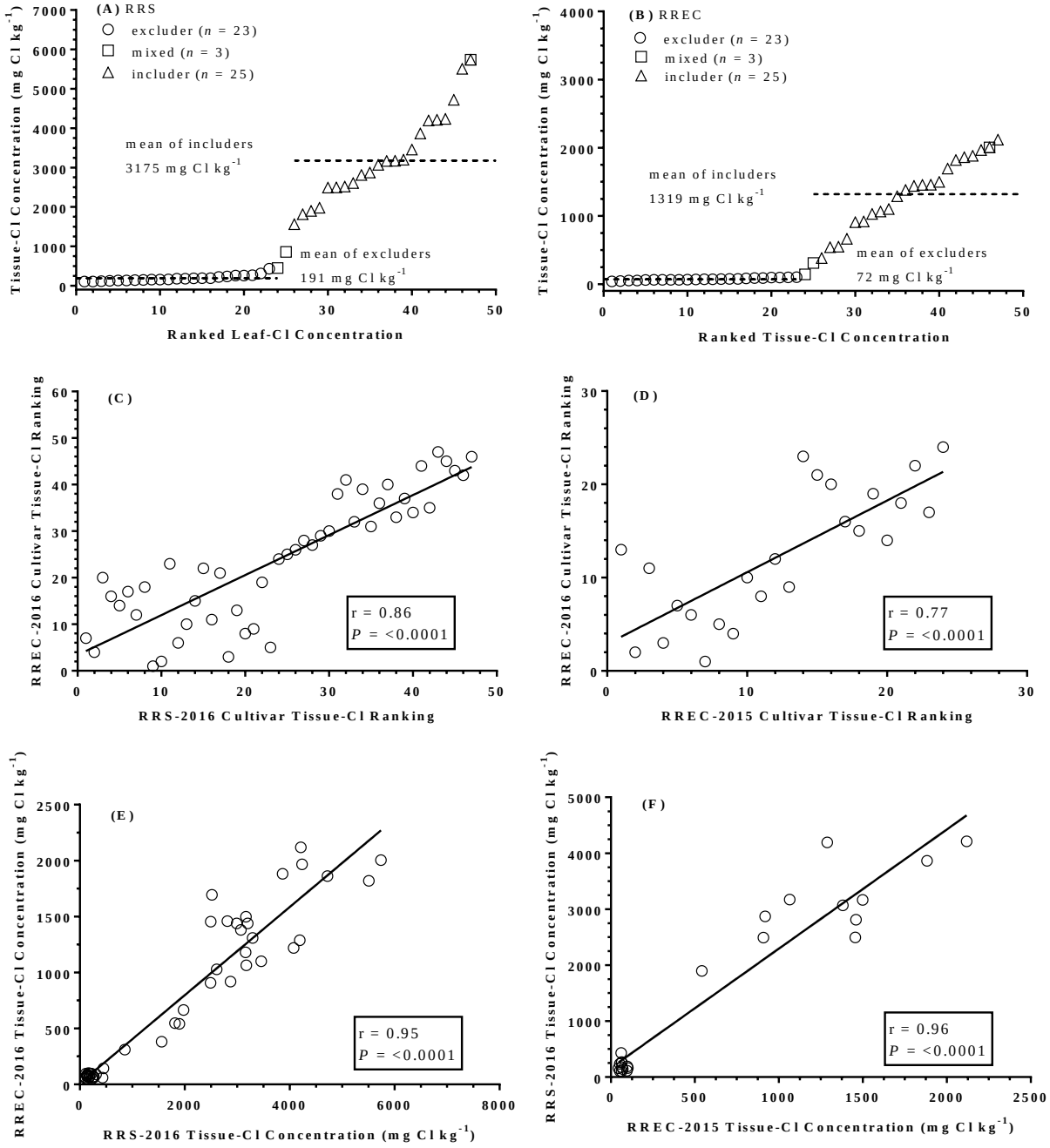
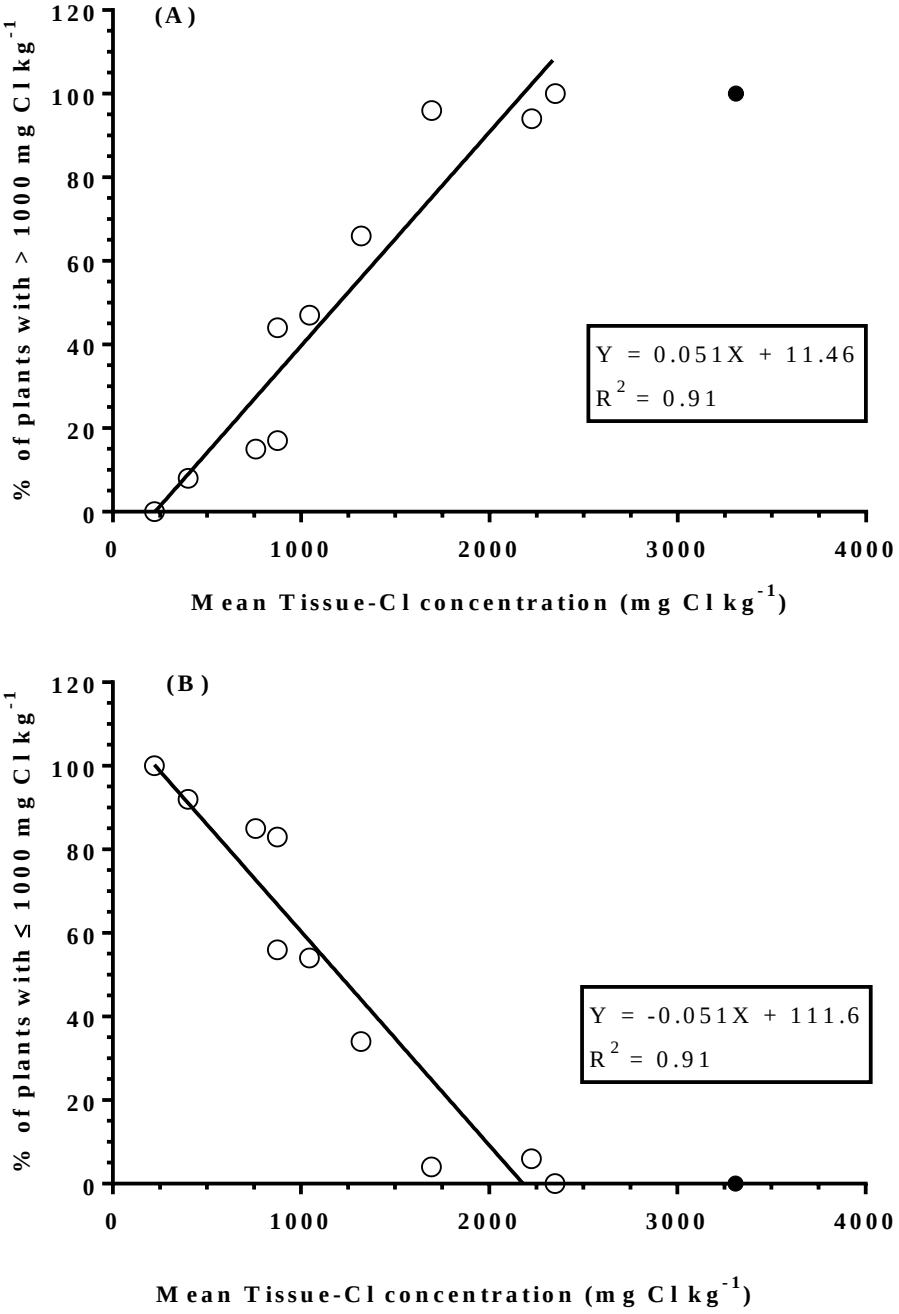


Fig. 3.4. The average percentage of individual plants having Cl concentrations (A) > 1000 mg Cl kg^{-1} and (B) ≤1000 mg Cl kg^{-1} regressed against mean composite leaf-Cl concentration of 48 individual plants of eleven soybean from a field trial located at the Pine Tree Research Station (PTRS) in 2016. The overall model p-value was <0.0001 and the intercept and slope coefficient p-values were <0.05. The filled circle represents an influential data point as determined by Cooks D statistic and was not omitted from the regression analysis.



Appendix 3.1. Cultivars planted in late IV maturity group and early V maturity group in 2015 and the early V maturity group in 2016 in the Arkansas Soybean Performance Tests.

Late Maturity Group IV (2015)		
Cultivar name	**Seed Company**	**Location**
Armor 48-C5	Armor Seed	Jonesboro, AR
Armor AR49X	Armor Seed	Jonesboro, AR
Armor 49X5L	Armor Seed	Jonesboro, AR
Armor AR4904	Armor Seed	Jonesboro, AR
Asgrow AG4835	Monsanto	St. Louis, MO
Asgrow AG4934	Monsanto	St. Louis, MO
AvDx-D814	AgVenture, Inc	Johnston, IA
AvDx-D914	AgVenture, Inc	Johnston, IA
CZ 4818LL	Bayer	Leverkusen, Germany
CZ 4959RY	Bayer	Leverkusen, Germany
Delta Grow DG 4825 RR2/STS	Delta Grow	England, AR
Delta Grow DG 4880 RR	Delta Grow	England, AR
Delta Grow DG 4935 RR2/STS	Delta Grow	England, AR
Delta Grow DG 4940 RR	Delta Grow	England, AR
Delta Grow DG 4967 LL	Delta Grow	England, AR
Delta Grow DG 4970 RR	Delta Grow	England, AR
Delta Grow DG 4977 LL/STS	Delta Grow	England, AR
Delta Grow DG 4981 LL/STS	Delta Grow	England, AR
Delta Grow DG 4985 RR2	Delta Grow	England, AR
Delta Grow DG 4990 LL	Delta Grow	England, AR
Delta Grow DG 4995 RR	Delta Grow	England, AR
Dyna-Gro S48RS53	Crop Production Services	Loveland, CO
Dyna-Gro S49LL34	Crop Production Services	Loveland, CO
Dyna-Gro S49RY25	Crop Production Services	Loveland, CO
Eagle Seed ES4840RY	Eagle Seed Company	Weiner, AR
Eagle Seed ES4960RY	Eagle Seed Company	Weiner, AR
Eagle Seed ES4998RR	Eagle Seed Company	Weiner, AR
Go Soy 483C	Stratton Seed Company	Carlisle, AR
Go Soy 4914GTS	Stratton Seed Company	Carlisle, AR
Go Soy 4915R2	Stratton Seed Company	Carlisle, AR
Go Soy Ireane	Stratton Seed Company	Carlisle, AR
HALO 4:80	Hornbeck Seed Company	DeWitt, AR
HALO 4:95	Hornbeck Seed Company	DeWitt, AR
HALO 4:98	Hornbeck Seed Company	DeWitt, AR
HBK 4950LL	Hornbeck Seed Company	DeWitt, AR
HBK 4953LL	Hornbeck Seed Company	DeWitt, AR
MORSOY XTRA 48X02	MorSoy Genetics	Cash, AR
Morsoy Xtra 49X85	MorSoy Genetics	Cash, AR
Mycogen 5N490R2	DOW	Midland, MI
NK S48-D9 Brand	Syngenta	Basel, Switzerland
Pioneer P48T53R	DuPont Pioneer	Johnston, IA
Pioneer P49T09BR	DuPont Pioneer	Johnston, IA

Appendix 3.1 (Cont.)

Cultivar name	Seed Company	Location
Pioneer P49T80R	DuPont Pioneer	Johnston, IA
Progeny P 4814LLS	Erwin-Keith, Inc	Wynne, AR
Progeny P 4850RYS	Erwin-Keith, Inc	Wynne, AR
Progeny P 4900RY	Erwin-Keith, Inc	Wynne, AR
Progeny P 4930LL	Erwin-Keith, Inc	Wynne, AR
R09-1589	University of Arkansas	Fayetteville, AR
REV® 48A46™	Terral Seed	Rayville, LA
REV® 49A14™	Terral Seed	Rayville, LA
REV® 49A55™	Terral Seed	Rayville, LA
REV® 49A75™	Terral Seed	Rayville, LA
REV® 49L29™	Terral Seed	Rayville, LA
REV® 49R94™	Terral Seed	Rayville, LA
S11-20337	University of Missouri	Columbia, MO
Schillinger 495.RC	eMerge Genetics	West Des Moines, IA
UA 5014C	University of Arkansas	Fayetteville, AR
USG 74B83RS	UniSouth Genetics, Inc	Dickson, TN
USG 74D95RS	UniSouth Genetics, Inc	Dickson, TN
USG 74G99L	UniSouth Genetics, Inc	Dickson, TN
USG 74K95RS	UniSouth Genetics, Inc	Dickson, TN
USG Ellis	UniSouth Genetics, Inc	Dickson, TN
Willcross WXE2495N	Willcross Seed	Chillicothe, MO
Willcross WXR2494NS	Willcross Seed	Chillicothe, MO

Appendix 3.1 (Cont.)

Early Maturity Group V (2015)		
Cultivar name	**Seed Company**	**Location**
Armor 50-R21	Armor Seed	Jonesboro, AR
Armor 51X5L	Armor Seed	Jonesboro, AR
Armor 53-L55	Armor Seed	Jonesboro, AR
Armor AR5205	Armor Seed	Jonesboro, AR
Armor AR53X	Armor Seed	Jonesboro, AR
Asgrow AG5233	Monsanto	St. Louis, MO
Asgrow AG5335	Monsanto	St. Louis, MO
CZ 5147LL	Bayer	Leverkusen, Germany
CZ 5150LL	Bayer	Leverkusen, Germany
CZ 5225LL	Bayer	Leverkusen, Germany
CZ 5242LL	Bayer	Leverkusen, Germany
Delta Grow DG 5067 LL	Delta Grow	England, AR
Delta Grow DG 5128	Delta Grow	England, AR
Delta Grow DG 5170 RR2	Delta Grow	England, AR
Delta Grow DG 5230 RR2	Delta Grow	England, AR
Delta Grow DG 5267 LL	Delta Grow	England, AR
Delta Grow DG 5367 LL	Delta Grow	England, AR
Dyna-Gro S52LL66	Crop Production Services	Loveland, CO
Dyna-Gro S52RY75	Crop Production Services	Loveland, CO
Eagle Seed ES5225RY	Eagle Seed Company	Weiner, AR
Eagle Seed ES5335RY	Eagle Seed Company	Weiner, AR
Go Soy 5115LL	Stratton Seed Company	Carlisle, AR
Go Soy 5215LL	Stratton Seed Company	Carlisle, AR
Go Soy 5315LL	Stratton Seed Company	Carlisle, AR
Go Soy Leland	Stratton Seed Company	Carlisle, AR
HALO 5:26	Hornbeck Seed Company	DeWitt, AR
Hutcheson	Virginia Polytechnic Institute	Blacksburg, VA
Mycogen 5N501R2	DOW	Midland, MI
Mycogen 5N522R2	DOW	Midland, MI
NK S52-Y2 Brand	Syngenta	Basel, Switzerland
Pioneer P50T15BR	DuPont Pioneer	Johnston, IA
Pioneer P50T64R	DuPont Pioneer	Johnston, IA
Pioneer P52T50R	DuPont Pioneer	Johnston, IA
Pioneer P53T73SR	DuPont Pioneer	Johnston, IA
Progeny P 5160LL	Erwin-Keith, Inc	Wynne, AR
Progeny P 5213RY	Erwin-Keith, Inc	Wynne, AR
Progeny P 5226RYS	Erwin-Keith, Inc	Wynne, AR
Progeny P 5333RY	Erwin-Keith, Inc	Wynne, AR
R09-430	University of Arkansas	Fayetteville, AR

Appendix 3.1 (Cont.)

REV® 51A56	Terral Seed	Rayville, LA
REV® 52A94™	Terral Seed	Rayville, LA
Cultivar name	**Seed Company**	**Location**
S11-16653	University of Missouri	Columbia, MO
S11-17025	University of Missouri	Columbia, MO
S11-20124	University of Missouri	Columbia, MO
S11-20195	University of Missouri	Columbia, MO
UA 5213C	University of Arkansas	Fayetteville, AR
USG 75G24L	UniSouth Genetics, Inc	Dickson, TN
Willcross WXE2535NS	Willcross Seed	Chillicothe, MO
Willcross WXR2524N	Willcross Seed	Chillicothe, MO

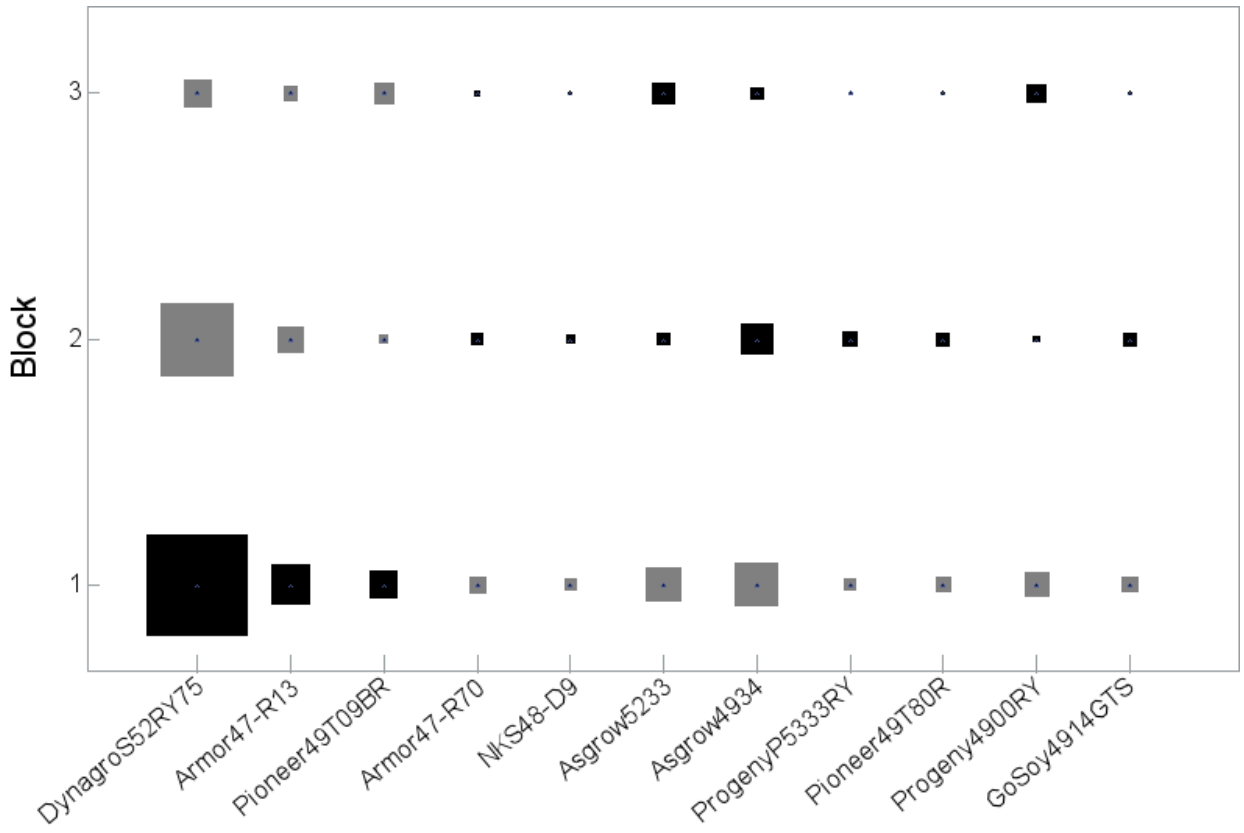
Appendix 3.1 (Cont.)

Early Maturity Group V (2016)		
Cultivar name	**Seed Company**	**Location**
Armor 53-D04	Armor Seed	Jonesboro, AR
Armor AR5206C	Armor Seed	Jonesboro, AR
Asgrow AG 53X6	Monsanto	St. Louis, MO
Asgrow AG 54X6	Monsanto	St. Louis, MO
AvDx-F216	AgVenture, Inc	Johnston, IA
Blue River 50SK7	Blue River Organic Seed	Ames, IA
CZ 5147 LL	Bayer	Leverkusen, Germany
CZ 5150 LL	Bayer	Leverkusen, Germany
CZ 5225 LL	Bayer	Leverkusen, Germany
CZ 5242 LL	Bayer	Leverkusen, Germany
CZ 5445 LL	Bayer	Leverkusen, Germany
Delta Grow DG5067 LL	Delta Grow	England, AR
Delta Grow DG5170 RR2/STS	Delta Grow	England, AR
Delta Grow DG5230 RR2	Delta Grow	England, AR
Delta Grow DG5461 LL	Delta Grow	England, AR
Dyna-Gro S52LL66	Crop Production Services	Loveland, CO
Dyna-Gro S52RY75	Crop Production Services	Loveland, CO
Eagle Seed ES5015RYX	Eagle Seed Company	Weiner, AR
Eagle Seed ES5225RY	Eagle Seed Company	Weiner, AR
Eagle Seed ES5420RYX	Eagle Seed Company	Weiner, AR
Go Soy 5115LL	Stratton Seed Company	Carlisle, AR
Go Soy 5214GTS	Stratton Seed Company	Carlisle, AR
Go Soy Leland	Stratton Seed Company	Carlisle, AR
Hutcheson	Virginia Polytechnic Institute	Blacksburg, VA
NK S52-Y2 Brand	Syngenta	Basel, Switzerland
Pioneer P50T64R	DuPont Pioneer	Johnston, IA
Pioneer P52T50R	DuPont Pioneer	Johnston, IA
Pioneer P53T73SR	DuPont Pioneer	Johnston, IA
Progeny P 5016RXS	Erwin-Keith, Inc	Wynne, AR
Progeny P 5226RYS	Erwin-Keith, Inc	Wynne, AR
Progeny P 5414LLS	Erwin-Keith, Inc	Wynne, AR
Progeny P 5417RX	Erwin-Keith, Inc	Wynne, AR
R09-430	University of Arkansas	Fayetteville, AR
R11-89RY	University of Arkansas	Fayetteville, AR
REV® 51A56™	Terral Seed	Rayville, LA
REV® 52A94™	Terral Seed	Rayville, LA
S11-17025	University of Missouri	Columbia, MO
S11-20124	University of Missouri	Columbia, MO

Appendix 3.1 (Cont.)

Cultivar name	**Seed Company**	**Location**
S12-4718	University of Missouri	Columbia, MO
UA 5014C	University of Arkansas	Fayetteville, AR
UA 5213C	University of Arkansas	Fayetteville, AR
UA 5414RR	University of Arkansas	Fayetteville, AR
UAX 51010	University of Arkansas	Fayetteville, AR
UAX 5102	University of Arkansas	Fayetteville, AR
USG 7506XTS	UniSouth Genetics, Inc	Dickson, TN
USG 7537XT	UniSouth Genetics, Inc	Dickson, TN
USG 7547XT	UniSouth Genetics, Inc	Dickson, TN

Appendix 3.2. Eleven cultivars represented in three blocks of 16 plants block^{-1} in the 2016 soybean Cl population study at the Pine Tree Research Station. The squares represent studentized residuals of the mean leaf-Cl concentration in each block. The size of the block represents the magnitude of the studentized residual and the color represent a postive (black) or negative (gray) value.



Chapter 4

Conclusions

Understanding how Cl accumulates in soybean and at what concentrations grain yield begins to decline from Cl toxicity are essential for the recognition and management of Cl toxicity. Use of amendments such as fertilizers containing Cl (e.g., muriate of potash) and irrigation water containing dissolved salts creates the potential for Cl toxicity which can be magnified by high temperatures and low precipitation. The overall research goal was comprised of two facets: i) identify a threshold leaf-Cl concentration at which yield loss occurs for Cl-excluder and –includer cultivars, and ii) understand why cultivar Cl ratings are inconsistent so that a more accurate method can be developed.

Experiments conducted across five site-years show the addition of Cl tended to decrease the yield of both Cl-excluder and –includer cultivars, however the magnitude of yield loss in Cl include cultivars was frequently greater than the yield loss in Cl excluder cultivars. Across site-years, the yield decline from CL toxicity was 4 to 20% for Cl includers compared to 0 to 8% for excluders. The leaf-Cl concentrations of Cl-includers were much higher compared to Cl excluders, suggesting toxic Cl concentrations thresholds would differ. In order to assess toxic concentrations, relative yield was calculated for each cultivar and regressed against leaflet-Cl concentration for each cultivar Cl rating. The relationship defined critical leaflet-Cl concentrations of 1885 mg Cl kg^{-1} for Cl excluder cultivars and 3923 mg Cl kg^{-1} for Cl includer cultivars with yield loss increasing linearly as leaflet-Cl concentration increased. Diagnostic leaf-Cl concentrations will be of value to detect Cl toxicity in soybean because the appearance of symptoms from chronic Cl accumulation does not usually appear until late reproductive growth (R5-6) at which point nothing can be done to manage the problem. The detection of Cl toxicity at a growth stage before the appearance of symptoms is crucial for developing strategies to mitigate yield loss.

The second objective of our research to investigate why cultivar Cl ratings are variable from one year to the next. Composite trifoliolate leaflet samples from a large number of soybean cultivars produced an incremental increase in leaf-Cl concentration when ranked in ascending order, with large amounts of variability around the mean concentration of cultivars labeled as Cl-includers. The range of leaflet-Cl concentrations showed no clear, separate grouping of includer, excluder, and mixed cultivars suggesting that many cultivars may be mixed populations. To investigate the source of this variability, a field study was conducted in which individual plants of multiple cultivars where collected to assess leaf-Cl concentration variability. Results strongly suggest that only a small portion of cultivars are a pure population of includer and excluder plants which take up similar amounts of Cl. Many cultivars likely contain both includer and excluder plants in varying population ratios that explains the continuous range of mean leaf-Cl concentrations of the more than 100 cultivars evaluated.

Individual plant leaf-Cl concentrations were examined to determine how many plants would need to be sampled in order to accurately assess the Cl trait distribution of that cultivar and develop a new rating system to represent the ratio based on mean leaf-Cl concentration. The results suggest 20 plants would be sufficient to provide a representative mean leaf-Cl concentrations of most cultivars. Our results suggest that field-grown plants included in the Arkansas Soybean Performance Tests can be successfully used to accurately assign a Cl tolerance ratings to new cultivars.

In summary, our research showed that accurate Cl tolerance ratings are perhaps the first step to more effectively managing Cl toxicity and the developed critical Cl concentrations will enable scientists to survey soybean-production areas to estimate how widespread yield loss from Cl toxicity is. It is well documented in the research that Cl-excluder cultivars produce greater

grain yields when grown in fields with salinity (Cl) problems compared to includer cultivars, making the selection of an excluder cultivar the first and most effective management strategy. The findings of this research provide the fundamental information to develop a more robust Cl rating system that will more accurately describe the Cl tolerance of the population of plants in each soybean cultivar.