

THE EFFECTS OF SALINITY ON HERBIVOROUS PESTS OF CORN AND SOYBEAN

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State University's regulations and meets the accepted standards for the degree of

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ABSTRACT

Many environmental factors, including soil characteristics, are critically important for plants, herbivorous arthropods, and their interactions. Despite increasing evidence that soil salinity can drastically impact plants, little is known about how salinity affects the herbivorous arthropod pests feeding on those plants we investigated how soil salinity affects two major crop pests: the two spotted spider mite (*Tetranychus urticae*) feeding on corn and soybean and the soybean aphid (*Aphis glycines*) on soybean. We quantified the impact of salinity by measuring demographic characteristics and behavior of pests on plants grown in soil with various levels of salinity. Overall, both crop pests performed better as salinity increased. These studies suggest that salinity can be just as important for herbivores as it is for plants. Moreover, the negative effects of soil salinity on crop plants in agroecosystems may be further compounded by a greater risk of pest problems.

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DEDICATION

I would like to dedicate this thesis to my husband J.J. Nelson for all of his support through graduate school.

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LIST OF ABBREVIATIONS

EC	Electrical Conductivity.
NGP.....	Northern Great Plains.
SBA.....	Soybean Aphid.
TSSM	Two Spotted Spider Mite.

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CHAPTER 1. THE EFFECTS OF SALINITY ON AN HERBIVOROUS CROP PEST (*TETRANYCHUS URTUCAE*) ON SOYBEAN AND CORN

Introduction

Abiotic factors including temperature, humidity, water, and soil characteristics can significantly impact herbivore-plant interactions. While it is clear that changes to these factors can be critical to such organisms and their interactions (Tylianakis et al., 2008), it is much less clear how a given herbivore will react when changing a particular abiotic factor. Some of this confusion is because abiotic factors can directly affect the consumer (herbivore), the resource (plant), and their interactions (Harmon & Barton, 2013). However, even in relatively simple cases where abiotic changes primarily influence the herbivore indirectly through the plant, we do not necessarily know what to expect. For example, two general ecological theories predict opposite results for how arthropods will respond to stressed plants (the plant stress hypothesis (White, 1969) vs. the plant vigor hypothesis (Price, 1991)). Therefore, to broadly understand the effects of changing abiotic conditions, we must continue to explore specific abiotic changes and their potential effects on plant-herbivore interactions.

Increasing soil salinity is a growing, global problem (Essa, 2002) that can have a tremendous impact on plants (Bernstein, 1975; Dagar 2011; Greenway and Munns 1980), and therefore may also be strongly affecting the herbivores that feed on those plants. The stress that salts induce into plants varies depending on species and cultivar but yields of most crops in the Northern Great Plains (NGP) decrease with increasing salinity (Bernstein 1975). Irrigation water can also contribute to soil salinity (Rengasamy 2010). Consequently, 35–40% of the world's total food and fiber production is estimated to be adversely affected by soil salinity (Corwin et al., 2007).

Despite the strong negative effect of increasing salinity on plants, few studies have investigated the effects of salinity on herbivorous arthropods feeding on terrestrial plants grown in different soil conditions (reviewed in Harmon and Daigh 2017). The few examples that already exist are not helpful for predicting effects on new systems as they have shown that herbivores can be positively (Aucejo-Romero et al., 2004), negatively (Araya et al. 1991), or not be affected (Younginger et al., 2009) by changes to soil salinity. Some of these differences may have to do with differences in the host plant they are feeding on (Araya et al. 1991), making host plant an important variable to consider and control for in experiments.

The purpose of this study is to investigate the effects of naturally occurring soil salinity on two spotted spider mite (TSSM), *Tetranychus urticae*, feeding on either corn (*Zea mays*) or soybean (*Glycine max*) host plants. We used sodium sulfate and magnesium sulfate, salts which naturally occur in agricultural fields in NGP, to experimentally induce saline treatments. Our specific objectives were to 1) determine how soil salinity impacts TSSM at an individual level on corn and soybean plants and 2) determine how soil salinity impacts TSSM at a population level on corn and soybean plants.

Materials and Methods

Two Spotted Spider Mites

TSSM is an important agricultural pest due to its worldwide distribution, rapid reproduction, and quick generation time (Jeppson et al. 1975). This species has been found on over 900 host plants including several economically important agricultural crops including corn, soybean, and cotton (Jeppson et al. 1975; Navajas, 1998; Dennehy and Granett 1984). Feeding by TSSM can result in a reduction of photosynthesis, leaf drop, and ultimately plant death (Helle & Sabelis, 1985).

The life cycle of TSSM consists of 5 stages: egg, larva, protonymph, deuteronymph, and adult (Shih et al.,1976). For the majority of mite species optimum temperature ranges from 23-29°C (Boudreaux 1963). Within these ranges mites can complete their life cycle within 7-12 days (Boudreaux 1963).

Colonies

TSSM were originally collected from the USDA-Agricultural Research Service (ARS), Northern Crop Science Laboratory greenhouse in Fargo, North Dakota in June 2013. Mite colonies were maintained on susceptible soybean plants (variety RG607RR NDSU Research Foundation, Fargo, ND) and corn plants (variety 45Q93 from Peterson Farm Seed) in separate colonies. TSSM used in experiments were collected from colonies on the same plant type to avoid any effects due to switching plant host. Plants and mites were contained in cages (model # BD44545F, MegaView Science Co., Taichung, Taiwan, China) at 21-24°C, 60-80% RH, under a photoperiod of 14:10(L/D) at the NDSU Agricultural Experiment Station Research Greenhouse Complex in Fargo, North Dakota. Mite colonies were renewed once each week by removing the most heavily infested plants and replacing them with clean soybean or corn plants.

Greenhouse experiments (common methodology)

The goal of our greenhouse experiments was to test TSSM performance on corn and soybean plants grown in soils that were manipulated to contain a range of salinity levels. We used the same basic methodology for all four of our greenhouse experiments. Experimental plants were grown in the same greenhouse conditions as the mite colonies and used the same plant varieties. However, altered how experimental plants were grown to create our salinity treatments.

Soybean plants were grown in 1kg pots lined with a plastic bag to keep the salts from leaching out when pots were watered. PRO-MIX Premier BX Mycorrhizae Pro soil (Riviere-du-Loup, Quebec, Canada) was used as growth media and three seeds were added per pot. Pots were thinned to one plant each upon emergence. Soybean plants were watered gravimetrically two times each week (550 grams). When determining soil salinity, the amount of water in the soil is as important as the amount of salt (Bernstein 1975), for this reason consistent watering was performed. We used relatively young soybean plants for the experiment (vegetative growth stages V1 to V3 as defined by Fehr & Caviness (1977)).

Corn plants used in experiments were grown similarly with a couple exceptions. We used larger, 2 kg pots that were lined with a plastic bag, but with the same soil as the soybean. Corn was also thinned to 1 plant per pot upon emergence. Corn plants were watered gravimetrically two times each week (1500 grams). We also used young corn plants for experiments (vegetative growth stages V2-V5).

To provide information that was as relevant as possible to growers in our region we chose salts found in our region. Plant tolerances to salinity can vary depending on the type of salt (Bernstein, 1975), so using different salts could influence both the plant and the herbivore. Many previous studies have used sodium chloride (NaCl) to induce salinity stress (Aucejo-Romero et al., 2004; Bowdish & Stiling, 1998; Cakmak & Demiral, 2007; Dunn, et al., 1998; Hemminga, M. A., Van Soelen, 1992; Moon & Stiling, 2000, 2002a, 2002b) however, NaCl isn't the only salt contributing to salinity problems in agricultural systems. In our greenhouse experiments we used sodium sulfate (anhydrous ACS grade from VWR International) and magnesium sulfate (heptahydrate ACS grade from VWR International) to experimentally manipulate target levels of

salinity as those are two of the most common, moderately soluble salts naturally found within agricultural fields in North Dakota (Franzen, 2003).

We established five treatments: no salts added with an electrical conductivity (EC) near 0, which served as the control, and targeted EC values of 2,4,6, and 8 (EC saturated paste) which match values found in some North Dakota production fields (Butcher et al 2016). To create our treatments, we added specific amounts of both salts to the pot in which the plant was grown (Table 1). Salts were added and mixed with the soil in the bottom $\frac{3}{4}$ portion of the pot. More soil was added to the top of the pot and seeds were planted in the top $\frac{1}{4}$ portion of soil to prevent scorching or burning due to salinity, which would decrease germination.

Table 1.1. Mass of salts added and measured salinity values for soybean (1kg pot) and corn (2kg pot).

Soybean 1 kg pot		Corn 2 kg pot		Measured Salinity value EC _{1:5} dS m ⁻¹
Grams Na ₂ SO ₄ per pot	Grams MgSO ₄ •7 H ₂ O per pot	Grams Na ₂ SO ₄ per pot	Grams MgSO ₄ •7 H ₂ O per pot	
0	0	0	0	0.84
1.34	1.16	5.36	4.64	2.37
3.08	2.67	12.3	10.7	4.66
4.69	4.06	18.8	16.2	6.05
6.25	5.41	25.0	43.3	8.07

We used EC_{1:5} to measure soil salinity levels (Ju et al. 2007, Shi et al. 2009). This procedure was performed by taking five grams of dried, homogenized soil from each pot and mixing it with 25mL of deionized water. After samples were mixed they were allowed to rest for 30 minutes before being mixed a second time. After the samples rested for an additional 30 minutes they were tested with a SensIon5 Conductivity Meter, Hach Company, Loveland, CO. This procedure was performed on four samples from each EC treatment and the values of those samples were averaged, this averaged value was used for statistical analysis.

We performed experiments using two different soils. Once we used the greenhouse media mentioned above and the other time we used a natural field-sourced loamy sand textured Glyndon soil (Coarse-silty, mixed, superactive, frigid Aeric Calciaquoll) from soil collected near Hunter, North Dakota (experiments as in Langseth 2015; Appendix). The overall results of the two experiments were the same, but here we report only the packaged soil results (other in Appendix). We feel that the natural soil results complement the results we report here, but we were less comfortable giving them equal weight as there were some questions about how the method of processing the natural soil may have been unintentionally influencing the long term growth of plants grown in our experiments.

Fecundity experiments

Immediately before beginning this experiment, we took plant measurements to help us understand how our salinity treatments impacted the performance of experimental plants. Plant measurements included: plant height and length and width of the focal leaf used in the fecundity experiments. To calculate a rough approximation of the leaf area for each plant type we used the length and width measurement along with the equation of an ellipse (soybean) or rectangle (corn).

Host plant quality can directly affect herbivore fecundity (Awmack & Leather, 2002). Fecundity can therefore be a good measure of how herbivorous arthropods are impacted by differences in hosts. To determine how soil salinity impacts TSSM we first measured TSSM fecundity on plants grown in different concentrations of soil salinity (described above).

In this experiment three adult female TSSM were transferred with a fine camel hair brush onto a single leaf of a soybean plant (stage V2-V4) or onto a corn plant (stage V6-V8) grown in one of the five salinity treatments. Mites were enclosed within a clip cage (1-7/16"

model # 1458, Bioquip products, Rancho Dominguez, CA) that was positioned to include mostly leaf but also a gap off of the leaf so TSSM could move between the top and bottom sides of the leaf. Because eggs hatch about four days after they are laid (Shih et al.,1976) the spider mites were kept on the single leaf for three days to accumulate as many eggs possible without eggs starting to hatch. After three days the plant was harvested and all mites and eggs on the plant were counted under a microscope. The fecundity experiment was carried out on corn and on soybean plants separately. Individual plants were the unit of replication, with 10 replications per treatment, for both corn and soy (N=50 for each plant).

We used linear regression in JMP (SAS Institute, 2010) to analyze all our results. We performed a separate analysis for each of the four plant measurements (height or leaf area crossed by corn or soybean), with each analysis using the plant measurements as the dependent variable and the EC_{1.5} measurement as the independent variable. We used the same basic analysis to look at the fecundity results. In this case TSSM fecundity (how many eggs were laid) on each plant was compared to the salinity treatment with separate regression equations performed for each crop (TSSM on corn and TSSM soybean). Because the number of adults at the end of the experiment varied considerably in the corn experiment, the number of adults was also included as a covariate for the analysis of corn. The three adult spider mites used in the soy clip cages almost all survived, so it was not helpful to include the term for analyzing soybean results.

Population growth experiments

We also performed complementary experiments to look at TSSM performance at a slightly greater temporal and spatial scale. Experimental plants were used as before, but now small populations of TSSM were tested on entire plants for approximately one full generation

(seven days). Using a full generation exposes mites to salinity during all developmental stages from egg to adult, meaning that changes to fecundity (as measured above) would be accounted for as well as any other potential differences in mortality and development.

For the population growth experiment seven adult female TSSM were transferred with a fine camel hair brush onto the leaf of a corn (stage V7-V9) or soybean plant (stage V3-V6), and the entire plant was enclosed in a tube cage. Cages were composed of a thin plastic sheet 10cm in diameter and 40cm in height with two holes covered in thrips proof mesh to allow for airflow, without allowing the mites to escape. This arena allowed mites to move freely across the entire plant. Mites remained on the corn or soybean plant for seven days, at which time the plant was harvested and all adult mites, eggs, and nymphs were counted under a microscope. The population growth experiment was carried out on individual corn and on soybean plants separately. Each individual plant was the unit of replication, there were 10 replications per treatment, of each plant type, but one plant in the soybean experiment and two plants in the corn experiment had to be excluded due to contamination (N=49 total in soy and N=48 in corn).

Linear regression analyses were again performed in JMP (SAS Institute, 2010). TSSM population size (total number of mites in all life stages after seven days) on each plant was the dependent variable and the $EC_{1.5}$ measurement was the independent variable. Separate regression equations were again used for each crop.

Field fecundity experiments

We performed the same basic fecundity experiment described above, but in agricultural fields, to look at TSSM performance across natural gradients of salinity under field conditions. Within production agricultural fields, we ran experiments on individual plants that were located in different areas of the field with different levels of salinity. To quantify these differences in

salinity, soil samples were taken prior to or during the field experiments by hand with an auger at a depth of 0-15.24 cm to confirm EC values. Samples were submitted to Agvise Laboratories (Northwood, ND, USA) and tested for EC saturated paste (EC_e) values, these values were used for analyses. EC_e values in the field ranged from 0.40-5.52 $dS\ m^{-1}$ at the 0-15 cm depth in the 2014 growing season.

All field sites were located in Richland County, ND, and management practices varied slightly by field (Butcher 2016). In August and September of 2014 we ran the three-day fecundity experiments with TSSM in three soybean fields with a soil type mapped as Wyndmere loam (Coarse-loamy, mixed, superactive, frigid Aeric Calciaquoll; USDA-NRCS, 1999a; USDA-NRCS Web Soil Survey, 2014). Sandy loam fields planted with soybean received a total of 38.2 cm of rainfall with an estimated 94.6 cm of potential evapotranspiration (PET) during the 2014 growing season (North Dakota Agricultural Weather Network, NDAWN, 2014). The field experiments were repeated in July and August 2015. Three fields with silty clay loam soil were planted with soybean in 2015. These fields received 36.6 cm rainfall with an estimated PET of 116.3 cm in 2015. Soil samples were taken while experiments were running, to test for salinity levels as in 2014. EC_e values in the field ranged from 0.51-4.42 $dS\ m^{-1}$ at a 0-15 cm depth in the 2015 growing season.

We also ran the fecundity experiment in corn fields in both growing seasons, but unfortunately the experiments failed. The adult female spider mites did not successfully establish on most of the field corn plants, making the results of the experiment meaningless. Therefore, we only show the results from our field experiments in soybeans.

To perform our fecundity experiment we prepared mites from our colony and transported them to the field to infest plants. Three adult female spider mites from the colony were

transferred to a small clean leaf piece of either corn or soybean. The leaf piece was placed on a chunk of moist cotton contained within a small plastic cup. These cups were kept on ice packs in coolers to prevent the mites from overheating during transport. Coolers were taken out into the fields and the leaf piece containing the mites was transferred onto the field plant using tweezers. Then a clip cage as above was placed over the leaf piece preventing the mites from escaping. The clip cages remained on the plants for three days, just as in the greenhouse experiments. Then the leaf of the field plants with the clip cage was destructively sampled and the infested leaves collected and transported back to the lab where samples were examined for TSSM under a dissecting microscope. The number of adults, eggs, and juveniles was quantified and recorded.

To analyze our field data, we used a general linear model focused on the interaction between salinity level and the field that the work was performed on (SAS Institute, 2010). Since we performed the experiment in three fields in each of two years, we accounted for variation in both field and year by nesting field within year and including year and field (year) as categorical independent variables in the model. We also included salinity and the interaction between salinity and field (year) as additional independent variables. This allowed us to account for different relationships between TSSM performance and salinity in each of our fields. In this experiment we measured performance by calculating the number of eggs present at the end of the experiment divided by the number of adults present at the end of the experiment and using this as our dependent variable. The values of the three clip cages in each plot were averaged to produce a single value for each plot and a linear regression was performed on these plot values.

Results

Greenhouse fecundity experiments

We consistently found that salinity had a negative effect on plant growth (Figure 1.1). Soybean plant height decreased with salinity (Figure 1.1A) ($F_{1,56}=39.8$, $p<0.0001$). Similarly, the surface area of the leaves on soybean plants decreased with salinity (Figure 1.1B) ($F_{1,56}=39.8$, $p<0.0001$). Likewise, in corn the plant height decreased with salinity (Figure 1.1C) ($F_{1,48}=89.2$, $p<0.0001$) as did surface area (Figure 1.1D) ($F_{1,48}=262$, $p<0.0001$).

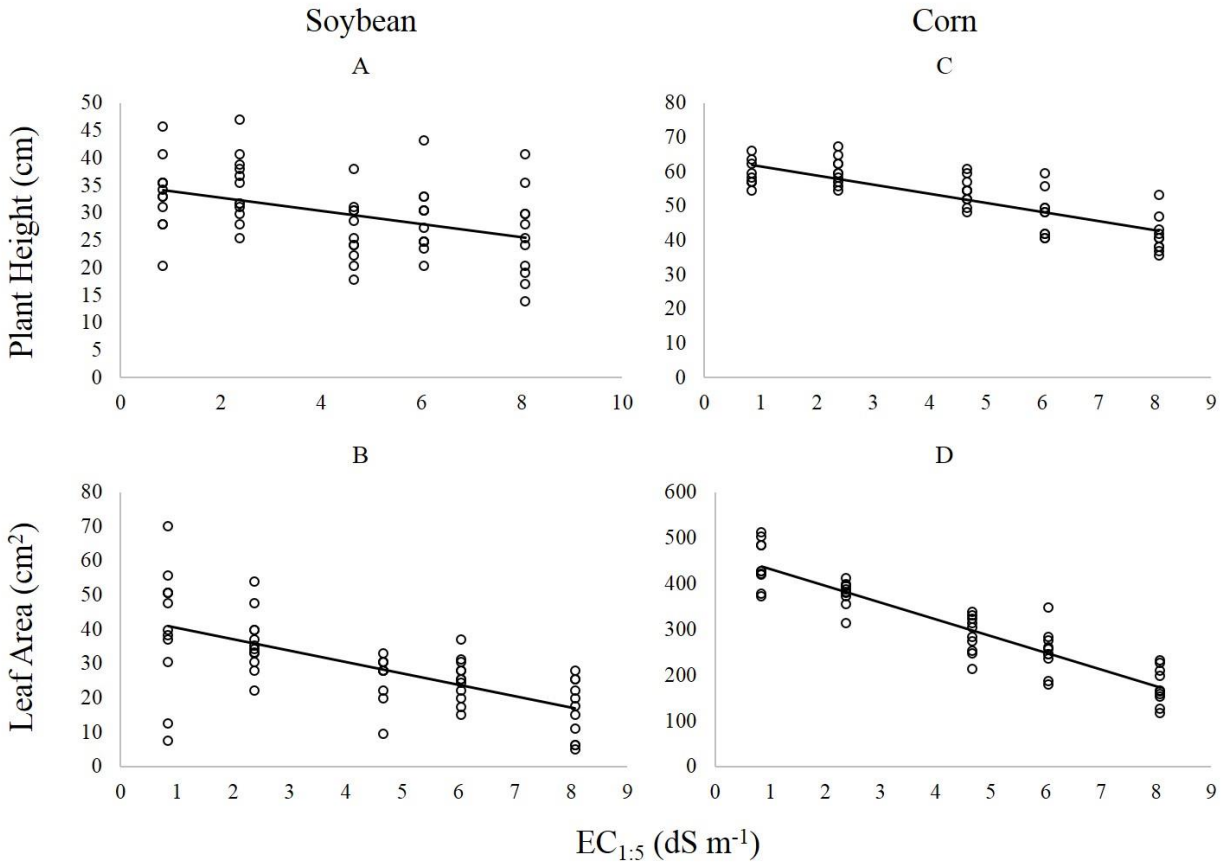


Fig. 1.1. Plant measurements as a function of salinity
Plant measurements for soybean (A-B) and corn (C-D) as a function of salinity $EC_{1:5}$ ($dS\ m^{-1}$) for three-day two spotted spider mite experiment. Plant measurements included the height of the plant (A and C) and the leaf area of the infested leaf used in the three-day fecundity experiment (B and D).

We found that TSSM fecundity increased with greater soil salinity on both soybean and corn plants (Figure 1.2). TSSM females laid more eggs in higher saline treatments compared to the lower saline treatments and this occurred for both TSSM feeding on soybeans (Figure 1.2 A) ($F_{1,48}=22.3$, $p<0.0001$) and corn (Figure 1.2 B) ($F_{1,47}=4.99$, $p=0.0303$).

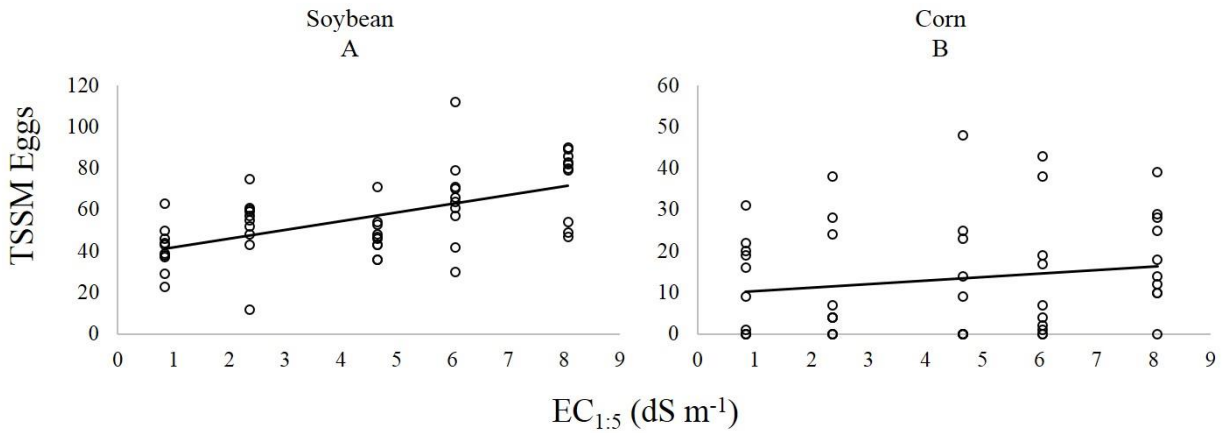


Fig. 1.2. Three-day two spotted spider mite fecundity as a function of salinity. Number of two spotted spider mite eggs laid per plant on soybean and corn as a function of salinity EC_{1:5} (dS m⁻¹).

We found that TSSM population growth also increased significantly with soil salinity on both soybean and corn plants (Figure 1.3). The total number of TSSM, including eggs, nymphs, and adults, increased with salinity for soybean plants (Figure 1.3 A) ($F_{1,47}=19.7$, $p<0.0001$) and for corn plants (Figure 1.3 B) ($F_{1,46}=11.5$, $p=0.0014$).

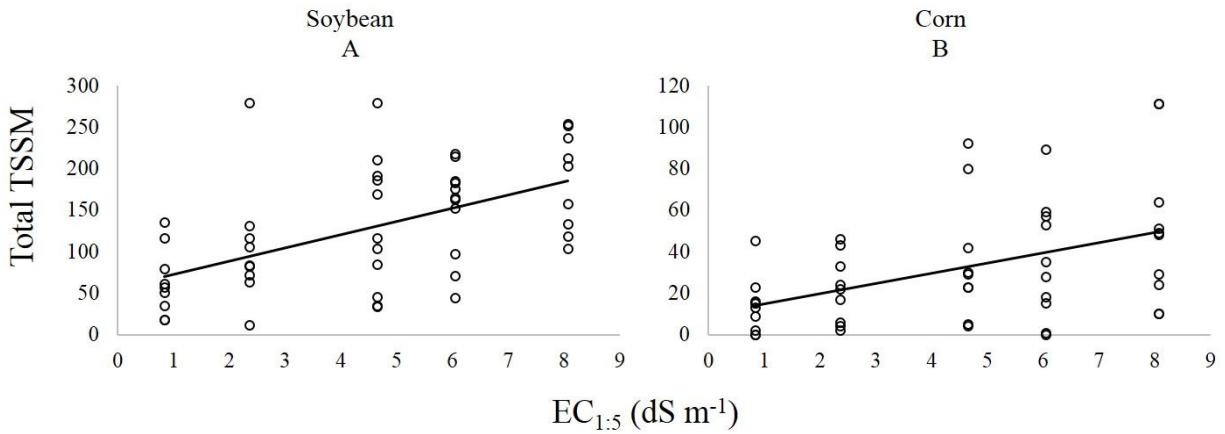


Fig. 1.3. Seven-day two spotted spider mite population growth as a function of salinity
 Total number of two spotted spider mite (all life stages) per plant as a function of salinity EC_{1:5} (dS m⁻¹).

We found that for the most part, TSSM fecundity tended to be higher in parts of the soybean field with greater soil salinity compared to parts of the same field with lower levels of salinity (Figure 1.4). However, this relationship between salinity and TSSM was not consistent across fields leading to a significant interaction between field and salinity level ($F_{5,68}=2.78$, $p=0.0351$). This interaction is likely to be caused by the number of eggs laid per female TSSM tending to increase with salinity in 5 of 6 fields (Figure 1.4A-E) but having a strong negative trend with salinity in one field (Figure 1.4F).

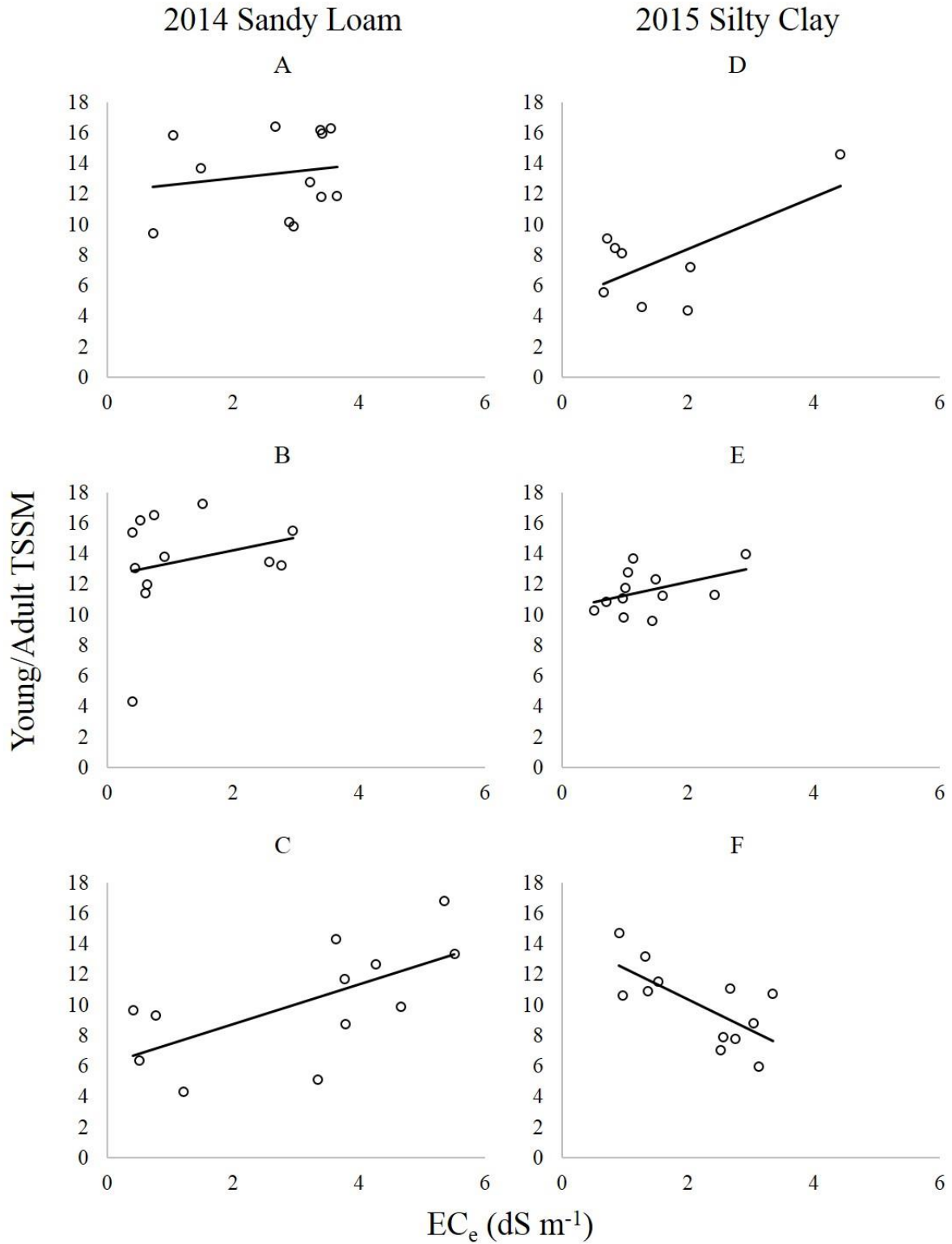


Fig. 1.4. Three-day two spotted spider mite fecundity (field) as a function of salinity Number of eggs laid per plant as a function of measured salinity EC_e (dS m⁻¹) in each of six fields (A-E).

Discussion

We showed that experimentally increasing soil salinity had a positive impact on TSSM. In our greenhouse experiments both corn and soybean plants were negatively affected by increasing soil salinity while mite fecundity increased with salinity. Likewise, population growth experiments exhibited a clear increase with higher salinity in both crops. Our results were slightly more variable in the field where for most soybean fields TSSM fecundity was usually higher within patches that were naturally greater in salinity compared to other parts of the field that were lower in salinity.

The plant stress hypothesis predicts that herbivores feeding on stressed plants will do better. This hypothesis was formulated by White while he was working in a system where plants were stressed by drought conditions, which lead to outbreaks in herbivore populations (White 1969). White suggested that stress can induce increases in available plant nitrogen and this could result in improved growth and reproduction of herbivores and thus promote population outbreaks. The plant vigor hypothesis predicts the exact opposite, that plant stress negatively impacts herbivores and that they are expected to perform well on vigorously growing plant tissue. This hypothesis was founded after an increasing number of studies performed failed to support the plant stress hypothesis (Price 1991). Allelochemicals elevate and plant growth is often reduced in times of plant stress, which could compromise any benefit of available nitrogen to herbivores. Our results seem to be evidence of the plant stress hypothesis, but other work done doesn't necessarily follow this.

A great deal of variation exists in the results of the few experiments done on salinity effects on herbivores feeding on terrestrial plants (Aucejo-Romero et al. 2004, Araya et al. 1991, Cakmak and Demiral 2007, Harmon and Daigh 2017, Martel 1998). These studies have

suggested two reasons for explaining this variation: species-specific differences in plant hosts and species-specific differences in herbivores. We compared the effects of salinity on two different host plants for the same herbivore and found consistent results. Positive effects of salinity were also found in the same species of mite on a third host plant (Aucejo-Romero et al., 2004) and for another species of herbivore in the same genus feeding on a fourth species of host plant (Cakmak & Demiral, 2007). Together this may indicate that TSSM has a particular and relatively consistent response to salinity.

Our study did not investigate why TSSM do better on saline-stressed plants. However, we know that abiotic factors like high temperature and low moisture positively impact TSSM populations and make them more prone to outbreaks (English-Loeb, 2014). From a plant perspective, we also know that saline stress can produce similar physiological responses as drought conditions (Bernstein 1975). It is possible, therefore, that TSSM is responding to these same type of conditions in saline stress and drought stressed plants. If true, this may indicate that herbivorous species that respond strongly to drought conditions would be very likely candidates to also respond strongly to salinity.

More generally, our findings are consistent with the plant stress hypothesis, which predicts that herbivorous arthropods will perform better on stressed plants (White, 1969). Reviewing the mechanisms that are connected to the plant stress hypothesis could help lead to potential mechanisms for explaining the results in our system. For example, plants experiencing drought stress can have lower levels of defensive compounds, which can lead to an increase in herbivore populations (Rhoades 1979, 1983). In addition, stressed plants can experience changes in nutrient availability. One example of this is an increase in soluble nitrogen, which often

enables herbivores to perform better (White, 1984). Future studies would help detangle such possible mechanisms behind this and similar studies.

Understanding how salinity influences herbivores feeding on terrestrial plants is important for predictions about managing herbivorous pests in agroecosystems struggling with salinity. Integrated pest management recommendations are usually dependent on growth rates of pest populations (Radcliffe et al., 2009), therefore factors that fundamentally change those growth rates will affect the accuracy and validity of economic thresholds and similar recommendations. In addition, salinity issues can vary spatially (Bernstein 1975, Harmon and Daigh 2017), this means that specific areas within a field that have higher salinity could be hot spots for TSSM, especially if these areas correspond with edges or other areas with relatively quicker establishment. These areas could provide a challenge and opportunity for sampling efforts; if such areas are known as higher risk, they could be useful for determining if TSSM has entered a field, yet they are not necessarily representative of pest densities throughout the field. Moreover, herbivorous pest populations are not solely based on the interactions of the herbivore and the plant. Natural enemies can play an important role in regulating pest populations, yet we do not know if these enemies are also being affected by soil salinity, just as they are known to be effected by changes in other abiotic factors (Tylianakis et al., 2008). Adding a trophic level to this study system will be important to help aid in our understanding of whole-system effects.

Herbivorous insects, like all organisms, face a daunting array of variable and changing abiotic factors. Understanding the nature of changing abiotic factors is fundamental to understanding the nature of insects and their role in agroecosystems. While it has been difficult to find many sweeping universal truths, general patterns connecting insects to their changing environment are still crucial for advancing scientific understanding and effectively applying that

information. Finding particular species or groups of species that are more or less sensitive to a particular abiotic variable may be one such important general pattern that helps us develop broader insights into the connections between herbivores and abiotic factors.

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CHAPTER 2. THE EFFECTS OF SALINITY ON SOYBEAN APHID (*APHIS GLYCINES*)

Introduction

Abiotic factors are an important cornerstone of ecology (Price et al. 2011). Changes in such abiotic conditions have raised many questions and concerns, while prompting a great deal of research investigating how these changes impact crop production (Tester and Bacic 2005) and plants in general (Jamieson et al. 2012). Given these potential effects on plants, we would expect that many of the herbivores that rely on impacted crops will also be impacted (Fink and Volkl 1995, Boullis et al. 2015). Despite this potential importance, herbivorous insects have received only 10% of the attention that plants have received in this area (Jamieson et al. 2012).

Developing clear connections between changing abiotic factors and herbivorous pests is crucial, especially when we already know that a specific factor can be important to the host crop.

One such example of a changing abiotic factor that can impact crop plants is soil salinity. Soil salinization can be defined as any accumulation of soluble salts that may be harmful to plants (Bernstein 1975). Shifts in climate and agronomic practices have increased the amount of salt-affected soils (Metternicht and Zinck 2003), and such saline soils often has a negative impact on crop yield and other plant fitness characteristics (Bernstein 1975, Butcher 2016). Given the relationship between soil salinity and plant health, it's reasonable to expect that soil salinity may strongly affect herbivores as well.

Soil salinity can have large negative effects on soybean plants (Abel and MacKenzie 1964, Bernstein and Ogata 1966, Essa 2002, Langseth 2015, Butcher 2016), but we know little about the herbivores that feed on soybean and how they are influenced by salinity. We recently showed that soil salinity positively affects two spotted spider mite feeding on soybean (Chp. 1).

However, nothing is yet known of salinity effects on soybean aphid (SBA), the most important pest of soybeans in North America (Ragsdale et al. 2007).

We do, however, know that many other abiotic factors influence SBA. Temperature has a substantial effect on SBA fecundity, population growth rates, and life expectancy (McCornack et al. 2004). Temperature can also interact with host plant resistance to influence SBA (Whalen and Harmon 2015). Ultraviolet radiation can also impact SBA populations, although that effect may be mediated by SBA behavior (Burdick et al. 2015). Abiotic factors in the soil can also affect soybean aphids as soils deficient in potassium produced better host plants for SBA (Walter and DiFonzo 2007). These studies demonstrate that abiotic conditions can alter growth rates and performance of SBA, which are critical to know for their effective management.

Although most research on abiotic factors focus on changes to population growth or demographic parameters like fecundity, they can also influence an organism's behavior (Harmon and Barton 2013), including their movement and distribution. For example, herbivorous arthropods can move and distribute based on the quality of the host plant they are on (Hóðar et al. 2002). This movement can incur costs in terms of energy and feeding time, which are especially detrimental to sedentary insects like the apterous (wingless) soybean aphid (Nelson 2007, Whalen and Harmon 2012). Such movement can also be important for understanding risks from a pest as it helps determine colonization, population spread, and even virus transmission (Kennedy 1976, Collinge 2000).

The purpose of this study was to investigate the effects of soil salinity on the soybean aphid. We used sodium sulfate and magnesium sulfate, which are naturally occurring salts in agricultural fields of the Northern Great Plains, to experimentally induce a range of saline conditions in a controlled greenhouse. The specific objectives were to 1) determine how soil

salinity impacts SBA at a population level, 2) determine how soil salinity impacts SBA individuals, and 3) determine how soil salinity impacts distribution of SBA.

Methods

The goal of our greenhouse experiments was to test SBA performance on soybean plants grown in soil that we manipulated to be across a range of salinity levels. We used the same basic methodology for all greenhouse experiments (see Methods section of Chapter 1). In brief, we added known quantities of salts to pots with a prepacked growing medium in a controlled greenhouse. We established five treatments ($EC_{1.5}=0, 2, 4, 6, \text{ and } 8$) by adding different amount of salts. The different amounts were added to establish a range of salinity that reflected the range of salinity values observed in production fields of the Northern Great Plains (Hadrich 2012). Soybeans were planted and grown in these pots before being used in our experiments.

Soybean Aphid Colony

Soybean aphids used in our experiments were from an established greenhouse colony. This colony originated in the summer of 2008 from soybean plots near Prosper, North Dakota Agricultural Experiment Station fields. Colonies were refreshed periodically with field collected aphids from soybean fields in the same location to maintain genetic diversity. Aphids were maintained on susceptible soybean plants variety (RG607RR NDSU Research Foundation, Fargo, North Dakota). Plants and aphids were contained in a Bugdorm thrips proof cages (Taiwan model # BD44545F dimensions 47.5x47.0x47.5) with 150x150 nylon mesh at 21-24°C, 60-80% RH, under a photoperiod of 14:10(L/D) at the NDSU Agricultural Experiment Station Research Greenhouse Complex in Fargo, North Dakota. SBA colonies were renewed once each week by removing the most heavily infested plants and replacing them with clean soybean plants.

Population growth experiment (Seven-day SBA)

To determine how soil salinity impacts SBA, we first measured SBA population growth on plants grown in different levels of soil salinity. A small group of adults were placed onto each plant and populations were allowed to grow for approximately one full generation (seven days) (McCornack et al. 2004). This time frame exposes aphids to salinity through their entire development from nymph to adult, allowing all life stages to be exposed to the differences in soil salinity. We used a straightforward and established methodology (Whalen and Harmon 2015) to test differences in SBA population growth. Seven adult female SBA were transferred with a fine camel hair brush onto the leaf of a soybean plant. All plants used in this experiment were planted on the same day, but differences in treatments meant that the plants' growth stage varied from V3-V6. Once infested, the entire plant was enclosed in a tube cage which allowed aphids to move freely across the entire plant. Cages were composed of a thin plastic sheet 10cm in diameter and 40cm in height, with two holes covered in mesh allowing airflow, but preventing escape allowing the aphids. The cage was supported in the middle with a ring of PVC pipe. Aphids remained on the soybean plant for seven days, at which time the plant was harvested and all adult aphids and nymphs were counted under a microscope. Each individual plant was the unit of replication, and there were 10 replications for each of the 5 salinity treatments. Data were analyzed using a regression (SAS Institute 2010) where the total number of aphids was the dependent variable and $EC_{1.5}$ measurement as the independent variable.

Fecundity experiment (three-day SBA)

Herbivore fecundity is directly affected by host plant quality (Awmack and Leather 2002) and it is often an important factor influencing population growth. To help better understand our

results from the population level experiment, we performed a complementary experiment to look at a more specific measure of SBA performance at a smaller temporal and spatial scale.

We designed a three-day experiment to measure SBA fecundity across plants grown in different salinity conditions. In this experiment three adult female SBA were transferred with a fine camel hair brush onto a single leaf of a soybean plant (stage V1-V3) grown in one of the five salinity treatments. Aphids were enclosed within a clip cage (2.54cm inside diameter model # 1458, Bioquip products, Rancho Dominguez, CA) that was positioned to include mostly leaf but also a gap off of the leaf so SBA could move between the top and bottom sides of the leaf. Aphids were kept on the single leaf for three days, at which time the plant was harvested. All adults and nymphs on the plant were counted under a microscope. Individual clip cages (each on its own soybean plant) were the unit of replication, with 10 replications per treatment. Data were again analyzed using a regression (SAS Institute 2010), in this case the total number of offspring laid over the entire experiment was the dependent variable and the $EC_{1.5}$ measurement as the independent variable.

Longevity experiment

Both of the previous experiments provide important information about how salinity can influence SBA, but not necessarily the complete story of salinity's impact. We performed another complementary experiment to look at the long-term (lifetime) effects of salinity on SBA. To do this, we designed an experiment that recorded the lifespan and reproductive output of individual female aphids born and kept on plants grown in different salinity conditions. Specifically, we used soybean plants grown in one of two treatments: a control (or low salinity) and high salinity. Plants were grown using the same methodology as previous experiments but only included the no salts added control ($EC=0$) and the highest salinity treatment ($EC=8$). We

started with 15 soybean plants in each of two treatments, but three of the control plants were excluded when the focal aphid went missing.

Aphids were added to the experiment by first infesting each plant with one adult soybean aphid placed in a clip cage (as in Fecundity experiment). Plants used in this experiment were in a fairly young vegetative stage (V2-V4 growth stage). Adults were given one day to lay nymphs, after which the adult and all but one newly born aphid were removed. The remaining focal aphid was observed three times each week for its entire life. During each observation we recorded whether the aphid was still alive, if the aphid had nymphs, and how many nymphs had been laid. When new offspring were found in the cage, we removed those nymphs to avoid potential effects from crowding. The experiment was terminated after 47 days at which point all aphids had died.

To determine effects of salinity in our experiment, we used ANOVA (SAS Institute 2010) with salinity treatment (high vs control) as the independent variable and total nymphs laid, longevity, length of reproductive period, or age at first reproduction as dependent variables. Each response variable was analyzed with a separate ANOVA. We also used a repeated measures analysis (SAS Institute 2010) to look at fecundity patterns through time. Our primary interest was in a potential time*treatment interaction which would indicate if differences in fecundity across the two treatments varied over the course of the aphid's life.

Choice experiment

In addition to demographic and population level differences in salinity, we wanted to investigate whether SBA had a behavioral response to plants grown in different salinity conditions. To do this we set up a choice experiment in which an infested plant from the SBA colony was placed between a soybean plant grown in a high salinity ($EC_{1.5} \text{ dS m}^{-1} = 8.07$) and a control plant grown with no added salts. Differences in the number of adult aphids on high

salinity plants vs control at the end of the experiment would indicate that salinity generated movement in the form of differential attraction to one plant type, repulsion from one plant type, or retention on one plant type. Differences in the number of juvenile SBA could be a function of juveniles moving and/or adults having different numbers of nymphs on either high or low salinity plants.

We performed the experiment by first connecting the leaves of both the high salinity and control plants to the source plant so that aphids could freely walk between plants. We used colonies of apterous (wingless) aphids so that all movement would be through walking. Aphids were given three days to redistribute on the two uninfested experimental plants after which time the plants were harvested and aphids on each plant were counted under the microscope. In this experiment the source plant, high salinity plant, and control plant were all enclosed in a thrips proof mesh cage and comparisons were made between the high salinity and control plants within a given cage. We set up 20 cages that each included all three of those plants.

To account for the fact that high salinity plants and control plants in the same cage were not independent from each other, we used a paired t-test to look at the difference in aphids on control plants vs high salinity plants. We measured both the number of adult (or late instar) aphids and the number of juvenile aphids and performed separate analyses for each of these plus total aphids.

Results

We found that SBA population growth increased significantly with soil salinity on soybean plants (Figure 2.1). The total number of SBA increased as salinity increased ($F_{1,48}=72.3$, $p<0.0001$), with the EC 8 treatment yielding the highest population.

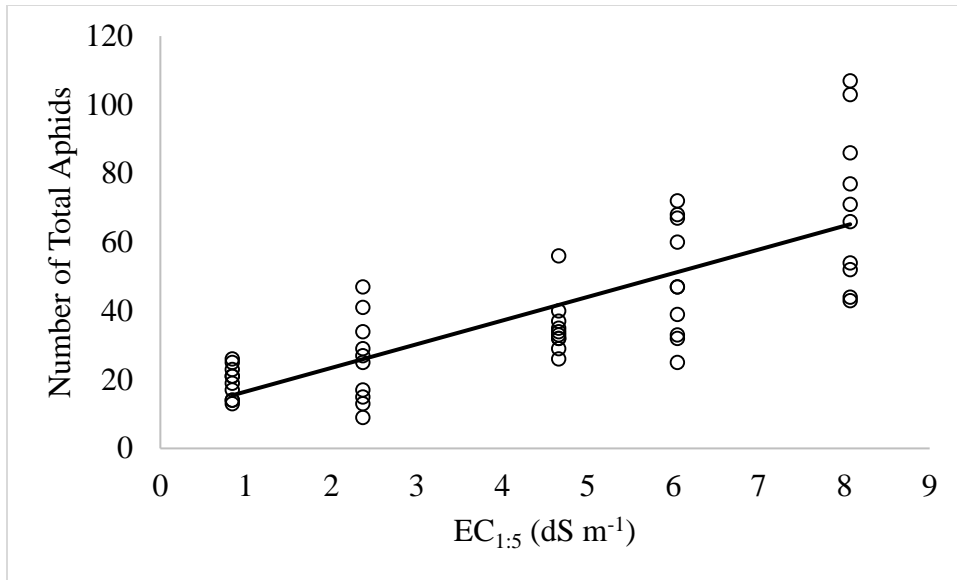


Fig. 2.1. Seven-day population growth as a function of salinity
 Total number of aphids (all life stages) per plant as a function of salinity EC_{1.5} (dS m⁻¹) after seven days.

Similarly, we found that SBA fecundity increased with soil salinity (Figure 2.2). SBA females produced more offspring per individual as salinity increased (F_{1,48}=43.7, p<0.0001).

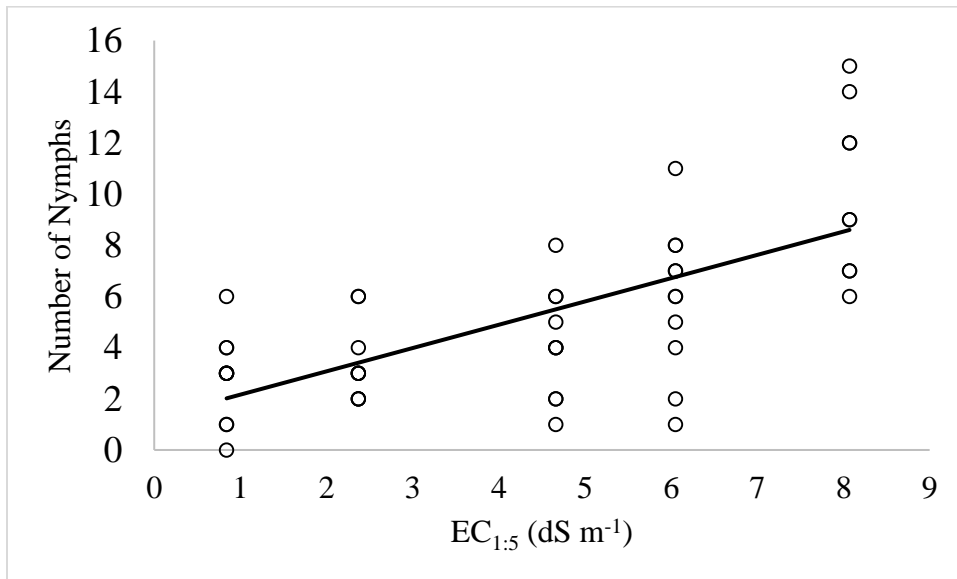


Fig. 2.2. Three-day aphid fecundity as a function of salinity
 Total number of juvenile aphids (nymphs) per plant as a function of salinity EC_{1.5} (dS m⁻¹).

In the longevity experiment, as in the other two experiments, most demographic parameters were improved in the high salinity treatment compared to the control treatment. This

includes measures of reproduction. The total number of nymphs a soybean aphid had over its entire lifetime was almost twice as great for an aphid in the high salinity treatment compared to the control treatment (Average±S.E.: high salinity 38.5±2.55 nymphs vs. control 20.0±2.31 nymphs; $F_{1,25}=27.6$ $p<0.0001$). When looking over the entire reproductive period (Figure 2.3), there were always as many or more nymphs laid in the high salinity treatment as in the control. However, how many more aphids were laid in the control did vary with time leading to a significant interaction between time and salinity treatment (Repeated measure $F_{8,18}=3.30$ $p=0.0166$).

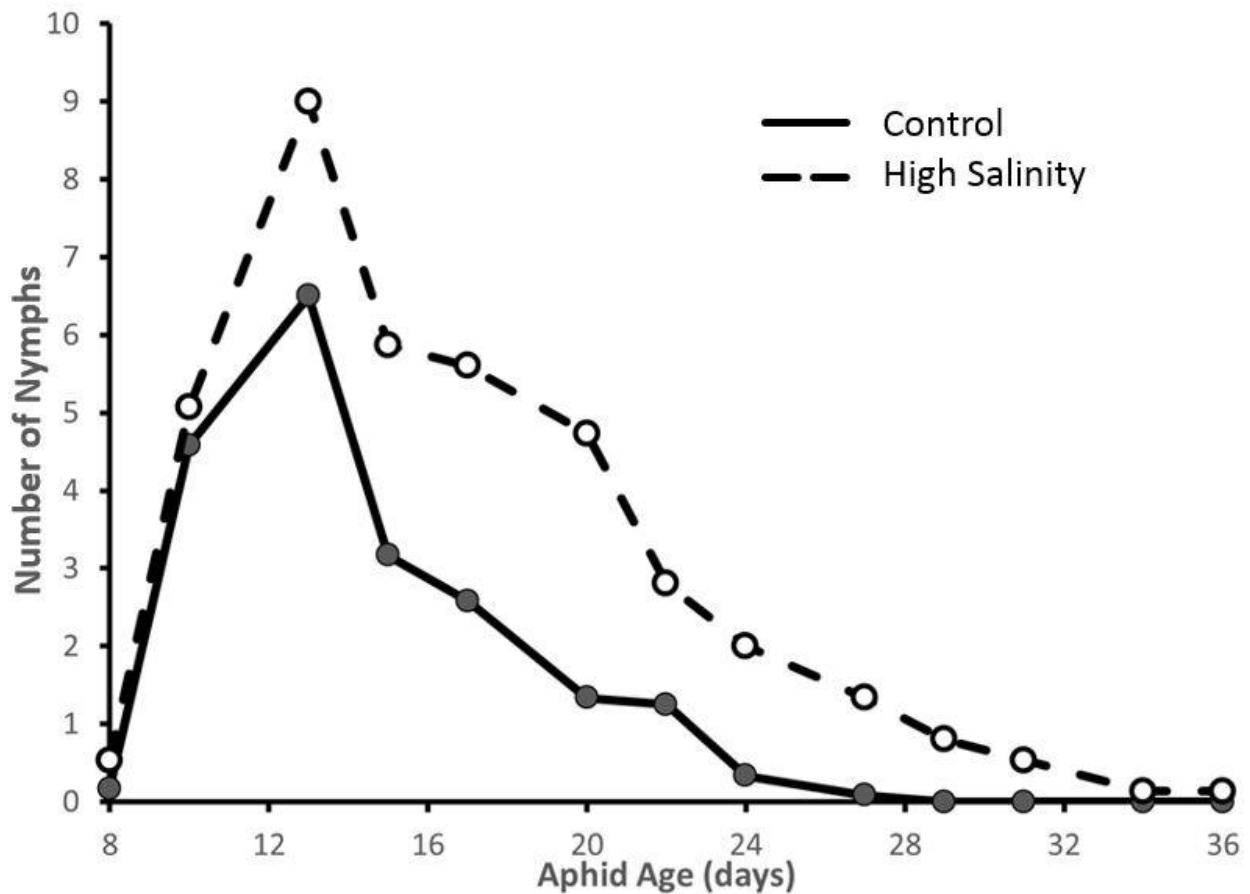


Fig. 2.3. Average soybean aphid reproduction as a function of the aphid's age. Aphids grown on control plants (no salts added) are shown with a solid line and closed circle. Aphids grown on plants in high salinity soil ($EC_{1.5}$ ($dS\ m^{-1}$) = 8.07) are shown with a dashed line and open circle. No nymphs were laid before the observation on day 8 or after day 36.

Salinity also had some effects on aphid longevity. Aphids in the high salinity treatment lived more than 50% longer than aphids in the control treatment (Average±S.E.: high salinity 34.0±2.13 d vs. control 21.7±1.70 d; $F_{1,25}=19.0$ $p=0.0002$). This difference in longevity was primarily during the reproductive period of the aphid's life. The total number of days aphids laid nymphs was greater in the high salinity treatment compared to the control (Average±S.E.: high salinity 18.1±1.34 vs. control 9.67±1.08; $F_{1,25}=22.1$ $p<0.0001$). There was not a difference in the aphid's age when they had their first nymph (Average±S.E.: high salinity 8.40±0.363 vs. control 8.83±0.167; $F_{1,25}=1.00$ $p=0.327$). It is worth noting that salinity may have impacted development in a way that was smaller than we could determine with our observation period.

In the choice experiment, we found that aphid numbers were drastically higher on high salinity plants compared to control plants. After three days, the total number of aphids was almost five times higher on the high salinity plants compared to the controls (Average±S.E.: high salinity 586.8±102.2 vs. control 123±24.4; $t_{19}=4.35$ $p=0.0035$). This effect is primarily driven by an enormous difference in the number of juvenile aphids (Average±S.E.: high salinity 500.1±87.5 vs. control 111±23.1; $t_{19}=4.22$ $p=0.0005$) which may have preferentially moved to the high salinity plants or been born there at a much greater rate (as seen in previous experiments). However, adult aphids were also found preferentially on the high salinity plant (Average±S.E.: high salinity 88.0±22.4 vs. control 11.9±2.42; $t_{19}=3.40$ $p=0.0031$). This difference should only be due to differential movement since the experiment was not long enough for aphids to lay nymphs on the new plants that grew to adults.

Discussion

Soil salinity had a consistently positive effect on soybean aphids. Their population growth increased with salinity as measured by the number of aphids we found on plants grown

across a range of saline soils. Such differences in population growth can manifest through a number of different demographic changes, therefore we designed and carried out complementary experiments to gain a clearer understanding of the effects of salinity on SBA. Our second experiment showed that SBA fecundity increased with salinity. Our third experiment showed that SBA produced more offspring throughout their lives and that SBA lived longer on host plants grown in saline conditions compared to SBA reared on non-saline plants. All three of these factors can lead to greater population growth on plants grown in higher salinity. Moreover, our fourth experiment determined that SBA differentially moved and distributed in response to the soil salinity of its host plants. In areas with both high and low salinity, this may further influence SBA populations if the aphids preferentially distribute on the higher salinity plants where they can perform better.

Although the few experiments performed in agroecosystems with salinity have failed to show a consistent effect across herbivores (e.g., Araya et al. 1991, Aucejo-Romero et al. 2004, Younginger et al. 2009) this set of experiments showed similar results to those with two-spotted spider mites (Chapter 1). Fecundity and population growth of TSSM increased as the concentration of salts increased for soybean plants. While it is only two examples, it is worth hypothesizing if herbivores on soybeans have a consistent response to salinity.

However, no such generality seems to exist for aphids. Some previous studies found results similar to ours and showed salinity to have positive effects on aphids (Braun and Fluckiger 1984, Spencer and Port 1988, Polack et al. 2011). Yet, another study showed that no effect was found in a controlled experiment with NaCl (Spencer and Port 1988), and another showed a negative impact of salinity on aphids (Araya et al. 1991). While still a relatively small

sample size, these results could mean that effects of salinity in plant-aphid systems may depend more on the particular species of plant or aphid.

While most previous studies of salinity measured demographic and population level effects, movement and distribution are also incredibly important to consider when studying herbivore-plant interactions, especially with crop pests. Movement can impact individual fitness, population dynamics, and species distribution (Bowler and Benton 2005). This is especially true when plant quality motivates herbivore movement and gives the insect a chance to increase their fitness by finding a more suitable host (Whalen and Harmon 2012). While we did not directly observe individuals moving in our experiment, we know that the adults in our experiment must have come from the original source plant. Thus, there was something about the higher salinity plant that either attracted the aphid or decreased their willingness to leave and find a different host.

This movement behavior could have important implications in population growth and infestation within soybean fields. In agricultural fields, soil salinity can have a patchy distribution, with areas of high salinity located near areas of low salinity. Given the differences we found in both fitness and movement, saline areas within a field could become hot spots where SBA populations increase at a much greater rate than other areas. Such areas would have important consequences for scouting efforts while also potentially providing areas where aphid populations grow quickly and spread to the rest of the field. Thus salinity could be an important factor in determining the risk of SBA being a problem in a given field. Since salinity can also decrease crop yield (Abel and MacKenzie 1964, Bernstein and Ogata 1966, Essa 2002, Langseth 2015, Butcher 2016), our results may help encourage producers to manage saline issues by indicating the multiple ways that salinity can negatively impact crops.

Pests like SBA are, however, potentially influenced by additional factors that would need to be considered in relation to salinity. For example, many studies have been performed on natural enemies and SBA densities (Donaldson et al. 2007). While we don't yet know what impacts salinity has on the natural enemies of these herbivores the results from our experiments could suggest important implications. If SBA in the field exhibit higher densities in high salinity areas, natural enemies could be drawn into these spots, have high rates of reproduction, and then spread out to have a greater effect on aphids throughout the area. In addition, if SBA in saline areas live longer, this could potentially increase parasitoid populations by giving aphid parasitoids more time to find the aphids and complete development (Ballman et al. 2012).

Our study demonstrates that salinity can have a positive impact on SBA across a wide range of aphid characteristics. This study furthers the knowledge of how terrestrial herbivores respond to soil salinity while demonstrating the number of ways herbivores can be influenced by changing abiotic variables altering their host plants. By looking for such knock-on effects, we can take a whole-systems perspective to abiotic changes. Not only can we investigate the primary direct response to the central host plant, but we can see how other organisms that are dependent on that plant also change. This can help lead to richer and more meaningful predictions of how changing abiotic factors affect many different aspects of ecological systems.

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APPENDIX. SPIDER MITE PERFORMANCE ACROSS A RANGE OF SALINITY IN A NATURAL SOIL

Methods

Just as in the previously discussed greenhouse experiments, the goal of these experiments was to test TSSM performance on corn and soybean plants grown in soil across a range of salinity levels. The primary difference is that this set of experiments used a naturally collected soil (soil collected and treated as in Langseth 2015). Different soil types can have different interactions with both plants and salts (Bernstein, 1975), so we present these results as complementary evidence as to the robustness strength of our results.

There were a few minor differences in the methodology of these experiments to those reported in the main text. First, the salinity levels in the experiment were slightly lower as we did not include the same highest salinity treatment. Second, the data from these experiments were collected as part of a larger greenhouse experiment that included a no mite treatment. Plant performance data across treatments with and without TSSM can be found in Langseth (2015). Third, despite multiple efforts, we were not able to grow corn adequately in any treatment of the plant-level population study, so we exclude that study. Fourth, a nutrient solution was added to the natural soil (Langseth 2015). Finally, the soybean clip cage experiment was done with 10 plants placed inside one of 4 larger mesh cages (Bugdorm pop up rearing and observation cage 1466CB 24x36, MegaView Science, Taiwan). While this extra step was meant to reduce contaminants such as thrips, it primarily results in extra variation in the microclimate from large cage to large cage.

As in the previous experiment we used linear regression to look for the effects of TSSM performance (eggs laid or total population size) across salinity level (SAS Institute, 2010). For

the independent variable we use the average $EC_{1:1}$ values measured across experimental pots in corn and soybean (Langseth 2015). The clip cage experiment in soybean included an additional blocking variable to account for variation in the larger mesh cages plants were placed. It also included the number of remaining adults as a co-variate. The other two experiments had very little variation in the number of adults remaining at the end of the experiment and were not set up with the additional blocking structure, so neither of those terms were useful to the analyses.

Results

All three experiments performed in the natural soil had the same general results as the four greenhouse experiments reported in the main text, as salinity increases, so does TSSM performance (Figures A1-A3). The clip cage experiment in soybeans had a good deal of variation that seemed to be attributed to both the extra cages and the variable number of adult spider mites that survived for the length of the experiment. However, salinity still had a positive effect on TSSM fecundity (Figure A1; $F_{1,33}=6.38$ $p=0.0165$). Clip cages performed on corn similarly showed a positive effect of salinity on fecundity (Figure A2; $F_{1,38}=2.25$ $p=0.0307$). The strongest pattern of salinity, in terms of magnitude and lower variance was in the longer plant-level experiment performed on soybeans (Figure A3; $F_{1,38}=18.6$ $p<0.0001$).

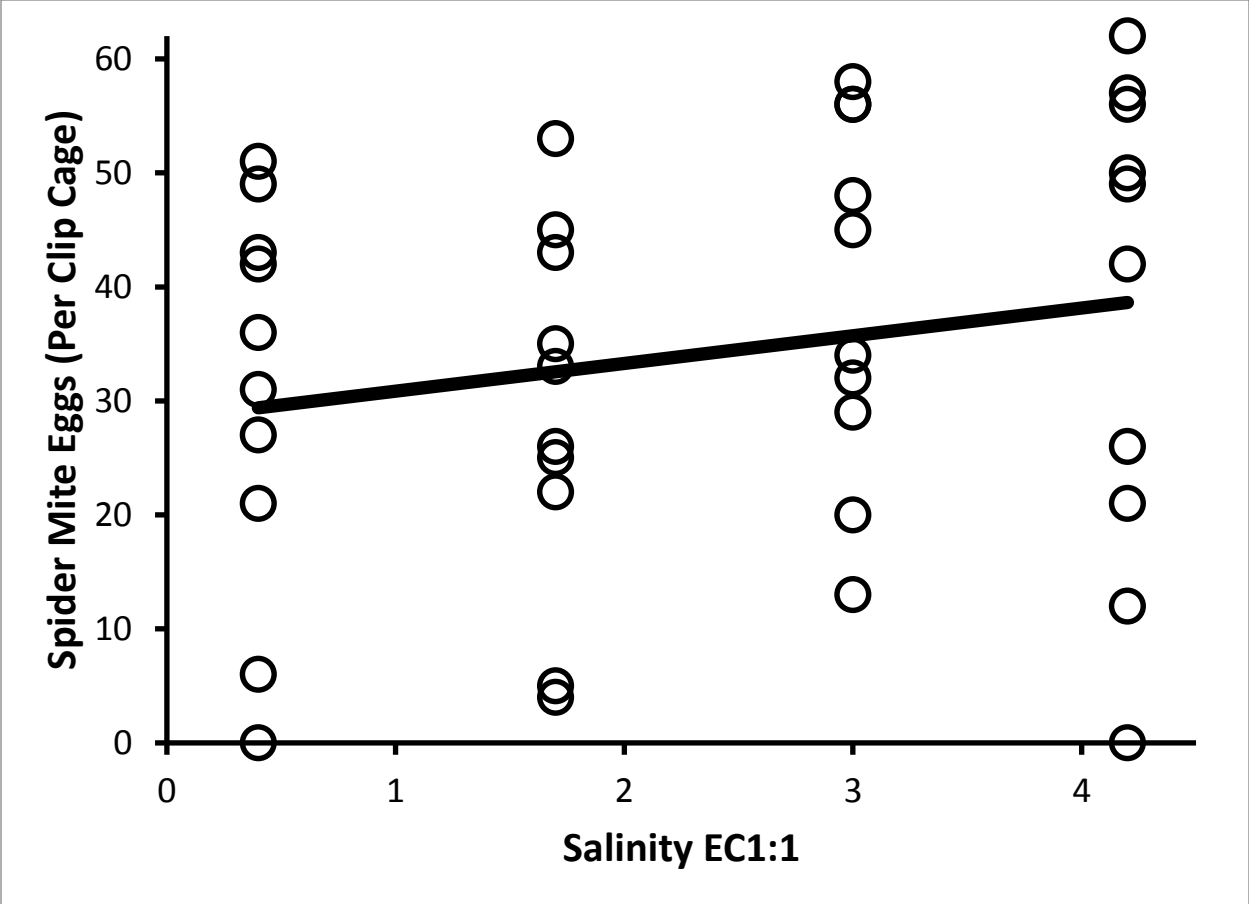


Fig. A1. Three-day two spotted spider mite fecundity on soybean as a function of salinity in a natural soil

Number of eggs laid per soy plant grown in a natural soil as a function of the salinity value for that pot.

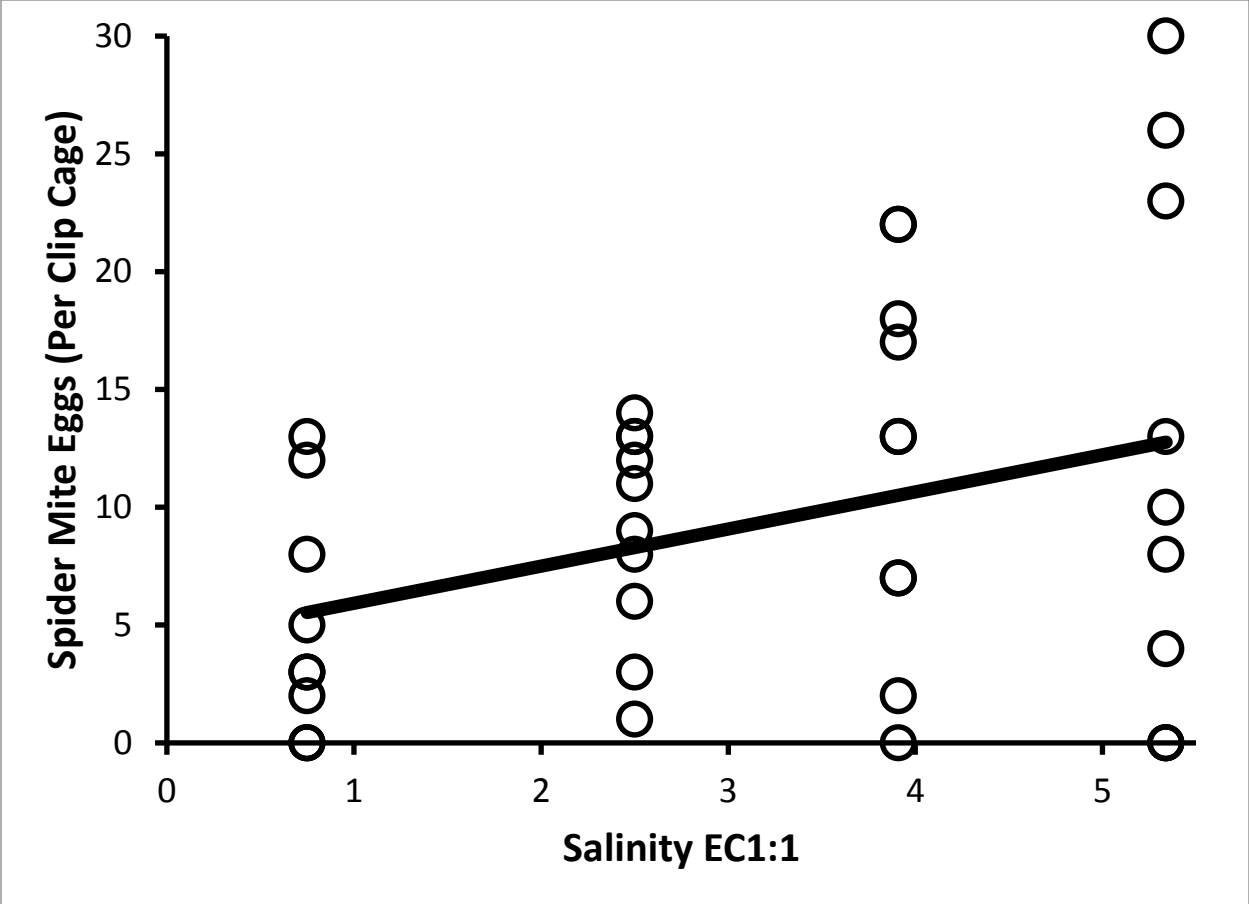


Fig. A2. Three-day two spotted spider mite fecundity on corn as a function of salinity in a natural soil
 Number of spider mite eggs laid per corn plant grown in a natural soil as a function of the salinity value for that pot.

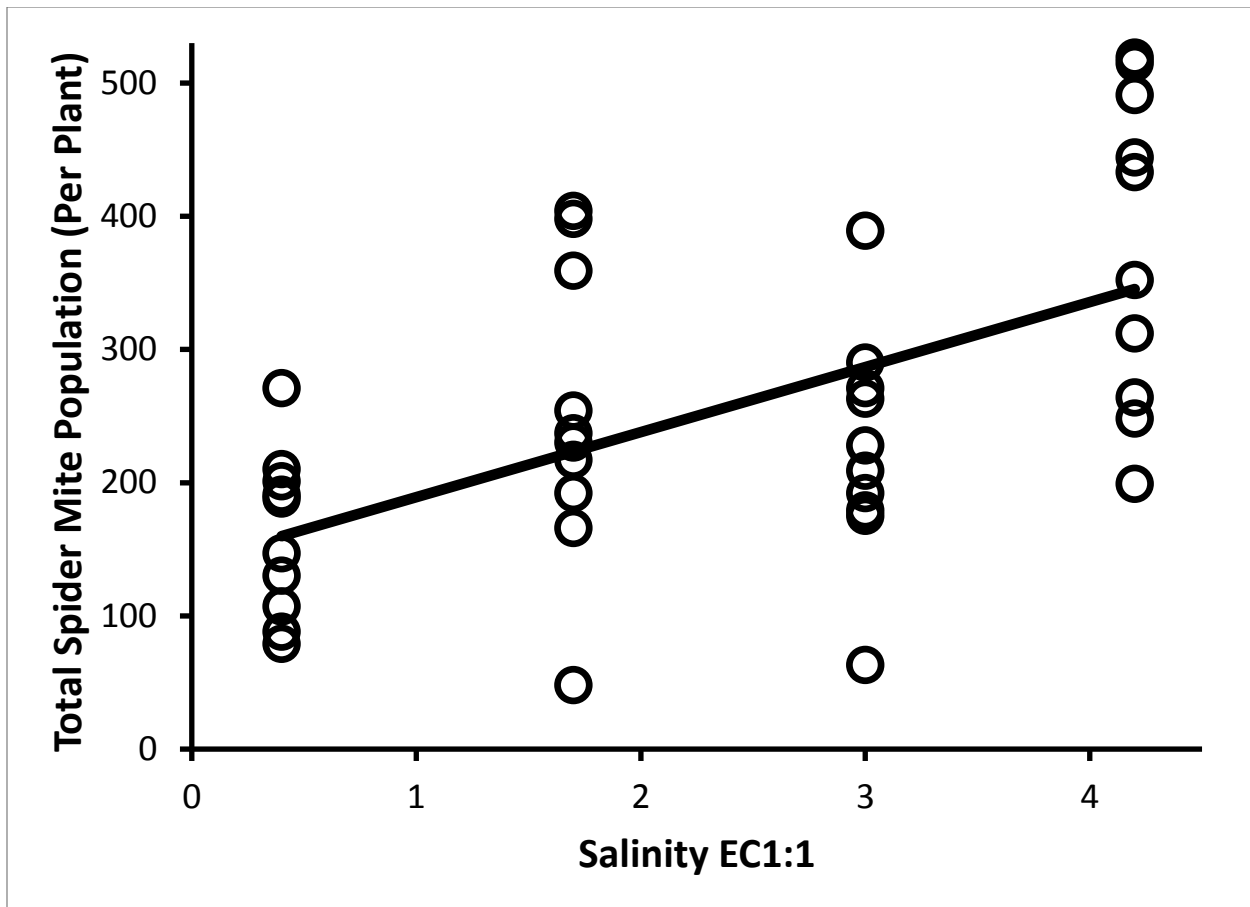


Fig. A3. Seven-day two spotted spider mite population growth on soybean as a function of salinity in a natural soil
 Total number of two spotted spider mites (all life stages) per soy plant grown in a natural soil as a function of the salinity value for that pot.

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