Effects of western corn rootworm larval feeding, drought, and their interaction on maize performance and rootworm development

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By

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The undersigned, appointed by the dean of the Graduate School, have examined the

dissertation entitled

EFFECTS OF WESTERN CORN ROOTWORM LARVAL FEEDING, DROUGHT, AND THEIR INTERACTION ON MAIZE PERFORMANCE AND ROOTWORM DEVELOPMENT

Presented by Mervat Ahmed Badawy Mahmoud

a candidate for the degree of

Doctorate of Philosophy

and hereby certify that, in their opinion, it is worthy of acceptance.

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Dr. Robert E. Sharp

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DEDICATION

To my father: Ahmad Badawy (RIP)

To my mother: Rasmia Mahdy (Allah Bless Her)

To my brothers: Rafik, Emad, Kamal

To my sisters: Ahlam, Fadia, Faten, Azza

To my 19 (so far) nieces and nephews

You are my support system. I would never be who I am today without you being in my life. No words can express my

Love, Respect, and Appreciation

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ABSTRACT

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte can affect water relations and yield of its host, maize (*Zea mays* L.), under normal soil moisture. Drought stress negatively affects plant growth and yield. Anecdotal data have suggested that the effect of western corn rootworm is greater under drought and the effect of drought is greater under rootworm infestations, but few experiments have controlled both moisture and rootworm levels. We hypothesized that if drought and rootworms both occur, there would be a negative synergistic effect on maize growth and yield. To test this hypothesis, a series of greenhouse and field experiments were performed. Greenhouse experiments tested only one maize line at three different moisture levels and three different western corn rootworm infestation levels. This was done not only with neonate larvae, but also second instar larvae and eggs in separate, full experiments. Overall, the greenhouse results indicated that under the conditions of these experiments, the effect of drought was greater than the effect of western corn rootworm and the interactions between soil moisture level in western corn rootworm infestation level did not affect plant traits such as water potential, stomatal conductance, shoot air-dried weight, and root air-dried weight in most of the trials. Rootworms did add some interesting complexity to the greenhouse experiment. For instance, plants without western corn rootworm were more stressed than the moderate western corn rootworm infestation for the drought treatments in the third greenhouse experiment. Drought also impacted western corn rootworm larvae in the same experiment, but only when the larvae were already stressed at the highest infestation level. Field studies also were conducted in 2012, 2013, and 2014 with treatments varying soil moisture levels, western corn rootworm infestation levels, and maize hybrids with and without tolerance to drought and rootworm pressure. In 2012 and 2013, western corn rootworm infestation significantly impacted yield, but its impact on yield was much less than the effect of drought. When under drought and rootworm pressure, the Bt+AQUAmax hybrid with tolerance to western corn rootworm and drought was generally higher yielding and significantly less water-stressed than other hybrids. Root damage ratings were not significantly impacted by drought or its interactions with western corn rootworm infestation. Both drought and western corn rootworm affected water potential, stomatal conductance, root complexity, and beetle emergence. The magnitude of the effect of drought versus western corn rootworm infestation level varied depending on the factor being evaluated, but in general drought had a greater effect on maize growth factors. We must reject our hypothesis that rootworms and drought have a negative synergistic effect on maize growth and yield under the conditions of our study.

CHAPTER I: INTRODUCTION AND LITERATURE REVIEW

Introduction

Maize (*Zea mays* L.) is one of the most important food crops in the world. Maize, rice, and wheat together, deliver 30% of the food calories to more than 4.5 billion people in 94 developing countries (Shiferaw at al. 2011). It is also used in ethanol production, many additional industrial products, and as a food source for livestock. The United States is the largest producer of maize in the world: in 2014, maize average yield was 10.7 metric ton/hectare (171.0 bushels per acre; USDA 2015). Maize, like any other plant, is exposed to challenges from many sources of biotic stress (e.g. insect herbivores, fungal, and bacterial pathogens) and abiotic stress (e.g. drought, flooding, heat), which affect both plant growth and yield. Each stress may cause economically important yield losses on its own while multiple stressors can cause greater loss. Maize global production loss caused by arthropods is estimated to be at least 12% (Vaadia 1985, Culy 2001), while abiotic stresses such as drought, heat, and salinity can cause 50% yield loss for most of the major crops (Wang et al. 2003). Differences between record and average yield can be as much as 4-fold and can be explained by the combined effects of insect damage, disease, and unfavorable environmental conditions (Boyer 1982). Improvements to average yields are needed to feed a growing human population. For maize in the United States, there are two major stressors that impact yield: drought and western corn rootworm. The purpose of this dissertation is to explore how these two stressors interact and determine how they impact yield under various conditions (severity of drought, western corn rootworm infestation level).

Drought is one of the most global of all of the climatic abiotic stresses (Wilhite 2000). It is defined as a continual and severe soil water deficiency that can lead to widespread impacts on vegetation, animals, and human beings (Boyer 1982, Boyer et al. 2013). Recent climate prediction models indicate that average earth surface temperatures will rise by 3-5 ^{o}C in the next 50-100 years, which consequently will increase the frequency of drought and heat wave events (Bates et al. 2008, Mittler and Blumwald 2010). This will significantly impact the global agricultural system (IPCC 2007). Crop and livestock losses due to drought in the United States are estimated to average $9.5 billion per drought event (NOAA 2012, Smith and Matthew 2015). Drought stress in 2012 caused a 13% maize yield loss when averaged across the United States compared to yield production in 2011 (USDA-NASS 2012). The impact of the 2012 drought in the United States was the most extensive in decades (NOAA 2014), and was estimated to cost $31 billion to United States agriculture (NOAA 2014).

Diabrotica virgifera virgifera LeConte (Chrysomelidae, Coleoptera), the western corn rootworm, is one of the most devastating insect pests of maize (*Zea mays* L.) in the United States (Branson and Krysan 1981, Krysan et al. 1986, Gray et al. 2009). The corn rootworm complex, specifically the western corn rootworm and the northern corn rootworm (*Diabrotica barberi* Smith and Lawrence), is estimated to cause an average annual loss of $1-2 billion in yield reduction and management costs in the United States Corn Belt (Metcalf 1986, Rice 2004, Mitchell 2011). Populations of the species utilizing maize as a host were first documented in eastern Colorado in 1909 (Gillette 1912). It soon became a pest of continuous maize production in parts of western Kansas, eastern Colorado, and southwestern Nebraska (Chiang 1973, Smith 1966). Over the years, the western corn rootworm has spread to the East Coast, north to Montana and Ontario, and it is also found in Texas and portions of neighboring states (Meinke et al. 2009).

The western corn rootworm is not only a pest in the United States; it is also an invasive and serious insect pest for maize in Europe. It was first detected near Belgrade in 1992 and by 2007 20 European countries had confirmed the presence of western corn rootworm (Sivcev et al. 1994, Barčić et al. 2003, Baufeld and Enzian 2005, Gray et al. 2009). Miller et al. (2005) documented that there were at least three introductions of western corn rootworm from North America to Europe, while Ciosi et al. (2008) determined that there were five introductions from North America to Europe. The cost of western corn rootworm damage in Europe is estimated to be € 472 million annually (Wesseler and Fall 2010).

Western Corn Rootworm Life History

The western corn rootworm is univoltine, i.e., it has one generation per year (Branson and Krysan 1981). The female typically deposits eggs from July through September (Shaw et al. 1978, Hein and Tollefson 1985, Levine and Oloumi-Sadeghi 1991) in moist soil (Kirk et al. 1968, Gustin 1979) at an egg depth from 5 to 36 cm (Ball 1957, Gustin 1979, Gray et al. 1992), depending on soil type, moisture, and depth of the cracks in the soil that sometimes result from drought. Eggs are often laid in soil pores or earthworm holes in moist soil (Kirk 1979, 1981a, 1981b) and overwinter there in diapause (Chiang 1973, Krysan and Branson 1977, Coats et al. 1986).

Larval hatch begins in late May or June, depending on soil temperature (Levine et al. 2002). Western corn rootworm larvae go through three instars. The development time of each instar will vary dramatically with temperature. At 21 ^{o}C, development time for males is 5.6, 4.9, and 11.2 d for 1st, 2nd, and 3rd instar larvae (Jackson and Elliott 1988). For females, development time is a little longer. Head capsule width is used to distinguish between the three instars and is 200-260, 300-400, and 440-560 µm in width for the first, second and third instar larvae, respectively (Pitre and Kantack 1962, Hammack et al. 2003). Larvae are responsible for locating host roots and typically neonate larvae begin to feed on the first host roots encountered (Clark et al. 2006). Larvae feed nearly exclusively on maize, but can survive on a number of other grasses (Branson and Ortman 1967, Wilson and Hibbard 2004, Clark and Hibbard 2004, Oyediran et al. 2004). The majority of the damage caused by western corn rootworm is a result of larval direct feeding on maize roots.

After larval feeding is complete, they pupate in the soil. Again, pupation time varies dramatically with temperature (Jackson and Elliott 1988), and then adults emerge. Males emerge before females and take 5 - 7 d post emergence to reach sexual maturity, while females are sexually mature shortly after emergence (Guess 1979, Quiring and Timmins 1990, Hammack 1995, Nowatzki et al. 2002). Western corn rootworm females are capable of producing from ~400 to more than 1,000 eggs (Boetel and Fuller 1997, Branson and Johnson 1973). Adults feed on leaves, pollen, and silks and can cause "silk-clipping", which interferes with pollination and results in poorly filled ears if heavy feeding occurs prior to pollen shed (Culy et al. 1992, Moeser and Vidal 2005, Gray et al. 2009). Western corn rootworm adults are not normally economically important because silk feeding does not matter if pollination has already occurred, but this feeding can interfere with pollination if they emerge in high densities prior to pollen shed (Spike and

Tollefson 1991, Al-Deeb et al. 2003). Generally, beetles are present from late June until frost (Spencer et al. 2009).

Western Corn Rootworm Larval Establishment and Movement

Soil traits such as bulk density, porosity, moisture, and texture affect western corn rootworm establishment and movement (Krysan 1999). Soil bulk density and compaction decrease western corn rootworm larval movement. First instar larval movement in sandy, silt loam soil was < 5 cm at a bulk density of 1.5 g/cm^3. However, it was almost 18 cm at a bulk density of 1.3 g/cm^3 (Strnad and Bergman 1987a). This is because soil compaction reduced pore space and restricted larval movement (Strnad and Bergman 1987a, Gustin and Schumacher 1989). Compacted soil also affected adult emergence, which was significantly less than from non-compacted soil (Ellsbury et al. 1994). Soil texture also plays a role in corn rootworm larval survival. Western corn rootworms survived better in clay soil than sandy soil and larvae moved from sandy soil to clay soil more than from clay to sandy soils (Turpin and Peters 1971). First instar larval movement decreased both in saturated and in very dry soil (MacDonald and Ellis 1990). MacDonald and Ellis (1990) found that movement of neonate western corn rootworm larvae was greatest in soil moisture between 24% and 30%. Saturated soil also decreased western corn rootworm adult emergence (Riedell and Sutter 1995). Soil moisture, then, not only impacts maize growth, but very dry (as occurs during drought) and very wet soil also impact western corn rootworm life history.

Western Corn Rootworm Host Location

Insect feeding behavior can be categorized in three steps: attraction, host plant recognition, and acceptance. In the case of western corn rootworm, neonate larvae are responsible for traveling through soil to locate host roots, and when they do, they tend to feed on the root immediately (Clark et al. 2006). Carbon dioxide (CO_2) is the primary component in the first step, attraction (Strnad et al. 1986, Strnad and Bergman 1987, Hibbard and Bjostad 1988, Bernklau and Bjostad 1998a, b). CO_2 is released by plant roots during respiration (Harris and van Bavel 1957). Bernklau and Bjostad (1998a) found that western corn rootworm larvae were attracted to CO_2 concentrations as low as 0.1%. Hibbard et al. (1994) documented that second instar western corn rootworm larvae choose long-chain free fatty acids plus CO_2 versus an equivalent amount of CO_2 without long chain free fatty acids. Bernklau and Bjostad (2008) documented the same compounds to be part of a blend of compounds that are feeding stimulants to neonate western corn rootworm. Bjostad and Hibbard (1992) documented that 6-methoxybenzoxazolinone (MBOA) is involved in host orientation by second instar larvae. While Bernklau and Bjostad (1998b) suggested that this was not the case for neonate larvae, Robert et al. (2012c) suggested that second instar larvae use its precursor, 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), to locate nutritious roots. Robert et al. (2012a, b) also utilized second instar larvae in a series of assays that suggested that (*E*)-β-caryophyllene (EβC) may be involved in western corn rootworm attraction to host plants. Western corn rootworm larvae were attracted to (*E*)-β-caryophyllene (Robert et al. 2012a). EβC is a sesquiterpene previously shown to recruit insect-killing nematodes towards roots damaged by western corn rootworm (Rasmann et al. 2005, Köllner et al. 2008, Hiltpold et al. 2010).

The second step in feeding behavior is host plant recognition. Host recognition is a behavior expressed by larvae after a short time of feeding and tasting a host plant, which indicates suitability of the host (Strnad and Dunn 1990, Bernklau et al. 2009). If a western corn rootworm larva is exposed to host roots, it exhibits a localized “tight turning” behavior characterized by relatively slow travel with increased turning and path crossings. This indicates the suitability of this host. However, if a western corn rootworm larva is exposed to non-host roots, it exhibits a ranging behavior, which is typified by increased rates of travel, fewer turns and path crossings that cover a greater overall area (Strnad and Dunn 1990). This indicates that the host is not suitable. Bernklau et al. (2009) demonstrated that the localized search (tight turning) behavior was elicited with solvent extracts from maize roots and suggested that the cues responsible for host recognition are chemical in nature. Bernklau et al. (2015) identified monogalactosyldiacylglycerol (MGDG) as a host recognition cue for western corn rootworm larvae. They found that western corn rootworm larvae exhibited the tight turning behavior in response to MGDG when combined with a blend of sugars from maize roots.

The third feeding behavior in the sequence is acceptance, and this is exhibited by continuous feeding of the larvae on the host for a period of time. Bernklau and Bjostad (2008) identified a blend of compounds extracted from germinating maize roots (glucose: sucrose: fructose, plus at least one free fatty acid (linoleic acid or oleic acid)). Bernklau and Bjostad (2008) found that this blend serves as a feeding stimulant and elicits vigorous feeding. Western corn rootworm larvae can differentiate between phagostimulants and deterrents using their mouthparts such as galea and maxillary palp (Eichenseer and Mullin 1996). Western corn rootworm larvae have chemosensory sensilla in the maxillary galea that help in tasting and sampling the host plant (Chyb et al. 1995). Host location is a critical component of larval behavior and will be altered by soil moisture extremes.

Western Corn Rootworm Feeding Behavior

The feeding behavior of western corn rootworm on maize root tissues differs depending on the larval developmental stage. Neonate western corn rootworm larvae often feed on the fine root hairs and then on newly emerging nodes of adventitious root axes (nodal root whorls) (Chiang 1973, Strnad and Bergman 1987b, Clark et al. 2006). Root hairs are extensions of root epidermal cells and increase the root surface area. Root hairs constitute up to 60% of the root surface area and improve water and nutrient acquisition ability from the soil (Taiz and Zeiger 2002). Adventitious root axes in maize arise from stem tissues and form rings of roots around a node. First instar western corn rootworm larvae tend to feed on the seminal roots and the first several adventitious root axes (1-5). Both first and second instar larvae significantly orient to the root tip more than the root base (Clark et al. 2006).

The second instar and third instar larvae feed almost exclusively on the root cortex tissue, an unspecialized cell layer that along with the epidermis (surface cells) that protects the vascular tissues (Riedell and Kim 1990). Western corn rootworm second instar and third instar larvae do not feed on the endodermis, the pith, or the vascular system (Riedell and Kim 1990). Riedell and Kim (1990) suggested that suberin was avoided during feeding. Suberin is a wax-like hydrophobic substance that acts as a barrier to water and solutes between the cortex and vascular tissues (Taiz and Zeiger 2002, Perumalla and Peterson 1986). Later instar larvae move toward newly developed adventitious root axes, often called nodal roots (Strnad and Bergman 1987b). Larvae from eggs artificially infested during anthesis to early reproductive stage of maize (much later than normal maize phenology during egg hatch), when all root nodes were already formed, are unable to complete development (Hibbard et al. 2008). Given this, Hibbard et al. (2008) suggested that western corn rootworm larvae not only prefer newly emerged nodal roots, but also may actually require them. Clark et al. (2006) found that western corn rootworm first instar larvae feed on susceptible maize lines starting with the root tips and continued to feed inside the root. Also, they found that in Bt lines, western corn rootworm larvae were biting sample roots and actively moving between root hairs and tips, but not actually feeding on the roots, suggesting that Cry3Bb1-expressing Bt corn was a less preferred host. One of the goals of the current thesis was not only to evaluate interactions of drought and western corn rootworm feeding for their effects on maize, but also the effect of drought on western corn rootworm life history, if any.

Plant Response to Drought

Physiological Traits

Plant response to drought depends on the level, the timing, and the duration of drought as well as soil type and plant species (Hsiao 1973, Kicheva et al. 1994). Physiological traits that change in plants in response to drought include tissue water potentials, stomatal conductance and hormone levels.

Water potential is the potential energy status of water in the atmosphere, the plant tissues, and the soil. It is an important plant trait that can be quantified and it is correlated with the level of water deficit. Specifically, it is the sum of the osmotic potential and turgor pressure (Tiaz and Zeiger 2002). Because water moves from high to low energy levels, tissue water potential gradients define the direction of water movement from one location in the plant to another (Tiaz and Zeiger 2002). Under water deficit stress, water potential decreases as a result of decreased turgor pressure and osmotic potential.

However, water potential is less impacted under water deficit in drought-tolerant genotypes (Bradford and Hsiao 1982, Grzesiak et al. 2006). Change in water potential affects growth of leaves (Boyer 1968, 1970, Acevedo et al. 1979, Westgate and Boyer 1985) and roots (Sharp and Davies 1985). Maize roots can maintain growth at lower water potentials that those that inhibit shoot growth. The primary roots of maize maintained elongation at a water potential of -1.6 MPa while shoot growth was inhibited at a water potential of -0.8 MPa (Sharp et al. 1988).

Stomata are responsible for gas exchange, i.e. CO_2 uptake and transpirational water loss, through the leaf (Farquhar and Sharkey 1982). Stomatal conductance is a measure of the rate of passage of water vapor and CO_2 (Bianchi 2007) and is a function of size, density, and degree of opening of stomata. Closing of the stomatal aperture helps plants tolerate drought by decreasing water loss and reducing rates of dehydration (Ackerson and Krieg 1977, Damour et al. 2010). Consequently, however, photosynthesis decreases under drought stress and this also affects plant growth (Farquhar and Sharkey 1982, Leuning 1990). The opening and closing mechanism of stomata is controlled by the turgor pressure of the guard cells which surround the stomatal pore (Taiz and Zeiger 2002, Farooq et al. 2009). Stomatal conductance itself affects water potential by changing the transpiration rate (Passioura 1980). CO_2 is also a major regulator of stomatal opening and closing (Medlyn et al. 1999, Schroeder et al. 2001, Ainsworth and Rogers 2007, Hu et al. 2010). Stomatal conductance is sensitive to temperature, humidity and wind speed (Jarvis and Morison 1981, Ball et al. 1987, Alphalo and Jarvis 1991, Monteith 1995).

Abscisic acid (ABA) is a phytohormone that has a variety of functions, including the induction of stomatal closure (Taiz and Zieger 2002). It plays a role in closing and opening of stomata by affecting calcium ion influx, depolarization of ion channels, and allowing potassium and chloride ion efflux, which also increases water efflux and decreases cell turgor (Kwak et al. 2008). Under soil water deficit, roots produce ABA, which stimulates the closure of the stomata (Sharp and Davies 1989). Under sufficient water, ABA introduced to the transpiration stream accumulates in the guard cell apoplast and causes stomatal closure (Zhang and Outlaw 2001). ABA-deficient mutant plants showed wilting symptoms and were stunted because the plants were unable to close the stomata to decrease transpiration (Finkelstein and Rock 2002). Pantin et al. (2013) proposed that ABA induced stomatal closure in a dual way by first its biochemical effects on the guard cell and the indirect effect of decreasing water permeability within the leaf vascular tissue.

Maize Growth

Water stress during seedling development delays leaf emergence, vegetative growth and root growth (Denmead and Shaw 1960, Abrecht and Carberry 1993, Ma et al. 2010). In maize, leaf area and extension are also affected by water deficit as a result of reductions in leaf turgor, and cell wall loosening (Sharp and Davies 1979, Van Volkenburgh and Boyer 1985). Stems and roots are also affected (Westgate and Boyer 1985, Sharp et al. 1988, Spollen et al. 1993).

Anthesis (tasseling, pollen shed, and ear production) and grain fill are considered the most economically important growth stages in maize because of their impact on ultimate yield. Drought at the beginning of anthesis affects female organs (ears) more than male floral development (Herrero and Johnson 1981). Under mild water stress (no visible wilting) and severe stress (visible wilting) the time interval between initial silking and initial pollen shed increased 3-4 d (Herrero and Johnson 1981). This decreased pollination and yield because delaying silking in time of pollen shed results in barrenness and poor filled ears. NeSmith and Ritchie (1992) documented that yield was reduced by 90% when severe water deficit starts just before tassel emergence and extends to the beginning of grain fill. Soil water deficit in early ear shoot and ovule development caused a grain yield reduction of 12-15% (Claassen and Shaw 1970). Prolonged drought during tasseling and ear formation can cause 66-93% yield loss (Çakir 2004). Yield loss varies with the severity and the timing of drought.

Plant response to drought varies with the degree of drought tolerance. Barnaby et al. (2013) reported the response of three maize hybrids with different degrees of tolerance to drought applied during the vegetative stage. The authors found that the highly tolerant maize line maintained greater stomatal conductance than the intermediate drought tolerant and susceptible hybrids. Also, they found that the root dry weight for the highly tolerant maize hybrid increased by 2% under drought while the intermediate drought-tolerant and susceptible maize hybrids roots dry weight decreased (Barnaby et al. 2013).

Plant Response to Western Corn Rootworm Feeding

Physiological traits

Western corn rootworm larval feeding damage to maize roots can severely interfere with nutrient and water uptake and negatively affect yield (Kahler et al. 1985, Riedell 1990, Gavloski et al. 1992, Hou et al. 1997). When averaged over many trials, a 15% yield loss resulted per node of adventitious maize root axes damaged by western corn rootworm larval feeding (Tinsley et al. 2013). Heavy larval infestations can cause extreme "goose-necking" or lodging of maize stalks because damage to adventitious root axes (nodal root whorls) including "brace roots" that develop from the first aboveground node, function as support for the plant. When pruned, this decreases stalk stability. Lodging affects photosynthesis because leaves are no longer oriented properly for interception of light (Riedell 1990, Spike and Tollefson 1991). Damaged maize roots are also more likely to suffer root fungal diseases (Palmer and Kommedahl 1969, Kurtz et al. 2010).

Although it was suggested that western corn rootworm feeding can affect maize root water relations (Owens et al. 1974) by decreasing stomatal conductance and photosynthesis, it has not been documented to decrease leaf water potential. On the contrary, leaf water potential was higher (plants less stressed) in response to western corn rootworm larval feeding under adequate soil moisture conditions (Riedell 1990), perhaps due to compensatory growth of the roots. Also, larval feeding did not affect maize sap flow (Gavloski et al. 1992), perhaps because the larvae generally avoid feeding on vascular tissue (Riedell and Kim 1990).

Western corn rootworm larval feeding decreased the photosynthetic rate of maize (Godfrey et al. 1993a). Hou et al. (1997) documented that western corn rootworm feeding decreased photosynthesis and stomatal conductance was also affected when soil moisture was controlled, the interaction of soil moisture and western corn rootworm larval feeding also had a significant negative effect on photosynthesis and stomatal conductance (Hou et al. 1997). Moderate western corn rootworm larval feeding imposed well before anthesis (V12 maize developmental stage), resulted in decreased stomatal conductance at tassel stage (Dunn and Frommelt 1998, Riedell and Reese 1999). The timing of western corn rootworm feeding and the amount of feeding damage will greatly affect plant response and survival (Godfrey et al. 1993a, b).

Western corn rootworm feeding promoted ABA-inducible gene expression in the roots and leaves of maize and reduced leaf water content by 2% compared with equivalent mechanical removal of maize roots (Erb et al. 2009). Erb et al. (2009) found that western corn rootworm feeding on maize roots caused ABA accumulation locally and systemically. Western corn rootworm larval feeding reduced leaf water content under medium and low water supply and ABA levels increased 40-fold when plants were not watered for 48 h compared with the non-infested and un-watered plants (Erb et al. 2011). This confirmed that western corn rootworm feeding disturbs maize water relations under water stress.

Maize growth

Western corn rootworm larval feeding can affect maize growth and yield. Western corn rootworm feeding decreased vegetative biomass and leaf area (Spike and Tollefson 1991, Godfrey et al. 1993b), shoot dry weight (Kahler et al. 1985), as well as fresh and dry root weight (Gavloski et al. 1992). Yield loss from maize lodging, caused by western corn rootworm larval feeding, occurs because goose-necked maize may not be oriented correctly for mechanical harvest (Riedell 1990, Spike and Tollefson 1991). Root regrowth after attack from western corn rootworm positively affects yield when soil moisture is low, but negatively affects yield when soil moisture is adequate (Gray and Steffey 1998).

Tinsley et al. (2015) reported yield data for various rootworm treatments in 2012, which was the driest year in decades in many parts of the United States. At a DeKalb, IL location, untreated hybrid maize average yield was 25.5 bushels/acre or 1.6 metric tons/hectare. The Bt hybrid with only the Cry34/35Ab1 gene had a yield of 149.3 bushels/acre or 9.4 metric tons/hectare. The maize hybrids that contain a pyramid of Cry3Bb1+Cry34/35Ab1 yielded 9.9 metric tons/hectare. The nontransgenic checks were generally the same genetic background as the Bt hybrid, so the dramatic yield difference of more than 120 bushels/acre (7.5 metric tons/hectare) could be primarily attributed to rootworm damage in this one location in a year with severe drought. Unfortunately, there was not a well-watered control, so the relative contribution of drought and rootworms could not be quantified from the Tinsley et al. (2015) report.

Drought Effect on Plant-Insect Interactions

The effect of drought on plant and insect relations is an evolving field. From analysis of 50 years of data of the psyllid *Cardiasphina deiisitexta* Taylor and its outbreaks on *Eucalyptus fasciculosa* F.V.M. in Australia, White (1969) found that psyillid abundance and outbreaks correlated with a high level of drought stress. From this, White (1969) proposed the “Plant Stress-Insect Performance Hypothesis” (PSH).

This hypothesis suggests that plants under prolonged drought stress become more suitable for insect herbivores because under drought stress free amino acids accumulate and free nitrogen increases as a result of physiological changes (such as impairment of protein synthesis). This greater availability of nitrogen presumably would improve insect growth and reproduction.

The failure of studies to support White's original hypothesis led Larsson (1989) to propose that drought also enhances the production of allelochemicals, secondary metabolites produced by the plants that have defensive action against herbivory, decreasing the benefit of the additional free nitrogen to insect herbivores. Although free amino acids and nitrogen increase in stressed plants, not all insect guilds benefit as suggested by White (1969, 1984). Sucking and piercing insects exhibited enhanced performance when fed on drought-stressed host plants compared to the chewing insects and gall formers (Larsson 1989).

To test the predictions of Larsson (1989), Huberty and Denno (2004) reviewed evidence from 116 observations reported in 82 published papers and found two factors that affect the outcome of drought effects on insects: the feeding guild of the insect involved and the pattern and the duration of stress. For the feeding guild, they found strong negative responses to drought stressed plants from sap feeders such as aphids, leafhoppers, and gall inducers such as sawflies. Free living chewing insects and leaf miners had an inconsistent response that could be positive, negative or no response at all. Only long-horned beetles and bark beetles showed a positive response to stressed plants and trees. Huberty and Denno (2004) suggested that the key is plant turgor pressure and water content. They suggested that positive turgor pressure is necessary for phloem feeders to extract the newly available nitrogen. Prolonged drought negatively affected turgor pressure, but a short period of drought stress followed by a period with no drought stress led to the recovery of cell turgor and thus allowed the sap feeder to benefit from the available nitrogen. From his analysis they proposed the “pulsed stress hypothesis”. They suggested that fluctuation of plant stress allows sap feeders to access and get benefit from the free nitrogen (Huberty and Denno 2004).

Roots can exhibit the same physiological changes under drought stress as exhibited by the aboveground parts, including free amino acid accumulation (Voetberg and Sharp 1991) and sugar accumulation (Mohammadkhani and Heidari 2008), and these changes should be advantageous for root herbivores. However, the degree that these factors are advantageous will depend on the availability, quality, and quantity of the nutritional factors involved. For instance, root toughness or lignification under drought (Fan et al. 2006) may affect negatively on insect feeding. Roots under stress from drought and feeding by root herbivores may release other secondary metabolites. For example, Vaughan et al. (2015) documented that drought-induced phytoalexins (Zealexins and Kauralexins) accumulation in the maize roots. Meanwhile, feeding by root herbivores increases nitrogen and carbon reallocation in the plant from the root to the shoot (Newingham et al. 2007, Robert et al. 2014). So, western corn rootworm larvae may be unable to get the benefits of concentrated amino acids under drought, but this information is not known at this time.

Objectives

From the previous research, it was documented that both drought and rootworms can cause yield losses on their own, but each is likely to be much more important when the other is present. A moderate rootworm infestation under well-watered conditions may have minimal yield loss, but the same infestation level under drought may have a severe yield loss without rootworm protection. Relatively little literature is available that documents the simultaneous effects of western corn rootworm and soil moisture on the physiology of maize.

The objectives of my Ph.D. research were to 1) quantify the effect of soil moisture and feeding damage by western corn rootworm larvae on the performance of maize and western corn rootworm under controlled conditions in the greenhouse and the field, and 2) quantify the effect of western corn rootworm larval feeding damage and drought on the performance of maize hybrids with and without drought and rootworm tolerance in the field.

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CHAPTER II: the effect OF western corn rootworm AND water deficits ON MAIZE PERFORMANCE UNDER CONTROLLED CONDITIONS

Introduction

There are numerous biotic factors (e.g. insect herbivores, pathogens) and abiotic factors (e.g. drought, temperature, floods) that influence plant growth and production. Maize (*Zea mays* L.), like other crops, is faced with challenges from many biotic and abiotic sources of stress which can affect biomass production and grain quality and quantity (yield). Individual stressors may cause economically important yield losses on their own while multiple stressors can cause much greater loss. Globally, maize production losses caused by arthropods is estimated to be at least 12% (Vaadia 1985, Culy 2001), while abiotic stresses such as drought, heat, and salinity may cause up to 50% yield loss for most of the major plant crops in some areas (Wang et al. 2003). The differences between record and average yields can be as much as four-fold and can be explained by the combined effects of insect damage, diseases, and unfavorable environmental conditions (Boyer 1982). Improvements to average yields are needed to feed a growing human population.

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte, is an example of an important biotic stress factor. It is generally considered to be the most serious insect pest of maize in the United States and has the potential to severely reduce maize grain yields. It can cause up to $2 billion annually in terms of yield loss and increased management costs in the United States (Mitchell 2011) and €472 million in Europe (Wesseler and Fall 2010). As implied by its common name, the major impact that western corn rootworm has on the growth and productivity of maize is delivered by its soil-dwelling larvae which feed on the roots. Severe root damage impacts the ability of the plant to access and take up water as well as nutrients (Owens et al. 1974, Spike and Tollefson 1989, Riedell 1990, 1992, Hou et al. 1997). However, leaf water potential has been observed to be higher (plants less stressed) in response to moderate western corn rootworm larval feeding (Riedell 1990), perhaps due to compensatory growth of the roots. Heavy larval infestations can cause extreme "goose-necking" or lodging of maize stalks because all roots function as support for the plant and damage to "brace roots" that develop from the first aboveground node and damage to other adventitious root axes (nodal root whorls) erodes that support and decreases stalk stability. Lodging also results in a reduction of photosynthesis because leaves are no longer oriented for the efficient interception of light (Riedell 1990, Spike and Tollefson 1991, Godfrey et al. 1993). An additional yield loss component results from the difficulties encountered during the mechanical harvest of lodged corn. Injured roots are also more likely to develop root fungal diseases, and facilitate the entry and infection by stalk fungal pathogens (Palmer and Kommedahl 1969, Kurtz et al. 2010). Furthermore, adult western corn rootworms feed on leaves, pollen, and silk and can cause "silk clipping", which reduces pollination resulting in lightly filled ears when heavy feeding occurs prior to pollen shed (Culy et al. 1992).

Drought is one of the most important abiotic factors affecting global crop productivity (Boyer 1982, Kramer and Boyer 1995). The average loss incurred during a single drought event in the United States was estimated at $9.5 billion from data spanning 1980 through 2013 (Smith and Mathews 2015, NOAA 2015). The 2012 drought in the

U.S. was the most intense and extensive in decades (Boyer et al. 2013). This drought was estimated to cost $31 billion for U.S. agriculture (NOAA 2014).

The impact of drought stress on plant growth and physiology depends on the timing, duration, and intensity of the stress (Sanchez-Diaz and Karmer 1971, Hsiao 1973, Saab and Sharp 1989). Loss of cell turgor resulting from drought decreases cell expansion, thereby affecting plant growth (Taiz and Zeiger 2002). Plant growth is inhibited under limited water conditions, but individual plant tissues respond differently to water deficit. Plant roots continue to grow under water deficit stress levels that inhibit growth of shoots and leaves (Westgate and Boyer 1985, Sharp et al. 1988, Spollen et al. 1993).

Soil moisture affects not only plants, but also soil insects. Initial host location, larval establishment on a host, and movement between maize roots by western corn rootworm larvae are dependent on specific soil moisture ranges (MacDonald and Ellis 1990, Strnad and Bergman 1987a, 1987b). MacDonald and Ellis (1990) found that movement of neonate western corn rootworm was greatest at soil moisture levels between 24% and 30% w/w and that movement was restricted in saturated (36% w/w) and very dry (<18% w/w) soils.

The relationship between soil moisture, western corn rootworm injury, and the impact of rootworm injury on maize is complicated (Spike and Tollefson 1989). Root regrowth after attack from western corn rootworm can positively affects yield when soil moisture is low, but can negatively affect yield when soil moisture is adequate (Gray and Steffey 1998). Supporting this, both stomatal conductance and leaf water potential under moderate infestation can be greater (indicating less stress) when under moderate western corn rootworm infestation than for uninfested plots (Chapter 3). However, other studies have demonstrated that the interaction of soil moisture and western corn rootworm larval feeding had a significant negative effect on photosynthesis and stomatal conductance (Hou et al. 1997). Moderate western corn rootworm larval feeding imposed well before anthesis (V12 maize developmental stage), resulted in decreased stomatal conductance (indicating greater stress) at the tassel stage (Dunn and Frommelt 1998, Riedell and Reese 1999). In the 2012 drought, maize yields at specific locations in Illinois were up to 53.5 fold greater for rootworm-protected hybrids than the same background genetics without the transgenes producing the Bt proteins that are toxic to rootworms (Tinsley et al. 2015). This level of yield loss is nearly 200 fold greater than would be expected from the same level of damage in a location with average moisture (Tinsley et al. 2013, 2015). However, there was no well-watered control included in this study, so the relative contributions of drought and rootworm damage could not be quantified.

With the potential for catastrophic interactions under specific field conditions (Tinsley et al. 2015), we wanted to quantify potential interactions between western corn rootworm infestation and water deficit stress under controlled conditions: comparing plants with and without western corn rootworm infestation under either well-watered or drought conditions. Soil moisture and western corn rootworm each affects maize performance when applied singly but, in light of previous data (Tinsley et al. 2015), our hypothesis was that if drought and rootworms both occur simultaneously, there will be a synergistic negative effect on maize performance. To test this hypothesis, our objective was to quantify the interaction by measuring maize performance when exposed to various western corn rootworm infestation and drought levels. In addition, we also monitored western corn rootworm development during the experiment to quantify insect performance in relation to soil water deficits.

Materials and Methods

A series of three independent greenhouse experiments were performed to assess the potential interactions between soil moisture and rootworm feeding on maize performance and western corn rootworm development. For all experiments, we used the maize hybrid B73×Mo17 because B73 is the inbred line that serves as the reference sequenced genome and many modern lines are based on its hybrid with Mo17. For each experiment, we manipulated soil moisture regimes and western corn rootworm infestation levels in a factorial arrangement as described below. The developmental stage of the released rootworms differed for each experiment to quantify the interaction of each stage with the effect of water deficit on maize performance. In the first experiment (Experiment 1), plants were infested with newly hatched (neonate) larvae. This experiment was conducted in three separate trials with five replications per trial. Two trials were conducted one week apart whilst the third trial was conducted ~50 d later. In the second experiment (Experiment 2), plants were infested with 2nd instar western corn rootworm larvae. As in Experiment 1, three trials were conducted with five replications of each treatment per trial. Again, two trials were conducted one week apart whilst the third trial was conducted 50 d later. Conducting multiple trials improved the logistics of daily pot weighing and allowed assessment of the repeatability of the study. In the third experiment (Experiment 3), plants were infested with western corn rootworm eggs. This experiment was conducted in one trial with ten replications.

Growing conditions. All experiments were performed using a 3×3 (three soil moisture levels × three western corn rootworm infestation levels) factorial treatment arrangement in a randomized complete block design. Maize seeds (B73×Mo17) were planted in 3.8-liter clay pots for Experiments 1 and 2 and 9.9 liter plastic pots in Experiment 3 (experiments described below). Drainage holes were covered with 114 μm stainless steel mesh screen (TWP, Berkley, CA) to prevent larvae from escaping (Clark and Hibbard 2004). All experiments were conducted using a 14:10 (L: D) photoperiod achieved with natural light and augmented with growth lights (400 watt high pressure sodium lamps, Voigt lighting industries, Philadelphia, PA). Greenhouse air temperature was recorded hourly for each experiment using a single-channel data logger with internal temperature sensor (HOBO, model H08-001-02, Bourne, MA); the greenhouse average daily temperatures were 25.5 ± 0.3 ^{o}C (28.7 ± 0.4 ^{o}C maximum, 23.4 ± 0.3 minimum). The growth medium was a 2:1 mixture (by volume) of autoclaved field-collected soil and ProMix® BX, general purpose potting soil (Premier Horticulture Inc., Quakertown, PA). Prior to planting, 2.0 kg (air-dried weight) of soil mix were added to the pots for Experiments 1 and 2 and 8.0 kg soil mix were added in Experiment 3. Each pot was planted with two maize seeds and following seedling establishment, plants were thinned to one plant per pot. Plants were fertilized after 15 and 30 d with the recommended indoor dose of Miracle-Gro® All Purpose Plant Food 24N-8P-16K (The Scotts Miracle-Gro Products, Inc. Marysville. OH).

Western corn rootworm infestation

Experiment 1: Neonate larvae. Western corn rootworm neonate larvae used for this study were from the primary, non-diapausing colony established by USDA-ARS

North Central Agricultural Research Laboratory, Brookings, SD. Ten d prior to infestation, eggs were placed in 15 cm×10 cm plastic oval containers (708 ml, The Glad Products Company, Oakland, CA). Containers were filled ~ 4 cm deep with a growth medium of 2:1 autoclaved soil and ProMix® (Premier Horticulture Inc., Quakertown, PA). Containers were placed in a growth chamber at 25 ^{o}C and monitored for hatching. Thirty days after planting, V5 stage plants (Ritchie et al. 1992) were infested with newly hatched, unfed neonate larvae by careful transference from the hatching containers using a camel hair brush into a depression in the soil 2.5 cm from the base of the maize shoot. Infestation levels used were no infestation (0), low infestation (20 individual larvae), and high infestation (70 individual larvae) of neonate larvae. After infestation, the small depression was covered with soil. The experiment ended 15 d after infestation when most larvae were third instar (larval recovery and other trait measurements are described below).

Experiment 2: 2nd instar larvae. Since neonate larvae consume only a small amount of root tissue compared to 2nd and 3rd instar larvae, we wanted to see if more damage during the experiment would reveal any interactions between western corn rootworm infestation and soil moisture levels. Five days before infestation, newly hatched neonate larvae were transferred with a camel hair brush to 15 × 10 cm plastic containers (The Glad Products Company, Oakland, CA) with germinated maize seedlings (V2) as a food source (100 neonates/container). After four days, larvae were recovered from the seedling mats using modified Tullgren funnels equipped with 60-W light bulbs. Seedlings were cut at their base and the soil/root mat was gently broken up to promote faster drying. The larvae were collected in 473 ml glass jars containing 5 cm water attached to the funnels. The next morning (after16-20 h), the recovered five-d old 2nd instar larvae were carefully transferred to the pots as described in Experiment 1. The three western corn rootworm infestation levels were no infestation (0), low infestation (10 larvae), and high infestation (40 larvae). To recover the larvae before pupation, the experiment was terminated 10 d post infestation (larval recovery and other trait measurements described below).

Experiment 3: Eggs, delayed drought. We designed this experiment to quantify maize response to soil moisture and western corn rootworm infestation over a longer period of feeding by western corn rootworm (from egg hatch through three larval instars and grain fill in plants) to more closely simulate field conditions. Soil moisture regimes were applied shortly after expected rootworm pupation so as to more closely match the staggered timing of herbivore emergence and drought conditions in the field.

Ten d after planting (V2 developmental stage), plants were infested with 0, 70, or 150 western corn rootworm eggs for the uninfested, low infestation, and high infestation treatments, respectively. Eggs were suspended in 0.15% agar (CAS9002-18-0, USB Corporation, Cleveland, OH) solution and placed in a depression in the soil near the base of the maize shoot using a pipette to facilitate larval access to the root. The depression was subsequently covered with soil. The hatch percentage and timing of hatching was monitored with a subsample of the eggs placed on moistened filter paper in Petri dishes near the pots: hatch percentage was (67%). Three weeks post infestation, pots were covered with mesh to prevent beetle escape and adult emergence was monitored (Meihls et al. 2008). For adult recovery, each pot was checked a minimum of twice per week for emergence until no adults were collected for two consecutive weeks. Beetles were stored in 95% ethanol until processing for head capsule width and dry weight measurements.

Soil moisture regimes

Three soil moisture regimes were used for each experiment: “very dry” which simulated a long-term drought, “well-watered” which simulated optimum soil moisture for maximum plant yield, and “moderately dry” which simulated moderate drought. These were achieved by careful monitoring of the pot weight and wilting symptoms of the plants during the experiments. Before starting the soil moisture regimes, plants were watered regularly to maintain optimum growth. In Experiments 1 and 2, all plants were irrigated to saturation 29 d post-planting. The next day, when plants were at the V5 stage (Ritchie et al. 1992), the initial wet weight of the whole pot (including the growing plant) was recorded using a digital scale (Model V51PH30, Hogentogler & Co. Inc., Columbia, MD). All pots were weighed daily to monitor soil moisture. Soil moisture deficit regimes began immediately after infestation with western corn rootworm larvae in the same day. For the well-watered treatment, water was added to maintain the initial total wet weight of the whole pot. For the moderately dry treatment, water was added only when 40% of the plants exhibited wilting symptoms such as curling or rolling of leaves. At that point, water was added to bring the weight back to the initial total wet weight of the whole pot. The time period between each irrigation event for the moderately dry treatment was between 3 - 4 d. For the very dry treatment, no water was added after infestation.

In Experiment 3, soil moisture regimes were initiated four weeks post infestation. All plants were irrigated to saturation. The next day, initial total wet weight was recorded and all pots were subsequently weighed every other day. For the well-watered treatment, water was added to bring the weight up to the initial total weight. The moderately dry and the very dry treatments were adjusted to more resemble conditions of a rain fed field. For the moderately dry treatment, pots received only 50% of the water required to bring the pot to the initial wet weight after 40% of the plants exhibited curling or rolling of leaves symptoms. For the very dry treatment, water was added only when all plants exhibited curling or rolling of the leaves for four consecutive days and only 25% of the water required to bring the pot up to the initial wet weight was added.

Measurements.

Leaf water potential. For Experiments 1 and 2, leaf water potential was measured on the final day of each trial. Pots were monitored for this trait using a pressure chamber (Model number 670, PMS Instruments, Albany, OR). Measurements were taken as close as possible to solar noon by cutting the tip of the flag leaf (10 cm). For Experiment 3, leaf water potential was measured six times throughout the experiment at solar noon. Measurements were made 15 and 30 d before initiating the soil moisture regime and four measurements were made after initiation of soil moisture treatments, each 15 d apart.

Stomatal conductance. In Experiments 1 and 2, abaxial stomatal conductance was measured using a leaf porometer (version 8, 2005-2010 Decagon Devices, Inc. Pullman, WA) twice daily for the duration of the experiments. The first measurement was made at 11:00 am, before adding water to any treatment, and the second was made at 4:00 pm. Readings were taken from the uppermost completely expanded developed (with a visible ligule) leaf on either side of the midvein. Experiment 3 extended for a longer duration, so stomatal conductance was measured six times during the experiment at 9 am 15 and 30 d before initiating the soil moisture regime and four sampling times were employed after initiation of soil moisture treatments, each 15 d apart, starting July 3.

Shoot air-dried weight. Shoot air-dried weight was measured by sampling all above ground biomass and allowing it to dry. For Experiments 1 and 2, where shoots were smaller, they were placed in a drying oven (Thelco model 16, GCA/ Precision Scientific Co., Chicago, IL) at 37.8 ^{o}C prior to weighing. For Experiment 3, shoots were air dried in the greenhouse with the cooling system turned off prior to weighing (scale model AB135-SFACT, Mettler Toledo Inc., Columbus, OH). Peak temperatures exceeded 50 ^{o}C under these conditions. For all experiments, shoot air-dried weight was monitored until it reached a constant weight, at which point it was recorded.

Root damage rating. For Experiment 3, at the termination of the experiment, roots were thoroughly washed from the attached soil and rated for western corn rootworm damage using the 0 to 3 node injury scale (NIS) (Oleson et al. 2005). On this scale, a rating of zero translates to no damage, and a rating of three translates to at least three nodal root whorls pruned to within 8 cm of the stalk, and any fraction of a node damaged gets that rating (a half a node of roots damaged gets a score of 0.5).

Root air-dried weight. After larval or adult recovery was completed in all experiments, plant roots were thoroughly washed to remove attached soil. For Experiments 1 and 2, roots were placed in an oven (Thelco model 16, GCA/ Precision Scientific Co., Chicago, IL) at 37.8 ^{o}C to dry. Root weights were monitored until they reached a constant weight. For Experiment 3, after rating roots for western corn root worm root damage, roots were air dried in the greenhouse with the cooling system turned off. Peak temperatures exceeded 50 ^{o}C under these conditions. Root weights were monitored (scale model AB135-SFACT, Mettler Toledo Inc., Columbus, OH) until they reached a constant weight and then recorded.

Larval recovery. In Experiments 1 and 2, larvae were recovered after cutting the plant shoot at the base. The whole below ground portion of the plant (root mass and surrounding soil) was placed inside a modified Tullgren funnel equipped with a 60 W light bulb. The soil around the root system was gently broken to speed the drying process by spreading the soil throughout the funnel. After 2 and 4 d, larvae were collected from 473 ml glass jars containing 5 cm of water from the bottom of the Tullgren funnels. Larval number was recorded and they were then stored in 95% ethanol until further processing.

Head capsule width and larval dry weight. Each sampled larva (or adult for Experiment 3) was measured for head capsule width using an ocular micrometer (10/21, Wild Co., Heerbrugg, Switzerland) mounted on a microscope (M3Z, Wild Co.). The remaining 95% ethanol was removed from the vials, and the open vials were placed in a desiccating oven (Thelco model 16, GCA/ Precision Scientific Co., Chicago, IL) at 37.8^{o}C for 7 d. Larval and adult dry weights were measured using an analytical scale (ER-182A, A & D Co., Tokyo, Japan).

Statistical analysis: Experiments 1 and 2. A test of homogeneity of the error mean square for the three trials was conducted first. For this, variance differences among trials were calculated to determine the proper transformation as outlined by Snedecor and Cochran (1989). The transformation $\log_{10}(x+1)$ or $\sqrt{(x + 0.5)}$ that stabilized variance among trials was chosen from the analysis. If both the untransformed data and both sets of transformed data did not show homogeneity of variance, a ranked transformation was used (Conover and Iman 1981). The water potential and larval recovery from Experiment 1 and shoot air-dried weight from Experiment 2 were analyzed as rank transformed data while all the other variables from both experiments were analyzed using $\log_{10}$ transformed data. For data with small values (2 and less) (larval dry weight, $\log_{10}$ (x + 0.1) was used and for data with larger means, $\log_{10}$ (x + 1) was used.

For all data with the exception of stomatal conductance, treatments were arranged as a 3 × 3 (soil moisture level × infestation level) factorial within each trial, and all data were analyzed using an analysis of variance (ANOVA) as a randomized complete block within each trial. After pooling trials together, the model statement contained the fixed effects of trial, soil moisture, infestation level, and all possible interactions. The random effect of replicate within trial was used for the dominator of *F* to test trial. All other fixed effects used the residual mean square. PROC MIXED of the SAS statistical packages (SAS Institute 2008) was used. Trial 3 was removed from the analysis of larval dry weight for Experiment 1 because of the large number of missing values for the very dry treatment for all infestation levels.

For stomatal conductance, data were analyzed using a repeated measures ANOVA design. The data were analyzed as a randomized complete block split-plot in time as outlined by Littell et al. (1998). The fixed effects were arranged as a 3 × 3 × 10 × 2 factorial (soil moisture level × infestation level × sample day × sample time). The combination of trial and replication were considered as a block. The linear statistical model contained the main plot effect of soil moisture level by infestation level, the subplot effect of sample time, and all possible interactions with the main plot effects. The denominator for the main plot effects was replication × trial within soil moisture and infestation levels. The denominator of *F* for the subplot effect was the residual mean square.

For Experiment 3, shoot air-dried weight, root air-dried weight, plant height, root damage ratings, adult recovery, and beetle size data were analyzed as above, except only one trial was conducted, so trial was removed from the model. Treatments were arranged as a 3 × 3 (soil moisture level × infestation level) factorial and all data were analyzed using an analysis of variance (ANOVA) as a randomized complete block. The model statement contained the fixed effects of soil moisture, infestation level, and their interaction. The random effect of replicate was used for the dominator of *F*. All other fixed effects used the residual mean square. PROC MIXED of the SAS statistical packages (SAS Institute 2008) was used. Leaf water potential and stomatal conductance data were analyzed as a repeated measures ANOVA design. The data were analyzed as a randomized complete block. The fixed effects were arranged as a 3 × 3 × 6 factorial (soil moisture level × infestation level × sample day). The linear statistical model contained the fixed effects of soil moisture, infestation level, and all possible interactions. The random effect of replicate × replicate within soil moisture and infestation levels was used for the dominator of *F*. All other fixed effects used the residual mean square.

Results

Experiment 1: Neonate larvae

Leaf water potential. Leaf water potential was significantly impacted by soil moisture level and soil moisture level × trial (Table 1). For all three trials, the plants from the very dry treatment were significantly more stressed (more negative leaf water potential) than the well-watered treatment, but the moderately dry treatment was significantly different between trials. All three soil moisture treatments were significantly different from each other in the combined analysis (Fig. 1A).

Stomatal conductance. Stomatal conductance was impacted by soil moisture level, sample day, sample time, the interaction of soil moisture level × sample day, sample day × sample time, and soil moisture level × sample day × sample time (Table 1). Neither western corn rootworm infestation level nor their interaction with any factor affected the stomatal conductance. In general, stomatal conductance went down over time and was lowest for the very dry treatment (Appendix Figs. 35S, 36S).

Shoot, root air-dried weights, and plant height. Soil moisture level, trial, and their interaction significantly impacted shoot and root air-dried weights (Table 1). In all three trials, shoots from the very dry treatment weighed significantly less than those from the well-watered treatment, but the moderately dry treatment varied in its significance between trials. For root air-dried weight, there was no significant difference between soil moisture levels for one of the trials, but there was for the other two trials, accounting for the effect of trial. Roots and shoots from the very dry treatment weighed significantly less than the other two treatments (Fig. 1B, C). Infestation level did not significantly impact shoot or root weight (Table 1). Soil moisture level, trial, and their interaction each had a significant impact on plant height (Table 8S). In all three trials, plant height from the very dry treatment was significantly shorter than those from the well-watered treatment, but the moderately dry treatment varied in its significance between trials (Appendix Fig. 37S).

Larval recovery, larval dry weight, and head capsule width. Larval recovery was significantly impacted by all factors except trial and the three way interaction of soil moisture level × infestation level × trial (Table 1). The third trial had almost no larval recovery from the very dry treatment, which accounts for the significance of the trial × soil moisture interaction. The soil moisture × infestation level interaction can be seen by the varying responses of each infestation level to the two soil moisture levels (Fig. 2A). For the very dry treatment, significantly fewer larvae were recovered from the high infestation level than from the other two soil moisture levels at the high infestation level. At the low infestation level, there were no differences between soil moisture levels in the number of larvae recovered (Fig. 2A). For larval dry weight, infestation level was the only factor providing a significant impact (Table 1). Larvae recovered from the lower infestation level weighed significantly more than those from the higher infestation level, and soil moisture level did not significantly impact this difference (Fig. 2B). For head capsule width, soil moisture level and infestation levels significantly impacted head capsule width, but not their interaction (Table 8S, Fig. 36S).

Experiment 2: 2nd instar larvae

Leaf water potential. Leaf water potential was significantly impacted by soil moisture level, trial, and their interaction (Table 2). Western corn rootworm infestation levels and all treatment interactions with infestation level did not significantly impact water potential (Table 2). For all three trials, plants in the very dry treatment were significantly more stressed than those from the well-watered treatment. However, leaf water potentials of plants in the moderately dry treatment varied between trials in terms of whether the data were closer to the well-watered or very dry treatment, accounting for the significant effect of trial. When the three trials were combined, the leaf water potential of plants between each soil moisture level was significantly different from each other (Fig. 3A).

Stomatal conductance. Stomatal conductance was impacted by soil moisture level, sample day, sample time, the interaction of soil moisture level × sample day, sample day × sample time, and soil moisture level × time × sample day. The only factors not to significantly impact stomatal conductance were western corn rootworm infestation level and all treatment interactions with infestation level (Table 2). Stomata were primarily closed during the last three sampling days for the uninfested and low infestation treatments at 11:00am (Appendix Fig. 38S A, B). Stomatal conductance was low for the very dry treatment at 4:00pm compared to the well-watered treatment (Appendix Fig. 39S).

Shoot air-dried weight and plant height. Shoot air-dried weight was impacted by soil moisture level, trial, and the interactions of soil moisture level × trial, and infestation level × trial (Table 2). Although the effect of soil moisture on shoot air-dried weight varied between trials for the moderately dry treatment, the well-watered treatments consistently weighed more than the very dry treatments for each trial. For the third trial, shoot air-dried weight was significantly greater than for the other two trials. Infestation level caused a small, statistically significant difference in shoot air-dried weight in the first trial, but not in the other two. When trials were combined, shoot dry weight in the very dry treatment was significantly less than both the well-watered and the moderately dry treatments, and this difference was not impacted by rootworm infestation level (Fig. 3B). For plant height, soil moisture level was the only significant factor (Table 9S). In all three trials, plant height from the very dry treatment was significantly shorter than those from the well-watered treatment, but the moderately dry treatment varied in its significance between trials (Appendix Fig. 40S).

Root air-dried weight. Root air-dried weight was impacted by trial and soil moisture level × trial. Roots in the third trial weighed more than double those in the other two trials. The effect of soil moisture on root air-dried weights varied considerably between trials. For the first trial, roots from the well-watered treatment weighed significantly less than those from the very dry and moderately dry treatments. For the final two trials, roots from the well-watered treatments weighed significantly more than roots from the very dry treatments. When the data from each trial were combined, no differences were found for root air-dried weights between soil moisture or infestation levels (Fig. 3C).

Larval recovery and larval dry weight. Larval recovery was impacted by infestation level and the three way interaction of soil moisture level × infestation level × trial. Trial did not impact the effect of soil moisture on larval recovery at the low infestation level, but did at the high infestation level. For the second trial only, the well-watered treatment had significantly more larvae recovered than from the very dry treatment which accounted for the significant three way interaction. When data for trials were combined, significantly more larvae were recovered from the high infestation than the low infestation level (Fig. 4A), but no other significant differences were found. Larval air-dried weight was impacted by infestation level and the interaction of soil moisture level × trial. For trial 1, larvae from the well-watered treatment were significantly smaller than from the very dry treatment. For trial 2, larvae from the well-watered treatment were significantly larger than from the moderately dry treatment. No differences were found in larval size between soil moisture levels for trial 3. When trials were combined, larvae from the high infestation treatments were significantly smaller than larvae from the low infestation level treatments (Fig. 4B), but soil moisture level did not impact this (Fig. 4B).

Experiment 3: Eggs and delayed drought

Leaf water potential. Leaf water potential was significantly impacted by soil moisture level, infestation level, sample day, and the interactions of soil moisture level × sample day, and infestation level × sample day (Table 3). As expected, leaf water potentials were the same for all treatments early in the experiment, i.e., prior to the initiation of drought (Fig. 5). Overall plant stress, as indicated by increasingly negative leaf water potential, was greater for maize plants without western corn rootworm infestation than for maize plants with western corn rootworm larvae feeding on their roots (Figs 5A), especially at the July 31 sample date. The same effect was observed for the moderately dry treatment (Fig. 5B). As expected, well-watered plants were less stressed than the other two treatments (Fig. 5C). The infestation level × sample day and soil moisture level × sample day interactions can be seen in the differences between the infestation level and the soil moisture level through the sampling days (Fig. 5).

Stomatal conductance. Soil moisture level, sample day, and the interaction of soil moisture level × sample day significantly impacted stomatal conductance (Table 3). Western corn rootworm infestation levels and their interactions did not have a significant impact on stomatal conductance. Overall, regardless of the western corn rootworm infestation level, the stomata were almost completely closed for all treatments for the last three sampling dates (Fig. 6).

Root damage rating. As expected, the main effect of infestation level significantly impacted the root damage rating, but soil moisture level and the interaction of soil moisture level and infestation level did not impact root damage (Table 3). Soil moisture levels were not imposed until after larval feeding was complete, so it is not surprising that they had no effect on root damage.

Shoot air-dried weight and root air-dried weight. Soil moisture level significantly impacted shoot and root air-dried weight, but western corn rootworm infestation levels and their interaction with soil moisture level did not (Table 3). The greatest shoot air-dried weight was recorded for plants under the well-watered treatment, which weighed significantly more than shoots from the other soil moisture treatments (Fig. 7B). Root air-dried weight was also greatest for plants under the well-watered treatment, which weighed significantly more than roots from the other soil moisture treatments.

Adult recovery. Neither soil moisture or infestation level, nor their interaction significantly impacted the number of beetles collected or their dry weight (Table 3). However, nominally more beetles emerged from the well-watered treatments than from the very dry treatments (Fig. 8).

Discussion

In the current study, maize growth was greatly impacted by soil moisture. In contrast, although western corn rootworm infestation occasionally affected some characteristics, the interaction between western corn rootworm infestation level and soil moisture level was not significant for any factors evaluated in any of the experiments except larval recovery in Experiment 1. Overall, the effect of soil moisture on maize growth and yield (biomass) was much greater than that for western corn rootworm. For some of the parameters evaluated in Experiments 1 and 2, the interaction between trial and soil moisture, or trial and infestation were significant. This primarily related to differences between the three trials for the moderately dry treatment.

Regardless of the lack of a significant overall interactive effect of soil moisture × western corn rootworm on maize growth, rootworms did add some interesting complexity results. For instance, in the soil moisture deficit treatments for Experiment 3, plants without western corn rootworm infestation were more stressed, as indicated by leaf water potential, than plants with western corn rootworm moderate and high infestation levels for most comparisons for the last three sample dates (Fig. 5A, B). Riedell and Reese (1999) reported that western corn rootworm larval feeding helped speed the growth of adventitious root axes in the nodes just above the damaged roots. In addition, they documented that there was compensatory root regrowth in response to larval feeding damage. This root growth helped to neutralize negative physiological effects on the plant resulting from western corn rootworm feeding (Riedell and Reese 1999). In addition to root regrowth, the location of western corn rootworm larval feeding likely also had an effect. Western corn rootworm larvae usually feed on the root cortex without reaching the endodermis or the vascular system (Riedell and Kim 1992). Therefore, flow through the vascular tissue should only be minimally affected, and this was previously documented by Gavloski et al. (1992). Rootworm feeding caused dramatically less water deficit stress than mechanically pruned roots when each appeared, to the human eye, to have caused a similar amount of damage (Gavloski et al. 1992, Riedell 1990). Overall, root regrowth triggered by larval feeding and the avoidance of vascular tissues during feeding may explain the water potential reduced stress for plants with a water deficit and moderate rootworm infestation (Fig. 5).

Both water potential and stomatal conductance measurements have been correlated with drought. In general, it would be expected that stomata should close and thus decrease stomatal conductance and delay a reduction in leaf water potential under water deficit (Taiz and Zeiger 2002). In Experiment 3, stomatal conductance was affected by soil moisture level, sample day, and the interaction between these two factors. The effect of sample day can be seen with the stomata exhibiting almost complete closure during the last three sampling days (Fig. 6). Unexpectedly, the stomata were closed for all plants under all moisture levels and infestation levels (Fig. 6). Stomata would be expected to be open for the well-watered treatment (MacRobbie 1998, Schroeder et al. 2001, Taiz and Zeiger 2002), and we do not fully understand why they were not. It is possible that western corn rootworm feeding decreased stomatal conductance. Riedell (1990) documented that stomatal conductance decreased in the infested plants under adequate soil moisture. However, we do not have a good explanation for why stomata were also closed for the well-watered treatment without rootworm infestation. It should be also noted that many of the plants in Experiment 3 were infested with spider mites before the experiment ended. It has been documented that spider mites can cause a significant reduction in stomatal conductance of soybean when under soil moisture stress (Haile and Higley 2003), and spider mite populations typically increase under drought stress (Gillman et al. 1996). Accordingly, spider mites may have confounded stomatal conductance data also.

Another complexity of the current experiment was the observed effect of soil moisture on western corn rootworm growth and development. Soil moisture level, infestation level, trial, and all interactions significantly impacted the number of larvae recovered in Experiment 1. Significantly fewer larvae were recovered from the very dry soil than from the well-watered and moderately dry soil when the infestation level was high (Fig. 2A). Larvae from all soil moisture levels weighed significantly less at the high infestation level than at the low infestation level (Fig. 2B). Larvae from the high infestation level were generally more stressed, as indicated by reduced weight gains, and perhaps more so when under soil moisture deficit conditions, both of which resulted in significant mortality of neonate larvae (Fig. 2A). MacDonald and Ellis (1990) also documented that neonates are impacted by dry soil. Second instar larvae have been reported to be more tolerant of environmental stresses (Olmer and Hibbard 2008), and consistently, only infestation level (not water deficit) impacted the number of larvae recovered when larger, more tolerant second instar were used in Experiment 2 (Fig. 4).

In conclusion, although we have documented interactions between soil moisture and western corn rootworm for the number of larvae recovered in Experiment 1, we did not document any interaction for the traits associated with maize growth. Experiment 3 was added and designed to better represent field conditions so as to more effectively observe the accumulated effect of western corn rootworm feeding on maize growth and development through to grain fill. Eggs were infested rather than larvae to simulate a broader egg hatch time frame and soil moisture regimes were initiated later, as would more typically occur under drought condition in field. Under these conditions no interactions between western corn rootworm infestation level and soil moisture level on maize characteristics. However, there were significant effects on western corn rootworm, for example on larval mortality. Overall, under the conditions of these experiments, drought had a much greater effect on maize performance than western corn rootworm even when both were present. It is possible that interactions of western corn rootworm and drought that might, in fact, occur in the field cannot be duplicated in the greenhouse with a single maize hybrid. Many factors affect maize and western corn rootworm performance including plant tolerance to both drought and western corn rootworm. Further investigations were performed in the field (Chapter 3) to investigate the effect of western corn rootworm and drought on the performance of maize using hybrids with and without tolerance for drought and western corn rootworm.

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Table 1. Analysis of variance results for Experiment 1, in which plants were infested with western corn rootworm neonate larvae.

Factors	Effect	*DF*	*F value*	*Pr > F*
Leaf Water Potential[a]	**Moisture**	2,95	141.79	<.0001
	Infestation	2,95	0.78	0.4632
	Moisture × Infestation	4,95	1.10	0.3588
	Trial	2,12	0.01	0.9936
	Moisture × Trial	4,95	5.33	0.0006
	Infestation × Trial	4,95	0.99	0.4185
	Moisture × Infestation × Trial	8,95	0.96	0.4700
Stomatal Conductance	**Moisture**	2,86	10.02	0.0001
	Infestation	2,86	0.03	0.9726
	Moisture × Infestation	4,86	0.43	0.7830
	Day	9,1628	45.11	<.0001
	Moisture × Day	18,1628	8.11	<.0001
	Infestation × Day	18,1628	0.75	0.7578
	Moisture × Infestation×Day	36,1628	1.05	0.3923
	Time	1,1628	15.20	0.0001
	Moisture × Time	2,1628	0.40	0.6732
	Infestation × Time	2,1628	0.28	0.8940
	Moisture × Infestation × Time	4,1628	0.17	0.9560
	Day × Time	9,1628	3.18	0.0008
	Moisture × Day × Time	18,1628	1.92	0.0116
	Infestation × Day × Time	18,1628	0.85	0.6350
	Moisture × Infestation × Day × Time	36,1628	0.40	0.9994
Shoot Air-Dried Weight	**Moisture**	2,95	162.96	<.0001
	Infestation	2,95	0.28	0.7557
	Moisture × Infestation	4,95	1.16	0.3315
	Trial	2,12	183.56	<.0001
	Moisture × Trial	4,95	13.42	<.0001
	Infestation × Trial	4,95	0.54	0.7036
	Moisture × Infestation × Trial	8,95	1.49	0.1720
Root Air-Dried Weight	**Moisture**	2,94	33.44	<.0001
	Infestation	2,94	1.01	0.3687
	Moisture × Infestation	4,94	0.80	0.5293
	Trial	2,12	21.43	0.0001
	Moisture × Trial	4,94	10.39	<.0001
	Infestation × Trial	4,94	0.14	0.9675
	Moisture × Infestation × Trial	8,94	0.71	0.6861

Cont. Table 1

Factors	Effect	*DF*	*F value*	*Pr > F*
Larval Recovery[a]	**Moisture**	2,60	9.33	0.0003
	Infestation	1,60	21.15	<.0001
	Moisture × Infestation	2,60	5.65	0.0056
	Trial	2,12	3.78	0.0532
	Moisture × Trial	4,60	5.32	0.0010
	Infestation × Trial	2,60	5.55	0.0062
	Moisture × Infestation × Trial	4,60	0.81	0.5221
Larval Dry Weight	**Moisture**	2,32	2.30	0.1169
	Infestation	1,32	35.09	<.0001
	Moisture × Infestation	2,32	0.28	0.7559
	Trial	1,8	0.93	0.3639
	Moisture × Trial	2,32	2.30	0.1165
	Infestation × Trial	1,32	0.44	0.5118
	Moisture × Infestation × Trial	2,32	0.23	0.7946

[a]Data were rank-transformed because both transformed (log and square root) and untransformed data did not show homogeneity of variance. Data for all other factors were log-transformed to meet normality assumptions.

Table 2. Analysis of variance results for Experiment 2, in which plants were infested with western corn rootworm second instar larvae.

Factors	Effect	*DF*	*F value*	*Pr > F*
Leaf Water Potential	**Moisture**	2,96	193.09	<.0001
	Infestation	2,96	0.11	0.8966
	Moisture × Infestation	4,96	0.13	0.9720
	Trial	2,12	18.23	0.0002
	Moisture × Trial	4,96	11.64	<.0001
	Infestation × Trial	4,96	0.90	0.4664
	Moisture × Infestation × Trial	8,96	0.68	0.7048
Stomatal Conductance	**Moisture**	2,64	86.29	<.0001
	Infestation	2,64	0.93	0.4004
	Moisture × Infestation	4,64	1.24	0.3043
	Day	8,1204	21.80	<.0001
	Moisture × Day	16,1204	8.69	<.0001
	Infestation × Day	16,1204	1.56	0.0737
	Moisture × Infestation×Day	32,1204	1.03	0.4271
	Time	1,1204	8.30	0.0040
	Moisture × Time	2,1204	4.59	0.0104
	Infestation × Time	2,1204	1.80	0.1650
	Moisture × Infestation × Time	4,1204	1.60	0.1729
	Day × Time	8,1204	14.33	<.0001
	Moisture × Day × Time	16,1204	2.16	0.0050
	Infestation × Day × Time	16,1204	1.01	0.4395
	Moisture × Infestation × Day × Time	32,1204	1.28	0.1370
Shoot Air-Dried Weight[a]	**Moisture**	2,95	72.20	<.0001
	Infestation	2,95	2.24	0.1116
	Moisture × Infestation	4,95	1.56	0.1918
	Trial	2,12	38.36	<.0001
	Moisture × Trial	4,95	4.52	0.0022
	Infestation × Trial	4,95	3.64	0.0084
	Moisture × Infestation × Trial	8,95	0.87	0.5422
Root Air-Dried Weight	**Moisture**	2,89	1.08	0.3434
	Infestation	2,89	1.26	0.2884
	Moisture × Infestation	4,89	0.84	0.5017
	Trial	2,12	74.52	<.0001
	Moisture × Trial	4,89	7.07	<.0001
	Infestation × Trial	4,89	0.40	0.8088
	Moisture × Infestation × Trial	8,89	1.66	0.1198

Cont. Table 2

Factors	Effect	*DF*	*F value*	*Pr > F*
Larval Recovery	**Moisture**	2,60	1.29	0.2836
	Infestation	1,60	91.07	<.0001
	Moisture × Infestation	2,60	1.03	0.3627
	Trial	2,12	2.51	0.1229
	Moisture × Trial	4,60	0.55	0.6996
	Infestation × Trial	2,60	0.39	0.6786
	Moisture × Infestation × Trial	4,60	3.23	0.0181
Larval Dry Weight	**Moisture**	2,56	1.12	0.3344
	Infestation	1,56	38.49	<.0001
	Moisture × Infestation	2,56	0.50	0.6092
	Trial	2,12	1.46	0.2698
	Moisture × Trial	4,56	3.91	0.0071
	Infestation × Trial	2,56	1.76	0.1815
	Moisture × Infestation × Trial	4,56	1.92	0.1205

[a]Data were rank-transformed because both transformed (log and square root) and untransformed data did not show homogeneity of variance. Data for all other factors were log-transformed to meet normality assumptions.

Table 3. Analysis of variance for Experiment 3, in which plants were infested with western corn rootworm eggs.

Factors	Effect	*DF*	*F value*	*Pr > F*
Leaf Water Potential	**Moisture**	2,72	31.47	<.0001
	Infestation	2,72	3.71	0.0292
	Moisture × Infestation	4,72	0.13	0.9722
	Day	5,391	75.46	<.0001
	Moisture × Day	10,391	9.12	<.0001
	Infestation × Day	10,391	2.50	0.0064
	Moisture × Infestation × Day	20,391	0.42	0.9877
Stomatal Conductance	**Moisture**	2,72	9.97	0.0002
	Infestation	2,72	0.80	0.4549
	Moisture × Infestation	4,72	1.20	0.3195
	Day	5,311	49.20	<.0001
	Moisture × Day	10,311	6.64	<.0001
	Infestation × Day	10,311	0.49	0.8945
	Moisture × Infestation×Day	20,311	1.17	0.2743
Root Damage Rating	**Moisture**	2,44	0.19	0.8259
	Infestation	1,44	6.57	0.0139
	Moisture × Infestation	2,44	0.83	0.4407
Shoot Air-Dried Weight	**Moisture**	2,71	25.77	<.0001
	Infestation	2,71	0.39	0.6754
	Moisture × Infestation	4,71	1.23	0.3057
Root Air-Dried Weight	**Moisture**	2,70	29.84	<.0001
	Infestation	2,70	1.04	0.3576
	Moisture × Infestation	4,70	0.22	0.9256
Adult Recovery	**Moisture**	2,44	2.19	0.1240
	Infestation	1,44	0.36	0.5516
	Moisture × Infestation	2,44	2.84	0.0689
Adult Dry Weight	**Moisture**	2,8	2.33	0.1592
	Infestation	1,8	3.18	0.1123
	Moisture × Infestation	2,8	0.59	0.5789

All data were log-transformed to meet normality assumptions.

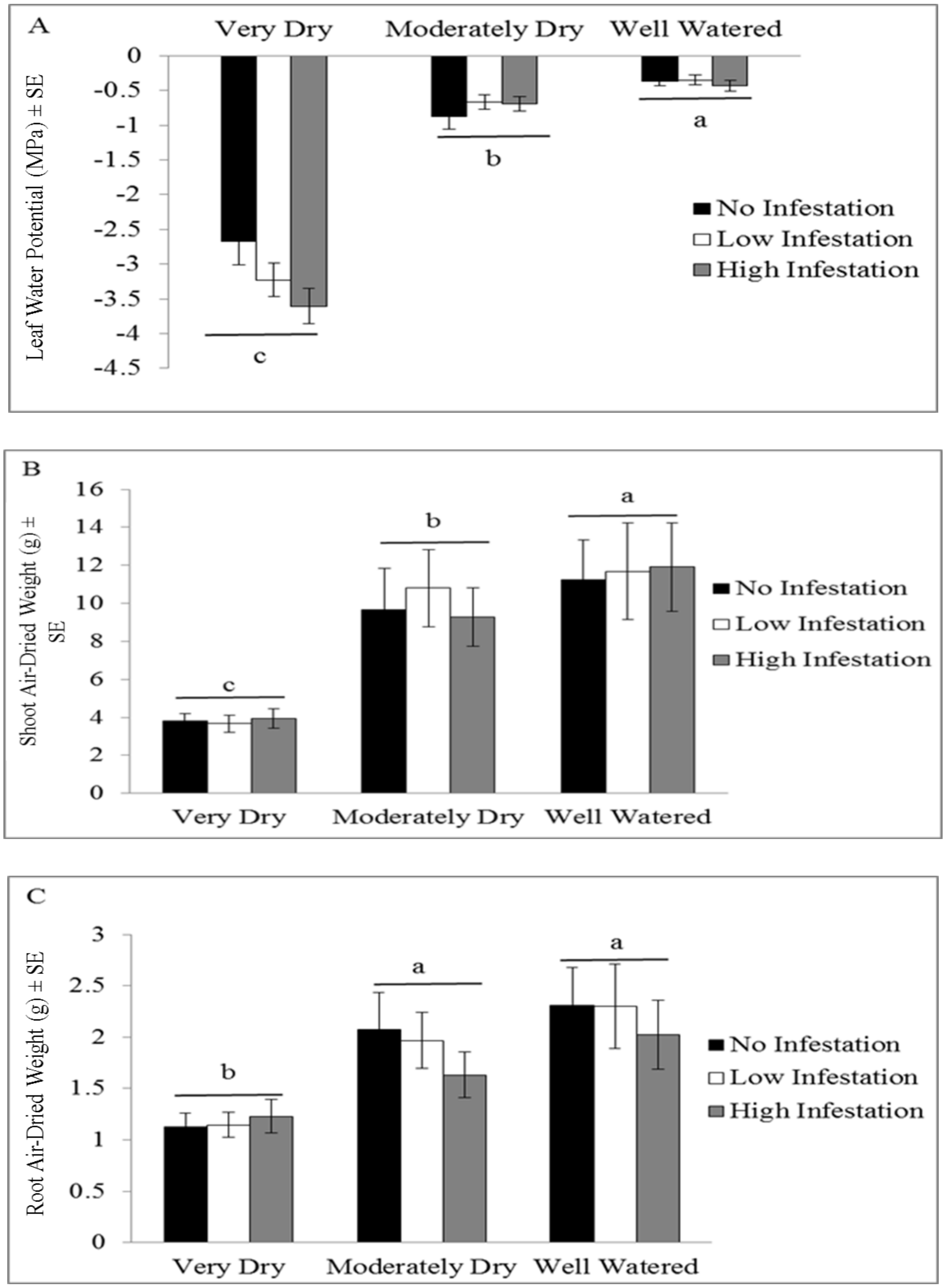

Fig. 1. Average leaf water potential (A), shoot air-dried weight (B), and root air-dried weight (C) of maize plants infested with western corn rootworm neonate larvae in Experiment 1. Although untransformed data are shown, analyses were performed using rank transformed data (A) and log-transformed [$\log_{10}$ (x +1)] data (B, C). Lines over bars indicate the main effect of soil moisture level. Lines with different letters are significantly different ($P \leq 0.05$).

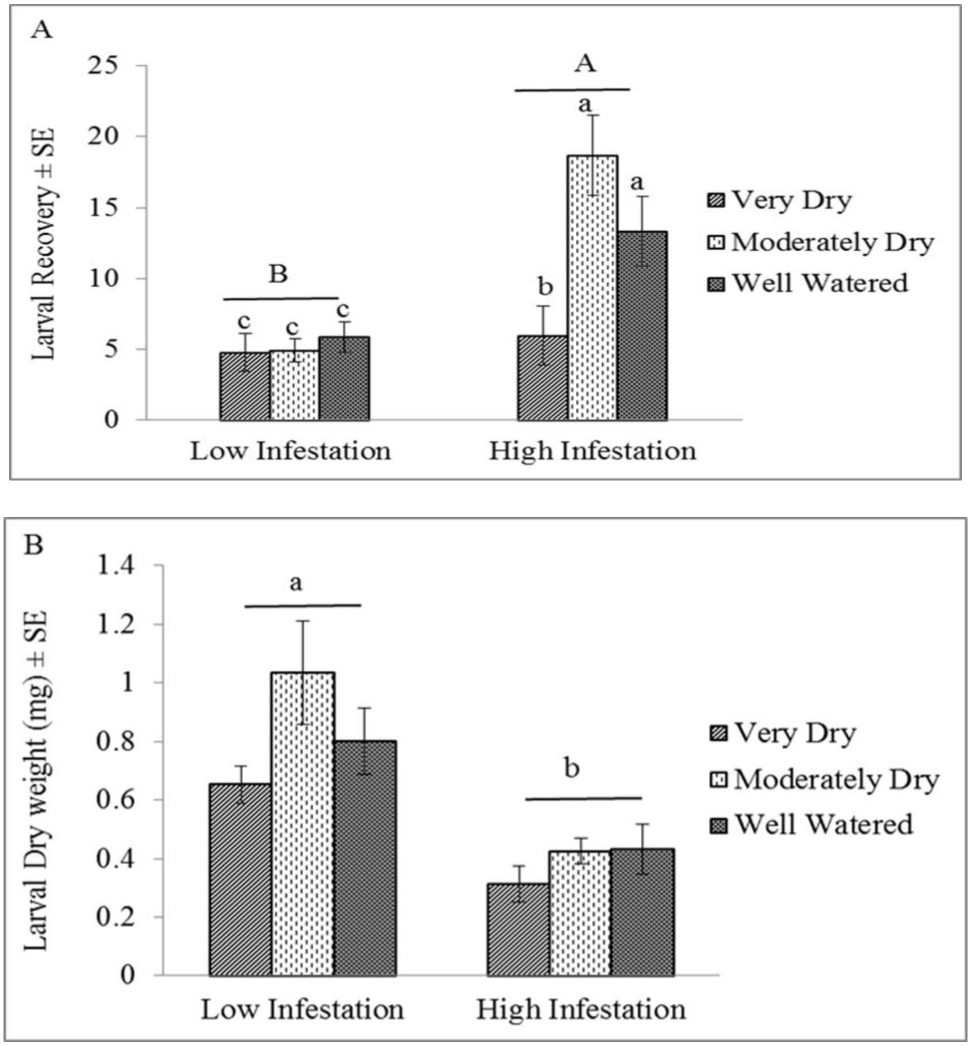

Fig. 2. Average number of western corn rootworm larvae recovered (A), and larval dry weight (B) from Experiment 1. Although untransformed data are shown, analyses were performed using rank transformed data (A) and log-transformed [$\log_{10}$ (x +0.1)] data (B). Lines over bars indicate the main effect of western corn rootworm infestation level. Lowercase letters in (A) indicate comparisons between soil moisture levels within western corn rootworm infestation level. Lines or bars with different letters are significantly different ($P \leq 0.05$).

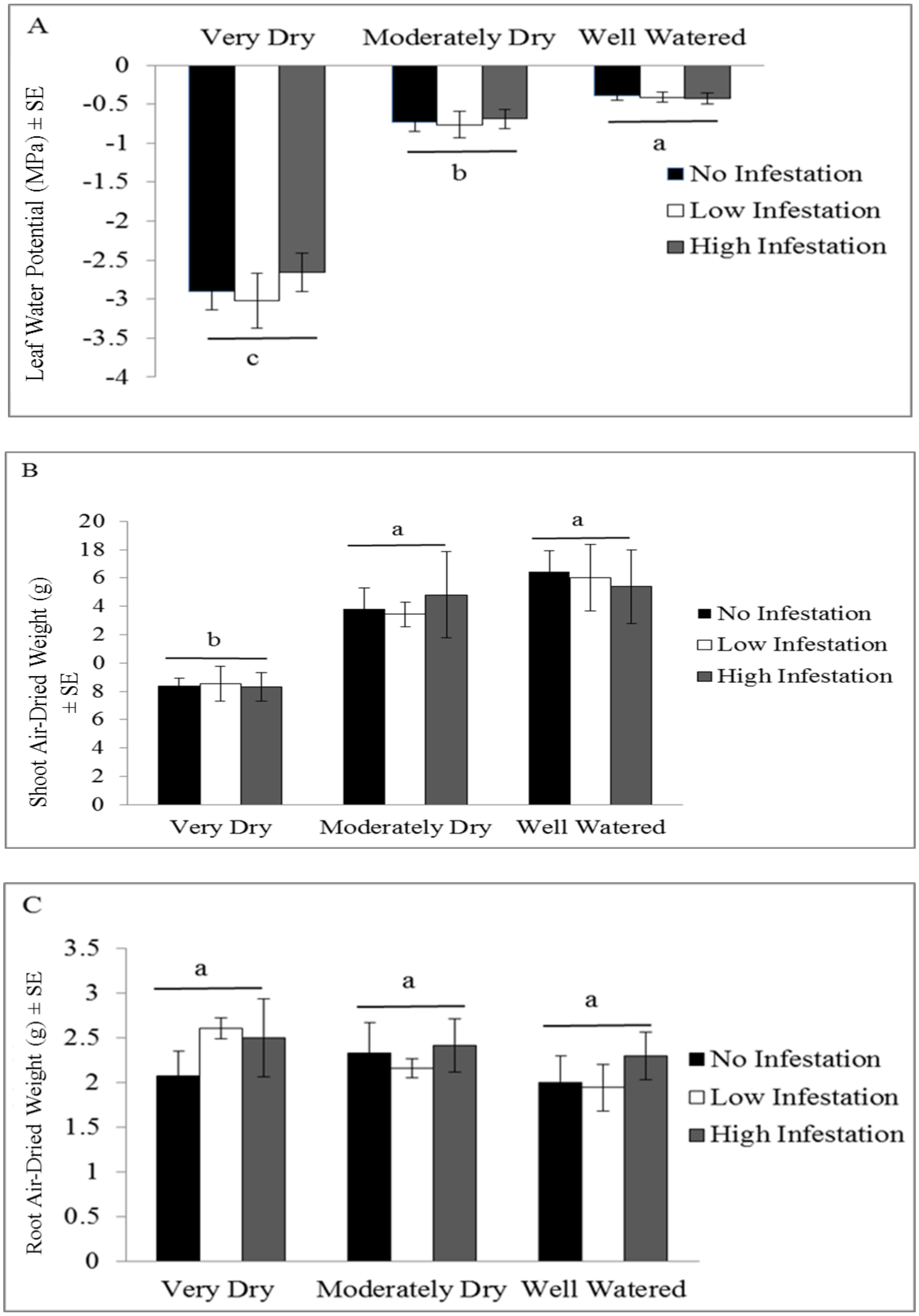

Fig. 3. Average leaf water potential (A), shoot air-dried weight (B), and root air-dried weight (C) of maize plants infested with western corn rootworm second instar larvae in Experiment 2. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)] data (A, C) and rank transformed data (B). Lines over bars indicate the main effect of soil moisture level. Lines with different letters are significantly different ($P \leq 0.05$).

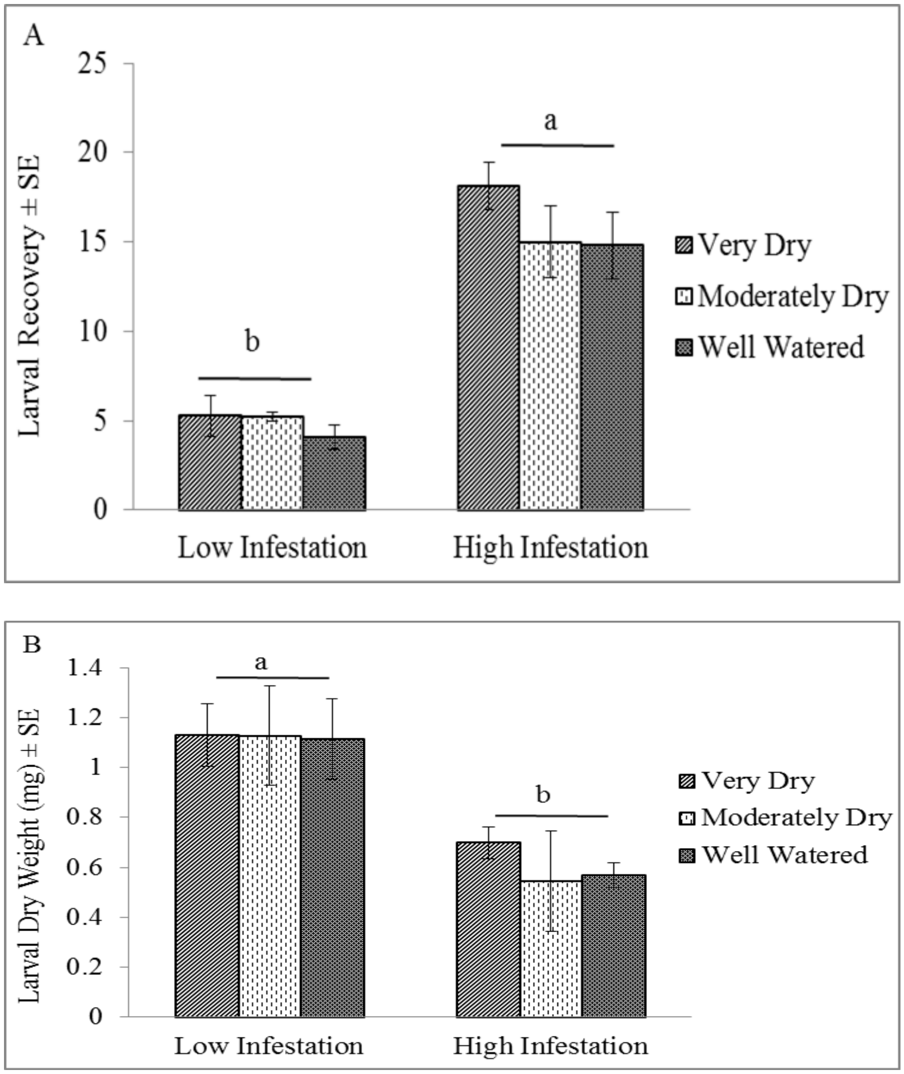

Fig. 4. Average number of western corn rootworm larvae recovered (A) and larval dry weight (B) from Experiment 2. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)] data (A) and [$\log_{10}$ (x +0.1)] (B). Lines over bars indicate the main effect of western corn rootworm infestation level. Lines with different letters are significantly different ($P \leq 0.05$).

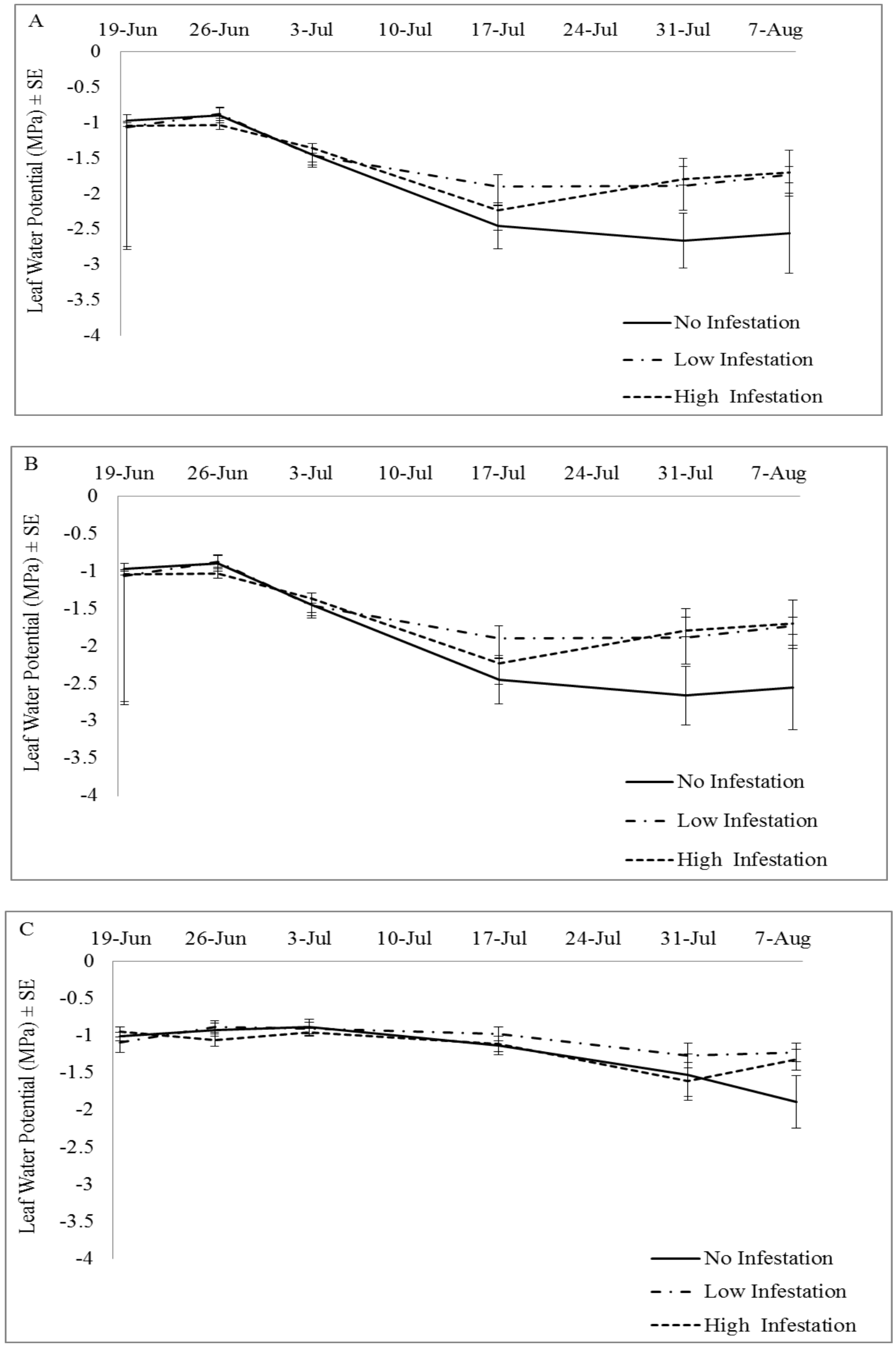

Fig. 5. Average leaf water potential of maize plants with or without infestation with western corn rootworm eggs under very dry (A), moderately dry (B), and well-watered (C) soil moisture regimes in Experiment 3. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)] data.

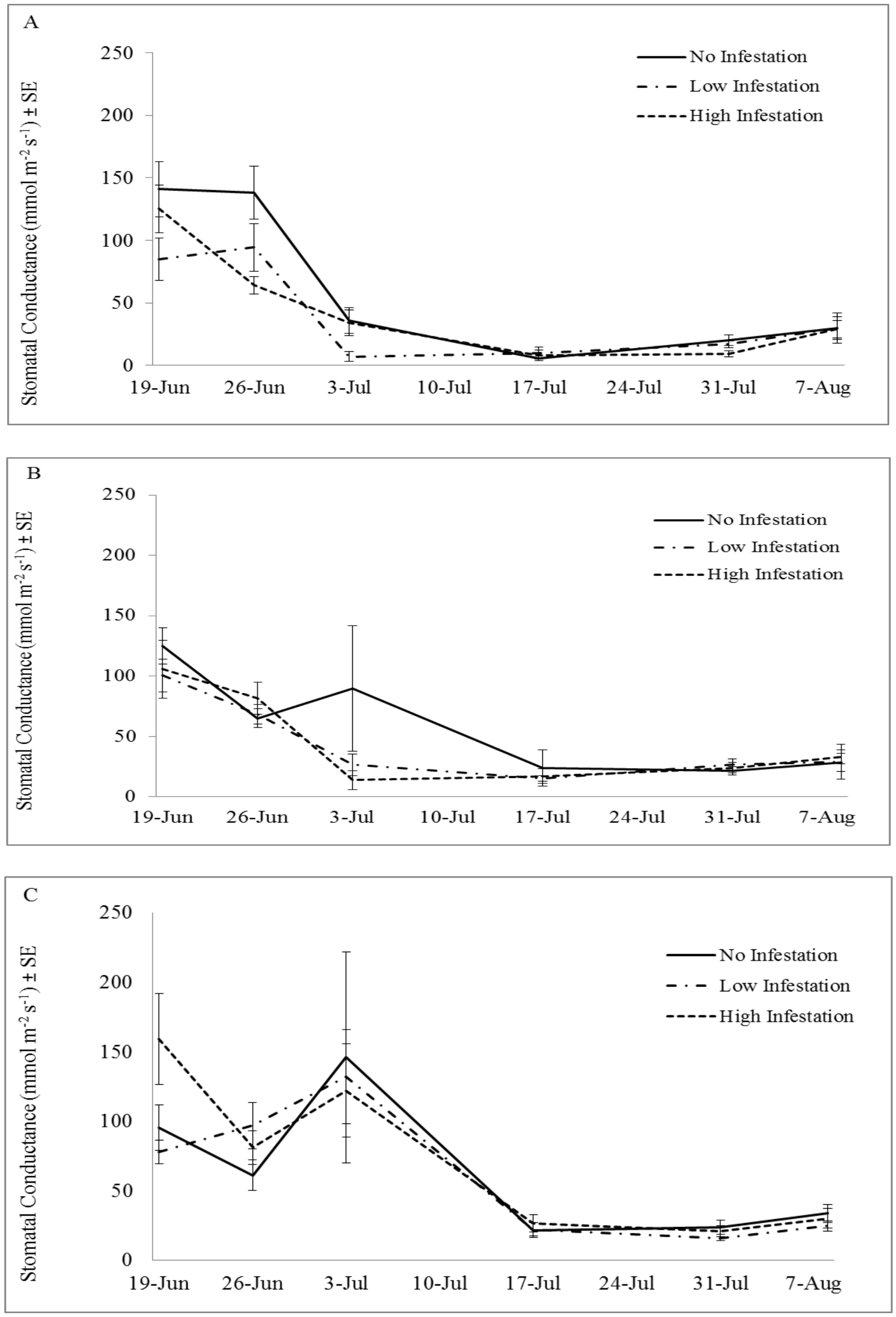

Fig. 6. Average stomatal conductance of maize plants with or without infestation of western corn rootworm eggs under very dry (A), moderately dry (B), and well-watered (C) soil moisture regimes in Experiment 3. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)] data.

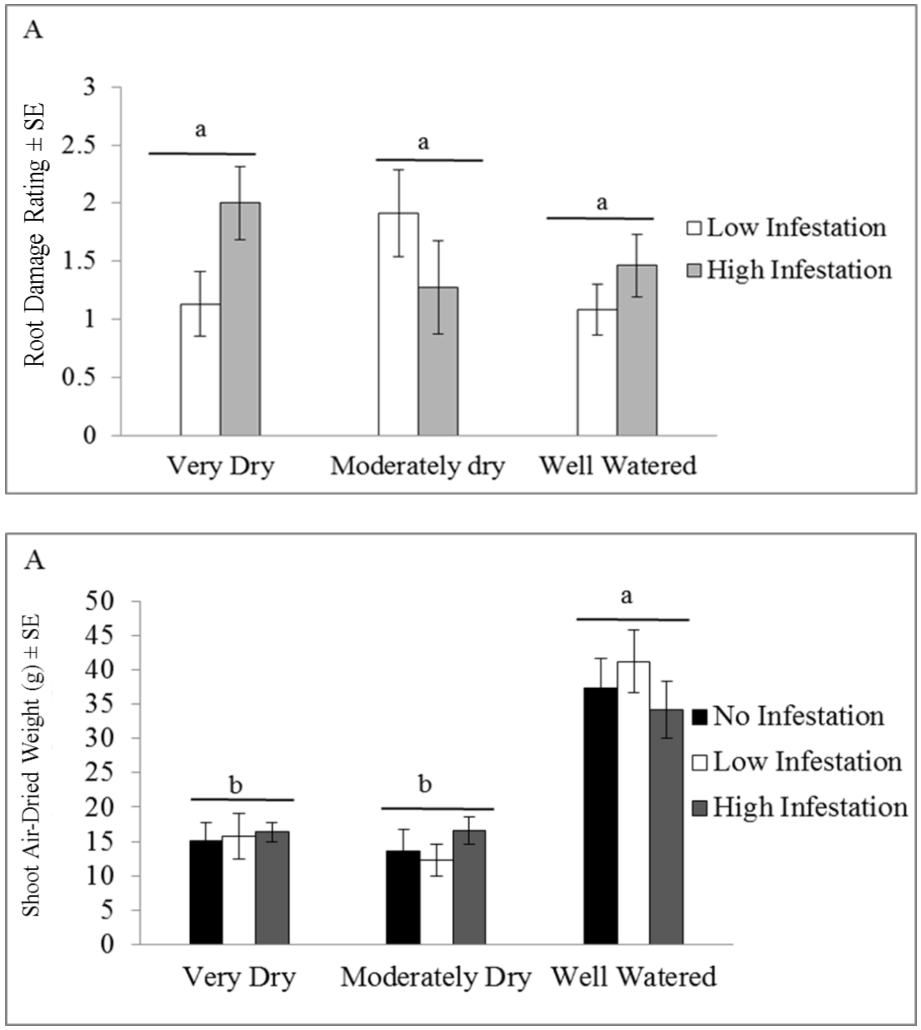

Fig. 7. Average root damage rating (A) and shoot air-dried weight (B) of maize plants with or without infestation of western corn rootworm eggs in Experiment 3. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)] data. Lines over bars indicate the main effect of western corn rootworm infestation level. Lines with different letters are significantly different ($P \leq 0.05$).

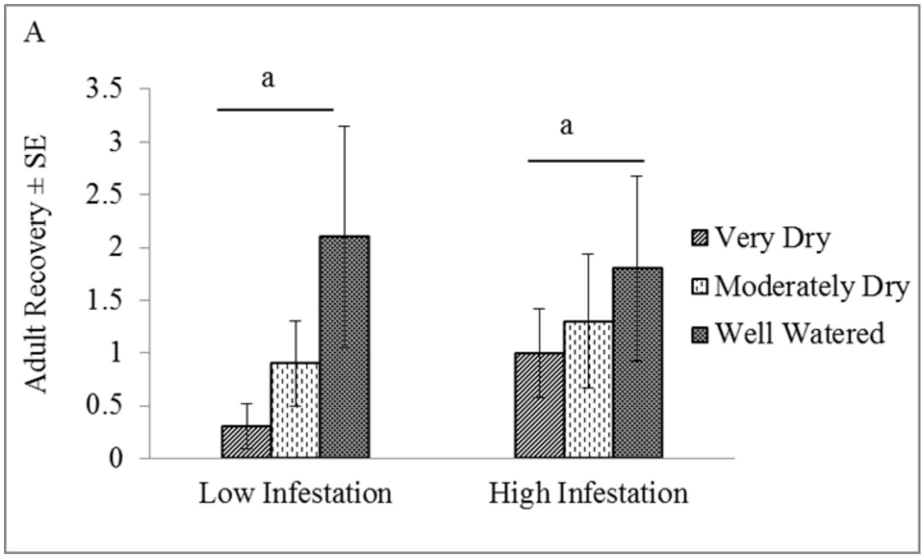

Fig. 8. Average western corn rootworm adult number recovered from plants with or without infestation of western corn rootworm eggs in Experiment 3. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)] data. Lines over bars indicate the main effect of western corn rootworm infestation level. Lines with different letters are significantly different ($P \leq 0.05$).

CHAPTER III: INTERACTIVE effects of WESTERN CORN ROOTWORM and DROUGHT ON MAIZE HYBRIDS WITH AND WITHOUT DROUGHT- AND ROOTWORM-TOLERANCE IN THE FIELD

Introduction

Diabrotica virgifera virgifera LeConte, the western corn rootworm, is the most economically damaging insect pest of maize (*Zea mays* L.) in the United States. Eggs overwinter in the soil, and to survive, the newly hatched larvae must locate roots of host plants, including maize and a few other grasses (Clark and Hibbard 2004, Oyediran et al. 2004). Neonate larvae typically begin feeding on the first host roots that they encounter (Clark et al. 2006). Larvae develop through three instars and during that time they tend to move to and feed on the succulent new growth of nodal root axes that arise from the stem nodes (Strnad and Bergman 1987b, Schumann et al. 2013).

Western corn rootworm larval feeding impacts maize yield in several ways. Larval feeding can interfere with the ability of the plants to take up water (Gavloski et al., 1992, Hou et al. 1997, Erb et al. 2009, 2011), thus resembling drought stress and limiting plant productivity. Heavy larval infestations can also cause “goose necking” or lodging of maize because damage to “brace roots” (nodal roots that develop from aboveground stem nodes) and damage to other nodal root axes erodes the structural support delivered by these roots. The decrease in stalk stability that causes lodging adds an additional yield loss component as a result of difficulties in mechanical harvesting (Spike and Tollefson 1988, 1989, 1991). When averaged over 19 location-years in Illinois, a 15% yield loss was associated with one full node of damage (Tinsley et al. 2013). Damaged maize roots are also more likely to be infected by root and stalk fungal diseases (Palmer and

Kommedahl 1969, Kurtz et al. 2010). Finally, adult western corn rootworm also impact maize as they feed on leaves, pollen, and silks, and, if heavy feeding occurs prior to pollen shed, can cause "silk-clipping”, which reduces pollination and results in lightly filled ears (Culy et al. 1992).

To help manage the impact of damage by the western corn rootworm, the Environmental Protection Agency (EPA) first approved genetically modified maize expressing a Cry3Bb toxin from *Bacillus thuringiensis* (Bt) in 2003 (EPA 2003). When consumed by susceptible insect larvae, Bt toxins in the roots bind to receptors in the insect midgut and cause pores to form which lead to larval death (Schnepf et al. 1998). Since 2003, several additional Bt toxins (Cry34/35Ab1, mCry3A, and eCry3.1Ab) have been developed to target the western corn rootworm, and pyramids (transgenic plants that express more than one toxin targeting the same pest) were first commercialized to manage western corn rootworm in 2008 (Tabashnik and Gould 2012). In 2014, 80% of the maize planted in the United States was genetically modified to produce one or more Bt toxins (USDA-ERS 2015).

Globally, drought is one of the most important climatic natural hazards affecting crop production (Boyer 1982, Wilhite 2000, Boyer et al. 2013). The average cost in terms of crop losses and livestock food due to drought in the United States was estimated to be $9.5 billion per drought event (Smith and Mathews 2015, NOAA 2012). The impact of the 2012 drought in the United States was the most extensive in decades (Boyer et al. 2013). This drought was estimated to result in a loss of $31 billion to United States agriculture (NOAA 2014, 2015). On average, maize yields have been rising steadily over the years, but the 2012 drought in the U.S. caused a 13% yield loss when averaged across the country (USDA-NASS 2012). In some areas such as Indiana, maize grain yield losses were 38% below the trend line of expected yield (http://www.kingcorn.org/news/imeless/YieldTrends.htm).

Native and transgenic traits with enhanced drought tolerance have been developed in maize in an effort to combat the sustained yield loss from drought. In 2011, native drought-tolerant maize hybrids, developed using advanced conventional breeding techniques, became commercially available (http://voices.nationalgeographic.com/2013/09/20/fighting-drought-with-a-new-super-corn/). In 2013, the first transgenic drought-tolerant maize hybrid was also commercialized (http://corn.osu.edu/newsletters/2013/c.o.r.n.-newsletter-2013-07/drought-tolerant-corn-hybrids).

Drought stress caused by low water availability in the soil affects plant growth and physiology to different extents depending on the timing, duration, and intensity of the water-deficit stress (Sanchez-Diaz and Kramer 1971, Hsiao 1973, Saab and Sharp 1989). Plant water deficit decreases cell turgor and, therefore, inhibits cell expansion and thus reduces plant growth (Taiz and Zeiger 2002). Individual plant tissues respond in different ways to a water deficit stress. Roots maintain growth under a water-deficit stress that results in the complete inhibition of shoot and leaf growth (Westgate and Boyer 1985, Sharp et al. 1988, Spollen et al. 1993).

Plant responses to insects and drought may be linked, so there is the possibility that both have the potential to influence plant response to the other. Root regrowth after feeding by western corn rootworm positively affected yield when soil moisture was low, but negatively affected yield when soil moisture was adequate (Gray and Steffey 1998). This may be due to the fact that the additional roots from regrowth help to aquire water in a low soil moisture environment, but when ‘normal’ roots are adequate for aquiring needed moisture, then the extra carbon channeled into root regrowth would ultimately have a negative impact on yield. Maize plants were less water stressed when infested with a moderate level of western corn rootworm compared with uninfested plants under very dry soil as indicated by higher leaf water potential (Chapter 2, Fig. 5). Other interactions with rootworm feeding also occur. Erb et al. (2009) demonstrated that rootworm larval feeding decreased the water content of leaves concomitant with an induction of the production of abscisic acid (ABA). ABA is a phytohormone that induces stomatal closure (Taiz and Zieger 2002) to help slow water loss from plants under water-deficit stress. Soil moisture content also plays a role in the interaction between maize and western corn rootworm. Larval mobility, which is important for initial establishment of western corn rootworm on a host root (Ellsbury et al. 1994, Strnad and Bergman 1987b) and also for movement between roots (larvae tend to move to new whorls of nodal roots as they emerge from the stem; Strnad and Bergman 1987a), is affected by soil moisture content. MacDonald and Ellis (1990) found that movement of neonate larvae was more extensive in soil with moisture contents between 24% and 30% w/w and that it was restricted in both saturated (36% w/w) and very dry (<18% w/w) soils.

Individually, both drought and rootworm feeding can generate yield losses in maize, but yield loss is potentially greater and thus more economically important if both stressors are present. Occasional anecdotal evidence had suggested that yield can be dramatically decreased when significant rootworm pressure is found with drought (for example, Tinsley et al. 2015). A moderate rootworm infestation under well-watered conditions may cause minimal yield loss, but the same infestation level under severe drought may cause a 50% yield loss or more without protection from rootworm infestation by use of insecticides or transgenic hybrids expressing an insect toxin (Tinsley et al. 2015). However, to date, experiments documenting the potential effect of these stress interactions have not included a well-watered control to characterize the individual contributions of drought and western corn rootworm and the interaction between the two in generating a reduction in yield.

In Chapter 2, a single hybrid under greenhouse conditions was evaluated under differing soil moisture levels and western rootworm infestation levels, but a significant interaction between soil moisture levels and western rootworm infestation levels was not found, in general. Anecdotal evidence for this interaction in previous studies (Tinsley et al. 2015) was from field experiments, so we wanted to include a well-watered control in field experiments of our own. Rather than using a single hybrid, as in Chapter 2, the goal of the current work was to quantify the interaction between western corn rootworm and drought for its effect on maize hybrids in the field with and without expressed drought- and rootworm-tolerance. We hypothesized that if both drought and rootworms occur; there will be a synergistic and negative effect of these factors on maize yield in susceptible hybrids. We also hypothesized that drought- and rootworm-tolerant hybrids will be less affected and have higher yield than non-tolerant hybrids under combined drought and western corn rootworm stress.

Materials and Methods

Experimental design. The study was conducted in 2012, 2013, and 2014 at the University of Missouri, Hinkson Valley Farm in Boone Co., MO, USA. The soil type at this location is Haymond loam comprised of 50% sand, 38.5% silt, and 12.5% clay as determined by the University of Missouri Soil Testing Facility, Columbia, MO. The sandy soil environment ensures increased drainage rates and soil drying, and thereby facilitated the establishment of drought conditions. Prior to this study, the site was planted in maize, adding the possibility of the presence of a persistent low level population of extant western corn rootworm. Agronomic practices, such as herbicide, tillage, fertilizer, etc., were applied as recommended for optimal yield under local conditions.

The experimental design was a completely randomized split split plot in space. The main plot was soil moisture level. The subplot contained the effects of infestation level and the interaction of soil moisture level × infestation level. The sub sub plot contained the effect of maize lines and all possible interactions with the main plot and subplot. Treatments were arranged in a 2 × 2 × 5 factorial arrangement (2 soil moisture levels × 2 western corn rootworm infestation levels × 5 maize lines). Soil moisture levels were well-watered plots (Drought-), which were planted in an open field and were adjacent to drought-stressed (Drought+) plots, which were situated under a rainout shelter (see below). Western corn rootworm infestation levels (see below) were a moderate infestation (WCR+) and zero (WCR-) artificially infested eggs in 2012 and 2013. An additional high infestation level (WCR++) was added in 2014, resulting in a 2 × 3 × 5 factorial arrangement in 2014. Each year, five maize lines were planted: B73×Mo17; Pioneer's Bt (33P83XR), targeting western corn rootworm with the Cry34/35Ab1 protein; the non-transgenic near-isoline to the Bt (33P81R); a conventional drought-tolerant line AQUAmax (P1151R); and a trait pyramid line AQUAmax line+Bt (P1151XR). In 2014, the Bt (P0987AMX) and the non-transgenic near-isoline

(P0987AM) were substituted for 33P83XR and 33P81R, respectively, due to the unavailability of seed. Eight maize kernels were planted in each plot, with each plot being a 1.5 m portion of a maize row. There were four replications under the rainout shelter (Drought+) and eight well-watered replications (Drought-) in the adjacent field from which yield and plant damage assessments were measured each year. In 2013, an additional destructive measurement (larval recovery) was added which required four additional replications of all treatments to study the effect of drought on larval performance. The larval sampling procedure did not allow use of these specific plots for any other measurement.

Corn rootworm infestations. In 2012 and 2013, ~800 viable eggs per 30.5 cm of maize row were used, while in 2014, ~1000 and 10000 viable eggs per 30.5 cm were used for the low infestation (WCR+) and high infestation (WCR++) treatments, respectively. Each year, western corn rootworm infestation was targeted to the V2 maize developmental stage (Ritchie et al. 1992). Infestation timing varied from 10-14 d after planting, depending on the year. Eggs were suspended in 0.15 % agar (USB Corporation, Cleveland, OH) to deliver eggs to the soil for infestation. In 2012 and 2013, eggs were applied using a tractor-mounted delivery system modified from Moellenbeck et al. (1994). In 2014, eggs were manually infested into the soil by excavating a small trench on each side of the plant row and delivering an egg suspension into the trench using a syringe. The different infestation rates were randomly distributed and hand infestation ensured that the exact egg infestation level was delivered to each of the designated plots. The trench was subsequently covered with soil.

Soil moisture levels. Each year, the well-watered treatment (Drought-) was watered according to conventional agricultural practices for irrigated high yielding maize in Missouri, and received ~25-50 mm water per week, depending on growth stage. The drought treatment (Drought+) was established by planting maize under a rainout shelter that shielded the plants from precipitation during predicted rain events. The shelter, of our design, was built in 2011 (Fig. 9): it is 9.30 m wide, 15.24 m long, 5.18 m tall at the center ridge, and 2.13 m tall at the outside edges. The shelter is supported by steel and has a moveable transparent plastic cover (PAK 1212C Clear Barrier, Hummert International, St. Louis, MO), which is supported at the top and on the sides by cables at 0.564 m intervals. The end of the cover is connected to a 3-cm diameter pipe around which the cover rolls when retracted. The plastic is manually retracted or deployed using a winching system, and the cover is held in place with tie down straps when deployed. For our study, the area under the shelter was exposed during good weather and sheltered by deploying the plastic roof when a rain event was predicted. Average monthly precipitation and air temperature from May to September in 2012, 2013, and 2014 were monitored by a weather station (http://aes.missouri.edu/sanborn/weather/sanreal.stm) ~2.5 km from the research site (Table 4). An extreme drought in 2012 necessitated some irrigation for the plants in all treatments (including Drought+) to ensure plant survival. In 2013, the soil was completely saturated by heavy rains ~17 d after planting (7 d post rootworm infestation).

Each year, the plants and insect infestations were established prior to initiating the water-deficit treatment, since drought in Missouri is more common later in the growing season (July and August). In 2012, drought was initiated by withholding water, and employing the rain-out shelter during predicted rain events starting 30 d post infestation. In 2013, rain was withheld from the drought plots starting 25 d post infestation. In 2014, the drought treatment was initiated 32 d post infestation. Each year, upon initiation of the drought treatment, the western corn rootworm larvae were in early to late third instar, as calculated using soil temperature degree-day models.

Measurements

Yield. The three largest ears, determined visually, were harvested from each plot to assess yield. However, some plots did not produce three ears (especially in 2012). In this case, all available ears were harvested. Ears were harvested into small mesh bags (Sacramento Bag Manufacturing Co., Woodland, CA), dried, and the kernels shelled from the cob using a maize sheller (Silver Machine Shop, Champaign, Ill). Debris was cleaned from kernels and total dry weight was recorded using a digital scale (Model V51PH30, Hogentogler & Co. Inc., Columbia, MD). Prior to weighing, grain moisture was monitored throughout the drying process until a 15.5% moisture was obtained using moisture tester (Dickey John, model GAC2000, Churchill Industries, MN), when grain weight was recorded.

Leaf water potential. Leaf water potential measurements were made using a pressure chamber (PMS Instruments, model 670, Albany, Oregon, USA). In 2012, the flag leaf was chosen for these measurements. In 2013 and 2014, a leaf from the 6^{th} or 7^{th} set of leaves from the bottom of the plant (V6, V7; Ritchie et al. 1992) was sampled for consistency between sampled plots across sampling dates. Measurements were taken from one leaf from one plant in each of the plots. Leaf water potential measurements were initiated at solar noon after initiating drought. Biweekly measurements were made four times in 2012 and 2014, and five times in 2013. Measurements were taken from four replications from plants grown under the rain out shelter and from four out of the eight replications from the well-watered plots. The leaf was excised 15 cm from the tip with scissors and placed in a sealable bag with a moistened paper towel to create a 100% humidity environment for the short period prior to measurement.

Stomatal conductance. Abaxial stomatal conductance was measured using a leaf porometer (version 8, 2005-2010 Decagon Devices, Inc. Pullman, WA, USA). In 2012 and 2013, measurements were made 3 h after sunrise on sunny days only, since stomatal conductance is affected by overcast skies (Will and Teskey 1999). In 2014, measurements were taken at noon. As with the water potential measurements, the flag leaf was chosen for stomatal conductance in 2012, and a leaf from the 6^{th} or 7^{th} set of leaves from the bottom of the plant was sampled in 2013 and 2014 for consistency between sampled plots. The leaf-clip sensor was placed on the most expanded part of the leaf far from the main vein. Biweekly measurements were made four times in 2012 and 2014, and five times in 2013.

Root damage ratings. Roots from the same plants chosen for yield were rated for damage just after ears were harvested. Root harvests were done shortly after physiological maturity of the ears, as indicated by a “black layer” formation at the base of the kernels. Harvesting prior to complete drying ensured that the roots would still be viable to facilitate the rating. After cutting the stalks ~15 cm from ground level, the roots were excavated, thoroughly washed, and rated for western corn rootworm damage using the 0 to 3 node injury scale (Oleson et al. 2005).

Root complexity and root weight. Root complexity is the number of root branching points per soil volume (Novais 2011). It is an important factor in maize plant tolerance to certain biotic and abiotic factors stress. The greater the number of branching points, the more efficient the plant is in taking up water and nutrients from the soil. In our experiment, root complexity was determined by calculating the fractal dimension. Fractal dimension is a statistical evaluation of geometrical shapes which provide an indirect estimate of the number of branching points. It was employed in the statistical analysis to evaluate the root complexity following the procedures of Novais (2011). Root complexity as indicated by fractal dimension was measured in 2012 and 2013 using the same roots as those rated for western corn rootworm feeding damage. A maize root-imaging box employing a highly diffuse illumination to eliminate shadows, as described by Zhonga et al. (2009), was used to facilitate complexity measurements. The imaging box was fitted with a computer controlled digital camera, series of images was acquired by rotating the washed, excavated roots (Appendix Figs. 50S-59S). The images were subsequently processed using Matlab® (MATHWORKS) software package according to the methods of Zhonga et al. (2009) and Grift et al. (2011). After imaging, roots were air dried in a greenhouse and weighed using a digital scale (scale model AB135-SFACT, Mettler Toledo INC., Columbus, OH). Air-dried root weight was monitored throughout the drying process until a constant weight was obtained.

Larval recovery, larval head capsule width, and larval dry weight. In 2013 only, larvae were sampled as described by Hibbard et al. (2004). Briefly, whole root systems with attached soil were excavated and transferred to mesh bags (Sacramento Bag Manufacturing Co., Woodland, CA). The bags were brought to a greenhouse and hung individually over water pans (6.65 L) for 6 d with the cooling system turned off. Under this situation, the temperature reached as high as 50°C during the day. The high temperature and the drying soil caused the larvae to move out of the soil and fall into the water below. The larvae were collected twice a day and preserved in 95% ethanol until processing. The larvae were examined under a microscope to differentiate between southern corn rootworm (*Diabrotica undecimpunctata howardii* Smith and Lawrence) and western corn rootworm: southern corn rootworm larvae were counted and discarded. Each western corn rootworm larva was examined to determine head capsule width using an ocular micrometer (10/21, Wild Co., Heerbrugg, Switzerland) mounted on a microscope (M3Z, Wild Co.). Subsequently, the remaining 95% ethanol was poured off and the open vials were placed in a desiccating oven (Thelco model 16, GCA/ Precision Scientific Co., Chicago, IL) at 37.8°C for 7 d. Air-dried larval weight was measured using an analytical scale (ER-182A, A & D Co., Tokyo, Japan).

Adult recovery. Adult emergence traps were placed over one maize plant per plot well before predicted emergence of the first beetles, as calculated by soil temperature degree day models (Wilde 1971, Levine et al. 1992). The emergence trap design was adapted from Hein et al. (1985) with modifications from Pierce and Gray (2007). The trap components included a wooden frame (76.2 × 45.7 cm) enclosed in a wire mesh screen with two holes cut into the center wooden support. The shoot was gently pulled through one of the holes and tied off using a gauze sock and a cable tie. A funnel was placed in the second hole and a jar was placed open side down over the funnel. A metal trim extended below the wooden frame 5 cm into the soil. Adults were collected two to three times per week and preserved in 95% ethanol until processing. Emergence traps were deployed until two weeks after the last adult was collected. In 2014, emergence traps were not deployed because density-dependent mortality was predicted at the high infestation level (Hibbard et al. 2010). In 2012 and 2013 the total number of beetles was recorded. The head capsule width for each adult was measured using an ocular micrometer (10/21, Wild Co., Heerbrugg, Switzerland) mounted on a microscope (M3Z, Wild Co.). Subsequent to head capsule measurement, the remaining 95% ethanol was poured off and the open vials were placed in a desiccating oven (Thelco model 16, GCA/ Precision Scientific Co., Chicago, IL) at 37.8^{o}C for 7 d. Adult dry weight was measured using an analytical scale (ER-182A, A & D Co., Tokyo, Japan).

Statistical Analysis. Yield, root measurements, and rootworm data for each year were analyzed separately using analysis of variance (ANOVA) for a completely randomized split split plot in space design using PROC MIXED procedure, SAS 9.2 (SAS 2008). Treatments were arranged in a 2 × 2 × 5 factorial arrangement (2 soil moisture levels × 2 western corn rootworm infestation levels × 5 maize lines). The main plot was soil moisture level. The subplot contained the effects of infestation level and the interaction of soil moisture level and infestation level. The sub sub plot contained the effect of maize lines and all possible interactions with the main plot and subplot. Fixed effects were arranged as a factorial arrangement of maize lines and infestation levels (five maize lines × two western corn rootworm infestation levels) and all possible interactions with the main plot (soil moisture level) for 2012 and 2013. In 2014, the subplot was a factorial arrangement of five maize lines × three western corn rootworm infestation levels, and all possible interactions with the main plot. Beyond the standard ANOVA, preplanned comparisons were made between maize lines while keeping infestation and soil moisture levels constant, between infestation levels while keeping soil moisture level and maize lines constant, and between soil moisture levels while keeping infestation level and maize lines constant. This was done using the LSMEANS output of PROC MIXED. All mean differences were tested using Fisher's protected least significant differences (LSD), which is produced from the LSMEANS Statement in the PROC MIXED, SAS 2.9 (SAS 2008).

Water potential and stomatal conductance data were analyzed as a completely randomized split split split plot in space to account for the repeated nature of the sampling throughout the year, using PROC MIXED procedure SAS9.2 (SAS 2008). The main plot was soil moisture level. The subplot contained the effects of infestation level and the interaction of soil moisture and infestation level. The sub sub plot contained the effect of maize lines and all possible interactions with the main plot and subplot. The sub sub sub plot contained the effect of sample day and all possible interactions with the main plot, the subplot, and the sub sub plot. The fixed effects were arranged as a $2 \times 2 \times 5 \times 4$ (in 2012), a $2 \times 2 \times 5 \times 5$ (in 2013), and a $2 \times 3 \times 5 \times 4$ (in 2014) factorial arrangement of soil moisture level (dry and well-watered), western corn rootworm infestation level (moderate and none), and maize lines (B73×Mo17, Bt, the non-transgenic near-isoline to the Bt, the non-transgenic AQUAmax, and Bt+AQUAmax). In 2012 and 2014, there were four sample days, while in 2013 there were five sample days. Beyond the standard ANOVA analysis, preplanned comparisons were made between maize lines whilst keeping infestation and soil moisture level constant, between infestation level keeping soil moisture level and maize lines constant, and between soil moisture level keeping infestation level and maize lines constant. All mean differences were tested using

Fisher's protected least significant differences (LSD), which is produced from the LSMEANS Statement in the PROC MIXED, SAS 2.9 (SAS 2008).

Results

Yield. Soil moisture level, infestation level, and maize lines all significantly affected yield in 2012 and 2013, while maize lines and the interaction between soil moisture level × infestation level, and soil moisture level × maize lines significantly affected yield in 2014 (Tables 5, 6, 7). The interaction between soil moisture level × infestation level was marginally significant in 2012 ($P = 0.0518$). No other interactions were significant in 2012 and 2013. Overall, the average yield under drought was significantly and dramatically less than the average yields measured for well-watered plots in 2012 and 2013 (Figs. 10A, 11B, 12A, 13B), but the main effect of drought was not significant in 2014 (Table 7). Unexpectedly, the main effect of rootworm infestation generated an increased yield for the average of all maize lines under severe drought in 2012 (Fig. 10B). However, when evaluating individual lines, this difference was only significant for the Bt+AQUAmax line (Fig. 11A). In 2013, the main effect of rootworm significantly decreased overall yields (Fig. 12B), but this was not significant for any specific maize line with or without drought (Fig. 13A). In 2014, yield was significantly higher for the low infestation level than for the high infestation when well watered, but not when under water deficit, accounting for the drought level × infestation level interaction (Table 7, Fig. 15A). The 2014 soil moisture level × maize lines interaction can be seen in Fig. 14B, demonstrating the differing reactions to drought for each maize line. When rootworm and drought stress were both present, the highest yielding maize line was usually the one with tolerance to both stresses (Bt+AQUAmax) (Figs. 11C, 13C, 15B).

Leaf water potential. Sample day, maize lines, and the interactions of soil moisture level × sample day, infestation level × sample day, and infestation level × maize lines × sample day significantly impacted leaf water potential in 2012 (Table 5). The third sampling day gave the most negative leaf water potential for plants under drought and western corn rootworm infestation in 2012, accounting for the significant interaction between these treatments (Fig. 17). Bt+AQUAmax and AQUAmax had significantly less negative leaf water potentials than the Bt and non-transgenic isoline line in 2012 (Fig. 16B). There were inconsistent differences between western corn rootworm infestation level and soil moisture level on different sample dates (Figs. 17A, 17B). In 2013, soil moisture level, sample day, their interaction, and the interaction of infestation level × sample day significantly impacted leaf water potential (Table 6). In 2013, plots were saturated at the beginning of the season and the final sampling day yielded the most negative leaf water potentials (Figs. 18B, 19). The effect of maize line and all interactions with maize line on leaf water potential were not significant in 2013 (Table 6). Soil moisture level, infestation level, sample day, maize line, and the interactions of infestation level × sample day, soil moisture level × infestation level × sample day, soil moisture level × maize line, and infestation level × maize line significantly impacted leaf water potential in 2014 (Table 7). In 2014, the final sampling day again yielded the most negative leaf water potentials (Fig. 20B). Interestingly, moderate western corn rootworm infestation positively affected leaf water potential compared with no and high infestation plots when all sample dates and maize lines were combined (Fig. 20C). This effect was consistent between sample days except for the final day (Fig. 21A). AQUAmax and Bt+AQUAmax were the maize lines that exhibited the least negative leaf water potentials when averaged across sample days, soil moisture level, and infestation level (Fig. 21B).

Stomatal conductance. Sample day, soil moisture level × sample day, and infestation level × sample day interactions significantly impacted stomatal conductance in 2012 (Table 5). Stomata were almost completely closed on the third and fourth sampling days (Fig. 22). Unexpectedly, stomatal conductance was greater under western corn rootworm pressure, especially on the first and second sampling days (Fig. 22B), while there was no difference between infestation level for the fourth sampling day, which accounted for the significant interactions between sampling day and infestation level. In 2013, soil moisture level, sample day, and the interactions of soil moisture level × infestation level, soil moisture level × sample day, infestation level × sample day, maize line × sample day, and infestation level × maize line × sample day all significantly impacted stomatal conductance (Table 6). Stomatal conductance was reduced later in the season under drought (Figs. 23B, 24B, 25). Stomatal conductance was significantly greater with rootworm infestation than without rootworm infestation under drought conditions, but not when well-watered, which accounted for the interaction of infestation level × soil moisture level (Fig. 24A). Rootworm infestation had a minimal effect on stomatal conductance in 2013, with a positive effect on the first sample day and a negative effect on the final sample day accounting for the infestation level × sampling day interaction (Fig. 24B). B73×Mo17 had the least stomatal conductance compared with the other maize lines, especially in the fourth and fifth sampling days (Fig. 25B). In 2014, soil moisture level, sample day, maize lines, and the interaction of drought level × sample day, infestation level × sample day, soil moisture level × maize lines, sample day × maize lines, and drought level × sample day × infestation level all significantly impacted stomatal conductance (Table 7). Stomatal conductance was again reduced by drought (Figs. 26A 27B). Stomatal conductance was inconsistent between sampling days for the differing infestation levels (Fig. 27A).

Root damage ratings. Infestation level, maize lines, and the interaction of infestation level × maize lines significantly impacted root damage in 2012, 2013 and 2014 (Tables 5, 6, 7). Although the effect of drought was not significant in any year (Tables 5, 6, 7), the interaction of soil moisture level × maize line significantly impacted root damage in 2013 (Table 6, Fig. 29B). As expected, the Bt and Bt+AQUAmax hybrids were the least damaged when exposed to rootworm infestation (Figs. 28C, 29C, 30C). Natural infestations, which are often quite variable, played more of a role in 2013 than in the other two years as indicated by plant damage without artificial infestation of western corn rootworm (Fig. 29). In 2014, the moderate infestation level caused less damage compared to the moderate infestation level applied in 2012 and 2013 (Fig. 30A).

Root complexity (*fractal dimensions*). Soil moisture level, infestation level, maize lines, and the interaction of infestation level × maize lines significantly impacted root complexity measurements in 2012 (Table 5). In 2013, soil moisture level, infestation level, and maize lines again impacted root complexity, as did the interaction of soil moisture × infestation level and soil moisture level × maize lines (Table 6). Under severe drought in 2012, the main effect of drought significantly inhibited root complexity (lowered fractal dimension values), and this was also significant for B73×Mo17 with and without rootworm and for Bt maize without rootworm (Table 5, Fig. 31B). However, in

2013 when the soil was saturated early in the season and gradually dried, drought significantly increased root complexity for each maize line with and without rootworm infestation (Fig. 32B). As expected, the effect of western corn rootworm varied between maize lines. The Bt+AQUAmax and Bt had the greatest root complexity compared with the other maize lines when under severe drought and rootworm pressure in 2012 (Fig. 31C). In 2013, there were no differences between maize lines under drought with or without rootworm pressure (Fig. 32C), but interestingly, despite significant damage (Fig. 29C), the Bt and Bt+AQUAmax had the lowest root complexity under rootworm pressure when well-watered (Fig. 32C).

Larval recovery. The only factor to significantly affect the number of larvae collected was maize lines (Table 6). Larval recovery was similar with or without drought (Fig. 33A). As expected, the number of larvae collected from the two Bt expressing lines was lower, though not always significantly so than the other lines. Drought was the only significant factor impacting larval weight, with larvae significantly heavier on well-watered maize lines (Table 6, Fig. 33C). Soil moisture level and maize lines each significantly impacted the head capsule width of larvae recovered (Table 6). In general, larvae recovered from the two maize lines with Bt toxin had the smallest head capsule width (Fig. 33B).

Adult recovery. Soil moisture level, infestation level, maize lines, and the interactions of soil moisture level × infestation level and infestation level × maize lines significantly impacted adult recovery in 2012, while maize lines, soil moisture level × maize line, infestation level × maize line and soil moisture level × infestation level × maize line significantly impacted adult recovery in 2013 (Tables 5, 6). Overall in 2012, the number of adult beetles collected from the well-watered plots was significantly greater than from the drought plots (Fig. 34A). In general, the number of beetles recovered from Bt and Bt+AQUAmax was lower than from the non-transgenic lines (Fig. 34). Beetle emergence was more variable in 2013, with almost no adult emergence from plots with Bt expressing maize. Significantly more beetles emerged from B73×Mo17 without drought than with drought (Fig. 34B).

Discussion

The 2012 U.S. growing season reminded many of the impact of drought on agriculture. Tinsley et al. (2015) reported yield data for various rootworm treatments including those from fields in areas experiencing a severe drought in 2012. In the region of DeKalb, IL, they determined that untreated, non-Bt maize hybrids yield averaged 1.4 metric tons/hectare. In contrast, yield from transgenic lines protected from rootworm feeding by Bt toxins averaged 9.6 metric tons/hectare for the Cry34/35Ab1 single trait and 10.0 metric tons/hectare for the trait pyramid of Cry34/35Ab1 plus Cry3Bb1. The non-transgenic lines were in the same genetic background as the Bt hybrids, so the dramatic 7.14-fold yield difference of more than 8.6 metric tons/hectare can be primarily attributed to rootworm damage in this one location in a year with severe drought. In most years, yield differences with and without rootworm feeding are not nearly as dramatic. Given that a 15% yield loss would be expected from a single node of injury in an average year (Tinsley et al. 2013), the expected yield loss from a control treatment (non-transgenic) with 2.21 nodes of damage in an average year would be 2.21 × 15% = 33% less than fully protected plants (expected yield of 6.7 versus 10.0 metric tons/hector obtained in DeKalb in 2012 when protected from rootworm feeding). Actual yield (1.4

metric tons/hector) was 4.8-fold less than expected from an average moisture year with 2.2 nodes of damage. Obviously, the interaction of rootworm pressure and drought can result in a greater yield loss than expected when maize is impacted by the independent action of the different stressors. However, there was not a well-watered control treatment in any of the locations studies by Tinsley et al. (2015), so the relative contribution of drought and rootworms could not be quantified.

In the current experiment, drought and western corn rootworm negatively impacted maize growth and productivity when applied together, but in most of the situations evaluated, the effect of drought was much greater than the effect of western corn rootworm on both yield and other agronomic traits (Tables 5, 6). The environmental conditions were quite different for each season of this study and almost certainly impacted the experimental data. For instance, drought was becoming critical by the end of May in 2012, which was earlier than imposed experimentally. Many midwest areas were categorized as moderate to severe drought at that time, and these drought conditions did not improve (NOAA 2012). In 2013, excessive rainfall occurred early in the growing season, and the field site was saturated 17 d after planting (7 d after infestation with western corn rootworm eggs). The impact of drought under our rainout shelter took longer to materialize in 2013 because of this, but by the end of the season, a significant drought was achieved as indicated by leaf water potential (Fig. 18B). The 2014 growing season received adequate rainfall much of the season, requiring extensive use of the rainout shelter. As a result, an artifact from the extensive use of the cover of the rain-out shelter is possible (Vogel et al. 2013), although the shelter was designed to minimize this (translucent plastic and open sides and ends). In the 2012 growing season, the rain-out shelter was rarely extended because of the severe drought that year. In 2013, it was occasionally used, while in the 2014 growing season the rain-out shelter was used extensively. We did not measure photosynthetically active radiation and soil temperature, so we are uncertain to what extent, if any, traits measured in this study were impacted by the rainout shelter.

In 2012, yield was marginally impacted by the interaction of soil moisture level × western corn rootworm infestation level (Table 5). The average yield of the infested plants was greater than the yield of the un-infested plants when each were under severe drought in 2012 (Table 5, Fig. 10B, 11A). Gray and Steffey (1998) suggested that root regrowth after western corn rootworm larval injury positively affects yield in conditions of insufficient soil moisture. Root growth may have been stimulated by the moderate level of rootworm feeding in the 2012 experiment, and this might explain the increased yield. Similar results were obtained from greenhouse experiments in Chapter 2: the leaf water potential for plants infested with a moderate level of western corn rootworm were less water stressed than uninfested plants under very dry soil (Fig. 5). Contrary to Chapter 2 and 2012, for the current experiment in 2013, when plants were not stressed by water deficit early in the season, the effect of rootworm feeding negatively impacted yield when combining all maize lines and both drought levels (Table 6). Early season saturated soils in 2013 may have altered root development and reduced root expansion within the soil profile, even when triggered by rootworm feeding.

A significant response of maize water relations to drought was seen in 2012 when drought stress was severe and stomata were almost completely closed for both the final two sample days (Fig. 22). Closing of the stomata may have reduced plant stress as measured by leaf water potential on the fourth sample date (Fig. 16A). The stomatal conductance for the infested plants was greater than for the non-infested plants (Fig. 22B). As discussed previously for yield in 2012, this could have resulted from root regrowth triggered by western corn rootworm larval feeding (Gray and Steffey 1998). In 2014, western corn rootworm, drought, and their interaction impacted stomatal conductance for some but not all of the sample days (Fig. 27). It is possible that the reduction in stomatal conductance may be explained, in part, by regulation with abscisic acid (ABA). ABA is known to increase under water-deficit stress (Sharp and Davies 1989, Taiz and Zieger 2002). Erb et al. (2009) observed that western corn rootworm feeding promoted ABA-inducible gene expression in maize roots and leaves and significantly reduced leaf water content by 2% compared to equivalent damage mediated by mechanical removal of maize roots. Erb et al. (2011) also determined that the interaction of water status × western corn rootworm impacted ABA concentration in the leaves. ABA concentration for non-watered and uninfested plants was slightly elevated, while the concentration of ABA was 40-fold greater for the infested and non-watered plants. This may explain the negative effect of western corn rootworm on stomatal conductance on some of the sample days.

Root architecture can be affected by drought (Grzesiak et al. 2014) and insect feeding (Oleson et al. 2005), so root complexity (fractal dimensions) was also measured in response to drought and western corn rootworm feeding in 2012 and 2013. The fractal dimension value was reduced by the severe drought in 2012, but was increased by the drought treatment in 2013, which was less severe and developed later in the season (Table 6, Fig. 32). In contrast to some of the other hybrids, Bt+AQUAmax retained most of its root complexity even under western corn rootworm infestation and the severe drought of 2012 (Fig. 31). This confirmed that drought-tolerant maize hybrids had a greater root complexity under drought, which presumably resulted in an increased ability to take up water and nutrients.

The infestation levels chosen in the initial two years of the experiment were relatively moderate in order to evaluate the effect of drought on western corn rootworm development and beetle emergence. A moderate level of infestation ensured that density-dependent mortality of western corn rootworm larvae would not occur (Hibbard et al. 2010). Under these conditions, the number of beetles recovered was impacted by drought level, maize line, infestation level, and the interactions of drought × maize line and infestation level × maize lines (Table 5). The number of larvae recovered in 2013 was not impacted by drought (Table 6, Fig. 33A), but larval head capsule width was negatively impacted (Table 6, Fig. 33B). Agosti et al. (2009) found that the head capsule width of western corn rootworm adults collected from irrigated fields was greater than from dry fields. The significant effect of drought × maize line on beetle emergence was demonstrated by a dramatic drop in survival on B73×Mo17 with drought present versus when B73×Mo17 was well-watered (Fig. 34B). Mortality must have occurred between larval sampling and beetle emergence. Reduced larval health under drought is implied by their reduced weight (Fig. 33C).

In summary, both drought and western corn rootworm significantly impacted many of the agronomic traits of maize including yield, root complexity, leaf water potential, and stomatal conductance. Interactions between drought and western corn rootworm were less than predicted and did not support our hypothesis that drought and western corn rootworm negatively and synergistically affect maize growth and performance. Riedell and Kim (1992) documented that western corn rootworm larvae usually feed on the cortex and not on the vascular system, perhaps accounting for why the effect of rootworm damage on maize water relations was not nearly as strong as the effects of drought in this study. The magnitude of the effect of drought relative to the effect of western corn rootworm infestation varied depending on which factor was evaluated, but in general, drought had a greater negative impact on maize growth factors (Chapter 2, Chapter 3 Tables 2-6) and yield. In order to see the catastrophic effects of rootworm and drought that were seen in Tinsley et al. (2015), it is likely that severe and prolonged drought stress must be combined with very high western corn rootworm infestation levels. The infestation level used in the current study was apparently insufficient to see this effect in 2012, when drought stress was prolonged and severe. In 2014, we employed an extreme level of western corn rootworm pressure, but the effect of drought was not severe and did not even significantly impact yield that year when assessed as an independent stressor (Table 7).

Although we rejected our hypothesis that if drought and rootworms both occur simultaneously, there will be a synergistic negative effect on maize performance, Chapter 2 and the current chapter are of importance to maize breeders, entomologists, and growers because we have documented that under our conditions, the interaction was not significant. Growers should continue to protect maize from drought and rootworm as best as possible. Drought will continue to be one of the most important factors affecting maize yield and maize breeders, entomologists, and growers need to recognize drought as a more important problem for maize yield than western corn rootworm.

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Table 4. Monthly precipitation and average air temperature from May to September in 2012, 2013, and 2014.

Month	Total Precipitation mm/month			Average Temperature ^{o}C/month		
	2012	**2013**	**2014**	**2012**	**2013**	**2014**
May	25.91	259.08	44.45	21.8	17.9	19.5
June	41.66	36.58	149.6	25.3	23.3	23.6
July	21.85	37.08	44.45	29.7	24.7	23.4
August	46.74	56.64	85.09	25.9	24.3	25.1
September	43.69	68.59	164.33	19.7	22.0	19.8
Average	35.97	91.60	97.58	24.5	22. 4	22.28

Table 5. Analysis of variance for data of 2012 growing season.

Factor	Effect	*DF*	*F value*	*Pr > F*
Yield	**Moisture**	1,10	166.05	<.0001
	Infestation	1,90	9.68	0.0025
	Moisture × Infestation	1,90	3.89	0.0518
	Maize lines	4,90	3.47	0.0110
	Moisture × Maize lines	4,90	1.96	0.1072
	Infestation × Maize lines	4,90	0.90	0.4668
	Moisture × Infestation × Maize lines	4,90	2.01	0.1001
Leaf Water Potential	**Moisture**	1,6	2.47	0.1675
	Infestation	1,6	0.04	0.8413
	Moisture × Infestation	1,6	2.48	0.1667
	Day	3,182	41.23	<.0001
	Moisture × Day	3,182	11.92	<.0001
	Infestation × Day	3,182	6.51	0.0003
	Moisture × Infestation × Day	3,182	0.95	0.4183
	Maize lines	4,46	2.70	0.0419
	Moisture × Maize lines	4,46	1.35	0.2675
	Infestation × Maize lines	4,46	0.51	0.7293
	Moisture × Infestation × Maize lines	4,46	1.04	0.3970
	Maize lines × Day	12,182	1.65	0.0824
	Moisture × Maize lines × Day	12,182	0.58	0.8580
	Infestation × Maize lines × Day	12,182	1.94	0.0321
	Moisture × Infestation × Maize lines × Day	12,182	1.39	0.1753
Stomatal Conductance	**Moisture**	1,6	1.56	0.2576
	Infestation	1,6	1.02	0.3524
	Moisture × Infestation	1,6	0.99	0.3578
	Day	3,177	685.86	<.0001
	Moisture × Day	3,177	45.76	<.0001
	Infestation × Day	3,177	10.70	<.0001
	Moisture × Infestation × Day	3,177	0.79	0.5007
	Maize lines	4,48	0.50	0.7337
	Moisture × Maize lines	448	0.58	0.6809
	Infestation × Maize lines	448	0.65	0.6301
	Moisture × Infestation × Maize lines	448	0.99	0.4236
	Maize lines × Day	12,177	0.64	0.8068
	Moisture × Maize lines × Day	12,177	0.55	0.8805
	Infestation × Maize lines × Day	12,177	1.52	0.1205
	Moisture × Infestation × Maize lines × Day	12,177	0.64	0.8040

Cont. Table 5

Factor	Effect	*DF*	*F value*	*Pr > F*
Root Damage Rating	**Moisture**	1,10	2.80	0.1255
	Infestation	1,90	81.91	<.0001
	Moisture × Infestation	1,90	1.05	0.3080
	Maize lines	4,90	11.65	<.0001
	Moisture × Maize lines	4,90	0.57	0.6824
	Infestation × Maize lines	4,90	9.43	<.0001
	Moisture × Infestation × Maize lines	4,90	0.90	0.4659
Fractal Dimension	**Moisture**	1,10	63.74	<.0001
	Infestation	1,88	6.97	0.0098
	Moisture × Infestation	1,88	0.41	0.5212
	Maize lines	4,88	4.06	0.0046
	Moisture × Maize lines	4,88	0.90	0.4652
	Infestation × Maize lines	4,88	4.21	0.0036
	Moisture × Infestation × Maize lines	4,88	1.64	0.1718
Adult Recovery	**Moisture**	1,10	5.04	0.0487
	Infestation	1,90	88.21	<.0001
	Moisture × Infestation	1,90	5.36	0.0229
	Maize lines	4,90	2.68	0.0365
	Moisture × Maize lines	4,90	0.15	0.9617
	Infestation × Maize lines	4,90	3.33	0.0136
	Moisture × Infestation × Maize lines	4,90	0.66	0.9617

All data were log-transformed to meet normality assumptions

Table 6. Analysis of variance for data of 2013 growing season.

Factor	Effect	*DF*	*F value*	*Pr > F*
Yield	**Moisture**	1,60	160.96	<.0001
	Infestation	1,94	4.26	0.0418
	Moisture × Infestation	1,94	0.45	0.5046
	Maize lines	4,94	5.00	0.0011
	Moisture × Maize lines	4,94	1.44	0.2255
	Infestation × Maize lines	4,94	0.69	0.6007
	Moisture × Infestation × Maize lines	4,94	0.01	0.9997
Leaf Water Potential	**Moisture**	1,6	6.69	0.0414
	Infestation	1,6	5.25	0.0618
	Moisture × Infestation	1,6	0.08	0.7815
	Day	4,240	59.85	<.0001
	Moisture × Day	4,240	6.96	<.0001
	Infestation × Day	4,240	6.39	<.0001
	Moisture × Infestation × Day	4,240	2.17	0.1447
	Maize lines	4,48	0.14	0.0861
	Moisture × Maize lines	4,48	1.45	0.9651
	Infestation × Maize lines	4,48	1.35	0.2333
	Moisture× Infestation × Maize lines	4,48	0.76	0.2643
	Maize lines × Day	16,240	0.95	0.7247
	Moisture × Maize lines × Day	16,240	0.84	0.5149
	Infestation × Maize lines × Day	16,240	0.81	0.6339
	Moisture × Infestation × Maize lines × Day	16,240	1.39	0.6789
Stomatal Conductance	**Moisture**	1,6	18.66	0.0050
	Infestation	1,6	0.91	0.3773
	Moisture × Infestation	1,6	6.17	0.0476
	Day	4,240	84.91	<.0001
	Moisture × Day	4,240	7.93	<.0001
	Infestation × Day	4,240	3.62	0.0070
	Moisture × Infestation × Day	4,240	1.35	0.2521
	Maize lines	4,48	2.42	0.0613
	Moisture × Maize lines	4,48	1.06	0.3856
	Infestation × Maize lines	4,48	0.53	0.7168
	Moisture × Infestation × Maize lines	4,48	2.05	0.1022
	Maize lines × Day	16,240	2.31	0.0036
	Moisture × Maize lines × Day	16,240	1.19	0.2796
	Infestation × Maize lines × Day	16,240	2.09	0.0095
	Moisture × Infestation × Maize lines × Day	16,240	0.57	0.9035

Cont. Table 6

Factor	Effect	*DF*	*F value*	*Pr > F*
Root Damage Rating	**Moisture**	1,60	0.17	0.6932
	Infestation	1,94	61.76	<.0001
	Moisture × Infestation	1,94	0.12	0.7332
	Maize lines	4,94	8.36	<.0001
	Moisture × Maize lines	4,94	4.10	0.0042
	Infestation × Maize lines	4,94	9.52	<.0001
	Moisture × Infestation × Maize lines	4,94	0.54	0.7091
Fractal Dimension	**Moisture**	1,6	185.18	<.0001
	Infestation	1.94	9.86	0.0023
	Moisture × Infestation	1.94	7.27	0.0083
	Maize lines	4,94	4.99	0.0011
	Moisture × Maize lines	4,94	2.52	0.0466
	Infestation × Maize lines	4,94	2.09	0.0888
	Moisture × Infestation × Maize lines	4,94	1.15	0.3365
Larval Recovery	**Moisture**	1,6	0.86	0.3905
	Maize lines	4,24	6.01	0.0017
	Moisture × Maize lines	4, 24	1.02	0.4176
Larval Head Capsule Width	**Moisture**	1,6	14.11	0.0094
	Maize lines	4,21	6.10	0.0020
	Moisture × Maize lines	4,21	1.58	0.2154
Larval Dry Weight	**Moisture**	1,6	20.47	0.0040
	Maize lines	4,20	1.75	0.1785
	Moisture × Maize lines	4,20	0.43	0.7865
Adult Recovery	**Moisture**	1,10	0.03	0.8721
	Infestation	1,90	0.19	0.6646
	Moisture × Infestation	1,90	0.98	0.3256
	Maize lines	4,90	5.63	0.0004
	Moisture × Maize lines	4,90	5.49	0.0005
	Infestation × Maize lines	4,90	2.79	0.0309
	Moisture × Infestation × Maize lines	4,90	2.90	0.0260

All data were log-transformed to meet normality assumptions.

Table 7. Analysis of variance for data of 2014 growing season.

Factor	Effect	*DF*	*F value*	*Pr > F*
Yield	**Moisture**	1,10	0.03	0.8640
	Infestation	2,140	2.58	0.0792
	Moisture × Infestation	2,140	4.75	0.0101
	Maize lines	4,140	4.23	0.0029
	Moisture × Maize lines	4,140	5.40	0.0005
	Infestation × Maize lines	8,140	0.76	0.6350
	Moisture × Infestation × Maize lines	8,140	0.70	0.6942
Leaf Water Potential	**Moisture**	1,6	52.78	0.0003
	Infestation	2,12	10.80	0.0021
	Moisture × Infestation	2,12	3.81	0.0524
	Day	3,270	185.80	<.0001
	Moisture × Day	6,270	45.00	<.0001
	Infestation × Day	6,270	2.32	0.0334
	Moisture × Infestation × Day	6,270	0.92	0.4784
	Maize lines	4,72	5.41	0.0007
	Moisture × Maize lines	4,72	0.46	0.7625
	Infestation × Maize lines	8,72	2.26	0.0327
	Moisture× Infestation × Maize lines	4,48	2.40	0.0234
	Maize lines × Day	12,270	1.61	0.0880
	Moisture × Maize lines × Day	12,270	0.66	0.7905
	Infestation × Maize lines × Day	12,270	1.13	0.3079
	Moisture × Infestation × Maize lines × Day	12,270	0.62	0.9157
Stomatal Conductance	**Moisture**	1,6	33.28	0.0012
	Infestation	2,12	0.83	0.4577
	Moisture × Infestation	2,13	0.46	0.6408
	Day	3,269	50.21	<.0001
	Moisture × Day	3,269	32.97	<.0001
	Infestation × Day	3,269	4.02	0.0007
	Moisture × Infestation × Day	3,269	13.95	<.0001
	Maize lines	4,72	4.70	0.0020
	Moisture × Maize lines	4,72	2.98	0.0247
	Infestation × Maize lines	4,72	0.85	0.5583
	Moisture × Infestation × Maize lines	4,72	0.48	0.8641
	Maize lines × Day	12,269	1.99	0.0253
	Moisture × Maize lines × Day	12,269	1.17	0.3048
	Infestation × Maize lines × Day	24,269	0.36	0.9981
	Moisture × Infestation × Maize lines × Day	24,269	0.81	0.7283
Root Damage Rating	**Moisture**	1,21	0.03	0.8728
	Infestation	2,140	105.15	<.0001
	Moisture × Infestation	2,140	2.17	0.1183
	Maize lines	4,140	41.06	<.0001
	Moisture × Maize lines	4,140	1.55	0.1920
	Infestation × Maize lines	8,140	11.31	<.0001
	Moisture × Infestation × Maize lines	8,140	0.69	0.7041

All data were log-transformed to meet normality assumptions.

Fig. 9. Rain-out shelter used to prevent precipitation reaching the (Drought +) plots. A) cover retracted, B) cover extended.

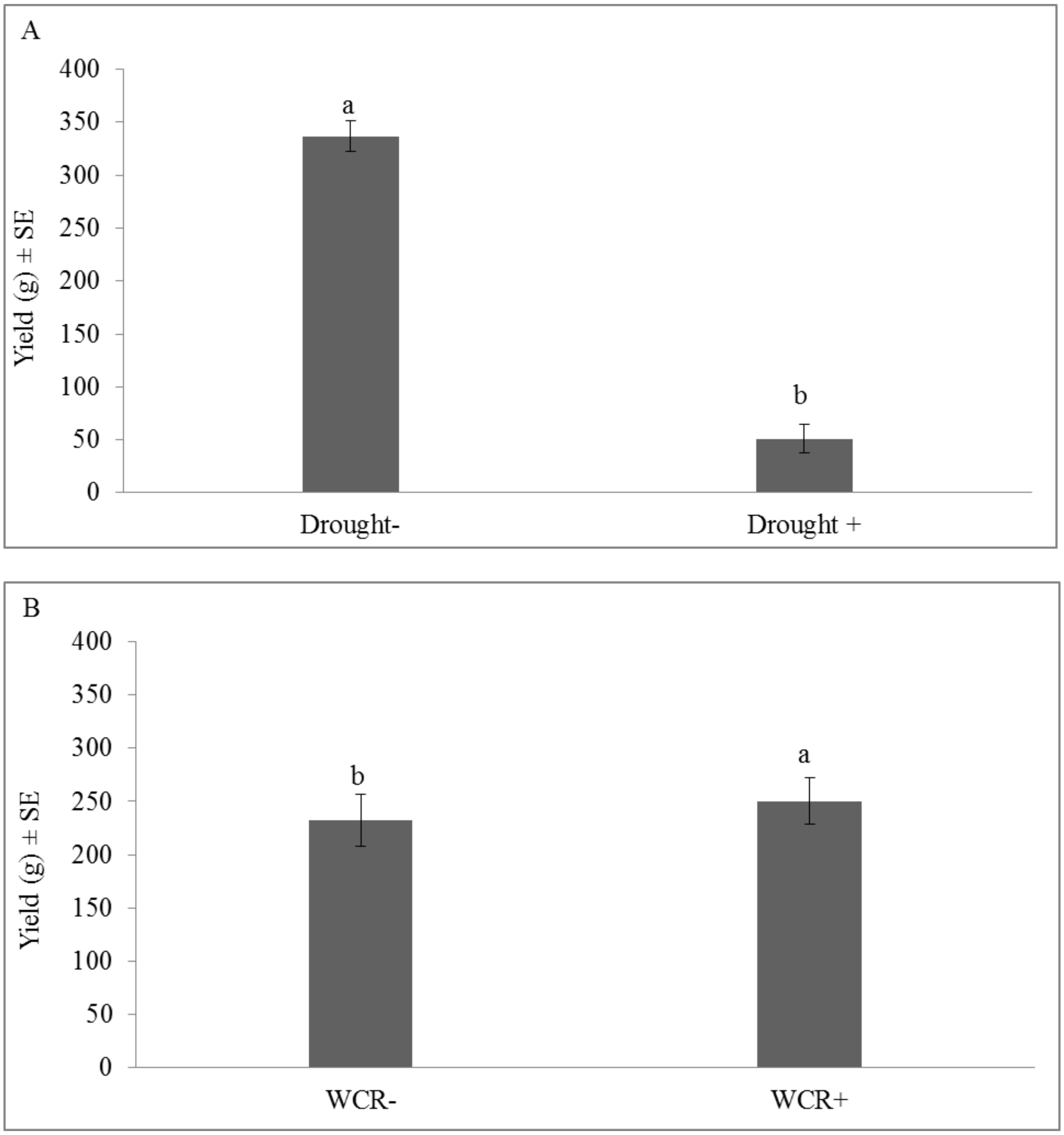


Fig. 10. The average total dry weight of maize kernels obtained from the largest three ears per plot for the 2012 growing season. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)] data. (A) Different letters indicate significant differences between soil moisture levels ($P \leq 0.05$). (B) Different letters indicate significant differences between western corn rootworm infestation levels ($P \leq 0.05$).

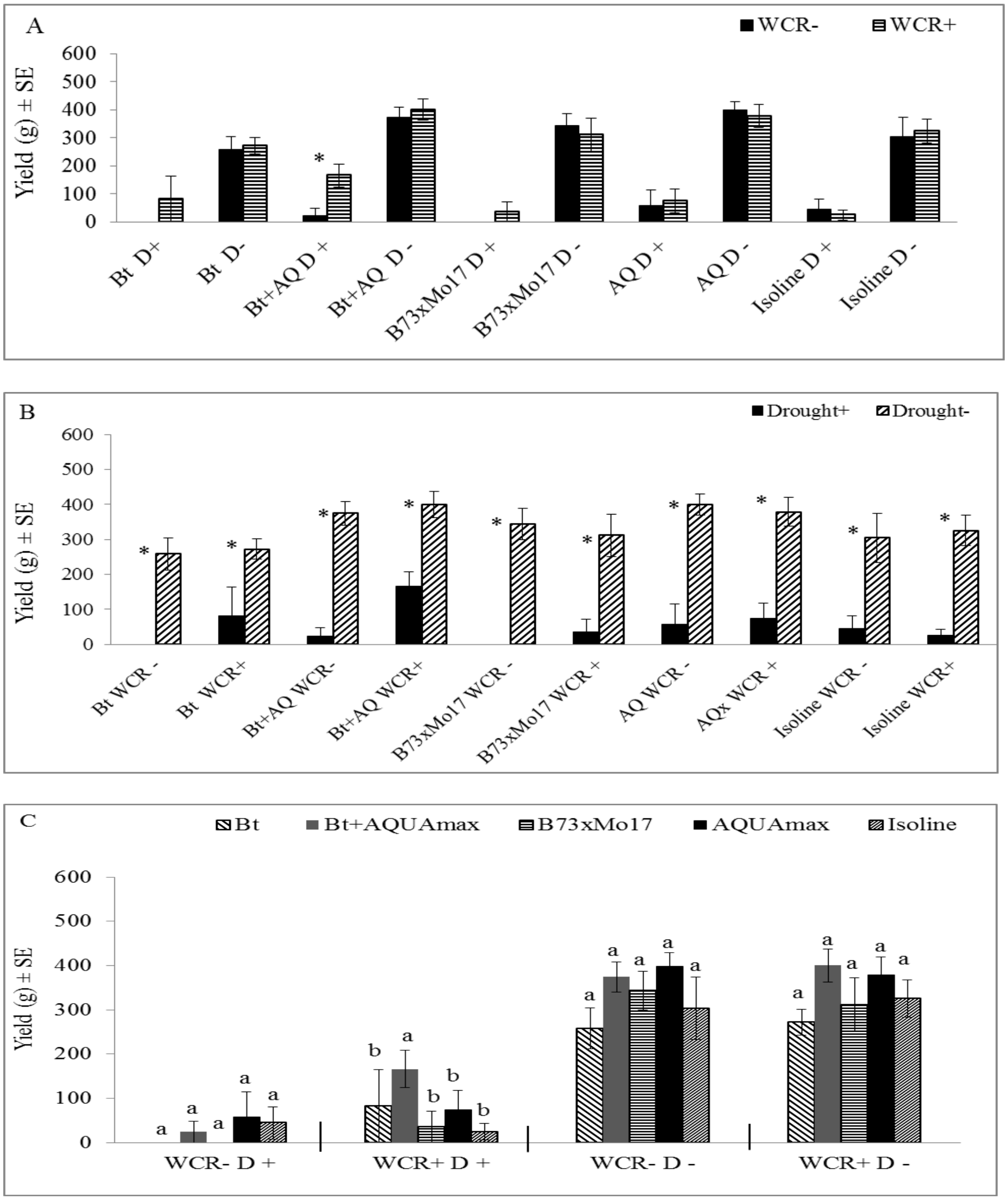


Fig. 11. The average total dry weight of maize kernels obtained from the largest three ears per plot for the 2012 growing season. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)] data. (A) Asterisks indicate significant differences between western corn rootworm infestation levels keeping maize line and soil moisture level constant ($P \leq 0.05$). (B) Asterisks indicate significant differences between soil moisture levels keeping maize line and western corn rootworm infestation level constant ($P \leq 0.05$). (C) Different letters indicate significant differences between maize line keeping infestation level and soil moisture level constant ($P \leq 0.05$). Abbreviations: (D-) well watered treatment; (D+) drought treatment; (Bt+AQ) Bt+AQUAmax; and (AQ) AQUAmax.

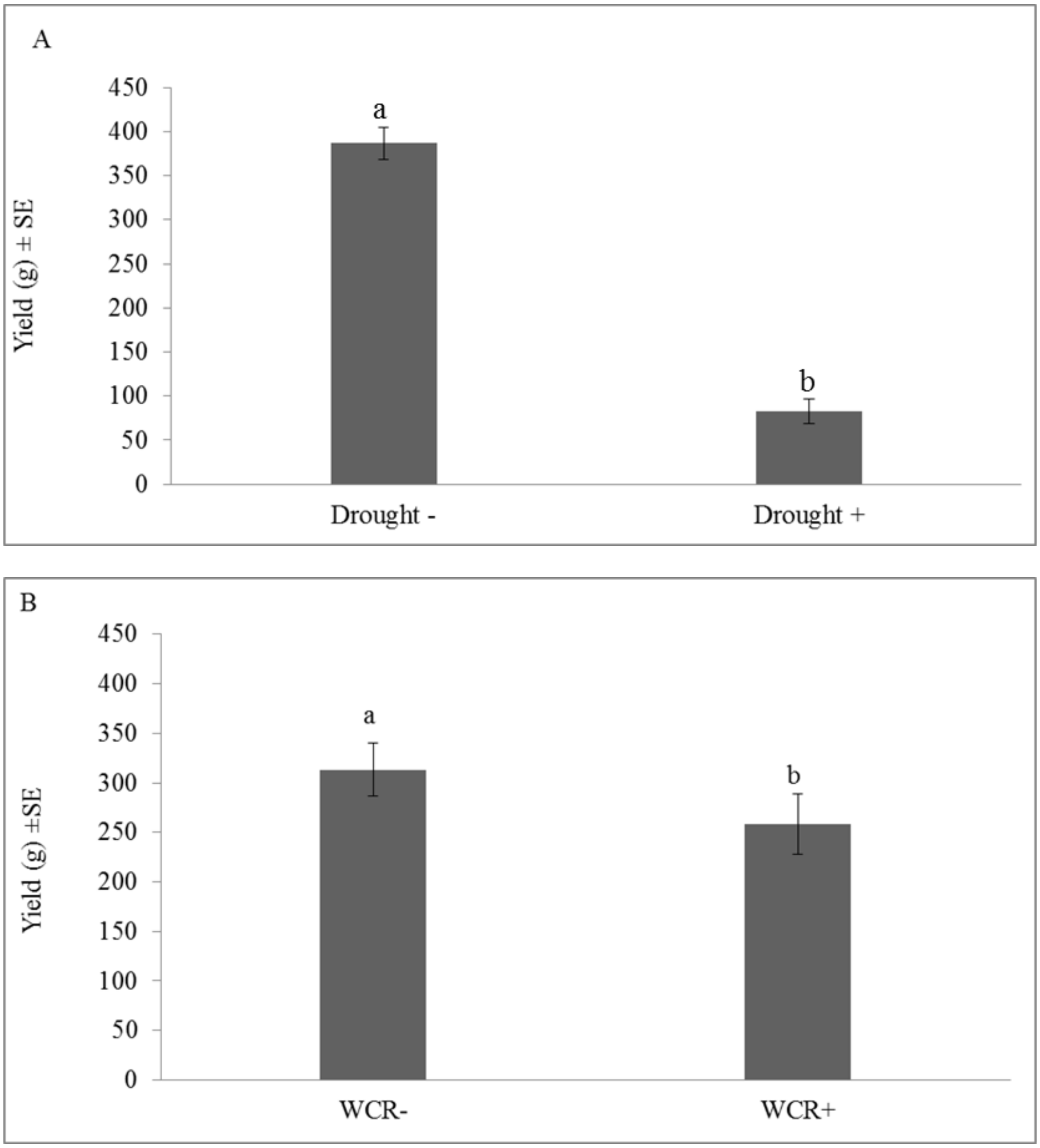


Fig. 12. The average total dry weight of maize kernels obtained from the largest three ears per plot for the 2013 growing season**.** Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)] data. (A) Different letters indicate significant differences between soil moisture levels ($P \leq 0.05$). (B) Different letters indicate significant differences between western corn rootworm infestation levels ($P \leq 0.05$).

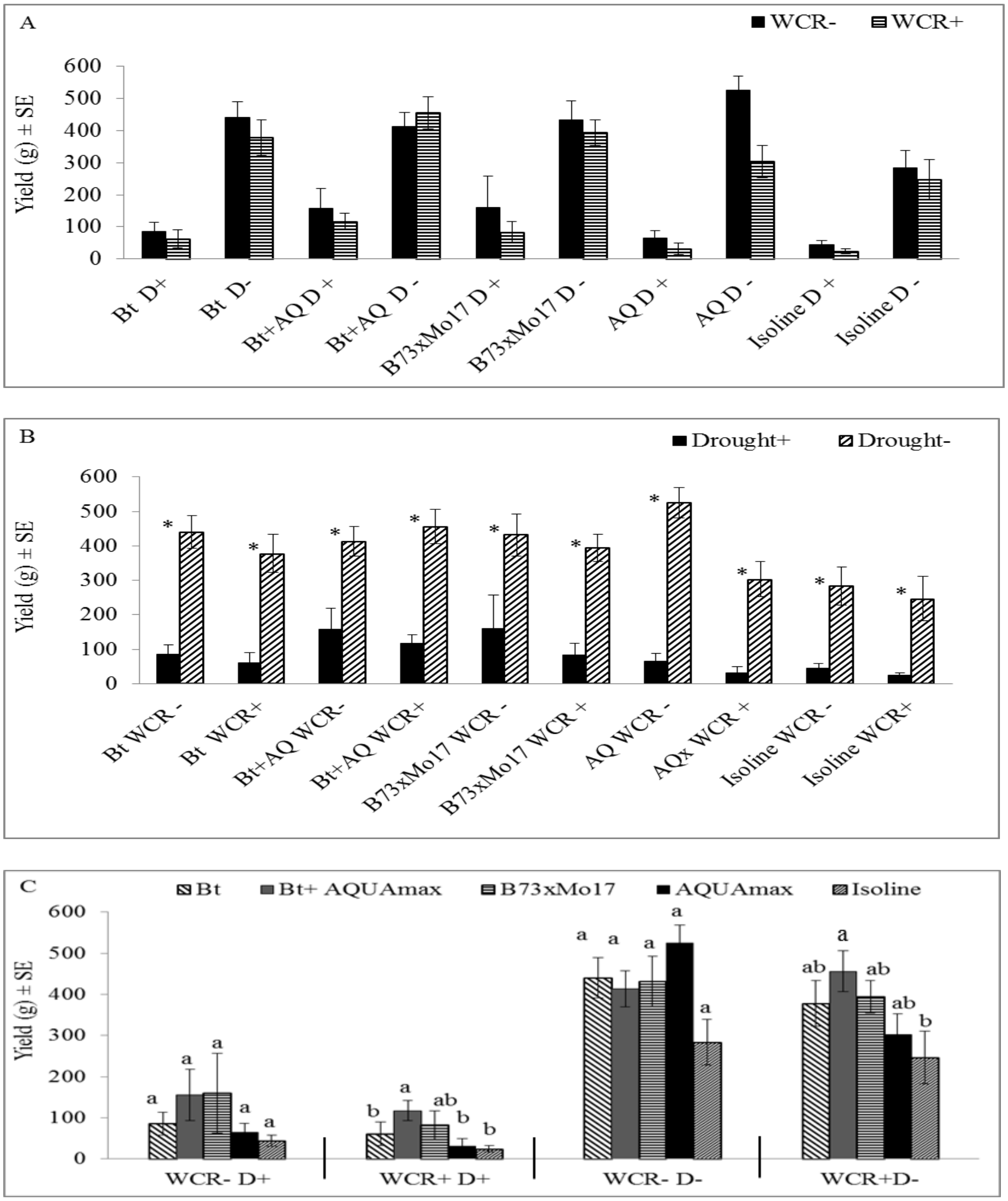


Fig. 13. The average total dry weight of maize kernels obtained from the largest three ears per plot for the 2013 growing season. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)] data. (A) Asterisks indicate significant differences between western corn rootworm infestation levels keeping maize line and soil moisture level constant ($P \leq 0.05$). (B) Asterisks indicate significant differences between soil moisture levels keeping maize line and western corn rootworm infestation level constant ($P \leq 0.05$). (C) Different letters indicate significant differences between maize lines keeping infestation level and soil moisture level constant ($P \leq 0.05$). Abbreviations: (D-) well watered treatment; (D+) drought treatment; (Bt+AQ) Bt+AQUAmax; and (AQ) AQUAmax.

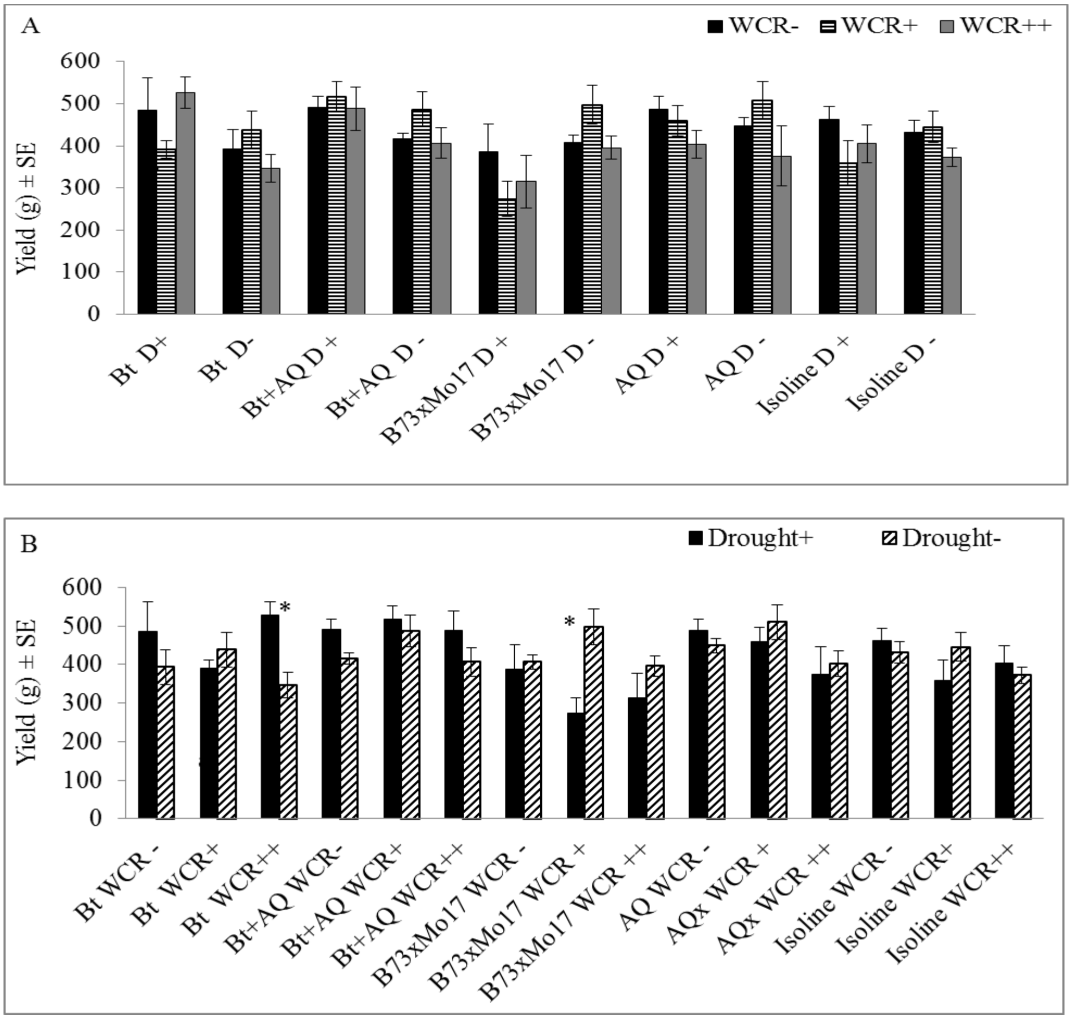
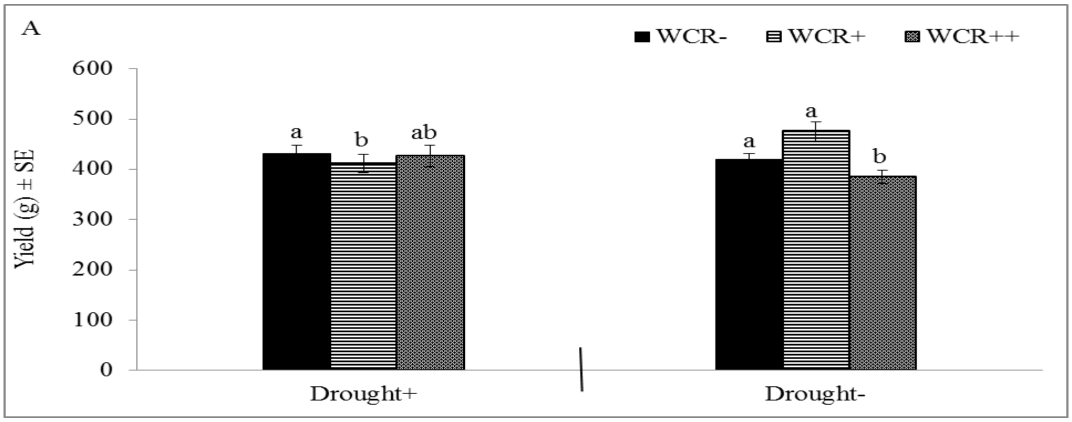
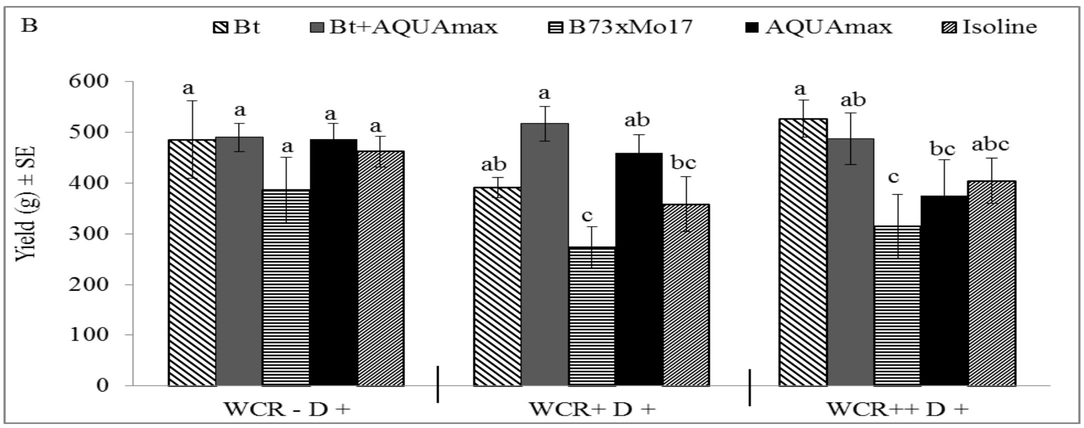


Fig. 14. The average total dry weight of maize kernels obtained from the largest three ears per plot for the 2014 growing season. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)] data. (A) Asterisks indicate significant differences between western corn rootworm infestation levels keeping maize line and soil moisture level constant ($P \leq 0.05$). (B) Asterisks indicate significant differences between soil moisture levels keeping maize line and western corn rootworm infestation level constant ($P \leq 0.05$). Abbreviations: (D-) well watered treatment; (D+) drought treatment; (Bt+AQ) Bt+AQUAmax; and (AQ) AQUAmax.





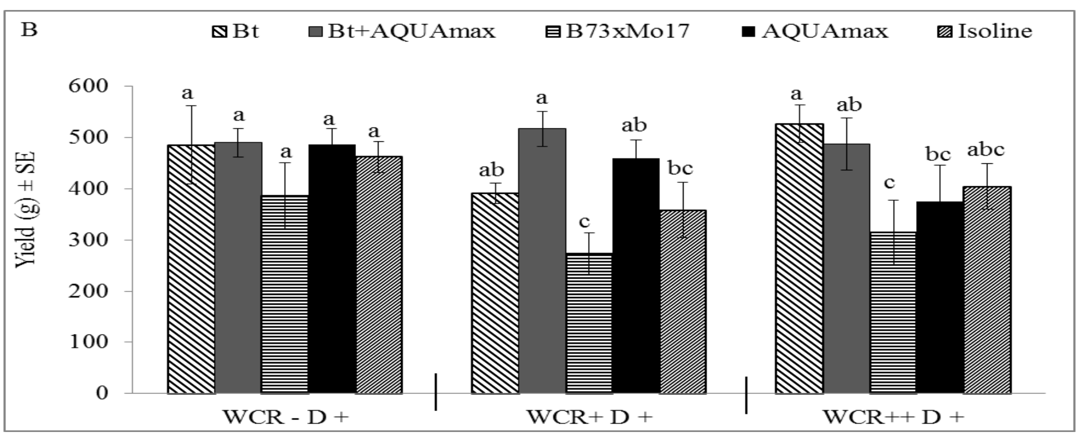


Fig. 15. The average total dry weight of maize kernels obtained from the largest three ears per plot for the 2014 growing season. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)] data. (A) Different letters indicate significant differences between western corn rootworm infestation levels keeping soil moisture level constant ($P \leq 0.05$). (B) Different letters indicate significant differences between maize lines keeping western corn rootworm infestation level and soil moisture level constant ($P \leq 0.05$). (C) Different letters indicate significant difference between maize lines keeping western corn rootworm infestation level and soil moisture level constant ($P \leq 0.05$).

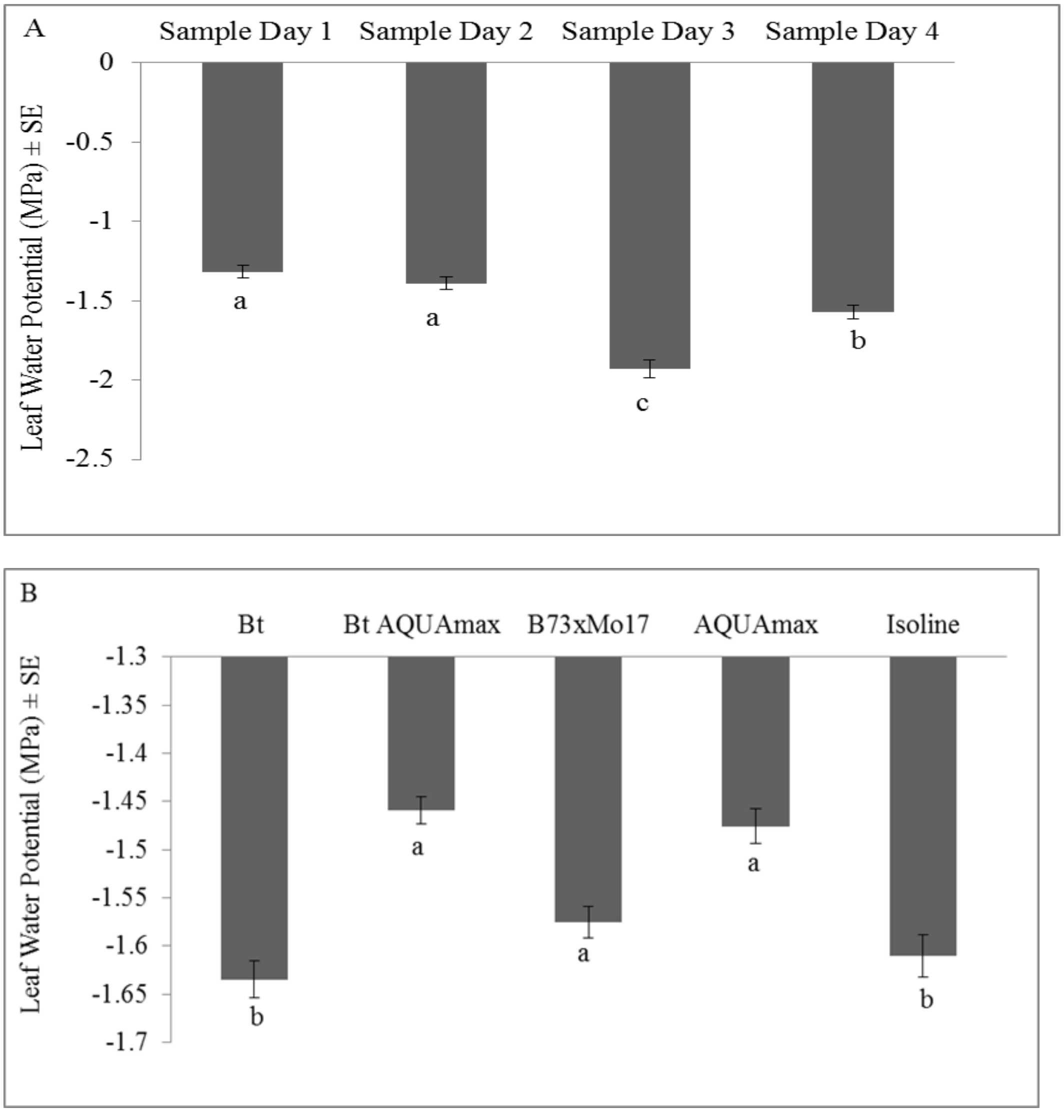


Fig. 16. The average leaf water potential from one leaf /plant/plot for the 2012 growing season. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)] data. (A) Different letters indicate significant differences between sample days ($P \leq 0.05$). (B) Different letters indicate significant differences between maize lines ($P \leq 0.05$).

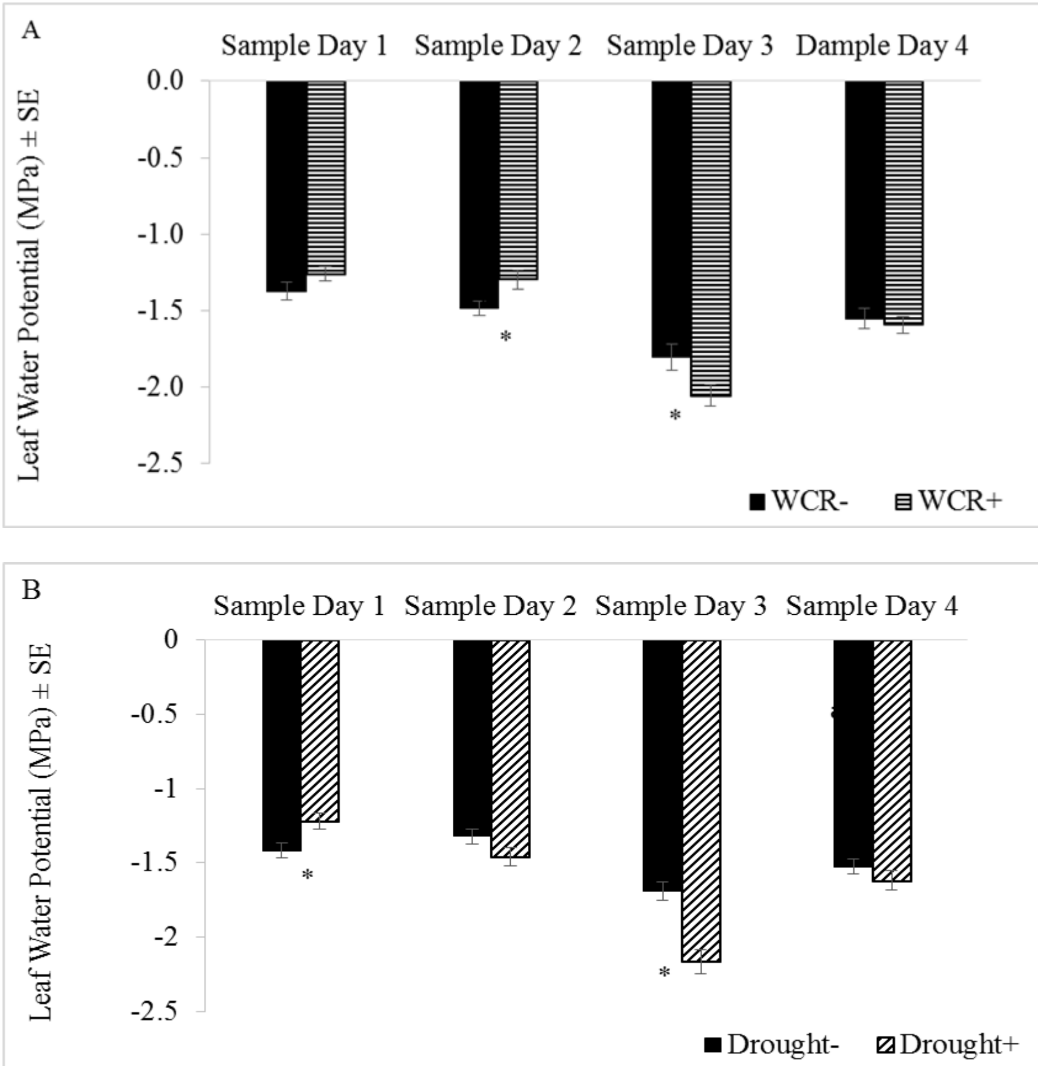


Fig. 17. The average leaf water potential from one leaf/plant/plot for the 2012 growing season. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)] data. (A) Asterisks indicate significant differences between western corn rootworm infestation levels within each sample day ($P \leq 0.05$). (B) Asterisks indicate significant difference between soil moisture levels within each sample day ($P \leq 0.05$).

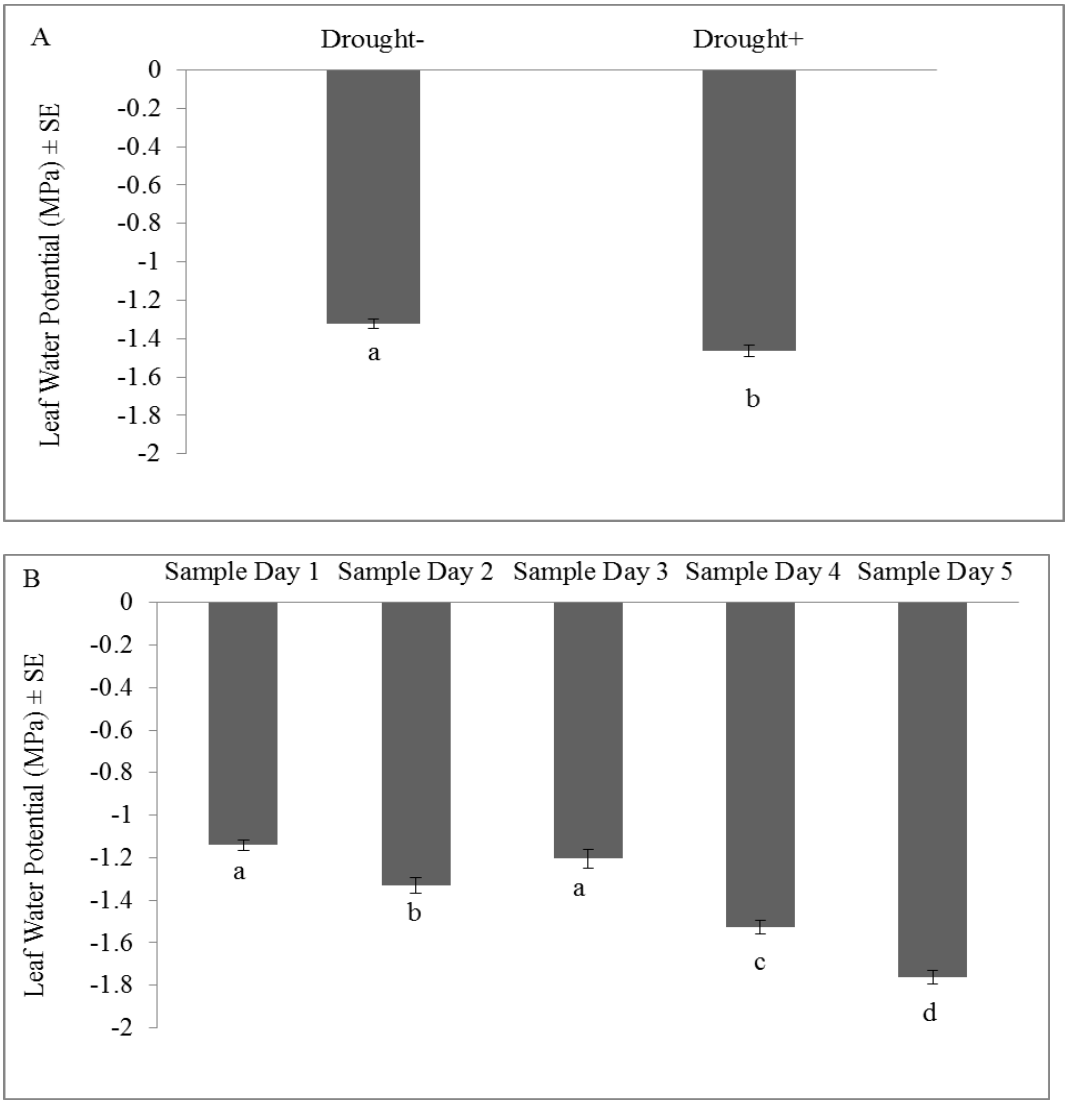


Fig. 18. The average leaf water potential for maize plants from one leaf/plant/plot for the 2013 growing season. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)] data. (A) Different letters indicate significant difference between soil moisture levels ($P \leq 0.05$). (B) Different letters indicate significant differences between sample days ($P \leq 0.05$).

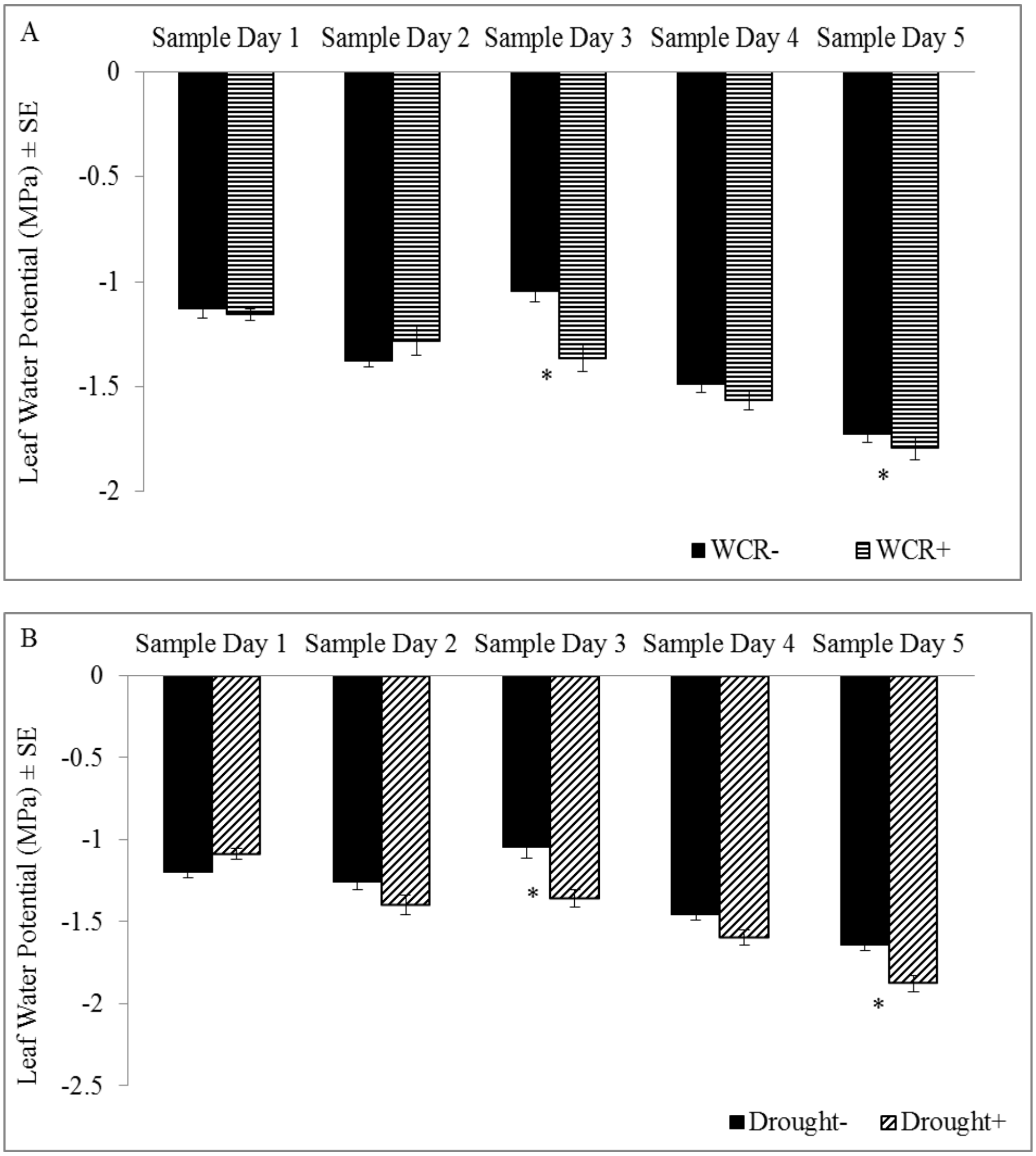


Fig. 19. The average leaf water potential for maize plants from one leaf/plant/plot for the 2013 growing season. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)] data. (A) Asterisks indicate significant differences between western corn rootworm infestation levels within each sample day ($P \leq 0.05$). (B) Asterisks indicate significant difference between soil moisture levels within each sample day ($P \leq 0.05$).

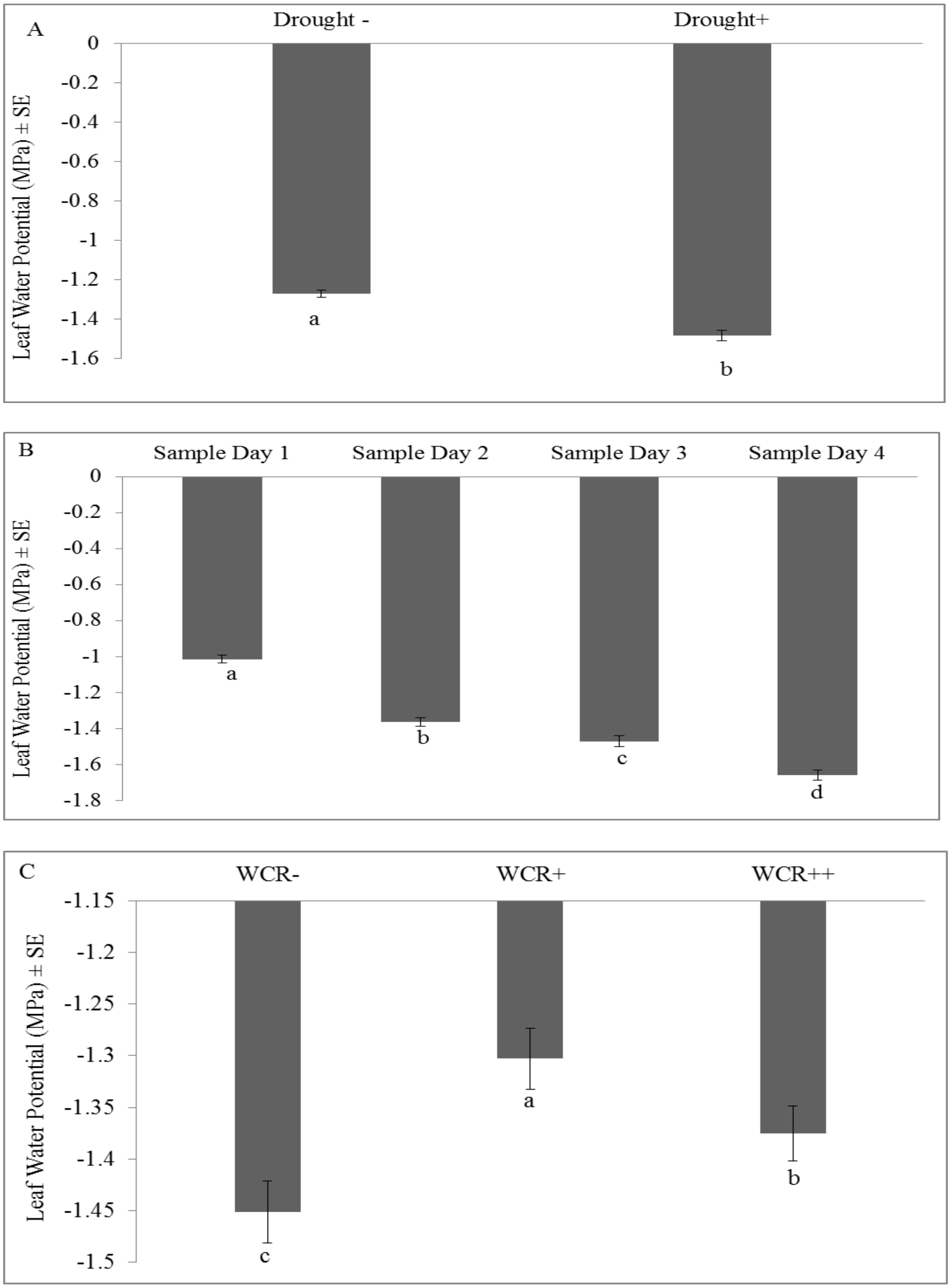


Fig. 20. The average leaf water potential for maize plants from one leaf/plant/plot for the 2014 growing season. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)] data. (A) Different letters indicate significant difference between soil moisture levels ($P \leq 0.05$). (B) Different letters indicate significant differences between sample days ($P \leq 0.05$). (C) Different letters indicate significant differences between western corn rootworm infestation levels ($P \leq 0.05$).

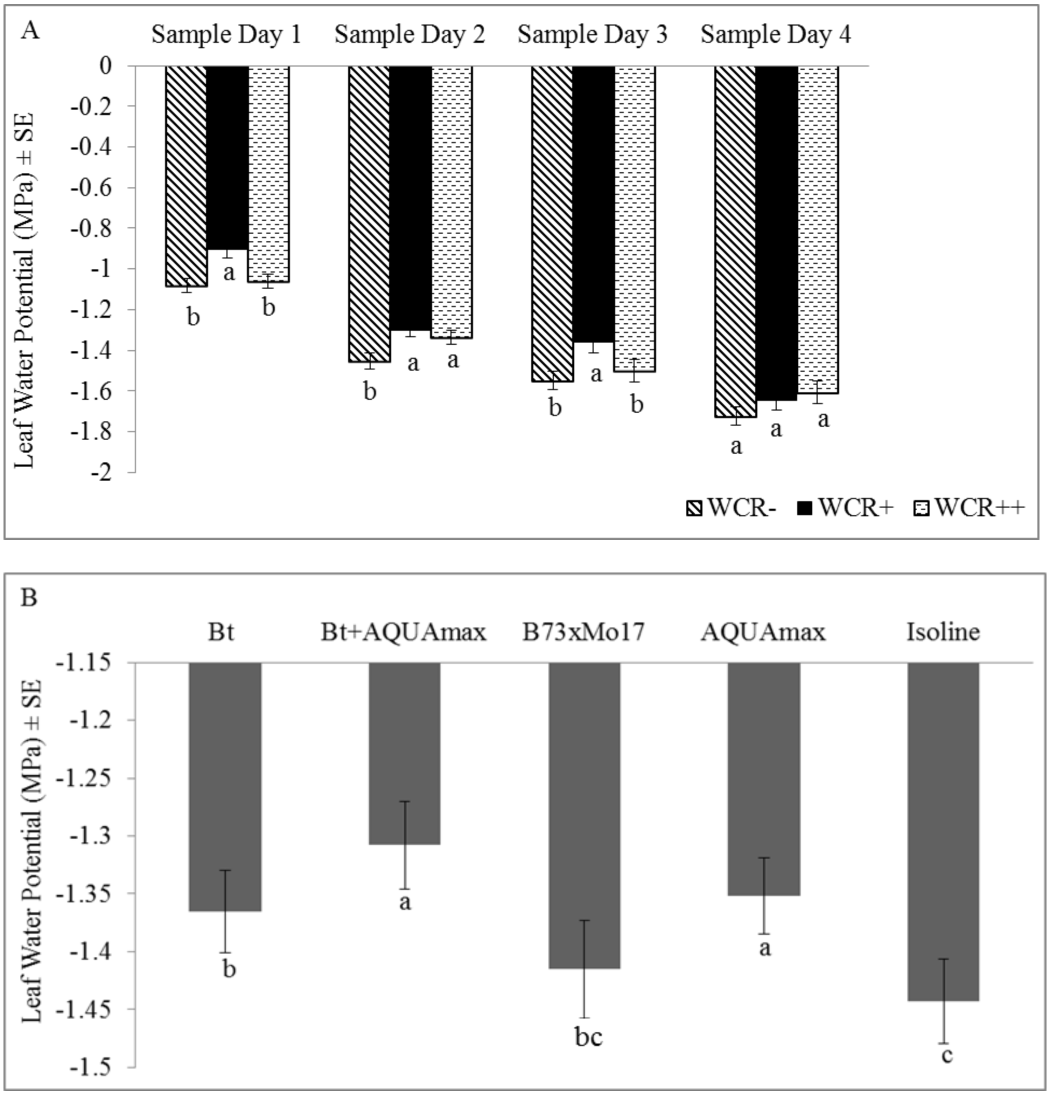


Fig. 21. The average leaf water potential for maize plants from one leaf/plant/plot for the 2014 growing season. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)] data. (A) Different letters indicate significant differences between infestation levels within each sample day ($P \leq 0.05$). (B) Different letters indicate significant differences between maize lines ($P \leq 0.05$).

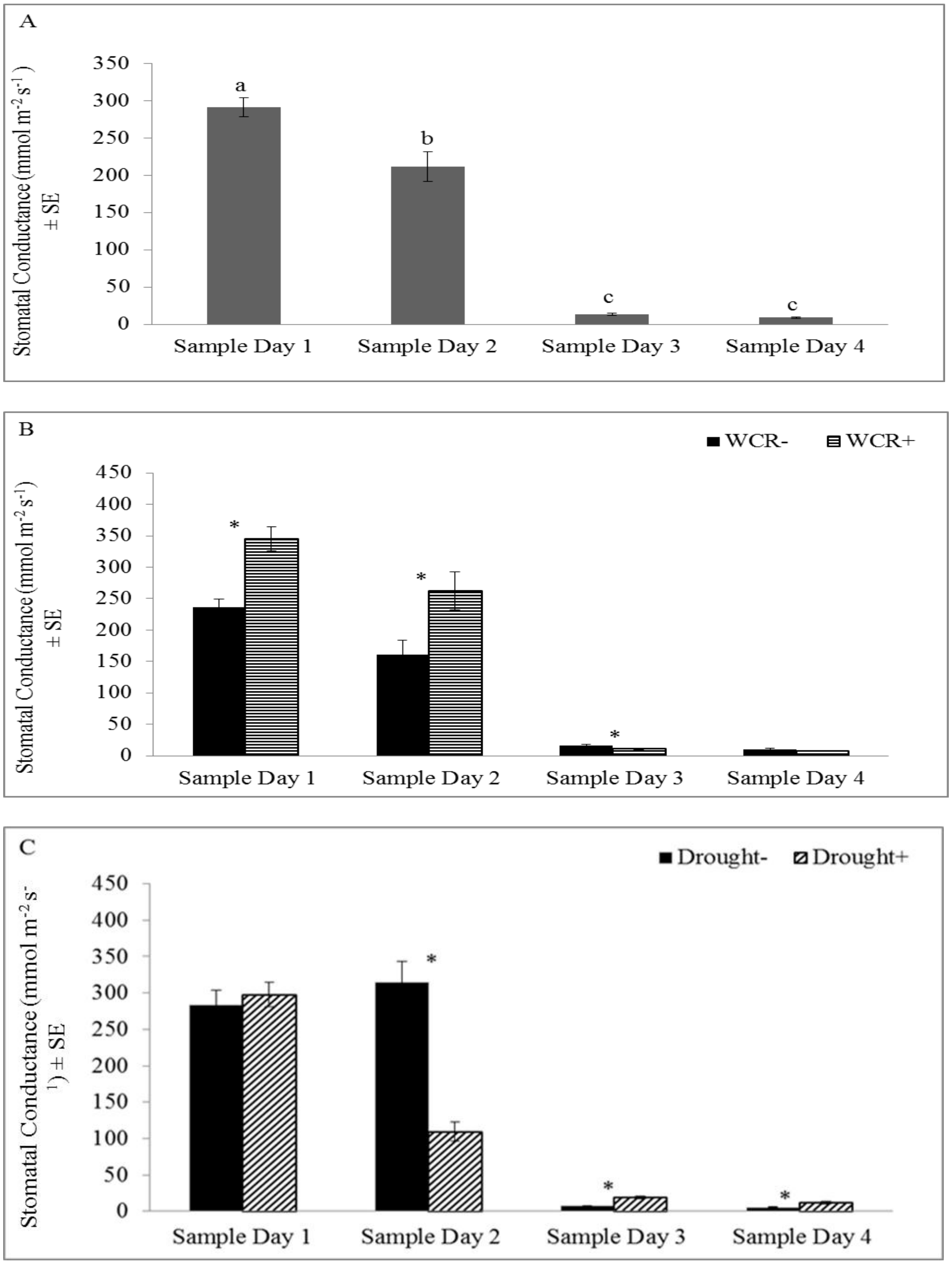
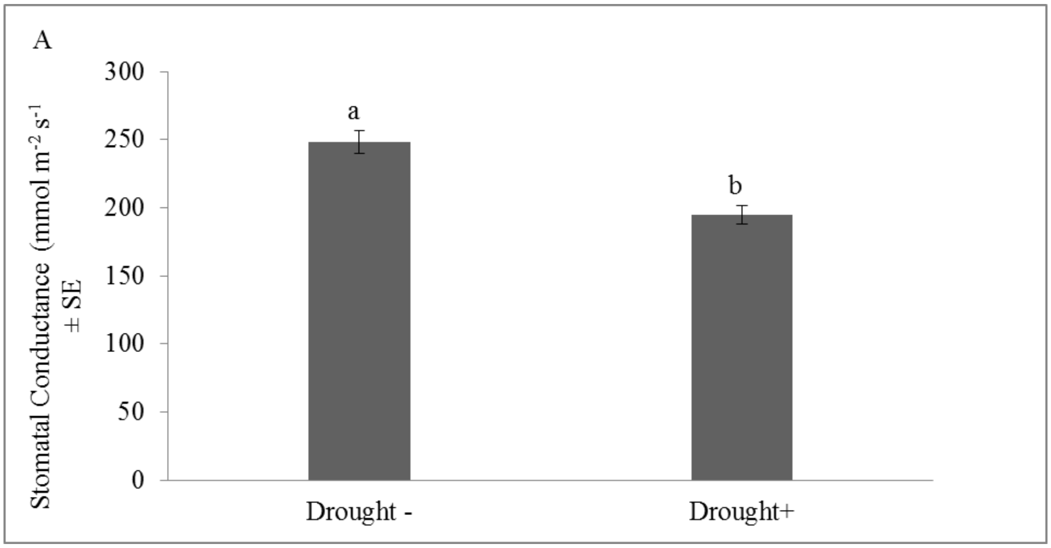


Fig. 22. The average stomatal conductance for maize plants from one leaf/plant/plot for the 2012 growing season**.** Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)] data. (A) Different letters indicate significant differences between sample days ($P \leq 0.05$). (B) Asterisks indicate significant differences between western corn rootworm infestation levels within each sample day ($P \leq 0.05$). (C) Asterisks indicate significant difference between soil moisture levels within each sample day ($P \leq 0.05$).



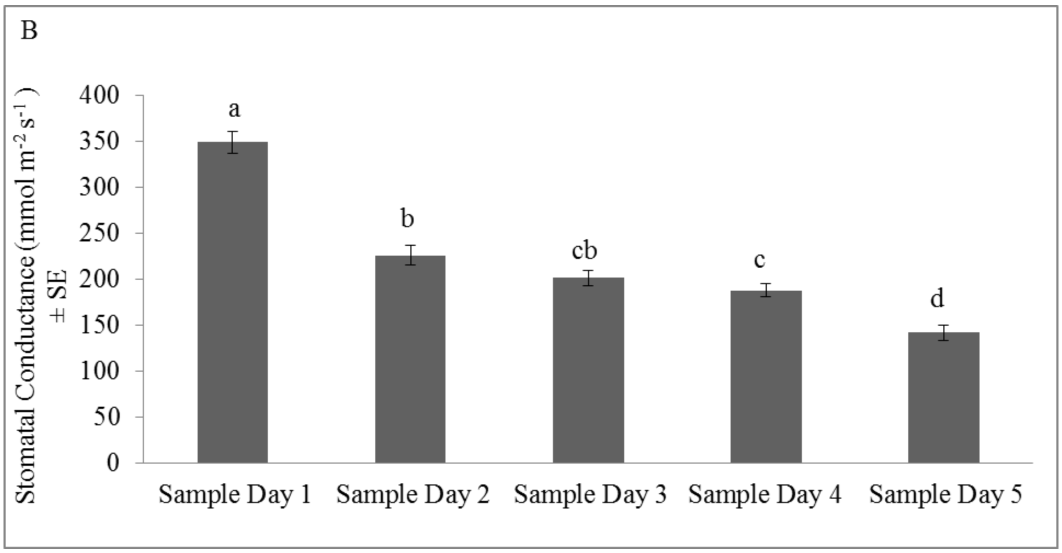


Fig. 23. The average stomatal conductance for maize plants from one leaf/plant/plot for the 2013 growing season. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)] data. (A) Different letters indicate significant difference between soil moisture levels ($P \leq 0.05$). (B) Different letters indicate significant differences between sample days ($P \leq 0.05$).

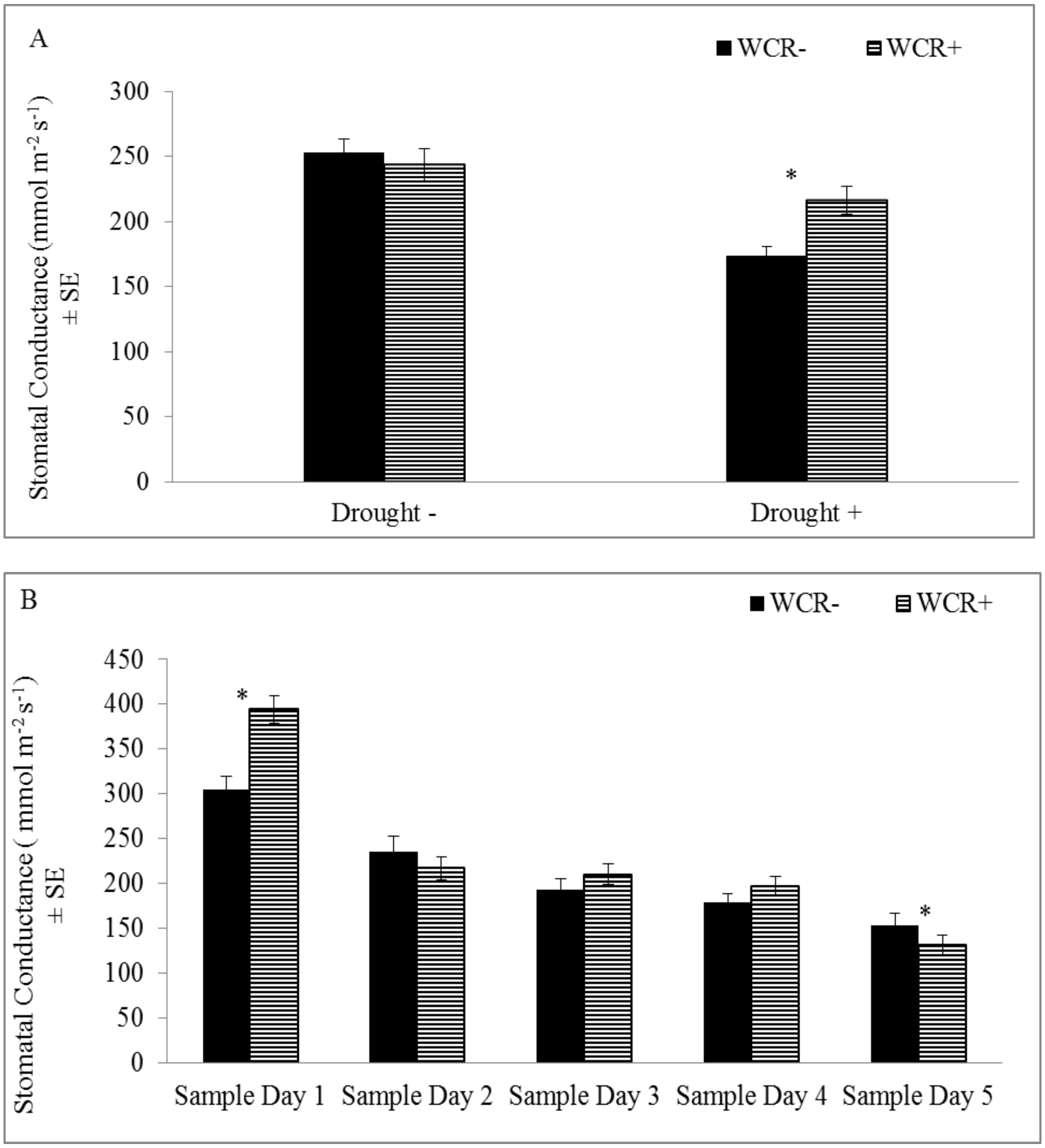


Fig. 24. The average stomatal conductance for maize plants from one leaf/plant/plot for the 2013 growing season. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)] data. (A) Asterisks indicate significant differences between western corn rootworm infestation levels keeping the soil moisture level constant ($P \leq 0.05$). (B) Asterisks indicate significant differences between western corn rootworm infestation levels within each sample day ($P \leq 0.05$).

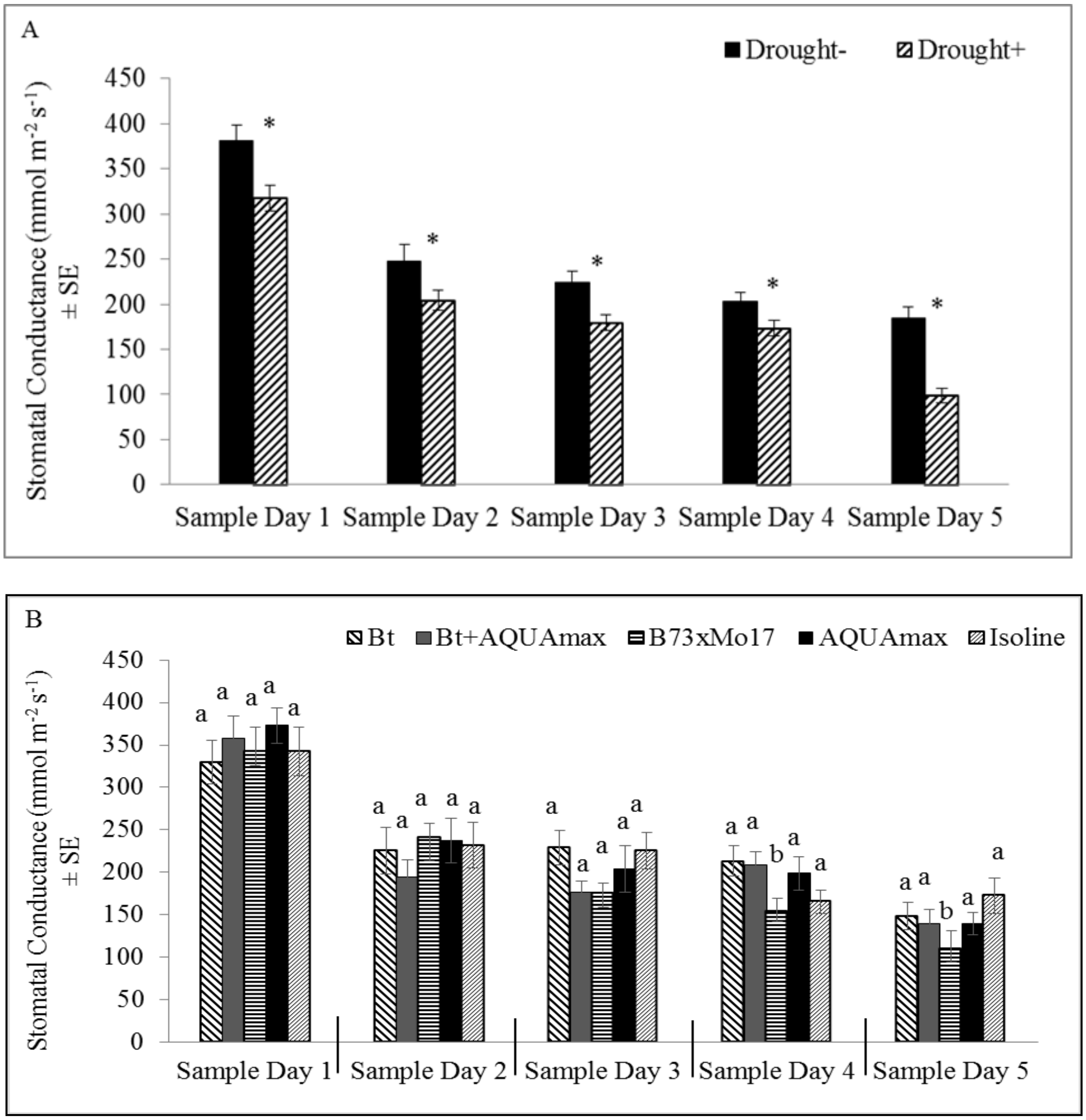


Fig. 25. The average stomatal conductance for maize plants from one leaf/plant/plot for the 2013 growing season. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)] data. (A) Asterisks indicate significant differences between soil moisture levels within each sample day ($P \leq 0.05$). (B) Different letter indicate significant differences between maize lines within each sample day ($P \leq$ 0.05).

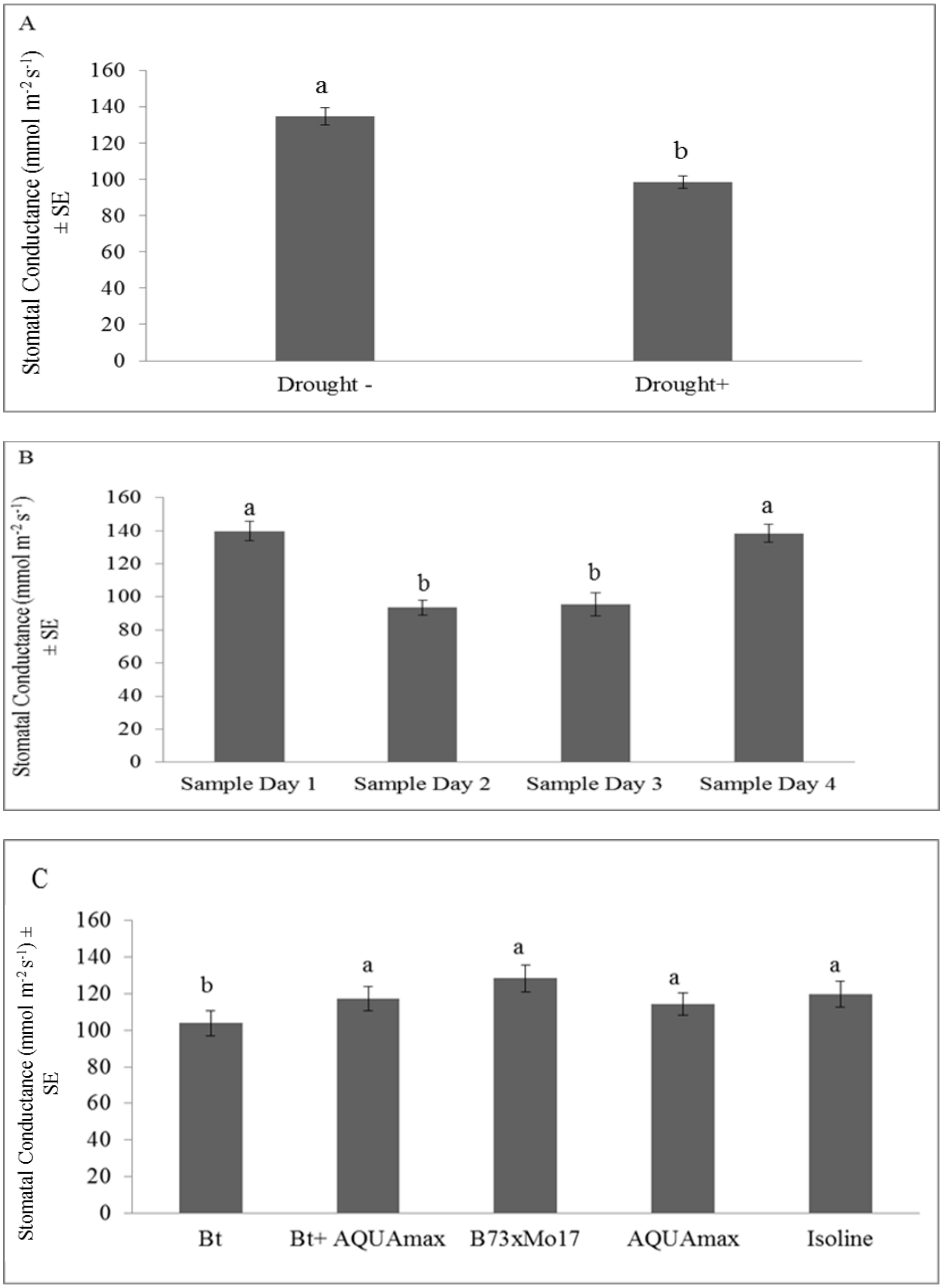
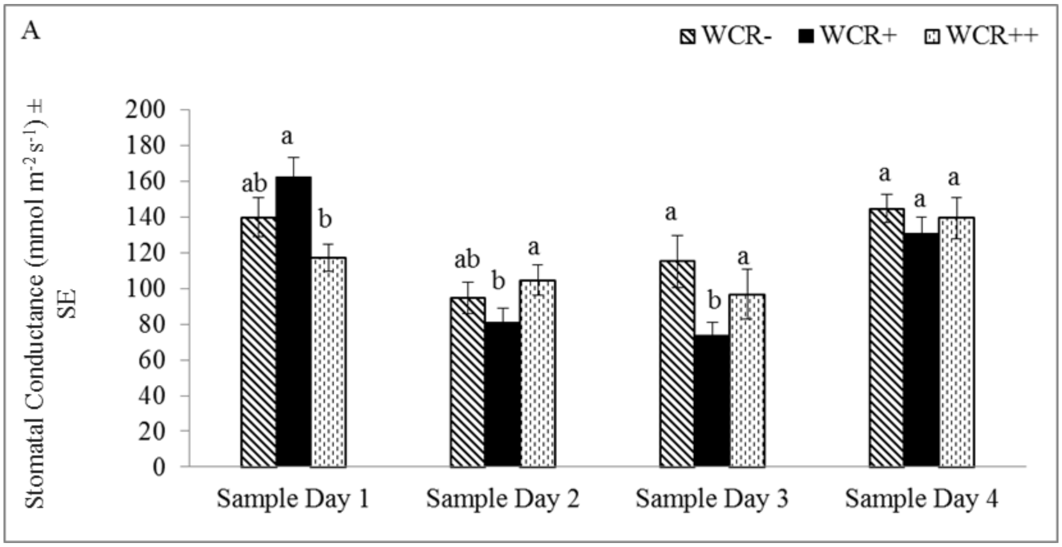


Fig. 26. The average stomatal conductance for maize plants from one leaf/plant/plot for the 2014 growing season. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)] data. (A) Different letters indicate significant differences between drought levels ($P \leq 0.05$). (B) Different letters indicate significant differences between sample days ($P \leq 0.05$). (C) Different letters indicate significant differences between maize lines ($P \leq 0.05$).



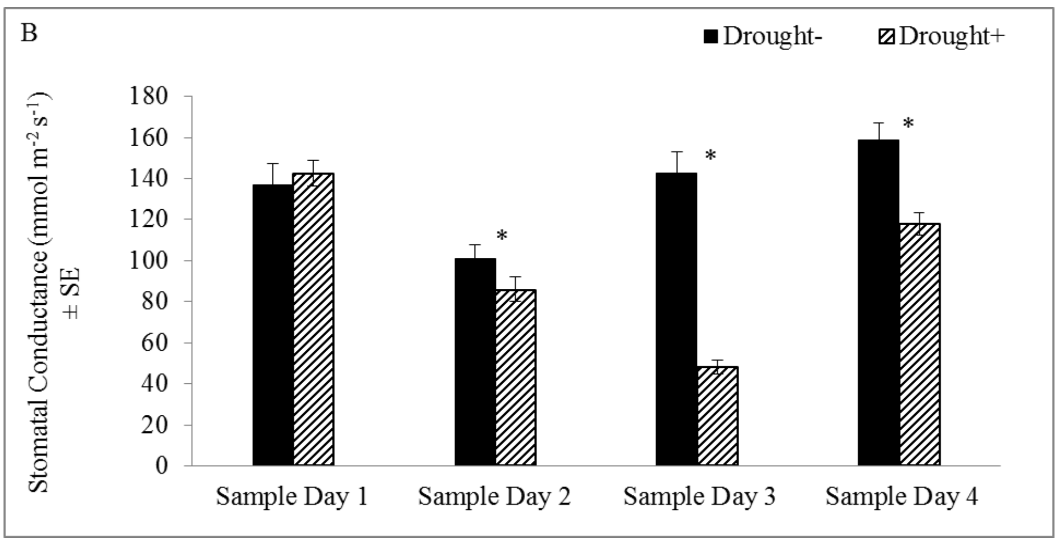


Fig. 27. The average stomatal conductance for maize plants from one leaf/plant/plot for the 2014 growing season. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)] data. (A) Different letters indicate significant differences between western corn rootworm infestation levels within each sample day ($P \leq 0.05$). (B) Asterisks indicate significant differences between soil moisture levels on each sample day ($P \leq 0.05$).

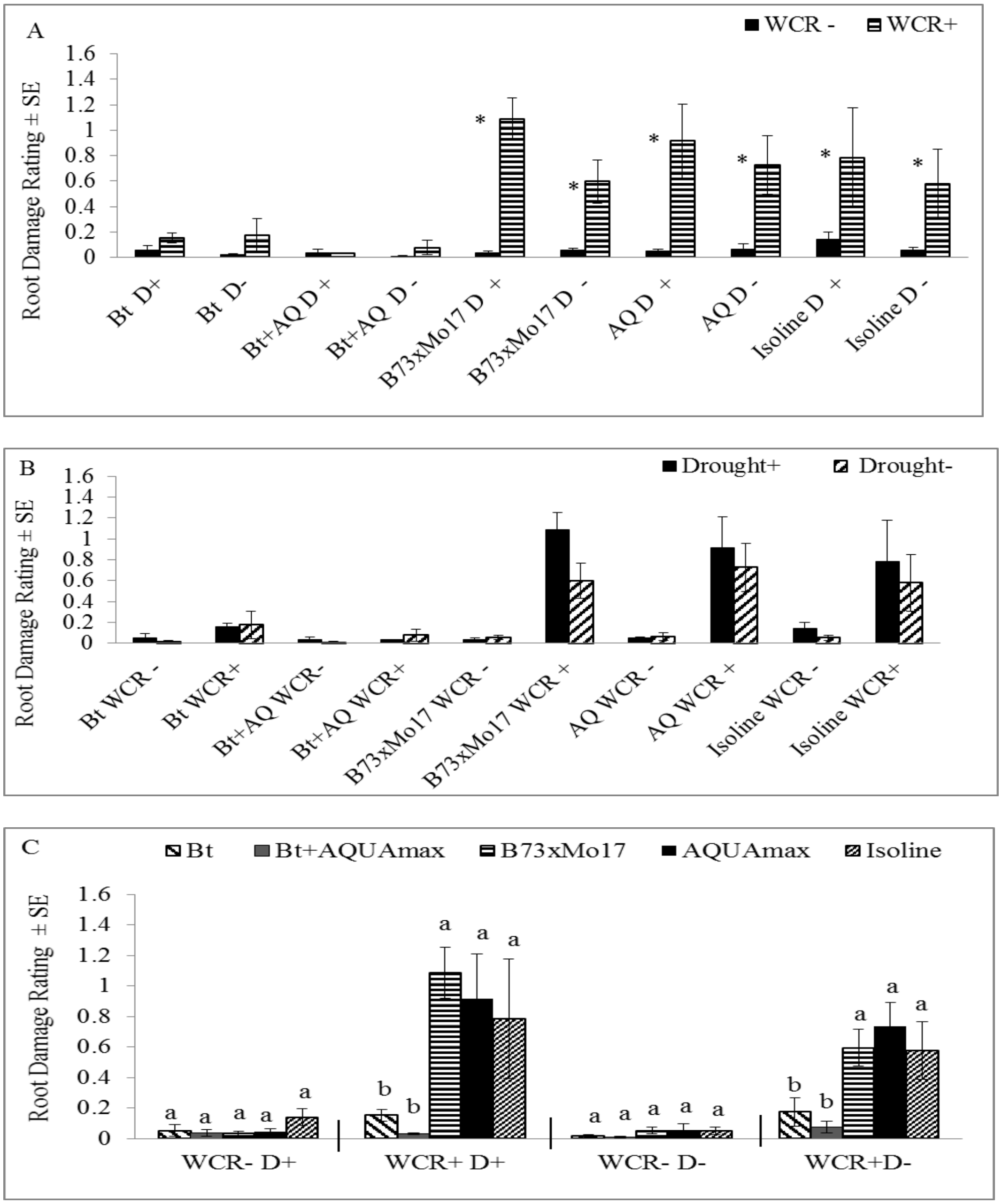
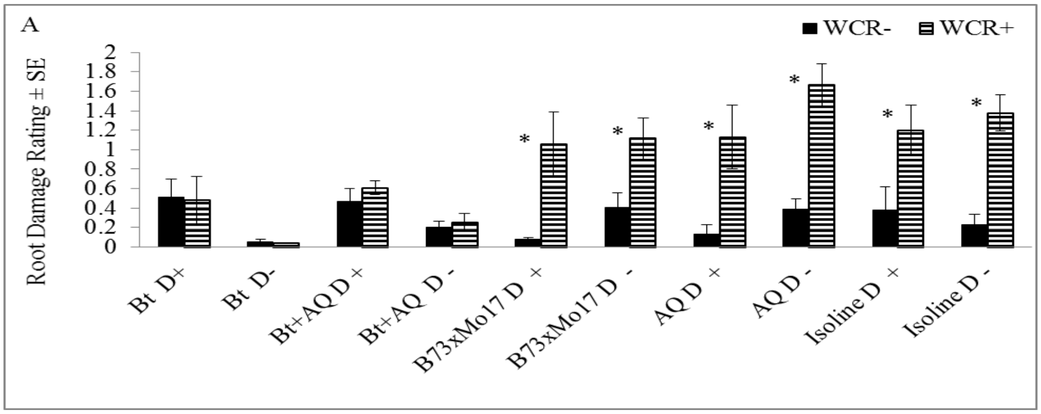
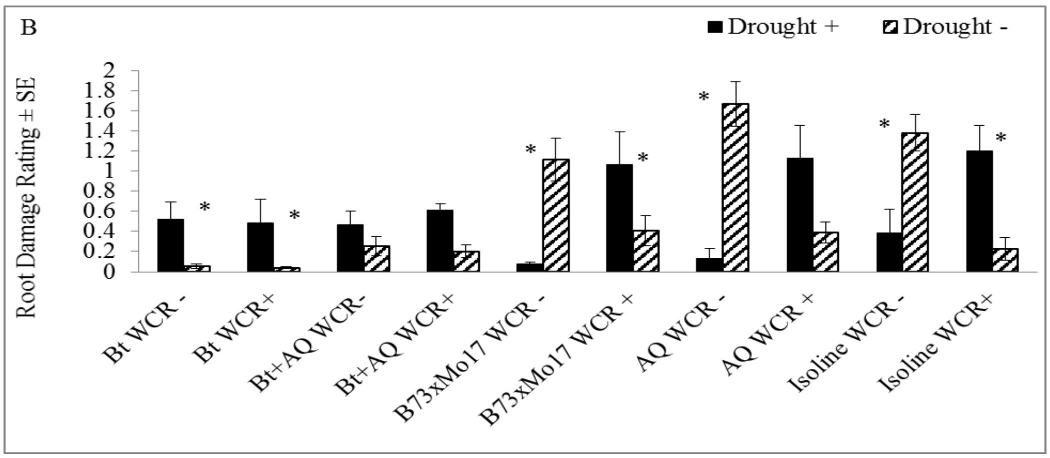


Fig. 28. The average root damage rating for three maize plant roots/plot for 2012 growing season. Although untransformed data are shown, analyses were performed using log-transformed [log10 (x +1)] data. (A) Asterisks indicate significant differences between western corn rootworm infestation levels, keeping maize line and soil moisture level constant ($P \leq 0.05$). (B) Asterisks indicate significant differences between soil moisture levels, keeping infestation level and maize line constant ($P \leq 0.05$). (C) Different letters indicate significant differences between maize lines, keeping western corn rootworm infestation level and soil moisture levels constant ($P \leq 0.05$). Abbreviations: (D-) well watered treatment; (D+) drought treatment; (Bt+AQ) Bt+AQUAmax; (AQ) AQUAmax.





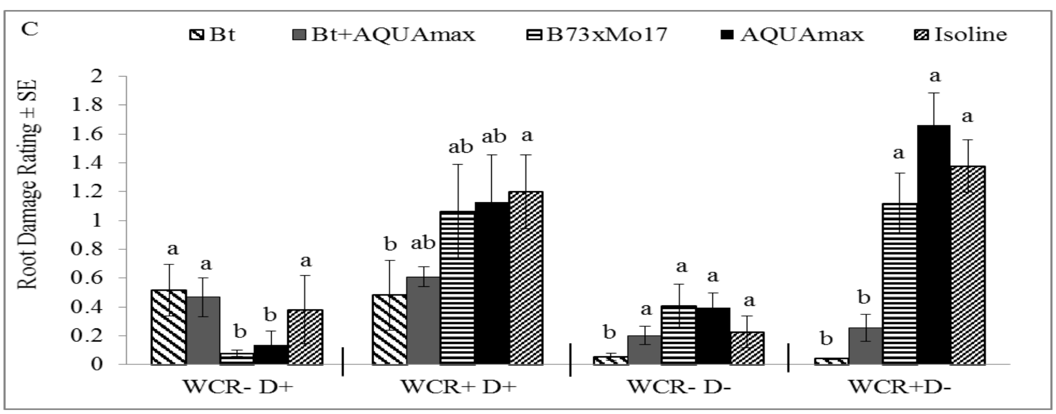


Fig. 29. Average root damage rating from three maize plant roots/plot for 2013 growing season. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)] data. (A) Asterisks indicate significant differences between western corn rootworm infestation levels, keeping maize line and soil moisture level constant ($P \leq 0.05$). (B) Asterisks indicate significant differences between soil moisture levels, keeping infestation level and maize line constant ($P \leq 0.05$). (C) Different letters indicate significant differences between maize lines, keeping western corn rootworm infestation level and soil moisture level constant ($P \leq 0.05$). Abbreviations: (D-) well watered treatment; (D+) drought treatment; (Bt+AQ) Bt+AQUAmax; (AQ) AQUAmax.

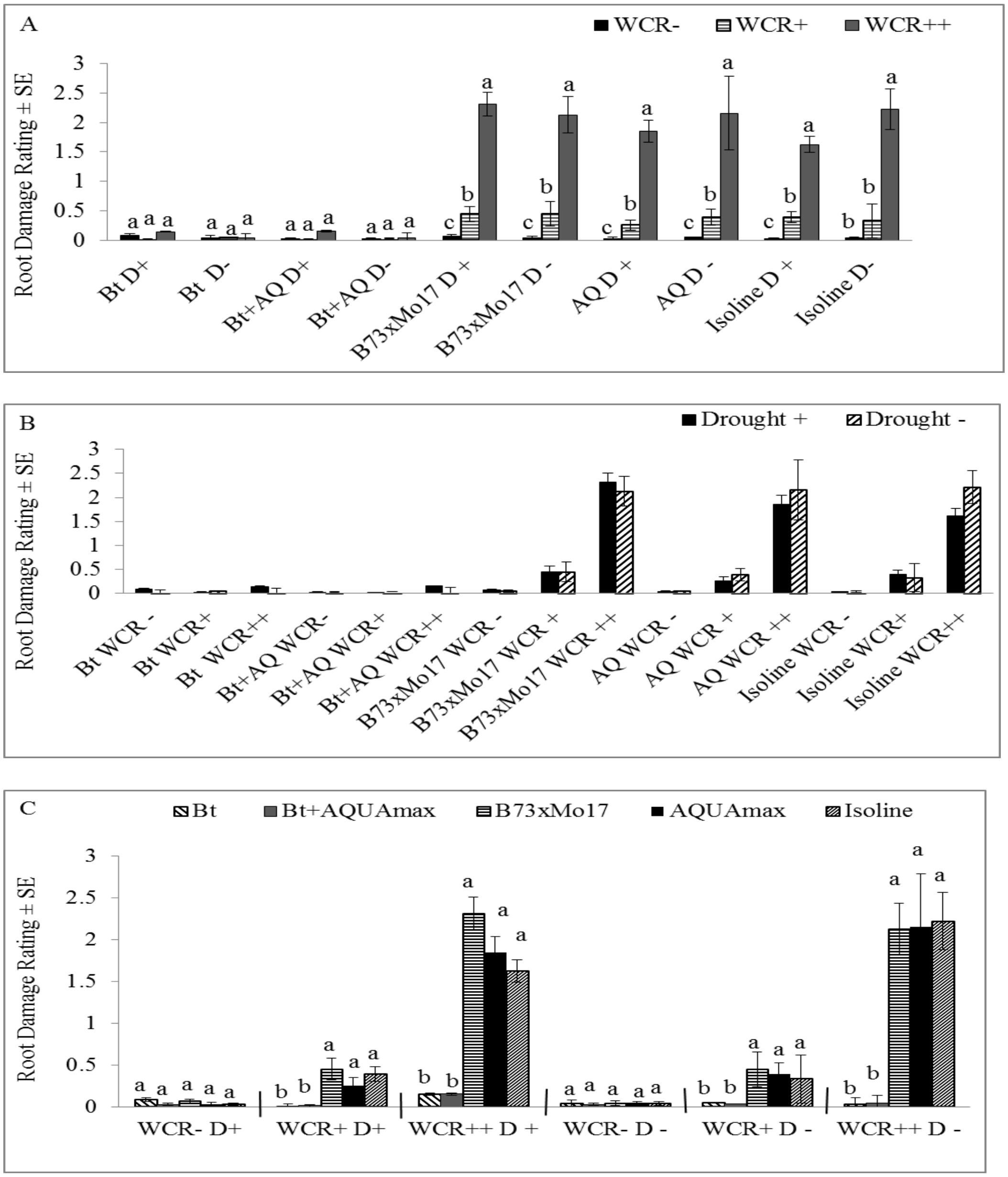


Fig. 30. Average root damage rating from three maize plant roots/plot for 2012 growing season. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}(x + 1)$] data. (A) Different letters indicate significant differences between western corn rootworm infestation levels, keeping maize line and soil moisture level constant ($P \leq 0.05$). (B) Asterisks indicate significant differences between soil moisture levels, keeping infestation level and maize line constant ($P \leq 0.05$). (C) Different letters indicate significant differences between maize lines, keeping western corn rootworm infestation level and soil moisture level constant ($P \leq 0.05$). Abbreviations: (D-) well watered treatment; (D+) drought treatment; (Bt+AQ) Bt+AQUAmax; (AQ) AQUAmax.

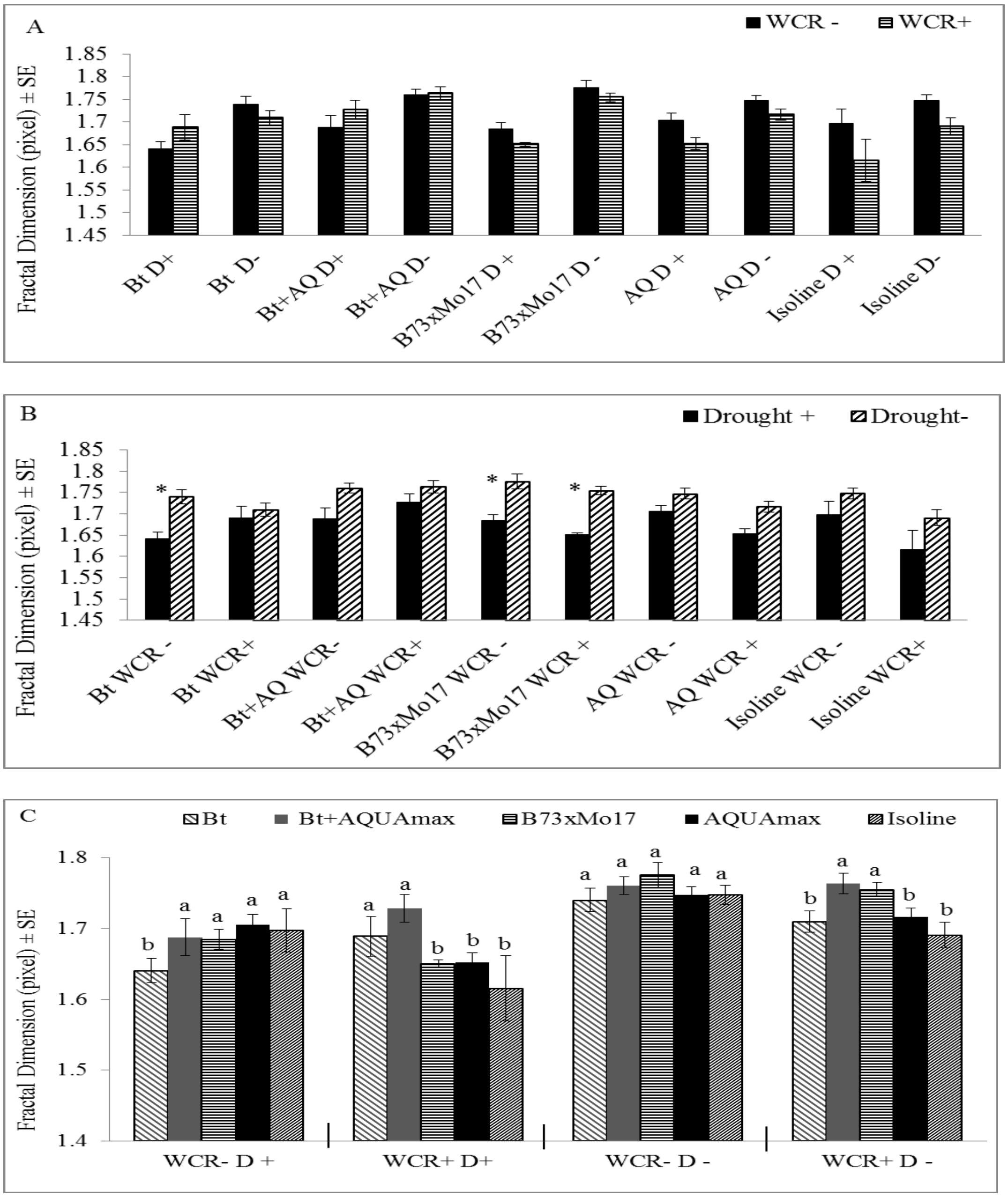


Fig. 31. Average fractal dimension from three maize plant roots/plot for 2012 growing season. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)] data. (A) Asterisks indicate significant difference between western corn rootworm infestation levels, keeping maize line and soil moisture level constant ($P \leq 0.05$). (B) Asterisks indicate significant differences between drought levels, keeping infestation level and maize line constant ($P \leq 0.05$). (C) Different letters indicate significant differences between maize lines, keeping western corn rootworm infestation level and soil moisture level constant ($P \leq 0.05$). Abbreviations: (D-) well watered treatment; (D+) drought treatment; (Bt+AQ) Bt+AQUAmax; (AQ) AQUAmax.

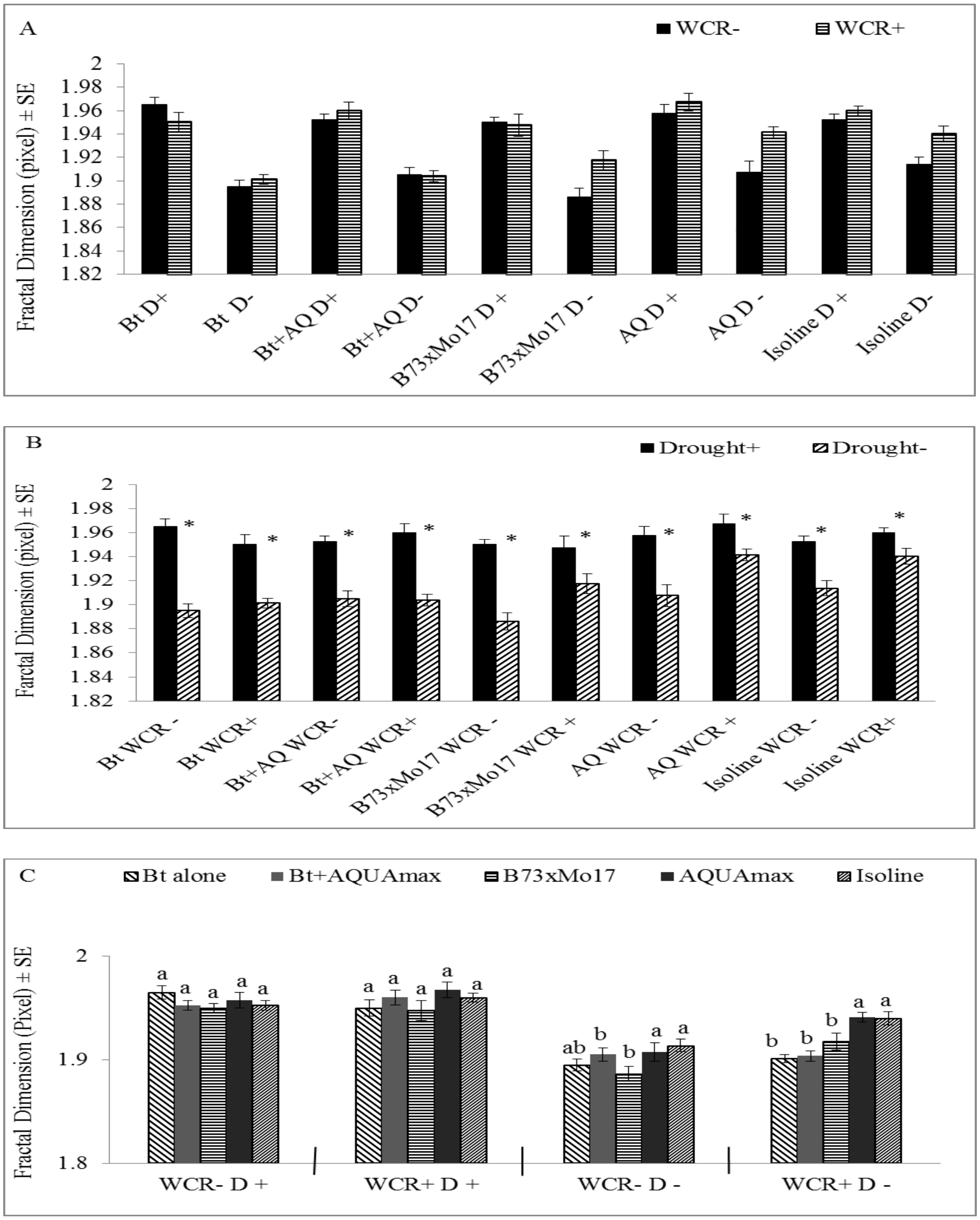


Fig. 32. Average fractal dimension from three maize plant roots/plot for 2013 growing season. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}(x+1)$] data. (A) Asterisks indicate significant differences between western corn rootworm infestation levels, keeping maize line and drought level constant ($P \leq 0.05$). (B) Asterisks indicate significant differences between soil moisture level, keeping infestation level and maize line constant ($P \leq 0.05$). (C) Different letters indicate significant differences between maize lines keeping western corn rootworm infestation level and soil moisture level constant ($P \leq 0.05$). Abbreviations: (D-) well watered treatment; (D+) drought treatment; (Bt+AQ) Bt+AQUAmax; (AQ) AQUAmax.

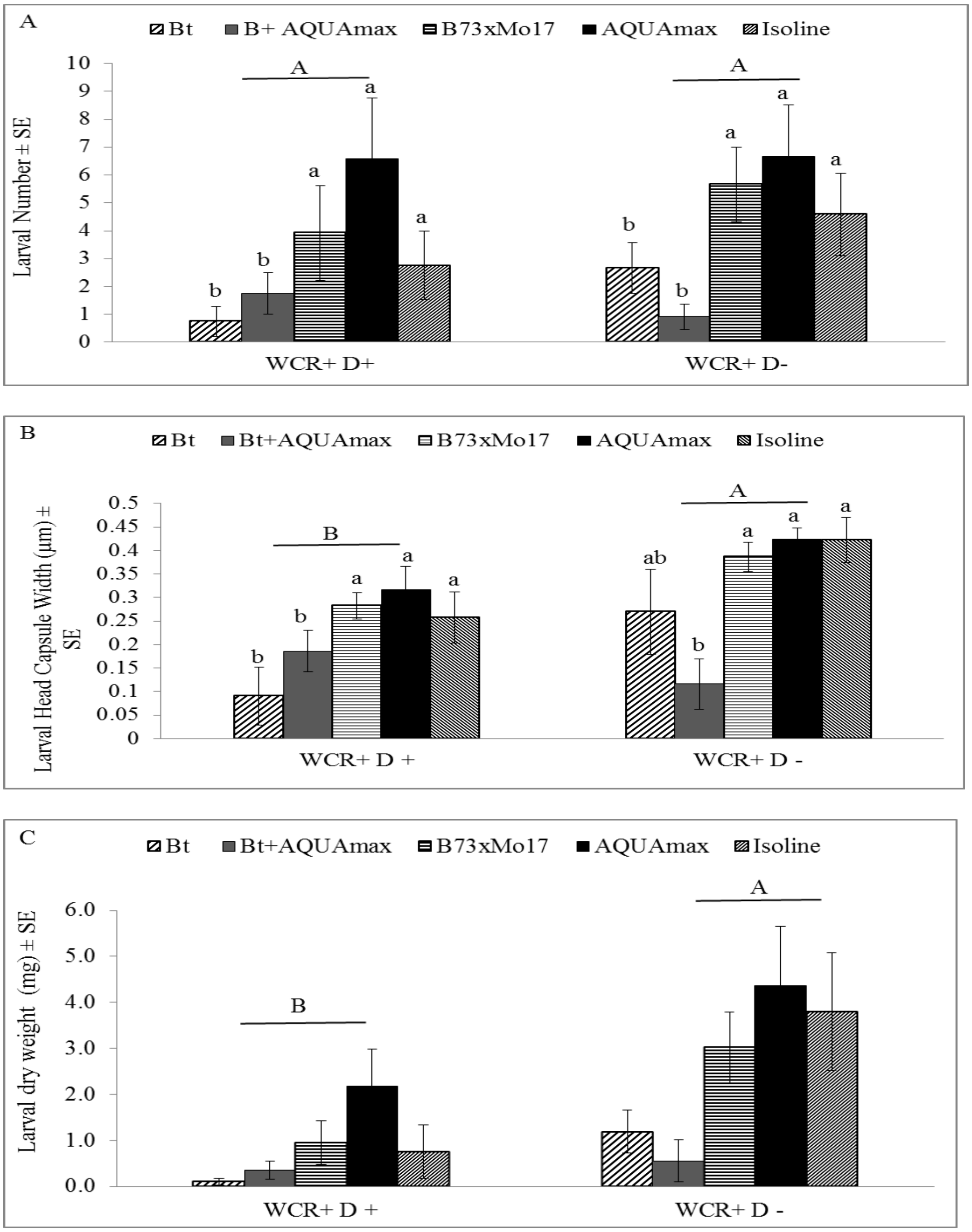


Fig. 33. Average number of recovered western corn rootworm larvae (A) larval head capsule width (B) and larval dry weight (C) of western corn rootworm recovered in 2013 rowing season. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}(x+1)$] data. Lines over bars indicate the main effect of western corn rootworm infestation level ($P \leq 0.05$). Lowercase letters indicate comparisons between soil moisture levels within western corn rootworm infestation level ($P \leq 0.05$).

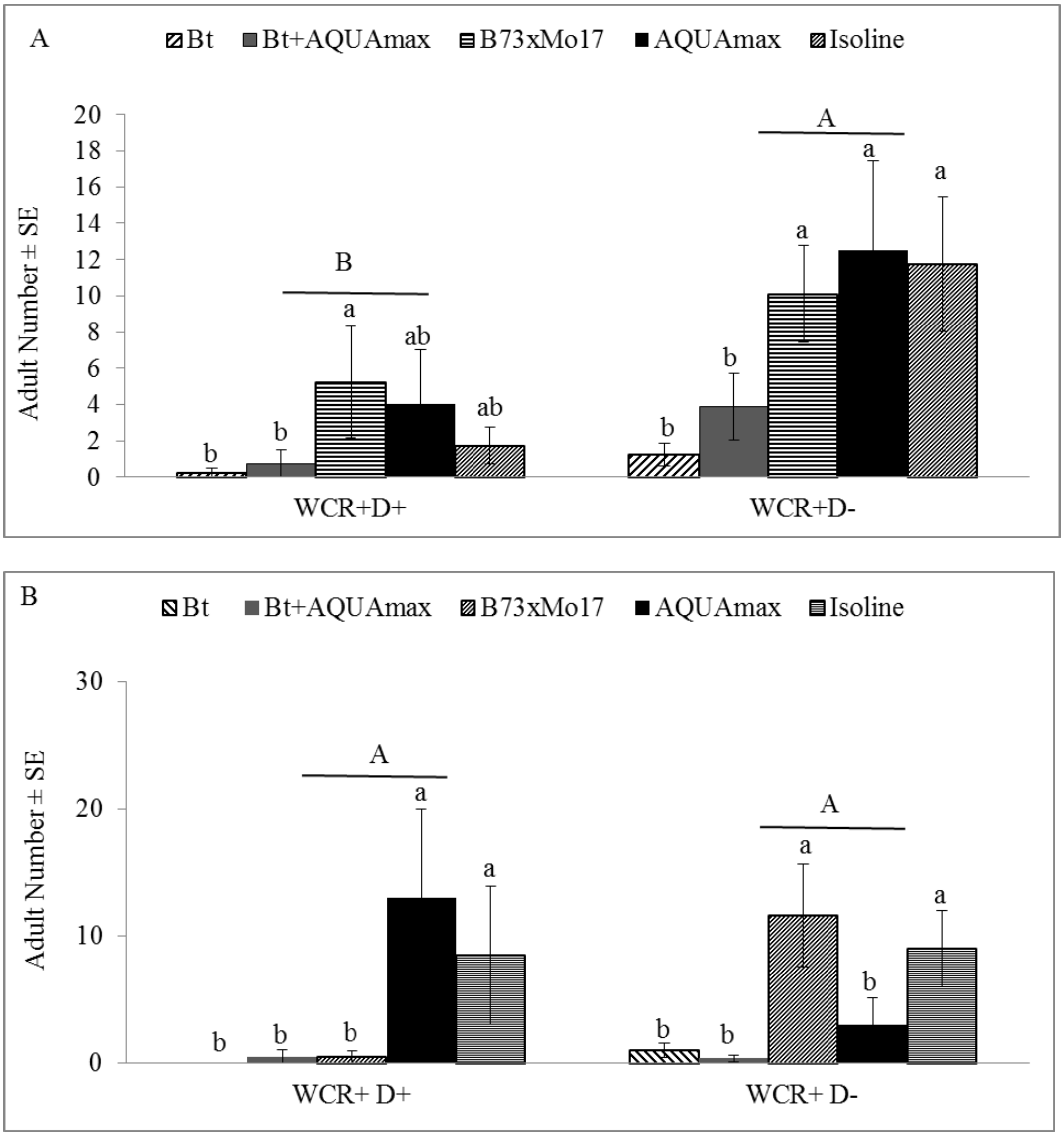


Fig. 34. Average number of recovered western corn rootworm adults (A) in 2012 (B) 2013 growing seasons. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)] data. Lines over bars indicate the main effect of western corn rootworm infestation level ($P \leq 0.05$). Lowercase letters indicate comparisons between soil moisture levels within western corn rootworm infestation level ($P \leq 0.05$).

Appendix

Appendix 1 – Chapter 2 supplemental material.

Table 8S. Analysis of variance results for Experiment 1, in which plants were infested with western corn rootworm neonate larvae.

Factors	Effect	*DF*	*F value*	*Pr > F*
Plant Height	**Moisture**	2,92	140	<.0001
	Infestation	2,92	0.05	0.9483
	Moisture*Infestation	4,92	0.45	0.7700
	Trial	2,12	9.92	0.0029
	Moisture*Trial	4,92	7.82	<.0001
	Infestation*Trial	4,92	1.29	0.2808
	Moisture*Infestation*Trial	8,92	1.16	0.3349
Head Capsule Width	**Moisture**	2,32	5.57	0.0084
	Infestation	1,32	15.77	0.0004
	Moisture*Infestation	2,32	1.83	0.1765
	Trial	1,8	0.29	0.6070
	Moisture*Trial	2,32	0.94	0.4006
	Infestation*Trial	1,32	0.01	0.9147
	Moisture*Infestation*Trial	2,32	0.45	0.6432

All data were log-transformed to meet normality assumptions.

Table 9S. Analysis of variance results for Experiment 2, in which plants were infested with western corn rootworm second instar larvae.

Factors	Effect	*DF*	*F value*	*Pr > F*
Plant Height	**Moisture**	2,96	4.52	0.0133
	Infestation	2,96	1.49	0.2510
	Moisture*Infestation	4,96	0.95	0.4401
	Trial	2,12	0.83	0.4591
	Moisture*Trial	4,96	1.05	0.3860
	Infestation*Trial	4,96	1.51	0.2043
	Moisture*Infestation*Trial	8,96	1.26	0.2754
Head Capsule Width	**Moisture**	2,55	0.12	0.8856
	Infestation	1,55	1.86	0.1778
	Moisture*Infestation	2,55	1.35	0.2690
	Trial	2,12	0.07	0.9339
	Moisture*Trial	4,55	1.35	0.2624
	Infestation*Trial	2,55	0.85	0.4312
	Moisture*Infestation*Trial	4,55	0.30	0.8747

All data were log-transformed to meet normality assumptions.

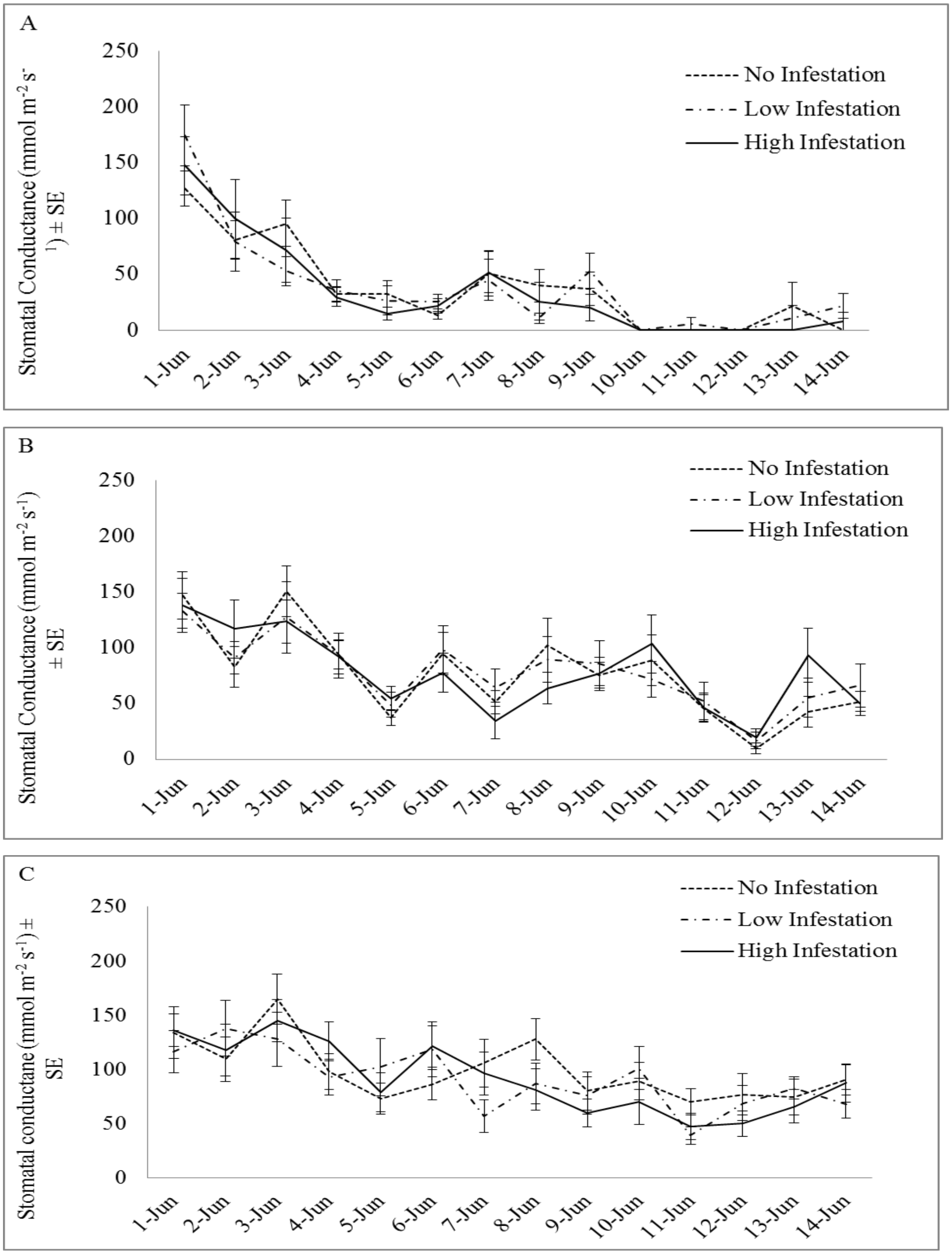


Fig. 35S. Average stomatal conductance at 11:00 am of maize plants under very dry (A), moderately dry (B), and well-watered soil moisture regimes (C) in Experiment 1 infested with neonate western corn rootworm larvae. The data are a combination of three trials. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)] data.

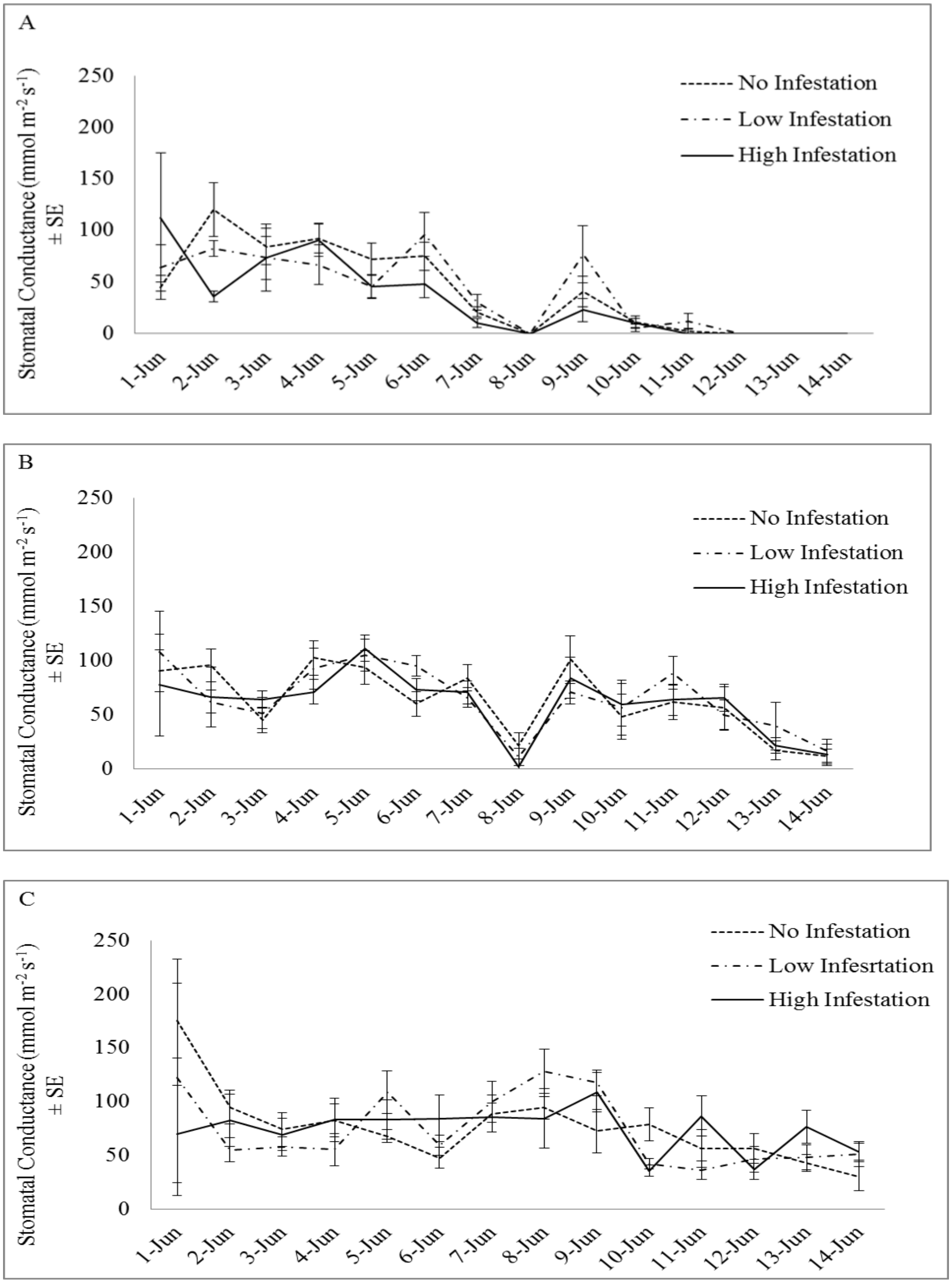


Fig. 36S. Average stomatal conductance at 4:00 pm of maize plants very dry (A), moderately dry (B), and well watered soil moisture regimes (C) in Experiment 1 infested with neonate western corn rootworm larvae. The data are a combination of three trials. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)] data.

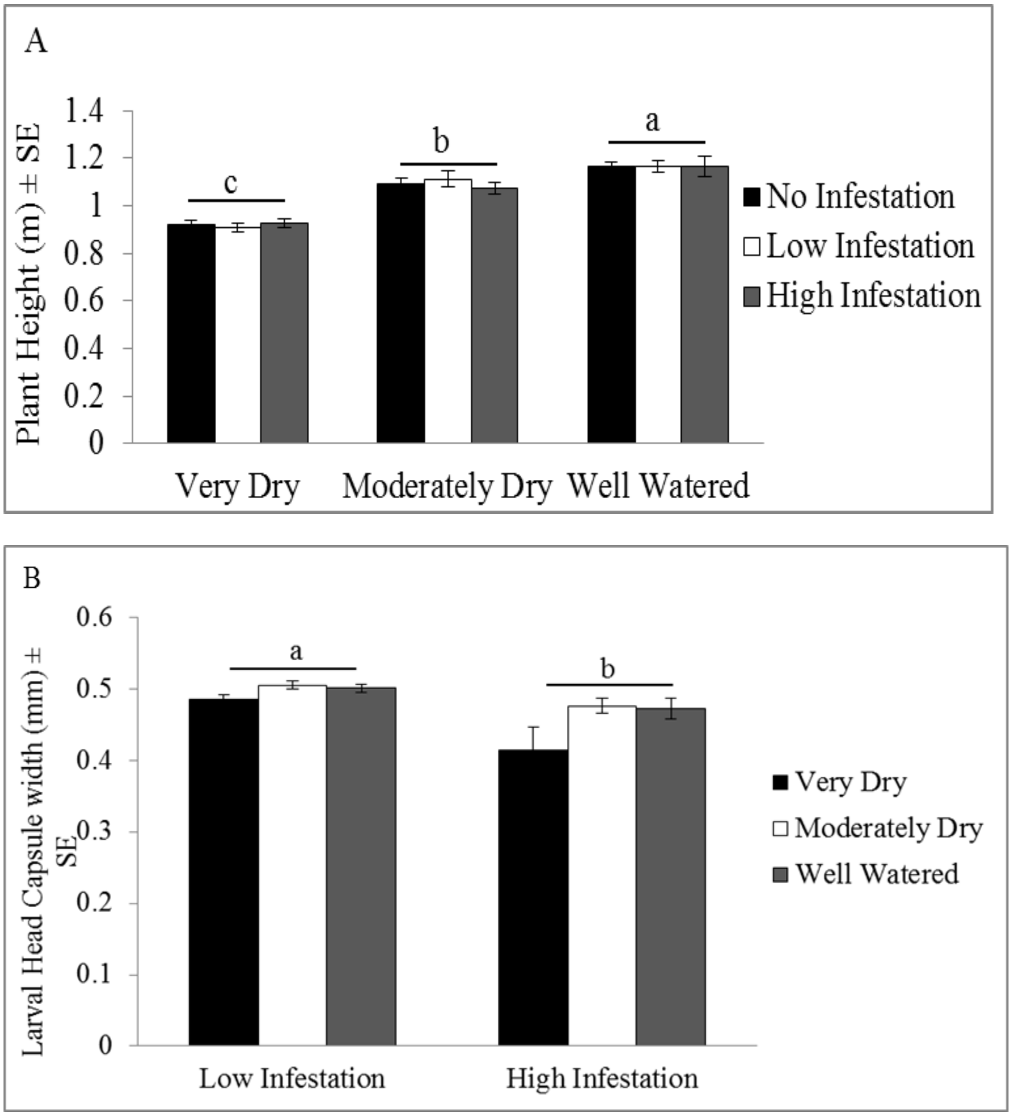


Fig. 37S. Average plant height of maize plants (A) and larval head capsule width for western corn rootworm neonate larvae (B) in Experiment 1**.** Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +0.1)] (A) or [$\log_{10}$ (x +0.01)] (B). Lines over bars indicate the main effect of soil moisture level in (A Lines over bars indicate the main effect of soil moisture level.). Lines over bars indicate the main effect of western corn rootworm infestation levels in (B). ($P \leq 0.05$) Plant height was measured from the shoot base to the flag leaf.

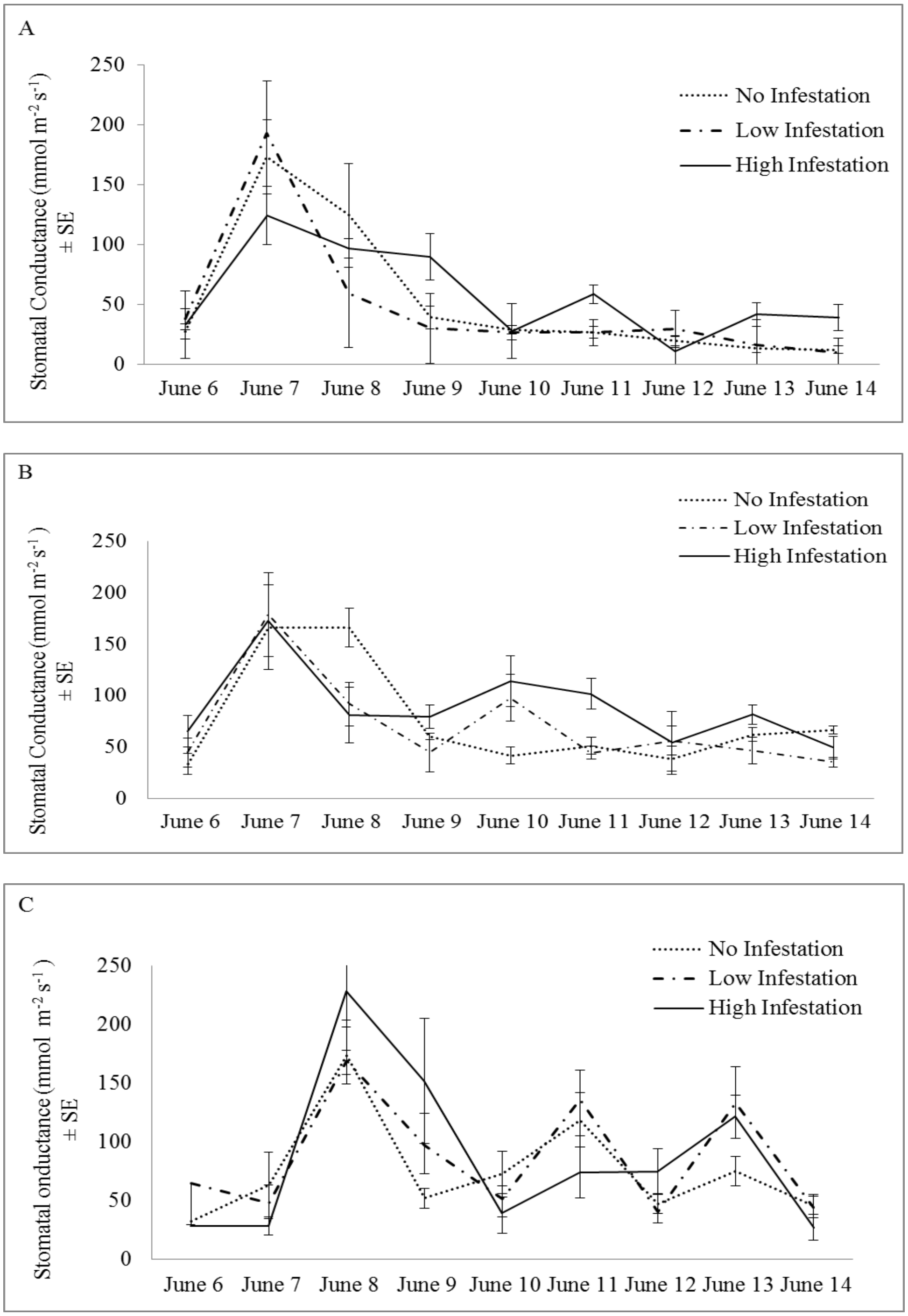


Fig. 38S. Average stomatal conductance at 11:00 am of maize under very dry (A), moderately dry (B), and well watered soil moisture regimes (C) in Experiment 2 infested with western corn rootworm second instar. The data are a combination of three trials. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)] data.

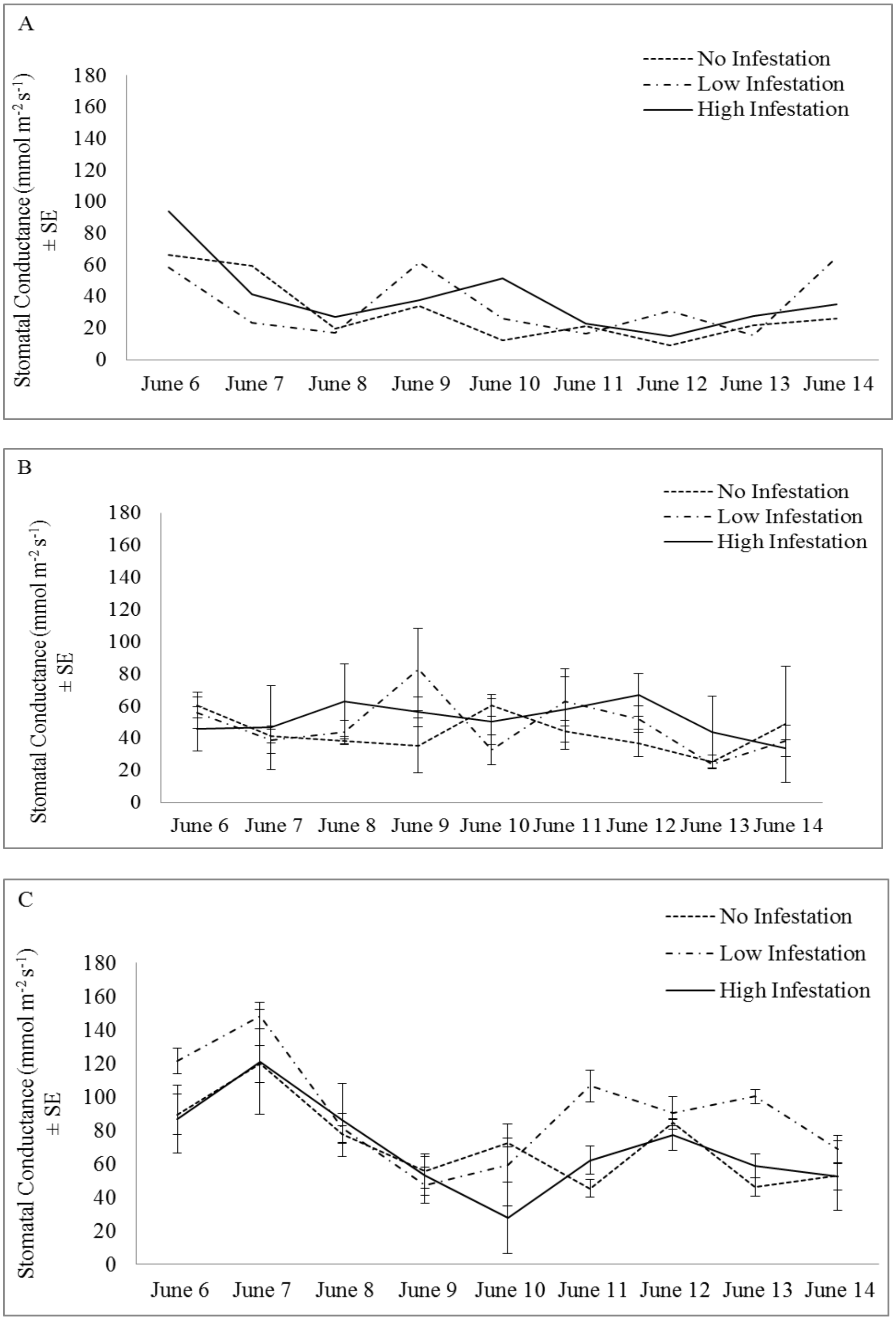


Fig. 39S. Average stomatal conductance at 4:00 pm of maize plants under very dry (A), moderately dry (B), and well watered soil moisture regimes (C) in Experiment 2 infested with western corn rootworm second instar. The data are a combination of three trials. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)] data.

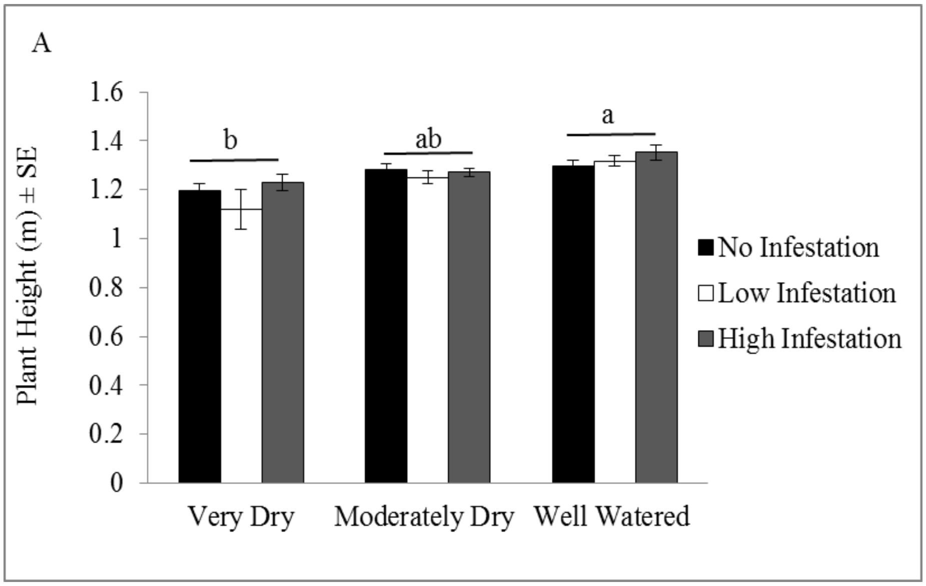


Fig. 40S. Average plant height of maize plants infested with western corn rootworm second instar larvae for Experiment 2**.** Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)] data. Lines over bars indicate the main effect of soil moisture level in (A). Plant height was measured from the shoot base to the flag leaf. ($P \leq 0.05$)

Appendix 2 – Chapter 3 supplemental material.

Table 10S. Analysis of variance results for 2012, 2013, and 2014 growing season.

Factor	Effect	*DF*	*F value*	*Pr > F*
	2012			
Root Dry Weight	**Drought**	1,10	56.26	<.0001
	Infestation	1,90	0.14	0.7132
	Drought × Infestation	1,90	3.14	0.0795
	Maize lines	4,90	3.09	0.0196
	Drought× Maize lines	4,90	2.13	0.0841
	Infestation× Maize lines	4,90	1.39	0.2425
	Drought× Infestation× Maize lines	4,90	0.27	0.8987
	2013			
Root Dry Weight	**Drought**	1,60	116.24	<.0001
	Infestation	1,94	0.08	0.7783
	Drought× * Infestation	1,94	2.06	0.1544
	Maize lines	4,94	6.07	0.0002
	Drought× * Maize lines	4,94	1.62	0.1757
	Infestation× * Maize lines	4,94	1.8	0.1357
	Drought× * Infestation× × Maize lines	4,94	0.62	0.6471
Root Regrowth	**Drought**	1,60	10.85	0.0165
	Infestation	1,94	1.78	0.1853
	Drought× Infestation	1,94	4.53	0.0359
	Maize lines	4,94	29.17	<.0001
	Drought× Maize lines	4,94	9.44	<.0001
	Infestation× Maize lines	4,94	2.74	0.0329
	Drought× Infestation× Maize lines	4,94	1.41	0.2381
	2014			
Root Dry Weight	**Drought**	1,100	34.46	0.0002
	Infestation	2,140	3.76	0.0256
	Drought× Infestation	2,140	9.23	0.0002
	Maize lines	4,140	6.58	<.0001
	Drought × Maize lines	4,140	0.34	0.8481
	Infestation * Maize lines	8,140	4.6	<.0001
	Drought× Infestation× Maize lines	8,140	1.53	0.1518
Root Regrowth	**Drought**	1,100	22.26	0.0008
	Infestation	2,140	6.74	0.0016
	Drought× Infestation	2,140	6.65	0.0017
	Maize lines	4,140	7.62	<.0001
	Drought× Maize lines	4,140	0.83	0.5099
	Infestation× Maize lines	8,140	0.91	0.5142
	Drought× Infestation× Maize lines	8,140	0.59	0.7876

All data were log-transformed to meet normality assumptions.

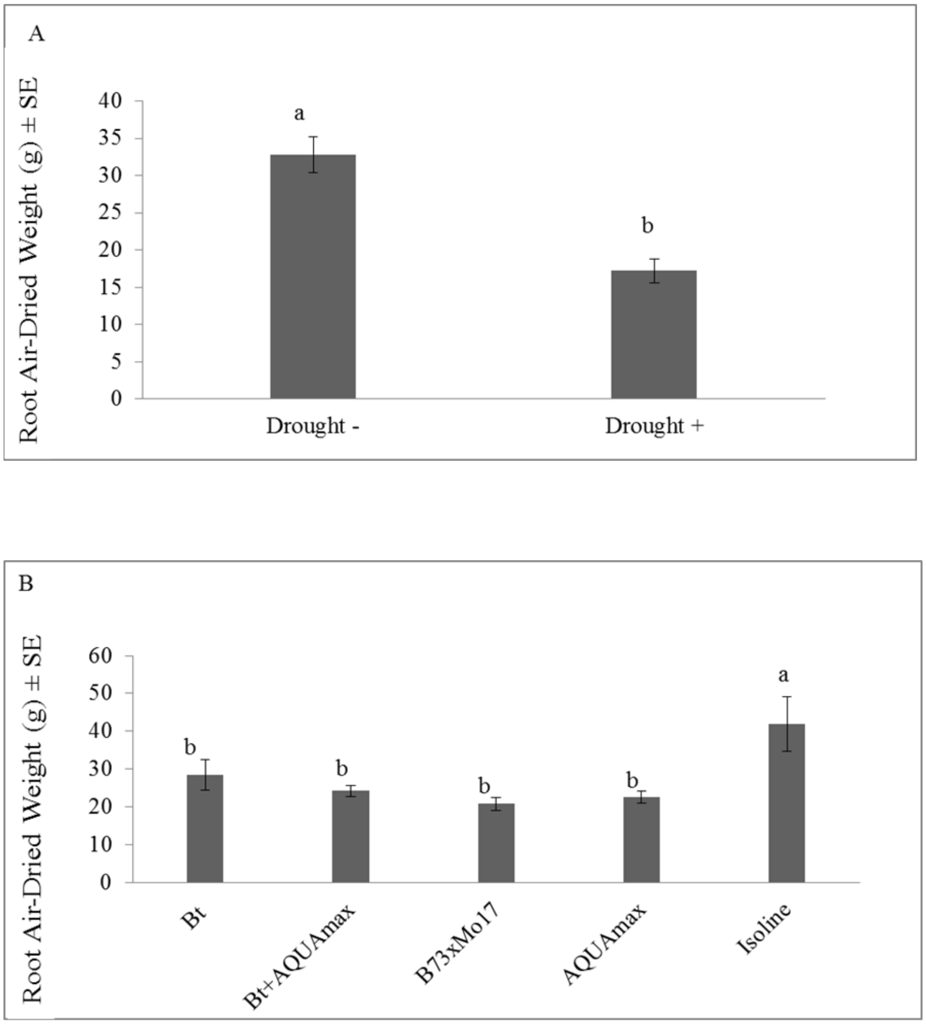


Fig. 41S. Average root dry weight of three maize plant roots per plot for 2012 growing season. (A) Different letters indicate significant differences between drought levels ($P \leq 0.05$). (B) Different letters indicate significant differences between maize lines ($P \leq 0.05$). Although untransformed data are shown, analyses were performed using log - transformed [$\log_{10}$ (x +1)] data.

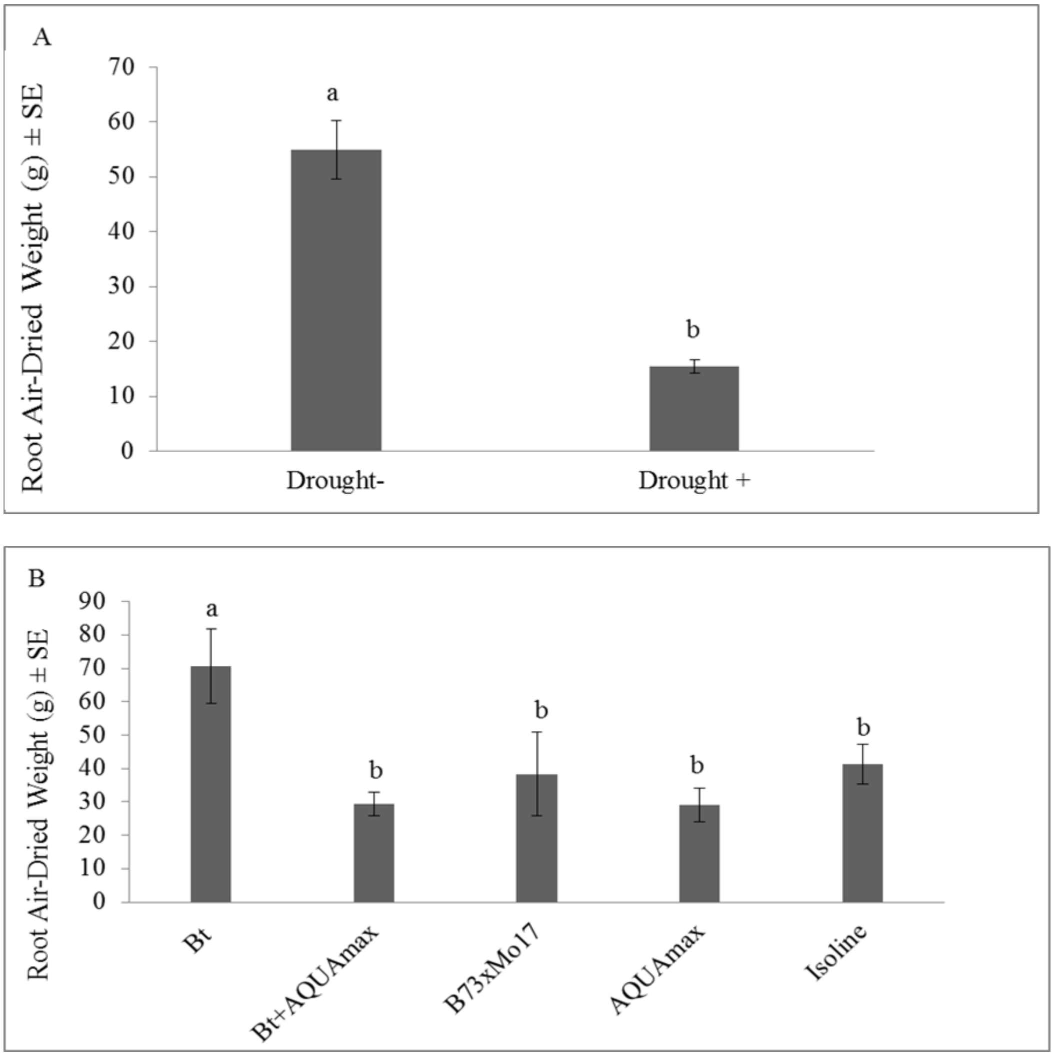
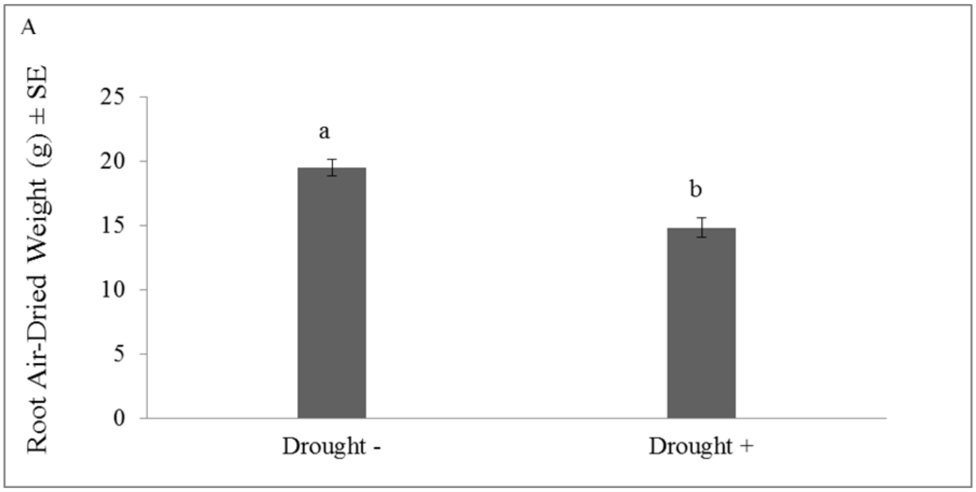


Fig. 42S. Average root dry weight of three maize plant roots per plot for 2013 growing season. (A) Different letters indicate significant differences between drought levels (*P* ≤ 0.05). (B) Different letters indicate significant differences between maize lines (*P* ≤ 0.05). Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)].



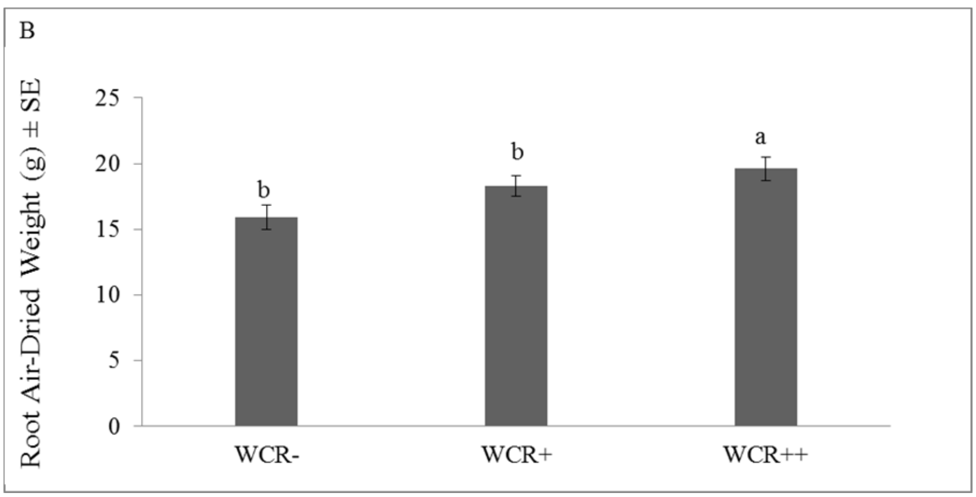


Fig. 43S. Average root dry weight of three maize plant roots per plot for 2014 growing season. (A) Different letters indicate significant differences between drought levels ($P \leq 0.05$). (B) Different letters indicate significant differences between western corn rootworm ($P \leq 0.05$). Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)].

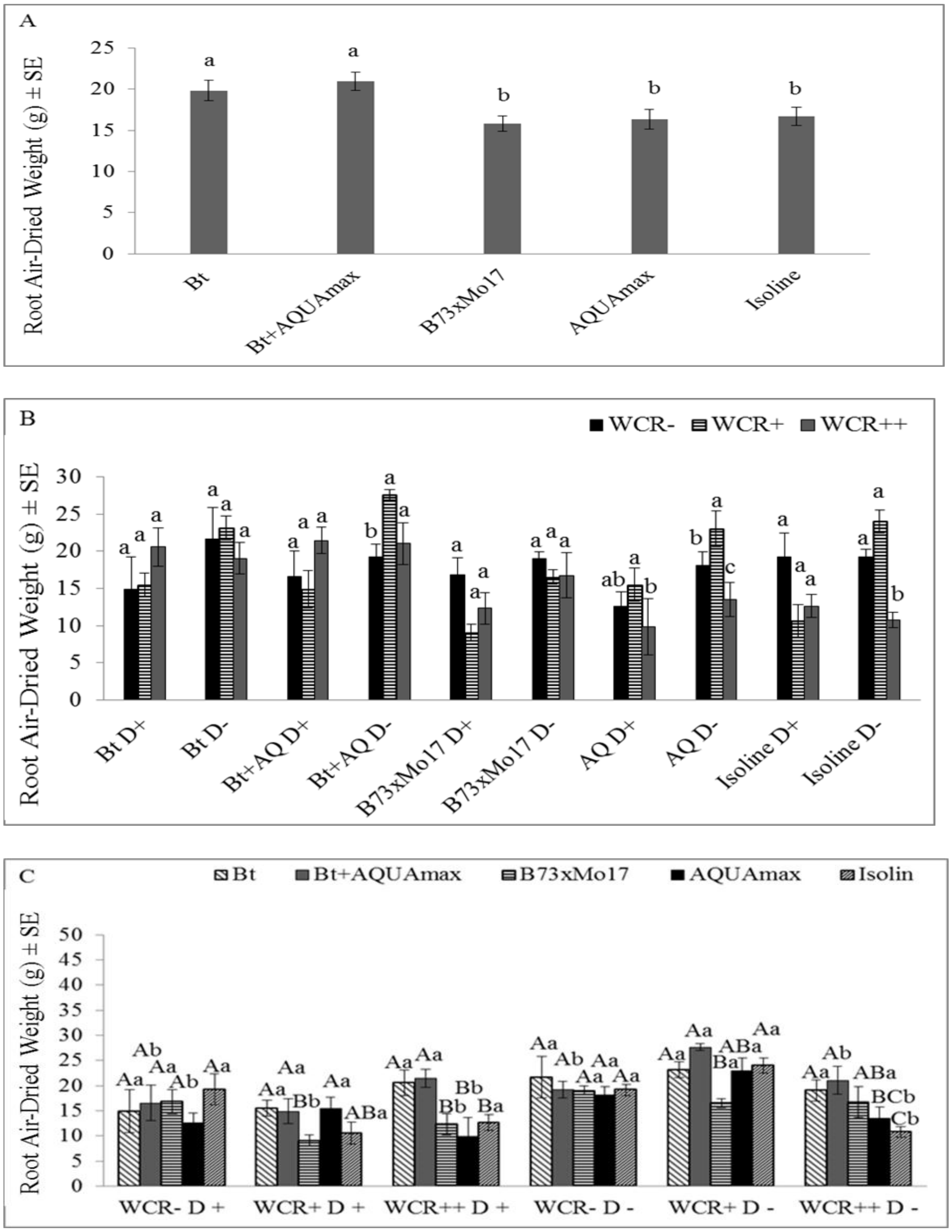


Fig. 44S. Average root dry weight of three maize plant roots per plot for 2014 growing season. (A) Different letters indicate significant differences between maize lines ($P \leq 0.05$). (B) Different letters indicate significant differences between western corn rootworm infestation levels keeping maize line and drought levels constant ($P \leq 0.05$). (C) Uppercase letter indicate comparisons between maize lines within drought level and western corn rootworm infestation level, and lowercase letters indicate differences between maize lines across the western corn rootworm infestation levels within the same drought level ($P \leq 0.05$). Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +1)]. Abbreviations: (D-) well watered treatment; (D+) drought treatment; (Bt+AQ) Bt+AQUAmax; and (AQ) AQUAmax.

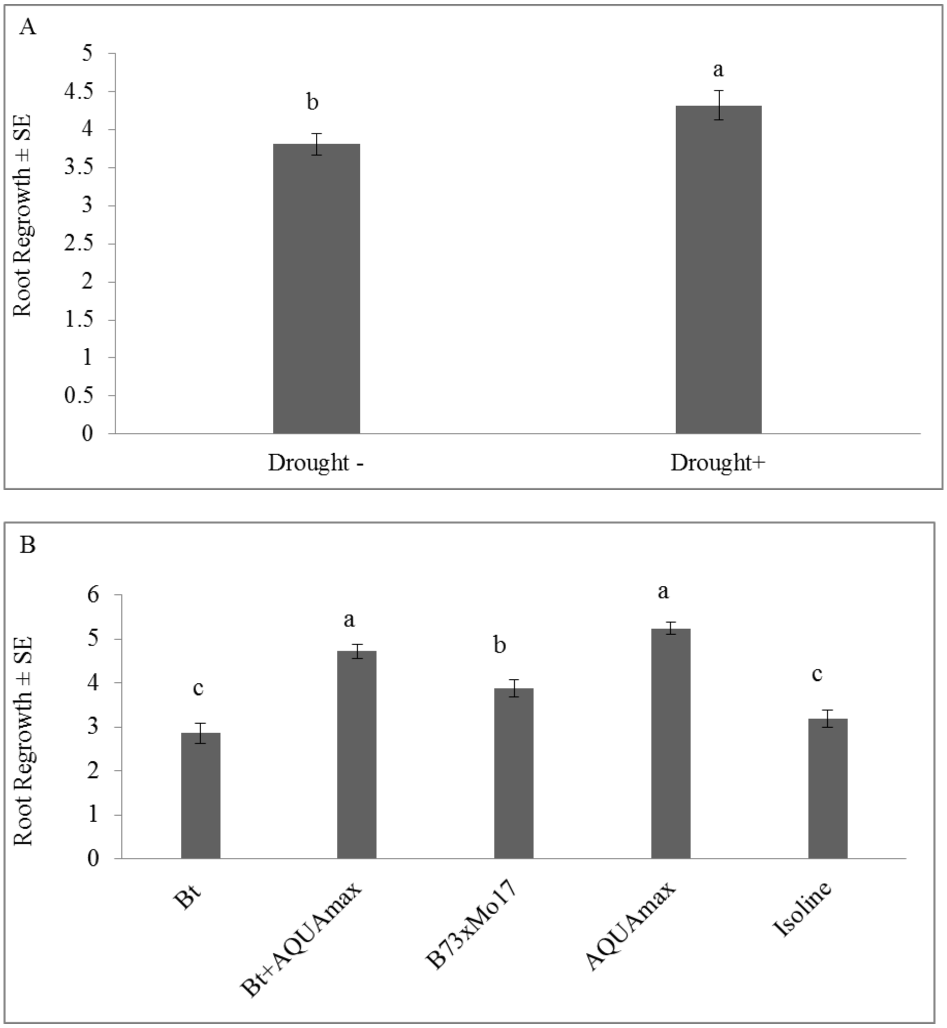


Fig. 45S. Average root regrowth of three maize plant roots per plot for the 2013 field growing season. (A) Different letters indicate significant differences between drought levels ($P \leq 0.05$). (B) Different letters indicate significant differences between maize lines ($P \leq 0.05$). The 1 scale indicated the greatest root system with secondary roots, the 6 scale indicated the poorest root system. Although untransformed data are shown, analysis were performed using log-transformed [$\log_{10}$ (x +1)] data.

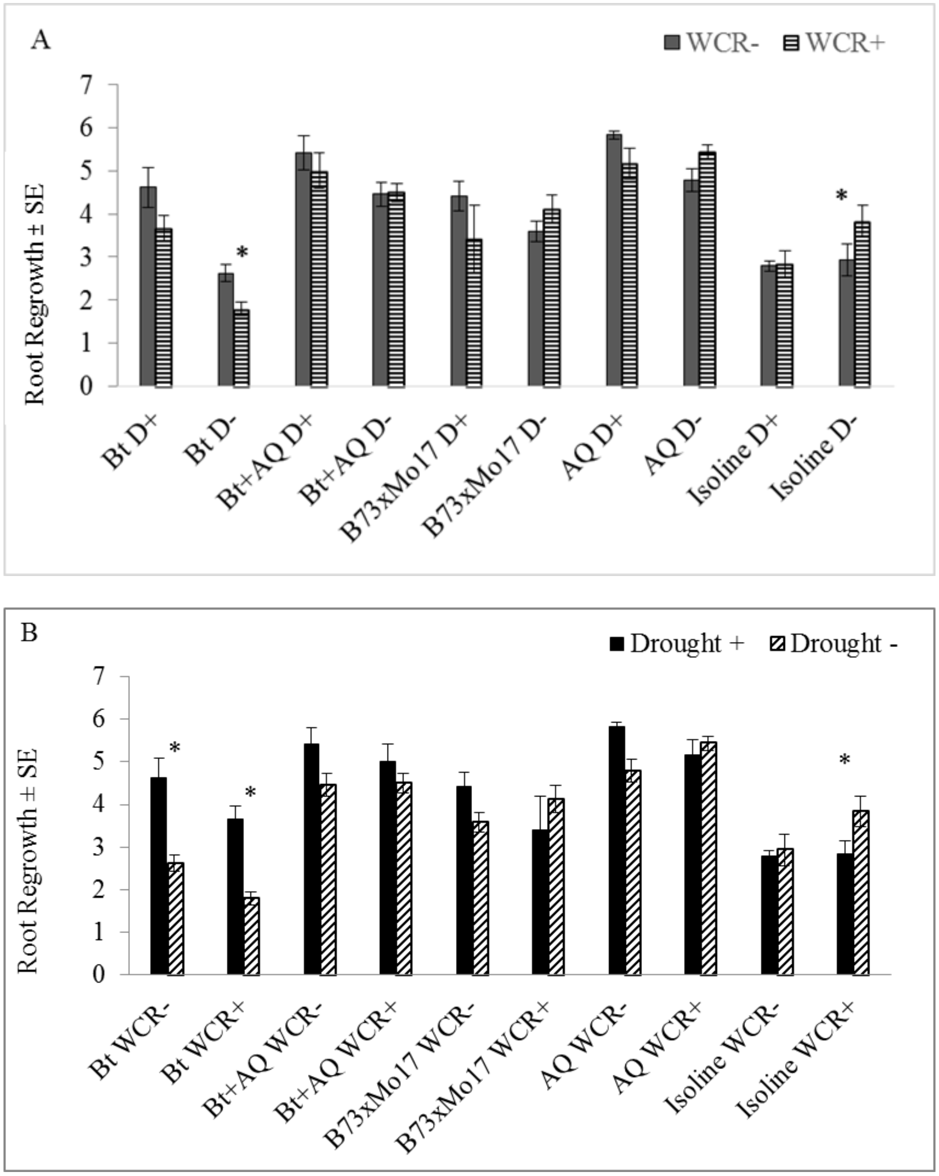


Fig. 46S. Average root regrowth of three maize plant roots per plot for the 2013 field growing season. (A) Asterisks indicate significant differences between western corn rootworm infestation levels keeping maize line and drought level constant ($P \leq 0.05$). (B) Asterisks indicate significant differences between drought levels keeping infestation level and maize line constant ($P \leq 0.05$). The 1 scale indicated the greatest root system with secondary roots, the 6 scale indicated the poorest root system. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +0.01)]. Abbreviations: (D-) well watered treatment; (D+) drought treatment; (Bt+AQ) Bt+AQUAmax; and (AQ) AQUAmax.

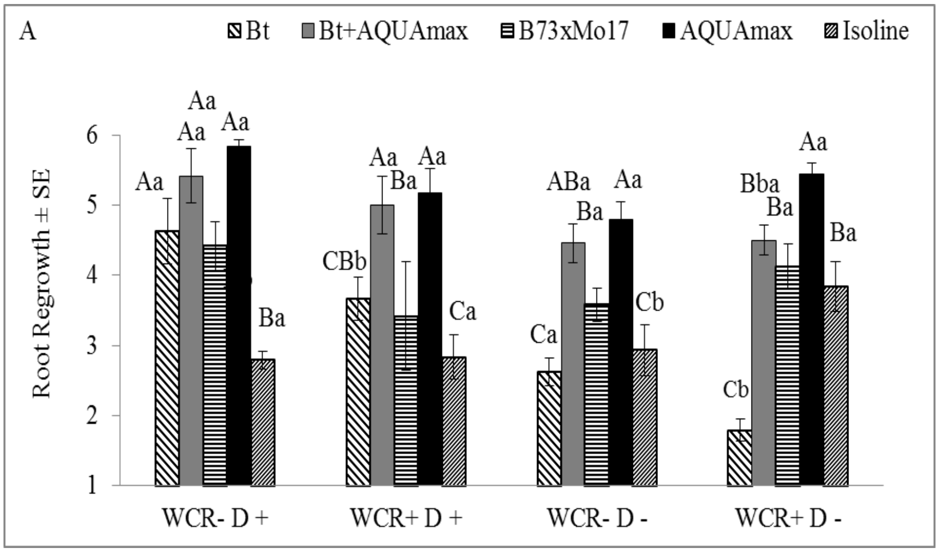


Fig. 47S. Average root regrowth of three maize plant roots per plot for the 2013 field growing season**.** Uppercase letter indicate differences between maize lines within drought level and western corn rootworm infestation level, and lowercase letters indicate differences between maize lines across the western corn rootworm infestation levels within the same drought level ($P \leq 0.05$). The 1 scale indicated the greatest root system with secondary roots, the 6 scale indicated the poorest root system. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +0.01)]. Abbreviations: (D-) well watered treatment; (D+) drought treatment.

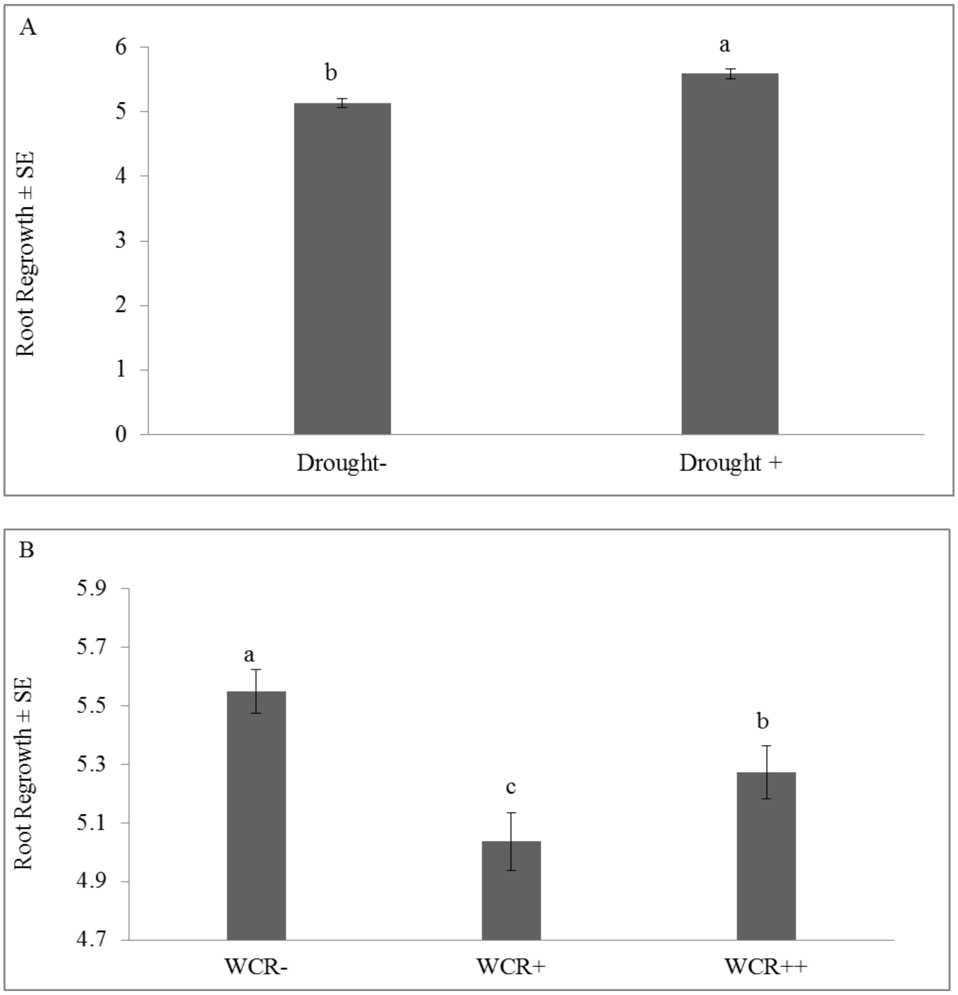
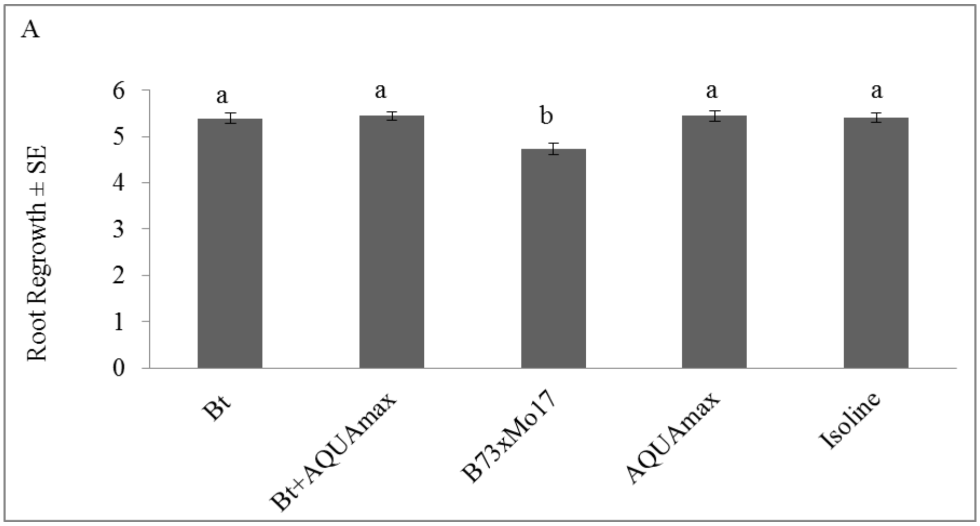


Fig. 48S. Average root regrowth of three maize plant roots per plot for 2014 growing season. (A) Different letters indicate significant differences between drought levels. (B) Different letters indicate significant differences between western corn rootworm infestation levels ($P \leq 0.05$). The 1 scale indicated the greatest root system with secondary roots, the 6 scale indicated the poorest root system. Although untransformed data are shown, analyses were performed using log-transformed [$\log_{10}$ (x +0.01)].



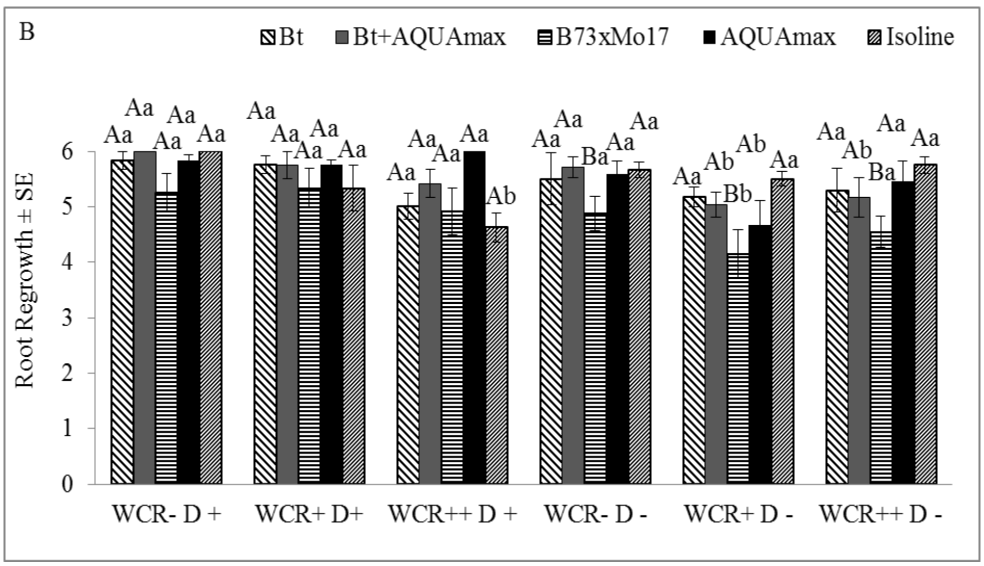


Fig. 49S. Average root regrowth of three maize plant roots per plot for 2014 growing season**.** (A) Different letters indicate significant differences between maize lines ($P \leq 0.05$). (B) Uppercase letter indicate differences between maize lines within drought level and western corn rootworm infestation level, and lowercase letters indicate comparisons between maize lines across the western corn rootworm infestation levels within the same drought level ($P \leq 0.05$). The 1 scale indicated the greatest root system with secondary roots, the 6 scale indicated the poorest root system. Although untransformed data are shown, analyses were performed using log [$\log_{10}$ (x +0.01)]. Abbreviations: (D-) well watered treatment; (D+) drought treatment.

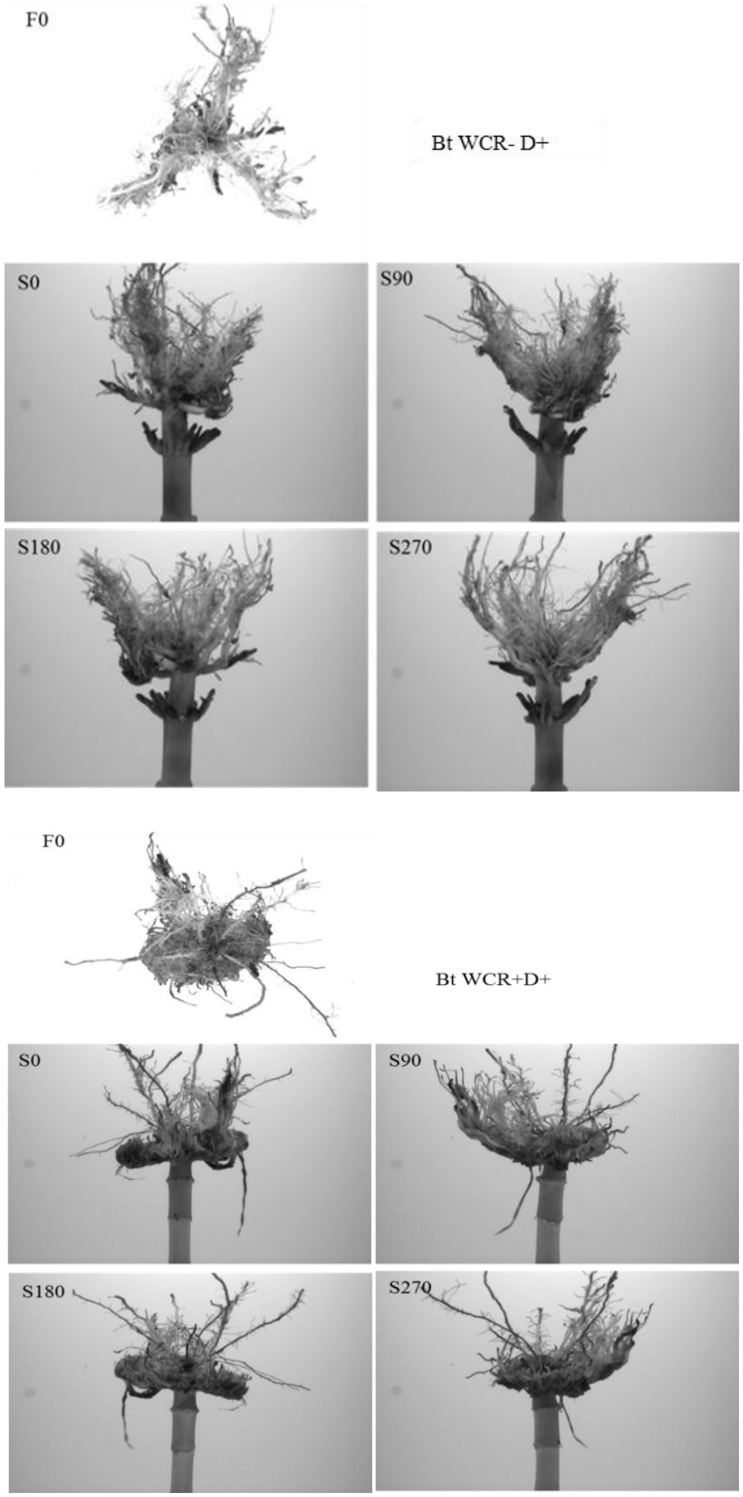
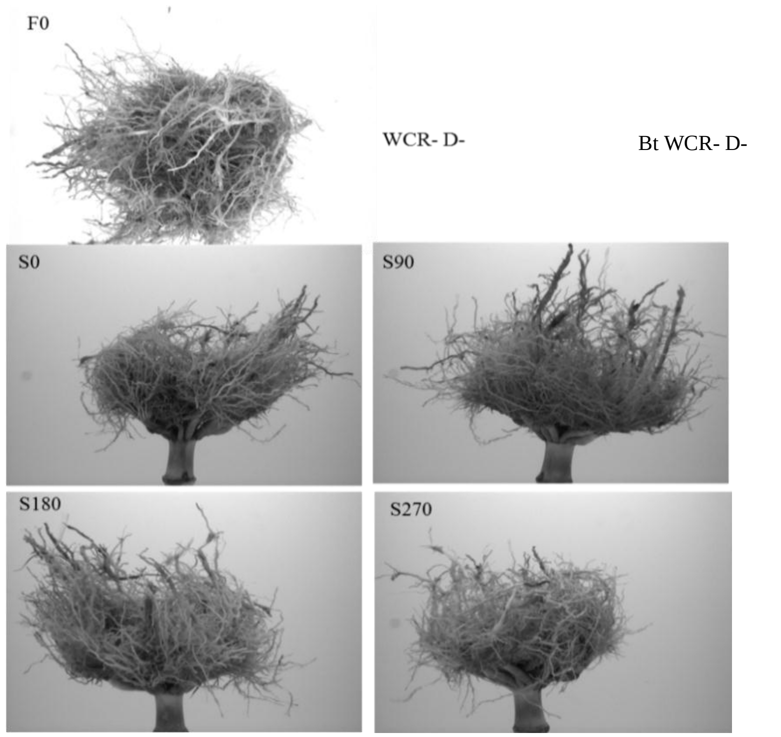


Fig. 50S. Images of Bt maize roots from different angles to measure root complexity. Abbreviation: (WCR-) no western corn rootworm; (D+) drought treatment; and (WCR+) moderate western corn rootworm infestation level.



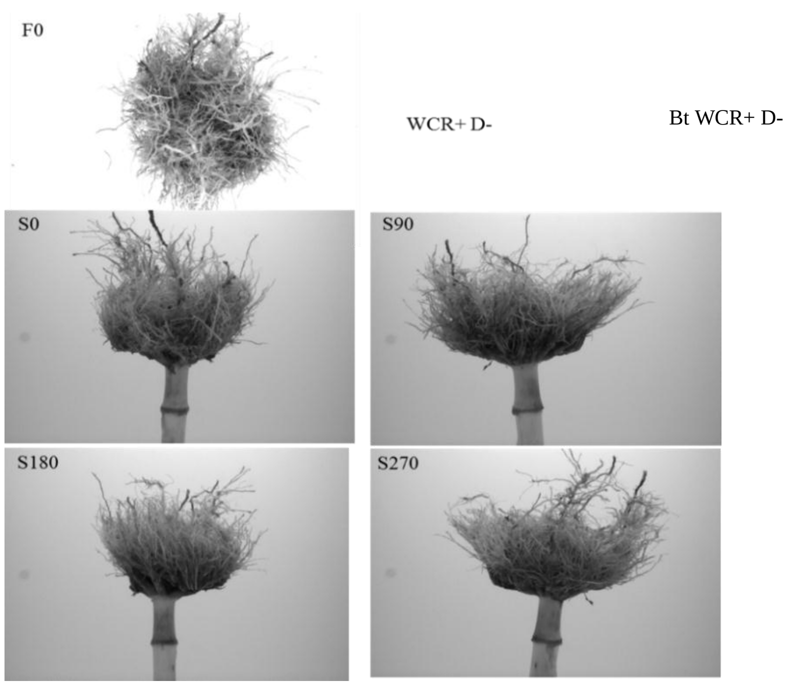


Fig. 51S. Images of Bt maize roots from different angles to measure root complexity. Abbreviations: (WCR-) no western corn rootworm; (D-) well watered treatment; and (WCR+) moderate western corn rootworm infestation level.

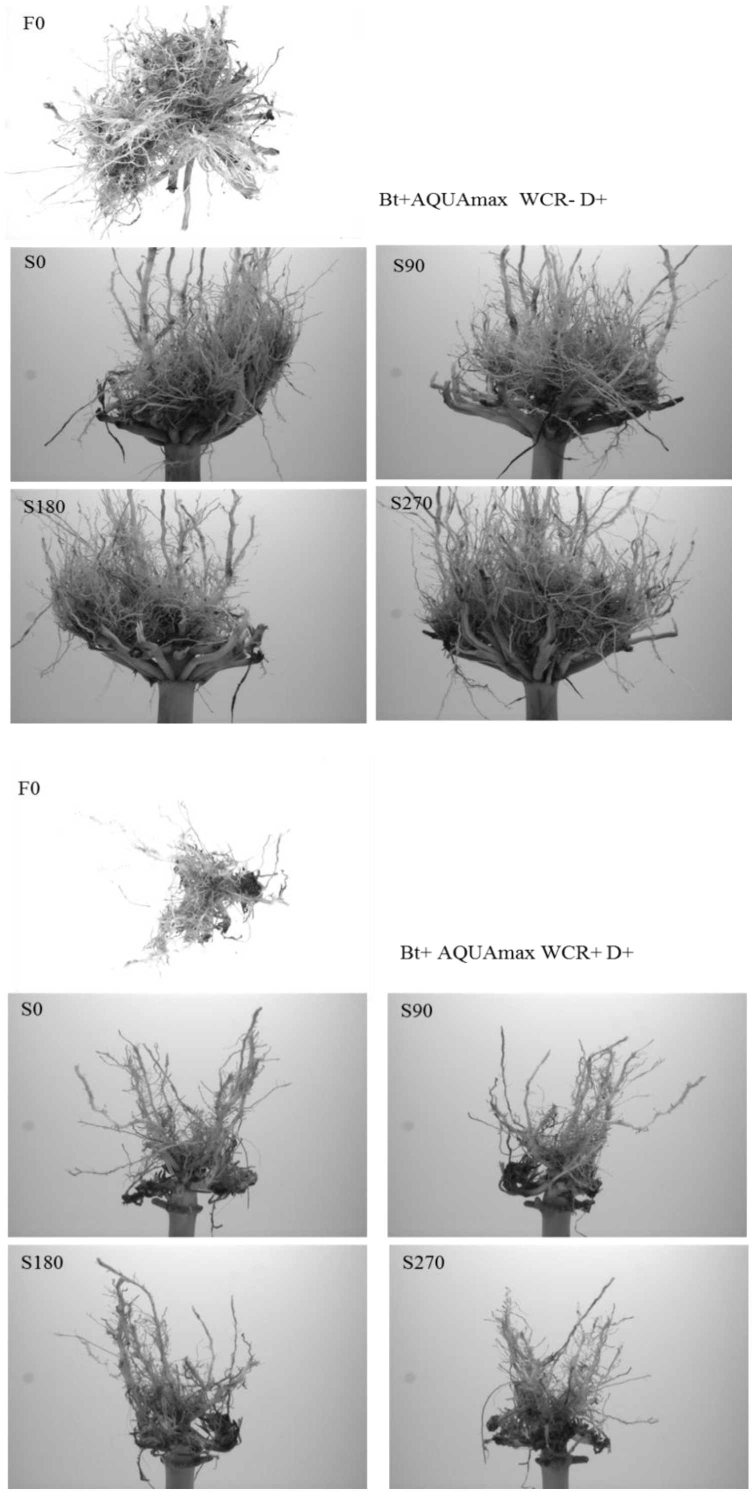
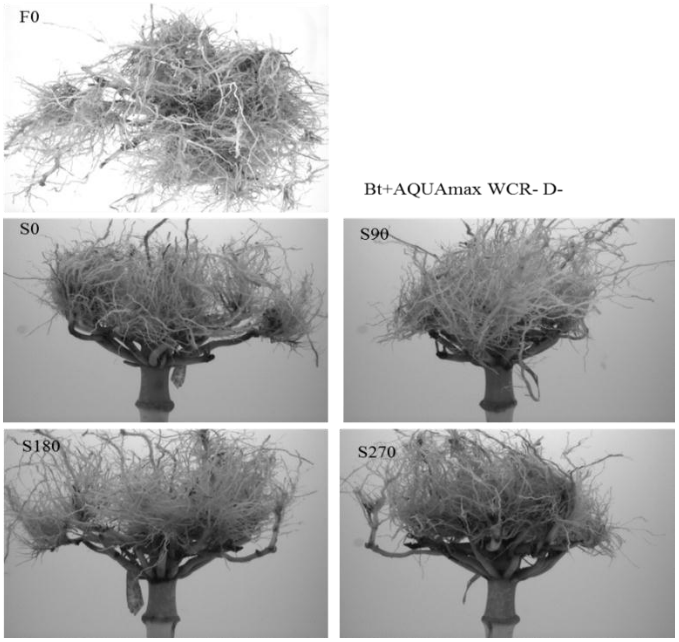


Fig. 52S. Images of Bt+AQUAmax maize roots from different angles to measure root complexity. Abbreviations: (WCR-) no western corn rootworm; (D+) drought treatment; and (WCR+) moderate western corn rootworm infestation level.



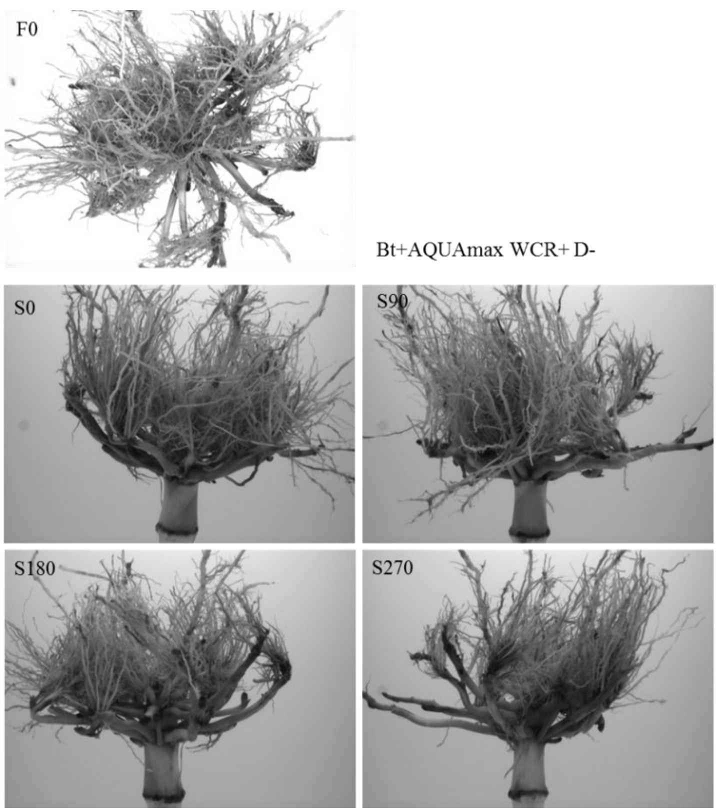


Fig. 53S. Images of Bt+AQUAmax maize roots from different angles to measure root complexity. Abbreviations: (WCR-) no western corn rootworm; (D-) well watered treatment; and (WCR+) moderate western corn rootworm infestation level.

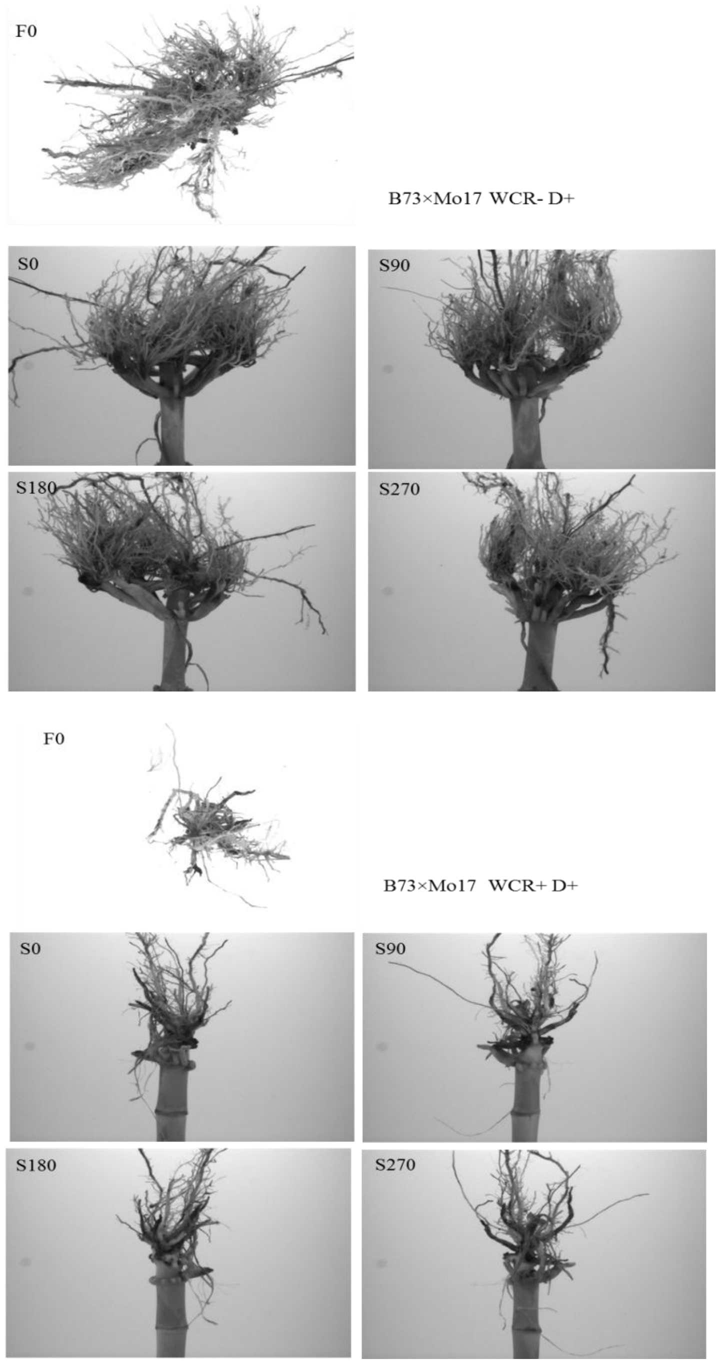


Fig. 54S. Images of B73×Mo17 maize roots from different angles to measure root complexity. Abbreviations: (WCR-) no western corn rootworm; (D+) drought treatment; and (WCR+) moderate western corn rootworm infestation level.

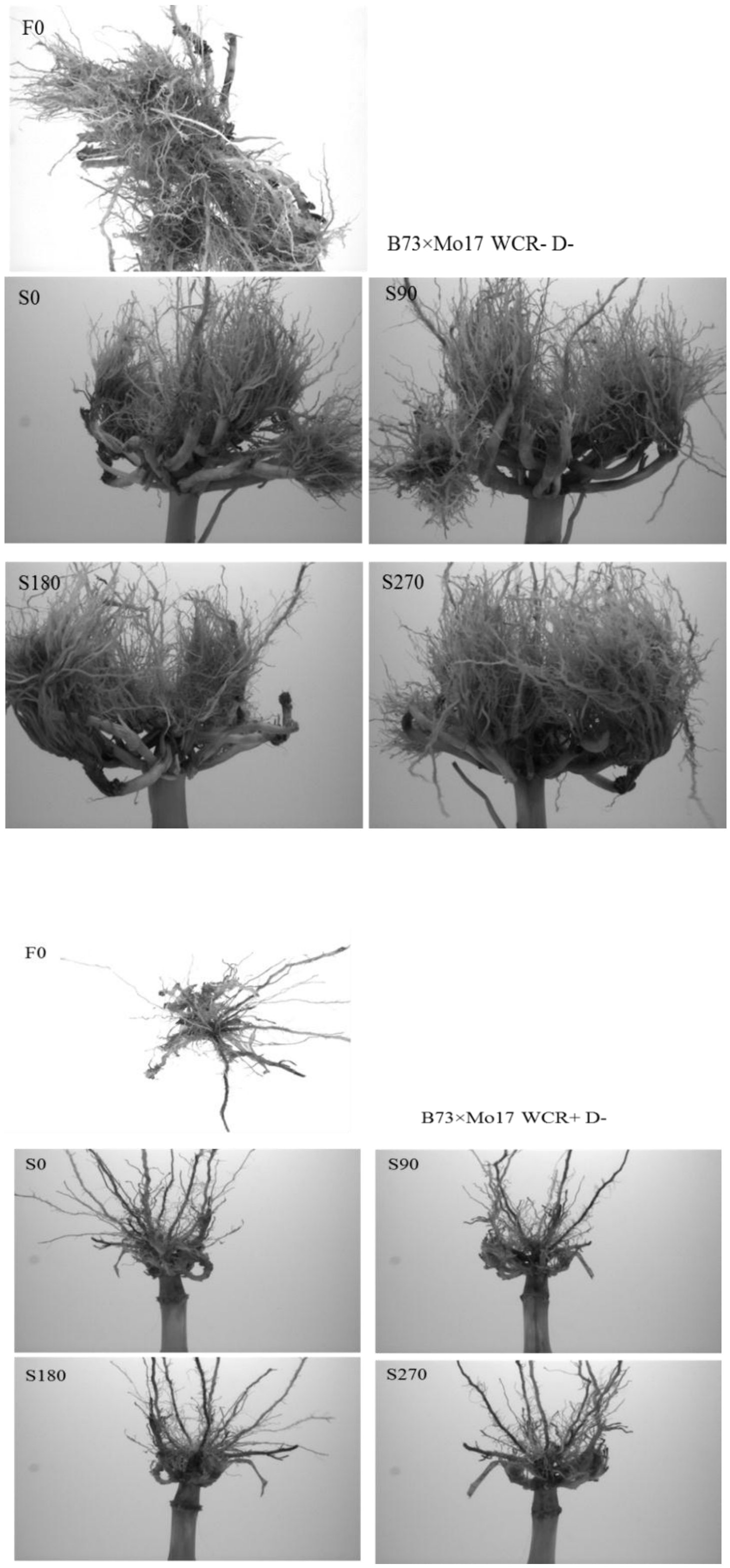


Fig. 55S. Images of B73×Mo17 maize roots from different angles to measure root complexity. Abbreviations: (WCR-) no western corn rootworm; (D-) well watered treatment; and (WCR+) moderate western corn rootworm infestation level.

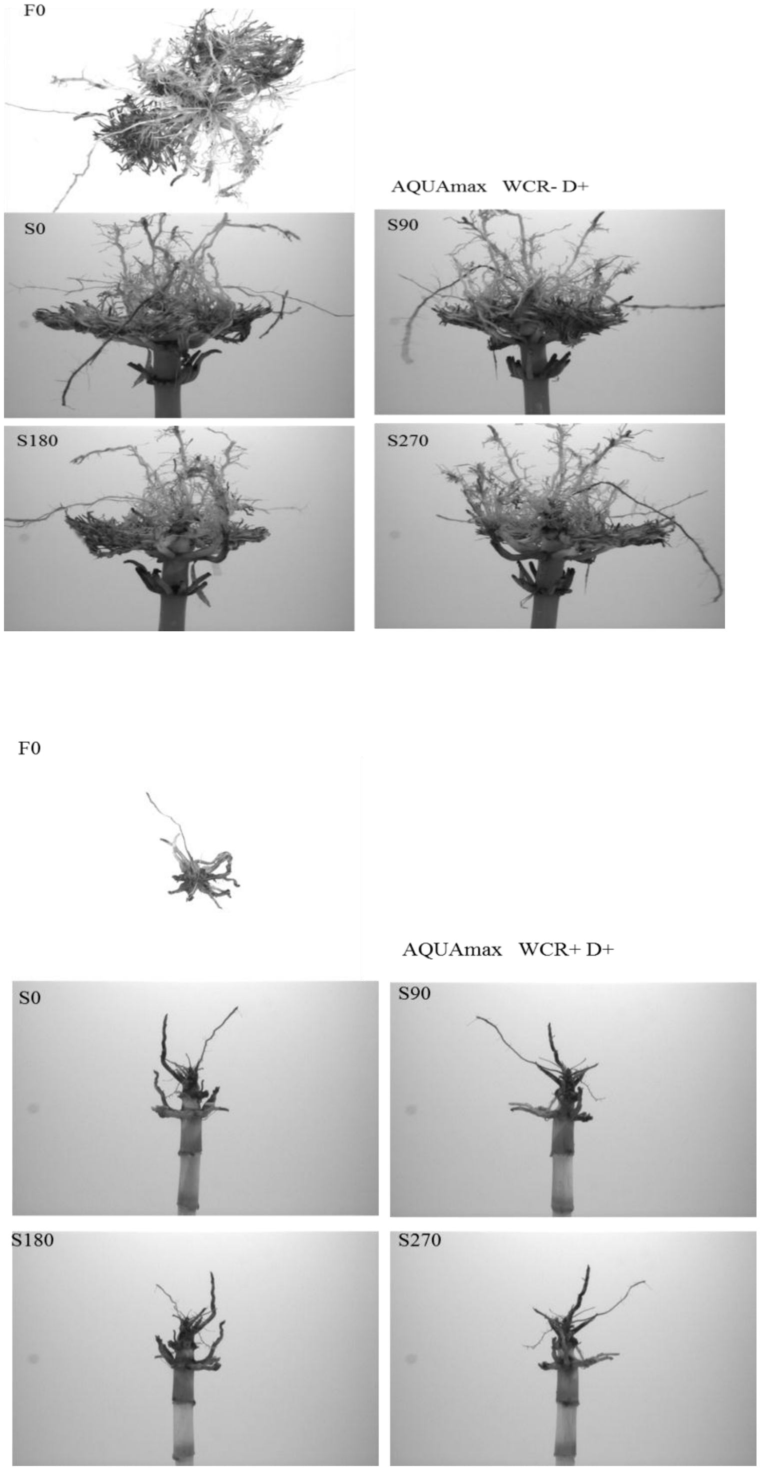


Fig. 56S. Images of AQUAmax maize roots from different angles to measure root complexity. (WCR-) no western corn rootworm; (D+) drought treatment; and (WCR+) moderate western corn rootworm infestation level.

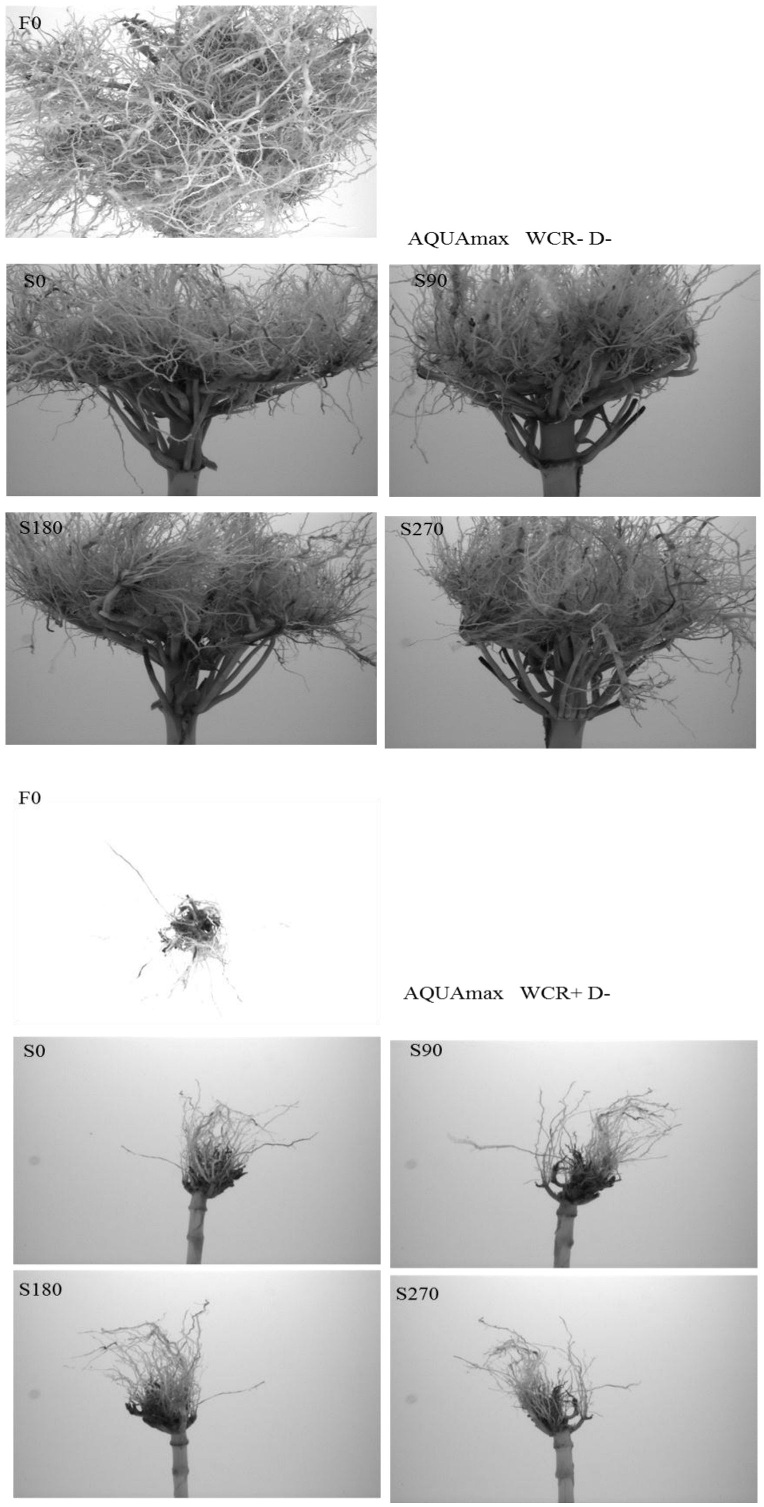


Fig. 57S. Images of AQUAmax maize roots from different angles to measure root complexity. Abbreviations: (WCR-) no western corn rootworm; (D-) well watered treatment; and (WCR+) moderate western corn rootworm infestation level.

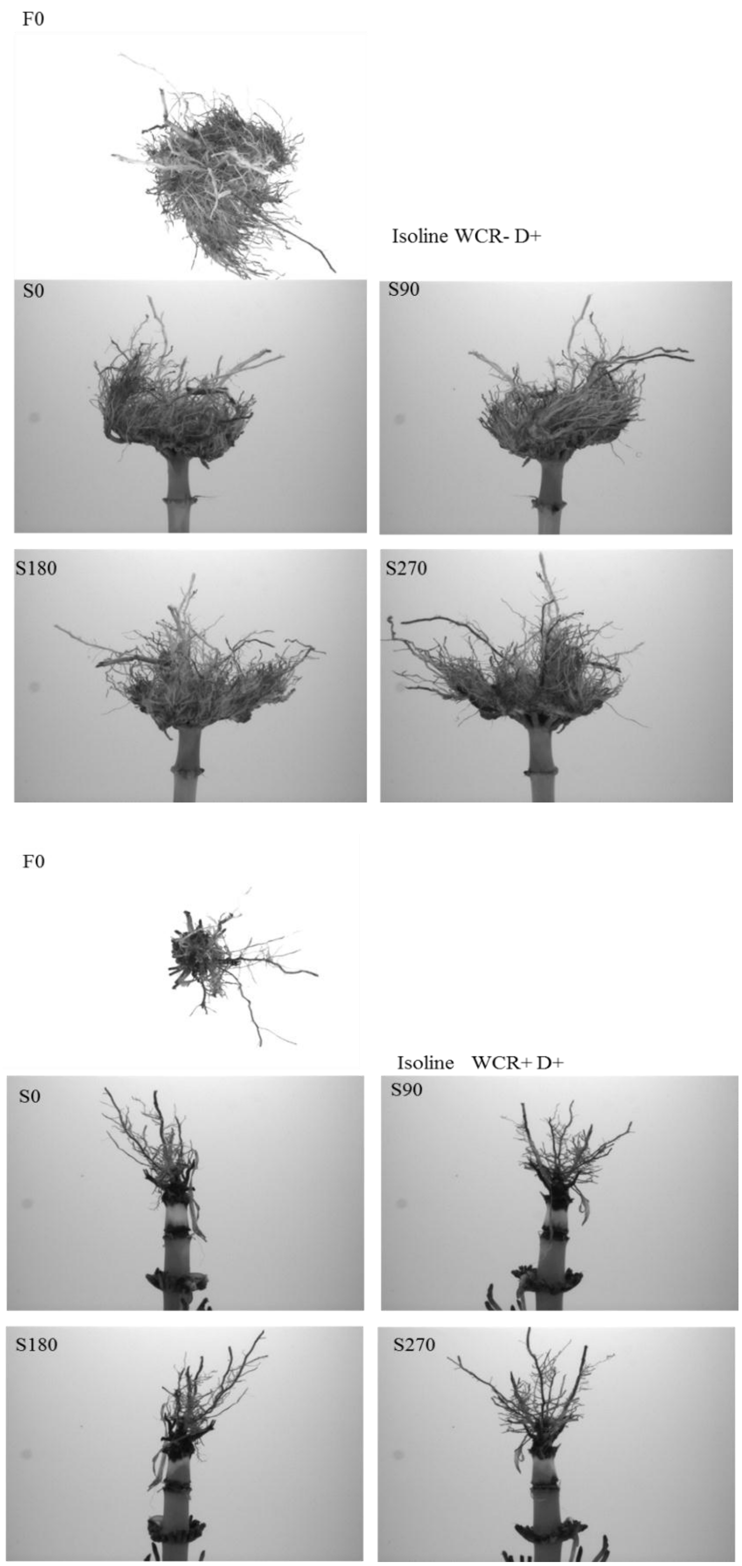


Fig. 58S. Images of isoline maize roots from different angles to measure root complexity**.** Abbreviation (WCR-) no western corn rootworm;(D-) well watered treatment; and (WCR+) moderate western corn rootworm infestation level.

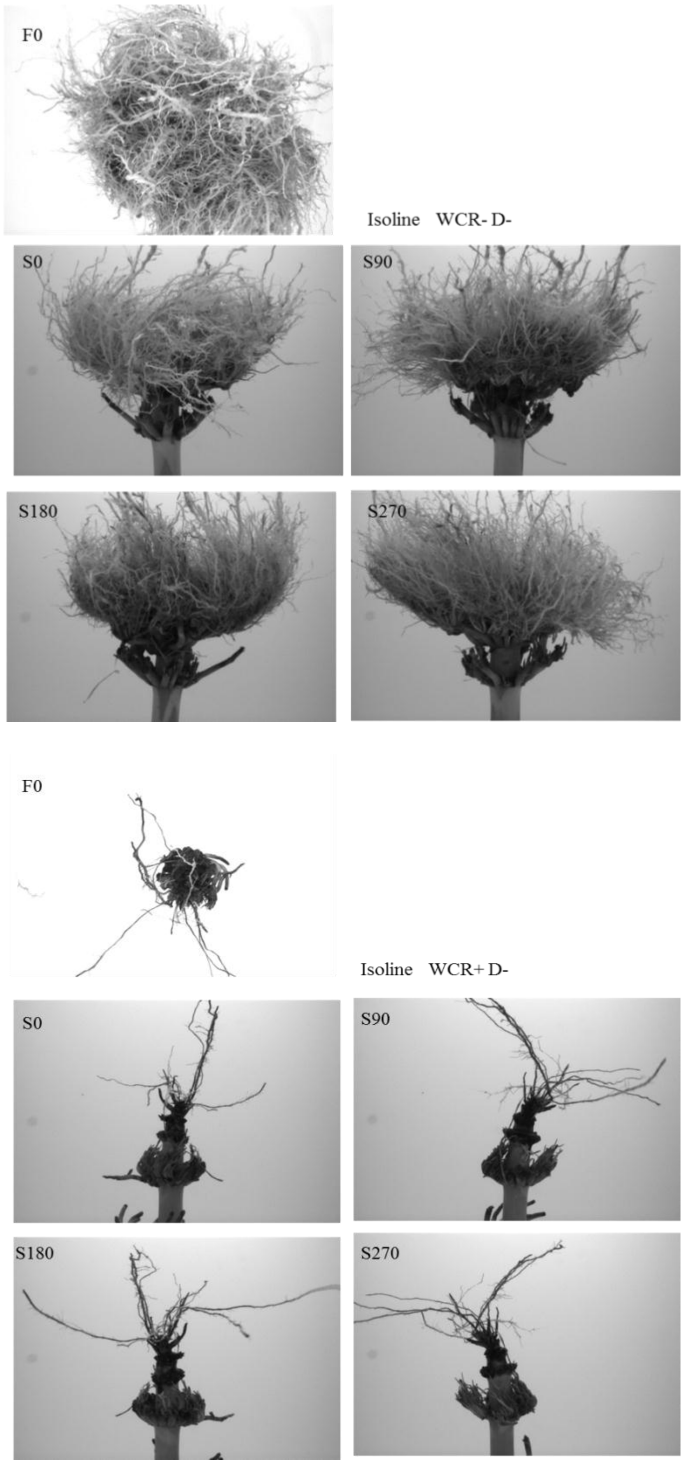


Fig. 59S. Images of isoline maize roots from different angles to measure root complexity**.** Abbreviations: (WCR-) no western corn rootworm; (D-) well watered treatment; (WCR+) moderate western corn rootworm infestation level.

VITAE

Mervat A. B. Mahmoud was born in Qena, Egypt. She graduated from South Valley University in Qena, Egypt, in May 2000 with her B.A. in Biology. She started her M.S. program in Dr. Mohammad Zaki lab during Jan. 2001, and her thesis was entitled, “Ecological Studies on Certain Pests and the Naturally Occurring Biological Control Agents of Order Hemiptera–Heteroptera in Qena”. Mervat graduated with her Master’s in Aug. 2005, and started her Ph.D. program with Dr. Bruce Hibbard in August 2010. Mervat gained her teaching experience by working for a brief time as a science teacher in Qeft High School, Qena, Egypt. During pursuing her Master degree at the South Valley University she was a teaching assistant for Entomology and Zoology courses for the undergraduate students. She continued as a teaching assistant in the university until she awarded the scholarship from the Egyptian government.