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Interactions Between Plant Water-Stress and Neonicotinoid Insecticides on Spider Mite Infestations in Corn

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INTERACTIONS BETWEEN WATER-STRESS AND NEONICOTINOID INSECTICIDES ON SPIDER MITE INFESTATIONS IN CORN

by

Alice Ruckert

A dissertation submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Ecology

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UTAH STATE UNIVERSITY
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2017
ABSTRACT

Interactions Between Plant Water-Stress and Neonicotinoid Insecticides on Spider Mite Infestations in Corn

by

Alice Ruckert, Doctor of Philosophy

Utah State University, 2017

Major Professor: Dr. Ricardo A. Ramirez
Department: Biology

Plant water-stress and the use of neonicotinoid insecticides are two abiotic factors that can independently trigger outbreaks of some agricultural pests through direct and plant-mediated mechanisms. Spider mites (Acari: Tetranychidae) are a pest of many crops and thrive under hot and dry conditions, during which generations are shorter, females are more fecund, and eggs hatch faster. Recent studies also show that neonicotinoids, a commonly used insecticide class, promote the development of spider mite infestations. Water-stress and the use of neonicotinoids often occur simultaneously, yet their interactive effects on spider mite outbreaks is unknown. Moreover, drought is expected to intensify in the Intermountain West in the near future and neonicotinoids are a primary insecticide class used in field crops. In chapter 2, I studied the combined effect of these two abiotic factors on Banks grass mites (BGM) in corn crops by conducting greenhouse and field experiments. Contrary to neonicotinoid insecticides, I found that water-stress alone increased BGM densities. However, the effect of water-stress on BGM was exacerbated when this factor was combined with the neonicotinoid active ingredients clothianidin and thiamethoxam.
Clothianidin, specifically, had a stronger and more consistent effect than thiamethoxam. Changes in plant defenses, mainly due to water-stress and spider mite herbivory, as well as changes of total protein concentration in response to spider mite herbivory combined with plant water-stress or neonicotinoids respectively, are possible mechanisms resulting in BGM outbreaks. In chapter 3, I tested whether drought-tolerant (DT) corn hybrids decrease BGM infestations when plants were water-stressed. In addition, in chapter 4, I examined whether exogenous application of jasmonic (JA) and salicylic (SA) acids, phytohormones involved in the biosynthesis of plant defenses, increase BGM resistance of corn in the presence of water-stress and neonicotinoids. I found that DT hybrids alleviated the effects of water-stress and reduced BGM populations, whereas applications of JA and SA did not decrease BGM infestations in the presence of water-stress and neonicotinoids.

My research represents an important step in the understanding of the mechanisms driving spider mite outbreaks under plant water-stress and the use of neonicotinoids, and provided practical tools to alleviate infestations in corn.

(163 pages)
Interactions Between Plant Water-Stress and Neonicotinoid Insecticides on Spider Mite Infestations in Corn

Alice Ruckert

Spider mites (Acari: Tetranychidae) are an important agricultural pest of many crops and landscape plants. They can reproduce rapidly and quickly develop resistance to many pesticides, making them difficult to manage. Plant water-stress and high temperatures promote spider mite infestations, while spider mite outbreaks can also result from neonicotinoid insecticide applications. Drought is predicted to increase in the Intermountain West due to increases in temperature and reduced frequency of precipitation events in the region, and neonicotinoids are currently one of the most widely used classes of insecticides in field crops. I studied the interactive effect of these two simultaneously occurring abiotic factors on spider mite outbreaks and plant biosynthesis of herbivore-related defense proteins. I also evaluated ways to alleviate spider mite outbreaks with drought-tolerant corn and the exogenous application of plant phytohormones involved in plant resistance toward biotic stressors. I found that plant water-stress increased spider mites and that neonicotinoids exacerbated the effect of water-stress. Although applications of plant hormones did not reduce the effect of water-stress and neonicotinoids, drought tolerant corn showed promise in reducing the effect of water-stress and spider mite outbreaks.
This work is dedicated to my family:

To my parents, who always encouraged me to discover the world, believe in myself, and stand up after every fall.

To Nonna Rosa, who is the smartest of our family, does math better than me, and rocks the world at 88 years-old.

To Joni and Thomas, who welcomed me as a daughter, spoiled me with delicious treats, and gave me the warmth of a family when I arrived to US.

To Faye, who became my second grandma, made me laugh with her dry humor, and always thought of me at my birthdays.

To Irene, who has always been there for me despite these 8984.968 km of distance, is a true and generous friend, and taught me to appreciate the pleasures of life.

And to my beloved husband Chris and daughter Sofia, who fill my day with joy and laughs, with optimism and faith, with tenderness and unconditional love.
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Alice Ruckert
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CHAPTER I

LITERATURE REVIEW

Spider mites

Spider mites are arthropods of the order Acari and belong to the family Tetranychidae, which includes about 900 species (Gutierrez et al. 1985) feeding on all major food crops and many ornamental plants (Pritchard and Baker 1955). Spider mites are parenchyma-sucking arachnids that feed on plant cell content by using their stylet. They usually attack leaves but can also feed on specific plant parts including cotyledons, fruits, flowers, fruit spurs, or tips of shoots (Tomczyk and Kropczyńska 1985). Due to their rapid rate of reproduction, spider mites can easily colonize plants by reaching very high densities in a relatively short period of time (Kennedy and Smitley 1985). If left uncontrolled, spider mites are capable of seriously damaging their host plants, especially in an agricultural ecosystem, where heavy fertilization, conditions of drought-stress, and the use of certain pesticides can trigger their outbreaks (Kennedy and Storer 2000).

_Tetranychus urticae_ (Koch) & _Oligonychus pratensis_ (Banks)

The twospotted spider mite (TSSM), _Tetranychus urticae_ Koch, which has a polyphagous feeding behavior, and the Banks grass mite (BGM), _Oligonychus pratensis_ Banks, which is a grass specialist, commonly attack corn (Tuttle and Baker 1968, Kant et al. 2008, Peairs 2010). These species are secondary pests of corn crops, but at times, they can become a serious issue, especially in arid regions of the western United States (Ehler 1974, Owens et al. 1976). TSSM and BGM, are known to thrive
under high temperatures and low humidity, which are common conditions in the West (Boudreaux 1958, Ferro and Chapman 1979).

**Morphology**

TSSM and BGM have a similar shape and size (they measure less than 1 mm), which make it difficult to distinguish the two species with the naked eye. TSSM has a creamy-pale color and two well-defined symmetrical black spots on the upper part of the abdomen (Tuttle and Baker 1968), while BGM is pale-green and has blackish spots extending along the entire sides of the abdomen (Auger et al. 2013). Males are commonly smaller, slender and have a pointed abdomen, while females are more robust (Mitchell 1973, Zhang and Jacobson 2000). Larvae have three pairs of legs, while proto- and deutonymphs, as well as adults have four pairs of legs.

**Life history**

Before reaching adulthood, tetranychid mites develop through five stages including an egg, larva, protonymph, and deutonymph. Development from egg to adult may vary from 6 to 10 days or more, and the total life span can last from 2 to 4 weeks (Crooker 1985). Spider mites overwinter as adult females or eggs on weeds, in the soil, or leaf litter. The total production of eggs by a single female in her life-time varies greatly from as few as 10 to 150 or more (5-6 per day on average), depending on the species, humidity, temperature and host (Crooker 1985). Females are arrhenotokous, where unfertilized eggs give rise to males that are haploid and fertilized eggs produce females that are diploid (Vale et al. 2004). Eggs are usually laid on plants, primarily on the undersides of leaves, but in some cases, on soil or near
plants (Van de Lustgraaf, 1977). The shape of the egg is spherical or onion-like, and the diameter varies from 110 to 160 µm, depending on the species (Crooker 1985). Eggs are translucent when first deposited, then they become more opaque and can assume different colors, such as pale green, brown, orange or red (Crooker 1985). The incubation period lasts on average 3-15 days, with high temperature levels promoting a faster hatch (Dittrich and Streibert 1969, Ferro and Chapman 1979, Crooker 1985).

Larvae are the same size as the eggs and are characterized by three pairs of legs. Larvae first molt to a protonymph, which then molts to a larger deutonymph, the last immature stage. The duration of each developmental stage was measured by Mondal and Ara (2006) for TSSM. These authors observed that, under optimal conditions of temperature and humidity, the egg to larva, larva to protonymph, protonymph to deutonymph, and deutonymph to adult stages took approximately 5, 2, 2, and 2 days respectively.

Nymphs have four pairs of legs and resemble the adult. Immature stages actively feed on plants, with the exception of a brief period of quiescence during which mites form new cuticle (Mitchell 1973). Males mature before females and they remain in the proximity of the other sex until emergence (Potter et al. 1976). The male often aids the females emerging as adults, by freeing them from the exuvium. Copulation takes place immediately after female emergence and lasts almost 1-2 minutes (Potter et al. 1976).

Tetranychid mites have developed mechanisms that enable their populations to spread over large areas, such as crawling from plant to plant through intertwined foliage and over the ground, and wind transportation through the production of silk webbing (Kennedy and Smitley 1985). Spider mites commonly spread from
overwintering sites along the field margins, where secondary plant hosts (either weeds or adjacent crops) are more likely to be located, and gradually move towards its center (Margolies et al. 1985).

Spider mite infestations start to appear in late spring, but populations can quickly build up later in the summer, when temperatures start to increase, rainfall events are less frequent, and the deposition of dust on leaves is higher, which decreases their visibility to natural enemies (Mckie and Johnson 2005). Spider mites, such as TSSM and BGM, tend to first colonize the lower portion of the plants on leaves near the mid-vein, and then slowly move upwards as mites become overcrowded and leaves senesce (Mitchell 1973). TSSM and BGM most commonly feed on the underside of the leaves. Some species, however, feed on both sides and, occasionally, the upper side is preferred (Tomczyk and Kropczyńska 1985).

TSSM tends to appear earlier in the season since it prefers slightly cooler temperatures (30-32 °C), while BGM flourishes under hotter weather conditions (35-37 °C) (Peairs 2010). Infestations are commonly found in corn from the tasseling phase through the grain filling growth stage. Rapid population buildup usually occurs after pollen shed (Ehler 1974, Feese and Wilde 1977, Chandler 1979). It has also been estimated that the percent field loss per unit for most independent variables (spider mite densities, permanence on the plant, percentage of the leaf area damaged) is usually very similar for TSSM and BGM (Archer and Bynum 1993).

**Plant injury**

Spider mites usually cause plant mechanical damage and biochemical alterations, visible as leaf chlorosis, desiccation and abscission, growth inhibition, and
reduced seed size (Tomczyk and Kropczyńska 1985, Schmidt 2014). TSSM and BGM, for example, cause chlorotic spots on corn leaves which can turn into necrotic areas, grain moisture reduction, kernel shrinkage and, in some cases, stalk rot and plant lodging (Bacon et al. 1962, Archer and Bynum 1990, 1993). Symptoms of spider mite attack, however, can vary and it may depend, among other factors, on the species, the characteristics of the leaves (i.e. anatomical structure or chemical composition), and the specific reactions in the plant (Tomczyk and Kropczyńska 1985). Spider mite saliva can also trigger a toxic effect on cells adjacent to damaged cells, causing the destruction of their chloroplasts, degeneration of the nucleus, and alterations in the structure of the cell walls (Tomczyk and Kropczyńska 1985). Previous studies have also shown that spider mite infested plants can have decreased levels of nitrogen, phosphorous, and potassium, as well as altered concentrations of proteins, carbohydrates and plant hormones, which can slow plant growth and reduce both productivity and the quality of the final product (Zukova 1963, Storms 1971, Herbert and Butler 1973, Golik 1975, Kolodziej et al. 1975).

**Effects of drought-stress on spider mites**

Spider mites have always been a common pest of field and orchard crops in arid and sub-arid regions of the United States such as the Intermountain West, where summer daytime temperatures are high (20-30 °C) and routine droughts occur (Karsten and MacAdam 2001). Uncontrolled secondary outbreaks of this pest, however, have started to appear in this region, where more frequent and persistent periods of drought have occurred. Numerous studies have shown that high temperatures and arid conditions promote spider mite outbreaks. During these

During events of drought, plants undergo morphological, physiological and biochemical changes, which can decrease plant resistance to pests and promote the development of phytophagous arthropod outbreaks (Jeppson et al., 1975, Christiansen et al. 1987, Mattson et al. 1987a, Chaves et al. 2003). Under conditions of drought-stress, plant tissues were found to have higher availability of nutrients, such as nitrogen and certain amino acids (Brodbeck and Strong 1987). Water-stress can reduce the biosynthesis of proteins, since growth is limited during periods of drought (White 1969, Hsiao 1973, Brodbeck and Strong 1987). Also, the concentration of soluble sugars, as well as other inorganic solutes (K+, Ca2+, Na+), can increase due to simple accumulation following water loss and plant activation of osmotic adjustments, aimed to reduce the water flow to intercellular spaces (Hsiao 1976, Jones and Rawson 1979, Morgan 1984, Blum 1988, Nielsen and Orcutt 1996, Mohammadkhani and Heidari, 2008). Drought-stressed plants can also have inferior levels of defensive compounds against pests and pathogens (Rhoades 1979, English-Loeb 1990, Herms and Mattson, 1992). For example, less glucosinolates, which are the main secondary compound in some Brassicaceae, were found in water deficient Alliaria petiolata (Haugen et al. 2008) and Brassica holboelli (Gutbrodt et al. 2011). However, the production of defensive compounds can increase, depending on the intensity of drought. As an example, even if plants reduce growth at moderate water-
stress, photosynthetic processes are maintained and carbohydrates continue to accumulate, thus the production of carbon-based secondary compounds can increase (Gershenzon 1984, Mattson and Haack 1987 a,b, Inbar et al. 2001).

Many herbivores, including spider mites, can benefit from conditions of moderate drought-stress or pulsed stress (due to irrigation or rainfall events interrupting prolonged dry periods), as some herbivorous pests require positive cell turgor pressure to extract nutrients (Holtzer et al. 1988, Archer et al. 1995, Huberty and Denno 2004). In addition, drought fosters the dispersion of dust from adjacent roads onto crops, which creates a more favorable habitat for the development of spider mites by reducing their visibility to natural enemies (Mckie and Johnson 2005).

**Spider mite management**

Corn is a very important crop in the United States because of the rapidly growing dairy industry’s need for maize-based grain and silage. In addition, the biofuel industry is expanding nationwide and causing considerable increase of maize grain demand (Nansen et al. 2009). Consequently, spider mite control is becoming a necessity. Utah, which is located in the Intermountain West, is one of the driest regions of the United States, and has approximately 24,300 hectares in corn production (USDA 2015). Monitoring for TSSM and BGM and understanding the key components of spider mite outbreaks, are important for successful management.

**Monitoring**

To prevent TSSM and BGM infestations from reaching economically damaging levels, careful monitoring of corn plants through their growing cycle is
recommended. Monitoring for these species starts after plant establishment and becomes more frequent over time, especially during tasseling and the plant reproductive phase, when populations can build up quickly (Chandler 1979). It is recommended that leaf samples be collected from the lower third of corn plants, where spider mite infestations start to develop. Leaves should be examined for yellowish-reddish brown spots, which is the typical evidence of mite injury, webbing and presence of mites (Archer and Bynum 1990; 1992, Ruckert et al. 2015).

The percent corn yield loss per unit for independent variables such as mite density, mite permanence on the plant, and percentage of the leaf damaged, is very similar for BGM and TSSM, hence the same economic threshold could be used for either mite species (Archer and Bynum, 1993).

Archer and Bynum (1990) developed a relatively simple guideline to calculate treatment thresholds for BGM. In their guideline, four variables were suggested to be evaluated before recommending chemical control. Specifically, cost of miticide treatment, expected crop market value per acre, percent of infested leaves per plant, and amount of leaf area damaged. Currently, however, no predictive models to time treatments for TSSM and BGM in corn fields are available. As a consequence, preventive control methods should be adopted, in addition to chemicals, to help avoid or decrease the development of spider mite resistant populations.

**Preventive control**

TSSM and BGM are known to overwinter on border vegetation or senescent crops, hence, weed management and prompt removal of host plants can help prevent spider mite contamination of adjacent corn fields, which mainly occurs by crawling or
by wind dispersal (Brandenburg and Kennedy 1982). TSSM, in fact, has been recorded on over 900 plant species (Buolland et al. 1998) and has a broader host range than BGM, due to its polyphagous feeding habit (Kant et al. 2008). BGM, instead, is found primarily on grasses, both wild and cultivated (Tuttle and Baker 1968).

Reducing the application of fertilizers can also decrease the risk of spider mite outbreaks, since high nitrogen concentrations were found to promote the development of spider mite populations by increasing the nutrient availability in plant tissues (Watson 1964). More frequent and uniform irrigation events during dry conditions can reduce the effect of drought-stress that appears to foster the development of spider mites (see effects of drought-stress on spider mites section). Moreover, overhead irrigation systems (pivot and sprinkles), can mechanically wash mites off from the plant canopy or delay their development (Filgueira 2000, Ruckert et al. 2015).

**Curative control**

*Biocontrol* Spider mites have several natural enemies which can successfully limit their development. Some of the most important are predaceous mites, such as Phytoseiulus spp. and Neoseiulus spp. (McMurtry et al. 1970). Predatory mites are about the same size as herbivorous mites, but have a tear-drop shape, longer legs, and are more active. Other predators include beetles of the genus Stethorus and Oligota, predatory thrips, predatory bugs such as damsel bugs, big-eyed bugs and minute pirate bugs, lacewing larvae, and earwigs (McMurtry et al. 1970). Some spiders from the families Argiopidae, Theridiidae and Linyphiidae were also found to prey on herbivorous mites (Chant 1956; 1957). Biological control of spider mites is available
on the market through the purchase of some of these predators, specifically: predatory mites, _Stethorus_ spp., _Orius_ spp., _Scolothrips sexmaculatus_ and _Chrysoperla_ spp. (Oatman and McMurtry 1966, Pickett and Gilstrap 1986a;b, García-Marí and González-Zamora 1999, Ruckert et al. 2015). The use of these beneficial arthropods can be a solution to reduce spider mite populations, however these predators can fly away or die by starvation if the prey abundance is low, or if the food source is not easily traceable in the plant canopy (Grevstad and Klepetka 1992). Moreover, the biological control of spider mites may not be feasible due to cost for large commercial operations, hence, it may work best for small farms and backyard gardeners.

Many of the natural enemies of spider mites are common residents of corn fields (Pickett and Gilstrap 1986a). As a consequence, it is important to use more selective insecticides that are less toxic to natural enemies and reduce the numbers of applications to prevent natural predator populations from dying off.

*Chemical control* Chemical applications for mites in corn is recommended near the tasseling phase, especially if the field has a history of spider mite outbreaks, the daily temperatures are expected to exceed 35°C, rainfall episodes are limited, drought-stress conditions are starting to appear, mite predator density is low, and feeding injury is evident in the first third of the plant (Peairs 2010, Ruckert et al. 2015). Chemical control of spider mites usually involves miticides or acaricides with contact or systemic activity. These products are usually more active on adults since, during molting, juveniles remain inactive beneath the former skin that acts as a protection and they do not feed immediately. In this case, active ingredients that kill by ingestion are temporarily ineffective (Mitchell 1973). The most common products
used against spider mites in commercial corn production include Oberon 2 SC (active ingredient spiromesifen), Comite II (active ingredient propargite), Onager (active ingredient hexythiazox), Zeal (active ingredient etoxazole), Hero (active ingredients bifenthrin and zeta-cypermethrin), Dimethoate (active ingredient dimethoate) and Portal (active ingredient fenpyroximate) (Sanderson and Edson 1964, Herron et al. 2003, Nauen et al. 2003, Riedl et al. 2006, Irigaray and Zalom 2007, Haviland et al. 2011). Oberon 2 SC acts by contact, has a translaminar movement, and affects all life stages, causing death by inhibiting the biosynthesis of lipids (Nauen et al. 2003, Cloyd et al. 2009). Comite II is a contact miticide effective against adults and juveniles, and acts as an ATP synthesis inhibitor (Martini et al. 2012). Onager and Zeal are both efficient against eggs and juveniles and act as growth regulators. However, Onager is a contact miticide, while Zeal works by contact and has a translaminar movement (Cloyd et al. 2009, Martini et al. 2012). Hero is efficient against adults and juveniles, and alters the sodium channels when directly applied on mites (Ahmad et al. 2002). Dimethoate is instead a systemic and a contact pesticide, killing both adults and juveniles by inhibiting the functions of cholinesterase (Uchida et al. 1965). Finally, Portal is a contact pesticide effective against all life stages, and inhibits the mitochondrial electron transportation (Stumpf and Nauen 2001).

reproduction (Van Leeuwen et al. 2010). TSSM is one of the most resistant arthropods to pesticides, including 92 unique active ingredients for which resistance has been reported (Whalon et al. 2008). As a result, the control of this species has become increasingly complicated in many agricultural systems (Whalon et al. 2008, Van Leeuwen et al. 2010). The first widespread event of spider mite resistance towards chemical control was observed in fruit and citrus orchards in 1950, a few years after the introduction of organophosphates. Resistance was achieved due to the development of acetylcholinesterase insensitivity, which was targeted by this insecticide class (Smissaert 1964, Cranham and Helle 1985). Dimethoate, which is frequently used in corn fields, is part of this chemical class and spider mites were found to be less susceptible to this active ingredient. More recently, Yang et al. (2002) have observed the development of resistance in TSSM and BGM following the use of bifenthrin (pyrethroid) and dimethoate. The mechanism of resistance is due to an increased enzymatic hydrolysis and oxidation of the insecticide by mites (Ay and Gurkan 2005, Van Leeuwen et al. 2005, Van Leeuwen and Tirry 2007). TSSM has recently showed resistance against bifenazate, an acaricidal active ingredient which is part of the hydrazine derivative group and acts by altering the electron transport chain (Dekeyser 2005, Van Leeuwen et al. 2008, Van Nieuwenhuyse et al. 2009). Episodes of cross-resistance were observed in TSSM towards chemical families such as quinazolines, pyrimidinamines, pyrazoles and pyridazinones, which inhibits mitochondrial electron transport. TSSM also showed resistance towards avermectins (abamectin), which are obtained from the soil-micro-organism Streptomyces avermitilis, and is thought to interfere with γ-aminobutyric acid and glutamate-gated chloride channels (Van Leeuwen et al. 2009; 2010). TSSM resistance has been
detected on organotin compounds too (chlorfenapyr, dicofol), and some growth regulators such as clofentezine, hexythiazox, and etoxazole (Van Leeuwen 2009).

Episodes of hormoligosis, in which sublethal quantities of pesticides induce reproductive stimulation in herbivorous arthropods, have been observed in spider mites. For example, Dittrich et al. (1974) found that residues of carbaryl and dioxacarb caused TSSM to lay more eggs than when the species was kept on untreated plants. TSSM also showed increased egg production when directly exposed to spray formulations of imidacloprid, or fed with treated bean plants (James and Price 2002). Other examples of hormoligosis were found by Jones and Parrella (1984) on red mite *Panonychus citri* after citrus plants were treated with malathion or permethrin.

Rotation to chemicals with different modes of action, reduced frequency of applications, and adhering to the doses on the product label are strongly encouraged. By respecting these practices, the development of spider mite resistance, as well as hormoligosis, can be deterred.

**Effects of neonicotinoid insecticides on spider mites**

Neonicotinoids are a class of systemic insecticides widely used in agriculture against a broad range of pests having piercing-sucking mouthparts or for cryptic chewing pests (Jeschke and Nauen 2008). The first neonicotinoid compound produced was imidacloprid in 1991, followed by six additional active ingredients including acetamiprid, nitenpyram, thiametoxam, thiacloprid, clothianidin and dinotefuran (Elbert et al. 2008). Neonicotinoids can be either translaminar, when the insecticide passes through the leaf tissue from one surface of a leaf to the other, or systemic,
when the insecticide is absorbed by the plant and transported to other plant tissues. These insecticides act as agonists at the post-synaptic membrane of the insect nicotinic acetylcholine receptor, mimicking the natural neurotransmitter acetylcholine and inducing a neural hyper-excitation, which leads to the insect's death (Matsuda et al. 2001). Neonicotinoids are not phytotoxic and can be directly applied in the soil through irrigation systems, by foliar spray application, or as seed treatments. Currently, neonicotinoids are widely used for seed treatment in corn, cotton, cereals, sugar beet, oilseed rape, soybean, and other crops (Elbert et al. 2008). It is thought that the application of neonicotinoids to seeds has marked an unprecedented shift towards large-scale use of these insecticides. Neonicotinic insecticides were also found to be the dominant class of insecticidal compounds used to treat corn seeds (Douglas and Tooker 2015).

Neonicotinoids have recently come under scrutiny for several unintended consequences on some arthropods, particularly bees and spider mites. These insecticides have a relatively long persistence in the soil and in the water, which prolongs the uptake by wild plants and subsequent crop plantings. As a consequence, arthropods are exposed to sub-lethal concentrations of the insecticidal active ingredients (Van der Sluijs et al. 2013). Recent studies have found that the nitro-containing neonicotinoids such as imidacloprid, clothianidin, thiamethoxam, nitenpyram and dinotefuran can severely affect pollinators by interfering with their memory, foraging behavior, nectar storing, and by reducing queen survival (Blacquière et al. 2012, Fischer et al. 2014, Scholer and Krischik 2014). Moser and Obrycki (2009) also found that clothianidin and thiamethoxam seed treatments cause
larval mortality of *Harmonia axyridis*, non-target coccinellids, when feeding on corn seedlings.

Spider mite outbreaks following neonicotinoid insecticide applications have also been observed. Sclar *et al.* (1998) first described the phenomenon of increased populations of honeylocust spider mites (*Platytetranychus multidigitali*) after the application of imidacloprid on honeylocust. The same active ingredient was also found to promote outbreaks of spruce spider mites (*Olygonychus ununguis*) on hemlock (Raupp *et al.* 2004), twospotted spider mites (*Tetranychus urticae*) on rose plants (Gupta and Krischik 2007), Schoenei spider mites (*Tetranychus schoenei*) on elms (Szczepaniec *et al.* 2011), and boxwood spider mites (*Eurytetranychus buxi*) on boxwood (Szczepaniec and Raupp 2012; 2013).

The occurrence of pest outbreaks due to the use of pesticides is usually associated with the unintended suppression of natural enemies. However, neonicotinoid insecticides were found to have little effect on spider mite predators (Szczepaniec *et al.* 2011). Specifically, no negative effect of imidacloprid was observed on predatory mites and lacewings. Neonicotinoids are thought to activate different plant-mediated mechanisms that lead to spider mite outbreaks. For example, Szczepaniec and Raupp (2013) showed that *E. buxi* females laid nearly 40% more eggs when feeding on foliage from treated elms, but their fecundity was not increased when mites where directly exposed to imidacloprid through dermal applications and then fed with untreated foliage. This result suggested that the increased reproductive performance was an indirect, plant-mediated effect of the neonicotinoid. In 2013, Szczepaniec *et al.* demonstrated that spider mite outbreaks could be related to an alteration of the biosynthesis of plant defensive compounds against pathogens and
herbivorous arthropods, due to the application of neonicotinoids. The study showed that active ingredients such as imidacloprid, clothianidin, and thiamethoxam altered the concentration of the phytohormone 12-oxo-phytodienoic acid, jasmonic acid (JA) and salicylic acid (SA), which are important precursors of plant defenses, in tomato, corn, and cotton. These neonicotinoids also suppressed the transcription of phenylalanine ammonia lyase (PAL), coenzyme A ligase (CoA ligase), trypsin protease inhibitor (TI) and chitinase (Szczepaniec et al. 2013), all regulated by SA (PAL, CoA ligase, chitinase) and JA (TI) (Shah 2003, Wu and Baldwin 2010). This physiological alteration was then followed by significant spider mite population growth in both greenhouse and field experiments. Moreover, Ford et al. (2010) discovered that some neonicotinoid active ingredients can induce SA-related plant responses in *Arabidopsis thaliana*. Specifically, imidacloprid was found to act as a SA mimic, via its metabolite 6-chloro-2-hydroxypyridinyl-3-carboxylic acid, whereas clothianidin induced endogenous SA biosynthesis via its metabolite 2-chlorothiazolyl-5-carboxylic acid (Ford et al. 2010). As a consequence, *A. thaliana* treated with imidacloprid and clothianidin became more resistant to powdery mildew, as SA is known to activate plant resistance to pathogens (Ford et al. 2010). Treatments with imidacloprid in sugar beets, cereals, and tobacco, were found to enhance plant resistance to beet mild yellow virus, barley yellow dwarf virus, and thrips and tomato spotted wilt virus respectively (Dewar 1992, Heatherington and Meredith 1992, Tatchell 1992, Rudolph and Rogers 2001). Karthikeyan et al. (2009) also observed that treatments with thiamethoxam significantly increased resistance of *Vigna mugo* to urdbean leaf crinkle virus.
In some plants, pathogen-associated defenses, which are linked to SA, and herbivore-related defenses, which can be primarily linked to JA, were found to have antagonistic interactions (cross talk) (Doares et al. 1995, Koornneef and Pieterse 2008). Thus, the induction of resistance towards pathogens, due to an increase of SA after the use of neonicotinoids, can eventually decrease JA and raise the susceptibility of plants to herbivory (Ford et al. 2010, Szczepaniec et al. 2013b).

Aside from the effects of neonicotinoids on spider mites, this class of insecticides can improve the productivity of the plant. Gupta and Krischik (2007) reported that rose plants treated with imidacloprid had a greater content of chlorophyll and a larger leaf area. Gonias and Oosterhuis (2006; 2008) found that imidacloprid-treated cotton had enhanced growth and yield, a higher number of fruiting positions, and increased lint yield. Gonias and Oosterhuis (2008) also showed that cotton plants had a higher chlorophyll fluorescence yield at high temperatures after imidacloprid applications, and that plants could better resist temperature stress. Preetha and Stanley (2012) observed a general increase in soluble protein content in thiamethoxam- and acetamiprid-treated cotton and okra, which increased the ability of the plants to fix carbon dioxide and promote photosynthesis. In the same study, acetamiprid was also found to boost the total chlorophyll content in okra.

**Research objectives**

Drought-stress and neonicotinoids affect the physiology of plants and may alter plant resistance against pests. Yet, the interaction between these simultaneously occurring abiotic factors on spider mites is not clear. So far, much work has focused on the cosmopolitan TSSM, while there has been less known about the specialist
BGM and its interactions with neonicotinoid treatments. We tested the combined effect of water-stress, which is prevalent in the Intermountain West and predicted to intensify (NOAA 2014), and the use of neonicotinoids, which are widely used as a seed treatment in corn, on the development of BGM through plant-mediated mechanisms. More precisely, field and greenhouse experiments were conducted to examine:

1. how water-stress and the use of neonicotinoid insecticides affect spider mite populations over time, and how these factors influence plant phenotypic and physiological responses in the presence and absence of spider mite herbivory (see Chapter 2; formatted according to PLOS ONE journal guidelines);

2. how the use of drought-tolerant corn hybrids impact growing BGM populations under conditions of water-stress (see Chapter 3; formatted according to Journal of Economic Entomology guidelines);

3. how exogenous application of JA and SA phytohormones on neonicotinoid-treated and water-stressed corn plants affect the development of BGM infestations (see Chapter 4; formatted according to Journal of Economic Entomology guidelines).

References


CHAPTER II

COMBINATION OF PLANT WATER-STRESS AND NEONICOTINOIDs CAN LEAD TO SECONDARY OUTBREAKS OF BANKS GRASS MITES

(*Oligonychus pratensis* Banks)

**ABSTRACT** Spider mites, a cosmopolitan pest of agricultural and landscape plants, thrive under hot and dry conditions, which could become more frequent and extreme due to climate change. Recent work has shown that neonicotinoids, a widely used class of systemic insecticides that have come under scrutiny for non-target effects, can elevate spider mite populations. Both water-stress and neonicotinoids independently alter plant resistance against herbivores. Yet, the interaction between these two factors on spider mites is unclear. We conducted a field study to examine the effects of water-stress (optimal irrigation = 100% estimated ET replacement, water stress = 25% of the water provided to optimally irrigated plants) and neonicotinoid seed treatments (control, clothianidin, thiamethoxam) on resident mite populations in corn (*Zea mays*, hybrid KSC7112). Our field study was followed by a manipulative field cage study and a parallel greenhouse study, where we tested the effects of water-stress and neonicotinoids on Banks grass mites (BGM) and plant responses. We found that water-stress and clothianidin consistently increased BGM densities, while thiamethoxam-treated plants only had this effect when plants were mature. Water-stress and BGM herbivory had a greater effect on plant defenses than neonicotinoids alone, and the combination of BGM herbivory with the two abiotic factors increased the concentration of total soluble proteins. These results suggest that spider mites may outbreak by combinations of changes in plant defenses and protein...
concentration triggered by water-stress and neonicotinoids, but the severity of the infestations varies depending on the active ingredient of the insecticide. Hence, it is important to understand the interactions between abiotic factors under these extreme conditions to anticipate and manage pest outbreaks.

INTRODUCTION

The use of neonicotinoid treated seeds has dramatically increased in the United States for the management of insect herbivores in field crops [1], partly due to the systemic and translaminar action of the insecticide, its long residual activity in plants, and reduced exposure of the operator to the insecticide [2-3]. Recently, neonicotinoid insecticides have come under scrutiny because of their effects on beneficial insects [4-12] and their association with outbreaks of spider mites (Acari: Tetranychidae) [13-18]. Spider mites are important agricultural pests that feed on all major food crops and many ornamental plants [19]. Neonicotinoids are not effective in suppressing spider mites [20] and little effect has been observed on the natural enemies of this pest [15,21-23]. Two mechanisms that can explain spider mite outbreaks following neonicotinoid applications include the increased production of eggs stimulated by the insecticide [17] and plant-mediated mechanisms thought to involve plant defenses [21]. Szczepaniec et al. [15], for example, found that neonicotinoids alter phytohormone 12-oxo-phytodienoic acid, which is an important precursor of plant defense regulators (e.g., jasmonic acid (JA) and salicylic acid (SA)) that can lead to increased susceptibility of plants to herbivores.

In the western U.S., the effects of neonicotinoids on spider mites are confounded with frequent and severe drought episodes. Under hot and dry conditions,
spider mite populations can increase exponentially and reach damaging levels [24-33]. Although changes in microhabitat (i.e., increased dust) that favor spider mites can lead to outbreaks during drought conditions, several plant physiological changes (i.e., higher nutrient availability and altered biosynthesis of plant defenses) can also occur that may further contribute to these outbreaks [34-45]. As a consequence, the severity of spider mite outbreaks from the use of neonicotinoids may be dictated by changes in water-stress, and the interaction between these two simultaneously occurring abiotic factors. However, the interactive effect of neonicotinoids and water-stress on the development of spider mites and the physiology of their plant host remain unknown.

Spider mites are a serious pest of corn, especially in the semiarid regions of the western U.S. [46], and their management is becoming increasingly difficult, due to their ability to develop pesticide resistance [47,48]. So far, work has focused mostly on the cosmopolitan twospotted spider mite (*Tetranychus urticae* Koch) (TSSM) and less is known about the Banks grass mite (*Oligonychus pratensis* Banks) (BGM), a grass specialist, and its response to water-stress and neonicotinoid treatments. We tested the combined effect of water-stress, which is prevalent in the Intermountain West and predicted to intensify [49], and neonicotinoids, widely used as a seed treatment in corn, on BGM, which primarily attacks this crop [26,50]. Field and greenhouse experiments examined (1) how water-stress and the use of neonicotinoid insecticides affect spider mite populations over time, and (2) how these factors influence plant phenotypic and physiological responses in the presence or absence of spider mite herbivory.
MATERIAL AND METHODS

Field Experiment 1: Effect of Water-Stress and Neonicotinoid Seed Treatments on Resident Spider Mite Populations

We conducted a 3×2 factorial experiment (three types of neonicotinoid seed treatments × two irrigation levels) with repeated measures to determine the effects of neonicotinoids and water-stress on resident spider mite populations in corn. This field experiment was conducted in 2013 at the Utah State University Greenville Research Station in Logan, UT, USA.

Experimental units were 2×1.5 m plots arranged in a completely randomized design within varied irrigation levels. Plots receiving the same irrigation treatment were spaced 2 m apart, while plots with different irrigation levels were 4 m apart, necessary to establish two distinct irrigation levels. Each plot was seeded with field corn (Zea mays hybrid. KSC7112, relative maturity 112 days; Bayer Crop Science, Raleigh-Durham, NC, USA), treated with the fungicide Evergol Energy at a rate of 65.19 ml/100 kg and treated with either clothianidin (‘Poncho’, 0.5 mg a.i./seed), thiamethoxam (‘Cruiser’, 0.5 mg a.i./seed), or no neonicotinoid (Control). Each plot contained 30 plants divided into 2 rows at a distance of 15 cm within rows and 75 cm between rows. A granular fertilizer (15N:9P:12K; Scotts Osmocote Plus) was applied 3 times (0.5 kg/sq. meter): prior to seeding, when plants had 8-10 fully developed leaves, and when the first corn silk appeared.

Water was provided to plants using drip tape (Toro EAP 5101245-600, 15 mm diameter, 0.10 mm thin black plastic, 30 cm emitters, Q-100: 2.8 × 10⁻⁵ m³ s⁻¹/30 m at 0.7 bar). All plots were watered the same during establishment; at three weeks after germination, the two irrigation treatments were initiated. Plants under optimal
irrigation were kept at field capacity and received 100% replacement of the total water lost by estimated evapotranspiration (ET), while water-stressed plants received 25% of the irrigation provided to optimally watered plots, and were approximately kept at the wilting point. ET was estimated according to Allen et al. [51], where the daily reference evapotranspiration (ETo) is multiplied by the crop coefficient (Kc). The total water provided to the plants was then calculated by subtracting the precipitation recorded within two consecutive irrigation events from the plant irrigation need accumulated in the same time period. To make sure that the two irrigation levels were maintained over time, visual signs of plant water-stress were monitored (leaf curling) as well as soil moisture, which was measured following the gravimetric sampling method [52]. Data on precipitation were obtained from an onsite weather station (Texas Electronics TR-525I Rain Gauge Tipping Bucket, Dallas, Texas, USA), while data on reference evapotranspiration were provided by Utah Agweather (https://climate.usurf.usu.edu/agweather.php). Each treatment combination was replicated 3 times (N = 18).

Plant samples were collected once a week for four weeks, from plant tasseling until the soft dough phase. One fully developed leaf per plant was collected from five destructively sampled plants at each collection date. At each sampling event, we selected the third leaf below the newly developing leaf from the top of each sampled plant, given that spider mites move upward as leaves become older and resource-deficient [53]. Leaf samples were transported to the laboratory on ice and stored in a freezer at 0°C.

Density of resident spider mites (BGM and TSSM) were recorded using a stereomicroscope (Leica S6 D Greenough). Counts were made on both sides of the
leaf along the mid-vein by cutting out 36 cm² subsamples (4 per leaf) across the leaf length. The density of mites (number of mites per cm²) was calculated by dividing the total number of individuals by the total area examined.

Field Experiment 2: Effect of Water-Stress and Neonicotinoid Seed Treatments on BGM and Plant Responses

Field experiment 2 was conducted to isolate the effects of water-stress and neonicotinoids on BGM specifically, and to evaluate changes in plant responses to each abiotic factor and spider mite herbivory. This complementary field experiment was conducted in 2013 (trial 1) and 2015 (trial 2) at the Greenville Research Station. The first trial was a 2×2×2 factorial experiment (two levels of neonicotinoid treatment × two irrigation levels × mite presence/absence) with repeated measures. The second trial, instead, was a 3×2×2 factorial experiment (three types of neonicotinoid treatment × two irrigation levels × mite presence/absence) with repeated measures.

Experimental units were 2×2×2 m lumite cages (mesh size 32×32) (Lumite, Alto, GA, USA). Each treatment was replicated five times in trial 1 (N=40), and three times in trial 2 (N=36). Cages were setup in a completely randomized design within each irrigation level. Each cage housed 30 corn plants (Zea mays hybrid. KSC7112, relative maturity 112 days; Bayer Crop Science, Raleigh-Durham, NC, USA), distributed into two rows at a distance of 15 cm within rows and 75 cm between rows. Cages excluded other herbivores and predators from the experiment. Plots receiving the same irrigation treatment were spaced 2 m apart, while cages with different irrigation levels were 4 m apart, necessary to establish two distinct irrigation levels.
In trial 1, half of the seeds were treated with clothianidin (‘Poncho’, 0.5 mg a.i./seed), while the other half were left untreated. In trial 2, which was conducted to evaluate the effect of multiple seasons, an additional neonicotinoid treatment, thiamethoxam (‘Cruiser’, 0.5 mg a.i./seed), was also tested. Irrigation treatments were setup as described in field experiment 1 using drip tape. Fertilizer was applied to all cages as previously discussed.

Four weeks after germination (vegetative stage V5), ten adult BGM females were applied to each plant with a fine paintbrush. BGM colonies were established from individuals collected in 2012 from commercial field corn and reared on untreated corn plants under laboratory conditions (16:8 h light:dark photoperiod and 28±2 °C). After one week of BGM establishment, the second and third fully developed leaves below the newly developing leaf were collected from each of four destructively sampled plants. Leaf samples were obtained each week for 5 weeks from four unique plants until tasseling. Leaves were stored in a freezer at 0°C so that BGM could be recorded and plant defenses compounds evaluated at a later date (see Plant Defense Bioassay section). BGM were counted on both sides of the leaf along the mid-vein by taking 0.785 cm² subsamples evenly distributed across the length of the leaf. Subsamples were 1.5 cm apart from each other. The density of BGM (number of BGM per cm²) was calculated by dividing the total number of individuals by the total area examined.

For trial 2, stem height, leaf number and area, leaf temperature, and yield (cob dry weight) were also measured, as well as the concentration of neonicotinoids in leaf tissue. Plant height was measured from the soil surface to the last fully developed leaf on the stalk, and all fully developed leaves were counted. Leaf area was calculated
using ImageJ Software (version 1.41, National Institutes of Health, Bethesda, Maryland, USA), while leaf temperature was measured using an infrared thermometer (Cen Tech, model number 69465, Temecula, CA, USA) following Stiefel [54]. Yield was determined by measuring the dry weight of the cob after desiccation in a drying oven at 60°C for 14 days. Finally, the concentration of clothianidin and thiamethoxam were quantified in leaf tissues not subjected to BGM herbivory using ELISA (Enzyme Linked Immunosorbent Assay, SmartAssay Series Test Kit, HORIBA, Ltd, Kyoto, Japan).

Greenhouse Experiments: Effect of Water-Stress and Neonicotinoid Seed Treatments on BGM and Plant Responses

Two parallel experiments were conducted at the Utah State University Research Greenhouse in Logan, UT. The first greenhouse experiment mirrored field experiment 2 (trial 2) and evaluated the combined effect of water-stress and neonicotinoids on BGM populations and plant defenses. Greenhouse experiment 1 was setup as a 3×2×2 factorial experiment (three types of neonicotinoid treatment × two irrigation levels × mite presence / absence) and each treatment was replicated three times (N=36). A second greenhouse experiment was conducted to evaluate the effect of water-stress and neonicotinoids on the fecundity of BGM. Greenhouse experiment 2 was setup as a 3×2 factorial experiment (three types of neonicotinoid treatment × two irrigation levels) with each treatment replicated four times (N=24).

Greenhouse experimental units were represented by plants grown within 5-liter capacity pots (22.5 cm top diameter, 16.5 cm base diameter, 17.8 cm depth), distributed in a completely randomized design. Each pot received a single seed (Zea
*maizehybrid.* KSC7112, relative maturity 112 days; Bayer Crop Science, Raleigh-Durham, NC, USA) grown within Sunshine soil mix #3 as substrate and under 14:10 h light:dark photoperiod. Fertilization was applied once at seeding with Osmocote time release fertilizer (14:14:14, N-P-K).

Similar to the field experiments, plants receiving optimal irrigation were kept at field capacity, which is equal to a volumetric water content (VWC) of 15% for sandy-loam soils, while plants under water-stress conditions were kept at 5%VWC, where the wilting point is reached in the same soil type [55]. VWC was measured with a soil moisture sensor probe (FieldScout TDR 100, Spectrum Technologies, Inc). The two irrigation treatments were initiated three weeks after germination.

In the first greenhouse experiment, ten adult BGM females were applied to each of three leaves per plant four weeks after germination. A plastic sleeve (ClearBags 33×10 cm micro perforated bags, model MPF1324) was used to enclose each leaf. After three weeks, leaves were collected and BGMs were counted on both sides of the entire leaf. BGM density was then estimated to the leaf level. Plant height, leaf number, leaf area, neonicotinoid concentration in leaf tissue, and plant defense proteins were recorded. Leaf temperature was also recorded, and measurements occurred just before each irrigation treatment within the plastic sleeve cover and on the adjacent leaf not enclosed in the sleeve for comparison.

In the second greenhouse experiment, a single adult unmated female BGM was transferred to each of five leaves per plant, six weeks after germination. These leaves were enclosed with a plastic sleeve, collected ten days later, and then the total number of eggs and juveniles was recorded. The collection timing prevented the start of a second BGM generation, as suggested by Mondal and Ara [56].
**Plant Defense Bioassays**

All collected leaves were analyzed for levels of total soluble proteins, as well as plant protein defense compounds including peroxidase (POD), polyphenol oxidase (PPO), trypsin protease inhibitor (TI), and chitinase (CHI). POD and CHI are directly related to the phytohormone pathways of JA, while PPO and TI are regulated by SA [57]. Proteins were extracted by macerating 15 mg of leaf material in 0.25 mL of 0.05M sodium phosphate buffer (pH 7.0) and analyzed following methods in Bradford [58]. POD, PPO, and CHI were measured using a microplate reader (Biotek EPOCH, Winooski, VT, USA), following methods in Moran and Cipollini [59] and dal Soglio et al. [60]. TI was measured by examining the diffusion of protein extracts through an agar substrate and analyzed following methods in Cipollini and Bergelson [61].

**STATISTICAL ANALYSIS**

For field experiment 1, a two-way ANOVA with repeated measures that included WATER (+,-) and PESTICIDE (control, clothianidin, thiamethoxam) as factors, was used to test differences in BGM density across the treatments.

For field experiment 2, trial 1 and 2 were analyzed together within a 2×2×2 factorial design (two levels of neonicotinoid treatment × two irrigation levels × two trials) with repeated measures. Our goal was to evaluate differences between clothianidin and control plants (non-neonicotinoid treated) on BGM density. Trial 2 was also analyzed separately within a 3×2 factorial design (three types of neonicotinoid treatment × two irrigation levels) with repeated measures, to evaluate differences between clothianidin and thiamethoxam.
For trial 2 of field experiment 2, a three-way ANOVA with repeated measures that included WATER (+, -), PESTICIDE (control, clothianidin and thiamethoxam) and HERBIVORY (+, -) as factors, was used to test differences of plant height, leaf area and number, leaf temperature, and yield across treatments.

To better interpret plant protein data, trial 1 of field experiment 2 was analyzed separately from trial 2, using a three-way ANOVA with repeated measures which included WATER (+, -), PESTICIDE (control, clothianidin) and HERBIVORY (+, -) as factors. Similar to trial 1, plant protein data of trial 2 were analyzed using the same statistical method, which accounted for an additional neonicotinoid treatment, thiamethoxam.

A one-way ANOVA with repeated measures, that included WATER (+, -) as a factor, was applied to test for differences in the neonicotinoid concentration in field experiment 2 (trial 2).

In greenhouse experiment 1, differences in BGM density, plant height, leaf area and number, leaf temperature, and plant proteins across the treatments were tested with a three-way ANOVA, that included WATER (+, -), PESTICIDE (control, clothianidin, thiamethoxam) and HERBIVORY (+, -) as factors. A two-sample t-test with unequal variances was used to test for differences in the neonicotinoid concentration of plant tissues.

In greenhouse experiment 2, a two-way ANOVA with repeated measures that included WATER (+, -) and PESTICIDE (control, clothianidin, thiamethoxam) as factors, was used to test differences in egg abundance across the treatments. To meet model assumptions, data were log transformed and analyzed using SAS (version 9.3;
SAS Institute Inc., Cary, NC, USA). Significant interaction terms were examined with Holm’s step-down Bonferroni.

RESULTS

Effect of Water-Stress and Neonicotinoid Seed Treatments on the Development of Mite Populations

Nearly 96% of the resident spider mite population was represented by BGM, while 4% was identified as TSSM. Water-stressed plants treated with either clothianidin or thiamethoxam had higher resident spider mite density than all other treatments (Field Experiment 1 - WATER×PESTICIDE: $F_{2, 44} = 8.65, P<0.001$; Holm’s step down Bonferroni comparisons $P<0.03$) (Fig. 1A).

BGM densities (Field Experiment 2) increased throughout the season, a result that was apparently driven when BGM were exposed to water-stressed plants treated with clothianidin (WATER×PESTICIDE×TIME: $F_{4, 101} = 2.74, P=0.03$) (Fig. 1B). Upon further evaluation of each neonicotinoid seed treatment (trial 2), however, BGM densities did not increase when exposed to thiamethoxam as they did with clothianidin and were similar to the control plants (WATER×PESTICIDE×TIME: $F_{8, 48} = 2.11, P=0.05$) (Fig. 1C).

In greenhouse experiment 1, water-stressed plants had more BGMs than well-watered plants, and the combination of water-stress and clothianidin increased BGM populations (WATER×PESTICIDE: $F_{2, 12} = 4.21, P=0.04$; Holm’s step down Bonferroni comparisons $P<0.02$) (Fig 2A). No difference was observed between the two irrigation levels in thiamethoxam-treated plants.
In greenhouse experiment 2, BGM deposited more eggs on water-stressed plants, particularly when they were treated with clothianidin (WATER×PESTICIDE: $F_{2, 18}=6.03, P<0.01$) (Fig 2B). We recorded approximately 22% more eggs on these plants than on plants provided optimal irrigation (Holm’s step down Bonferroni $P=0.04$). No difference in egg abundance was detected between the two irrigation levels on control and thiamethoxam-treated plants.

**Effect of Water-Stress and Neonicotinoid Seed Treatments on Plant Responses**

**Phenotypic Plant Responses**

Water-stressed plants had reduced plant height (field: WATER×DATE: $F_{1, 24}=104.74, P<0.0001$; greenhouse: WATER: $F_{1}=654.92, P<0.0001$), leaf number (field: WATER: $F_{1, 24}=104.74, P<0.0001$; greenhouse: WATER: $F_{1}=561.72, P<0.0001$), leaf area (field: WATER: $F_{1, 120}=19.54, P<0.0001$; greenhouse: WATER: $F_{1}=81.96, P<0.0001$), and yield (field: WATER: $F_{1, 24}=171.14, P<0.0001$). Specifically, yield was decreased by nearly 54%.

Feeding by BGM also decreased the leaf number (greenhouse: HERBIVORY: $F_{1}=4.96, P=0.04$), leaf area (field: HERBIVORY: $F_{1, 120}=11.64, P<0.001$; greenhouse: HERBIVORY: $F_{1}=15.70, P<0.001$), and yield (field: HERBIVORY: $F_{1, 24}=18.75, P<0.001$). Specifically, BGM reduced yields by nearly 20%. Moreover, the combination of BGM and thiamethoxam had significantly smaller cobs than thiamethoxam-treated plants without mites (Holm’s step down Bonferroni $P<0.001$).

Interestingly, water-stress and presence of BGM significantly increased the leaf temperature (field: WATER: $F_{1, 120}=18.43, P<0.0001$, HERBIVORY: $F_{1, 120}=9.68, P<0.01$; greenhouse: WATER: $F_{1, 69}=24.36, P<0.0001$ and HERBIVORY: $F_{1, 69}=24.36, P<0.0001$).
Water-stressed plants in the field and greenhouse had elevated leaf temperatures of 0.73±0.2°C and 1.05±0.2°C, respectively, compared to plants provided with optimal irrigation. BGM also increased leaf temperature by 0.5±0.3°C and 0.73±0.2°C in the field and greenhouse, respectively, compared to plants without BGM.

**Plant Proteins**

In the 2013 field experiment (trial 1), we found that the total protein concentration increased more rapidly throughout the season in plants treated with clothianidin than in non-treated control plants when BGM was present (PESTICIDE×HERBIVORY×TIME: $F_{2, 93} = 3.79, P = 0.03$) (Fig. 3A). In the absence of BGM, the total protein concentration increased at a similar rate in all plant treatments throughout the season.

In the 2015 season (trial 2), plants provided with optimal irrigation had a higher protein concentration when BGM were absent, however the effect was lost over time (WATER×HERBIVORY×TIME: $F_{2, 72} = 4.66, P = 0.01$) (Fig. 4A). In the presence of BGM, well-watered plants and water-stressed plants had instead a similar protein concentration throughout the season.

Similar to the 2013 field season, plants grown in the greenhouse that were treated with either clothianidin or thiamethoxam had a higher concentration of proteins than control plants when BGM were present, while no difference in the total protein concentration was observed across the treatments when BGM was absent (PESTICIDE×HERBIVORY: $F_{1, 24} = 4.29, P = 0.03$; Holm’s step down Bonferroni $P<0.05$) (Fig. 5). Moreover, water-stressed plants had more proteins than plants
provided with optimal irrigation in the presence of BGM, while no difference in the total protein concentration was found between the two irrigation levels when BGM was absent (WATER×HERBIVORY: $F_{1, 24} = 5.40, P = 0.03$; Holm’s step down Bonferroni $P < 0.05$) (Fig. 5A).

In the 2013 field experiment (trial 1), we found that plants provided with optimal irrigation had lower concentrations of POD than water-stressed plants throughout the season when BGM was absent. Instead the presence of BGM increased POD concentrations, however this result was observed in well-watered plants only (WATER×HERBIVORY×TIME: $F_{2, 90} = 2.49, P = 0.09$) (Fig. 3B). In the 2015 season (trial 2), POD concentrations were the same throughout the season for all plants receiving optimal irrigation. However, when plants were subjected to water-stress, POD concentrations increased in control and in thiamethoxam treatments, while POD concentrations remained the same in plants treated with clothianidin (WATER×PESTICIDE×TIME: $F_{4, 68} = 5.06, P < 0.01$) (Fig. 4B). Similar to the 2013 field season, BGM herbivory in the greenhouse increased POD concentration, particularly on well-watered plants, compared to plants provided with optimal irrigation and no herbivore pressure (WATER×HERBIVORY: $F_{1, 24} = 10.88, P < 0.01$; Holm’s step down Bonferroni $P < 0.01$) (Fig. 5B).

PPO concentrations in the 2013 field experiment decreased over time and the magnitude of this change was less for plants with BGM, as BGM herbivory appeared to decrease PPO within the first week, compared to plants without BGM (HERBIVORY×TIME: $F_{2, 93} = 36.04, P < 0.0001$; Holm’s step down Bonferroni $P < 0.0001$) (Fig. 3C). Alternatively, in 2015, PPO concentrations increased over time (TIME: $F_{2, 72} = 267.29, P < 0.0001$). We also found that in the absence of BGM, water-
stressed plants treated with clothianidin started with lower PPO concentrations than all other treatments. Instead, in the presence of BGM, water-stressed control plants and plants treated with thiamethoxam which were also water-stressed started with lower PPO concentrations than all the other treatments (WATER×PESTICIDE×HERBIVORY: $F_{2,24}= 8.86, P<0.01$) (Fig. 4C). Over time, however, PPO concentrations increased in all treatments and remained the same throughout the season (Fig. 4C). Changes in PPO concentrations were not evident in the greenhouse (Fig. 5C).

Similar to observations for PPO, the CHI concentrations in 2013 decreased over time and the magnitude of this change was less for plants with BGM, as the presence of BGM appeared to reduce CHI within the first week (HERBIVORY×TIME: $F_{2,62}=5.71, P<0.01$; Holm’s step down Bonferroni $P<0.05$) (Fig. 3D). In 2015, the absence of BGM increased CHI concentrations in plants receiving optimal irrigation more than water-stressed plants, but the effect was lost over time as CHI concentrations decreased and then stabilized. In the presence of BGM, no difference between the two irrigation levels was observed throughout the season (WATER×HERBIVORY×TIME: $F_{2,72}=6.15, P<0.01$) (Fig. 4D). In general, we found that control plants and thiamethoxam-treated plants appeared to have similar CHI concentrations regardless of mite herbivory, compared to clothianidin-treated plants. The greenhouse study also confirmed that water-stress significantly decreased CHI concentrations compared to plants provided with optimal irrigation (WATER: $F_{1,24}=8.87, P<0.01$) (Fig. 5D).

In 2013, water-stress appeared to reduce TI concentrations when BGM were absent, while in the presence of herbivory, concentrations of TI increased, especially
in water-stressed plants (WATER×HERBIVORY: $F_{1,93}=3.30, P=0.07$) (Fig. 3E). Similarly, in 2015, we found that water-stress alone initially decreased TI concentrations compared to plants provided with optimal irrigation, but the effect of water-stress was short lived as it dissipated over time (WATER×TIME: $F_{1,71}=3.48, P<0.05$). Contrary to the 2013 field season, the interactive effect of BGM herbivory and water-stress was not detected in 2015 (Fig. 4E). In the greenhouse, water-stressed control plants with BGM herbivory appeared to have less TI than when provided with optimal irrigation. Water-stressed plants that were treated with thiamethoxam and BGM herbivory, instead, appeared to have more TI than when plants were provided with optimal irrigation (WATER×PESTICIDE×HERBIVORY: $F_{2,24}=3.48, P<0.05$) (Fig. 5E).

**Neonicotinoid Concentration**

There was no difference in the concentration of clothianidin and thiamethoxam in leaf tissue between the two irrigation levels (field: WATER: $F_{1,4}=1.85 P=0.25$ and $F_{1,4}=0.01 P=0.93$ respectively; greenhouse: WATER: $T_4=3.18 P=0.66$ and $T_4=4.30 P=0.25$ respectively). In the field, however, clothianidin strongly decreased throughout the season from an average of 30±3.84 ppb to approximately 15±5.10 ppb (TIME: $F_{4,15}=2.99 P=0.05$), while thiamethoxam did not change over time and maintained an average of 16±1.06 ppb (TIME: $F_{4,16}=0.99 P=0.44$). In the greenhouse, the concentration of clothianidin was 4.90±0.36 ppb, and thiamethoxam was 3.52±0.43 ppb.
DISCUSSION

Spider mite outbreaks have been regularly observed on plants exposed to hot and dry conditions [24-32], or as a consequence of applying neonicotinoid insecticides [13-17, 21]. We found that the combination of water-stress and neonicotinoid use exacerbated spider mite density on corn. Surprisingly, BGM outbreaks were not stimulated by the use of neonicotinoids alone, compared to what has been previously observed for TSSM feeding on well-watered cotton and corn plants treated with clothianidin and thiamethoxam [15-17, 62]. BGM outbreaks occurred only when neonicotinoid-treated plants were water-stressed. The neonicotinoid clothianidin, in particular, had the strongest and most consistent effect on spider mites. This outcome does not appear to be a result of increased neonicotinoid concentration in leaf tissues of water-stressed plants, since non-stressed plants (optimal irrigation) had equal neonicotinoid concentrations in leaves. It is important to note that water-stress and neonicotinoid effects on spider mite outbreaks was not universal, because thiamethoxam-treated plants did not always result in spider mite outbreaks. Specifically, we observed that only the resident spider mite population increased when combining water-stress and thiamethoxam. Nauen et al. [63] found that thiamethoxam metabolizes to clothianidin over time and reaches toxicologically relevant concentrations, which can lead to hormoligosis. In field experiment 1, leaf samples were collected later in the season (between tasseling and the soft dough phase), while they were collected much earlier in all other experiments (from the beginning of the vegetative growth stage until tasseling). One possibility is that thiamethoxam-treated plants in field experiment 1 accumulated the drought interactive clothianidin compound in more mature plants, leading to increased spider
mite densities, but this was not specifically tested. Similar to our study, Szczepaniec et al. [15] found that thiamethoxam seed treatments did not elevate *Tetranychus cinnabarinus* (Boisduval) infesting cotton plants. However, thiamethoxam-treated plants appeared to have more mites than control at the end of the sampling period, but this result was not further discussed.

Overall, water-stress was a major factor elevating spider mite densities. We found that corn subjected to drought conditions generally had a higher leaf temperature than plants provided with optimal irrigation. Greater spider mite infestations commonly occur under high temperatures, where both BGM and TSSM lay more eggs and the overall generation time period is shorter [64,65]. In a study conducted under dryland conditions, Stiefel [54] demonstrated that drought-susceptible sorghum lines had a faster development of BGM infestations, due to greater leaf temperatures, than drought-resistant lines. It is possible that, by having a higher leaf temperature, water-stressed plants promoted BGM development, however this was not specifically tested. In our experiments, water-stress also altered the concentration of plant defense proteins. Similar to previous studies, water-stress increased the concentrations of POD and TI, as seen in corn and amaranth (*Amaranthus hypochondriacus*) plants respectively [36,40]. This was not surprising as, in some instances, plants can produce more herbivore-associated defenses when water-stressed [66], instead of generally decreasing the biosynthesis of secondary compounds [67]. Unlike what was observed in sesame (*Sesamum indicum*), Madagascar periwinkle (*Catharanthus roseus*), and tomato (*Solanum lycopersicum*), water-stress did not increase PPO and CHI concentrations, [37-39].
Clothianidin and thiamethoxam did not increase BGM densities when not combined with water-stress, which is in contradiction to Szczepaniec et al. [15], who found a stimulating effect of these two active ingredients on spider mite development in optimally irrigated plants. Szczepaniec et al. [15], however, focused on *T. cinnabarinus* and TSSM, where species specific effects may be a possible factor for not observing a similar effect of neonicotinoids when combined with optimal irrigation.

We did not find a clear correlation between spider mite outbreaks and the alteration of plant defense compounds due to neonicotinoids. Clothianidin and thiamethoxam are known to affect plant defense phytohormones by enhancing SA [68] and inhibiting the synthesis of JA from cross-talk [69]. We did not find a tradeoff in PPO and TI, which are regulated by SA, and POD and CHI, which are in turn regulated by JA [57], as cross-talk may predict. Instead, our results are similar to those of Szczepaniec et al. [15], who found that clothianidin did not alter the transcription of CHI (JA-related plant defense) and TI (SA related) in corn. Interestingly, thiamethoxam-treated plants were more similar to control plants than clothianidin-treated plants in terms of plant defense concentrations. For example, the concentration of POD and CHI, which are known to be directly related to spider mite feeding [15,70], increased over time in water-stressed control and thiamethoxam-treated plants, while their concentrations stayed constant in clothianidin-treated plants under the same water regime. This outcome may explain the increased BGM density on water-stressed plants treated with clothianidin, compared to control and thiamethoxam-treated plants that were also water-stressed and did not have an increase in BGM.
Similar to water-stress, BGM herbivory had a major role in the alteration of plant defenses. As previously observed in tomato and corn plants subjected to spider mite herbivory [15,70], the presence of BGM increased the concentration of POD and TI. Contrary to TSSM, which was found to trigger the expression of genes codifying for CHI [15], BGM did not appear to promote CHI concentrations. Interestingly, BGM herbivory also increased the total soluble protein concentration in plants when combined with water-stress and neonicotinoids respectively. On one side, water-stressed plants had more proteins than plants provided with optimal irrigation when BGM were present. On the other side, more proteins were found in plants treated with either clothianidin or thiamethoxam than in control plants when subjected to BGM herbivory. The effect of water-stress, which increases the concentration of amino acids in desiccating leaves due to enhanced degradation of ammonia [42], may be one contributing factor leading to the effect of spider mite herbivory, which is known to stimulate the accumulation of amino acids in neighboring cells, following increased osmotic tension and increased sink-demands by the spider mites [71-73]. The greater availability of amino-acids is one possibility that promoted the synthesis of stress proteins, which is commonly triggered in water-stressed plants, as observed in water-stressed corn seedlings [43]. Moreover, spider mites cause cell dehydration by sucking the cell content [74] which, in turn, can lead to additional synthesis of stress proteins. Spider mite feeding is also thought to induce the assimilation of nitrogen into organic nitrogen compounds such as amino acids and peptides, as a compensating mechanism that undamaged mesophyll cells have adapted for the loss of nutrients in damaged neighboring cells [71]. Clothianidin and thiamethoxam, which are N-nitroguanidines, may be factors that exacerbated this process of assimilation by
increasing the availability of inorganic nitrogen after the insecticides are degraded in the plant tissues.

Some of the variation in plant defenses that we observed between field seasons, can be attributed to soil type and environmental conditions, which can influence the magnitude of the effect of the water-stress severity and duration, as well as plant growth rate [45]. In the field specifically, although irrigation treatments were maintained, we recorded more than double the precipitation in 2015 (32.5 mm) than 2013 (14.5 mm). In addition, in 2013 and 2015, relative humidity was 49±1.7 % and 54±1.4 %, and air temperature was 23±0.4 °C and 20±0.3 °C, respectively. In a previous study, for example, Gouinguené and Turlings [75] found that differences in soil moisture and air temperature altered the emission of pest induced volatiles in young corn plants. Moreover, higher temperatures are known to interfere with the synthesis of proteins normally produced under non-stressful temperature conditions in favor of novel heat shock proteins [76].

In addition to environmental factors, plant development stage may contribute to the variation in plant defenses among the experiments. Plants in the field had a lower number of leaves and a shorter height than plants in the greenhouse at week 7 after germination, which may have caused a different biosynthesis of plant defenses despite the equal plant age. Commonly (but not always) more defense compounds are found in younger leaves than in older ones [77]. For example, a higher concentration of hydroxamic acids, which play an important role in resistance to western corn rootworm larvae, are commonly found in younger corn plants compared to older ones [78, 79]. Other pathogenesis related proteins, such as glucanase and chitinase, are not detectable in young tobacco leaves, but accumulate with leaf age [80]. In our case, we
observed an overall greater concentration of plant defense proteins on greenhouse plants than on equally old plants grown in the field.

When we looked at plant responses, we observed that water-stress decreased plant height, leaf area and leaf number, as well as cob dry weight, which commonly occurs in corn growing under drought conditions [81]. Clothianidin and thiamethoxam did not affect these plant responses, contrary to previous studies where increased leaf area and yield were measured in neonicotinoid-treated plants [16, 82, 83]. Similar to water-stress, BGM feeding had an additional major impact on plant responses. In the presence of BGM, plants had lower leaf area, leaf number, and yield, which is congruent to what was previously described by other studies [84-86]. Surprisingly, BGM feeding resulted in increased leaf temperature, regardless of the irrigation level.

Overall, our study shows that the combination of plant water-stress and neonicotinoids leads to BGM secondary outbreaks in corn, however the direct interaction may be an additive effect of two plant physiological pathways rather than trade-offs in any one alone. Water-stress appeared to affect some of the key plant defense responses, while neonicotinoids appeared to increase nitrogen concentrations in plants. We conclude that the combination of the two abiotic factors may play a major role in the development of BGM populations in corn through two independent plant pathways.

**CONCLUSIONS**

Neonicotinoids have come under scrutiny for several unintended consequences on non-target organisms such as pollinators [8-12], predators [7], and now the
outbreak of spider mites [13-17]. These effects, however, can be exacerbated by environmental factors such as drought. As observed in our study, spider mite density in corn was elevated by the combination of two abiotic factors, water-stress and neonicotinoids, which independently caused alterations in plant defense responses and total nitrogen concentrations. However, not all neonicotinoids act in the same way, as was seen with thiamethoxam use and inconsistent BGM outbreaks, compared to clothianidin use. Likewise, Moser and Obrycki [7] found that clothianidin seed treatments cause a greater larval mortality of non-target coccinellid larvae *Harmonia axyridis*, which feed on corn seedlings, than thiamethoxam-treated seeds. More focus has been placed on these different attributes of neonicotinoid compounds and their impact on non-targets. For example, nitro-containing neonicotinoids (imidacloprid, clothianidin, thiamethoxam, nitenpyram and dinotefuran) were found to be more toxic to pollinators than the cyano-group containing neonicotinoids (acetamiprid and thiacloprid), due to increased bee metabolism by cytochrome P450s and lower bee toxicity to the metabolites [7, 8-12]. Therefore, understanding the nuances of different neonicotinoid active ingredients is key to selecting insecticide active ingredients that are less harmful to non-target arthropods and have little or no effect on spider mites.

There are a number of ecologically-based pest management strategies for spider mite suppression, including reducing water-stress through proper irrigation, disruption of spider mites in plants with overhead irrigation, and the judicious use of miticides. Given the limitations on water resources, however, the development of commercially available drought-resistant plant hybrids may alleviate spider mite outbreaks in drought-stress conditions [54]. Overall, our new understanding of the interactions between abiotic factors, namely water-stress and neonicotinoid use, on
the development of spider mite infestations in corn, allows crop managers to anticipate and predict pest outbreaks and combine this with available pest management strategies.

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REFERENCES


Fig 2-1. Effect of water-stress and neonicotinoids on (A) resident mites and (B and C) BGM density. Field cage experiments with the introduction of BGM were conducted in 2013 (B) and 2015 (C). The x-axis represents the weekly collections of leaf samples from which spider mite were counted. Spider mite density was calculated to the level of the area observed on each leaf. Collection of samples started at the silking phase and terminated at the soft dough phase (A); collection of samples started at the vegetative stage V5 and ended at tasseling (B and C). Circle or “CON” represent control (no insecticide treatment); triangle or “CLO” represent plants treated with clothianidin; square or “THI” represent plants treated with thiamethoxam; optimal irrigation (100% estimated ET replacement) is represented by solid lines, black symbols, and “100%”; water-stress (25% of the total water provided to optimally irrigated plants) is represented by dashed lines, white symbols and “25%”. Means are +1SE.
Fig 2-2. Effect of water-stress and neonicotinoids on the (A) density and (B) fecundity of BGM. Ten adult BGM females were applied 4 weeks after corn plant germination and left develop for 3 weeks after which BGM density was calculated to the leaf level (greenhouse experiment trial 1). BGM fecundity was measured 10 days after 1 unmated adult BGM female was applied on individual leaves at 6 weeks from corn plant germination (greenhouse experiment trial 2). Soil volumetric water content (VWC) is expressed in %. Grey bars represent plants provided with optimal irrigation (15% VWC); white bars represent water-stressed plants (5% VWC); CON= control; CLO= clothianidin; THI= thiamethoxam. Lowercase letters represent step-down Bonferroni significant differences ($P<0.05$) within a treatment. Means are +1SE.
Fig 2-3. Effects of water-stress, neonicotinoids, and BGM herbivory on plant defense proteins (2013 field season). The x-axis represents weeks after the application of BGM on plants; the y-axis represents LOG concentration of each plant protein (POD, peroxidase; PPO, polyphenol oxidase; CHI, chitinase; TI, trypsin inhibitor). Collection of samples started at the vegetative stage V5 and ended at tasseling. Circle or “CON” represent control (no insecticide treatment); triangle or “CLO” represent plants treated with clothianidin; optimal irrigation (100% estimated ET replacement) is represented by solid lines, black symbols, and “100%”; water-stress (25% of water provided to optimally irrigated plants) is represented by dashed lines, white symbols, and “100%”. Means are ±1SE.
Fig 2-4. Effects of water-stress, neonicotinoids, and BGM herbivory on plant defense proteins (2015 field season). The x-axis represents weeks after the application of BGM on plants; the y-axis represents LOG concentration of each plant protein (POD, peroxidase; PPO, polyphenol oxidase; CHI, chitinase; TI, trypsin inhibitor). Collection of samples started at the vegetative stage V5 and ended at tasseling. Circle or “CON” represent control (no insecticide treatment); triangle or “CLO” represent plants treated with clothianidin; square or “THI” represent plants treated with thiamethoxam; optimal irrigation (100% estimated ET replacement) is represented by solid lines, black symbols, and “100%”; water-stress (25% of water provided to optimally irrigated plants) is represented by dashed lines, white symbols, and “25%”. Means are ±1SE.
Fig 2-5. Effects of water-stress, neonicotinoids, and BGM herbivory on plant defense proteins (greenhouse experiment). Measurements were conducted on 7-week old corn plants. Soil volumetric water content (VWC) expressed in %, where H-represents plants provided with optimal irrigation (15% VWC) and without BGM; L-represents water-stressed plants (5% VWC) without BGM; H+ represents plants provided with optimal irrigation (15% VWC) and with BGM; L+ represents water-stressed plants (5% VWC) with BGM; dark grey bar or “CON” represent control (no insecticide treatment), light grey bar or “CLO” represent plants treated with clothianidin; white bar or “THI” represent plants treated with thiamethoxam. Different capital letters represent a significant (P<0.05) main effect of water; different lowercase letters represent a significant effect (step-down Bonferroni test, P<0.05) of the 2-way interaction (WATER×HERBIVORY). The effect of the 3-way interaction (WATER×PESTICIDE×HERBIVORY) is not shown. Means are +1SE.
CHAPTER III

BANKS GRASS MITE (ACARI: TETRANYCHIDAE) SUPPRESSION ADDS TO BENEFIT OF DROUGHT-TOLERANT CORN HYBRIDS IN DROUGHT CONDITIONS

ABSTRACT Spider mite (Acari: Tetranychidae) outbreaks are common in corn crops grown under dry-land conditions of the arid West. This agricultural pest thrives under hot and dry weather, when juveniles quickly reach adulthood, females are more fecund, and eggs hatch faster. Increases in drought episodes are predicted with changing climate, with the possibility of triggering more serious spider mite infestations. Application of acaricides has become increasingly ineffective because spider mites quickly develop resistance, making it necessary to find alternatives to limit outbreaks. Development of drought-tolerant (DT) plant lines alleviates the challenges of growing crops in drought-stressed environments. However, it is not clear how DT plants grown under water-stress affect herbivores that thrive under conditions of drought. We conducted a greenhouse experiment to evaluate the effect of DT corn hybrids on the development of Banks grass mites (BGM), a primary pest of corn, under optimal irrigation and water-stress conditions. We followed this with a field study, where we tested the effect of DT corn hybrids on BGM (added to plants) and resident spider mites, under optimal-water and water-stress conditions throughout a season. We found that water-stressed DT hybrids had significantly less BGM and resident spider mites than water-stressed drought-susceptible hybrids. Interestingly, water-stressed DT hybrids had equal BGM abundance and density to drought susceptible and DT hybrids provided with optimal irrigation. We conclude that DT
corn hybrids may help alleviate spider mite outbreaks in water challenged areas, and
that crop managers may benefit from drought-tolerant plants against multiple
stressors.

INTRODUCTION

As a consequence of climate change, an increase in the frequency and
intensity of extreme warming events in water-challenged regions is predicted, which
may lead to an additional reduction of the water supply for agricultural needs
(Jamieson et al. 2012). Since water scarcity is a major constraint to agriculture, the
demand for crops that can handle this condition is increasing, and plant producers are
investing in the development of crop varieties with increased tolerance to this abiotic
stressor. Besides having adverse effects on plant survival, growth, and productivity,
water-stress has also been recognized as one of the major causes of herbivorous pest
outbreaks, particularly bark beetles, wood borers, and sap feeders (Koricheva et al.

Although water-stress can elevate the plant biosynthesis of allelochemicals in
some plants (Gershenzon 1984), this environmental condition can also cause the
opposite outcome, where plant defenses against stressors are decreased. Under water-
stress, plants can also have a greater availability of soluble sugars, amino acids, and
other inorganic solutes which, in addition to lower levels of plant defenses, are
thought to increase plant susceptibility to herbivore attack (White 1969, Hsiao 1973,
Hsiao 1976, Jones and Rawson 1979, Morgan 1984, Brodbeck and Strong 1987,
Mattson and Haack 1987, Blum 1988, Nielsen and Orcutt 1996, Mohammadkhani and
Heidari 2008, Mody et al. 2009). Outbreaks of the spruce beetle (*Dendroctonus*
*rufipennis* Kirbi) and mountain pine beetle (*Dendroctonus ponderosae* Hopkins) were observed following episodes of drought (Logan et al. 2003, Powell and Bentz 2009). Similar to these two pests, outbreaks of the wheat aphid (*Schizaphis graminum* Rondani) and the fall armyworm (*Spodoptera frugiperda* J. E. Smith) were observed in wheat and corn, respectively (Luginbill 1928, Dorschner et al. 1986). Spider mites (*Acari: Tetranychidae*), however, are known to be one of the agricultural pests that benefits the most from conditions of water-stress, during which they have a higher reproductive rate and fecundity, generations are shorter, and eggs hatch faster (Chandler et al. 1979, Ferro and Chapman 1979, Perring et al. 1984a; 1984b, 1986, Youngman and Barnes 1986, Quiros-Gonzalez 2000, Roy et al. 2003, Gotoh et al. 2014).

Spider mites have become difficult to manage, due to their development of resistance to many pesticides, and their control is currently limited to a few commercially available products, mainly acaricides (Croft and Baan 1988, Van Leeuwen et al. 2010, Khajehali et al. 2011). Stiefel (1992), however, found that the use of drought-resistant grain sorghum suppresses outbreaks of Banks grass mites (*Oligonychus pratensis* Banks) (BGM), a grass specialist which thrives under dry-land conditions (Blasi et al. 2015). Drought resistant sorghum lines maintained a lower leaf temperature during water-stress than drought-susceptible (DS) lines, resulting in a slower development of BGM, as the reproductive rate of this pest is positively correlated with temperature (Norman et al. 1984, Perring 1984a; 1984b, Youngman et al. 1988, Oi et al. 1989, Stiefel 1992).

Water-stress is a condition that frequently occurs in the western part of the United States and is predicted to become more severe, due to increases in temperature
and reduced frequency of precipitation events in the region (Cook et al. 2004). As a consequence, growers may predict more serious spider mite infestations. Currently, three corn hybrid technologies for drought tolerance are commercially available and include Pioneer Optimum AQUAmax™ (DuPont Pioneer, Johnston, IA, USA), SyngentaArtesian™ (Syngenta Seeds, Minnetonka, MN, USA), and Monsanto Genuinity™ DroughtGard™ (Monsanto Co., St. Louis, MO, USA) (Adee et al. 2016). The first two technologies were obtained through traditional breeding, while Monsanto's technology was achieved with the introduction of a transgenic trait, which activates the expression of a bacterial cold shock protein B (Nemali et al. 2015, Adee et al. 2016). Similar to Stiefel (1992), who tested the effect of drought resistant sorghum on BGM, we evaluated the effect of commercially available drought tolerant (DT) corn hybrids on the development of BGM infestations under water-stress conditions, by running a greenhouse experiment and a parallel field study.

MATERIALS AND METHODS

Greenhouse experiment

A 2×2 factorial experiment (two levels of drought-sensitivity (SENSITIVITY) × two irrigation levels (WATER)) in a completely randomized design was conducted to evaluate BGM populations on DT corn hybrids under water-stress conditions. This experiment was conducted in 2015 at the Utah State University Research Greenhouse in Logan, UT, USA.

Ten commercially available corn hybrids were used (Table 1). Half of these were DS, while the other half were DT. Two hybrids were an isogenic line and a genetically modified strain derived from it, while all the other hybrids had different
genotypes. The hybrids did not exceed a relative maturity of 116 days, which is common for northern regions, including the Intermountain West and Pacific Northwest.

A single seed of each hybrid was planted within a 5-liter capacity pot (22.5 cm top diameter, 16.5 cm base diameter, 17.8 cm depth) containing Sunshine soil mix #3, and plants were grown under greenhouse conditions (14:10 h light:dark photoperiod and 28±2°C). Fertilization was applied once at seeding with Osmocote time release fertilizer (14:14:14, N-P-K). Each combination of plant hybrid and irrigation level was replicated 4 times (N=80). Plants receiving optimal irrigation were maintained at 15% volumetric water content (VWC), which corresponds to field capacity conditions for sandy-loam soils (Walker and Skogerboe 1987). Plants under water-stress, instead, were at 5% VWC, which establishes wilting point conditions in the same type of substrate (Walker and Skogerboe 1987). Soil moisture was measured with a soil moisture meter (FieldScout TDR 100, Spectrum Technologies, Inc.). The two irrigation treatments were initiated two weeks after germination.

Twenty adult BGM females were applied to each of two leaves per plant, when plants were 6 weeks old. BGM colonies were collected in local corn fields in 2012 and reared on untreated corn plants under laboratory conditions (16:8h light:dark photoperiod and 28±2°C). Leaves subjected to BGM herbivory were enclosed with a perforated plastic sleeve (ClearBags 33×10 cm micro perforated bags, model MPF1324), and leaves were collected after two weeks. Abundance and density of BGM (adults and immatures) and eggs (individuals or eggs per cm² of leaf) were then recorded using a stereomicroscope (Leica S6 D Greenough).
To assess the amount of water-stress sustained by corn plants, leaf area and leaf temperature were measured. Leaf area was calculated using ImageJ Software (National Institutes of Health, Bethesda, Maryland, USA), while leaf temperature was measured according to Stiefel (1992) with an infrared thermometer (Cen Tech, model number 69465, Temecula, CA, USA). Leaf temperature was measured just before each irrigation event. For comparison, leaf temperature was recorded for leaves enclosed within the plastic sleeve and adjacent leaves not enclosed in the sleeve.

**Field experiment**

A 2×2 factorial (two levels of drought sensitivity (SENSITIVITY) × two irrigation levels (WATER)) field experiment in a completely randomized design was conducted in 2015 and 2016 at Utah State University’s Greenville Research Station in Logan UT, USA. This study mirrored the greenhouse experiment, and evaluated the effect of DT corn hybrids on the development of BGM populations added to plants, and resident spider mite populations across the season.

Each corn hybrid was represented by 2×1.5 m corn plots, setup in a completely randomized design within varied irrigation levels. Each combination of plant hybrid and irrigation level was replicated 3 times (N=60). Adjacent plots were 2 m apart, while plots with different irrigation levels were 4 m apart, necessary to establish two distinct irrigation levels. Every plot had 20 plants divided into two rows, at a distance of 15 cm on the row and 75 cm between the rows. A granular fertilizer (15N:9P:12K; Scotts Osmocote Plus) was applied 3 times (0.5 kg/sq. meter), once each prior to seeding, when the plants had 8-10 fully developed leaves, and when the first silk appeared.
Plants were watered using drip tape (Toro EA 5080644-200B, 16 mm diameter, 0.20 mm thin black plastic, 15 cm emitters, Q-100: $2.8 \times 10^{-5} \, \text{m}^3\text{s}^{-1}/30 \, \text{m at 0.6 bar}$. Plants received the same amount of water during their establishment and then, at three weeks after germination, the two irrigation treatments were initiated. Plants provided with optimal irrigation were kept at field capacity and received 100% replacement of the total water lost by estimated evapotranspiration (ET). Water-stressed plants were approximately kept at wilting point and received 25% of the water provided to fully irrigated plants. Soil moisture content, leaf temperature, leaf area, and plant height were recorded to monitor the severity of drought. ET was estimated according to Allen et al. [51], where the daily reference evapotranspiration (ETo) is multiplied by the crop coefficient (Kc). The total water provided to the plants was then calculated by subtracting the precipitation recorded within two consecutive irrigation events from the plant irrigation need accumulated in the same time period. Data on precipitation, air temperature, and relative humidity were obtained from a local onsite weather station (Texas Electronics TR-525I Rain Gauge Tipping Bucket, Dallas, Texas, USA), while data on reference evapotranspiration were obtained from Utah Agweather (https://climate.usurf.usu.edu/agweather.php).

For trial 1, twenty adult BGM females were applied to one leaf per plant, for a total of three randomly selected plants per plot (experimental unit), when plants were ten weeks old. Leaves were selected from the middle third of the plant and then enclosed within a perforated plastic sleeve (same as the greenhouse experiment). Leaves were collected two weeks after BGM application, after which the abundance and density of BGM (adults and immatures) and eggs were calculated following methods described in the greenhouse experiment.
For trial 2, resident spider mites were monitored once a week for a 5-week time period, from plant tasseling (eight weeks after plant germination) to the soft dough phase. At each collection date, two leaves were collected from four destructively sampled plants. Precisely, we selected the second and third leaves from the top newly developing leaf, to capture spider mite movement as they crawl up from resource-deficient older leaves (Wrensch and Young 1975). Abundance and density of resident spider mites (adults and immatures) and eggs were then calculated following the methods used for the greenhouse experiment.

To assess the amount of water-stress sustained by corn plants, leaf area and leaf temperature were recorded as previously described, while soil moisture was additionally measured following the gravimetric sampling method (Reynolds 1970). In the greenhouse experiment, we found that the plastic sleeve did not affect the leaf temperature, hence this variable was only measured on leaves that were not enclosed in the sleeve as to not disturb the mites within a sleeve. Corn cobs were also collected at physiological maturity and weighed after desiccation until no weight change was observed.

**STATISTICAL ANALYSIS**

Each hybrid was treated as a replicate, while plots were treated as sub-replicates and were combined into a single data point to distinguish between the two levels of drought sensitivity.

For the greenhouse experiment, a two-way ANOVA consisting of WATER (+,-) and drought SENSITIVITY (+,-), was used to test differences in abundance and density of BGM and BGM eggs. A two-way ANOVA consisting of WATER (+,-) and
drought SENSITIVITY (+,-) with repeated measures was measured to test for differences in leaf temperature. To evaluate the effect of the leaf enclosures (plastic sleeves) used to isolate spider mite on leaves, differences in the leaf temperature recorded on leaves within or outside the plastic sleeve were analyzed using a two-sample t-test with equal variances.

For the field experiments, we combined the two datasets from 2015 and 2016 and incorporated year into the model. Specifically, we used ANOVA with WATER (+,-), drought SENSITIVITY (+,-) and YEAR (2015, 2016) when we evaluated abundance and density of BGM and BGM eggs, as well as corn cob dry weight. A three-way ANOVA with repeated measures consisting of WATER (+,-), drought SENSITIVITY (+,-), and YEAR (2015, 2016) was used to test differences in resident spider mite population growth and leaf temperature across the treatments. When we found an effect of year, we analyzed each season (2015 and 2016) independently and discussed the analysis separately.

Abundance and density of resident spider mites and resident mite eggs were square-root transformed, while abundance and density of BGM and BGM eggs, as well as leaf temperature and corn cob dry weight, were log-transformed to correct for heterogeneous variances and non-normal distribution. Data were analyzed using SAS (version 9.3; SAS Institute Inc., Cary, NC, USA).

For the greenhouse experiment, we also analyzed differences among the hybrids within each seed company, to look at each specific hybrid individually. In this case, a two-way ANOVA consisting of WATER (+,-) and drought SENSITIVITY (+,-) was used to test for differences in BGM and BGM egg density and abundance. A two-way ANOVA consisting of WATER (+,-) and drought SENSITIVITY (+,-) with
repeated measures was also used to test for differences between leaf temperature. Data were log-transformed to correct for heterogeneous variances and non-normal distribution. Significant interaction terms were examined with Holm’s step-down Bonferroni.

**RESULTS**

*BGM and resident spider mites*

In the greenhouse, water-stressed DS hybrids had greater abundance and density of BGM than DT hybrids under the same water-stress condition (abundance: WATER×SENSITIVITY: $F= 11.19; df= 1, 76; P< 0.01$; density: WATER×SENSITIVITY: $F= 6.24; df= 1, 76; P= 0.01$) (Fig. 1). Surprisingly, water-stressed DT corn hybrids had equal BGM abundance and density to DS and DT hybrids provided with optimal irrigation. Moreover, water-stressed DS hybrids had greater BGM abundance and density than DS hybrids kept under optimal irrigation.

A significant effect of year was found when BGM abundance and density were examined for field trial 1; hence, each season was analyzed independently. Similar to the greenhouse, water-stressed DS hybrids had greater abundance and density of BGM than DT hybrids under the same water-stress condition, and water-stressed DT corn hybrids had equal BGM abundance and density to DS and DT hybrids provided with optimal irrigation (abundance: WATER×SENSITIVITY: $F= 0.77; df= 1,4; P= 0.42$ and $F= 5.72; df= 1,4; P= 0.08$ in 2015 and 2016 respectively; density: WATER×SENSITIVITY: $F= 1.30; df= 1,4; P= 0.32$ and $F= 5.52; df= 1, 4; P=0.08$ in 2015 and 2016 respectively) (Fig. 1). The interaction between water-stress
and plant sensitivity to drought, however, was more evident in 2016 than in 2015, even though we observed a very similar trend.

A significant effect of year was also found for field trial 2 when resident spider mite abundance and density were examined. Similar to BGM populations in the greenhouse and field trial 1, water-stressed DS hybrids had more resident spider mites than DT hybrids under water-stress conditions, while resident spider mite abundance and density on water-stressed DT corn hybrids was equal to DS and DT hybrids that were provided with optimal irrigation (abundance: \( F = 8.10; df = 1, 13.46; P = 0.01 \) and \( F = 9.06; df = 1, 8.964; P = 0.01 \) in 2015 and 2016 respectively; density: \( F = 4.12; df = 1, 13.61; P = 0.06 \) and \( F = 9.72; df = 1, 11.03; P = 0.01 \) in 2015 and 2016, respectively) (Fig 2). In addition, water-stressed DS hybrids had a greater abundance and density of resident mites than DS hybrids provided with optimal irrigation. Similar to field trial 1, the interaction between water-stress and plant sensitivity to drought was stronger in 2016, while the magnitude of change was less for 2015.

In the greenhouse experiment, BGM egg abundance was significantly higher on plants receiving optimal irrigation than water-stressed plants, while no difference in BGM egg density was found between the two irrigation levels (abundance: \( F = 7.07; df = 1, 76; P < 0.01 \); density: \( F = 0.89; df = 1, 76; P = 0.35 \)) (Fig. 1).

Contrary to the greenhouse experiment, an increased abundance and density of BGM eggs on water-stressed DS hybrids were observed in field trial 1 than on DT hybrids under the same water-stress condition (abundance: \( F = 4.49; df = 1, 12; P = 0.06 \); density: \( F = 5.76; df = 1, 12; \))
Similar to the observation on BGM (adults and immatures), water-stressed DT corn hybrids had equal BGM egg abundance and density to DS and DT hybrids provided with optimal irrigation. In the 2015 season, a higher BGM egg density was also observed on water-stressed plants than on plants provided with optimal irrigation, while in the 2016 season, water-stressed plants had equal BGM egg density to plants provided with optimal irrigation (density: WATER: $F= 23.04; df= 1, 4; P< 0.01$ and $F= 2.15; df= 1, 4; P= 0.22$ in 2015 and 2016, respectively) (Fig. 1).

A significant effect of year was found when resident mite eggs were analyzed for field trial 2. In the 2015 season, a higher abundance and density of resident mite eggs was recorded on DT hybrids provided with optimal irrigation than on DT hybrids under water-stress, while no difference was found between optimally irrigated DT hybrids, and DS hybrids kept under either optimal irrigation or water stress (abundance: WATER×SENSITIVITY: $F= 3.49; df= 1, 15.07; P= 0.08$ density: WATER×SENSITIVITY: $F= 14.03; df= 1, 7.289; P< 0.01$) (Fig. 2). In 2016, instead, the abundance and density of resident mite eggs increased throughout the season in water-stressed DS hybrids, while they remained constant in all other treatments (abundance: WATER×SENSITIVITY×TIME: $F= 3.03; df= 3, 24; P= 0.05$ density: WATER×SENSITIVITY×TIME: $F= 8.08; df= 3, 12.61; P< 0.01$) (Fig. 2).

**Plant responses**

Leaf area was strongly reduced by water-stress in the greenhouse (WATER $F= 31.71; df= 1, 76; P< 0.0001$) and in each of the field experiments, (trial 1: WATER: $F= 88.27; df= 1, 4; P< 0.001$ and $F= 21.92; df= 1, 4; P< 0.01$ in 2015 and
2016 respectively; trial 2: WATER: $F= 5.30; df= 1, 4; P= 0.08$ and $F= 28.33; df= 1, 16; P< 0.0001$ in 2015 and 2016 respectively).

No difference was found in the leaf temperature when measured inside and outside of the plastic sleeve ($t= 1.04; df= 458; P= 0.30$). On average, leaf temperature was affected by water-stress only, which increased it by approximately $0.79 \, ^\circ C \pm 0.2$ and ranged between ~22 and 32°C (WATER $F= 17.83; df= 1, 36; P< 0.001$). Similar to the greenhouse experiment, leaf temperature recorded in the field experiment was increased by water stress only and fluctuated between 13 and 28°C (WATER $F= 13.41; df= 1, 8; P< 0.01$). A significant difference, however, was found between the two seasons, hence this variable was analyzed separately for both years. Leaf temperature ranged between 12 and 21°C in 2015 and between 13 and 26°C in 2016. In both seasons, leaf temperature was elevated by water-stress upwards of $1.6\, ^\circ C \pm 0.4$ in 2015 and $1.14\, ^\circ C \pm 0.3$ in 2016 (WATER: $F= 9.25; df= 1, 4; P= 0.04$ and $F= 5.23; df= 1, 6.442; P= 0.06$ in 2015 and 2016 respectively).

Water-stressed DS corn hybrids produced significantly smaller cobs than DT hybrids under the same irrigation treatment, while no difference was observed between DS and DT hybrids when provided with optimal irrigation (WATER×SENSITIVITY: $F= 11.17; df= 1, 108; P< 0.01$).

*Differences among hybrids*

When Monsanto Genuinity™ DroughtGard™ hybrids were analyzed, there was a trend showing water-stressed DT hybrid (DKC66-42RIB) to have less BGM than the DS hybrid (DKC66-40RIB) under the same water conditions(density: WATER×SENSITIVITY: $F= 3.39; df= 1,12; P= 0.09$, abundance:
WATER×SENSITIVITY: \( F = 3.85; df = 1,12; P = 0.07 \). More BGM were generally found on water-stressed plants (density: WATER: \( F = 6.94; df = 1,12; P = 0.02 \), abundance: WATER: \( F = 0.06; df = 1,12; P = 0.80 \). No factor affected BGM egg abundance and BGM egg density. No difference in leaf temperature was also observed between the DS isoline and its genetically modified strain for drought tolerance. Leaf temperature was only increased by water-stress (WATER: \( F = 12.72; df = 1, 12; P < 0.01 \)).

In a comparison of PioneerOptimum AQUAmax™ hybrids, we did not find any significant difference in BGM density between DT hybrid P0157R and DS hybrid P9973R. Overall, a higher density of BGM was found on water-stressed plants than on plants provided with optimal irrigation (WATER: \( F = 1.93; df = 1, 12; P = 0.02 \). None of the other factors affected BGM abundance. BGM eggs were more numerous on well-watered plants than plants maintained under water-stress and on DS than on DT hybrid (density: WATER: \( F = 4.45; df = 1,12; P = 0.06 \), SENSITIVITY: \( F = 12.30; df = 1,12; P < 0.01 \); abundance: WATER: \( F = 11.05; df = 1,12; P < 0.01 \), SENSITIVITY: \( F = 14.26; df = 1,12; P < 0.01 \). Leaf temperature was raised by water-stress only (WATER: \( F = 7.75; df = 1, 12; P = 0.02 \)).

A significant lower number of BGM was commonly found on Syngenta Artesian™ DT hybrid G07B39-3111A when subjected to water-stress compared to water-stressed DS hybrids G16T00-3111, G11A40-3000GT, and G08X83 (WATER×SENSITIVITY: \( P < 0.05 \)). Water-stressed DT hybrid G07B39-3111A had similar BGM density and abundance to the level recorded for well-watered plants.

Syngenta Artesian™ DT hybrid G12J11-3011A had less BGM than DS hybrids G16T00-3111 and G11A40-3000GT when all hybrids were maintained under
water-stress (WATER×SENSITIVITY: \( P < 0.05 \)), and it had similar levels of BGMs as those recorded for optimally irrigated plants. DT hybrid G12J11-3011A had, instead, an equal BGM number to DS hybrid G08X83, regardless of the irrigation level (density: WATER×SENSITIVITY: \( F = 0.08; df = 1,12; P = 0.78 \), abundance: WATER×SENSITIVITY: \( F = 0.02; df = 1,12; P = 0.90 \)).

No difference was found in BGM abundance and density between DT hybrid G14H66-3010A and DS hybrids G16T00-3111, G11A40-3000GT, and G08X83, where increased BGM density and abundance were found on water-stressed plants than on plants provided with optimal irrigation only (WATER: \( P < 0.05 \)).

No factor affected BGM egg density and abundance when all DT Syngenta Artesian™ hybrids were compared to each DS hybrid. Leaf temperature, instead, was higher in all water-stressed plants than plants provided with optimal irrigation (WATER: \( P > 0.05 \)).

Environmental conditions

In 2015, we recorded significantly more precipitation (42 mm) than in 2016 (0.76 mm) (YEAR: \( T = 3.87; df = 71; P < 0.001 \)). A significant difference in air temperature and relative humidity was also found, where air temperature was 20.95±0.07°C and 22.21±0.08 °C, while relative humidity was 51.86±0.14 % and 44.72±0.15 %, in 2015 and 2016 respectively (air temperature: YEAR: \( T = -2.64; df = 121; P < 0.01 \); relative humidity: YEAR: \( T = 4.36; df = 121; P < 0.0001 \)).
DISCUSSION

Drought-resistant plants, which include mechanisms of drought tolerance and avoidance, are being developed to counter the effect of decreased summer water availability in water-challenged agricultural areas, but their effect on herbivorous pests has only recently been evaluated. Here, we found that water-stressed DT corn hybrids decreased the abundance and density of BGM and resident spider mites compared to DS hybrids under the same conditions of water-stress. Moreover, water-stressed DT corn hybrids alleviated spider mite infestations to the level of plants receiving optimal irrigation. In the 2015 field season, however, the magnitude of the effect was lower than in 2016. Although irrigation treatments were maintained, seasonal environmental conditions may have been a factor influencing the severity and duration of water-stress. In 2015, for example, significantly more precipitation was recorded than in 2016, as well as a generally higher humidity and a lower air temperature.

A similar outcome to the one observed for spider mite adults and immatures was also measured for egg abundance and egg density, which is in opposition with what was observed by Stiefel (1992), where different drought-resistant sorghum lines did not affect BGM fecundity. In addition, when hybrids were grown in the field, more mite eggs were generally found on water-stressed plants than on plants receiving optimal irrigation, as leaf temperatures increase due to reduced transpiration rates (Youngman et al. 1988, Oi et al. 1989, Stiefel 1992). The opposite outcome, instead, was observed in the greenhouse. We believe that greater humidity levels were reached in the greenhouse, hence, the simultaneous occurrence of high humidity and elevated
leaf temperatures recorded on water-stressed plants reduced spider mite egg hatch, as previously demonstrated by Perring et al. (1984b).

Stiefel (1992) found that, under water-stress conditions, drought-resistant sorghum lines had a lower leaf temperature than DS lines, which resulted in a slower BGM development rate. In our case, we did not find a significant difference in leaf temperature between DT and DS corn hybrids kept under water-stress conditions. We also did not find a significant difference in leaf temperature among the hybrids within each seed company. All plants simply recorded a higher leaf temperature when subjected to water-stress, as plants close their leaf stomata to conserve water, which can result in reduced transpirational cooling (Tanners 1963, Gates 1964, Wiegand et al. 1966, Jackson et al. 1981, Chandhuri et al 1986). Low leaf temperature is a result of greater water uptake, a mechanism of drought avoidance used by drought-resistant plants (such as the sorghum lines used by Stiefel (1992)) to cope with water-stress, but not by DT plants (such as many of the current corn hybrids) (Bing et al. 2016). Drought tolerance in fact, is a mechanism of drought resistance which makes plants less susceptible to water-stress through other means, such as cellular osmotic adjustments, increased biosynthesis of antioxidant enzymes to control the concentration of active oxygen species, greater production of abscisic acid to maintain root growth and stomatal closure, and higher tolerance to desiccation (delayed senescence) (Olsson 1995, Trypothy 2000, Barker et al. 2004, Chaves and Oliveira 2004, Ashraf 2007, Boyer et al. 2008, Miller 2010, Chen 2011, Bing et al. 2016).

Substantial variation in leaf temperature is also common within plant species, hybrids (crosses between two species or distinct parent lines), and plant lines (naturally occurring variation of individual plants within the same species) (Kizkham et al. 2015).
1984). Stiefel (1992) also observed differences in leaf temperature among drought-resistant sorghum lines at different stages of plant development, and under different environmental conditions, as well as within and between growing seasons, as leaf temperature depends on changes in abiotic factors such as solar radiation, precipitation, ambient temperature, and relative humidity. Other mechanisms may exist by the DT corn hybrids we tested to cope with water-stress, and were directly correlated to the observed lower spider mite abundance and density. In some instances, plants subjected to water-stress can produce more defenses against herbivorous pests (Gershenzon 1984), which could be enhanced in drought-resistant hybrids. Zhang et al. (2012), for example, found that the MYB protein TaPIMP1 provides simultaneous resistance to drought and the fungus *Bipolaris sorokiniana* in wheat, by regulating stress- and defense-related genes in abscisic and salicylic acid signal pathways. Other authors found that sorghum hybrids with delayed leaf senescence (stay-green hybrids) had higher concentrations of alcohol soluble carbohydrates in their leaf tissues, which caused a reduction of BGM damage (Foster et al. 1977, Perring 1982, Archer 1986b). Expression of the stay-green trait has also been reported in corn, and has been recognized as one of the mechanisms used by plants to cope with water-stress (Tollenaar and Daynard 1978, Al Hakimi 1995, Ma and Dwyer 1998, Rajcan and Tollenaar 1999a; 1999b, Borrell et al. 2000, Howard and Howarth 2000, He et al. 2001, 2002a; 2002b, Kumudini et al. 2002, Barker et al. 2004). Moreover, Mohammadkhani and Heidari (2008) found that soluble sugars play an important role in osmotic adjustment in water-stressed corn plants, especially in corn hybrids with a short relative maturity, such as those used in this study. These hybrids are known to accumulate high levels of soluble sugars in the leaves, which
then are moved to the stem at physiological maturity to protect from lodging, a serious problem of corn plants in short-season environments, such as the Intermountain West (Martimore and Ward 1964).

Mechanisms of drought-resistance that negatively impact one species could, however, positively affect other agricultural pests. High concentrations of soluble sugars that appear to reduce BGM damage, were found to promote the development of several lepidopterans, the grasshopper Locusta migratoria, and the spruce budworm Choristoneura fumiferana (Foster et al. 1977, Albert et al. 1982, Archer 1986b, Mattson and Haack 1987). Additionally, drought-resistant hybrids can alter the biology of natural enemies feeding on insect herbivores by changing the plant physical and physiological characteristics (Awmack et al. 2002). For example, a high density of leaf hairs, which is typically used by certain drought-resistant plants to reduce water loss by transpiration, was found to significantly reduce the predation rate of the predatory mite Phytoseiulus persimilis (Krips et al. 1999). Instead, when whiteflies were reared on nutritionally poor plants, lacewings, which commonly prey on this pest, were not able to complete their development (Legaspi 1996).

In some instances, the use of drought-resistant hybrids can also be counterproductive. Some drought-resistant sorghum hybrids, for example, were found to be less productive under non-stressed (irrigated) conditions than DS hybrids (Blum 1973). This effect was not observed in our study, however, it is important to consider that plants with drought-resistant traits may not be as productive as plants without those traits when conditions of water-stress do not occur, since drought can be severe in one season and non-existent in the next (Dow et al. 1984).
Drought episodes, however, are predicted to become more intense and recurrent in the West due to increases in temperature and reduced precipitation events in the region. As a consequence, seed suppliers are heavily investing in the development of new technologies aimed at creating plants that can withstand water-stress and still maintain high yields. Drought-resistance traits, however, could potentially offer protection to plants from herbivorous pests that outbreak under conditions of drought, but this ecological outcome has not been widely tested. Here we found that, under conditions of water-stress, DT corn hybrids reduced the population development of BGM. This result suggests that, although DT crop technology is not a pest management tool, it may have some major implications for pest management, particularly for pests that flourish under water-stress, including *S. graminum* (Rondani) in wheat and *S. frugiperda* (J. E. Smith) in corn.

Advanced breeding techniques and sophisticated genetic engineering have allowed the development of highly productive hybrids under hostile environmental conditions, by stacking different technologies (resistance to drought, pests, and herbicides) within the same plant, especially corn. These innovations offer a great potential in the management of agricultural crops, by providing a possible remedy to biotic and abiotic stressors.

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### TABLES AND FIGURES

Table 3-1. **Corn hybrids used in the experiments.**

<table>
<thead>
<tr>
<th>Marketer</th>
<th>Hybrid</th>
<th>Drought sensitivity</th>
<th>Relative Maturity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monsanto</td>
<td>DKC66-40RIB*</td>
<td>Susceptible</td>
<td>116</td>
</tr>
<tr>
<td>Pioneer</td>
<td>P9973R</td>
<td>Susceptible</td>
<td>99</td>
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<td>Susceptible</td>
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<td>G11A40-3000GT</td>
<td>Susceptible</td>
<td>111</td>
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<tr>
<td>Syngenta</td>
<td>G08X83</td>
<td>Susceptible</td>
<td>108</td>
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<td>G12J11-3011A</td>
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</tr>
<tr>
<td>Syngenta</td>
<td>G14H66-3010A</td>
<td>Tolerant</td>
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*DS isolate and its genetically modified strain for drought tolerance.
Fig. 3-1. Effect of drought-tolerant corn hybrids and water-stress on the development of BGM populations. Measurements recorded in the greenhouse experiment (first column, left) and in trial 1 of the field experiment (season 2015, second column; season 2016, third column). The y-axis represents, from top to bottom, BGM abundance, BGM density (expressed as number of BGM per sq. cm of leaf), egg abundance, and egg density (expressed as number of eggs per sq. cm of leaf). The x-axis shows the irrigation levels, where 15% represents the volumetric water content (VWC) indicating optimal irrigation for the greenhouse experiment, 5% represents the VWC indicating water-stress for the greenhouse experiment, 100% represents the estimated evapotranspiration replaced to provide optimal irrigation for the field experiment, and 25% represents the percentage of the water provided to optimally irrigated plants used to create water-stress for the field experiment. Dark circles or "T" represent drought-tolerant corn hybrids, while white circles or "S" represent drought-susceptible corn hybrids. Means are +1SE.
Fig. 3-2. Evaluation of the effect of drought-tolerant corn hybrids under conditions of water-stress on the development of resident spider mite populations. Measurements recorded in trial 2 of the field experiment (season 2015, left column; season 2016, right column). Collections occurred from plant tasseling (eight weeks after plant germination) to the soft dough phase. The y-axis represents, from top to bottom, resident spider mite abundance, resident spider mite density (expressed as number of mites per sq. cm of leaf), egg abundance, and egg density (expressed as number of eggs per sq. cm of leaf). The x-axis shows the weekly collections of leaf samples. Circles, solid lines, or "100%" represent optimal irrigation (100% estimated evapotranspiration (ET) replacement), triangles, dashed lines, or "25%" represent water-stress (25% of the water provided to optimally irrigated plants), "T" drought-tolerant corn hybrids, "S" drought-susceptible corn hybrids. Means are +1SE.
Fig. 3-3. Evaluation of the effect of drought-tolerant corn hybrids under conditions of water-stress and resident spider mite herbivory on the corn cob weight. Measurements recorded in the field experiment (season 2015, left; season 2016, right). The y-axis represents the corn cob dry weight (Kg). The x-axis shows the two irrigation levels, 100% estimated evapotranspiration (ET) replacement (optimal irrigation) and 25%, percentage of the water provided to optimally irrigated plants (water-stress). Dark circles or "T" represent drought-tolerant corn hybrids, while white circles or "S" represent drought-susceptible hybrids. Means are ±1SE.
CHAPTER IV

EFFECT OF EXOGENOUS PHYTOHORMONE APPLICATIONS ON BANKS GRASS MITE DEVELOPMENT ON WATER-STRESSED AND NEONICOTINOID-TREATED CORN

ABSTRACT Water-stressed plants and neonicotinoid insecticides, two simultaneously occurring abiotic factors, can trigger secondary outbreaks of spider mites (Acari: Tetranychidae), an important herbivorous pest of many plants. Mechanisms for decreased host plant resistance toward spider mites include an alteration of plant defenses and changes in plant nutrients. Exogenous applications of phytohormones, such as methyl jasmonate (MeJA) and methyl salicylate (MeSA), can enhance plant resistance to biotic stressors and decrease pests, including spider mites. However, it is not clear whether applications of phytohormones can improve Banks grass mite (BGM) resistance of corn plants that are water-stressed and treated with neonicotinoids. We conducted a greenhouse and field study where MeJA or MeSA was applied to corn plants treated with the neonicotinoid clothianidin (+, -), and subjected to water-stress (water stress = 25% of the water provided to optimally irrigated plants or 5% volumetric water content (VWC) for field and greenhouse, respectively; optimal irrigation = 100% estimated ET replacement and 15% VWC for field and greenhouse, respectively). We recorded these effects on BGM density and egg counts. We found applications of MeSA and water-stress increased BGM (adult and immature) and egg density, while MeJA applications resulted in fewer eggs. However, MeJA showed this effect when plants had optimal irrigation and no
clothianidin. Alternatively, no phytohormone was able to improve BGM suppression when plants were water-stressed and treated with clothianidin. While phytohormones, may assist in reducing spider mites, abiotic factors including water-stress and neonicotinoids may overwhelm the benefits of this integrated pest management approach.

INTRODUCTION

Abiotic factors such as plant water-stress and neonicotinoids have been implicated in the outbreak of spider mites (Acari: Tetranychidae), an important agricultural pest in a variety of cropping systems (Chandler et al. 1979, Price et al. 1982, Perring et al. 1986, Raupp et al. 2004, Gupta and Krischik 2007, Szczepaniec et al. 2011, Szczepaniec and Raupp 2012; 2013). Spider mites thrive in hot and dry conditions, during which their fecundity and reproductive rate increase (Ferro and Chapman 1979, Perring et al. 1984a; 1984b, Kennedy and Smitley 1985, Kennedy and Storer 2000, Roy et al. 2003, Gotoh et al. 2014). Additionally, females have increased oviposition when exposed to neonicotinoid-treated plants (Raupp et al. 2004, Gupta and Krischik 2007, Szczepaniec et al. 2011, Szczepaniec and Raupp 2012; 2013). Currently, the management of this pest is limited to the use of a few acaricides, due to spider mites quickly developing resistance to a growing number of pesticides (Croft and Baan 1988, Van Leeuwen et al. 2010, Khajehali et al. 2011). Spider mite outbreaks may become more frequent as drought events intensify in the western United States, and given neonicotinoids have become the most widely used class of insecticides for the control of many agricultural pests (Cook et al. 2004, NOAA 2014, Douglas and Tooker 2015).
Plant water-stress and neonicotinoids can lead to spider mite outbreaks due to a multitude of mechanisms. These two abiotic factors can alter the biosynthesis of plant defensive compounds, by interfering with jasmonic (JA) and salicylic acids (SA), important phytohormones that regulate plant resistance to biotic stressors (Uknes 1992; 1993, Thomma et al. 1998). Although exceptions exist, lower concentrations of plant defense chemicals were found in several water-stressed plants, including cotton (Olson et al. 2009), wild tobacco (Halpern et al. 2010), loblolly pine (Lombardero et al. 2000), garlic mustard (Gutbrodt et al. 2011), and apple trees (Gutbrodt et al. 2012). Moreover, neonicotinoid active ingredients, such as imidacloprid, clothianidin, and thiamethoxam decreased levels of 12-oxophytodienoic acid, an important precursor of plant defenses, in cotton, corn, and tomato (Szczepaniec et al. 2013). Szczepaniec et al. (2013) also observed that these neonicotinoid active ingredients suppressed the transcription of important herbivore-related defense compounds such as phenylalanine ammonia lyase (PAL), and some active proteinase inhibitors. Imidacloprid and clothianidin also induced global transcriptional responses similar to that of SA, which can lead to the down-regulation of JA, as increases in SA can decrease JA and vice-versa (Ford et al. 2010). Doares et al. (1995), for example, found that SA reduced the synthesis of JA-related proteinase inhibitors, a powerful inhibitor of major intestinal enzymes of herbivorous pests (Green and Ryan 1972). Alternatively, plant water-stress can enhance concentrations of JA, used by plants to protect from the damage of water-deficiency and can cause an inhibition of SA (Wang 1999, Brossa et al. 2011, de Ollas et al. 2013).

Given our knowledge of JA and SA’s role in host plant resistance, research efforts have evaluated exogenous applications of these phytohormones and their
derivatives (methyl jasmonate (MeJA) and methyl salicylate (MeSA)) in the management of herbivorous pests including spider mites. Applications of JA on a variety of plants, for example, were found to reduce spider mite feeding, fecundity, and egg survival (Omer et al. 2000; 2001, Thaler et al. 2002, Gols et al. 2003, Ament et al. 2004, Warabieda et al. 2005, Kawazu et al. 2013, Wei et al. 2014, Miyazaki et al. 2014). Exogenous applications of MeSA also reduced infestations of this pest by attracting natural enemies (James and Price 2004).

Abiotic factors, namely plant water-stress and neonicotinoids, can alter phytohormone concentrations resulting in a reduction in herbivore resistance. Exogenous applications of phytohormones, however, could potentially alleviate some of the effects of these abiotic stresses and lead to improved plant resistance to spider mites. Hence, we tested how the combination of phytohormones (MeJA, MeSA, control), neonicotinoid (+/−), and irrigation level (water-stressed or optimal irrigation) affected the grass specialist Banks grass mite (Oligonychus pratensis Banks) (BGM), developing on corn grown under greenhouse and field conditions.

**MATERIAL AND METHODS**

**Greenhouse experiment**

A 2×2×3 factorial experiment (two levels of pesticide × two irrigation levels × three phytohormone levels) in a completely randomized design was conducted in 2015 (trial 1) and 2016 (trial 2) at Utah State University’s Research Greenhouse, Logan, Utah. This experiment evaluated the effect of exogenous applications of MeJA and MeSA on the development of BGM populations on neonicotinoid-treated corn plants subjected to two irrigation levels (water-stress conditions or optimal irrigation).
Experimental units (N=72) were corn plants (*Zea mays* hybrid, KSC7112, relative maturity 112 days; Bayer Crop Science, Raleigh-Durham, NC, USA) grown from single seeds planted in 5-liter capacity pots (22.5 cm top diameter, 16.5 cm base diameter, 17.8 cm depth), containing Sunshine Soil mix #3 and grown under 14:10 h light:dark photoperiod and 28±2°C. Osmocote time-release fertilizer (14:14:14, N-P-K) was applied once at seeding. Half of the seeds were treated with the neonicotinoid active ingredient clothianidin (‘Poncho’, 0.5 mg a.i./seed) and Evergol Energy fungicide at a rate of 65.19 ml/100 kg, while the other half was treated with the fungicide only.

Plants receiving optimal irrigation were kept at field capacity, which is equal to a volumetric water content (VWC) of 15% for sandy-loam soils (Walker and Skogerboe, 1987). Plants under water-stress conditions were kept under a VWC of 5%, at which the wilting point is reached in the same soil type (Walker and Skogerboe, 1987). The soil water content was measured with a soil moisture sensor probe (FieldScout TDR 100, Spectrum Technologies, Inc) and the two irrigation treatments were started two weeks after plant germination.

Similar to Uppalapati et al. (2005), and Shetty et al. (2012), we used three different solutions containing MeJA (0.1 mM in 0.001% ethanol), MeSA (1 mM), and 0.001% ethanol, which was used as a mock treatment (phytohormone control), respectively. Ethanol was used to help MeJA dissolve in water, while MeSA was used in place of salicylic acid because it promotes an easier penetration into the leaf tissue and is converted to the free form faster (Forouhar et al. 2005). Four weeks after germination, an equal amount of each solution was independently sprayed with a hand
held atomizer onto plants of each respective treatment, until dripping was observed. Each treatment combination was replicated six times.

Phytohormone spray treatments were conducted outside of the greenhouse in isolation to avoid the risk of cross contamination. Plants were left to dry and then returned to the greenhouse. Twenty adult BGM females were then immediately applied to each plant. BGM were obtained from colonies originally collected in commercial field corn in 2012, and reared on untreated corn plants under laboratory conditions (16:8 h light:dark photoperiod and 28±2 °C). Each plant was then housed within a greenhouse anti-insect netting mesh (mesh size 0.29×0.8 mm) (Meyabond Industry & Trading (Beijing) Co., Ltd). After two weeks, all of the leaves were collected from each plant to determine the number of BGM (adults and juveniles) and eggs remaining using a stereomicroscope (Leica S6 D Greenough). Counts were divided by the total plant leaf area to determine the density (number per cm²). Leaf area was measured with ImageJ Software (National Institutes of Health, Bethesda, Maryland, USA).

Field experiment

A parallel 2×2×3 factorial experiment (two levels of pesticide × two irrigation levels × three phytohormone levels) was performed in 2016 (trial 1 and 2) at Utah State University’s Greenville Research Station in Logan, UT, USA.

Experimental units were 1×1×1 m lumite cages (N=48) (mesh size 32×32) (Lumite, Alto, GA, USA) setup in a completely randomized design within varied water levels, and treatments were replicated 4 times. Similar to Abreu and Munné-Bosch (2008), adjacent cages were 1.5 m apart to prevent phytohormone cross-
contamination. Cages with different water levels were 4 m apart, to maintain two distinct irrigation levels. Each cage had 8 corn plants divided into two rows, at a distance of 10 cm in the row and 30 cm between rows. Corn hybrid and seed treatments were the same as those reported in the greenhouse experiment.

Plants were fertilized at the seeding stage with a granular fertilizer (15N:9P:12K; Scotts Osmocote Plus), which was applied once prior to the seeding at 0.5kg/sq. meter.

Irrigation was performed using drip tape (Toro EA 5080644-200B, 16 mm diameter, 0.20 mm thin black plastic, 15 cm emitters, Q-100: $2.8 \times 10^{-5}$ m$^3$s$^{-1}$/ 30 m at 0.6 bar). Plants under optimal irrigation were kept at field capacity and received 100% of the total water lost by estimated evapotranspiration (ET). Plants subjected to water-stress were approximately kept at wilting point and received 25% of the total water provided to plants that were optimally irrigated. Irrigation events occurred the same days. ET was calculated following Allen et. al. (1998), where the daily reference evapotranspiration (ETo) is multiplied by the crop coefficient (Kc). The total water given to the plants was then estimated by subtracting the millimeters of precipitation registered within two consecutive irrigation events from the plant irrigation need accumulated in the same time period. Data on precipitation were obtained from an on-site weather station (Texas Electronics TR-525I Rain Gauge Tipping Bucket, Dallas, Texas, USA), and data on reference evapotranspiration were obtained from Utah Agweather (https://climate.usurf.usu.edu/agweather.php). Plants received the same amount of water for the first two weeks following germination, after which the two irrigation treatments were initiated. Visual signs of water-stress were monitored (leaf
curling and stunted growth), and soil moisture was measured following Reynolds (1970).

Phytohormones were sprayed onto plants of their respective treatment four weeks after germination, following the method used in the greenhouse. To prevent phytohormone cross-contamination, treatments were sprayed at a very close distance from the plant canopy, and at a moment of the day where wind was absent or imperceptible. Twenty adult BGM females were then transferred to each plant and, after two weeks, all leaves were collected to count spider mites (adults and juveniles) and eggs.

STATISTICAL ANALYSIS

The trials of the greenhouse experiments (trail 1 and trial 2), and the field experiments (trial 1 and trial 2), were analyzed together within a 2×2×3 factorial design for each experiment, having WATER (+,-), PESTICIDE (+,-), and PHYTOHORMONE (MeJA, MeSA, and mock solution) as experimental factors. Differences in egg density and BGM density across treatments were evaluated with a negative binomial generalized linear mixed model (GLMM), to account for data over-dispersion (Johnson et al. 2015). When we found a main effect of TRIAL, we analyzed each dataset independently and discussed the analysis separately.

GLMM was followed by Tukey's post hoc test when a significant PHYTOHORMONE main effect was observed. Data were analyzed using SAS (version 9.3; SAS Institute Inc., Cary, NC, USA).
RESULTS

When the two greenhouse trials were analyzed together for BGM egg density, we observed a significant 3-way interaction between water, pesticide, and phytohormone (egg density: \( \text{WATER} \times \text{PESTICIDE} \times \text{PHYTOHORMONE}: F = 3.61; df = 2, 110; P = 0.03 \)) (Fig. 1). To better interpret this interaction, we conducted separate analyses focusing on planned contrasts between each factor. First, we removed the effect of pesticide and focused on \( \text{PHYTOHORMONE} \times \text{WATER} \), and found that egg density was generally higher on water-stressed plants and on plants treated with MeSA, respectively (WATER: \( F = 88.74; df = 1, 128; P < 0.001 \); PHYTOHORMONE: \( F = 4.46; df = 2, 128; P = 0.01 \)). Overall, plants treated with MeJA had significantly lower egg density than plants treated with MeSA (Tukey comparisons \( P < 0.05 \)), yet similar to the mock treatment. Only when plants were not treated with clothianidin and had optimal irrigation, did MeJA decrease eggs more than MeSA or mock solution (Fig. 1). Focusing on \( \text{WATER} \times \text{PESTICIDE} \), we found that water-stress was the only factor enhancing egg density (WATER: \( F = 78.33; df = 1, 130; P < 0.001 \)). Finally, egg density was not affected when analyzing \( \text{PHYTOHORMONE} \times \text{PESTICIDE} \).

In the field experiment, more eggs were only found on water-stressed plants than on plants provided with optimal irrigation (egg density: WATER: \( F = 9.41; df = 1, 6; P = 0.02 \) and \( F = 13.30; df = 1, 6; P = 0.01 \) in trial 1 and 2 respectively) (Fig. 2).

In both trials of the greenhouse experiment, BGM density (adults and immatures) was significantly higher on water-stressed plants (BGM density: WATER: \( F = 16.86; df = 1, 51; P < 0.001 \) and \( F = 38.41; df = 1, 59; P < 0.0001 \) in trial 1 and 2 respectively) (Fig 1). In trial 2, plants that were not treated with clothianidin
had a greater BGM density than plants that were treated with this active ingredient (PESTICIDE: $F=4.75; df=1, 59; P=0.03$). Moreover, plants sprayed with MeSA had more BGM than plants treated with the mock solution and MeJA (BGM density: PHYTOHORMONE: $F=4.51; df=2, 59; P=0.02$; Tukey comparisons $P<0.04$) (Fig. 1). Equal BGM density was observed between plants treated with the mock solution and MeJA. In the field experiment, BGM density was not affected by any factor in both trial 1 and 2 (Fig 2).

**DISCUSSION**

Previous studies have shown that exogenous applications of JA and MeJA can decrease the development of spider mite infestations by reducing female fecundity, egg survival, and mite feeding, as well as increasing the time needed for embryonic development (Omer et al. 2000; 2001, Li et al. 2002, Thaler et al. 2002, Gols et al. 2003, Ament et al. 2004, Warabieda 2005, Schweighofer et al. 2007, Miyazaki et al. 2014). Similar to JA and its metabolite, MeSA can reduce spider mite infestations by attracting natural occurring predators, but when applied directly to plants, it rather elevates spider mite feeding preference and oviposition rates (de Boer and Dicke 2003, Ament et al. 2004, James and Price 2004). Similar to previous studies, we found that corn sprayed with MeJA had fewer BGM eggs compared to MeSA and control applications, but the effect of MeJA was not observed when plants were water-stressed and/or treated with the neonicotinoid active ingredient clothianidin. Alternatively, plants treated with MeSA had greater egg and BGM (adults and immatures) densities than plants treated with MeJA and the mock solution.
JA and its metabolite MeJA, are known to have a suppressive effect on spider mites, specifically *Tetranychus urticae* Koch, due to the induction of plant defenses such as proteinase inhibitors, lipoxygenase, oxydative enzymes, and anti-nutritive components (Stout and Duffey 1996, Thaler et al. 1996, Bi et al. 1997). In our case, however, the negative effect of MeJA on BGM was relatively weak and not consistent as the effect was only seen on the density of BGM eggs and not on the density of BGM adults and immatures. A possible reason may be that specialist species, such as BGM which primarily feeds on monocots (Blasi et al. 2015), are commonly less affected by a given plant defense compared to polyphagous species, such as *Tetranychus urticae* (Whittaker and Feeny 1971). However, we did not measure plant defenses, hence we do not know if the phytohormone treatments were actually effective at inducing plant defenses. Intraspecific genotypic variation with respect to plant-defense coping mechanisms is also known to exist among spider mites (Ozawa et al. 2017). As an example, red strains of *Tetranychus kanzaway* have a superior detoxification system than white strains when feeding on bean plants, which allows for an easier host adaptation and performance (Ozawa et al. 2017). Significant changes in gene expression for detoxification commonly occur in *Tetranychus urticae* upon host plant transfer, from most preferred to less suitable host plants, which affect the pest development differently (Grbic et al. 2011). Furthermore, herbivores with similar feeding modes can have opposite effects on plant defenses (Oliveira et al. 2016). As an example, *Tetranychus urticae* induces certain plant defenses in tomato plants, whereas *Tetranychus evansi*, a common pest of Solanaceae, typically reduces these defenses (Oliveira et al. 2016). For these reasons, BGM may not show strong effects toward host plant resistant changes, assuming phytohormone treatments
activated the biosynthesis of defenses in our corn plants, as we see in polyphagous herbivores.

MeJA generally had a weak effect on BGM, and another possible factor could be related to an insufficient dose of phytohormone applied to plants, or the method we used to apply MeJA was not ideal. Baldwin (1988), for example, suggested that the best way to prime a response in *Nicotiana attenuata* that would be as effective as the one elicited by a standardized leaf-wounding protocol, was to add MeJA to the soil by mixing the phytohormone with irrigation water or injecting MeJA with a syringe below the soil surface. We, however, are confident that the application was appropriate, as we adopted methods that had been previously used with success and at similar rates.

Water-stress is known to decrease the concentration of herbivore-related defensive compounds in some plants (Lombardero et al. 2000, Olson et al. 2009, Halpern et al. 2010, Gutbrodt et al. 2011; 2012). From our findings, exogenous applications of MeJA did not alleviate BGM densities in water-stressed corn plants. JA is a common precursor used by plants to produce defenses against herbivorous arthropods (Ford et al. 2010) and to protect from the damage of water-deficiency by activating oxygen-scavenging enzymes and reducing transpiration and membrane-lipid peroxidation (Wang 1999, Brossa et al. 2011, de Ollas et al. 2013). Under simultaneous conditions of drought and pest herbivory, JA can become a limiting resource for plants and, as suggested by the growth-differentiation balance hypothesis (GDB), trade-offs between growth maintenance and defense can occur (Herms and Mattson 1992). Specifically, plant resistance to biotic stress can decline in some plants when subjected to increasing intensities of drought (Herms and Mattson 1992).
Several studies found a reduction of defensive compounds in drought-stressed plants, such as for loblolly pine (Lombardero et al. 2000), cotton (Olson et al. 2009), wild tobacco (Halpern et al. 2010), garlic mustard (Gutbrodt et al. 2011), and apple trees (Gutbrodt et al. 2012). In some instances, however, growth is more costly to the plants under water-stress conditions and, as a consequence, defenses are expected to increase (Gershenzon 1984).

MeJA also did not suppress BGM egg density when plants were treated with clothianidin. This active ingredient can induce the biosynthesis of SA through its metabolite 2-chlorothiazolyl 5-carboxylic acid (Ford et al. 2010). By increasing SA, clothianidin can inhibit the transcription of JA-related defenses against herbivorous pests, as the signaling pathways of these two phytohormones are mutually antagonistic (Ford. et al. 2010). The concentration of JA and SA in plant tissue, however, were not measured here.

Similar to Zhang et al. (2009) and Wei et al. (2014), who found that SA increased *Tetranychus urticae* feeding preference and oviposition rates in Lima bean plants, MeSA applications led to increases in BGM in our study. Integrated pest management programs, however, have included SA as a management tool to control spider mites, as MeSA-containing volatile blends were found to attract natural enemies following spider mite herbivory in several plants including lima bean, grape, tomato, and hop (de Boer and Dicke 2003, Ament et al. 2004, James and Price 2004). The use of SA and its derivative need to be carefully evaluated, as this phytohormone was also found to reduce resistance to water-stress in some plants, particularly corn (Németh et al. 2002, Khan et al. 2003).
Results observed in the greenhouse were not observed in the field and, similar to Miyazaki et al. (2013), the density of BGM and their eggs was higher on corn plants grown under greenhouse conditions than on plants grown in the field. In the greenhouse, for example, plants were exposed to minimum and maximum daily air temperatures comprised between 26 and 30°C. In the field, instead, plants were exposed to more fluctuating temperatures between minimum and maximum daily temperatures from 6 to 22°C and from 22 to 36°C, respectively. Divergence between greenhouse and field experiments can also be attributed to additional differences in growing conditions, such as radiation, effects of wind buffeting, relative air humidity, soil type, fertility, and soil moisture content. As an example, Gouinguené and Turlings (2002) observed that different climatic and plant nutrient conditions changed the amount and composition of induced plant volatiles emitted by corn plants to protect them from pests, whereas Wang et al. (2001) found that concentrations of abscisic and JA in *Iris hexagona* generally increased in response to salinity, while indole-3-acetic and SA declined.

This represents the first study evaluating the effect of plant phytohormone derivatives, specifically MeJA and MeSA, as a possible tool to alleviate spider mite outbreaks triggered by abiotic factors, namely water-stress and the use of neonicotinoids. We focused on BGM, a species less studied than the polyphagous twospotted spider mite *Tetranychus urticae* (Koch), and a grass specialist. We expanded our observations of spider mites to monocots, as previous studies have focused on dicots, including cotton (Omer et al. 2001, Miyazaki et al. 2014), strawberry (Warabieda et al. 2005), tomato (Li et al. 2002, Thaler et al 2002, Ament et al. 2004), lima beans (Gols et al. 2003), grapevine (Omer et al. 2000), and
*Arabidopsis thaliana* (Schweighofer et al. 2007). Moreover, while most of these studies were conducted in laboratory or greenhouse studies, we expanded our investigation to field conditions.

Contrary to previous work, which observed strong negative effects of JA and MeJA on the performance of *Tetranychus urticae*, we rather observed a relatively weak response of BGM to this phytohormone (Omer et al. 2000; 2001, Li et al. 2002, Thaler et al. 2002, Gols et al. 2003, Ament et al. 2004, Warabieda 2005, Miyazaki et al. 2014). MeJA, however, can still be an additional approach to the management of spider mite infestations, as exogenous applications of JA and its derivatives were found to enhance the attraction of *Phytoseiulus persemilis*, a specialist enemy of spider mites (Dicke et al. 1999, Gols et al. 1999; 2003). Similar to JA and MeJA, MeSA-containing volatile blends are emitted by plants after spider mite wounding and were found to attract spider mite natural enemies (Dicke 1998, de Boer and Dicke 2003, Ament et al. 2004, James and Price 2004). In addition to biocontrol benefits, exogenous applications of SA and other salicylates were also found to increase plant resistance to water-stress in *Phaseulus vulgaris, Lycopersicum esculentum* and *Commelina communis* (Larque-Saavedra 1978; 1979, Senaratna et al. 2000).

However, in some instances, as well as in our study, SA can promote the formation of spider mite infestation and increase feeding and oviposition rate when directly applied to plants (Zhang et al. 2009, Wei et al. 2014). Furthermore, SA was found to lower corn tolerance for water-deficiency (Németh et al. 2002, Khan et al. 2003). As a consequence, the benefits of SA as a possible tool to improve the efficacy of biocontrol may be outweighed by the improved performance of spider mites when
directly exposed to this phytohormone and the increased susceptibility of corn plants to water-stress.

The use of JA, SA, and their derivatives to suppress the development of spider mites and other pests has not been widely adopted and is still under investigation. Specifically, more research needs to be conducted on the ecological consequences of JA and SA exogenous applications on different spider mite and plant species, as well as the plant-mediated mechanisms induced by JA and SA that allow for the control of certain herbivorous pests, and the phytohormone interaction with different abiotic factors. Our current findings suggest that phytohormones may be less effective against specialist herbivores and do not appear to alleviate the abiotic stress responses of plants that lead to pest outbreaks.

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REFERENCES


**Fig. 4-1.** Effect of MeJA and MeSA exogenous applications on the development of BGM on neonicotinoid-treated corn plants grown in a greenhouse, and receiving optimal irrigation or kept under water-stress conditions. The first graph (top left) represents the results obtained in trial 1 and 2 combined together for egg density (eggs per sq. cm of total plant leaf area). The bottom two graphs represent the results obtained in trial 1 and 2 for BGM density (BGM per sq. cm of total plant leaf area), respectively. Counts were made on 6-week old plants. The x-axis represents the different combinations of irrigation and neonicotinoid treatments. 'C' represents plants not treated with neonicotinoid insecticide, 'CL' represents plants treated with clothianidin, '15%' is the volumetric water content (VWC) under which plants provided with optimal irrigation were kept, '5%' is the VWC under which water-stressed plants were kept. The y-axis represents the egg density (eggs per sq. cm$^2$ of plant total leaf area) (top) and the BGM density (BGM per sq. cm$^2$ of plant total leaf area) (bottom). White bars are phytohormone control plants treated with 0.001% ethanol solution, light grey bars are plants treated with MeJA, dark grey bars are plants treated with MeSA. *Different capital letters* represent a significant (*P*<0.05) main effect of water. Means are ±1SE.
Fig. 4-2. Effect of MeJA and MeSA exogenous applications on the development of BGM on neonicotinoid-treated corn plants grown in the field, and receiving optimal irrigation or kept under water-stress conditions. For trial (right) and trial 2 (left), the x-axis represents the different combinations of irrigation and neonicotinoid treatments. 'C' represents plants not treated with neonicotinoid insecticide, 'CL' represents plants treated with clothianidin, '100%' represents plants provided with optimal irrigation (100% estimated evapotranspiration (ET) replacement), '25%' represents water-stressed plants that received 25% of the water provided to optimally irrigated plants. The y-axis represents the egg density (eggs per sq. cm² of the plant total leaf area) (top) and the BGM density (BGM per sq. cm² of the plant total leaf area) (bottom). Counts were made on 6-week old plants. White bars are phytohormone control plants treated with 0.001% ethanol, light grey bars are plants treated with MeJA, dark grey bars are plants treated with MeSA. Different capital letters represent a significant (P<0.05) main effect of water. Means are +1SE.
CHAPTER V

GENERAL SUMMARY AND CONCLUSIONS

General summary

Abiotic factors, such as plant water-stress and the use of neonicotinoid insecticides, have been implicated in the outbreak of several agricultural pests, particularly spider mites (Acari: Tetranychidae), through direct and indirect (plant-mediated) mechanisms. Increases in temperature and reduced frequency of precipitation events in the region, has led to predictions that drought events will be more frequent in the Intermountain West and elsewhere. Meanwhile, neonicotinoids have become a common pest management tool in many agricultural systems, yet their consequences for plant-arthropod interactions are only partially understood.

In my first study, I investigated how plant water-stress and the application of neonicotinoids, two simultaneously occurring abiotic factors in corn crops, affected the development of spider mites and the biosynthesis of plant herbivore-related defenses. I found that plant water-stress and the neonicotinoid active ingredient clothianidin consistently increased BGM densities on field corn plants. Thiamethoxam, instead, a second active ingredient commonly used in corn, exacerbated the effect of water-stress only after plants reached maturity, suggesting that different neonicotinoid active ingredients affect spider mites and interfere with plant physiology differently. Water-stress and spider mite herbivory had a greater effect on plant defenses than neonicotinoids alone, while combinations of spider mite herbivory with plant water-stress or neonicotinoids increased the concentration of proteins in leaf tissues.
In my second study, I evaluated drought-tolerant (DT) corn hybrids as a possible strategy to decrease spider mite infestations on water-stressed plants. I found that the development of spider mite outbreaks in field corn with water-stress, can be decreased by using DT corn hybrids. Specifically, corn varieties without drought-tolerance had increased BGM when water-stressed compared to corn with optimal irrigation. Moreover, DT corn hybrids that were water-stressed had equal BGM densities to optimally irrigated corn with and without the DT trait.

In my third study, I tested whether exogenous applications of methyl jasmonate (MeJA) and methyl salicylate (MeSA), phytohormones involved in plant resistance against pests and pathogens, decrease spider mite infestations in corn exposed to water-stress and treated with neonicotinoids. These two abiotic factors can alter the biosynthesis of plant defenses against biotic stressors, while studies have shown that exogenous applications of MeJA and MeSA can enhance plant resistance to pests and pathogens. I found that MeJA reduced the fecundity of BGM on plants that were provided with optimal irrigation and not treated with neonicotinoids, compared to plants that were water-stressed and/or when clothianidin was applied. However, these results were not consistent across all the experiments and the overall response of BGM to MeJA was relatively weak. Greater egg and BGM (adult and immature) density were found when plants were treated with MeSA or kept under water-stress.

Conclusions

The data collected in this study represents an important step in the understanding of the mechanisms driving spider mite outbreaks with conditions of
plant water-stress and the use of neonicotinoid insecticides. My research is innovative because it examined the combined effect of these two simultaneously occurring abiotic factors, commonly investigated separately, on spider mite outbreaks and plant biosynthesis of herbivore-related defenses. My research also focused on BGM, a less studied spider mite species that primarily develops in field corn, and the outbreak response to clothianidin and thiamethoxam, two neonicotinoid active ingredients commonly used in corn as a seed treatment. In addition, my investigations examined these interactions over the entire development of corn plants, while others have traditionally examined these factors with younger plants.

From my work, it is clear that plant water-stress and neonicotinoid use can lead to spider mite outbreaks and this can persist throughout the corn-growing season. Given these results, growers can anticipate spider mite outbreaks under these conditions and plan their management accordingly. For instance, thiamethoxam appeared to have less of an effect on spider mite outbreaks with water-stress conditions and may be considered for seed treatment rather than clothianidin. Growers may also consider the use of drought-resistant plants as a possible tool to slow the buildup of spider mite infestations. While most of the development of drought-resistant plants is directed toward irrigation challenges, my work suggests growers may find these same hybrids are also a new sustainable approach for spider mite management. However, more research is needed to understand the relationship between pest resistance and drought-resistance. While exogenous applications of MeJA have been shown to decrease infestations of the polyphagous twospotted spider mite, it does not appear to be an approach that would successfully control BGM and alleviate the effects of water-stress and neonicotinoids on this pest. Overall, my
research provides a strong contribution to the study of plant-arthropod interactions caused by simultaneously occurring abiotic factors. My research also offers some practical and sustainable solutions to spider mite management in field corn crops, and concurrently opens the doors to further investigations on the ecological implications deriving from the adoption of these new tools for spider mite control.
CURRICULUM VITAE

Alice Ruckert

EDUCATION

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<tr>
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<th>Degree</th>
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<tr>
<td>Utah State University, Department of Biology</td>
<td>Logan, UT</td>
<td>Ph.D. in Ecology</td>
<td>April 2017</td>
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<tr>
<td>University of Pisa (Italy) &amp; Sant’Anna School of Advanced Studies</td>
<td>Pisa, Italy</td>
<td>M.S. in Agriculture and Applied Sciences</td>
<td>2009-2011</td>
</tr>
<tr>
<td>University of Pisa (Italy) &amp; Sant’Anna School of Advanced Studies</td>
<td>Pisa, Italy</td>
<td>B.S. in Agriculture and Applied Sciences</td>
<td>2006-2009</td>
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RESEARCH EXPERIENCE

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<tr>
<td>Utah State University</td>
<td>Logan, UT</td>
<td>PhD Researcher with Dr. Ricardo Ramirez</td>
<td>2012-2017</td>
</tr>
<tr>
<td>New Zealand Institute Plant and Food Research Ltd.</td>
<td>Christchurch, NZ</td>
<td>Intern with Dr. Max Suckling</td>
<td>2011-2012</td>
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</table>

Doctoral theses research conducted with Dr. Ricardo A. Ramirez. Interactive effect of plant water-stress and the use of neonicotinoid insecticides on spider mite (Acari: Tetranychidae) outbreaks in corn. Conducted greenhouse and manipulative field experiments, measured plant physical and physiological responses to biotic and abiotic stressors, and found alternative ways to control spider mite infestations in dry-land conditions.
Performed analysis of electroantennography on *Bactericera cockerelli* (potato psyllid) and on some leaf roller moths (Lepidoptera: Tortricidae), common pests of fruit and ornamental trees, aimed at identifying attractive chemical blends to be used for mating disruption.

**University of Pisa**

*Undergraduate and Master's Researcher* with Dr. Andrea Lucchi  *2006-2011*

Studied mechanisms of vibrational communication in the planthopper *Scaphoideus titanus* (Hemiptera: Auchenorrhincha), and the effect of vibrational disturbance on the biodiversity and abundance of insect pests in vineyards, with particular emphasis on the taxonomic group of the *Auchenorrhincha*, their predators, and parasitoids.

**LEADERSHIP & TRAINING EXPERIENCE**

**Ramirez Lab, Department of Biology, Utah State University**  *2012-present*

Responsible of laboratory safety and adherence to safe lab practices, maintained and calibrated equipment, and evaluated instrumentation for effectiveness.

**Ramirez Lab, Department of Biology, Utah State University**  *2012-present*

Hired, trained, and supervised the lab's part-time and full-time staff person (approx. 30 technicians).

**Consultation Field Visits**  *2013-present*

Provided assistance to Utah corn growers with spider mite identification and management.

**Department of Biology, Utah State University**  *2012-present*

Mentored undergraduate students as they progressed in their academic path.
Reviewed and edited documents (research posters, articles, and presentations) for accuracy and completeness.

TEACHING EXPERIENCE

ECOL 2220: General ecology  Fall 2016

Teaching assistant

In the course, adaptation, population growth, species interactions, biodiversity, and ecosystem function are explored for a wide variety of organisms and ecosystems. Oversaw group problem sets, corrected homework and exams. Class size: 150 students.

BIOL 1620: General biology  2014-2017

Laboratory Teaching assistant

This course focuses on ecology and evolution, and is designed to developing the skills and competencies needed for biological research. Gave lectures and led laboratory demonstrations to illustrate course concepts, oversaw group projects, and corrected homework and exams. Class size: 90 students.

PUBLICATIONS


Ruckert, A. and Ramirez, R.A. Interactions between neonicotinoids and water stress lead to Banks grass mite (Oligonychus pratensis Banks) outbreaks in corn. (in preparation).


PROFESSIONAL PRESENTATIONS


Symposium: Are We Stressed Enough Yet? Interdisciplinary Partnerships to Evaluate the Consequences of Plant Abiotic and Biotic Stresses.


HONORS AND AWARDS

- Entomological Society of America’s USDA AFRI Student Travel Grant, $500 (2015);
- Pacific Branch-ESA Student Travel Award, $200, Entomological Society of America (2015);
- 2nd place PhD 10-minute paper competition, ESA 99th Annual Meeting of the Pacific Branch (2015);
- Ecology Center Graduate Research Award, $3600, Utah State University (2015);
- Invited to speak at the XXV International Congress of Entomology 2016, Orlando, Florida, USA, September 25-30 (2016);
- Sant’Anna School of Advanced Studies 5-year scholarship for students maintaining at least a 3.0 GPA, Pisa, Italy (2006-2011).

OUTREACH EXPERIENCE

**Entomological Society of America Pacific Branch**

Check-in desk volunteer.  
April 2015

**USU Entomology club**

Organized insect collection tours and taught Entomology classes to children from 5 to 10 years old.  
2014-2015

**Mentally Disabled Student Outreach Program**

Participated in activities organized for mentally disabled students.  
2013
TECHNICAL SKILLS

- **Software**

- **Languages**
  Italian (native); fluent in English (written and verbal).

PROFESSIONAL SOCIETIES

- **Entomological Society of America** - student member

- **USU Ecology Center** - active member

- **USU Entomology club** - active member

PERSONAL TRAITS

Determined, committed, reliable, kind, respectful, grounded.

HOBBIES

Travelling, attending scientific conferences, reading, having a healthy life style and, last but not least, spending time with my beautiful family.