MANAGEMENT OF *FUSARIUM OXYSPORUM* F. SP. *NIVEUM* AND *MELOIDOGYNE INCOGNITA* IN WATERMELON

by

KASMITA KARKI

(Under the Direction of Bhabesh Dutta and Abolfazl Hajihassani)

ABSTRACT

Commercial production of watermelon *(Citrullus lanatus* var. *lanatus*) is adversely affected by soil-borne pathogens, *Fusarium oxysporum* f. sp. *niveum* (FON) and southern root-knot nematode (RKN, *Meloidogyne incognita*). We studied if micronutrient manipulation could induce systemic resistance in FON and RKN pathosystems. Expression of defense genes in salicylic acid- and jasmonic acid-pathway was significantly affected with Fe, Mn and Zn manipulation. RNA-seq revealed multiple differentially expressed genes (DEGs) in plants that were treated with high concentration of Zn. These DEGs were enriched in hormone signal transduction and MAPK signaling pathway suggesting potential induction of systemic resistance. Further, the efficacy of fumigant and soil-applied fungicides were evaluated for the management of FON under field conditions. Our results showed that the fumigant Pic-clor 60 at 336.26 kg/ha when applied in combination with either prothioconazole (Proline, Bayer Crop Science) or pydiflumetofen (Miravis, Syngenta) resulted in significant reduction of disease incidence. Overall, the results of these studies can be incorporated in time into the integrated disease management options for watermelon against FON and RKN.

INDEX WORDS: *Fusarium oxysporum* f. sp. *niveum*, *Meloidogyne incognita*, induced resistance, RNA-seq, Pic-clor 60, prothioconazole, pydiflumetofen

MANAGEMENT OF *FUSARIUM OXYSPORUM* F. SP. *NIVEUM* AND *MELOIDOGYNE INCOGNITA* IN WATERMELON

by

KASMITA KARKI

B.S., Tribhuvan University, Nepal, 2016

A thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2020

MANAGEMENT OF *FUSARIUM OXYSPORUM* F. SP. *NIVEUM* AND

MELOIDOGYNE INCOGNITA IN WATERMELON

by

KASMITA KARKI

Major Professor:	Bhabesh Dutta
	Abolfazl Hajihassani
Committee:	Tim Coolong
	Chandrasekar Kousik

Electronic Version Approved:

Ron Walcott
Dean of the Graduate School
The University of Georgia
December 2020

DEDICATION

This work is dedicated to my family.

ACKNOWLEDGEMENTS

I would like to express my appreciation to the faculty, staff and graduate students in the Department of Plant Pathology, University of Georgia for their guidance and support throughout my time in University of Georgia. I would like to thank my advisors, Dr. Bhabesh Dutta and Dr. Abolfazl Hajihassani for offering me this learning opportunity and guidance throughout this research project. I greatly appreciate my committee members, Dr. Tim Coolong and Dr. Chandrashekar Kousik, for their expertise and guidance in this project, and assistance in completing my thesis. I would also like to thank Dr. Mihir Mandal for offering his expertise and guidance. Finally, my sincerest gratitude is extended to my family for their love and support.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Watermelon background, history, production

Watermelon (*Citrullus lanatus* var. *lanatus* (Thunb.) Matsum. & Nakai) is a dicot in the genus *Citrullus* Schrad. ex Eckl. et Zeyh. of the botanical family Cucurbitaceae (Schaffer and Paris 2003). It is an annual plant with long, weak, climbing type stems, which are five-angled (five-sided) and up to 3 m long (Rubatzky and Yamaguchi 1997). The leaves are broad, thick, hairy, pinnately lobed, and alternate; when they age, they become stiff and rough (Butterwick 2018). The leaves are 60 to 200 mm long and between 40 and 150 mm wide. The plant develops branching tendrils. Plants are monoecious bearing alternating staminate (male) flowers appearing first and pistillate (female) flowers later with ratios in favor of maleness (e.g. 7 staminate: 1 pistillate) on 40-mm-long stalks (Kyriacou et al. 2018). Various insects, especially bees, are essential to aid in pollination. The USDA recommends that at least three beehives should be used per hectare (One hive per 4,000 m^2) to pollinate conventional, seeded varieties in commercial plantings. As the pollen in seedless hybrids is sterile, planting pollinizer rows of varieties with viable pollen is required. Even so, the supply of viable pollen is reduced, and pollination is much more critical in the production of the seedless variety. Hence, the recommended number of hives per hectare (pollinator destiny) increases to nine hives per acre (One hive per 1,300 m^2). Watermelon fruits are a modified form of berry called pepo with a thick rind (exocarp) and fleshy center (mesocarp and endocarp) (Butterwick 2018). They are round, oval, or elongated, ranging from 1.5 to 15 kg. The rind is light to dark green and either solid in color or with stripes

of various patterns. The flesh may vary from white, green, yellow, orange to red, though consumers associate the internal quality with deep red, pink, or intense yellow, in addition to sweetness and texture (Kyriacou et al. 2018).

Watermelon has been cultivated since 2000 BCE, and its origins can be traced back to western Africa (Chomicki and Renner 2015). The crop was introduced to India by at least 800 CE, and to China by 900 CE. It was then extended to southeastern Asia, Japan, Europe, and the western hemisphere in the 1500s (Kyriacou et al. 2018). Initially, seedless watermelons were developed by Japanese scientists in 1939. They were able to grow seedless triploid hybrids that at first were not commonly used because they did not have adequate resistance to diseases (Andrus et al. 1971). The seedless varieties became more popular in the 21st century. Its demand has increased in the United States over the years, which is shown by the increase in the share of seedless watermelon in total watermelon shipments from 51% in 2003 to nearly 85% in 2014 (AgMRC 2018; Andrus et al. 1971).

Watermelons are currently produced in 119 countries, with approximately 3.2 million ha being harvested annually (FAO 2018). Nearly 104 million tons of watermelons are produced annually worldwide; China is the largest producer (FAO 2018). The United States is the seventh-largest watermelon producer in the world with annual production and a farm gate value of 2.18 million tons and $657 million, respectively (USDA-NASS 2019). Currently, at least 44 states in the United States grow watermelon commercially, and around 50% of the total national production is produced in the southeastern United States, including Georgia, Florida, South Carolina, and North Carolina. With a production area of 8,903 hectares, Georgia is the third-largest watermelon producing state (USDA-NASS 2019) in the United States.

Fusarium wilt of watermelon and *Fusarium oxysporum* f. sp. *niveum*

Fusarium wilt of watermelon caused by *Fusarium oxysporum* Schltdl.: Fr. f. sp. *niveum* (FON) W. C. Snyder and H. N. Hansen, is one of the most economically damaging pathogens in watermelon worldwide (Martyn 2014). It is a soil-borne pathogen that was described in the early 1890s and was first studied in detail by Erwin F. Smith (Smith 1894). There are four races of FON (0, 1, 2, and 3) that are differentiated according to virulence on differential cultivars. The varieties 'Sugar Baby' and 'Black Diamond' are susceptible to all FON races. 'Charleston Gray' is resistant to race 0 but susceptible to races 1, 2, and 3. 'Calhoun Gray' is resistant to race 0 and 1 but susceptible to race 2 and 3. 'PI-296341-FR' is resistant to races 0, 1, 2 but susceptible to race 3 (Table 1.1) (Zhou et al. 2010). Race 2 is the most important race as it is widely distributed within the United States, including Georgia (Petkar et al. 2019; Zhou and Everts 2004; Zhou et al. 2010). FON race 3 has also been identified in Florida, Georgia, and Maryland (Amaradasa et al. 2018; Petkar and Ji 2017; Zhou et al. 2010).

Symptoms. FON can infect the host at any stage of plant development. Symptoms of Fusarium wilt in the seedling stage include dieback and scorched appearance. In mature plants, foliage discoloration is followed by loss of turgor pressure, general chlorosis of tissue and wilting of the vine. However, the severity of the disease depends on the environmental factors, the age of the plant during infection, soil pathogen density and FON aggressiveness (Kleczewski and Egel 2011; Martyn 1987; Martyn and Bruton 1989). The first symptom often appears in the field as a unilateral wilting; the one-side or one runner of the plant wilts but the other side or runners appear healthy. This unilateral wilt symptom can be seen in plants three to four weeks after transplantation in the field. Symptoms include a gradual change in the appearance of the crown leaf from dull gray-green to yellow, permanent wilting of the vines, and eventual death.

Vascular necrosis of the stem at the base of the plant is a characteristic symptom of Fusarium wilt. Under damp conditions, white to pink fungal hyphae may be visualized emerging from necrotic tissues.

Pathogen biology. Only the asexual reproductive stage (anamorphic) of FON is known as the sexual stage (teleomorphic), has not been identified. Asexual reproductive structures are micro-, and macroconidia produced in an asexual fruiting system called a sporodochium. Microconidia are aseptate, develop on short conidiophores, are oval to kidney-shaped, and are formed on the aerial structures of mycelia (Kleczewski and Egel 2011). Macroconidia are fusiform (canoe-shaped) with three to five cells, and often develop in the masses of hyphae known as sporodochia. Both microconidia and macroconidia can infect the roots; however, the primary function of macroconidia is to support fungal survival as it possesses the ability to form chlamydospores (asexual resting structures). Chlamydospores are thick-walled survival spores that provide primary means of protection against adverse conditions. There are two types of chlamydospores known to FON; one forms with the macroconidium and one forms within the mycelium. Chlamydospores formed from mycelia typically occur individually or in pairs and may be either intercalary (within mycelium) or terminal (at the ends).

Disease cycle and epidemiology. The primary infections start with an infection-hyphae germinating from either chlamydospores or macroconidia and may occur at any point in plant development (Egel and Martyn 2007; Lin et al. 2010; Wehner 2008). The fungal hyphae can penetrate the root-cortex using cell wall degrading enzymes and plant saponin to overcome the primary plant resistance barriers such as cell walls and constitutive phytoalexins (Bruton 1998; Idnurm and Howlett 2001; Inoue et al. 2002). Upon penetration, the fungus grows within the xylem and produces microconidia, which are translocated to the upper part. Microconidia bind to

the xylem walls and sporadically germinate to form mycelia and other infection structures (Egel and Martyn 2007; Wehner 2008). The watermelon plant attempts to avoid the systemic spread of the pathogen by blocking xylem vessels by using specific structures called tyloses resulting from the invagination of xylem parenchymal cells. As a consequence, the structures hinder the flow of water and thus cause wilting symptoms (Egel and Martyn 2007; Lin et al. 2010). However, this theory has been contrasted by a recent study done by Sun et al. (2017) with *Fusarium oxysporum* f. sp. *cucumerinum*, where it was demonstrated that the wilt associated with Fusarium infection is not due to water scarcity but to an increase of pathogen-produced toxins. This has not yet been proven in watermelon, but the pathosystem of these two crops are likely similar. After plant death, substantial colonization of the plant surface is observed, and the fungus forms chlamydospores that remain viable in the soil for an extended period (Egel and Martyn 2007).

FON infections are favored by temperatures of approximately 27℃ and light, sandy, slightly acidic soils with high-to-moderate organic matter (Boyhan et al. 2003; Bruton 1998; Egel and Martyn 2007; Wehner 2008). Infection is delayed at temperatures above 33℃ (Bruton 1998), often resulting in plants that are chlorotic and stunted but not wilted (Egel and Martyn 2007). FON can survive as a saprophyte in soil and on plant debris for more than 10 years due to its ability to form thick-walled chlamydospores (Martyn 1996; Zhang et al. 2015). These pathogen propagules may be distributed inside and through agricultural fields by soil, water, plant debris, infected transplants and farm equipment (Bruton 1998; Egel and Martyn 2007; Wehner 2008). Additionally, FON is also transmitted by seeds, potentially infested through the pericarp and peduncle of immature fruit and pistils of watermelon flowers (Petkar and Ji 2017).

Root-knot nematodes

Root-knot nematodes (*Meloidogyne* Goldi (Tylenchida: Meloidogynidae), are highly adaptable, obligate plant parasites with global distribution (Jones et al. 2013). *Meloidogyne* is derived from two Greek terms meaning “apple-shaped” and “female” characterized by the swollen female in the nematode life cycle (Karssen and Moens 2006; Moens et al. 2009). Initially, all the root-knot nematodes were regarded as one species but later, several early contradictory reports of resistance or susceptibility to plant hosts as well behavioral patterns in the literature emerged (Berkeley 1855; Chitwood 1949; Moens et al. 2009). It was not until 1949 that Chitwood differentiated and described four widespread and common *Meloidogyne* species: *Meloidogyne incognita*, *M. arenaria*, *M. javanica*, and *M. hapla* (Chitwood 1949). The genus *Meloidogyne* has 98 species (Jones et al. 2013). A variety of economically important crops are attacked by root-knot nematodes (Sikora et al. 2018); one of them is watermelon. A survey conducted in southern Georgia in 2018 found that root-knot nematodes were present in 50% of the fields planted with watermelon with majority of the fields infected with *M. incognita* followed by *M. arenaria* (Hajihassani et al. 2020). Root-knot nematode infection has been reported to cause around 20% yield loss in watermelon (Lynch and Carpenter 1999).

Symptoms. The primary symptom to identify root-knot nematodes is the presence of galls on infected roots. Galls formed by cellular hypertrophy are the exterior appearance of giant cell formations on the roots. Galls often coalesce, and the number and size of galls per root system depend on host susceptibility and soil population density. Galls disrupt the normal root function, causing plants to grow poorly or sometimes, leading to death under heavy infestation (Palomares-Rius et al. 2017). Watermelon plants infected by the nematodes usually show

symptoms such as leaf discoloration, root deformation due to the development of galls, and stunted growth.

Life cycle of root-knot nematodes. All root-knot nematodes have a similar life cycle with three basic life stages: egg, juvenile and adult stage. A female lays 300-500 eggs on the surface of the root of a susceptible host. The eggs are covered with a gelatinous mass released from rectal glands that protect them from environmental extremes and attack from microbes (Moens et al. 2009). When conditions are favorable, embryogenesis takes place inside the egg resulting in the first-stage juvenile followed by a molt to the infective second-stage juvenile (J2) larvae that hatch from the egg (Perry et al. 2009). According to Curtis et al. (2009), the hatching of J2 larvae from egg and movement to a host root tip is dependent on temperature, moisture, oxygen availability, physiological barriers, root exudates and other unknown factors. The J2 larvae invade the root behind the root end and intercellularly migrates through the root before a permanent feeding site is established. Root-knot nematodes penetrate the host cell wall with a specialized feeding structure (stylet) followed by injection of secretory protein, which induces giant cells. Giant cells undergo repetitive mitosis without cytokinesis, and are multinucleated with several sets of chromosomes in each nucleus (Jones and Goto 2011). Each giant cell may have an increase of up to 600 times the number of copies of plant genes, providing a considerable amount of protein for the nematode to be ingested (Perry et al. 2009). Once a giant cell initiates and the J2 begin to develop within this permanent feeding site, the nematodes become sedentary and enlarges, having to undergo three more molts to become an adult. If the juvenile differentiates into an adult male, the nematode either exits and mate with a female, or as in the case of *M. incognita,* the male leaves the root and die. If the juvenile differentiates into an adult female, it will continue to feed and enlarge, allowing the nematode posterior end to split

through the root epidermis where several hundred eggs are to be deposited (Perry et al. 2009). Since *M. incognita* reproduces through mitotic parthenogenesis, males are vestigial and are not always found in routine soil samples. Owing to the brief life period of root-knot nematodes (approximately 20-30 days), multiple life cycles can occur within one crop season, increasing the population exponentially by the end of the season.

Pathological interrelations between *Fusarium oxysporum* f. sp. *niveum* and southern root-knot nematodes in watermelon

Co-infection of a host by *Fusarium oxysporum* f. sp. *niveum* and root-knot nematodes may lead to different outcomes; inducing wilt symptoms in a wilt-resistant plant, altering the non-host response to induce a symptom, increasing incidence and severity in a susceptible cultivar but having no effect on resistant ones, and having no impact on either a susceptible or resistant cultivar (Keinath and Agudelo 2018). FON has been stated to interact with southern root-knot nematode [RKN; *Meloidogyne incognita* (Kofoid and White) Chitwood] (Davis 2007; Hua et al. 2019; Sumner and Johnson 1973). The relationship between FON and RKN in watermelon was first examined by Sumner and Johnson in 1973, according to which the presence of nematodes enhanced the wilt symptoms caused by FON. Similar findings were obtained in another study in which susceptibility to Fusarium wilt was enhanced in the presence of RKN (Hua et al. 2019). However, in later reported research, Fusarium wilt severity did not vary for grafted vs. non- grafted watermelon (with susceptible scion) when inoculated with FON or both FON and RKN (Keinath and Agudelo 2018; Keinath et al. 2019; Seo and Kim 2017). These findings suggested that the relationships between FON and RKN should be further explored in-depth, including the identification of various influences that could potentially influence both pathogens.

Management of FON and RKN in watermelon

Host resistance (FON). Fusarium wilt management in watermelon by the employment of host resistance was first started in the 1900s with the introduction of a resistant diploid (seeded) cultivar (Martyn 2014). Prior to the shift to triploid (seedless) cultivar, diploid cultivars such as 'Conquerer' and several others were used in the United States (Guner and Wehner 2004). However, the majority of triploid watermelon cultivars currently used have little or no resistance to Fusarium wilt (Everts and Hochmuth 2011).

Host resistance (RKN). No cultivated watermelons are known to be resistant against RKN (Winstead and Riggs 1959). All 10 watermelon cultivars evaluated against *M. incognita* in Puerto Rico were susceptible (Montalvo and Esnard 1994). A core collection of *Citrullus* spp. from a U.S. Plant Introduction unit was evaluated for response to *M. incognita* and *M. arenaria* races 1 and 2 in greenhouse tests by Thies and Levi (2003, 2007). In those studies, several accessions of *C. lanatus* var. *citroides* were identified as moderately resistant to *M. incognita* and *M. arenaria*. However, they have not been widely used in breeding programs.

Cultural control (FON). The amount of FON in the soil can be reduced with soil solarization, where the soil is covered with plastic mulch and the ground below the plastic reaches temperatures high enough to kill the pathogen (Egel et al. 2008). Also use of cover crops such as *Vicia villosa* (hairy vetch) (Zhou and Everts 2004) and *Trifolium incarnatum* (crimson clover) (Himmelstein et al. 2014) has been studied. Himmelstein et al. (2014) demonstrated that the magnitude of the suppression was higher with *V. villosa* than with *T. incarnatum*. Further, intercropping watermelon with aerobically produced rice demonstrated a reduction in Fusarium

wilt (Ren et al. 2008). Another important management strategy is crop rotation and using fallow periods. Study of FON populations in fallow fields over time found that levels dropped by 20%, 40%, and 50%, respectively, after 1, 2 and 3 years of fallow, respectively (Wu et al. 2013). Due to the ability of FON to live in soil for a long period of time, the standard suggestion is that watermelons should be planted in the field only 5-7 years after the previous watermelon crop. However, these cultural management approaches are laborious, expensive, impractical in terms of time and resources, and may not sufficiently control FON.

Cultural control (RKN). At the farm level, transportation of nematodes to an non-infested field can be avoided by cleaning all agricultural machines and tools. Bridge (1996) advised uprooting plants after each harvest and exposing the roots to solar radiation to kill nematodes in root tissues. Removal of residual roots in the soil reduced *Meloidogyne* populations by 90% compared to leaving them in the soil; however, it is labor-intensive (Kenneth and Stephen 1998). Fallowing for 2 weeks under some conditions has provided equal or better control than rotation with a cover crop or non-host (Kinloch and Dunavin 1993). Additionally, flooding creates an anaerobic condition that kills RKN juveniles (Padgham et al. 2003). Flooding alternated with drying, 2-3 weeks cycles seem to be more effective than continuous flooding (Noling 1999). A temperature of around 45°C is considered lethal for plant-parasitic nematodes (Sikora et al. 2018). Soil temperature can be increased by soil solarization through mulching/tarping the soil surface with clear plastic to trap solar energy. Although this can control root-knot nematode damage effectively, the soil biota populations and their activities are negatively affected by repeated treatment (Scopa et al. 2008). Short cycle susceptible crops such as *Solanum nigrum* (black nightshade) and *Lactuca sativa* (lettuce) have been used as a trap crop for root-knot control in various parts of the world (Sikora et al. 2018). Similarly, in the southern

US, a number of non-host crops such as *Mucuna pruriens* (velvet bean), *Canavalia ensiformis* (horse bean) and *Aeschynomene americana* (joint vetch) have been successfully used for RKN control (McSorley et al. 1994a; McSorley et al. 1994b). *Tagetes erecta* (Mexican marigold), *T. signata* (signet marigold), *T. patula* (French marigold), *Sesasum indicum* (sesame), *Ricinus communis* (castor bean), *Crotalaria juncea* (sunn hemp), *Crotalaria longirostrata* (chipilin) have been studied to be effective antagonistic crops that suppress RKN (Sikora et al. 2018).

Biological control (FON). Fusarium wilt of watermelon can also be suppressed by employing biological control microorganisms. *Penicillium oxalicum* application to seeds and seedlings of watermelon reduced the incidence of FON (De Cal et al. 2009). *Paenibacillus polymyxa* and *T. harzianum* incorporated into bio-organic fertilizer reduced Fusarium wilt of watermelon by 59-73% in the field and 60-100% for pot experiments (Ling et al. 2011; Qiu et al. 2012). However, the commercial use of biological products remains low regardless of the evidence of some disease reduction (Zhou and Everts 2006).

Biological control (RKN). Biological methods employ bacterial or fungal antagonist agents that infect eggs, juveniles, or adults of RKN. *Paecilomyces, Pochonia* and *Verticilium* genera are nematode egg-parasitic fungi. *Paecilomyces lilacinus* has been proven to control successfully, *M. javanica* and *M. incognita* on several vegetables (Collange et al. 2011). *Pochonia chlamydosporia* also parasitizes endo-parasitic nematode eggs but not juveniles; hence it might not reduce initial plant infections (Manzanilla-Lopez et al. 2013). Other beneficial fungi that have toxic effects on nematodes include *Aspergillus*, *Actyllina*, *Arthobotrys*, *Catenaria*, *Hirsute*, *Purpureocillium*, *Dactylellina*, and *Trichoderma* (Abd-Elgawad and Askary 2018; Timper 2014). Several *Aspergillus* species (*A. niger*, *A. fumigates*, *A. terreus*, and *A. allahabadii*) were highly toxic to *M. incognita* (Affokpon et al. 2011; Goswami and Tiwari 2007; Tripathi et

al. 2006). Also, *Trichoderma viride, T harzianum, T. lignorum, T. asperellum, T. brevicompactum* have been studied to control RKN in vegetable crops (Sikora et al. 2018). *Pasteuria, Pseudomonas, Burkholderia, Arthrobacter, Serratia, Achromobacter,* and *Rhizobium* in bacterial genera are known to have nematicidal property (Forghani and Hajihassani 2020). *Bacillus firmus* is a naturally occurring soil bacterium that has been developed commercially as BioNem ® and Flocter ® for suppression of RKN (Keren-Zur et al. 2000; Sikora et al. 2018). *Pasteuria penetran*s and *Pseudomonas fluorescens* are another two most studied antagonistic bacteria in vegetables (Stalin et al. 2007; Stirling 2014).

Grafting (FON). Formae speciales of *F. oxysporum* are host specific and hence, FON infection does not occur in the rootstock of other cucurbits. The grafting strategy started in Japan, where watermelon cuttings were grafted onto *Cucurbita moschata* rootstock. Interspecific hybrid squash (*Cucurbita maxima* Duch. Ex Lam. × *C. moschata* Duch. Ex Poir) and bottle gourd (*Lagenaria siceraria* (Molina) Standl.) are the most widely used rootstocks for FON management (Keinath and Hassell 2014a; King et al. 2008; Miguel et al. 2004). These rootstocks are resistant to both FON races 1 and 2 (Keinath and Hassell 2014a; Yetişir et al. 2003). However, this rootstock is vulnerable to attack by *M. incognita* ((Huitrón et al. 2007; Oda and Lee 2003) and some growers are reluctant to use this measure as grafted plants are considerably more expensive than non-grafted ones (Keinath and Hassell 2014b). A newly released rootstock, Carolina Strongback (CSB), which is resistant to FON (Wechter et al. 2016), has shown resistance to *Meloidogyne* spp. when compared with interspecific hybrid [*Cucurbita maxima* (Duchesne) × *C. moschata* (Duchesne)] rootstock 'Carnivor' that is susceptible to nematodes, used as control (Smith et al. 2019). Cultivar CSB is a cross of U.S. Vegetable Laboratory (USVL) 246 and USVL252, two citron selections with resistance to FON races 1 and

2 (Smith and Freeman 2017; Wechter et al. 2016). A rootstock that is resistant to both FON race 2 and RKN would offer similar benefits provided by interspecific hybrid squash rootstocks without the drawbacks of susceptibility to RKN. Keinath et al. (2019) demonstrated that the seedless triploid 'cv. Fascination' scion grafted on to the CSB rootstock displayed improved resistance to root-knot galling and Fusarium wilt incidence when compared to a traditional rootstock (bottle gourd or *C. moschata* × *C. maxima* hybrids) and non-grafted plants, respectively. Overall, the introduction of the CSB rootstock may enable growers to overcome not only FON, but also reduce nematode damage, which has been a significant limitation of traditional rootstocks in the southeastern United States.

Grafting (RKN). Germplasm lines derived from *Citrullus amarus* (*C. lanatus* var. *citroides*) accessions have performed well as rootstocks for grafted watermelon (Chomicki and Renner 2015). These accessions have shown resistance to FON as well as some RKN species in both greenhouse and field studies (Huitrón et al. 2007; Levi et al. 2017; Thies et al. 2015; Thies et al. 2010; Thies et al. 2016; Thies and Levi 2003, 2007). Additionally, *Cucumis pustulatus*, has shown potential as a rootstock for RKN management in watermelon (Liu et al. 2015). Further, an accession of *C. amarus* (BGV0005167) with resistance to *M. arenaria, M. incognita* and *M. javonica* without influencing watermelon fruit quality has been reported (García-Mendívil et al. 2019).

Chemical control (FON). Previously, management of Fusarium wilt has mainly relied on fumigation with methyl bromide (MBr). Following the Montreal protocol, which mandated the reduction of MBr use, efforts have been made to see alternatives to MeBr (Antoniou et al. 2014). Soil applied fungicides, Proline (a. i. prothioconazole: Bayer CropScience, Research Triangle Park, NC), Actigard (acibenzolar-S-methyl; Syngenta Crop Protection, Greensboro,

NC), and Topsin M (thiophanate-methyl; United Phosphorous, Inc, King of Prussia, PA) have been found to be effective against FON in controlled studies (Everts et al. 2014). However, of these, only Proline is labeled for Fusarium wilt management on watermelon.

Chemical control (RKN). Similar to FON, MBr was the primary method for controlling RKN in watermelon for decades, but since it has been phased out, alternative soil fumigants such as 1,3-D dichloropropene, methyl iodide, tetrathiocarbonate, metam sodium, dazomet and chloropicrin used for RKN management (Sikora et al. 2018). However, these fumigants are expensive, more challenging to apply (as they require specialized application equipment and buffer zones), are highly volatile, and have worker safety issues (Morris et al. 2015). Some of the available fumigants for *Meloidogyne* spp. management are Telone II (a.i. 1,3-D dichloropropene, Corteva Agriscience™, Agriculture Division of DowDuPont™, Indianapolis, IN), Vapam (metam sodium, Amvac Chemical Corporation, Los Angeles, CA), and chloropicrin (trichloronitromethane, TriEst Ag group, Inc., Greensville, NC) (Hajihassani 2018). Non-fumigant nematicides that are registered for use in watermelon include Nimitz (a.i. fluensulfone, Adama, Raleigh, NC), Vydate (oxamyl, Corteva Agriscience™, Agriculture Division of DowDuPont™, Indianapolis, IN), Velum Prime (fluopyram, Bayer CropScience, Research Triangle Park, NC), and Movento (spirotetramat, Bayer CropScience, Research Triangle Park, NC) (Hajihassani 2018).

As mentioned above, current disease management approaches available for FON and RKN do not adequately prevent yield losses in watermelon. Additionally, the challenge of management increases when both pathogens are present together. To avoid crop loss, a combination of various disease control strategies must be utilized. This thesis will address research to evaluate additional management strategies, including the use of micronutrients,

chemicals and fumigants against FON and RKN. This will help increase the options available to the producers and will help improve disease management in watermelon.

Justification

Mineral nutrition and its effect on plant diseases. Mineral nutrients are necessary for the growth and production of plants and microorganisms and are essential factors in biochemical pathways for the interaction of plant diseases (Agrios 2005; Datnoff et al. 2007). Plants uptake mineral nutrients for healthy growth. The primary macronutrients that are utilized in large quantities are nitrogen (N), phosphorus (P), and potassium (K); the secondary macronutrients that are taken up in moderate amounts are calcium (Ca), magnesium (Mg) and sulfur (S); and the micronutrients that are taken up in minute quantities are iron (Fe), manganese (Mn), boron (B), molybdenum (Mo), copper (Cu), zinc (Zn), chlorine (Cl), and nickel (Ni). The nutritional condition of the host plant influences interactions with pests and pathogens, either in a negative or positive manner. Mineral nutrients influence plant resistance mechanism by formation of mechanical barriers (fibers, silicon), primarily through the development of thicker walls and by synthesis of natural defense metabolites such as callose, glucosinolates, lignin, phenols, and phytoalexins against pathogens (Datnoff et al. 2007; Marschner 2012). Disease severity can be affected by all the essential nutrients (Huber and Graham 1999). However, there is no general rule, as a particular nutrient can reduce the severity of a disease, but it can also increase the severity of the incidence of other diseases or have an opposite effect in a different environment (Graham and Webb 1991; Huber 1980; Marschner 1995). In other words, the impact of a particular nutrient cannot be generalized for all plant-pest/pathogen systems, either globally or individually (Agrios 2005; Huber 1980).

Although plant disease resistance is genetically controlled (Agrios 2005), it is affected by the environment conditions and, in particular, by nutrient deficiencies and toxicities (Krauss 1999; Marschner 1995). Induced resistance (IR) has been proposed as one of the explanations for the interaction between specific nutrients and the increase and decrease in plant disease. Induced resistance is a plant-based defense system that is elicited by specific environmental stimuli, which enables them to resist biotic challenges (Van Loon et al. 1998). This defense system reacts with physiological changes to the external attack, triggered by the production of proteins and chemicals that activate the defense system of the plant. Researchers have found that interaction with pathogenic and non-pathogenic organisms can cause two primary defense mechanisms in plants: systemic acquired resistance (SAR) and induced systemic resistance (ISR) (Vallad and Goodman 2004; Yan et al. 2002). SAR is commonly activated by local pathogen attacks, which correlate with the salicylic acid (SA) involvement and PR genes activation (Vallad and Goodman 2004). ISR is triggered when the plant interacts with plant growth-promoting rhizobacteria (PGPR) or non-pathogenic rhizosphere fungi, which activate the jasmonic acid (JA)/ethylene (ET) pathway (Shoresh et al. 2010).

The involvement of mineral nutrients in inducing resistance has been studied in several plant-pathogen systems. Foliar sprays of P have shown to confer local and systemic protection against foliar diseases such as powdery mildew in cucumber, mango, grape, apple wheat, and peppers; rust on maize and others (Reuveni et al. 1998). Many investigations have reported K salts as a chemical agent for the induction of plant resistance against powdery mildew diseases on cucumber (Reuveni et al. 2000), pepper (Reuveni et al. 1998), tomato (Ehret et al. 2002) and sugar beet (Mosa 2002). Liljeroth et al. (2016) observed that potassium phosphite (K_3O_3P) when used in combination with fungicides, increased the efficacy of fungicides against late blight

disease of potato. High N supply decreases the severity of infection caused by *Fusarium oxysporum* (Woltz and Engelhard 1973), *Alternaria solani* (Blachinski et al. 1996), and *Xanthomonas* sp. (Chase 1989). Combined treatment of $CaCl_2$ and SA improved the self-defense capability of tomato against *Botrytis Cinerea* (Li et al. 2016). Some studies revealed that the application of Ca at the post-harvest stage was successful in controlling fungal infections; *Colletotrichum gloeosporiodes* disease on papaya (Ayón-Reyna et al. 2017; Madani et al. 2014) and dragon fruits (Awang et al. 2011; Ghani et al. 2011). Similarly, the effect of Mg has been investigated in some studies to reduce disease severity in crops such as rice, wheat, citrus, potato, poppy and peanut (Jones and Huber 2007; Moreira et al. 2015). S fertilizer application reduced the incidence and severity of several fungal diseases, with the reduction of disease attributed to the induction of sulfur-induced resistance (Haneklaus et al. 2007). Several studies have reported on the role of silicon (Si) in plant-microbe interactions and enhanced host resistance to a variety of microbial pathogens through inducing defensive reactions (Cai et al. 2008; Ghareeb et al. 2011; Rémus-Borel et al. 2005; Ye et al. 2013).

These influences of nutrients to increase plant resistance against diseases are, however, nonspecific; examples can also be found for the increase in disease incidence and severity. N treatments have been linked with the rise in diseases caused by *Puccinia graminis, Blumeria graminis, Tobacco mosaic virus, Pseudomonas syringae and Didymella bryoniae* (Büschbell and Hoffmann 1992; Hoffland et al. 2000; Howard et al. 1994; Singh 1970). P tends to increase the susceptibility of plants to viruses in general (Prabhu et al. 2007). K application has been shown to increase downy mildew in muskmelon, caused by *Pseudoperonospora cubensis* , and microdochium patch caused by *Microdochium nivale* in creeping bentgrass (Bains and Jhooty 1976; Bier et al. 2018). Ca is used by the pathogen *Colletotrichum trifolli* and assists in tissue

maceration and increased disease severity (Kiraly 1976). Similarly, Mg is also associated with an increase in the bacterial spot of pepper and tomato caused by *Xanthomonas euvesicatoria* and peanut pod rot caused by *Fusarium*, *Pythium*, and *Rhizoctonia* spp., (Csinos and Bell 1989; Halleck and Garren 1968; Woltz and Jones 1979).

Micronutrients are equally essential in controlling the plant diseases, as they are also known to reduce disease severity by inducing the resistance within the plant (Dordas 2008). Micronutrient cofactors are essential to activate enzymes that generate secondary inhibitory metabolites against crop diseases (Servin et al. 2015). For instance, Mn, Fe, and Zn enhance disease resistance by activating the host defensive enzymes phenylalanine ammonia lyase and polyphenol oxidases (Dordas 2008; Duffy 2007; Evans et al. 2007; Fones and Preston 2013; Huber 2007; Römheld and Marschner 1991). In this thesis, one of our objectives is to examine the effect of different level of these micronutrients on plant induced resistance against FON and RKN in watermelon.

Fumigants. Soil fumigation is a process through which liquid is injected into the soil to provide protection against pest. When the liquid is injected, it volatilizes and spreads quickly as a gas. Currently available MeBr alternatives, 1,3-dichlorooropropene, chloropicrin, and metam sodium are not as effective as MeBr against FON (Egel and Martyn 2013; Everts and Himmelstein 2015). As mentioned earlier, Proline, a demethylation inhibiting fungicide [DMI; Fungicide Resistance Committee (FRAC) code 3], is the only fungicide labeled for use on watermelon to manage FON (Miller et al. 2020). A new succinate dehydrogenase inhibitor fungicide (SDHI; FRAC code 7), pydiflumetofen (Miravis, Syngenta Inc.), has recently been developed and is likely to be labeled for use on watermelon to manage Fusarium wilt (Rapicavoli et al. 2018). The efficacy of these two chemicals was studied by Miller et al. (2020) against FON

in North Carolina. Their study demonstrated Miravis as an effective management option for Fusarium wilt of watermelon as all FON isolates from the state screened sensitive to the fungicide. Another study conducted in Georgia also revealed that all FON isolates tested to be sensitive to Proline (Petkar et al. 2017). However, with limited options available, fungicide resistance might become a significant concern for watermelon growers, and current chemical management practices are not effective in heavily infested fields. Hence, one of the objectives of this study is to examine the interaction between fumigant and fungicides in an effort to develop options for better management of FON in watermelon.

Objectives

The overall goal of this thesis was to evaluate the disease management options against *Fusarium oxysporum* f. sp. *niveum* and *Meloidogyne incognita* in watermelon. Specific objectives are as follows:

1. Determine the role of micronutrients on induced resistance in watermelon against *Fusarium oxysporum* f. sp. *niveum* and *Meloidogyne incognita* infection under hydroponics conditions.
2. Evaluate if micronutrient affects transcript-level changes in watermelon in relation to *Fusarium oxysporum* f. sp. *niveum* and *Meloidogyne incognita* infection under hydroponics conditions.
3. Field evaluation of the efficacy of Pic-clor-60 [choloropicrin pre-mixed with 1,3 dicholoropropene (Telone II)] and soil-applied fungicides against Fusarium wilt severity in watermelon.

Literature cited

Abd-Elgawad, M. M., and Askary, T. H. 2018. Fungal and bacterial nematicides in integrated nematode management strategies. Egypt. J. Biol. Pest Control 28:74.

Affokpon, A., Coyne, D. L., Htay, C. C., Agbèdè, R. D., Lawouin, L., and Coosemans, J. 2011. Biocontrol potential of native *Trichoderma* isolates against root-knot nematodes in West African vegetable production systems. Soil Biol. Biochem. 43:600-608.

AgMRC. 2018. Watermelon. Available at https://www.agmrc.org/commodities-products/vegetables/watermelon. Accessed 2020-03-24.

Agrios, G. N. 2005. Plant Pathology. 5th ed. Elsevier Academic Press, San Diego.

Amaradasa, B., Beckham, K., Dufault, N., Sanchez, T., Ertek, T., Iriarte, F., Paret, M., and Ji, P. 2018. First report of *Fusarium oxysporum* f. sp. *niveum* race 3 causing wilt of watermelon in Florida, USA. Plant Dis. 102:1029-1029.

Andrus, C. F., Seshadri, V., and Grimball, P. C. 1971. Production of seedless watermelons. Agric. Res. Service, US Dept. of Agri. Technical Bull. No. 1425.

Antoniou, P., Tjamos, E., and Giannakou, J. 2014. Low-cost and effective approaches of soil disinfestation of plastic house or open field crops in Greece. Proc. Acta Hort. 1044:29-41.

Awang, Y., Azlan, M., Ghani, A., Sijam, K., and Mohamad, R. 2011. Effect of calcium chloride on anthracnose disease and postharvest quality of red-flesh dragon fruit (*Hylocereus polyrhizus*). Afr. J. Microbiol. Res 5:5250-5259.

Ayón-Reyna, L. E., González-Robles, A., Rendón-Maldonado, J. G., Báez-Flores, M. E., López-López, M. E., and Vega-García, M. O. 2017. Application of a hydrothermal-calcium

chloride treatment to inhibit postharvest anthracnose development in papaya. Postharvest Biol. Technol. 124:85-90.

Bains, S., and Jhooty, J. 1976. Effect of mineral nutrition of muskmelon on downy mildew caused by *Pseudoperonospora cubensis*. Plant Soil 29:105.

Berkeley, M. 1855. Vibrio forming cysts on the roots of cucumbers. Gardener's Chron. Agric. Gaz. 14:220.

Bier, P. V., Persche, M., Koch, P., and Soldat, D. J. 2018. A long term evaluation of differential potassium fertilization of a creeping bentgrass putting green. Plant Soil 431:303-316.

Blachinski, D., Shtienberg, D., Dinoor, A., Kafkafi, U., Sujkowski, L., Zitter, T., and Fry, W. 1996. Influence of foliar application of nitrogen and potassium on *Alternaria* diseases in potato, tomato and cotton. Phytoparasitica 24:281-292.

Boyhan, G., Langston, D., Granberry, D., Lewis, P., and Linton, D. 2003. Resistance to Fusarium wilt and root-knot nematode in watermelon germplasm. Cucurbit Genet. Coop. Rpt. 26:18-25.

Bridge, J. 1996. Nematode management in sustainable and subsistence agriculture. Annu. Rev. Phytopathol. 34:201-225.

Bruton, B. 1998. Soilborne diseases in cucurbitaceae: pathogen virulence and host resistance. Pages 143-166 in: Cucurbitaceae'98. J. McCreight, ed. Am. Soc. Hortic. Sci., Alexandria, VA.

Büschbell, T., and Hoffmann, G. 1992. The effects of different nitrogen regimes on the epidemiological development of pathogens on winter wheat and their control. J. Plant Dis. Prot. 99:381-403.

Butterwick, M. 2018. Cucurbitaceae gourd family. Available at https://canotia.org/volumes/Canotia12-Cucurbitaceae-Butterwick-8-13-16.pdf. Accessed 2020-03-24.

Cai, K., Gao, D., Luo, S., Zeng, R., Yang, J., and Zhu, X. 2008. Physiological and cytological mechanisms of silicon-induced resistance in rice against blast disease. Physiol. Plant. 134:324-333.

Chase, A. 1989. Effect of nitrogen and potassium fertilizer rates in severity of *Xanthomonas blight* of *Syngonium podophyllum*. Plant Dis. 73:972-975.

Chitwood, B. 1949. 'Root-knot nematodes'. Part 1. A revision of the genus *Meloidogyne Goeldi*, 1887. Proc. Helminth. Soc. Wash. 16:90-114.

Chomicki, G., and Renner, S. S. 2015. Watermelon origin solved with molecular phylogenetics including Linnaean material: another example of museomics. New Phytol. 205:526-532.

Collange, B., Navarrete, M., Peyre, G., Mateille, T., and Tchamitchian, M. 2011. Root-knot nematode (*Meloidogyne*) management in vegetable crop production: The challenge of an agronomic system analysis. Crop Prot. 30:1251-1262.

Csinos, A., and Bell, D. 1989. Pathology and nutrition in the peanut pod rot complex. Pages 124-136 in: Soilborne plant pathogens: management of diseases with macro- and microelements. A. W. Engelhard, ed., APS, St. Paul, MN.

Curtis, R. H., Robinson, A. F., and Perry, R. N. 2009. Hatch and host location. Pages 139-162 in: Root-knot nematodes. R. N. Perry, M. Moens and J. L. Starr, eds., CAB International, Wallingford, UK and Cambridge, USA.

Datnoff, L. E., Elmer, W. H., and Huber, D. M. 2007. Mineral nutrition and plant disease. APS, St. Paul, MN.

Davis, R. F. 2007. Effect of *Meloidogyne incognita* on watermelon yield. Nematropica 37:287-294.

De Cal, A., Sztejnberg, A., Sabuquillo, P., and Melgarejo, P. 2009. Management Fusarium wilt on melon and watermelon by *Penicillium oxalicum*. Biol. Control 51:480-486.

Dordas, C. 2008. Role of nutrients in controlling plant diseases in sustainable agriculture. A review. Agron. Sustain. Dev. 28:33-46.

Duffy, B. 2007. Zinc and plant disease. Pages 155-176 in: Mineral nutrition and plant disease. L. E. Datnoff, W. H. Elmer and D. M. Huber, eds. APS, St. Paul, MN. .

Egel, D., and Martyn, R. 2007. Fusarium wilt of watermelon and other cucurbits. Online publication. The Plant Health Instructor. doi:10.1094/PHI-I2007-0122-01.

Egel, D., and Martyn, R. 2013. Fusarium wilt of watermelon and other cucurbits. Online publication. Plant Health Instruct. doi: 10.1094/PHI-I-2007-0122-01.

Egel, D. S., Martyn, R., and Gunter, C. 2008. Planting method, plastic mulch, and fumigation influence growth, yield, and root structure of watermelon. HortScience 43:1410-1414.

Ehret, D., Utkhede, R., Frey, B., Menzies, J., and Bogdanoff, C. 2002. Foliar applications of fertilizer salts inhibit powdery mildew on tomato. Can. J. Plant Pathol., 24:437-444.

Evans, I., Solberg, E., and Huber, D. 2007. Copper and plant disease. in: Mineral nutrition and plant disease, vol. 177. L. E. Datnoff, W. H. Elmer and D. M. Huber, eds. APS, St. Paul, MN.

Everts, K., and Hochmuth, M. 2011. Field evaluation of triploid cultivars for resistance to Fusarium wilt of watermelon in Delaware, 2010. Plant Dis. Manage. Rep 5:V175.

Everts, K. L., and Himmelstein, J. C. 2015. Fusarium wilt of watermelon: Towards sustainable management of a re-emerging plant disease. Crop Prot. 73:93-99.

Everts, K. L., Egel, D. S., Langston, D., and Zhou, X.-G. 2014. Chemical management of Fusarium wilt of watermelon. Crop Prot. 66:114-119.

FAO. 2018. Countries by Commodities –Top Yield – Watermelons. Available at http://www.fao.org/faostat/en/#data/QC Accessed 2020-04-14.

Fones, H., and Preston, G. M. 2013. The impact of transition metals on bacterial plant disease. FEMS Microbiol. Rev. 37:495-519.

Forghani, F., and Hajihassani, A. 2020. Recent advances in the development of environmentally benign treatments to control root-knot nematodes. Front. Plant Sci. 11.

García-Mendívil, H. A., Munera, M., Giné, A., Escudero, N., Picó, M. B., Gisbert, C., and Sorribas, F. J. 2019. Response of two *Citrullus amarus* accessions to isolates of three species of *Meloidogyne* and their graft compatibility with watermelon. Crop Prot. 119:208-213.

Ghani, M. A. A., Awang, Y., and Sijam, K. 2011. Disease occurrence and fruit quality of pre-harvest calcium treated red flesh dragon fruit (*Hylocereus polyrhizus*). Afric. J. Biotech. 10:1550-1558.

Ghareeb, H., Bozsó, Z., Ott, P. G., Repenning, C., Stahl, F., and Wydra, K. 2011. Transcriptome of silicon-induced resistance against *Ralstonia solanacearum* in the silicon non-accumulator tomato implicates priming effect. Physiol. Mol. Plant Pathol. 75:83-89.

Goswami, J., and Tiwari, D. 2007. Management of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *lycopersici* Disease Complex on Tomato by *Trichoderma harzianum*, *Tinopsora longifolia* and *Glomus fasciculatum*. Pestic. Res. J. 19:51-55.

Graham, R. D., and Webb, M. J. 1991. Micronutrients and disease resistance and tolerance in plants. Pages 329-370 in: Micronutrients in agriculture. J. J. Mortvedt, F. R. Cox, L. M.

Shuman and R. M. Welch, eds. Soil Science Society of America Inc, Madison, Wisconsin.

Guner, N., and Wehner, T. C. 2004. The genes of watermelon. HortScience 39:1175-1182.

Hajihassani, A. 2018. Chemical nematicides for control of plant-parasitic nematodes in Georgia vegetable crops. UGA Extension. Available at https://secure.caes.uga.edu/extension/publications/files/pdf/B%201502_1.PDF Accessed 2020-11-15.

Hajihassani, A., Forghani, F., and Marquez, J. 2020. Managing root-knot nematodes in Georgia watermelons. UGA Extension. Available at https://extension.uga.edu/publications/detail.html?number=B1529 Accessed 2020-04-14.

Halleck, D. L., and Garren, K. H. 1968. Pod breakdown, yield, and grade of Virginia type peanuts as affected by Ca, Mg, and K sulfates. Agron. J. 60:253-257.

Haneklaus, S., Bloem, E., and Schnug, E. 2007. Sulfur and Plant Disease. Pages 101–118 in: Mineral Nutrition and Plant Disease. L. E. Datnoff, W. H. Elmer and D. M. Huber, eds. APS, St. Paul, MN.

Himmelstein, J., Maul, J., and Everts, K. 2014. Impact of five cover crop green manures and Actinovate on Fusarium wilt of watermelon. Plant Dis. 98:965-972.

Hoffland, E., Jeger, M. J., and van Beusichem, M. L. 2000. Effect of nitrogen supply rate on disease resistance in tomato depends on the pathogen. Plant Soil 218:239-247.

Howard, D., Chambers, A., and Logan, J. 1994. Nitrogen and fungicide effects on yield components and disease severity in wheat. J. Prod. Agric. 7:448-454.

Hua, G. K. H., Timper, P., and Ji, P. 2019. *Meloidogyne incognita* intensifies the severity of Fusarium wilt on watermelon caused by *Fusarium oxysporum* f. sp. *niveum*. Can. J. Plant Pathol. 41:261-269.

Huber, D. 1980. The role of mineral nutrition in defense. Pages 381-406 in: Plant disease: an advanced treatise: how plants defend themselves. J. G. Horsfall and E. B. Cowling, eds., Academic Press, New York.

Huber, D. M. 2007. Nitrogen and plant disease. Pages 31-44 in: Mineral nutrition and plant disease. L. E. Datnoff, W. H. Elmer and D. M. Huber, eds. APS, St. Paul, MN. .

Huber, D. M., and Graham, R. D. 1999. The Role of Nutrition in Crop Resistance. Page 169 in: Mineral nutrition of crops: fundamental mechanisms and implications, vol. 18. Z. Rengel, ed., Food Products Press, New York.

Huitrón, M. V., Diaz, M., Diánez, F., and Camacho, F. 2007. The effect of various rootstocks on triploid watermelon yield and quality. J. Food Agric. Environ. 5:344.

Idnurm, A., and Howlett, B. J. 2001. Pathogenicity genes of phytopathogenic fungi. Mol. Plant Pathol. 2:241-255.

Inoue, I., Namiki, F., and Tsuge, T. 2002. Plant colonization by the vascular wilt fungus *Fusarium oxysporum* requires FOW1, a gene encoding a mitochondrial protein. Plant Cell 14:1869-1883.

Jones, J. B., and Huber, D. M. 2007. Magnesium and plant disease. in: Mineral nutrition and plant disease. L. E. Datnoff, W. H. Elmer and D. M. Huber, eds. APS, St. Paul, MN.

Jones, J. T., Haegeman, A., Danchin, E. G., Gaur, H. S., Helder, J., Jones, M. G., Kikuchi, T., Manzanilla-López, R., Palomares-Rius, J. E., and Wesemael, W. M. 2013. Top 10 plant-parasitic nematodes in molecular plant pathology. Mol. Plant Pathol. 14:946-961.

Jones, M. G., and Goto, D. B. 2011. Root-knot nematodes and giant cells. Pages 83-100 in: Genomics and molecular genetics of plant-nematode interactions. J. Jones, G. Gheysen and Fenoll C, eds. Springer, Berlin.

Karssen, G., and Moens, M. 2006. Root-knot Nematodes, 59-90. Page 447 in: Plant Nematology R. N. Perry and M. Moens, eds., CAB International, Wallingford, UK.

Keinath, A., and Hassell, R. 2014a. Control of Fusarium wilt of watermelon by grafting onto bottlegourd or interspecific hybrid squash despite colonization of rootstocks by *Fusarium*. Plant Dis. 98:255-266.

Keinath, A. P., and Hassell, R. L. 2014b. Suppression of Fusarium wilt caused by *Fusarium oxysporum* f. sp. *niveum* race 2 on grafted triploid watermelon. Plant Dis. 98:1326-1332.

Keinath, A. P., and Agudelo, P. A. 2018. Retention of resistance to *Fusarium oxysporum* f. sp. *niveum* in cucurbit rootstocks infected by *Meloidogyne incognita*. Plant Dis. 102:1820-1827.

Keinath, A. P., Wechter, W. P., Rutter, W. B., and Agudelo, P. A. 2019. Cucurbit rootstocks resistant to *Fusarium oxysporum* f. sp. *niveum* remain resistant when coinfected by *Meloidogyne incognita* in the field. Plant Dis. 103:1383-1390.

Kenneth, R. B., and Stephen, R. K. 1998. Developing sustainable systems for nematode management. Annu. Rev. Phytopathol. 36:165-205.

Keren-Zur, M., Antonov, J., Bercovitz, A., Feldman, K., Husid, A., Kenan, G., Marcov, N., and Rebhun, M. 2000. *Bacillus firmus* formulations for the safe control of root-knot nematodes. Pages 47-52 in: Proc. of the Brighton Crop Prot. Conf. on Pests and Dis. Vol. 2A.

King, S. R., Davis, A. R., Liu, W., and Levi, A. 2008. Grafting for disease resistance. HortScience 43:1673-1676.

Kinloch, R., and Dunavin, L. 1993. Summer cropping effects on the abundance of *Meloidogyne arenaria* race 2 and subsequent soybean yield. J. Nematol. 25:806.

Kiraly, Z. 1976. Plant disease resistance as influenced by biochemical effects of nutrients in fertilizer. Pages 33-46 in: Fertilizer use and Plant Health. International Potash Institute, Atlanta, GA.

Kleczewski, N. M., and Egel, D. S. 2011. A diagnostic guide for Fusarium wilt of watermelon Online Plant Health Prog.. http://dx.doi.org/10.1094/PHP-2011-1129-01-DG.

Krauss, A. 1999. Balanced nutrition and biotic stress. in: IFA Agricultural conference on managing plant nutrition, 29 June-2 July, Barcelona, Spain.

Kyriacou, M. C., Leskovar, D. I., Colla, G., and Rouphael, Y. 2018. Watermelon and melon fruit quality: The genotypic and agro-environmental factors implicated. Sci. Hortic. 234:393-408.

Levi, A., Jarret, R., Kousik, S., Wechter, W. P., Nimmakayala, P., and Reddy, U. K. 2017. Genetic resources of watermelon. Pages 87-110 in: Genetics and Genomics of Cucurbitaceae. R. Grumet, N. Katzir and J. Garcia-Mas, eds. Springer, Switzerland.

Li, L., Guo, P., Jin, H., and Li, T. 2016. Different proteomics of Ca2+ on SA-induced resistance to *Botrytis cinerea* in tomato. Hortic. Plant J. 2:154-162.

Liljeroth, E., Lankinen, Å., Wiik, L., Burra, D. D., Alexandersson, E., and Andreasson, E. 2016. Potassium phosphite combined with reduced doses of fungicides provides efficient protection against potato late blight in large-scale field trials. Crop Prot. 86:42-55.

Lin, Y. H., Chen, K. S., Chang, J. Y., Wan, Y. L., Hsu, C. C., Huang, J. W., and Chang, P. F. L. 2010. Development of the molecular methods for rapid detection and differentiation of *Fusarium oxysporum* and *F. oxysporum* f. sp. *niveum* in Taiwan. New biotechnol. 27:409-418.

Ling, N., Huang, Q., Guo, S., and Shen, Q. 2011. *Paenibacillus polymyxa* SQR-21 systemically affects root exudates of watermelon to decrease the conidial germination of *Fusarium oxysporum* f. sp. *niveum*. Plant Soil 341:485-493.

Liu, B., Ren, J., Zhang, Y., An, J., Chen, M., Chen, H., Xu, C., and Ren, H. 2015. A new grafted rootstock against root-knot nematode for cucumber, melon, and watermelon. Agron. Sustain. Dev. 35:251-259.

Lynch, L., and Carpenter, J. 1999. The economic impacts of banning methyl bromide: Where do we need more research. in: 1999 Annual Research Conference on Methyl Bromide Alternatives and Emissions Reductions. http://mbao.org/mbrpro99.html.

Madani, B., Mohamed, M. T. M., Biggs, A. R., Kadir, J., Awang, Y., Tayebimeigooni, A., and Shojaei, T. R. 2014. Effect of pre-harvest calcium chloride applications on fruit calcium level and post-harvest anthracnose disease of papaya. Crop Prot. 55:55-60.

Manzanilla-Lopez, R. H., Esteves, I., Finetti-Sialer, M. M., Hirsch, P. R., Ward, E., Devonshire, J., and Hidalgo-Díaz, L. 2013. *Pochonia chlamydosporia*: advances and challenges to improve its performance as a biological control agent of sedentary endo-parasitic nematodes. J. Nematol. 45:1.

Marschner, H. 1995. Mineral nutrition of higher plants. Academic Press, London.

Marschner, H. 2012. Marschner's mineral nutrition of higher plants. 3rd Ed. Academic Press, Cambridge.

Martyn, R. 1987. *Fusarium oxysporum* f. sp. *niveum* race 2, a new race of the watermelon wilt pathogen in the United States. Plant Dis. 71:233-236.

Martyn, R. 1996. Fusarium wilt of watermelon Pages 13-14 in: Compendium of cucurbit diseases. T. A. Zitter, D. L. Hopkins and C. E. Hopkins, eds. The American Phytopathological Society, St. Paul, MN.

Martyn, R., and Bruton, B. 1989. An initial survey of the United States for races of *Fusarium oxysporum* f. sp. *niveum* HortScience 24:696-698.

Martyn, R. D. 2014. Fusarium wilt of watermelon: 120 years of research. Hortic. Rev. 42:349-442.

McSorley, R., Dickson, D., and De Brito, J. 1994a. Host status of selected tropical rotation crops to four populations of root-knot nematodes.

McSorley, R., Dickson, D., De Brito, J., and Hochmuth, R. 1994b. Tropical rotation crops influence nematode densities and vegetable yields. J. Nematol. 26:308.

Miguel, A., Maroto, J., San Bautista, A., Baixauli, C., Cebolla, V., Pascual, B., Lopez, S., and Guardiola, J. 2004. The grafting of triploid watermelon is an advantageous alternative to soil fumigation by methyl bromide for control of Fusarium wilt. Scientia Horticulturae 103:9-17.

Miller, N. F., Standish, J. R., and Quesada-Ocampo, L. M. 2020. Sensitivity of *Fusarium oxysporum* f. sp. *niveum* to prothioconazole and pydiflumetofen In vitro and efficacy for Fusarium wilt management in watermelon. Plant Health Prog. 21:13-18.

Moens, M., Perry, R. N., and Starr, J. L. 2009. *Meloidogyne* species–a diverse group of novel and important plant parasites. Page 483 in: Root-knot nematodes, vol. 1. R. N. Perry, M. Moens and J. L. Starr, eds., CAB International, Wallingford, UK.

Montalvo, A. E., and Esnard, J. 1994. Reaction of ten cultivars of watermelon (*Citrullus lanatus*) to a Puerto Rican population of *Meloidogyne incognita*. J. Nematol. 26:640.

Moreira, W. R., Bispo, W. M. d. S., Rios, J. A., Debona, D., Nascimento, C. W. A., and Rodrigues, F. Á. 2015. Magnesium-induced alterations in the photosynthetic performance and resistance of rice plants infected with *Bipolaris oryzae*. Scientia Agricola 72:328-333.

Morris, K. A., Langston, D. B., Dickson, D. W., Davis, R. F., Timper, P., and Noe, J. P. 2015. Efficacy of fluensulfone in a tomato–cucumber double cropping system. J. Nematol. 47:310.

Mosa, A. 2002. Management of sugar beet powdery mildew by foliar spraying of potassium phosphate salts. Arab Univ. J. Agric. Sci., 10: 1043-1057. .

Noling, J. W. 1999. Nematode management in strawberries. ENY-031. IFAS extension, University of Florida, Gainesville, FL Available at http://edis.ifas.ufl.edu/pdffiles/NG/NG03100.pdf Accessed 2020-04-14.

Oda, J. L. M., and Lee, M. 2003. Grafting of herbaceous vegetable and ornamental crops. Hort. Rev. (Amer. Soc. Hort. Sci.) 28:61-124.

Padgham, J., Abawi, G., and Duxbury, J. 2003. Survival and infectivity of *Meloidogyne graminicola* in flooded and non-flooded soils. Nematol. Medit. 31:225-230.

Palomares-Rius, J. E., Escobar, C., Cabrera, J., Vovlas, A., and Castillo, P. 2017. Anatomical alterations in plant tissues induced by plant-parasitic nematodes. Front. Plant Sci. 8:1987.

Perry, R. N., Moens, M., and Starr, J. L. 2009. Root-knot nematodes. CAB International, Wallingford, UK.

Petkar, A., and Ji, P. 2017. Infection courts in watermelon plants leading to seed infestation by *Fusarium oxysporum* f. sp. *niveum*. Phytopathology 107:828-833.

Petkar, A., Langston, D. B., Buck, J. W., Stevenson, K. L., and Ji, P. 2017. Sensitivity of *Fusarium oxysporum* f. sp. *niveum* to prothioconazole and thiophanate-methyl and gene mutation conferring resistance to thiophanate-methyl. Plant Dis. 101:366-371.

Petkar, A., Harris-Shultz, K., Wang, H., Brewer, M. T., Sumabat, L., and Ji, P. 2019. Genetic and phenotypic diversity of *Fusarium oxysporum* f. sp. *niveum* populations from watermelon in the southeastern United States. PLoS One 14.

Prabhu, A., Fageria, N., Berni, R., and Rodrigues, F. 2007. Phosphorous and plant disease. Pages 45-55 in: Mineral nutrition and plant disease. L. E. Datnoff, W. H. Elmer and D. M. Huber, eds. American Phytopathological Society (APS Press).

Qiu, M., Zhang, R., Xue, C., Zhang, S., Li, S., Zhang, N., and Shen, Q. 2012. Application of bio-organic fertilizer can control Fusarium wilt of cucumber plants by regulating microbial community of rhizosphere soil. Biol. Fertility Soils 48:807-816.

Rapicavoli, J., Whitten Buxton, K. R., and Hadden, J. F. 2018. Miravis®: A new fungicide for control of Fusarium wilt in cucurbits. Phytopathology 108:S1.152 (Abstract).

Rémus-Borel, W., Menzies, J. G., and Bélanger, R. R. 2005. Silicon induces antifungal compounds in powdery mildew-infected wheat. Physiol. Mol. Plant Pathol. 66:108-115.

Ren, L., Su, S., Yang, X., Xu, Y., Huang, Q., and Shen, Q. 2008. Intercropping with aerobic rice suppressed Fusarium wilt in watermelon. Soil Biol. Biochem. 40:834-844.

Reuveni, M., Oppenheim, D., and Reuveni, R. 1998. Integrated control of powdery mildew on apple trees by foliar sprays of mono-potassium phosphate fertilizer and sterol inhibiting fungicides. Crop Prot. 17:563-568.

Reuveni, R., Dor, G., Raviv, M., Reuveni, M., and Tuzun, S. 2000. Systemic resistance against *Sphaerotheca fuliginea* in cucumber plants exposed to phosphate in hydroponics system, and its control by foliar spray of mono-potassium phosphate. Crop Prot. 19:355-361.

Römheld, V., and Marschner, H. 1991. Function of micronutrients in plants. Pages 297-328 in: Micronutrients in agriculture, vol. 4. J. J. Mortvedt, ed. Soil Sci. Soc. Amer. Inc. Madison Wisconsin, USA.

Rubatzky, V. E., and Yamaguchi, M. 1997. Cucumber, melons, watermelons, squash, and other cucurbits. Pages 577-639 in: World vegetables. principles, production and nutritive values. 2nd Ed. Chapman & Hall.

Schaffer, A. A., and Paris, H. S. 2003. Melons, squashes, and gourds. Pages 3817-3826 in: Encyclopedia of Food Sciences and Nutrition, 2nd Ed. B. Caballero, ed. Academic Press, Oxford.

Scopa, A., Candido, V., Dumontet, S., and Miccolis, V. 2008. Greenhouse solarization: effects on soil microbiological parameters and agronomic aspects. Sci. Hortic 116:98-103.

Seo, Y., and Kim, Y. H. 2017. Pathological interrelations of soil-borne diseases in cucurbits caused by *Fusarium* species and *Meloidogyne incognita*. Plant Pathol. J. 33:410.

Servin, A., Elmer, W., Mukherjee, A., De la Torre-Roche, R., Hamdi, H., White, J. C., Bindraban, P., and Dimkpa, C. 2015. A review of the use of engineered nanomaterials to suppress plant disease and enhance crop yield. J. Nanopart. Res. 17:92.

Shoresh, M., Harman, G. E., and Mastouri, F. 2010. Induced systemic resistance and plant responses to fungal biocontrol agents. Annu. Rev. Phytopathol. 48:21-43.

Sikora, R. A., Coyne, D., Hallmann, J., and Timper, P. 2018. Plant parasitic nematodes in subtropical and tropical agriculture. CABI, New York.

Singh, R. 1970. Influence of nitrogen supply on host susceptibility to Tobacco mosaic virus infection. Phyton. Horn 14:37-39.

Smith, C., and Freeman, J. 2017. Assessment of watermelon rootstock resistance to *Fusarium oxysporum* f. sp. *niveum* when subjected to *Meloidogyne incognita*. HortScience 52:S73.

Smith, C. L., Freeman, J. H., Kokalis-Burelle, N., and Wechter, W. P. 2019. Screening Cucurbit Rootstocks for Resistance to Meloidogyne spp. and Rotylenchulus reniformis. HortScience 54:125-128.

Smith, E. F. 1894. The watermelon disease of the South. Pages 289-290 in: Proc. Am. Assoc. Adv. Sci.

Stalin, C., Ramakrishnan, S., and Jonathan, E. 2007. Management of root knot nematode *Meloidogyne incognita* in bhumyamalaki (*Phyllanthus amarus*) and makoy (*Solanum nigrum*). Biomed 2:119-122.

Stirling, G. R. 2014. Biological control of plant-parasitic nematodes: soil ecosystem management in sustainable agriculture. CABI, Wallingford.

Sumner, D. R., and Johnson, A. W. 1973. Effect of root-knot nematodes on Fusarium wilt of watermelon. Phytopathology 63:857-861.

Sun, Y., Wang, M., Li, Y., Gu, Z., Ling, N., Shen, Q., and Guo, S. 2017. Wilted cucumber plants infected by *Fusarium oxysporum* f. sp. *cucumerinum* do not suffer from water shortage. Ann. Bot. 120:427-436.

Thies, J. A., and Levi, A. 2003. Resistance of watermelon germplasm to the peanut root-knot nematode. HortScience 38:1417-1421.

Thies, J. A., and Levi, A. 2007. Characterization of watermelon (*Citrullus lanatus var. citroides*) germplasm for resistance to root-knot nematodes. HortScience 42:1530-1533.

Thies, J. A., Ariss, J. J., Hassell, R. L., Buckner, S., and Levi, A. 2015. Accessions of *Citrullus lanatus* var. *citroides* are valuable rootstocks for grafted watermelon in fields infested with root-knot nematodes. HortScience 50:4-8.

Thies, J. A., Ariss, J. J., Kousik, C. S., Hassell, R. L., and Levi, A. 2016. Resistance to southern root-knot nematode (*Meloidogyne incognita*) in wild watermelon (*Citrullus lanatus var. citroides*). J. Nematol. 48:14.

Thies, J. A., Ariss, J. J., Hassell, R. L., Olson, S., Kousik, C. S., and Levi, A. 2010. Grafting for management of southern root-knot nematode, *Meloidogyne incognita*, in watermelon. Plant Dis. 94:1195-1199.

Timper, P. 2014. Conserving and enhancing biological control of nematodes. J. Nematol. 46:75.

Tripathi, P. K., Singh, C., Prasad, D., and Singh, O. 2006. Use of fungal bio-agents for the management of *Meloidogyne incognita* infecting tomato. Ann. of Plant Prote. Sci. 14:194-196.

USDA-NASS. 2019. Quick Stats. USDA National Agricultural Statistics Service. Available at https://data.nal.usda.gov/dataset/nass-quick-stats. Accessed 2020-03-24.

Vallad, G. E., and Goodman, R. M. 2004. Systemic acquired resistance and induced systemic resistance in conventional agriculture. Crop Sci. 44:1920-1934.

Van Loon, L., Bakker, P., and Pieterse, C. 1998. Systemic resistance induced by rhizosphere bacteria. Annu. Rev. Phytopathol. 36:453-483.

Wechter, W. P., McMillan, M. M., Farnham, M. W., and Levi, A. 2016. Watermelon germplasm lines USVL246-FR2 and USVL252-FR2 tolerant to Fusarium oxysporum f. sp. niveum race 2. HortScience 51:1065-1067.

Wehner, T. C. 2008. Watermelon. Pages 381-418 in: Vegetables I. F. Nuez, ed., Springer, Berlin.

Winstead, N., and Riggs, R. 1959. Reaction of watermelon varieties to root-knot nematodes. Plant Dis. Rep. 43:909-912.

Woltz, S., and Engelhard, A. 1973. Fusarium wilt of chrysanthemum: effect of nitrogen source and lime on disease development. Phytopathology 63:155-157.

Woltz, S., and Jones, J. P. 1979. Effects of magnesium on bacterial spot of pepper and tomato and on the *in vitro* inhibition of *Xanthomonas vesicatoria* by streptomycin. Plant Dis.Rep. 63:182-184.

Wu, H. S., Gao, Z. Q., Zhou, X. D., Shi, X., Wang, M. Y., Shang, X. X., Liu, Y. D., Gu, D. L., and Wang, W. Z. 2013. Microbial dynamics and natural remediation patterns of Fusarium-infested watermelon soil under 3-yr of continuous fallow condition. Soil Use Manage 29:220-229.

Yan, Z., Reddy, M., Ryu, C.-M., McInroy, J. A., Wilson, M., and Kloepper, J. W. 2002. Induced systemic protection against tomato late blight elicited by plant growth-promoting rhizobacteria. Phytopathology 92:1329-1333.

Ye, M., Song, Y., Long, J., Wang, R., Baerson, S. R., Pan, Z., Zhu-Salzman, K., Xie, J., Cai, K., and Luo, S. 2013. Priming of jasmonate-mediated antiherbivore defense responses in rice by silicon. P. Natl. Acad. Sci. USA 110(38):E3631–E3639.

Yetişir, H., Sari, N., and Yücel, S. 2003. Rootstock resistance to Fusarium wilt and effect on fruit yield and quality of watermelon. Phytoparasitica 31:163-169.

Zhang, M., Xu, J., Liu, G., Yao, X., Li, P., and Yang, X. 2015. Characterization of the watermelon seedling infection process by *Fusarium oxysporum* f. sp. *niveum*. Plant Pathol. 64:1076-1084.

Zhou, X., and Everts, K. 2004. Suppression of Fusarium wilt of watermelon by soil amendment with hairy vetch. Plant Dis. 88:1357-1365.

Zhou, X., and Everts, K. 2006. Suppression of Fusarium wilt of watermelon enhanced by hairy vetch green manure and partial cultivar resistance. Plant Health Prog. 7:23.

Zhou, X., Everts, K., and Bruton, B. 2010. Race 3, a new and highly virulent race of *Fusarium oxysporum* f. sp. *niveum* causing Fusarium wilt in watermelon. Plant Dis. 94:92-98.

Table 1.1. Reactions of differential cultivars of watermelon when inoculated with different races of *Fusarium oxysporum* f. sp. *niveum*.

Genotype	Race			
	0	1	2	3
Sugar Baby, Black Diamond	S[a]	S	S	S
Charleston Gray	R	S	S	S
Calhoun Gray	R	R	S	S
PI-296341-FR	R	R	R	S

[a]S refers to a genotype susceptible to that race, whereas R refers to a genotype resistant to that FON race.

CHAPTER 2

DETERMINE THE ROLE OF MICRONUTRIENTS ON INDUCED RESISTANCE IN WATERMELON AGAINST FUSARIUM OXYSPORUM F. SP. NIVEUM AND MELOIDOGYNE INCOGNITA IN WATERMELON

Karki, K., Coolong, T., Mandal, M., Kousik, C., Gitaitis, G., Hajihassani, A., and Dutta, B. 2020. To be submitted to Plant Disease.

Abstract

Fusarium oxysporum f. sp. *niveum* (FON) and southern root-knot nematode (RKN, *Meloidogyne incognita*) are devastating soil-borne pathogens of cultivated watermelon in the southeastern United States. The effects of controlled, micronutrient applications [iron (Fe), manganese (Mn) and zinc (Zn)] via hydroponics on induced resistance (IR) genes [salicylic acid (SA) pathway genes (*PR1, PR5,* and *NPR1*) and jasmonic acid (JA) pathway genes (*VSP*, *PDF*, and *LOX*)] in watermelon seedlings were investigated. Plants were treated with micronutrients stated above at higher (3X) and lower levels (0.5X) of the standard-dose in a Steiner solution (X= standard dose) for 7 days and the expression levels of the induced resistance genes were evaluated. A sub-set of micronutrient treated plants were inoculated on 8th day with either FON or RKN or both to investigate the differential expression of genes mentioned prior at 3-days post-inoculation. It was observed that IR genes were not upregulated with any of the micronutrient treatments at 7-day post-treatment. However, upon pathogen inoculation, both SA and JA genes were activated and the expression levels varied considerably with different micronutrient treatments at 3-day post-inoculation (equivalent to 11-day post micronutrient treatments). For genes in the JA pathway, *PDF* was upregulated in high Fe treated plants upon FON inoculation, whereas *VSP* was upregulated in low Mn treated plants upon RKN inoculation. For genes in the SA pathway, *PR1* was upregulated in plants treated with low Zn only following co-inoculation with both FON and RKN. These observations suggest that soil micronutrients play an essential role in the induction of resistance via SA and JA pathways against FON and RKN.

Introduction

Watermelon (*Citrullus lanatus* var. *lanatus* (Thunb.) Matsum. & Nakai) is one of the most popular vegetables in the world with a total production of approximately 104 million tons (FAO 2018). The United States is the seventh-largest watermelon producer in the world with annual production and a farm gate value of 2.18 million tons and $657 million, respectively (USDA-NASS 2019). Approximately 50% of the total national production is produced in the southeastern United States, including Georgia, Florida, South Carolina, and North Carolina. With 8,903 hectares of watermelon cultivated area, Georgia is the third-largest watermelon producing state (USDA-NASS 2019). Watermelon diseases cause both economic and quality-related losses in the southern U.S. annually (Romay et al. 2014). In recent years, soil-borne pathogens have become more prevalent due to the loss of methyl bromide (an effective soil fumigant) and also partly because of limited land resources for rotation (Thies et al. 2015).

Fusarium wilt of watermelon, a disease caused by *Fusarium oxysporum* Schltdl.:Fr. f. sp. *niveum* (FON) W. C. Snyder and H. N. Hansen, is one of the most economically important diseases of watermelon worldwide (Martyn 2014). FON can infect and induce symptoms in watermelon plants at any growth stage (Beattie and Doolittle 1951). Yield losses of more than 80% have been reported under favorable conditions (Petkar et al. 2017). This soil-borne pathogen produces asexual reproductive structures including both macro- and micro-conidia. FON can survive as a saprophyte in soil and plant debris for more than 10 years due to its ability to form thick-walled chlamydospores (Martyn 1996; Zhang et al. 2015). FON is also transmitted by seeds, potentially infested through the pericarp and peduncle of immature fruit and pistils of watermelon flowers (Petkar and Ji 2017). Four physiological races of FON (0, 1, 2, 3) have been reported (Zhou et al. 2010). These races are identified based on their aggressiveness to overcome

specific resistance in a set of differential cultivars. Race 2 is the most important race as it is widely distributed within the U.S., including Georgia (Martyn 2017; Petkar et al. 2019; Zhou and Everts 2004; Zhou et al. 2010). Fusarium wilt in the U.S. had once been managed using diploid cultivars such as 'Conqueror' and several others before the shift to triploid cultivars (Guner and Wehner 2004). There is some level of variation in resistance among triploid cultivars (Egel and Hoke 2010). However, a triploid cultivar with a high level of resistance to race 2 has not yet been deployed (Wechter et al. 2012). To complicate the issue of resistance, FON race 3 has been identified in Florida, Georgia, and Maryland (Amaradasa et al. 2018; Petkar and Ji 2017; Zhou et al. 2010). Seedless cultivars do not possess resistance to race 3, making it a great threat to watermelon production. Grafting of susceptible watermelon scions onto a rootstock of interspecific hybrid squash (*Cucurbita maxima* Duch. Ex Lam. × *C. moschata* Duch. Ex Poir) and bottle gourd (*Lagenaria siceraria* (Molina) Standl.) can be a viable option. The rootstocks are resistant to both FON races 1 and 2 (Keinath and Hassell 2014a; Yetişir et al. 2003). Even so, some growers are reluctant to use this measure as grafted plants are considerably more expensive than non-grafted ones (Keinath and Hassell 2014b).

FON has also been reported to interact with southern root-knot nematode [(RKN, *Meloidogyne incognita* (Kofoid and White) Chitwood)] (Davis 2007; Hua et al. 2019; Sumner and Johnson 1973). RKN is an obligatory parasite that induces galls on the infected root system of a susceptible host. Galls disrupt the vascular system of plants making them grow poorly and can lead to death under heavy infestation (Dale 1973; Newton et al. 2012). RKN has a cosmopolitan distribution and a wide range of hosts (Jepson 1987). A survey conducted in southern Georgia in 2018 found that root-knot nematodes were present in 50% of the fields planted with watermelon (Hajihassani et al. 2020). No commercially available watermelon

cultivars are resistant to RKN and yield losses due to RKN has been estimated to reach approximately 20% in situations with high RKN populations (Davis 2007; Lynch and Carpenter 1999; Thies et al. 2010). In addition, the interspecific hybrid squash and bottle gourd root-stocks used in grafted watermelons against FON are susceptible to RKN (Thies et al. 2016). The interaction between FON and RKN in watermelon was first studied by Sumner and Johnson in 1973, according to which the presence of nematodes increased wilt symptoms caused by FON. Similar results were obtained in another study, where susceptibility to Fusarium wilt was enhanced in the presence of *M. incognita* (Hua et al. 2019). However, in later published studies, Fusarium wilt severity did not differ for grafted vs. non- grafted watermelon (with susceptible scion) when inoculated with FON or FON and RKN (Keinath and Agudelo 2018; Keinath et al. 2019; Seo and Kim 2017). These observations indicated that interactions between FON and RKN should be further investigated in detail including evaluation of different factors that can potentially impact both pathogens. Nevertheless, the management of both pathogens is crucial for sustainable watermelon production.

Host resistance is the most successful strategy for plant disease management but currently available commercial watermelon cultivars lack resistance to both FON (race 2 and race 3) and RKN. Plants possess several endogenous defense genes against infections by plant pathogens, that can be induced by biotic and abiotic agents through various mechanisms (Chinnasri et al. 2006; Siddiqui and Shaukat 2002). Induced resistance regulates the expression of defense genes by a variety of mechanisms including those dependent on the signaling molecules, salicylic acid (SA) and jasmonic acid (JA). Induced systemic resistance (ISR) in watermelon against FON by *Bacillus velezensis* F21 is mediated by simultaneous activation of the SA- and JA-mediated defense signaling pathways (Jiang et al. 2019). In another study, resistance to RKN was

increased in watermelon when plants were exposed to red light as a result of coordinated regulation of SA-, JA- and redox signaling (Yang et al. 2018). *PR1* and *PR5* are two important markers involved in SA-dependent signaling (Cao et al. 1994). Also, the accumulation of proteins produced by pathogenesis-related genes (*PR* genes) has been reported to minimize pathogen populations and disease onset in non-infected plant parts of an infected plant (Ali et al. 2018). Non-expressor pathogenesis-related gene 1 (*NPR1*) is a global transcription factor in the SA-dependent pathway (Fu et al. 2012; Wu et al. 2012) and is necessary for regulating pathogenesis defense responses (Delaney et al. 1995). Plant defensins (*PDFs*), vegetative storage proteins (*VSPs*) and lipoxygenases (*LOXs*) are key genes involved in the JA- dependent signaling pathway (Benedetti et al. 1995; Heitz et al. 1997; Penninckx et al. 1998). The transcriptional activation of these genes represents a crucial part of the plants' defense mechanism against pathogens following pathogen or elicitor perception.

Management of plant nutritional status is crucial for the management of plant diseases, as robust nutrition mediates responses to crop susceptibility and resistance to diseases (Servin et al. 2015). Micronutrient cofactors are often essential for the activation of host defense responses following tissue infection (Servin et al. 2015), which imparts critical importance to soil and plant micronutrients. Poschenrieder et al. (2006) proposed that plants absorb high concentrations of metals from the substrate as a self-defense mechanism against pathogens. Fe, Mn, and Zn have been reported to enhance disease resistance by activating the host defense response (Duffy 2007; Huber 2007; Römheld and Marschner 1991). Further, the effect of soil micronutrients in plant defense genes against pathogens has been studied previously. Selph et al. (2014) developed a model in onion, which significantly correlated the incidence of sour skin with soil and tissue Cu, Fe, Mn, and Zn concentrations. Sour skin incidence in onion sampled from areas with high soil

Cu: Fe concentrations ratio was low compared to samples from the area with low Cu: Fe concentrations ratio. Further, transcriptome analysis conducted on the samples from the same experiment indicated upregulation (>5000 fold) of *PR1* in onion tissues collected from high soil Cu: Fe concentrations ratio compared to low Cu: Fe concentrations ratio (Watson-Selph et al. 2016). A field study in tobacco also showed a significant correlation between tomato spotted wilt (TSW) and soil Cu: Fe values (Dutta et al. 2017; Gitaitis et al. 2009). Rooks et al. (2015) developed a risk model based on this study that successfully predicted TSW risk before planting in 2014 and 2015. Gene expression analysis indicated a 650-fold increase in *NPR1* in plant tissue samples from the low-risk zone compared to the high-risk zone (Rooks et al. 2015). Another model was developed in pepper consisting of concentrations of Cu, Mn, K, and the Fe: Zn ratio with bacterial leaf spot (BLS) (Dutta et al. 2017). The authors reported that BLS risk could be predicted based on micronutrient concentrations in soil prior to pepper planting. They also reported that defense genes related to SA pathway are significantly up-regulated in pepper tissues from predicted low risk vs. predicted high risk BLS sites.

Although the above studies demonstrated several roles soil micronutrients might play on induced resistance genes and plant diseases, it is often difficult to deduce as to what role individual micronutrients may play in a complex-soil medium. Hence, experiments should be conducted in a soil-less medium (e.g., hydroponics) that may aid in reducing or negating the interactive effects that may often be present in a soil medium. Since FON and RKN are soil-borne pathogens, it is hypothesized that soil micronutrients may play an important role in disease severity when plants become infected with these pathogens. However, limited information is available on this relationship. In this study, we utilized a hydroponics system to investigate if Fe, Mn, and Zn (higher and lower than the standard dose) treatments can differentially affect

induced resistance (IR) genes in the SA (*PR1*, *NPR1*, and *PR5*) and JA (*LOX*, *VSP*, and *PDF*) pathways in pre- and post-pathogen infection events (FON or RKN or FON+RKN) in watermelon.

Materials and methods

Preparation of inoculum of FON and RKN. An isolate of FON was collected from symptomatic watermelon plants in Georgia, U.S., and was identified as race 2 by inoculating a set of watermelon differentials (Petkar and Ji 2017; Petkar et al. 2017). A pure culture of FON was maintained on Potato Dextrose Agar (PDA) at 25ºC for 7 days. Five mycelial plugs (0.7-cm diameter) were removed from the edge of the colony and were transferred to a 500 mL flask containing 200 mL of potato dextrose broth. Cultures were incubated at room temperature on a rotary shaker at 160 rpm. After 2 weeks, the culture was poured through two layers of sterile cheesecloth and microconidia were collected. Using a hemocytometer, the resulting concentration of the microconidia suspension was adjusted to a final concentration of 1×10^5 microconidia/ml (Turechek and Stevenson 1998).

A population of *M. incognita* race 3 (RKN) maintained on eggplant (*Solanum melongena* L.) was kindly provided by Dr. Richard Davis (USDA-ARS, Tifton, Georgia) and Nematology Lab (Tifton campus). Eggplants were maintained in a greenhouse at 22–30°C. Infested plant roots were cut, washed in tap water and placed in a mist chamber for 2–9 days for eggs to hatch. Freshly hatched second-stage juveniles (J2) were collected every 3 days after incubation until 9 days. The J2s were also collected from the soil in which the culture was maintained by the sugar centrifugation method (Freckman et al. 1977). The resulting *M. incognita* (J2) collected were diluted in tap water to get a final concentration of 6000 J2/ml.

Hydroponics set-up under greenhouse conditions. Two independent greenhouse experiments were designed for use with a hydroponics system to evaluate the effects of micronutrients on induced resistance in watermelon against FON or RKN or co-inoculation of FON and RKN. Watermelon seeds (cv. Sugar Baby) were sown into sheets of 2.54 cm^2/cell Rockwool cubes (Grodan Inc., Hedehusene, Denmark) and covered with a thin layer of vermiculite. The sheet was kept moist with water as needed. After germinating, seedlings were watered as needed and fertilized once with a Steiner universal nutrient solution modified for this study (Table 2.1). For this manuscript, we will refer to "the modified Steiner universal nutrient solution" (Steiner 1984) as a "Steiner solution". Seedlings were maintained for three weeks on Rockwool and then were transferred to plastic containers (43.05 cm W × 30.48 cm D × 19.81 cm H) containing the Steiner solution amended with the respective micronutrient treatments (Table 2.1). The micronutrient treatments included: high Fe (3X concentration of Fe in Steiner solution), low Fe (0.5X concentration of Fe in Steiner solution), high Zn (3X concentration of Zn in Steiner solution), low Zn (0.5X concentration of Zn in Steiner solution), high Mn (3X concentration of Mn in Steiner solution), low Mn (0.5X concentration of Mn in Steiner solution), and Steiner [X concentration of Fe (3 ppm), Mn (1 ppm) and Zn (0.4 ppm)]. The concentration (ppm) of individual ingredients are listed in Table 2.1. The micronutrient treatments were prepared in 8L of deionized (dH_2O) water in each plastic container.

Three seedlings along with Rockwool were placed equidistant in a nine-well styrofoam tray with holes at the bottom so that roots could suspend down. The Styrofoam tray was then placed over the plastic container filled with micronutrient treatments, as indicated above. The seedlings were placed so that the roots were suspended in hydroponics solutions (Fig. 2.1). Solutions were aerated through a 15.24cm aquarium air-stone fitted with plastic-tubes (0.3-mm

diameter) and an air-pump (Pentair aquatic eco-systems Inc., Apopka, USA) (Fig. 2.1). Plastic containers were maintained to the original volume (8L) by adding dH_2O every two days. Seedlings were treated with different micronutrient treatments for 7 days (Table 2.1). At 8-day post-treatment (DPT), the micronutrient treatments were either inoculated with FON or RKN or co-inoculated with both FON and RKN. For FON inoculation, a 1 ml suspension containing 5×10^5 microconidia/ml was applied at the base of watermelon seedling. For nematode inoculation, a 1 ml suspension containing 6000 active RKN J2s was added at the base of the seedling. For treatments that were inoculated with both FON and RKN, a similar inoculation approach was adopted and the inocula were applied simultaneously.

Plants were maintained in nutrient solution for 23 days post-inoculation with a total experimental duration of 30 days (7 days pre-inoculation+ 23 days post-inoculation) at 28°C mean greenhouse temperature. The pH of the nutrient solution ranged between 5.1-6.5 throughout the experiment. The replicate of each micronutrient treatment was comprised of a plastic container with three seedlings each (Figure 2.1). Three replicates per treatment were used in an experiment and two independent experiments were conducted. Treatments were arranged in a completely randomized design.

Mineral analysis. Samples of nutrient solution (20 mL) were collected from each container at 7-DPT and stored at 4°C. Concentrations of Fe, Mn, and Zn (ppm) were determined for three replicates per treatment using inductively coupled plasma atomic emission spectroscopy (ICP-AES) at the Waters Agricultural laboratories (Camilla, USA). Leaf tissues were collected from 10 leaves at 30-DPT from one plant per replicate per treatment totaling three plants per treatment. Leaf samples were dried for 3 days in a forced air-oven (Grieve Corp., Round Lake,

USA) at 55℃. Foliar nutrient concentrations for N, P, K, Ca, Mg, S, Cu, B, Fe, Mn, and Zn were determined by employing the ICP procedure as described above.

Relative expression of SA- and JA-genes in watermelon seedlings grown in specific micronutrient solutions. Leaf samples were collected to determine the relative expression of SA- and JA-genes in watermelon seedlings grown in various micronutrient treatments before inoculation. Leaf samples (*n*=3 per replicate/treatment) were collected by cutting the 3rd or 4th leaf from the terminal with a pair of sterile scissors 7-DPT. Samples were immediately stored in liquid nitrogen and later transferred to a -80ºC freezer at the UGA Tifton Campus laboratory until needed for further analysis. Leaves were ground in liquid nitrogen and total RNA was extracted from 100 mg of ground leaf tissue using the manufacturer's protocol (RNeasy Plant Mini Kit). Concentrations were determined using a NanoDropTM Lite (Thermo Scientific., Wilmington, DE, USA). The RNA quality was confirmed by the value of the A260/A280 absorbance ratio which was between 2.1 and 2.2 for all samples indicating that the RNA was relatively free of protein contamination (Djami-Tchatchou and Straker 2012). Total RNA samples were aliquoted and stored at −80°C for later use. First-strand cDNA was synthesized from 500 ng of total RNA by reverse transcription using the iScript cDNA synthesis kit (Bio-Rad Laboratories Inc., Hercules, CA). qPCR was conducted on a Smart Cycler System (Cepheid, Sunnyvale, CA) using SsoFast EvaGreen Supermix (Bio-Rad Laboratories Inc.) and specific primer pairs for the marker genes (Table 2.2). These genes were selected based on their involvement in plant defense response mechanisms. For real-time PCR, the resulting first strand of cDNA was diluted (1:10). Two microliters of diluted cDNA (5 ng) were amplified in 10 µl of PCR master mix containing 5 µl of SsoFast EvaGreen Supermix (Bio-Rad Laboratories Inc.) and 0.4 µl each of 25 µM primers per sample. The *ß-Actin* gene was used as an internal control, and

was assessed using primers: (forward primer, 5' -CCATGTATGTTGCCATCCAG-3', reverse primer, 3'-GGATAGCATGGGGTAGAGCA-5') (Kong et al. 2014). Plants in the Steiner treatment were considered as non-treated control. Relative fold changes of target genes were calculated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001; Schmittgen and Livak 2008). Relative gene expression was then compared for each treated (*n*=48; 24 biological replicates and 2 technical replicates) and non-treated (*n*=12; 6 biological replicates and 2 technical replicates) plants.

Relative expression of SA- and JA-genes in watermelon seedlings, grown in specific micronutrient solutions and inoculated with FON, RKN, or both. The effects of micronutrient treatment and pathogen inoculation alone or in-combination on the relative expression of SA- and JA-induction pathway genes in watermelon seedlings were investigated. Leaf samples (*n*=3 per replicate/treatment) were collected by cutting 3rd or 4th leaf from the terminal with a pair of sterile scissors from plants at 3 days post-inoculation (DPI) (equivalent to 11 DPT). Non-inoculated plants grown on the Steiner treatment were considered as the non-treated control. From each replicate, two technical replicates per sample were utilized for gene expression analysis. Further procedures spanning from the extraction of RNA to the determination of relative gene expression were performed in the same manner as mentioned above.

FON recovery and RKN gall rating. Visual symptoms of wilting were not observed in plants throughout the experiment. At the end of the experiment, one plant from each container (*n*=3 containers/treatment), inoculated with FON alone, or co-inoculated with RKN and non-inoculated plants in the Steiner solution, were checked for the presence of FON. Stem pieces (0.5-cm-long) were cut from the base of the main stem of each plant using a pair of scissors

sterilized by dipping in 70% ethanol. Stem pieces were surface-disinfested for 1.5 min with 0.6% sodium hypochlorite, rinsed in sterile water and placed on a semi-selective peptone pentachloronitrobenzene agar medium. Plates were incubated at 25℃ for 7 days. Fungal isolates were then microscopically identified based on morphological criteria (Leslie and Summerell 2008) and further were confirmed as FON by PCR assay with FON specific primers Fon-1/Fon-2 (Lin et al. 2010). Percentage of plants infested with FON as determined by morphological and PCR confirmatory assays were recorded. For plants inoculated with RKN, 10 cm of roots closest to the base of plants were sampled and evaluated as galls were observed only in this portion. Rockwool plugs were removed from the root system, and roots were washed and rated visually for the number of galls using 0-5 galling index (GI) as follows; 0-0 galls; 1=1-2 galls; 2=3-10 galls; a3= 11-30 galls; 4=31-100 galls; 5> 100 galls (Taylor and Sasser 1978).

Statistical analysis. Prior to analysis, data were log-transformed [$\log_{10}$ (x+1)] wherever appropriate to normalize the data distribution. Untransformed arithmetic means are presented. Data from two trials were combined after ensuring no significant interaction of trial-treatment was present at $P \leq 0.05$. Data obtained from the trial were subjected to statistical analysis using one-way Analysis of Variance (ANOVA) procedure and treatments were compared by Tukey-Kramer test at $P<0.05$ level in SAS 9.4®.

Results

Mineral content in nutrient solutions. At 0-DPT, the concentration of Fe applied in the high Fe treatment was significantly ($P<0.05$) higher than in the low Fe and Steiner treatments. Similar observations were also made at 7-DPT, where the high Fe treatment had significantly greater Fe concentrations compared to other treatments (Table 2.3). With respect to Mn

availability in solutions, higher concentrations were observed for the high Mn treatment compared to the low Mn and Steiner treatments at 0 and 7 DPT (Table 2.3). Zn availability also differed significantly among Zn treatments at both 0 and 7 DPT (Table 2.3). The high Zn treatment had significantly higher Zn concentrations in hydroponics solutions compared to the low Zn and Steiner.

Mineral content in leaf tissues. In non-inoculated plants, Fe concentrations were significantly higher in plants treated with high Fe compared to non-inoculated plants grown in the standard Steiner solution. With RKN inoculation, the Fe concentration was significantly higher than high Fe and Steiner in plants treated with low Fe (Table 2.4). Plants inoculated with FON and FON+RKN did not have significantly different Fe concentrations than the non-inoculated control regardless of Fe level in the nutrient solution. Foliar Fe concentrations were significantly lower in RKN inoculated plants compared to non-inoculated control plants when grown under high Fe levels ($P<0.05$) (Table 2.4)

When micronutrient treatments were compared for the non-inoculated treatments, Mn concentration was significantly higher in plants treated with high Mn compared to those grown with the Steiner or low Mn. In plants inoculated with either FON or RKN alone, the concentration of Mn in plants treated with low Mn was similar to the plants treated with high Mn (Table 2.4). For plants grown under low Mn concentrations, those that were inoculated with FON+RKN, had significantly lower foliar Mn concentrations compared to those inoculated with only RKN, but not those inoculated with FON or the non-inoculated control (Table 2.4).

Foliar Zn concentrations in non-inoculated plants were significantly greater in the high Zn treatment compared to Steiner treatment. However, accumulation of Zn in foliage was not significantly different among inoculated and non-inoculated Zn treatments (Table 2.4). Foliar

concentrations of N, P, K, Ca, Mg, S, Cu, and B (data not shown) were not significantly different among treatments ($P<0.05$).

Relative expression of SA- and JA-genes in watermelon seedlings grown in different micronutrient solutions. Quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR) was used to determine the transcript levels of six genes (*PR1, NPR1, PR5, LOX, VSP,* and *PDF*), which are considered as key genes in plant induced resistance (Benedetti et al. 1995; Cao et al. 1994; Heitz et al. 1997; Penninckx et al. 1998). Transcripts of the above-stated genes were detected in plants treated with micronutrient treatments (Table 2.1) at 7-DPT and compared with non-treated control plants in Steiner (Fig. 2.2).

Watermelon plants treated with high Fe for 7 days significantly downregulated *PDF, PR1,* and *PR5* genes prior to inoculation. When high- and low Fe treated plants were compared, relative expression of *PR1* gene was significantly lower in plants treated with high Fe (Fig. 2.2A). The expression of *PR5* gene was significantly downregulated in low Fe treated plants but expression levels were not significantly different between high- and low Fe (Fig. 2.2A). Relative expression of *NPR1, PDF, PR1,* and *PR5* genes was significantly downregulated by the Mn treatment (Fig. 2.2B). Relative expression of *PR1* and *VSP* genes was significantly higher for high Mn compared to low Mn treated plants. Expression of *NPR1, PDF, PR1,* and *PR5* genes was significantly downregulated by Zn treatments (Fig. 2.2C). When high- and low Zn treated plants were compared, *VSP* gene expression was significantly higher in the low Zn treatment compared to the high Zn treatment (Fig. 2.2C).

Relative expression of SA- and JA-genes in watermelon seedlings, grown in different micronutrient solutions and inoculated with FON, RKN, or both.

The *PDF* gene was significantly upregulated in plants treated with high Fe in response to FON inoculation (Fig. 2.3A). Plants inoculated with FON in the Steiner treatment demonstrated downregulation of the *LOX* gene (Fig. 2.3A). RKN inoculation resulted in significant downregulation of the *NPR1* gene in the plants treated with high Fe (Fig. 2.3D). *NPR1* and *PR1* genes were downregulated in low Fe treated plants with co-inoculation of both FON and RKN (Fig. 2.3G). When Fe treated plants were compared, expression of the *PDF* gene was higher in plants treated with high Fe with FON inoculation, and the same was true for the *PR1 and VSP* genes after inoculation with both FON and RKN (Fig. 2.3A and G).

FON inoculation in plants treated with Steiner and high Mn resulted in downregulation of *LOX* gene (Fig. 2.3B). The *LOX* gene was also downregulated with RKN inoculation in plants treated with Steiner (Fig. 2.3E). RKN inoculation significantly upregulated *VSP* gene expression in plants treated with low Mn (Fig. 2.3E). When Mn treated plants were compared, the relative expression of *LOX*, *PDF* and *PR5* genes was higher in plants treated with low Mn with FON inoculation (Fig. 2.3B). Inoculation of RKN resulted in higher expression of *LOX, NPR1* and *VSP* genes in the same treatment (Fig. 2.3E). After inoculation of FON and RKN both, expression of *PR1* and *PR5* were again higher in plants treated with low Mn (Fig. 2.3H).

The *PR1* gene was significantly upregulated in plants treated with low Zn in response to co-inoculation with both FON and RKN (Fig. 2.3I). Co-inoculation with both FON and RKN resulted in significant downregulation of the *PR5* gene in plants treated with high Zn (Fig. 2.3I). When Zn treated plants were compared, relative expression of the *LOX* gene was significantly higher in plants treated with high Zn compared to Steiner and low Zn with FON inoculation (Fig. 2.3C). *LOX* and *PR5* gene expressions were higher in plants treated with high Zn after RKN

inoculation (Fig. 2.3F). However, co-inoculation with both FON and RKN reduced the expression of these genes in the high Zn treatment (Fig. 2.3I).

FON recovery and RKN gall rating. FON was re-isolated from 100% of the tested plants that were inoculated with FON or co-inoculated with RKN in both trials but not from non-inoculated control or RKN only inoculated plants. Isolates were tested for FON by conventional PCR as described earlier (Lin et al. 2010). One hundred percent of the putatively isolated FON colonies from infected plants were confirmed as FON using the PCR assay. No difference in plants being colonized by FON for all treatments and no Fusarium wilt symptoms were observed. Also, no differences in root galling between treatments either. Galls were present only on plants inoculated with RKN or RKN co-inoculated with FON but were absent on non-inoculated, or FON alone inoculated plants.

Discussion

Soil micronutrients play an essential role in the expression of plant defense genes against plant diseases (Dutta et al. 2017; Gitaitis et al. 2009; Rooks et al. 2015; Watson-Selph et al. 2016). However, limited information is available on the role of individual micronutrient in plant defense responses in watermelon against soil pathogens FON and RKN. In this study, the influence of the level of micronutrients, Fe, Mn, and Zn, were monitored through relative gene expression in watermelon leaves before and after inoculation with FON and RKN **(**single and mixed) in a hydroponics system. We observed that plants treated with Fe, Mn, and Zn at higher and lower doses than those found in standard Steiner solution for 7 days demonstrated downregulation of induced resistance genes; *PR1, PR5, NPR1,* and *PDF* (Fig. 2.2A-C). IR genes were not activated even at 11 days in those treatments (Appendix, Fig. A.1A-C). However,

upregulation was observed in some micronutrient-pathogen treatments 3-day post-inoculation of the pathogen (equivalent to 11-day post micronutrient treatments). This result is in line with the study done by Mathys et al. (2012), where no activation of the JA-pathway in the *Trichoderma hamatum* T382 pre-inoculated *Arabidopsis* plants was observed without a subsequent infection with *Botrytis cinera*.

Although previous reports evaluated expression of these genes in response to pathogens or micronutrients, none of the studies evaluated these variables in combination. In this study, we provide evidence that watermelon plants respond differentially to distinct micronutrients and pathogen combinations. For example, we observed upregulation in the following micronutrient-pathogen treatments; *PDF* gene in high Fe treated plants with FON inoculation, *VSP* gene in low Mn treated plants with RKN inoculation, and *PR1* gene in low Zn treated plants with co-inoculation of both FON and RKN. These observations indicate that genes in JA (*PDF* and *VSP*) and SA pathway (*PR1*) in watermelon plants respond differentially with respect to different micronutrients and pathogen combination treatments. *PDF* and *VSP* genes are regulated by JA, a key compound of the JA-signaling pathway, and are induced during soil-borne pathogen infection (Jain et al. 2018; Tamaoki et al. 2013). Jain et al. (2018) reported the upregulation of the *VSP* gene in soybean treated with rhizobacterial isolate *Bacillus* sp. strain SJ-5 (MCC-2607) against *Rhizoctonia solani* and *F. oxysporum*. *VSP* is present in the host-vegetative tissues and has displayed mutagenic and phosphatase potential against herbivores (Liu et al. 2005; Staswick 1994). Plant defensins (*PDFs*) are small, basic, cysteine-rich peptides with antimicrobial activity against a wide variety of microorganisms (De Coninck et al. 2013; de Oliveira Carvalho and Moreira Gomes 2011). Dos Santos et al. (2003) demonstrated induced expression of *PDF1.2* and nine other genes upon defense-gene expression analysis of *A.*

thaliana parasitized by *Orobanche ramose*. Pathogenesis-related proteins are induced by products made in the SA pathway and help defend plants against plant pathogens (Vijayan et al. 1998; Wang and Wu 2013). The *PR1* gene is an essential marker of the SA-mediated defense response (Rairdan and Delaney 2002; Van Loon 1997). The up-regulation of defense-related genes such as *PR1* and *PR2* was associated with lesion limitation in the roots of soybean seedlings inoculated with *Phytophthora sojae* (Upchurch and Ramirez 2010). Similarly, increased resistance of watermelon to FON was observed in a wheat intercropping system because of the enhanced relative expression of the defense-related genes including the *PR1* and increased activities of PR enzymes (Lv et al. 2018).

Induced resistance mediated by several genes governing the SA- and JA-pathways can also be downregulated with specific micronutrient and pathogen combinations. We observed downregulation of plant-induced resistance genes in response to inoculation with FON, RKN or both. The *LOX* gene was downregulated in plants treated with Steiner only, and when grown with high Mn and inoculated with FON, and also in plants treated with the Steiner solution only but inoculated with RKN. The *NPR1* gene was downregulated in plants treated with high Fe after RKN inoculation and also in plants treated with low Fe in response to co-inoculation of FON and RKN. *PR1* gene was downregulated in the plants treated with low Fe in response to the co-inoculation with FON and RKN. Inoculation of FON and RKN significantly downregulated expression of the *PR5* gene in plants treated with high Zn. The downregulation of these induced resistance genes in response to the pathogens (FON or RKN or both) infecting watermelon in a particular micronutrient treatment may affect their defense response by enhancing host-susceptibility.

We observed a significant connection between the availability of micronutrients in nutrient solution and foliar concentrations of those nutrients. The availability of each micronutrient was adjusted in each treatment at the beginning of the experiment. This was confirmed by the analysis of the nutrient solution at 7-DPT, which showed differences in the level of micronutrients in each solution were maintained for most treatments (Table 2.3). The concentration of micronutrients in leaf tissue at 30-DPT varied according to the level of micronutrient applied but in some cases was also affected with pathogen inoculation. Foliar concentrations for Fe, Mn, and Zn were highest in plants treated with the high level of the respective micronutrients. However, foliar micronutrients in non-inoculated plants grown in the low-level treatments did not differ from those in the standard treatments. The differences between Fe, Mn, and Zn concentrations in the low and control (Steiner) treatments were smaller than those between the high and control treatments. This suggests that without the presence of another factor (RKN or FON), the difference between Fe, Mn, and Zn concentrations between the low and control solutions may not be large enough to obtain significant differences in foliar nutrient levels under the conditions of our trial. Our study demonstrates that inoculation of RKN reduces leaf Fe concentration in plants treated with high Fe, and inoculation with FON and RKN reduces leaf Mn concentration in plants treated with low Mn compared to non-inoculated control plants treated with Steiner (Table 2.4). The concentration of foliar nutrient may vary with plant biomass; however, there was no substantial difference between treatments for fresh and dry biomass. This suggests that the maintenance of Fe and Mn concentrations within watermelon plants may be involved in an induced resistance strategy against pathogen infection.

In the present study, we found the highest expression of the genes in the leaf tissue of high Fe treated plants compared to low-and standard-Fe (Steiner). Fe is essential for various

innate host defense mechanisms (Schaible and Kaufmann 2004). In *Arabidopsis* (Chen et al. 2014; Segond et al. 2009) and wheat (Liu et al. 2007), direct involvement of Fe in defense response was observed by cellular relocation of Fe to infection sites that coincided with local reactive oxygen species (ROS) production. Maintaining adequate Fe concentrations in maize was found to delay and partially suppresses the fungal infection process and the biotrophic growth phase of *Colletotrichum graminicola* (Ye et al. 2014). Mn is involved in the production of phenolic compounds and plant induced resistance mechanisms (Fernando et al. 2009). It is a cofactor of superoxide dismutase (SOD) that participates in plant defense against oxidative stress produced by the elevated level of reactive forms of oxygen and free radicals (ROS), which are harmful to plants (Millaleo et al. 2010). Effects of increased Mn availability on disease severity have been found to vary among different plant species with different diseases (Thompson and Huber 2007). In our study, we observed that relative expression of induced resistance genes was higher in the plants treated with low Mn after pathogen inoculation.

Relative expression of defense genes was higher with plants grown in the high Zn treatment in response to inoculation with either FON or RKN. However, expression was higher in plants treated with low Zn after co-inoculation of both FON and RKN. Zn fertilization has been reported to reduce symptoms of disease in many cases (Grewal et al. 1996; Li et al. 2016; Machado et al. 2018). The variability we observed in plants inoculated with FON and RKN might be due to protective concentration of Zn against one pathogen might have induced increased susceptibility to another pathogen as reported by Helfenstein et al. (2015). We did not observe a significant difference in terms of the symptom development and severity in plants inoculated with FON and RKN. Wilting symptoms due to FON infection likely were not observed because the plants had constant access to moisture as the roots were merged in the

nutrient solutions. Symptom differences were also not observed among treatments in terms of wilting and gall rating caused by RKN inoculation. The development of symptoms might have been affected by the hydroponics system in which plants were grown. Conversely, the induction of resistance genes (JA and SA pathways) might not be strong enough that would translate to phenotypic differences among different micronutrients (type and concentrations).

Overall, these results suggest a possible association between the availability of specific nutrients and the induction of plant defense genes in watermelon against FON and RKN. This study indicates potential cross-talk between pathways affected by intracellular nutrient concentrations, including micronutrient homeostasis and pathways governed by induced resistance against FON and RKN infections, which may directly affect induced resistance responses in watermelon. Detailed study is essential to understand how nutrient concentrations in substrate or leaf tissue of watermelon affect the expression of induced resistance genes, and also if FON and RKN induce the plant response by itself or in the micronutrient treatment-mediated manner.

Literature cited

Ali, S., Ganai, B. A., Kamili, A. N., Bhat, A. A., Mir, Z. A., Bhat, J. A., Tyagi, A., Islam, S. T., Mushtaq, M., and Yadav, P. 2018. Pathogenesis-related proteins and peptides as promising tools for engineering plants with multiple stress tolerance. Microbiol. Res. 212:29-37.

Amaradasa, B., Beckham, K., Dufault, N., Sanchez, T., Ertek, T., Iriarte, F., Paret, M., and Ji, P. 2018. First report of *Fusarium oxysporum* f. sp. *niveum* race 3 causing wilt of watermelon in Florida, USA. Plant Dis. 102:1029-1029.

Beattie, J., and Doolittle, S. 1951. Watermelons. US Government Printing Office, Washington, DC.

Benedetti, C. E., Xie, D., and Turner, J. G. 1995. COI1-dependent expression of an *Arabidopsis* vegetative storage protein in flowers and siliques and in response to coronatine or methyl jasmonate. Plant Physiol. 109:567-572.

Cao, H., Bowling, S. A., Gordon, A. S., and Dong, X. 1994. Characterization of an *Arabidopsis* mutant that is nonresponsive to inducers of systemic acquired resistance. Plant Cell 6:1583-1592.

Chen, C. C., Chien, W. F., Lin, N. C., and Yeh, K. C. 2014. Alternative functions of Arabidopsis Yellow Stripe-Like3: from metal translocation to pathogen defense. PLoS One 9.

Chinnasri, B., Sipes, B., and Schmitt, D. 2006. Effects of inducers of systemic acquired resistance on reproduction of *Meloidogyne javanica* and *Rotylenchulus reniformis* in pineapple. J. Nematol. 38:319.

Dale, P. 1973. Elimination of root-knot nematodes from roses by chemical bare-root dips. N.Z. J. Exp. Agric. 1:121-122.

Davis, R. F. 2007. Effect of *Meloidogyne incognita* on watermelon yield. Nematropica 37:287-294.

De Coninck, B., Cammue, B. P., and Thevissen, K. 2013. Modes of antifungal action and in planta functions of plant defensins and defensin-like peptides. Fungal Biol. Rev. 26:109-120.

de Oliveira Carvalho, A., and Moreira Gomes, V. 2011. Plant defensins and defensin-like peptides-biological activities and biotechnological applications. Curr. Pharm. Des. 17:4270-4293.

Delaney, T., Friedrich, L., and Ryals, J. 1995. Arabidopsis signal transduction mutant defective in chemically and biologically induced disease resistance. Proc. Natl. Acad. Sci. U. S. A. 92:6602-6606.

Djami-Tchatchou, A., and Straker, C. 2012. The isolation of high quality RNA from the fruit of avocado (*Persea americana* Mill.). S. Afr. J. Bot. 78:44-46.

Dos Santos, C. V., Letousey, P., Delavault, P., and Thalouarn, P. 2003. Defense gene expression analysis of *Arabidopsis thaliana* parasitized by *Orobanche ramosa*. Phytopathology 93:451-457.

Duffy, B. 2007. Zinc and plant disease. Pages 155-176 in: Mineral nutrition and plant disease. L. E. Datnoff, W. H. Elmer and D. M. Huber, eds. APS, St. Paul, MN. .

Dutta, B., Langston, D. B., Luo, X., Carlson, S., Kichler, J., and Gitaitis, R. 2017. A risk assessment model for bacterial leaf spot of pepper (*Capsicum annuum*), caused by

Xanthomonas euvesicatoria, based on concentrations of macronutrients, micronutrients, and micronutrient ratios. Phytopathology 107:1331-1338.

Egel, D., and Hoke, S. 2010. Evaluation of triploid watermelon for resistance to Fusarium wilt, 2010. Plant Dis. Manage. Rep 4:V135.

FAO. 2018. Countries by Commodities –Top Yield – Watermelons. Available at http://www.fao.org/faostat/en/#data/QC Accessed 2020-04-14.

Fernando, D., Baker, A., and Woodrow, I. 2009. Physiological responses in *Macadamia integrifolia* on exposure to manganese treatment. Aust. J. Bot. 57:406-413.

Freckman, D., Kaplan, D., and Van Gundy, S. 1977. A comparison of techniques for extraction and study of anhydrobiotic nematodes from dry soils. J. Nematol. 9:176.

Fu, Z. Q., Yan, S., Saleh, A., Wang, W., Ruble, J., Oka, N., Mohan, R., Spoel, S. H., Tada, Y., and Zheng, N. 2012. NPR3 and NPR4 are receptors for the immune signal salicylic acid in plants. Nature 486:228-232.

Gitaitis, R., Nischwitz, C., Mullis, S., Csinos, A., Burrell, D., and Pearce, M. 2009. Soil fertility related to Tomato spotted wilt virus in tobacco Phytopathology 99:S198. (Abstract).

Grewal, H. S., Graham, R. D., and Rengel, Z. 1996. Genotypic variation in zinc efficiency and resistance to crown rot disease (*Fusarium graminearum* Schw. Group 1) in wheat. Plant Soil. 186:219-226.

Guner, N., and Wehner, T. C. 2004. The genes of watermelon. HortScience 39:1175-1182.

Hajihassani, A., Forghani, F., and Marquez, J. 2020. Managing root-knot nematodes in Georgia watermelons. UGA Extension. Available at https://extension.uga.edu/publications/detail.html?number=B1529 Accessed 2020-04-14.

Heitz, T., Bergey, D. R., and Ryan, C. A. 1997. A gene encoding a chloroplast-targeted lipoxygenase in tomato leaves is transiently induced by wounding, systemin, and methyl jasmonate. Plant Physiol. 114:1085-1093.

Helfenstein, J., Pawlowski, M. L., Hill, C. B., Stewart, J., Lagos-Kutz, D., Bowen, C. R., Frossard, E., and Hartman, G. L. 2015. Zinc deficiency alters soybean susceptibility to pathogens and pests. J. Plant Nutr. and Soil Sci. 178:896-903.

Hua, G. K. H., Timper, P., and Ji, P. 2019. *Meloidogyne incognita* intensifies the severity of Fusarium wilt on watermelon caused by *Fusarium oxysporum* f. sp. *niveum*. Can. J. Plant Pathol. 41:261-269.

Huber, D. M. 2007. Nitrogen and plant disease. Pages 31-44 in: Mineral nutrition and plant disease. L. E. Datnoff, W. H. Elmer and D. M. Huber, eds. APS, St. Paul, MN. .

Jain, S., Vaishnav, A., Varma, A., and Choudhary, D. K. 2018. Comparative expression analysis of defence-related genes in *Bacillus*-treated *Glycine max* upon challenge inoculation with selective fungal phytopathogens. Curr. Sci. 115:1950.

Jepson, S. B. 1987. Identification of root-knot nematodes (*Meloidogyne* species). CAB International, Wallingford, UK.

Jiang, C., Guo, J., Yao, X., Mi, D., Li, Z., Yang, B., Qi, Y., and Zheng, Y. 2019. Comparative transcriptome analysis reveals the biocontrol mechanism of *Bacillus velezensis* F21 against Fusarium wilt on watermelon. Front. Microbiol. 10:652.

Keinath, A., and Hassell, R. 2014a. Control of Fusarium wilt of watermelon by grafting onto bottlegourd or interspecific hybrid squash despite colonization of rootstocks by *Fusarium*. Plant Dis. 98:255-266.

Keinath, A. P., and Hassell, R. L. 2014b. Suppression of Fusarium wilt caused by *Fusarium oxysporum* f. sp. *niveum* race 2 on grafted triploid watermelon. Plant Dis. 98:1326-1332.

Keinath, A. P., and Agudelo, P. A. 2018. Retention of resistance to *Fusarium oxysporum* f. sp. *niveum* in cucurbit rootstocks infected by *Meloidogyne incognita*. Plant Dis. 102:1820-1827.

Keinath, A. P., Coolong, T. W., Lanier, J. D., and Ji, P. 2019. Managing Fusarium wilt of watermelon with delayed transplanting and cultivar resistance. Plant Dis. 103:44-50.

Kong, Q., Yuan, J., Gao, L., Zhao, S., Jiang, W., Huang, Y., and Bie, Z. 2014. Identification of suitable reference genes for gene expression normalization in qRT-PCR analysis in watermelon. PLoS One 9.

Leslie, J. F., and Summerell, B. A. 2008. The fusarium laboratory manual. Blackwell Publishing, Ames, IA. .

Li, Z., Fan, Y., Gao, L., Cao, X., Ye, J., and Li, G. 2016. The dual roles of zinc sulfate in mitigating peach gummosis. Plant Dis. 100:345-351.

Lin, Y. H., Chen, K. S., Chang, J. Y., Wan, Y. L., Hsu, C. C., Huang, J. W., and Chang, P. F. L. 2010. Development of the molecular methods for rapid detection and differentiation of *Fusarium oxysporum* and *F. oxysporum* f. sp. *niveum* in Taiwan. New biotechnol. 27:409-418.

Liu, G., Greenshields, D. L., Sammynaiken, R., Hirji, R. N., Selvaraj, G., and Wei, Y. 2007. Targeted alterations in iron homeostasis underlie plant defense responses. J. Cell Sci. 120:596-605.

Liu, Y., Ahn, J.-E., Datta, S., Salzman, R. A., Moon, J., Huyghues-Despointes, B., Pittendrigh, B., Murdock, L. L., Koiwa, H., and Zhu-Salzman, K. 2005. *Arabidopsis* vegetative storage protein is an anti-insect acid phosphatase. Plant Physiol. 139:1545-1556.

Livak, K. J., and Schmittgen, T. D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. Methods 25:402-408.

Lv, H., Cao, H., Nawaz, M. A., Sohail, H., Huang, Y., Cheng, F., Kong, Q., and Bie, Z. 2018. Wheat intercropping enhances the resistance of watermelon to Fusarium wilt. Front Plant Sci 9:696.

Lynch, L., and Carpenter, J. 1999. The economic impacts of banning methyl bromide: Where do we need more research. in: 1999 Annual Research Conference on Methyl Bromide Alternatives and Emissions Reductions. http://mbao.org/mbrpro99.html.

Machado, P. P., Steiner, F., Zuffo, A. M., and Machado, R. A. 2018. Could the supply of boron and zinc improve resistance of potato to early blight? Potato Res. 61:169-182.

Mandal, M. K., Suren, H., Ward, B., Boroujerdi, A., and Kousik, C. 2018. Differential roles of melatonin in plant-host resistance and pathogen suppression in cucurbits. J. Pineal Res. 65:e12505.

Martyn, R. 1996. Fusarium wilt of watermelon Pages 13-14 in: Compendium of cucurbit diseases. T. A. Zitter, D. L. Hopkins and C. E. Hopkins, eds. The American Phytopathological Society, St. Paul, MN.

Martyn, R. D. 2014. Fusarium wilt of watermelon: 120 years of research. Hortic. Rev. 42:349-442.

Martyn, R. D. 2017. Fusarium wilt of watermelon. Pages 38-40 in: Compendium of Cucurbit Diseases and Pests, 2nd Ed. A. P. Keinath, W. M. Wintermantel and T. A. Zitter, eds. American Phytopathological Society, St. Paul, MN.

Mathys, J., De Cremer, K., Timmermans, P., Van Kerkhove, S., Lievens, B., Vanhaecke, M., Cammue, B., and De Coninck, B. 2012. Genome-wide characterization of ISR induced in *Arabidopsis thaliana* by *Trichoderma hamatum* T382 against *Botrytis cinerea* infection. Front. Plant Sci. 3:108.

Millaleo, R., Reyes-Díaz, M., Ivanov, A., Mora, M., and Alberdi, M. 2010. Manganese as essential and toxic element for plants: transport, accumulation and resistance mechanisms. J. Soil Sci. Plant Nutr. 10:470-481.

Newton, A. C., Torrance, L., Holden, N., Toth, I. K., Cooke, D. E., Blok, V., and Gilroy, E. M. 2012. Climate change and defense against pathogens in plants. Adv. Appl. Microbiol. 81:89-132.

Penninckx, I. A., Thomma, B. P., Buchala, A., Métraux, J.-P., and Broekaert, W. F. 1998. Concomitant activation of jasmonate and ethylene response pathways is required for induction of a plant defensin gene in *Arabidopsis*. Plant Cell 10:2103-2113.

Petkar, A., and Ji, P. 2017. Infection courts in watermelon plants leading to seed infestation by *Fusarium oxysporum* f. sp. *niveum*. Phytopathology 107:828-833.

Petkar, A., Langston, D. B., Buck, J. W., Stevenson, K. L., and Ji, P. 2017. Sensitivity of *Fusarium oxysporum* f. sp. *niveum* to prothioconazole and thiophanate-methyl and gene mutation conferring resistance to thiophanate-methyl. Plant Dis. 101:366-371.

Petkar, A., Harris-Shultz, K., Wang, H., Brewer, M. T., Sumabat, L., and Ji, P. 2019. Genetic and phenotypic diversity of *Fusarium oxysporum* f. sp. *niveum* populations from watermelon in the southeastern United States. PLoS One 14.

Poschenrieder, C., Tolrà, R., and Barceló, J. 2006. Can metals defend plants against biotic stress? Trends Plant Sci. 11:288-295.

Rairdan, G. J., and Delaney, T. P. 2002. Role of salicylic acid and NIM1/NPR1 in race-specific resistance in *Arabidopsis*. Genetics 161:803-811.

Romay, G., Lecoq, H., and Desbiez, C. 2014. Cucurbit crops and their viral diseases in Latin America and the Caribbean islands: A review. J. Plant Pathol. 96:227-242.

Römheld, V., and Marschner, H. 1991. Function of micronutrients in plants. Pages 297-328 in: Micronutrients in agriculture, vol. 4. J. J. Mortvedt, ed. Soil Sci. Soc. Amer. Inc. Madison Wisconsin, USA.

Rooks, S., Watson-Selph, A., Dutta, B., Gitaitis, R., Nischwitz, C., Mullis, S., Culbreath, A., and Csinos, A. 2015. Role of minor nutrients on tomato spotted wilt of tobacco (*Nicotania tabacum*) and superoxide-dismutase and NPR1 genes Phytopathology 105:S2.9 (Abstract)

Schaible, U. E., and Kaufmann, S. H. E. 2004. Iron and microbial infection. Nat. Rev. Microbiol. 2:946-953.

Schmittgen, T. D., and Livak, K. J. 2008. Analyzing real-time PCR data by the comparative C T method. Nat. Protoc. 3:1101.

Segond, D., Dellagi, A., Lanquar, V., Rigault, M., Patrit, O., Thomine, S., and Expert, D. 2009. NRAMP genes function in *Arabidopsis thaliana* resistance to *Erwinia chrysanthemi* infection. Plant J. 58:195-207.

Selph, A., Dutta, B., Burrell, D., and Gitaitis, R. 2014. Effect of crop rotation and of soil and plant tissue micronutrients on sour skin incidence in Vidalia onion Phytopathology 104:S2.9 (Abstract)

Seo, Y., and Kim, Y. H. 2017. Pathological interrelations of soil-borne diseases in cucurbits caused by *Fusarium* species and *Meloidogyne incognita*. Plant Pathol. J. 33:410.

Servin, A., Elmer, W., Mukherjee, A., De la Torre-Roche, R., Hamdi, H., White, J. C., Bindraban, P., and Dimkpa, C. 2015. A review of the use of engineered nanomaterials to suppress plant disease and enhance crop yield. J. Nanopart. Res. 17:92.

Siddiqui, I., and Shaukat, S. 2002. Rhizobacteria-mediated induction of systemic resistance (ISR) in tomato against *Meloidogyne javanica*. J. Phytopathol. 150:469-473.

Staswick, P. 1994. Storage proteins of vegetative plant tissues. Annu. Rev. Plant Biol. 45:303-322.

Steiner, A. A. 1984. The universal nutrient solution. Pages 633-649 in: Proceedings of the Sixth International Congress on Soilless Culture, Wageningen, The Netherlands.

Sumner, D. R., and Johnson, A. W. 1973. Effect of root-knot nematodes on Fusarium wilt of watermelon. Phytopathology 63:857-861.

Tamaoki, D., Seo, S., Yamada, S., Kano, A., Miyamoto, A., Shishido, H., Miyoshi, S., Taniguchi, S., Akimitsu, K., and Gomi, K. 2013. Jasmonic acid and salicylic acid activate a common defense system in rice. Plant Signal. Behav. 8:e24260.

Taylor, A., and Sasser, J. 1978. Biology, identification and control of some root-knot nematodes (*Meloidogyne* spp.) Raleigh (NC): Department of Plant Pathology and Genetics, North Carolina State University and USAID:111.

Thies, J. A., Ariss, J. J., Hassell, R. L., Buckner, S., and Levi, A. 2015. Accessions of *Citrullus lanatus* var. *citroides* are valuable rootstocks for grafted watermelon in fields infested with root-knot nematodes. HortScience 50:4-8.

Thies, J. A., Ariss, J. J., Kousik, C. S., Hassell, R. L., and Levi, A. 2016. Resistance to southern root-knot nematode (*Meloidogyne incognita*) in wild watermelon (*Citrullus lanatus var. citroides*). J. Nematol. 48:14.

Thies, J. A., Ariss, J. J., Hassell, R. L., Olson, S., Kousik, C. S., and Levi, A. 2010. Grafting for management of southern root-knot nematode, *Meloidogyne incognita*, in watermelon. Plant Dis. 94:1195-1199.

Thompson, I. A., and Huber, D. M. 2007. Manganese and plant disease. Pages 139-153 in: Mineral nutrition and plant disease. L. E. Datnoff, W. H. Elmer and D. M. Huber, eds. American Phytopathological Society (APS Press).

Turechek, W., and Stevenson, K. 1998. Effects of host resistance, temperature, leaf wetness, and leaf age on infection and lesion development of pecan scab. Phytopathology 88:1294-1301.

Upchurch, R. G., and Ramirez, M. E. 2010. Defense-related gene expression in soybean leaves and seeds inoculated with *Cercospora kikuchii* and *Diaporthe phaseolorum* var. *meridionalis*. Physiol. Mol. Plant P. 75:64-70.

USDA-NASS. 2019. Quick Stats. USDA National Agricultural Statistics Service. Available at https://data.nal.usda.gov/dataset/nass-quick-stats. Accessed 2020-03-24.

Van Loon, L. 1997. Induced resistance in plants and the role of pathogenesis-related proteins. Eur. J. Plant Pathol. 103:753-765.

Vijayan, P., Shockey, J., Lévesque, C. A., and Cook, R. J. 1998. A role for jasmonate in pathogen defense of *Arabidopsis*. Proc. Natl. Acad. Sci. U.S.A. 95:7209-7214.

Wang, L., and Wu, J. 2013. The essential role of jasmonic acid in plant–herbivore interactions–using the wild tobacco *Nicotiana attenuata* as a model. J. Genet. 40:597-606.

Watson-Selph, A., Gitaitis, R., and Dutta, B. 2016. Micronutrients may affect plant disease severity by interacting with components of the systemic acquired resistance pathway. Phytopathology 106:S2.6. (Abstract)

Wechter, W. P., Kousik, C., McMillan, M., and Levi, A. S. 2012. Identification of resistance to *Fusarium oxysporum* f. sp. *niveum* race 2 in *Citrullus lanatus* var. *citroides* plant introductions. HortScience 47:334-338.

Wu, Y., Zhang, D., Chu, J. Y., Boyle, P., Wang, Y., Brindle, I. D., De Luca, V., and Després, C. 2012. The *Arabidopsis* NPR1 protein is a receptor for the plant defense hormone salicylic acid. Cell Rep. 1:639-647.

Yang, Y. X., Wu, C., Ahammed, G. J., Wu, C., Yang, Z., Wan, C., and Chen, J. 2018. Red light-induced systemic resistance against root-knot nematode is mediated by a coordinated regulation of salicylic acid, jasmonic acid and redox signaling in watermelon. Front. Plant Sci. 9:899.

Ye, F., Albarouki, E., Lingam, B., Deising, H. B., and von Wirén, N. 2014. An adequate Fe nutritional status of maize suppresses infection and biotrophic growth of *Colletotrichum graminicola*. Physiol. Plant. 151:280-292.

Yetişir, H., Sari, N., and Yücel, S. 2003. Rootstock resistance to Fusarium wilt and effect on fruit yield and quality of watermelon. Phytoparasitica 31:163-169.

Zhang, M., Xu, J., Liu, G., Yao, X., Li, P., and Yang, X. 2015. Characterization of the watermelon seedling infection process by *Fusarium oxysporum* f. sp. *niveum*. Plant Pathol. 64:1076-1084.

Zhou, X., and Everts, K. 2004. Suppression of Fusarium wilt of watermelon by soil amendment with hairy vetch. Plant Dis. 88:1357-1365.

Zhou, X., Everts, K., and Bruton, B. 2010. Race 3, a new and highly virulent race of *Fusarium oxysporum* f. sp. *niveum* causing Fusarium wilt in watermelon. Plant Dis. 94:92-98.

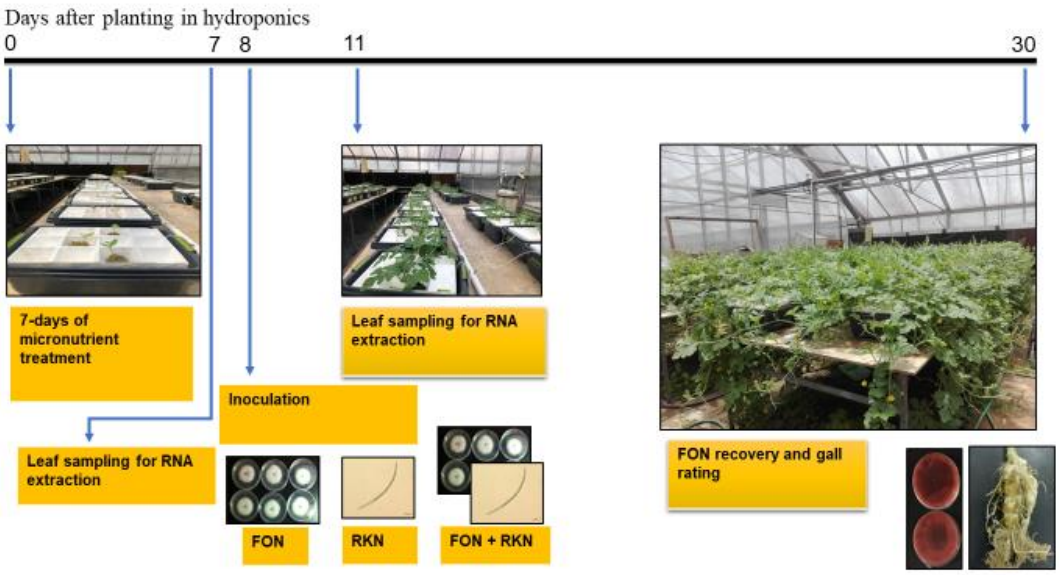


Figure 2.1. Schematic representation of the timeline (in days) of the greenhouse experiment and associated sampling to evaluate the effects of micronutrients (Fe or Mn or Zn) on induced resistance in watermelon seedlings when challenged inoculated with *Fusarium oxysporum* f. sp. *niveum* (FON) and *Meloidogyne incognita* (RKN) or both.

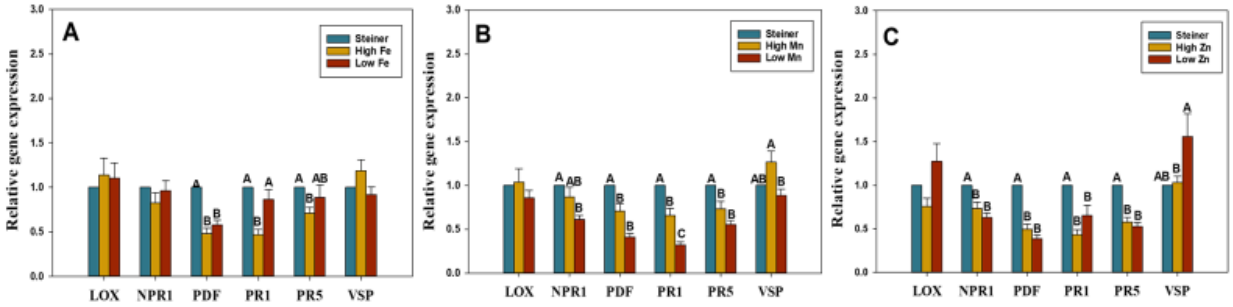


Figure 2.2. Relative expression of nonexpressor pathogenesis-related gene 1 (*NPR1*), pathogenesis-related protein 1 (*PR1*), pathogenesis-related protein 5 (*PR5*), lipoxygenase (*LOX*), plant defensin (*PDF*), and vegetative storage protein (*VSP*) genes by qRT-PCR in watermelon leaves at 7 day-post treatment with micronutrients (Fe, Mn and Zn). Watermelon seedlings (cv. Sugar Baby; 3-weeks old) were either treated with Fe or Mn or Zn at high (3X), low (0.5X) and standard concentration (X, Steiner) for 7 days. Data are the mean fold changes ± SE in gene transcript levels in tissues from micronutrient treated plants relative to tissues from non-treated control plants in Steiner. Letters indicate a significant difference between treatments with the Tukey-Kramer test ($P<0.05$).

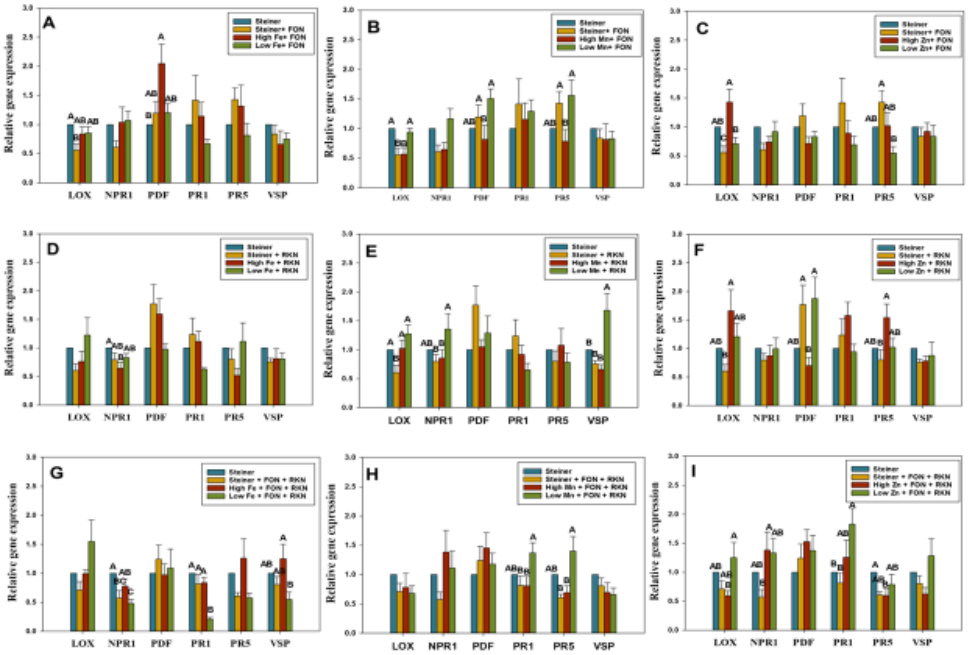


Figure 2.3. Relative expression of *NPR1*, *PR1*, *PR5*, *LOX*, *PDF* and *VSP* genes by qRT-PCR in leaves of watermelon plants at 11 day-post treatment with different micronutrients (Fe, Mn and Zn) at high (3X), low (0.5X) and standard concentration (X, Steiner) via hydroponics system and 3 day-post inoculation with 1ml of 5×10^5 microconidia of *Fusarium oxysporum* f.sp. *niveum* (FON) or 1ml of 6000 active J2s of *Meloidogyne incognita* (RKN) or both. Data are the mean fold changes ± SE in genes transcripts levels of tissues from inoculated plants in micronutrient treatments relative to tissues from non-inoculated control plants in Steiner. Letters indicate a significant difference between treatments with the Tukey-Kramer test (P<0.05).

Table 2.1. Mineral concentrations (mg. L^{-1}) in nutrient solutions used in this study.

Micronutrient treatments	N	P	K	Ca	Mg	B	Cu	Mo	Fe	Mn	Zn
Steiner[a]	256	48	304	180	48	1	0.2	0.1	3	1	0.4
High Fe	256	48	304	180	48	1	0.2	0.1	**9**	1	0.4
Low Fe	256	48	304	180	48	1	0.2	0.1	**1.5**	1	0.4
High Mn	256	48	304	180	48	1	0.2	0.1	3	**3**	0.4
Low Mn	256	48	304	180	48	1	0.2	0.1	3	**0.5**	0.4
High Zn	256	48	304	180	48	1	0.2	0.1	3	1	**1.2**
Low Zn	256	48	304	180	48	1	0.2	0.1	3	1	**0.2**

[a]Steiner solution is modified from Steiner's universal nutrient solution with composition (mg.L^{-1}); N-168 **[$NH_4H_2PO_4$, KNO_3, $Ca(NO_3)_2$]**, P-31 **($NH_4H_2PO_4$)**, K-273 **(KNO_3)**, Ca-180 **[$Ca(NO_3)_2$]**, Mg-48 **($MgSO_4$)**, B-0.44 **(H_3BO_3)**, Cu-0.02 **($CuSO_4$)**, Mo-0.1 **($Na_2MoO_4 \cdot 2H_2O$)**, Fe- 2 to 4 **(Fe Chelate; Sequestrene 330)**, Mn-0.62 **($MnCl_2$)** and Zn-0.11 **($ZnSO_4 \bullet 7H_2O$)**.

Table 2.2. The list of genes, primer sequences, and quantitative polymerase chain reaction (PCR) conditions used in this study.

Genes	Forward Primer Sequence (5'-3')	Reverse Primer Sequence (3'-5')	Comments	PCR conditions
NPR1	CGCTGCCGATATG CATGTGA	GTCAACCTTCAGC AAGTTGCCA	This study	95℃ for 2 min; 35 cycles of 95℃ for 20 s, 62 ℃ for 30 s, and 72 ℃ for 60 s; and final extension of 72℃ for 6 min
PR1	GACTCGCCTCAAGA CTTTGT	GATGCGTTGGTTG GCATATTG	Mandal et al., 2018	95℃ for 3 min; 40 cycles of 95℃ for 10 s, 60 ℃ for 30 s, and 72 ℃ for 30 s; and final extension of 72℃ for 6 min
PR5	CCTGGAGCGTCAAA GTCATTTA	CTCCAGTTAAGCA GGTGATACG	Mandal et al., 2018	95℃ for 3 min; 40 cycles of 95℃ for 10 s, 60 ℃ for 30 s, and 72 ℃ for 30 s; and final extension of 72℃ for 6 min
LOX	TCTCAACTGTGCT CCCATTC	GGAAGCAGTGGC TTTGAATTAC	This study	95℃ for 3 min; 40 cycles of 95℃ for 10 s, 60 ℃ for 30 s, and 72 ℃ for 30 s; and final extension of 72℃ for 6 min
PDF	GCGAAGGTGTGCG AGAA	CATGGCAAGCTC CATGTTTG	This study	95℃ for 3 min; 40 cycles of 95℃ for 10 s, 60 ℃ for 30 s, and 72 ℃ for 30 s; and final extension of 72℃ for 6 min
VSP	ACCAAGGGAAGTC AGCAATAC	CCGAAACTGACG TACCCAATAA	This study	95℃ for 3 min; 40 cycles of 95℃ for 10 s, 60 ℃ for 30 s, and 72 ℃ for 30 s; and final extension of 72℃ for 6 min

Table 2.3. Mean concentrations (mg. L^{-1}) of Fe, Mn and Zn in nutrient solutions available at 0 and 7 day-post hydroponics treatment.

Micronutrient	Micronutrient treatment	Day 0 (mg. L^{-1}; applied)	Day 7 (mg. L^{-1})
Fe	High Fe	9.0a[1]	9.28±0.29a
	Steiner	3.0b	2.35±0.33b
	Low Fe	1.5c	1.40±0.05c
Mn	High Mn	3.0a	2.25±0.12a
	Steiner	1.0b	0.57±0.08b
	Low Mn	0.5c	0.29±0.03c
Zn	High Zn	1.2a	1.03±0.05a
	Steiner	0.4b	0.43±0.08b
	Low Zn	0.2c	0.34±0.07b

[1]Means ± standard error followed by different letters in same column under same section for each micronutrient are significantly different with the Tukey-Kramer test (P<0.05).

Table 2.4. Final Fe, Mn and Zn concentrations of foliar tissue in hydroponics system inoculated with *Fusarium oxysporum* f.sp *niveum* (FON), *Meloidogyne incognita* (RKN) or both under greenhouse conditions.

Foliar Fe (mg.kg^{-1} dry weight)			
		Micronutrient treatment	
Pathogen	High Fe	Low Fe	Steiner
FON	154.41±6.35AB	149.19±15.34	136.80±15.89
RKN	137.40±7.27Bb	183.09±13.03a	138.59±10.37b
FON and RKN	141.64±8.33AB	172.19±17.99	135.80±11.40
Control	181.31±14.21Aa	159.38±5.49ab	136.43±7.45b
Foliar Mn (mg.kg^{-1} dry weight)			
		Micronutrient treatment	
Pathogen	High Mn	Low Mn	Steiner
FON	197.81±18.44a	173.53±28.11ABab	100.16±9.74b
RKN	175.86±11.79a	180.93±25.67Aa	110.41±7.62b
FON and RKN	180.86±33.21a	98.39±13.09Bb	113.92±9.86ab
Control	270.61±49.43a	114.29±5.41ABb	110.70±14.44b
Foliar Zn (mg.kg^{-1} dry weight)			
		Micronutrient treatment	
Pathogen	High Zn	Low Zn	Steiner
FON	110.66±8.94	104.73±24.37	96.09±13.042
RKN	109.67±8.69	119.71±22.84	90.38±13.97
FON and RKN	105.39±15.02	87.47±15.07	93.16±7.44
Control	130.25±10.08a	92.93±9.99ab	85.17±11.64b

Means within the same treatment class (Foliar Fe, Foliar Mn, and Foliar Zn) followed by different uppercase (column-wise comparison in each section) and lowercase (row-wise comparison in each section) letters are significantly different at $P < 0.5$ according to Tukey-Kramer test.

CHAPTER 3

EVALUATE IF MICRONUTRIENT AFFECT TRANSCRIPT-LEVEL CHANGES IN WATERMELON IN RELATION TO FUSARIUM OXYSPORUM F. SP. NIVEUM AND MELOIDOGYNE INCOGNITA UNDER HYDROPONICS CONDITIONS

Karki, K., Coolong, T., Mandal, M., Kousik, C., Myers, B., Hajihassani, A., and Dutta, B. 2020. To be submitted to Plant Disease.

Abstract

Zinc (Zn) accumulation and deficiency affect plant response to pests and diseases differently in varying pathosystem. The concentrations of Zn in plants aid in priming defense signaling pathways and help in enhanced structural defenses against plant pathogens. Studies are lacking on how concentrations of Zn in watermelon plant influence defense against two important soil-borne pathogens: *Fusarium oxysporum* f. sp. *niveum* (FON) and southern root-knot nematode (RKN, *Meloidogyne incognita*). In this study we present a comparative transcriptomics evaluation of watermelon plants in response to different levels of Zn (High: 1.2ppm; and low: 0.2ppm). In addition, we evaluated as to how transcript level-responses will differ in watermelon plants when infected with either FON or RKN or both under high- and low-Zn treatment regimes in a controlled hydroponics system. Higher numbers of differentially expressed genes (DEGs) were observed in high Zn-treated than in low Zn-treated non-inoculated plants (high Zn: low Zn :: 333:14), in plants inoculated with FON alone (high Zn: low Zn :: 524:104), and in plants inoculated with RKN alone (high Zn: low Zn :: 2908:2). However, in the co-inoculated system, low Zn treatment had 268 DEGs and high Zn treatment only had one DEG. In addition, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis revealed that most DEGs could be significantly enriched in hormone signal transduction and MAPK signaling pathway suggesting an induction of systemic resistance with high Zn concentrations. Taken together, this study substantially expands transcriptome data resources and suggests a molecular framework for watermelon-Zn interaction in different pathosystem.

Introduction

The Fusarium wilt fungus (*Fusarium oxysporum* f. sp. *niveum*, FON) and the root-knot nematode (*Meloidogyne incognita* (Kofoid and White) Chitwood; RKN) are important soil-borne pathogens causing severe damage in watermelon production throughout the world (Davis 2007; Lynch and Carpenter 1999; Martyn 2014; Thies et al. 2010). FON can infect and induce symptoms in watermelon plants at any growth stage (Beattie and Doolittle 1951). Symptoms of Fusarium wilt in the seedling stage include dieback and scorched appearance. In mature plants, foliage discoloration is followed by loss of turgor pressure, general chlorosis of tissue and unilateral wilting of the vine. As the disease progresses, complete vine wilting is often experienced. FON has four physiological races (0, 1, 2, and 3) based on variability in aggressiveness on differential cultivars (Zhou et al. 2010). RKN is an obligatory parasite that induces galls on the infected root system of a susceptible host. Galls disrupt the vascular system of plants making them grow poorly and can lead to death under heavy infestation (Dale 1973; Newton et al. 2012). Pre-plant fumigant application with methyl bromide was effective in reducing both FON and RKN in the watermelon production system. Phasing out of MeBr in accordance with the Montreal Protocol (1993) resulted in limited options for managing both soil-borne pathogens.

Triploid seedless watermelon cultivars that are currently cultivated widely, do not possess a high level of resistance against FON race 2 (Wechter et al. 2012) and to complicate the issue FON race 3 has been identified in several states of the U.S. There is only one fungicide Proline (prothioconazole; Bayer Crop Science) labeled for use on watermelon to manage FON (Miller et al. 2020). Increased selection pressure on FON pathogen populations might lead to increased resistance to the fungicides. Grafting susceptible watermelon scions to interspecific hybrid

squash (*Cucurbita maxima* Duch. Ex Lam. × *C. moschata* Duch. Ex Poir) and bottle gourd (*Lagenaria siceraria* (Molina) Standl.) can be a viable option for FON management. One of the most important drawbacks with bottle gourd and *C. moschata* × *C. maxima* hybrids rootstocks is their susceptibility to root-knot nematode and also, the grafted plants are considerably more expensive than the non-grafted ones (Keinath and Hassell 2014). Therefore, an alternative method of FON management is required that along with the available options can provide integrated management of this disease.

Plant disease resistance is affected by the plants' genetics and by the environment in which it is grown, including nutrient deficiencies and toxicity (Agrios 2005; Krauss 1999; Marschner 1995). Induced resistance has been proposed as one of the explanations for the interaction between specific nutrients and the increase and decrease in plant disease (Dordas 2008). Induced resistance is a plant-based defense system that is elicited by specific environmental stimuli, which enables them to resist biotic challenges (Van Loon et al. 1998). This defense responses include oxidative burst, changes in cell composition and synthesis of antimicrobial compounds such as phytoalexins (Walters et al. 2005). The involvement of mineral nutrients in inducing resistance has been studied in several plant-pathogen systems. For instance, many investigations have reported potassium salts as a chemical agent for induction of plant resistance against powdery mildew on cucumber (*Cucumis sativus*) (Reuveni et al. 2000), pepper (*Capsicum annuum*) (Reuveni et al. 1998), tomato (*Solanum lycopersicum*) (Ehret et al. 2002) and sugar beet (*Beta vulgaris*) (Mosa 2002). However, the impact of a particular nutrient cannot be generalized for all plant-pest/pathogen systems, either globally or individually (Agrios 2005; Huber 1980). Potassium application has also been shown to increase downy mildew in muskmelon (*Cucumis melo*), caused by *Pseudoperonospora cubensis*, and microdochium patch

caused by *Microdochium nivale* in creeping bentgrass (*Agrotis stolonifera*) (Bains and Jhooty 1976; Bier et al. 2018). Therefore, a more detailed understanding of the relationship between plant responses to nutritional status and biotic stresses is essential.

Zinc plays a crucial role in the plant response to pests and diseases. Plants naturally absorb high concentrations of metals such as Zn from the substrate as a self-defense mechanism directly against pathogens and herbivores (Babitha et al. 2002; Rolke et al. 2004; Stolpe et al. 2017). Metal ions may activate defense reactions through a signaling pathway or by plant fortification. Several studies have demonstrated that Zn fertilization decreases disease severity of Phytophthora root rot and common leaf spot disease in alfalfa (*Medicago sativ*a L.), peach gummosis in peach (*Prunus persica*) and early blight in potato (*Solanum tuberosum*) (Grewal et al. 1996; Li et al. 2016; Machado et al. 2018). However, a protective concentration of Zn against certain pathogens can also induce an increased susceptibility to another pathogen on the same host (Helfenstein et al. 2015). It is currently not well understood how watermelon responds to FON and RKN infections under conditions with varying levels of Zn. In our previous study, we observed that differing levels of Zn can affect induced resistance genes in salicylic acid (SA) and jasmonic acid (JA) pathway. In the present study, hydroponic experiment was conducted to investigate the transcriptomic changes induced by varying level of Zn with or without challenge inoculation with either FON or RKN or both.

Materials and methods

Experimental set-up. Seeds of watermelon cultivar “Sugar Baby”, which is susceptible to both FON and RKN, were sown into sheets of 2.54 cm^2/cell Rockwool cubes (Grodan Inc., Hedehusene, Denmark) and covered with a thin layer of vermiculite. The sheet was kept moist

with water as needed. After germinating, seedlings were watered as needed and fertilized once with a “Steiner solution”, which was modified from the Steiner universal nutrient solution. The composition of the Steiner solution was as follows ($mg.L^{-1}$): N-256 $mg.L^{-1}$ [$NH_4H_2PO_4$, KNO_3, $Ca(NO_3)_2$], P-48 $mg.L^{-1}$ ($NH_4H_2PO_4$), K-304 (KNO_3), Ca-180 $mg.L^{-1}$ [$Ca(NO_3)_2$], Mg-48 $mg.L^{-1}$ ($MgSO_4$), B-1 $mg.L^{-1}$ (H_3BO_3), Cu-0.2 $mg.L^{-1}$ ($CuSO_4$), Mo-0.1 $mg.L^{-1}$ ($Na_2MoO_4 \cdot 2H_2O$), Fe- 3 $mg.L^{-1}$ (Fe Chelate; Sequestrene 330), Mn-1 $mg.L^{-1}$ ($MnCl_2$) and Zn-0.4 $mg.L^{-1}$ ($ZnSO_4 \bullet 7H_2O$). Seedlings were maintained for three weeks on Rockwool and then were transferred to plastic containers (43.05 cm W × 30.48 cm D × 19.81 cm H) containing 8L solution of respective micronutrient treatments. The micronutrient treatments were prepared by modifying the Zn composition in the Steiner solution mentioned above. The micronutrient treatments included: high Zn [HZ: 3X concentration of Zn in Steiner solution, (1.2ppm)], low Zn [LZ: 0.5X concentration of Zn in Steiner solution, (0.2ppm)], and Steiner [Stnr: X concentration of Zn (0.4 ppm)].

Three seedlings along with Rockwool were placed equidistant in a nine-well styrofoam tray with holes at the bottom so that roots could suspend down and be in contact with the nutrient solution. The styrofoam tray was then placed over the plastic container filled with micronutrient treatments, as indicated above. Solutions were aerated through a 15.2 cm aquarium air-stone fitted with plastic-tubes (0.3-mm diameter) and an air-pump (Pentair aquatic eco-systems Inc., Apopka, USA). Plastic containers were maintained to the original volume (8L) by adding dH_2O every two days. At 8-day post-treatment (DPT), the micronutrient treatments were either inoculated with FON or RKN or co-inoculated with both FON and RKN. For FON inoculation, a 1ml suspension containing 5×10^5 microconidia/ml (race 2) was applied at the base of watermelon seedling. For nematode inoculation, a 1ml suspension containing 6000 active RKN

J2s (race 3) was added at the base of the seedling. For treatments that were inoculated with both FON and RKN, a similar inoculation approach was adopted and the inocula were applied simultaneously. Plants were maintained at 28°C mean greenhouse temperature. Three replicates (plastic container with three seedlings each) per treatment were used in the experiment and treatments were arranged in a completely randomized design.

RNA extraction, quantification and integrity determination. Leaf samples (*n*=3 per replicate/treatment) were collected by cutting the 3rd or 4th leaf from the terminal with a pair of sterile scissors at 7 days post treatment (dpt) and at 11 dpt [3 days post-inoculation (dpi))]. Samples were immediately stored in liquid nitrogen and later transferred to a -80ºC freezer at the UGA Tifton Campus laboratory until needed for further analysis. Leaves were ground in liquid nitrogen and total RNA was extracted from 100 mg of ground leaf tissue using the manufacturer's protocol (RNeasy Plant Mini Kit). Concentrations were determined using a NanoDropTM Lite (Thermo Scientific., Wilmington, DE, USA) and a Qubit® RNA Assay Kit and a Qubit® 2.0 Fluorometer (Life Technologies, Frederick, MD, USA). A total of 45 RNA samples were sent to Novogene Corporation Inc. Sacramento, CA, USA for library construction, sequencing and bioinformatic analysis. The RNA purity was checked using the NanoPhotometer®Spectrophotometer (IMPLEN, CA, USA). RNA integrity was confirmed (RIN>7) using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, USA) with a minimum RNA integrated number of 8.

Library preparation and transcriptome sequencing. A total amount of 1µg RNA per sample was used to generate RNA-seq libraries using the NEBNext®Ultra^{TM} RNA library Prep Kit for Illimina® (NEB, USA) following the recommendations of the manufacturer, and index codes were added to attribute sequences to each sample. Briefly, mRNA was purified from total

RNA utilizing poly-T oligo-attached magnetic beads. Fragmentation was performed using divalent cations under elevated temperature in NEBNext First Strand Synthesis Reaction Buffer (5X). First-strand cDNA was synthesized using random hexamer primer and M- MuLV Reverse Transcriptase (RNAse H). Second strand cDNA synthesis was subsequently performed using DNA Polymerase I and RNase H. Remaining overhangs were converted into blunt ends through exonuclease/polymerase activities. Following the adenylation of 3' ends of DNA fragments, NEBNext Adaptor with hairpin loop structure was ligated to prepare for hybridization.

In order to select cDNA fragments of preferably 150-200 bp in length, the library fragments were purified using the AMPure XP system (Beckman Coulter, Beverly, USA). Then 3 µl USER Enzyme (NEB, USA) was used with size-chosen, adapter ligated cDNA at 37 ℃ for 15 min followed by 5 min at 95 ℃ before PCR. PCR was then performed with Phusion High-Fidelity DNA polymerase, Universal PCR primers, and Index (X) Primer. Finally, PCR products were purified (AMPure XP system) and the quality of the library was assessed on the Agilent Bioanalyzer 2100 system. The clustering of index-coded samples was performed on the cBOt Cluster Generation System using the P.E. Cluster Kit cBot- H.S. (Illumina) as instructed by the manufacturer. After cluster generation, the preparation of the library was sequenced on the Illumina platform and 125 bp/150 bp paired-end reads were generated.

Sequence read cleanup and mapping to genome. First, raw data (raw reads) of the fastq format were processed. Clean reads were obtained by removing reads containing the adapter, reads containing poly-N and low-quality readings (<Q20) from raw data. At the same time, the percentages of reads with Q20, and Q30 were calculated. All downstream analyses were based on clean, high-quality ≥Q20 data. Reference genome and gene model annotation files were downloaded directly from the genome website

(ftp://cucurbitgenomics.org/pub/cucurbit/genome/watermelon/WCG/v2/). Index of the reference genome was developed using hisat2 2.1.0 and paired-end clean reads were aligned to the reference genome Charleston Gray (Wu et al. 2019) using HISAT2.

Quantification of gene expression levels and differential expression of analysis. FeatureCounts v1.5.0-p3 was used to count the reads numbers mapped to each gene (Liao et al. 2014). Fragments Per Kilobase of transcript sequence per Millions or FPKM of each gene was then calculated based on the length of the gene and reads count mapped to this gene. Differential expression analysis was performed using the DESeq2 R package (1.14.1). The resulting *P* values were adjusted using the Benjamini & Hochberg method for controlling the false discovery rate (FDR). Corrected *P*-value of 0.05 and a log2 (foldchange) of 1 were set as the threshold for significantly differential expression.

GO and KEGG enrichment analysis of DEGs. Gene Ontology (GO) enrichment analysis of differentially expressed genes was performed by the clusterProfiler R package, in which gene length bias was corrected. GO terms with corrected *P*-value less than 0.05 were considered to be significantly enriched for a given set of genes. For pathway mapping, the Kyoto Encyclopedia of Genes and Genomics (KEGG) orthology database was adopted (http://www.genome.jp/kegg/). We used clusterProfiler R package to test the statistical enrichment of differential expression genes in KEGG pathways.

Results

Overview of the RNA-Seq Results. In order to explore the transcript level changes in watermelon mediated with different levels of Zn and pathogen inoculation (FON or RKN or both), 45 cDNA libraries were constructed. Three biological replicates were used for each

treatment. A total of raw reads 800,231,485 were generated. After removing the low-quality reads and trimming the adapter sequences, we obtained 787,477,379 (98.41%) clean reads. Using HISAT2 software, 765227330 (97.30%) clean reads were mapped to the watermelon genome, and 724923705 (92.15%) unique reads were mapped to the reference genome. The Q20 percentage was over 98%, and the Q30 percentage was over 98.49% (Table 3.1).

Differently expressed genes (DEGs). A comparative gene expression analysis of high Zn (HZ) and low Zn (LZ) treated plants under inoculated [FON (F) or RKN (R) or both(F_R)] and non-inoculated system was conducted. The non-inoculated micronutrient control (Stnr) was used as a control nutrient solution. In non-inoculated plants, the number of DEGs in plants treated with high Zn was higher than plants treated with low Zn at both 7 and 11 dpt (Fig. 3.1). At 7 dpt, there were eight DEGs in plants treated with high Zn and six DEGs in plants treated with low Zn. High Zn treated plants had five upregulated genes and three downregulated genes. In low Zn treated plants, three genes were upregulated and three were downregulated. At 11 dpt, there were 333 DEGs in plants treated with high Zn and 14 DEGs in plants treated with low Zn. In high Zn treated plants, 113 genes were upregulated and 220 genes were downregulated whereas low Zn treated plants had 13 upregulated genes and one downregulated gene.

In plants inoculated with FON alone, a similar pattern was observed, DEGs in plants treated with high Zn (524) was higher than Steiner (100) and low Zn (104) treated plants. In high Zn treated plants inoculated with FON, 447 genes were upregulated and 77 genes were downregulated. Steiner treated plants inoculated with FON had 77 upregulated genes and 23 downregulated genes. Low Zn treated plants inoculated with RKN had 79 upregulated genes and 25 downregulated genes.

Results showed a higher number of DEGs in high Zn (2908) treated plants compared to Steiner (0) and low Zn (2) also in RKN alone inoculated plants. Among the 2908 DEGs in high Zn treated plants inoculated with RKN, 1522 genes were upregulated, and 1386 genes were downregulated. The two genes in low Zn treated plants inoculated with RKN were upregulated.

In plants inoculated with both FON and RKN, low Zn (489) treated plants had the highest number of DEGs, followed by Steiner (19) and high Zn (1). The one DEG in high Zn treated plants inoculated with FON and RKN was upregulated. There were 11 upregulated and 8 downregulated genes in Steiner treated plants inoculated with FON and RKN. In low Zn treated plants inoculated with FON and RKN, 268 genes were upregulated and 221 genes were downregulated.

GO enrichment analyses. GO terms with corrected *P*-value less than 0.05 were considered significantly enriched by differential expressed genes and are shown in Fig. 3.2. Significantly enriched GO terms were observed only at 11 dpt. In the biological process, movement of cell or subcellular component, microtubule-based process and microtubule-based movement were the significantly enriched terms common in high Zn treated plants inoculated with FON alone, low Zn treated plants inoculated with FON alone, high Zn treated plants inoculated with RKN alone, and low Zn treated plants inoculated with FON and RKN (Fig. 3.2A). In the cellular component, thylakoid part, thylakoid and photosystem II were significantly enriched terms in both high Zn treated plants and high Zn treated plants inoculated with RKN alone. In high Zn treated plants inoculated with FON alone, chromosome, chromosomal part and chromatin were the most enriched terms (Fig. 3.2B).

Moreover, in molecular function, exopeptidase activity was the only significantly enriched term in low Zn treated plants (Fig. 3.2C). In high Zn treated plants inoculated with FON alone,

transcription factor activity sequence-specific DNA binding, nucleic acid binding transcription factor activity and protein dimerization activity were most enriched terms. The most enriched term in Steiner treated plant inoculated with FON alone were transcription factor activity sequence-specific DNA binding, nucleic acid binding transcription factor activity and sequence-specific DNA binding. Similarly, tubulin binding, protein complex binding and motor activity were most enriched terms in low Zn treated plants inoculated with FON alone. High Zn treated plants inoculated with RKN alone showed the most enrichment of cytoskeletal protein binding, macromolecular complex binding and protein complex binding. In low Zn treated plants inoculated with FON and RKN, pyrophosphatase activity, nucleoside-triphosphatase activity and hydrolase activity acting on acid anhydrides in phosphorus-containing anhydrides were the most enriched terms.

KEGG classification of DEGs. KEGG enrichment results showed that DEGs were classified into multiple signaling pathways (Table 3.2). Significant KEGG (corrected *P*-value less than 0.05) enrichment was observed in plants treated with high Zn at 7 dpt. At 11 dpt plants in treatments: high Zn inoculated with FON alone, low Zn inoculated with FON alone, and high Zn inoculated with RKN alone showed significant enrichment. Peroxisome and glycosaminoglycan degradation were significantly enriched with two and one DEGs, respectively, in plants treated with high Zn at 7 dpt. Two pathways, plant hormone signal transduction and plant-pathogen interaction, were significantly enriched in high Zn treated plants inoculated with FON alone. In plants treated with low Zn that were inoculated with FON, alpha-Linolenic acid metabolism, valine, leucine and isoleucine biosynthesis and linoleic acid metabolism were significantly enriched. Metabolic pathways, carbon fixation in photosynthetic organisms, photosynthesis, pentose phosphate pathway, starch and sucrose metabolism,

ascorbate and aldarate metabolism, carbon metabolism, photosynthesis-antenna proteins and carotenoid biosynthesis were enriched in high Zn treated plants inoculated with RKN alone.

Investigation of DEGs associated with plant phytohormone signaling pathway in plants treated with high and low Zn. Phytohormone signaling has been shown to have crucial regulatory role in planta (Flors et al. 2008). We have therefore studied the effect of different levels of Zn on changes in the phytohormone signaling pathway. As shown in Fig. 3.3, many genes involved in the synthesis and regulation of abscisic acid, auxin, brassinosteroid, cytokinin, ethylene, jasmonic acid and salicylic acid were affected.

Jasmonic acid is a major class of plant hormones involved in mediating plant response to biotic and abiotic stress. Four genes predicted to be jasmonate zim domain protein (JAZ: ClCG07G014490, ClCG07G005870, ClCG06G001800, ClCG03G014890) and one gene predicted to be transcription factor MYC2-like protein (MYC2: ClCG10G001590) were induced. Number as well as expression of these genes was higher in high Zn treatments (high Zn inoculated with FON- and RKN-alone) (Fig. 3.3).

Salicylic acid is a key phytohormone that induces plants to produce systemic acquired resistance (SAR) to defend against pathogens (Kumar 2014). Nonexpressor of pathogenesis-related genes 1 (NPR1: ClCG04G004170) and pathogenesis-related protein1 (PR1: ClCG02G007190) are key regulators in the SA-dependent pathway (Nobuta et al. 2007). In high Zn treated plants inoculated with RKN alone, one NPR1 gene was significantly upregulated, while one PR-1 gene was significantly downregulated. Ethylene plays a vital role in plant development and the initiation of defense mechanisms against pathogens (Blanco-Ulate et al. 2013). Only one gene associated with the ethylene signaling pathway, ethylene-responsive

transcription factor (ERF1/2: ClCG05G004370) was induced in high Zn treated plants inoculated with RKN alone.

Genes related to abscisic acid, auxin, brassinosteroid and cytokinin signaling pathways were differentially expressed with the difference in Zn level. In abscisic acid pathway, serine/threonine-protein kinase SRK2 (SnRK2: ClCG00G003890), abscisic acid receptor PYR/PYL family (PYR/PYL: ClCG05G017790, ClCG05G000910), and protein phosphatase 2C (PP2C: ClCG03G001230, ClCG03G016180, ClCG05G008820) were affected. Both the genes related to abscisic acid pathway were downregulated in plants treated with high Zn while in high Zn treated plants inoculated with RKN 60 % of these genes were upregulated.

In auxin signaling pathway, SAUR family protein (SAUR: ClCG05G018470), auxin responsive GH3 gene family (GH3: ClCG07G012040, ClCG05G015030, ClCG05G010400, ClCG01G017190), auxin-responsive protein IAA (AUX/IAA: ClCG11G006270, ClCG11G003560, ClCG07G013340, ClCG07G000600, ClCG06G011420, ClCG06G008840, ClCG05G004670, ClCG01G018130), auxin transporter-like protein 3 (AUX1: ClCG02G011740, ClCG02G004210), and auxin response factor (ARF: ClCG09G009470, ClCG05G018490, ClCG07G011440) were affected. A maximum number of genes were affected in the high Zn treatments inoculated with FON- and RKN-alone. In plants treated with high Zn one gene out of two was upregulated. In high Zn treated plants inoculated with FON, all six genes were upregulated. In high Zn treated plants inoculated with RKN, 12 out of 15 genes were upregulated. Both low Zn treated plants inoculated with FON and Steiner treated plants inoculated with FON had one upregulated gene. In low Zn treated plants inoculated with FON and RKN, two out of four genes were upregulated. In the Brassinosteroid pathway, cyclin D3 (CYCD3: ClCG08G016160, ClCG05G020230, ClCG02G006220, ClCG05G010220) and

brassinosteroid signaling positive regulator family protein (BZR1/2: ClCG08G015300) were affected, and all of these genes were upregulated. Similarly, histidine phototransfer protein (AHP: ClCG09G000190), Arabidopsis histidine kinase 2/3/4 (cytokinin receptor) (CRE1: ClCG11G001010) and type-B Arabidopsis response regulator (B-ARR: ClCG05G002710) were affected in the cytokinin pathway.

Investigation of DEGs associated with MAPK signaling pathway in plants treated with high and low Zn. Previous studies have demonstrated that the MAPK signaling pathway widely exists in eukaryotic organisms and participates in plant growth and responds to abiotic and biotic stress. As shown in Fig. 3.4, a total of 26 genes in MAPK signaling were significantly affected. Results showed, effect in terms of number and induction level of genes was higher in high Zn treatments: (high Zn, high Zn inoculated with FON alone and high Zn inoculated with RKN alone). Genes such as WRKY DNA-binding protein 33 (WRKY33: ClCG03G000130, ClCG10G022500), respiratory burst oxidase protein D (RbohD: ClCG10G013100), MYC2 (ClCG10G001590, ClCG07G007900) and 1-amicocyclopropane-1-carboxylate synthase 6 (ACS6: ClCG07G007900) were significantly upregulated in high Zn treated plants inoculated with FON- and RKN-alone.

Discussion

In the present study, a whole transcriptome analysis was performed using RNA-seq in inoculated and non-inoculated (FON or RKN or both) watermelon plants in response to high Zn and low Zn level. We found higher number of DEGs in high Zn - than in low Zn-treated plants that were not inoculated (when at 7 dpt or 11 dpt), also in plants inoculated with FON or RKN alone. However, the effect of high Zn was not consistent in co-inoculated system, low Zn treated

plants inoculated with FON and RKN had higher DEGs than high Zn treated plants inoculated with FON and RKN. These findings indicate that high Zn treatment can alter differential expression of genes in watermelon, but the level of expression is not consistent across FON and RKN or co-infection system. DEGs for GO and KEGG pathway enrichments were also analyzed. We identified that expression level of many genes associated with phytohormone signaling pathway and MAPK signaling pathway were affected in high Zn treated plants particularly in high Zn treated plants inoculated with FON- or RKN-alone.

Hormone signaling pathways play significant roles in regulating the interaction between plants and microorganisms (Vrabka et al. 2019; Xie et al. 2018). We observed increased expression of JA pathway genes JAZ and MYC2 in high Zn treated plants inoculated with FON- and RKN-alone. The core signal transduction mechanism of JA signaling comprises JAZ and MYC. Specific JAZ/TFs are generated by JAZ and different TFs that explicitly regulate many downstream responses (Chini et al. 2016). The JAZ-MYC module triggers defense response or inhibit plant growth against pathogen infection by increasing the concentration of defense compounds (Havko et al. 2016). Gallego et al. (2017) also found that surplus Zn can potentiate the plant defense responses, especially in the synthesis of JA and its signaling pathway, thus improving plant resistance in *Arabidopsis thaliana* against *Alternaria brassicicola*. In addition, we observed increased expression of SA gene, NPR1, in high Zn treated plants inoculated with RKN. NPR1 plays an integral part in the efficacy of plant defense response. It activates PR gene expression by recruiting TGA transcription factors (Després et al. 2000). Arabidopsis NPR1 mutants showed decreased PR gene expression and increased susceptibility to pathogens (Roetschi et al. 2001). Also, ERF1/2 gene involved in ethylene signaling was upregulated in high Zn treated plants inoculated with RKN. Activation of ERF genes is known to enhance plant

disease resistance (Pré et al. 2008). This could mean that high Zn concentration in watermelon plants modulated induced resistance in watermelon plants against FON and RKN. It is further justified by number of genes involved in synthesis and regulation of SA, JA, abscisic acid, auxin, brassinosteroid and cytokinin that were significantly affected in high Zn treated plants inoculated with FON- and RKN-alone.

Plant MAPKs participate in plant growth, development, and responses to endogenous and environmental cues, Studies have shown that MAPKs could be activated by external sensors for cellular reactions (Wu and Tang 2004). Study conducted by Bi and Zhou (2017) demonstrated that several pathogen-secreted effectors inhibit the MAPK cascade which confirms that involvement of MAPKs in plant-pathogenic interactions and its role in plant response to pathogen invasion. Our study demonstrated that many genes in MAPK pathway were significantly affected in high Zn treated plants inoculated with FON- and RKN-alone. The genes significantly upregulated were WRKY33, RbohD, MYC2, and ACS6 genes. Genes in WRKY family have been confirmed to perform important regulatory functions to modulate pathogen-triggered cellular responses in a variety of plants, and most WRKY factors participate in the salicylic acid signaling pathway. RbohD is responsible for ROS production which is involved in regulation of immune function against various pathogens (Mittler et al. 2004; Torres et al. 2002). As mentioned earlier, MYC2 is triggers defense response via jasmonic acid pathway. Similarly, ACS regulates synthesis of ethylene which plays a positive role in plant resistance against fungal and bacterial pathogens (Guan et al. 2015; Wang et al. 2002).

In summary, we observed that high Zn level affects plant-pathogen interaction in watermelon by regulating many crucial plant defense pathways. Multiple genes in several hormone signaling pathways, including salicylic acid, jasmonic acid, ethylene, abscisic acid,

auxin, brassinosteroid and cytokinin were strongly altered. However, this effect was limited to FON and RKN solo inoculated system. In FON and RKN co-inoculated system, the induction of resistance with high Zn concentration was not observed. It is possible that the disease pressure was too high and was able to counteract the host resistance. Pathogens are also known to evolve strategies to counteract Zn-related plant defense (Cabot et al. 2019). Further study is essential to optimize the level of Zn that can successfully reduce disease severity in field level. To our knowledge, this is the first study to analyze the effect of Zn level on watermelon.

Literature cited

Agrios, G. N. 2005. Plant Pathology. 5th ed. Elsevier Academic Press, San Diego.

Babitha, M., Bhat, S., Prakash, H., and Shetty, H. 2002. Differential induction of superoxide dismutase in downy mildew-resistant and-susceptible genotypes of pearl millet. Plant Pathol. 51:480-486.

Bains, S., and Jhooty, J. 1976. Effect of mineral nutrition of muskmelon on downy mildew caused by *Pseudoperonospora cubensis*. Plant Soil 29:105.

Beattie, J., and Doolittle, S. 1951. Watermelons. US Government Printing Office, Washington, DC.

Bi, G., and Zhou, J.-M. 2017. MAP kinase signaling pathways: A hub of plant-microbe interactions. Cell Host Microbe 21:270-273.

Bier, P. V., Persche, M., Koch, P., and Soldat, D. J. 2018. A long term evaluation of differential potassium fertilization of a creeping bentgrass putting green. Plant Soil 431:303-316.

Blanco-Ulate, B., Vincenti, E., Powell, A. L., and Cantu, D. 2013. Tomato transcriptome and mutant analyses suggest a role for plant stress hormones in the interaction between fruit and Botrytis cinerea. Front. Plant Sci 4:142.

Cabot, C., Martos, S., Llugany, M., Gallego, B., Tolrà, R., and Poschenrieder, C. 2019. A role for zinc in plant defense against pathogens and herbivores. Front. Plant Sci 10:1171.

Chini, A., Gimenez-Ibanez, S., Goossens, A., and Solano, R. 2016. Redundancy and specificity in jasmonate signalling. Curr. Opin. Plant Biol. 33:147-156.

Dale, P. 1973. Elimination of root-knot nematodes from roses by chemical bare-root dips. N.Z. J. Exp. Agric. 1:121-122.

Davis, R. F. 2007. Effect of *Meloidogyne incognita* on watermelon yield. Nematropica 37:287-294.

Després, C., DeLong, C., Glaze, S., Liu, E., and Fobert, P. R. 2000. The *Arabidopsis* NPR1/NIM1 protein enhances the DNA binding activity of a subgroup of the TGA family of bZIP transcription factors. Plant Cell 12:279-290.

Dordas, C. 2008. Role of nutrients in controlling plant diseases in sustainable agriculture. A review. Agron. Sustain. Dev. 28:33-46.

Ehret, D., Utkhede, R., Frey, B., Menzies, J., and Bogdanoff, C. 2002. Foliar applications of fertilizer salts inhibit powdery mildew on tomato. Can. J. Plant Pathol., 24:437-444.

Flors, V., Ton, J., Van Doorn, R., Jakab, G., García-Agustín, P., and Mauch-Mani, B. 2008. Interplay between JA, SA and ABA signalling during basal and induced resistance against *Pseudomonas syringae* and *Alternaria brassicicola*. Plant J. 54:81-92.

Gallego, B., Martos, S., Cabot, C., Barceló, J., and Poschenrieder, C. 2017. Zinc hyperaccumulation substitutes for defense failures beyond salicylate and jasmonate signaling pathways of *Alternaria brassicicola* attack in Noccaea caerulescens. Physiol. Plant. 159:401-415.

Grewal, H. S., Graham, R. D., and Rengel, Z. 1996. Genotypic variation in zinc efficiency and resistance to crown rot disease (*Fusarium graminearum* Schw. Group 1) in wheat. Plant Soil. 186:219-226.

Guan, R., Su, J., Meng, X., Li, S., Liu, Y., Xu, J., and Zhang, S. 2015. Multilayered regulation of ethylene induction plays a positive role in Arabidopsis resistance against Pseudomonas syringae. Plant Physiol. 169:299-312.

Havko, N. E., Major, I. T., Jewell, J. B., Attaran, E., and Howe, G. A. 2016. Control of carbon assimilation and partitioning by jasmonate: An accounting of growth–defense tradeoffs. Plants 5:7.

Helfenstein, J., Pawlowski, M. L., Hill, C. B., Stewart, J., Lagos-Kutz, D., Bowen, C. R., Frossard, E., and Hartman, G. L. 2015. Zinc deficiency alters soybean susceptibility to pathogens and pests. J. Plant Nutr. and Soil Sci. 178:896-903.

Huber, D. 1980. The role of mineral nutrition in defense. Pages 381-406 in: Plant disease: an advanced treatise: how plants defend themselves. J. G. Horsfall and E. B. Cowling, eds., Academic Press, New York.

Keinath, A. P., and Hassell, R. L. 2014. Suppression of Fusarium wilt caused by *Fusarium oxysporum* f. sp. *niveum* race 2 on grafted triploid watermelon. Plant Dis. 98:1326-1332.

Krauss, A. 1999. Balanced nutrition and biotic stress. in: IFA Agricultural conference on managing plant nutrition, 29 June-2 July, Barcelona, Spain.

Kumar, D. 2014. Salicylic acid signaling in disease resistance. Plant Sci. 228:127-134.

Li, Z., Fan, Y., Gao, L., Cao, X., Ye, J., and Li, G. 2016. The dual roles of zinc sulfate in mitigating peach gummosis. Plant Dis. 100:345-351.

Liao, Y., Smyth, G. K., and Shi, W. 2014. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics 30:923-930.

Lynch, L., and Carpenter, J. 1999. The economic impacts of banning methyl bromide: Where do we need more research. in: 1999 Annual Research Conference on Methyl Bromide Alternatives and Emissions Reductions. http://mbao.org/mbrpro99.html.

Machado, P. P., Steiner, F., Zuffo, A. M., and Machado, R. A. 2018. Could the supply of boron and zinc improve resistance of potato to early blight? Potato Res. 61:169-182.

Marschner, H. 1995. Mineral nutrition of higher plants. Academic Press, London.

Martyn, R. D. 2014. Fusarium wilt of watermelon: 120 years of research. Hortic. Rev. 42:349-442.

Miller, N. F., Standish, J. R., and Quesada-Ocampo, L. M. 2020. Sensitivity of *Fusarium oxysporum* f. sp. *niveum* to prothioconazole and pydiflumetofen In vitro and efficacy for Fusarium wilt management in watermelon. Plant Health Prog. 21:13-18.

Mittler, R., Vanderauwera, S., Gollery, M., and Van Breusegem, F. 2004. Reactive oxygen gene network of plants. Trends Plant Sci. 9:490-498.

Mosa, A. 2002. Management of sugar beet powdery mildew by foliar spraying of potassium phosphate salts. Arab Univ. J. Agric. Sci., 10: 1043-1057. .

Newton, A. C., Torrance, L., Holden, N., Toth, I. K., Cooke, D. E., Blok, V., and Gilroy, E. M. 2012. Climate change and defense against pathogens in plants. Adv. Appl. Microbiol. 81:89-132.

Nobuta, K., Okrent, R., Stoutemyer, M., Rodibaugh, N., Kempema, L., Wildermuth, M., and Innes, R. 2007. The GH3 acyl adenylase family member PBS3 regulates salicylic acid-dependent defense responses in *Arabidopsis*. Plant Physiol. 144:1144-1156.

Pré, M., Atallah, M., Champion, A., De Vos, M., Pieterse, C. M., and Memelink, J. 2008. The AP2/ERF domain transcription factor ORA59 integrates jasmonic acid and ethylene signals in plant defense. Plant Physiol. 147:1347-1357.

Reuveni, M., Oppenheim, D., and Reuveni, R. 1998. Integrated control of powdery mildew on apple trees by foliar sprays of mono-potassium phosphate fertilizer and sterol inhibiting fungicides. Crop Prot. 17:563-568.

Reuveni, R., Dor, G., Raviv, M., Reuveni, M., and Tuzun, S. 2000. Systemic resistance against *Sphaerotheca fuliginea* in cucumber plants exposed to phosphate in hydroponics system, and its control by foliar spray of mono-potassium phosphate. Crop Prot. 19:355-361.

Roetschi, A., Si-Ammour, A., Belbahri, L., Mauch, F., and Mauch-Mani, B. 2001. Characterization of an *Arabidopsis–Phytophthora* pathosystem: resistance requires a functional PAD2 gene and is independent of salicylic acid, ethylene and jasmonic acid signalling. Plant J. 28:293-305.

Rolke, Y., Liu, S., Quidde, T., Williamson, B., Schouten, A., Weltring, K. M., Siewers, V., Tenberge, K. B., Tudzynski, B., and Tudzynski, P. 2004. Functional analysis of H2O2-generating systems in *Botrytis cinerea*: the major Cu-Zn-superoxide dismutase (BCSOD1) contributes to virulence on French bean, whereas a glucose oxidase (BCGOD1) is dispensable. Mol. Plant Pathol. 5:17-27.

Stolpe, C., Giehren, F., Krämer, U., and Müller, C. 2017. Both heavy metal-amendment of soil and aphid-infestation increase Cd and Zn concentrations in phloem exudates of a metal-hyperaccumulating plant. Phytochemistry 139:109-117.

Thies, J. A., Ariss, J. J., Hassell, R. L., Olson, S., Kousik, C. S., and Levi, A. 2010. Grafting for management of southern root-knot nematode, *Meloidogyne incognita*, in watermelon. Plant Dis. 94:1195-1199.

Torres, M. A., Dangl, J. L., and Jones, J. D. 2002. Arabidopsis gp91phox homologues AtrbohD and AtrbohF are required for accumulation of reactive oxygen intermediates in the plant defense response. Proceedings of the National Academy of Sciences 99:517-522.

Van Loon, L., Bakker, P., and Pieterse, C. 1998. Systemic resistance induced by rhizosphere bacteria. Annu. Rev. Phytopathol. 36:453-483.

Vrabka, J., Niehaus, E.-M., Münsterkötter, M., Proctor, R. H., Brown, D. W., Novák, O., Pěnčik, A., Tarkowská, D., Hromadová, K., and Hradilová, M. 2019. Production and role of hormones during interaction of *Fusarium* species with maize (Zea mays L.) seedlings. Front. Plant Sci 9:1936.

Walters, D., Walsh, D., Newton, A., and Lyon, G. 2005. Induced resistance for plant disease control: maximizing the efficacy of resistance elicitors. Phytopathology 95:1368-1373.

Wang, K. L.-C., Li, H., and Ecker, J. R. 2002. Ethylene biosynthesis and signaling networks. Plant Cell 14:S131-S151.

Wechter, W. P., Kousik, C., McMillan, M., and Levi, A. S. 2012. Identification of resistance to *Fusarium oxysporum* f. sp. *niveum* race 2 in *Citrullus lanatus* var. *citroides* plant introductions. HortScience 47:334-338.

Wu, S., Wang, X., Reddy, U., Sun, H., Bao, K., Gao, L., Mao, L., Patel, T., Ortiz, C., and Abburi, V. L. 2019. Genome of 'Charleston Gray', the principal American watermelon cultivar, and genetic characterization of 1,365 accessions in the US National Plant Germplasm System watermelon collection. Plant Biotechnol. J. 17:2246-2258.

Wu, Y. S., and Tang, K. X. 2004. MAP kinase cascades responding to environmental stress in plants. Acta Bot. Sin 46:127-136.

Xie, S., Jiang, H., Ding, T., Xu, Q., Chai, W., and Cheng, B. 2018. *Bacillus amyloliquefaciens* FZB42 represses plant miR846 to induce systemic resistance via a jasmonic acid-dependent signalling pathway. Mol. Plant Pathol. 19:1612-1623.

Zhou, X., Everts, K., and Bruton, B. 2010. Race 3, a new and highly virulent race of *Fusarium oxysporum* f. sp. *niveum* causing Fusarium wilt in watermelon. Plant Dis. 94:92-98.

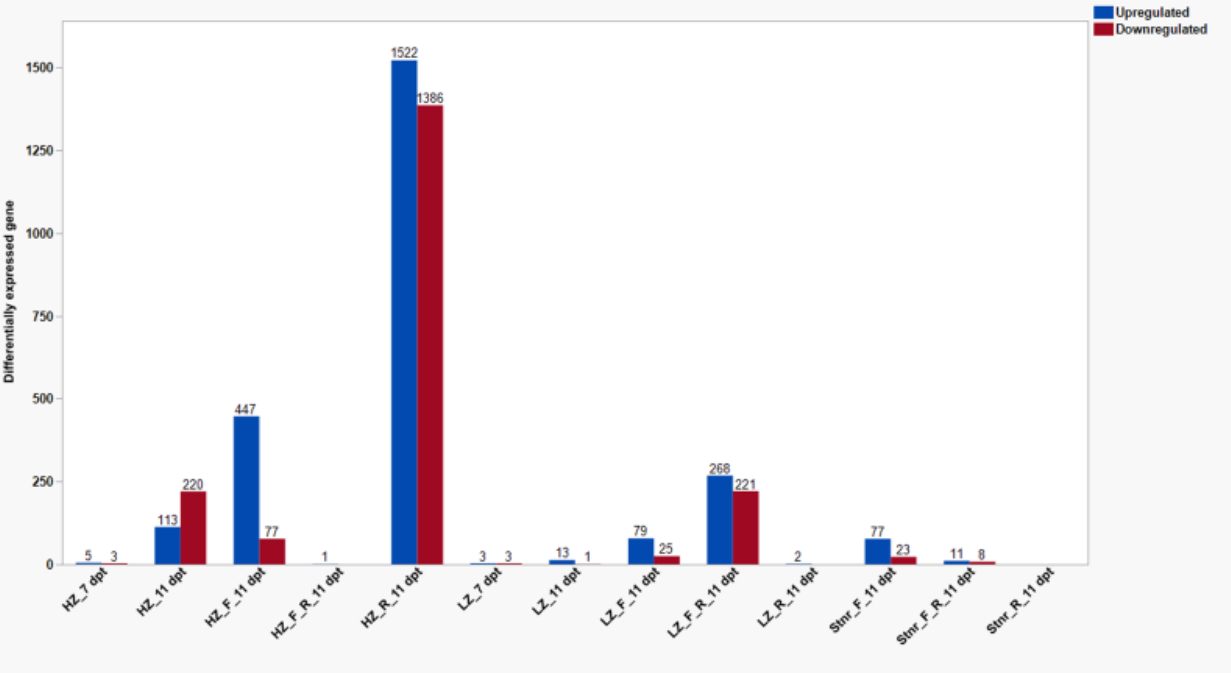
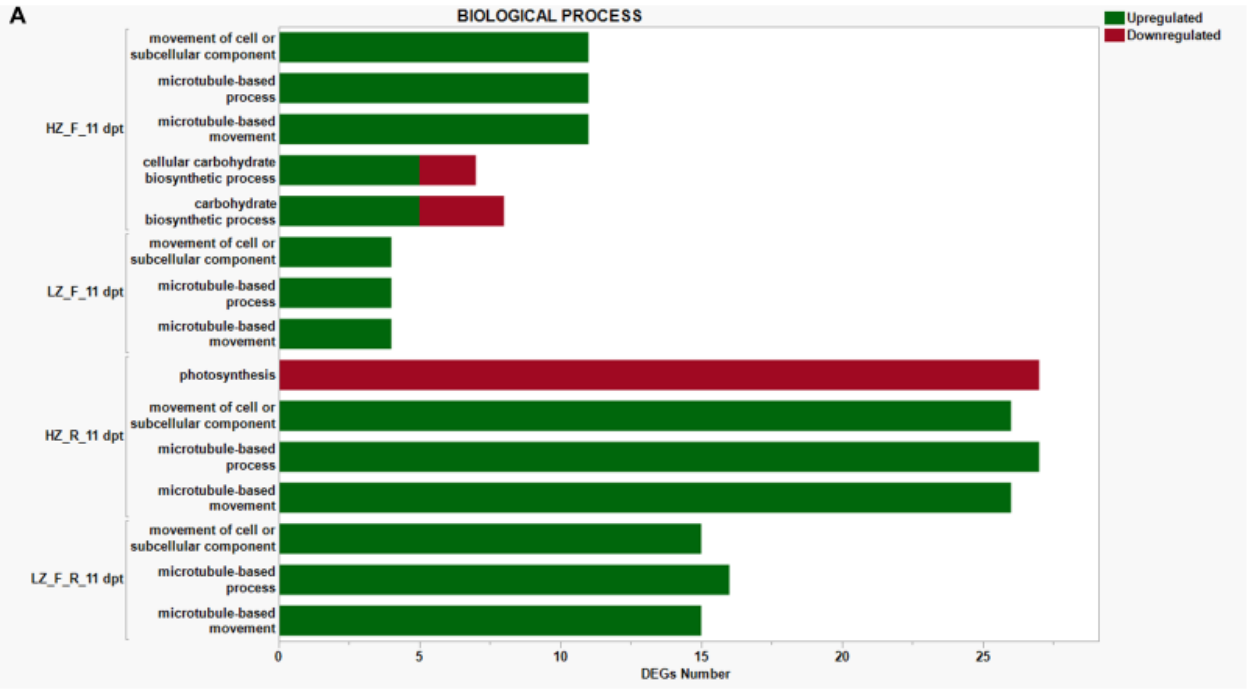
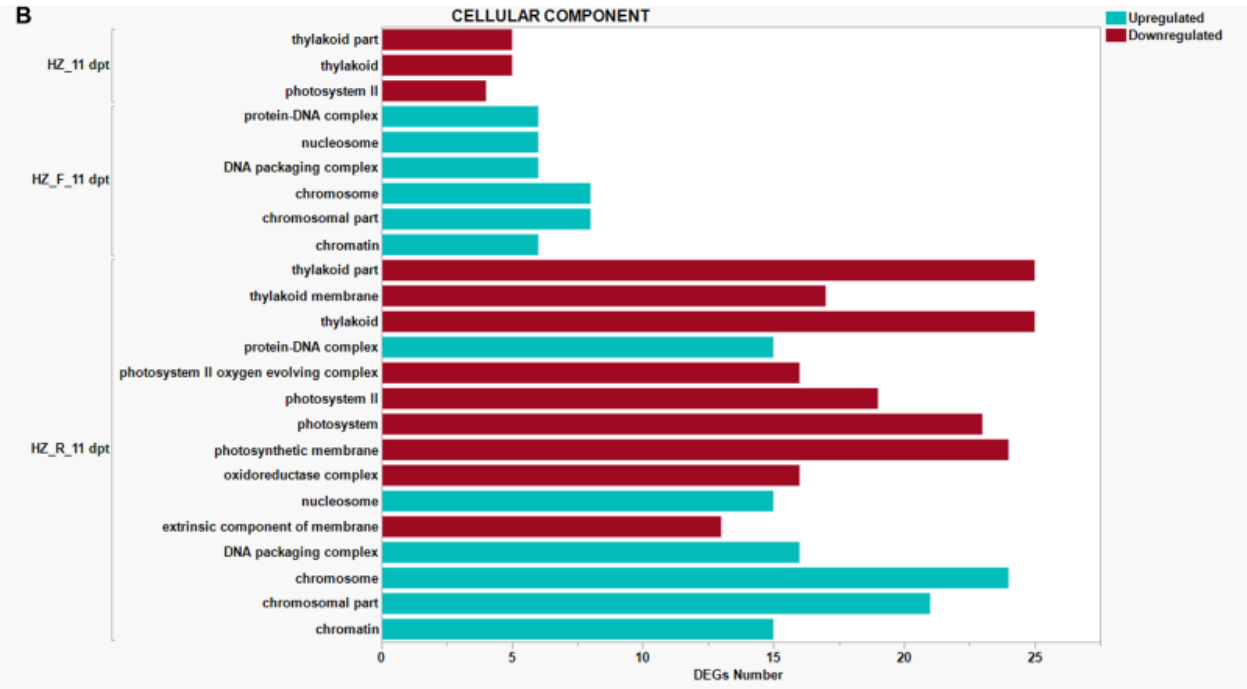


Figure 3.1. Differentially expressed genes (DEGs) at 7 and 11 days post-treatment (dpt), compared with non-inoculated micronutrient control (Stnr) in high Zn (HZ) and low Zn (LZ) treated plants inoculated with *Fusarium oxysporum* f. sp. *niveum* (F) or *Meloidogyne incognita* (R) or both (F_R).


A
BIOLOGICAL PROCESS
Upregulated
Downregulated
HZ_F_11 dpt
movement of cell or subcellular component
microtubule-based process
microtubule-based movement
cellular carbohydrate biosynthetic process
carbohydrate biosynthetic process
LZ_F_11 dpt
movement of cell or subcellular component
microtubule-based process
microtubule-based movement
HZ_R_11 dpt
photosynthesis
movement of cell or subcellular component
microtubule-based process
microtubule-based movement
LZ_F_R_11 dpt
movement of cell or subcellular component
microtubule-based process
microtubule-based movement
0
5
10
15
20
25
DEGs Number


B
CELLULAR COMPONENT
Upregulated
Downregulated
HZ_11 dpt
thylakoid part
thylakoid
photosystem II
HZ_F_11 dpt
protein-DNA complex
nucleosome
DNA packaging complex
chromosome
chromosomal part
chromatin
HZ_R_11 dpt
thylakoid part
thylakoid membrane
thylakoid
protein-DNA complex
photosystem II oxygen evolving complex
photosystem II
photosystem
photosynthetic membrane
oxidoreductase complex
nucleosome
extrinsic component of membrane
DNA packaging complex
chromosome
chromosomal part
chromatin
0
5
10
15
20
25
DEGs Number

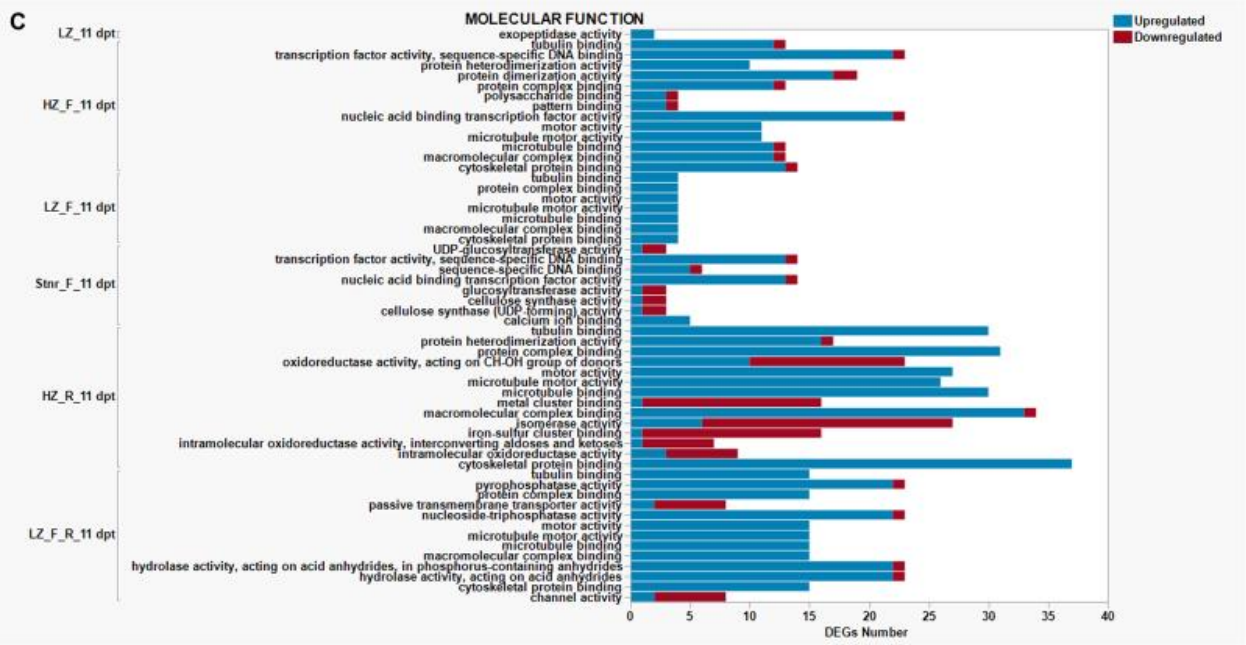


Figure 3.2. Gene Ontology enrichment analysis of (A) biological process (BP), (B) cellular component (CC), and (C) molecular function (MF) for upregulated and downregulated genes between high Zn (HZ) or low Zn (LZ) treated plants inoculated with *Fusarium oxysporum* f. sp. *niveum* (F) or *Meloidogyne incognita* (R) or both (F_R) and non-inoculated micronutrient control (Stnr) at 11 days post treatment (dpt) and 3 days post inoculation.

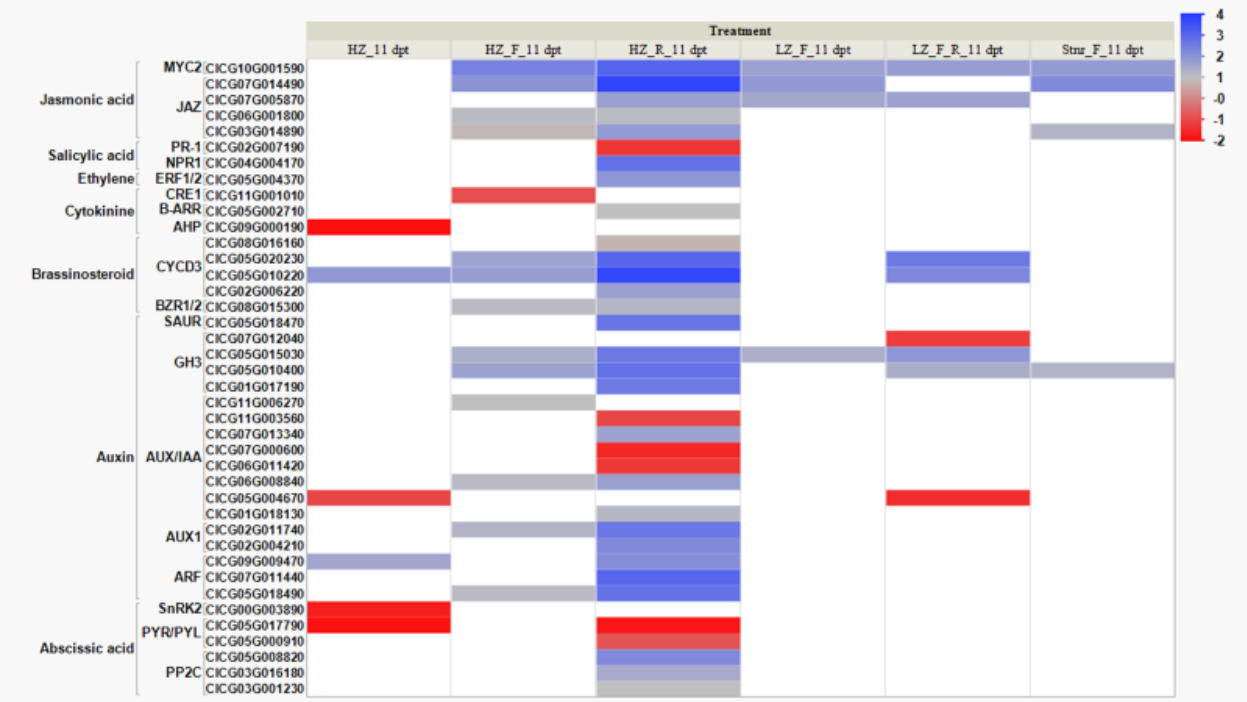


Figure 3.3. Expression profile of hormone related genes affected by high Zn (HZ) and low Zn (LZ) in plants inoculated with *Fusarium oxysporum* f. sp. *niveum* (F) or *Meloidogyne incognita* (R) or both (F_R) compared to non-inoculated micronutrient control (Stnr) at 11 days post treatment (dpt) and 3 days post inoculation. The heat map is drawn through the value of log2 (fold change).

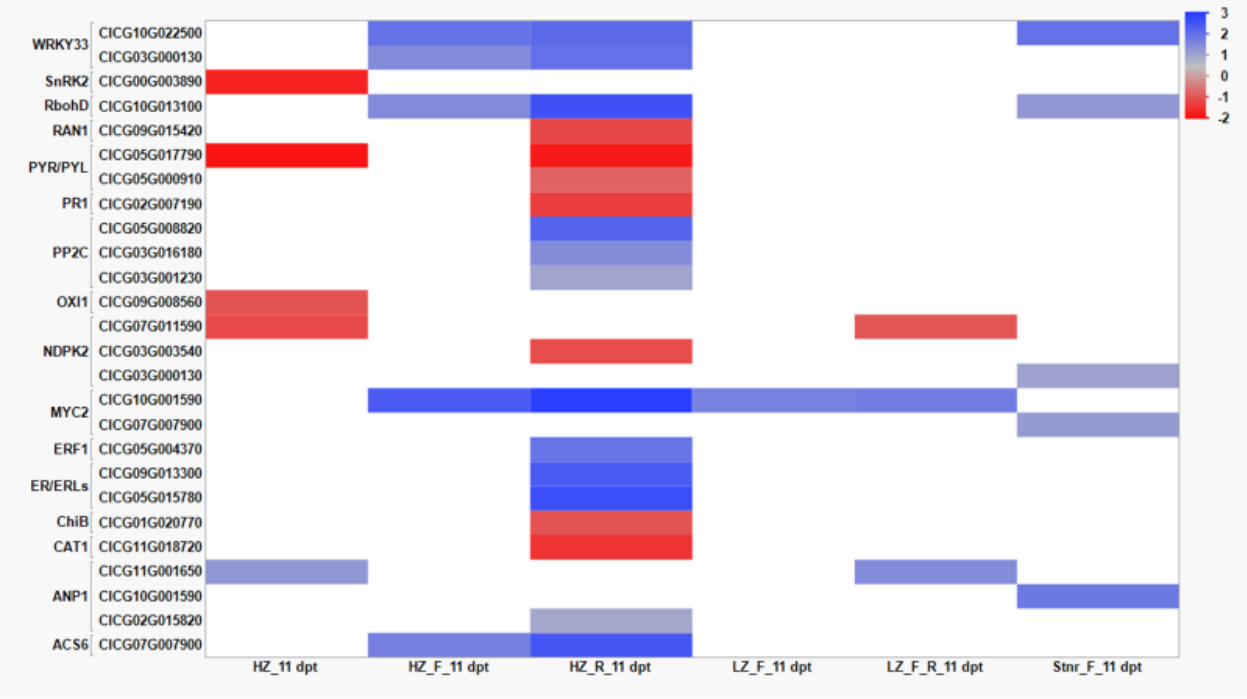


Figure 3.4. Expression profile of genes involved in MAPK signaling affected by high Zn (HZ) and low Zn (LZ) in plants inoculated with *Fusarium oxysporum* f. sp. *niveum* (F) or *Meloidogyne incognita* (R) or both (F_R) compared to non-inoculated micronutrient control (Stnr) at 11 days post treatment (dpt) and 3 days post inoculation. The heat map is drawn through the value of log2 (fold change).

Table 3.1. Overview of the watermelon leaf RNA sequencing (RNA-Seq) data.

Sample name	Raw reads	Raw bases	Clean reads	Total mapped reads	Uniquely mapped reads	Q20 (%)	Q30 (%)
HZ_7 dpt	59687502	8.93	58729353 (98.39%)	55127160 (97.29%)	52483640 (92.49%)	98.31	94.70
LZ_7 dpt	50014715	7.50	49203661 (98.38%)	47956039 (97.52%)	45459273 (92.44%)	98.32	94.77
Stnr_7 dpt	49986762	7.50	49117792 (98.26%)	49335419 (97.24%)	46613488 (91.88%)	98.27	94.67
HZ_11 dpt	51629032	7.76	50690973 (98.18%)	49414844 (97.5%)	47171062 (93.07%)	98.15	94.34
HZ_F_11 dpt	53908944	8.10	53109847 (98.52%)	51636022 (97.22%)	49002564 (92.26%)	98.22	94.54
HZ_F_R_11 dpt	53586318	8.05	52878509 (98.68%)	51426192 (96.99%)	48472116 (91.42%)	98.38	94.94
HZ_R_11 dpt	59980358	9.00	59047384 (98.45%)	56309037 (96.75%)	52972370 (91.19%)	98.24	94.49
LZ_11 dpt	51350437	7.70	50617202 (98.57%)	49331625 (97.44%)	46616093 (92.09%)	98.36	94.87
LZ_F_11 dpt	49014948	7.33	48293772 (98.53%)	46823518 (97.53%)	44562924 (92.83%)	98.23	94.51
LZ_F_R_11 dpt	54588526	8.20	53737007 (98.44%)	50680264 (97.12%)	47754893 (91.61%)	98.37	94.86
LZ_R_11 dpt	49512342	7.43	48642963 (98.24%)	49121924 (97.64%)	46768239 (92.97%)	98.32	94.74
Stnr_11 dpt	53059770	7.96	52281673 (98.53%)	50265130 (97.17%)	47274700 (91.37%)	98.00	93.96
Stnr_F_11 dpt	49591765	7.47	48726254 (98.25%)	45602076 (97.69%)	43141210 (92.41%)	98.24	94.58
Stnr_F_R_11 dpt	51176967	7.67	50322421 (98.33%)	68103179 (97.49%)	65281192 (93.26%)	98.36	94.84
Stnr_R_11 dpt	63143099	9.50	62078568 (98.31%)	44094901 (96.95%)	41349941 (90.92%)	98.32	94.76

Numbers indicate the average of the three biological replicates.

Table 3.2. Pathways and gene count of major changes in KEGG enrichment in DEGs.

KEGGID	Description	*P* value	Count
HZ_7 dpt			
csv04146	Peroxisome	0.002165	2
csv00531	Glycosaminoglycan degradation	0.01651	1
HZ_F_11 dpt			
csv04075	Plant hormone signal transduction	1.41E-05	14
csv04626	Plant-pathogen interaction	0.0007	9
LZ_F_11 dpt			
csv00592	alpha-Linolenic acid metabolism	0.00213	3
csv00290	Valine, leucine and isoleucine biosynthesis	0.00363	2
csv00591	Linoleic acid metabolism	0.00417	2
HZ_R_11 dpt			
csv00710	Carbon fixation in photosynthetic organisms	1.73E-07	25
csv00195	Photosynthesis	1.58E-06	21
csv00030	Pentose phosphate pathway	0.00015	17
csv00500	Starch and sucrose metabolism	0.00021	31
csv00053	Ascorbate and aldarate metabolism	0.00052	15
csv01200	Carbon metabolism	0.00138	48
csv00196	Photosynthesis - antenna proteins	0.00246	8
csv00906	Carotenoid biosynthesis	0.00327	11

CHAPTER 4

FIELD EVALUATION OF THE EFFICACY OF PIC-CLOR 60 [CHLOROPICRIN PRE-MIXED WITH 1, 3 DICHLOROPROPENE (TELONE II)] AND SOIL-APPLIED FUNGICIDES AGAINST FUSARIUM WILT SEVERITY IN WATERMELEON

Karki, K., Grant, J., da Silva, A., Coolong, T., Hajihassani, A., and Dutta, B. 2020. To be submitted to Plant Disease.

Abstract

Watermelon (*Citrullus lanatus* var. *lanatus*) yield losses due to Fusarium wilt are increasing in the United States, mainly due to the emergence of the new races of *Fusarium oxysporum* f. sp. *niveum* (FON). One of the potential management strategies to reduce Fusarium wilt is the use of pesticides; fumigants and soil-applied fungicides. Three field experiments were conducted in 2018-2020 to evaluate fumigant Pic-clor 60 (choloropicrin pre-mixed with 1,3 dicholoropropene) along with post-plant soil-applied fungicides (prothioconazole and pydiflumetofen) for their effectiveness against Fusarium wilt. The field was fumigated 21 days before transplanting, and three applications of either fungicide were made at 14-day intervals via a drip irrigation system starting a day after transplanting. In 2018, based on the disease ratings at harvest maturity, all treatments significantly reduced final disease incidence compared to non-treated control, and the highest reduction was observed when the fumigant was applied at 336.26 kg/ha along with either fungicide. The application of fumigant, together with either fungicide, had higher disease reduction compared to their stand-alone application. Also, Pic-clor 60 at 336.26 kg/ha provided a higher reduction in disease incidence than Pic-clor 60 at 280.21 kg/ha. In 2019, treatment effects on Fusarium wilt incidence were only significant for the mid-season ratings. Fusarium wilt incidence was significantly lower for the treatment with Pic-clor 60 at 224.17 kg/ha and bio-control than fungicide pydiflumetofen alone. However, none of the treatments reduced the disease incidence significantly compared to control. In 2020, results showed a similar trend to 2018, where Pic-clor 60 at 336.26 kg/ha in combination with either prothioconazole or pydiflumetofen had significantly lower final disease incidence. The reduction in disease incidence in Pic-clor 60 at 336.26 kg/ha was higher compared to a lower application rate of 280.21 kg/ha, either when applied alone or in combination with either fungicide. The

results presented here are the first to demonstrate that the fumigant Pic-clor 60 along with soil-applied fungicides, pydiflumetofen or prothioconazole may provide an effective management option for Fusarium wilt of watermelon.

Introduction

Watermelon (*Citrullus lanatus* var. *lanatus*) is an important crop currently produced in 119 countries around the world (FAO 2018). The United States, with an annual production and a farm gate value of 2.18 million tons and $657 million, respectively, is the seventh-largest watermelon producer in the world (USDA-NASS 2019). It is cultivated on 8,903 hectares in Georgia, making it the third-largest watermelon producing state (USDA-NASS 2019). The production of watermelon in the southeastern United States especially in Georgia has been severely affected by Fusarium wilt (Dutta et al. 2018). This disease is caused by the soil-borne fungal pathogen *Fusarium oxysporum* f. sp. *niveum* (FON). This pathogen causes pre- or post-emergence damping-off of young seedlings and wilting of mature plants in the field. Under conditions favorable for the disease, yield losses of more than 80% can occur. In recent years, outbreaks of Fusarium wilt in the southeastern United States including Georgia have been economically damaging in watermelon (Keinath et al. 2019). Several management strategies, including seed treatments, crop rotation, weed control, and chemical management options are available, but in most cases, they are inadequate to manage FON epidemics (Everts and Himmelstein 2015). Limited benefits can be observed in fields with low disease pressure; however, in heavily infested fields and a conducive environment, current chemical management practices are not effective (Keinath 2019). Because of the destructive nature of this disease, watermelon growers in the southeastern United States often abandon FON infested fields.

Field management of Fusarium wilt in watermelon is challenging as the pathogen can survive in soil for years in the form of chlamydospores (Egel and Martyn 2013). Furthermore, FON has the capacity of developing new races that are able to overcome host resistance (Egel and Martyn 2013). The most economical approach to manage FON would be to plant resistant cultivars. However, cultivars resistant to all four races of FON (races 0, 1, 2, and 3) are not commercially available. Soil fumigation has been the primary chemical management practice for Fusarium wilt. Previously, management of Fusarium wilt relied on the use of methyl bromide (MeBr) as a soil-fumigant (Martyn 2014). Following the Montreal Protocol, which mandated the reduction of MeBr use, efforts have been made to sought alternatives (Antoniou et al. 2014). Currently, available MeBr alternatives include chloropicrin, 1,3-dichloropropene, and metam sodium are not as effective as MeBr at reducing Fusarium wilt incidence (Egel and Martyn 2013; Everts and Himmelstein 2015). Moreover, chloropicrin is not commonly used; instead, commercial vegetable growers utilize 1,3-dichloropropene as their preferred choice of the fumigant. However, 1,3-dichloropropene is only effective on plant-parasitic nematodes (*Meloidogyne* sp.) but not effective on soil-borne fungal pathogens, including FON. This could be partly responsible for the re-emergence and widespread distribution of FON in the southeastern United States, particularly in Georgia.

Prothioconazole (Proline, Bayer Crop Science), a demethylation inhibiting fungicide [DMI; Fungicide Resistance Action Committee (FRAC) code 3], is the only fungicide labeled for use on watermelon to manage FON (Miller et al. 2020). A new succinate dehydrogenase inhibitor fungicide (SDHI; FRAC code 7), pydiflumetofen (Miravis, Syngenta), has recently been developed and is likely to be labeled for use on watermelon to manage Fusarium wilt (Rapicavoli et al. 2018). According to a recent finding, pydiflumetofen and prothioconazole were

effective in reducing FON incidence and severity in a multi-year field trial (Miller et al. 2020). The authors also observed that 100% of the FON isolates collected from North Carolina were sensitive to pydiflumetofen (Miller et al. 2020). In a separate study, it was observed that FON isolates collected in Georgia were also sensitive to prothioconazole (Petkar et al. 2017).

However, with limited options available, it is imperative to evaluate if fumigant and post-plant soil-applied fungicides (prothioconazole and pydiflumetofen) can be utilized for FON management. This approach has not been evaluated in FON management before, and in this manuscript, we provide evidence from a three-year study of the reduction of Fusarium wilt incidence in a naturally infested field using Pic-clor-60 [choloropicrin pre-mixed with 1,3 dicholoropropene (Telone II)] and the post-plant soil-applied fungicides (prothioconazole and pydiflumetofen).

Materials and methods

Site description. Three field trials were conducted in 2018-2020 at the Crisp County UGA Extension field site (31°57'25.37"N; 83°48'43.02”W) at Cordele, GA. Climate in the region is classified as a Cfa (humid subtropical) with warm temperatures and heavy rainfall events during the summer but cool dry periods during the winter (Koppen 1931). This field site was classified with a Tifton loamy sand soil (USDA 1983) and the soil-series were siliceous, thermic psammentic paleudults. It has a history of FON infestation only and has been under continuous watermelon production since 2010. Nematode populations (including *M. incognita*) were below action thresholds. Three different races of FON were identified from this site; races 1, 2 and 3. This field site also had a low nutsedge infestation.

Seedling development in the greenhouse. Watermelon seedlings 'cv. Crimson Sweet' and SP-6 pollinizers were grown in a 128-cell seedling tray filled with fine-grade, composted pine bark mixed with vermiculite in a 3:1 ratio. Seedlings were grown for 5-weeks under greenhouse conditions of 24℃ and 55% R.H and were irrigated with overhead-irrigation. Seedlings were not treated with any fungicides in the greenhouse.

Field trials. Prior to fumigation, the area was harrowed, roto-tilled and plots (15 m long and 1.8 m wide) were prepared. An alley space of 3 m was kept between the plot ends. Plots were fumigated with Pic-clor-60 at 336.26 kg/ha and 280.21 kg/ha in both 2018 and 2020. In 2019 a lower rate of Pic-clor-60 (224.17 kg/ha) was used. Following fumigation, plots were covered with a totally impermeable film (TIF) using Rain-Flo 2600 raised mulch layers (Martins Products Supplies, Shippensburg, PA). A single line of drip irrigation tubing (30.5 cm emitter spacing, 1 L/h per 30.5 m at 8 psi; John Deere T-Tape, Moline, IL) was placed 2-4 cm underneath the plastic mulch. Twelve seedlings of watermelon, five-week-old, were planted in each plot at a 0.6 m spacing after 21 days of fumigant application. Transplanting was done on April 18, April 25, and April 10 in 2018, 2019, and 2020, respectively. Foliar and soilborne diseases other than Fusarium, insects and weeds were controlled using the University of Georgia Extension recommendations. All treatments were arranged in a randomized complete block design with five replicates per treatment.

The fungicide programs included three applications, each of either prothioconazole (as Proline) or pydiflumetofen (as Miravis) at 14-day intervals via a drip irrigation system. The first application of both fungicides was made 1day after transplanting. The other two applications were made at a two-week interval at 29 and 43 days after transplanting (DAT). In 2019, a bio-control treatment consisting of a mixture of *Bacillus amyloliquefaciens* Strain PTA-4838* (as

Aveo EZ, Valent,) at 0.05 L/ha and *Glomus intaradices*, *G. mosseae*, *G. aggregatum* and *G. etunicatum* (as EndoMaxx, Valent) at 0.01 L/ha was tested for its efficacy against FON. The bio-control treatment was applied 30 DAT via a drip irrigation system. Specific treatments that were administered in 2018-2020 is given below.

Field trial (2018). Seven treatments that were evaluated include i) pydiflumetofen 0.63 L/ha (three post-plant applications via drip irrigation system); ii) prothioconazole at 0.42 L/ha (three post-plant applications via drip irrigation system); iii) Pic-clor 60 at 280.21 kg/ha; iv) Pic-clor 60 at 336.26 kg/ha; v) Pic-clor 60 at 336.26 kg/ha and three-post-plant applications of pydiflumetofen 0.63 L/ha via drip irrigation system; vi) Pic-clor 60 at 336.26 kg/ha and three-post-plant applications of prothioconazole at 0.42 L/ha via drip irrigation system; and vii) non-treated check.

Field trial (2019). Eight treatments that were evaluated include i) pydiflumetofen at 0.63 L/ha (three post-plant applications via drip irrigation system); ii) prothioconazole at 0.42 L/ha (three post-plant applications via drip irrigation system); iii) Bio-control agents (AveoEZ at 0.05 L/ha and EndoMaxx at 0.01 L/ha, one post-plant application via drip irrigation system); iv) Pic-clor 60 at 224.17 kg/ha and three-post-plant applications of prothioconazole at 0.42 L/ha via drip irrigation system; v) Pic-clor 60 at 224.17 kg/ha and three-post-plant applications of pydiflumetofen 0.63 L/ha via drip-irrigation system; vi) Pic-clor 60 at 224.17 kg/ha and Bio-control agents (AveoEZ at 0.05 L/ha and EndoMaxx at 0.01 L/ha, one post-plant application via drip irrigation system); vii) Pic-clor 60 at 224.17 kg/ha; and viii) non-treated check.

Field trial (2020). Nine treatments evaluated in a field trial in 2020 were ; i) pydiflumetofen 0.63 L/ha (three post-plant applications via drip irrigation system); ii) prothioconazole at 0.42 L/ha (three post-plant applications via drip irrigation system); iii) Pic-

clor 60 at 280.21 kg/ha; iv) Pic-clor 60 at 336.26 kg/ha; v) Pic-clor 60 at 336.26 kg/ha and three-post-plant applications of pydiflumetofen 0.63 L/ha via drip irrigation system; vi) Pic-clor 60 at 280.21 kg/ha and three-post-plant applications of prothioconazole at 0.42 L/ha via drip irrigation system; vii) Pic-clor 60 at 280.21 kg/ha and three-post-plant applications of pydiflumetofen 0.63 L/ha via drip irrigation system; viii) Pic-clor 60 at 336.26 kg/ha and three-post-plant applications of prothioconazole at 0.42 L/ha via drip irrigation system; and ix) non-treated check.

Data collection. For the three years studied, the weather conditions of maximum and minimum air temperature, solar radiation, and rainfall was recorded during the watermelon season using an automated weather stations from the Georgia Automated Environmental Monitoring Network (UGA 2019). Fusarium wilt incidence (%) as the number of plants with visible wilting symptoms was recorded at the end of the season prior to harvest for the 2018 trial. For 2019 and 2020, the mid-season incidence of Fusarium wilt along with the final incidence at the end of the season prior to harvest was recorded. Apart from the visual Fusarium wilt incidence, percent vascular discoloration for each plant per plot per treatment was recorded after the final visual rating for 2019 and 2020 trails. For vascular discoloration, a cross-section of 10-cm stem tissue sections were cut at the collar region of the plant and visually evaluated as positive or negative, as indicated in Fig. 4.1. Representative samples of stem tissues displaying vascular discoloration from each treatment were also evaluated for presence of fungi by incubating on potato dextrose agar medium, as described previously by (Petkar and Ji 2017). After 7-10 days of incubation at 28°C, resulting fungal isolates were microscopically identified based on morphological criteria (Leslie and Summerell 2008) and FON suspects were confirmed by PCR assay using FON specific primers Fon-1/Fon-2 (Lin et al. 2010).

Data analysis. Results were analyzed separately for each trial after ensuring significant interaction of trial-treatment was present at $P \leq 0.05$. Data were subjected to statistical analysis using the Analysis of Variance (ANOVA) procedure and treatments were compared by the Student-Newman-Keuls test at $P = 0.05$ in SAS 9.4® (SAS Institute, Cary, NC).

Results

Field trial (2018). In 2018, daily average air temperature was 24 °C from transplanting to harvest, while maximum air temperature was 36 °C and minimum air temperature was 5 °C. Rainfall events accumulated 513 mm and were well distributed during the watermelon season, still there were daily rainfall events for 15 d during flowering, from 15/05/2018 to 29/05/2018, that accumulated 190 mm (Fig. 4.2).

All treatments significantly reduced the final Fusarium wilt incidence compared with the non-treated control ($P<0.05$) (Table 4.1). No significant differences were observed among fungicide alone treatments (pydiflumetofen and prothioconazole) and fumigant alone treatment, Pic-clor 60 at 280.21 kg/ha. However, Pic-clor 60 applied alone at 336.26 kg/ha had a significant reduction in disease incidence compared to Pic-clor 60 applied alone at 280.21 kg/ha or fungicide alone treatments (pydiflumetofen and prothioconazole). Fungicides, pydiflumetofen, or prothioconazole, when applied together with Pic-clor 60 at 336.26 kg/ha, showed the highest reduction of disease incidence compared to other treatments. However, they were not significantly different when compared with each other.

Field trial (2019). In 2019, daily average air temperature was 25 °C from transplanting to harvest, while maximum air temperature was 37 °C and minimum air temperature was 5 °C.

Rainfall events accumulated 433 mm and were well distributed during the watermelon season (Fig. 4.2).

Treatment effects on Fusarium wilt incidence were only significant ($P < 0.05$) for the mid-season season ratings in 2019 (Table 4.2). Fusarium wilt incidence was significantly lower for the treatment that was comprised of Pic-clor 60 (224.17 kg/ha) and bio-control agents. Although, numerical reduction in disease incidence was observed for the Pic-clor 60 and pydiflumetofen or Pic-clor 60 and prothioconazole compared with pydiflumetofen or prothioconazole only treatment, they were not significantly different from each other. Application of Pic-clor 60 only treatment numerically but not significantly reduced Fusarium wilt incidence compared to non-treated check. Disease incidence rating at harvest maturity did not show significant reduction in Fusarium wilt incidence ($P = 0.578$). No significant differences in vascular discoloration were observed among treatments ($P = 0.933$).

Field trial (2020). In 2020, daily average air temperature was 23 °C from transplanting to harvest, while maximum air temperature was 36 °C and minimum air temperature was 3 °C. Rainfall events accumulated 557 mm and were well distributed during the watermelon season, except for a 5 d rainy period that accumulated 145 mm right after transplanting, from 19/04/2020 to 23/04/202 (Fig. 4.2).

All treatments significantly reduced final Fusarium wilt incidence compared with the non-treated control ($P < 0.05$) except for the Pic-Clor 60 alone treatment at 220.22 kg/ha (Table 4.3). Pic-clor 60 at 280.2 kg/ha had significantly higher disease incidence compared to the Pic-clor 60 at 336.2 kg/ha. No significant differences were observed among treatments with fungicide alone (pydiflumetofen or prothioconazole) and the fungicide treatment combined with fumigant Pic-Clor 60 at both 280.21 kg/ha (with pydiflumetofen at 0.63 L/ha or with

prothioconazole at 0.42 L/ha) and 336.26 kg/ha (with pydiflumetofen at 0.63 L/ha or with prothioconazole at 0.42 L/ha). Although, a numerical reduction was more pronounced with Pic-clor 60 at 336.26 kg/ha applied together with either fungicides (pydiflumetofen or prothioconazole) compared with other treatments. Pic-clor 60 at 336.26 kg/ha applied together with either fungicides (pydiflumetofen or prothioconazole) provided higher reduction in disease incidence compared to stand alone Pic-clor 60 (at 336.26 kg/ha or at 280.21 kg/ha). Pic-clor 60 at 280.21 kg/ha applied together with fungicides (with pydiflumetofen at 0.63 L/ha or with prothioconazole at 0.42 L/ha) significantly reduced disease incidence compared with Pic-clor 60 at 280.2 kg/ha alone treatment.

Vascular discoloration was significantly reduced with all treatments in comparison to the non-treated control. No significant differences were observed among treatments with fungicide alone (pydiflumetofen and prothioconazole), stand-alone fumigant (Pic-clor 60 at 280.21 kg/ha and Pic-clor 60 at 336.26 kg/ha) and the fungicide treatment combined with fumigant Pic-clor 60 at 280.21 kg/ha (pydiflumetofen at 0.63 L/ha or prothioconazole at 0.42 L/ha). However, significant reduction in vascular discoloration was observed with Pic-clor 60 at 336.26 kg/ha together with fungicides (pydiflumetofen at 0.63 L/ha or prothioconazole at 0.42 L/ha) compared with other treatments.

FON recovery. FON was re-isolated from 100% of the tested plants that displayed vascular discoloration. Isolates were assayed for FON identity by conventional PCR, as described earlier (Lin et al. 2010). One hundred percent of the putatively isolated FON colonies from infected plants were confirmed as FON using the PCR assay.

Discussion

This study evaluated the combination of chemical treatments, fumigant Pic-clor 60 and post-plant, soil-applied fungicides (prothioconazole and pydiflumetofen) for Fusarium wilt management in watermelon. For the three watermelon seasons studied, weather conditions were optimum for crop development and did not affect treatments performance. Daily average air temperatures were within the optimum range of 21 °C to 30 °C for watermelon development. Rainfall events were well distributed throughout the season and accumulated higher volumes than the historical crop water demand of 416, 419, and 406 mm for the same period in 2018, 2019, and 2020, respectively (da Silva et al. 2019). Irrigation events were still required in no rainy periods.

The fumigant, Pic-clor 60 at 336.26 kg/ha when applied together with a fungicide either pydiflumetofen or prothioconazole, significantly reduced the incidence of Fusarium wilt of watermelon. To achieve season-long reductions in disease, it was necessary to apply the fumigant at a higher rate and soil-applied fungicide or fungicides more than once. However, we also found that the observed disease reduction was not consistent across trials, indicating that other factors such as soil pathogen distribution within a field, edaphic factors, or a combination of other environmental factors not evaluated in our study might affect the performance of these chemicals. Local and regional variations in pathogen inoculum and their sensitivity to these fungicides present another challenge in managing Fusarium wilt disease with chemicals. Pathogenic formae speciales of FON are not distributed uniformly between regions or within a field (Netzer 1976; Zhou and Everts 2003, 2007). Additionally, FON survives by forming chlamydospores, thus being shielded from chemical contact (Smith 2007).

Pic-clor 60 is a combination of chloropicrin (60%) and 1,3-Dichloropropene (40%). Chloropicrin has effectively reduced fungal pathogens in many vegetable crops, and increased yield and quality (Hutchinson 2005; Sydorovych et al. 2008). Chloropicrin is often combined with other fumigants to increase the spectrum of control (US-EPA 2005). In the case of Pic-clor 60, the chloropicrin (60%) is combined with the 1,3-dichloropropene (40%), which is considered a nematicide. In this study, when the efficacy of stand-alone Pic-clor 60 at three different rates was compared, a higher dosage of Pic-clor 60, 336.26 kg/ha could provide more effective control of FON than lower rates 280.22 kg/ha and 224.17 kg/ha. This provides an additional compound besides 1, 3- dichloropropene which is considered as a nematicide. Pic-clor 60 was reported to be effective in controlling plant-parasitic nematodes, such as *Belonolaimus longicaudatus* (Watson and Desaeger 2019), *Pratylenchus penetrans* (Walters et al. 2017), and root-knot nematodes (*Meloidogyne* spp.) (Nnamdi et al. 2020) but also fungal pathogens, such as *Phytophthora rubi* (Walters et al. 2017), and weeds, such as nutsedge (Stevens et al. 2016). Nevertheless, there is little literature available assessing its effect against FON in watermelon.

Prothioconazole (Proline) is demethylation inhibiting (DMI) fungicide (Fungicide Resistance action committee [FRAC] code 3) labeled for use on watermelon to manage Fusarium wilt. Another fungicide, pydiflumetofen (Miravis; SDHI; FRAC code 7), is labeled for foliar use against certain fungal foliar diseases but not yet labeled for soil-use. Our result is in line with the field experiments conducted by Miller et al. (2020), where they observed, soil-applied prothioconazole and pydiflumetofen reduced final disease incidence and thus identified pydiflumetofen as an effective management option for Fusarium wilt. Our results also showed, bio-control agents *Bacillus amyloliquefaciens* Strain PTA-4838*, *Glomus intaradices*, *G. mosseae*, *G. aggregatum* and *G. etunicatum* reduced Fusarium wilt incidence. Reduction in

disease incidence was more pronounced with Pic-clor 60 and bio-control than bio-control alone in non-fumigated treatment. It is possible that use of Pic-clor 60 resulted in the reduction of soil-microflora populations that competed with the bio-control agents for nutrition and space. This aided the bio-control to further establish in soil and resulted in better colonization of the rhizosphere of watermelon. However, since it was tested in only one trial further field studies are essential to test its effectiveness against FON.

Although prothioconazole and pydiflumetofen stand-alone successfully reduced the disease incidence compared to control treatments, location of application together with Pic-clor 60 at 336.26 kg/ha, showed the highest reduction of Fusarium wilt incidence. Pic-clor 60, when followed with additional chemical treatment, produced better results in other pathogen systems such as root-knot nematode in tomato (Castillo et al. 2018). Pic-clor 60, followed by fluensulfone, decreased population densities of root-knot nematode by 88% at harvest in tomato (Castillo et al. 2018). Field experiments conducted in cut flowers, delphinium (*Delphinium elatum*), ranunculus (*Ranunculus asiaticus*), and in strawberry (*Fragaria* × *ananassa*) reported that the use of Allyl isothiocyanate (AITC) as a stand-alone treatment provided no consistent weed or pathogen control efficacy but when combined with 1,3- dichloropropene, chloropicrin, and metam sodium there was effective control of soil-borne diseases and weeds (Hoffmann et al. 2020).

Further study and evaluation of these chemicals, taking into account different climate, soil types, and IPM practices, will add value to the current knowledge, considering current limited management options and the challenge that FON represents. Also, for future experiments, plant mortality and FON populations in the soil could be appropriate measures for evaluating soil treatments against the pathogen. The ability to use a combination of fumigants

and fungicides applied through a drip irrigation system, in combination with the use of cultural practices and host resistance, could provide growers with multifaceted and improved management programs for Fusarium wilt of watermelon.

Literature cited

Antoniou, P., Tjamos, E., and Giannakou, J. 2014. Low-cost and effective approaches of soil disinfestation of plastic house or open field crops in Greece. Proc. Acta Hort. 1044:29-41.

Castillo, G. X., Ozores-Hampton, M., and Gine, P. A. N. 2018. Fluensulfone: A new "tool in the tool box" to manage plant-parasitic nematodes in vegetable production. EDIS 2018.

da Silva, A. L. B. R., Coolong, T., and Smith, E. 2019. Irrigating app. Available at http://www.irrigating.uga.edu. Accessed 10-01-2020

Dutta, B., Searcy, J., and Coolong, T. 2018. Fusarium wilt of watermelon in Georgia. UGA Extension. Available at https://secure.caes.uga.edu/extension/publications/files/pdf/B%201485_1.PDF Accessed 2020-09-09.

Egel, D., and Martyn, R. 2013. Fusarium wilt of watermelon and other cucurbits. Online publication. Plant Health Instruct. doi: 10.1094/PHI-I-2007-0122-01.

Everts, K. L., and Himmelstein, J. C. 2015. Fusarium wilt of watermelon: Towards sustainable management of a re-emerging plant disease. Crop Prot. 73:93-99.

FAO. 2018. Countries by Commodities –Top Yield – Watermelons. Available at http://www.fao.org/faostat/en/#data/QC Accessed 2020-04-14.

Hoffmann, M., Ajwa, H. A., Westerdahl, B. B., Koike, S. T., Stanghellini, M., Wilen, C., and Fennimore, S. A. 2020. Multitactic preplant soil fumigation with allyl isothiocyanate in cut flowers and strawberry. HortTechnology 1:1-8.

Hutchinson, C. M. 2005. Evaluation of chloropicrin soil fumigation programs for potato (*Solanum tuberosum* L.) production. Pages 129-131 in: Proceedings of the Florida State Horticultural Society.

Keinath, A. P. 2019. Integrated management for Fusarium wilt of watermelon. Clemson (SC): Clemson Cooperative Extension, Land-Grant Press by Clemson Extension. Available at https://lgpress.clemson.edu/publication/integrated-management-for-fusarium-wilt-of-watermelon/ Accessed 2020-09-09.

Keinath, A. P., Coolong, T. W., Lanier, J. D., and Ji, P. 2019. Managing Fusarium wilt of watermelon with delayed transplanting and cultivar resistance. Plant Dis. 103:44-50.

Koppen, W. 1931. Grundriss der Klimakunde. Walter de Gruyter, Berlin, Germany.

Leslie, J. F., and Summerell, B. A. 2008. The fusarium laboratory manual. Blackwell Publishing, Ames, IA. .

Lin, Y. H., Chen, K. S., Chang, J. Y., Wan, Y. L., Hsu, C. C., Huang, J. W., and Chang, P. F. L. 2010. Development of the molecular methods for rapid detection and differentiation of *Fusarium oxysporum* and *F. oxysporum* f. sp. *niveum* in Taiwan. New biotechnol. 27:409-418.

Martyn, R. D. 2014. Fusarium wilt of watermelon: 120 years of research. Hortic. Rev. 42:349-442.

Miller, N. F., Standish, J. R., and Quesada-Ocampo, L. M. 2020. Sensitivity of *Fusarium oxysporum* f. sp. *niveum* to prothioconazole and pydiflumetofen In vitro and efficacy for Fusarium wilt management in watermelon. Plant Health Prog. 21:13-18.

Netzer, D. 1976. Physiological races and soil population level of Fusarium wilt of watermelon. Phytoparasitica 4:131-136.

Nnamdi, C., Hajihassani, A., and Grey, T. 2020. Evaluation of fumigants on the control of southern blight, southern root-knot nematode, and weeds in pepper, 2019. . Plant Dis. Manag. Rep. 14: N001.

Petkar, A., and Ji, P. 2017. Infection courts in watermelon plants leading to seed infestation by *Fusarium oxysporum* f. sp. *niveum*. Phytopathology 107:828-833.

Petkar, A., Langston, D. B., Buck, J. W., Stevenson, K. L., and Ji, P. 2017. Sensitivity of *Fusarium oxysporum* f. sp. *niveum* to prothioconazole and thiophanate-methyl and gene mutation conferring resistance to thiophanate-methyl. Plant Dis. 101:366-371.

Rapicavoli, J., Whitten Buxton, K. R., and Hadden, J. F. 2018. Miravis®: A new fungicide for control of Fusarium wilt in cucurbits. Phytopathology 108:S1.152 (Abstract).

Smith, S. N. 2007. An overview of ecological and habitat aspects in the genus Fusarium with special emphasis on the soil-borne pathogenic forms. Plant Pathol. Bull. 16:97-120.

Stevens, M. C., Freeman, J. H., and Dittmar, P. J. 2016. Impact of totally impermeable film on the efficacy of 1, 3-dichloropropene and chloropicrin mixtures for the control of nutsedge. Weed Technol. 30:910-918.

Sydorovych, O., Safley, C. D., Welker, R. M., Ferguson, L. M., Monks, D. W., Jennings, K., Driver, J., and Louws, F. J. 2008. Economic evaluation of methyl bromide alternatives for the production of tomatoes in North Carolina. HortTechnology 18:705-713.

UGA. 2019. Cordele, Georgia climate data. Available at http://www.georgiaweather.net/?content=calculator&variable=CC&site=CORDELE 08-01-2020.

US-EPA. 2005. Overview of the use and usage of soil fumigants.32.

USDA-NASS. 2019. Quick Stats. USDA National Agricultural Statistics Service. Available at https://data.nal.usda.gov/dataset/nass-quick-stats. Accessed 2020-03-24.

USDA. 1983. Soil survey. Web soil survey - soil survey of Crisp County, Georgia. Available at https://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx Accessed 10-01-2020.

Walters, T. W., Bolda, M., and Zasada, I. A. 2017. Alternatives to current fumigation practices in western states raspberry. Plant Health Progress 18:104-111.

Watson, T. T., and Desaeger, J. A. 2019. Evaluation of non-fumigant chemical and biological nematicides for strawberry production in Florida. Crop Protect. 117:100-107.

Zhou, X., and Everts, K. 2003. Races and inoculum density of *Fusarium oxysporum* f. sp. *niveum* in commercial watermelon fields in Maryland and Delaware. Plant Dis. 87:692-698.

Zhou, X., and Everts, K. 2007. Characterization of a regional population of *Fusarium oxysporum* f. sp. *niveum* by race, cross pathogenicity, and vegetative compatibility. Phytopathology 97:461-469.

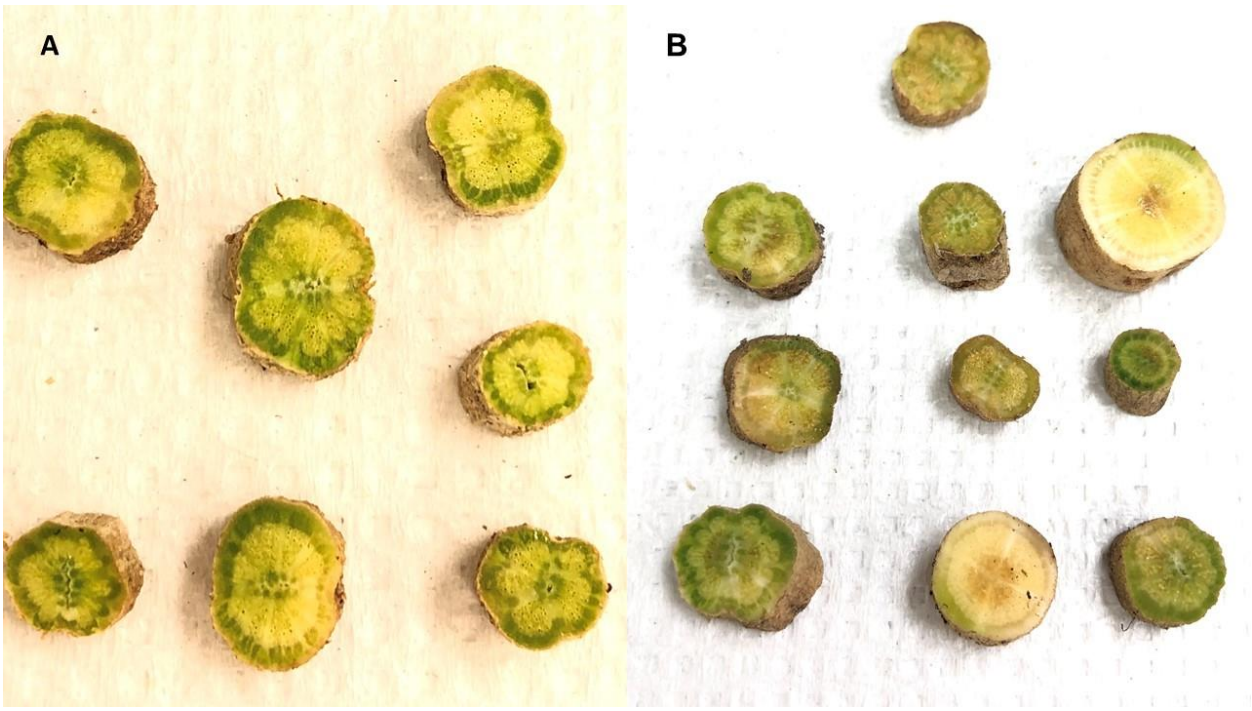


Figure 4.1. Vascular discoloration rating of Fusarium wilt of watermelon caused by *Fusarium oxysporum* f. sp. *niveum* A) Negative B) Positive.

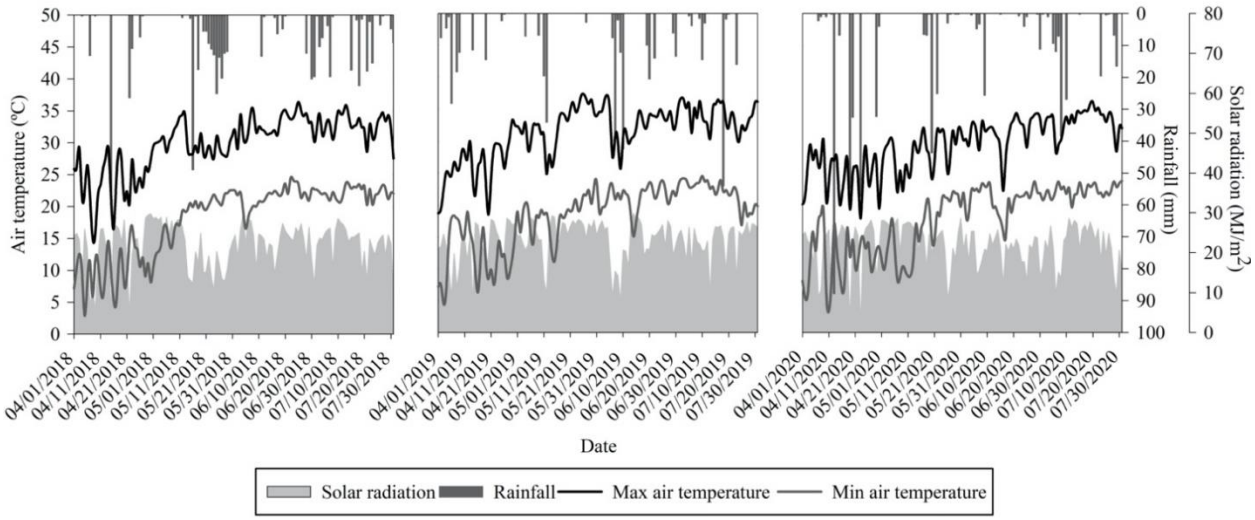


Figure 4.2. Weather conditions of maximum and minimum air temperature, solar radiation, and rainfall in 2018, 2019, and 2020. Data retrieved from the Georgia Automated Environmental Monitoring Network at Cordele, GA.

Table 4.1. Evaluation of Pic-clor 60 and post-plant soil-applied fungicides on Fusarium wilt incidence in watermelon (2018).

Treatments and rate/ha	Disease incidence[a]
Non-treated	62.8 a[b]
Pydiflumetofen, 0.63 liters	48.5 b
Prothioconazole, 0.42 liters	44.5 b
Pic-clor 60, 280.21 kg	37.2 b
Pic-clor 60, 336.26 kg	26.2 c
Pic-clor 60, 336.26 kg + pydiflumetofen, 0.63 liters	14.2 d
Pic-clor 60, 336.26 kg +prothioconazole, 0.42 liters	11.5 d
P-value	<0.05

[a]Fusarium wilt incidence was determined (57 days after transplanting: at harvest maturity) as the percentage of plants with symptoms to the total number of plants in a bed.

[b]Data are means of five replicates. Means within a column with the same letter are not significantly different (P =.05, Student-Newman-Keuls).

Table 4.2. Evaluation of Pic-clor 60 and post-plant soil-applied fungicides on Fusarium wilt incidence in watermelon (2019).

Treatments and rate/ha	**Disease incidence**		
	Mid-season[a]	**At harvest[b]**	**Vascular discoloration[c]**
Non-treated	15.3 ab[d]	17.7	32.6
Pydiflumetofen, 0.63 liters	23.4 a	25.4	28.0
Prothioconazole, 0.42 liters	16.5 ab	19.8	38.8
Bio-control[e]	22.9 ab	26.2	33.4
Pic-clor 60, 224.17 kg	7.8 ab	13.7	38.0
Pic-clor 60, 224.17 kg + bio-control	6.1 b	15.4	32.2
Pic-clor 60, 224.17 kg + pydiflumetofen, 0.63 liters	8.7 ab	19.9	36.7
Pic-clor 60, 224.17 kg+ prothioconazole, 0.42 liters	13.2 ab	20.8	28.9
P-value	0.014	0.578	0.933

[a]Mid-season rating was taken at 35 days after transplanting as the percentage of plants with symptoms to the total number of plants in a bed.

[b]Final disease incidence was taken at 85 days after transplanting as the percentage of plants with symptoms to the total number of plants in a bed.

[c]Percent vascular discoloration was rated as the percentage of plant roots in a bed vascular discoloration. Vascular discoloration was determined visually by taking a cross-section of 10-cm (in length) stem tissue at the collar region of the plant.

[d]Data are means of five replicates. Means within a column with the same letter are not significantly different (*P* =.05, Student-Newman-Keuls).

[e]Bio-control treatment included a mixture of *Bacillus amyloliquefaciens* Strain PTA-4838* at 0.05 L/ha and *Glomus intaradices*, *Glomus mosseae, Glomus aggregatum* and *Glomus etunicatum* at 0.01 L/ha.

Table 4.3. Evaluation of Pic-clor 60 and post-plant soil-applied fungicides on Fusarium wilt incidence in watermelon (2020).

Treatments and rate/ha	**Disease Incidence[a]**	**Vascular discoloration[b]**
Nontreated	62.5 a[c]	89.7 a
Pydiflumetofen, 0.63 liters	20.0 bcd	49.7 bc
Prothioconazole, 0.42 liters	15.0 cd	44.9 bc
Pic-clor 60, 280.21 kg	50.0 a	58.7 b
Pic-clor 60, 280.21 kg + pydiflumetofen, 0.63 liters	27.5 bc	49.7 bc
Pic-clor 60, 280.21 kg + prothioconazole, 0.42 liters	35.0 b	52.2 bc
Pic-clor 60, 336.26 kg	27.5 bc	38.4 c
Pic-clor 60, 336.26 kg + pydiflumetofen, 0.63 liters	5.0 d	22.3 d
Pic-clor 60, 336.26 kg+ prothioconazole, 0.42 liters	2.5 d	24.8 d
P-value	0.001	0.001

[a]Final disease incidence was taken at 83 days after transplanting as the percentage of plants with symptoms to the total number of plants in a bed.

[b]Percent vascular discoloration was rated as the percentage of plant roots in a bed vascular discoloration. Vascular discoloration was determined visually by taking a cross-section of 10-cm (in length) stem tissue at the collar region of the plant.

[c]Data are means of five replicates. Means within a column with the same letter are not significantly different (P =.05, Student-Newman-Keuls).

CHAPTER 5

CONCLUSIONS

The Fusarium wilt fungus (*Fusarium oxysporum* f. sp. *niveum*, FON) and the root-knot nematode (*Meloidogyne incognita* (Kofoid and White) Chitwood; RKN) are important soil-borne pathogens causing severe damage in watermelon production throughout the world (Davis 2007; Lynch and Carpenter 1999; Martyn 2014; Thies et al. 2010). FON can infect and induce symptoms in watermelon plants at any growth stage (Beattie and Doolittle 1951). Under favorable condition, yield losses of more than 80% have been reported (Petkar et al. 2017). Moreover, yield losses due to RKN has been estimated to reach approximately 20% in situations with high RKN populations (Davis 2007; Lynch and Carpenter 1999; Thies et al. 2010). Management of both pathogens is crucial for sustainable watermelon production.

In this study, the influence of the level of micronutrients, Fe, Mn, and Zn, were monitored through relative gene expression in watermelon leaves before and after inoculation with FON and RKN **(**single and mixed) in a hydroponics system. We observed that plants treated with Fe, Mn, and Zn at higher and lower doses than those found in standard Steiner solution resulted in differential expression of genes in both SA and JA pathway. Upregulation was observed only in some micronutrient-pathogen treatments such as: *PDF* gene in high Fe treated plants with FON inoculation, *VSP* gene in low Mn treated plants with RKN inoculation, and *PR1* gene in low Zn treated plants with co-inoculation of both FON and RKN. These observations

indicate that genes in JA (*PDF* and *VSP*) and SA pathway (*PR1*) in watermelon plants respond differentially with respect to different micronutrients and pathogen combination treatments. Overall, these results suggest a possible association between the availability of specific nutrients and the induction of plant defense genes in watermelon against FON and RKN. Detailed study is essential to understand how different levels of nutrient concentrations affect the expression of induced resistance genes in watermelon.

To better understand the interaction between watermelon and Zn in FON and RKN pathosystems, RNA-based sequencing (RNAseq) was used to identify transcript level changes induced by different level of Zn in watermelon. Higher numbers of differentially expressed genes (DEGs) were observed in high Zn-treated than in low Zn-treated non-inoculated plants (high Zn: low Zn :: 333:14), in plants inoculated with FON alone (high Zn: low Zn :: 524:104), and in plants inoculated with RKN alone (high Zn: low Zn :: 2908:2). However, in the co-inoculated system, low Zn treatment had 268 DEGs and the high Zn treatment only had one DEG. Further, GO and KEGG enrichment analysis revealed that most DEGs in high Zn treatment could be significantly enriched in hormone signal transduction and MAPK signaling pathway suggesting potential induction of systemic resistance. This could mean that high Zn treatment is modulating genes related crucial disease resistance and signal transduction pathways against FON and RKN in watermelon. Further research is essential to optimize the level of Zn necessary to induce systemic resistance in watermelon that can successfully be translated into a management strategy.

Another focus of this study was to evaluate the combination of chemical treatments, fumigant Pic-clor 60 and post-plant soil-applied fungicides (prothioconazole and pydiflumetofen) for Fusarium wilt management in watermelon. The fumigant, Pic-clor 60 at

336.26 kg/ha when applied together with a fungicide either pydiflumetofen or prothioconazole, significantly reduced the incidence of Fusarium wilt of watermelon in two of the three field trials. Prothioconazole and pydiflumetofen stand-alone application also successfully reduced the disease incidence compared to control treatments; however, application of either fungicides together with Pic-clor 60 at 336.26 kg/ha, showed the highest reduction of FON incidence. In summary, this study provides new insights into the management of FON and RKN in watermelon using micronutrient manipulation of host by inducing genes related to plant defense. Additionally, use of fumigant and soil-applied fungicides in a program to reduce disease incidence of FON. Overall, the outcome of these studies may in time can be incorporated into the integrated disease management options in watermelon against FON and RKN in the future.

Literature cited

Beattie, J., and Doolittle, S. 1951. Watermelons. US Government Printing Office, Washington, DC.

Davis, R. F. 2007. Effect of *Meloidogyne incognita* on watermelon yield. Nematropica 37:287-294.

Lynch, L., and Carpenter, J. 1999. The economic impacts of banning methyl bromide: Where do we need more research. in: 1999 Annual Research Conference on Methyl Bromide Alternatives and Emissions Reductions. http://mbao.org/mbrpro99.html.

Martyn, R. D. 2014. Fusarium wilt of watermelon: 120 years of research. Hortic. Rev. 42:349-442.

Petkar, A., Langston, D. B., Buck, J. W., Stevenson, K. L., and Ji, P. 2017. Sensitivity of *Fusarium oxysporum* f. sp. *niveum* to prothioconazole and thiophanate-methyl and gene mutation conferring resistance to thiophanate-methyl. Plant Dis. 101:366-371.

Thies, J. A., Ariss, J. J., Hassell, R. L., Olson, S., Kousik, C. S., and Levi, A. 2010. Grafting for management of southern root-knot nematode, *Meloidogyne incognita*, in watermelon. Plant Dis. 94:1195-1199.

APPENDIX

APPENDIX TO CHAPTER 2

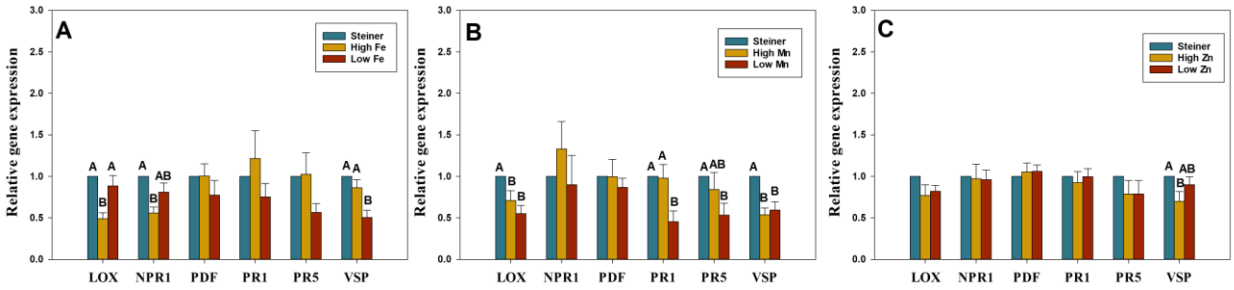


Figure A.1. Relative expression of *NPR1*, *PR1*, *PR5*, *LOX*, *PDF* and *VSP* genes by qRT-PCR in watermelon leaves at 11 day-post treatment with micronutrients (Fe, Mn and Zn) via hydroponics system. Watermelon seedlings (cv. Sugar Baby; 3-weeks old) were either treated with Fe or Mn or Zn at high (3X), low (0.5X) and standard concentration (X, Steiner) for 11-days. Data are the mean fold changes ± SE in gene transcript levels in tissues from micronutrient treated plants relative to tissues from non-treated control plants in Steiner. Letters indicate a significant difference between treatments with the Tukey-Kramer test (P<0.05).