PHYTOHORMONES ASOCIATED WITH A BACTERIAL DISEASE OF CREEPING BENTGRASS (*AGROSTIS STOLONIFERA* L.) CAUSED BY *ACIDOVORAX AVENAE* SUBSP. *AVENA*

By

Sha Liu

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Crop and Soil Sciences – Master of Science

2016

ABSTRACT

PHYTOHORMONES ASOCIATED WITH A BACTERIAL DISEASE OF CREEPING BENTGRASS (*AGROSTIS STOLONIFERA* L.) CAUSED BY *ACIDOVORAX AVENAE* SUBSP. *AVENAE*

By

Sha Liu

Creeping bentgrass (*Agrostis stolonifera*) putting greens have been plagued by bacterial etiolation disease caused by *Acidovorax avenae* subsp. *avenae* (*Aaa*). Based on the visual symptoms of the disease we aimed to determine whether disease symptoms could be associated with bacterial manipulation of the phytohormone balance in creeping bentgrass tissues. *In vitro* and *in vivo* studies of this pathosystem for phytohormone analysis were performed. *Aaa* and a non-pathogenic, negative control *Pseudomonas aureofaciens* (*Pa)* were cultured at room temperature (20-22 °C) for 14 days. For *in vivo* studies, *Aaa* infected and uninfected creeping bentgrass 'Tyee' and 'Penn A-4' were grown in hydroponics under optimal 23/20°C day/night temperatures and heat stress 35/30 °C conditions in growth chambers. Bacterial culture or plant samples were taken for analysis of phytohormones: gibberellic acid isoforms (GA1, GA3, GA4, and GA20), jasmonic acid (JA), salicylic acid (SA), indole-3-acetic acid (IAA), zeatin riboside (ZR), and abscisic acid (ABA). GA1, GA3, GA4 and IAA were detected in some *Aaa* cultures but not in *Pa*. 'Penn-A4' was more *Aaa* sensitive under high temperature than 'Tyee'. 'Tyee' infected with *Aaa* at high temperatures showed higher JA content in all plant tissues, higher SA in stolons and roots, and less GA3 and GA20 in leaf and stolon tissues than 'Penn-A4' in the same conditions. Based on these results we hypothesized that pre-treating plants with SA or JA could be effective in reducing disease symptoms of *Aaa* in creeping bentgrass. The treatments included foliar application of 10 µmol/L SA, 20 µmol/L SA, 0.5 mM JA in 0.02% ethanol and 2 mM JA in 0.02% ethanol prior to exposure to heat stress (35°C) and optimal temperature (23 °C). Physiological measurements included turf quality, leaf and root electrolyte leakage, chlorophyll content, photochemical efficiency and root viability. The results demonstrate that SA application may reduce disease symptoms of both cultivars under both temperature treatments. The effect of 20 µmol/L SA was the most significant among treatments, especially under optimal temperature. JA application could also reduce *Aaa* disease severity especially under optimal temperature; however, the effects of 2 mM JA were more significant in cultivar ‘Tyee’ than ‘Penn-A4’. These results suggest that under controlled conditions SA and JA may induce tolerance of creeping bentgrass to *Aaa* and reduce disease damage. Differential GA isoform accumulation produced by *Aaa* could contribute to disease severity and JA and SA accumulation may contribute to disease tolerance.

DEDICATED
To
My parents Xiaoping and Guitian Liu, and all my friends,
Thank you so much for your support

ACKNOWLEDGEMENTS

During my graduate experience, I always gain supports from the Department of Plant, Soil and Microbial Science and Turf science program. I especially thank my advisor and professor Dr. Emily Merewitz, who is my mentor and help me not only in academics but also in daily life. I would also like to thank to my graduate committee Dr. Joseph Vargas and Dr. James Crum, for the opportunity to learn and develop skills in plant pathology and soil science.

I would like to thank my lab colleagues, Dr. Sanal Krishnan, Yingmei Ma and Kevin Laskowski, for always giving me a hand when I get troubled with the experiments. I am grateful to Nancy Dykema, Adam Palmatier, Ron Detweiler and Yan Liu for their friendly support and advice in plant pathology study. Thanks to all undergraduates and interns who help us to maintain lab and facilitate in every aspect of researches.

I appreciate to my dear parents, for sending me their concerns and supports cross time zones. I would like to thank my boyfriend Bo Meng and other friends surrounding me. Thank you for being here by my side, and help me to reach this accomplishment. Thank you for your support, understand, friendship and love.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

BACKGROUND

Bacterial etiolation and decline of creeping bentgrass

The first bacterial decline problem on creeping bentgrass was reported by Vargas (1981), and the infection was caused by *Xanthomonas campestris* pv. *graminis* (Roberts and Vargas, 1984). *Acidovorax* species have also been previously reported causing bacterial brown stripe on creeping bentgrass in Japan (Furuya et al., 2009). Recently, creeping bentgrass etiolation and decline was determined to be associated with the genus *Acidovorax*. Early symptoms include foliar chlorosis and can become severely necrotic as symptoms progress. Damage is most commonly seen on highly managed putting greens under high temperature conditions. Symptoms caused by *Aaa* are similar to those caused by *Xanthomonas translucens* pv. *poae* on annual bluegrass (Giordano et al., 2010). In creeping bentgrass, Giordano et al. (2012) observed bacterial streaming in infected plants and amplified a portion of the 16S ribosomal DNA. Koch's postulates were used to show that *Aaa* is a causal agent of the disease. Roberts et al., (2014) isolated bacteria from etiolated samples and found *A. avenae*, *Pseudomonas* spp., *Pantoea* spp. and *X. translucens* may also be associated with the disease.

Acidovorax species

Aaa has been reported to infect a broad range of hosts. The genus *Acidovorax* contains many plant pathogenic species that cause extensive damage to important economic crops including corn, pearl millet, and rice. Three major subspecies exist in *Acidovorax avenae*, including *A. avenae* subsp. *avenae*, and *A. avenae* subsp. *cattleyae*. *Acidovorax avenae* subsp. *avenae* was once classified as a *Pseudomonas* sp. and it is pathogenic to members of the *Gramineae* (Che et al., 1999; Willems et al., 1992). The bacterium can attack young rice leaves and can also cause grain discoloration in mature rice (Webster and Gunnell, 1992). The disease can cause water-soaked stripes on the leaves and sheaths (Kadota 1996). *Aaa* has been reported to infect maize through leaf stomata, resulting in narrow and white leaf lesions (Gitaitis et al., 1981). In pearl millet (*Penisetum glaucum*) the infection likely occurs through hydathodes and induces reddish-brown stripes on leaves (Gitaitis et al., 2002). *Aaa* is a seed-borne pathogen and the infection of *Aaa* in rice has been found in a broad range of geographical regions (Shakya et al., 1985). *Aaa* may not be able to survive well in soil or plant debris. The infection of *Aaa* is thought to spread via farm instruments (Gitaitis et al., 1978) as well as internal transmission in latently infected plants. High humidity and high temperatures are thought to be favorable conditions to trigger disease development caused by *Aaa* (White et al., 1994).

Phytohormones play a role in plant defense responses

Based on the visual disease symptoms of *Aaa*, we hypothesized that a phytohormones could play a major role in bacterial etiolation of creeping bentgrass. We further speculate that the phytohormone gibberellic acid (GA) could be produced by the bacteria and cause the abnormal etiolation symptom, since the primary function of GA in plants is to promote cellular elongation. The symptoms of bacterial etiolation in creeping bentgrass are similar to bakanae of rice seedlings, where the fungus *Gibberella fujikuroi* can produce GA to trigger an increase in height in the seedlings (Kurosawa 1926). The biosynthesis of GAs is complex and various isoforms of GA exist. The most active isoforms in plants are GA1 and GA4; Various soil microorganisms can produce GA3 (MacMillan 2001).

Measuring phytohormone profiles in this research is important since GAs often interact with other phytohormones, especially under pathogen attack, and could play a role in plant susceptibility to *Aaa* under high temperatures. Components of GA signaling pathway appear to integrate SA and JA pathways in plant defense response (Achard et al. 2006). Similarly, auxins and ABA also are important in disease responses and are associated with GA signling. Thus, understanding whether the accumulation of specific hormones is differential during *Aaa* disease or due to high temperature stress will help us to reveal the mechanism behind bacterial etiolation of creeping bentgrass. Here, a brief background on each hormone is provided.

Auxins

Auxins can regulate plant development and show direct and indirect effects on the regulation of pathogen resistance responses (Swarup and Péret, 2012; Kazan and Manners, 2009). The plant innate immune system relies in the detection of pathogen-associated molecular patterns (PAMPs) and This resistance response is called PAMP-triggered immunity (PTI) (Dodds and Rathjen, 2010). Auxins can negatively affect plant defense by interacting with PTI (Robert-Seilaniantz et al., 2011). Auxin–aspartic acid (IAA–Asp) has been reported to exacerbate necrotrophic fungus *Botrytis cinerea* disease symptoms in infected plants (Gonzalez-Lamothe et al., 2012).

Exogenous application of auxin has been shown to promote *Pst* DC3000 disease (Navarro et al. 2006). Auxin-treated rice was observed showing enhanced susceptibility to *Xanthomonas oryzae* pv. *oryzae* (Ding et al., 2008). In contrast, reduction in *P. syringae* pv. *maculicola* 4326 growth has been found in Auxin resistant axr2-1 mutants of Arabidopsis, which indicate that blocking auxin responses can increase resistance in plants (Wang et al. 2007). These results indicate that auxin plays a role in the attenuation of plant disease defense responses.

Auxins regulation of plant development can also cause indirect effects on plant defense response. For instance, IAA application can reduce rice resistance to *Xanthomonas oryzae* pv. *oryzae.* The possible reason of pathogen growth may be caused by cell wall expansion and loosening which are activated by IAA (Ding et al., 2008).

Abscisic acid

Abscisic acid is an essential phytohormone that regulates plants abiotic stress (Shinozaki and Yamaguchi-Shinozaki, 2007). In addition, ABA plays a role as a positive or a negative regulator of plant defense (Ton et al., 2009). Abscisic acid can positively regulate the resistance to some necrotrophs. For instance, ABA-insensitive mutants was found more susceptible to *Pseudomonas syringae* than wild-type plants in Arabidopsis (*Arabidopsis thaliana*). Exogenous application of ABA protects Arabidopsis against *A.brassicicola* and *P. cucumerina*indicating (Ton and Mauch-Mani 2004). In addition, ABA activates stomatal closure that acts as a barrier against bacterial infection (Melotto et al. 2006).

However, ABA has been shown to negatively regulate plant defense against various pathogens. The wild type plants increase disease severity to *Fusarium oxysporum* compared to ABA-deficient mutant of Arabidopsis (*aba2-1*) (Anderson et al. 2004). Also, enhanced resistance to *Pseudomonas syringae* has been observed in ABA-impaired mutants in tomato (*sitiens*) *c*ompared to the wild type plants. The exogenous application of ABA reduced the resistance of rice plants to *Magnaporthe grisea* (Koga et al.2004). An antagonistic interaction between SAR and ABA signaling in Arabidopsis has been indicated, by which ABA treatment showed to suppress SAR induction (Yasuda et al. 2008).In addition, ABA can also regulate resistance protein (R-protein) activity. ABA application can reduce the nuclear accumulation of SNC1 (suppressor of *npr1-1*, constitutive1) and RPS4 (resistant to *Pseudomonas syringae* 4) enhancing disease susceptibility to *P. syringae* (Mang et al., 2012).

Salicylic acid

SA plays a role in the activation of defense responses against biotrophic and hemi-biotrophic pathogens (Grant and Lamb 2006). It also regulates systemic acquired resistance

(SAR) (Glazebrook, 2005). In addition, SA is also important to regulate plant immunity. SA can interact with JA and auxin signaling pathways to trigger the efficient resistance responses against pathogens (Thaler et al., 2012). NPR1 (non-expressor of plant resistant genes 1) is an important regulatory component of SA signaling, which interacts with TGACG sequence-specific binding protein (TGA) that can activate SA-responsive plant resistant genes (*PR* genes). In the presence of SA, NPR1 complex redox dissociate and monomers translocate to the nucleus. NPR1 plays an important role in SA-JA interaction. For example, in *npr1* mutant *Arabidopsis*, SA-mediated suppression of JA responsive gene expression was decreased compared to wild type plants (Spoel et al. 2007). SAR can activate systemic resistance to distal parts of the plant through the salicylic acid (SA)-mediated signaling pathway (Glazebrook 2005). SAR induced by SA can trigger systemic resistance to distal parts of the plant (Glazebrook 2005). Wild *Arabidopsis* plants were more resistant than SA-deficient mutant (*sid2-1)* mutant (Nawrath and Métraux, 1999). Exogenous application of SA has been shown to reduce plant disease severity. Iwai et al. (2007) found that SA application could protect rice seedlings from blast fungus.

Jasmonic acid

JA plays a role in plant defense responses against insects and microbial pathogens. Previous studies have demonstrated that pathogen infection and tissue damage could promote local JA content, and of *PR* genes could be induced exogenous application of JA (Wasternack 2007). Exogenous application of JA results in enhanced resistance to herbivore attacks (Howe and Jander 2008). In *Arabidopsis,* JA signaling is induced in response to against *Frankliniella occidentalis* thrips (De Vos et al., 2005). Besides insects, JA can also induce defense response to microbial pathogens. Exogenous application of JA can reduce the growth of semi-biotrophic oomycete *Phytophthora infestans* (Cohen et al., 1993). JA-deficient mutants in *Arabidopsis* reduced resistance against necrotrophic bacteria *Erwinia carotovora*

(Pozo et al., 2005). JA treatments has been reported to against semi-biotrophic fungus *Tilletia. laevia* in wheat (*Triticum. aestivum*) against (Lu et al., 2006). JA treatment can also induce expression of specific *PR* genes to protect rice (*Oryza sativa*) against biotrophic blast disease (*Magnaporthe. oryzae*, Mei, et al 2006).

SA and JA signaling pathways negatively interact in many aspects. WRKY33 as a positive regulator of JA-related genes, is component involved in mediating the antagonism between SA and JA, and it is also a repressor of the SA pathway (Birkenbihl et al., 2012). What is more, SA induce down-regulation of JA-signaling, and increase the susceptibility of *wrky33* mutant plants to necrotrophic fungi (Sánchez-Vallet et al., 2012). Mitogen activated protein kinase 4 (MPK4) also involved in mediating SA and JA interaction, which acts as a negative regulator of SA signaling and positive regulator of JA signaling in *Arabidopsis*. The *Arabidopsis mpk4* mutants were found reduced expression of JA responsive genes and the resistance to *Alternaria. brassicicola* compared to wild plants (Brodersen et al. 2006). In contrast, SA content and expression of SA induced *PR* genes are increased in *mpk4* mutants against *Pseudomonas syringae pv. tomato* (Petersen et al., 2000).

Recent evidence reveals that monocots and dicots may be differential in SA and JA signaling. A coordinate or parallel interaction between SA and JA in plant defense responses has been supported by Tamaoki et al. (2013). JA-dependent signaling can mediate parts of SA-upregulated genes in rice (*Oryza sativa L.*). A better understanding of JA and SA signaling in response to abiotic and biotic stress in monocot species is needed.

Gibberellic acid

Gibberellins including GA promotes plant growth and regulate various developmental processes. DELLA proteins act as repressors of GA responses. GA- responsive genes can be stimulated by degradation of negative regulators of DELLA proteins via a ubiquitin E3 ligase

SCF complex and the 26S proteasome (Griffiths et al. 2006). GAs can be produced not only by higher plants, fungi and bacteria (MacMillan 2001).

DELLA proteins and other GA signaling components are involved in plant immune responses (Navarro et al. 2008). DELLA proteins integrate SA and JA plant defense response pathways and promote resistance to necrotrophs (Achard et al. 2006). Since GA stimulates degradation of DELLA proteins, it is likely that GA promotes resistance to biotrophs and susceptibility to nectrotrophs. GA treatments resulted in enhanced susceptibility to necrotrophic pathogen *Alternaria brassicicola* by degrading DELLA proteins. To investigate the reason of DELLA proteins regulation of defense responses, researchers found that DELLA proteins regulate the levels of ROS after pathogen infection (Achard et al. 2008).

Elongated Uppermost Internode (EUI) as a GA deactivating enzyme modulate bioactive GA levels and involve in disease defense against bacterial pathogens in rice Yang et al. (2008). Overexpress EUI in rice showed reduced GAs content and increased resistance to *Magnaporthe oryzae*; however,the *eui* mutants which loss function of EUI , showed increased levels of GAs and reduced resistance (Yang et al. 2008).

GA perception mutants have also been shown to affect defense responses in plant. Probenazole inducible 1 (PBZ1) can be induced by rice blast pathogen, and the of PBZ1 was increased in gid1 mutant which is defective in GA reception. High content of PBZ1 results in strong resistance to blast disease in rice (Tanaka et al. 2006).

Although adequate evidence demonstrates that GA and its signaling components can regulate plant defense responses, the mechanism of GA action on defense responses has not been fully elucidated.

Disease Management

Up to now, there are no effective chemical methods to control or prevent this disease reported for turfgrass management. In rice, it is recommended to maintain proper levels of temperature and humidity in seedlings and choose healthy seeds to control bacterial brown stripe of rice caused by *Aaa* (Kadota 1996). Resistant cultivars have been found in sugarcane and maize by disease resistance screening (Mariotti et al., 1991; Pataky et al., 1997). In creeping bentgrass, a growth chamber study showed that the antibiotics oxyteracycline and streptomycin sulfate can reduce bacterial decline caused by *Aaa*, but only oxytetracycline could maintain healthy plants compared to untreated control. Antibiotic use was a way to provide further evidence that a bacterium is the causal agent of the disease. In other words, this was not tested as a practical management strategy for wide scale field use. Additionally, application of the plant growth regulater (PGR) trinexapac-ethyl (TE) was found to increase disease incidence (Giordano 2014). A better understanding of the plant-bacterial-environment interactions will allow for a better understanding of disease development and will allow turfgrass scientists to make better recommendations to turfgrass managers regarding this disease.

Summary and Major Objectives

Previous research shows that *Aaa* can infect weakened creeping bentgrass, under favorable environmental conditions; however, interactions of bacterial pathogen occurrence with changes in hormone content, with cultivar variation in susceptibility, environmental stress are unclear. Therefore, our major goals of the research are to better understand if and why heat stress may make creeping bentgrass more susceptible to bacterial infection and to better understand the mechanism behind the etiolation symptom related to endogenous and exogenous phytohormone content. With this research, we hope to reveal valuable information that may lead to better disease management practices and remediation methods that can be applied to this and other bacterial diseases.

LITERATURE CITED

LITERATURE CITED

Achard, P., Cheng, H., De Grauwe, L., Decat, J., Schoutteten, H., Moritz, T., Van Der Straeten, D., Peng, J., Harberd, N.P. 2006. Integration of plant responses to environmentally activated phytohormonal signals. Science 311:91–94.

Achard, P., Renou, J.P., Berthome, R., Harberd, N.P., Genschik, P. 2008. Plant DELLAs restrain growth and promote survival of adversity by reducing the levels of reactive oxygen species. Curr Biol. 18:656–660.

Anderson, J.P., Badruzsaufari, E., Schenk, P.M. 2004. Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defence gene expression and disease resistance in *Arabidopsis*. Plant Cell. 16:3460–3479.

Birkenbihl, R.P., Diezel, C., Somssich, I.E. 2012. *Arabidopsis* WRKY33 is a key transcriptional regulator of hormonal and metabolic responses toward *Botrytis cinerea* infection. PlantPhysiol. 159:266–285.

Brodersen, P. and Petersen, M. 2006. *Arabidopsis* MAP kinase 4 regulates salicylic acid- and jasmonic acid/ethylene-dependent responses via EDS1 and PAD4. Plant J 47:532–546.

Che, F.S., Iwano, M., Tanaka, N., Takayama, S., Minami, E., Shibuya, N., Kadota, I., and Isogai, A. 1999. Biochemical and morphological features of rice cell death induced by Pseudomonas avenae. Plant & Cell Physiology. 40:1036-1045.

Cohen, Y., Gisi, U., Niderman, T. 1993. Local and systemic protection against Phytophthora infestans induced in potato and tomato plants by jasmonic acid and jasmonic methyl ester. Phytopathology. 83:1054–62

Dernoedon, P. 1998. Summer bentgrass decline complex may be more physiological than pathological. Turfax. 6:4-5.

De Vos, M., Van Oosten, V.R., Van Poecke, R.M. 2005 Signal signature and transcriptome changes of *Arabidopsis* during pathogen and insect attack. Mol Plant Microbe Interact. 18:923–937.

Ding, X., Cao, Y., Huang, L., Zhao, J., Xu, C., Li, X. 2008. Activation of the indole-3-acetic acid amido synthetase GH3-8 suppresses expansin expression and promotes salicylate- and jasmonate-independent basal immunity in rice. Plant Cell. 20:228-240.

Dodds, P.N. and Rathjen J.P. 2010. Plant immunity: towards an integrated view of plant–pathogen interactions. Nat. Rev. Genet. 11:539–548

Gitaitis, R., Samuelson, D., and Strandberg, J. 1981. Scanning electron microscopy of the ingress and establishment of *Pseudomonas alboprecipitans* in sweet corn leaves. Phytopathology. 71:171-175.

Gitaitis, R., Stall, R., and Strandberg, J. 1978. Dissemination and survival of *Pseudomonas alboprecipitans* ascertained by disease distribution. Phytopathology. 68:227-231.

Gitaitis, R., Walcott, R., Culpepper, S., Sanders, H., Zolobowska, L., Langston, D., 2002. Recovery of *Pantoea ananatis*, causal agent of center rot of onion, from weeds and crops in Georgia, USA. Crop Protection. 21: 983-989.

Giordano, P.R. 2014. Identification and characterization of a new bacterial disease of creeping bentgrass (Agrostis stolonifera L.) caused by *Acidovorax avenea* subsp. *avenae*. Ph.D. Thesis. Michigan State University, Michigan, USA, pp.106-107.

Giordano, P.R., Chaves, A.M., Mitkowski, N.A. and Vargas, J.M. 2012. Identification, characterization, and distribution of *Acidovorax avenae* subsp. *avenae* associated with creeping bentgrass etiolation and decline. Plant Disease. 96:1736-1742. DOI:10.1094/pdis-04-12-0377-re.

Giordano, P.R., Vargas, J.M., Detweiler, A.R., Dykema, N.M. and Yan, L. 2010. First report of a bacterial disease on creeping bentgrass (*Agrostis stolonifera*) caused by *Acidovorax* spp. in the United States. Plant Disease. 94:922-922. DOI: 10.1094/pdis-94-7-0922b.

Glazebrook, J. 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu Rev Phytopathol. 43:205–227.

Gonzalez-Lamothe, R., El Oirdi, M., Brisson, N., and Bouarab, K. 2012. The conjugated auxin indole-3-acetic acid-aspartic acid promotes plant disease development. Plant Cell. 24:762–777

Grant, M. and Lamb, C. 2006. Systemic immunity. Curr Opin Plant Biol. 9:414–420.

Griffiths, J., Murase, K., Rieu, I., Zentella, R., Zhang, Z.L., Powers, S.J., Gong, F., Phillips, A.L., Hedden, P., Sun, T.P., and Thomas, S.G. 2006. Genetic characterization and functional analysis of the GID1 gibberellin receptors in *Arabidopsis*. Plant Cell. 18:3399–3414.

Howe, G.A. and Jander, G. 2008. Plant immunity to insect herbivores. Annu Rev Plant Biol. 59:41–66.

Iwai, T., Seo, S., Mitsuhara, I., and Ohashi, Y. 2007. Probenazole-induced accumulation of salicylic acid confers resistance to Magnaporthe grisea in adult rice plants. Plant Cell Physiol. 48:915-924.

Kazan, K. and Manners, J.M. 2009. Linking development to defense: auxin in plant–pathogen interactions. Trends Plant Sci. 14: 373–382

Kadota, I. 1996. Studies on the pathogen of bacterial brown stripe of rice *Oryza sativa* and its ecology in Japan. Bull. Hokuriku Nat. Agric. Exp. Stn.

Koga, H., Dohi, K., Mori, and M. 2004. Abscisic acid and low temperatures suppress the whole plant-specific resistance reaction of rice plants to the infection of *Magnaporthe grisea*. Physiol Mol Plant Pathol. 65:3–9.

Kurosawa, E. 1926. Experimental studies on the nature of the substance excreted by the “bakanae” fungus. in Japanese Hist. Soc. Formosa. 16:213-227.

Lu, Z.X., Gaudet, D., Puchalski, B., Despins, T., Frick, M., and Laroche, A. 2006. Inducers of resistance reduce common bunt infection in wheat seedlings while differentially regulating defence-gene expression. Physiol. Mol. Plant Pathol. 67:138-148.

MacMillan, J. 2001. Occurrence of gibberellins in vascular plants, fungi, and bacteria. J Plant Growth Regul 20:387–442

Mang, H.G., Qian, W., Zhu, Y., Qian, J., Kang, H.G., and Klessig, D.F. 2012. Abscisic acid deficiency antagonizes high-temperature inhibition of disease resistance through enhancing nuclear accumulation of resistance proteins SNC1 and RPS4 in *Arabidopsis*. Plant Cell. 24:1271–1284.

Mariotti, S., Caturegli, P., Barbesino Del Prete, G.F., and Pinchera, A. 1991. Circulating soluble interleukin-2 receptor concentration is increased in both immunogenic and nonimmunogenic hyperthyroidism. J. Endocr. Invest. 14: 777–781

Mei, C.S., Qi, M., Sheng, G.Y., and Yang, Y.N. 2006. Inducible overexpression of a rice allene oxide synthase gene increases the endogenous jasmonic acid level, PR gene expression, and host resistance to fungal infection. Mol. Plant-Microbe Interact. 19:1127-1137.

Melotto, M., Underwood, W., Koczan, J., Nomura, K., and He, S.Y. 2006. Plant stomata function in innate immunity against bacterial invasion. Cell 126:969–980.

Navarro, L., Dunoyer, P., Jay, F., Arnold, B., Dharmasiri, N., and Estelle, M. 2006. A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. Science. 312 436–439

Navarro, L., Bari, R., Achard, P., Lison, P., Nemri, A., Harberd, N.P., and Jones, J.D. 2008. DELLAs control plant immune responses by modulating the balance of jasmonic acid and salicylic acid signaling. Curr Biol. 18:650–655.

Nawrath, C. and Métraux, J.P. 1999. Salicylic acid induction-deficient mutants of *Arabidopsis* express *PR-2* and *PR-5* and accumulate high levels of camalexin after pathogen inoculation. Plant Cell. 11:1393–1404.

Pataky, J., Du Toit, L., and Kerns, M. 1997. Bacterial leaf blight on shrunken-2 sweet corn. Plant Disease. 81:1293-1298.

Petersen, M., Brodersen, P., Naested, H.2000. *Arabidopsis* map kinase 4 negatively regulates systemic acquired resistance. Cell 103:1111–1120.

Piskurewicz, U., Jikumaru, Y., Kinoshita, N. 2008. The gibberellic acid signaling repressor RGL2 inhibits Arabidopsis seed germination by stimulating abscisic acid synthesis and ABI5 activity. Plant Cell. 20:2729–2745.

Pozo, M.J., Van Loon, L.C., Pieterse, C.M.J. 2005. Jasmonates—signals in plant-microbe interactions. J Plant Growth Regul 23:211–222.

Roberts, D.L. and Vargas, J.M. 1984 Antigenic relatedness of the north american Toronto bentgrass bacterium to *Xanthomonas campestris* pv. *graminis* from Europe. Phytopathology. 74:813.

Roberts, J.A. 2014. Investigating the Cause and Developing Management Options to Control Bacterial Etiolation in Creeping Bentgrass Putting Greens. Ph.D. Thesis. North Carolina State University, North Carolina, USA, pp.86-105.

Robert-Seilaniantz, A., Grant, M., and Jones, J.D. 2011. Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism. Annu. Rev. Phytopathol. 49 317–343

Ross, J.J., Miraghazadeh, A., Beckett, A.H., Quittenden, L.J. and McAdam, E.L. 2016. Interactions between gibberellins and other hormones, in Annual Plant Reviews, Volume 49: Gibberellins, The (eds P. Hedden and S. G. Thomas), John Wiley & Sons, Ltd, Chichester, UK. doi: 10.1002/9781119210436.ch8

Sánchez-Vallet, A., Lopez, G., Ramos, B., Delgado-Cerezo, M., Riviere, M. P., and Llorente, F. 2012. Disruption of abscisic acid signalling constitutively activates *Arabidopsis* resistance to the necrotrophic fungus *Plectosphaerella cucumerina*. Plant Physiol. 160 2109–2124

Shakya, D., Vinther, F., and Mathur, S. 1985. World wide distribution of a bacterial stripe pathogen of rice identified as *Pseudomonas avenae*. Journal of Phytopathology. 114:256-259.

Shinozaki, K., Yamaguchi-Shinozaki, K. 2007. Gene networks involved in drought stress response and tolerance. J. Exp. Bot. 58 221–227

Spoel, S.H., Johnson, J.S., Dong, X. 2007. Regulation of tradeoffs between plant defenses against pathogens with different life styles. Proc. Natl. Acad. Sci. U.S.A. 104:18842–18847.

Swarup, R., Péret, B. 2012. AUX/LAX family of auxin influx carriers – an overview. Front. Plant Sci. 3:225 10.3389/fpls.2012.00225

Taiz, L., Zeiger, E. 2010. Plant physiology. Sunderland, MA: Sinauer Associates.

Tamaoki, D., Seo, S., Yamada, S., Kano, A., Miyamoto, A., Shishido, H. 2013. Jasmonic acid and salicylic acid activate a common defense system in rice. Plant Signal. Behav 8: e24260 doi: 10.4161/psb.24260.

Tanaka, N, Matsuoka, M. 2006. Gid1, a gibberellin-insensitive dwarf mutant, shows altered regulation of probenazole-inducible protein (PBZ1) in response to cold stress and pathogen attack. Plant Cell Environ 29:619–631.

Thaler, J. S., Humphrey, P.T., Whiteman, N.K. 2012. Evolution of jasmonate and salicylate signal crosstalk. Trends Plant Sci. 17 260–270

Ton, J., Flors V., Mauch-Mani, B. 2009. The multifaceted role of ABA in disease resistance. Trends Plant Sci. 14 310–317

Ton, J., Mauch-Mani, B. 2004. Beta-amino-butyric acid-induced resistance against necrotrophic pathogens is based on ABA-dependent priming for callose. Plant J 38:119–130.

Vargas, J.M. 1981. Management of Turfgrass Diseases. Burgess Publishing Co., Minneapolis, MN.

Wang, D., Pajerowska-Mukhtar, K., Culler, A. H., Dong, X. 2007. Salicylic acid inhibits pathogen growth in plants through repression of the auxin signaling pathway. Curr. Biol. 17 1784–1790

Wasternack, C. 2007. Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. Ann Bot (Lond) 100:681–697

Webster, R.K., Gunnell, P.S. 1992. Compendium of Rice Diseases. The American Phytopathological Society, St. Paul, MN.

White, D., Pataky, J., and Stall, R. 1994. Unusual occurrences of bacterial leaf blight on maize and sorghum in Central Illinois. Plant Disease. 78:640.

Willems, A., Goor, M., Thielemans, S., Gillis, M., Kersters, K., and De Ley, J. 1992. Transfer of several phytopathogenic Pseudomonas species to *Acidovorax* as *Acidovorax avenae* subsp. *avenae* subsp. nov., comb. nov., *Acidovorax avenae* subsp. *citrulli*, *Acidovorax avenae* subsp. *cattleyae*, and *Acidovorax konjaci*. Int J Syst Bacteriol. 42:107-119.

Yang, D.L., Li, Q., Deng, Y.W., Lou, Y.G., Wang, M.Y., Zhou, G.X., Zhang, Y.Y., He, Z.H. 2008. Altered disease development in the *eui* mutants and *eui* overexpressors indicates that gibberellins negatively regulate rice basal disease resistance. Mol Plant. 1:528–537

Yasuda, M., Ishikawa, A., Jikumaru, Y., Seki, M., Umezawa, T., Asami, T., Maruyama-Nakashita, A., Kudo, T., Shinozaki, K., Yoshida, S., Nakashita, H. 2008. Antagonistic interaction between systemic acquired resistance and the abscisic acid-mediated abiotic stress response in *Arabidopsis*. Plant Cell. 20:1678–1692.

CHAPTER ONE

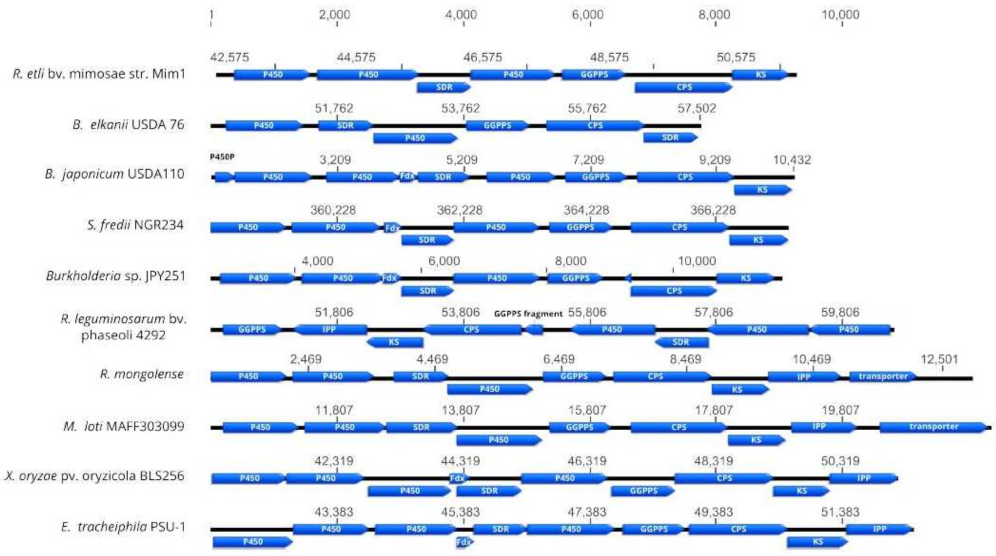
DETECTION OF GIBBERELLIN BIOSYNTHESIS GENES IN *ACIDOVORAX AVENAE* SUBSP. *AVENAE*

Gibberellin Biosynthesis in bacteria

Gibberellins are formed from geranylgeranyl diphosphate (GGPP) via a set of reactions catalyzed by different enzymes, including consecutively acting diterpene cyclases, cytochromes P450, geranyltranstransferase (GGPPS) and isopentenyl pyrophosphate (IPP). During this process, short-chain dehydrogenases/reductases (SDR), kaurene synthases(KS), and copalyl diphosphate synthases (CPS) are also important enzymes in GA biosynthesis pathway (Morrone et al., 2009; Marcassa 2014). The homologs of operons in GA biosynthesis pathway have been reported by Marcassa (2014) (Figure 1.1.); however, GA biosynthesis pathways in bacteria has not been fully elucidated.

The operon associated with GA biosynthesis has been evaluated in the soybean symbiont *Bradyrhizobium japonicum* USDA 110. Gibberellin biosynthesis by bacteria may be specifically linked to biological nitrogen fixation (BNF) in *B. japonicum* (Carvalho et al., 2014). There is a known link between the operon's promoter and the regulatory signal cascade in the microbe during nodule organogenesis and BNF. A schematic diagram showing the operon as it is found in *Bradyrhizobium* (Figure 1.1., Marcassa 2014). Farnesyl pyrophosphate synthase (FPPS) shares similar function with GGPPS (Tully and Keister, 1993).

Figure 1.1. Gibberellin Biosynthesis Operon in some proteobacteria (Marcassa 2014)



Whole genome sequencing

The whole genome sequence of *Aaa* used in this study was reported by Giordano (2014). DNA was extracted from *Acidovorax* spp using the Qiagen DNeasy Plant Mini Kit according to the manufacturer's instructions. Genomic DNA was sequenced and assembled by the Michigan State University Research Technology Support Facility. Standard Illumina TruSeq DNA Sample Prep Kit v2 was used for sequencing libraries. Real Time Analysis (RTA) was used to perform on-board base calling and image analysis.

Trimmomatic (v0.30) was used to do the adapter and quality trimming. By using Burrows-Wheeler Aligner (BWA, v0.7.5a), non-overlapping read pairs and these pseudo long reads were aligned to the *Acidovorax avenae* reference genome (subsp. *avenae* ATCC 19860, NCBI accession # NC_015138.1). Genome Analysis Toolkit (GATK v2.4-9) was used to perform variant calling and filtering. Variant calling was performed by using the UnifiedGenotyper tool of GATK with ploidy set to one. Velvet (v1.2.7) was used to perform De novo assembly. In steps of 10, a range of kmers were scanned from 23 to 93 for each strain. From the set of assemblies for each strain, based on maximization of the length of long scaffolds the optimal kmer assembly was selected to minimize the total number of scaffolds making up most of the assembly (Giordano 2014).

Protein-nucleotide alignment of GA biosynthesis genes associated with *Aaa*

To determine whether there are GA biosynthesis genes in *Aaa*, we searched the *Aaa* genome using potentially homologous sequences, which are well-known enzymes and precursors of GA biosynthesis pathway in other bacteria. The Basic Local Alignment Search Tool (BLAST) was used for comparing nucleotides of RNA sequences between *Aaa* strains and other bacteria based on the amino-acid sequences of different proteins working in GA biosynthesis in NCBI database. Protein-nucleotide 6-frame translation (tblastn) was conducted to search translated nucleotide databases using a protein query. GA biosynthesis operons were selected from three bacteria *Bradyrhizobium japonicum* (USDA110), *Sinorhizobium fredii* (NGR234), and *Mesorhizobium loti* (MAFF303099). *Aaa* strain ATCC 19860 (isolate MSU 1, MSU 4 and MSU 13) and ICMP 3183 (isolate URI 1) and were used in this study.

A small set of one strain of predicted *Aaa* protein sequences aligned with translated sequences of related bacterial organisms (Table 1). Gibberellin biosynthesis proteins with *Aaa* strain ATCC 19860 sequence by tblastn. Homologs of SDR in *B. japonicum* and *M. loti*, GGPPS in *S. fredii* and *M. loti*, and FPPS in *B. japonicum* have been found in *Aaa*. The match spanned almost the entire length of our query sequence. The S scores and expect value (E-value) indicate the alignments are acceptable.

Table 1. Significant alignments of gibberenllin biosynthesis proteins with the ATCC 19860 query sequence by tblastn from data presented in Chapter One.

Aaa strain	Protein	Source of proteins	Score(S)	Query cover	Expect(E) value
ATCC 19860	SDR	*Bradyrhizobium japonicum*	110	92%	1e-27
ATCC 19860	SDR	*Mesorhizobium loti*	114	76%	4e-28
ATCC 19860	GGPPS	*Mesorhizobium loti*	150	82%	3e-41
ATCC 19860	GGPPS	*Sinorhizobium fredii*	146	81%	2e-39
ATCC 19860	FPPS	*Bradyrhizobium japonicum*	153	84%	8e-42

The genetic regulation of GA biosynthesis in bacteria is complicated. Homologs of three essential emzymes facilitating the GA biosynthesis pathway have been found in *Aaa*, which indicate *Aaa* could have the ability to produce GA. However, most parts of the GA biosynthesis pathway in *Aaa* has not been fully explained. The results encourage us to evaluate whether *Aaa* can produce phytohormones in pure cultures.

LITERATURE CITED

LITERATURE CITED

Carvalho, T.L.G., Balsemão-Pires, E., Saraiva, R.M., Ferreira, P.C.G. and Hemerly, A. S. 2014. Nitrogen signalling in plant interactions with associative and endophytic diazotrophic bacteria. Journal of Experimenal Botany, First published online: August 11, 2014, 1-12. http://dx.doi.org/10.1093/jxb/eru319.

Giordano, P.R. 2014. Identification and characterization of a new bacterial disease of creeping bentgrass (Agrostis stolonifera L.) caused by *Acidovorax avenea* subsp. *avenae*. Ph.D. Thesis. Michigan State University, Michigan, USA, pp.106-107.

Marcassa, A. 2014. Gibberellin Biosynthesis in Bradyrhizobium japonicum USDA110. Master thesis. University of Waterloo, Waterloo, Ontario, Canada, pp. 7-62.

Morrone, D., Chambers, J., Lowry, L., Kim, G., Anterola, A., Bender, K., and Peters, R.J. 2009. Gibberellin biosynthesis in bacteria: separate ent-copalyl diphosphate and ent-kaurene synthases in Bradyrhizobium japonicum. FEBS Lett.583: 475-480.

Tully, R.E., and Keister, D.L. 1993. Cloning and mutagenesis of a cytochrome P450 locus from Bradyrhizobium japonicum that is expressed anaerobically and symbiotically. Appl. Environ. Microbiol. 59:4136-4142.

CHAPTER TWO

PHYTOHORMONES ASSOCIATED WITH BACTERIAL ETIOLATION DISEASE IN CREEPING BENTGRASS

INTRODUCTION

The turfgrass industry has recently been plagued by a highly damaging new bacterial disease caused by *Acidovorax avenae* subsp. *avenae* (*Aaa*) called bacterial etiolation. It can cause severe damage to creeping bentgrass putting greens on golf courses (Giordano et al., 2010). The symptoms of the disease include chlorosis, necrosis, and etiolation of plant tissues. Creeping bentgrass is a cool-season turfgrass species that is highly susceptible to various abiotic and biotic stresses in the summer. *Aaa* is most problematic to creeping bentgrass putting greens during periods of above optimal high temperatures in summer months (30-40°C). Treatments with plant growth regulators (PGRs) can inhibit GA biosynthesis and PGR treatment has been found to increase creeping bentgrass susceptibility to *Aaa* (Roberts et al., 2015). Also, Using PGRs with different modes of action including early-GAbiosynthesis inhibitor, could reduce the potential of bacterial etiolation development in creeping bentgrass (Roberts et al., 2016). Thus, there could be an intimate link between this disease, heat stress, and plant growth regulator associated changes in GA content. A better understanding of this link and the phytohormones involved could lead to better recommendations for use of PGRs and a reduction of *Aaa* disease incidence.

The first report of *Aaa* being able to produce phytohormones concluded that *Aaa* is capable of producing GA3 by enzyme linked immunosorbent assay (ELISA) (Roberts et al., 2014). In this study, researchers only detected a single hormone and single isoform; however, many isoforms of GA and other plant hormones are able to be produced by plant pathogens. Additionally, more advanced techniques to investigate full hormone profiles are available such as ultra-performance liquid chromatography (UPLC). Several different bacterial species are known to produce GA including *Acetobacter* spp., *Bacillus* spp., *Herbaspirillum* spp., and *Rhizobium* spp. (MacMillan 2001). However, less information is available for *Acidovorax* production of GA or other phytohormones and this is the first investigation of hormone profiles in a bacterial pathogen of creeping bentgrass. An evaluation of hormone profiles using UPLC may shed light on the association of this disease with GA regulation.

Determining phytohormone profiles in creeping bentgrass and *Aaa* interactions is important due to the widespread use of PGRs in use in the turfgrass industry and the link of this disease to PGR application. Previous studies have suggested trinexapac-ethyl (TE), one of the most utilized plant PGRs in the turfgrass industry, can cause bacterial etiolation outbreak of creeping bentgrass (Giordano et al., 2012). TE inhibits late in the GA biosynthesis pathway at several points such as inhibiting 3β-hydroxylase conversion of GA20 to GA1 (Adams et al., 1992; Rademacher 2000). TE is commonly applied to reduce turfgrass vertical growth to reduce labor costs associated with mowing and for other purposes such as for flower inhibition (Fagerness and Yelverton 2001). Since PGRs seem to influence the severity of the *Aaa* disease and GA is involved in cross-talk with several other hormone pathways controlling abiotic and biotic defense signaling pathways, an evaluation of full phytohormone profiles in the *Aaa* and in plant-bacteria interactions is needed.

GA is involved in cross-talk with SA and JA via the systemic acquired resistance (SAR) and induced systemic resistance (ISR) pathways, respectively (Yang et al., 2012). SAR and ISR are two pathways that convey signals to systemically activate defense responses in response to a local disease infection site or a disease priming event (Choudhary et al., 2007). Inhibition of GA has resulted in differentially accumulated SA and JA in kentucky bluegrass (Krishnan et al., 2015). Thus, the ISR and SAR pathways may be significantly affected by PGRs application to creeping bentgrass and could play a role in *Aaa* sensitivity or tolerance.

Additionally, GA interacts with various other hormones that could play a role in this disease system, such as auxins and ABA. Whether *Aaa* produced phytohormones are associated with creeping bentgrass disease symptoms and why *Aaa* is more successful at causing disease under high temperatures has not yet been fully elucidated.

Therefore, our major goals of the research are to better understand if and why heat stress may make creeping bentgrass more susceptible to bacterial infection and to better understand the mechanism behind disease symptoms. Additionally, there is evidence of variation in disease tolerance of creeping bentgrass cultivars, but no resistant cultivars have been found to date. The physiological mechanisms behind variation in creeping bentgrass responses to pathogenic bacterial infection are not known. Additionally, we have found that the *Aaa* genome contains sequences that are homologous to bacterial GA biosynthesis genes. Therefore, we hypothesize that 1) *Aaa* may be capable of producing other phytohormones in addition to GA3; 2) phytohormone profiles in plant tissue is significantly affected by high temperature and bacterial infection; 3) differences in phytohormone accumulation between *Aaa* sensitive and tolerant creeping bentgrass cultivars could play a role in disease susceptibility or resistance. Thus, the objectives of the study were to determine phytohormone profiles in pure cultures of bacteria and to evaluate phytohormones in two cultivars of infected and uninfected creeping bentgrass treated with TE and differing in disease susceptibility under optimal or heat stress conditions.

MATERIALS AND METHODS

Bacterial Culture

Four distinct strains of *Aaa* were isolated at Michigan State University (MSU) and the University of Rhode Island (URI) (Giordano et al., 2012). The strain TX-1 of *Pa* was isolated at MSU as described by Powell et al., (2000). All bacterial strains were grown in nutrient broth containing 0.5 g enzymatic digest of gelatin and 0.3 g beef extract in 100 mL Erlenmeyer flasks on a rotary shaker set to 100 rpm at room temperature (20-22 °C).

For hormone profile analysis, 1.0 mL samples from each four independent bacterial cultures (four replications of each isolate) were taken at 2, 5, 8 and 14 days of culture into 1.5 mL tubes, and diluted to 10^{-6} colony forming unit (CFU) based on a growth curve. Culture medium was centrifuged at 20817 G for 20 min at 4 °C and the supernatant of pure bacterial culture was discarded. Bacterial cell pellets were extracted by consecutively adding 0.5 ml of ethyl acetate three times. Extracts were evaporated in a rotary evaporator (Heidolph Laborota 4000; Cole-Parmer, IL) and the residues were dissolved in 500 µL absolute methanol (Mazzella et al., 2004). Phytohormones were analyzed as described below.

Plant Material and Growth Conditions

Creeping bentgrass cultivars 'Tyee' and 'Penn-A4' were seeded at the rate of 0.45 kg/93 m^2 in sand in 11.4 cm pots and were established in a greenhouse for 8 weeks with daily watering and weekly fertilization. Plants were regularly trimmed to 2 cm height. After they were established, plants were separated and propagated into clonal tillers. They were then transferred to a hydroponic system in a controlled environmental growth chamber using the methods described in Merewitz et al. (2011). Growth chamber conditions were maintained a 12 h photoperiod at 900 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of photosynthetically active radiation (PAR), 65% relative humidity, and a day/night temperature of 23/20 °C. Plants were inserted into 2.54 cm diameter holes in foam boards with plastic covers. The boards were floated on the nutrient solution in black plastic tanks (71 x 51 x 15 cm). The hydroponic solution was aerated via a tube connected to a pump (115 V, 60 Hz, Tetra Whisper; Blacksburg, VA). The solution was changed weekly and solution pH was monitored and adjusted to a pH of 6.0 every 3 days. Once established, plants were kept trimmed to a height of 5 cm. All plants were sprayed with trinexapac-ethyl (Syngenta Crop Protection, Greensboro, NC) at the rate of 0.79 L ha^{-1} and applied twice before the experiment.

The second spray was applied two weeks later and 48 h before the bacterial treatments.

Temperature and Bacteria Treatments

After hydroponic plants were established, 16 plants were kept in each hydroponic tank. Four tanks were placed in an optimal temperature growth chamber at 23/20°C day/night temperature and four tanks were moved to a high temperature chamber at 35/30°C for the duration of the experiment (20 days). For each cultivar, one of the tanks in each chamber was infected with *Aaa* bacteria (*Aaa* treatment) and the other was not (control). Both chambers were equal in size, RH, photoperiod, and light levels are the same as described above. For infected plants, plant nutrient solution was replaced with bacterial culture of the pure MSU-13 strain of *Aaa* at a concentration of 10^{-6} CFU one day after the measurement day (0, 5, 15 and 20 days). One day before subsequent treatment, bacterial culture was replaced with nutrient solution. Nutrient solution for control plants were changed every 3 d. The canopies of bacteria-treated plants were trimmed to a height of 3 cm with scissors soaked in MSU-13 *Aaa* suspension 2 days before the experiment. During the treatment with bacterial culture about 0.5 cm of root tips were pruned with sterilized scissors every day to ensure that bacteria could enter roots through wounds. Control plant canopy and roots were also cut in the same manner with sterile scissors rinsed with de-ionized water.

Physiological Evaluation of Plants

Electrolyte leakage (EL), turf quality ratings (TQ), and chlorophyll content (CHL) were measured on plant leaves or canopies every 5 days during the study. EL gives an indication of cellular membrane stability. Approximately 10 leaves were rinsed and submerged in 10 mL of de-ionized water after taken from each plant. The samples were then placed on a shaker for 24 h and the initial conductivity (C_i) was measured by conductivity meter (YSI Model 3200; Yellow Springs, OH, USA). The leaf tissues were then boiled for 20 min and put on the shaker for another 24 h to measure the maximum conductivity (C_{max}). Percent EL was calculated as C_i/C_{max} x 100 (Blum and Ebercon, 1981). Total CHL of plant tissue was extracted in the dark for 72 h in dimethyl sulphoxide. The absorbance of the leaf extract was measured in a spectrophotometer (Genesys 10S UV-VIS; Thermo Fisher Scientific, Madison, WI) at 663 and 645nmHL (Hiscox and Israelstam 1979). CHL was calculated based on the equation described in Arnon (1949). TQ was visually rated on a scale of 1–9 (9 representing a fully turgid, dense green canopy, and 1 representing necrotic plants) by evaluating the color, density, and uniformity of the grasses (Turgeon 2008).

Root health was evaluated by determining root EL (REL) and root viability. REL was measured as described by Huang et al. (1998). 200 mg of roots were taken from individual plant and washed thoroughly with de-ionized water and EL was measured as described above. Root viability was estimated by measuring the activity of dehydrogenase by using the triphenyltetrazolium chloride (TTC) reduction technique (Knievel 1973; McMichael and Burke 1994). The activity was based on the dry weight of each root sample, which was determined after drying in an 80 °C oven for 72 h (Merewitz et al., 2011).

Phytohormone analysis

Extracts of bacteria and plant tissue were analyzed by the method described by Liu and others (2012). Plant tissue extracts were processed as described by Krishnan and Merewitz (2015). The internal standards for liquid chromatography (LC) analysis included 100 nmol of deuterium-labeled ABA. LC was carried out using an Ultra Performance Liquid Chromatography- tandem mass spectrometer [(UPLC/MS/MS) Waters Quattro Premier XE ACQUITY® Tandem Quadrupole; Waters, Milford, MA]. Desolvation temperature was 342-350 °C, and the ionization mode was ES-. Cone gas flow was kept 38-50 L/hr, while desolvation gas flow was 717-800 L/hr. Methanol and 0.1% formic acid were selected as solvents with a C18 column (5cmx2.1mmx2.7µm; ascentis express, Sigma-Aldrich, St. Louis, MO). External standards for GA isoforms (GA1, GA3, GA4 and GA20) were diluted to 1.25 μM. Standards for ABA, SA, JA, IAA, and zeatin riboside (ZR) were diluted to 12.5 μM, 25 μM, 50 μM, 100 μM and 200 μM, respectively.

Measurements of bacterial culture study were conducted on October 18th,2014 (experiment 1), and repeated on December 5th, 2014 (experiment 2). Hydroponics studies were first conducted on March 17th, 2015 (experiment 1), and then repeated on May 20th,2015 (experiment 2). Physiological indices were taken on day 0 of both studies to make sure materials of repeated studies had the same initial conditions.

Experimental Design and Statistical Analysis

The bacterial culture experimental design was a completely randomized design with four replications of each bacterial strain. The hydroponic plant experiment was a split-split-plot design with four replicates with temperature as the whole plot, bacterial treatments as the sub-plot and cultivar as the sub-sub-plot. Bacterial treatment was randomly assigned within one chamber room, and cultivars were randomly arranged within a tank. Treatment effects were determined by analysis of variance (ANOVA) according to the mixed procedure within the Statistical Analysis System software (SAS 9.2; Cary, NC). ANOVA results are shown in the appendix. Statistical interactions and means among temperature, bacteria, and cultivar treatments were separated by Fisher's protected LSD test ($P \leq 0.05$).

RESULTS

Bacterial phytohormone production

Aaa in pure culture was found to produce GA1, GA3, and IAA whereas the negative control *Pa* isolate TX-1 did not produce these hormones (Figure 2.1.). GA1 content increased with days of culture in all isolates of *Aaa* in experiment 1(Figure 2.1. A). Differences between isolates were significant on day 14 where isolate MSU-4 showed significantly higher GA1 content than other *Aaa* isolates and TX-1 in experiment 2 (Figure 2.1. E). GA3 was detected in isolates MSU-1, MSU-4 and MSU-13 (Figure 2.1. B and F). The maximum production of GA3 was detected on day 5 in both experiments. Isolate MSU-13 showed significantly higher GA3 content on days 5, 8, and 14 compared to other isolates, and showed maximum GA3 content (259.8 µM mL^{-1}) on day 5 in experiment 1 (Figure 2.1. B). Relatively low levels of GA4 (less than 12 µM mL-^{1}) were detected in all four *Aaa* isolates (Figure 2.1. C and G). Isolate URI-1 showed significantly higher GA4 content than other isolates on days 2, 5 and 8 in experiment 1 (Figure 2.1. C); however, MSU-13 showed highest GA4 content on day 5 in experiment 2 (Figure 2.1. G). Isolate MSU-13 and isolate URI-1 produced more IAA than other isolates in both experiments (Figure 2.1. D and H). The highest IAA content (212.1 µM mL^{-1}) was detected in isolate MSU-13 on day 2 in experiment 1 (Figure 2.1. D). URI-1 showed higher IAA content than other isolates in experiment 2 on days 2, 5 and 14 (Figure 2.1. H).

Figure 2.1. Phytohormone production by *Acidovorax avenae subsp. avenae* (*Aaa*) isolates (MSU-1, MSU-13, MSU-4 and URI-1) and *Pseudomonas aureofaciens* (*Pa*) isolate (TX-1).

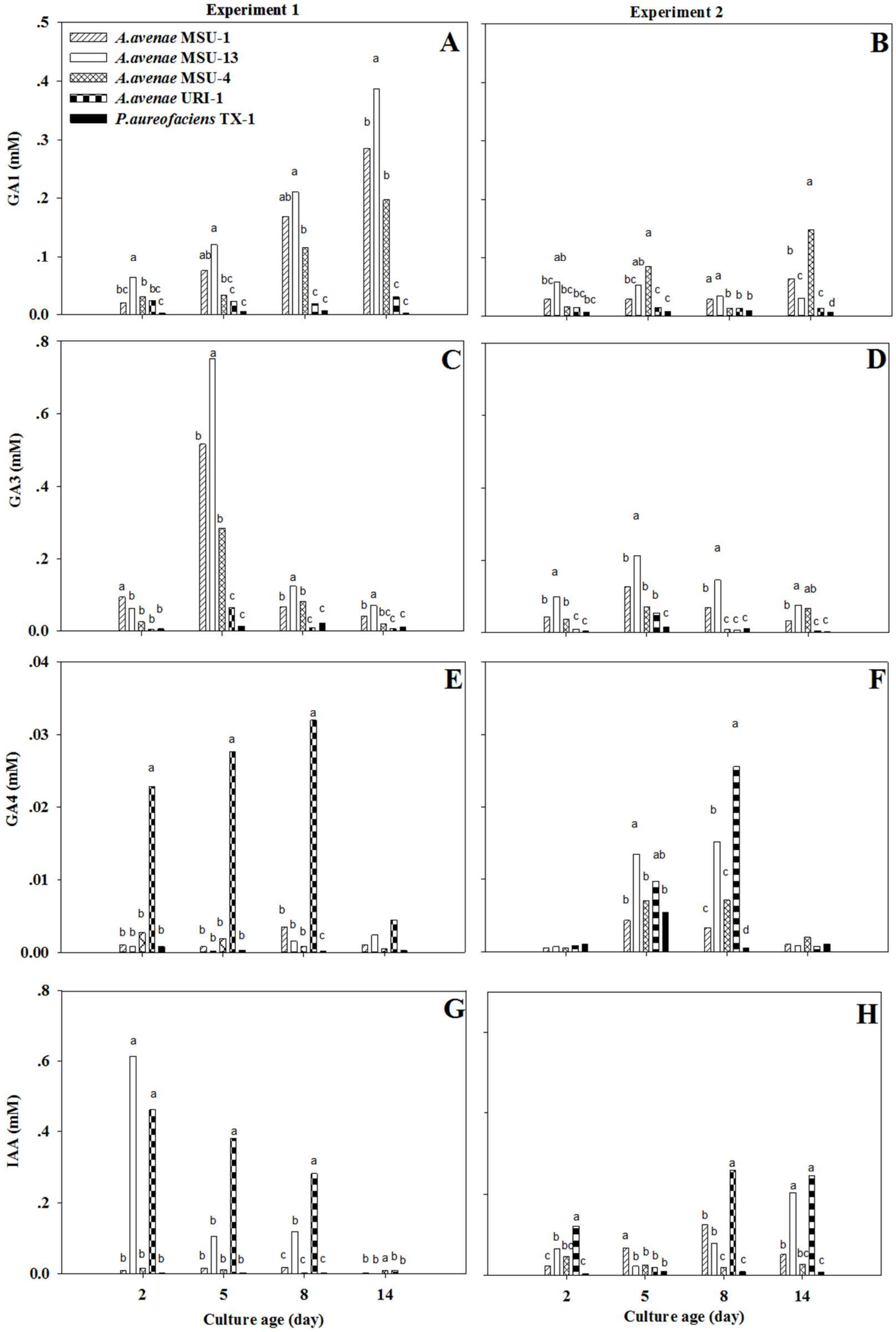


Figure 2.1. Phytohormones identified include gibberellic acid-1 (GA1) in **A)** experiment 1 and **B)** experiment 2, gibberellic acid-3 (GA3) in **C)** experiment 1 and **D)** experiment 2, gibberellic acid-4 (GA4) in **E)** experiment 1 and **F)** experiment 2, and indole 3 acetic acid (IAA) in **D)**

Figure 2.1. (cont'd)

experiment 1 and **H)** experiment 2. Different letters indicate statistically different values between isolates on a given day, based on LSD values determined by Fisher's protected t test ($P \leq 0.05$).

Physiological evaluation of plants

Without bacterial treatments, plants exposed to high temperature compared to optimal temperatures did not show significant differences in leaf EL (Figure 2.2. A and D). Compared to control plants increases of EL were more pronounced in infected plants of both cultivars at both temperatures. Higher EL was exhibited by infected plants at high temperature than at optimal temperature. In control plants, 'Penn-A4' at the high temperature showed higher EL than other treatments on days 10, 15 and 20 in experiment 1 (Figure 2.2. A). EL of infected 'Penn-A4' was higher than infected 'Tyee' on days 5, 10 and 15 under optimal temperature. 'Penn-A4' treated with bacteria under high temperature showed the highest leaf EL compared to other treatments except for day 10. For instance, on day 15 under bacteria and high temperature treatments, 'Penn-A4' had a leaf EL of 41.8%, whereas 'Tyee' had a leaf EL of 35.4% (Figure 2.2. D).

CHL of plants without bacterial treatment under optimal temperature treatments did not change significantly over time throughout the study (Figure 2.2. B and E). Without bacterial treatment, both cultivars exhibited less CHL at high temperature compared to optimal temperature on days 15 and 20 in experiment 2 (Figure 2.2. E). Infected plants of both cultivars exhibited a more rapid decline in CHL at high temperature than at optimal temperature in experiment 1(Figure 2.2. B). Infected 'Penn-A4'showed lower CHL than infected 'Tyee' at both temperatures on all measure days, except for day 20 at optimal temperature.

TQ did not statistically significant different among treatments under optimal temperatures without *Aaa* treatment (Figure 2.2. C and F). TQ ratings declined in both cultivars at high temperature treatment with and without *Aaa* treatment, with a greater decline in plants treated with *Aaa* (Figure 2.2. C). Infected 'Penn-A4' had significantly lower TQ than infected 'Tyee' under high temperature treatment on days 15 and 20 (Figure 2.2. F).

Figure 2.2. Electrolyte leakage (% EL), chlorophyll content (CHL) and turf quality rating (TQ) of 'Penn-A4' and 'Tyee' creeping bentgrass in response to bacterial and temperature treatments.

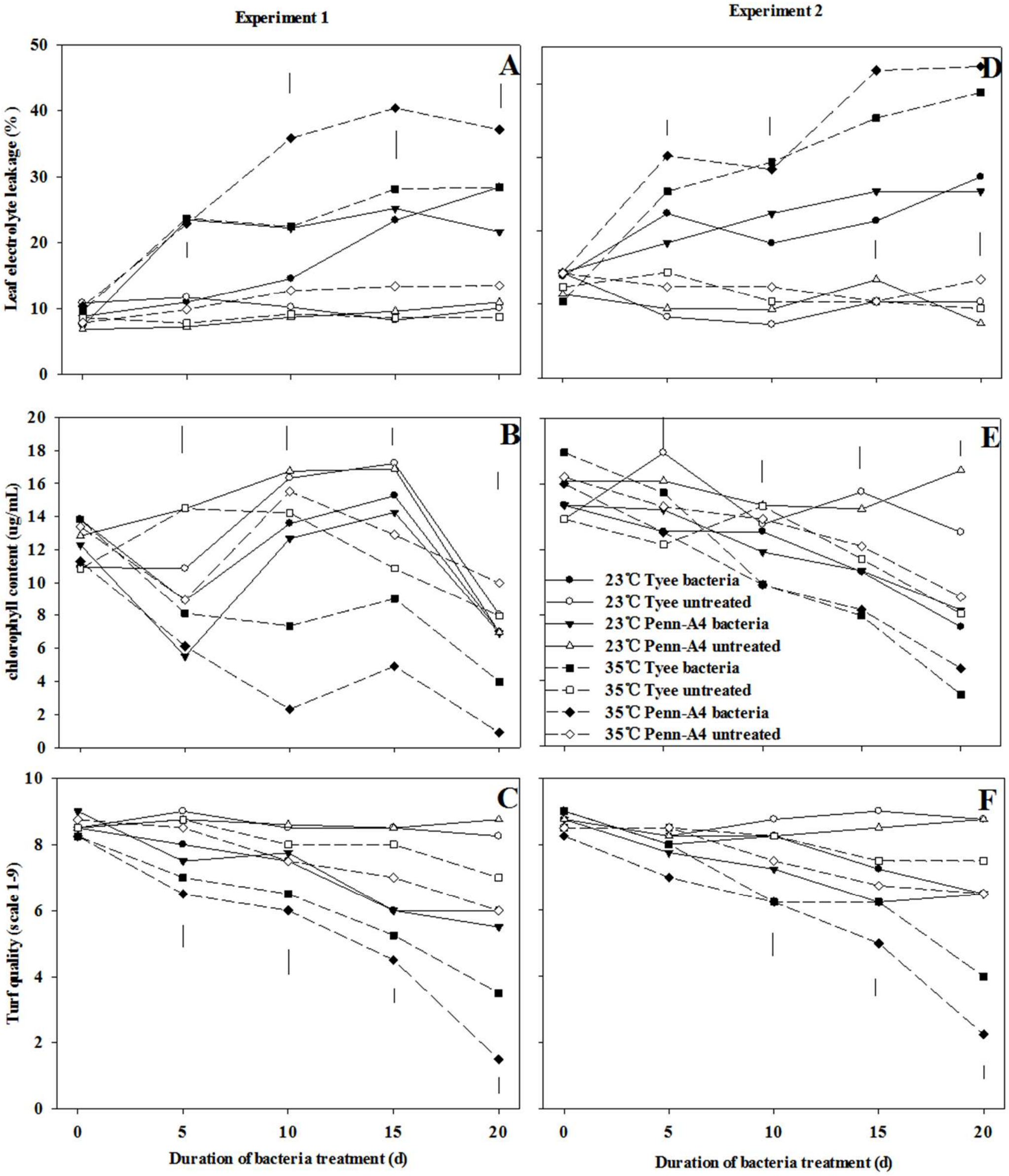


Figure 2.2. A. Electrolyte leakage (% EL) in the experiment 1. **B.** Electrolyte leakage (% EL) in the experiment 2. **C.** Chlorophyll content in the experiment 1. **D.** Chlorophyll content in the experiment 2. **E.** Turf quality ratings (TQ, 1–9 scale; 1 = worst, 9 = best) in the experiment 1. **F.** Turf quality ratings in the experiment 2.

LSD bars are present on days when statistically significant differences were observed among treatments (P ≤ 0.05).

Root health as measured by REL was also affected by bacterial infection, high temperature and cultivar difference (Figure 2.3. A and C). Both cultivars showed higher REL under high temperature than optimal temperature with and without bacterial infection throughout the study in experiment 1 (Figure 2.3. A). Under both temperature conditions, infected plants exhibited higher REL than control plants (Figure 2.3. A and C). However, under optimal conditions, infected 'Penn-A4' had significantly higher REL than infected 'Tyee' in experiment 2 on days 15 and 20 (Figure 3 C).

Without bacterial infection, RV was higher in 'Tyee' than 'Penn-A4' at optimal temperature (Figure 2.3. B and D). Uninfected 'Penn-A4' showed higher RV than uninfected 'Tyee' at high temperature in experiment 2 (Figure 2.3. D). With bacterial infection, plants exhibited lower RV at high temperature compared to optimal temperature in experiment 1 on days 10,15 and 20 (Figure 3B). No significant differences in RV was detected between infected cultivars at high temperature. However, RV of infected 'Tyee' was higher than infected 'Penn-A4' on days 10 and 20. For instance, on day 20, at optimal temperature, RV of infected 'Penn-A4' and 'Tyee' were 7.9 and 21.7 OD•g^{-1} respectively.

Figure 2.3. Root electrolyte leakage (% REL) and Root viability (RV), of 'Penn-A4' and 'Tyee' creeping bentgrass exposed to bacterial and temperature treatments.

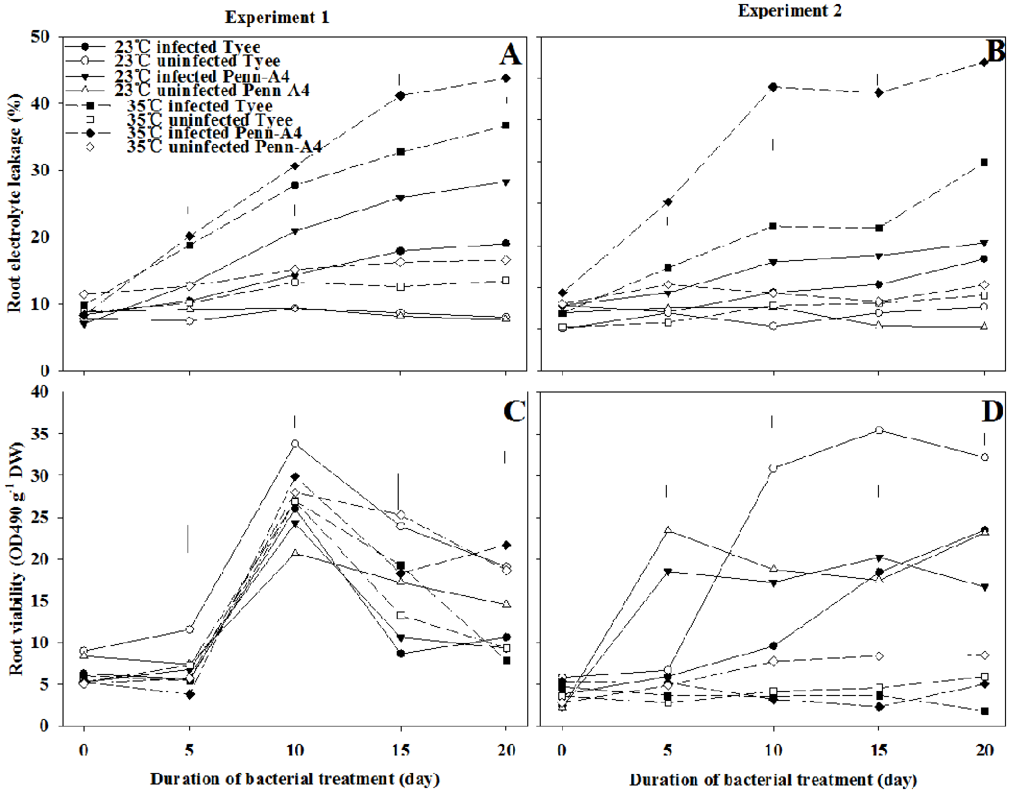


Figure 2.3. A. Root electrolyte leakage (% REL) in the experiment 1. **B.** Root electrolyte leakage (% REL) in the experiment 2. **C.** Root viability (RV) in the experiment 1. **D.** Root viability (RV) in the experiment 2.
LSD bars are present on days when statistically significant differences were observed among treatments ($P \leq 0.05$). No LSD bar indicates lack of significant differences on that day.

Phytohormone analysis of plant tissues

In leaves without *Aaa* treatment, both cultivars contained higher SA at high temperature than at optimal temperature at day 20 (Figure 2.4). Infected 'Tyee' at optimal condition contained more leaf SA than 'Penn-A4' under the same conditions. In stolons, at high temperature, infected 'Tyee' had more SA than 'Penn-A4' in experiment 1 on days 5 and 10 (Figure 2.4. B). 'Penn-A4' exhibited an increase in SA in leaves and roots due to elevated temperature; however, 'Penn-A4' did not exhibit a significant increase in SA in response to bacterial infection (Figure 2.4 A and C). In contrast, uninfected ‘Tyee’ did not exhibit significant differences in SA content of leaves or roots in response to high temperature but SA content was responsive to bacterial infection in experiment 2 (Figure 2.4. D and F). Infected ‘Tyee’ at high temperature showed more SA content in roots compared to ‘Penn-A4’ under the same conditions on days 10, 15, and 20.

Higher JA content was detected in leaves and stolons of both cultivars at high temperature than at optimal temperature without bacteria treatment in experiment 1 (Figure 2.5. A and C). In infected plants at optimal temperature, JA content was higher in ‘Tyee’ than ‘Penn-A4’ at 10, 15, and 20 days in leave; in stolons, however, ‘Penn-A4’ had more JA content than ‘Tyee’ except on days 10 and 15. At high temperature, infected ‘Tyee’ accumulated more JA compared to infected ‘Penn-A4’ in leaves, stolons, and roots in experiment 2 (Figure2.5. D, E and F).

Figure 2.4. Endogenous hormone salicylic acid (SA) contents of 'Penn-A4' and 'Tyee' creeping bentgrass exposed to bacterial and temperature treatments in repeated experiments in leaf, stolon, and root.

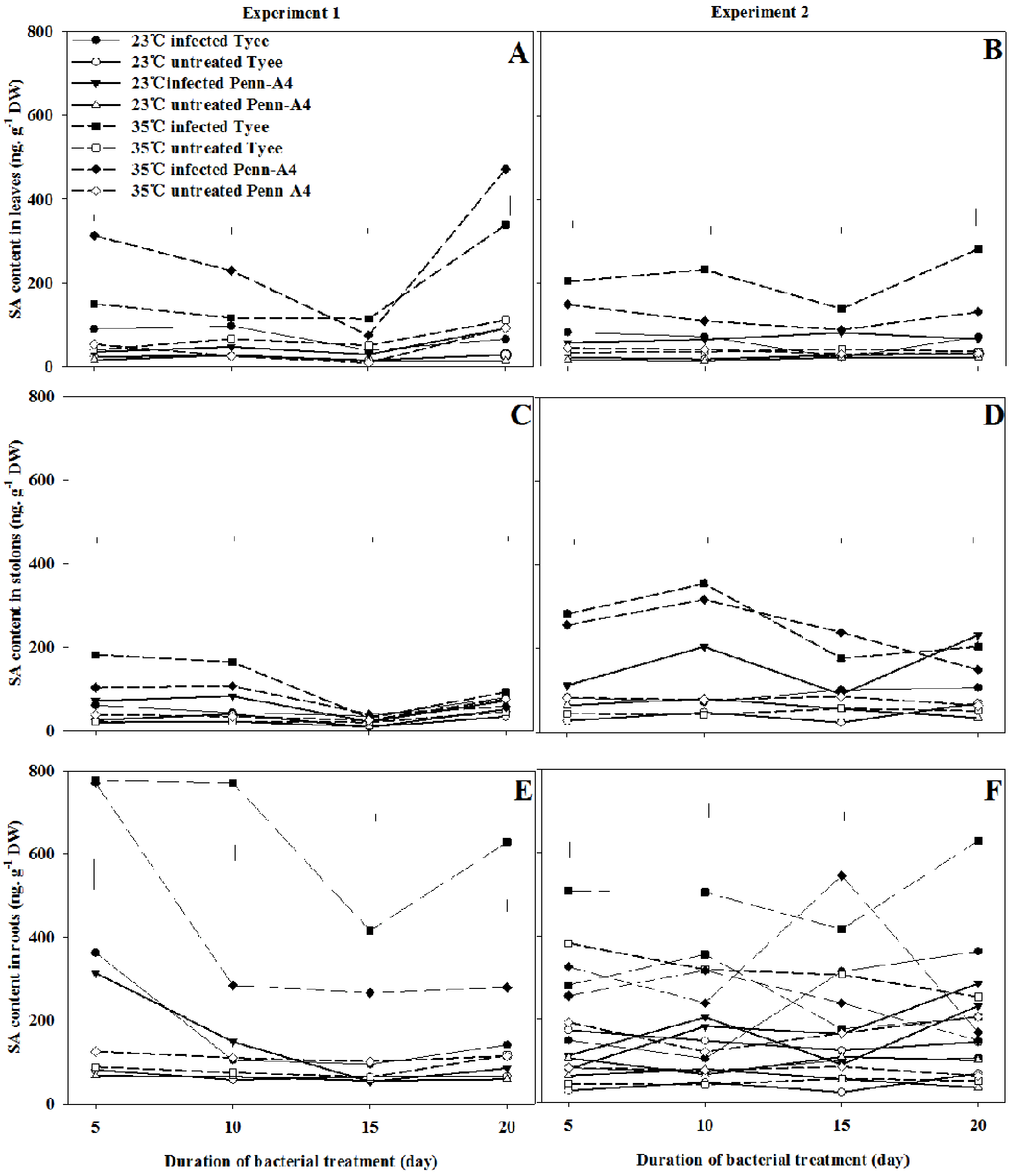


Figure 2.4. A. Salicylic acid (SA) contents in leaves in the experiment 1. **B.** Salicylic acid (SA) contents in leaves in the experiment 2. **C.** Salicylic acid (SA) contents in stolons in the experiment 1. **D.** Salicylic acid (SA) contents in stolons in the experiment 2. **E.** Salicylic acid (SA) contents in roots in the experiment 1. **F.** Salicylic acid (SA) contents in roots in the experiment 2.

LSD bars are present on days when statistically significant differences were observed among treatments ($P \leq 0.05$).

Figure 2.5. Endogenous hormone jasmonic acid (JA) contents of ‘Penn-A4’ and ‘Tyee’ creeping bentgrass exposed to bacterial and temperature treatments in repeated experiments in leaf, stolon, and root.

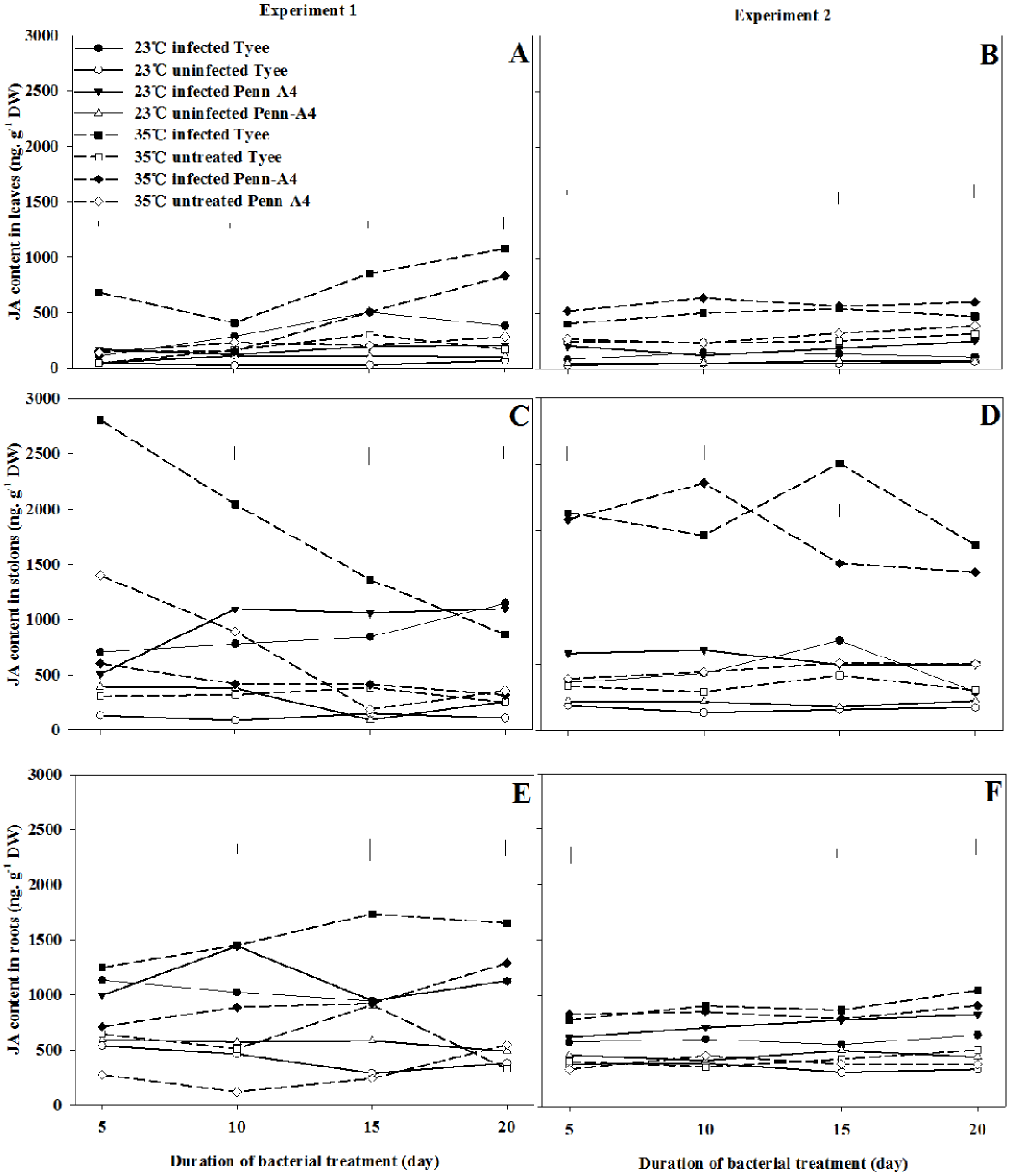


Figure 2.5. A. Jasmonic acid (JA) contents in leaves in the experiment 1. **B.** Jasmonic acid (JA) contents in leaves in the experiment 2. **C.** Jasmonic acid (JA) contents in stolons in the experiment 1. **D.** Jasmonic acid (JA) contents in stolons in the experiment 2. **E.** Jasmonic acid (JA) contents in roots in the experiment 1. **F.** Jasmonic acid (JA) contents in roots in the experiment 2.

LSD bars are present on days when statistically significant differences were observed among treatments ($P \leq 0.05$).

Infection generally increased GA1 content in leaves and roots of both cultivars, but not in stolons (Figure 2.6.). Without infection, plants at high temperature showed lower GA1 than optimal temperature in experiment 2 (Figure 2.6.D, E and F). Infected ‘Penn-A4’ at optimal temperature showed the highest GA1 content compared to other treatments in leaves in experiment 1 (Figure 2.6.A). Higher GA1 content was detected in infected ‘Tyee’ at high temperature compared to infected ‘Penn-A4’ in stolons and roots (Figure 6B and C).

Uninfected leaves showed higher GA3 at optimal temperature than high temperature (Figure 7A and D). With bacterial treatment, GA3 content was higher in ‘Tyee’ than ‘Penn-A4’ at optimal temperature in stolons and roots, but not leaves in experiment 1 (Figure 2.7.B and C). Infected ‘Penn-A4’ showed highest GA3 content in stolons and roots than other treatments on days 15 and 20 in experiment 2 (Figure 2.7.E and F).

Figure 2.6. Endogenous hormone gibberellic acid-1 (GA1) contents of 'Penn-A4' and 'Tyee' creeping bentgrass exposed to bacterial and temperature treatments in repeated experiments in leaf, stolon, and root.

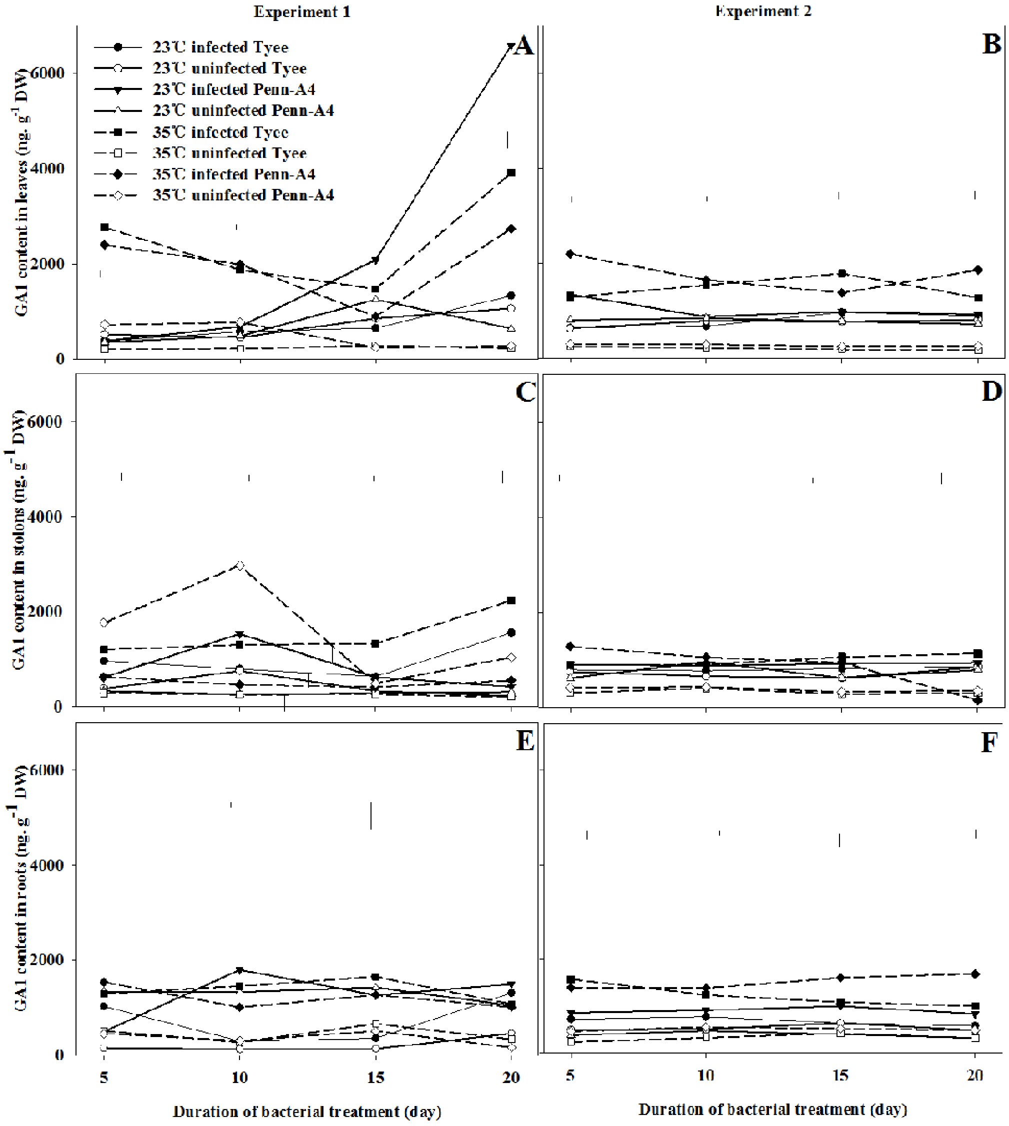


Figure 2.6. A. Gibberellic acid-1 (GA1) contents in leaves in the experiment 1. **B.** Gibberellic acid-1 (GA1) contents in leaves in the experiment 2. **C.** Gibberellic acid-1 (GA1) contents in stolons in the experiment 1. **D.** Gibberellic acid-1 (GA1) contents in stolons in the experiment 2. **E.** Gibberellic acid-1 (GA1) contents in roots in the experiment 1. **F.** Gibberellic acid-1 (GA1) contents in roots in the experiment 2.

LSD bars are present on days when statistically significant differences were observed among treatments ($P \leq 0.05$).

Figure 2.7. Endogenous hormone gibberellic acid-3 (GA3) contents of ‘Penn-A4’ and ‘Tyee’ creeping bentgrass exposed to bacterial and temperature treatments in repeated experiments in leaf, stolon, and root.

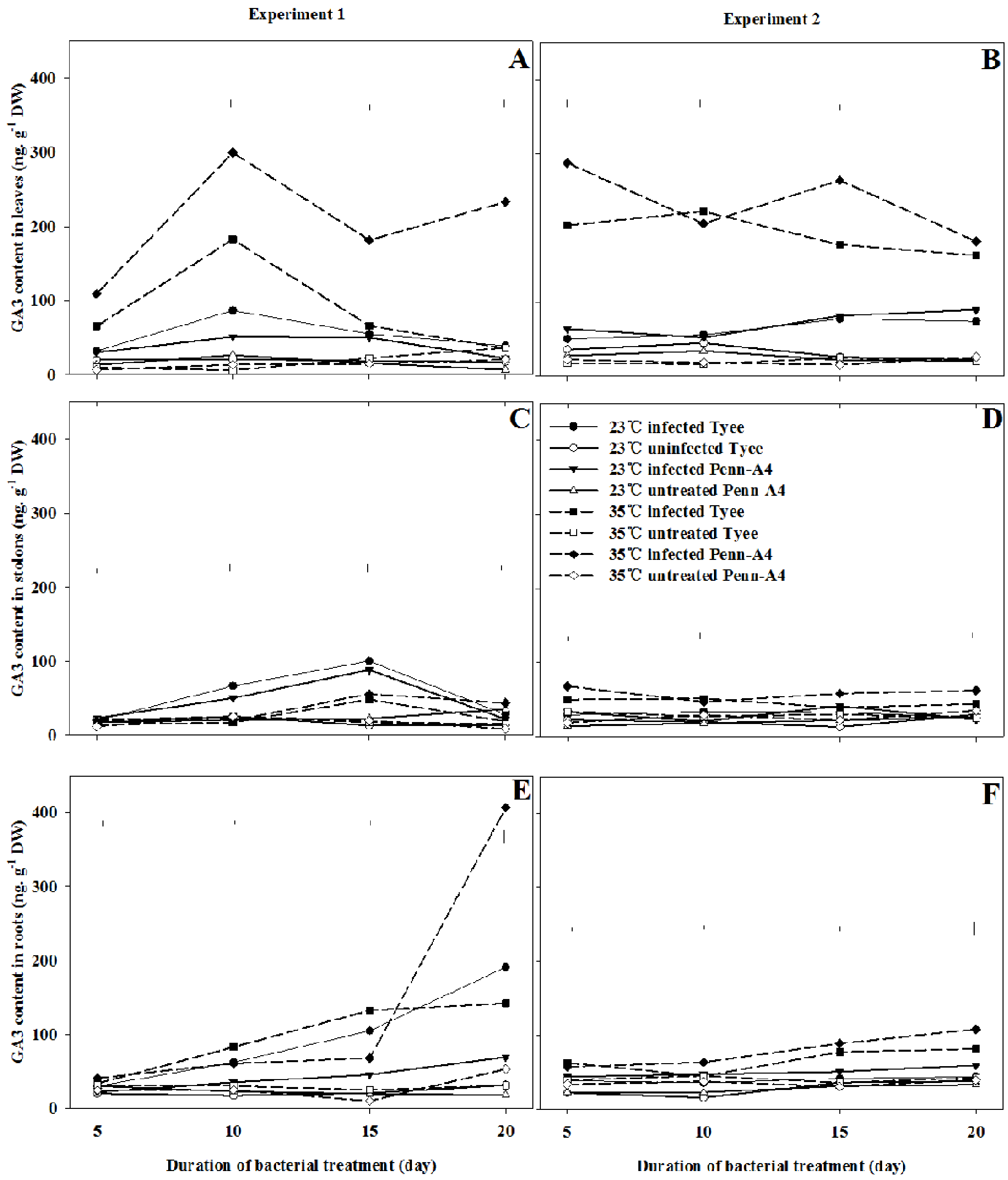


Figure 2.7. A. Gibberellic acid-3 (GA3) contents in leaves in the experiment 1. **B.** Gibberellic acid-3 (GA3) contents in leaves in the experiment 2. **C.** Gibberellic acid-3 (GA3) contents in stolons in the experiment 1. **D.** Gibberellic acid-3 (GA3) contents in stolons in the experiment 2. **E.** Gibberellic acid-3 (GA3) contents in roots in the experiment 1. **F.** Gibberellic acid-3 (GA3) contents in roots in the experiment 2.

LSD bars are present on days when statistically significant differences were observed among treatments ($P \leq 0.05$).

Without infection, plants exhibit more GA4 at optimal temperature than high temperature in experiment 2 (Figure 2.8. D, E and F). At high temperature, uninfected ‘Tyee’ showed less GA4 than uninfected ‘Penn-A4’ in leaves and stolons in experiment 1 (Figure 2.8. A and B). At high temperature, infected plants of both cultivars had more GA4 than uninfected plants in roots (Figure 2.8 C).

Without infection, plants exhibit more GA20 at optimal temperature than high temperature in stolons (Figure 2.9. B and E). In leaves of both cultivars, at high temperature, GA20 content was higher in infected plants than uninfected plants (Figure 2.9. D). Infected ‘Penn-A4’ at high temperature had more GA20 accumulation compared to ‘Tyee’ in leaves and stolons (Figure 2.9. A, B, D and E). GA20 content in roots was not significantly affected by bacteria, cultivar, or temperature treatment (Figure 2.9. C and F).

Figure 2.8. Endogenous hormone gibberellic acid-4 (GA4) contents of ‘Penn-A4’ and ‘Tyee’ creeping bentgrass exposed to bacterial and temperature treatments in repeated experiments in leaf, stolon, and root.

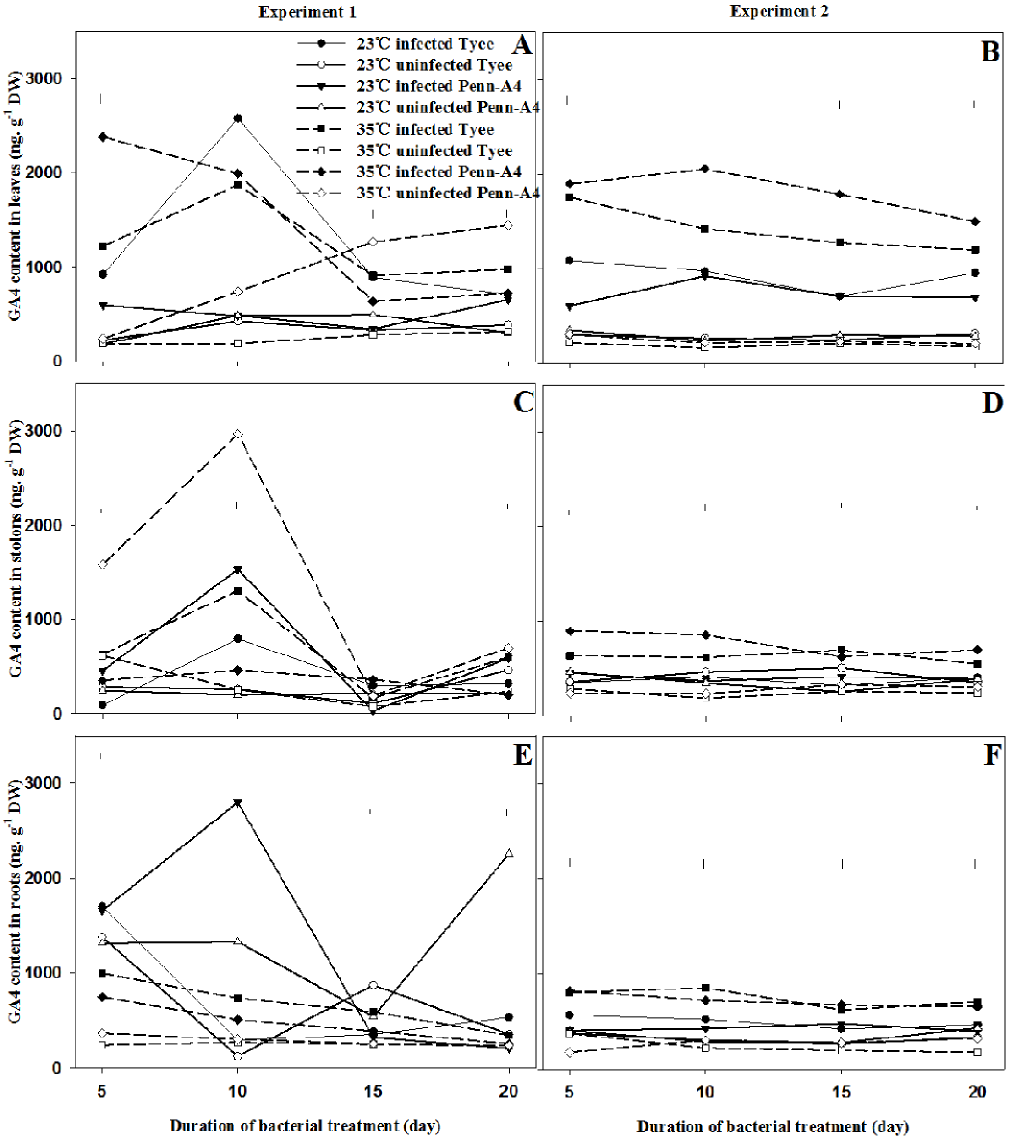


Figure 2.8. A. Gibberellic acid-4 (GA4) contents in leaves in the experiment 1. **B.** Gibberellic acid-4 (GA4) contents in leaves in the experiment 2. **C.** Gibberellic acid-4 (GA4) contents in stolons in the experiment 1. **D.** Gibberellic acid-4 (GA4) contents in stolons in the experiment 2. **E.** Gibberellic acid-4 (GA4) contents in roots in the experiment 1. **F.** Gibberellic acid-4 (GA4) contents in roots in the experiment 2.

LSD bars are present on days when statistically significant differences were observed among treatments ($P \leq 0.05$).

Figure 2.9. Endogenous hormone gibberellic acid-20 (GA20) contents of 'Penn-A4' and 'Tyee' creeping bentgrass exposed to bacterial and temperature treatments in repeated experiments in leaf, stolon, and root.

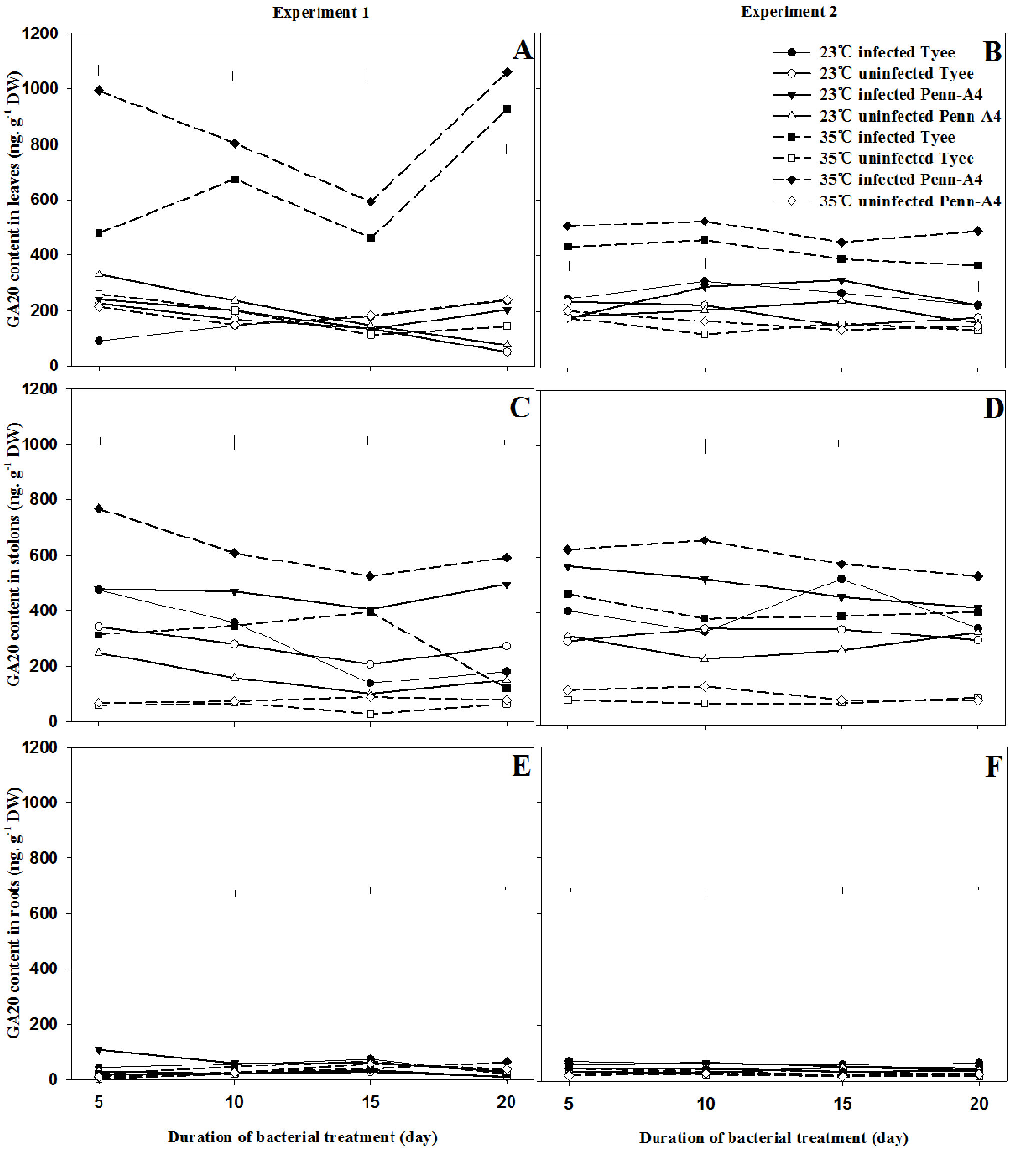


Figure 2.9. A. Gibberellic acid-20 (GA20) contents in leaves in the experiment 1. **B.** Gibberellic acid-20 (GA20) contents in leaves in the experiment 2. **C.** Gibberellic acid-20 (GA20) contents in stolons in the experiment 1. **D.** Gibberellic acid-20 (GA20) contents in stolons in the experiment 2. **E.** Gibberellic acid-20 (GA20) contents in roots in the experiment 1. **F.** Gibberellic acid-20 (GA20) contents in roots in the experiment 2.

LSD bars are present on days when statistically significant differences were observed among treatments ($P \leq 0.05$).

ABA content was higher at high temperature compared to optimal temperature without bacterial infection in plant leaf tissues on days15 and 20 (Figure 10). Infected ‘Penn-4’ showed higher ABA content compared to ‘Tyee’ at both temperature treatments on day 20 in solons in experiment 1 (Figure 10B). Infected ‘Penn-4’ showed higher ABA content compared to ‘Tyee’ at high temperature in leaves and roots on day 20 in experiment 1 (Figure 10A and C). At high temperature, infected plants of both cultivars showed more ABA than at optimal temperature in experiment 2 on days 10, 15 and 20 (Figure 10D, E and F).

Uninfected plants of both cultivars showed more ZR at optimal temperature than high temperature in leaves in experiment 1 (Figure 11A). Uninfected ‘Penn-A4’ showed more ZR than uninfected ‘Tyee’ at high temperature in stolons and roots in experiment 1 (Figure 11B and C). Uninfected ‘Tyee’ exhibited highest ZR content than other treatments in roots in experiment 2 (Figure 11F). Infected ‘Tyee’ showed more ZR than infected ‘Penn-A4’ in leaves and stolons at high temperature in experiment 2 (Figure 11D and E).

Figure 2.10. Endogenous hormone abscisic acid (ABA) contents of ‘Penn-A4’ and ‘Tyee’ creeping bentgrass exposed to bacterial and temperature treatments in repeated experiments in leaf, stolon, and root.

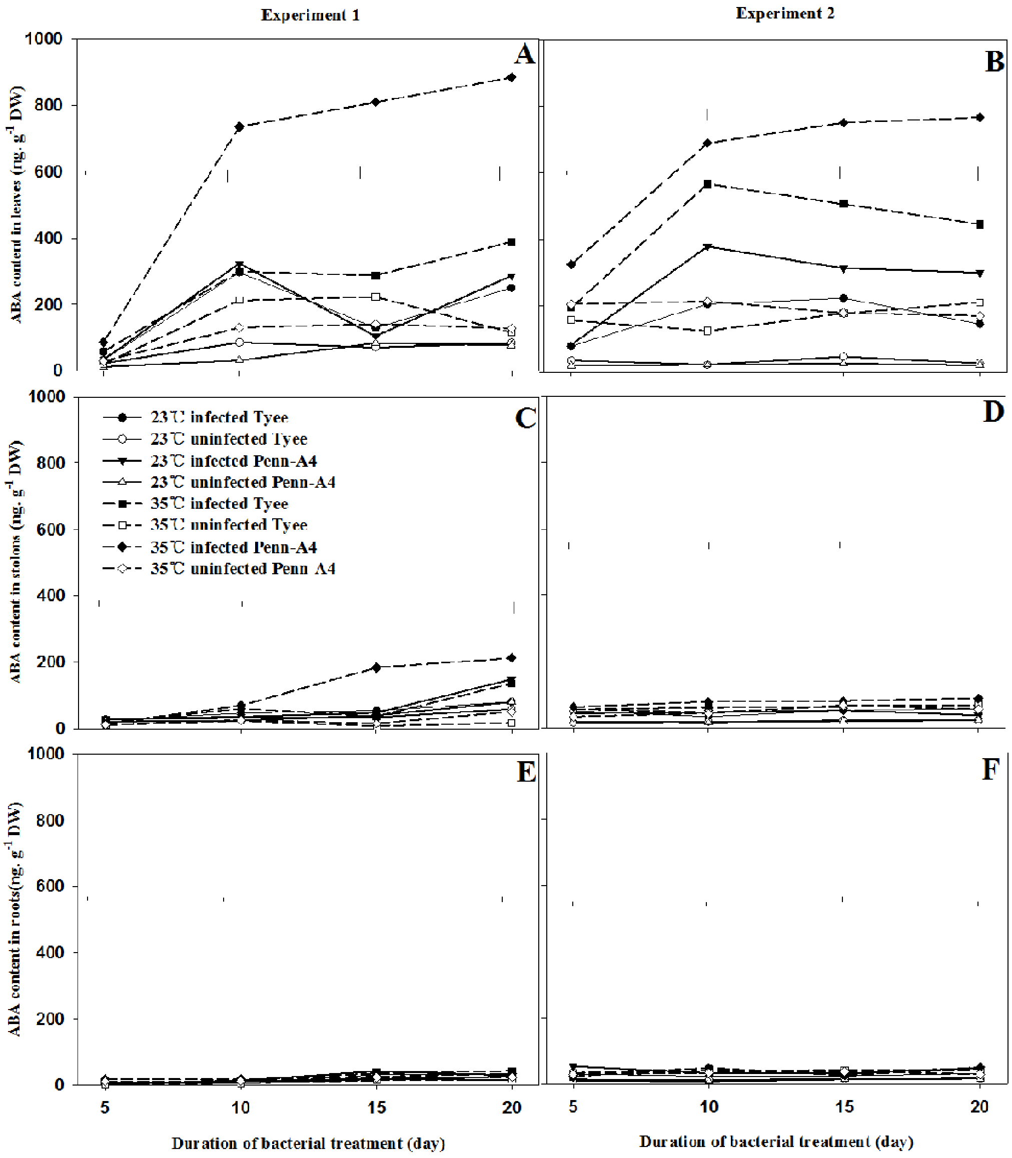


Figure 2.10. A. Abscisic acid (ABA) contents in leaves in the experiment 1. **B.** Abscisic acid (ABA) contents in leaves in the experiment 2. **C.** Abscisic acid (ABA) contents in stolons in the experiment 1. **D.** Abscisic acid (ABA) contents in stolons in the experiment 2. **E.** Abscisic acid (ABA) contents in roots in the experiment 1. **F.** Abscisic acid (ABA) contents in roots in the experiment 2.

LSD bars are present on days when statistically significant differences were observed among treatments ($P \leq 0.05$).

Figure 2.11. Endogenous hormone zeatin riboside (ZR) contents of 'Penn-A4' and 'Tyee' creeping bentgrass exposed to bacterial and temperature treatments in repeated experiments in leaf, stolon, and root.

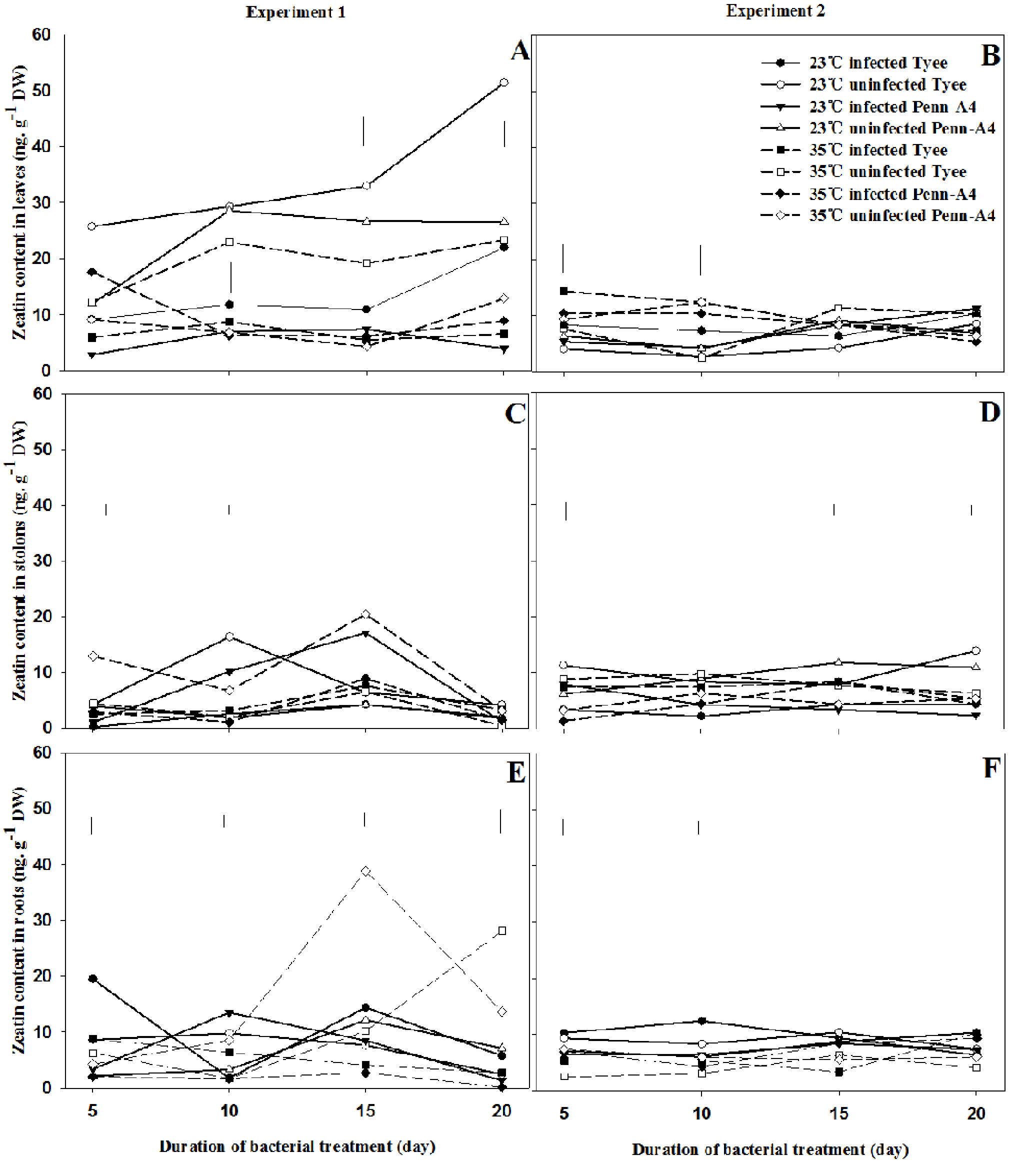


Figure 2.11. A. Zeatin riboside (ZR) contents in leaves in the experiment 1. **B.** Zeatin riboside (ZR) contents in leaves in the experiment 2. **C.** Zeatin riboside (ZR) contents in stolons in the experiment 1. **D.** Zeatin riboside (ZR) contents in stolons in the experiment 2. **E.** Zeatin riboside (ZR) contents in roots in the experiment 1. **F.** Zeatin riboside (ZR) contents in roots in the experiment 2.
LSD bars are present on days when statistically significant differences were observed among treatments ($P \leq 0.05$). No LSD bar indicates lack of significant differences on that day.

DISCUSSION

Aaa in pure culture produced GA3, GA1, and IAA whereas the non-plant pathogenic *Pa* did not. Previous research on *Aaa* using an ELISA method determined that *Aaa* is able to produce GA3 (Roberts et al., 2014). In our study, we used UPLC as a more sensitive method to detect full phytohormone profiles and showed that *Aaa* in pure culture has the ability to also produce GA1 and IAA. Many microorganisms are known to produce phytohormones that contribute to disease progression and symptom development (Ortíz-Castro et al., 2009). Several studies have characterized GAs in microbes. In *Azospirillum* spp., for instance, GA_1 and GA_3 were detected in gnotobiotic cultures of *A. brasilense* (Janzen et al., 1992). In addition to *Azospirillum* spp., GAs have also been found in *Acetobacter diazotrophicus*, *Herbaspirillum seropedicae* (Bastián et al., 1998) and *Bacillus* sp. (Gutiérrez-Mañero et al., 2001). A closely related bacterium, *Acidovorax avenae* subsp. *citrulli,* which is a pathogen of watermelon (*Citrulus lanatus*), has also been shown to produce IAA (Oliveira et al., 2007). The determination of these hormones specifically in *Aaa* cultures is important due to their potential to cause disease symptoms when synthesized by the bacteria in plant tissues. Additionally, since GA1 and GA3 have different levels of activity in promoting stem elongation, this identification of GA1 in this system is also important in order for changing potential disease management strategies or selection of PGR chemistries.

Variation in phytohormone production did exist among *Aaa* isolates in this study. *Aaa* isolate MSU-13 was the only isolate able to produce all three phytohormones GA1, GA3, and IAA. Variation in the biochemistry of bacterial isolates is known to occur in *Acidovorax* spp. and many other bacteria. For instance, isolates of the closely related *Acidovorax avenae* subsp. *citrulli* was found to have variation in antibiotic resistance (Bull et al., 2001). The differential ability of *Aaa* strains to cause disease could be positively associated with phytohormone production. In other words, *Aaa* isolate MSU-13 tended to cause greater disease symptoms in our preliminary tests than the other isolates used in the pure culture study. Thus, the MSU-13 isolate was used in the *in vivo* studies discussed below.

Based on the plant physiological responses we observed, bacterial infection can be influenced by plant cultivar. 'Tyee' was more tolerant than 'Penn-A4' based on lower EL and REL, higher CHL and TQ in infected 'Tyee' than in infected 'Penn-A4'. It was demonstrated that the cultivars had differential levels of phytohormones under high temperature and *Aaa* infection, which could play a role in disease susceptibility or tolerance. How each hormone responded to high temperature with and without bacteria treatment in the more *Aaa* tolerant and sensitive cultivars is discussed below.

In response to high temperature without bacteria treatment, SA levels were enhanced leaves in both cultivars. Increases in SA are most directly associated with the induction of SAR for pathogen responses but can also be found during abiotic stress (Metraux et al., 1990; Fragnière et al., 2011). The increase in SA content can also be inhibited by high temperatures, since at a certain high temperature level, increases in SA were blocked under high temperatures in tobacco plants during infection with tobacco (*Nicotiana tabacum*) mosaic virus but were restored when plants were moved to optimal temperatures (Malamy et al., 1992). The results here are consistent with previous work that showed higher levels of SA in creeping bentgrass leaves under heat stress (30°C) compared to optimal temperature (23°C) (Krishnan, Ma, and Merewitz 2016).

With high temperature and bacteria treatment, SA content in stolons and roots was higher in 'Tyee' compared to 'Penn-A4'. As *Aaa* is taken up by the roots, SA accumulation in root tissue could be associated with a disease response. Since 'Tyee' exhibited a greater tolerance to this disease, a greater increase in SA levels in roots and stolons could be associated with a greater level of tolerance to *Aaa*. Other plants species have demonstrated that leaf levels of SA play a role in disease tolerance. Younger potato (*Solanum tuberosum*) leaves containing higher levels of SA were more resistant to potato blight (*Phytophthora infestans*) infection than older leaves. Moreover, higher levels of SA were detected in potato varieties with race-nonspecific resistance to *P. infestans* than susceptible ones (Coquoz et al., 1995). The cultivar of rice, 'C101LAC', is more resistant to rice blast fungus (*Magnaporthe oryzae*) than 'CO39.' 'C101LAC'exhibits a faster response to exogenous SA application and generated a stronger antioxidant system than susceptible 'CO39' against *M. oryzae* (Li et al., 2012). Previous studies have shown that SA application to roots can effectively control root diseases. Dipping roots of tomato (*Solanum lycopersicum*) seedlings in a high concentration of SA reduced root rot incidence and severity caused by *Rhizoctonia solani*, *Fusarium solani,* and *Sclerotium rolfsii* (El-Mohamedy et al., 2014). In a study of another bacterial pathogen, potato soft rot (*Dickeya solani*), exogenous SA application reduced disease symptoms in potatoes grown in tissue culture (Czajkowski et al., 2015). Further research would be needed to determine whether SA levels is associated with *Aaa* tolerance in creeping bentgrass and whether supplementation with SA would reduce *Aaa* incidence.

JA is also related to pathogen defense since JA is a key regulator of the ISR pathway and plays a signaling role primarily in response to infection with necrotrophic pathogens (Glazebrook 2005 and Van der Ent et al., 2009). When plants are exposed to elevated temperature, JA biosynthesis is often stimulated (Herde et al., 1996). In this study, we found more JA content under high temperature than optimal temperature in both infected and uninfected plants of both cultivars. Drought stress caused the accumulation of JA in creeping bentgrass at a 20-40% loss of water from leaves (Krishnan and Merewitz 2015). In another study, JA content was elevated in both trimmed and untrimmed bentgrass due to high temperature (Krishnan et al., 2016). In regards to JA responses due to high temperature and pathogen infection, we found a higher content of JA in infected 'Tyee' than infected 'Penn-A4' at high temperature in leaves, stolons, and roots. Accumulation of JA has also been found in many other disease systems in response to pathogen infection such as infection of *Pseudomonas syringae* pv. *maculicola* in and *Phytophthora infestans* in potatoes (Landgraf et al., 2002 and Halim et al., 2004). Moreover, JA is essential for plant defense against root rot fungi (*Pythium mastophorum)* and JA-mediated responses help Arabidopsis to defend against *P. mastophorum* (Vijayan et al., 1998). JA levels were elevated in a resistant wheat cultivar (*Triticum aestivum* L.) after it was infected with the Fusarium head blight fungus (*Fusarium graminearum*) (Ding et al., 2011). A more apparent increase of JA could be associated with *Aaa* tolerance since the more tolerant 'Tyee' had greater shifts in JA content compared to the sensitive 'Penn-A4'; however, additional work is needed to clarify this.

The results show high temperature can increase the susceptibility of both a tolerant and sensitive cultivar of creeping bentgrass to *Aaa* infection. During times of heat stress plants often reduce or cease to grow in order for a metabolic shift towards stress defense. The reduction in growth is often regulated by GAs and GA biosynthesis can be repressed (Huot et al., 2014). Conversely, the content of GAs is often found to be elevated during heat stress. Elevated temperature promoted GA1 accumulation in six genotypes of near-isogenic wheat (Pinthus et al., 1989) and high temperature promoted accumulation of GA1, GA3, GA4, and GA20 in creeping bentgrass (Krishnan et al., 2016). Higher GA3 and GA20 were found in plant leaves under high temperature compared to in plants under optimal temperature. It is important to note that GA isoform levels were likely affected in all plants in this study as they were all under TE treatment; however, *Aaa* bacteria may be able to take advantage of the heat-induced stimulation of plant synthesized GA by heat stress in order to proliferate and cause disease symptoms.

In regards to how TE may promote *Aaa* infection, the mechanism is not yet clear based on the results of this study. Through the multistep and complicated process of GA biosynthesis, the most biologically active GAs are typically GA1 (derived from GA20), GA3, and GA4 as well as other GAs. GA20 can be converted to GA1 by GA3 oxylase (Olszewski et al., 2002) and also be converted to GA3 (Spray et al., 1996). *Aaa* has the ability to produce GA3 and GA1. TE can repress the conversion of GA53 to GA19, GA19 to GA20, GA20 to GA1, and GA1 to GA8. All of these steps are late in the GA biosynthesis pathway, which conceptually should inhibit the accumulation of most of the active forms of GAs such as GA3 and GA1 in plants. The accumulation of GA1 in infected plants may be produced by *Aaa* (Figure 12) (Hedden and Thomas 2012). Regarding the GA biosynthesis in *Aaa*-infected plants, the accumulation of GA1 and GA3 in infected plants could be produced by *Aaa*. The production of GAs by *Aaa* could stimulate etiolation and increase disease incidence and severity; however, it is not clear why repression of GAs by TE coupled with promotion of GAs by heat stress and GA production by *Aaa* promotes disease in creeping bentgrass. Additional work would be needed to better understand this complex GA regulation. Another possibility is that the reduction of GA in plant tissues caused by TE application is not the main or only factor that causes increased susceptibility to *Aaa*. In fact, other physiological consequences of TE application could play a role in TE-induced susceptibility to *Aaa*; for instance, TE becomes an acid when dissolved in plant tissues (Rademacher, 2000). *Acidovorax* spp. prefer an acidic growth environment to grow and reproduce (Atlas, 2004). Furthermore, GA inhibition in creeping bentgrass has been shown to reduce JA accumulation, which could play a role in disease susceptibility (Krishnan et al., 2015). Thus, future work could be performed to determine whether GA, GA crosstalk with JA, or pH plays more of a role in this pathosystem.

GA levels are also closely associated with ABA in plants. ABA levels could also play a role in the contrasting GA content results in the studies discussed above (Chandler and Robertson 1994). High temperature can stimulate ABA biosynthesis, which can inhibit GA actions in plants (Toh et al., 2008). The high accumulation of ABA in plants treated with high temperature in our study, could be associated with GA accumulation and disease incidence.

ABA is important for mediating abiotic stress responses (Tuteja 2007). Accumulation of ABA was more readily apparent due to bacterial infection than due to high temperature in ‘Penn-A4’. ABA in ‘Penn-A4’ was upregulated by *Aaa* in leaves, while uninfected ‘Penn-A4’ at high temperature did not have any increase in ABA content. ABA is not only important for regulating abiotic stress responses, but also plays an important role in plant immunity (Cao et al., 2011). Accumulation of ABA increases the susceptibility of plants to several pathogens like *Xanthomonas oryzae* pv. *oryzae* by suppressing SA-mediated defense genes (Xu et al., 2013). Fan et al. (2009) also showed that ABA is involved in defense signaling networks or interact with effectors of defenses. More accumulation of ABA was found in infected ‘Penn-A4’ indicated that “Penn-A4” is more susceptible to *Aaa*. This could partially explain why *Aaa* disease is more prevalent under high temperature conditions.

Conclusions

Aaa has the ability to produce phytohormones that could be associated with etiolation. Interestingly, the etiolation symptom remained elusive in our growth chamber study whereas chlorosis and necrosis were readily apparent as were shifts in phytohormones that conceptually could cause etiolation. Thus, much more is needed to be learned about how this etiolation symptom develops and why it only develops under certain conditions. Regardless, this study has shown that high temperature exacerbated *Aaa* necrosis and chlorosis disease symptoms of creeping bentgrass. Creeping bentgrass ‘Tyee’ was more resistant to *Aaa* infection than ‘Penn-A4’ which could be associated with differential accumulation of SA and JA in plant tissues to mount a defense response. We now also have a better understanding of GA involvement in this pathogen system. All of these hormone results will lead to better disease management practices and remediation methods that can be applied to this and other bacterial diseases. Ongoing studies aim to isolate etiolated tissue for determining phytohormone responses, to evaluate phytohormone gene expression in *Aaa*, and to determine whether SA and JA could be used exogenously for better creeping bentgrass tolerance of *Aaa*.

LITERATURE CITED

LITERATURE CITED

Adams, R., Kerber, E., Pfister, K., Weiler, E.W., 1992. Studies on the action of the new growth retardant CGA 163'935 (cimectacarb). Plant Growth Regulation. 14th Inter. Conf. Plant Growth Substances: 818–827.

Arnon, D.I., 1949. Copper enzyme polyphenoloxides in isolated chloroplast in *Beta vulgaris*. Plant Physiology. 24, 1-15.

Atlas, R.M., 2004. Handbook of Microbiological Media, third ed. CRC press. pp. 2056
Bastian, B.C., LeBoit, P.E., Hamm, H., Brocker, E.B., and Pinkel, D., 1998. Chromosomal gains and losses in primary cutaneous melanomas detected by comparative genomic hybridization. Cancer Res. 58, 2170–2175.

Blum, A. and Ebercon, A., 1981. Cell membrane stability as a measure of drought and heat tolerance in wheat. Crop Sci. 21, 43–47.

Bull, H.J., Lombardo, M.J., and Rosenberg, S.M., 2001. Stationary-phase mutation in the bacterial chromosome: Recombination protein and DNA polymerase IV dependence. Proc Natl Acad Sci USA. 98, 8334-8341.

Cao, F., Yoshioka, K., and Desveaux, D., 2011. The roles of ABA in plantpathogen interactions. J. Plant Research. 124, 489–499.

Chandler, P., and Robertson, M., 1994. Gene expression regulated by abscisic acid and its relation to stress tolerance. Ann. Rev. Plant Physiol. Plant Mol. Biol. 45, 113-141.

Choudhary, D.K., Prakash, A., and Johri, B.N., 2007. Induced systemic resistance (ISR) in plants: mechanism of action. Indian J. Microbiol. 47, 289-97

Coquoz, J.L., Buchala, A., Meuwly, P., and Métraux, J.P., 1995. Arachidonic acid induces local but not systemic synthesis of salicylic acid and confers systemic resistance in potato plants to Phytophthora infestans and Alternaria solani. Phytopathology. 85, 1219 – 1224.

Czajkowski, R., Van Der Wolf, J.M., Krolicka, A., Ozymko, Z., Narajczyk, M., Kaczynska, N., and Lojkowska, E., 2015. Salicylic acid can reduce infection symptoms caused by *Dickeya solan*i in tissue culture grown potato (*Solanum tuberosum* L.) plants. European Journal of Plant Pathology. 141, 545-558

Ding, L., Xu, H., Yi, H., Yang, L., Kong, Z., Zhang, L., Xue, S., Jia, H., and Ma, Z., 2011. Resistance to hemi-biotrophic F. graminearum infection is associated with coordinated and ordered expression of diverse defense signaling pathways. PloS. 6(4): e19008. doi: 10.1371/journal.pone.0019008.

El-Mohamedy, R.S.R., Jabnoun-Khiareddine, H., and Daami-Remadi, M., 2014. Control of root rot diseases of tomato plants caused by *Fusarium solani*, *Rhizoctonia solani* and *Sclerotium rolfsii* using different chemical plant resistance inducers. Tunisian Journal of Plant Protection. 9, 45-55.

Fagerness, M.J. and Yelverton, F.H., 2001. Plant growth regulator and mowing height effects on seasonal root growth of Penncross creeping bentgrass. Crop Sci. 41, 1901–1905.

Fan, J., Hill, L., Crooks, C., Doerner, P., and Lamb, C., 2009. Abscisic acid has a key role in modulating diverse plant-pathogen interactions. Plant Physiol. 150, 1750–1761.
Fragnière, C., Serrano, M., Abou-Mansour, E., Métraux, J.P., and L'Haridon, F., 2011. Salicylic acid and its location in response to biotic and abiotic stress. FEBS Lett. 585, 1847–1852

Giordano, P.R., Vargas, J.M., Detweiler, A.R., Dykema, N.M. and Yan, L., 2010. First report of a bacterial disease on creeping bentgrass (*Agrostis stolonifera*) caused by *Acidovorax* spp. in the United States. Plant Disease. 94(7), 922.

Giordano, P.R., Chaves, A.M., Mitkowski, N.A., and Vargas, J.M. 2012. Identification, characterization, and distribution of *Acidovorax avenae* subsp. *avenae* associated with creeping bentgrass etiolation and decline. Plant Dis. 96, 1736-1742.

Glazebrook, J., 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annual Review of Phytopathology. 43, 205 – 227.

Gutiérrez-Mañero, F.J., Ramos, B., Probanza, A., Mehouachi, J., and Talon, M., 2001. The plant growth promoting rhizobacteria Bacillus pumilus and Bacillus licheniformis produce high amounts of physiologically active gibberellins. Physiol Plant. 111, 206–211. doi: 10.1034/j.1399-3054.2001.1110211.x.

Halim, V.A., Hunger, A., Macioszek, V., Landgraf, P., Nürnberger, T., Scheel, D., and Rosahl, S., 2004. The oligopeptide elicitor Pep-13 induces salicylic acid-dependent and -independent defense reactions in potato. Physiological and Molecular Plant Pathology. 64, 311 – 318.

Hedden, P. and Thomas, S.G., 2012. Gibberellin biosynthesis and its regulation. Biochemical Journal. 444 (1), 11-25.

Herde, O., Atzorn, R., Fisahn, J., Wasternack, C., Willmitzer, L., and Pena-Cortes, H., 1996. Localized wounding by heat initiates the accumulation of proteinase inhibitor II in abscisic acid-deficient plants by triggering jasmonic acid biosynthesis. Plant Physiol. 112, 853–860.

Hiscox, J.D. and Israelstam, G.F., 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. Can. J. Bot. 57, 1332-1334.

Huang, B. and Fry. J.D., 1998. Root anatomical, physiological and morphological responses to drought stress for tall fescue cultivars. Crop Science. 38, 1017-1022.

Huot, B., Yao, J., Montgomery, B.L., and He, S.Y., 2014. Growth-defense tradeoffs in plants: A balancing act to optimize fitness. *Molecular Plant. 7*, 1267-1287.

Janzen, R., Rood, S., Dormar, J., and McGill, W., 1992. Azospirillum brasilense produces gibberellins in pure culture and chemically-medium and in co-culture on straw. Soil Biol Biochem. 24, 1061–1064.

Knievel, D.P., 1973. Procedure for estimating ratio of living to dead root dry matter in the root core sample. Crop Science. 13, 124-126.

Krishnan, S., Ma, Y., and Merewitz, E., 2016. Leaf trimming and high temperature regulation of phytohormones and polyamines in creeping bentgrass leaves. Journal of the American Society for Horticultural Science. 141, 66-75.

Krishnan, S. and Merewitz, E., 2015. Drought stress and trinexapac-ethyl modify phytohormone content within kentucky bluegrass leaves. Journal of Plant Growth Regulation. 34, 1-12.

Landgraf, P., Feussner, I., Hunger, A., Scheel, D., and Rosahl, S., 2002. Systemic accumulation of 12-oxo-phytodienoic acid in SAR-induced potato plants. European Journal of Plant Pathology. 108, 279 – 283.

Li, Y., Zhang, Z., Nie, Y., Zhang, L., and Wang, Z., 2012. Proteomic analysis of salicylic acid-induced resistance to *Magnaporthe oryzae* in susceptible and resistant rice. Proteomics. 12, 2340–2354. doi:10.1002/pmic.201200054

Liu, H., Li, X., Xiao, J., and Wang, S., 2012. A convenient method for simultaneous quantification of multiple phytohormones and metabolites: application in study of rice-bacterium interaction. Plant methods. 8, 2.

MacMillan, J., 2001. Occurrence of gibberellins in vascular plants, fungi, and bacteria. Journal of Plant Growth Regulation. 20:387-442.

Malamy, J., Hennig, J., and Klessig, D.F., 1992. Temperature-dependent induction of salicylic acid and its conjugates during the resistance response to tobacco mosaic virus infection. Plant Cell. 4:359-366.

Mazzella, N., Molinet, J., Syakti, A.D., Dodi, A., Doumenq, P., Artaud, J., and Bertrand, J.C., 2004. Bacterial phospholipid molecular species analysis by ion-pair reversed-phase HPLC/ESI/MS. Journal of Lipid Research. 45, 1355-1363.

McMichael, B.L., and Burke, J.J., 1994. Metabolic activity of cotton roots in response to temperature. Environmental and Experimental Botany. 34, 201-206.

Merewitz, E.B., Gianfagna, T., and Huang, B., 2011. Photosynthesis, water use, and root viability under water stress as affected by expression of SAG12-ipt controlling cytokinin synthesis in *Agrostis stolonifera.* J Exp Bot. 62(1), 383–395.

Metraux, J.P., Signer, H., Ryals, J., Ward, E., Wyss-Benz, M., Gaudin, J., Raschdorf, K., Schmid, E., Blum, W., and Inverardi, B., 1990. lncrease in salicylic acid at the onset of systemic acquired resistance in cucumber. Science. 250, 1004-1006.

Oliveira, J.C, Silveira, E.B., Mariano, R.L.R., Cardoso, E., and Viana, I.O., 2007. Characterization of strains of *Acidovorax avenae* subsp. *citrulli*. Fitopatol Bras. 32, 480–7.

Olszewski, N., Sun, T., and Gubler, F., 2002. Gibberellin signaling: biosynthesis, catabolism, and response pathways. Plant cell supplement. S61-S80.

Ortíz-Castro, R., Contreras-Cornejo, H.A., Macías-Rodríguez, L., and López-Bucio J., 2009.

The role of microbial signals in plant growth and development. Plant signal behav. 4, 701-712.

Pinthus, M.J., Gale, M.D., Appleford, N.E.J., and Lenton, J.R. 1989. Effect of temperature on gibberellin (GA) responsiveness and on endogenous GA_1 content of tall and dwarf wheat genotypes. Plant Physiol. 90, 854–859.

Powell, J.F., Vargas, J.M., Nair, M.G., Detweiler, A.R., and Chandra, A., 2000. Management of dollar spot on creeping bentgrass with metabolites of *Pseudomonas aureofaciens* (TX- 1). Plant Dis. 84, 19-24.

Rademacher, W., 2000. Growth retardants: effects on gibberellin biosynthesis and other metabolic pathways. Ann. Rev. Plant Physiol and Plant Mol. Biol. 51, 501-531.

Roberts, J.A., 2014. Investigating the Cause and Developing Management Options to Control Bacterial Etiolation in Creeping Bentgrass Putting Greens. Ph.D. Thesis. North Carolina State University, North Carolina, USA, pp.86-105.

Roberts, J.A., Kerns, J.P., and Ritchie, D.F., 2015. Bacterial etiolation of creeping bentgrass as influenced by biostimulants and trinexapac-ethyl. Crop Protection. 72, 119-126.

Roberts, J.A., Ritchie, D.F., and Kerns, J.P., 2016. Plant growth regulator effects on bacterial etiolation of creeping bentgrass putting green turf caused by *Acidovorax avenae*. Plant Dis. 100, 577-582.

Spray, C.R., Kobayashi, M., Suzuki, Y., Phinney, B.O., Gaskin, P., and MacMillan, J., 1996. The *dwarf-1* (*d1*) mutant of*Zea mays* blocks three steps in the gibberellin-biosynthetic pathway. Proc Nat Acad Sci USA. 93, 10515–10518

Toh, S., Imamura, A., Watanabe, A., Nakabayashi, K., Okamoto, M., Jikumaru, Y., Hanada, A., Aso, Y., Ishiyama, K., Tamura, N., Iuchi, S., Kobayashi, M., Yamaguchi, S., Kamiya, Y., Nambara, E., and Kawakami N., 2008. High temperature-induced abscisic acid biosynthesis and its role in the inhibition of gibberellin action in *Arabidopsis* seeds. Plant Physiol. 146, 1368–1385.

Turgeon, A.J., 2008. Turfgrass management, eighth ed. Upper Saddle River, Pearson Prentice Hall, NJ, pp.122.

Tuteja, N., 2007. Abscisic Acid and abiotic stress signaling. Plant Signal Behav. 2, 135–8.

Van der Ent, S., Van Wees, S.C., and Pieterse, C.M. 2009., Jasmonate signaling in plant interactions with resistance-inducing beneficial microbes. Phytochemistry. 70(13-14), 1581-8. doi: 10.1016/j.phytochem.2009.06.009.

Vijayan, P., Shockey, J., Levesque, C.A., Cook, R.J., and Browse, J., 1998. A role for jasmonate in pathogen defense of Arabidopsis. Proc Natl Acad Sci USA. 95, 7209-7214.

Xu, J., Audenaert, K., Höfte, M., and De Vleesschauwer, D., 2013. Abscisic acid promotes susceptibility to the rice leaf blight pathogen Xanthomonas oryzae pv. oryzae by suppressing salicylic acid-mediated defenses. PLoS ONE 8:e67413. 10.1371/journal.pone.0067413

Yang, D.L., Yao, J., Mei, C.S., Tong, X.H., Zeng, L., Li, Q., Xiao, L., Sun, T., Li, J.,Deng, X., Lee, C.M., Thomashow, M.F., Yang, Y., He, Z., and He, S.Y., 2012. Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade PNAS. doi:10.1073/pnas.1201616109

CHAPTER THREE

JASMONIC AND SALICYLIC ACID TREATMENTS REDUCE BACTERIAL ETIOLATION DISEASE SYMPTOMS OF CREEPING BENTGRASS

INTRODUCTION

Jasmonic acid (JA) and salicylic acid (SA) play important roles in signaling plant defense systems against pathogen attacks and in response to abiotic stress (Bari and Jones 2009). Recently, it has become clear that defense response systems associated with JA and SA in dicots and monocots may be differential. In monocots, it is likely that JA and SA coordinately induce a defense response via a parallel model rather than an antagonistic type model as in dicots. SA has long been associated with resistance against biotrophic type pathogens and JA with necrotrophic pathogens; however, those associations do not seem to hold true in monocots (Tamaoki and others 2013). Even in dicots, the effects of SA and JA in plants could be more complex than previously thought (Mur and others 2006; Tsuda and others 2009). A better understanding of JA and SA effects on monocot species is needed. Additionally, little to no information is available for bacterial pathogens of creeping bentgrass or other valuable turfgrass species. *Aaa* is a biotrophic pathogen or creeping bentgrass. Whether both SA and JA may play a role in disease symptom development of this disease will help to further support the parallel model of SA and JA signaling.

Creeping bentgrass (*Agrostis stolonifera*) is one of the most widely used turfgrasses on golf course greens, tees, and fairways but can be highly susceptible to various abiotic and biotic stresses (Dernoeden 2013). Bacterial etiolation caused by *Acidovorax avenae* subsp. *avenae* (*Aaa*) can cause severe damage to creeping bentgrass putting greens on golf courses (Giordano and others 2010). Creeping bentgrass is a cool-season turfgrass species and bacterial etiolation caused by *Aaa* is most problematic during periods of above optimal high temperatures (30-

40 °C). Visual symptoms of the disease include chlorosis, necrosis, and etiolation of plant tissues, which reduces turf quality. Physiological plant symptoms include an increase in leaf and root electrolyte leakage, a reduction in chlorophyll content, and reduced root viability. Cultivar variation in susceptibility to *Aaa* and physiological damage caused by *Aaa* has been found. Creeping bentgrass cultivar 'Tyee' is more tolerant of *Aaa* than 'Penn-A4' (Liu and others 2017).

Plant growth regulators (PGRs), which inhibit gibberellic acid (GA) biosynthesis, have also been found to increase creeping bentgrass susceptibility to *Aaa* (Roberts and others 2015), which may be associated with the mode of action of the GA inhibitor (Roberts and others 2016). PGR application coupled with heat stress have been shown to increase *Aaa* disease incidence. The close linkage of this disease with PGR use and the unique etiolation symptoms have indicated that this disease is likely to be highly associated with bacterial synthesis of phytohormones or significant shifts in plant phytohormone profiles. Certain pathogenic strains of *Aaa* are capable of producing GA1, GA3, and indole-3-acetic acid (Liu and others 2017). Creeping bentgrass phytohormone profiles are also significantly affected by *Aaa* infection and are differential in 'Tyee' compared to 'Penn-A4.' With high temperature and bacterial treatment, creeping bentgrass cultivar 'Tyee' had greater SA accumulation in stolons and roots than 'Penn-A4'. Higher content of JA was found in infected 'Tyee' than infected 'Penn-A4' at high temperature in leaves, stolons, and roots. PGR application has also been found to reduce the accumulation of jasmonic acid (JA) under abiotic stress conditions (Krishnan and Merewitz, 2015). This repression of JA could play a role in the increased sensitivity of PGR-treated creeping bentgrass to *Aaa*. Therefore, SA and JA levels in plants could be associated with *Aaa* tolerance or susceptibility, particularly in plants treated with PGRs. PGR application causing a reduction in a JA responses and/or cultivar variation in JA or SA production could play a role in disease susceptibility (Liu and others 2017).

Integrated management of plant diseases requires the exploitation of host defense responses to control plant diseases (Sundin and others 2016). Therefore, the objective of the study was to determine whether treatment of creeping bentgrass with JA or SA prior to *Aaa* exposure can reduce disease incidence in an *Aaa* sensitive and a more tolerant creeping bentgrass cultivar. If exogenous treatment with SA or JA is effective in reducing disease incidence, this would serve as additional evidence that these hormones are highly important in creeping bentgrass tolerance of *Aaa* and possibly related bacteria or pathogens. With this research, we hope to better understand the plant-bacterial interaction of this disease and reveal simple, integrated management strategies that could be recommended to turf managers and to manage *Aaa* disease.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Creeping bentgrass cultivars 'Tyee' and 'Penn-A4' were seeded at the rate of 0.45 kg/93 m^2 in sand in 11.4 cm pots and were established in a greenhouse for 8 weeks with daily watering and weekly fertilization. Plants were regularly trimmed to 2 cm height. After they were established, plants were separated and propagated into clonal tillers. They were then transferred to a hydroponic system in a controlled environmental growth chamber using the methods described in Merewitz and others (2011). Growth chamber conditions were maintained a 12 h photoperiod at 900 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of photosynthetically active radiation (PAR), 65% relative humidity, and a day/night temperature of 23/20 °C. Plants were inserted into 2.54 cm diameter holes in foam boards with plastic covers. The boards were floated on the nutrient solution in black plastic tanks (71 x 51 x 15 cm). The hydroponic solution was aerated via a tube connected to a pump (115 V, 60 Hz, Tetra Whisper; Blacksburg, VA). The solution was changed weekly and solution pH was monitored and adjusted to a pH of 6.0 every 3 days. Once established, plants were kept trimmed to a height of 5 cm. The hydroponic system and experimental design has been used previously (Liu and others 2017). Trinexapac-ethyl (Syngenta Crop Protection, Greensboro, NC) was sprayed on all plants at the rate of 0.79 L ha^{-1} and applied twice, every other week. The second spray occurred 48 h before the experimental treatments.

Experimental Treatments

Prior to temperature and bacterial infection, the plants were exposed to six chemical treatments: 1) SA at a concentration 10 µmol/L; 2) SA at a concentration 20 µmol/L; 3) de-ionized water; 4) 0.5 mM JA in 0.02% ethanol; 5) 2 mM JA in 0.02% ethanol; 6) control de-ionized water with 0.02% ethanol. The rates for SA were selected based on previous research in creeping bentgrass (Larkindale and Huang 2004). JA application rates were based on previous research in wheat seedlings (Qiu and others 2014). Plants were given chemical pre-treatments by spraying 50 mL of the appropriate solution to the foliage of the plant three times daily for two days, and for a final time just prior to exposure to high temperatures and bacteria treatments.

For temperature and bacterial treatments, 16 plants were kept in each hydroponic tank. Four tanks were placed in an optimal temperature growth chamber at 23/20 °C day/night temperature and four tanks were moved to a high temperature chamber at 35/30 °C for the duration of the experiment (12 days). Both chambers were equal in size, RH, photoperiod, and light levels are the same as described above. For infected plants, plant nutrient solution was replaced with bacterial culture of the pure MSU-13 strain of *Aaa* at a concentration of 10^{-6} CFU one day after the measurement day (0, 5, 15 and 20 days). One day before subsequent treatment, bacterial culture was replaced with nutrient solution. Nutrient solution for control plants were changed every 3 days. The canopies of bacteria-treated plants were trimmed to a height of 3 cm with scissors soaked in MSU-13 *Aaa* suspension 2 days before the experiment. Plants were sprayed with 100 mL MSU-13 *Aaa* suspension right after trimming. During the experiment, about 0.5 cm of root tips were pruned with sterilized scissors every day to ensure that bacteria could enter roots through wounds.

Physiological Evaluation of Plants

Electrolyte leakage (EL), turf quality ratings (TQ), chlorophyll content (CHL), photochemical efficiency (F_v/F_m), root leakage (REL) and root viability (RV) were measured on plant leaves and roots every 3 d during the study. EL indicate cellular membrane stability in leaves. Approximately 10 leaves were taken from each plant and rinsen de-ionized water to avoid the inference of chemical treatments. Samples then were submerged in 10 mL of de-ionized water and shaken for 24 h. The conductivity meter (YSI Model 3200; Yellow Springs, OH, USA) was used to measure the initial conductivity (Ci). Leaf tissues were then boiled for 20 min and put on the shaker for another 24 h to measure the maximum conductivity (Cmax). Percent EL was calculated as Ci/Cmax x 100 (Blum and Ebercon 1981).

TQ was visually rated and evaluated by the color, density, and uniformity of the grasses (Turgeon 2008). The rating scale is from 1 to 9 (9 representing a fully turgid, dense green canopy, and 1 representing necrotic plants).

Leaf senescence was evaluated by leaf chlorophyll content (CHL) and photochemical efficiency (F_v/F_m). Total CHL was extracted in dimethyl sulphoxide in the dark for 72 h. The spectrophotometer (Spectronic Genesys 2; Spectronic Instruments, Rochester, NY, USA) was used to measure the absorbance at 663 nm and 645 nm. CHL was calculated using the formula described in Arnon (1949). F_v/F_m was evaluated by using a chlorophyll fluorescence meter (Fim 1500; Dynamax, Houston, TX, USA). F_v/F_m represents a ratio of the variable fluorescence (F_v) to the maximal fluorescence (F_m). Leaf clips were adapted to darkness for 30 min prior to reading the F_v/F_m ratio with the fluorescence meter (Krause and others 1989).

Root health was evaluated by determining root EL (REL) and root viability (RV). REL was measured as described by Huang and others (1998). 200 mg of roots were taken from individual plant and washed thoroughly with de-ionized water and EL was measured as described above. RV was estimated by measuring the activity of dehydrogenase by using the triphenyltetrazolium chloride (TTC) reduction technique (McMichael and Burke 1994). The activity was based on the dry weight of each root sample, which was determined after drying in an 80 °C oven for 72 h (Merewitz and others 2011).

Hydroponics studies were first conducted on July 20th, 2016 (experiment 1), and then repeated on October 7th, 2016 (experiment 2). Physiological indices were taken on 0 day of both studies to make sure materials of repeated studies had the same initial conditions.

Experimental Design and Statistical Analysis

The experiment was a completely randomized block design with four individual plants serving as the replications per treatment. Treatment effects were determined by analysis of variance (ANOVA) according to the general linear model procedure within the Statistical Analysis System software (SAS 9.2; Cary, NC). Results of ANOVA are shown in the appendix. Statistical interactions and means among temperature, bacteria, and cultivar treatments were separated by Fisher’s protected LSD test ($P \leq 0.05$).

RESULTS

Leaf EL was increased under bacterial treatment (Figure 3.1.). At the optimal temperature, plants of both cultivars treated with either JA or SA showed significantly lower leaf EL than control plants in experiment 1 (Figure 3.1. C and G). Plants treated with SA at either concentration showed significantly lower leaf EL than other treatments in both cultivars at the optimal temperature in experiment 2 (Figure 3.1. D and H). ‘Penn-A4’ plants treated with 20 μmol/L SA showed the significantly lowest leaf EL throughout experiment 2 at optimal temperatures (Figure 3.1. H). Plants treated with 2 mM JA had the significantly lowest leaf EL on days 9 and 12 in ‘Tyee’ at the optimal temperature in experiment 1 (Figure 3.1. C). At the high temperature, plants of both cultivars treated with SA at a concentration 20 μmol/L showed significantly lowest leaf EL on days 6, 9 and 12 (Figure 3.1. A, B, E and F).

Figure 3.1. Electrolyte leakage (% EL) of 'Penn-A4' and 'Tyee' creeping bentgrass in response to bacterial, temperature, and phytohormone treatments.

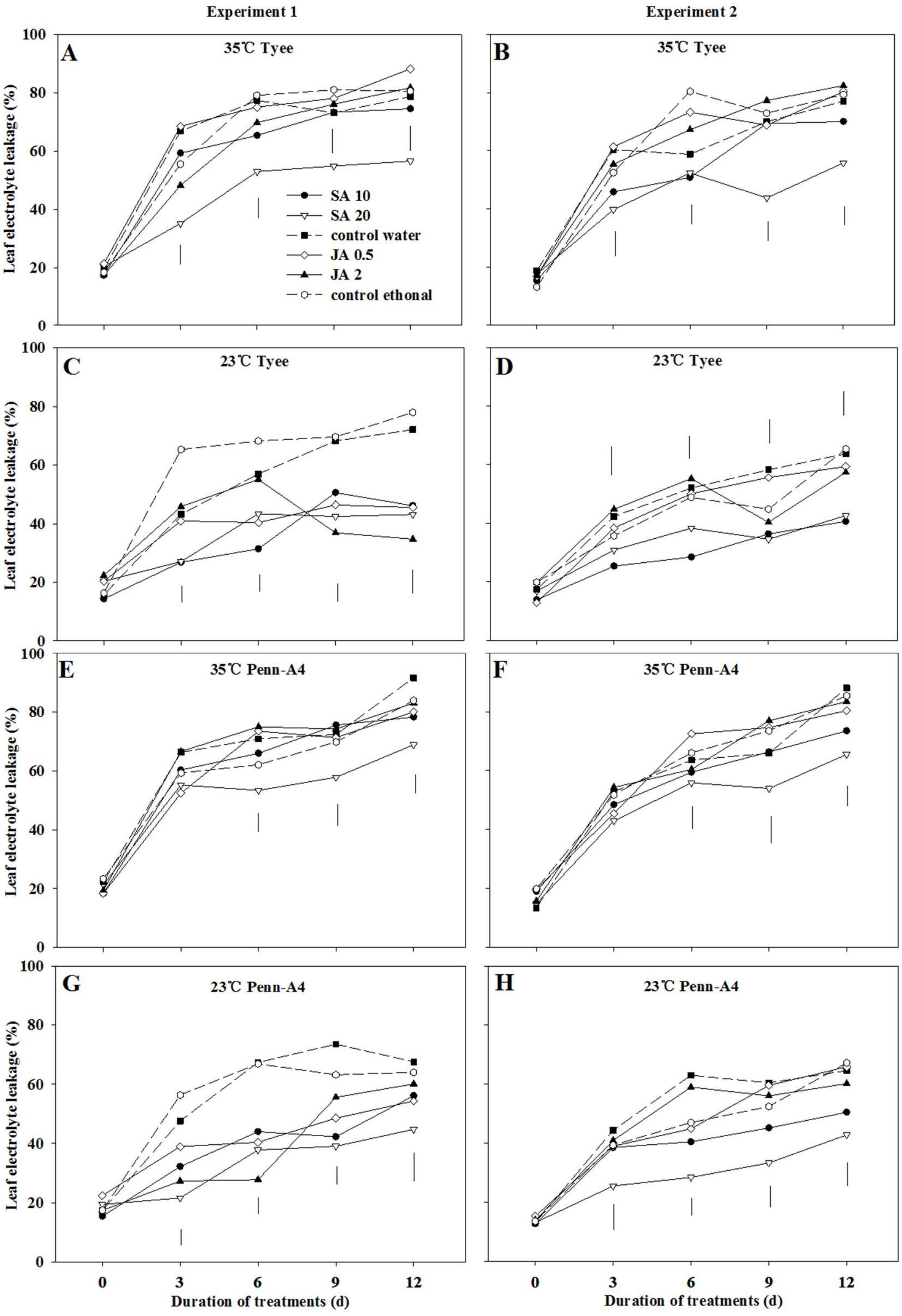


Figure 3.1.A. Electrolyte leakage (% EL) of 'Tyee' exposed to bacterial treatments at 35°C in the experiment 1; **B**. EL of 'Tyee' exposed to bacterial treatments at 35°C in the experiment 2;

Figure 3.1. (cont'd)

C. EL of 'Tyee' at 23°C in the experiment 1; **D**. EL of 'Tyee' at 23°C in the experiment 2; **E.** EL of 'Penn-A4' at 35°C in the experiment 1; **F**. EL of 'Penn-A4' at 35°C in the experiment 2; **G.** EL of 'Penn-A4' at 23°C in the experiment 1; **H**. EL of 'Penn-A4' at 23°C in the experiment 2. LSD bars are present on days when statistically significant differences were observed among treatments ($P \leq 0.05$).

CHL of plants was decreased under bacterial treatment (Figure 3.2.). At the optimal temperature, 'Tyee' treated with SA at both concentrations showed significantly higher CHL than other treatments on days 6, 9 and 12 in the experiment 1 (Figure 3.2. C). 'Tyee' treated with SA at a concentration 20 μmol/L had significantly highest CHL on days 9 and 12 at optimal temperature (Figure 3.2. C and D). Plants treated with either SA or JA had significantly higher CHL than control groups in 'Penn-A4' at the optimal temperature throughout the study in experiment 1; however, 'Penn-A4' treated with 10 μmol/L SA had the significantly highest CHL (Figure 3.2. G). Plants treated with 20 μmol/L SA showed significantly highest CHL in 'Penn-A4' at the optimal temperature on days 3, 9 and 12 in experiment 2 (Figure 3.2.H). At high temperature, plant treated with either SA or JA showed significantly higher CHL than control plants in 'Penn-A4' throughout the study (Figure 3.2. E and F). In 'Tyee', plants treated with SA at either concentration had significantly higher CHL than control plants on days 6, 9 and 12 at the high temperature (Figure 3.2. A and B).

Figure 3.2. Chlorophyll content (CHL) of 'Penn-A4' and 'Tyee' creeping bentgrass in response to bacterial, temperature, and phytohormone treatments.

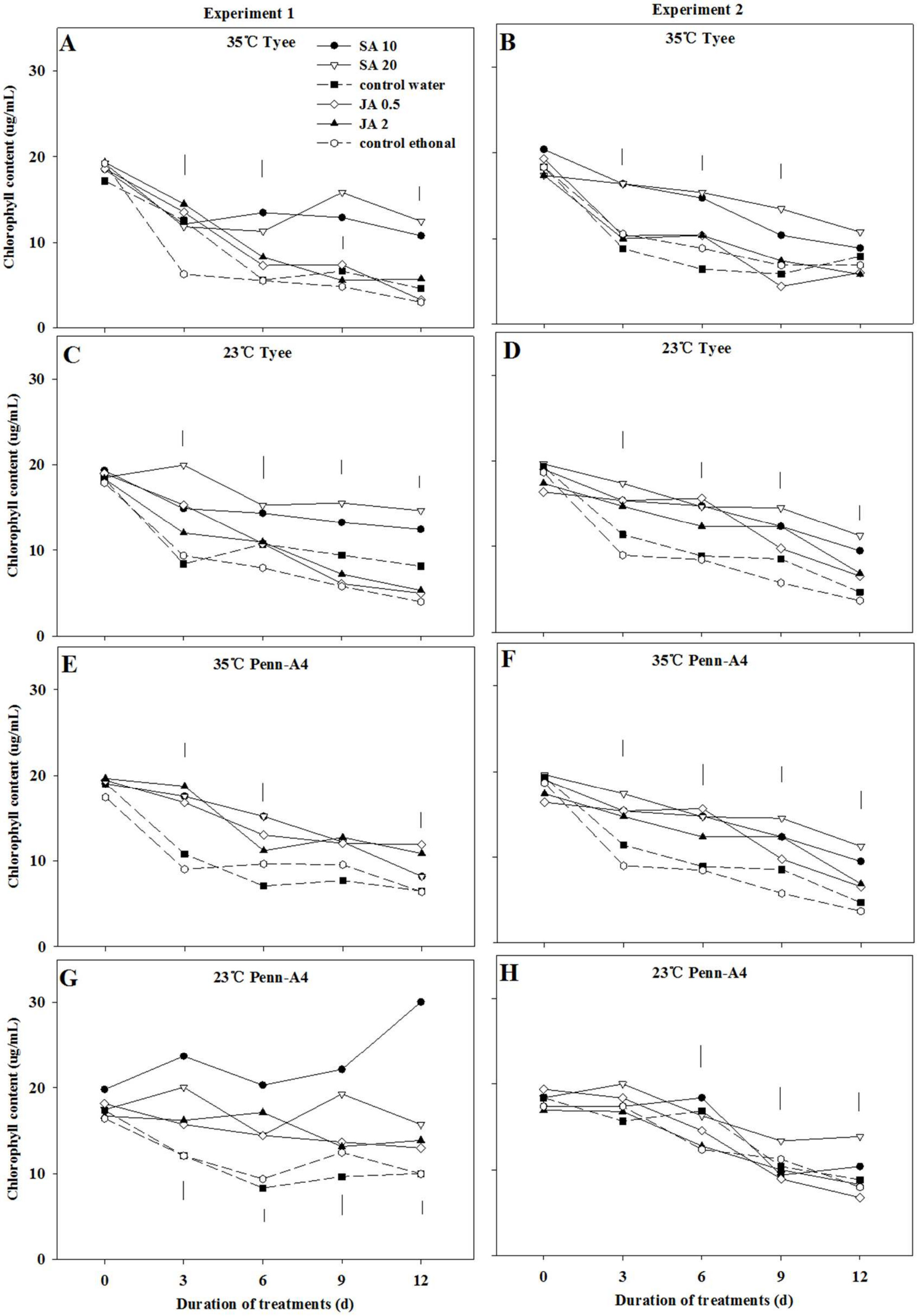


Figure 3.2. A. Chlorophyll content (CHL) of 'Tyee' exposed to bacterial treatments at 35°C in the experiment 1; **B**. CHL of 'Tyee' exposed to bacterial treatments at 35°C in the experiment 2; **C**. CHL of 'Tyee' at 23°C in the experiment 1; **D**. CHL of 'Tyee' at 23°C in the experiment

Figure 3.2. (cont'd)

2; **E.** CHL of 'Penn-A4' at 35°C in the experiment 1; **F**. CHL of 'Penn-A4' at 35°C in the experiment 2; **G.** CHL of 'Penn-A4' at 23°C in the experiment 1; **H**. CHL of 'Penn-A4' at 23°C in the experiment 2. LSD bars are present on days when statistically significant differences were observed among treatments ($P \leq 0.05$).

TQ was decreased due to bacterial infection (Figure 3.3.). At optimal temperatures, TQ was statistically higher in plants treated with either SA or JA than control plants in cultivar 'Tyee' on days 6, 9 and 12 in experiment 1 (Figure 3.3. C). 'Tyee' treated with 20 µmol/L SA or 2 mM JA showed significantly higher TQ than other treatments on days 6, 9 and 12 at the optimal temperature in the experiment 2 (Figure 3.3. D). In 'Penn-A4', TQ was significantly higher in plants treated with SA compared to all other treatments throughout the study at optimal temperatures in experiment 1(Figure 3.3. G). 'Penn-A4' treated with 20 µmol/L SA showed the statistically highest TQ on days 9 and 12 in experiment 2 (Figure 3.3. H). At high temperature, 'Tyee' treated with either SA or JA showed significantly higher TQ than controls on days 9 and 12 (Figure 3.3. A and B). SA treated 'Penn-A4' a showed statistically highest TQ on days 9 and 12 at the high temperature (Figure 3.3. E and F).

Bacterial treatment reduced F_v/F_m in both cultivars at both temperatures (Figure 3.4). At the optimal temperature, SA treated plants had significantly higher F_v/F_m in 'Tyee' than other treatments on days 6, 9 and 12 (Figure 3.4C). Plants treated with either 2 mM JA or 20 µmol/L SA showed statistically highest F_v/F_m in 'Tyee'at the optimal temperature on days 6, 9 and 12 in the experiment 2 (Figure 3.4D). In 'Penn-A4', plants treated with SA at a concentration 20 µmol/L showed statistically highest F_v/F_m on days 9 and 12 at optimal temperature (Figure 3.4G and H). At the high temperature, plants treated with 20 µmol/L SA showed the statistically highest F_v/F_m in both cultivars on days 9 and 12 (Fig 3.4. A, B, E and F). In 'Penn-A4', plants treated with either SA or JA showed significantly statistically higher F_v/F_m than control plants at high temperature on days 9 and 12 (Figure 3.4E).

Figure 3.3. Turf quality rating (TQ) of ‘Penn-A4’ and ‘Tyee’ creeping bentgrass in response to bacterial, temperature, and phytohormone treatments.

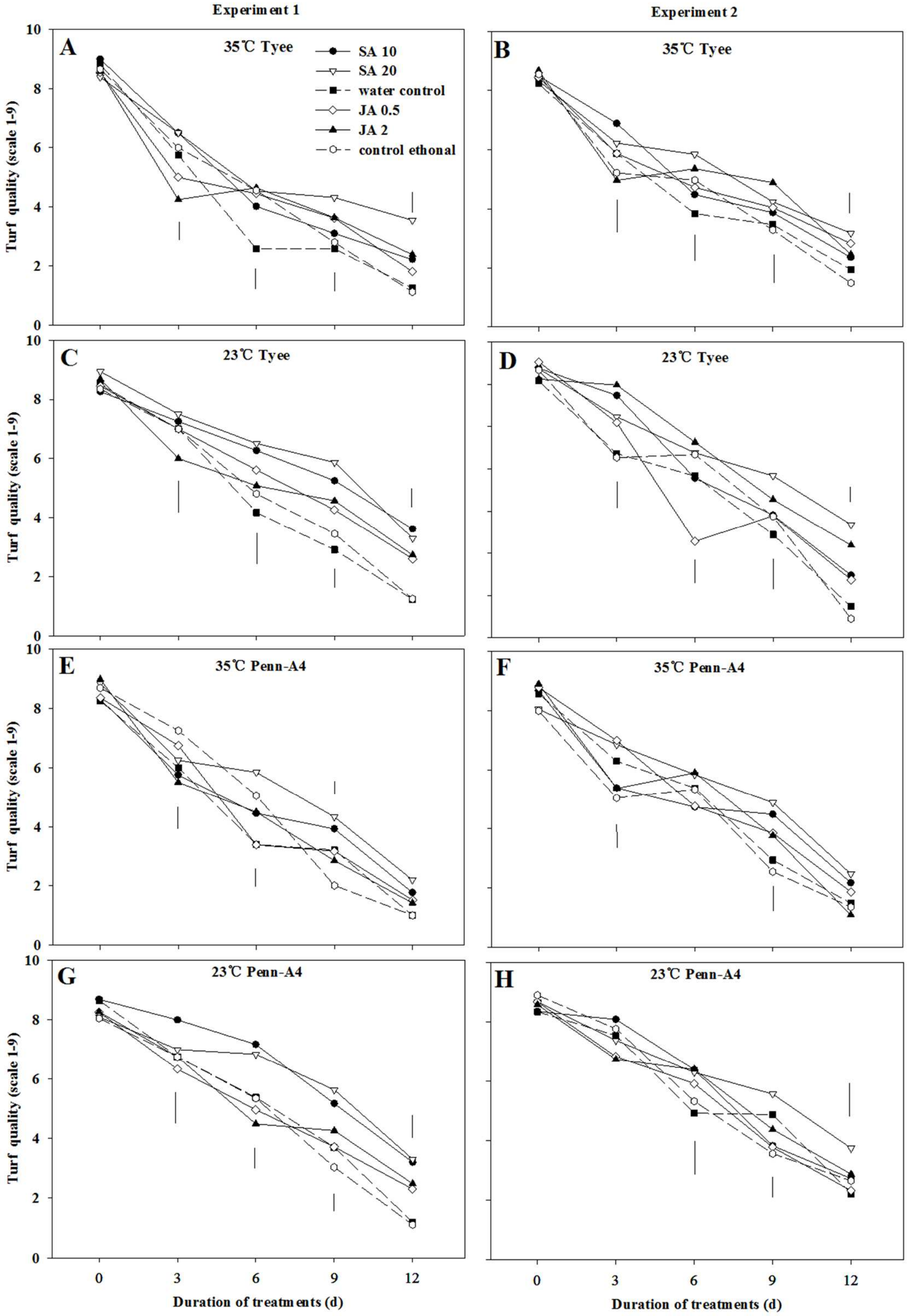


Figure 3.3. A. Turf quality ratings (TQ, 1–9 scale; 1 = worst, 9 = best) of ‘Tyee’ exposed to

Figure 3.3. (cont'd)

bacterial treatments at 35°C in the experiment 1; **B**. TQ of 'Tyee' exposed to bacterial treatments at 35°C in the experiment 2; **C**. TQ of 'Tyee' at 23°C in the experiment 1; **D**. TQ of 'Tyee' at 23°C in the experiment 2; **E.** TQ of 'Penn-A4' at 35°C in the experiment 1; **F**. TQ of 'Penn-A4' at 35°C in the experiment 2; **G.** TQ of 'Penn-A4' at 23°C in the experiment 1; **H**. TQ of 'Penn-A4' at 23°C in the experiment 2.
LSD bars are present on days when statistically significant differences were observed among treatments ($P \leq 0.05$).

Figure 3.4. Photochemical efficiency (Fv/Fm) of 'Penn-A4' and 'Tyee' creeping bentgrass in response to bacterial, temperature, and phytohormone treatments.

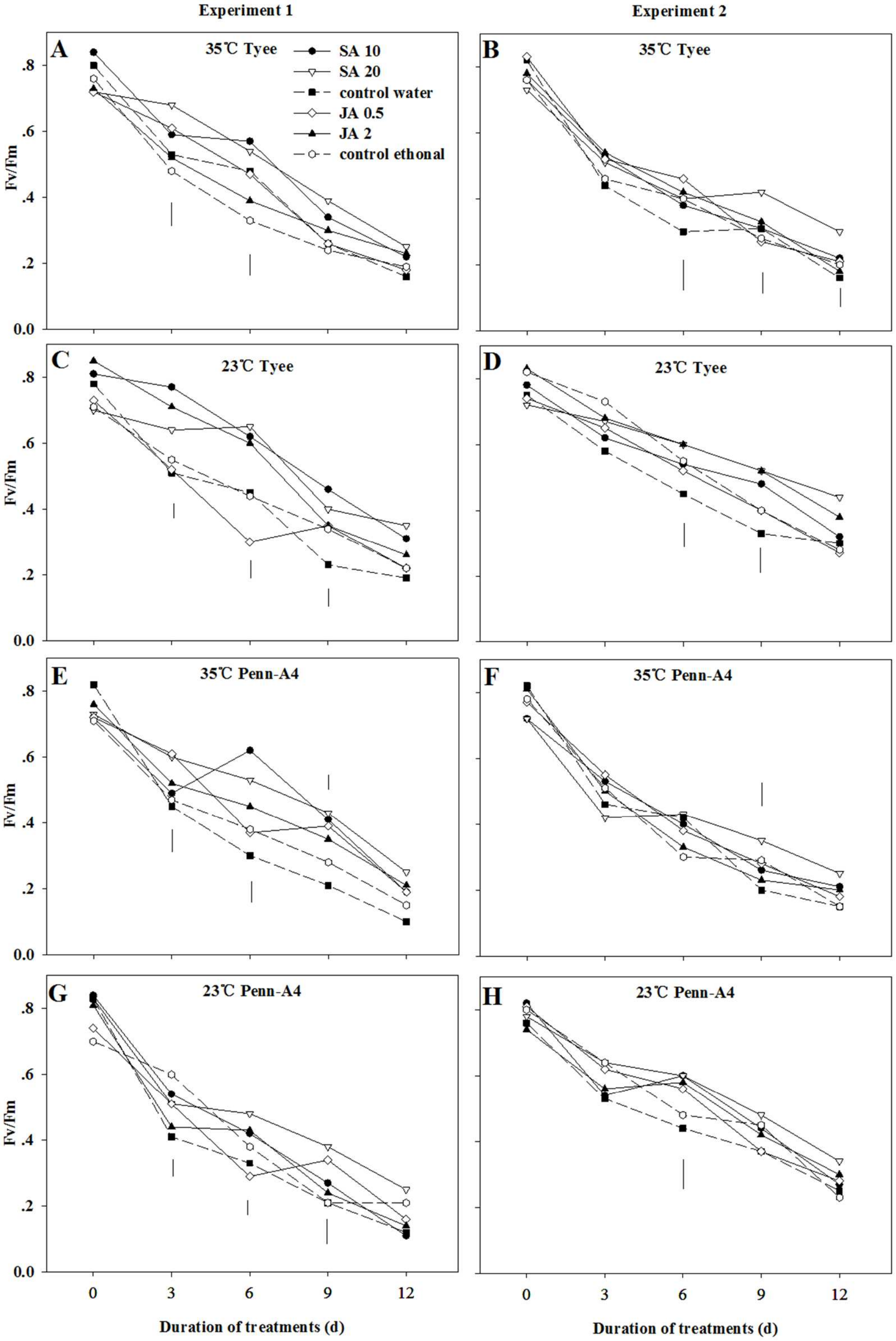


Figure 3.4. A. Photochemical efficiency (F_v/F_m) of of 'Tyee' exposed to bacterial treatments at 35°C in the experiment 1; **B**. F_v/F_m of 'Tyee' exposed to bacterial treatments at 35°C in the experiment 2; **C**. F_v/F_m of 'Tyee' at 23°C in the experiment 1; **D**. F_v/F_m of 'Tyee' at 23°C in the experiment 2; **E.** F_v/F_m of 'Penn-A4' at 35°C in the experiment 1; **F**. F_v/F_m of 'Penn-A4'

Figure 3.4. (cont'd)

at 35°C in the experiment 2; **G.** F_v/F_m of 'Penn-A4' at 23°C in the experiment 1; **H**. F_v/F_m of 'Penn-A4' at 23°C in the experiment 2.
LSD bars are present on days when statistically significant differences were observed among treatments ($P \leq 0.05$).

REL was increased throughout the study. At the optimal temperature, 'Tyee' treated with either SA or JA showed significantly lower REL than control groups on days 9 and 12 in the experiment 1 (Figure 3.5. C). In 'Tyee', plant treated with SA at a concentration 20 μmol/L showed significantly lowest REL at optimal temperature throughout the study in the experiment 2 (Figure 3.5. D). In 'Penn-A4', plants treated with SA at either concentration showed significantly lower REL than other treatments at optimal temperature on days 9 and 12 in the experiment 2 (Figure 3.5. H); however, plants treated with SA at a concentration 20 μmol/L showed significantly lowest REL on days 9 and 12 in the experiment 1 (Figure 3.5. G). At the high temperature, REL was significantly lower in plants treated with SA in 'Tyee' than other treatments on days 6, 9 and 12 (Figure 3.5. A and B). In 'Penn-A4', REL was the significantly lowest in plants treated with SA at a concentration 20 μmol/L at the high temperature throughout the study (Figure 3.5. E and F).

RV was reduced by bacterial infection throughout the study (Figure 3.6.). At the optimal temperature, plants treated with both SA and JA showed significantly higher RV than control plants in 'Penn-A4', while plants treated with SA at a concentration 20 μmol/L showed significantly highest RV (Figure 3.6. G and H). In 'Tyee', RV was significantly higher in plants treated with SA at a concentration 20 μmol/L than other treatments at optimal temperature on days 6, 9 and 12 (Figure 3.6. C and D). At the high temperature, plants treated with SA at a concentration 20 μmol/L showed the significantly highest RV in 'Tyee' throughout the study (Figure 3.6. A and B). In 'Penn-A4', RV was significantly higher in plants treated with SA and plants treated with 2 mM JA on day 12 at the high temperature in the experiment 1 (Figure 3.6. E). 'Penn-A4' treated with SA had significantly higher RV than control groups at the high temperature on days 9 and 12 in the experiment 2 (Figure 3.6. F).

Figure 3.5. Root electrolyte leakage (% REL) of 'Penn-A4' and 'Tyee' creeping bentgrass in response to bacterial, temperature, and phytohormone treatments

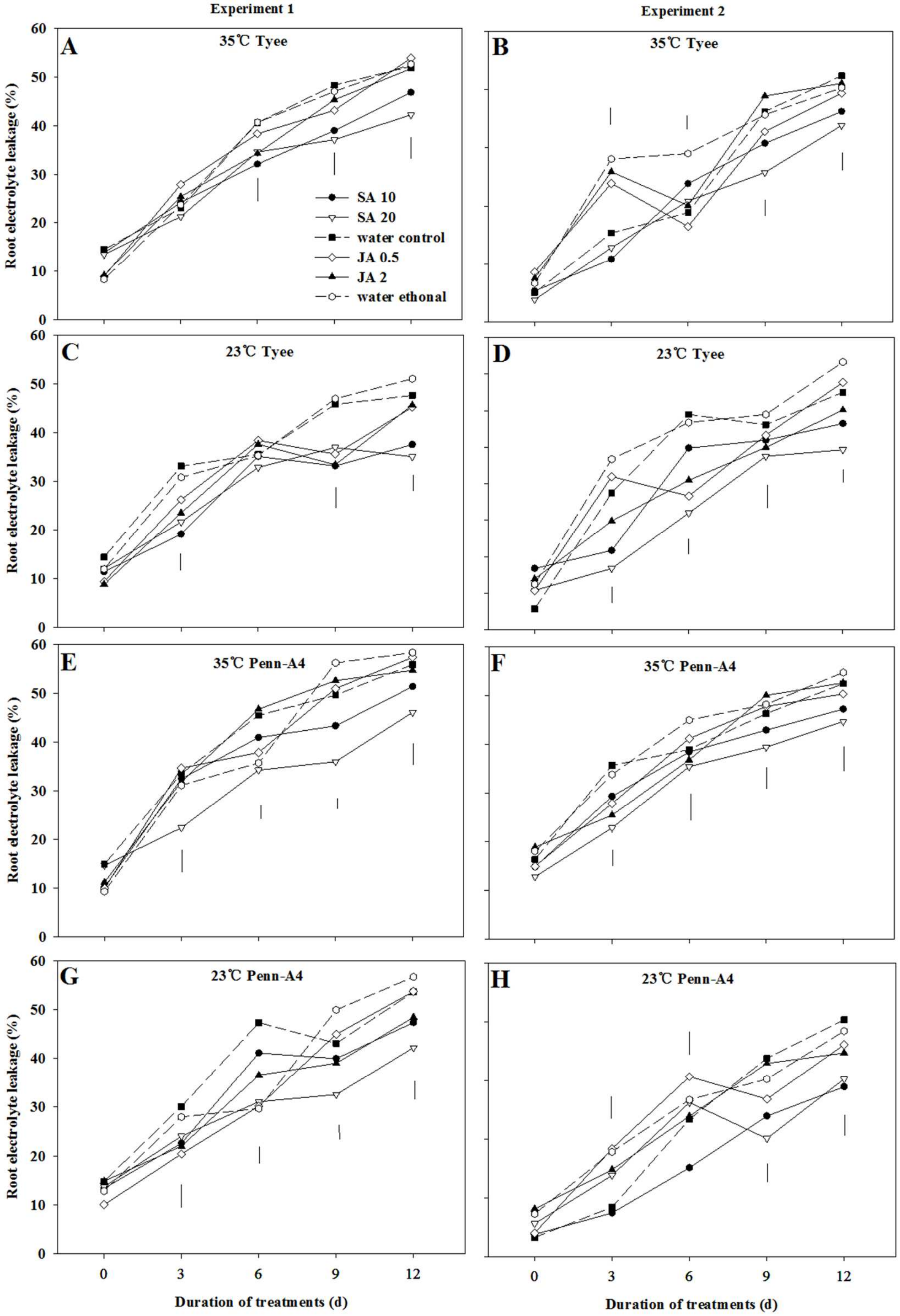


Figure 3.5. A. Root electrolyte leakage (% REL) of of 'Tyee' exposed to bacterial treatments at 35°C in the experiment 1; **B**. REL of 'Tyee' exposed to bacterial treatments at 35°C in the

Figure 3.5. (cont'd)

experiment 2; **C**. REL of 'Tyee' at 23°C in the experiment 1; **D**. REL of 'Tyee' at 23°C in the experiment 2; **E.** REL of 'Penn-A4' at 35°C in the experiment 1; **F**. REL of 'Penn-A4' at 35°C in the experiment 2; **G.** REL of 'Penn-A4' at 23°C in the experiment 1; **H**. REL of 'Penn-A4' at 23°C in the experiment 2.
LSD bars are present on days when statistically significant differences were observed among treatments ($P \leq 0.05$).

Figure 3.6. Root viability (RV) of 'Penn-A4' and 'Tyee' creeping bentgrass in response to bacterial, temperature, and phytohormone treatments.

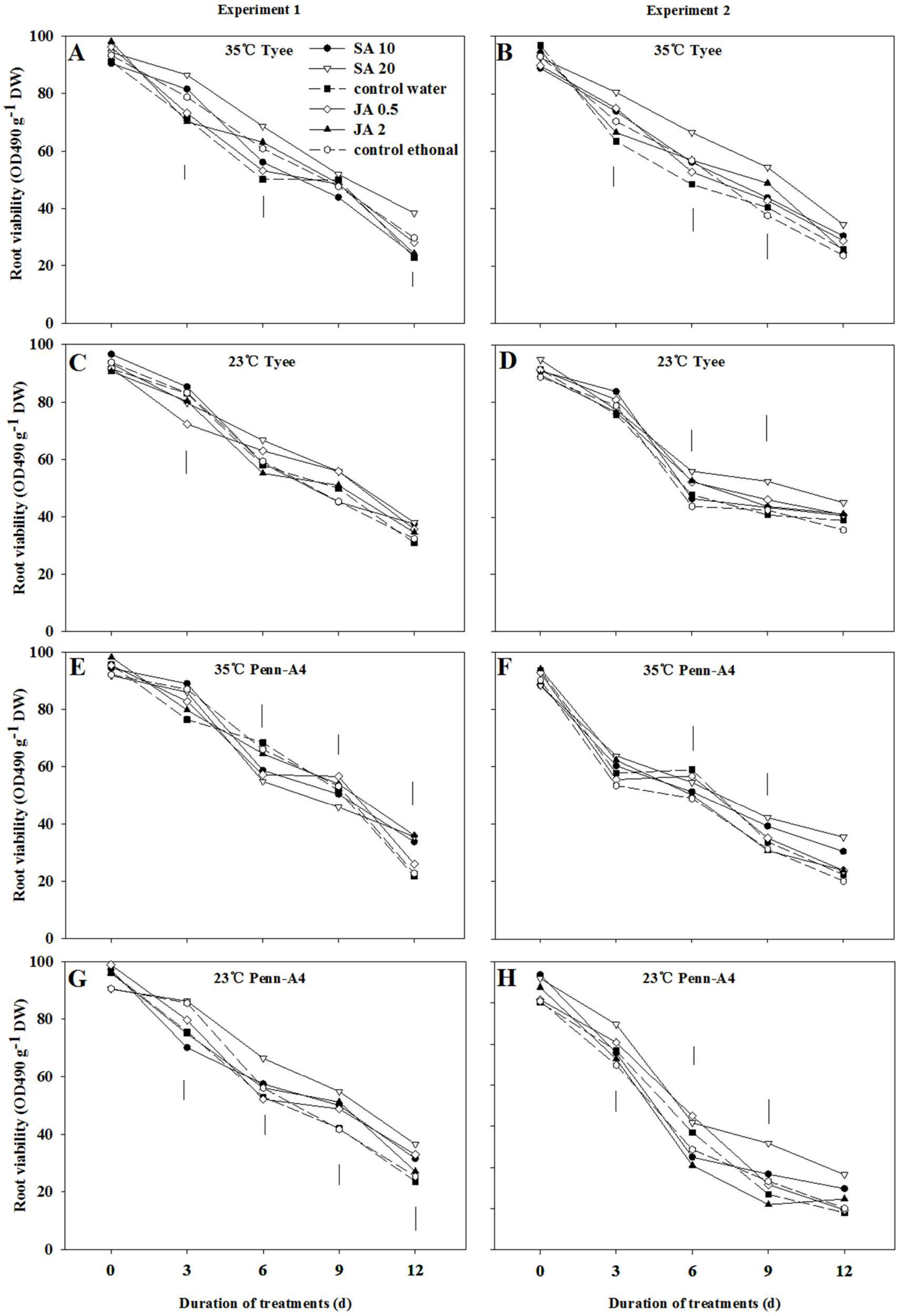


Figure 3.6. A. Root viability (RV) of of 'Tyee' exposed to bacterial treatments at 35°C in the

Figure 3.6. (cont'd)

experiment 1; **B**. RV of 'Tyee' exposed to bacterial treatments at 35°C in the experiment 2; **C**. RV of 'Tyee' at 23°C in the experiment 1; **D**. RV of 'Tyee' at 23°C in the experiment 2; **E.** RV of 'Penn-A4' at 35°C in the experiment 1; **F**. RV of 'Penn-A4' at 35°C in the experiment 2; **G.** RV of 'Penn-A4' at 23°C in the experiment 1; **H**. RV of 'Penn-A4' at 23°C in the experiment 2.
LSD bars are present on days when statistically significant differences were observed among treatments ($P \leq 0.05$).

DISCUSSION

Acidovorax pathogens cause disease in a wide range of economically important monocotyledonous and dicotyledonous plants, including rice, watermelon, corn, as well as grass species. *Aaa* infects rice seedlings resulting in brown stripes on sheaths. Other symptoms include the elongation of the mesocotyl, irregular curvature of leaf sheaths, and the inhibition of seed germination (Kadota and Ohuchi 1983). The most typical symptoms produced on grass hosts by *Aaa* consist of leaf streaks and stripes, often extending into the leaf sheaths with occasional development of stalk rot (Saddler and others 1995). Severely affected plants are necrotic, and the infections often cause an overall inhibition of germination (Xie and others 1998).

In bacterial etiolation of creeping bentgrass, symptoms include chlorosis and necrosis of leaf tissue. A unique etiolated appearance is also observed in stem tissue. These symptoms caused by *Aaa* occur in conjunction with a significant shift in the phytohormone profiles of plants. In pure culture, certain strains of *Aaa* are capable of producing GA1, GA4, and IAA (Liu and others 2017). The bacterial production of phytohormones is likely coupled with the plant mounting a defense response and shifting endogenous hormone production, particularly of JA and SA. JA and SA were detected at higher levels in a more *Aaa* tolerant creeping bentgrass compared to a sensitive one (Liu and others 2017), it follows that exogenous application of JA or SA may be an effective means of managing this newly identified disease.

JA and SA play important roles in transducing the activation of plant defense systems against pathogen attacks (Diaz and others 2002; Mitchell and Walters 1995). JA and SA defense signaling pathways interact antagonistically but can also interact together in a complex manner. There appears to be differences in monocots and dicots in SA and JA signaling (Tamaoki and others 2013; Koornneef and Pieterse 2008). JA-dependent signaling can mediate parts of SA-upregulated genes in rice (*Oryza sativa* L.) suggesting that JA and SA signaling interacts coordinately in rice defense responses (Tamaoki and others 2013). JA biosynthesis mutants in Arabidopsis are altered in their resistance against necrotrophic bacteria *Erwinia carotovora* (Pozo and others 2005). Regardless of the signaling mechanism, it is accepted that these hormones play an important role in plant tolerance of abiotic and biotic stresses.

SA and JA have been used to chemically prime plants for stress defense, largely based on the life cycle and strategy of each tested pathogen. SA is typically associated with tolerance of biotrophic pathogens, whereas JA plays a role in tolerance to necrotrophic pathogens (Glazebrook 2005) Recently however, the lines of these associations are becoming increasingly blurred. In potato and tomato plants, JA treatment decreased germination and growth of semi-biotrophic oomycete P*hytophthora infestans* (Cohen and others 1993). Exogenous application of JA was found to protect wheat against semi-biotrophic fungus *Tilletia laevia* (Lu and others 2006). In rice, JA treatment provided protection against biotrophic blast disease (*Magnaporthe. oryzae*), by inducing the activation of defense response genes PR1a, PR1b, PR2, PR3, PR5, and PR10 (Mei and others 2006). Exogenous application of SA can induce pathogenesis-related (PR) genes and activate SAR in a wide variety of plant species (Mauch and others 2000). Application of exogenous SA has been found to protect rice seedlings from rice blast fungus (Iwai and others 2007). SA treatment can induce wheat resistance to *Gaeumannomyces graminis* var. *tritici* by accumulation of free SA in wheat roots (Khaosaad and others 2007). SA treatment has also been shown to help barley against powdery mildew (*B. graminis f.* sp. *hordei*) disease (Beßer and others 2000).

In turfgrasses, less is known about the plant immune responses displayed by pathogens with various virulence strategies. The high input of pesticides common to turfgrass management makes it essential to have integrated management strategies. Little information is available for the efficacy of use of SA and JA in a diverse set of plant pathogens. Additionally, *Aaa* is a relatively newly characterized disease for which recommendations of management strategies are lacking. Our results indicate that both SA and JA are effective in reducing creeping bentgrass symptoms of *Aaa* infection. Similarly, SA pre-treatment reduced the disease incidence and severity of gray leaf spot in perennial ryegrass (*Lolium perenne* L.) caused by *Magnaporthe oryzae* (Rahman and others 2015).

The effect of 20 µmol/L SA against *Aaa* was more significant and consistent under the optimal temperatures than at high temperatures. In other plant species, SA content in plants are influenced by high temperatures. Elevated temperature (32 °C) blocked the increase in SA levels during the resistance response of tobacco (*Nicotiana tabacum*) to tobacco mosaic virus (TMV) infection. Also, the exogenous SA to infected plants at this temperature was not sufficient to induce hypersensitive response (HR) (Malamy and others 1992). High temperature increased the accumulation of SA in creeping bentgrass (Krishnan and others 2016). Yalpani and others (1991) found that HR and the induction of the PR-1 genes were blocked at high temperature, when SA was applied to tobacco (*Nicotiana tabacum*) against TMV. There are common pathways and components in response to different biotic and abiotic stress, and SA signaling in thermotolerance could interact with SAR (Pastori and Foyer 2002). Based on our results and results from previous studies, it is not clear why SA was more effective under optimal temperatures compared to high temperatures; however, it could be that the plant is not at as great of a disadvantage under optimal temperature and *Aaa* compared to high temperature and *Aaa* treatment.

Cultivar differences in response to JA or SA were found in this study. Both JA and SA improved

Aaa tolerance; however, 2 mM JA was more effective in reducing *Aaa* symptom expression in cultivar ‘Tyee’ compared to ‘Penn-A4’. This is consistent with previous work by Hsiang and others (2014) that found cultivars of creeping bentgrass vary significantly in their response to ISR activators and have a major impact on effectiveness of these activators (Tung 2011). Cultivar resistances in many crops are closely associated with effective biochemical and cellular defenses against pathogen infection (Miles and others 2011). The different response to JA treatments against pathogens could associated with differential JA-induced transcriptional regulation of PR genes expression in different cultivars (Lee and others 2014).

Here, we found that both SA and JA exogenous applications could reduce *Aaa* severity of creeping bentgrass. SA at the concentration of 20 µmol/L exhibited the greatest *Aaa* suppression among treatments. The effect of 2 mM JA was most significant in cultivar ‘Tyee’ under optimal temperature. These results will lead to better disease management practices and remediation methods that can be applied to *Aaa* and other bacterial diseases on turfgrass. Ongoing studies aim to further study temperature and cultivar influences on SA and JA applications and creeping bentgrass tolerance of *Aaa*. The different rates of JA and SA used in the study prevented a direct comparison of effectiveness of JA to SA. The parallel model of disease response pathways signaled by JA and SA could indicate that JA may play more of an important role in disease responses than previously thought. Thus, future work to directly compare these hormones for applied use in turfgrass management may be warranted, particularly in a diverse set of cultivar.

APPENDIX

Table 2. Analysis of variance (AOV) table for leaf electrolyte leakage within cultivars, temperature and bacterial treatments from data reported in Chapter Two.

		Duration of treatments (d)									
		Experiment 1					Experiment 2				
Source of variation	df	0	5	10	15	20	0	5	10	15	20
Replicate	3										
Temperature (T)	1	NS	*	*	*	*	NS	*	*	*	*
Error(T)	3										
Bacteria(B)	1	NS	NS	*	*	*	NS	*	*	*	*
BxT	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(B)	6										
Cultivar	1	NS	NS	*	*	*	NS	NS	NS	*	*
CxT	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxB	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxTXB	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(C)	12										
Total	31										

*and NS indicate significance at P=0.05, and not significant at P=0.05, respectively

Table 3. Analysis of variance (AOV) table for chlorophyll content within cultivars, temperature and bacteria treatments from data reported in Chapter Two.

		Duration of treatments (d)									
		Experiment 1					Experiment 2				
Source of variation	df	0	5	10	15	20	0	5	10	15	20
Replicate	3										
Temperature (T)	1	NS	NS	NS	*	*	NS	NS	NS	*	*
Error(T)	3										
Bacteria(B)	1	NS	*	*	*	NS	NS	NS	NS	*	*
BxT	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(B)	6										
Cultivar	1	NS	NS	*	*	*	NS	NS	NS	NS	*
CxT	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxB	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxTXB	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(C)	12										
Total	31										

*and NS indicate significance at P=0.05, and not significant at P=0.05, respectively

Table 4. Analysis of variance (AOV) table for turf quality within cultivars, temperature and bacteria treatments from data reported in Chapter Two.

		Duration of treatments (d)									
		Experiment 1					Experiment 2				
Source of variation	df	0	5	10	15	20	0	5	10	15	20
Replicate	3										
Temperature (T)	1	NS	NS	*	*	*	NS	*	*	*	NS
Error(T)	3										
Bacteria(B)	1	NS	*	*	*	*	NS	NS	*	*	*
BxT	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(B)	6										
Cultivar	1	NS	*	*	*	*	NS	NS	*	*	*
CxT	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxB	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxTxB	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(C)	12										
Total	31										

*and NS indicate significance at P=0.05, and not significant at P=0.05, respectively

Table 5. Analysis of variance (AOV) table for root electrolyte leakage within cultivars, temperature and bacteria treatments from data reported in Chapter Two.

		Duration of treatments (d)									
		Experiment 1					Experiment 2				
Source of variation	df	0	5	10	15	20	0	5	10	15	20
Replicate	3										
Temperature (T)	1	NS	NS	*	*	*	NS	*	*	*	NS
Error(T)	3										
Bacteria(B)	1	NS	*	*	*	*	NS	NS	*	*	*
BxT	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(B)	6										
Cultivar	1	NS	*	*	*	*	NS	NS	*	*	*
CxT	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxB	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxTxB	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(C)	12										
Total	31										

*and NS indicate significance at P=0.05, and not significant at P=0.05, respectively

Table 6. Analysis of variance (AOV) table for root viability within cultivars, temperature and bacteria treatments from data reported in Chapter Two.

		Duration of treatments (d)									
		Experiment 1					Experiment 2				
Source of variation	df	0	5	10	15	20	0	5	10	15	20
Replicate	3										
Temperature (T)	1	NS	NS	*	*	*	NS	*	*	*	NS
Error(T)	3										
Bacteria(B)	1	NS	*	*	*	*	NS	NS	*	*	*
BxT	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(B)	6										
Cultivar	1	NS	*	*	*	*	NS	NS	*	*	*
CxT	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxB	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxTxB	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(C)	12										
Total	31										

*and NS indicate significance at P=0.05, and not significant at P=0.05, respectively

Table 7. Analysis of variance (AOV) table for salicylic acid content within plant tissues, cultivars, temperature and bacteria treatments from data reported in Chapter Two.

Duration of treatments (d)

		leaf								stolon								root							
		Experiment 1				Experiment 2				Experiment 1				Experiment 2				Experiment 1				Experiment 2			
Source of variation	df	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20
Replicate	3																								
Temperature (T)	1	*	*	*	*	*	*	*	*	*	*	NS	NS	NS	*	*	*	*	*	*	*	*	*	*	*
Error(T)	3																								
Bacteria(B)	1	*	*	*	*	*	*	*	*	*	*	NS	NS	*	*	*	*	*	*	NS	NS	*	*	*	*
BxT	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(B)	6																								
Cultivar	1	*	NS	NS	*	*	*	*	*	*	*	NS	NS	NS	*	NS	*	NS	*	*	*	NS	NS	*	*
CxT	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxB	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxTxB	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(C)	12																								
Total	31																								

*and NS indicate significance at P=0.05, and not significant at P=0.05, respectively

Table 8. Analysis of variance (AOV) table for jasmonic acid content within plant tissues, cultivars, temperature and bacteria treatments from data reported in Chapter Two.

Duration of treatments (d)

		leaf								stolon								root							
		Experiment 1				Experiment 2				Experiment 1				Experiment 2				Experiment 1				Experiment 2			
Source of variation	df	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20
Replicate	3																								
Temperature (T)	1	NS	NS	*	*	*	*	*	*	NS	NS	NS	NS	*	*	*	*	NS	NS	NS	NS	*	*	*	*
Error(T)	3																								
Bacteria(B)	1	NS	NS	*	*	NS	*	NS	NS	NS	NS	*	*	*	*	*	*	*	*	NS	*	NS	*	*	*
BxT	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(B)	6																								
Cultivar	1	NS	NS	*	*	*	*	NS	NS	NS	*	*	NS	NS	*	*	*	NS	*	NS	*	NS	NS	NS	NS
CxT	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxB	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxTxB	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(C)	12																								
Total	31																								

*and NS indicate significance and not significant at P=0.05, respectively

Table 9. Analysis of variance (AOV) table for gibberellic acid 1 content within plant tissues, cultivars, temperature and bacteria treatments from data reported in Chapter Two.

Duration of treatments (d)

		leaf								stolon								root							
		Experiment 1				Experiment 2				Experiment 1				Experiment 2				Experiment 1				Experiment 2			
Source of variation	df	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20
Replicate	3																								
Temperature (T)	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(T)	3																								
Bacteria(B)	1	*	*	*	*	*	*	*	*	NS	*	*	NS	*	*	NS	*	NS	*	*	*	NS	NS	*	*
BxT	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(B)	6																								
Cultivar	1	*	*	NS	NS	*	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	*	*	NS	NS	NS	NS	NS	*
CxT	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxB	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxTxB	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(C)	12																								
Total	31																								

*and NS indicate significance and not significant at P=0.05, respectively

Table 10. Analysis of variance (AOV) table for gibberellic acid 3 content within plant tissues, cultivars, temperature and bacteria treatments from data reported in Chapter Two.

Duration of treatments (d)

		leaf								stolon								root							
		Experiment 1				Experiment 2				Experiment 1				Experiment 2				Experiment 1				Experiment 2			
Source of variation	df	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20
Replicate	3																								
Temperature (T)	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(T)	3																								
Bacteria(B)	1	*	*	*	*	*	*	*	*	NS	*	*	NS	*	*	NS	*	NS	*	*	*	NS	NS	*	*
BxT	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(B)	6																								
Cultivar	1	*	*	NS	NS	*	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	*	*	NS	NS	NS	NS	NS	*
CxT	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxB	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxTxB	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(C)	12																								
Total	31																								

*and NS indicate significance and not significant at P=0.05, respectively

Table 11. Analysis of variance (AOV) table for gibberellic acid 4 content within plant tissues, cultivars, temperature and bacteria treatments from data reported in Chapter Two.

Duration of treatments (d)

		leaf								stolon								root							
		Experiment 1				Experiment 2				Experiment 1				Experiment 2				Experiment 1				Experiment 2			
Source of variation	df	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20
Replicate	3																								
Temperature (T)	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(T)	3																								
Bacteria(B)	1	*	*	*	*	*	*	*	*	NS	*	*	NS	*	*	NS	*	NS	*	*	*	NS	NS	*	*
BxT	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(B)	6																								
Cultivar	1	*	*	NS	NS	*	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	*	*	NS	NS	NS	NS	NS	*
CxT	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxB	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxTxB	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(C)	12																								
Total	31																								

*and NS indicate significance and not significant at P=0.05, respectively

Table 12. Analysis of variance (AOV) table for gibberellic acid 20 content within plant tissues, cultivars, temperature and bacteria treatments from data reported in Chapter Two.

Duration of treatments (d)

		leaf								stolon								root							
		Experiment 1				Experiment 2				Experiment 1				Experiment 2				Experiment 1				Experiment 2			
Source of variation	df	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20
Replicate	3																								
Temperature (T)	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(T)	3																								
Bacteria(B)	1	*	*	*	*	*	*	*	*	NS	*	*	NS	*	*	NS	*	NS	*	*	*	NS	NS	*	*
BxT	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(B)	6																								
Cultivar	1	*	*	NS	NS	*	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	*	*	NS	NS	NS	NS	NS	*
CxT	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxB	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxTxB	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(C)	12																								
Total	31																								

*and NS indicate significance and not significant at P=0.05, respectively

Table 13. Analysis of variance (AOV) table for abscisic acid content within plant tissues, cultivars, temperature and bacteria treatments from data reported in Chapter Two.

Duration of treatments (d)

		leaf								stolon								root							
		Experiment 1				Experiment 2				Experiment 1				Experiment 2				Experiment 1				Experiment 2			
Source of variation	df	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20
Replicate	3																								
Temperature (T)	1	*	*	*	*	*	*	*	*	*	*	NS	NS	NS	*	*	*	*	*	*	*	*	*	*	*
Error(T)	3																								
Bacteria(B)	1	*	*	*	*	*	*	*	*	*	*	NS	NS	*	*	*	*	*	*	NS	NS	*	*	*	*
BxT	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(B)	6																								
Cultivar	1	*	NS	NS	*	*	*	*	*	*	*	NS	NS	NS	*	NS	*	NS	*	*	*	NS	NS	*	*
CxT	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxB	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxTxB	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(C)	12																								
Total	31																								

*and NS indicate significance at P=0.05, and not significant at P=0.05, respectively

Table 14. Analysis of variance (AOV) table for zeatin riboside content within plant tissues, cultivars, temperature and bacteria treatments from data reported in Chapter Two.

Duration of treatments (d)

		leaf								stolon								root							
		Experiment 1				Experiment 2				Experiment 1				Experiment 2				Experiment 1				Experiment 2			
Source of variation	df	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20
Replicate	3																								
Temperature (T)	1	NS	NS	*	*	*	*	*	*	NS	NS	NS	NS	*	*	*	*	NS	NS	NS	NS	*	*	*	*
Error(T)	3																								
Bacteria(B)	1	NS	NS	*	*	NS	*	NS	NS	NS	NS	*	*	*	*	*	*	*	*	NS	*	NS	*	*	*
BxT	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(B)	6																								
Cultivar	1	NS	NS	*	*	*	*	NS	NS	NS	*	*	NS	NS	*	*	*	NS	*	NS	*	NS	NS	NS	NS
CxT	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxB	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxTxB	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(C)	12																								
Total	31																								

*and NS indicate significance and not significant at P=0.05, respectively

Table 15. Analysis of variance (AOV) table for leaf electrolyte leakage within cultivars, temperature and bacteria treatments from data reported in Chapter Three.

		Duration of treatments (d)									
		Experiment 1					Experiment 2				
Source of variation	df	0	5	10	15	20	0	5	10	15	20
Replicate	3										
Temperature (T)	1	NS	*	*	*	*	NS	*	*	*	*
Error(T)	3										
Chemical treatment (Ch)	5	NS	NS	*	*	*	NS	*	*	*	*
Cultivar (C)	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
TxCh	5										
CxT	1	NS	NS	*	*	*	NS	NS	NS	*	*
CxCh	5	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxTxCh	5	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(C)	66										
Total	95										

*and NS indicate significance at P=0.05, and not significant at P=0.05, respectively

Table 16. Analysis of variance (AOV) table for turf quality within cultivars, temperature and bacteria treatments from data reported in Chapter Three.

		Duration of treatments (d)									
		Experiment 1					Experiment 2				
Source of variation	df	0	5	10	15	20	0	5	10	15	20
Replicate	3										
Temperature (T)	1	NS	*	*	*	*	NS	*	*	*	*
Error(T)	3										
Chemical treatment (Ch)	5	NS	NS	*	*	*	NS	*	*	*	*
Cultivar (C)	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
TxCh	5										
CxT	1	NS	NS	NS	NS	NS	NS	NS	NS	*	*
CxCh	5	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxTxCh	5	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(C)	66										
Total	95										

*and NS indicate significance at P=0.05, and not significant at P=0.05, respectively

Table 17. Analysis of variance (AOV) table for chlorophyll content within cultivars, temperature and bacteria treatments from data reported in Chapter Three.

		Duration of treatments (d)									
		Experiment 1					Experiment 2				
Source of variation	df	0	5	10	15	20	0	5	10	15	20
Replicate	3										
Temperature (T)	1	NS	*	*	*	*	NS	*	*	*	*
Error(T)	3										
Chemical treatment (Ch)	5	NS	*	*	*	*	NS	*	*	*	*
Cultivar (C)	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
TxCh	5										
CxT	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxCh	5	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxTxCh	5	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(C)	66										
Total	95										

*and NS indicate significance at P=0.05, and not significant at P=0.05, respectively

Table 18. Analysis of variance (AOV) table for photochemical efficiency within cultivars, temperature and bacteria treatments from data reported in Chapter Three.

		Duration of treatments (d)									
		Experiment 1					Experiment 2				
Source of variation	df	0	5	10	15	20	0	5	10	15	20
Replicate	3										
Temperature (T)	1	NS	NS	NS	*	*	NS	NS	NS	*	*
Error(T)	3										
Chemical treatment (Ch)	5	NS	NS	*	*	*	NS	*	*	*	*
Cultivar (C)	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
TxCh	5										
CxT	1	NS	NS	NS	*	*	NS	NS	NS	*	*
CxCh	5	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxTxCh	5	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(C)	66										
Total	95										

*and NS indicate significance at P=0.05, and not significant at P=0.05, respectively

Table 19. Analysis of variance (AOV) table for root electrolyte leakage within cultivars, temperature and bacteria treatments from data reported in Chapter Three.

		Duration of treatments (d)									
		Experiment 1					Experiment 2				
Source of variation	df	0	5	10	15	20	0	5	10	15	20
Replicate	3										
Temperature (T)	1	NS	*	*	*	*	NS	*	*	*	*
Error(T)	3										
Chemical treatment (Ch)	5	NS	NS	*	*	*	NS	*	*	*	*
Cultivar (C)	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
TxCh	5										
CxT	1	NS	NS	*	*	*	NS	NS	NS	*	*
CxCh	5	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxTxCh	5	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(C)	66										
Total	95										

*and NS indicate significance at P=0.05, and not significant at P=0.05, respectively

Table 20. Analysis of variance (AOV) table for root viability within cultivars, temperature and bacteria treatments from data reported in Chapter Three.

		Duration of treatments (d)									
		Experiment 1					Experiment 2				
Source of variation	df	0	5	10	15	20	0	5	10	15	20
Replicate	3										
Temperature (T)	1	NS	NS	NS	NS	*	NS	NS	NS	NS	*
Error(T)	3										
Chemical treatment (Ch)	5	NS	NS	*	*	*	NS	*	NS	*	*
Cultivar (C)	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
TxCh	5										
CxT	1	NS	NS	NS	NS	*	NS	NS	NS	*	*
CxCh	5	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CxTxCh	5	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error(C)	66										

Total	95	

*and NS indicate significance at P=0.05, and not significant at P=0.05, respectively

LITERATURE CITED

LITERATURE CITED

Arnon DI (1949) Copper enzyme polyphenoloxides in isolated chloroplast in Beta vulgaris. Plant Physiology 24:1-15.

Bari R, Jones JD (2009) Role of plant hormones in plant defence responses. Plant Mol Biol 69(4):473-88.

Beßer K, Jarosch B, Langen G, Kogel KH (2000) Expression analysis of genes induced in barley after chemical activation reveals distinct disease resistance pathways. Mol. Plant Pathol 1:277-286.

Blum A, Ebercon A (1981) Cell membrane stability as a measure of drought and heat tolerance in wheat. Crop Sci 21:43–47.

Cohen Y, Gisi U, Niderman T (1993) Local and systemic protection against Phytophthora infestans induced in potato and tomato plants by jasmonic acid and jasmonic methyl ester. Phytopathology 83:1054–62

Dernoeden PH (2013) Creeping bentgrass management. CRC Press, Boca Raton, FL.
Diaz J, ten Have A, van Kan JA (2002) The role of ethylene and wound signaling in resistance of tomato to Botrytis cinerea. Plant Physiol 129:1341–1351.

Giordano PR, Vargas JM, Detweiler AR, Dykema NM, Yan L (2010) First report of a bacterial disease on creeping bentgrass (*Agrostis stolonifera*) caused by *Acidovorax* spp. in the United States. Plant Dis 94(7):922.

Glazebrook J (2005) Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu. Rev. Phytopathol 43:205-227.

Hsiang T, Goodwin PH, Gao W (2014) Variation in responsiveness of *Agrostis* cultivars to defense activators.Eur. J. Turfgrass Science 45(2/15):47-48.

Huang B, Fry JD (1998) Root anatomical, physiological and morphological responses to drought stress for tall fescue cultivars. Crop Science 38:1017-1022.

Iwai T, Seo S, Mitsuhara I, Ohashi Y (2007) Probenazole-induced accumulation of salicylic acid confers resistance to Magnaporthe grisea in adult rice plants. Plant Cell Physiol 48:915-924.

Kadota I, Ohuchi A (1983) Symptoms of bacterial brown stripe of rice seedlings in nursery boxes. Annals of the Phytopathological Society of Japan 49:561-564.

Khaosaad T, Garcia-Garrido JM, Steinkellner S, Vierheilig H (2007) Take-all disease is systemically reduced in roots of mycorrhizal barley plants. Soil Biology & Biochemistry 39: 727–734.

Koornneef A, Pieterse CM (2008) Cross talk in defense signaling. Plant Physiol 146:839-44.

Krause GH, Somersalo S, Osmond CB, Briantais JM, Schreiber U (1989) Fluorescence as a tool in photosynthesis research: application in studies of photoinhibition, cold acclimation and freezing stress and discussion. Philos Trans R Soc Lond B Biol Sci 323: 281–293.

Krishnan SK, Merewitz E (2015) Drought stress and trinexapac-ethyl modify phytohormone content within kentucky bluegrass leaves. J. Plant Growth Regul 34:1-12. DOI: 10.1007/s00344-014-9434-0.

Krishnan S, Ma Y, Merewitz E (2016) Leaf trimming and high temperature regulation of phytohormones and polyamines in creeping bentgrass leaves. Journal of the American Society for Horticultural Science. 141, 66-75.

Larkindale J, Huang B (2004) Thermotolerance and antioxidant systems in *Agrostis stolonifera*: involvement of salicylic acid, abscisic acid, calcium, hydrogen peroxide, and ethylene. J. Plant Physiol., 161 (2004), pp. 405–413.

Lee YH, Kim SH, Yun BW, Hong JK (2014) Altered cultivar resistance of kimchi cabbage seedlings mediated by salicylic acid, jasmonic acid and ethylene. Plant Pathol J 30:323–329. doi: 10.5423/PPJ.NT.06.2014.0053.

Liu S, Vargas J, Merewitz E (2017) Phytohormones associated with bacterial etiolation disease in creeping bentgrass. Environmental and Experimental Botany 133:35-49.

Lu ZX, Gaudet D, Puchalski B, Despins T, Frick M, Laroche A (2006) Inducers of resistance reduce common bunt infection in wheat seedlings while differentially regulating defence-gene expression. Physiol. Mol. Plant Pathol 67:138-148.

Malamy J, Hennig J, Klessig DF (1992) Temperature-dependent induction of salicylic acid and its conjugates during the resistance response to tobacco mosaic virus infection. Plant Cell 4:359–66.

Mauch F, Mauch-Mani B, Gaille C, Kull B, Haas D, Reimmann C (2000) Manipulation of salicylate content in Arabidopsis thaliana by the expression of an engineered bacterial salicylate synthase. Plant J 25:67–77.

McMichael BL, Burke JJ (1994) Metabolic activity of cotton roots in response to temperature. Environmental and Experimental Botany 34:201-206.

Mei CS, Qi M, Sheng GY, Yang YN (2006) Inducible overexpression of a rice allene oxide synthase gene increases the endogenous jasmonic acid level, PR gene expression, and host resistance to fungal infection. Mol. Plant-Microbe Interact 19:1127-1137.

Merewitz EB, Gianfagna T, Huang B (2011) Photosynthesis, water use, and root viability under water stress as affected by expression of SAG12-ipt controlling cytokinin synthesis in *Agrostis stolonifera.* J Exp Bot 62(1): 383–395.

Miles TD, Day B, Schilder AC (2011) Identification of differentially expressed genes in a resistant versus a susceptible blueberry cultivar after infection by *Colletotrichum acutatum*.Mol Plant Pathol 12(5):463-77.

Mitchell AF, Walters DR (1995) Systemic protection in barley against powdery mildew infection using methyl jasmonate. Asp Appl Biol 42:323–326.

Mur LAJ, Kenton P, Atzorn R, Miersch O, Wasternack C (2006). The outcomes of concentration-specific interactions between salicylate and jasmonate signaling include synergy, antagonism, and oxidative stress leading to cell death. Plant Physiol. 140:249–262.
Pastori GM, Foyer CH (2002) Common components, networks and pathways of cross-tolerance to stress. The central role of 'redox' and abscisic acid-mediated controls. Plant Physiol 129:460–468.

Pozo MJ, Van Loon LC, Pieterse CMJ (2005) Jasmonates—signals in plant-microbe interactions. J Plant Growth Regul 23:211–222.

Qiu Z, Guo J, Zhu A, Zhang L, Zhang M (2014) Exogenous jasmonic acid can enhance tolerance of wheat seedlings to salt stress. Ecotoxicol Environ Saf. 104:202-8.

Rahman A, Uddin W, Wenner NG (2015) Induced systemic resistance responses in perennial ryegrass against Magnaporthe oryzae elicited by semi-purified surfactin lipopeptides and live cells of Bacillus amyloliquefaciens. Mol. Plant Pathol 10.1111/mpp.12209.

Roberts JA, Kerns JP, Ritchie DF (2015) Bacterial etiolation of creeping bentgrass as influenced by biostimulants and trinexapac-ethyl. Crop Protection 72:119-126.

Roberts JA, Ritchie DF, Kerns JP (2016) Plant growth regulator effects on bacterial etiolation of creeping bentgrass putting green turf caused by *Acidovorax avenae*. Plant Dis 100: 577-582.

Saddler G, O'GRADY E, Spence J (1995) Characterization of a pseudomonad-like bacterium pathogenic for Anthurium species 1. EPPO Bulletin 25:211-217.

Sundin GW, Castiblanco LF, Yuan X, Zeng Q, Yang C (2016) Bacterial disease management: challenges, experience, innovation and future prospects. Challenges in Bacterial Molecular Plant Pathology. Mol Plant Pathol doi: 10.1111/mpp.12436.

Tamaoki D, Seo S, Yamada S, Kano A, Miyamoto A, Shishido H (2013). Jasmonic acid and salicylic acid activate a common defense system in rice. Plant Signal. Behav 8: e24260 doi: 10.4161/psb.24260.

Tsuda K, Sato M, Stoddard T, Glazebrook J, Katagiri F (2009) Network Properties of Robust Immunity in Plants. PLoS Genet 5: e1000772.

Tung J (2011) The effectiveness of induced plant disease resistance: genotypic variation and quantification by chlorophyll fluorescence. M.Sc. Thesis, University of Guelph. 192 pp.

Turgeon AJ (2008) Turfgrass management. Pearson Prentice Hall, Upper Saddle River, New Jersey.

Xie GL, Sun XL, Mew T (1998) Characterization of *Acidovorax avenae* subsp. *avenae* from rice seeds. Chinese Journal of Rice Science 12:165-171.

Yalpani N, Silverman P, Wilson TMA, Kleier DA, Raskin I (1991) Salicylic acid as a systemic signal and an inducer of pathogenesis-related proteins in virus-infected tobacco. Plant Cell 3: 809-818.