Crop Protection 109 (2018) 9–16

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Jasmonic and salicylic acid effects on bacterial etiolation and decline disease of creeping bentgrass☆

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ARTICLE INFO

Keywords:
Creeping bentgrass
Acidovorax avenae
Jasmonic acid
Salicylic acid
Turfgrass
Turfgrass pathogen

ABSTRACT

Creeping bentgrass (*Agrostis stolonifera*), an important turfgrass on golf course putting greens can become plagued by bacterial etiolation disease caused by *Acidovorax avenae* subsp. *avenae* (*Aaa*). There are no known pesticides that have been shown to be effective in managing this disease. This series of hydroponic growth chamber studies investigated whether pre-treating plants with SA or JA could be effective in reducing disease symptoms of *Aaa* in creeping bentgrass 'Penn-A4' (*Aaa* sensitive) and 'Tyee' (more tolerant). 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) with or without *Aaa* inoculation. Physiological measurements included turf quality (TQ), leaf and root electrolyte leakage, chlorophyll content (CHL), photochemical efficiency and root viability. No significant differences were detected in either cultivar due to chemical or heat treatment alone, which indicates chemical treatments had specific effects on plant responses to bacteria and were not confounded by plant heat stress symptoms. SA application reduced *Aaa* disease symptoms of both cultivars under both optimal and high temperature treatments. At both 23 °C and 35 °C, the severity of disease in plants treated with SA was less than in control plants based on all measurements in our experiment. Creeping bentgrass 'Tyee' treated with JA showed significantly higher TQ than control plants at 35 °C. 'Penn-A4' treated with JA only showed significantly higher CHL than control at 23 °C. SA and JA treatments could be viable methods to induce creeping bentgrass tolerance of bacterial pathogens; however, field testing of these methods is needed.

1. Introduction

Creeping bentgrass (*Agrostis stolonifera*) is a cool-season turfgrass grown on golf course greens, tees, fairways and it is also used as a forage grass (Balasko et al., 1995). It 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, particularly on golf course putting greens (Giordano et al., 2010). Bacterial etiolation caused by *Aaa* is most problematic during periods of above optimal temperatures for creeping bentgrass growth (30–40 °C, Giordano, 2014). Visual symptoms of this disease include chlorosis, necrosis, and etiolation of plant tissues, which reduces turf appearance and functionality. Physiological plant symptoms include an increase in leaf and root electrolyte leakage, a reduction in chlorophyll content, and reduced root viability (Giordano et al., 2012; Liu et al., 2017). Moderate cultivar variation in susceptibility to *Aaa* and physiological damage caused by *Aaa* has been found (Liu et al., 2017), but there are no known cultivars resistant to the bacteria. Additionally, there are no known management strategies to prevent or reduce disease symptoms. Therefore, identification of compounds that may reduce disease symptoms are needed, particularly those that may boost plant natural defense mechanisms.

Two plant hormones that may naturally boost plant disease defenses are jasmonic acid (JA) and salicylic acid (SA), which are major signaling agents in the induced systemic resistance (ISR) and systemic acquired resistance (SAR) plant defense pathways (Bari and Jones, 2009; Yang et al., 2012). SAR and ISR are two pathways that can increase plant tolerance to subsequent disease infections (Choudhary et al., 2007). Recently, it has become clear that defense response systems associated with JA and SA in dicots and monocots may be differential (Tamaoki et al., 2013). In dicots, the effects of SA and JA in plants could be more complex than previously thought (Mur et al., 2006; Tsuda et al., 2009). Thus, a better understanding of JA and SA effects on monocot species such as grasses and diseases associated with grass species is needed (Liu, 2016). Additionally, plant bacterial species are notoriously hard to control. Integrated and sustainable management

☆ The text of this manuscript is a partial reprint of the material as it appears in a master's thesis Liu (2016).

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https://doi.org/10.1016/j.cropro.2018.02.023
Received 6 February 2018; Accepted 26 February 2018

of plant diseases requires the exploitation of host defense responses (Sundin et al., 2016). Thus, research on the use of natural plant hormones that could pre-condition plants for enhanced tolerance to bacterial species is also needed.

Creeping bentgrass phytohormone profiles are significantly affected by *Aaa* infection and are differential in 'Tyee' compared to 'Penn-A4.' With high temperature and bacterial treatment, creeping bentgrass 'Tyee' had greater SA accumulation in stolons and roots than 'Penn-A4'. At high temperature, infected 'Tyee' showed higher content of JA than infected 'Penn-A4' (Liu et al., 2017). Comparable results have been found for fungal diseases. Young potato (*Solanum tuberosum*) leaves more resistant to potato blight (*Phytophthora infestans*) was associated with higher SA levels than disease sensitive leaves (Coquoz et al., 1995). When infected with the Fusarium head blight fungus (*Fusarium graminearum*), the resistant wheat cultivar (*Triticum aestivum* L.) showed higher JA content than the sensitive cultivar (Ding et al., 2011). In response to abiotic stress, both JA and SA can increase heat tolerance in Arabidopsis (*Arabidopsis thaliana,* Clarke et al., 2009). In grass species, heat stress induced the accumulation of JA and SA (Krishnan and Merewitz, 2015). Since higher levels of JA and SA was merely correlated to greater tolerance of *Aaa* in Liu et al. (2017), it is warranted to apply JA and SA exogenously to determine whether theses plant hormones may explicitly play a role in *Aaa* tolerance. More research is needed on JA and SA effects on bacterial species, particularly because there are no good chemical control options for most bacterial diseases.

Plant growth regulators (PGRs), which inhibit gibberellic acid (GA) biosynthesis, have been found to increase creeping bentgrass susceptibility to *Aaa* (Roberts et al., 2015), which may be associated with the mode of action of the GA inhibitor (Roberts et al., 2016). GA inhibiting PGRs have also been found to reduce the accumulation of JA under abiotic stress conditions (Krishnan and Merewitz, 2015). Thus, repression of JA endogenously could play a role in the increased sensitivity of PGR-treated creeping bentgrass to *Aaa* and plants may benefit from an exogenous supplement. We hypothesized that supplementation of JA to PGR treated plants could reduce any effects of a possible repression of JA by PGRs.

Therefore, the objective of the study was to determine whether pretreatments of JA and SA to PGR-treated creeping bentgrass could reduce disease severity in *Aaa* sensitive and tolerant creeping bentgrass cultivars. If exogenous treatment with SA or JA is effective in reducing disease severity, this would serve as evidence that these hormones are highly important in creeping bentgrass tolerance of *Aaa,* and possibly other pathogens, and could be incorporated into integrated turf management practices.

2. Materials and methods

2.1. Plant material and growth conditions

Creeping bentgrass 'Tyee' and 'Penn-A4' were seeded at a rate of 0.45 kg/93 m^2 in sand in 11.4 cm pots. They were then allowed to establish in a greenhouse for 8 weeks. During establishment, they were maintained as needed with daily watering, weekly fertilization, and regularly leaf trimming (2 cm in height). After they were established, plants were separated and propagated into clonal tillers. They were then transferred to a hydroponic system in a growth chamber using the methods described in Merewitz et al. (2011). Growth chamber conditions were maintained as described in Liu (2016) with a 12 h photoperiod at 900 μmol m^{-2}·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 and were floated on the nutrient solution in black plastic tanks (71 × 51 × 15 cm). The hydroponic solution in each tank was aerated via 2 tubes connected to pumps (115 V, 60 Hz, Tetra Whisper; Blacksburg, VA). The solution was changed weekly and the solution pH was monitored and adjusted using sodium hydroxide to a pH of 6.0 every 3 days. Once established in hydroponics, plants were maintained at a height of 3 cm by hand trimming. Trinexapac-ethyl (TE, Syngenta Crop Protection, Greensboro, NC) was sprayed on all plants at the rate of 0.79 L ha^{-1}. The hydroponic system, TE treatment of all plants to promote disease, and experimental design has been used previously (Liu et al., 2017). The first spray was applied two weeks before the experimental treatments. The second spray occurred 48 h before the experimental treatments. TE application is required to see a significant degree of disease symptoms, particularly under controlled conditions. All plants were treated with TE to eliminate TE as an experimental factor.

2.2. Experimental treatments

A non-inoculated control hydroponic experiment and a hydroponic experiment with bacterial inoculations were conducted separately to eliminate any possibility of bacterial contamination among hydroponic systems. A total of 96 plants were placed in 6 tanks for each experiment, so each tank contained a total of 16 individual plants. At two days prior to any temperature or disease treatment, the plants were exposed to different concentrations of SA (20 μmol/L and 10 μmol/L) and JA (0.5 Mm and 2 mM in 0.02% ethanol). De-ionized water and 0.02% ethanol were used as control. The application rates of SA and JA were based on previous research (Larkindale and Huang, 2004; Qiu et al., 2014). Chemical treatments were applied three times daily for two days, by spraying 50 mL of the appropriate solution on the foliage of the plant.

Temperature treatments included 1) optimal temperature (23/20 °C day/night) or 2) heat treatment (35/30 °C day/night). Disease treatments included 1) no bacterial inoculation or 2) bacterial inoculations. Three hydroponic tanks were placed in an optimal temperature growth chamber and three tanks were moved to a high temperature chamber for the duration of the experiment (12 days). Both growth chambers have equal conditions as described above. *Aaa* MSU-13 was used in this study and is a one of the more virulent strains of *Aaa* we have isolated (Liu et al., 2017). The inoculation process was similar to the method described in Liu et al. (2017) with modifications. Briefly, to inoculate plants with bacteria, the entire canopy of each plant was trimmed to a height of 2 cm with scissors soaked in *Aaa* MSU-13 suspension. Subsequent to trimming, plant canopies were evenly sprayed with 100 mL MSU-13 *Aaa* suspension until run-off occurred from leaf surfaces. During the 0–12 day optimal or high temperature treatment period, about 0.5 cm of root tips of all plants were pruned with sterilized scissors every day to ensure that bacteria could enter roots through wounds for those that were treated with bacteria. Control non-inoculated plants were also trimmed in the same manner in order to not have any confounding effects of any change in plant physiology due to root trimming. All experiments were repeated as discussed in the experimental design section. Experiments were first conducted on July 20th, 2016 and then repeated on October 7th, 2016.

Physiological Evaluation of Plants

Physiological measurements were described in Liu (2016). Briefly, every 3 days during the study, turf quality ratings (TQ), chlorophyll content (CHL), Electrolyte leakage (EL), and photochemical efficiency (F_v/F_m) were measured on leaves; Root leakage (REL) and root viability were mearsured on roots. EL was used to evaluate cellular membrane stability in leaves and roots. Approximately 10 leaves were taken from individual plant, rinsed in de-ionized water, submerged in 15 mL tubes with 10 mL of de-ionized water, and shaken for 24 h. The initial conductivity (Ci) was measured on a conductivity meter (YSI Model 3200; Yellow Springs, OH, USA). Leaf tissues were then autoclaved for 20 min and shaked for another 24 h to measure the maximum conductivity (Cmax). Perce C_i/C_{max} x 100 was used to calculate the percentage of EL (Blum and Ebercon, 1981). REL was measured as described above. The only difference was taking 200 mg of roots instead of leaves (Huang and Fry, 1998).