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Evaluation of the control effect of SAR inducers against citrus Huanglongbing applied by foliar spray, soil drench or trunk injection

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Abstract

Huanglongbing (HLB) or greening disease, associated with the bacterial pathogen *Candidatus* Liberibacter asiaticus (Las), is currently the most devastating citrus disease worldwide and no cure is available. Inducers of systemic acquired resistance (SAR) are effective and sustainable to combat various plant diseases. In this study, the SAR inducers acibenzolar-S-methyl (ASM), imidacloprid (IMI), 2,6-dichloroisonicotinic acid (INA), and salicylic acid (SA), applied individually by foliar spray, soil drench or trunk injection at various rates and frequencies, were evaluated for control of HLB in a 3-year field trial with mature Hamlin sweet orange trees in central Florida, USA in the 2016, 2017, and 2018 crop seasons. Six foliar sprays, six soil applications, and three trunk injections of ASM, IMI, INA, or SA per year were conducted with the untreated as a negative control. HLB disease severity, Las titers, pre-harvest fruit drop, yield and fruit quality were investigated for the treatments. By the end of the 2018 season, all trunk injection treatments at 0.25 g/tree and foliar sprays of INA or SA (but not ASM or IMI) at 0.5 g/tree significantly reduced disease severity, Las population, and fruit drop, and increased fruit yield; whereas all foliar spray treatments at 0.25 g/tree, trunk injection treatments at 0.125 g/tree, and soil drench treatments at 0.25 or 0.5 g/tree did not provide effective control of HLB. Additionally, all trunk injection treatments at 0.25 g/tree had shown a significant decrease in fruit drop and increase of fruit yield starting from 2016 after 1 year of applications, whereas foliar sprays of INA or SA at 0.5 g/tree exhibited similar effects at 2018 after 3 years of applications. None of the SAR inducer treatments had significant effect on fruit quality. Economic analysis suggested that the trunk injection treatments at 0.25 g/tree might produce financial benefits. Overall, this study presents useful information for management of citrus HLB with SAR inducers.

Keywords: Citrus, Disease control, Huanglongbing (HLB), Induced plant resistance, Liberibacter, Systemic acquired resistance (SAR), Salicylic acid

Background

Citrus huanglongbing (HLB), also called citrus greening disease, is currently the most destructive citrus disease affecting the global citrus industry and causes significant economic losses worldwide (Blaustein et al. 2018; Wang 2019; Andrade et al. 2020). HLB is associated with the three phloem-colonizing and uncultured bacterial species of '*Candidatus* Liberibacter': '*Ca*. L. asiaticus' (Las) in Asia, Africa and the Americas, '*Ca*. L. africanus' (Laf) in Africa and limited areas of Asia, and '*Ca*. L. americanus' (Lam) in Brazil (Bové 2006; Gottwald et al. 2007; Wang 2020). Among them, Las is the most prevalent species worldwide (Bové 2006; Gottwald 2010; Wang et al. 2017b; Andrade et al. 2020). Las is naturally

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transmitted to citrus by the Asian citrus psyllid (ACP, *Diaphorina citri* Kuwayama) during feeding on citrus plants (Bové 2006). HLB disease symptoms include leaf mottling, yellow shoots, dead and dying twigs, deformed and discolored fruits, premature fruit drop, root decline, and eventually tree mortality (Bové 2006; Gottwald 2010; Johnson et al. 2014). Most commercial citrus varieties are susceptible to Las infection, with few, such as Sugar Belle, showing tolerance to HLB (Clark et al. 2018; Wang 2019; Andrade et al. 2020). Since it was first identified in East Asia over 100 years ago, HLB has spread to most citrus producing areas in the world, including Asia, Africa, and the Americas, except Australia and the Mediterranean region (Wang 2019). In the US, HLB is present in most citrus producing states including the top three producers: Florida, California, and Texas. HLB is endemic in Florida and the estimated losses to the Florida citrus industry caused by HLB exceed $8 billion (Singerman and Futch 2018). HLB was first detected in Texas in January 2012 and HLB-positive trees were approximately 38.7% in 2017 (Sétamou et al. 2020). HLB is also threatening the California citrus industry (Graham et al. 2020) with HLB first reported in a residential property in Hacienda Heights, Los Angeles Co. in March 2012 (Kumagai et al. 2013). Approximately 2000 trees in residential areas have tested positive for Las. Importantly, the first Las-positive ACP was found in a commercial citrus grove in California in August 2020 (California Citrus Pest and Disease Prevention Program 2020).

Currently, there are no practical cures for HLB (Andrade et al. 2020). Different HLB management strategies are being implemented in different citrus producing areas depending on the overall HLB incidence in the corresponding region. For example in China, a comprehensive HLB control strategy is being used including control of the insect vector ACP, and eradication of symptomatic trees, as well as the use of pathogen-free seedlings for new plantings (Wang et al. 2017a; Blaustein et al. 2018; Andrade et al. 2020). However, in many citrus producing areas where HLB is endemic or has high incidence, such as Florida, inoculum eradication is largely abandoned (Graham et al. 2020). Intriguingly, despite that Las has not been cultured in artificial media, promising advances have been made that might help develop long-term solutions to control HLB, including understanding the virulence mechanisms of Las (Duan et al. 2009; Jain et al. 2018; Pitino et al. 2018; Andrade et al. 2019; Andrade and Wang 2019; Chen et al. 2020; Clark et al. 2020; Pandey et al. 2020; Pang et al. 2020; Thapa et al. 2020a; Thapa et al. 2020b; Vasconcelos et al. 2020), improving Las detection (Kim and Wang 2009; Pagliaccia et al. 2017; Pandey and Wang 2019; Gottwald et al. 2020), and developing tolerant or resistant citrus varieties using traditional and novel approaches (Jia and Wang 2014; Rawat et al. 2015; Deng et al. 2019; Jia et al. 2019; Jia and Wang 2020). In addition, in HLB-endemic regions, such as Florida, the HLB management focus has shifted to how to improve and sustain the productivity at the presence of HLB. Therefore, multiple approaches were tested to mitigate HLB damage and losses caused by HLB, including maintaining optimal tree growth conditions (Gottwald et al. 2012; Stansly et al. 2014), targeting Las (Akula et al. 2011; Hu and Wang 2016; Barnett et al. 2019; Hussain et al. 2019; Li et al. 2019b), eliciting plant defenses (Zhang et al. 2014; Canales et al. 2016; Li et al. 2016; Li et al. 2017; Hu et al. 2018; Li et al. 2019a), thermotherapy (Thapa et al. 2020a; Thapa et al. 2020b), and biological control (Riera et al. 2017; Wang et al. 2017b; Blaustein et al. 2018).

Induced resistance, either locally or systemically, has been shown to be effective for control of numerous plant diseases (Durrant and Dong 2004; Graham and Myers 2011; Justyna and Ewa 2013; Walters et al. 2013; Pieterse et al. 2014; Riera et al. 2018; Frąckowiak et al. 2019). Specifically, systemic acquired resistance (SAR) has attracted much attention because of its long-lasting control of a broad spectrum of plant diseases (Durrant and Dong 2004). SAR requires specific signaling pathways that involve the signal molecule salicylic acid (SA) and the accumulation of pathogenesis related (PR) proteins (Durrant and Dong 2004). SAR may be activated by plant pathogens or chemical inducers (Walters et al. 2013; Aranega-Bou et al. 2014). Many plant defense inducers, including acibenzolar-S-methyl (ASM), β-aminobutyric acid (BABA), benzothiadiazole (BTH, synonym of ASM), and 2, 6-dichloroisonicotinic acid (INA), have been evaluated extensively for their efficacy on disease control under natural conditions (Vallad and Goodman 2004; Walters et al. 2013; Li et al. 2016, 2017; Frąckowiak et al. 2019). Numerous earlier studies have shown that SAR inducers provided inconsistent effectiveness in protection against various plant pathogens under field conditions, varying from lack of control to 99% from field to field, depending on pathogens, host genotypes, nutritional conditions, environmental conditions as well as application time, frequency, and rate, and mode of delivery (Vallad and Goodman 2004; Walters et al. 2013; Li et al. 2016; Frąckowiak et al. 2019). Such relatively large variability in their efficacy underlines the importance of optimizing application methods of plant defense inducers under local environmental conditions.

Several earlier studies have noted that application methods greatly affected plant defense inducers' effectiveness in disease control, with trunk injection and root/soil application superior to foliar spray (Dekkers et al. 2004; Graham and Leite 2004; Francis et al. 2009; Graham and Myers 2011; Aćimović and Meredith 2017). For example, foliar application of ASM was effective against citrus canker under greenhouse conditions but not in field trials

(Graham and Leite 2004), while soil application of ASM provided effective protection from canker on flushes of young grapefruit trees in the field, despite the efficacy depended on rate, frequency, and timing of application (Graham and Myers 2011). Delivery of the SAR-activator candidate Regalia/MBI-10612 (5 and 12% extract of plant *Reynoutria sachalinensis*) into apple plants by trunk injection increased the efficacy in fire blight control in comparison to foliar spray (Aćimović and Meredith 2017). Altogether, these studies indicate that optimizing application methods of plant defense inducers may achieve a better efficacy in disease control.

Previous research has demonstrated that treatment of citrus plants with SAR inducers resulted in enhanced resistance to both bacterial and fungal diseases. For example, soil application of ASM, INA, and imidacloprid (IMI) provided effective control of citrus canker in greenhouse assays (Francis et al. 2009). Soil-applied ASM, IMI, and thiamethoxam provided effective protection from canker on foliar flushes of young citrus trees under epidemic conditions (Graham and Myers 2011, 2013). Foliar spray of citrus with BTH induced resistance to citrus scab, melanose and *Alternaria* brown spot (Agostini et al. 2003). Furthermore, foliar spray of BABA, BTH, and INA on citrus, individually or in combination, were effective in slowing down HLB disease progression (Li et al. 2016). Trunk injection of the SAR inducers ASM, SA, oxalic acid, and potassium phosphate significantly suppressed Las titer and disease progress of HLB in the field (Hu et al. 2018). These studies suggest that trunk injections of SAR inducers seem to provide better treatment effects than foliar sprays in the control of citrus HLB. However, to date, no investigations have been conducted to evaluate and compare the efficacy of SAR inducers applied via different methods at a comparable rate and frequency, and consequently, to optimize their application for effective control of citrus HLB.

The purpose of this study was to evaluate the effects of the four SAR inducers ASM, IMI, INA, and SA, applied individually by foliar spray, soil drench, or trunk injection at various rates and frequencies, on HLB disease progression and fruit production under field conditions, to optimize application of SAR inducers for the management of citrus HLB.

Results

Effect of SAR inducer treatments on HLB disease progression and Las titers

For all treatments, the HLB symptoms, such as blotchy mottle, loss of foliage, dead and dying twigs, generally became more severe, showing a gradual increase in the HLB severity over time (Fig. 1). However, six treatments showed various effectiveness in reduction of HLB disease severity (Fig. 1).

The HLB disease severity (expressed as standardized area under the disease progress stair, sAUDPS) over the experiment duration was significantly ($P < 0.05$) reduced by foliar sprays of INA (0.5 g/tree) and SA (0.5 g/tree), and trunk injections of ASM, IMI, INA, or SA at 0.25 g/tree compared with the untreated control (UTC) (Fig. 2). The Las bacterial titers under these six treatments were also significantly ($P < 0.05$) lower than that of the UTC at the end of the experiments (Table 1). From the initiation to end of the tests, the mean values of Las titer in the INA (0.5 g/tree) or SA (0.5 g/tree) spray-treated trees increased from 1.21×10^7 and 0.87×10^7 to 3.05×10^7 and 2.12×10^7 cells/g plant tissue, respectively, whereas that of the UTC increased from 0.75×10^7 to 7.63×10^7 cells/g plant tissue. In the ASM, IMI, INA, or SA injection (0.25 g/tree)-treated trees, Las titers were not significantly altered but maintained a relatively stable level at 1.05 to 1.75×10^7 cells/g plant tissue over time (Table 1). These observations indicated that the ASM, IMI, INA, and SA injections at 0.25 g/tree had better efficacy of suppressing Las growth and HLB disease development than the foliar sprays at 0.5 g/tree. HLB disease severity was also reduced by foliar sprays of INA (0.25 g/tree) and SA (0.25 g/tree) compared with the UTC, but the differences were not statistically significant ($P > 0.05$) (Fig. 2). The Las titer in the INA (0.25 g/tree)- or SA (0.25 g/tree)-sprayed tree was not significantly ($P > 0.05$) different from that of the UTC over time (Table 1). Applications of the SAR inducers via soil drench at 0.25 or 0.5 g/tree or via trunk injections at 0.125 g/tree were not effective in reduction of HLB symptom expressions or Las titer compared with the UTC in this field trial (Figs. 1, 2 and Table 1).

Effect of SAR inducer treatments on fruit drop, yield and quality

The pre-harvest fruit drop, fruit yield and quality data were collected for all treatments from the beginning (2015) to the end (2018) of the field trial. In September 2017, the hurricane Irma caused severe fruit drop of all trees in this grove, and consequently, no differences in fruit drop or yield were observed among the treatments (Tables 2 and 3). Therefore, the 2017 data were omitted for further discussion.

Prior to the era of HLB, production for Hamlin sweet oranges in Florida on 13-year-old trees averaged more than 112 kg per tree (Roka et al. 2000). Yields for all treatments during the trial were substantially below the historical level (Table 2). One month after the initiation of treatment applications in 2015, no significant differences ($P > 0.05$) were observed in fruit yield among the treatments including the UTC. But in both 2016 and 2018, the INA (0.5 g/tree) or SA (0.5 g/tree) sprays and the ASM, IMI, INA, or SA injections at 0.25 g/tree resulted in a significantly ($P < 0.05$) higher yield compared

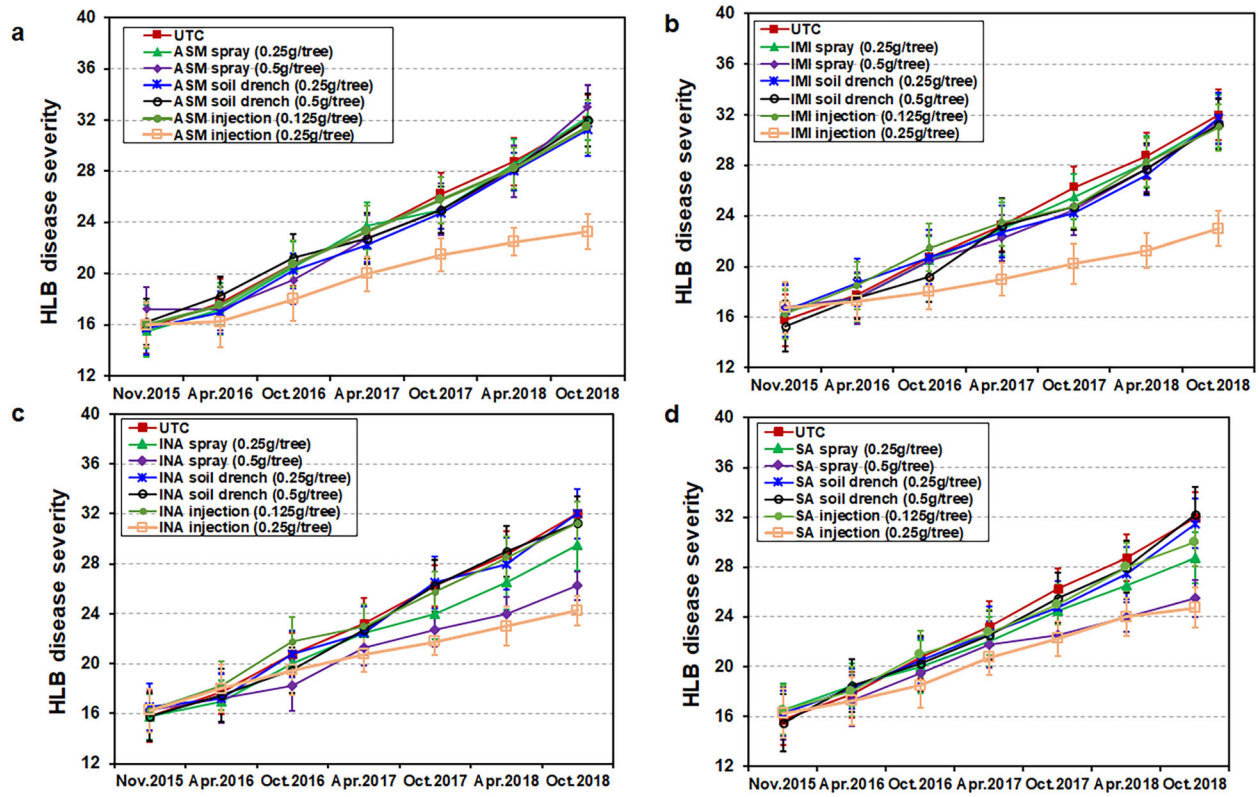

Fig. 1 HLB disease severity progression of Hamlin sweet orange trees following treatments with SAR inducers. **a** Disease progression under ASM treatments. **b** Disease progression under IMI treatments. **c** Disease progression under INA treatments. **d** Disease progression under SA treatments. The mean of disease severity was calculated from evaluations of the treated trees. Disease severity scores were shown as means and error bars (standard deviation) ($n = 6$). The untreated control (UTC, spray with water only) was included in each panel for comparison purpose

with the UTC; among these six treatments, there were no significant differences in yield in either 2016 or 2018 (Table 2). The average yield of the six treatments ranged from 52.7 to 60.7 kg/tree in 2016 and from 60.5 to 63.3 kg/tree in 2018, whereas that of the UTC was 47.1 and 48.5 kg/tree in 2016 and 2018, respectively (Table 2).

When compared year by year, the ASM, IMI, INA, or SA injections at 0.25 g/tree exhibited a significantly ($P < 0.05$) higher yield in both 2016 and 2018 than 2015 (Table 2), indicating a significant fruit yield increase after 1-year or longer applications; whereas the INA (0.5 g/tree) or SA (0.5 g/tree) sprays showed a significantly higher yield in 2018 (but not in 2016) than in 2015 (Table 2), suggesting a significant yield increase after continuous applications for 3 years.

Similarly, in 2015, no significant differences ($P > 0.05$) were observed in pre-harvest fruit drop among all treatments including the UTC. But in both 2016 and 2018, the INA (0.5 g/tree) or SA (0.5 g/tree) sprays and the ASM, IMI, INA, or SA injections at 0.25 g/tree resulted in a significantly ($P < 0.05$) fewer fruit drop compared with the UTC (Table 3). When compared year by year, the ASM, IMI, INA, or SA injections at 0.25 g/tree had a significantly lower number of fruit drop in both 2016 and 2018 than 2015 (Table 3), indicating a significant decrease of fruit drop after 1-year or longer applications; whereas the INA (0.5 g/tree) or SA (0.5 g/tree) sprays showed a significant decrease in fruit drop in 2018 (but not in 2016) than 2015 (Table 3), suggesting that a significant effect on fruit drop after continuous applications for 3 years. These observations demonstrated that the ASM, IMI, INA, or SA injections at 0.25 g/tree were more effective in reducing pre-harvest fruit drop and sustaining yield than the INA (0.5 g/tree) or SA (0.5 g/tree) sprays.

Foliar sprays of ASM, IMI, INA, or SA at 0.25 g/tree, soil applications of the SAR inducers, or trunk injections at 0.125 g/tree did not show a positive effect on fruit yield (Table 2) or pre-harvest fruit drop (Table 3). In addition, none of the treatments had a statistically significant positive effect on fruit quality, with regard to percent juice content, fruit brix, acidity, or fruit brix acidity ratio; although ASM, IMI, INA, or SA injections at 0.25 g/tree resulted in a lower acidity and higher brix acidity ratio compared with the UTC, the differences were not statistically significant ($P > 0.05$) (Table 4).

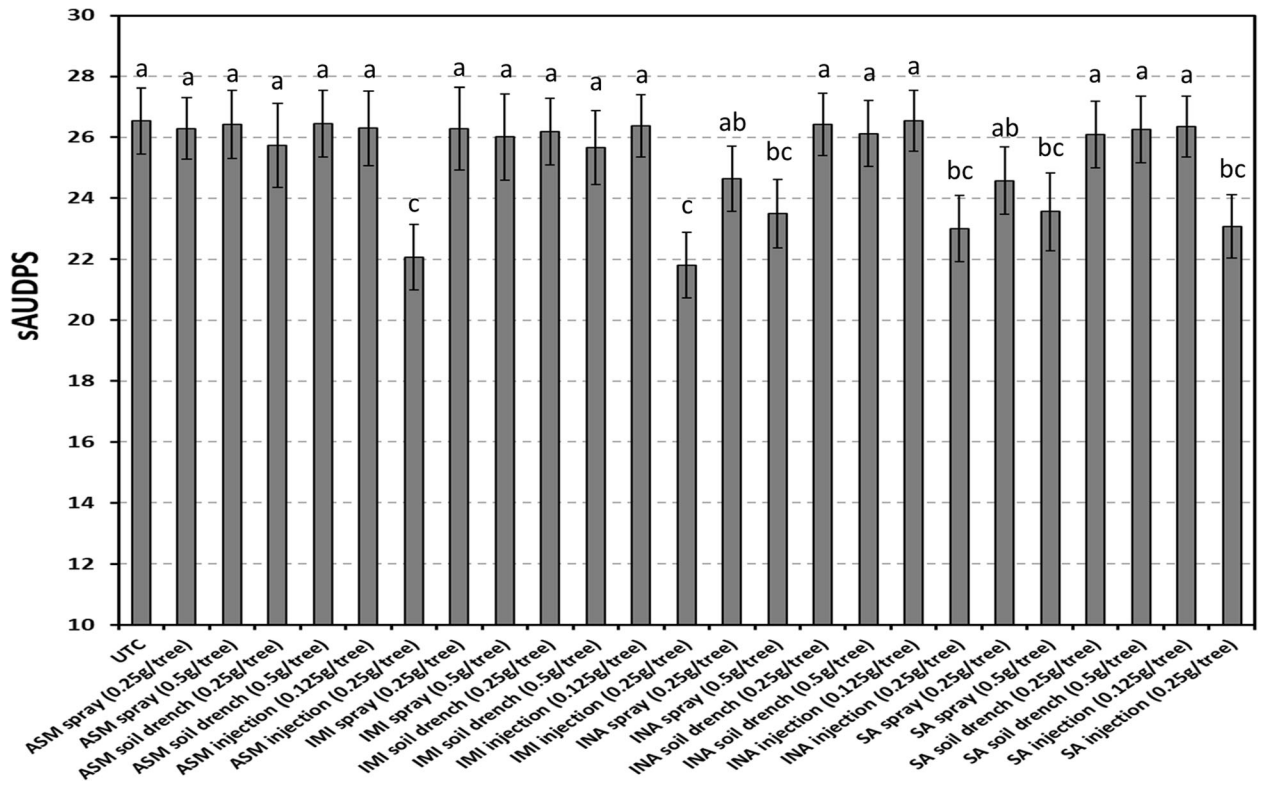

Fig. 2 HLB disease severity, expressed as standardized area under the disease progress stairs (sAUDPS), achieved by various SAR inducer treatments at the end of a three-year field trial (2015–2018). Bars represent standard deviations of the mean ($n = 6$). Different letters on the bars indicate significant difference among the treatments based on Tukey's HSD test ($\alpha = 0.05$)

When compared year by year, the ASM, IMI, INA, or SA injections at 0.25 g/tree had a lower acidity and higher brix acidity ratio in 2016, 2017, and 2018 than 2015, but the differences were not statistically significant ($P > 0.05$) (Table 4).

Growth performance of citrus treated with SAR inducers

Following foliar spray, soil application or trunk injection of ASM, IMI, INA or SA during the spring, summer, and fall flushes, all treated trees produced vigorous new shoots with normal leaves. No symptoms of discoloring, stunting and other abnormal appearance were observed on new flushes or shoots in the treated trees over the course of 3 years. These observations indicated that the SAR inducers did not have a phytotoxic effect at the rates tested. However, in 5 of the 48 trees (10.4%) receiving trunk injection treatments, the wounds caused by drilling holes did not heal completely within 4 months of injection, with cracking in the area around drilling site, indicating certain mechanical injuries to the trunk. These observations suggested that fewer injections per crop season are needed to alleviate the potential harmful effects.

Economic analysis

Economic feasibility of the six treatments showing improvement of fruit yield, i.e., the INA (0.5 g/tree) or SA (0.5 g/tree) sprays and the ASM, IMI, INA, or SA injections at 0.25 g/tree, was evaluated by comparing the revenue with the cost for the 2016 and 2018 growing seasons. Higher yields for treated trees resulted in greater gross values of the fruit harvested from treated trees for all the six treatments in both years (Table 5). The revenue produced from harvested fruit was greater than the estimated costs for ASM, IMI, INA, or SA-injected (at 0.25 g/tree) trees in both years and improvements in revenue relative to untreated control were observed consistently for these four treatments (Table 5). However, the costs for the INA (0.5 g/tree) spray treatment were greater than the revenue generated from harvested fruit in both 2016 and 2018, and no increased revenue was observed for this treatment in either year. Similarly, in 2016, the revenue of the SA (0.5 g/tree) spray treatment was offset by the cost and there was no increased revenue for this treatment; whereas, in 2018, the revenue was greater than the cost of the SA (0.5 g/tree) spray treatment and increased revenue was observed for the treatment (Table 5).

Table 1 *Candidatus* Liberibacter asiaticus (Las) titers in leaf samples of Hamlin sweet orange trees treated with various SAR inducers in a three-year field trial (2015–2018)

Treatment	Las titer (Log_{10}[cells/g of leaf tissue]) [z]						
	Nov.2015	Apr.2016	Oct.2016	Apr.2017	Oct.2017	Apr.2018	Oct.2018
UTC	6.87 $_{a}^{D}$	7.16 $_{a}^{CD}$	7.37 $_{a}^{BC}$	7.42 $_{a}^{BC}$	7.51 $_{a}^{ABC}$	7.64 $_{a}^{AB}$	7.88 $_{a}^{A}$
ASM spray (0.25 g/tree)	6.92 $_{a}^{D}$	7.11 $_{a}^{CD}$	7.29 $_{a}^{CD}$	7.33 $_{a}^{BC}$	7.47 $_{a}^{ABC}$	7.56 $_{a}^{AB}$	7.78 $_{ab}^{A}$
ASM spray (0.5 g/tree)	6.84 $_{a}^{D}$	7.06 $_{a}^{CD}$	7.15 $_{a}^{CD}$	7.30 $_{a}^{BC}$	7.44 $_{a}^{ABC}$	7.67 $_{a}^{AB}$	7.76 $_{ab}^{A}$
ASM soil drench (0.25 g/tree)	7.07 $_{a}^{D}$	7.21 $_{a}^{CD}$	7.26 $_{a}^{CD}$	7.47 $_{a}^{BC}$	7.51 $_{a}^{ABC}$	7.64 $_{a}^{AB}$	7.87 $_{a}^{A}$
ASM soil drench (0.5 g/tree)	6.96 $_{a}^{D}$	7.11 $_{a}^{CD}$	7.22 $_{a}^{CD}$	7.28 $_{a}^{BC}$	7.42 $_{a}^{ABC}$	7.66 $_{a}^{AB}$	7.79 $_{a}^{A}$
ASM injection (0.125 g/tree)	6.81 $_{a}^{C}$	7.09 $_{a}^{BC}$	7.27 $_{a}^{B}$	7.39 $_{a}^{B}$	7.55 $_{a}^{AB}$	7.71 $_{a}^{A}$	7.85 $_{a}^{A}$
ASM injection (0.25 g/tree)	7.13 $_{a}^{A}$	7.17 $_{a}^{A}$	7.23 $_{a}^{A}$	7.25 $_{a}^{A}$	7.27 $_{a}^{A}$	7.17 $_{b}^{A}$	7.21 $_{c}^{A}$
IMI spray (0.25 g/tree)	6.91 $_{a}^{C}$	7.15 $_{a}^{BC}$	7.22 $_{a}^{AB}$	7.32 $_{a}^{AB}$	7.49 $_{a}^{AB}$	7.60 $_{a}^{A}$	7.78 $_{ab}^{A}$
IMI spray (0.5 g/tree)	7.02 $_{a}^{C}$	7.24 $_{a}^{BC}$	7.29 $_{a}^{BC}$	7.35 $_{a}^{BC}$	7.52 $_{a}^{AB}$	7.59 $_{a}^{AB}$	7.82 $_{a}^{A}$
IMI soil drench (0.25 g/tree)	7.11 $_{a}^{D}$	7.19 $_{a}^{CD}$	7.30 $_{a}^{CD}$	7.38 $_{a}^{BC}$	7.54 $_{a}^{ABC}$	7.68 $_{a}^{AB}$	7.89 $_{a}^{A}$
IMI soil drench (0.5 g/tree)	6.93 $_{a}^{D}$	7.04 $_{a}^{CD}$	7.25 $_{a}^{CD}$	7.33 $_{a}^{BC}$	7.52 $_{a}^{ABC}$	7.57 $_{a}^{AB}$	7.85 $_{a}^{A}$
IMI injection (0.125 g/tree)	7.10 $_{a}^{D}$	7.24 $_{a}^{CD}$	7.31 $_{a}^{CD}$	7.44 $_{a}^{BC}$	7.57 $_{a}^{ABC}$	7.66 $_{a}^{AB}$	7.83 $_{a}^{A}$
IMI injection (0.25 g/tree)	7.06 $_{a}^{A}$	7.15 $_{a}^{A}$	7.21 $_{a}^{A}$	7.17 $_{a}^{A}$	7.28 $_{a}^{A}$	7.36 $_{ab}^{A}$	7.28 $_{c}^{A}$
INA spray (0.25 g/tree)	6.95 $_{a}^{C}$	7.21 $_{a}^{BC}$	7.32 $_{a}^{AB}$	7.42 $_{a}^{AB}$	7.53 $_{a}^{AB}$	7.60 $_{a}^{A}$	7.79 $_{a}^{A}$
INA spray (0.5 g/tree)	7.08 $_{a}^{B}$	7.19 $_{a}^{B}$	7.26 $_{a}^{AB}$	7.31 $_{a}^{AB}$	7.34 $_{a}^{AB}$	7.41 $_{a}^{A}$	7.43 $_{bc}^{A}$
INA soil drench (0.25 g/tree)	7.17 $_{a}^{D}$	7.25 $_{a}^{CD}$	7.32 $_{a}^{CD}$	7.43 $_{a}^{BC}$	7.55 $_{a}^{ABC}$	7.69 $_{a}^{AB}$	7.87 $_{a}^{A}$
INA soil drench (0.5 g/tree)	6.91 $_{a}^{D}$	7.17 $_{a}^{CD}$	7.27 $_{a}^{CD}$	7.39 $_{a}^{BC}$	7.52 $_{a}^{ABC}$	7.65 $_{a}^{AB}$	7.81 $_{a}^{A}$
INA injection (0.125 g/tree)	6.84 $_{a}^{E}$	7.12 $_{a}^{DE}$	7.29 $_{a}^{CD}$	7.42 $_{a}^{BCD}$	7.55 $_{a}^{ABC}$	7.67 $_{a}^{AB}$	7.86 $_{a}^{A}$
INA injection (0.25 g/tree)	7.10 $_{a}^{A}$	7.21 $_{a}^{A}$	7.17 $_{a}^{AB}$	7.29 $_{a}^{AB}$	7.33 $_{a}^{AB}$	7.37 $_{ab}^{A}$	7.34 $_{bc}^{A}$
SA spray (0.25 g/tree)	6.88 $_{a}^{D}$	7.01 $_{a}^{CD}$	7.27 $_{a}^{BC}$	7.35 $_{a}^{BC}$	7.46 $_{a}^{AB}$	7.61 $_{a}^{AB}$	7.81 $_{a}^{A}$
SA spray (0.5 g/tree)	6.93 $_{a}^{B}$	7.13 $_{a}^{B}$	7.24 $_{a}^{AB}$	7.28 $_{a}^{AB}$	7.31 $_{a}^{A}$	7.37 $_{ab}^{A}$	7.48 $_{bc}^{A}$
SA soil drench (0.25 g/tree)	7.14 $_{a}^{D}$	7.21 $_{a}^{CD}$	7.32 $_{a}^{BCD}$	7.39 $_{a}^{BC}$	7.56 $_{a}^{ABC}$	7.69 $_{a}^{AB}$	7.86 $_{a}^{A}$
SA soil drench (0.5 g/tree)	6.85 $_{a}^{D}$	7.01 $_{a}^{CD}$	7.15 $_{a}^{CD}$	7.31 $_{a}^{BC}$	7.55 $_{a}^{AB}$	7.66 $_{a}^{AB}$	7.82 $_{a}^{A}$
SA injection (0.125 g/tree)	7.07 $_{a}^{D}$	7.22 $_{a}^{CD}$	7.33 $_{a}^{BCD}$	7.43 $_{a}^{BC}$	7.57 $_{a}^{AB}$	7.65 $_{a}^{AB}$	7.85 $_{a}^{A}$
SA injection (0.25 g/tree)	7.12 $_{a}^{A}$	7.24 $_{a}^{A}$	7.19 $_{a}^{A}$	7.21 $_{a}^{A}$	7.27 $_{a}^{A}$	7.22 $_{b}^{A}$	7.24 $_{c}^{A}$

[z] Data shown are the means of six replicates ($n = 6$). Values in the same row followed by different superscript uppercase letters are significantly different within each treatment ($P < 0.05$). Values in the same column followed by different subscript lowercase letters are significantly different at each sampling time ($P < 0.05$)

Discussion

The present study evaluated the control effect of plant SAR inducers applied by foliar spray, soil drench, or trunk injection against citrus HLB under field conditions. The results indicated that trunk injection of the SAR inducers ASM, IMI, INA, or SA at 0.25 g/tree with three applications per year and foliar spray of INA or SA at 0.5 g/tree with six applications per year suppressed Las population growth within infected trees and reduced disease severity and pre-harvest fruit drop, and sustained fruit yield. Harnessing induced resistance for practical plant disease control represents a sustainable approach to crop protection (Durrant and Dong 2004). Therefore, the findings of this study may be useful for developing management programs with SAR inducers for effective control of citrus HLB. It is important to note that the present study was performed in a single grove of Hamlin sweet orange. Its performance in large-scale commercial citrus groves remains to be evaluated. More studies at multiple locations with various citrus cultivars or varieties under diverse climate conditions are required to adequately evaluate effects of such SAR inducer treatments for the control of citrus HLB.

Earlier studies have demonstrated that the efficacy of SAR inducers against citrus canker and HLB varied widely from limited to moderate under field conditions, depending on method, rate, frequency, and timing of application (Graham and Leite 2004; Graham and Myers 2011, 2013; Li et al. 2016, 2017; Hu et al. 2018). Results of this field trial appeared consistent with those earlier reports. When applied by foliar spray, two (INA and SA) of the four SAR inducers (0.5 g/tree) are effective for control of citrus HLB, but not at a lower rate (0.25 g/tree). When applied by trunk injection, all the four SAR inducers (0.25 g/tree), but not at a lower rate (0.125 g/tree), showed relatively fast and consistent effectiveness

Table 2 Fruit yield of Hamlin sweet orange trees treated with various SAR inducers in a three-year field trial (2015–2018)

Treatment	Yield (kg/tree) [z]			
	2015	2016	2017	2018
UTC	$50.7 \pm 5.9_{a}^{A}$	$47.1 \pm 5.3_{b}^{A}$	$41.5 \pm 5.8_{a}^{B}$	$48.5 \pm 6.5_{b}^{A}$
ASM spray (0.25 g/tree)	$52.1 \pm 5.2_{a}^{A}$	$48.5 \pm 5.6_{b}^{A}$	$40.4 \pm 7.2_{a}^{B}$	$48.9 \pm 6.2_{b}^{A}$
ASM spray (0.5 g/tree)	$51.4 \pm 6.7_{a}^{A}$	$48.8 \pm 7.3_{b}^{A}$	$42.5 \pm 6.1_{a}^{B}$	$50.5 \pm 5.7_{b}^{A}$
ASM soil drench (0.25 g/tree)	$49.5 \pm 5.3_{a}^{A}$	$47.7 \pm 6.2_{b}^{A}$	$40.8 \pm 5.9_{a}^{B}$	$47.7 \pm 6.2_{b}^{A}$
ASM soil drench (0.5 g/tree)	$50.2 \pm 5.9_{a}^{A}$	$47.5 \pm 5.7_{b}^{A}$	$41.4 \pm 6.5_{a}^{B}$	$49.1 \pm 6.6_{b}^{A}$
ASM injection (0.125 g/tree)	$51.1 \pm 5.5_{a}^{A}$	$50.4 \pm 4.8_{b}^{A}$	$39.7 \pm 5.6_{a}^{B}$	$48.4 \pm 5.2_{b}^{A}$
ASM injection (0.25 g/tree)	$49.8 \pm 6.4_{a}^{B}$	$57.6 \pm 6.9_{a}^{A}$	$43.5 \pm 5.3_{a}^{C}$	$62.2 \pm 6.1_{a}^{A}$
IMI spray (0.25 g/tree)	$49.2 \pm 6.3_{a}^{A}$	$48.3 \pm 6.1_{b}^{A}$	$39.5 \pm 5.6_{a}^{B}$	$50.4 \pm 5.8_{b}^{A}$
IMI spray (0.5 g/tree)	$52.7 \pm 5.1_{a}^{A}$	$50.1 \pm 5.3_{b}^{A}$	$42.6 \pm 6.4_{a}^{B}$	$52.2 \pm 6.9_{b}^{A}$
IMI soil drench (0.25 g/tree)	$48.5 \pm 5.3_{a}^{A}$	$46.7 \pm 6.0_{b}^{A}$	$40.4 \pm 5.5_{a}^{B}$	$48.9 \pm 6.3_{b}^{A}$
IMI soil drench (0.5 g/tree)	$50.6 \pm 5.9_{a}^{A}$	$48.2 \pm 5.8_{b}^{A}$	$39.9 \pm 6.2_{a}^{B}$	$48.4 \pm 5.7_{b}^{A}$
IMI injection (0.125 g/tree)	$47.7 \pm 6.6_{a}^{A}$	$49.4 \pm 5.4_{b}^{A}$	$42.2 \pm 6.4_{a}^{B}$	$49.6 \pm 6.1_{b}^{A}$
IMI injection (0.25 g/tree)	$51.9 \pm 6.1_{a}^{B}$	$58.7 \pm 5.9_{a}^{A}$	$43.6 \pm 6.5_{a}^{C}$	$60.5 \pm 5.8_{a}^{A}$
INA spray (0.25 g/tree)	$49.5 \pm 6.3_{a}^{A}$	$47.8 \pm 5.7_{b}^{A}$	$39.1 \pm 5.2_{a}^{B}$	$51.6 \pm 6.5_{b}^{A}$
INA spray (0.5 g/tree)	$51.9 \pm 5.2_{a}^{B}$	$52.7 \pm 6.5_{ab}^{B}$	$41.2 \pm 5.9_{a}^{C}$	$60.7 \pm 6.3_{a}^{A}$
INA soil drench (0.25 g/tree)	$48.6 \pm 6.1_{a}^{A}$	$47.7 \pm 6.4_{b}^{A}$	$39.3 \pm 4.5_{a}^{B}$	$49.9 \pm 5.8_{b}^{A}$
INA soil drench (0.5 g/tree)	$50.3 \pm 5.9_{a}^{A}$	$48.2 \pm 5.9_{b}^{A}$	$40.6 \pm 5.2_{a}^{B}$	$48.1 \pm 5.5_{b}^{A}$
INA injection (0.125 g/tree)	$49.2 \pm 5.5_{a}^{A}$	$51.4 \pm 5.6_{b}^{A}$	$43.2 \pm 6.9_{a}^{B}$	$50.6 \pm 5.7_{b}^{A}$
INA injection (0.25 g/tree)	$51.1 \pm 6.4_{a}^{B}$	$60.7 \pm 6.5_{a}^{A}$	$43.2 \pm 6.9_{a}^{C}$	$59.8 \pm 5.3_{a}^{A}$
SA spray (0.25 g/tree)	$50.5 \pm 7.1_{a}^{A}$	$47.4 \pm 5.8_{b}^{A}$	$39.3 \pm 5.1_{a}^{B}$	$49.9 \pm 6.2_{b}^{A}$
SA spray (0.5 g/tree)	$53.3 \pm 6.5_{a}^{B}$	$54.6 \pm 6.7_{ab}^{B}$	$43.4 \pm 4.6_{a}^{C}$	$63.3 \pm 5.8_{a}^{A}$
SA soil drench (0.25 g/tree)	$51.6 \pm 6.4_{a}^{A}$	$48.7 \pm 6.3_{b}^{A}$	$40.5 \pm 4.7_{a}^{B}$	$50.9 \pm 5.4_{b}^{A}$
SA soil drench (0.5 g/tree)	$50.2 \pm 5.5_{a}^{A}$	$48.6 \pm 5.7_{b}^{A}$	$40.8 \pm 5.6_{a}^{B}$	$51.2 \pm 6.3_{b}^{A}$
SA injection (0.125 g/tree)	$48.6 \pm 6.2_{a}^{A}$	$49.7 \pm 5.5_{b}^{A}$	$42.3 \pm 5.9_{a}^{B}$	$49.8 \pm 5.5_{b}^{A}$
SA injection (0.25 g/tree)	$51.5 \pm 5.6_{a}^{B}$	$59.9 \pm 6.1_{a}^{A}$	$43.9 \pm 7.7_{a}^{C}$	$62.4 \pm 5.7_{a}^{A}$
13-year old healthy tree [y]	112.6			

[z] Data shown are the means ± SD of six replicates ($n = 6$). Values in the same row followed by different superscript uppercase letters are significantly different within each treatment ($P < 0.05$). Values in the same column followed by different subscript lowercase letters are significantly different at each sampling time ($P < 0.05$). [y] averaged fruit yield of 13-year old, healthy Hamlin sweet orange trees in Florida reported by Roka et al. (2000) prior to the era of HLB

of disease control (Figs. 1, 2 and Tables 1, 2 and 3). In contrast, soil applications of the SAR inducers did not have significant control effect against HLB. Multiple factors might be responsible for such a varying efficacy. Among others, foliar spray and soil drench may be a significant limiting factor that results in poor intake of SAR inducers by a citrus plant. Many SAR inducers such as ASM and SA are highly photodegradable and biodegradable (Silva et al. 2007; Sleiman et al. 2017) and vulnerable to other environmental conditions including wash-off by rainfall, whereas soil particles might absorb SAR inducers that reduces the uptake by roots. Trunk injection is a target-precise, efficient method for delivery of compounds into plants, and, may avoid problems of degradation and poor uptake of SAR inducers associated with foliar spray and soil drench (Aćimović et al. 2015). However, current injection technologies and equipment are labor intensive and costly, and consequently, may not be practical for large-scale applications in HLB endemic areas, such as Florida. Therefore, development of automated injection systems with high efficiency and low cost is needed for commercial citrus production. Meanwhile, how to improve the uptake efficacy of SAR inducers via foliar spray needs further investigation.

SAR inducers might suppress Las growth via two different mechanisms (Table 1). First, it may induce the production of antimicrobials. Earlier studies have demonstrated that a high and persistent upregulation of expression of PR genes *PR1, 2, 3* in citrus in response to ASM, IMI, INA and SA (Dekkers et al. 2004; Francis et al. 2009; Li et al. 2017; Hu et al. 2018). The PR proteins PR1, PR2 and PR3 have antimicrobial activities against various plant pathogenic fungi, bacteria and oomycetes (Mauch et al. 1988; Alexander et al. 1993;

Table 3 Pre-harvest fruit drop of Hamlin sweet orange trees treated with various SAR inducers in a three-year field trial (2015–2018)

Treatment	No. of fruit dropped [z]			
	2015	2016	2017	2018
UTC	$136 \pm 29_{a}^{B}$	$149 \pm 32_{a}^{B}$	$214 \pm 43_{a}^{A}$	$148 \pm 35_{a}^{B}$
ASM spray (0.25 g/tree)	$128 \pm 26_{a}^{B}$	$139 \pm 28_{a}^{B}$	$217 \pm 47_{a}^{A}$	$137 \pm 33_{a}^{B}$
ASM spray (0.5 g/tree)	$139 \pm 32_{a}^{B}$	$145 \pm 31_{a}^{B}$	$229 \pm 45_{a}^{A}$	$147 \pm 32_{a}^{B}$
ASM soil drench (0.25 g/tree)	$133 \pm 27_{a}^{B}$	$148 \pm 34_{a}^{B}$	$237 \pm 46_{a}^{A}$	$146 \pm 29_{a}^{B}$
ASM soil drench (0.5 g/tree)	$129 \pm 24_{a}^{B}$	$141 \pm 28_{a}^{B}$	$221 \pm 41_{a}^{A}$	$135 \pm 31_{a}^{B}$
ASM injection (0.125 g/tree)	$135 \pm 32_{a}^{B}$	$124 \pm 30_{a}^{B}$	$216 \pm 38_{a}^{A}$	$129 \pm 33_{a}^{B}$
ASM injection (0.25 g/tree)	$143 \pm 28_{a}^{B}$	$78 \pm 21_{b}^{C}$	$235 \pm 45_{a}^{A}$	$69 \pm 24_{b}^{C}$
IMI spray (0.25 g/tree)	$126 \pm 29_{a}^{B}$	$139 \pm 33_{a}^{B}$	$219 \pm 36_{a}^{A}$	$144 \pm 30_{a}^{B}$
IMI spray (0.5 g/tree)	$138 \pm 31_{a}^{B}$	$124 \pm 28_{a}^{B}$	$228 \pm 41_{a}^{A}$	$152 \pm 37_{a}^{B}$
IMI soil drench (0.25 g/tree)	$143 \pm 30_{a}^{B}$	$132 \pm 31_{a}^{B}$	$242 \pm 48_{a}^{A}$	$137 \pm 31_{a}^{B}$
IMI soil drench (0.5 g/tree)	$132 \pm 28_{a}^{B}$	$147 \pm 35_{a}^{B}$	$233 \pm 44_{a}^{A}$	$144 \pm 33_{a}^{B}$
IMI injection (0.125 g/tree)	$145 \pm 34_{a}^{B}$	$131 \pm 29_{a}^{B}$	$226 \pm 39_{a}^{A}$	$150 \pm 38_{a}^{B}$
IMI injection (0.25 g/tree)	$128 \pm 29_{a}^{B}$	$71 \pm 20_{b}^{C}$	$215 \pm 37_{a}^{A}$	$79 \pm 21_{b}^{C}$
INA spray (0.25 g/tree)	$121 \pm 29_{a}^{B}$	$138 \pm 32_{a}^{B}$	$237 \pm 48_{a}^{A}$	$139 \pm 35_{a}^{B}$
INA spray (0.5 g/tree)	$137 \pm 30_{a}^{B}$	$145 \pm 35_{a}^{B}$	$219 \pm 46_{a}^{A}$	$70 \pm 25_{b}^{B}$
INA soil drench (0.25 g/tree)	$129 \pm 28_{a}^{B}$	$139 \pm 32_{a}^{B}$	$240 \pm 48_{a}^{A}$	$142 \pm 31_{a}^{B}$
INA soil drench (0.5 g/tree)	$140 \pm 29_{a}^{B}$	$151 \pm 31_{a}^{B}$	$226 \pm 44_{a}^{A}$	$133 \pm 30_{a}^{B}$
INA injection (0.125 g/tree)	$132 \pm 31_{a}^{B}$	$127 \pm 27_{a}^{B}$	$215 \pm 35_{a}^{A}$	$127 \pm 34_{a}^{B}$
INA injection (0.25 g/tree)	$145 \pm 32_{a}^{B}$	$72 \pm 20_{b}^{C}$	$230 \pm 43_{a}^{A}$	$77 \pm 24_{b}^{C}$
SA spray (0.25 g/tree)	$136 \pm 27_{a}^{B}$	$148 \pm 32_{a}^{B}$	$242 \pm 49_{a}^{A}$	$129 \pm 35_{a}^{B}$
SA spray (0.5 g/tree)	$129 \pm 30_{a}^{B}$	$140 \pm 34_{a}^{B}$	$227 \pm 44_{a}^{A}$	$81 \pm 22_{b}^{C}$
SA soil drench (0.25 g/tree)	$133 \pm 27_{a}^{B}$	$148 \pm 34_{a}^{B}$	$237 \pm 46_{a}^{A}$	$146 \pm 29_{a}^{B}$
SA soil drench (0.5 g/tree)	$142 \pm 32_{a}^{B}$	$133 \pm 27_{a}^{B}$	$222 \pm 48_{a}^{A}$	$133 \pm 33_{a}^{B}$
SA injection (0.125 g/tree)	$126 \pm 28_{a}^{B}$	$140 \pm 31_{a}^{B}$	$235 \pm 44_{a}^{A}$	$142 \pm 35_{a}^{B}$
SA injection (0.25 g/tree)	$141 \pm 31_{a}^{B}$	$77 \pm 23_{b}^{C}$	$225 \pm 42_{a}^{A}$	$69 \pm 21_{b}^{C}$

[z] Data shown are the means ± SD of six replicates ($n = 6$). Values in the same row followed by different superscript uppercase letters are significantly different within each treatment ($P < 0.05$). Values in the same column followed by different subscript lowercase letters are significantly different at each sampling time ($P < 0.05$)

Rauscher et al. 1999; Maxson-Stein et al. 2002; Walters and Fountaine 2009). Furthermore, ASM spray-induced PR1 expression in tomato was positively associated with field control efficacy of *Xanthomonas* leaf spot and *Pseudomonas* bacterial speck tomato (Herman et al. 2007). Upregulation of PR2 expression was correlated with reduction of canker lesions on citrus leaves (Francis et al. 2009). These earlier reports suggested that the PR proteins might have antibacterial activity against bacterial pathogens including Las. Second, SAR inducers themselves might have direct antimicrobial activities against Las under certain conditions. For example, SA has antibacterial activity against *Agrobacterium tumefaciens* (Yuan et al. 2007; Anand et al. 2008) and *Rhizobium meliloti* (Martínez-Abarca et al. 1998) at relatively lower concentrations (5 to 25 μM = 0.69 to 3.45 μg/mL) in vitro. Las is a closely relative of *Agrobacterium* and *Rhizobium*, belonging to the family *Rhizobiaceae* (Duan et al. 2009). It is possible that application of the SAR inducers, and the consequent accumulation of SA in a citrus tree may have inhibitory effect on Las. Las encodes a functional SA hydroxylase that degrades SA, thus affecting its efficacy in inducing SAR in Las-infected citrus plants (Li et al. 2017). Trunk injection or multiple foliar sprays of SA at a relatively higher rate seems to be able to overcome the SA degradation by the enzyme.

In this study, all SAR inducer treatments did not result in significant reduction of Las titers in treated trees when compared year by year and the Las titer was consistently maintained in a relative high level of 10^7 cells/g of leaf tissue throughout the experiments (Table 1). The results of this study were consistent with those in earlier studies that SAR inducer treatments had limited effect in reduction of the titers of bacterial pathogens within infected plants (Aćimović et al. 2015; Li et al. 2016; Hu et al. 2018). The antimicrobials induced by SAR inducers

Table 4 Quality of fruit harvested from Hamlin sweet orange trees treated with SAR inducers in a three-year field trial (2015–2018) [z]

Treatment	Percent juice content				Brix				Acidity (% w/w)				Brix acidity ratio			
	2015	2016	2017	2018	2015	2016	2017	2018	2015	2016	2017	2018	2015	2016	2017	2018
UTC	55.4 $_{a}^{A}$	53.8 $_{a}^{A}$	54.0 $_{a}^{A}$	55.7 $_{a}^{A}$	11.2 $_{a}^{A}$	11.7 $_{a}^{A}$	11.4 $_{a}^{A}$	11.8 $_{a}^{A}$	0.64 $_{a}^{A}$	0.63 $_{a}^{A}$	0.66 $_{a}^{A}$	0.68 $_{a}^{A}$	17.6 $_{a}^{A}$	18.2 $_{a}^{A}$	17.4 $_{a}^{A}$	17.3 $_{a}^{A}$
ASM spray (0.25 g/tree)	53.6 $_{a}^{A}$	54.6 $_{a}^{A}$	53.9 $_{a}^{A}$	54.4 $_{a}^{A}$	11.3 $_{a}^{A}$	11.9 $_{a}^{A}$	11.2 $_{a}^{A}$	11.5 $_{a}^{A}$	0.65 $_{a}^{A}$	0.69 $_{a}^{A}$	0.64 $_{a}^{A}$	0.67 $_{a}^{A}$	17.4 $_{a}^{A}$	17.9 $_{a}^{A}$	18.3 $_{a}^{A}$	17.5 $_{a}^{A}$
ASM spray (0.5 g/tree)	53.8 $_{a}^{A}$	54.7 $_{a}^{A}$	55.4 $_{a}^{A}$	54.8 $_{a}^{A}$	11.8 $_{a}^{A}$	11.1 $_{a}^{A}$	11.4 $_{a}^{A}$	11.7 $_{a}^{A}$	0.68 $_{a}^{A}$	0.61 $_{a}^{A}$	0.64 $_{a}^{A}$	0.65 $_{a}^{A}$	17.5 $_{a}^{A}$	18.3 $_{a}^{A}$	17.8 $_{a}^{A}$	18.2 $_{a}^{A}$
ASM soil drench (0.25 g/tree)	55.3 $_{a}^{A}$	52.2 $_{a}^{A}$	53.9 $_{a}^{A}$	54.4 $_{a}^{A}$	11.1 $_{a}^{A}$	12.0 $_{a}^{A}$	11.5 $_{a}^{A}$	11.8 $_{a}^{A}$	0.61 $_{a}^{A}$	0.69 $_{a}^{A}$	0.66 $_{a}^{A}$	0.68 $_{a}^{A}$	17.8 $_{a}^{A}$	17.5 $_{a}^{A}$	17.2 $_{a}^{A}$	17.6 $_{a}^{A}$
ASM soil drench (0.5 g/tree)	54.2 $_{a}^{A}$	55.1 $_{a}^{A}$	53.2 $_{a}^{A}$	55.0 $_{a}^{A}$	11.0 $_{a}^{A}$	11.7 $_{a}^{A}$	11.4 $_{a}^{A}$	11.9 $_{a}^{A}$	0.62 $_{a}^{A}$	0.68 $_{a}^{A}$	0.64 $_{a}^{A}$	0.67 $_{a}^{A}$	17.6 $_{a}^{A}$	17.3 $_{a}^{A}$	17.7 $_{a}^{A}$	18.2 $_{a}^{A}$
ASM injection (0.125 g/tree)	54.6 $_{a}^{A}$	52.8 $_{a}^{A}$	54.9 $_{a}^{A}$	53.4 $_{a}^{A}$	12.1 $_{a}^{A}$	11.3 $_{a}^{A}$	11.7 $_{a}^{A}$	11.8 $_{a}^{A}$	0.68 $_{a}^{A}$	0.62 $_{a}^{A}$	0.67 $_{a}^{A}$	0.65 $_{a}^{A}$	18.5 $_{a}^{A}$	17.7 $_{a}^{A}$	18.1 $_{a}^{A}$	18.3 $_{a}^{A}$
ASM injection (0.25 g/tree)	55.1 $_{a}^{A}$	53.6 $_{a}^{A}$	54.4 $_{a}^{A}$	55.2 $_{a}^{A}$	11.8 $_{a}^{A}$	11.4 $_{a}^{A}$	11.6 $_{a}^{A}$	11.7 $_{a}^{A}$	0.67 $_{a}^{A}$	0.60 $_{a}^{A}$	0.62 $_{a}^{A}$	0.63 $_{a}^{A}$	17.9 $_{a}^{A}$	18.9 $_{a}^{A}$	18.7 $_{a}^{A}$	18.6 $_{a}^{A}$
IMI spray (0.25 g/tree)	53.4 $_{a}^{A}$	54.6 $_{a}^{A}$	55.0 $_{a}^{A}$	54.5 $_{a}^{A}$	11.4 $_{a}^{A}$	11.6 $_{a}^{A}$	11.1 $_{a}^{A}$	11.6 $_{a}^{A}$	0.66 $_{a}^{A}$	0.68 $_{a}^{A}$	0.65 $_{a}^{A}$	0.67 $_{a}^{A}$	17.4 $_{a}^{A}$	17.9 $_{a}^{A}$	17.2 $_{a}^{A}$	17.7 $_{a}^{A}$
IMI spray (0.5 g/tree)	53.9 $_{a}^{A}$	55.1 $_{a}^{A}$	54.6 $_{a}^{A}$	53.8 $_{a}^{A}$	11.5 $_{a}^{A}$	11.1 $_{a}^{A}$	11.6 $_{a}^{A}$	11.2 $_{a}^{A}$	0.64 $_{a}^{A}$	0.63 $_{a}^{A}$	0.67 $_{a}^{A}$	0.65 $_{a}^{A}$	17.9 $_{a}^{A}$	17.3 $_{a}^{A}$	17.4 $_{a}^{A}$	17.2 $_{a}^{A}$
IMI soil drench (0.25 g/tree)	55.1 $_{a}^{A}$	53.7 $_{a}^{A}$	54.4 $_{a}^{A}$	55.0 $_{a}^{A}$	12.0 $_{a}^{A}$	11.5 $_{a}^{A}$	11.4 $_{a}^{A}$	11.6 $_{a}^{A}$	0.69 $_{a}^{A}$	0.66 $_{a}^{A}$	0.64 $_{a}^{A}$	0.67 $_{a}^{A}$	17.4 $_{a}^{A}$	17.8 $_{a}^{A}$	17.7 $_{a}^{A}$	17.3 $_{a}^{A}$
IMI soil drench (0.5 g/tree)	54.4 $_{a}^{A}$	52.8 $_{a}^{A}$	53.5 $_{a}^{A}$	54.1 $_{a}^{A}$	11.4 $_{a}^{A}$	12.1 $_{a}^{A}$	11.5 $_{a}^{A}$	11.7 $_{a}^{A}$	0.64 $_{a}^{A}$	0.69 $_{a}^{A}$	0.65 $_{a}^{A}$	0.68 $_{a}^{A}$	17.8 $_{a}^{A}$	17.5 $_{a}^{A}$	17.7 $_{a}^{A}$	17.2 $_{a}^{A}$
IMI injection (0.125 g/tree)	53.2 $_{a}^{A}$	54.9 $_{a}^{A}$	53.5 $_{a}^{A}$	54.5 $_{a}^{A}$	11.6 $_{a}^{A}$	11.7 $_{a}^{A}$	11.3 $_{a}^{A}$	11.8 $_{a}^{A}$	0.66 $_{a}^{A}$	0.68 $_{a}^{A}$	0.64 $_{a}^{A}$	0.67 $_{a}^{A}$	17.6 $_{a}^{A}$	17.2 $_{a}^{A}$	17.6 $_{a}^{A}$	17.7 $_{a}^{A}$
IMI injection (0.25 g/tree)	54.9 $_{a}^{A}$	53.8 $_{a}^{A}$	54.2 $_{a}^{A}$	55.1 $_{a}^{A}$	11.4 $_{a}^{A}$	11.3 $_{a}^{A}$	11.5 $_{a}^{A}$	11.4 $_{a}^{A}$	0.66 $_{a}^{A}$	0.60 $_{a}^{A}$	0.62 $_{a}^{A}$	0.62 $_{a}^{A}$	17.3 $_{a}^{A}$	18.7 $_{a}^{A}$	18.5 $_{a}^{A}$	18.3 $_{a}^{A}$
INA spray (0.25 g/tree)	55.0 $_{a}^{A}$	53.3 $_{a}^{A}$	54.6 $_{a}^{A}$	53.2 $_{a}^{A}$	11.2 $_{a}^{A}$	11.5 $_{a}^{A}$	11.6 $_{a}^{A}$	11.3 $_{a}^{A}$	0.65 $_{a}^{A}$	0.66 $_{a}^{A}$	0.68 $_{a}^{A}$	0.64 $_{a}^{A}$	17.2 $_{a}^{A}$	17.4 $_{a}^{A}$	17.2 $_{a}^{A}$	17.6 $_{a}^{A}$
INA spray (0.5 g/tree)	53.5 $_{a}^{A}$	52.4 $_{a}^{A}$	54.9 $_{a}^{A}$	53.5 $_{a}^{A}$	11.9 $_{a}^{A}$	11.1 $_{a}^{A}$	11.4 $_{a}^{A}$	11.7 $_{a}^{A}$	0.68 $_{a}^{A}$	0.63 $_{a}^{A}$	0.66 $_{a}^{A}$	0.65 $_{a}^{A}$	17.5 $_{a}^{A}$	17.7 $_{a}^{A}$	17.3 $_{a}^{A}$	18.0 $_{a}^{A}$
INA soil drench (0.25 g/tree)	54.4 $_{a}^{A}$	55.1 $_{a}^{A}$	53.6 $_{a}^{A}$	54.7 $_{a}^{A}$	11.6 $_{a}^{A}$	11.7 $_{a}^{A}$	11.2 $_{a}^{A}$	11.5 $_{a}^{A}$	0.67 $_{a}^{A}$	0.68 $_{a}^{A}$	0.63 $_{a}^{A}$	0.66 $_{a}^{A}$	17.3 $_{a}^{A}$	17.1 $_{a}^{A}$	17.8 $_{a}^{A}$	17.4 $_{a}^{A}$
INA soil drench (0.5 g/tree)	54.1 $_{a}^{A}$	53.7 $_{a}^{A}$	54.5 $_{a}^{A}$	53.8 $_{a}^{A}$	11.8 $_{a}^{A}$	11.1 $_{a}^{A}$	12.0 $_{a}^{A}$	11.4 $_{a}^{A}$	0.69 $_{a}^{A}$	0.63 $_{a}^{A}$	0.69 $_{a}^{A}$	0.64 $_{a}^{A}$	17.1 $_{a}^{A}$	17.6 $_{a}^{A}$	17.4 $_{a}^{A}$	17.8 $_{a}^{A}$
INA injection (0.125 g/tree)	53.8 $_{a}^{A}$	54.8 $_{a}^{A}$	53.2 $_{a}^{A}$	54.3 $_{a}^{A}$	11.2 $_{a}^{A}$	12.1 $_{a}^{A}$	11.3 $_{a}^{A}$	11.7 $_{a}^{A}$	0.64 $_{a}^{A}$	0.68 $_{a}^{A}$	0.64 $_{a}^{A}$	0.66 $_{a}^{A}$	17.5 $_{a}^{A}$	17.8 $_{a}^{A}$	17.6 $_{a}^{A}$	17.7 $_{a}^{A}$
INA injection (0.25 g/tree)	54.7 $_{a}^{A}$	53.9 $_{a}^{A}$	54.2 $_{a}^{A}$	55.0 $_{a}^{A}$	12.0 $_{a}^{A}$	11.6 $_{a}^{A}$	11.5 $_{a}^{A}$	11.8 $_{a}^{A}$	0.69 $_{a}^{A}$	0.62 $_{a}^{A}$	0.61 $_{a}^{A}$	0.63 $_{a}^{A}$	17.4 $_{a}^{A}$	18.6 $_{a}^{A}$	18.8 $_{a}^{A}$	18.7 $_{a}^{A}$
SA spray (0.25 g/tree)	54.9 $_{a}^{A}$	53.5 $_{a}^{A}$	55.1 $_{a}^{A}$	54.2 $_{a}^{A}$	11.6 $_{a}^{A}$	12.1 $_{a}^{A}$	11.4 $_{a}^{A}$	11.5 $_{a}^{A}$	0.66 $_{a}^{A}$	0.69 $_{a}^{A}$	0.64 $_{a}^{A}$	0.65 $_{a}^{A}$	17.6 $_{a}^{A}$	17.5 $_{a}^{A}$	17.8 $_{a}^{A}$	17.7 $_{a}^{A}$
SA spray (0.5 g/tree)	53.8 $_{a}^{A}$	54.4 $_{a}^{A}$	54.2 $_{a}^{A}$	55.3 $_{a}^{A}$	11.9 $_{a}^{A}$	11.4 $_{a}^{A}$	10.9 $_{a}^{A}$	11.6 $_{a}^{A}$	0.68 $_{a}^{A}$	0.65 $_{a}^{A}$	0.62 $_{a}^{A}$	0.66 $_{a}^{A}$	17.5 $_{a}^{A}$	17.4 $_{a}^{A}$	17.6 $_{a}^{A}$	17.5 $_{a}^{A}$
SA soil drench (0.25 g/tree)	54.2 $_{a}^{A}$	53.7 $_{a}^{A}$	54.6 $_{a}^{A}$	53.9 $_{a}^{A}$	10.9 $_{a}^{A}$	11.3 $_{a}^{A}$	11.5 $_{a}^{A}$	11.2 $_{a}^{A}$	0.63 $_{a}^{A}$	0.66 $_{a}^{A}$	0.64 $_{a}^{A}$	0.63 $_{a}^{A}$	17.3 $_{a}^{A}$	17.1 $_{a}^{A}$	18.0 $_{a}^{A}$	17.7 $_{a}^{A}$
SA soil drench (0.5 g/tree)	54.7 $_{a}^{A}$	55.1 $_{a}^{A}$	54.3 $_{a}^{A}$	53.9 $_{a}^{A}$	11.7 $_{a}^{A}$	11.3 $_{a}^{A}$	12.0 $_{a}^{A}$	11.5 $_{a}^{A}$	0.68 $_{a}^{A}$	0.64 $_{a}^{A}$	0.69 $_{a}^{A}$	0.66 $_{a}^{A}$	17.2 $_{a}^{A}$	17.7 $_{a}^{A}$	17.4 $_{a}^{A}$	17.4 $_{a}^{A}$
SA injection (0.125 g/tree)	53.5 $_{a}^{A}$	54.7 $_{a}^{A}$	55.2 $_{a}^{A}$	54.1 $_{a}^{A}$	11.6 $_{a}^{A}$	11.1 $_{a}^{A}$	11.5 $_{a}^{A}$	11.7 $_{a}^{A}$	0.66 $_{a}^{A}$	0.63 $_{a}^{A}$	0.65 $_{a}^{A}$	0.66 $_{a}^{A}$	17.6 $_{a}^{A}$	17.5 $_{a}^{A}$	17.7 $_{a}^{A}$	17.7 $_{a}^{A}$
SA injection (0.25 g/tree)	54.4 $_{a}^{A}$	53.8 $_{a}^{A}$	54.7 $_{a}^{A}$	54.1 $_{a}^{A}$	11.8 $_{a}^{A}$	11.3 $_{a}^{A}$	11.4 $_{a}^{A}$	11.6 $_{a}^{A}$	0.68 $_{a}^{A}$	0.61 $_{a}^{A}$	0.62 $_{a}^{A}$	0.63 $_{a}^{A}$	17.4 $_{a}^{A}$	18.5 $_{a}^{A}$	18.4 $_{a}^{A}$	18.3 $_{a}^{A}$

[z] Data shown are the means of six replicates ($n = 6$). Values in the same row followed by same superscript uppercase letters are not significantly different within each treatment for each parameter of fruit quality ($P > 0.05$). Values in the same column followed by same subscript lowercase letters are not significantly different at each sampling time for different treatments ($P > 0.05$)

in treated plants might not reach sufficiently high concentrations to kill or inhibit the growth of a large portion of bacterial cells in individual plants. Another potential explanation is that the HLB damages for the tested trees are too severe to respond to and recover from the treatments. Our previous study demonstrates that induction of plant defense by chemical inducers is more effective to young trees with mild HLB symptoms than older trees with severe HLB symptoms (Li et al. 2016).

The significant reduction of pre-harvest fruit drop by trunk-injected SAR inducers at 0.25 g/tree (Table 3) might be attributed to their suppressive effects on Las population growth in HLB diseased citrus trees. Excessive pre-harvest fruit drop, usually happening three to 4 months prior to harvest, is positively correlated with HLB disease severity (Tang et al. 2019). Earlier work has speculated that altered balance among the plant hormones abscisic acid, auxin, ethylene, and jasmonic acid caused by Las infection played a critical role in increasing pre-harvest fruit drop in HLB disease trees (Martinelli et al. 2012; Nehela et al. 2018). Most recently, the oxidative stress induced by Las infection was suggested to result in cell wall modification, causing cell separation, and eventually promote pre-harvest fruit drop in infected plants (Tang and Vashisth 2020). Furthermore, it was proposed that the HLB-tolerant 'LB8–9' mandarin might have an advanced antioxidant system to mitigate the pathogen-induced oxidative stress, thereby contributing to its significantly decreased pre-harvest fruit drop relative to the HLB-susceptible Hamlin sweet orange (Tang and Vashisth 2020). Whether the SAR inducers tested in this study improve the antioxidant system to mitigate the Las infection-induced pre-harvest fruit drop needs to be investigated.

Previous studies show that some plant defense inducers applied to citrus are effective in improvement of fruit quality, with a higher juice content and/or higher brix acidity ratio (Li et al. 2016; Hu et al. 2018). The results of this study indicate all the SAR inducers applied had trivial positive effect on fruit quality. ASM, IMI, INA, or SA injections at 0.25 g/tree resulted in a lower

Table 5 Economic analysis of SAR inducer treatment of citrus trees [a]

Year	Treatment	Material cost ($US/ha)	Application cost ($US/ha)	Picking & Transport cost ($US/ha)	Total cost ($US/ha)	Production total (kg/ha)	Average delivered-in price ($US/kg fruit)	Gross income ($US/ha)	Net income ($US/ha)	Added revenue ($US/ha)
2016	UTC	0.00	0.00	1598.10	1598.10	17,756.7	0.27	4794.31	3196.21	0.00
	INA spray (0.5 g/tree)	705.65	333.58	1788.11	2827.34	19,867.9	0.27	5364.33	2536.99	− 659.22
	SA spray (0.5 g/tree)	380.15	333.58	1716.86	2430.59	19,076.2	0.27	5150.57	2719.98	− 476.23
	ASM injection (0.25 g/tree)	409.99	166.79	1954.37	2531.15	21,715.2	0.27	5863.10	3331.95	135.74
	IMI injection (0.25 g/tree)	77.31	166.79	1991.69	2235.79	22,129.9	0.27	5975.07	3739.28	543.07
	INA injection (0.25 g/tree)	155.51	166.79	2059.55	2381.85	22,883.9	0.27	6178.65	3796.80	600.59
	SA injection (0.25 g/tree)	74.14	166.79	2032.41	2273.34	22,582.3	0.27	6097.22	3823.88	627.67
2018	UTC	0.00	0.00	1645.61	1645.61	18,284.5	0.29	5302.51	3656.90	0.00
	INA spray (0.5 g/tree)	705.65	333.58	2059.55	3098.78	22,883.9	0.29	6636.33	3537.55	−119.35
	SA spray (0.5 g/tree)	380.15	333.58	2147.77	2861.50	23,864.1	0.29	6920.59	4059.09	402.19
	ASM injection (0.25 g/tree)	409.99	166.79	2110.45	2687.23	23,449.4	0.29	6800.33	4113.10	456.2
	IMI injection (0.25 g/tree)	77.31	166.79	2052.77	2296.87	22,808.5	0.29	6614.47	4317.60	660.7
	INA injection (0.25 g/tree)	155.51	166.79	2029.01	2351.31	22,544.6	0.29	6537.93	4186.62	529.72
	SA injection (0.25 g/tree)	74.14	166.79	2117.23	2358.16	23,524.8	0.29	6822.19	4464.03	807.13
Cumulative	UTC	0.00	0.00	3243.71	3243.71	36,041.2	0.28	10,096.82	6853.11	0.00
	INA spray (0.5 g/tree)	1411.30	667.16	3847.66	5926.12	42,751.8	0.28	12,000.66	6074.54	− 778.57
	SA spray (0.5 g/tree)	760.30	667.16	3864.63	5292.09	42,940.3	0.28	12,071.16	6779.07	−74.04
	ASM injection (0.25 g/tree)	819.98	333.58	4064.82	5218.38	45,164.6	0.28	12,663.43	7445.05	591.94
	IMI injection (0.25 g/tree)	154.62	333.58	4044.46	4532.66	44,938.4	0.28	12,589.54	8056.88	1203.77
	INA injection (0.25 g/tree)	311.02	333.58	4088.56	4733.16	45,428.5	0.28	12,716.58	7983.42	1130.31
	SA injection (0.25 g/tree)	148.28	333.58	4149.64	4631.50	46,107.1	0.28	12,919.41	8287.91	1434.8

[a]Production was calculated as kg fruit per tree (the mean value per year was used), multiplied by the numbers (377) of trees per hectare. Treatment costs included the materials for SAR inducer applications and the costs of applying these treatments per crop season. Costs of materials were based on quotes from chemical supply companies in 2015 (ASM: $US 145.00 per 100 g; IMI: $US 27.34 per 100 g; INA: $US 55.00 per 100 g; SA: $US 141.10 per 500 g; Induce adjuvant: $US 7.39 per 1.0 L). Application costs for treatments were $US 68.92/ha for 934.7 L per ha (100 gal/acre) air-blast nutrient or pesticide applications. Picking & Transport costs were calculated on the basis of the mass of fruit harvested from each treatment and assuming $US 3.50 per 40.82 kg (Singerman and Futch 2018). Gross income was based on the average delivered-in price per year and the mean delivered-in price for cumulative estimates. Net income is the gross income minus input costs per hectare. Added revenue is the net income relative to untreated control

acidity and higher brix acidity ratio compared with the UTC or compared among years, but the differences were not statistically significant (Table 4). The discrepancy between this study and previous studies may be because of the differences in tree age, HLB disease severity, physiological conditions of the treated citrus trees, and the differences in local climate conditions. The spray treatment of INA or SA (0.5 g/tree) increased yield compared with the untreated control at the end of this trial, but its cost was over the revenue and might not be profitable in its present form (Table 5). Trunk injection of ASM, IMI, INA, or SA at 0.25 g/tree consistently increased yields compared with the untreated control in both 2016 and 2018, and the estimated revenue exceeded the total cost, with an assumed application cost equal to spray (Table 5). Therefore, these treatments might be profitable with the prerequisite that automated injection systems with high efficiency and low cost are available for commercial citrus production as discussed earlier.

Conclusions

This study showed that three trunk injections of the SAR inducers ASM, IMI, INA, or SA at a relatively low rate (0.25 g/tree) annually exhibited significant positive effects to slow down HLB disease progression and sustain fruit yield. Trunk injection of SAR inducers is more effective in controlling HLB than foliar spray and soil drench, and might be profitable. Further investigations are required to optimize injection systems for large-scale applications and to optimize SAR inducer uptake via foliar spray for HLB disease control. Finally, this study provides useful information regarding developing management programs with SAR inducers for effective control of citrus HLB.

Methods

Field sites and experimental design

The field trial was conducted with 17-year-old Hamlin sweet orange (*Citrus sinensis* [L.] Osbeck) on Swingle citrumelo (*C. paradisi* Macf. 'Duncan' grapefruit × *Poncirus trifoliata* [L.] Raf.) rootstock trees planted in 1999 in a citrus grove at Citrus Research and Education Center (CREC) in Lake Alfred, Florida from 2015 to 2018. The grove was established on the well-drained soil of Astatula fine sand (Typic Quartzipsamment) and with all trees being HLB positive. The grove received standard commercial care including normal irrigation and fertilization as well as conventional pest management practices throughout the experiments. One-week prior to the start of the trial, a field survey was carried out to determine the HLB severity via visual assessment and Las titers using quantitative real-time PCR (qPCR) assays. Trees with similar HLB severity were selected for the tests. Treatments were arranged in a randomized complete block design (RCBD) with 25 treatments (including an untreated control) replicated three times in blocks of two contiguous trees. Eight foliar spray treatments were ASM (Actigard 50 WG, 50% a. i., Syngenta Crop Protection), IMI (Admire Pro, 42.8% a.i.; Bayer Crop Science), INA (Astatech Inc., PA), and SA (Sigma-Aldrich, St. Louis, MO) at 0.25 and 0.5 g/tree per application that were applied six times per year. Eight soil drench treatments included ASM, IMI, INA, and SA at 0.25 and 0.5 g/tree per application in six applications per year. Eight trunk injection treatments consisted of ASM, IMI, INA, and SA at 0.125 and 0.25 g/tree per application in three applications per year. Products were mixed with water. The untreated control trees received a water-only spray treatment at each foliar spray time. The materials, application rates, and number and dates of application for each treatment were listed in Table 6.

Treatment applications

Six sprays per year were conducted by bi-monthly spraying ~ 2.0 L of the treatment solution with 2.5 mL/L of Induce non-ionic spreader-sticker adjuvant (Helena Chemical Company) onto each tree at sunny days. Treatments were initiated during the fall flush in early November 2015. Individual treatments were applied with a power-pumped sprayer at 400 kPa of air pressure until runoff to ensure complete coverage.

Six soil applications per year were made bi-monthly as soil drenches at 4.0 L/tree to the soil surface in a crescent within 75 to 100 cm of the trunk on the top side of the bed to minimize runoff at sunny days during the spring, summer, summer-fall, and fall flush. Treatments were initiated during the fall flush in early November 2015. To facilitate infiltration, microsprinkler irrigation was applied for a few minutes to prewet the soil surface before drenching.

For trunk injection, one injection port per tree positioned at approximately 20 cm above bud union was made by drilling 20–30 mm into the xylem tissue with a 7.14 mm drill bit. The port was sealed with Arborplug® No. 3 using Arborplug® setter and a rubber hammer (Arborjet Inc., Woburn, MA, USA). Treatment solution in a pressurized bottle was injected into the trunk through the port using Arborjet tree I.V. at the recommended pressure (~ 345 kPa) during sunny days. The area surrounding drilling site was sprayed with 20 mL of Mefenoxam solution (337.5 ml/L RidomilGold®SL, Syngenta Crop Protection) at the maximum label rate (2337 mL per hectare per application) for citrus based on 346 trees per hectare to prevent infection by *Phytophthora* spp. Each treatment required 12–24 h to inject the solution (400 mL) into the tree. Three injections per year of different treatments were conducted when new flush

Table 6 Treatments applied to Hamlin sweet orange trees in a three-year field trial (2015–2018) at CREC-Lake Alfred, FL

Treatment[a]	Applications			Application dates		
	Number[b]	Rate[c] (g/tree)	Volume (mL/tree)	2015 & 2016	2017	2018
UTC	6	–	2000	11/2, 2/2, 4/5, 6/3, 8/5, 10/10, 12/8	2/1, 4/6, 6/8, 8/10, 10/12, 12/7	2/5, 4/3, 6/7, 8/9, 10/12
ASM spray	6	0.25	2000	11/2, 2/2, 4/5, 6/3, 8/5, 10/10, 12/8	2/1, 4/6, 6/8, 8/10, 10/12, 12/7	2/5, 4/3, 6/7, 8/9, 10/12
ASM spray	6	0.5	2000	11/2, 2/2, 4/5, 6/3, 8/5, 10/10, 12/8	2/1, 4/6, 6/8, 8/10, 10/12, 12/7	2/5, 4/3, 6/7, 8/9, 10/12
ASM soil drench	6	0.25	4000	11/2, 2/2, 4/5, 6/3, 8/5, 10/10, 12/8	2/1, 4/6, 6/8, 8/10, 10/12, 12/7	2/5, 4/3, 6/7, 8/9, 10/12
ASM soil drench	6	0.5	4000	11/2, 2/2, 4/5, 6/3, 8/5, 10/10, 12/8	2/1, 4/6, 6/8, 8/10, 10/12, 12/7	2/5, 4/3, 6/7, 8/9, 10/12
ASM trunk injection	3	0.125	400	11/3, 2/3, 6/4, 10/11	2/2, 6/9, 10/13	2/6, 6/8, 10/15
ASM trunk injection	3	0.25	400	11/3, 2/3, 6/4, 10/11	2/2, 6/9, 10/13	2/6, 6/8, 10/15
IMI spray	6	0.25	2000	11/2, 2/2, 4/5, 6/3, 8/5, 10/10, 12/8	2/1, 4/6, 6/8, 8/10, 10/12, 12/7	2/5, 4/3, 6/7, 8/9, 10/12
IMI spray	6	0.5	2000	11/2, 2/2, 4/5, 6/3, 8/5, 10/10, 12/8	2/1, 4/6, 6/8, 8/10, 10/12, 12/7	2/5, 4/3, 6/7, 8/9, 10/12
IMI soil drench	6	0.25	4000	11/2, 2/2, 4/5, 6/3, 8/5, 10/10, 12/8	2/1, 4/6, 6/8, 8/10, 10/12, 12/7	2/5, 4/3, 6/7, 8/9, 10/12
IMI soil drench	6	0.5	4000	11/2, 2/2, 4/5, 6/3, 8/5, 10/10, 12/8	2/1, 4/6, 6/8, 8/10, 10/12, 12/7	2/5, 4/3, 6/7, 8/9, 10/12
IMI trunk injection	3	0.125	400	11/3, 2/3, 6/4, 10/11	2/2, 6/9, 10/13	2/6, 6/8, 10/15
IMI trunk injection	3	0.25	400	11/3, 2/3, 6/4, 10/11	2/2, 6/9, 10/13	2/6, 6/8, 10/15
INA spray	6	0.25	2000	11/2, 2/2, 4/5, 6/3, 8/5, 10/10, 12/8	2/1, 4/6, 6/8, 8/10, 10/12, 12/7	2/5, 4/3, 6/7, 8/9, 10/12
INA spray	6	0.5	2000	11/2, 2/2, 4/5, 6/3, 8/5, 10/10, 12/8	2/1, 4/6, 6/8, 8/10, 10/12, 12/7	2/5, 4/3, 6/7, 8/9, 10/12
INA soil drench	6	0.25	4000	11/2, 2/2, 4/5, 6/3, 8/5, 10/10, 12/8	2/1, 4/6, 6/8, 8/10, 10/12, 12/7	2/5, 4/3, 6/7, 8/9, 10/12
INA soil drench	6	0.5	4000	11/2, 2/2, 4/5, 6/3, 8/5, 10/10, 12/8	2/1, 4/6, 6/8, 8/10, 10/12, 12/7	2/5, 4/3, 6/7, 8/9, 10/12
INA trunk injection	3	0.125	400	11/3, 2/3, 6/4, 10/11	2/2, 6/9, 10/13	2/6, 6/8, 10/15
INA trunk injection	3	0.25	400	11/3, 2/3, 6/4, 10/11	2/2, 6/9, 10/13	2/6, 6/8, 10/15
SA spray	6	0.25	2000	11/2, 2/2, 4/5, 6/3, 8/5, 10/10, 12/8	2/1, 4/6, 6/8, 8/10, 10/12, 12/7	2/5, 4/3, 6/7, 8/9, 10/12
SA spray	6	0.5	2000	11/2, 2/2, 4/5, 6/3, 8/5, 10/10, 12/8	2/1, 4/6, 6/8, 8/10, 10/12, 12/7	2/5, 4/3, 6/7, 8/9, 10/12
SA soil drench	6	0.25	4000	11/2, 2/2, 4/5, 6/3, 8/5, 10/10, 12/8	2/1, 4/6, 6/8, 8/10, 10/12, 12/7	2/5, 4/3, 6/7, 8/9, 10/12
SA soil drench	6	0.5	4000	11/2, 2/2, 4/5, 6/3, 8/5, 10/10, 12/8	2/1, 4/6, 6/8, 8/10, 10/12, 12/7	2/5, 4/3, 6/7, 8/9, 10/12
SA trunk injection	3	0.125	400	11/3, 2/3, 6/4, 10/11	2/2, 6/9, 10/13	2/6, 6/8, 10/15
SA trunk injection	3	0.25	400	11/3, 2/3, 6/4, 10/11	2/2, 6/9, 10/13	2/6, 6/8, 10/15

[a] UTC: untreated control, ASM: acibenzolar-*S*-methyl, IMI: imidacloprid, INA: 2,6-dichloroisonicotinic acid, SA: salicylic acid
[b] Number of applications per year
[c] Amount of treatment (a.i.) per tree per application

was present in spring, summer, and fall. Treatments were initiated during the fall flush in early November 2015.

HLB disease severity assessment

Disease severity data were collected by visual assessments as described by Gottwald et al. (2007) on a 6-month interval from November 2015 through October 2018. Briefly, the canopy was divided horizontally and vertically into 8 sections and disease severity was scored for each section using a scale from 0 to 5 that indicates the proportion of limbs expressing HLB symptoms, where 0 = no limbs; 1 = 1 to 20% limbs; 2 = 21 to 40% limbs; 3 = 41 to 60% limbs; 4 = 61 to 80% limbs; and 5 = 81 to 100% limbs. An overall severity rating of 0 to 40 for each tree was obtained by summarizing these 8 scores of an individual tree and used to calculate the value of disease severity, expressed as the standardized area under the disease progress stair (sAUDPS) as described by Simko and Piepho (2012).

Quantitative real-time PCR assays for evaluation of Las titers in citrus leaves

To estimate the Las titer in treated trees, eight leaves with mottling symptoms were randomly collected from each tree to pool together as one biological replicate. For each pooled sample, 100 mg of midribs was excised for DNA extraction. DNA was extracted from macerated midrib tissues using the Wizard Genomic DNA purification kit (Promega) following the manufacturer's recommendations. The air-dried DNA pellet was dissolved in 50–100 μL of DNA rehydration solution (Promega) and

kept at − 20 °C. Quantitative real-time PCR (qPCR) was performed with the Las specific primer-probe set "CQULA04F-CQULAP10-CQULA04R" (Wang et al. 2006; Trivedi et al. 2009) using an ABI PRISM 7500 sequence detection system (Applied Biosystems, Foster City, CA). qPCR was performed in 20 μL of RT-qPCR reaction mixture that contained 10 μL of 2 × Quantitect probe PCR master mix (Qiagen, Valencia, CA), 500 nM of each primer, 200 nM of probe, and 100 ng of DNA template. The qPCR reactions included 95 °C denaturation for 15 min, followed by 40 cycles of 94 °C for 15 s and 60 °C for 1 min. All DNA samples were run in triplicate. The cycle threshold (Ct) values were obtained by adjusting threshold to the recommended level of 0.02 and were converted to the estimated bacterial titers with a standard equation as described by Li et al. (2016).

Fruit drop, yield and quality analyses

Pre-harvest fruit dropped was counted when fruit was harvested in each crop season. Fruits were harvested and weighed in December 2015, 2016, 2017, and 2018. The yield data were collected for each treated tree. For each treatment, a bag of fruit sample (~ 8.5 kg) was randomly sampled from each of three selected trees and subjected to fruit quality analysis using a standard procedure (Gottwald et al. 2012). Juice content and fruit acidity were expressed as percent juice and percent citric acid. Total soluble solids were expressed as fruit brix (the measure of sugar content in fruit, i.e., 1 g of sugar/100 g of juice is equivalent to 1° of Brix). Thereafter, fruit brix and acidity ratio were calculated accordingly.

Economic analysis

The costs and benefits of treatments were estimated by multiplying yield (kg fruit/ha) by the published values of marketable fruit per ha for the 2016 and 2018 growing seasons. The costs included the materials for SAR inducers and the costs of applying these treatments per growing season. Material costs were based on quotes from chemical supply companies in 2015 (ASM: $US 145.00 per 100 g; IMI: $US 27.34 per 100 g; INA: $US 55.00 per 100 g; SA: $US 141.10 per 500 g; Induce adjuvant: $US 7.39 per 1.0 L). Application costs for spray treatments were $US 68.92/ha for 934.7 L per ha (100 gal/acre) air-blast nutrient or pesticide applications (Tansey et al. 2017). No published value for application cost of trunk injection was available at the time this work, so the application cost of trunk injection was estimated with that of spray. Costs also included transport and picking costs of harvested oranges ($US 3.50 per 40.82 kg) (Singerman and Futch 2018). Mean delivered-in prices for Hamlin oranges for processing in Florida were $US 0.27/ kg fruit ($US 11.16 per 40.82 kg) for 2016–2017 (National Agricultural Statistics Service 2018) and $US 0.29 /kg fruit ($US 11.97 per 40.82 kg) for 2018–2019 (National Agricultural Statistics Service 2020). Net income was calculated by subtracting total cost from the gross income per ha for each treatment. Added revenue was obtained by subtracting the net income of untreated control from the net income of each treatment.

Statistical analysis

All data analyses were performed using SAS V9.4 (SAS Institute Inc., Cary, NC). The data were first tested for normality and homogeneity of variance using the Shapiro-Wilk test and Levene's test, respectively. Prior to analysis, Las titers were $\log_{10}$ transformed to satisfy assumptions of normality and homoscedasticity. The main effects of treatment and time on Las titer, fruit drop, yield, and fruit quality were determined using repeated measures analysis of variance (ANOVA) with the MIXED procedure in SAS, with the first order autoregressive (AR (1)) covariance structure option based on the Akaikei and Bayesian information criteria. When treatment effects were significant ($P < 0.05$), mean separation was made by pairwise comparisons using the LSMEANS statement with the Tukey honestly significant difference ($\alpha = 0.05$) adjustment. When interactions of factors were significant ($P < 0.05$), the SLICE option of the LSMEANS statement was used to identify the significant factor effect. A one-way ANOVA was performed to determine any differences in treatment effects on sAUDPS and the treatment means were separated using Tukey's HSD test ($\alpha = 0.05$).

Abbreviations

ANOVA: Analysis of variance; ASM: Acibenzolar-S-methyl; BTH: Benzothiadiazole; CREC: Citrus Research and Education Center; FL: Florida; HLB: Huanglongbing; IMI: Imidacloprid; INA: 2,6-dichloroisonicotinic acid; Las: *Candidatus* Liberibacter asiaticus; Laf: *Candidatus* Liberibacter africanus; Lam: *Candidatus* Liberibacter americanus; PR: Pathogenesis-related; qPCR: Quantitative real-time PCR; SA: Salicylic acid; SAR: Systemic acquired resistance; sAUDPS: Standardized area under the disease progress stairs

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Authors' contributions

NW and JL conceived and designed the experiments. JL, VK, ZP, SD, DL, YH, JX, DP, TL, and NW carried out the experiments. JL and NW analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

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