Impact of Foliar Application of Acibenzolar-*S*-Methyl on Rose Rosette Disease and Rose Plant Quality

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Abstract

Rose rosette disease (RRD) caused by rose rosette emaravirus (RRV) is a major issue in the U.S. rose industry with no effective method for its management. This study evaluated the effect of foliar application of Acibenzolar *S*-methyl (ASM), a plant systemic acquired resistance inducer in reducing RRD disease severity on *Rosa* species cv. Radtkopink (Pink Double Knock Out®) under greenhouse condition, and the effect of ASM on plant growth under commercial nursery production conditions. ASM at 50 or 100 mg/L at weekly intervals significantly reduced RRD severity compared to the untreated control in two of the three greenhouse trials ($P < 0.05$). The plants in these trials were subsequently pruned and observed for symptoms, which further

indicated that application of ASM at 50 or 100 mg/L lowered disease severity compared to the untreated control ($P < 0.05$) in these two trials. Plants treated with ASM at 50 or 100 mg/L had delayed incidence of RRD compared to the non-treated controls. Plants treated with ASM at 50 or 100 mg/L rate in all three trials either did not have RRV present or the virus was present in fewer leaf samples than untreated controls as indicated by RT-qPCR analysis. Overall, plants treated with ASM at 50 mg/L had 36-43% reduced RRD incidence compared to the water control. The treatment of two cultivars of rose, 'Radtkopink' and 'Meijocos' (Pink Drift®), with weekly foliar applications of ASM at three rates (0.5, 0.75 and 1.0 oz/A) indicated that ASM had no negative effect on flowering or plant growth at even the highest rate.

Keywords: Plant activator, systemic acquired resistance inducer, Rose viruses, *Phyllocoptes fructiphilus*

Introduction

Roses are one of the most popular ornamental flowering shrubs in the United States. In the U.S., the total wholesale production of roses accounted for $204 million (United States Department of Agriculture 2015). Rose rosette disease (RRD), caused by rose rosette emaravirus (RRV), has become the most devastating disease on roses, in recent years, causing huge economic losses to rose nurseries, landscape industries and home gardeners (Laney et al. 2011). RRV is transmitted by the eriophyid mite species *Phyllocoptes fructiphilus* Keifer (Acari: Eriophyidae) (Amrine 1988; Laney et al. 2011; Di Bello et al. 2015; Di Bello et al. 2017). Additionally, budding or grafting also can transmit the virus. The symptoms associated with RRD are highly variable and include lateral shoot growth, excessive thorniness, witches' broom, leaf proliferation and

distortion, mosaic, and red pigmentation of the leaves and the stems. RRD can also lead to the death of the plant (Amrine 2002). The virus infects many rose species and cultivars (Babu et al. 2017), and it is widespread through much of the wild and cultivated rose population of the Midwestern, Southern, and Eastern U.S. With increased interstate commerce and no known resistant commercial cultivars (Byrne et al. 2018), the risk for spread of RRD to areas where the RRV/eriophyid mite vector is not established also has increased. The disease affects all the traditional rose varieties including the Knock Out® series, which is of high relevance to the shrub rose industry. RRD now threatens the U.S. rose industry and has the potential to affect the nursery and landscape industry severely.

In Florida, RRD was confirmed in 2013 and 2014 for the first time in three counties. However, *P. fructiphilus* was not found in these infected roses (Babu et al. 2014). The first confirmation of the disease was in plants that were shipped from Oklahoma, where the disease and the vector, *P. fructiphilus,* are both widespread both in commercial production and in the landscape (Olson and Rebek 2014). The early discovery of infected plants introduced from outside the state of Florida led to their destruction. RRV currently is not known to be established in Florida. However, a survey conducted in Georgia, Alabama and Mississippi in 2017, confirmed that RRV and *P. fructiphilus* are widespread in the landscape in these neighboring states (Solo et al. 2020). Most recently, *P. fructiphilus* was confirmed in the landscape in North Florida (Martini et al. 2019; Fife et al. 2020). Even though the disease is not established in Florida, with the presence of RRV in all neighboring states and *P. fructiphilus* in all neighboring states and in North Florida, the shipment of roses from other states with RRV is a risk to the rose industry in Florida.

Currently, a significant limitation in preventing RRD is the absence of any chemical and biological control options for preventative management of the disease during early production age (1-2 years old). Many synthetic phytohormone analogs have been developed to elicit plant defense responses prior to pathogen attack. Among them, Acibenzolar *S*-methyl (ASM; Actigard™, Syngenta 2015) is a systemic acquired resistance inducer labelled on vegetables and fruit trees that is known to activate the plant defense system by increasing the transcription of stress related genes. ASM is labelled for use on crops for the management of plant diseases caused by bacterial, fungal and oomycete pathogens. Additionally, ASM by itself or integrated with other methods has been shown to protect plants from *Tomato spotted wilt virus* in tomato and tobacco (Mandal et al. 2008; Momol et al. 2004), *Iris yellow spot virus* in tobacco (Tripathi and Pappu 2015), *Cucumber mosaic virus* and *Cucurbit yellow stunting disorder virus* (Kenney et al. 2020). We hypothesize that ASM application will provide protection to roses from RRD by preventing disease incidence or reducing disease severity.

The objectives of this study were to 1) evaluate the effect of ASM in reducing RRD severity and incidence; and 2) to evaluate the effect of ASM on plant growth and flowering. The goal of this research is to analyze the potential for ASM as a tool in protecting roses against RRD during early stages in their production.

Materials and Methods

Evaluating the efficiency of budding in transmitting RRV on different rose species.

Rosa species cv. Radtkopink (Pink Double Knock Out®) Shrub type (1.5 years old) and Patio type (5 years old), Seven sister rose (*Rosa multiflora*; 1 year old), and Cherokee rose (*Rosa laevigata;* 1 year old) were used to evaluate the efficiency of budding in transmitting RRV.

Budding was used as the inoculation procedure instead of viruliferous *P. fructiphilus* due to quarantine regulations established in Florida during the period of the study. The collected varieties (n = 5) were budded with two to three buds from a mother plant (Pink Double Knock Out®) shrub type with a RRV disease severity of 100%. The plants were monitored for symptoms starting from two days after the budding until nine months. The presence of RRV in the leaves was confirmed by RRV specific RT-qPCR or RT-PCR (Babu et al. 2016; Laney et al. 2011).

Acibenzolar *S*-methyl (ASM) as a protectant in reducing RRD severity and incidence. Three trials were conducted in the RRV quarantine greenhouse facility at the University of Florida, North Florida Research and Education Center located in Quincy, FL. Pink Double Knock Out® rose plants (shrub type) that were 1.5 years old were used for trials 1 and 3; plants that were 1-year old were used for trial 2. The roses were maintained as per production guidelines in southern and central U.S. (Dunwell et al. 2014; Knox et al. 2012); and were tested negative for RRV initially (data not shown). Trial 1 consisted of three treatments with three replicates per treatment. Treatments were ASM - foliar (50 and 100 mg/L) and a control treated with water only (NTC = water treatment). The foliar application was sprayed to run-off. The plants were arranged in a randomized complete block design. The plants were treated initially with ASM or NTC at weekly intervals for two weeks. This was followed by budding the plants one week after the second ASM application with buds from a RRV infected mother plant with a disease severity of 100%, and leaves were confirmed positive for RRV by RT-qPCR (Babu et al. 2016). A total of three buds were used per plant. After budding, the application of ASM - foliar (50 and 100 mg/L) and NTC were continued at weekly intervals for 11 more applications. The plants were monitored for symptom expression starting one week after budding in all trials. The

disease severity for all trials was assessed at weekly intervals starting from the day of initial symptom expression in any of the plants based on Horsfall-Barratt (H-B) scale (Horsfall and Barratt 1945). Trial 2 consisted of the same treatments listed above with three plants per treatment. Trial 3 consisted of the same treatments listed above with eight plants per treatment. The H-B values from all trials were converted to midpoint averages and area under disease progression curve (AUDPC) was calculated (Horsfall and Barratt 1945; Paret et al. 2013). To ensure that plants that were asymptomatic or having reduced disease severity represent a long-term effect, the roses in all trials were pruned to a height of 20 cm after one month from the end of the trial to remove all branches and leaves. The pruner was sanitized with 70% ethanol between plants. Disease severity assessments were conducted starting one week after pruning and data collected at weekly intervals for 6 (trial 1), 7 (trial 2) and 4 (trial 3) times. Subsequently, the plants that remained asymptomatic at the end of the trials were kept for 18-34 months for the monitoring of expression of any symptoms. The details of the treatments and number of plants are summarized in Table 1.

RRV-specific RT-qPCR analysis of plants treated with Acibenzolar S-methyl (ASM)

The plants in all the trials were tested for the presence or absence of virus using established protocol for the RRV specific RT-qPCR (Babu et al. 2016) using SuperScript® III Platinum® one-step qRT-PCR Kit w/ROX (Invitrogen, Life Technologies, Grand Island, NY). Testing was performed using total RNA extracted from 100 mg of leaf tissues with the Qiagen RNeasy plant mini kit (Qiagen Inc., Valencia, CA). For trial 1, the first and second testing were performed 13 and 17 weeks after budding, in trials 2 and 3, 13 and 20 weeks, respectively after budding. In trial 1, multiple leaf samples including symptomatic, asymptomatic, young and old leaves were used in the analysis. The details of the samples including distance from the bud, and symptom

variations are reported in Table 2. In trials 2 and 3, limited leaf samples were used from treatments showing prominent RRV symptoms while majority of the samples were from treatments showing no or low disease severity (Table 3).

Impact of Acibenzolar *S*-methyl on rose plant quality, flowering and phytotoxicity

Two rose cultivars, Pink Double Knock Out® (shrub type) and Pink Drift® (both 1.5 years old), were treated weekly with foliar application of ASM at 0.5 (low rate; 50 mg/L), 0.75 (medium rate; 75 mg/L) and 1.0 oz./A (high rate; 100 mg/L)) and non-treated control (water). A backpack sprayer (15 L, piston) with a plastic adjustable nozzle was used for application of water and ASM (Solo Inc., Newport News, VA). Two trials, each containing 40 Pink Double Knock Out roses® and 40 Pink Drift® roses were evaluated for plant responses to ASM at Monrovia Nursery, Cairo, Georgia in Summer 2016. Trial 1 included 13 consecutive weekly treatments, and trial 2 for 12 weeks. The experiments were organized into two replications of 20 plants of each cultivar per trial for a total 160 rose plants evaluated. The cultivars were maintained separately, but individuals of each cultivar were randomized within each block based on treatment. All experimental rose plants received continued care by the commercial nursery identical to all other roses undergoing general nursery production (i.e. irrigation, fertilizer, pre-emergent herbicides, fungicides, and insecticides). Impact of ASM on roses was assessed weekly by counting open flowers, measuring plants (length, width, height), and rating phytotoxicity. Flower counts and plant volume were assessed weekly. Phytotoxicity was rated using a 1 to 5 scale in which 1 = no damage, 2 = slight spotting or discoloration of foliage or flowers, 3 = slight distortion of leaves and flowers and spotting/discoloration of foliage or flowers, 4 = slight stunting of growth, distortion of leaves and flowers, and spotting/discoloration of foliage or flowers, and 5 =

extensive damage, stunting of growth and distortion of leaves or flowers and spotting/discoloration of foliage or flowers.

Statistical analysis. The data from objective 1 was analyzed using SPSS version 25 (IBM Corporation, Armonk, NY). The data were analyzed first for normal distribution using the Shapiro-Wilk test. The data with normal distribution were analyzed by ANOVA. If statistical differences were noted, the means of treatments were separated using Student Newman Keuls Test ($\alpha = 0.05$), The data not normally distributed, were analyzed by the Independent Samples Kruskall-Wallis Test. If statistical differences were noted ($\alpha = 0.05$), the means were compared using Pairwise Comparisons between treatments with significance values adjusted by the Bonferroni correction for multiple tests ($\alpha = 0.05$). The data from objective 2 were analyzed using SigmaPlot version 13 (Systat Software Inc., San Jose, CA). The data were first analyzed for normal distribution. For understanding the effect of ASM on plant growth, the growth rate in 12 weeks was compared with two-way ANOVA (Growth × Cultivar × ASM) on log-transformed data. To assess unwanted effects of ASM on roses, plant height, length and width, and the numbers of flowers per plant were analyzed with ANOVA for repeated measures, to avoid pseudo replication. The effect of ASM applications on visible phytotoxic damages were assessed with a visual ranking, and then analyzed with a Kruskal-Wallis test, as the data were not normally distributed. When the ANOVA indicated a significant difference ($\alpha = 0.05$), a Tukey test was conducted to determine treatment differences.

Results

Efficiency of budding in transmitting RRV on different rose cultivars.

The budding experiment on all the four different rose cultivars produced prominent RRD symptoms (Fig. Supp.1), exhibited at different time intervals. Among the cultivars, Pink Double Knock Out® rose (shrub type) and Seven Sister rose showed symptoms within 1-2 weeks of budding, and Pink Double Knock Out® patio rose showed symptoms in about 1 month. Cherokee rose plants took more than 3 months to show symptoms. Symptoms also varied among different cultivars. Among cultivars, the most prominent symptoms of RRV, including witches' broom, abnormal redness, distortion and thorniness, were evident in Pink Double Knock Out® rose (shrub type), while the Pink Double Knock Out® Patio rose predominantly had abnormal redness and witches' broom. The Seven Sister rose mainly showed abnormal redness only. The Cherokee rose showed delayed symptom expression at ~ 3 months, with swollen bud growth. RRV-specific RT-qPCR or RT-PCR analysis on all the plant cultivars tested positive for RRV (Table Supp. 1).

Acibenzolar *S*-methyl (ASM) as a protectant in reducing RRD incidence and severity on Pink Double Knock Out® rose plants.

In all three trials the water control had very high severity of RRD as indicated by the AUDPC data (Figs. 1, 2, 3 and 4). In trial 1, before and after pruning, 100% of plants treated with ASM at 50 and 100 mg/L did not show symptoms, while 100% of plants treated with water exhibited symptoms (Table 1). For trial 1, before pruning, the AUDPC data was not normally distributed ($F(2,6) = 0.659$, $P = 0.0001$), and there were statistical differences between the treatments ($H = 7.624$, $P = 0.022$). Roses with applications of ASM at 50 mg/L (Mean rank = 3.50) and 100 mg/L (Mean rank = 3.50) had no symptoms of RRD, and both treatments were significantly different from the water control (Mean rank = 8.00) at $P = 0.050$ (Figs. 1A, 2). There were no statistical differences in AUDPC for ASM at 50 mg/L and 100 mg/L ($P = 1.000$). For trial 1, after pruning,

the AUDPC data were not normally distributed (F (2,6) = 0.619, P = 0.0001), and there were statistical differences between the treatments (H = 7.624, P = 0.022). At the end of this experiment water controls had very high severity of RRD as indicated by the AUDPC data while plants applied with ASM at 50 and 100 mg/L had no symptoms of RRD (Figs. 1B, 2). The asymptomatic plants treated with ASM (50 and 100 mg/L) and monitored for 34 months from the start of the experiment showed no visible symptoms of RRD (data not shown). In case of trial 1, the statistical difference in the AUDPC data between each treatment pairs were same for before and after pruning (Fig. 1A, 1B).

For trial 2, before pruning 100% of plants treated with ASM at 50 mg/L did not show symptoms, while 100% of plants treated with ASM at 100 mg/L, and 67% of plants treated with water exhibited symptoms (Table 1). The AUDPC data were not distributed normally (F (2,6) = 0.775, P = 0.011), and there were no significant differences between treatments (Fig. 3A; P = 0.129). The lowest AUDPC calculations were for plants treated with ASM at 50 mg/L. For trial 2, after pruning 100% of plants treated with ASM at 50 mg/L did not show symptoms, while 100% of plants treated with ASM at 100 mg/L and water exhibited symptoms (Table 1). The AUDPC data were normally distributed (F (2,6) = 0.871, P = 0.125), but there were no statistical differences between the treatments (Fig. 3A; P = 0.143). Like before pruning, the lowest AUDPC data were for plants treated with ASM at 50 mg/L, and no RRD symptoms were noted in this treatment. The asymptomatic plants treated with ASM at 50 mg/L and monitored for 18 months from the start of the experiment showed no visible symptoms of RRD (data not shown).

For trial 3, before pruning 75% of plants treated with ASM at 100 mg/L exhibited symptoms, while 100% of plants treated with water and ASM at 100 mg/L exhibited symptoms (Table 1). For trial 3, before pruning, the AUDPC data were normally distributed (F (2, 21) =

0.947, P = 0.237), and there were statistical differences between the treatments (P = 0.0001). ASM at 50 and 100 mg/L concentrations had significantly lower AUDPC measurements compared to the untreated control (P < 0.5; Fig. 4A). ASM at this 100 mg/L had significantly lower AUDPC calculations than ASM at 50 mg/L (P < 0.5). For trial 3, after pruning 100% of plants treated with water and ASM at 50 and 100 mg/L exhibited symptoms (Table 1). Only ASM at 100 mg/L had statistically lower AUDPC calculations than the water control (P < 0.5); however, AUDPC calculations for plants treated with ASM at 50 mg/L were not significantly different from plants treated with ASM at 100 mg/L and the water control (Fig. 4B).

For all trials combined, the non-treated control plants had no incidence of RRD for an average 0.5 weeks before pruning, and 0.4 weeks after pruning (Fig. 5). For plants treated with ASM at 50 mg/L, the average numbers of weeks with no incidence of RRD were 3.4 weeks before pruning, and 3.1 weeks after pruning. For plants treated with ASM at 100 mg/L, the average numbers of weeks with no incidence of RRD were 3.7 weeks before pruning, and 2.3 weeks after pruning.

RT-qPCR analysis of RRV levels in roses (Pink Double Knock Out®) treated with ASM during the period of the trials.

All leaves from the non-treated controls in trial 1 at the two sampling intervals were symptomatic and produced a positive amplification curve for RRV in 100% of the samples (n = 12/12, Table 2). All leaves from plants treated with ASM at 50 and 100 mg/L were asymptomatic and negative for RRV at both time points of analysis in 100% of the samples (n = 52). In trial 2, all symptomatic leaves from the water controls were positive for RRV which represented 67% of the samples (n = 4/6; Table 3). The two asymptomatic leaves from the water control were negative for RRV. All leaves from plants treated with ASM at 50 mg/L were

asymptomatic and negative for RRV in 100% of the samples (n = 10/10). All symptomatic leaves (75% of the samples) from ASM at 100 mg/L tested were positive for RRV (n=6/8). The two asymptomatic leaves from this treatment were negative for RRV. In trial 3, all leaves from the non-treated controls were symptomatic and produced a positive amplification curve for RRV in 100% of the samples (n = 6/6; Table 3). All leaves from plants treated with ASM at 50 mg/L were symptomatic and positive for RRV in 100% of the samples (n = 7/7). Leaves from plants treated with ASM at 100 mg/L were negative for RRV in 50% of the samples (n = 9/18).

When averages Ct (cycle threshold) values of all samples (positive or negative for RRV) were evaluated, significant differences were noted in trial 1, first and second testing dates for the water control (Mean rank/s = 25.50 and 32.50) compared to ASM at 50 or 100 mg/L (Mean rank/s = 11.50 and 15.00) (P = 0.0001) (Table 2). In trial 2, the Ct value of ASM at 50 mg/L (Mean rank = 7.50) was significantly lower than ASM at 100 mg/L (Mean rank = 17.50) (P = 0.003) (Table 3). No significant differences were noted in the Ct values between treatments in trial 3. The average Ct value of samples from the water control, which came positive for RRV in trial 1 for the first testing date, was 23.30, and for the second testing date was 23.21 (Table 2). In trial 2, the average Ct value of samples from non-treated control which came positive for RRV was 18.49 compared to ASM 100 mg/L at a higher Ct value of 24.66 (Table 3). In trial 3, the average Ct value of samples from water control that were positive for RRV was 23.27 compared 24.68 for ASM 50 mg/L and 24.67 for ASM 100 mg/L (Table 3). However, there were no significant differences between treatments for Ct values of RRV-positive samples.

Effect of ASM on rose plant width, length, height, and flower number; and phytotoxicity on Pink Double Knock Out® and Pink Drift® roses.

No significant effects of cultivar and ASM rates were found on rose growth (Table 4, Fig. 6). The height, length nor the width of the plants was affected by ASM application (Fig. 7; $P = 0.05$). Similarly, there was no significant difference among treatments regarding the numbers of flowers per plant ($P < 0.05$, Figs. 8A and 8B). However, the visual assessment of phytotoxic symptoms showed a significant difference, with more phytotoxic symptoms present on plants with the highest concentration of ASM (Figs. 8C and 8D). For Pink Double Knock Out® rose, we found a significant difference only at 49 days after application ($H = 12.582$, $P = 0.006$). There was more phytotoxic effect at the highest rate of ASM (1.0 oz/A) compared to the control ($P = 0.008$); however, other treatments did not differ from the control. For Pink Drift® roses, we found an increase of phytotoxic symptoms at 28 ($H = 9.894$, $P = 0.019$), 49 ($H = 10.273$), 56 ($H = 15.206$, $P = 0.002$), and 63 days ($H = 10.186$, $P = 0.017$) after the start of the treatments (Fig. 8).

Discussion

Despite being described since the middle of the past century, RRD is still a relatively new disease in the horticultural industry. This is because initially RRV was envisioned as a potential biological control agent to mitigate the spread of the noxious multiflora rose (Epstein and Hill 1999). In fact, a decline in the distribution of multiflora rose has been observed due to the effects of the virus (Banasiak and Meiners 2009). However, RRD has spread recently in commercial nurseries and caused a widespread death of commercial roses in the Midwestern and eastern regions of the United States.

There are currently few options to control RRD in nurseries. First, only 7% of rose cultivars are tolerant to RRD, and there is no known resistance among commercial rose cultivars

297 (Byrne et al. 2018). Second, there is no cure for RRD, and miticide treatments to control *P.*
298 *fructiphilus* are not entirely effective. Biological control of the vector has not been achieved,
299 even if Phytoseiidae (predatory mites) of the genus *Neoseiulus* and *Phytoseius* have been found
300 in association with *P. fructiphilus* in multiflora roses (Jesse et al. 2006). Predatory mites have
301 been used to control two spotted spider mites *Tetranychus urticae* Koch in roses (Zhang and
302 Sanderson 1995; Nicetic et al. 2001). However, there is no information of the potential of these
303 predatory mites and how seasonal fluctuations in its numbers could affect the control of *P.*
304 *fructiphilus* in a perennial crop like rose. Therefore, in the absence of resistant cultivars and
305 effective control of mites, there is a critical need in developing new methods to manage RRD
306 and prevent infection with the virus.
307 With the limitations in preventative measures and the absence of effective chemical and
308 biological control options for RRD, we investigated the use of Acibenzolar *S*-methyl (ASM;
309 Actigard™), a systemic acquired resistance inducer. In this study, the effect of ASM in reducing
310 RRD severity and incidence of infected roses, and the effects of ASM on plant growth, flowering
311 and phytotoxicity were evaluated. This study indicates that rose plants (Pink Double Knock
312 Out®) treated with ASM at 50 mg/L overall across all trials had 36-43% reduced RRD incidence
313 compared to the water control. ASM also has potential to reduce RRD symptom severity and
314 incidence at 50 or 100 mg/L rates. This impact was confirmed by pruning the plants and
315 conducting further evaluation of treated plants after pruning. This experiment demonstrated that
316 the impact of ASM on disease severity and incidence remained for the life of the experiment.
317 However, in trial 2 ASM at 100 mg/L showed no effect in reduction in RRD disease severity
318 compared to the water control and ASM at 50 mg/L. This may be due to the use of high
319 concentrations of ASM on 1-year-old plants causing plant stress that may cause it to be less

tolerant to pathogen compared to 1.5 year old ones used for trial 1 and trial 3. On labelled crops, ASM use rates typically starts low and increases as plants age (Syngenta 2015) and this approach may have to be tested in the future on roses. While no visual phytotoxicity was noted in trial 2, field trials on rose plant quality and flowering with ASM at the highest rate of 1.0 oz/A showed some marginal effects on plant quality with repeated applications. Interestingly, ASM also has been shown to reduce yield in cantaloupe in fields with lower disease levels of *Erwinia tracheiphila* and cucumber beetles, the insect vector of bacterial wilt of cantaloupe, while at high bacterial wilt severity and cucumber beetle presence ASM was effective in reducing the disease (Egel et al. 2018). In our studies, trial 2 had lower disease severity maximums (AUDPC of 652 – before pruning, and 814 – after pruning) in water control compared to trial 1 (1680 – before pruning and 3486 – after pruning) and trial 3 (2232 – before pruning and 1543 – after pruning) for non-treated. The lower disease severity in trial 2 and plant age compared to trial 1 and 3 independently or in combination, may have contributed to the higher AUDPC for ASM at 100 mg/L in trial 2.

The RRV-specific RT-qPCR were negative for roses treated with ASM at 50 or 100 mg/L in trial 1, and ASM at 50 mg/L in trial 2, indicating that either the roses were not infected, or the virus titer was reduced to an undetectable level. Finally, the roses treated with ASM were not impacted by the treatment, with similar growth among treatments, similar flower production, and no increase in phytotoxicity up to 0.75 oz/A rate. However, an increase in phytotoxicity was observed at 1.0 oz/A. Similar observation has been seen on foliar applications of ASM causing phytoxicity on tobacco (LaMondia 2002, Mandal et al. 2008)

The research presented is the first demonstration of a potential chemical option to manage RRD. However, all our studies were conducted using buds from infected plants as the

source for RRV while in nature, RRV is spread primarily by *P. fructiphilus*. Also, the studies of impact of ASM on RRD incidence and severity were conducted on a single rose cultivar Pink Double Knock Out®. It would be important to evaluate the impact of ASM in reducing RRD in the presence of *P. fructiphilus* under field conditions on different cultivars. Currently, management of RRD requires a multistep approach that includes pruning, sanitation, removal of wild roses in the area, mixed planting and sanitation, with removal of diseased foliage (Olson et al. 2015). In addition, the erection of protective barriers has been shown to decrease mite migration, because most eriophyid mites disperse primarily through passive aerial dispersal (de Lillo and Skoracka 2010). Currently, there are no methods capable of preventing RRV transmission. This research shows the potential role that SAR inducers could play as a component in the development of integrated management system.

ASM has been previously found to be a potential management option to prevent different types of plant virus including *Tomato spotted wilt virus*, *Cucumber mosaic virus* or *Cucurbit chlorotic yellows virus* (Smith-Beckera et al. 2003; Mandal et al. 2008; Takeshita et al. 2013). In order to prevent RRV infection, the timing of application is crucial. Previous research on ASM indicated that early treatment of the plants is critical to prevent infection with a pathogen (Cisno et al. 2001; Baysal et al. 2003). Indeed, systemic acquired resistance is associated with various cellular defense responses that need to be activated before pathogen infestation. These include accumulation of reactive oxygen species, enhanced activity of various defense-related enzymes, and synthesis of pathogenesis-related proteins such as hydrolytic enzymes, chitinases and β-1,3-glucanases and phytoalexins (Ryals et al. 1996; Conrath et al. 2001; Baysal et al. 2003; Fu and Dong 2013).

Further experiments are needed to determine if ASM is also impacting the population of *P. fructiphilus*. Previous experiments on other crops demonstrated that mite pests might be affected by the application of a salicylic acid analogue. Strawberries treated with ASM had the same proportion of damage attributed to mites (Tomazeli et al. 2016); however, neither the mite species nor the density of mites were determined. Conversely, a study conducted in an apple orchard showed that ASM significantly reduced the population of the European red mite (*Panonychus ulmi* Koch) without impacting the population of predatory mites (Warabieda 2015). Other plant defense inducers also demonstrated some effects on mite populations, including methyl jasmonate that reduced two-spotted spider mite *T. urticae* populations in ornamental plants (Rohwer and Erwin 2010). In strawberry, the direct application of salicylate acid decreased the population of *T. urticae,* and the mites avoided leaves treated with salicylate acid compared to the untreated control (Favaro et al. 2019). Also, an increase of methyl salicylate in headspace volatiles emitted by the plant after application of a salicylic acid analogue has been found (Hayat et al. 2010; Patt et al. 2018). Methyl salicylate is an important volatile for the recruitment of natural enemies, including predatory mites (De Boer and Dicke 2004). However, the effect of ASM on the eriophyid mite vector, natural enemies and other pests in roses is not known at this point.

In conclusion, ASM has the potential to be a useful systemic acquired resistance inducer to reduce disease severity, and incidence of infection in roses. In our study, the spray program was discontinued in all the trials after 11 applications of ASM. The plants in the trial 1 treated with ASM at 50 and 100 mg/L stayed healthy for several months after the ASM program was discontinued and same for ASM at 50 mg/L in trial 2. This indicates that if below detectable levels of virus load is achieved, plants will remain asymptomatic after treatments. However,

388 drawing a definitive conclusion from this study alone is not possible due to the fact that this
389 study was conducted in controlled conditions as a first attempt to see whether RRD severity
390 reduction is possible with ASM. In case of its potential field use, it should be further ascertained
391 whether ASM use could mask the potential presence of the virus in the background and could
392 manifest symptoms after purchase by a consumer in the absence of further treatments. This
393 would also be highly relevant for consideration in potential use of ASM for planting material that
394 is shipped outside U.S or coming into U.S. where masking of symptoms during shipment could
395 lead to plants bypassing visual inspection in the port of entry. The risks involved in this situation
396 needs to be studied in-depth before any potential use/no-use descriptions. Further studies on the
397 effect of ASM on RRD in the presence of *P. fructiphilus* under field conditions could lead to
398 generating information on the utility of ASM as a component in an IPM program along with
399 other approaches including conventional chemical miticides, biocontrol agents, mechanical
400 preventive measures and improved rose varieties for management of RRD.

401 **References**

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Figures:

Fig. 1. Area Under Disease Progress Curve (AUDPC) indicating severity of rose rosette disease (RRD) on Pink Double Knock Out® roses (n = 3) treated with Acibenzolar *S*-methyl (ASM; Actigard™), two times before budding with rose rosette emaravirus-infected buds followed by 11 applications of ASM at weekly intervals in trial 1. **A.** Before pruning and **B.** After pruning. The data was analyzed by Independent Samples Kruskall-Wallis Test at P = 0.05 and significant values for Pairwise Comparisons were adjusted by the Bonferroni correction for multiple tests (P = ≤ 0.05). Different letters indicate statistical differences between treatments, and error bars represent standard error of the mean.

Fig. 2. Symptoms of rose rosette disease (RRD) on Pink Double Knock Out® roses treated with Acibenzolar *S*-methyl (ASM; Actigard™) in trial 1.

Fig. 3. Area Under Disease Progress Curve (AUDPC) indicating severity of rose rosette disease (RRD) on Pink Double Knock Out® roses (n = 3) treated with Acibenzolar *S*-methyl (ASM; Actigard™), two times before budding with rose rosette emaravirus-infected buds followed by 11 applications of ASM at weekly intervals in trial 2. **A.** Before pruning and **B.** After pruning. The data was analyzed by Independent Samples Kruskall-Wallis Test at P = 0.05. Same letters indicate no statistical differences between treatments, and error bars represent standard error of the mean.

Fig. 4. Area Under Disease Progress Curve (AUDPC) indicating severity of rose rosette disease (RRD) on Pink Double Knock Out® roses (n = 8) treated with Acibenzolar *S*-methyl (ASM;

Actigard™), two times before budding with rose rosette emaravirus-infected buds followed by 11 applications of ASM at weekly intervals in trial 3. **A.** Before pruning and **B.** After pruning. The data was analyzed by one-way ANOVA and means were separated by Student-Newman-Keuls test at P = 0.05. Different letters above the bars indicate statistical differences between treatments, and error bars represent standard error of the mean.

Fig. 5. Average number of weeks that Pink Double Knock Out® roses (n = 14) exhibited no incidence of rose rosette disease (RRD) after treatment with Acibenzolar *S*-methyl (ASM; Actigard™) before pruning (dark grey) and after pruning (light grey). The assessments are based on the delay in symptom expression from the first week of symptom development on any plant in any of the treatments including non-treated control across all trials. Trial 1 had 7 weeks of assessment before, and 6 after pruning, trial 2 had 7 weeks of assessment before, and 6 weeks after pruning, and trial 3 had 8 weeks of assessment before, and 3 after pruning. The error bars represent standard error of the mean.

Fig. 6. Average growth rate of Pink Double Knock Out® and Pink Drift® rose depending of different rates of Acibenzolar *S*-methyl (ASM; Actigard™). The growth rate was calculated as the proportional gain in size of the rose plants between the start and the end of the experiment. The error bars represent standard error of the mean.

Fig. 7. Average height, length, and width of Pink Double Knock Out® (A, C, E) and Pink Drift® rose (B, D, F) growth over time affected by Acibenzolar *S*-Methyl (ASM; Actigard™) application rate. The error bars represent standard error of the mean.

595 **Fig. 8**. Average flower production and phytotoxicity symptoms of Pink Double Knock Out® (A,
596 C) and Pink Drift® rose (B, D) over time depending of different Acibenzolar *S*-Methyl (ASM;
597 Actigard™) application rate. O: $P < 0.10$, *: $P < 0.05$, **: $P < 0.01$. The error bars represent
598 standard error of the mean.
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617 **Table 1.** Number of plants with symptoms of rose rosette disease on Pink Double Knock Out
618 roses treated with Acibenzolar S-methyl (ASM; Actigard™), before and after pruning.

	Number of symptomatic plants/total plants tested			
	Trial 1	Trial 2	Trial 3	Total (%)
Before pruning				
Water control	3/3	2/3	8/8	13/14 (92.9%)
ASM at 50 mg/L	0/3	0/3	8/8	8/14 (57.1%)
ASM at 100 mg/L	0/3	3/3	6/8	9/14 (64.3%)
After pruning				
Water control	3/3	3/3	8/8	14/14 (100%)
ASM at 50 mg/L	0/3	0/3	8/8	8/14 (57.1%)
ASM at 100 mg/L	0/3	3/3	8/8	11/14 (78.6%)

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624 **Table 2.** RT-qPCR analysis on Pink Double Knock Out roses treated with Acibenzolar S-methyl (ASM; Actigard™), two times before
625 budding with rose rosette emaravirus-infected buds followed by 11 applications of ASM at weekly intervals in trial 1.
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Trial 1	**First testing date:** 13 weeks after budding					**Second testing date:** 17 weeks after budding				
	Replicate -sample number	**Bud number, leaf position**	**Symptoms [a]**	**Ct value[b]**	**RT-qPCR[b]**	**Replicate -sample number**	**Bud number, leaf position**	**Symptoms [a]**	**Ct value**	**RT-qPCR**
Controls				**23.30[c]**	**100%[e]**				**23.31**	**100%**
				23.30[d]					**23.31**	
	C1-1	1, bud sprout	R, Th	25.23	+	C1-1	1, bud sprout	R, Cru, Th,	24.43	+
	C1-2	3, bud sprout	R, Th, D, Cr	19.63	+	C1-2	Top non-budded branch	R, Cru, Th,	30.40	+
	C2-1	2, 8 cm above bud	Red	23.51	+	C2-1	2, 4.5 cm below bud	R, Cru, Th, Cur	22.45	+
	C2-2	Top Non-budded branch	Slight red	34.86	+	C2-2	2, bud sprout	R, Cru, D	19.45	+
	C3-1	1, bud sprout	Unusual red on bottom	19.08	+	C3-1	Top non-budded branch	R, Cru	21.36	+
	C3-2	1, 5 cm above bud	R, Cru, Cur	17.51	+	C3-2	1, 6 cm above bud	R, Cru, Th	21.15	+
ASM - 50 mg/L				**0.00**	**0%**				**0.00**	**0%**
				0.00					**0.00**	
	L1-1	1, bud sprout	Green	0.00	-	L1-1	1, bud sprout	Green	0.00	-
	L1-2	1, 5 cm above bud	Green	0.00	-	L1-2	1, 5 cm above bud	Green	0.00	-
	L1-3	Top non-budded branch	Green	0.00	-	L1-3	2, 1 cm above bud	Green	0.00	-
	L2-1	1, bud sprout	Green	0.00	-	L1-4	3, 3.5 cm above bud	Green	0.00	-
	L2-2	1, 6 cm above bud	Green	0.00	-	L1-5	Top non-budded branch	Green	0.00	-
	L2-3	Top non-budded branch	Green	0.00	-	L2-1	1, bud sprout	Green	0.00	-
	L3-1	1, bud sprout	Green	0.00	-	L2-2	2, 2 cm above bud	Green	0.00	-
	L3-2	1, 5.5 cm above bud	Green	0.00	-	L2-3	2, 6.5 cm above bud	Green	0.00	-
	L3-3	1, 6.5 cm above bud	Green	0.00	-	L2-4	3, 2 cm above bud	Green	0.00	-
	L3-4	Top non-budded branch	Green	0.00	-	L2-5	Top non-budded branch	Green	0.00	-
						L3-1	1, 3 cm above bud	Green	0.00	-
						L3-2	2, 6 cm above bud	Green	0.00	-
						L3-3	3, 4.5 cm above bud	Green	0.00	-
						L3-4	Top non-budded branch	Green	0.00	-
ASM - 100 mg/L				**0.00**	**0%**				**0.00**	**0%**
				0.00					**0.00**	
	H1-1	1, alive, 3.5 cm above bud	Green	0.00	-	H1-1	1, alive, 0.5 cm above bud	Green	0.00	-

‘	H1-2	2, 1 cm above bud	Green	0.00	-	H1-2	1, 5 cm above bud	Green	0.00	-
	H1-3	3, 2 cm above bud	Green	0.00	-	H1-3	2, 0.5 cm below bud	Green	0.00	-
	H1-4	Top non-budded branch	Green	0.00	-	H1-4	3, 3.5 cm above bud	Green	0.00	-
	H2-1	1, bud sprout	Green	0.00	-	H1-5	Top non-budded branch	Green	0.00	-
	H2-2	1, 9 cm above bud	Green	0.00	-	H2-1	1, bud sprout	Green	0.00	-
	H2-3	1, 11 cm above bud	Green	0.00	-	H2-2	1, 8 cm above bud	Green	0.00	-
	H2-4	Top non-budded branch	Green	0.00	-	H2-3	2, 8 cm above bud	Green	0.00	-
	H3-1	1, bud sprout	Green	0.00	-	H2-4	3, 1 cm above bud	Green	0.00	-
	H3-2	2, 4 cm above bud	Green	0.00	-	H2-5	Top non-budded branch	Green	0.00	-
	H3-3	3, 7 cm above bud	Green	0.00	-	H3-1	1, bud sprout	Green	0.00	-
	H3-4	Top non-budded branch	Green	0.00	-	H3-2	1, 4.5 cm above bud	Green	0.00	-
						H3-3	2, 2 cm above bud	Green	0.00	-
						H3-4	3, 6 cm above bud	Green	0.00	-
						H3-5	Top non-budded branch	Green	0.00	-

627 [a] R = redness; Th = thorniness; Cru = crumbling; Cur = curling, and D = distorted.
628 [b] RT-qPCR was conducted using methods described in Babu et a. 2016 and Ct values were generated.
629 [c]Average Ct value of all samples that were positive or negative for RRV in RT-qPCR
630 [d]Average Ct value of all samples that were positive for RRV in RT-qPCR
631 [e]Average percentage of leaves tested that were positive for RRV in RT-qPCR
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641 **Table 3.** RRV RT-qPCR analysis on Pink Double Knock Out roses treated with Acibenzolar S-methyl (ASM; Actigard™), two times
642 before budding with rose rosette emaravirus-infected buds followed by 11 applications of ASM at weekly intervals in trials 2 and 3.

	Trial 2 Testing date: 13 weeks after budding				**Trial 3 Testing date:** 20 weeks after budding			
Treatment	**Replicate-sample number**	**Symptoms [a]**	**Ct value[b]**	**RT-qPCR[b]**	**Replicate-sample number**	**Symptoms [a]**	**Ct value**	**RT-qPCR**
Controls			**12.33[c] 18.49[d]**	**67%[e]**			**23.27 23.27**	**100%**
	C1-1	Green (NS)	0.0	-	C1-1	R, Cru, Th (S)	23.17	+
	C1-2	Green (NS)	0.0	-	C1-2	R, Cru, Th (S)	20.35	+
	C2-1	R, Cru, Th (S)	19.81	+	C2-1	R, Cru, Th, Cur (S)	32.17	+
	C2-2	Cru, D (S)	21.35	+	C2-2	R, Cru, D (S)	26.35	+
	C3-1	Unusual red, Th (S)	15.34	+	C3-1	R, Cru (S)	22.31	+
	C3-2	R, Cru, Cur (S)	17.45	+	C3-2	R, Cru, Th (S)	15.26	+
ASM - 50 mg/L			**0.00 -**	**0%**			**24.68 24.68**	**100%**
	L1-1	Green (NS)	0.00	-	L1-1	R, D (S)	32.19	+
	L1-2	Green (NS)	0.00	-	L1-2	Unusual red (S)	25.64	+
	L1-3	Unusual red (NS)	0.00	-	L2-1	R, Cur, D (S)	23.12	+
	L2-1	Green (NS)	0.00	-	L2-2	R, Cru, Th (S)	17.85	+
	L2-2	Green (NS)	0.00	-	L2-3	R (S)	34.18	+
	L2-3	R, Cru (NS)	0.00	-	L3-1	R, Cru, D (S)	19.64	+
	L3-1	Green (NS)	0.00	-	L3-2	D, Th (S)	20.12	+
	L3-2	R, D (NS)	0.00	-				
	L3-3	Green (NS)	0.00	-				
	L3-4	Green (NS)	0.00	-				
ASM - 100 mg/L			**18.50 24.66**	**75%**			**12.33 24.67**	**50%**
	H1-1	R (NS; natural)	0.00	-	H1-1	R, Cru (S)	0.00	-

H1-2	Green (NS)	0.00	-	H1-2	R (NS; natural)	0.00	-
H1-3	R, Cru, D (S)	29.35	+	H1-3	Green (NS)	0.00	-
H1-4	R, Cru (S)	32.45	+	H2-1	R, Cru, Th (S)	18.75	+
H2-1	R (S)	24.16	+	H2-2	R, Cru, D (S)	25.64	+
H2-2	Cur, D (S)	19.82	+	H3-1	Green (NS)	0.00	-
H3-1	Unusual red (S)	17.33	+	H3-2	R (NS)	0.00	-
H3-2	Unusual red (S)	24.86	+	H3-3	R, Cur (S)	0.00	-
				H3-4	Green (NS)	0.00	-
				H3-5	Unusual red (S)	0.00	-
				H4-1	Th (S)	17.61	+
				H4-2	Green (NS)	0.00	-
				H5-1	R, Cru (S)	34.18	+
				H5-2	R (S)	30.42	+
				H6-1	R, Cru, D (S)	28.45	+
				H7-1	D, Unusual red (S)	27.16	+
				H8-1	R, Th (S)	17.35	+
				H8-2	R, Cru, Cur (S)	22.46	+

[a] R = redness; Th = thorniness; Cru = crumbling; Cur = curling, D = distorted, S = symptomatic, NS = asymptomatic. Not all plants with redness or crumbling are symptomatic as it looked like natural or other causes.
[b]RT-qPCR was conducted using methods described in Babu et a. 2016 and Ct values were generated.
[c]Average Ct value of all samples that were positive or negative for RRV in RT-qPCR
[d]Average Ct value of all samples that were positive for RRV in RT-qPCR
[e]Average percentage of leaves tested that were positive for RRV in RT-qPCR

644 **Table 4.** Effect of Acibenzolar S-Methyl (ASM; Actigard™) on rose cultivars on log
645 transformed data

	Df	Sum Sq.	Mean Sq.	F	value Pr(>F)
Growth x Cultivar	1	0.0142	0.014197	1.225	0.2700
Growth x ASM	3	0.0781	0.026026	2.247	0.0852
Growth x Cultivar x ASM	3	0.0246	0.008193	0.707	0.5491
Residuals	151	1.7493	0.011585		

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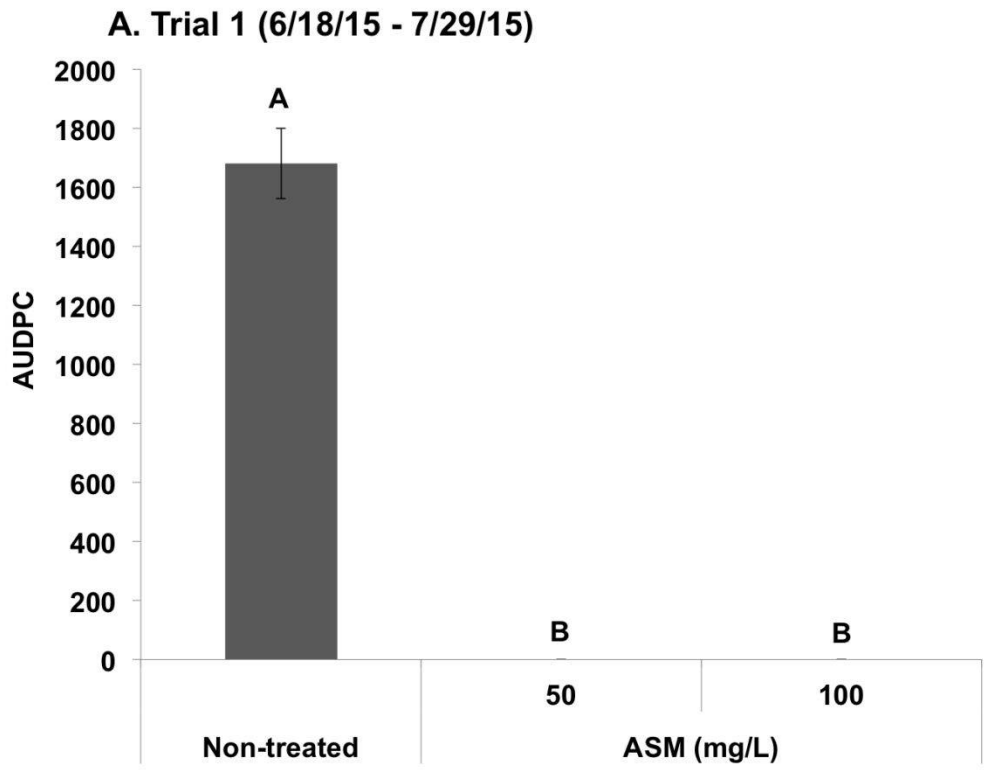
666 **Fig. 1**



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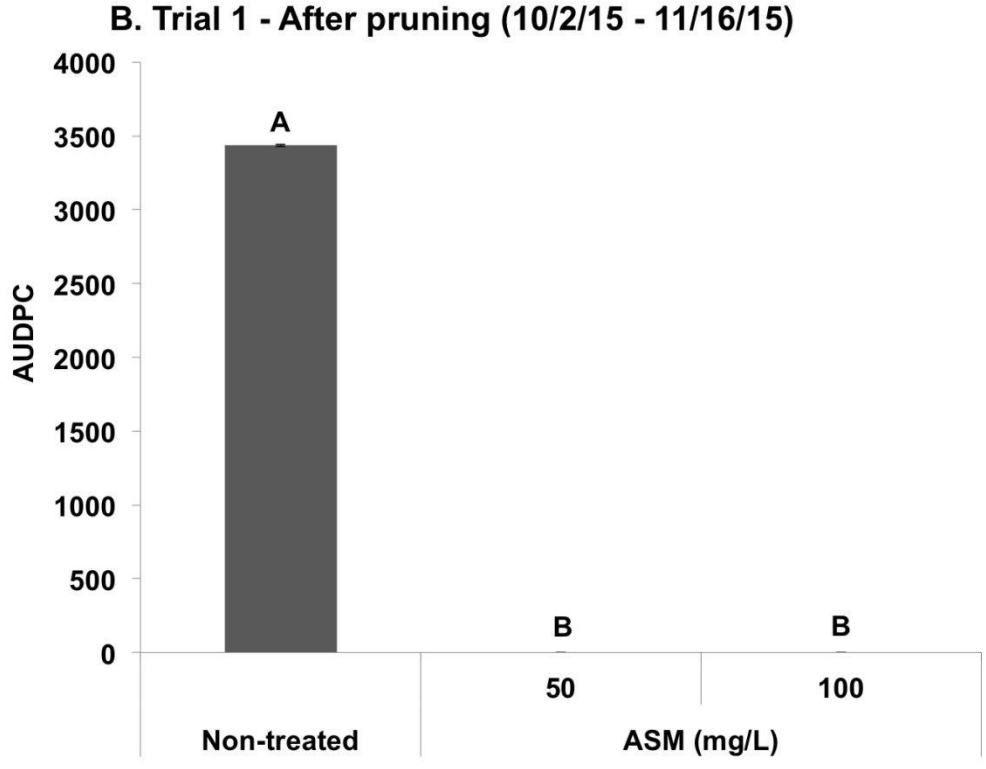

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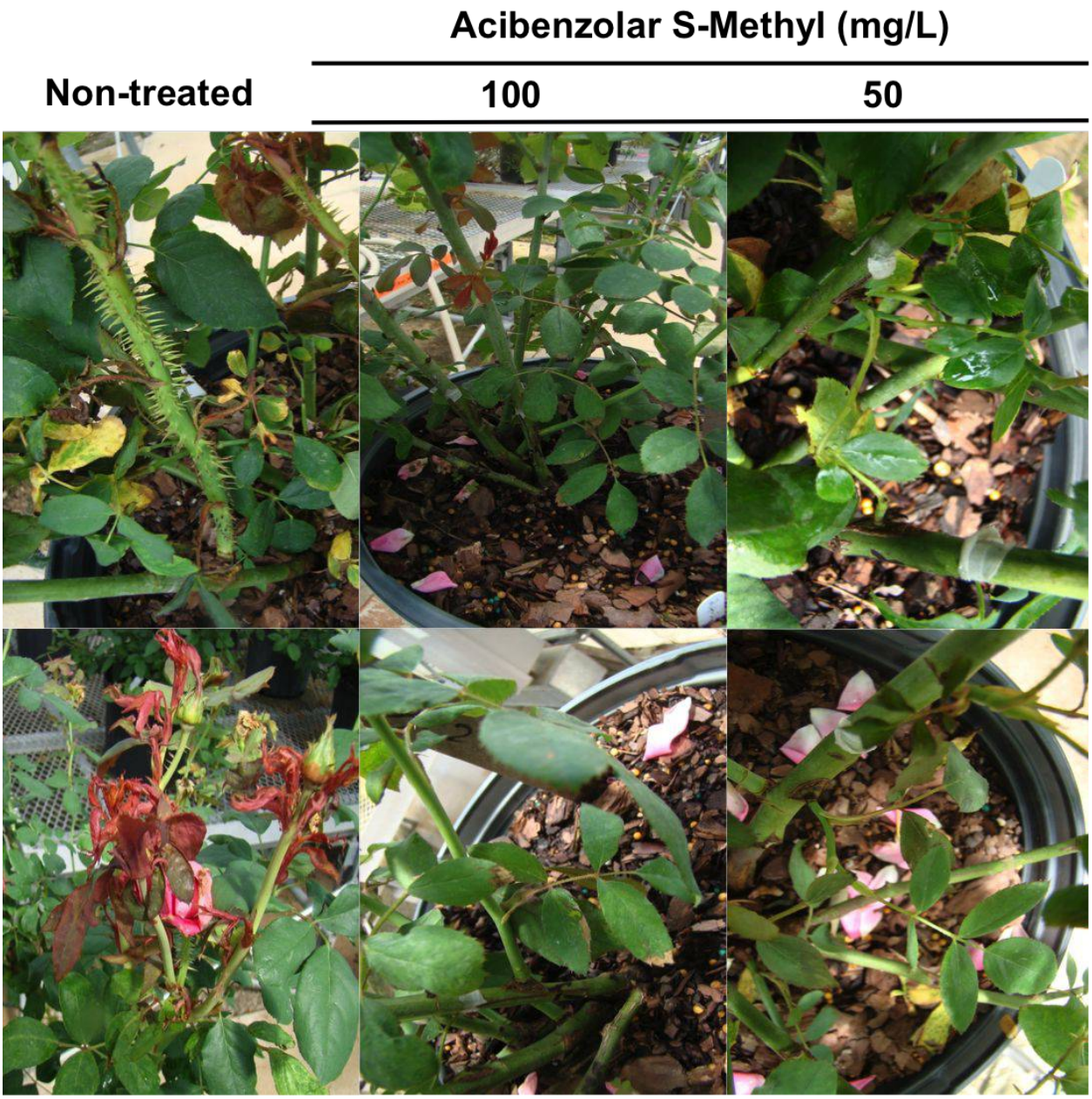
669 **Fig. 2.**



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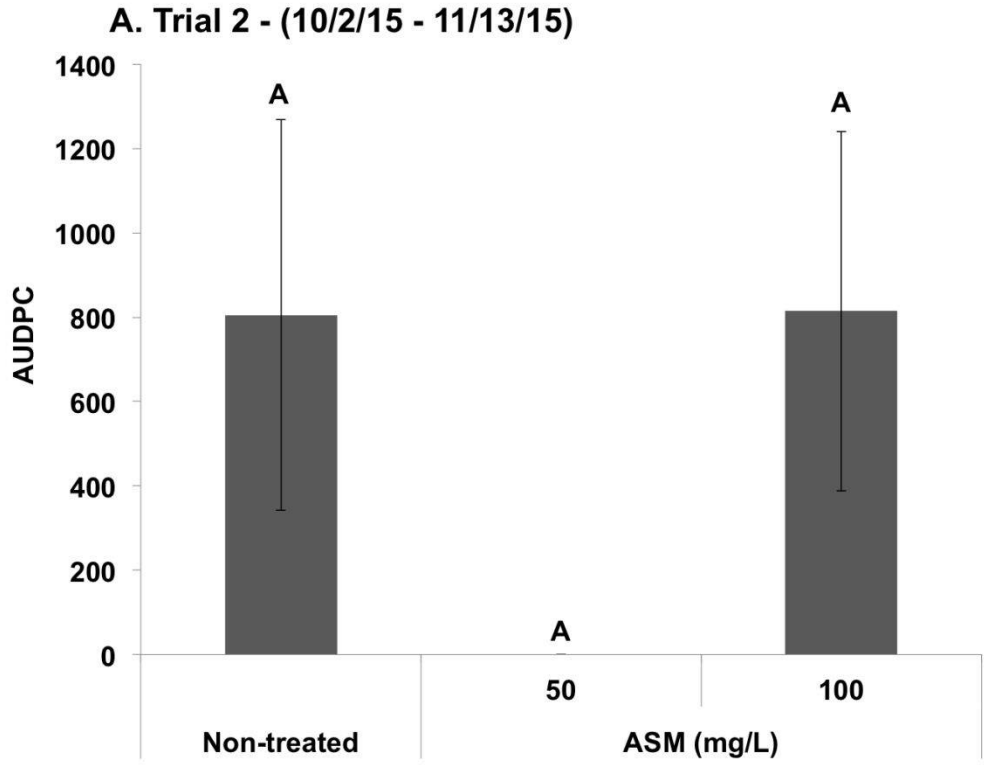
676 **Fig. 3**



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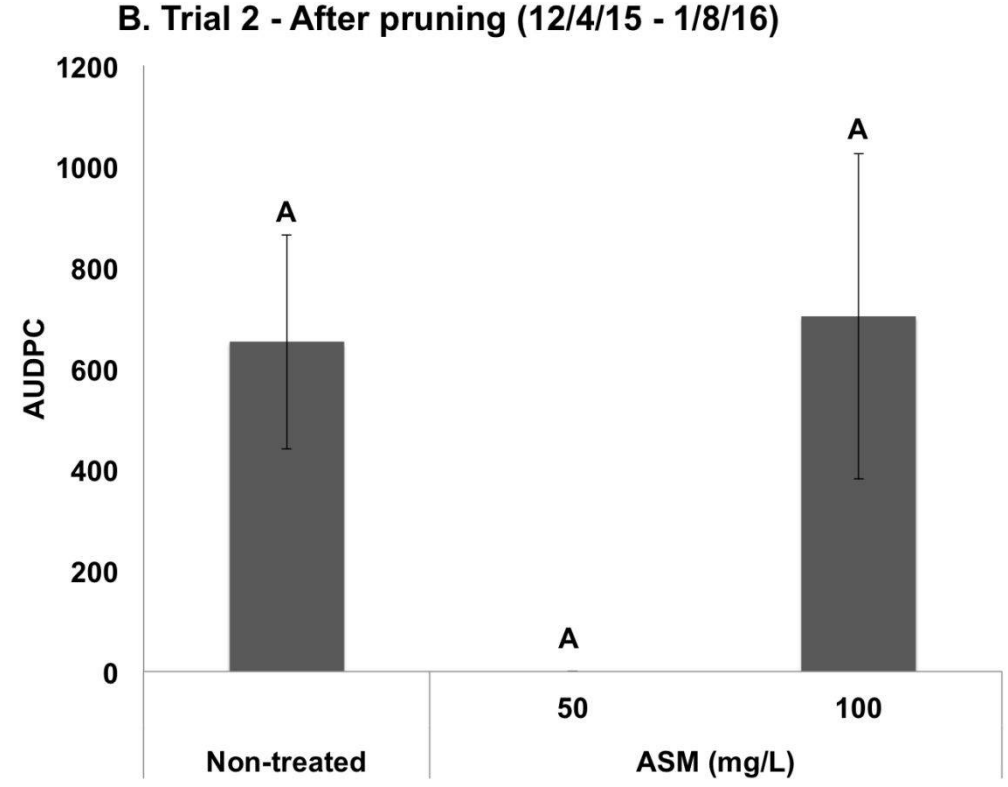

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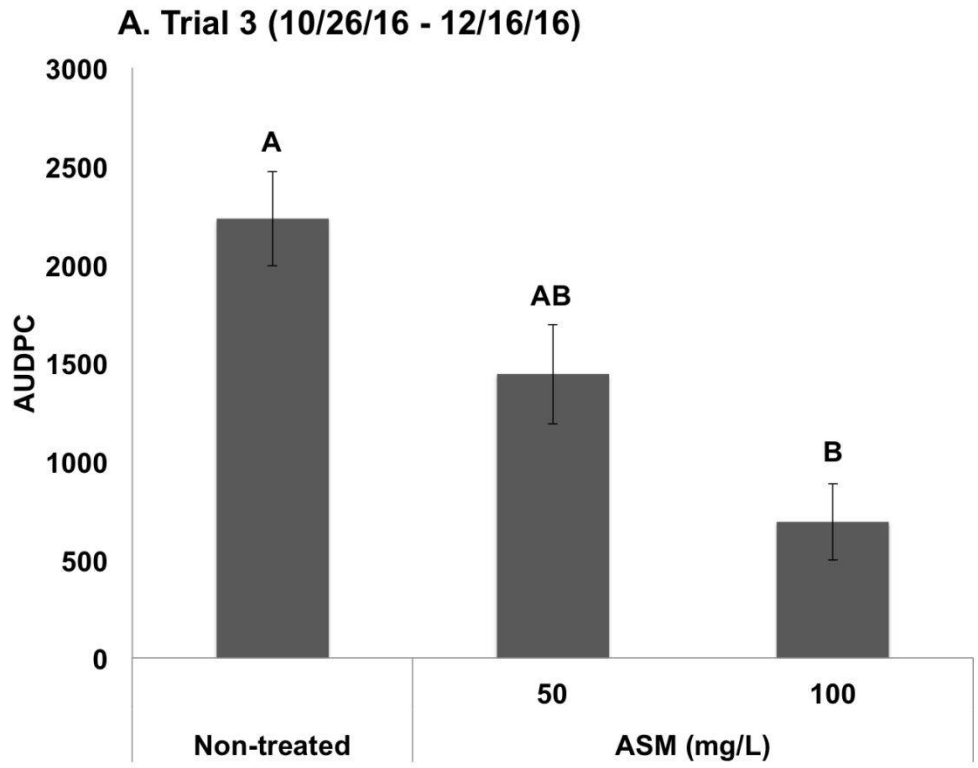
679 **Fig. 4**



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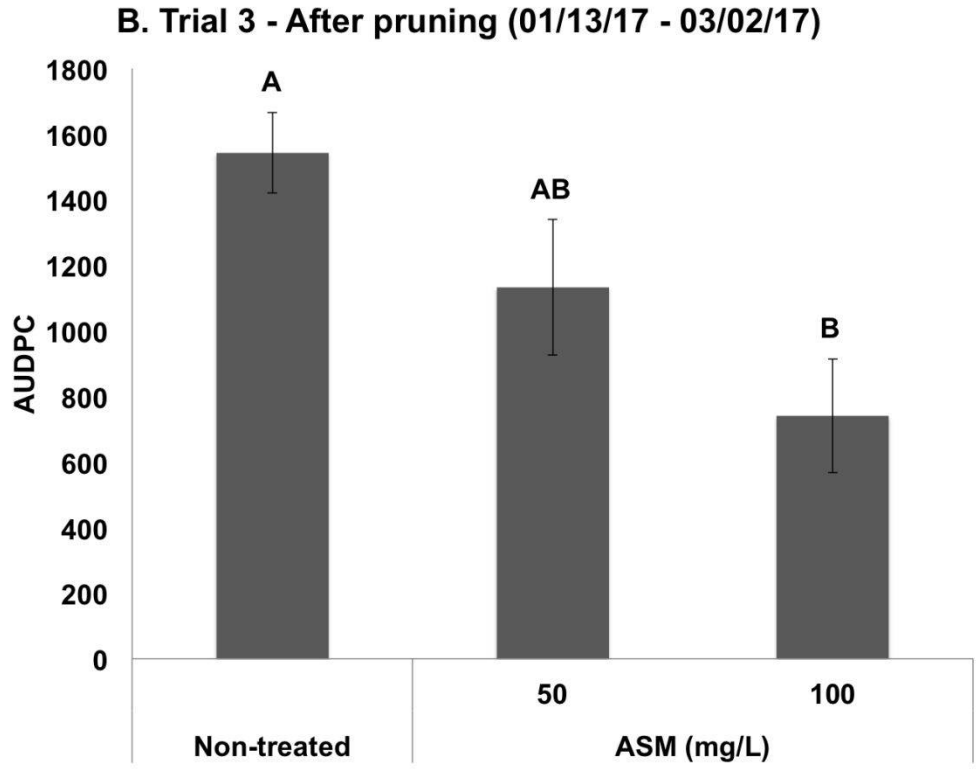

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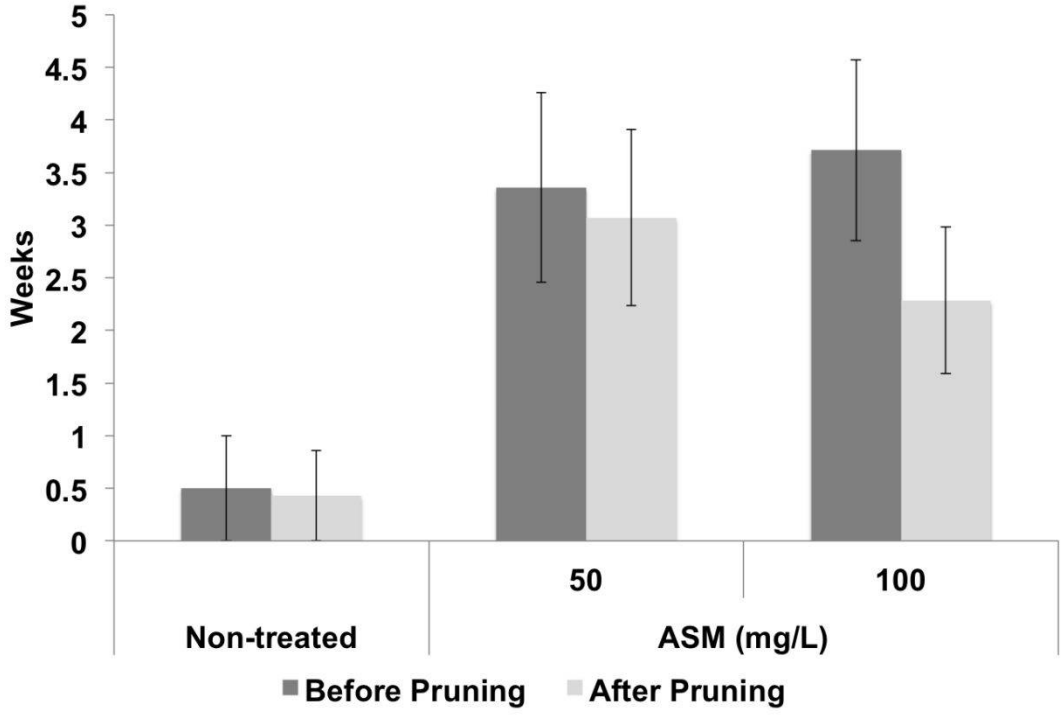
682 **Fig. 5**



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696 **Fig. 6**

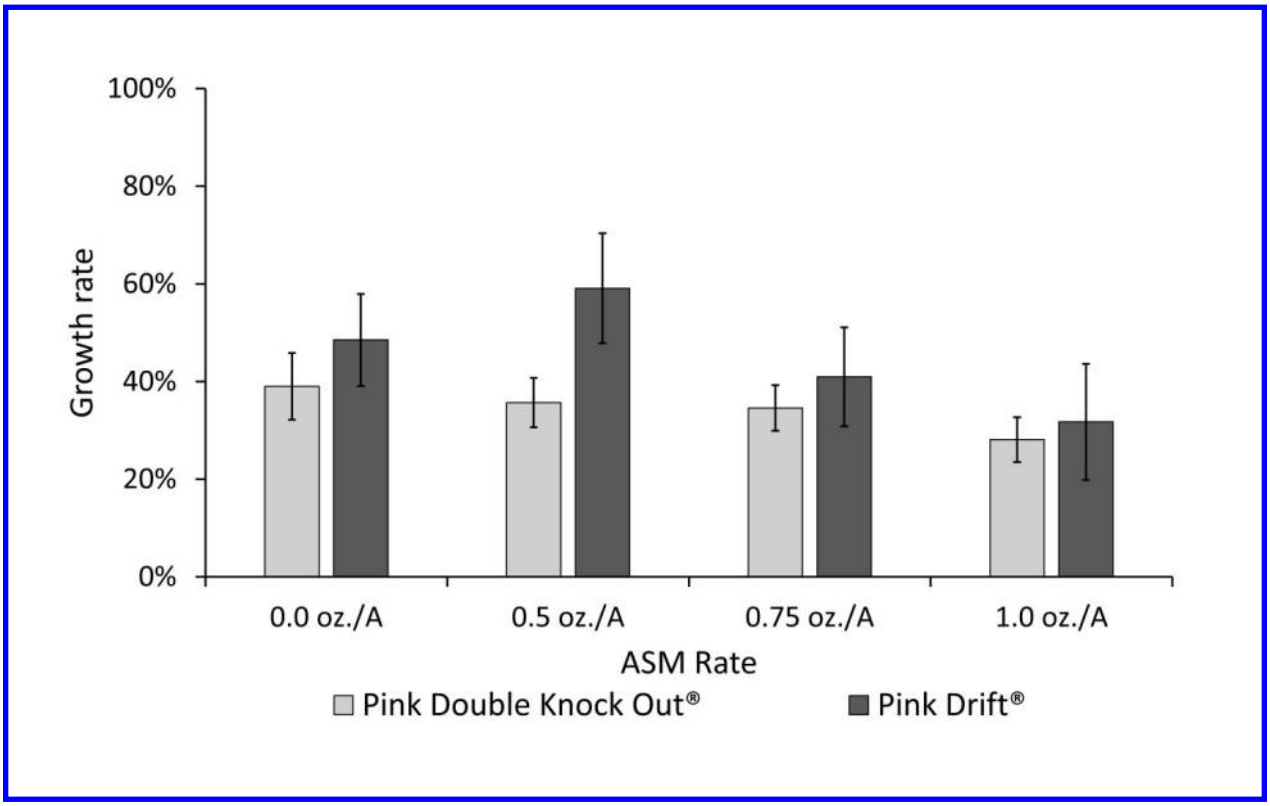

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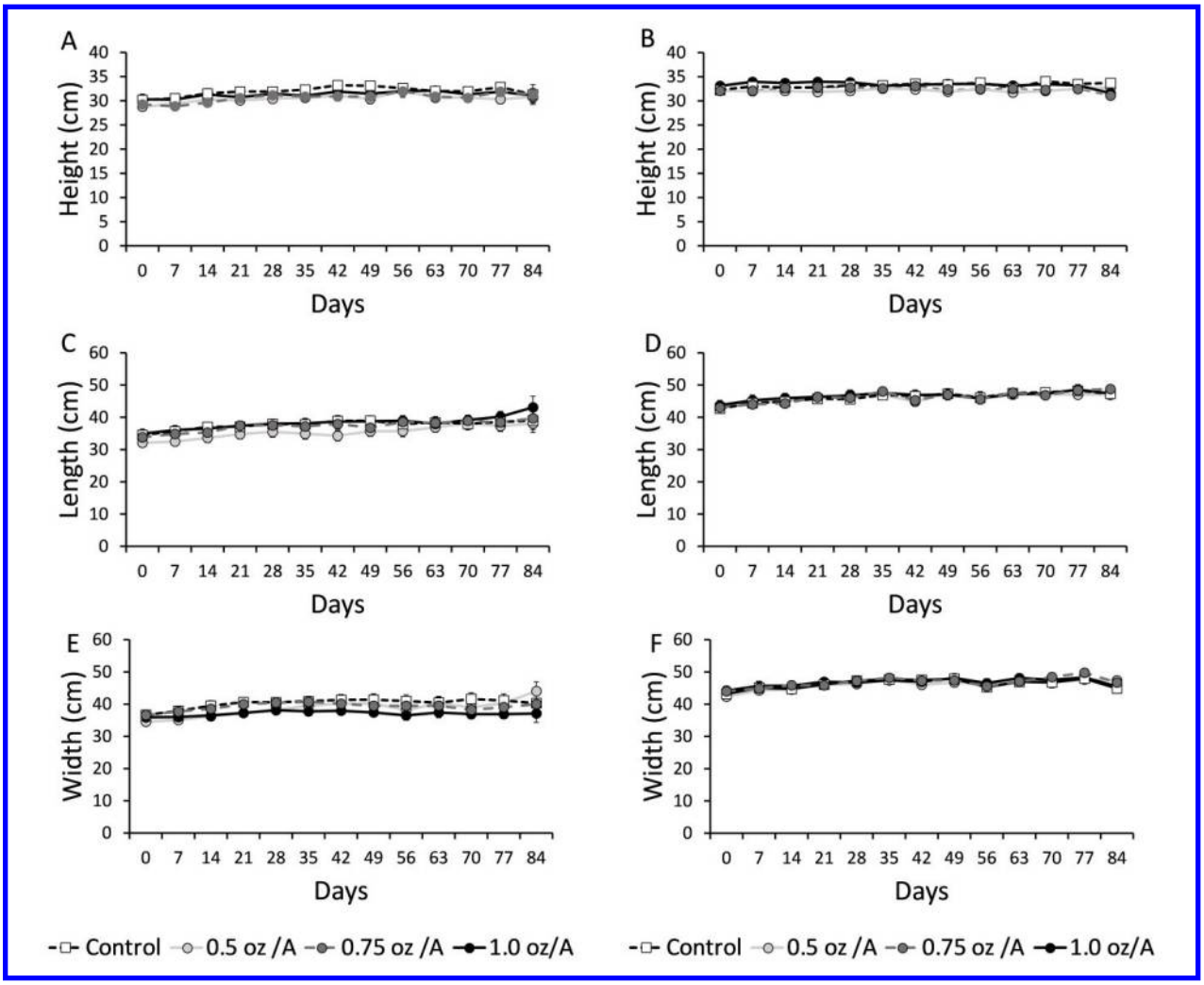
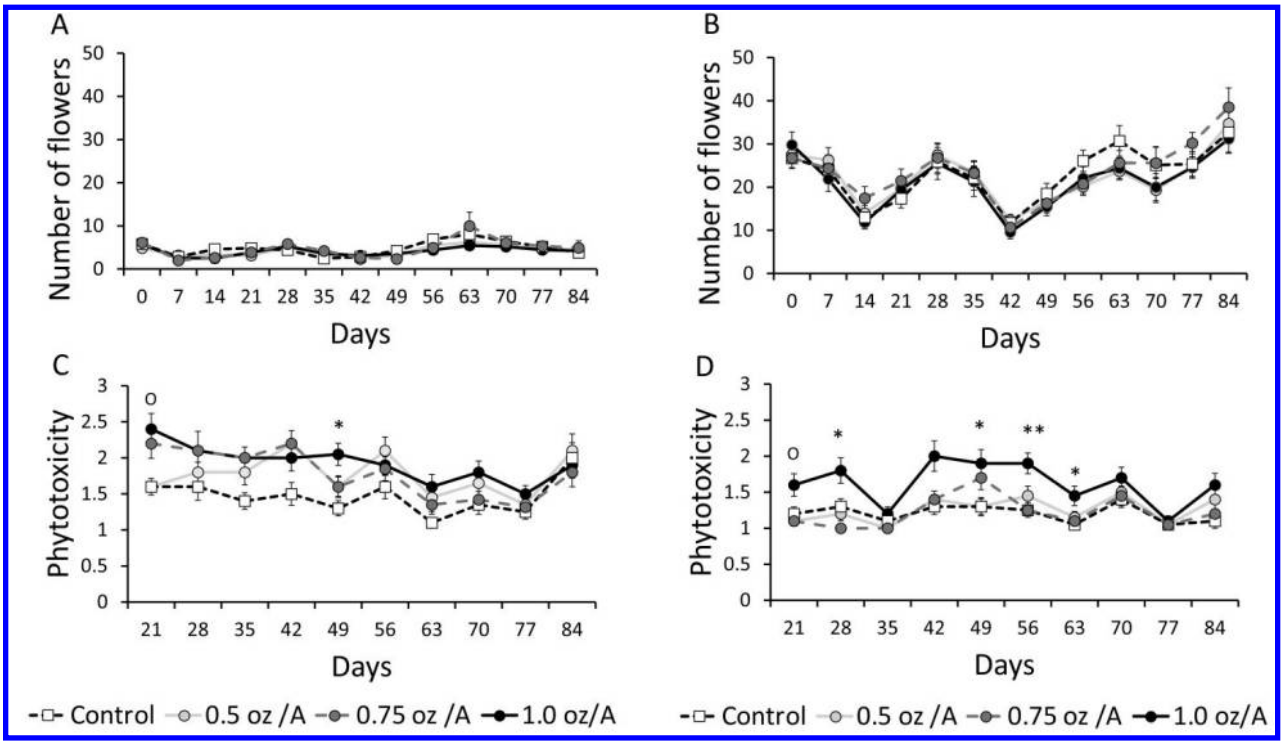
Fig. 7


A
Height (cm)
Days
B
Height (cm)
Days
C
Length (cm)
Days
D
Length (cm)
Days
E
Width (cm)
Days
F
Width (cm)
Days
Control
0.5 oz /A
0.75 oz /A
1.0 oz/A
Control
0.5 oz /A
0.75 oz /A
1.0 oz/A

719 **Fig. 8**



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733 **Supplementary Figure**

734 **Fig. 1.** Symptoms of rose rosette emaravirus on rose varieties after budding from an infected

735 rose. A. Pink Double Knock Out (shrub rose), B. Cherokee rose, C. Seven Sister rose, and D.

736 Pink Double Knock Out patio rose.

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Supplementary Table

Table 1. Presence of rose rosette emaravirus in symptomatic plants after budding and confirmed by RT-PCR or RT-qPCR analysis

Cultivar	Replicate	Molecular testing			
		PCR (Laney et al. 2011)		RT-qPCR (Babu et al. 2016) Average Ct values (SD[b])	
		Non-symptomatic leaves (before budding)	Symptomatic leaves (after budding)	Non-symptomatic leaves (before budding)	Symptomatic leaves (after budding)
Pink Double Knock Out (shrub rose)	1	-	+	0.0	19.65 (0.11)
	2	-	+	0.0	21.31 (0.18)
	3	-	+	0.0	14.95 (0.09)
	4	-	+	0.0	19.03 (0.26)
	5	-	+	0.0	29.50 (0.28)
Cherokee rose	1	-	+	NT[a]	NT
	2	-	+	NT	NT
Seven Sister rose	1	-	+	0.0	19.09 (0.19)
	2	-	+	0.0	21.41 (0.08)
	3	-	+	0.0	20.64 (0.67)
	4	-	+	0.0	25.86 (0.11)
	5	-	+	0.0	16.43 (0.44)
Pink Double Knock Out (patio rose)	1	-	+	NT	NT

[a] NT = Not tested
[b] SD = Standard deviation