Research Report

Title: Effect of bacterial endophyte treatments on strawberry growth and yield

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Public Abstract

The Institute for Advanced Learning and Research in Danville, VA has established an endophytic bacterial library of ~ 2000 strains isolated from plants grown in Virginia and characterized their plant growth promotive and biocontrol properties. We evaluated the effects of fall season application of three Bacillus strains, *Bacillus velezensis* #308, *B. velezensis* #585, and *B. velezensis* #619, on strawberry 'Chandler' plug transplants during the 2018-19 growing season at three grower sites in randomized complete block design and compared those to the non-treated control. Although not statistically different, the marketable yield on a per plant basis increased by 8% at the Chesapeake location, by 12% at the Fredericksburg location and by 15% at Mecklenburg county for plug plants inoculated with *B. velezensis* #619. In the 2019-20 growing season, field trials focused on evaluating *B. velezensis* #619 effect on strawberry crop yield with one and two applications of the bacterium. 'Chandler' and 'Liz' strawberry were evaluated at the Hampton Roads Agricultural Research and Extension Center (AREC) and at the Fredericksburg location 'Chandler' was evaluated. The study was a randomized complete block design with three treatments consisting of (i) an untreated control, (ii) strawberry plug plants inoculated with bacterial endophyte, *B. velezensis* #619 strain right before transplanting, and (iii) strawberry plug plants inoculated with *B. velezensis* #619 strain before transplanting and also applied once over the spring season. Neither the treatment by variety interaction nor the main effects of treatment and variety, was significant for marketable and total yield at the Hampton Roads AREC. 'Chandler' marketable fruit was 10.8% and 9.3% higher in *Bacillus velezensis* #619 treatment applied in fall and spring and applied in fall, respectively than that of non-treatment control at the AREC. At Braehead farms, there was a significant treatment effect for marketable yield and 'Chandler' marketable fruit was 20.9% and 7.8% higher in *Bacillus velezensis* #619 treatment applied in fall and spring, and applied only in fall, over non-treated control, respectively. Additional studies are needed to optimize the application dosage of *Bacillus velezensis* #619 in strawberry production.

Introduction

The strawberry industry in the U.S. currently faces many challenges that need overcoming to promote long-term sustainability and viability. One of the primary challenges is the loss of methyl bromide (MB). Methyl bromide has been discontinued as a soil fumigant in the U.S. for open-field production. In California, its loss has resulted in yield reductions of up to 15% and an increase in pest pressure (Goodhue et al., 2005). No economic data from the loss of MB exists for our region. However, currently about half of the strawberry growers in Virginia do not fumigate due to multiple reasons: the lack of technical know-how to fumigate, inadequate pest efficacy and yield outcome achieved with alternative fumigants, and regulatory constraints including the need for maintaining buffer zones around fumigated land (Christman and Samtani, 2019).

The Institute for Advanced Learning and Research (IALR) is a non-profit organization in Southern Virginia, with the mission to boost local economic development. Striving to meet this goal, the Plant Endophyte Research Center in IALR uses biology knowledge and technology to improve plant performance, helping farmers achieve more profits by developing low-input and sustainable cropping systems utilizing beneficial bacterial endophytes. In strawberry production, several reports showed positive effects on inhibition of pathogen growth and reducing disease occurrence. For example, Freeman et al. (2004) used Trichoderma strains to control gray mold and anthracnose. Dara (2016) reported that endophytic *Beauveria bassiana* negatively affects green peach aphids.

From a funded Virginia Specialty Crop Block Grant in 2017, we tested three bacterial endophyte strains in a randomized complete block design with triplicates at three farms in Virginia located in the City of Chesapeake, the City of Fredericksburg and one in Buffalo Junction, respectively. These trials were done in the 2018-19 growing season and treatments were selected based on the screening done for plant-endophyte interactions under greenhouse and laboratory studies at IALR. Treatments in the field included strawberry plug plants inoculated with either i) IALR isolated *Bacillus velezensis* #308, ii) *Bacillus velezensis* #585, iii) *Bacillus velezensis* #619, iv) combination of the three bacterial strains, or v) a nontreated control. All replicates had twelve plants of 'Chandler' that were in two-row beds spaced 14 inches apart in a staggered manner. The plug plants were inoculated with their respective treatments by soaking them overnight in bacterial endophyte culture before transplanting in open-field. Depending on the grower location, berries were harvested once or twice per week and separated into the marketable and non-marketable category. Although there were no statistically significant differences among treatments on marketable and total yield, at all three locations, the plants responded positively to the #619 strain. The marketable yield on a g/plant basis increased by 8% at the Chesapeake location, by 12% at the Fredericksburg location and by 15% at the Buffalo Junction location for plug plants inoculated with *Bacillus velezensis* #619 (data not shown). *B. velezensis* is an aerobic, endospore-forming bacterium known to promote plant growth and suppress pathogens (Rabbee et al., 2019). Metabolites produced by *B. velezensis* can trigger resistance in plants by which plants defend themselves (Rabbee et al., 2019). This positive influence of the *B. velezensis* #619 strain on strawberry yield prompted us to further focus on a) evaluating this endophyte and refine its dose rate and b) see if different strawberry genotypes respond differently to the endophyte. The objectives of this proposed study were to (i) further evaluate the effect of bacterial endophyte

treatments on strawberry growth, yield and fruit quality in open-field annual hill plasticulture production and (ii) extend the findings of these studies via on-farm research and outreach activities.

Materials and Methods

Trials were initiated in Sept 2019 at the Hampton Roads AREC in Virginia Beach, at a grower farm in Gretna (Pittsylvania Co.), one in the City of Fredericksburg, and one in Buffalo Junction (Mecklenburg Co.). Preplant fertilizers were added to the strawberry beds based on recommendations of Virginia Tech soil testing laboratory. The study was a randomized complete block design with three treatments consisting of (i) an untreated control, (ii) strawberry plug plants inoculated with bacterial endophyte, *B. velezensis* #619 strain before transplanting and (iii) strawberry plug plants inoculated with *B. velezensis* #619 strain before transplanting and also applied once in the spring season by adding 50 ml of bacterial culture per planting hole. Each replicate had 12 strawberry plants of 'Chandler' and 6 strawberry plants of 'Liz', and each treatment was replicated thrice. Strawberry 'Chandler' plants were transplanted from late September to the first week in October. In addition, at the AREC, the treatments were evaluated on Liz variety with 6 plants per replicate. Due to Covid-19 situation, yield data could not be collected at two of the four sites at which trials were initiated. Yield data were collected at the Hampton Roads AREC and in the City of Fredericksburg. Strawberry yield was collected twice per week. Fruits were sorted into marketable and non-marketable category at the time of weighing. Non-marketable fruits comprised of diseased, rotten, misshapen, animal bitten, insect damaged, or small fruits (<10 g fruit weight). Additionally, strawberry stand count and qualitative plant health rating (1 to 10) were recorded once a month at the Hampton Roads AREC site. At the AREC, strawberry plant canopy readings were taken once in November (early season), once in February (mid-season) and once in March to monitor the strawberry plant development. Fruit diameter for 'Chandler' were taken on five marketable fruits per replicate once per week during the harvest season and the fruit diameter readings were averaged for the season. Data were analyzed using SAS v. 9.4.

Results and Discussion:

At the AREC, neither the treatment by variety interaction nor the main effects of treatment and variety were significant for marketable and total yield. For the non-marketable yield, only the variety effect was significant and 'Chandler' had more non-marketable berries than 'Liz'. Non-marketability of fruits was due to numerous factors described in Materials and Methods section. The average health rating and the crop stand count for the season were not influenced by treatment by variety interaction or the main effects of treatment and variety. Fruit diameter for 'Chandler' was not influenced by any treatments. At the Hampton Roads AREC, although not statistically different, 'Chandler' marketable fruit was 10.8% and 9.3% higher in *Bacillus velezensis* #619 treatment applied in fall and spring and applied only in fall than that of non-treatment control, respectively. The canopy readings were not influenced by the two-way interactions or the main effect, except for the canopy data in March indicated Chandler variety had larger diameter than Liz.

At Braehead farms, marketable fruit was 20.9% and 7.8% higher in *Bacillus velezensis* #619 treatment applied in fall and spring, and applied only in fall, over non-treated control respectively (Table 1). There were no differences among non-marketable and total yield for the season. Developments and findings of the study were presented at the 2020 American Society for Horticultural Science Annual Meeting and at the NCCC 212: Small Fruit and Viticulture Research Multistate Research Coordinating Committee and Information Exchange Group.

Table 1. Total season yield in Fredericksburg, VA at the 2019-2020 growing season.

Treatment	**Marketable yield**	**Non-marketable yield**	**Total yield (Market + Non-marketable yield)**
	----------g/plant----------		
B. velezensis #619 (Fall + Spring)	519.6 a	144.2	663.8
B. velezensis # 619 (Fall)	463.2 ab	145.5	608.7
Non-treated control	429.8 b	155.5	585.2
$P \leq 0.05$	0.0425	0.1425	0.0655