Effect of probiotic bacteria, anaerobic soil disinfestation and mustard cover crop biofumgation on soilborne disease suppression and yield of strawberry

This study was conducted in grower cooperator Joyce Shafer's "Shafer Heritage" Farm in Preston county, WV by growing strawberry plug plants on beneficial microbes-inoculated or regular planting mix followed by transplanting in fruiting field plots that either were bio-fumigated with mustard cover crop, anaerobically disinfested (ASD) or left untreated.

Methods:

Production of treated plug plant

In the first phase, plug plants were produced from nursery supplied tips at the plug plant production facility of the grower cooperator Mr. Reuben Martin located at 8564 Olde Scotland Rd, Shippensburg, PA 17257. Plug production system was slightly modified to meet certified organic production standard and our pre-planned treated plant production requirements. Johnny's 512 Organic Mix was used for all plug plant production. Planting mix inoculation was done at the WVU Evansdale greenhouse by laying the mix on greenhouse bench in thin layers followed by spraying suspension of TerraGrow to ensure homogeneous distribution of probiotic bacteria (Fig. 1).

Fig. 1. Planting mix inoculation with TerraGrow before using for growing plug plants

Medium was then remixed and put in one pile, taken to Shippensburg, PA where plug plants were grown. Inoculated mix was dispensed evenly in 18 plug trays with 10% extra kept aside. A total of 900 strawberry plug plants of CV Chandler was grown on pasteurized medium that was inoculated with TerraGrow (TG) while 500 was grown on non-pasteurized/regular planting mix but treated with TG. Plastic plug trays (fifty cells) were used to grow plug plants. Strawberry tips

were immersed in TG suspension for one minute just prior to sticking in plug cells. Plug cells were filled with extra mix as needed and placed under mist following the protocol describe by Rowley et al. (2010). A total of 1200 plug plants without treatment were grown on regular mix destined for the non-treated, bio-fumigated and anaerobically soil disinfested (ASD) plots in fruiting field. Thus, combining the plug production and fruiting field, we had six different treatments to determine individual treatment effect or synergistic effect of plug and fruiting field treatment with unique treatment acronyms as follows; i) plug plants grown on pasteurized planting mix treated with TG and planted in non-treated fruiting field plots (Effect of TG with higher colonization potential -PM+TG+NF); ii) plug plants grown on non-pasteurized/regular planting mix treated with TG and planted in ASD plots (Synergistic effect of TG with regular colonization potential and ASD - RM+TG+ASD); iii) plug plants grown on non-treated planting mix and planted in ASD fruiting field plots (Effect of ASD - RM+NT+ASD); iv) plug plants grown on non-pasteurized planting mix treated with TG and planted in non-treated fruiting field plots (Effect of TG with regular colonization potential - RM+TG+NF); v) plug plants grown on regular non-treated planting mix and planted in 'Caliente 199' mustard cover crop plots (Effect of biofumigation - RM+NT+MCC); vi) plug plants grown on non-treated planting mix and planted in non-treated fruiting field plots (Non-treated check - RM+NT+NF).

Mustard cover crop was grown in randomly distributed plots up to flowering followed by tissue maceration and incorporation in the soil (Fig. 2). A total of 1200 plug plants without treatment were grown on regular mix destined for the non-treated, bio-fumigated and anaerobically soil disinfested (ASD) plots in fruiting field. Thus, combining the plug production and fruiting field, we had six different treatments to determine individual treatment effect or synergistic effect of plug and fruiting field treatment with unique treatment acronyms as follows; i) plug plants grown on pasteurized planting mix treated with TG and planted in non-treated fruiting field plots (Effect of TG with higher colonization potential -PM+TG+NF); ii) plug plants grown on non-pasteurized/regular planting mix treated with TG and planted in ASD plots (Synergistic effect of TG with regular colonization potential and ASD - RM+TG+ASD); iii) plug plants grown on non-treated planting mix and planted in ASD fruiting field plots (Effect of ASD - RM+NT+ASD); iv) plug plants grown on non-pasteurized planting mix treated with TG and planted in non-treated fruiting field plots (Effect of TG with regular colonization potential - RM+TG+NF); v) plug plants grown on regular non-treated planting mix and planted in 'Caliente 199' mustard cover crop plots (Effect of biofumigation - RM+NT+MCC); vi) plug plants grown on non-treated planting mix and planted in non-treated fruiting field plots (Non-treated check - RM+NT+NF).

A total of 1200 plug plants without treatment were grown on regular mix destined for the non-treated, bio-fumigated and anaerobically soil disinfested (ASD) plots in fruiting field. Thus, combining the plug production and fruiting field, we had six different treatments to determine individual treatment effect or synergistic effect of plug and fruiting field treatment with unique treatment acronyms as follows; i) plug plants grown on pasteurized planting mix treated with TG and planted in non-treated fruiting field plots (Effect of TG with higher colonization potential - PM+TG+NF); ii) plug plants grown on non-pasteurized/regular planting mix treated with TG and planted in ASD plots (Synergistic effect of TG with regular colonization potential and ASD - RM+TG+ASD); iii) plug plants grown on non-treated planting mix and planted in ASD fruiting field plots (Effect of ASD - RM+NT+ASD); iv) plug plants grown on non-pasteurized planting mix treated with TG and planted in non-treated fruiting field plots (Effect of TG with regular

colonization potential - RM+TG+NF); v) plug plants grown on regular non-treated planting mix and planted in 'Caliente 199' mustard cover crop plots (Effect of biofumigation - RM+NT+MCC); vi) plug plants grown on non-treated planting mix and planted in non-treated fruiting field plots (Non-treated check - RM+NT+NF).

Fig. 2. Mustard biofumigation of field plot; flail mowed tissues are incorporated in designated plots with a walk behind roto tiller.

Anaerobic soil disinfestation

ASD on selected plots was done according to Shennan et al. (2017) in 3 steps as described in the introduction section with minor modifications. Briefly, 50:50 mix of Brassica juncea/Sinapis alba seed meal was used at the rate of 4500 kg/ha as the labile carbon source for ASD. Triumph Italia brand Brassica juncea was procured as Biofence (Agrium Italia, Livorno, Italy) while and applied to the plots that were marked to receive the treatment by random distribution. Like mustard cover crop, mustard meal was either manually raked or rototilled thoroughly to mix with the soil followed by preparing the raised plastic mulched bed with a bed maker where a drip irrigation line was set in the middle of the bed at the same time. These plots were irrigated through drip line up to saturation of soil to make an anaerobic soil environment. The plastic mulch was perforated three weeks after incorporation of mustard meal to ensure release of residual toxic gas. Seedlings were transplanted in the holes 48 h after opening holes through the plastic.

Fig. 3. Mustard meal incorporation in field plots for anaerobic soil disinfestation (ASD)

Field trial set up

The second phase of the research was conducted in the fruiting field with three side by side raised plastic mulched beds with a buried drip irrigation line in the middle of each bed. All treatments were replicated three times in a randomized complete block design. Each replicate plot was 6.1 m long, 68.5 cm wide and 15 cm high, with a 1.5 m center-to-center bed spacing. The black root rot susceptible but popular strawberry cultivar Chandler was used in the study. These plots were planted on each bed in two staggered rows 60 cm apart within rows and 45.7 cm apart between rows with plug plants grown as previously described. Therefore, each treatment unit comprised 20 plants. Plots were staggered across the experimental site with 1.5 m of a non-planted area between plots and within adjacent beds to minimize inter-plot interference. Plots that received mustard bio-fumigation and ASD treatments were prepared accordingly to match the projected planting date of yr-1. These processes were completed 21 days before planting. Immediately after planting, plants were irrigated manually with a watering can as well

as through drip irrigation line to prevent desiccation and aid in establishment. Blood meal was applied manually in the fall after planting, spring of Yr-1 and Yr-2 at the rate of 2 g/plant. No other chemical fertilization or pesticides were applied, to keep plant management consistent with the regulation of USDA certified organic system. Weeding was done as needed for the weeds grew through planting holes and row middles. Irrigation was provided through the drip tape as needed.

Soil nutrient analyses before and after treatment:

Soil sample was collected from the whole experimental unit before initiating any treatment in the early spring of Yr-1 by taking multiple samples that were mixed together to have a composite sample to represent the baseline nutrient status of the plot. Soil samples were taken again by uprooting plants from each replicate plot of each treatment. Plants were then carefully shaken to collect rhizosphere soil as samples for nutrient analysis together with pH and organic matter content. All samples were analyzed at the WVU soil testing laboratory. Mehlich-3 method (Mehlich, 1984) was used for available P, K, P saturation index and LR Mehlich-1 for pH determination. Organic matter content was determined by following Hoogsteen et al. (2015).

Weed population assessment in treated plots

As plastic mulch was used to cover the beds as well as row middles, only weed growth was observed through the holes used for planting strawberry plugs. No weeding was done during fall of after planting. Higher number of weeds were noticed during the spring of Yr-1 when weeds were uprooted and counted from each planting holes. Number of weeds from all 20 holes were counted and averaged for each replicate of each treatment.