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Cucurbit Grafting - Management of Cucumber Beetle Complex and Bacterial Wilt in High -Tunnel Cucurbit Production

Introduction

The cucumber beetle complex causes serious damage, both direct and indirect to cucurbit crops such as cucumbers, melons and squash. Overwintering adults cause feeding damage on young plants, larvae in the soil feed on plant roots and second-generation adults cause feeding damage on plant leaves, flowers and fruit. Even more severe is the indirect damage sustained from the cucumber beetle complex through transmission of the bacterial pathogen *Erwinia tracheiphila*, which causes bacterial wilt (BW), leading to plant death. The hosts of *Erwinia tracheiphila* are wild and cultivated cucurbits, with cucumbers being the most susceptible hosts (Nadarasah and Stavrinides, 2011).

In Indiana cucurbit crops, two species of cucumber beetle present the most challenges for sustainable production. These are the striped cucumber beetle, *Acalymma vittatum* and the spotted cucumber beetle, *Diabrotica undecimpunctata howardi* (Ingwell, 2015). Adults of the two species are easily identified by their markings. The spotted cucumber beetle (SpCB) is somewhat larger with dark black spots; whereas, the striped cucumber beetle is marked with long black stripes down its back (Snyder, 2015). While both beetles feed on cucurbits, the striped cucumber beetle (StCB) has a much stronger preference for cucurbits and rarely feeds on other plants (Snyder, 2015). Conversely, the spotted cucumber beetle feeds on over 200 different crop and non-crop plants (Snyder, 2015). StCB and SpCB acquire *E. tracheiphila* by feeding on infected cucurbits, which then migrates to the insect gut epithelium. Infected beetles then vector the pathogen to healthy plants via fecal droppings (frass), which leach into the lesions created by feeding (Nadarasah and Stavrinides, 2011). Once inside the plant, the pathogen spreads and multiplies in the xylem and reduces water transport which leads to the subsequent wilt of the plant.

To help meet the demand for locally-grown and organic food, greenhouse and high-tunnel cultivation methods are being added to the production matrix to help extend the growing season and increase crop output (Carey, 2009). High-tunnel cultivation is widely used for cucurbit production. With an extended growing season, cucurbits are at greater risk for pest and pathogen damage. In addition, pesticide restrictions further complicate control options for successful pest management. A sound management approach to the disease is to reduce or prevent cucumber beetle feeding (Foster, 2016). Therefore, continued high-tunnel cucurbit production success is dependent on developing sustainable management strategies for controlling the cucumber beetle complex (Ingwell, 2015).

Due to the susceptibility of pest infestation and pathogen transmission, cucurbit pest management in high-tunnel production remains challenging. The objective of this two year project, undertaken by Laura Ingwell, Post Doc Research Associate at Purdue University, is to "identify the vectoring Diabrotica species and their role in BW epidemiology in high tunnels, as well as test novel management strategy alternatives for organic growers" (Ingwell, 2015). As part of that project, my research focused on examining the effectiveness of cucurbit grafting as a means of establishing resistance of cucurbit feeding by the cucumber beetle complex.

Vegetable grafting represents a viable means for establishing disease resistance, extending crop production and allowing a varietal matrix of vegetable selection (Ingwell, 2015). Grafting is the combination of two distinct plants into one and can be used to manage disease and increase crop yield. Grafting as a disease management tactic has rapidly expanded in vegetable production. Factors that have led to increased expansion of grafting include: increased pathogen inoculum densities due to intensification of production practices, reliance on susceptible cultivars to meet specific market demands, increased use of organic practices and the rapid adoption of high tunnel production systems (Louws and Rivard, 2010). Interspecific grafting is a common grafting approach used with cucurbits, where combinations of rootstock and scion that are of different species are used. With interspecific grafting, the use of a rootstock with resistance to a specific pathogen can be used to aid in the reduction of pathogen transmission (Louws and Rivard, 2010). Cucumbers and melons are highly susceptible to BW; whereas, other cucurbits such as squash and watermelon are resistant to or have a high tolerance for the bacterial pathogen. Therefore, rootstocks from watermelon and squash may serve as a viable means for reducing the feeding preferences of the cucumber beetle complex on cucumbers and melons; thereby, reducing the incidence of BW in high tunnel cucurbit production. In theory, the newly grafted plant will acquire resistance from watermelon and squash rootstocks and produce a scion that is more resistant to bacterial wilt.

Hypothesis

If sound cucurbit grafting techniques are employed and the selected varieties of rootstocks (watermelon and squash) and scions (cucumber and cantaloupe) are compatible, then rootstock from resistant cucurbits will reduce the incidence of feeding and BW transmission by the cucumber beetle complex.

Methods and Procedures

Cucurbit Planting

Under a greenhouse setting, 576 cucurbit seeds were planted, which included Spacemaster cucumber, Minnesota Midget cantaloupe, Big Red watermelon hybrid and Buffy Winter squash. Two 72 cell count trays of each cucurbit were planted and watered with automatic misters twice per day for 10 minute intervals. With seedling growth and cotyledon development, plants were transferred to 48 cell count trays. Cucumber and zucchini were also planted to serve as food for resident beetle population.

Grafting Methods

Cucumbers and melons are highly vulnerable to BW; whereas, watermelon and squash are resistant or show a low susceptibility (respectively) to the disease and therefore, served as preferred grafting selections (Diver, 2008). The survival rate of grafted plants depends on compatibility between scion and rootstock, quality and age of seedlings, quality of the joined section and post-graft management (Davis, 2008). Grafting trials were performed in the Entomology Environmental Lab greenhouse at Purdue University. Initially, two grafting methods were considered and performed: the tongue approach method (Fig. 1) and the one cotyledon method (Fig. 2). However, the one cotyledon method (Fig. 2) was utilized as it is the preferred approach when rootstocks have thin stems, i.e. watermelon and cucumbers (Davis, 2008). This method was also suitable for the cucurbits selected since the rootstocks and scions were of similar size. Grafted cucurbits were planted in 6 cell count trays and housed in the greenhouse. Examples of the two grafting methods are detailed in Figures 1 and 2 below.

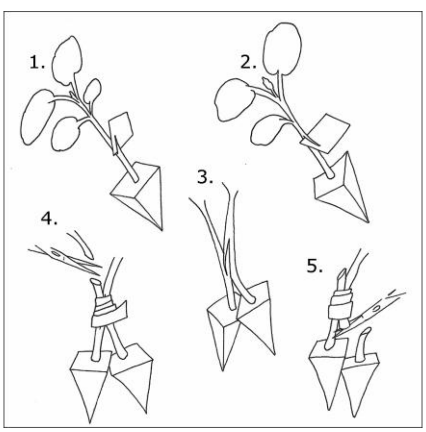


FIG. 1. **The tongue approach grafting method.** Step 1, preparing the rootstock; Step 2, preparing the scion; Step 3, joining the scion to the rootstock; Step 4, securing the joining with a metal strip; and Step 5, removing the scion roots. (Davis, A.R. et al., 2008)*

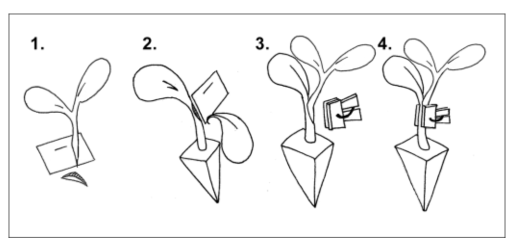


FIG. 2. **The one cotyledon grafting method**. Step 1, preparing scion; Step 2, preparing the rootstock; Step 3, joining the plants; Step 4, securing the joined region with a grafting clip. (Davis, A.R. et al., 2008)*

*Taken from: Davis, A.R. et al. (2008) *Cucurbit grafting*. Critical Reviews in Plant Sciences. 27:1

For this project, the following cucurbits were used in the grafting process:

- Spacemaster Cucumber - scion
- Minnesota Midget Cantaloupe - scion
- Big Red Watermelon Hybrid - rootstock
- Buffy Winter Squash - rootstock

Spacemaster cucumber was self-grafted and grafted onto rootstock of Buffy and Big Red. Likewise, Midget cantaloupe was grafted onto rootstock of Buffy, Big Red and self-grafted. Sixty grafted plants were deemed viable for the experiment based on grafting success and overall plant health.

Beetle Collection

Approximately 150 - 200 live striped and spotted cucumber beetles were collected from Meigs horticultural farm, part of the Throckmorton Purdue Agricultural Center (TPAC) in late August, 2016. Beetles were taken from cucumber, squash and pumpkin plants grown in both field and high tunnel settings at Meigs. Spotted beetle populations were abundant; however, striped beetle density was sparse with the SpCB representing the majority collected. Two collection methods were utilized. Beetles were removed from plants via insect vacuuming, using a vacuum (D-Vac suction sampler), as well as a manual apparatus consisting of a mason jar, funnel and duct tape which captured the insects when the plants were manually tapped. Collected beetles (StBC and SpBC) were maintained in one large mesh cage in Dr. Ian Kaplan's Insect Ecology lab. Beetle collection and maintenance modeled methods used by Mitchell and Hanks (2009) with regards to caging, colony additions and cucurbit feeding (Mitchell and Hanks, 2009).

Beetle Assays- Late August, 2016

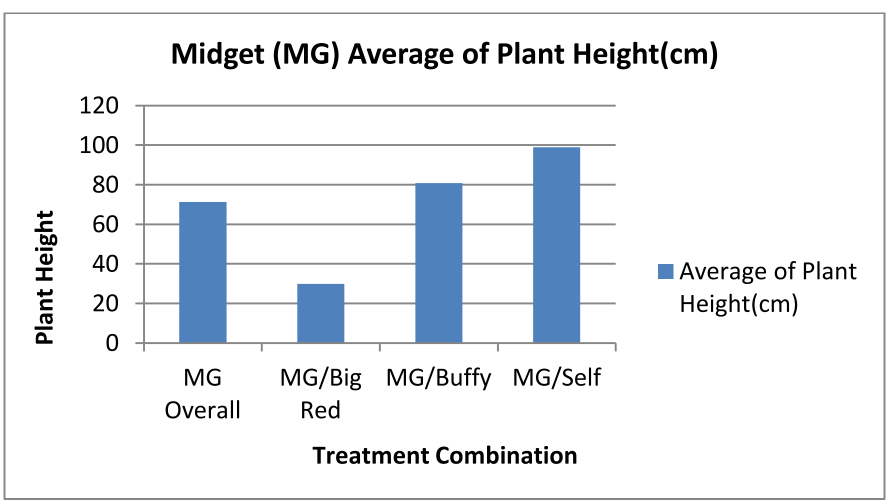
The experiment was structured using 60 grafted cucumber and melon plants and 60 cucumber beetles. Due to limitations on availability of StBC (preferred cucumber beetle), SpBC were used for the majority of the assays. Each of the ten cages contained one of each of the 3 grafted cucumber treatment combinations: Spacemaster/Self, Spacemaster/Buffy and Spacemaster/Big Red, along with 3 cucumber beetles. Likewise, the other 10 cages each contained 3 grafted melon treatment combinations: one each of Midget/Self, Midget/Buffy and Midget/Big Red, along with 3 cucumber beetles. The format for the experiment was randomized block design, i.e. there was no predetermined selection or pairing of individual treatment combinations. All treatment combinations were randomly selected for each cage, to represent the 3 different treatments per cage. Over a two-week period, plants received regular watering and were observed daily for growth, flower production, aphid infestation and occurrence of beetle feeding damage.

Results

Plant Height Distribution - Midget

The Midget/Self treatment combination served as a measurement standard for determining growth pattern since the self variety graft should present the same or similar growth as a non-grafted Midget melon. Average plant height for the Midget/Self treatment combination was 98.91cm as shown in Figure 3 below. Average plant height measurement for the other 2 treatment combinations, Midget/Buffy and Midget/Big Red, exhibited similar height or were significantly shorter in height, 80.76cm and 29.78 respectively as detailed in Figure 3 below.

Figure 3



ANOVA Midget Height

The Anova Analysis of Variance below (Table 1) also shows that there was a significant difference (Prob > F of <.0001) in plant height for the graft combinations of Midget.

Table 1

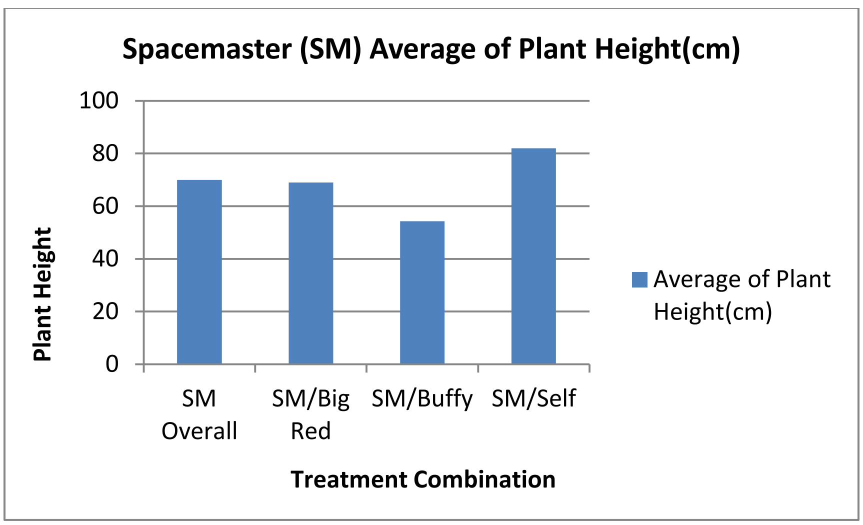
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Plant ID	2	24034.581	12017.3	67.5709	<.0001*
Error	26	4624.029	177.8		
C. Total	28	28658.610			

Plant Height Distribution - Spacemaster

The Spacemaster/Self treatment combination also served as a measurement standard for determining growth pattern among the Spacemaster treatment combinations. Average plant height for the Spacemaster/Self treatment combination was 81.97cm; whereas, Spacemaster/Big Red and Spacemaster/Buffy were not significantly shorter at 68.98cm and 54.29cm respectively as shown in Figure 4 below.

Figure 4



ANOVA Spacemaster Height

The Anova Analysis of Variance below (Table 2) shows that there was not a significant difference (Prob > F of 0.0509) in plant height for the graft combinations of Spacemaster. All plants were roughly of the same height (Figure 4 above) as depicted by the proximately to the mean.

Table 2

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Plant ID	2	3171.775	1585.89	3.3802	0.0509
Error	26	11260.046	469.17		
C. Total	28	11431.821			

Plant Height Distribution Analysis

Based on the data collected, graft treatment combination may contribute to plant height/growth. The source for the variance in plant height is inclusive. There was a significant difference in Midget plant height as seen in Figure 3 above. Since watermelon presents a slower growth pattern as compared to cantaloupe, Big Red watermelon hybrid rootstock may have also contributed to the shorter plant height exhibited with the Midget/Big Red treatment combination. As for Spacemaster plant height, there was no significant difference in height between the 3 treatment combinations as represented in Figure 4 above.

Beetle Preference - Area Removed: Midget

Upon review of the Nonparametric Comparisons for each pair using Wilcoxon Method (Table 3), there was a significant difference with regard to feeding (area removed) between Midget/Self and Midget/Big Red grafts as indicated by the p-value of 0.0262 which is lower than the alpha value of 0.05.

Table 3

Nonparametric Comparisons For Each Pair Using Wilcoxon Method

q*	Alpha
1.95996	0.05

Level	-Level	Score Mean Difference	Std Err Dif	Z	p-Value
Midget/Self	Midget/Big Red	4.961111	2.231301	2.223416	**0.0262***
Midget/Self	Midget/Buffy	3.700000	2.338466	1.582234	0.1136
Midget/Buffy	Midget/Big Red	0.950000	1.642415	0.578416	0.5630

Beetle Preference - Area Removed: Spacemaster

Upon review of the Nonparametric Comparisons for each pair using Wilcoxon Method (Table 4), there was no significant difference with regard to feeding (area removed) between any of the Spacemaster graft combinations as indicated by the p-values. None of the p-values were below the alpha value of 0.05.

Table 4

Nonparametric Comparisons For Each Pair Using Wilcoxon Method

q*	Alpha
1.95996	0.05

Level	-Level	Score Mean Difference	Std Err Dif	Z	p-Value
Spacemaster/Self	Spacemaster/Buffy	3.52143	2.431471	1.44827	0.1475
Spacemaster/Self	Spacemaster/Big Red	3.00000	2.606672	1.15089	0.2498
Spacemaster/Buffy	Spacemaster/Big Red	-1.21429	2.353758	-0.51589	0.6059

<u>Discussion</u>

The goal of this project was two-fold: the successful grafting of cucumber and melon scions with compatible cucurbit rootstocks and the reduction of BW incidence through selected cucurbit grafting to develop feeding resistance of the cucumber beetle complex. The data from the Nonparametric Comparisons for each pair using the Wilcoxon Method for Minnesota Midget showed there was a significant difference in area removed (mm^2) between Midget/Self and Midget/Big Red as depicted again in the bar graph below (Figure 5). One assumption could be that the beetles preferred to feed on Midget/Self grafts over Midget/Big Red. However, it is inconclusive as to if this difference was due to resistance factors from Big Red or if it was due to Midget/Big Red grafts being smaller plants, having less available leaf area to be consumed as compared to Midget/Self grafts. Conversely, with regard to the Spacemaster graft combinations shown in the second graph below (Figure 6), the data analysis did not support any significant difference in beetle feeding preference as feeding was relatively uniform amongst the graft combinations. The graph of Beetle Preference on Midget Grafts (Figure 5) reiterates that there is a significant difference between Midget/Big Red (A) and Midget/Self (B), yet no significant difference between either graft combination (A or B) and the middle graft combination, Midget/Buffy (AB).

After review of the results and to improve future experiments, 20-30 beetles per cage are recommended to more accurately depict a natural feeding environment in a field or greenhouse setting. While the use of spotted cucumber beetles was not a huge hindrance to the successful completion of the experiment, stripped cucumber beetles would be the ideal choice for the inoculation assays as they are monophagous and are the best vectors of BW.

Figure 5

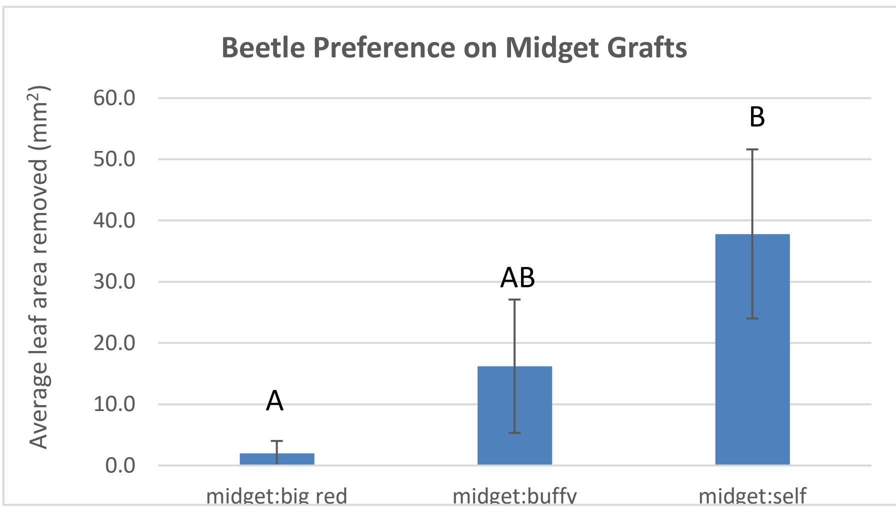
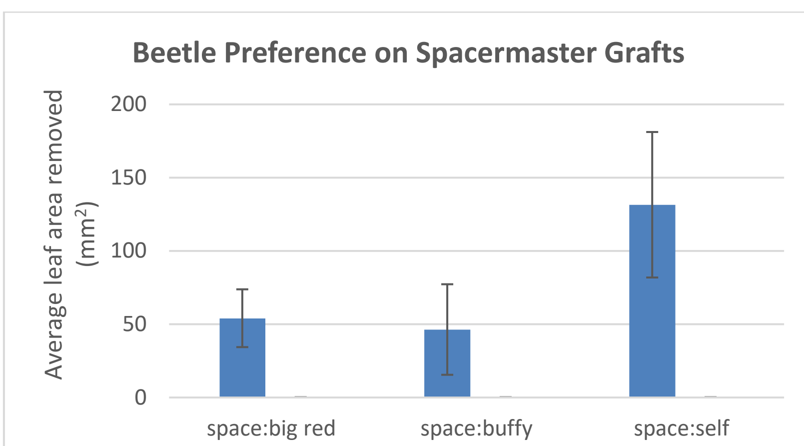


Figure 6



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