BIOAg PROJECT PROGRESS REPORT 2011

TITLE: Grafting Vegetables for Soil-Borne Disease Resistance

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KEY WORDS
Grafting, Verticillium wilt, tomato, eggplant, watermelon, rootstock

ABSTRACT
Tomato, eggplant, and watermelon can be significantly impacted by Verticillium wilt, a soil-borne disease common throughout Washington. Verticillium microsclerotia are known to persist in soil for over 13 years. Symptoms impact plants later in development after most production costs have been incurred, resulting in a 25-100% crop loss in some years even when ground has been fumigated prior to planting. Grafting vegetable crops onto resistant rootstock is a cultural control method that provides an organic and sustainable alternative to soil fumigation. Grafting has been used successfully in Asia for nearly 100 years, but is only now being adopted in the U.S. Grafting adds to production costs but is cost effective where disease pressure is high, such as in high tunnels and hoophouses, when high-value crops are often grown with minimal crop rotation. In Washington, both conventional and organic growers have expressed interest in grafting as an IPM strategy. This project examined the effects of grafting on disease resistance of heirloom tomato, eggplant, and watermelon in open-field production in Washington via on-farm and on-station trials. This project addressed the BIOAg priority area No. 1 by investigating a biologically intensive approach to disease management.

To increase grafting success, a healing chamber study was set-up and we found that an effective design involves a height of approximately 0.6 m covered with plastic and shade cloth to maintain 85-95% humidity, and temperatures maintained between 20-25 °C. In 2010 and 2011, grafting Cherokee Purple tomato with Maxifort or Beaufort rootstock did not affect total or marketable fruit number and weight. There were no Verticillium wilt symptoms observed in tomato in this study either year. In contrast, grafting Epic eggplant onto ‘Beaufort’ rootstock resulted in more vigorous plants with generally higher yields and lower severity of Verticillium wilt, even though ‘Beaufort’ rootstock was not found to be resistant to V. dahliae. Eggplant grafted onto Solanum aethiopicum had lower vigor and generally higher Verticillium wilt severity than other treatments, thus this rootstock is not recommended for grafting eggplant in the Pacific Northwest. Crisp’n Sweet watermelon grafted onto ‘Emphasis’ and ‘Strong Tosa’ rootstock had significantly less Verticillium wilt severity than non-grafted and self-grafted watermelon, however there were no differences in total and marketable fruit number and weight of fruit. Plant damage from high winds in the Columbia Basin in 2011 and cool 2010 and 2011 growing seasons in western Washington resulted in inconclusive harvest data for this study. Further research is needed to better understand the effect of grafting on watermelon yield and Verticillium wilt severity in the Pacific Northwest.
**PROJECT DESCRIPTION**

This study was conducted at WSU Mount Vernon NWREC and in cooperating growers’ fields with high *Verticillium* wilt pressure. In 2010, resistant commercial rootstocks and seeding dates were identified, and grafting techniques tested and selected for each crop. In 2011, due to healing chamber issues during grafting in 2010, a greenhouse study was conducted in January-February to determine the optimal healing chamber design for successful grafting of tomato, eggplant, and watermelon. Percent survival of the three crops was measured in three healing chamber designs with differing dimensions, materials, and humidity treatments (Johnson and Miles, 2011). Results from this study were used to select a healing chamber design for 2011 field trials.

For field trials each year, we grafted approximately 300 plants each of heirloom tomatoes, eggplants, and triploid watermelons. Tomato, eggplant, and watermelon were each grafted with two rootstocks with the goal of finding resistance to *V. dahliae*. For tomato, treatments were: non-grafted Cherokee Purple (CP), self-grafted CP, CP:Maxifort grafted plants, and CP:Beaufort grafted plants. For eggplant, treatments were: non-grafted Epic, self-grafted Epic, Epic:Beaufort grafted plants, and Epic:*S. aethiopicum* grafted plants. For watermelon, treatments were: non-grafted Crisp’n Sweet (CnS), self-grafted CnS, CnS:Emphasis grafted plants, and CnS:Strong Tosa grafted plants. Both years, the trial was planted with five replications with 6 plants per plot, so total number of plants per trial location was 120. Field trials were at on-farm sites in the Columbia Basin and at WSU Mount Vernon NWREC each year.

In 2010, on-farm sites were Alan Schreiber’s farm in Eltopia, WA, for tomato and eggplant, and the OSU Hermiston REU and Sam Pollock’s farm in Hermiston for watermelon. In 2011, all on-farm studies were at Alan Schreiber’s farm. In March of each year, soil samples were collected at prospective on-farm field block locations, assays were conducted to determine *V. dahliae* inoculum density in the soil, and results were used to select field sites with high disease pressure. In 2010, tomato and eggplant were transplanted on June 7 and watermelon were transplanted on June 8, and in 2011 all crops were transplanted on May 10. High winds in 2011 following transplanting caused significant mortality in the watermelon trial, and plants were replaced on June 8. However, there were not enough plants to replace all treatments and trial replications were reduced from five to two. At WSU Mount Vernon NWREC, tomato, eggplant and watermelon were transplanted on June 10 to June 14 in 2010, and June 3 to June 14 in 2011. In addition, we conducted a greenhouse inoculation study from September through November 2011 with eggplant and *Verticillium* isolates that we obtained at WSU Mount Vernon NWREC in 2010. Results from this study will not be available until Spring 2012.

In all field studies both years, plant health, vigor, and *Verticillium* wilt severity were rated biweekly in the field. Tomato and eggplant yield (fruit number and weight) were measured weekly following first harvest, and watermelon yield was measured up to three times until the end of August. Fruit quality of tomato and watermelon was measured through soluble solids (°Brix), firmness, and lycopene content. Average fruit diameter, length, and weight were recorded for all three crops. Whole plants were harvested in late August or early September and whole-plant dry weight was measured where possible. Plants were assayed for *Verticillium* wilt in the laboratory. Isolates obtained from stem assays were sent to WSU Puyallup for positive identification through DNA sequencing.

Expected outcomes from this project include: 1) An effective disease control method for *Verticillium* wilt that can be utilized by organic and other growers; 2) An expansion of crop options in the Pacific Northwest due to availability of a new, effective disease control method; and 3) The opportunity for a new agricultural industry to develop – the production of grafted transplants and rootstocks.
**Outputs**

**Work Completed:**

*Columbia Basin tomato trial.* Verticillium wilt symptoms were not observed in plants in either 2010 or 2011, and no *Verticillium* sp. conidia or microsclerotia were isolated from sampled stems. In 2010, there were no significant differences in total yield and total number of fruit, marketable yield and marketable number of fruit, and mean fruit weight among treatments. In 2011, there were no significant differences in marketable number of fruit and mean fruit weight between treatments. ‘Beaufort’ grafted tomato did have significantly greater marketable yield than ‘Maxifort’ and non-grafted tomato (P=0.0056), but was not significantly greater than self-grafted tomato (P=0.1101).

*Columbia Basin eggplant trial.* In 2010, there were no significant differences in number of marketable fruit (P=0.1437) or mean fruit weight (P=0.1731) among treatments. ‘Epic’ eggplant grafted onto ‘Beaufort’ rootstock had higher marketable fruit yield than non-grafted eggplant and significantly higher marketable fruit yield than self-grafted eggplant and eggplant grafted onto *Solanum aethiopicum* (P=0.042). ‘Beaufort’ grafted eggplant was significantly more vigorous than non-grafted, self-grafted, and *S. aethiopicum* grafted eggplant (P=0.0012). However, there was no significant difference in dry weight among treatments (P=0.1734). Non-grafted and self-grafted eggplant were also significantly more vigorous than *S. aethiopicum* grafted eggplant (P=0.0025 and 0.0165, respectively). ‘Beaufort’ grafted and non-grafted eggplant had significantly less Verticillium wilt severity (as determined by visual rating of interveinal leaf chlorosis) than *S. aethiopicum* grafted and self-grafted eggplant (P=0.0005).

In 2011, ‘Epic’ eggplant grafted onto ‘Beaufort’ rootstock exhibited less Verticillium wilt severity than self-grafted eggplant (P=0.0541) and significantly less Verticillium wilt severity than non-grafted eggplant and eggplant grafted onto *Solanum aethiopicum* (P=0.0445). ‘Beaufort’ grafted eggplant were also more vigorous with significantly greater mean dry plant weight than all other treatments (P=0.0101). ‘Beaufort’ grafted eggplant had significantly greater plant height than all other treatments except self-grafted eggplant, as measured on Aug. 2 and Aug. 16 (P<.0001). ‘Beaufort’ grafted eggplant had significantly greater total marketable yield than self-grafted and *S. aethiopicum* grafted eggplant (P=0.0212), but there were no significant differences in total marketable fruit number or mean fruit size among treatments. Verticillium conidia were isolated from infected stems of all four treatments and confirmed as *V. dahliae* through DNA sequencing at the WSU Puyallup Plant and Insect Diagnostic Laboratory. Although ‘Beaufort’ grafted eggplant did not show resistance to the *V. dahliae* strain present at Schreiber and Sons Farm in Eltopia, ‘Beaufort’ rootstock is advertised as resistant to Verticillium wilt by De Ruiter Seed. Given our observations of significantly less Verticillium wilt severity in ‘Beaufort’ grafted eggplant, we conclude that ‘Beaufort’ rootstock is tolerant but not resistant to *V. dahliae* populations in this location. It is important to note that *V. dahliae* strains differ in host specificity and pathogenicity, therefore, it is difficult to make comparisons between locations.

*Columbia Basin watermelon trial.* In 2010 and 2011, no statistically significant differences were observed in Verticillium wilt severity, marketable yield, or fruit quality and characteristics among treatments. In 2010, all treatments had similar total marketable fruit yield (26.0-27.4 kg-plant⁻¹), although self-grafted and ‘Emphasis’ grafted watermelon had higher numbers of total marketable fruit (12.3 fruit-plant⁻¹ and 11.5 fruit-plant⁻¹, respectively) than non-grafted and ‘Strong Tosa’ grafted watermelon (10.0 fruit-plant⁻¹ and 9.5 fruit-plant⁻¹, respectively). Thus, mean fruit weight of ‘Strong Tosa’ and non-grafted watermelon (14.9 kg and 13.5 kg, respectively) were greater than for ‘Emphasis’ and self-grafted watermelon (11.7 kg-plant⁻¹ and 11.3 kg-plant⁻¹, respectively).
In 2011, grafted watermelon were damaged by unusually high winds after transplanting, reducing total complete replications from 2 to 5 and resulting in an experimental design that could not accurately be statistically analyzed. Nevertheless, we collected yield, fruit characteristic, and disease data throughout the growing season. We observed highest total marketable yield in ‘Strong Tosa’ grafted watermelon (8.98 kg-plant⁻¹) followed by non-grafted watermelon (8.46 kg-plant⁻¹) and self-grafted watermelon (5.9 kg-plant⁻¹), with ‘Emphasis’ grafted watermelon having the lowest marketable yield (4.3 kg-plant⁻¹). Non-grafted watermelon had the highest total number of marketable fruit (5.2 fruit-plant⁻¹) followed by ‘Strong Tosa’ grafted watermelon (3.8 fruit-plant⁻¹) and self-grafted watermelon (2.8 fruit-plant⁻¹), and finally ‘Emphasis’ grafted watermelon (1.5 fruit-plant⁻¹). However, ‘Emphasis’ grafted watermelon had the greatest mean fruit weight (6.8 kg), followed by ‘Strong Tosa’ grafted watermelon (4.9 kg) and self-grafted watermelon (4.2 kg), with non-grafted watermelon having the lowest mean fruit weight (3.2 kg-plant⁻¹). Results in 2010 results are not consistent with results in 2011, likely due to low sample number in 2011. No significant Verticillium wilt symptoms were observed in either 2010 or 2011, possibly due to host specificity of V. dahliae at the field sites; therefore, it was not possible to analyze disease severity. No V. dahliae conidia or microsclerotia were observed in 2010, however, Verticillium sp. conidia were isolated from infected stems of non-grafted ‘Crisp’n Sweet’ watermelon in 2011 and confirmed as V. dahliae through DNA sequencing at the WSU Puyallup Plant and Insect Diagnostic Laboratory.

Mount Vernon NWREC tomato trial. In 2010 and 2011, Verticillium wilt symptoms were not observed in any treatments during the growing season and Verticillium sp. was not isolated from sampled stems. In 2010, the trial was severely impacted by late blight (Phytophthora infestans), and we were not able to collect accurate harvest data. In 2011, there were no significant differences in total number of fruit harvested (P=0.1565), total fruit weight (P=0.0525), marketable number of fruit (P=0.9197), marketable weight (P=0.3346), or mean fruit size (P=0.6061) among treatments.

Mount Vernon NWREC eggplant trial. In both 2010 and 2011, grafted eggplant exhibited considerable Verticillium wilt symptoms and there were significant differences among treatments. ‘Beaufort’ grafted eggplant did not exhibit Verticillium wilt symptoms and no Verticillium microsclerotia or conidia were isolated from sampled stems. However, Verticillium sp. isolates were obtained from self-grafted ‘Epic’, non-grafted ‘Epic’, and ‘Epic’ grafted onto Solanum aethiopicum. Due to unusually cool growing conditions during 2010 and 2011, there was insufficient harvest to analyze difference in yield and fruit characteristics between treatments.

Mount Vernon NWREC watermelon trial. In both 2010 and 2011, grafted watermelon exhibited considerable Verticillium wilt symptoms and there were significant differences among treatments. Both years ‘Emphasis’ and ‘Strong Tosa’ grafted watermelon had significantly less disease severity than non-grafted and self-grafted watermelon (P=0.0002 and P=.0001, respectively). There was no significant difference in disease severity between ‘Emphasis’ and Strong Tosa’ grafted watermelon (P=0.1734 and P=0.7072, respectively) or non-grafted and self-grafted watermelon (P=0.3343 and P=0.6665, respectively). Also both years Verticillium microsclerotia were observed in stems from self-grafted watermelon, non-grafted watermelon, and ‘Strong Tosa’ grafted watermelon. These microsclerotia were isolated from self-grafted and ‘Strong Tosa’ grafted watermelon, and were confirmed as V. dahliae through DNA sequencing at the WSU Puyallup Plant and Insect Diagnostic Laboratory. Non-grafted watermelon were severely infected with Verticillium wilt, and mostly dead at the time of the stem assay; because necrotic stems had been colonized by saprophytic organisms, we were unable to obtain a clean isolate although verticillate conidia and microsclerotia were observed in the stem tissue. Verticillium tricorpus was isolated from the outside of an ‘Emphasis’ grafted watermelon stem and confirmed through DNA sequencing at the WSU Puyallup Plant and Insect Diagnostic Laboratory. However, V.
tricorpus is commonly found in soil and is currently thought to be saprophytic or only weakly pathogenic.

Publications, Handouts, Other Text & Web Products:

Outreach & Education Activities: In 2010, the project overview was presented at the PNVA conference on November 18 in Kennewick, and the project research proposal was presented at the WSU Horticulture Department seminar on December 2 in Pullman. In 2011, a hands-on grafting workshop was presented on Orcas Island on June 10 in cooperation with WSU San Juan County Extension. Grafted vegetables were featured at WSU Mount Vernon during the Tilth Farm Walk on June 13 and NARF field day July 14. A project overview was presented at the Skagit Men’s Garden Club on February 3 in Mount Vernon, the Master Gardener State Conference on September 22 in Ocean Shores, at the WSU Mount Vernon Graduate Student Symposium on October 27 in Mount Vernon, at The Tilth Conference on November 11 in Yakima, and at the Sno-Valley Tilth meeting on November 14 in Carnation. Preliminary results were presented at the annual ASHS conference on September 26 in Waimea, Hawaii, and at the PNVA annual conference on November 17 in Kennewick. An online presentation series on vegetable grafting was produced and posted on-line at http://vegetables.wsu.edu/graftingVegetables.html#presentations using Adobe Presenter to incorporate voice and animation. Published article on healing chamber environments for vegetable grafting in HortTechnology (Johnson and Miles, 2011).
**IMPACTS**

- **Short-Term:** We will establish successful grafting techniques for each of the three crops and make this information available to growers and suppliers. Currently, there are several guidelines available that contain conflicting information.

- **Intermediate-Term:** Growers will view grafted vegetables as a viable alternative to manage *Verticillium* wilt by the end of 2012. Growers who utilize grafted transplants will reduce fumigation applications by 2013. Seed and transplant suppliers will have rootstocks and grafted transplants available for growers in Washington by 2013.

- **Long-Term:** By 2016 fumigation will be used less frequently by conventional growers to control *Verticillium* in watermelon and eggplant crops in Washington; organic growers will recognize *Verticillium* wilt symptoms and utilize appropriate control practices including grafting and rotation (if possible); and new effective, affordable and available rootstocks will be identified for use in grafted eggplant, watermelon and tomatoes.

**ADDITIONAL FUNDING APPLIED FOR / SECURED**

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**GRADUATE STUDENTS FUNDED**

Sacha Johnson, MS, WSU, Horticulture and Landscape Architecture

**RECOMMENDATIONS FOR FUTURE RESEARCH**

Investigate healing chamber environment for watermelon to develop guidelines for successful grafting.

Investigate resistant rootstocks for watermelon at WSU Mount Vernon NWREC, where disease pressure is very high.

Investigate cucumber grafting.