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Summary

The use of grafted tomato for commercial production has been implemented worldwide, where soilborne disease pressure is high. Grafting with resistant rootstock is used to manage *Fusarium*, *Verticillium*, Root-knot nematodes, and bacterial wilt in several Asian, Mediterranean, and northern European countries. However, this technique is relatively unknown in the United States. Recently, direct-marketing avenues for small, sustainable farmers have increased, and consumer-based demand for vine-ripened organic heirloom varieties has made this specialty crop especially important. These cultivars are open-pollinated, and are typically very susceptible to an array of soilborne and foliar diseases. A research program was initiated to investigate the potential of grafting as an integrated pest management strategy to reduce soilborne disease and increase crop productivity for organic heirloom tomato production.

Because this research relies heavily on well-developed international techniques and practices, an extension objective was implemented to disseminate information regarding grafting benefits and techniques, and to facilitate local adoption of this technology. An eight-page extension article focused on grafting technique was published through the North Carolina Cooperative Extension Service, and has been distributed widely at both the regional and national level. It is also available on Cary Rivard's website, which is dedicated to tomato grafting research. Cary has been invited to speak at a number of grower conferences throughout the southern region and nationally as well.

During 2005 and 2006, field trials were initiated to determine the capability of grafting to reduce soilborne disease incidence in heirloom tomato. Bacterial wilt (caused by *Ralstonia solanacearum*) is a devastating soilborne disease in eastern North Carolina. CRA 66 and Hawaii 7996 genotypes were highly effective at reducing bacterial wilt in naturally-infested soils when utilized as a resistant rootstock for heirloom fruit production. No evidence of wilt was seen among resistant rootstock treatments when terminal disease incidence among non-grafted treatments was 75%, and 79% in 2005 and 2006, respectively. Heirloom scion grafted onto rootstock-specific cultivar, 'Maxifort', showed no symptoms of fusarium wilt (caused by *Fusarium oxysporum* f.sp. *lycopersic*i), and non- and self-grafted controls had 45-50% disease incidence. In the mountain region of NC, verticillium wilt is an especially severe problem for tomato growers as crop rotation is not typically employed. Grafting with 'Maxifort' showed high potential as a management tool for this disease based upon increased vigor under continuous and rotational treatments.

Several field trials in 2005 and 2006 investigated the ability of rootstock-specific hybrids to increase crop productivity under organic management practices in a growing

environment with little soilborne disease. Grafting with 'Maxifort' and 'Robusta' did not enhance yields when implemented into a typical on-farm organic production setting. Evaluation of alternative training systems indicated the importance of added vigor by 'Maxifort' through enhanced yields under "twin-headed" management in 2005. In 2006, yields were not increased under alternative training methods as compared to standard training system, but grafting with 'Maxifort' rootstock showed enhanced crop productivity among both training systems (P=0.005).

Introduction

Grafting is a valuable technique to reduce damage caused by biotic and abiotic stressors in agricultural systems. Americans have typically limited the use of this practice to perennial crops such as fruits and ornamentals, where the graft is made during the dormant season. However, the worldwide use of soft-tissue grafting is evident, as increased sustainability as well as productivity has become more important worldwide in fruit-bearing vegetable production systems. The cultivation of grafted vegetable plants began in Korea and Japan at the end of the 1920's when watermelon plants were grafted onto squash rootstock. Since this time, this technique has spread throughout Asia and Europe. Currently, 81% of Korean and 54% of Japanese vegetable cultivation uses grafting (Rivero, 2003).

Grafting can take place on a number of crops. However, because of the added expense, it is typically associated with melons, cucurbits, and members of the *Solanaceae* family such as eggplant and tomato. In many Mediterranean countries, this technique is being used to control root-knot nematodes, bacterial wilt, and other soil-borne pathogens, as an alternative to methyl bromide applications for eggplant as well as melon and tomatoes (Ioannou, 2001).

There are a variety of methods for grafting vegetable crops. Tube grafting has caught on as the primary method for vegetable grafting on the farm as it can be easily carried out with small healing chambers, and typical success rates for this technique are between 85 and 90 percent (Oda, 1995). Tube grafting takes place when the scion and rootstock are severed as seedlings and reattached with a small, silicon tube or clip. This technique has been highly effective as it can be carried out when plants are very small, thereby eliminating the need for large healing chambers while increasing throughput.

Grafted vegetable crops have been used extensively in high tunnel production systems as a way to increase sustainability. Because these operations typically do not use extensive crop rotation, high levels of soilborne disease can lead to significant yield losses. Conventional "non-grafted" operations rely heavily upon chemical fumigants to decrease soilborne disease levels. As a result, the implementation of grafting can reduce disease levels in operations where fumigants are either unwanted or unavailable (Oda, 1999).

Although grafting has become increasingly important for all soilborne diseases, it is fundamental in reducing damage caused by bacterial wilt (*Ralstonia solanacearum*) of tomato. This particular disease requires long rotation intervals to successfully eliminate primary inoculum. Furthermore, breeders continue to struggle as resistance often leads to poor fruit quality. Thus far, attempts to uncouple these traits have been relatively unsuccessful. Grafting has been essential in Asian horticultural production for eliminating bacterial wilt incidence in solanaceous crops (Oda, 1999). It has also been

used in tropical environments, like Brunei, where bacterial wilt incidence is so high that tomatoes cannot be planted unless the soil is sterilized or resistant rootstocks are implemented (Peregrine, 1982). In India, CRA 66 rootstocks were used to reduce bacterial wilt in tomatoes, and plant survivability rates at 1st harvest increased from 54.5% in the control to 100% (Tikoo, 1979). By the end of the season, none of the control plants had survived while 100% of the grafted plants continued to produce. Furthermore, the yield of the tomatoes with resistant rootstocks was 4 times that of the susceptible lines. This particular line was also identified for use against bacterial wilt in Germany and similar results were found (Grimault, 1994). Several Hawaiian lines (Hawaii 7996-7998) have been identified as suitable candidates for resistance to bacterial wilt (Oda, 1999). This technique could be a very valuable tool for eliminating bacterial wilt in tomato, pepper, and eggplant production systems.

Although the use of grafted vegetables is associated with disease reduction and/or abiotic stress, yield may be increased even without the presence of these identified stressors. Yields increased by as much as 106% compared to the control in watermelon production systems in Australia (Yetisir, 2003). Data which further supports this idea is sparse. However, in cases where lines have been bred specifically to be used as rootstocks, yield increases are evident. This trend indicates the need for further research and development of this technique. By increasing yields even without the presence of specific disease pressure, grafting can be an economically viable method for improving production.

Agriculture in America is changing. Everyday, organic practices are more widely encouraged in US markets. Furthermore, the reappearance of the farmer's market as a viable marketing tool, has made small-scale sustainable farming ever more popular. Heirloom tomatoes make up a significant portion of these markets, but their production can vary based upon the presence or absence of disease epidemics in the field. The occurrence of bacterial wilt and other soilborne diseases consistently plague heirloom tomato growers in the southeastern United States. This problem will continue to grow as Early Blight, caused by *Alternaria solani*, has forced many organic tomato production systems to move inside of greenhouses and other tunnel systems. This practice may ultimately lead to increased severity of bacterial wilt and other soilborne diseases as crop rotation is limited in these instances.

Because this technique follows sustainable as well as certified organic practices, it is essential to integrate this method into our organic production systems. Grafting can be used by organic growers as a way to combat bacterial wilt as well as a number of other soilborne pathogens. Using grafted transplants also coincides with economic and social sustainability in agriculture as well as environmental sustainability. With the growing availability and encouragement of local markets, the use of greenhouses and other intensified farming methods has increased in the United States. This system leads to many of the same problems that brought about grafting in other countries, and it is fundamental that we bring these ideas to the forefront in our own.

Objectives

The objectives of this project were three-fold:

1) An evaluation of rootstock/scion combinations was carried out in North Carolina through field trials. Grafted heirloom tomatoes were planted fields where

bacterial wilt incidence is historically high. Data was collected pertaining to disease incidence as well as yield and fruit quality. Furthermore, production techniques were analyzed in an effort to increase yield on a per-plant basis, thereby offsetting the added cost of using grafted transplants. The expected outcomes of this aspect are increased disease resistance in areas where bacterial wilt pressure is high and increased productivity even in the absence of disease. The practical application of this technique and its success was evaluated and manipulated in order to cater to the success of North Carolina farmers.

2) To determine the dynamics of induced resistance mechanisms when heirloom scion are grafted onto rootstocks. The grafting process imposes a severe wounding event upon the plant, and wounding has been associated with an induced defense response in tomato. In order to understand how grafting affects this phenomenon, and the expression of defense genes associated with wounding was investigated. This work will lead to a knowledge base that may impact breeding and other cultural techniques.

3) To communicate the results and ideas behind this research through extension and education. An active role was taken in an effort to introduce farmers as well as extension agents to this technology. Demonstration plots and field days were used to illustrate the benefits of grafting directly. Grower workshops and training seminars were conducted to show farmers how to graft their own transplants. Finally, we constructed an extension factsheet that describes both the grafting technique and identifies resistant lines suitable for rootstock.

Materials and Methods

All grafted and non-grafted plants were produced in NCSU greenhouse facilities. The bacterial wilt and organic crop productivity on-farm trials were set up in a randomized complete block design with four replications. Seven plants were used per plot, and typical cultural practices were employed. The mountain trial and CEFS trial was set up in split plot design with four replications. At CEFS, alternative training systems were employed, whereby each plant was trained in way that produced a twin-row of stakes down the length of the bed. Once the twin-row was established, vines were trellised in a normal fashion, similar to the Florida stake-and-weave method. All results were analyzed using ANOVA, and significant findings were identified using a protected LSD test (P=0.05).

For the induced resistance study, plants were raised in a growth chamber at the NCSU Phytotron. Plants were grafted, and tissue from grafted and non-grafted plants was destructively sampled at 24 h, 48 h, 4 d, 8 d, 16 d, and 24 d after grafting. Plant tissue was immediately frozen in liquid nitrogen, and RNA was extracted using the Qiagen RNeasy isolation kit. RNA was reverse-transcribed and real-time PCR was used to monitor the induction of *PIN II*, a gene known to be associated with wounding in tomato. *Actin 2/8* was used as an endogenous normalizer gene, and relative quantification analysis of *PIN II* induction was carried out.

Results and Discussion / Milestones

Bacterial Wilt Trials - Field trials were implemented in 2005 and 2006 to investigate the efficacy of grafting susceptible heirloom cultivars onto resistant rootstock under naturally-infested field conditions. Heavy bacterial wilt disease incidence was observed among non-grafted controls (75% and 79% incidence in 2005 and 2006, respectively),

and grafted treatments with resistant genotypes CRA 66 and Hawaii 7996 showed no symptoms of wilt in both years. Yield in 2005 was significantly higher in Hawaii 7996 rootstock treatments compared to non-grafted control (P=0.04). In 2006, yields were not collected due to hurricane events that occurred early in the fruiting season. CRA 66 and Hawaii 7996 genotypes used as resistant rootstock for heirloom tomato production were highly effective at preventing bacterial wilt from endemic populations of *R. solanacearum* in eastern NC. These lines may be extremely important for future rootstock breeding programs.

Organic Crop Productivity Trials - In 2006, field trials were initiated to determine the efficacy of using commercial rootstock, 'Maxifort' and 'Robusta', to increase crop productivity for organic heirloom production. In Alamance County, Fusarium wilt (FW), caused by *Fusarium oxysporum* f.sp. *lycopersici*, occurred. Self-grafted and non-grafted controls were susceptible and generated similar terminal incidence values of 50% and 46%, respectively. 'Maxifort' rootstock completely controlled the incidence of FW and 'Robusta' offered moderate control. Cumulative marketable and total yields were not impacted by FW incidence or rootstock treatment. Earlier yields were seen with 'Robusta' rootstock and 'Maxifort' delayed peak harvest in Alamance County. A third trial was located at the Center for Environmental Farming Systems in Goldsboro, NC. This trial was located at the Small Farm Unit, which has been managed organically since 1994. 'Maxifort' showed 50% higher yield than the non-grafted and self-grafted controls and was statistically significant (P=0.01). The results of these studies are inconclusive as to whether or not grafting with vigorous rootstock will be able to increase yield where low disease pressure from soilborne plant pathogens is evident.

Crop Rotation Trial - A crop rotation trial at the Mountain Horticultural Crops Research Station (Buncombe Co.) was conducted on land with a history of Verticillium wilt (race 2). Continuous production (12 years) reduced biomass compared to the crop rotation (3 year) (P=0.003). 'Maxifort' rootstock improved growth compared to self-grafted and non-grafted controls within each rotational treatment (P=0.0003). Under continuous management, 'Maxifort' had similar or better plant vigor than non- and self-grafted treatments under rotation. Grafting with vigorous rootstock may be an alternative way of managing Verticillium wilt by giving a growth advantage over non-grafted plants.

Induced Resistance Experiment – An experiment was carried out to determine the gene expression dynamics of *proteinase inhibitor II* (*PIN II*) as a result of grafting. *PIN II* expression was elevated as a result of grafting alone, indicated by the elevated expression of *PIN II* in the self-grafted treatments. Furthermore, grafting with various rootstock showed that the quantitative expression of this gene was affected by rootstock genotype. Gene expression of PIN II was highest at 4 days, and had returned back to normal levels at 16 days.

Impact of Results / Outcomes

Our findings illustrate the efficacy of rootstock to manage soilborne disease. We have identified two lines (CRA 66 and Hawaii 7996) with excellent resistance to bacterial wilt. These lines will be very important for future rootstock breeding programs in the

US. Currently, rootstock-specific hybrids are available in the US, but very few of these lines have bacterial wilt resistance. This disease is especially important throughout the southeast, and the adoption of grafting for tomato production relies on the ability to manage this disease. Furthermore, this data represents the best management tool for bacterial wilt to-date. Traditional breeding methods have not been able to produce cultivars that contain good fruit quality characteristics and resistance to this disease, and it is hypothesized that these traits are linked. Even chemical fumigation shows poor efficacy against bacterial wilt as the bacterium can quickly re-colonize fumigated land from below the fumigation zone. Clearly, grafting is a viable management tool for bacterial wilt.

We have also identified rootstock than can be utilized to manage fusarium wilt in naturally-infested conditions. This pathogen is particularly difficult to manage for heirloom growers due to lack of genetic resistance and the pathogen's ability to survive in the soil for a long period time (5-10 years). Although our evidence is preliminary, it appears that grafting may be a viable management tool for verticillium wilt (race 2) as well. This pathogen is extremely difficult to manage for organic and conventional growers as the only effective management tool is soil fumigation. There is no genetic resistance known in tomato to this pathogen, and crop rotations must be greater than 5 years to reduce pathogen populations. 'Maxifort' provides an alternative management strategy as the added vigor of this rootstock appears to compensate for the functional effect of the pathogen.

The results of the *PIN II* study highlight the importance of rootstock genotype for induced resistance. In our findings, it was shown that rootstock genotype quantitatively affected the expression of *PIN II*, a gene known to be associated with reduced herbivory by insects. However, the functional affect is still unknown. Rootstock breeders could utilize this information and the techniques developed in this study to select for induced resistance as a character trait in future breeding programs.

Economic Analysis

Our project did not have an economic analysis component

Publication / Outreach

This project has generated a tremendous amount of interest among sustainable and organic farmers throughout the US. An aggressive extension and education component was undertaken to disseminate information generated from this work and to teach growers how to graft on their own farm. Grafting workshops and seminars were given at local, regional, and national venues. Furthermore, internships and undergraduate research projects were utilized to further the spread of this information.

Publications:

- Rivard, C.R. and F.J. Louws, 2007. Disease Management and Crop Productivity Utilizing Grafted Tomatoes. *Proceedings from the 2007Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions*. San Diego, CA USA.
- Rivard, C.R. and F.J. Louws, 2007. Elevation of proteinase inhibitor II (*PIN II*) expression in tomato as a response to grafting. *Phytopathology* 97:S99 (Meeting Abstract).
- Rivard, C.R. and F.J. Louws, 2007. Grafting for soilborne disease management in organic heirloom tomato production. *Phytopathology* 97:S99 (Meeting Abstract).

- Rivard, C.R. and F.J. Louws, 2006. Grafting: An Integrated Approach for Soilborne Disease Management. *Proceedings from the 21st Annual Tomato Disease Workshop*. Fletcher, NC USA.
- Rivard, C.R. and F.J. Louws, 2006. Grafting for Disease Resistance in Heirloom Tomatoes. *Ag-675: Extension Factsheet.* College of Agriculture and Life Sciences, North Carolina Cooperative Extension Services. http://www4.ncsu.edu/~clrivard/TubeGraftingTechnique.pdf
- Rivard, C.R. and F.J. Louws, 2006. Grafting Provides a Multi-strategic Management Tool for Heirloom Tomato Production Systems. *Phytopathology* 96:S98 (Meeting Abstract).

Grower and Professional Presentations:

- **22nd Annual Southeast Vegetable & Fruit Expo** – 12/13/2007. Myrtle Beach, SC
  - "Using High Tunnels and Organic Practices to Produce Grafted Heirloom Tomatoes" – High Tunnel session.
- **Carolina Farm Stewardship Assoc Sustainable Agriculture Conf** – 11/10/2007. Durham, NC
  - "Grafting and High Tunnels for Organic Heirloom Tomato Production" – SARE Extension Track.
- **2007 Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions** – 10/31/2007. San Diego, CA
  - "Disease Management and Crop Productivity Utilizing Grafted Tomatoes" – Grafting and Other International Alternatives Session.
- **22nd Annual Tomato Disease Workshop** – 10/26/2007. Williamsburg, VA
  - "Grafting and High Tunnel Tomato Research" – Advanced disease management workshop.
- **Seasons of Sustainable Agriculture Fall Festival** – 10/15/2007. Goldsboro, NC
  - "Tomato Grafting Workshop" – Field day celebrating the Center for Environmental Farming Systems.
- **Seasons of Sustainable Agriculture Tomato Grafting Workshop** – 10/03/2007. Goldsboro, NC
  - Advanced half-day grower workshop including grafting technique and research
  - Participants were given hands-on training in grafting technique
- **American Phytopathological Society** – 07/30/2007. San Diego, CA
  - "Elevation of proteinase inhibitor II (*PIN II*) expression in tomato as a response to grafting" – Host Resistance / Molecular Genetics session
  - "Grafting for soilborne disease management in organic heirloom tomato production" – Poster Presentation
- **Chatham County Tomato Grafting Workshop** – 03/15/2007. Pittsboro, NC
  - Advanced half-day grower workshop including grafting technique and research
  - Participants were given hands-on training in grafting technique
- **Mid-Atlantic Fruit and Vegetable Convention** – 01/31/2007. Hershey, PA
  - "Tomato Grafting for Soilborne Disease Resistance" – Cultivar Showcase.
- **21st Annual Southeast Vegetable & Fruit Expo** – 12/13/2006. Myrtle Beach, SC
  - "Tomato Grafting: Benefits and Techniques of and Emerging Technology" – New Vegetable Technologies Session.
- **21st Annual Tomato Disease Workshop** – 11/09/2006. Fletcher, NC
  - "Grafting: An Integrated Approach for Soilborne Disease Management" – Advanced disease management workshop.
- **Seasons of Sustainable Agriculture Fall Festival** – 10/16/2006. Goldsboro, NC
  - "Tomato Grafting Workshop" – Field day celebrating 10th anniversary for the Center for Environmental Farming Systems.
- **National SARE Conference** – 09/16/2006. Oconomowoc, WI
  - "Grafting Provides Sustainable and Profitable Technology in Organic Heirloom Tomato Production Systems" – Poster Presentation.
- **MHCR&EC Field Day** – 09/03/2006. Fletcher, NC
  - "Evaluation of Grafted Tomato Under Various Management Regimes" – Research Trial Update.
- **American Phytopathological Society** – 07/30/2006. Quebec City, Canada
  - "Grafting Provides a Multi-strategic Management Tool for Heirloom Tomato Production systems" – Integrated Pest Management Session.

- **NC Cooperative Extension Regional Greenhouse Workshop** – 02/02/2006. Fletcher, NC
  - "Vegetable Grafting Provides Disease Management and Increased Crop Productivity for Greenhouse Production" – Advanced Grower Workshop.
- **Carolina Farm Stewardship Assoc Sustainable Agriculture Conf** – 11/05/2005. Durham, NC
  - "Inducing Disease Resistance and Increased Yields in Heirloom Tomato Production Systems Through Grafting" – Advanced Organic Grower Workshop.
- **Center for Environmental Farming Systems Field Day** – 07/30/2005. Goldsboro, NC
  - "Grafting Heirloom Tomato Seedlings for Increased Productivity on the Organic Small Farm Unit" – Presentation of intern research projects.

Guest lectures for undergraduate education:

- Vegetable Food Production (HS 431) – (11/01/07).
- Greenhouse Food Production (HS 590C) – (02/15/07).
- Vegetable Food Production (HS 431) – (11/02/06).

Undergraduate Research Program

- **Tristan Underwood – Department of Biology Undergraduate Research Project,** 2006.
  - "Growth Rates and Nutrient Uptake of Grafted and Non-grafted Heirloom Tomato"
  - Provided guidance on basic experimental design and analysis for phytotron study.
  - Successfully trained student on tomato grafting technique and initiated greenhouse assays evaluating rootstock for ability to increase plant vigor and nutrient uptake.
- **Patsy Wilson and Amanda Watson – CEFS Summer Internship,** 2005.
  - "Grafting Heirloom Tomato Seedlings for Increased Productivity on the Organic Small Farm Unit"
  - Guided interns on basic experimental design and analysis for field research plot.
  - Successfully trained interns on tomato grafting technique and initiated greenhouse assays evaluating rootstock for bacterial wilt resistance.

Farmer Adoption

Growers both in the southeast and throughout the US are extremely interested in grafting, especially for heirloom tomato production. Many growers have recently adopted this practice into their farming system. We have received many emails from growers that have found our work through the website, and have started grafting on their own. The success of the extension component was highlighted best at the Chatham County workshop. After we had given the participants the opportunity to graft tomatoes on their own, each one of them raised their hand when the event coordinator asked it they would be willing to try grafting at their farm. This is very encouraging, as many growers were afraid to try this technique on their own.

Areas Needing Additional Study

The bacterial wilt trials were highly encouraging for growers who are forced to manage this disease. However, the lines utilized in this study are breeders' lines, and are not commercially available. In 2007, we evaluated several commercially-available rootstock hybrids, and have identified one of these for resistance to bacterial wilt. In 2008, this evaluation will be repeated and verified.

Clearly, the advantage of grafting for organic heirloom growers can be seen in conditions where soilborne disease pressure is high. However, the economic importance of grafting under little disease pressure is not consistent. If growers can get elevated yields even under little disease pressure, then the added cost of grafting might be compensated by increased revenue. This trend was not consistent in our trials and

continues to be a question for future research. One aspect of this is cultural management. Our current tomato production system is based around the fact that transplants can be produced with little expense. Plant spacing and cultural practices may be able to find ways to optimize production on a per-plant basis, rather than per-acre. This type of research will be crucial to determine the most important economic questions associated with grafting.

The verticillium wilt trials also showed lots of promise for future work. Verticillium wilt is a severe problem for large-scale conventional growers in NC and nationwide, and if grafting could be a viable alternative to soil fumigation, then we could reduce the amount of toxic chemicals being released in our environment. In 2007, a verticillium wilt trial was carried out, and increased yield was seen. This trial will be repeated in 2008 for verification and further analysis. Because this disease is so problematic for large-scale growers, the environmental impact of these findings may be insurmountable.