

Development and Assessment of Bacterial Wilt and Downy Mildew Resistant Cucumber and Melon Seedstocks (Year One)- Final Report by Edmund Frost

Cucurbit downy mildew is a central limiting factor for cucumber and melon production in the Eastern U.S., especially for mid- and late-season plantings. The disease affects foliage, and can quickly kill or cause severe damage to plants, drastically reducing yields and fruit quality. The problem is especially impactful for organic farms, because organic-approved sprays have limited effectiveness against the disease.

Most popular cucumber varieties in the U.S. were resistant to downy mildew (DM) prior to 2004, when a mutation in the disease organism caused that resistance to break down. A few varieties with some resistance to the new strain are now available, but there is a need for better resistance and for more options. (For example, there is only one DM resistant pickling variety on the market, and some varieties promoted as resistant haven't held up in our trials).

Most melon varieties are likewise susceptible to downy mildew, making mid- and late-season plantings problematic to grow with organic management.

Bacterial wilt, a disease transmitted by cucumber beetles, can cause widespread vine wilting and plant mortality, and is another especially serious management challenge for organic growers, including at our farm.

This project represents a continuation and intensification of work started by Common Wealth Seed Growers in 2014, when we received a SARE grant to trial dozens of cucumber, melon and winter squash seedstocks from around the world, with a focus on downy mildew (read more at projects.sare.org/project-reports/fs13-273/). By crossing standout cucumber seedstocks that stood out in the trial, and starting on selection work, we came into 2018 with F3 seed of a population with promise for resistance to both downy mildew and bacterial wilt, as well as for fruit quality and productivity.

Our project had two main goals:

- Identify cucumber and melon varieties that can grow well in the presence of both downy mildew and bacterial wilt.
- Make progress in developing new cucumber varieties that have resistance to both diseases.

The work consisted of four related research and breeding trials conducted on certified organic land at Twin Oaks Seed Farm in Louisa, Virginia in 2018:

- 1) An early-planted variety trial focused on bacterial wilt.
- 2) A late-planted variety trial focused on downy mildew.

- 3) A small early-planted cucumber breeding trial.
- 4) A larger late-planted cucumber breeding trial.

While some challenges were encountered, especially in the downy mildew trial, all four trials yielded useful and actionable results. Following is a presentation of the methods, data and conclusions from each element of the project.

Bacterial Wilt Trial

See Figure 1 for data from the bacterial wilt trial.

Entries in the bacterial wilt (BW) trial included 13 melons (*Cucumis melo*), 24 cucumbers (*Cucumis sativus*), and two *Cucumis anguria* gherkin varieties. Five replications of most entries were planted (some entries had very limited seed availability so we planted less).

The trial was transplanted in the field on May 20th. Each plot had three plants, and each plot was trained and evaluated separately. Ripe fruits were harvested regularly, but yields were not measured (yields were measured in the DM trial – see next section). We evaluated BW impact approximately every 10 days from July 8th - August 11th, rating plots on a 1-6 scale. 1 indicated no damage, while a rating of 5 indicated severe vine dieback, and a rating of 6 indicated that a plant had died from BW. The attached data table (Figure 1) summarizes these ratings by reporting the percentage of plots of each entry that experienced severe BW damage (a rating of 5 or 6).

Marketmore 76 cucumber stood out as the cucumber least affected by BW, showing no severe damage in any of the plots. Sumter and County Fair (which is billed as BW resistant) showed the most BW damage for cucumbers (60% of plots had severe damage). Common Wealth Seed Growers' (CWSG) downy mildew resistant breeding population showed average or slightly better than average BW resistance (1 out of 5 plots showed severe damage). Wautoma BWR and Dekah BWR also showed no severe damage, but these had only one and two plots, respectively, in the trial.

Trifecta was the least BW-affected cantaloupe melon in the trial, showing no severe damage. There were also two non-sweet melon entries that showed no damage. PI 124112 showed the most damage (severe damage in all plots). This is significant because PI 124112 is often cited as a good source of downy mildew resistance. Care should be taken to evaluate BW resistance of breeding lines that incorporate PI 124112. Tai Nang 2 (PI 436531), from Taiwan, also showed a high level of damage, with severe damage in 60% of plots.

Trifecta and Marketmore 76 both displayed low BW susceptibility in 2014 trials at Twin Oaks Seed Farm, while PI 124112 displayed a high level of damage, and Tai Nang 2 displayed a moderately high level of damage (the primary focus of the 2014

trials was downy mildew, but bacterial wilt was also noted). The 2018 results confirm these observations.

The method we used in the bacterial wilt trial has good potential to assess field tolerance to BW, especially given the corroboration from 2014 trials, but more research is needed to confirm results (we are doing a 2019 cucumber BW trial). More widespread BW trialing using similar methods and evaluating more varieties would be quite useful in assessing BW resistance/tolerance of cucumber and melon varieties. Since yield is not measured such trials would be relatively easy to carry out.

Downy Mildew Trial

See Figure 1 for summarized data from the downy mildew trial; see figure 2 for more detailed data.

The downy mildew (DM) trial was transplanted in the field on July 5th and 6th. Plants were covered with row cover until flowering to exclude cucumber beetles. It was our intention in the trial to plant four replications of six cucumber entries and six melon entries. We also planted single plots of several other varieties and two replicates of several experimental cucumber lines from Cornell (for which we had limited seed). Unfortunately, due to an early damping off problem in the cucumbers associated with over-watering and high heat, some cucumber plots had to be hoed under and replanted. While we kept data on the replanted plots, it is not appropriate to compare that data to the transplanted plots. Additionally, some of the cucumber plots that were kept had fewer plants than intended (as few as 6 instead of the intended 9 plants). To address this issue, cucumber yields are reported on a per-plant basis in this narrative. See figure 2 for information on plant numbers and per-plot yields.

Plots were trained and evaluated separately. Yields, and marketable yields, were measured throughout the season. DM foliage ratings were recorded on five dates between August 22nd and September 25th, using a 1-9 scale, with 1 indicating no damage and 9 indicating that all plants in the plot were dead from downy mildew. When assessing downy mildew resistance it is important to also look at yields, because plants that aren't putting as much energy into fruit production are better able to withstand the disease. What we seek is not varieties that have good foliage at the direct expense of yields, but for varieties that can withstand DM while also yielding normally.

Our results showed clear difference in foliage ratings between varieties, and while yield data was more variable between plots of the same varieties in some cases, overall we also obtained meaningful results on yields.

Marketmore 76, our DM-susceptible control, yielded a median of 3.8 pounds per plant, and had a median foliage rating of 9 on 9/13. Several varieties and lines did significantly better.

Common Wealth Seed Growers' DMR (Downy Mildew Resistant) cucumber population showed the best and most consistent DM foliage ratings and yields in the trial, with a median foliage rating of 5 on 9/13, and a median per-plant yield of 11.1 pounds. Median percent marketability was above average at 80%. Ghost In the Wind (an unreplicated entry) showed comparable foliage ratings (with a rating of 5 on 9/13) and good yields (9.9 pounds per plant), with a marketability of 66%. County Fair, and two experimental DMR lines from Cornell, 17-7416-2 and 12-7417-1, showed good yields (9.2, 10.3 and 9.3 pounds per plant respectively), moderate foliage ratings in late August and moderate to poor foliage ratings later on (8, 7 and 8 median foliage rating on 9/13). Median percent marketable fruits was 75%, 74% and 82% respectively.

DMR 401, Shandong Si Gua 1210, and Jin Yang showed inconsistent results between plots in terms of yields, although foliage ratings were consistent between plots. This could be due to incidental difference in fertility or transplant quality, or could indicate variability in the seedstocks. Both entries of DMR 401 showed that marketable fruit percentages are a problem for the variety, with an average of 51% marketable fruits. The better performing DMR 401 plot showed very high yields of 14.5 pounds per plant. The 9/13 foliage rating for both plots was 7.5. Shandong Si Gua 1210 showed good foliage ratings, with an average rating of 6 on 9/13. DMR 264 from Cornell showed fairly good foliage ratings (6.5 on 9/13) but relatively low yields (median of 4.3 pounds per plant).

Yield and quality measurements in the melon downy mildew trial were heavily impacted by excessive and constant rain (associated with hurricane Florence and other storms) during the month of September that caused most melons to rot in the field before ripening, and that lowered brix levels. (Note that the cucumber trial was much less affected because most of the harvests occurred before this rainy period). The three varieties best able to handle these extreme conditions were Trifecta, Athena, and an experimental Athena-type line from Jason Cavatorta (there was only one plot of this line). These three varieties had the highest measurable yield (35.6 median, 32.2 median and 49.0 pounds per plot respectively; median DM ratings on 9/13 were 6.5, 7 and 5 respectively); however, brix levels for most of these melons were below marketable standards. Edisto 47 also had good DM foliage ratings (median rating of 5 on 9/13), but a higher incidence of fruits that rotted before ripening led to low yield numbers. Seminole melon showed good foliage ratings but extremely low yields. PI 124112 and Tai Nang 2 showed very high levels of bacterial wilt damage despite use of row cover, to the extent that it was hard to evaluate DM damage.

Two *Cucumis anguria* gherkin varieties were also entered in the DM trial. These both showed excellent DM resistance, with PI 196477 showing better yields and

better percent marketability, and West Indian Gherkin showing better DM foliage ratings (PI 196477 also did better in the BW trial, with no occurrence of severe BW). Per-plant yields for West India Gherkin and PI 196477 were 4.4 pounds and 5.0 pounds respectively, and foliage ratings on 9/13 were 1.5 and 2.5 respectively.

CWSG DMR Cucumber Breeding Trials

See Figure 3 for data and notes from the late breeding trial.

CWSG's DMR breeding population was developed from a three-way cross between Shandong Si Gua 1210 (PI 432885), PI 426170 from Luzon in the Philippines, and a U.S. variety called Homemade Pickles. The F3 seedstock entered in the variety trials described above was selected from an F2 breeding trial late in 2017, primarily for DM resistance and non-bitterness.

This F3 selection, along with three other selections made in the same 2017 breeding trial, were direct seeded on May 1st as an early breeding trial. 45 plants were evaluated for bacterial wilt occurrence, yield, shape, earliness, and non-bitterness. Five plants were selected and open-crossed seeds were saved in mid July.

A second breeding trial was direct seeded on July 24th using the seeds selected in the early breeding trial. We planted four 180-foot rows. We also started replacement plants on July 27th because of concern about the impact of a heavy rain on emergence. Two of the rows didn't come up well, and we replanted those rows with the transplants (the other two rows had somewhat low but still adequate emergence). We ended up with 102 direct seeded plants and 131 transplanted plants, 233 in total, spaced 2.75 feet apart with some gaps.

We started harvesting and evaluating fruits in mid September, tracking harvests with hash marks on survey flags placed next to each plant, and also recording notes on flavor, sweetness, crispness, shape, size, presence of bitterness, and percent marketability. We also took DM foliage ratings on October 1st. BW was only minimally present in the trial, and plants displaying any vine dieback from BW were culled.

Weather in September was extremely wet due to rainfall associated with hurricane Florence and other storms, but most of the plants still grew and yielded well.

See figure 3 for notes and data from the late breeding trial. Notes and data are presented for the 57 plants among the population that most stood out for the categories evaluated.

On September 24th we started hand pollinating, crossing promising plants to each other. We successfully matured seed from a majority of the most promising crosses, and sent the seed to Hawaii for increase over the winter. We now have seed quantities of each of the promising lines (crosses) selected in 2018 that enable us to

do extensive trials and breeding trials this year. In our 2019 breeding work we are moving forward with 12 crosses from 2018.

While the original goal of this project was to breed a pickling cucumber, plants in the breeding trial showed promise for developing both pickling and slicing varieties. Crosses made reflected these two divergent goals, and in 2019 we have pursued separate pickler and slicer breeding trials.

Outreach

Our outreach around this project consisted of a field day, speaking engagements, and online publication of results.

About 25 people, mostly local vegetable farmers, attended our field day on August 28th, which was focused on the downy mildew variety trial. Participants were able to see the clear difference in foliage between varieties – susceptible varieties like Marketmore 76 and Sumter were nearly dead from DM, while resistant varieties like DMR 401, CWSG's DMR cucumber, and Ghost in the Wind were going strong. An early presentation of trial data was available to participants, as were cucumber samples.

I presented project results over the fall and winter of 2018-2019, including at conferences hosted by Carolina Farm Stewardship Association, Virginia Association for Biological Agriculture, and Organicology.

A summary of the project results and a slideshow presentation about the project are posted on our website at www.commonwealthseeds.com/research. We also did outreach via email and social media.

Discussion and Next Steps

Common Wealth Seed Growers has also received a 2019 grant from OFRF to continue the project. This year we decided to focus our efforts on cucumber. Due to the success of CWSG's DMR cucumber in the DM trial, we are especially focusing on research and breeding work related to this population and the lines selected from it in 2018. An aspect of the 2019 project that is different from 2018 is that we are working with numerous farms and research sites throughout the Southeast and beyond to provide a broader assessment of the selected lines. At our farm we are following up with another bacterial wilt trial, and with late breeding trials of both slicers and picklers.

Despite setbacks caused by damping off, the results of the 2018 cucumber DM trial showed clear difference in foliage ratings between susceptible and resistant varieties. Results were more variable for yields, but still showed difference. In 2019 we are conducting replicated DM trials at two University research stations,

University of Massachusetts and North Carolina A&T, as well as single-entry trials at four southeast organic farms. We expect these trials to expand on and possibly corroborate the results of our 2018 trial. Multiple lines from CWSG's DMR cucumber breeding project are being tested in these trials, which will help in decisions about the ongoing breeding work.

The melon DM trial took place in extremely wet conditions, causing extensive fruit rot. More widespread or repeated late-planted trials are needed to determine the best melon varieties to use in late-planted, high DM conditions. However I think it is likely that breeding work using crosses between the best performing varieties would be a promising way forward as well.

Our 2019 breeding trial features 12 selections made in 2018, and approximately 400 plants total. The method we used in 2018 of evaluating plants and then crossing the best ones to each other was a success in terms of efficiently isolating the best-adapted genetics in the trial. It is an efficient process because it does not require self-pollinating each plant in the trial. Additionally it is a method that avoids potential genetic bottlenecking that can occur when plants are self-pollinated.

In 2019 we are only doing a late breeding trial, rather than two generations in the same season. This is possible because we had our best 2018 selections increased in a winter growout in Hawaii in order to have enough seed for the 2019 variety trials and for the breeding trial. Since DM resistance is a central goal and DM is only present in the mid and late season, the late breeding trial is most important. While we succeeded in doing two generations in 2018, timing was tight, and might not be successful in some years. This year we planted the late breeding trial slightly earlier, which will better ensure that we can get viable seed. Additionally, the seed increase in Hawaii was not complete until mid May, at which point it was too late to plant an early breeding trial here.

If 2019 trial results successfully confirm that our lines have good DM resistance, we hope to begin releasing varieties from the project in late 2020.

Pictures - See Commonwealthseeds.com/research for a slideshow presentation about on-farm plant breeding and trialing that focuses on the project.

Field Day in Downy Mildew Trial, August 28th



Downy Mildew Trial, September 3rd



Early Breeding Trial



Late Breeding Trial, September 18th



Late Breeding Trial, October 3rd



Hand Pollination, Late Breeding Trial



Record Keeping Flag, Late Breeding Trial



Bacterial Wilt Trial, July 17th



**Breeding and Trialing Field Day (organized by NOVIC), September 10th,
in Melon Downy Mildew Trial**



Marketmore 76, August 31st



CWSG DMR Cucumber, August 31st

