January 18, 2023

COLD-HARDY PCNA HYBRID PERSIMMON PROJECT UPDATE

Persimmon season is over, and many of us are in the middle of a biting winter. Yet days are already lengthening, and we are eagerly looking for warmer weather and waking trees.

Persimmons have been a favorite crop of mine for some time. Perhaps you have heard about my breeding goals with this fruit…perhaps not. But either way, I wish to provide a brief update on the developments of 2023 and the outlook for 2024. First, however, let me take just a few minutes to explain our project for those who have not heard just yet…

😊 Breeding Goals! 😊

For many people, persimmons are one of the simplest and tastiest fruit crops to grow. They resist many common fruit tree diseases and they increase consumer access to local, nutritious food. The public is acquiring a taste for all types of persimmons, but many people consider non-astringent persimmons (such as 'Fuyu') are to be the most desirable. Unlike astringent cultivars, non-astringent varieties can be shipped and eaten while they are still firm. Yet from the perspective of a grower, the main disadvantage of these non-astringent cultivar is their susceptibility to cold weather. All of the commonly-available non-astringent varieties of persimmon will die if temperatures dip much below zero Fahrenheit.

Breeders such as David Lavergne, Cliff England, Don Compton, and others and others have labored to develop persimmon varieties that produce desirable fruit and also tolerate the winter conditions found throughout much of the temperate United States. In many respects their work has been a great success. Growers in zone 6 now have a wide array of cold-hardy hybrids between *Diospyros kaki* and *Diospyros virginiana* that will easily survive cold snaps that wipe out Asian persimmons. But to date, growers do not have any access to cold-hardy hybrids possessing the non-astringent phenotype! There are simply no non-astringent hybrids available. Enthusiasts like myself want to see this change! In spite of setbacks, we are working toward this goal!

😀 Genetic theory 😀

In December of 2022 I brushed the finishing touches on a SARE grant application designed to address this problem. Cliff England (Sandgap, KY), Matt Renkoski (Osage Beach, MO), Mike Hater (Nashville, IN) and Darren Bender-Beauregard (Paoli, IN) had agreed to lend support in a variety of ways. The application requested SARE funding to conduct a two-year controlled breeding project which would utilize the germplasm of the JT-02 hybrid and an array of non-astringent *Diospyros kaki* pollen donors. Let me explain why we chose these breeding parents.

Those who are familiar with the JT-02 hybrid will recall that Jerry Lehman was instrumental in working with Japan on this unique F1 hybrid between the American cultivar 'Josephine' and the Asian pollination-constant non-astringent (PCNA) cultivar 'Taishu.' A couple things make the JT-02 hybrid unique. 1) This cultivar is a true F1 cross, which is quite rare among hybrids. 2) The JT-02 hybrid has 50% PCNA parentage, which means it has a good dose of non-astringent genetics! And 3) The JT-02 hybrid is an extremely cold-hardy cultivar. In fact, it has retained cold hardiness akin to *D. virginiana*, even though it's a hybrid.
For the above reasons, we determined that crossing JT-02 x PCNA gave us the best-known chance of obtaining a prized variety with hybrid genetics, cold-hardiness, *and* non-astringent fruit! It's basically a backcross—with the goal of recombining the recessive PCNA alleles (genes) into a single, cold-hardy cultivar. This was the project we started this past spring.

😊 2023 Progress 😊

We were received grant approval in February of 2023 and excitedly got to work! I spent hours communicating with growers nationwide to secure pollen from PCNA-type cultivars for use in breeding. We knew that this task would be complicated by the fact that most PCNA cultivars do not produce male flowers. Those that do produce male flowers only do so sporadically. Although I communicated with an estimated 35 growers about this need, the USDA repository at Davis was the only reliable pollen source I found. From the USDA, I was secured pollen from the PCNA cultivars Hanagosho, Okugosho, and F-444. Even though the pollen was supposed to be overnighted, it took a couple days to reach us. As soon as I did receive it, I promptly transferred the pollen to frozen storage.

In May 2023, I took some days off work and traveled to the farms of Matt Renkoski, Darren Bender-Beauregard, and Cliff England. Each of these gentlemen were cultivating mature specimens of the JT-02 hybrid, and each was willing to utilize their specimens as pollen parents in the current breeding research. In addition to petri dishes and other needed supplies, I carried a portable DC-powered freezer to keep pollen in cold storage during travel.

Soon it was time to start work in the field! In order to prevent contamination from foreign pollen, I was required to bag unopened flowers with empty tea bags prior to pollination so that insects would not have access to them. Since persimmon blooms open before dawn, I frequently bagged my candidate blossoms in the evening in preparation for the next day. One or two mornings also found me up early in the dark hours of the morning bagging blooms before pollinators had become active.

The pollen we received from the USDA came in test tubes the form of dried anthers, and before use, the pollen grains had to be separated from the anthers. After some experimentation in the field, I discovered that an organza jewelers' bag and petri dish helped to make very efficient use of the small quantity of pollen we had. I dumped the dry anthers into the jewelers' bag and shook the bag carefully over a clean petri dish. Pollen grains would fall into the dish and create a thin, yellow layer on the clear plastic. At this point the pollen was ready to apply to the receptive blossoms.

I carefully inspected each bloom that had been bagged the night before to determine which ones had opened and become receptive to pollen. These flowers were then unbagged, stripped of petals, and dipped carefully into the petri dish of pollen. The pollen quickly adhered to the tacky surface of the stigma. Blooms were then promptly re-bagged. By dipping the stigmas directly into the petri dish (and skipping the paint brush method of pollen application), I was able to get many, many crosses from a small quantity of pollen. Each cross was tagged with flagging tape and labeled to document the donor pollen parent. In total, we conducted upwards of 180 controlled crosses amongst the 3 participating farms this past spring.

😖 Reality Check 😖

From the 180 crosses we conducted this spring, a fair number of fruits developed. We had some fruit abort over the summer—as is typical—and by harvesttime we had 40 hand-pollinated fruits to harvest. But as we opened these fruits and began extracting the seeds, we discovered something that sent us back to the drawing board for a little bit—most of the fruits were completely devoid of seeds! Of 40 fruits, we obtained 12 seeds. What was going on!?

This was a disappointment, needless to say, but it prompted us to reevaluate our breeding structure. Where was the missing link? Specifically, we looked at three primary factors that could have affected our results. 1) Hybrid sterility, 2) technician error, and 3) pollen inviability. On December 3, we held a zoom meeting with more than half a dozen U.S.-based *Diospyros* breeders or enthusiasts to discuss the challenge and identify the point of breakdown. In this meeting a participant shared that his JT-02 is reliably generating seeds in California via open pollination with *D. kaki*. This confirmed that we are likely not dealing with any sterility/incompatibility problem. Technician error is another possible factor. I personally conducted most of the controlled crosses, and while I have not conducted *Diospyros* crosses before, I have communicated with persimmon breeders in preparation for this project and I have also conducted many successful hand pollinations in other crops (*Zinnia*, *Viola*, *Citrullus*, *Solanum*, *Cucumis*, and *Cucurbita*). I believe our timing and application protocol was in line with standard industry principles. That leaves us with the third issue: inviable pollen.

The USDA was incredibly helpful in providing pollen this spring, but I have my doubts about the viability of the pollen we received. The pollen was shipped overnight to us, but the package was delayed by a day in shipment. Further, the USDA collected pollen on multiple days, so pollen was stored in refrigeration during the intervening holding period. On the inside of one vial of pollen that we received, I also spotted a small amount of condensation. These factors collectively cause me to question the viability of the pollen we received. I currently believe that pollen viability was responsible for the issues we dealt with—at least this past year.

😎 Looking Ahead 😎

The genetic basis of our project is solid, but the logistics of creating the cross have been challenging. With this in mind, I am pursuing three directions for this project in 2024.

First, I hope to continue experimenting with various chemicals to induce male blooms in *D. kaki*. This is not directly related to breeding a PCNA hybrid, but it is highly relevant to persimmon breeding in general. I have spoken with Japanese persimmon breeders over email, and they currently have no successful method for chemically inducing male blooms. This practice in persimmons deserves more research, because it is done successfully on other crops and chemical induction of male blooms would be a huge breeding breakthrough for persimmons. The primary chemicals I am working with are silver thiosulfate and gibberellic acid.

Second, I have recently studied the use of genetic markers in persimmon. Kanzaki et al. (2010) have done research into markers that can be used to identify astringent phenotypes early in development. I also intent to review any genetic markers associated with persimmon tree sex. I have initiated communication with a lab in California who may be able to run genetic marker tests for us.

And finally, I also have spoken with Joan Benjamin at the North-Central SARE and explained that I wish to adjust the breeding structure of this project slightly. The goals of the project aren't changing—just the strategy. Instead of spending time and money to crisscross the country and make a limited number of hybridizations at farms in various states, I am proposing that we plant a small orchard down south that would be planted with breeding in mind. Breeding work can progress more easily in the south because cultivars of *Diospyros kaki* will not winterkill, and also because the native southern population of persimmon has 60 chromosomes instead of the typical 90 chromosomes. This means that the wild specimens will not cross into plantings of hybrid trees and contaminate the genetics. This long-term strategy could potentially pull out the stops in generating *large populations* of hybrid crosses, because we can let the insects do the pollinating. Anyone involved in breeding knows how important *population size* is. Joan at SARE was agreeable to idea, and she requested that I submit a brief proposal of the idea for her team to evaluate and (likely) approve.

I have communicated with Anthony Czaja in southern Alabama who is very interested in persimmon culture and persimmon development. He has agreed to make his land available for research and breeding purposes, and as I communicate with SARE, I am simultaneously in the process of hammering out a plan of action with Anthony.

😊 Farewell 😊

My observation is that more and more people are seeking to grow persimmons throughout North America, and it's exciting to be working with the crop. The press is talking about persimmons, and lively discussion about this wonderful fruit continues across online platforms and elsewhere. I am thankful for the support of people at NAFEX and abroad as we pursue this worthwhile project. Persimmons are a miracle of Creation, and we are blessed to be involved with the future of this fruit.

If anyone has questions, thoughts, or ideas, don't hesitate to email: forwestonadams@gmail.com. Thanks to each of you for your interest in persimmons as a sustainable and unusual fruit crop. Your support is greatly appreciated.

References

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This project was developed with support from the Sustainable Agriculture Research and Education (SARE) program, which is funded by the U.S. Department of Agriculture -- National Institute of Food and Agriculture (USDA-NIFA). Any opinions, findings, conclusions or recommendations expressed within do not necessarily reflect hthe view of the SARE program or the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.