Title: Evaluation of Self- and Cross-Fertility and Increased Heat Tolerance of Primocane-Fruiting Blackberry Genotypes

Progress Report

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Research Proposal

Name, Mailing and Email Address of Principal Investigator(s):
John R. Clark
Dept of Horticulture
University of Arkansas
316 Plant Science
Fayetteville, AR 72701
jrclark@uark.edu

Elena Garcia
Dept of Horticulture
University of Arkansas
316 Plant Science
Fayetteville, AR 72701
megarcia@uark.edu

Objectives:
1. To evaluate self and cross fertility of named and advanced-selection fertility in primocane-fruiting (PF) genotypes to determine if these genotypes can potentially be grown in solid blocks without other blackberry genotypes as pollen sources. Additionally a limited number of floricane-fruiting (FF) genotypes will be included to evaluate self and cross compatibility of these among PF and with FF types.
2. To evaluate the fertility of flowers borne on flori- and primocanes to determine differences in flowers on these different cane types.
3. To determine if newer PF genotypes have increased tolerance for reproductive capability in high heat conditions based on pollen exposure to high temperatures in artificial screening conditions.

Justification and Description:
The first PF blackberry cultivars 'Prime-Jan'® and Prime-Jim'® were released by the University of Arkansas in 2004 (Clark et al., 2005). The benefits of PF can include: extended harvest season with potentially two crops per year if canes are overwintered; decreased pruning costs by mowing down canes in winter; elimination of winter injury; growth in non-traditional areas (low- no chill; severe winter); avoidance of over-wintering pests, such as anthracnose and red-necked cane borer; and "off-season" production of fruit in fall.

However, these genotypes have several limitations including lack of tolerance to high temperatures during flowering and fruiting on primocanes as observed in Arkansas early in their evaluation (Clark et al., 2005; Drake and Clark, 2003). When grown in a more moderate climate such as the Willamette Valley of Oregon, the PF performance was much better and this was believed to be due to more favorable temperatures during primocane bloom (Clark et al., 2005). Therefore the potential of these cultivars for the southern U.S. is limited commercially, and they are only recommended for home-garden use. Further work by Stanton et al. (2007) indicated that high temperature (35.0C) had major impacts on fruit set and other aspects of reproductive performance compared to more moderate temperatures of 23.9 and 29.4C. The greatest impact was on the male components of flowers.

Further breeding at the University of Arkansas has resulted in genotypes with much-improved fruit set in high-heat field conditions, although none of these have been released for public use. It is also thought that these improved selections have increased fruit set potential overall, independent of environment, based on field observations over several fruiting seasons. No information exists on what the basis of this improved set, be it heat tolerance, increased fertility or other factors.

The original source of PF was the wild, diploid selection designated 'Hillquist', originally found in Virginia. This genotype was used in early breeding at the University of Arkansas but offspring did not express PF. One selection, A-593, was later selfed and offspring found to express the PF trait (Ballington and Moore, 1995). Subsequent work supported the hypothesis that the PF trait is recessive (Lopez-Medina et al., 2000). A-593 is a tetraploid, based on its successful breeding behavior with other tetraploids and is hypothesized to have resulted from a maternal unreduced gamete in the Hillquist x Brazos cross made in 1967 at UA (Moore, 1997). Other selections in the Arkansas program were developed from the original source using A-593 and these led to the commercial releases along with many additional PF selections in the last 10 years.

Perry and Moore (1985) evaluated the self and cross compatibility of a range of tetraploid blackberry genotypes (all FF) and they found no differences in fruit set in eight cultivars whether selfed or pollinated with another cultivar. However, they evaluated A-593 in this study and found reduced fruit set when selfed for this genotype compared to the other floricane-fruiting (FF) tetraploids. This finding has raised the question if PF genotypes have reduced set when self-pollinated, which could be a major concern if a single PF genotype was grown in large block of plants as FF blackberries are currently produced. Also, it is of interest if subsequent breeding with PF types has resulted in increased fruitfulness (possibly self compatibility) and contributed to increased heat tolerance and fertility as reflected in increased fruit with the newer genotypes.

In Stanton et al.'s report (2007), they reported major effects of high temperatures on the male components of 'Prime-Jim' and 'Prime-Jan'. In their study pollen was stored at varying temperatures to simulate varying field temperatures. At 23.9C, pollen retained 50% of its original functionality after 32 hours. At 35.0C, germination was negligible after only 16 hours. This method needs to be evaluated further to determine if genotypes

can be screened for heat tolerance as a potential method to help evaluate breeding progress for heat tolerance in PF blackberry.

Methodologies

Study 1. Evaluation self and cross fertility among genotypes on flori- and primocanes.

The following genotypes were used:
'Prime Jim'
APF-31
APF-45
APF-59
APF-77
'Navaho'
'Natchez'
'Ouachita'
Of these genotypes, APF-31, 45, 59, and 77 have shown enhanced fruit set in hot conditions of primocane flowering of 2005-2007 compared to Prime-Jim and Prime-Jan and this is the reason for their inclusion in the study. Navaho has been reported to have reduced fertility (Hellman and Clark, 1984), Ouachita more fertile, and Natchez fully fertile (J.R.Clark, personal observation) as evidenced by drupelet development, providing a range of fertility for the comparison FF types.

One or two 6 m plots of each genotype growing at the University of Arkansas Fruit Research Station, Clarksville were used. Four pollination treatments were applied: undisturbed open-pollinated, self-pollinated, cross-pollinated with a within-cane-type pollen (PF x PF or FF x FF) and cross pollinated with a dissimilar-cane-type pollen source cross (PF x FF, FF x PF). The study was conducted on floricanes for all genotypes (in April) and only the PF types on primocanes (from late June to early August). Five to ten flowers per treatment were used for each replication and there were 10 replications with a target minimum of 50 flowers used for each treatment combination. Flowers were emasculated before anthesis and covered with protective bags to prevent errant pollen coming in contact with emasculated flowers. Each flower was pollinated once as receptivity was determined by appearance, usually within a one to four-day period. Open-pollinated flowers were neither emasculated nor pollinated but were allowed to be pollinated naturally. Collected pollen was applied to emasculated flowers by hand. APF-31 pollen was used as the common PF pollen source and Natchez as the FF source. APF-31 crossing flowers was pollinated byAPF-77 and Natchez by 'Ouachita' to cross-pollinate the common type pollen parents.

Measurements were taken on both floricane flowers and fruit as well as primocane flowers and fruit and included: date of bloom, flower number/inflorescence, percent flower set (number of fruit/ number of flowers pollinated), days from pollination to maturity, total fruit number, visual rating of drupelet set, and average berry weight. Data analysis was by SAS.

Study 2. Evaluation of heat tolerance of pollen.

Pollen was collected from the PF genotypes and plots during primocane flowering. Pollen was collected from 10-25 flowers based on pollen yield to attain an adequate supply for further testing.

Pollen viability was determined by staining. Pollen was tapped onto a microscope slide, and lactophenol cotton blue stain was added. Samples were covered and allowed to stain at least ten minutes. Three counts of 100 grains per sample will be taken. Pollen was classified as viable if it was stained. The procedure was done four times for each genotype.

For pollen germination, the procedure used by Stanton et al. (2005) was used. Pollen was germinated on Brewbaker & Kwack's medium, which contains sucrose, boric acid, calcium nitrate, magnesium sulfate heptahydrate, and potassium nitrate in distilled water. Extracted pollen was tapped onto petri dishes containing sterile medium, closed, and then incubated at 23.0C, 29.4C and 35.0C for 48 hours. After incubation, plates were examined under a microscope, and three counts of 100 grains were taken. Pollen was counted as germinated if the pollen tube was at least as long as the diameter of the grain. The procedure was done four times for each genotype. Comparison of germination among genotypes will be done and data will analyzed by SAS.

Results

Study 1. Evaluation self and cross fertility among genotypes on flori- and primocanes.

Pollination of floricanes began on 23 April 2008 and concluded 12 July 2008. Cooler than average temperatures in the spring delayed flowering slightly. Harvest began 6 June 2008 and was completed 21 July 2008. Cool temperatures and frequent rain characterized the fruit development period. Disease concerns were more than usual due to the rain, with the primary disease being anthracnose that was noted on APF-45, 'Prime Jim', and 'Natchez'. This disease accounted for the loss of numerous flowers. Overall plant health was poor in 'Prime Jim' and 'Natchez'. However, plant health was largely related to the specific plots used for these genotypes for this study.

Logit analysis was used to analyze percent flower set. Replications with no flowers pollinated were treated as missing data. For this variable, genotype and pollination treatment each had a significant effect, but there was no interaction between the two. Open pollination had the highest proportion of flower set (92.%), followed by similar cane type cross, dissimilar cane type cross, and self pollination. Self pollination had the lowest proportion of flower set (82.8%), but this was not significantly lower than the other two emasculated pollination treatments. Open pollination was not significantly different from similar cane type cross pollination, but was significantly higher than self and dissimilar cane type cross pollination.

Days to maturity was the only variable to show a significant interaction between genotype and pollination treatment (P=0.02). APF-45 cross pollinated with dissimilar

cane type pollen ripened significantly earlier (5-9 days) than the other pollination treatments within APF-45; open pollinated berries took the longest to mature (51 days). 'Prime Jim' similar cane type cross pollinated berries ripened significantly earlier than open pollinated and dissimilar cane type pollinated berries. 'Prime Jim' self pollination days of maturity were not significantly different from the other three treatments. The other six genotype*treatment interactions showed no significance.

APF-31 and APF-59 had significantly larger berries than the other six genotypes. APF-77, 'Navaho', and 'Ouachita' had the smallest berries. Among pollination treatments, open pollinated berries were the largest. There were no differences between the other three pollination treatments. No interaction between genotype and pollination treatment was found for berry weight.

Pollination treatment had the same effect on visual drupelet set rating. Among genotypes, APF-45, APF-31, and APF-77 had the highest average rating. 'Prime Jim' was significantly lower than the five other PF genotypes. 'Navaho' was rated higher than 'Natchez' and 'Ouachita'. There was no interaction between genotype and pollination treatment in visual drupelet set rating.

Primocane pollination began on 7 July 2008 and concluded 5 August 2008. First day of harvest was 11 August 2008. Harvest continued until 9 September 2008. Rainfall was average for this period, as was temperature. There were six days over 35C. No disease or insect pressure was noted. There were visual heat effects on emasculated and unemasculated flowers, especially on APF-45 and 'Prime Jim'. Data analysis is underway from this study thus results are not available for this report.

Study 2. Evaluation of heat tolerance of pollen.

Pollen was collected weekly for four consecutive weeks, beginning 22 August 2008. Temperature and rainfall were average during this period. Pollen was successfully germinated at all three temperature regimes. There were differences in viability and germination numbers among genotypes and temperatures, but data has not yet been fully analyzed. It is unknown whether or not these differences are significant.

Conclusions

Overall, self pollination fertility showed no disadvantage compared to cross fertility among the genotypes examined. It appears that solid blocks of these genotypes could be successfully planted.

The initial results from this research show recent PF genotypes have improved fruit set compared to 'Prime Jim'. Primocane data needs to be analyzed before comment can be made on improved heat tolerance and fertility differences between primocane and floricanes flowers.

Impact Statement

The evaluation of pollen fertility of several flori- and primocane-fruiting blackberry gentoypes was examined in a field study. The preliminary results indicate that self pollination was similar to cross pollination for fruit set, and that berry weight was not affected by self vs out-crossed pollen. These results indicate that solid blocks of primocane-fruiting blackberries should be adequately fruitful for cropping in commercial production plantings.

Citations from this work: None

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