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# Evaluation of chilling requirements for six Arkansas blackberry cultivars utilizing stem cuttings

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Dayanee Yazzetti\* and John R. Clark§

## ABSTRACT

Woody perennial plants including blackberries (*Rubus* subgenus *Rubus*) require certain amounts of chilling or rest hours below 7°C during the dormant season for successful bud break the following year. Arkansas-developed blackberry cultivars are being grown in various climates worldwide and all cultivars need chilling requirement estimates for accurate recommendations of adaptation. Determining chilling requirement using stem cuttings collected from field-grown plants rather than whole plants is a desirable system. We conducted a study to evaluate both artificial and field chilling of six cultivars. For the artificial-chilling study, 12-node stem cuttings were collected 2 days after the first killing frost. These were then placed in a moist medium in a walk-in cooler at 3°C. At 100 hour chilling intervals, five cuttings of each cultivar were placed under an intermittent mist system. For the field-chilling study, a biopneumometer was placed in the field to measure chill, and ten 12-node stem cuttings of each cultivar were collected at 100-hour intervals of chilling up to 1000 hours below 7°C and placed under mist. For both studies the mist bench was located in a heated greenhouse (min. temperature of 15°C), and cuttings were placed according to a completely random design. Budbreak was recorded weekly. Studies were analyzed separately by SAS. Results for Study One, artificial-chilling, were inconclusive due to a lack of clear differentiation among the cultivars and chilling intervals. Study Two, using field-chilling, showed a significant chilling interval x cultivar interaction. 'Arapaho' appeared to have a chilling requirement of 400 to 500 hours, 'Kiowa' 200 hours, 'Shawnee' 400 to 500 hours, and 'Chickasaw' possibly 600 to 700 hours. The cultivars Choctaw and Apache did not provide clear chilling interval differentiation in the study. Our results indicate that the use of stem cuttings receiving field chilling to evaluate chilling requirement of blackberry cultivars has merit and can be a successful method in this research area.

\* Dayanee Yazzetti graduated in May 2001 with a B.S. degree in horticulture.

§ John R. Clark, faculty sponsor, is a professor in the Department of Horticulture.

## **INTRODUCTION**

Woody perennial plants such as blackberry require certain amounts of chilling or rest during the dormant season for successful budbreak and normal shoot and flower development to occur during the next season. Rest period is defined as the duration that a plant must be exposed to cold temperatures at or below 7°C, while chilling requirement is the amount of cold needed to satisfy that rest period and is species and often cultivar specific (Ryugo, 1998). Failure to meet this requirement results in reduced and erratic budbreak, poor shoot growth, reduced flowering, and reduced fruit yields the next year.

Blackberry cultivars released from the University of Arkansas breeding program include 'Shawnee' (Moore et al., 1985), 'Choctaw' (Moore and Clark, 1989), 'Navaho' (Moore and Clark, 1989), 'Arapaho' (Moore and Clark, 1993), 'Kiowa' (Moore and Clark, 1996), 'Apache' (Clark and Moore, 1999), and 'Chickasaw' (Clark and Moore, 1999). Arkansas developed blackberry cultivars are being grown not only in Arkansas but worldwide, in locations with different amounts of chilling than where they originated. Chilling requirement estimates are needed for all cultivars to ensure accurate recommendations of adaptation. Limited formal research has been performed on chilling requirement of blackberry cultivars. Drake and Clark (2000), reported chilling requirement of 'Arapaho' was 400 to 500 hours and 'Navaho' was 800 to 900 hours using whole plants in a study with controlled artificial chilling of constant 3°C.

In the fall of 2000-2001, we conducted two studies to evaluate the use of stem cuttings to estimate chilling of six blackberry cultivars. The first study (Study One) was conducted to determine the feasibility of using artificial chilling to fulfill chilling requirements of stem cuttings. The objective of Study Two was to determine the feasibility of using blackberry stem cuttings receiving natural chilling to identify chilling requirement.

## **MATERIALS AND METHODS**

### *Study One*

Fifty 12-node, lateral-branch stem cuttings of 'Apache', 'Arapaho', 'Chickasaw', 'Choctaw', 'Kiowa', and 'Shawnee' were collected from a mature planting located at the University of Arkansas Agricultural Research and Extension Center, Fayetteville, 2 days

after the first killing frost, 12 Oct. 2000. The cuttings were then placed in a moist sawdust medium in a walk-in cooler at 3°C. At 100 hour chilling intervals, five cuttings of each cultivar were removed from the cooler and placed under an intermittent mist system in a completely random design. The mist bench was located in a heated greenhouse with a daily minimum temperature of 15°C and a daily maximum temperature of 25°C.

### *Study Two*

In order to measure natural field chilling, a biophenometer was placed in the planting to record the number of hours below 7°C. Ten stem cuttings from lateral branches of mature canes of each of the cultivars mentioned above were collected from the field at 100-hour intervals of chilling up to 1000 hours. However, due to a severe ice storm in December, the 900 hour chilling interval cuttings were not taken due to the inability to collect the cuttings. Also, 'Arapaho' cuttings were only collected for 100 to 600 hours of chilling due to the shortage of lateral branches in the planting for this cultivar. Following collecting, the field cuttings were placed in the same greenhouse under an intermittent mist system in a completely random design. For both studies, incandescent lighting was provided to lengthen the daylength to 16 hours in the greenhouse.

Data collection for both studies consisted of a budbreak count of each cutting of each cultivar weekly for 10 weeks. A bud was considered broken when the first leaf became visible as it unfolded from the bud. Budbreak data after 10 weeks for each study were analyzed separately by SAS (SAS, 1989) and standard errors of the means calculated.

## **RESULTS AND DISCUSSION**

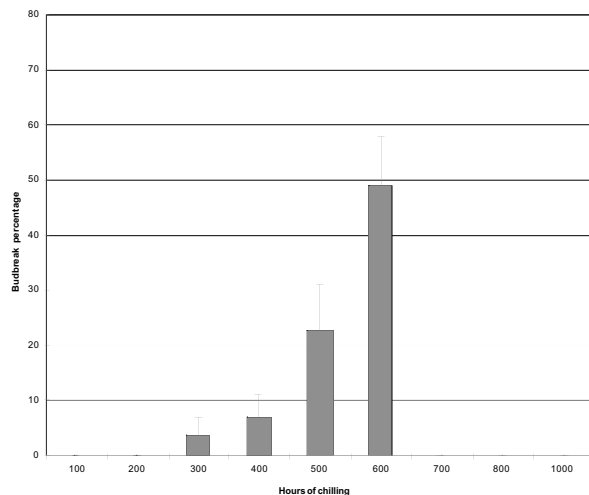
### *Study One*

The data analysis for Study One indicated a significant chilling interval x cultivar interaction, indicating that the cultivars did not have the same budbreak for all chilling intervals. For 100 to 600 hours, all cultivars except 'Kiowa' had 15% budbreak or less, indicating chilling differentials did not appear to be delineated using the artificial chilling method (data not shown). Substantial budbreak was experienced at several higher chilling (above 600 hours) levels for 'Choctaw', 'Apache', and 'Shawnee'. However, 'Arapaho' had very low budbreak for all the intervals except 900 to 1000 hours, and this result contradicts that of Drake and Clark (2000), who estimated 'Arapaho' chilling of 400

to 500 hours. 'Kiowa' behaved differently from all the other cultivars, showing no lower than 20% budbreak across all intervals and increasing up to 70% for the 1000 hour chilling interval. The lack of comparable findings for 'Arapaho' as reported before, and the lack of clear differentiation among the chilling intervals of the cultivars, indicated that this method was likely not a reliable method for chilling requirement estimates.

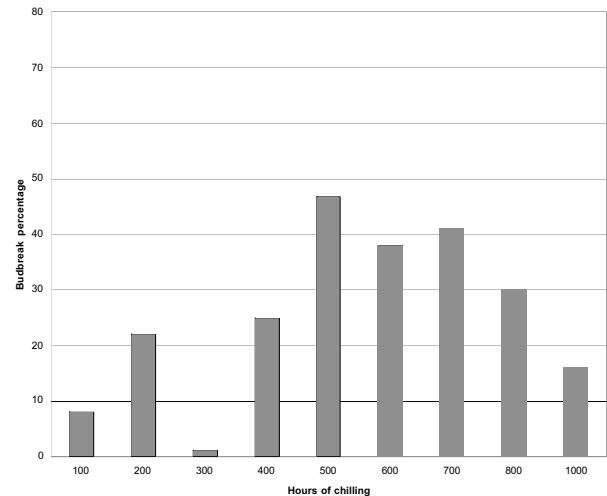
### Study Two

The chilling interval x cultivar interaction was significant for this study, indicating that budbreak differed among the cultivars for the various chilling intervals. 'Arapaho' was the only cultivar with a known chilling requirement used in the study, and it had a substantial increase in budbreak between 400 and 500 hours, consistent with the findings of Drake and Clark (2000) (Fig. 1). This finding was very important as it shows that this method of chilling determination appeared to



**Fig. 1.** Budbreak of 'Arapaho' blackberry after 10 weeks of forcing in a heated greenhouse following 100 through 1000 hours of chilling, below 7°C.

be successful for this cultivar. 'Kiowa' had substantial budbreak at 200 hours, and at most other chilling intervals (Fig. 2). There was a reduction in budbreak at 300 hours for 'Kiowa', due to the death of several cuttings collected for this chilling interval contributing to the low budbreak value. There was a substantial reduction for 'Kiowa' at the 800 and 1000 hours, likely due to winter injury sustained from extreme low temperature (-16.7°C) during this chilling interval. Based on these finding it appears that 'Kiowa' has the



**Fig. 2.** Budbreak of 'Kiowa' blackberry after 10 weeks of forcing in a heated greenhouse following 100 through 1000 hours of chilling, below 7°C.

lowest chilling requirement of the Arkansas cultivars, and this may be a low as 200 hours.

Field observations of 'Choctaw' in more subtropical climates of the world have shown it to have a lower chilling requirement than other Arkansas cultivars released prior to 1989 (J.N. Moore, personal communication). In Study Two, 'Choctaw' showed no budbreak until 400 hours, with higher budbreak at other chilling intervals (data not shown). Budbreak never exceeded 32% for 'Choctaw' at any interval, however, which was lower than most other cultivars. We conclude that for 'Choctaw' data were inconclusive in substantiating the low chilling observations that have been reported previously.

'Shawnee' has been the most widely grown Arkansas blackberry cultivar, with widespread planting of this cultivar in the southern U.S. Occurrences of evidence of lack of chill have not been reported (J.N. Moore, personal communication). In our study, 'Shawnee' appeared to have a chilling requirement of 400 to 500 hours due to the greatly increased budbreak between these two intervals (Fig. 3). Since most of southern states receive this amount or more chilling, one would expect a cultivar to not experience chilling requirement shortfalls at this chilling level. The chilling requirement seen in our data support this observation. The two newest Arkansas cultivars, 'Apache' and 'Chickasaw', have no chilling observations available. 'Chickasaw' had substantial budbreak at 700 hours of 50%, a major increase in budbreak compared to lower chilling intervals (data not shown). This suggests

## Meet the Student-Author

I am a 1997 graduate of Mena High School and a 2001 graduate of the University of Arkansas with a bachelor's degree in horticulture. I have been actively involved with the Horticulture Club since my arrival on campus four years ago. I have been the recipient of numerous scholarships, among them being the Gerber Endowment Scholarship and the Arkansas State Horticulture Society Scholarship. I have also been awarded the 1st Place Undergraduate Research Presentation by the Southern Region American Society for Horticultural Science, and the Gamma Sigma Delta's 2nd Place Award in their annual Undergraduate Research Presentation competition.

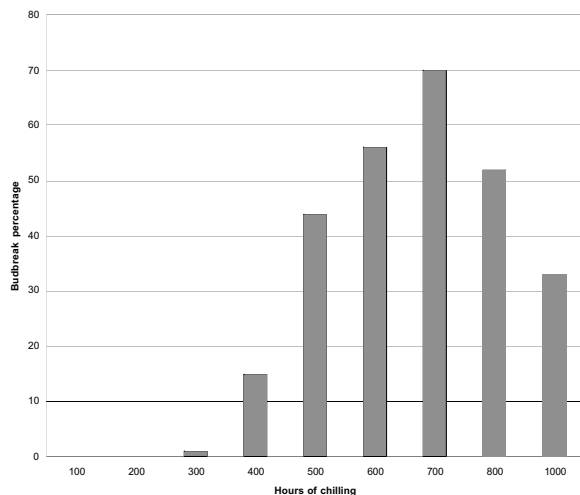
I will begin attending Graduate School in the Fall of 2001 here at the University of Arkansas.

While working on my internship at the UofA's Fruit Substation in Clarksville, Dr. Clark suggested the idea for my research. Since little research had been done on the chilling requirements of Arkansas-developed blackberries, I was intrigued by the opportunity of being involved in potentially groundbreaking research.



*Dayanee Yazzetti*

'Chickasaw' has a chilling requirement possibly between 600 to 700 hours. Budbreak did not remain as high for 'Chickasaw' at 800 and 1000 chilling intervals,



**Fig. 3.** Budbreak of 'Shawnee' blackberry after 10 weeks of forcing in a heated greenhouse following 100 through 1000 hours of chilling, below 7°C.

which again might be due to winter injury to some buds. Finally, 'Apache' had low budbreak at all chilling intervals, with the highest level at 800 hours of 20% (data not shown). It was anticipated that 'Apache' would have chilling near to that of 'Navaho' (800 to 900 hours as found by Drake and Clark, 2000), as 'Navaho' is one of its parents. Due to the low budbreak at all intervals, we feel our results are inconclusive in estimating chill requirement for 'Apache'.

The major premise of our studies was to determine if the use of stem cuttings would be successful in differentiating chilling requirements of blackberries. Stem cuttings are much easier to use for chilling requirement determinations as they can be collected from field-grown plants and forced to budbreak after collection. Conversely, using whole plants for this type of research requires that potted plants be grown for a season prior to exposure to chilling, and then that the whole plants be used for budbreak measurements after chilling treatment intervals are provided. This is a much more laborious and expensive process. Also, before or near the release of a new cultivar there is often a very limited

number of plants available, and having whole plants to use in a chilling determination study is usually not possible. However, using stem cuttings, which are much more plentiful in research plots, would be much more practical. Therefore, the evaluation of stem cuttings was deemed necessary as a method to investigate.

The use of artificial chilling on blackberry stem cuttings (Study One) was deemed unsuccessful in our study due to the lack of differentiation among most cultivars, and the low budbreak at all of the lower chilling intervals except for 'Kiowa'. This could be due to several reasons. It is possible that the cuttings were collected prior to the onset of dormancy of the plants. When dormancy actually begins is always a question, and we are not aware of an absolute way to know this. Our collection was based on the occurrence of the first killing frost on 12 Oct., which we hoped would be the beginning of dormancy or rest period. However, if the plants were not physiologically in or near dormancy at this time, this could affect subsequent ability of the plant to show response to chilling to satisfy the chilling or rest period requirement, and this could have contributed to our inconclusive results. Also, the plant material may require attachment to an entire plant to allow the measurement of chilling to fulfill the rest period, and this may not have been possible when the stem cuttings were removed from the plant. Whether the reasons are those discussed here, or the results were due to other causes, we feel that artificial chilling of stem cuttings was not a reliable method to measure chilling requirement of blackberries.

Conversely, the field-chilling study (Study Two) provided results that we feel allowed the differentiation of chilling requirement of most cultivars. Previous research by Drake and Clark (2000) showed a difference among two Arkansas cultivars in chilling requirement, and field observations in areas of low chill had also indicated cultivar chilling requirement differences. Our first noteworthy finding, that of a similar estimate of chilling response of 'Arapaho' stem cuttings exposed to field chilling compared to that found by Drake and Clark (2000) using whole plants of 400 to 500 hours, provided confidence in the stem cutting method we used.

A very apparent additional finding in Study Two was the unusual budbreak at low chilling level for 'Kiowa'. This cultivar was released in 1996, and has not been planted as widely as yet as cultivars such as 'Shawnee', 'Choctaw' or 'Arapaho'. Therefore, reports

from growers and researchers have not surfaced as to its chilling response, possibly because of the rather short period of time 'Kiowa' has been planted on a widespread basis. It was observed in the testing of 'Kiowa' prior to its release that it had earlier spring budbreak compared to 'Shawnee' and 'Choctaw' (Moore and Clark, 1996), and this might reflect either a lower chilling requirement or a lower heat requirement for bud development. Our data support the idea that this could be due to a lower chilling requirement, as our study did not measure differential heat requirement conditions. Additionally, a reason that no chilling concerns have been observed by early evaluators of 'Kiowa' may be due to the fact that it has had good budbreak in all areas grown, both low and high chill locations, due to its low chilling requirement. We conclude that 'Kiowa' likely has the lowest chilling requirement of all cultivars tested in our study.

We expected a low chilling requirement response for 'Choctaw' based on field observations of its reliable budbreak in locations of low chill. Our data were disappointing as we observed rather low budbreak at all chilling intervals, and therefore the differentiation of these was not reliable. Reasons for this were not clear, but could include the possibility of cold injury to buds during the study, or could be related to heat requirement to begin growth. 'Choctaw' has been observed to be the least hardy (most susceptible to winter injury) of the Arkansas cultivars (J.N. Moore, personal communication), and it is possible some bud injury occurred early in the fall. However, bud injury was not evaluated at collection thus this suggestion cannot be confirmed. The heat requirement for growth to begin has not been measured for any Arkansas blackberry cultivars, thus it is not possible to speculate if this was involved in our study, as the environment in which the cuttings were forced was thought to be warm enough to contribute to budbreak for all cultivars.

'Shawnee' response was very much as expected, as a chilling requirement of 400 to 600 hours was suspected for this cultivar based on field performance. Our finding of a requirement of 400 to 500 hours fell within this expected range, and the budbreak levels were among the highest of all cultivars after these chilling treatments. This provided further confidence in our method.

Finally, the results for 'Chickasaw' indicate that it might have a higher chilling requirement than 'Shawnee' by 200 hours. Further research and observa-

tion should be done on this cultivar to substantiate the chilling requirement of this new cultivar. 'Apache', with budbreak below 20% at all intervals, needs further investigation to determine chilling requirement. Why differentiation of chilling was not achieved in our study with this cultivar is not understood, as we were not aware of any limitations this cultivar had, such as winter injury of buds prior to collection, heat requirements, or other causes.

In conclusion, our results indicate that for the majority of the cultivars evaluated in our study, the use of stem cuttings receiving field chilling was a successful method of chilling requirement determination. We suggest that this investigation be repeated to verify this, and that bud viability of cultivars be determined prior to forcing to verify that winter injury does not contribute to reduced budbreak. Additionally, with other fruit crops, including peaches (*Prunus persica* Batsch.), it has been reported that different temperatures contribute to efficiency of chilling requirement fulfillment (Richardson et al., 1974). With peaches, temperatures between 7 and 0°C provided the most efficient chilling, while temperatures below 0°C contributed to little chill requirement fulfillment. The efficiency of chilling of various temperature ranges should also be investigated on blackberry to determine if a similar response is involved.

### **ACKNOWLEDGMENTS**

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