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Evaluation of Chilling Requirements for Six Arkansas Blackberry Cultivars Utilizing Stem Cuttings

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EVALUATION OF CHILLING REQUIREMENTS FOR SIX ARKANSAS BLACKBERRY CULTIVARS UTILIZING STEM CUTTINGS

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Abstract:

Woody perennial plants including blackberries (Rubus subgenus Rubus Watson) require certain amounts of chilling or rest hours below 7T during the dormant season for successful bud break the following year. Blackberry cultivars developed in Arkansas are being grown in various climates worldwide, and all cultivars need chilling requirement estimates for accurate recommendations of adaptation. Determining chilling requirements 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 30C. At- 100 hour chilling intervals, five cuttings of each cultivar were placed under an intermittent mist system. For the field-chilling study, a biophenometer 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 intermittent mist. For both studies the mist bench was located in a heated greenhouse (min. temperature of 15T), 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 their 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' probably near that of Shawnee. 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.

Introduction

Woody perennial plants such as blackberry *(Rubus* subgenus *Rubus)* 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 Arkansas Agricultural Experiment Station 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). Cultivars developed in Arkansas are being grown worldwide in environments with chilling conditions that differ from the original breeding locale. Chilling-requirement estimates are needed for all cultivars to ensure effective adaptation recommendations. Limited formal research has been performed on chilling requirements 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 that evaluated the use of stem cuttings to estimate chilling requirements 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.

Material and Methods

***Study One*:** Fifty 12-node, lateral-branch stem cuttings of Apache, Arapaho, Chickasaw, Choctaw, Kiowa, and Shawnee were collected from mature plants located at the University of Arkansas Agricultural Research and Extension Center, Fayetteville, 2 days after the first killing frost of 12 Oct. 2000. The cuttings then were placed in a moist sawdust medium in a walk-in cooler at 3'C. At I 00-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 IVC 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 70C. 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 2000, the 900-hour chilling interval cuttings were not taken because of the inability to collect the cuttings. Also, Arapaho cuttings were only collected for 100 to 600 hours of chilling due to a 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 prolong the day length 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 were calculated.

Results

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 that chilling differentials did not appear to be delineated using the artificial chilling method (data not shown). Substantial budbreak was experienced at several higher chilling levels (above 600 hours) for Choctaw, Apache, and Shawnee. However, Arapaho had very low budbreak for all the intervals except 900 to 1000 hours; 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 a comparable finding 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 to obtain 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 because it shows that this method of chilling-requirement determination appeared to be successful for this cultivar. Kiowa had substantial budbreak at 200 hours, and at most other chilling intervals (Fig. 2). A reduction in budbreak occurred at 300 hours for Kiowa, because the death of several cuttings collected for this chilling interval contributed to the low budbreak value. A substantial budbreak reduction was noted for Kiowa at the 800 and 1000 hour intervals, likely due to winter injury sustained from extreme low temperature (-16.7°C) during this chilling interval. Based on these findings, it appears that Kiowa has the lowest chilling requirement of the Arkansas cultivars, and it may be as 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 a lower percentage than most other cultivars produced. We conclude that data were inconclusive in substantiating the low chilling observations for Choctaw reported previously. Shawnee has been the most widely grown Arkansas blackberry cultivar, with widespread planting of this cultivar in the southern U.S. Evidence of lack of chill has 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 southern states receive this amount or more of chilling, one would expect a cultivar not to experience chilling-requirement shortfalls at this chilling level. The chilling requirements seen in our data support this observation. The two newest Arkansas cultivars, Apache and Chickasaw, have no chilling observations available. Chickasaw had its highest budbreak of 50% at 700 hours, a major increase in budbreak compared to the lowest chilling intervals (data not shown). It had increased budbreak at 400 to 600 hours also compared to the lower chilling intervals also. This suggests Chickasaw probably has a chilling requirement near that of Shawnee, or possibly slightly higher. Budbreak did not remain as high for Chickasaw at 800- and 1000-hour chilling intervals, which again might be due to winter injury of some buds. Finally, Apache had low budbreak at all chilling intervals, with the highest budbreak level at 800 hours of 20% (data not shown). It was anticipated that Apache would have a chilling requirement 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.

Discussion

The major objective of our studies was to determine if the use of stem cuttings would be successful in differentiating chilling requirements different blackberry cultivars. Stem cuttings are much easier to use for chilling requirement determinations because 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 be used for budbreak measurements after chilling-treatment intervals are provided. This is a much more labor intensive and expensive process. Also, before or near the release of a new cultivar there are often a very limited number of plants available, and having whole plants to use in a chilling determination study is usually not possible.

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 the dormancy period 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. 2000, which we hoped would be the beginning of dormancy or the rest period. However, if the plants were not physiologically in or near dormancy at this time, this could have affected the 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 collected plant material may require attachment to an entire plant under normal circumstances to allow the measurement of chilling to fulfill the rest period, and this may have been disrupted 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 requirements of different blackberry cultivars.

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

A very apparent additional finding in Study Two was the unusual budbreak at a low chilling level for Kiowa. This cultivar was released in 1996, and as yet has not been planted as widely as Shawnee, Choctaw, or Arapaho. Therefore, growers and researchers have not reported as yet on its chilling response. During the testing of Kiowa prior to its release, Moore and Clark (1996) observed that it had earlier spring budbreak compared to Shawnee and Choctaw, and this characteristic of Kiowa might reflect either a lower chilling requirement or a lower heat requirement for bud development. Our data support the idea that this characteristic could be due to a lower chilling requirement, as our study did not measure differential heat requirement conditions. Additionally, one reason that no chilling concerns have been observed by early evaluators of Kiowa may be due to the fact that it has produced good budbreak in all areas in which it has been 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 intervals 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 a sufficient 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 of this study period. However, bud injury was not evaluated at collection. The heat requirements for growth to begin have not been measured for any Arkansas blackberry cultivars, thus it is not possible to speculate if this variable 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.

The Shawnee response was very much as expected, since 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 result provided further confidence in our method.

Finally, the results for Chickasaw indicate that it likely has a similar chilling requirement to that of Shawnee or possibly slightly higher. Further research and observation 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 its chilling requirement. Why differentiation of chilling estimates was not achieved in our study with this cultivar is not understood, as we were not aware of any limitations of this cultivar, such as winter injury of buds prior to collection, heat requirements, or other factors.

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 conclusion, 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 O°T provided the most efficient chilling, while temperatures below O°C contributed to little chilling-requirement fulfillment. The chilling efficiency of various temperature ranges should also be investigated in blackberrys to determine if a similar response is involved.

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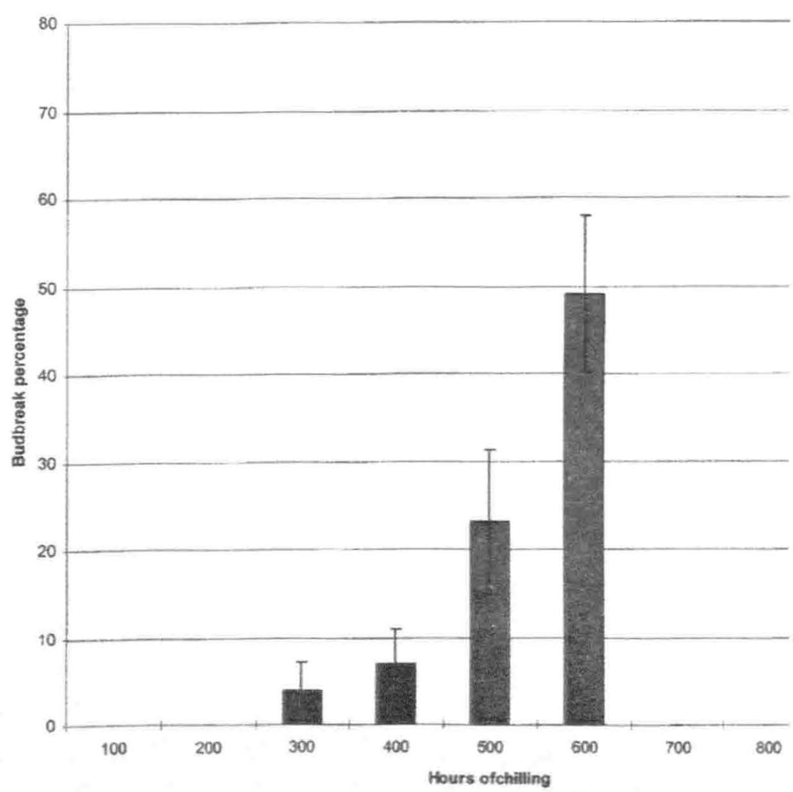

Fig. 1. Budbreak of Arapaho blackberry after 10 weeks of forcing in a heated greenhouse allowing 100 through 1000 hours of chilling, below 7°C.

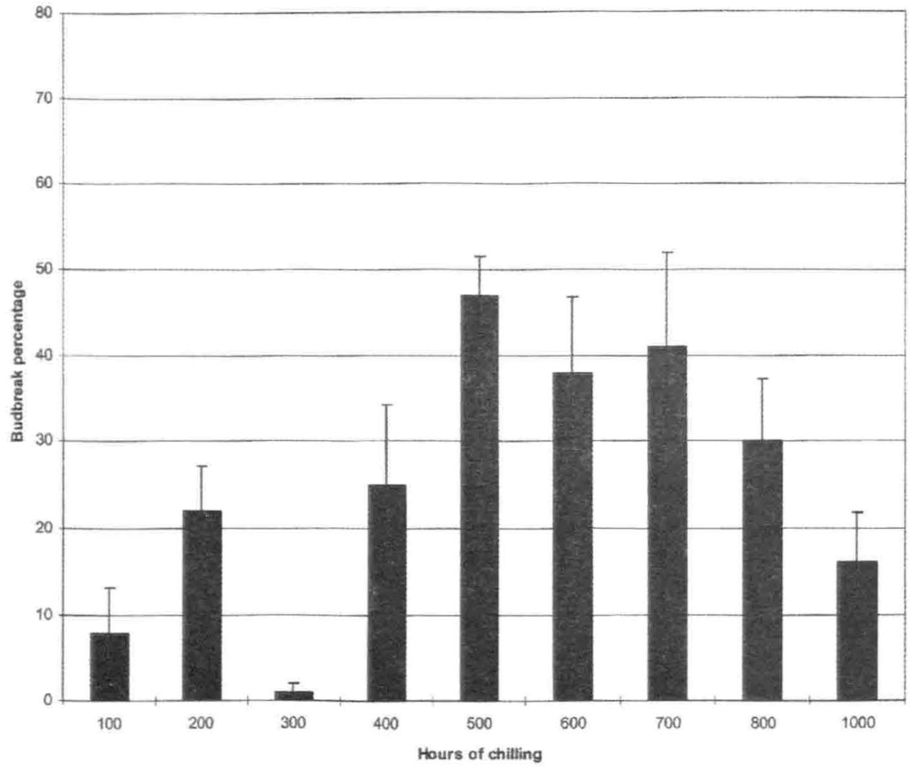

Fig. 2. Budbreak of Kiowa blackberry after 10 weeks of forcing in a heated greenhouse following 100 thr-ough 1000 hours of chilling, below 7°C.

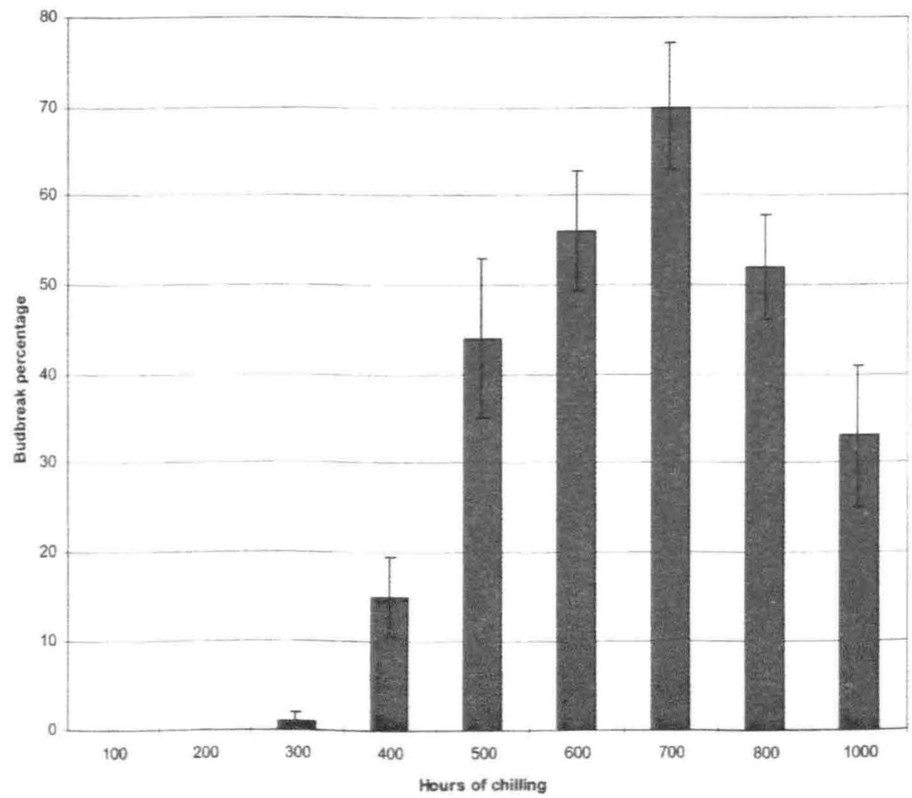

Fig.3. Budbreak of Shawnee blackberry after 10 weeks of forcing n a heated greenhouse following 100 through 1000 hours of ch8olling, below 7°C.

Faculty Comments:

Ms. Yazzetti's project mentor, John Clark, said in his recommendation:

> I am writing in support of Dayanee Yazzetti in her submission of a manuscript on blackberry chilling for *Inquiry*. I was Dayanee's special problem advisor for this study and I can attest that she did a fine job with the study. Work on the project began last October, and extended until just before spring break. Her findings provided some interesting and new data on the topic of chill requirements for blackberries in both methodology and cultivar aspects of this topic. Additionally she has presented part of the results of her work at a regional meeting of the American Society for Horticultural Science (placing first in her competition), and here on campus at the Gamma Sigma Delta Student Presentation Competition (placing second). Thus I deem her project a success in all its aspects.

Dave Hensley, Professor and Head of the Department of Horticulture, in recommending the inclusion of Ms. Yazzetti's paper in this journal made the following comments:

> The work is of high quality, is relevant in the field, and is publishable in one of our discipline's journals. Ms. Yazzetti has already received an award for presenting the work at a professional conference. The manuscript has been reviewed positively by various horticulture faculty. I think Dayanee Yazzetti would well represent the research activities of undergraduates in agriculture and urge you to give her serious consideration.