Response Of Blueberry To Day Length During Propagation

Internal report for Young Plant Research Center
Not for publication or reproduction in part or full without permission of the authors.
Paul Fisher and Ernesto Fonseca, University of Florida, pfisher@ufl.edu, 352 226 4410.
April 4, 2009

EXECUTIVE SUMMARY

Propagation of blueberry crops can be negatively affected by flower initiation during winter months when day length (photoperiod) is naturally short. Research by Dr. Darnell at the University of Florida showed that flower initiation occurs in southern highbush blueberries under short days, and flower bud development is then enhanced under long days. After flower bud initiation occurs, subsequent reproductive growth results in development of flower buds instead of leaves at the shoot apex, resulting in less leaf growth and potential for future generations of tip cuttings taken from liner trays or stock plants. Some flower initiation appears to also occur under long days, but we did not see flower buds successfully form. Juvenility is also important, and tissue culture (juvenile) plants are less sensitive to day length than tip cuttings.

During winter months, based on our trials and previous research, it is advised to keep liner trays under long days (night interruption lighting from 10 p.m. to 2 a.m. at 10 foot-candles) if the grower plans to harvest cuttings from these plants. The more generations of cuttings are grown under short days after tissue culture, or the longer that a liner tray stays under short days before having tip cuttings, the more likely that flower buds will be an issue. Applications of Florel (ethephon) could also help maintain vegetative growth under long or short days, but EPA label restrictions must be followed.

Cuttings of the southern highbush blueberry cultivars ‘Emerald’ and ‘Jewel’ were propagated under three photoperiod conditions including natural day lengths, short days (black cloth pulled from 5 p.m. to 8 a.m.) and long days (black cloth, plus incandescent lights from 10 a.m. to 2 p.m.). There was no difference in root growth during propagation of unrooted cuttings and tissue culture plants between long, short and natural day conditions. However, cuttings propagated under long days were observed to be greener than cuttings under short days. Continued exposure to long days helped keep plants vegetative. Florel sprays at 500 ppm reduced the number of reproductive shoot tips, especially in combination with long days. Although we did not test this, production of a vegetative blueberry liner without flower buds might also improve early growth and establishment for the finished plant customer in the spring.

OBJECTIVE:

The objective of this study was to evaluate effects of photoperiod and Florel spray applications on propagation of blueberry cutting during winter months.

BACKGROUND

In early December 2008, we observed flower bud initiation on liners propagated during the fall, both from plants propagated at the University of Florida, and also from a commercial supplier. This flower bud initiated while the stock plant (a rooted liner tray stuck at the end of July) was under natural days. Note the aborted leaf on the left and the bud forming at the apex. The uppermost leaf immediately below the flower bud is aborted.

When we took tip cuttings from these plants, even cuttings that appeared to be vegetative, we found that the light environment the cutting was growing under (short or long days) did not make a difference – flower initiation occurred on the stock plant liner tray. The first observable stage in reproductive growth can be seen below on a cutting under mist, where the top leaf has died. A flower bud could eventually form there above the aborted leaf.

On a few rooted liners, by the beginning of December open flowers had formed on liners that had been stuck in week 30 (end of July).

Research by Dr. Darnell at the University of Florida showed that flower initiation occurs in southern highbush blueberries under short days, and that subsequent flower bud development is then enhanced under long days. We therefore focused on day length and Florel (ethephon) applications as two tools that could be used to control flowering.

The published literature also discusses differences in flowering responses between blueberry cultivars, so the results reported here for Emerald and Jewel may differ from other cultivars, although we suspect there are similar overall trends.

MATERIALS AND METHODS

Two shipments of Emerald and Jewel blueberry tissue culture cuttings and liners were evaluated for these experiments. Jewel and Emerald are southern highbush cultivars (http://edis.ifas.ufl.edu/HS215) bred by Dr. Paul Lyrene at the University of Florida.

The first shipment of liners was received November 11, 2008. The liner trays had been planted on week 36 (Sep 1, Jewel) or week 33 (Aug 10, Emerald) and were grown under natural days by the commercial grower, AgriStarts in Apopka Florida.

The second shipment of liner trays, planted on weeks 39 to 44 2008 (Jewel and Emerald) and grown under natural days by the commercial grower, was received January 8, with.

Details of these two shipments are in the table on the next page.

First shipment

Cultivar	Type	Cuttings per tray, with 1 tray per block	Number of trays / Photoperiod	Photoperiod environments	Total Cuttings	Stick week at Agri Starts	Sticking date at UF ---------- Evaluation Dates at UF
Jewel	Tissue Culture	21	3 Natural Days 3 Long Days 3 Short Days	• Natural days • Short days (black cloth 5 p.m to 8 a.m. • Long days (black cloth plus night interruption)	189		Stuck Nov 12 1st evaluation Dec 11 2nd evaluation Feb 10
Emerald	Tissue Culture	21	3 Natural Days 3 Long Days 3 Short Days		189		
Jewel	Tip Cutting	12	3 Natural Days 3 Long Days 3 Short Days		108	36 36 36	Stuck Nov 17 1st evaluation Dec 15 2nd evaluation Feb 10
Emerald	Tip Cutting	12	3 Natural Days 3 Long Days 3 Short Days		108	33 33 33	

Second shipment

Cultivar	Type	Cuttings per tray, with 2 trays per block	Number of trays / Photoperiod	Photoperiod environments	Total Cuttings	Stick week at Agri Starts	Grown on at UF as rooted liners. Evaluation Date at UF
Jewel	Rooted liner from tip cutting	12	3 Long Days 3 Short Days	• Short days (black cloth 5 p.m to 8 a.m. • Long days (black cloth plus night interruption)	72	42 39 39	Received Jan 8 Evaluated Feb 10
Emerald	Rooted liner from tip cutting	12	3 Long Days 3 Short Days		72	44 43 41	

Experiments were run under three different photoperiods (natural day length, short day (black cloth pulled from 5 p.m. to 8 a.m.), and long day (black cloth, plus incandescent lights from 10 p.m. to 2 a.m. at 8 foot-candles)). In the second group of experiments, only the short and long day treatments were compared.

Photoperiod environments were set up in one greenhouse for mist propagation, and a second greenhouse with hand irrigation for growing on rooted liners. Each lighting environment was replicated three times in each greenhouse. The photo below shows how benches were blocked out with long and short day environments on the same bench with black cloth in between.

Photoperiod effect on rooting of cuttings

Liners and tissue culture cuttings of Emerald and Jewel blueberries arrived November 11, 2008 for rooting evaluation. The tissue culture cuttings were stuck on November 12. Tip cuttings from the liners of the same two blueberry varieties were harvested and stuck on November 17. Cuttings were stuck into partial trays – tissue-culture plants in Preforma 288-cell trays, and tip cuttings in Preforma 72-cell trays. Tip cuttings had 5 leaves, with the bottom 2 removed, and were treated with Hormodin 2 powder before sticking. There were 21 tissue culture cuttings per tray, or 12 tip cuttings per tray. There were three trays per cultivar per light treatment, on replicated benches (63 tissue culture plants and 36 tip cuttings per light zone per cultivar).

Cuttings were kept under mist at 15-20 min / 2 sec during the day and at 45 min/ 2sec at night, which included ZeroTol at 1:5000, until roots formed. After rooting, cuttings were removed from mist and hand-watered with 17-4-17 neutral fertilizer at 75 ppm N and micronutrients at 1 ppm Fe with each irrigation. These cuttings were planted in three different environments, including natural day lengths, short days (black cloth pulled from 5 p.m. to 8 a.m.) and long days (black cloth, plus incandescent lights from 10 a.m. to 2 p.m. Shading was needed to eliminate light pollution.

One month after sticking, all cuttings were evaluated for number of primary roots, longest root length, number of leaves per plant, and number orange and red leaves.

Florel and photoperiod effect on rooted liners (two spray applications of Florel)

After the rooting evaluation experiment, the rooted liners were pinched and used to conduct a separate experiment evaluating the effect of Florel and photoperiod on branching (number of shoot tips) and flower initiation (number of shoot tips showing abortion of the top leaf) of blueberry cuttings.

On January 10, trays were split into equal groups of 6 plants. Half of the trays received a Florel spray application at 500 ppm on Jan 10 2009 and a second Florel spray at 500 ppm two weeks later on Jan 21 2009. One half tray of 6 plants in each replicate was used as a control treatment, without Florel.

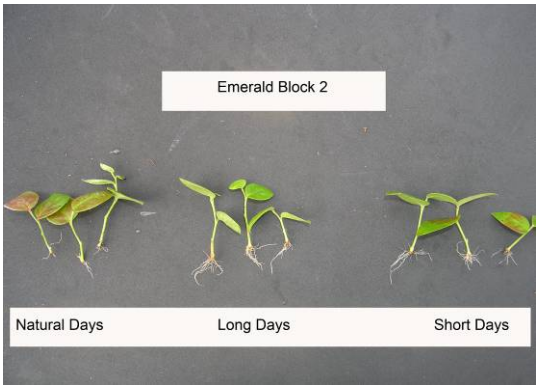
Florel and photoperiod effect on rooted liners (single spray application of Florel)
On January 10, 2009, rooted cuttings from the second shipment received a single application of Florel at 500 ppm. Two replicate 12-count partial trays per block in each light treatment (6 trays total) received an application of Florel and a corresponding number of 12-count partial trays did not receive Florel as a control treatment.

Statistically, data were analyzed using SAS with ANOVA as a split-plot design, with photoperiod as the main plot, and cultivar, cutting type, and growth retardant as sub-plots.

RESULTS AND DISCUSSION

Photoperiod effect on rooting of cuttings
The photos and charts below show that there was no significant difference in root systems between the three photoperiod treatments for either the tip cuttings or tissue culture cuttings.

Tip Cutting



Tissue culture cutting

However, tip cuttings grown under long days had greener foliage than those grown under natural days.

NATURAL DAYS

LONG DAYS

Average number of primary roots, leaves per plant, longest root, and orange or red leaves for blueberries propagated under mist.

Average Number of Primary Roots per plant				
	Cultivar	Natural D	Long D	Short D
Tissue Culture	Emerald	4.9	3.8	3.5
	Jewel	3.2	2.5	2.7
Tip Cutting	Emerald	3.8	3.1	2.6
	Jewel	3.2	4.7	3.4

Average Number of leaves per plant				
No Leaves/ plant	Cultivar	Natural D	Long D	Short D
Tissue Culture	Emerald	11.3	11.2	11.5
	Jewel	10.7	10.5	7.4
Tip Cutting	Emerald	2.4	2.6	2.6
	Jewel	2.7	2.8	3.2

Average Length of Longest Root (cm)				
	Cultivar	Natural D	Long D	Short D
Tissue Culture	Emerald	1.8	1.2	1.6
	Jewel	1.2	1.0	1.7
Tip Cutting	Emerald	1.0	1.0	0.9
	Jewel	1.5	1.7	1.7

Average Number of leaves/cutting with orange or red color				
	Cultivar	Natural D	Long D	Short D
Tissue Culture	Emerald	2.0	0.7	3.3
	Jewel	3.6	2.5	5.5
Tip Cutting	Emerald	1.7	0.6	1.2
	Jewel	1.4	1.2	1.9

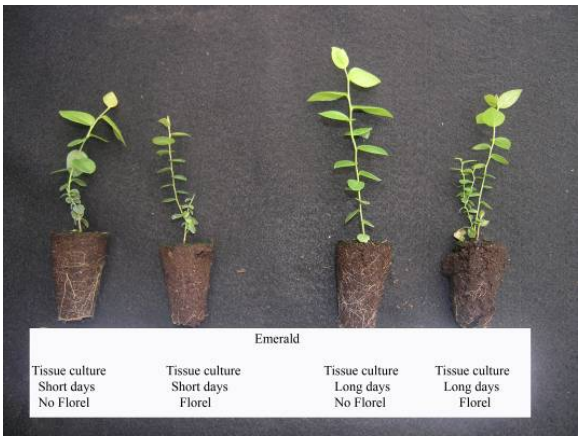
Florel and Photoperiod Effect on Rooted Liners (Two Florel Applications at 500 ppm)

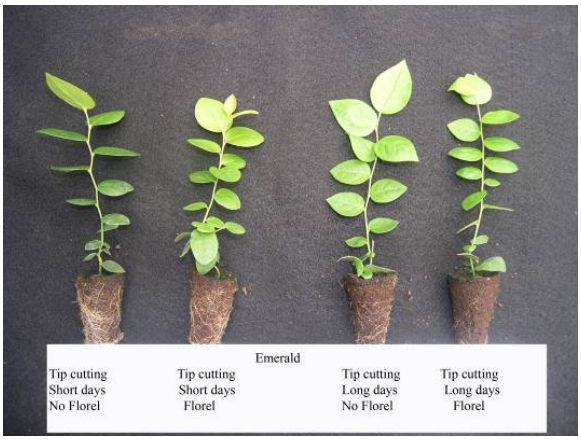
Shoots of Jewel tended to be 1-cm taller than Emerald cuttings, at 5.9 cm compared with 7.0 cm. Tip cuttings were also 0.7 cm taller than tissue-culture cuttings. Tip cuttings grown under long days had the tallest cuttings. Plants grown with Florel were observed to have smaller leaves, although this was not quantified.

Florel applications increased branching by 15%, with a greater effect on tissue culture plants than tip cuttings.

Jewel had 43% more vegetative shoot tips than Emerald, but the same number of reproductive shoots. Florel increased the number of vegetative shoot tips by 35% and decreased the number of reproductive tips by 32%. Tissue culture cuttings produced 60% more vegetative tips and 18% fewer reproductive tips than tip cuttings.

The combination of long days and Florel resulted in plants with the fewest reproductive shoot tips, compared with either long or short days and no Florel.





Percent of vegetative and reproductive tip cuttings produced following two applications of Florel to rooted liners.

Emerald Tissue culture (averages)

Florel Application	Long D	Shor D	Natural D
Number of shoot tips	2.1	1.3	1.8
% of vegetative tips	70%	71%	91%
% of reproductive tips	30%	29%	9%
Maximum length (cm)	7.1	5.4	5.8

No Florel Application	Long D	Shor D	Natural D
Number of shoot tips	1.6	1.6	1.8
% of vegetative tips	43%	52%	64%
% of reproductive tips	57%	48%	36%
Maximum length (cm)	7.4	5.9	6.6

Jewel Tissue Culture (averages)

Florel Application	Long D	Shor D	Natural D
Number of shoot tips	1.4	1.6	1.8
% of vegetative tips	84%	71%	61%
% of reproductive tips	16%	29%	39%
Maximum length (cm)	6.5	4.3	5.8

No Florel Application	Long D	Shor D	Natural D
Number of shoot tips	1.7	1.2	1.4
% of vegetative tips	52%	55%	44%
% of reproductive tips	48%	45%	56%
Maximum length (cm)	6.6	5.7	6.2

Emerald Tip Cutting (averages)

Florel Application	Long D	Shor D	Natural D
Number of shoot tips	1.3	1.2	1.2
% of vegetative tips	46%	29%	14%
% of reproductive tips	54%	71%	86%
Maximum length (cm)	7.4	4.1	4.4

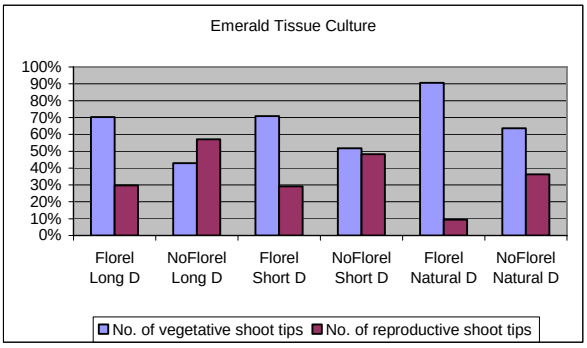
No Florel Application	Long D	Shor D	Natural D
Number of shoot tips	1.3	1.1	1.6
% of vegetative tips	25%	10%	55%
% of reproductive tips	75%	90%	45%
Maximum length (cm)	7.3	5.3	6.8

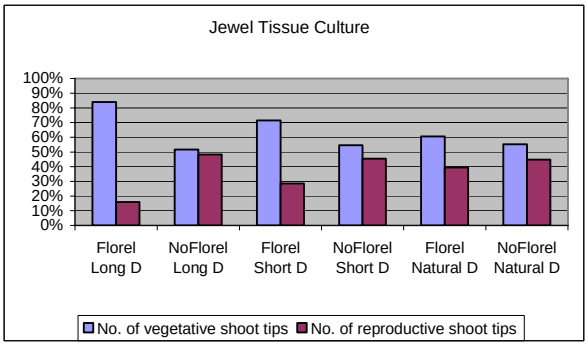
Jewel Tip Cutting (averages)

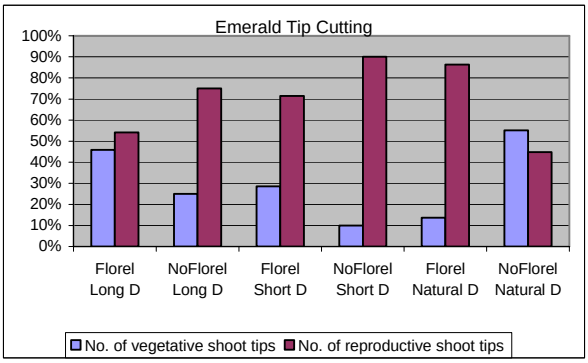
Florel Application	Long D	Shor D	Natural D
Number of shoot tips	1.6	2.0	1.7
% of vegetative tips	71%	64%	61%
% of reproductive tips	29%	36%	39%
Maximum length (cm)	10.9	7.6	7.7

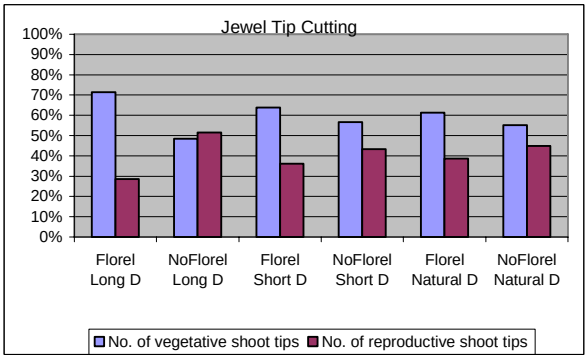
No Florel Application	Long D	Shor D	Natural D
Number of shoot tips	1.8	1.7	1.6
% of vegetative tips	48%	57%	55%
% of reproductive tips	52%	43%	45%
Maximum length (cm)	9.4	6.5	6.8

Percent of vegetative and reproductive shoot tips produced following two applications of Florel to rooted liners.









Florel and Photoperiod Effect on Rooted Liners (Single Florel Application at 500 ppm)
In this experiment with tip cuttings only, long days increased shoot length but plants receiving Florel actually were 1.2 cm longer than plants without Florel.

Florel again increased the proportion of vegetative shoot tips and decreased the proportion of reproductive tips. Emerald produced more reproductive tips and fewer vegetative tips than Jewel. Photoperiod, cultivar, and Florel did not affect the total number of shoot tips.

Percent of vegetative and reproductive shoot tips per cutting following a single application of Florel to rooted liners.

Emerald Tip Cutting (averages)

Florel Application	Long D	Shor D	Natural D
Number of shoot tips	2.2	2.1	2.2
% of vegetative shoot tips	58%	52%	69%
% of reproductive shoot tips	42%	48%	31%
Maximum length (cm)	10.1	8.7	12.0

No Florel Application	Long D	Shor D	Natural D
Number of shoot tips	2.1	1.8	2.1
% of vegetative shoot tips	45%	34%	42%
% of reproductive shoot tips	55%	66%	58%
Maximum length (cm)	11.4	8.6	10.7

Jewel Tip Cutting (averages)

Florel Application	Long D	Shor D	Natural D
Number of shoot tips	2.3	2.5	2.1
% of vegetative shoot tips	67%	69%	58%
% of reproductive shoot tips	33%	31%	42%
Maximum length (cm)	13.5	10.0	10.6

No Florel Application	Long D	Shor D	Natural D
Number of shoot tips	2.1	2.1	2.0
% of vegetative shoot tips	65%	53%	58%
% of reproductive shoot tips	35%	47%	42%
Maximum length (cm)	10.1	6.4	10.3

Later observations from the Florel and day length trials (April 1 2009)

On April 1 2009, we evaluated flowering on plants from the Florel and photoperiod treatments in both experiments. The observations are revealing, and help interpret the earlier results.

- In no cases did tissue culture cuttings show flower buds, indicating that they were still vegetative and possibly juvenile.
- However, tissue culture plants in all photoperiods had a mix of reproductive and vegetative shoot tips.
- During the experiments from Nov to February, we had rarely seen actual flower buds on plants from tip cuttings, and never on the tissue culture plants (only on a few of the tip cuttings). However, on April 1 we observed flower buds and even fruit on plants grown from tip cuttings under natural and short day lengths.
- Under natural day lengths, there were closed and open flower buds and a few fruit on liners grown from tip cuttings, especially for Emerald.
- Under short days, we saw the most open flowers and fruit, especially for Emerald.
- Under long days, there were no open flowers, but there were some tight flower buds. Only on one plant did we see a fruit, from an old side shoot near the base of a plant.
- There was no obvious carryover effect from the Florel in terms of flowering.

So if there were no flower buds on the tissue culture plants, why did we see reproductive shoot tips on tissue culture plants during the experiment, and on tip cutting plants under long days? We believe that the way we quantified flower initiation in our experiments, as the number of shoot tips showing abortion of the top leaf, is very important to interpret our data presented above.

Our hypothesis is as follows

- Blueberries have a long-day leaf number, just like poinsettia and chrysanthemum (two other plants that initiate flowering under short days). Even under non-inductive (long-day) photoperiods and with juvenile tissue culture plants, once a certain number of leaves unfold on a side shoot (which is probably cultivar-specific), a flower bud is formed which aborts unless it is under conditions inductive to flower development. A shoot that forms a flower

bud that aborts will not form a flower, but it does not make a good vegetative cutting because it will not continue to grow unless a new shoot emerges from a leaf axil. We did indeed observe older stunted side shoots on the tissue culture plants that had aborted leaves at their apex.

- Furthermore, liners grown from tip cuttings were all under short days before the start of the experiments, and the long day treatment presumably did not prevent flower development because plants had already initiated flower buds under natural days. That is consistent with Dr. Darnell's research on flower initiation and development in southern highbush blueberry.

CONCLUSIONS

There was no clear difference in root systems of blueberry cuttings propagated under long, natural or short days, although cuttings under long days were greener than under short days.

It is important to maintain Jewel and Emerald under long days during fall to spring to avoid flower initiation. If reproductive bud set occurs under short or natural day propagation, long day treatment following propagation will not stop flower initiation but is likely to reduce the formation of open flowers.

Tissue cultured plants stuck on Nov 12 showed evidence of flower bud initiation, but had not formed flower buds by April 1, indicating that flower buds may have formed and aborted because of long-day leaf number was reached.

Florel applications reduced the number of reproductive shoot tips.

Overall, a combination of long day lighting and Florel applications (probably applied multiple times) would probably lead to the most vegetative and well-branched cutting. Florel is listed as a fruit eliminator on "ornamental trees and shrubs" . It would be necessary to check EPA label restrictions on applying Florel for this purpose.

REFERENCES

http://www.lal.ufl.edu/academics/faculty/spann/PDF/5.pdf
http://books.google.com/books?id=ONJkN-jZu2EC&pg=PA357&lpg=PA357&dq=blueberry+flower+initiation+photoperiod+temperature&source=web&ots=cHmLx_ULir&sig=aSK8ukS2O-uNpjX1cSAXLYBJpAs&hl=en&sa=X&oi=book_result&resnum=1&ct=result#PPA358,M1,
UF cultivars
http://edis.ifas.ufl.edu/HS215
http://www.montereylawngarden.com/products/labels/

Acknowledgements

Thanks to AgriStarts for providing tissue culture plants and liners, and help with growing protocols. We thank Dr. Darnell and Dr. Lyrene at the University of Florida IFAS for sharing their technical expertise on blueberry physiology and horticulture. Funding for this study was provided by the Young Plant Research Center donor companies (AgriStarts, Altman Plants, Blackmore Co., D.S. Cole Growers, Ellegaard, Fafard, Four Star Greenhouses, Greencare Fertilizers, Knox Nursery, Kube-Pak Corp., Lucas Greenhouses, Pindstrup, Pleasant View Gardens, Premier Horticulture, Quality Analytical Laboratories, Spring Meadow Nursery, Smith Gardens, Sun Gro Horticulture, Twyford International, Wagner Greenhouses, and Welby Gardens for supporting this research).