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RESEARCH PAPER

Partial inhibition of flowering in young highbush blueberries with gibberellins

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Abstract

W. Lindberg, E. Hanson, and G.A. Lobos. 2014. Partial inhibition of flowering in young highbush blueberries with gibberellins. Cien. Inv. Agr. 41(3):349-356. Preventing young blueberry (*Vaccinium corymbosum* L.) plants from fruiting can increase their vegetative growth. In previous studies, gibberellin (GA) applications reduced flowering in highbush blueberry, but the response was variable and cultivar dependent and was studied mostly using potted nursery plants. The purpose of this work was to determine whether floral induction can be inhibited by GA in plants established in the field, particularly on newer cultivars now being widely planted. Foliar sprays were applied in several experiments with different application intervals and concentrations; the efficacy was determined by counting the number of initiated floral meristems. GA applications in July and August were more inhibitive than those in September and October, while cultivars did not vary in their response. GA significantly reduced flower bud numbers in three separate studies, but the greatest reduction (49%) required repeated applications from July to October. The results indicate that GA may have limited commercial utility for preventing fruiting in highbush blueberries under field conditions. However, further studies are needed to determine how vegetative growth is affected by the partial inhibition of flowering.

Key words: Floral induction, GA_3, GA_{4+7}, ProGibb, ProVide, *Vaccinium corymbosum.*

Introduction

Global production of highbush blueberries (*Vaccinium corymbosum* L.) increased 30% from 2005 to 2010, and acreage continues to expand in most production areas. In Michigan, new acreage has recently been planted with some older cultivars (Bluecrop, Elliott) and with newer cultivars, such as Aurora, Draper and Liberty (Hancock *et al*., 2008). In short growing regions, such as Michigan, blueberry plants may not reach full fruit production until 7-10 years after planting (Strik, 2007). Slow establishment rates delay returns on investments in new fields and deter growers from replacing older plants with newer cultivars that have higher yield potential and superior fruit (Hancock *et al*., 2008).

The growth of new plants can be accelerated by preventing fruit production, as young highbush blueberries allocate over 50% of photosynthates

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to fruiting (Pritts and Hancock, 1985). Manual removal of blueberry floral meristems (buds) for two years after planting increases shoot length and mass (Strik and Buller, 2005) and reduces the exposure of plants to pollen borne virus diseases (Bristow and Martin, 1999). The manual removal of flower buds is recommended for the first two seasons (Pritts and Hancock, 1992), but this process is labor intensive.

Gibberellin (GA) applications reduced floral induction in highbush blueberry (Black and Ehlenfeldt, 2007; Retamales *et al*., 2000) and several woody fruit crops (Lenahan *et al*., 2006; Lobos and Yuri, 2006). Most of the studies on blueberries were conducted on potted nursery plants. Preventing flowering in nurseries has the potential added benefit of preventing the exposure of plants to pollen-born virus diseases, such as blueberry shock (Bristow and Martin, 1999) and blueberry leaf mottle (Childress and Ramsdell, 1987). The results indicated that floral induction was inhibited by GA_3 and GA_{4+7} at concentrations of 150 to 400 mg L^{-1}. Inhibition ranged from 0 to 98% and varied by cultivar (Black and Ehlenfeldt, 2007; Retamales *et al*., 2000). Although single applications inhibited floral induction (Retamales *et al*., 2000), multiple sprays applied over several weeks were most effective (Black and Ehlenfeldt, 2007). When plants established in the field were treated, floral induction was reduced in one study but not in another (Retamales *et al*., 2000).

GA is expected to be most inhibitory when applied during floral induction (Retamales *et al*., 2000), but the time of floral induction in highbush blueberries is not clearly defined. Blueberries produce episodic and sympodial vegetative flushes. Each terminates with the abortion of the apical meristem and can be followed by other flushes originating from axillary buds (Gough *et al*., 1978). The first histological evidence of floral differentiation in 'Bluecrop' in Rhode Island occurred in late July or early August (Gough *et al*., 1978). Tamada (1997) concluded from anatomical observations that floral differentiation on primary shoots of 'Jersey' in Japan progressed for two to six weeks after apical abortion, which corresponded with mid-July to the end of August (day lengths of 14.1 to 12.8 h). If this is true for all shoots, floral induction could occur from July to October depending on the number and timing of growth flushes. In fact, flower buds are found in varying positions along all growth flushes (Gough *et al*., 1978). However, floral induction is also influenced by day length and temperature (Lobos *et al*., 2009 and 2013). In controlled environments, floral induction was strongly suppressed by long 14- to 16-h photoperiods **(**Hall *et al*., 1963; Bañados and Strik, 2006) and high temperatures (Spann *et al*., 2004).

It is hypothesized that multiple GA sprays on juvenile blueberry plants (2-3 years-old) will strongly inhibit floral induction, as was previously achieved on nursery plants. The purpose of this work was to determine the most effective spray timings for young blueberries established in the field, particularly for cultivars that are now being widely planted.

Materials and methods

Study 1

Commercial blocks of 'Elliott', 'Liberty', and 'Aurora' planted in the spring of 2009 as three-year-old nursery plants in Gobles, MI (42 º22' N, 85 º54' W) were used. Plots containing five plants of similar size and vigor were assigned to one of seven treatments, with six replicates in a randomized complete block design. Treatments consisted of GA_{4+7} (ProVide, Valent BioSciences Corp. Libertyville, IL, USA) or GA_3 (ProGibb, Valent BioSciences Corp.) applied early (10 and 17 Aug. 2009), mid-season (26 Aug. and 3 Sept., 2009), or late (13 and 21 Sept., 2009) and a water-sprayed control. The products were applied at 400 mg L^{-1} active ingredient (a.i.) with a handheld sprayer to the point of runoff. The effect on floral induction was determined by counting the number of floral buds per plant once they began to swell in April 2010.

Study 2

This experiment was conducted in commercial fields near Lacota, Michigan (42° 24' N, 86° 07' W) that were planted in 2008 ('Elliott') or 2009 ('Draper', 'Aurora', 'Liberty') as two-year-old plants. Plants of similar size and vigor were assigned one of five treatments, in a randomized complete block design with eight single-bush replicates. The treatments were a water-sprayed control and 400 mg L^{-1} a.i. GA_{4+7} applied at two-week intervals either early (21 July to 1 Sept., 2010), late (8 Sept. to 20 Oct., 2010), or early and late (21 July to 20 Oct., 2010). The fifth treatment consisted of sprays on the early and late dates at 200 mg L^{-1} a.i.

Flower bud numbers per plant were recorded in April, 2011. On May 13, 2011, the length of dead branches was recorded to assess winter cold injury, and the number of flowers per bud was determined on five randomly selected buds per plant. The berry number per bush was recorded on July 7, 2011. To estimate bush size, plant height and width (narrowest and widest dimensions) were measured. Canopy volume was calculated by multiplying these three dimensions.

Study 3

This study was conducted in separate rows of 'Liberty' and 'Draper' that were planted as two-year-old plants in 2008 at the Michigan State University Horticultural Teaching and Research Center in Holt, Michigan (42º 67' N, 84º 48' W). The treatments included a water sprayed control and 400 mg L^{-1} GA_{4+7} applied at weekly intervals either early (25 July to 30 Aug., 2011) or late (2 Sept. to 9 Oct., 2011). The treatments were replicated on eight single-bush plots in a randomized complete block design. Flower buds per plant were counted after leaves abscised in Nov. 2011. Average shoot length was also recorded by randomly selecting one main branch per plant and by measuring the length of each shoot.

Data from all of the studies were analyzed with SAS 9.2 (SAS Institute, Cary, NC, USA) as randomized complete block designs with the cultivar as the main factor and the growth regulator treatment and/or timing as sub-plot factors. PROC Glimmix was used to determine statistical significance for floral meristems and flowers per meristem, while PROC Mixed was used to determine significance for shoot length and canopy volume. When significant interactions ($P \leq 0.05$) were found, mean separation was performed with PDIFF in LSMEANS statement. Temperature data were recorded by an automated weather station located within 4, 5, and 0.1 km from study sites 1, 2, and 3, respectively.

Results

Study 1

Flower bud numbers were affected by GA application timing (Table 1) but not form (GA_3 or GA_{4+7}) or the interactions between GA timing, form, and cultivar. All GA application times

Table 1. Effect of 400 mg L^{-1} GA applied during 2009 on floral meristem numbers in 2010. The data are presented as the mean±SE of three cultivars (Aurora, Elliott, and Liberty) and two GA forms (GA_3 and GA_{4+7}).

Application dates[1]	Flower buds $plant^{-1}$
Control	347±34 a[2]
Early (Aug. 10 and 17)	269±17 b
Mid-season (26 Aug. and 3 Sep.)	197±14 c
Late (13 and 21 Sep.)	272±19 b
Factorial treatment analysis (P value)	
Cultivar (C)	0.014
GA form (F)	0.28
Application time (T)	<0.001
C × F	0.48
C × T	0.104
F × T	0.45
C × F × T	0.196

[1]Average temperature and day length for the 14-d period following the first application; the treatments were Early (21.8 ºC, 14.0 h), Mid-season (17.3 ºC, 13.2 h), and Late (17.9 ºC, 12.2 h).
[2]Means followed by the same letter are not significantly different ($P \leq 0.05$)

reduced the flower bud number relative to the control, but the mid-season timing (26 Aug. and 3 Sept.) provided a greater reduction (43%) than the early or late timing (22 and 21%, respectively).

Study 2

The time of GA application affected the number of flower buds and berries per plant, while the GA rate affected the number of flowers per bud (Table 2). The effects of concentration alone and all interactions between cultivar, time, and concentration were not significant (Table 2). The greatest reductions in flower bud and berry numbers resulted from earlier applications (49% reduction) and early plus late timings (42%). The 400 mg L^{-1} rate reduced the number of flowers per bud relative to the control. Treatments did not affect the length of dead branches per bush (overall mean 32 cm) or canopy volume (39 m^3) (data not shown).

Study 3

The analysis of variance indicated that flower bud numbers were significantly affected by GA. Weekly applications of GA_{4+7} in July and August resulted in fewer flower buds per plant compared to non-treated plants and those treated in September and October. The interaction of the GA application time and cultivar was not significant, indicating that 'Liberty' and 'Draper' responded similarly. Selected main branches contained an average of 23 shoots that averaged 8.4 cm in length and were not affected by treatments (data not shown).

Table 2. Effect of GA_{4+7} application time and concentration during 2010 on flower buds, flowers per bud and berry numbers in 2011. The data are presented as the mean±SE of four cultivars (Elliott, Draper, Aurora, and Liberty).

Treatment[1]	Flower buds plant^{-1}	Flowers bud^{-1}	Berries plant^{-1}
Application time			
Control	92±7.2 a[2]	4.8±0.34	182±21 a
Early (21July to 1 Sep., 2010)	57±6.1 b	4.2±0.42	132±19 bc
Late (8 Sep. to 20 Oct., 2010)	76±6.9 a	4.5±0.34	162±22 ab
Early and late (all dates)	47±4.0 b	4.2±0.26	105±11 c
Concentration (mg L^{-1})			
0	92±7.2	4.8±0.3 a	182±21
200	53±4.9	4.7±1.9 ab	124±17
400	58±3.9	4.1±2.2 b	137±11
Factorial analysis (P value)			
Cultivar (C)	0.004	<0.0001	<0.0001
Rate (R)	0.30	0.02	0.07
Application time (T)	0.011	0.11	0.02
C × R	0.57	0.523	0.20
C × T	0.53	0.81	0.89
R × T	0.58	0.49	0.59

[1]Applications were made at two-week intervals using 200 or 400 mg L^{-1} active ingredients and a handheld sprayer to the point of runoff. The controls were treated with water. Average temperature and day length for the period from the first application date until 7 d after the last application were 23.7 ºC and 14.0 h (Early), 14.7 ºC and 11.9 h (Late), and 19.2 ºC and 13.0 h (Early plus Late).
[2]Means within a column group that are not followed by the same letter are significantly different (P≤0.05).

Discussion

These results indicate that GA was only partially effective at preventing floral induction on highbush blueberry in field plantings. Maximum inhibition (49%) was achieved with eight applications at two-week intervals from late July and October (Table 2). Weekly sprays over approximately the same time period resulted in a 44% reduction (Table 3). Black and Ehlenfeldt (2007) achieved as high as 95% inhibition with weekly sprays from July through September. It is not clear why inhibition was greater in this early study than the current studies, but it may relate to the plants that were used in the study. Black and Ehlenfeldt (2007) utilized one-year-old rooted cuttings with approximately 20 flower buds per plant, whereas the current studies were conducted on older bushes producing 90 to 350 flower buds. Two previous studies on older field-grown plants showed that GA had either little or no effect on flower bud numbers (Retamales *et al*., 2000). Perhaps older plants or those established in the field are less responsive to GA than younger nursery plants. Mature cherry shoots were less responsive to GA than juvenile shoots (Oliveira and Browning, 1993).

These trials were conducted on the most commonly planted cultivars in Michigan today. Cultivars responded similarly to GA treatments in each study (Tables 1, 2, 3) even though they ranged in harvest times from relatively early ('Draper') to very late ('Aurora' and 'Elliott'). Retamales *et al*. (2000) and Black and Ehenfeldt (2007) reported some minor differences in responses to GA when comparing different groups of cultivars. Overall, genotypes do not appear to vary greatly in their response to GA.

As reported by Retamales *et al*. (2000) and Black and Ehlenfeldt (2007), GA_3 and GA_{4+7} were equally effective in reducing floral induction (Table 1). Concentrations in the current studies (200 or 400 mg L^{-1} GA) were similar to those used previously. Black and Ehlenfeldt (2007) found that 400 mg L^{-1} was more effective than 200 mg L^{-1} but not different than 600 mg L^{-1}. Retamales *et al*. (2000) reported that 150 and 300 mg L^{-1} were equally effective. In apples, the inhibition of floral induction was dependent on GA concentrations in a linear manner (Greene, 1993).

Table 3. Effect of GA_{4+7} applied during 2011 on floral meristem numbers in 2012. The data are presented as the mean±SE of two cultivars (Draper and Liberty).

Treatment [1]	Flower buds plant^{-1}
Control	218±34 a[2]
Early (July 25 to Aug. 30, 2011)	121±30 b
Late (Sept. 2 to Oct. 9, 2011)	194±23 a
Factorial analysis (P value)	
Cultivar (C)	<0.001
Treatment (T)	0.016
C × T	0.2898

[1]Applications were made at 7 d intervals using 400 mg L^{-1} active ingredient and a handheld sprayer to the point of runoff. The controls were treated with water. Average temperature and day length for the period between the first and last application dates were 22.0 ºC and 13.9 h (Early) and 15.5 ºC and 12.2 h (Late).
[2]Means followed by the same letter are not significantly different ($P \leq 0.05$).

The timing of GA sprays affected flower bud numbers in each study. Sprays in late August to early September (Table 1) and late July to late August (Tables 2 and 3) were most effective. This generally agrees with early reports that optimum applications times were mid-August to early September (Black and Ehlenfeldt, 2007; Retamales *et al*., 2000). Because GA sprays are expected to be most inhibitive when applied during floral induction (Black and Ehlenfeldt, 2007), the fact that multiple GA sprays over several weeks provide maximum inhibition indicates that induction occurs over several weeks. Blueberries can produce multiple shoot flushes that terminate in apical abortion. In Michigan, the growth of primary flushes tends to terminate in June or early July, and later, flushes may grow into September, depending on the cultivar, environment, and plant health (Gough *et al*., 1978). If flower bud induction occurs for 2-6 week after apical abortion (Tamada, 1997), induction may occur

from July to October, depending on the timing of growth flushes.

Photoperiod also affects floral induction in highbush blueberries. Induction was inhibited by 14- to 16-h photoperiods and was promoted below 12 h (Hall *et al*., 1963; Bañados and Strik, 2006), but cultivars may vary somewhat in their response (Hall *et al*., 1963). The day length was longer than 14 h until Aug. 7 in the current studies and until Aug. 1 in studies by Black and Ehlenfeldt (2007), so the most effective GA application times were generally at lengths of less than 14 h. However, GA applied before August, when days were longer than 14 h, also reduced flower bud numbers (Black and Ehlenfeldt, 2007; Retamales *et al*., 2000). In fact, flower bud numbers were reduced in half-high blueberries treated in early July when the day length was 15 h (Retamales *et al*., 2000). In addition, blueberries held in continuous 16-h days and treated with GA during bloom (to induce parthenocarpy) contained fewer flower buds the next season (Mainland and Eck, 1969). This suggests that induction can occur when days are longer than 14 h or, as proposed by Samach and Smith (2013), the GA application retains activity for several weeks after application.

Temperature also influences floral induction and perhaps responses to GA treatments. In short, during inductive photoperiods, southern highbush blueberries initiated numerous floral buds at 21 ºC but few at 28 ºC (Spann *et al*., 2004). During short photoperiods, lowbush blueberries produced more flower buds at 21 ºC than at 10 ºC (Hall and Ludwig, 1961). The photoperiod alone may be insufficient to predict when floral induction occurs in blueberries.

These studies indicate that GA was only partly effective at inhibiting floral induction in several blueberry cultivars established in the field. Multiple sprays may cost several hundred dollars per acre at current GA prices and application costs; therefore, this may not be a practical commercial approach for increasing vegetative growth by preventing fruiting in new plantings. Although manually removing all flower buds during the establishment years increases plant growth (Strik and Buller, 2005), the effect of partial prevention on growth is unknown. Further studies are needed to determine the effect of partially inhibiting fruiting on growth rates of bushes during the establishment years.

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Resumen

W. Lindberg, E. Hanson y G.A. Lobos. 2014. Inhibición parcial de la floración en plantas jóvenes de arándanos con giberelinas. Cien. Inv. Agr. 41(3):349-356. La inhibición de la fructificación en plantas jóvenes de arándanos (*Vaccinium corymbosum* L.) puede mejorar el crecimiento vegetativo. En estudios previos, en su mayoría en plantas de vivero en macetas, de mostró que la aplicación de giberelinas (GA) redujo la floración en arándanos de arbusto alto, pero la respuesta fue variable y dependiente del cultivar. El objetivo de este trabajo fue determinar si la inducción floral puede ser inhibida por GA en plantas jóvenes en campo, especialmente en nuevos cultivares que están siendo ampliamente plantados. En diversos experimentos se hicieron aplicaciones foliares con distintos intervalos y concentraciones; la eficacia se midió contando el número de meristemas diferenciados. Aplicaciones de GA en julio y agosto inhibieron más que aquellas de septiembre y octubre, mientras que el cultivar no fue dependiente. GA disminuyó significativamente el número de yemas florales en los

tres estudios, pero el mayor efecto (49%) requirió de aplicaciones consecutivas desde julio a octubre. Los resultados indican que GA puede tener una limitada utilidad comercial para prevenir la fructificación en arándanos de arbusto alto en condiciones de campo. Sin embargo, se requiere de mayor investigación para determinar como el crecimiento vegetativo es afectado por una inhibición parcial de la floración.

Palabras clave: Inducción floral, GA_3, GA_{4+7}, ProGibb, ProVide, *Vaccinium corymbosum*.

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