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Effects of Timing of CPPU Applications on Rabbiteye Blueberries

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Abstract. **A series of field and greenhouse experiments were conducted at two locations in Georgia to determine how rabbiteye blueberries (*Vaccinium ashei* Reade) respond to different timings of application of the growth regulator [N-(2-chloro-4-pyridyl)-N′-phenylurea (CPPU)]. In all tests, a CPPU rate of 15 mg·L^{-1} was used, and timings of applications were based on days after flowering (DAF). Overall, results indicate that a positive response in fruit set or berry size can occur with applications of CPPU depending on cultivar and timing of application. As with many growth regulators, the effect can vary, and these experiments indicate that the timing of CPPU application is critical in achieving the most desirable response. Collectively, data suggest an optimum window of application of CPPU to rabbiteye blueberries is between 7 and 21 DAF with the most probable success being from an application made around 14 ± 3 DAF.**

Poor fruit set and small berry size can limit production of rabbiteye blueberries (*Vaccinium ashei* Reade) grown in the southeastern United States (Scherm et al., 2001). Some factors that affect fruit set include pollinator population during bloom, cross-pollinating activity and pollen transfer during bloom, and subfreezing temperatures during, or after, bloom, which damage ovules and ovaries thereby inhibiting seed and fruit development (Brevis and NeSmith, 2005; Brevis et al., 2006a, 2006b; NeSmith et al., 1999). Much research over the past decade has focused on use of gibberellic acid (GA_3) to overcome some of the fruit set problems in blueberries grown in the lower southeastern United States (NeSmith et al., 1995, 1999; NeSmith and Krewer, 1992, 1997a, 1997b, 1999). Although GA_3 has been successfully used to increase fruit set, there have been problems regarding late ripening and reduced fruit size associated with its use (NeSmith and Krewer, 1999; Williamson et al., 1995).

Use of the cytokinin compound N-(2-chloro-4-pyridyl)-N′-phenylurea (CPPU) has resulted in increased fruit size and fruit set in a number of fruit crops, including table grapes (*Vitis* spp.), kiwifruit (*Actinidia chinensis* Planch and *A. deliciosa*), apples [*Malus domestica* (Borkh.) Mansf.], table olives *(Olea eropaea* L.), and Japanese persimmon (*Diospyros khaki* Thunb.) (Antognozzi et al., 1993a, 1993b; Greene, 1989, 1993; Looney, 1993; Reynolds et al., 1992; Sugiyama and Yamaki, 1995). Recently, research has shown that CPPU may also be beneficial in rabbiteye blueberry production (NeSmith, 2002; NeSmith and Adair, 2004). As with other growth regulators (NeSmith, 2005; NeSmith and Krewer, 1992), the timing of CPPU application with respect to stage of plant development will likely govern its effectiveness. The objective of this research was to examine fruit set and berry weight of 'Brightwell', 'Climax', and 'Tifblue' rabbiteye blueberries under field and greenhouse conditions to determine the optimum timing for CPPU applications.

Materials and Methods

This research was conducted under both field and greenhouse conditions during 2003 at two locations in Georgia. The greenhouse experiments were conducted at The University of Georgia Griffin Campus, and the field experiments were conducted in Griffin and at The University of Georgia Blueberry Research Farm in Alapaha, GA. The following are descriptions of the different experiments.

Greenhouse experiments. Two-year-old 'Brightwell', 'Climax', and 'Tifblue' rabbiteye blueberry plants grown in pine bark in 3-L containers were used for this experiment. Plants were subjected to natural chilling (outside conditions) at Griffin, GA. Flower development of plants was monitored and dates of 50% flowering were determined. The plants were in a nursery environment with many other cultivars during the flowering period. Thus, substantial crosspollination occurred for all treatments as a result of the presence of native pollinators and domestic honeybees near the growing site. CPPU timing treatments were based on the number of days after flowering (DAF). CPPU applications were made at 0, 7, 14, 21, and 28 DAF. Two exceptions were that 'Brightwell' and 'Climax' did not receive 0 DAF treatments. For all cultivars, an open-pollinated control treatment receiving no CPPU was also established. All CPPU applications were applied at a concentration of 15 mg·L^{-1}, which was in the range known to show positive benefits in rabbiteye blueberry (NeSmith and Adair, 2004). The growth regulator applications consisted of spraying whole plants to the point of drip using a back pack sprayer. All sprays used the nonionic surfactant Ortho X-77 (Valent USA Corp., Walnut Creek, CA) at 0.25% (v/v). There were five replications (single plants) of each CPPU treatment and the open-pollinated control. After flowering stopped, all plants were moved into a greenhouse for the remainder of the experiment to prevent fruit loss from bird feeding damage. Fruit set was determined by counting the number of flowers per shoot on three shoots per plant and dividing this into the total number of fruit that remained 8 weeks later. Berry weight was determined for each fruit individually as it was harvested to obtain an overall average berry weight per plant.

Field experiments. Field experiments were conducted in Alapaha, GA, with 'Brightwell' and 'Climax' and in Griffin, GA, with 'Tifblue'. All plants were of nominal size with those at Alapaha being 3 years old and those in Griffin being 8 years old. The rows of 'Brightwell' and 'Climax' plants used were in a three-row pattern of 'Brightwell': 'Climax': 'Austin', so natural pollination conditions were excellent. In Griffin, the 'Tifblue' plants were grown in a small block with other cultivars nearby to facilitate cross-pollination. At both sites, six treatments were established. The treatments were: 1) natural pollination alone (no CPPU); 2) CPPU applied 0 DAF; 3) CPPU applied 7 DAF; 4) CPPU applied 14 DAF; 5) CPPU applied 21 DAF; and 6) CPPU applied 28 DAF. All plants were exposed to bees and other pollinators, including the CPPU-treated plants. In Alapaha, plots consisted of 20 plants in a row, and CPPU applications were made with an air-blast sprayer at a spray delivery rate of 374 L·ha^{-1}. There were four replications of each treatment on both 'Brightwell' and 'Climax' at the location. In Griffin, plots were single plants, and CPPU was applied using a backpack sprayer (plants were sprayed to the point of drip). There were five replications (single plant) of each of the CPPU timing treatments and natural pollinated controls at the Griffin site. For all treatments at both sites, CPPU was applied at a concentration of 15 mg·L^{-1} and all sprays used a nonionic surfactant at 0.25% (v/v).

For all treatments at both sites, fruit set of plants was determined by tagging three shoots per plant (in each rep) and counting the number of flowers per shoot and later counting the number of fruit. Berry weight was determined for three samples of 50 fruit per plant (for each repetition) as they ripened. In Griffin, yields were not taken from the plants; however, in Alapaha, all plots were machine-harvested three to four times to determine total yield.

For data analyses, means and SEs were calculated for treatments and cultivars in the

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individual experiments for comparison. To view an overall trend for timing of application, data for all cultivars and experiments were averaged and regression analysis was done over mean values.

Results

Greenhouse experiments. 'Brightwell', 'Climax', and 'Tifblue' plants treated with CPPU at different DAF under greenhouse conditions all showed an increase in fruit set in response to CPPU, especially for the timings of 7 and 14 DAF (Table 1). The cultivars Brightwell and Tifblue showed a declining response to CPPU after timings exceeded 14 DAF; however, 'Climax' continued to show an increase in fruit set even up to the 28 DAF treatment.

Under greenhouse conditions, berry weight response to CPPU was variable among cultivars. 'Brightwell' had a 19% to 28% increase in berry weight depending on timing of CPPU application. 'Climax' showed little or no response with respect to berry weight, except for the 14 DAF application, which decreased berry weight. Reasons for this are not clear. 'Tifblue' berry weight also showed very little response to CPPU with only a slight trend for an increase for the 21 DAF application.

Field experiments. Table 2 shows fruit set and berry weight for the three rabbiteye blueberry cultivars in response to CPPU timing under field conditions. All cultivars had an increase in fruit set in response to at least one of the CPPU applications. 'Brightwell' had increased fruit set when application was made 14 DAF. 'Climax' fruit set increased over the control for all CPPU timings with the greatest response occurring for the 21 DAF application. 'Tifblue' fruit set increased when CPPU was applied at 7 and 14 DAF, and the response tended to decline after those dates.

Berry weight response to CPPU timing under field conditions also varied for the cultivars. 'Brightwell' berry weight increased 15% to 19% when CPPU was applied 14 to 28 DAF as compared with the control. 'Climax' berry weight increased slightly in response to CPPU with the greatest increase occurring with the 21 DAF application. 'Tifblue' berry weight also increased only slightly depending on CPPU timing with the largest increase in weight occurring for applications made 7 to 14 DAF.

Yields of 'Brightwell' and 'Climax' plots at Alapaha were taken in 2003 using a mechanical harvester (Table 3). Although the data were highly variable as a result of variations in plant sizes and health in the various plots, there was a trend for yield increases depending on the cultivar and CPPU timing. 'Brightwell' yields tended to be highest in response to the 14 DAF CPPU treatment. The 0 and 7 DAF CPPU treatments for 'Brightwell' actually showed a trend of yield decline. For 'Climax', all CPPU timings tended to increase yields as compared with the control with the largest increases occurring with the 14 to 21 DAF timings. Yields were not obtained for the 'Tifblue' plots in Griffin.

Table 1. Percent fruit set and average berry weight for Brightwell, Climax, and Tifblue rabbiteye blueberries in response to time of CPPU application [days after flowering (DAF)] under greenhouse conditions in Griffin, GA, during 2003.[z]

	Cultivar		
Timing treatment	Brightwell	Climax	Tifblue
	Fruit set (%)[y]		
Control (no CPPU)	45.0 ± 5.4	58.4 ± 3.9	35.2 ± 3.1
CPPU 0 DAF	—	—	57.3 ± 3.5
CPPU 7 DAF	80.7 ± 2.6	64.7 ± 5.3	64.9 ± 3.0
CPPU 14 DAF	75.6 ± 4.4	72.6 ± 3.6	73.5 ± 2.8
CPPU 21 DAF	59.2 ± 8.4	91.1 ± 2.4	35.0 ± 2.5
CPPU 28 DAF	43.3 ± 8.4	93.1 ± 1.7	31.7 ± 3.9
	Berry weight (g)[x]		
Control (no CPPU)	1.26 ± 0.04	1.49 ± 0.07	1.37 ± 0.06
CPPU 0 DAF	—	—	1.32 ± 0.08
CPPU 7 DAF	1.62 ± 0.13	1.47 ± 0.12	1.43 ± 0.13
CPPU 14 DAF	1.50 ± 0.11	1.20 ± 0.07	1.37 ± 0.07
CPPU 21 DAF	1.55 ± 0.09	1.44 ± 0.04	1.50 ± 0.08
CPPU 28 DAF	1.51 ± 0.13	1.57 ± 0.08	1.31 ± 0.09

[z]Control plants and all CPPU plants were exposed to natural pollination. CPPU was applied at 15 $mg \cdot L^{-1}$ at each time of application.
[y]Value represents mean ± SE with n = 15.
[x]Value represents mean ± SE with n = 5.

Table 2. Percent fruit set and average berry weight for Brightwell, Climax, and Tifblue rabbiteye blueberries in response to time of CPPU application [days after flowering (DAF)] under field conditions in Alapaha, GA (Brightwell and Climax) and Griffin, GA (Tifblue) during 2003.[z]

	Cultivar		
Timing treatment	Brightwell	Climax	Tifblue
	Fruit set (%)[y]		
Control (no CPPU)	28.6 ± 3.9	27.7 ± 3.4	49.9 ± 7.1
CPPU 0 DAF	14.4 ± 2.8	52.5 ± 5.1	70.2 ± 6.8
CPPU 7 DAF	26.0 ± 3.5	54.9 ± 6.0	75.0 ± 4.7
CPPU 14 DAF	44.7 ± 5.3	54.2 ± 6.1	54.6 ± 7.1
CPPU 21 DAF	32.8 ± 3.4	70.5 ± 5.1	51.1 ± 4.0
CPPU 28 DAF	38.0 ± 4.2	46.3 ± 5.7	63.7 ± 6.2
	Berry weight (g)[x]		
Control (no CPPU)	1.41 ± 0.05	1.28 ± 0.02	1.42 ± 0.02
CPPU 0 DAF	1.49 ± 0.04	1.33 ± 0.03	1.59 ± 0.03
CPPU 7 DAF	1.46 ± 0.04	1.27 ± 0.03	1.61 ± 0.02
CPPU 14 DAF	1.61 ± 0.04	1.27 ± 0.03	1.60 ± 0.02
CPPU 21 DAF	1.63 ± 0.04	1.43 ± 0.03	1.52 ± 0.02
CPPU 28 DAF	1.68 ± 0.04	1.42 ± 0.03	1.59 ± 0.03

[z]Control plants and all CPPU plants were exposed to natural pollination. CPPU was applied at 15 $mg \cdot L^{-1}$ at each time of application.
[y]Value represents mean ± SE with n = 15 to 24.
[x]Value represents mean ± SE with n = 50 to 60.

Table 3. Machine harvested total yield of Brightwell and Climax rabbiteye blueberries in response to time of CPPU application [days after flowering (DAF)] under field conditions in Alapaha, GA, during 2003.[z]

	Cultivar	
	Brightwell	Climax
Timing treatment	Yield ($kg \cdot ha^{-1}$)[y]	
Control (no CPPU)	1545 ± 407	732 ± 160
CPPU 0 DAF	1220 ± 400	1138 ± 165
CPPU 7 DAF	1138 ± 244	976 ± 178
CPPU 14 DAF	1870 ± 102	1220 ± 325
CPPU 21 DAF	1544 ± 163	1301 ± 240
CPPU 28 DAF	1464 ± 235	1145 ± 158

[z]Control plants and all CPPU plants were exposed to natural pollination. CPPU was applied at 15 $mg \cdot L^{-1}$ at each time of application.
[y]Value represents mean ± SE with n = 4.

Discussion

Overall, the data from these field and greenhouse studies with the rabbiteye blueberry cultivars Brightwell, Climax, and Tifblue suggest that a positive response in fruit set or berry size can occur with applications of CPPU in many instances. As with many growth regulators, the effect can vary (NeSmith, 2005), and these data indicate that proper timing of CPPU application is critical in achieving the most desirable response. Figure 1 and Figure 2 depict the average response of fruit set and berry weight in

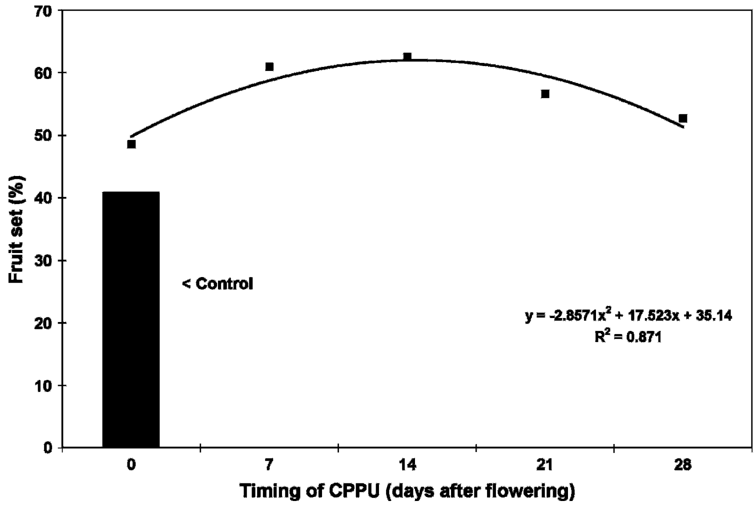

Fig. 1. Fruit set response to CPPU applications made different days after flowering. These are data averaged over all greenhouse and field experiments for all cultivars. Note vertical bar represents a control treatment that received no CPPU. Concentration of CPPU for all applications was 15 $mg \cdot L^{-1}$.

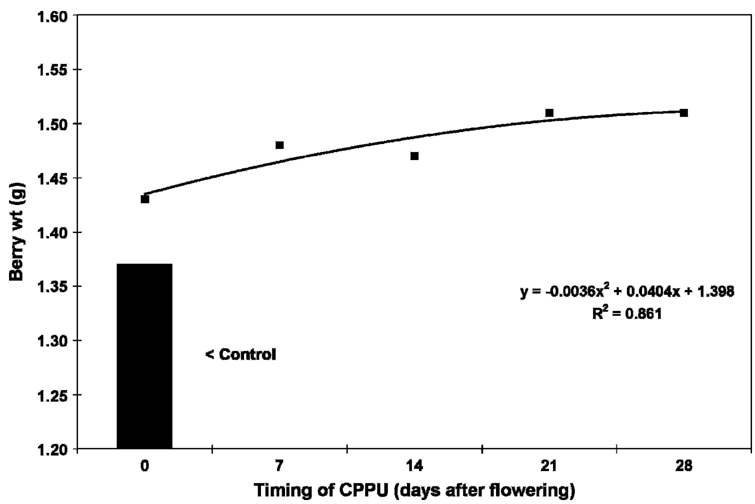

Fig. 2. Berry weight response to CPPU applications made different days after flowering. These are data averaged overall greenhouse and field experiments for all cultivars. Note vertical bar represents a control treatment that received no CPPU. Concentration of CPPU for all applications was 15 $mg \cdot L^{-1}$.

response to CPPU timing, respectively, for all cultivars and experiments. The vertical bar in each graph represents the open-pollinated control treatments that received no CPPU. These analyses were done simply to provide an overall view of the trend of response to CPPU application timing treatments. Collectively, the data suggest an optimum window of application of CPPU to rabbiteye blueberries is between 7 and 21 DAF with the most probable success being from an application made around 14 ± 3 DAF. It appears that CPPU applications made during actual bloom time (0 DAF) provide little benefit and may even possibly be harmful as a result of burning of blooms. Recent research with southern highbush blueberries (*V. corymbosum* L.) have indicated that burning of blooms and other plant tissue can occur when using CPPU (Williamson and NeSmith, 2007). Also, in the current studies, those applications late in the season (past 21 DAF) were of little or no use in most instances.

It appears that CPPU may have potential for use in rabbiteye blueberry production. It may be especially useful under conditions of poor fruit set. Additional research is needed to further examine the growth regulator's value in blueberries, including use in instances after freeze damage. There may be benefits for postfreeze rescue, similar to those found for gibberellic acid (NeSmith et al., 1995, 1999). However, caution is advised in using the growth regulator as a result of previously reported damage that can occur. Any use should be on a trial basis only until more is known about how different cultivars react to the product.

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