

## Repeated applications of CPPU on highbush blueberry cv. Duke increase yield and enhance fruit quality at harvest and during postharvest

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Applications of N-(2-chloro-4-pyridyl)-N'-phenylurea (CPPU) can increase blueberry (*Vaccinium corymbosum* L.) yield and fruit size, but their impact on postharvest is unknown. We studied repeated CPPU applications effects on yield and quality (harvest, postharvest), over 2 yr on mature 'Duke' plants in South-Central Chile. The first year, 5 or 10 mL L<sup>-1</sup> CPPU was applied at 3, 10, and/or 17 d after full bloom (DAFB) plus a non-sprayed control. The second year, 5 or 10 mL L<sup>-1</sup> CPPU were sprayed 10 and 17 DAFB plus a control. The first year, only 10 mL L<sup>-1</sup> CPPU sprayed 3+17 DAFB increased yield (32.5% > control); 10 mL L<sup>-1</sup> CPPU applied 10 or 3+17 DAFB had highest fruit diameter; and 10 mL L<sup>-1</sup> CPPU at 17 DAFB or at 3+10+17 DAFB had highest soluble solids. Overall, 10 mL L<sup>-1</sup> CPPU applied 3+17 DAFB, was the best treatment for year one, since it increased fruit yield and diameter, while soluble solids and postharvest weight loss were similar to control. The second year, 10 mL L<sup>-1</sup> CPPU reduced fruit coloration (blue color coverage index: BCCI) and soluble solids, but not firmness at harvest. This rate increased berry weight (24.2%) and fruit wax (59% > wax coverage index: WCI) at harvest. Harvest and postharvest WCI increased consistently as CPPU rate increased. CPPU reduced fruit rotting (15% at 45+5 evaluation). During storage, CPPU-treated-fruit had a slower decrease in firmness (30.5% < control at 30+1), but no difference at 30+5. CPPU-treated-fruit usually had higher post harvest soluble solids. Ten mL L<sup>-1</sup> CPPU retarded color evolution at harvest and at 30+1, but not at 30+5, 40+1 or 40+5.

**Key words:** Cytokinin, firmness, fruit rotting, fruit wax, growth regulators, long-term cold storage, *Vaccinium corymbosum*.

### INTRODUCTION

The worldwide area planted with blueberries (*Vaccinium corymbosum* L.) has greatly increased in the last two decades (Brazelton, 2009). This improved availability of fruit has increased the demand for higher quality fruit with consumer preference being mainly decided by visual, textural, and flavor-related quality parameters. Visual quality includes fruit color and size. In blueberries it has been shown that fruit size is a better indicator of sensory visual quality than the intensity of color (Saftner et al., 2008). In addition, large fruit are easier and cheaper to harvest (Strik et al., 2003). Fruit size varies among cultivars and is also influenced by management practices (irrigation, pruning, and growth regulators).

Application of growth regulators can alter fruit size expansion through flower bud inhibition, flower and/or fruit thinning, or increased fruit size. Currently there are no effective thinners for blueberries (Retamales and Hancock, 2012). Gibberellic acid (GA<sub>3</sub>) has been shown to significantly inhibit flower bud formation and increase

fruit size (Retamales et al., 2000; Black and Ehlenfeldt, 2007), but the industry has shown little interest in using this technology since application is near the harvest time of the previous season and this makes difficult to establish the final fruit count. Previous trials in other fruit crops (kiwifruit, apples, table grapes, olives, and persimmon), have demonstrated that a synthetic cytokinin known as CPPU (N-[2-chloro-4-pyridyl]-N'-phenylurea), markedly enhances fruit size when applied near bloom (Greene, 1989; Reynolds et al., 1992; Antognozzi et al., 1993a; 1993b; Sugiyama and Yamaki, 1995). CPPU applications have been tested extensively on rabbiteye blueberries (*Vaccinium virgatum* Aiton) both in greenhouse and fields in different seasons, varieties and rate/timing combinations (Merino et al., 2002; NeSmith, 2002; NeSmith and Adair, 2004; NeSmith, 2005; Serri and Hepp, 2006; Williamson and NeSmith, 2007; NeSmith, 2008). In rabbiteye varieties ('Brightwell', 'Climax' 'Bluebelle', 'Powderblue', 'Premier', and 'Tifblue') CPPU application increased berry size (5-25%) and fruit set (20%) (NeSmith and Adair, 2004; NeSmith, 2005; 2008). The effect on fruit set was greater in poor fruit set situations, such as low bee activity or when little overlap in bloom date occurred among varieties (NeSmith, 2008).

Only single CPPU application trials have been reported on northern highbush blueberries. The optimum window of application of CPPU in blueberries was established to

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be 7 to 21 d after 50% bloom, with the highest success being from an application made around  $14 \pm 3$  d after 50% bloom (NeSmith, 2008). In various blueberry producing areas, the bloom extends for a long period (Lyrene and Muñoz, 1997; Retamales and Hancock, 2012). The effectiveness of growth regulators depends markedly on the phenological phase of the reproductive organs (Stover and Greene, 2005), which implies that the performance of CPPU application in blueberries could be improved by repeated applications.

Despite the large number of studies on the effect of CPPU on blueberries, there have been no reports on the impact of CPPU applications on the postharvest life of the fruit. Given the recent expansion in international trade of blueberries it is important that management practices do not reduce the postharvest life of the fruit. This is particularly important in the case of Chilean blueberries where 30-60 d postharvest are required considering that nearly 95% of the fruit is shipped by boat to distant markets (Moggia et al., 2009).

In this context, the objective of this research was to study the effect of repeated CPPU sprays on blueberry yields and fruit quality both at harvest and after prolonged cold storage.

## MATERIALS AND METHODS

### Plant material, climatic conditions and CPPU applications

Trials were done with plants from a commercial planting located in Linares ( $35^{\circ}57'56''$  S;  $71^{\circ}40'73''$  W), Chile. Plants of similar size, fruit load and condition that had been established in 2003 at  $2.8 \times 0.8$  m were chosen. Plants had no signs or symptoms of diseases or pests. They were drip irrigated as required with one line of emitters on each side of the plant; emitters were distanced at 40 cm within the line. Plants were planted on 20 cm high and 80 cm

wide ridges. Blueberry fields were managed according to standards used for fresh export of blueberries.

In both seasons there was no rain within 72 h of CPPU applications or during the harvest period. Average minimum temperatures during the season ranged from 2 °C (bloom period) to 12 °C (harvest period). Average maximum temperatures varied from 15 °C (bloom period) to 26 °C (harvest season).

Sprays were done with a fan nozzle using a 15 L capacity knapsack sprayer (SOLO, Santiago, Chile). In each season, sprayer calibration was done before application to ensure adequate coverage.

### Experimental conditions for first season: 2008-2009

The treatments applied were the combination of two CPPU (CPPU 0.1 SL, ANASAC, Santiago, Chile) doses (5 or 10 mL ai L<sup>-1</sup>), and different number of applications (1, 2, or 3) during the season, applied at 3, 10, and/or 17 DAFB. Thus, this set of trials considered 15 treatments (14 with CPPU + 1 control with no application; Table 1).

Six hand harvests were done every 5-7 d. Fruit were hand harvested at the peak of the season when they had at least 50% of the fruit surface covered with blue color. Total weight of harvested fruit was determined at each harvest with an electronic balance (LSV, Veto, Santiago, Chile).

Soluble solids (Atago Digital refractometer, Pocket PAL-1; Tokyo, Japan) and diameter (Bull Tools, China) were measured in harvests 2 and 4, on a total of 40 fruit chosen at random per replicate and per treatment.

To estimate weight loss in storage, a sample of 100 fruit per replicate and treatment were randomly collected in harvest 3. These fruit were stored within 3 h at 4 °C and 80-85% RH for 25 d. The initial and final weight of those fruit was measured with the electronic balance.

### Experimental conditions for second season: 2009-2010

Three treatments were included for this season: CPPU

**Table 1. Effects of the application of CPPU in different dosages, timing, and number of applications on yield, average fruit diameter (five harvests), and soluble solids (two harvests) of mature 'Duke' plants. Treatment: Application dates = 3, 10, and/or 17 d after full bloom (DAFB), and doses = 5 or 10 mL L<sup>-1</sup>. First season.**

Date (DAFB)/ dose (mL L <sup>-1</sup> )	Yield per plant	Difference from control	Fruit diameter	Difference from control	Soluble solids	Difference from control
	kg	%	mm	%	°Brix	%
Control	5.75bcd	0.0	13.2fg	0.0	11.0cd	0.0
3/5	6.17abc	7.5	13.2fg	0.2	10.8cde	-1.6
10/5	6.87abc	19.6	13.1fg	-0.2	10.3ef	-5.7
17/5	7.08ab	23.3	13.3ef	1.1	10.9cd	-1.0
3, 10/5	6.98ab	21.5	13.5cde	2.5	10.7de	-2.7
3, 17/5	5.95abc	3.5	13.6bcd	3.2	11.2bc	2.3
10, 17/5	5.16cd	-10.2	13.4cdef	1.6	10.7cde	-2.0
3, 10, 17/5	6.45abc	12.3	13.0gh	-1.4	10.7de	-2.4
3/10	5.72bcd	-0.4	12.9h	-2.2	10.8cde	-1.5
10/10	6.12abc	6.5	14.2a	7.5	10.8cd	-1.1
17/10	5.34bcd	-7.0	13.3def	1.2	11.8a	7.3
3, 10/10	5.85bcd	1.8	13.6bc	3.3	11.1bcd	1.3
3, 17/10	7.61a	32.5	13.8b	4.7	11.2bc	2.2
10, 17/10	6.12abc	6.5	13.2efg	0.4	9.8f	-10.1
3, 10, 17/10	4.18d	-27.3	12.8h	-3.2	11.5ab	5.0
Significance	**	-	***	-	***	-

Means followed by the same letter, within columns, are not significantly different according to Duncan's multiple range test ( $P \leq 0.05$ ).

\*\*, \*\*\*: significant at  $P \leq 0.01$  or  $0.001$ , respectively. A minus sign before each figure indicates a negative effect.

applied at 10 and 17 DAFB at either 10 or 5 mL L<sup>-1</sup>, plus a control (no application).

Fruit were hand harvested at the peak of the season. Fruit weight was determined with electronic balance. Evaluations were done at harvest, after 30 d cold storage (1 °C) + 1 d at room temperature (30+1), as well as 30+5, 45+1, and 45+5. On each evaluation, 200 fruit per replicate were used to determine wax coverage and color. A wax coverage index (WCI) was calculated similarly to the superficial apple scald index reported by Lurie et al. (1989). For that three categories were established based on visual assessment of the fruit: Cat. 1 (67-100% wax coverage), Cat. 2 (34-66 wax coverage), and Cat. 3 (0-33 wax coverage). Then, the percentage of fruit in each category was multiplied by a factor which corresponded to 3, 2, and 1, respectively. The formula used to calculate WCI was:

$$(\% \text{fruit Cat.1} \times 3) + (\% \text{fruit Cat.2} \times 2) + (\% \text{fruit Cat.3} \times 1) / 3$$

Additionally, on each evaluation, blue color coverage (BCC) was rated as: range 1 (90-100% BCC), range 2 (80-89% BCC), range 3 (70-79% BCC), range 4 (60-69% BCC), or range 5 (50-59% BCC). Then, as done previously for WCI, a BCC index (BBCI) was calculated considering the proportion of fruit in each range (% fruit R1, to % fruit R5), and multiplied by a factor which corresponded to 5, 4, 3, 2 or 1, for R1 to R5, respectively. Thus, the formula used to calculate BBCI was as follows:

$$[(\% \text{fruit R1} \times 5) + (\% \text{fruit R2} \times 4) + (\% \text{fruit R3} \times 3) + (\% \text{fruit R4} \times 2) + (\% \text{fruit R5} \times 1)] / 5$$

Firmness was measured with a FirmTech 2 (BioWorks, Wamego, Kansas, USA) on 60 fruit per replicate. As reported by Ehlenfeldt and Martin (2002) and Saftner et al. (2008), the equipment was set up with maximum and minimum compression forces of 200 g (1.96 N) and 15 g (0.15 N), respectively; also, the speed of the piston was configured at 6 mm s<sup>-1</sup>. Soluble solids were assessed using 12 samples per replicate with a digital refractometer (Atago Model Pocket PAL-1, Tokyo, Japan). Rotting was evaluated visually on 200 fruit per replicate; values were expressed as a percentage.

### Experimental design, set up and data analysis

A completely randomized design was used with four replicates of four plants each. The two plants in the center of each experimental unit were used for fruit sampling.

Data were subjected to ANOVA. For variables that were significant, mean separation ( $p \leq 0.05$ ) was done with Duncan's (in season 1) and LSD (in season 2). All analyses were performed with Statgraphics Centurion statistical software (Statpoint, Warrenton, Virginia, USA).

## RESULTS

### First season: 2008-2009

Only the application of 10 mL L<sup>-1</sup> CPPU when done at both 3 and 17 DAFB significantly increased fruit yield

per plant with respect to control (32.5% greater; Table 1). As compared to the control, fruit diameter was both negatively (- sign) and positively affected by CPPU treatments, with the highest positive impact with 10 mL L<sup>-1</sup> CPPU applied 10 DAFB (7.5% > control) and 10 mL L<sup>-1</sup> CPPU applied 3 and 17 DAFB (4.7% > control). The majority of treatments (9 out of 14) had negative impacts on soluble solids, with the highest significant and positive effect over the control being shown after applying 10 mL L<sup>-1</sup> CPPU at 17 DAFB (7.3% > control) and the application of 10 mL L<sup>-1</sup> CPPU sprayed 3, 10, and 17 DAFB (5% > control) (Table 1). The triple application of CPPU had the lowest yield, although not significantly lower than control (27.5% < control); however, fruit diameter for this treatment was significantly lower than control (3.2% < control). There were no differences among treatments in weight loss after 25 d at 4 °C; values ranged between 6.7% for control fruit and 5.0% for 10 mL L<sup>-1</sup> CPPU applied at 10 and 17 DAFB (data not shown). Overall, the treatment of 10 mL L<sup>-1</sup> applied both at 3 and 17 DAFB to 'Duke' appeared as the most promising, since it increased both fruit yield and diameter, and had soluble solids similar to the control fruit (Table 1).

### Second season: 2009-2010

**Evaluations at harvest.** As compared to control the most evident effect for CPPU at high rate (10 mL L<sup>-1</sup>) were increased berry weight (24.3%; Table 2) and fruit wax coverage (Table 3; Figure 1). CPPU at 10 mL L<sup>-1</sup> reduced both fruit color (Table 4) and soluble solids at harvest (data not shown). Fruit firmness at harvest was not affected by treatments (Table 2).

**Table 2. Effect of dose of CPPU (5 or 10 mL L<sup>-1</sup>) applied at 10 and 17 d after full bloom on 'Duke' blueberry plants in terms of fruit weight and firmness at harvest (0) or after 30 d cold storage plus 1 or 5 d at room temperature (25 °C) (30+1 or 30+5). Second season.**

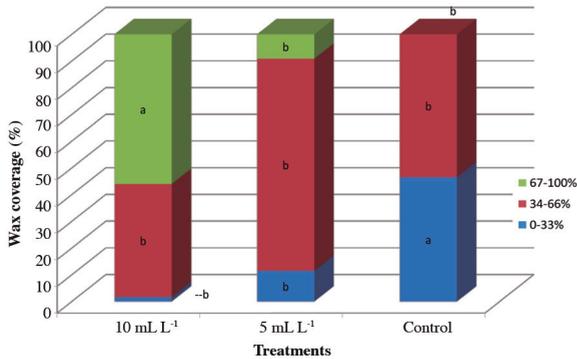
Dose	Fruit firmness			
	0 d	0 d	30+1 d	30+5 d
mL L <sup>-1</sup>	g	g	g mm <sup>-1</sup>	g mm <sup>-1</sup>
10	2.20a	204.5	151.9a	89.5a
5	1.87b	206.5	143.2a	77.9b
Control	1.77c	212.0	115.5b	83.5ab
p-value	0.00001	0.916	0.028	0.007

For each evaluation date, means with similar letters do not differ significantly according to LSD test ( $p \leq 0.05$ ).

**Table 3. Effect of CPPU dose (5 or 10 mL L<sup>-1</sup>) applied at 10 and 17 d after full bloom on wax coverage index (WCI) of 'Duke' blueberry fruits. WCI values near 100 represent higher proportion of fruit with 67-100% wax coverage. Measurements done at harvest (0) or after 30 or 45 d cold storage (1 °C) plus 1 or 5 d at room temperature (25 °C) (30+1, 30+5, 40+1, 40+5). Second season.**

Dose	WCI at various dates (d)				
	0	30 + 1	30 + 5	45 + 1	45 + 5
mL L <sup>-1</sup>					
10	81.6a	84.5a	84.7a	83.8a	83.0a
5	66.7b	66.6b	65.4b	67.1b	66.5b
Control	51.2c	52.4c	50.9c	52.3c	50.5c
p-value	0.00006	0.000001	0.000001	0.000001	0.000001

For each evaluation date, means with similar letters do not differ significantly according to LSD test ( $p \leq 0.001$ ).



Similar letters for each category indicate they do not differ significantly according to LSD test ( $p \leq 0.05$ ).

**Figure 1.** Effect of CPPU sprays (5 or 10 mL L<sup>-1</sup>) on the proportion of 'Duke' fruit in three wax coverage categories at harvest (Cat. 1 = 67-100%; Cat. 2 = 34-66%; Cat. 3 = 0-33%). Second season.

**Table 4.** Effect of CPPU dose (5 or 10 mL L<sup>-1</sup>) applied at 10 and 17 d on blue color coverage index (BCCI) on 'Duke' blueberry fruits. BCCI values near 100 represent higher proportion of fruit with 90-100% bloom coverage. Measurements done at harvest (0) or after 30 or 45 d cold storage (1 °C) plus 1 or 5 d at room temperature (25 °C) (30+1, 30+5, 40+1, 40+5). Second season.

Dose	Color index at various dates (d)				
mL L <sup>-1</sup>	0	30 + 1	30 + 5	45 + 1	45 + 5
10	93.4b	97.6b	97.0	96.4	98.3
5	97.5a	99.4a	99.3	98.8	98.2
Control	98.3a	98.4ab	99.3	99.2	99.1
p-value	0.0001	0.03	0.07	0.07	0.3

For each evaluation date, means with similar letters do not differ significantly according to LSD test ( $p \leq 0.001$ ).

**Postharvest evaluations.** Wax coverage index increased throughout storage on CPPU-treated fruit. The magnitude of the effect was directly related to CPPU concentration; thus, WCI was highest with 10 mL L<sup>-1</sup>, intermediate with 5 mL L<sup>-1</sup>, and lowest for the control (Table 3).

Fruit color development was significantly reduced by 10 mL L<sup>-1</sup> CPPU at harvest, and this trend was again observed at the 30+1 evaluation, but no differences among treatments were found at 30+5, 40+1 and 40+5 (Table 4).

The application of CPPU significantly reduced fruit rotting (Table 5). No rotting was detected after 30+1 d; however in the other postharvest evaluations (30+5, 45+1, 45+5), rotting was significantly and consistently reduced by CPPU treatments, with lowest decay observed at the highest CPPU rate (Table 5). In comparison to control, fruits treated with CPPU had reduced firmness loss at 30+1 (< 30.5%) but not at 30+5 (Table 2).

**Table 5.** Effect of dose of CPPU (5 or 10 mL L<sup>-1</sup>) applied at 10 and 17 d after full bloom on 'Duke' blueberries fruit rotting (%) after 30 or 45 d cold storage plus 1 or 5 d at room temperature (25 °C) (30+1 or 30+5). Second season.

Dose	Rotting (%) at various dates (d)			
mL L <sup>-1</sup>	30 + 1	30 + 5	45 + 1	45 + 5
10	0	1.5a	6.9a	10.3a
5	0	3.9b	11.1b	13.9b
Control	0	8.6c	20.3c	25.9c
p-value		0.0002	0.0001	0.0001

For each evaluation date, means with similar letters do not differ significantly according to LSD test ( $p \leq 0.05$ ).

## Yield and fruit size at harvest

In our trials, the only treatment that significantly increased fruit yield per plant with respect to control (32.5% greater) was 10 mL L<sup>-1</sup> CPPU when applied on two occasions, at 3 and 17 DAFB. Serri and Hepp (2006) applied 10 mL L<sup>-1</sup> CPPU once between 10 and 15 d after 50% bloom to 'Elliott' and 'Lateblue' northern highbush blueberries in Chile; they obtained a 20% and 50% increase in fruit weight, respectively. Within each variety, they reported that the number of fruits were similar in the CPPU and control treatments (no CPPU applied), which implies that yield increases for both varieties in response to CPPU were mainly due to improvements in fruit weight. In our trial for the first season, the largest increases in fruit diameter were obtained with the application of 10 mL L<sup>-1</sup> at 10 DAFB and also 10 mL L<sup>-1</sup> at 3 and 17 DAFB, with 7.5% and 4.7% greater diameter than control fruit. Our results for the second season confirmed these results as fruit weight was increased 24% by 10 mL L<sup>-1</sup> CPPU applied at 10 and 17 DAFB.

## Fruit wax coverage

It would be expected that increases in fruit size due to CPPU applications would decrease wax deposits as fruit expands. On the other hand, Williamson and NeSmith (2007) demonstrated that CPPU increases fruit size through cell expansion and division. This increase in cell number could be related to greater wax deposition on the fruit surface, as it has been reported that epidermal cells are linked to lipid synthesis (Post-Beittenmiller, 1996). In our trials, WCI was higher in fruit that received CPPU sprays and this was related to CPPU concentrate.

## Fruit quality at harvest

A slight reduction in the accumulation of soluble solids and development of color was detected at harvest in CPPU-treated-fruit (< 1.5%). CPPU-treated-fruit showed greater soluble solids in postharvest. Perkins-Veazie et al. (1995) found that soluble solids did not differ greatly among clones and between fresh fruit and that stored for 21 d at 5 °C plus 1 d at 20 °C. In part, the slower build up of soluble solids and color evolution could be due to the delay in ripening which is usually observed after CPPU sprays (Williamson and NeSmith, 2007).

## Postharvest fruit quality

To our knowledge no reports have been published on the effect of CPPU on decay of blueberries. Our results show that CPPU significantly reduced decay in postharvest (up to 15% with highest dose). Even though it was shown many years ago that the main point for infections to enter into blueberry fruit was the stem-end scar (Cappellini and Ceponis, 1977), it is possible that this increased wax coverage could provide CPPU-treated-fruit with a

physical barrier to drastically reduce pathogen infection (Duan et al., 2008). This greater wax coverage, caused by CPPU treatments, could also explain why fruit with greater diameter had similar fruit weight loss in storage during the first season.

Although in our study fruit firmness dropped during cold storage and shelf life, the effect should not be attributed to CPPU, since when firmness levels after shelf life (30+5) were compared with those at harvest, the reduction in firmness for control fruit (61%) was similar to that of 5 mL L<sup>-1</sup> CPPU-treated-fruit (62%) and higher than 10 mL L<sup>-1</sup> fruit (41%). This loss in firmness during storage of highbush blueberries was observed previously by Chiabrando et al. (2009). Firmness was slightly lower in our trials than those reported previously for 'Duke' (Yang et al., 2009), although they stored fruit only up to 3 wk. Also, even though these authors used the same firmness measuring equipment that we employed in our trials (FirmTech 2), they did not report the settings in their device, and this technical feature has shown to influence firmness readings (Ehlenfeldt and Martin, 2002).

## CONCLUSIONS

In conclusion these trials provide evidence that depending on rate, dose and number of sprays, repeated CPPU applications can increase yield and diameter of 'Duke' highbush blueberry. The beneficial effects of this growth regulator were extended to the postharvest period, with greater wax deposition and, probably as a consequence, a significant and consistent reduction in both fruit rotting and weight loss. Even though positive results were obtained over two seasons, these trials need to be validated in other highbush blueberry cultivars.

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