CPPU Influences Fruit Quality, Fruit Set, Return Bloom, and Preharvest Drop of Apples

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Abstract. CPPU was applied to whole spur 'Delicious' apple (*Malus domestica* Borkh.) trees in central Washington at 0,6.25,12.5,25, or 50 mg-liter⁻¹ at full bloom (FB) or FB plus 2 weeks. At both application times, the flesh firmness of treated fruit linearly increased with increasing concentration. CPPU applied at 0,5,10,15, or 20 mg-liter⁻¹ to spur 'Delicious' trees in Massachusetts at king bloom resulted in a linear increase in flesh firmness at harvest and following 28 weeks in air storage at 0C. CPPU did not affect the incidence of senescent breakdown, decay, or cork spot. Fruit length : diameter (L/D) ratios generally increased at all doses. Fruit weight was not influenced at either location. All CPPU concentrations reduced return bloom on 'Delicious' apples in Massachusetts in 1989. Of the 10, 20, or 40 mg-liter⁻¹ treatments for 'Empire' apples, only CPPU at 40 mg-liter⁻¹ reduced return bloom. CPPU applied to 'Empire' apples in Massachusetts did not effect fruit set, soluble solids concentration, L/D, or firmness; however, fruit weight increased linearly with concentration. CPPU applied at 100 mg-liter⁻¹ retarded preharvest fruit drop of 'Early McIntosh' in Massachusetts for ≈ 7 days but was not as effective as NAA at 20 mg-liter⁻¹. In a larger semicommercial trial, 'Delicious' fruit treated with CPPU at 5,10, or 15 mg-liter⁻¹ at FB, petal fall (PF), or PF plus 1 week, respectively, were harvested and graded over a commercial packing line. Malformities caused by CPPU at the highest doses reduced packout, although all CPPU application rates reduced the percent fruit culled due to poor color. CPPU increased packed fruit size, since the size of fruit (64 mm in diameter) in the >150-fruit/box size decreased, while the size of fruit (72 mm in diameter) in the 100- and 130-fruit/box sizes increased. Treated fruit stored for 7 months at 1C were firmer than nontreated controls. Chemical names used: N-(2-chloro-4-pyridyl)-N'-phenylurea (CPPU); 1 naphthalene-acetic acid (NAA).

Diphenylurea, a synthetic cytokinin, has shown considerable cytokinin-like activity (Shantz and Steward, 1955). Bruce et al. (1965) showed that substituting diphenylurea for zeatin (a true cytokinin) resulted in compounds with a wide spectrum of activity similar to that of zeatin compounds. Several substituted phenylurea compounds, including CPPU (also known as fenclopyr, KT-30, 4-PU, and CN-11-3 183; SKW Trostberg AG, Trostberg, Germany), have been shown to have a high cytokinin activity by the tobacco callus proliferation assay (Okamoto et al., 1981; Takahashi et al., 1978; Thomas and Katterman, 1986). Kurosaki et al. (1981) showed that CPPU has

a common active site with purine cytokinins such as zeatin. This result suggests that CPPU reacts using mechanisms similar to those used by true cytokinins, even though CPPU compounds seem to have few structural features in common with true cytokinin compounds.

Cytokinins have been used together with gibberellins to influence fruit shape in apples by increasing the length : diameter (L/D) ratio (Martin et al., 1970; Williams and Stahly, 1969). More recently, CPPU increased fruit size of apples, grapes (*Vitis vinifera* L.), and kiwifruit [*Actinidia deliciosa* (A. Chev.) C.F. Liang et R. Ferguson] (Nickel, 1985, 1986) and increased L/D of 'Delicious' (Curry and Williams, 1987), 'Empire' (Greene and Curry, 1989), and 'Mutsu' and 'Tsugaru' (Ogata et al., 1989) apples. Greene (1989) found that CPPU at 10 or 100 mg-liter⁻¹ reduced 'McIntosh' fruit set and return bloom but increased size and firmness.

The purpose of this study was to examine further the effects of CPPU on fruit size, shape, quality, and storage potential, and to evaluate its influence on fruit abscission and return bloom.

Fruit quality of 'Oregon Spur Delicious' in

Washington. The experimental site was a uniform block of 9-year-old 'Oregon Spur Delicious'/MM.111 trees located in central Washington. Trees were planted in a light sandy loam soil and irrigated by a permanent set of under-tree sprinklers. At the balloon stage of flower development, 66 trees were selected and randomly assigned to one of seven chemical treatments to be applied at full bloom (FB) or FB plus 2 weeks for a total of 14 treatments. Included in each treatment was a nonionic surfactant, Regulaid (Kalo Laboratories, Kansas City, MO.), at 0.125%. The treatments were (in mg-liter⁻¹) as follows: 1) nontreated, Regulaid only; 2) CPPU, 6.25; 3) CPPU, 12.5; 4) CPPU, 25; 5) CPPU, 50; 6) CPPU, 12.5, plus gibberellic acid (GA₃), 12.5; and 7) 6-benzyladenine (BA) plus GA₃, [Promalin (Abbott Laboratories, North Chicago, Ill.)], 25. Application was stopped before the drip point to simulate a spray volume of ≈ 306 liters-ha⁻¹. There were three whole-tree replications per treatment. The first treatments were applied on 5 May 1986. At commercial harvest in September, 40 fruit from each tree were harvested, beginning at the end of the limbs selected from the north side of the tree. In the laboratory, all fruit were weighed and L/D was measured. Flesh firmness was determined on opposite sides of each apple using an electronic penetrometer with a 10-mm probe (model LPT-1; Lake City Electronics, Kelowna, B.C.). Soluble solids concentration (SSC) was determined for each apple using a hand refractometer, and total acidity was quantified by titrating a 10-ml juice sample to pH 8.2.

Effect of CPPU on spur 'Delicious'/M.26 in Massachusetts. A block of 8-year-old spur 'Delicious'/M.26 at the Univ. of Massachusetts Horticultural Research Center (UMHRC), Belchertown, was grouped into six uniform blocks of six trees each. When king blossoms opened, one tree in each block was treated to the drip point with CPPU at 0,5,10, 15, or 20 mg-liter⁻¹ plus 0.05% Buffer-X. One tree in each block was sprayed with Promalin at 25 mg-liter⁻¹. Treatments were replicated six times as single-tree treatments. All trees were thinned with NAA at 3 mg-liter⁻¹ plus 1-naphthalenyl methylcarbamate (carbaryl) at 600 mg-liter⁻¹. At the commercial harvest date, 30 fruit were selected at random from the periphery of the tree. All harvested fruit were weighed, L/D was measured, and the seeds were counted. Ten fruit representative of a sample were selected, and flesh firmness was determined on two sides of each fruit using an Effegi penetrometer (Effegi, Alfonsine, Italy). SSC was measured in juice collected from the pressure test using a hand refractometer. Twenty kilograms of fruit per replication also were placed in air storage at 0C for 28 weeks. When the fruit were removed from storage, flesh firmness was determined as described for harvest evaluation. Fruit were allowed to remain at 20C for 21 days, after which 60 fruit were evaluated for storage disorders. The following spring, two ≈ 3.8 -cm-diameter limbs on each tree were tagged. When blossoms were in the pink stage, all blossom clusters on tagged limbs were counted.

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Effect of CPPU on 'Empire' apples in Massachusetts. Five uniform limbs on each of seven mature 'Empire'/MM.106 trees at UMHRC were tagged before bloom and all blossom clusters counted. On 4 June, 17 days after FB, 0.05% Buffer-X plus CPPU was applied to the drip point at 0, 10, 20, or 40 mg-liter⁻¹ to one limb on each tree. At the end of June drop, fruit on the tagged limbs were counted. At the commercial harvest, 40 apples were harvested from each limb. Fruit weight, shape, and quality were determined as described for spur 'Delicious' in Massachusetts.

Effect of CPPU on preharvest drop of 'Early McIntosh' in Massachusetts. Mature 'Early McIntosh'/M.7 growing at UMHRC were used in this study. Four limbs, 3.8 to 4.8 cm in diameter, per tree were selected, tagged, and all fruit counted on 1 Aug. On 2 Aug., two limbs per tree were sprayed to the drip point with CPPU at 50 or 100 mg-liter⁻¹, and a third limb was sprayed with NAA at 20mg-liter⁻¹. All treatments included 0.05% Buffer-X. The fourth limb on each tree was not sprayed and served as the control. All fruit persisting through the same season on the tagged limbs were counted periodically, and the percent drop calculated was based on the number of fruit present on 1 Aug.

Commercial grading of 'Hi-Early Delicious' treated with CPPU in Washington. Blocks of 0.4 ha were selected from a uniform 16-ha block of 20-year-old 'Hi-Early Delicious'/seedling trees in central Washington. The soil was a sandy loam, and trees were irrigated with overhead sprinklers. Trees were treated in Spring 1989 and treatments consisted of the following: 1) nontreated control, Regulaid only; 2) Promalin at 25 mg-liter⁻¹ applied at FB; 3) CPPU at 5 mg-liter⁻¹ applied at FB; 4) CPPU at 10 mg-liter⁻¹ applied at petal fall (PF); and 5) CPPU at 15 mg-liter⁻¹ applied at PF plus 1 week. All treatments were applied to 0.4 ha of fruit with a commercial airblast sprayer. Treatments 2 and 3 were applied at a spray volume of ≈ 153 liters-ha⁻¹, and treatments 4 and 5 at 306 liters-ha⁻¹ to compensate for increased foliage in the canopy. Treatments were applied at ≈ 0600 HR in dry weather with ambient temperature between 6 and 10C. All other commercial practices were the same for the whole block. The fruit were picked at normal commercial harvest and the bins tagged according to treatment. Fruit were placed in air storage at 0C until they were run through a commercial packing line on 16 Dec. 1989. Cull types were determined by examining 100 fruit from the cull bin after fruit from each treatment were packed. Also, one box (20 kg) of size 100 fruit (72 mm in diameter) from each of the CPPU treatments was kept in air storage at 0C. In April, 80 fruit per treatment were placed in a room at 20C for 7 days before firmness testing, as previously described.

In Spring 1990, 20 limbs were selected randomly from trees in each block, their circumferences measured, and all blossom clusters counted.

Data from all replicated experiments were analyzed as a one-way analysis of variance in a randomized complete-block or completely

randomized design. Means were separated by regression using SAS's General Linear Model procedure (SAS Institute, 1985) or by Duncan's multiple range test.

The large semicommercial trial was not a replicated experiment, but was designed to simulate commercial treatments. We fully realize the shortcomings of the data gathered from this endeavor and the interpretations derived therefrom. However, the data were included in an effort to substantiate results from the smaller replicated studies.

Fruit quality of 'Oregon Spur Delicious' in Washington. CPPU applied at FB or 2 weeks later did not affect fruit weight, SSC, or fruit acidity (Table 1). The optimum CPPU concentration for increasing fruit L/D was 12.5 to 25.0 mg-liter⁻¹. At 50 mg-liter⁻¹, CPPU may have increased fruit diameter more than fruit length (data not shown). This difference resulted in a significant quadratic regression. Flesh firmness increased linearly with concentrations at both application times. Adding GA₄₊₇ to CPPU had little influence other than slightly reducing titratable acidity compared with that of fruit treated with CPPU alone at FB plus 2 weeks.

Effect of CPPU on spur 'Delicious'/M.26 in Massachusetts. CPPU applied to 'Delicious' in Massachusetts did not affect fruit weight, SSC, or seed count (data not shown). L/D increased in all treatments compared with the

control, and flesh firmness at harvest linearly increased with increasing concentrations. This increase was similar to that which occurred in trials in Washington (Table 2). Although Promalin increased L/D, it was not as effective as CPPU. Following storage, CPPU-treated fruit were firmer than nontreated controls (Table 3). Flesh firmness of Promalin-treated fruit was similar to that of control fruit at harvest and following storage (Tables 2 and 3). The only influence of treatment on storage disorders was a slight increase in scald caused by CPPU (Table 3). All CPPU concentrations reduced return bloom in 1989.

Effect of CPPU on 'Empire' apples in Massachusetts. CPPU applied to 'Empire' apples increased fruit weight linearly but did not affect fruit set, SSC, L/D, or firmness (data not shown). The only treatment that reduced return bloom in 1989 below the control's was the highest CPPU application rate-40 mg-liter⁻¹; return bloom value for this CPPU application rate was $\approx 20\%$ of the control's value.

Effect of CPPU on preharvest drop of 'Early McIntosh' in Massachusetts. Six days following treatment, CPPU applied at 100 mg-liter⁻¹ and NAA applied at 20 mg-liter⁻¹ had reduced fruit drop of 'Early McIntosh' by 47% and 83%, respectively, compared with the control (Table 4). After 14 days, however, the beneficial effects of CPPU had diminished,

Table 1. Effect of CPPU and Promalin applied at full bloom or at full bloom plus 2 weeks on fruit characteristics of 'Oregon Spur Delicious'/MM.111 apples in Washington.

Compound and concn (mg-liter ⁻¹)	Fruit wt (g)	Flesh firmness (N)	Soluble solids concn (%)	Acidity (%)	L/D ²
<i>Full bloom</i>					
CPPU					
1) 0	219	62	13.2	0.171	1.03
2) 6.25	214	65	13.3	0.170	1.05
3) 12.5	223	67	13.7	0.172	1.08
4) 25.0	227	67	13.6	0.158	1.08
5) 50.0	216	73	13.8	0.161	1.05
6) + GA ₄₊₇ , both 12.5	240	67	13.9	0.152	1.08
Promalin					
7) 25.0	200	64	13.9	0.179	1.06
Contrasts (significance)					
Linear	NS ²	*	NS	NS	NS
Quadratic	NS	NS	NS	NS	*
Cubic	NS	NS	NS	NS	NS
T1 vs. T7	*	NS	*	NS	*
T3 vs. T6	NS	NS	NS	NS	NS
<i>Full bloom + 2 weeks</i>					
CPPU					
1) 0	209	62	13.2	0.171	1.03
2) 6.25	202	64	13.9	0.149	1.03
3) 12.5	234	67	13.6	0.192	1.08
4) 25.0	248	66	13.9	0.191	1.05
5) 50.0	225	72	13.0	0.180	1.07
6) + GA ₄₊₇ , both 12.5	246	65	13.4	0.175	1.09
Promalin					
7) 25.0	225	65	13.6	0.173	1.03
Contrasts (significance)					
Linear	NS	*	NS	NS	NS
Quadratic	NS	NS	NS	NS	NS
Cubic	NS	NS	NS	NS	*
T1 vs. T7	NS	NS	NS	NS	NS
T3 vs. T6	NS	NS	NS	*	NS

¹L/D = Length : diameter ratio.

²Mean of 120 observations.

NS, Non-significant or significant at $P = 0.05$.

whereas NAA continued to retard fruit drop by 46%. No treatment affected fruit drop during the next 16 days (data not shown).

Commercial grading of 'Hi-Early Delicious' treated with CPPU in Washington. There were ≈ 25 bins (≈ 450 kg) of fruit harvested from each treatment group. The percentage of fruit actually packed from each group was 85% for the control and Promalin treatments; 85% and 82%, respectively, for CPPU at 5 and 10 mg-liter⁻¹ and 73% for CPPU at 15 mg-liter⁻¹.

Reduced pack out at the highest CPPU concentrations was due mainly to an increase in malformed fruit frequency (Fig. 1). Fruit were classified as malformed if one side of the fruit was out of proportion or if the calyx end was uneven. Since cell division slows after anthesis, we believed that a higher concentration may be required at the later application time. However, the 15-mg-liter⁻¹ concentration was excessive. Even fruit treated with 10 mg-liter⁻¹ had 6% more deformed and misshapen fruit than the control.

CPPU treatments seemed to shift the mean fruit size to smaller fruit (Fig. 2). A Kolmogorov-Smirnov test, however, indicated no significant differences between any of the treatments and the control (data not shown). The control and Promalin treatments had the most fruit in the 80-, 88-, and >150-fruit/box sizes, whereas there were fewer fruit in the >150-, 100-, and 113-fruit/box sizes from the CPPU-treated trees. Results of blossom counts in 1990 indicated that none of the treatments influenced return bloom (data not shown).

CPPU seemed to increase the percentage of fruit surface that was red, since all CPPU treatments increased the percentage of Washington Extra Fancy fruit and reduced that of U.S. Fancy fruit (Fig. 3). Additionally, there were fewer culls due to poor color in the CPPU-treated fruit group (Fig. 1).

Fruit from air storage at 0C were evaluated in April after 7 days of storage at 20C. CPPU increased flesh firmness. Mean values for firmness were 53, 62, 62, and 62 N, respec-

tively, for the control and CPPU at 5, 10, and 15 mg-liter⁻¹, respectively.

Promalin is used commercially to elongate 'Delicious' apples. CPPU increased 'Delicious' L/D in Washington similarly to Promalin, and, in Massachusetts, CPPU was superior to Promalin. Using CPPU at concentrations of up to 15 mg-liter⁻¹ may increase L/D the most. However, reduced return bloom (Table 3) and an unacceptably high incidence of malformed fruit at harvest when using these high application rates necessitates applying CPPU at rates below 10 mg-liter⁻¹.

CPPU increased 'Empire' fruit size. This response confirms reports that CPPU increased 'Mutsu' and 'Tsugaru' (Otaga et al., 1989), 'Granny Smith' (Nickel, 1986), and 'McIntosh' (Greene, 1989) apple fruit size. Fruit size is frequently increased commercially by reducing crop load using chemical or hand thinning. CPPU can thin (Greene, 1989), but in this investigation it increased fruit size in the absence of thinning. There was, however, a tendency toward decreased fruit set. Therefore, the increase in 'Empire' fruit size must be attributed directly to the chemical. CPPU thinned 'McIntosh', but it increased fruit size so much that Greene (1989) concluded that at least part of the increased fruit size must be attributed directly to CPPU. The lack of effect of CPPU on 'Delicious' fruit size in two experiments suggests that the effect of CPPU on increasing fruit size may depend on cultivar or weather.

CPPU seemed to increase red pigmentation in the commercial field trial. Sansavini (1990, personal communication) reported a similar increase in redness in 'Delicious' when CPPU was applied at 20 mg-liter⁻¹ 25 days after FB. However, Devlin and Koszanski (1988) found that anthocyanin and flavin pigments in cranberry (*Vaccinium macrocarpon* Ait.) decreased following CPPU treatment, and Greene (1989) reported that red pigmentation decreased in 'McIntosh' apples receiving CPPU at 100 mg-liter⁻¹ but not 10 mg-liter⁻¹. Therefore, CPPU's effect on color may depend on specific cultivar, concentration, and environmental conditions.

Regardless of application time, CPPU increased 'Delicious' flesh firmness. This response confirms a previous report in which CPPU increased 'McIntosh' flesh firmness (Greene, 1989). Other compounds, including butanedioic acid mono-(2,2-dimethylhydrazide) (daminozide) (Greene et al., 1977) and BA (Greene and Autio, 1989), increase flesh firmness at harvest. However, unlike fruit treated previously with daminozide or BA, flesh firmness increases caused by CPPU did not dissipate, but persisted after storage.

Greene (1989) observed at harvest that fruit treated with CPPU were difficult to separate from the spur. He suggested that CPPU may prevent preharvest drop. CPPU retarded 'Early McIntosh' apple preharvest drop in this investigation, although it was not as effective as NAA—a chemical used commercially to

Table 2. Effect of CPPU applied at full bloom on fruit characteristics of spur 'Delicious'/MM.26 apples in Massachusetts.

Compound and concn (mg-liter ⁻¹)	L/D ^z	Flesh firmness (N) ^y
CPPU		
1) 0	0.95	69
2) 5	1.04	73
3) 10	1.02	74
4) 15	1.04	77
5) 20	1.02	77
Promalin		
6) 25	1.00	69
Contrasts (significance)		
Linear	*	*
Quadratic	*	NS
Cubic	NS	NS
T1 vs. T6	*	NS
T6 vs. T2 - T5	*	*

^zMean of 180 observations; L/D = length : diameter ratio.

^yMean of 120 observations.

NS, *Nonsignificant or significant at $P = 0.05$.

Table 3. Effect of CPPU and Promalin applied in 1988 on fruit quality criteria following 28 weeks in air storage and on return bloom in 1989 of spur 'Delicious' apples in Massachusetts.

Compound and concn (mg-liter ⁻¹)	Flesh firmness ^z (N)	Scald ^y (%)	Blossom clusters (1989)/cm ² limb cross-section ^x
CPPU			
1) 0	46	0	12.0
2) 5	50	2.0	5.7
3) 10	52	3.7	1.8
4) 15	53	3.4	2.1
5) 20	54	6.8	5.6
Promalin			
6) 25	47	2.4	7.7
Contrasts (significance)			
Linear	*	*	*
Quadratic	*	NS	*
Cubic	NS	NS	NS
T1 vs. T6	NS	NS	NS
T6 vs. T2 - T5	*	NS	*

^zMean of 120 observations.

^yMean of six observations.

^xMean of 12 observations.

NS, *Nonsignificant or significant at $P = 0.05$.

Table 4. Effect of CPPU or NAA applied on 2 Aug. on preharvest drops of 'Early McIntosh'.

Compound and concn (mg-liter ⁻¹)	Fruit drop after days indicated ^z			
	3	8	11	14
Control	23.0 a ^y	37.1 a	39.1 a	56.0 a
CPPU				
50	14.1 a	32.2 a	46.1 a	58.6 a
100	9.5 b	19.6 bc	37.1 a	52.3 a
NAA				
20	3.4 b	6.2 c	15.4 b	30.3 b

^zMean of seven observations.

^yMean separation by Duncan's multiple range test, $P = 0.05$.

Fig. 1. Cull analysis of fruit from 20-year-old 'Hi-Early'/seedling trees treated with CPPU at 5, 10, or 15 mg-liter⁻¹ or Promalin at 25 mg-liter⁻¹.

Fig. 2. Size distribution of fruit from 20-year-old 'Hi-Early'/seedling trees treated with CPPU at 5, 10, or 15 mg-liter⁻¹ or Promalin at 25 mg-liter⁻¹. Box size indicates the number of fruit in an 18-kg container.

Fig. 3. Color grade of fruit from 20-year-old 'High-Early' seedling trees treated with CPPU at 5, 10, or 15 mg-liter⁻¹ or Promalin at 25 mg-liter⁻¹. Requirements for percent surface pigmentation are as follows: Washington Extra Fancy (WAXF), 66% deep red; U.S. Extra Fancy (USXF), 66% deep red or pink-red; and U.S. Fancy, 40% deep red or pink-red.

retard preharvest drop-should be. More frequently, cytokinins increase rather than retard abscission. BA promotes abscission of developing apple fruit (Greene and Autio, 1989; McLaughlin and Greene, 1984). *N*-phenyl-*N'*-1,2,3-thiadiazol-5-ylurea (thidiazuron), a phenylurea similar to CPPU that has cytokinin activity, promotes leaf abscission in cotton (*Gossypium hirsutum* L.) (Suttle, 1985).

Luckwill (1969) first suggested that cytokinins may stimulate flower bud formation in fruit spurs. The cytokinins that have been reported to increase flower bud formation are *N*-substituted purines and not phenylurea compounds like CPPU. Greene and Autio (1989), Greene et al. (1990), and McLaughlin and Greene (1984) have confirmed that BA increases flower bud formation in apples. In this study, CPPU inhibited 'Empire' flower bud formation. This result confirms an earlier report that CPPU also inhibited 'McIntosh' return bloom (Greene, 1989).

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