

Exhibit P1
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**Fruit Set, Berry Size, and Yield of Three Rabbiteye
Blueberry Cultivars in Response
to CPPU Application**

A 2001 Research Report

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Research efforts in Georgia over the past ten years with the growth regulator gibberellic acid (GA_3) have overcome some of the fruit set problems with rabbiteye blueberry and have led to significant yield increases (NeSmith et al., 1995; NeSmith et al., 1999; NeSmith and Krewer, 1992, 1997a, 1997b, 1999). Even though research has shown positive benefits from using GA_3 in many instances, there are still some problems with small, late ripening fruit when using the growth regulator (NeSmith and Krewer, 1999). The cytokinin compound N-(2-chloro-4-pyridyl)-N'-phenylurea (CPPU) has shown some positive results in increasing fruit size and fruit set in a number of fruit crops including table grapes, kiwifruit, apples, table olives, and Japanese persimmon (Antognozzi et al., 1993a and 1993b; Greene, 1989 and 1993; Looney, 1993; Reynolds et al., 1992; Sugiyama and Yamaki, 1995). Recently, preliminary research at The University of Georgia has shown that CPPU may also be beneficial in rabbiteye blueberry production (NeSmith, 1999). The objective of this research was to test the usage of CPPU, along with GA_3 , in field grown rabbiteye blueberries during the 2001 growing season for potential benefits of increased fruit set, berry size, and yields.

Materials and Methods

This research was conducted at the University of Georgia's Blueberry Research Farm near Alapaha, Ga. The three cultivars used were 'Bluebelle', 'Tifblue', and 'Climax'. Treatments were: 1) control (no CPPU and no GA_3); 2) CPPU only; 3) GA_3 only. GA_3 treatments consisted of two applications (10 to 14 days apart) of 32 g/acre of GA_3 and a non-ionic surfactant at 0.25%, applied with an airblast sprayer at a volume of 50 gal/acre beginning at 30 to 50% bloom for a cultivar. CPPU was a one-time application utilizing a solution containing 10 mg/L of CPPU and a non-ionic surfactant at 0.25%, applied with an airblast sprayer at a volume of 50 gal/acre 10 to 14 days after 50% bloom. There were three replications of each treatment with 'Bluebelle' and 'Tifblue', and two replications with 'Climax'. Also, GA_3 was not applied to 'Climax' due to the lack of a sufficient number of available plants. All plants were mature, having been established for 12 years or more. The 'Bluebelle' and 'Tifblue' plants used were very vigorous, and the 'Climax' plants were moderately vigorous. Plants were sprayed from both sides with the growth regulators, and border rows existed between replications. Each treatment was applied to 10 to 12 plants together in a row for each replication.

Data taken from treatments for all cultivars consisted of fruit set, berry size, and yields. Prior to treatments, branches were tagged and flower bud numbers were determined. The average number of flowers per bud was also determined for 100 buds for each treatment at each farm. A total of 12 branches was tagged for each treatment of each cultivar in each replication. Fruit set was calculated from the flower bud counts and subsequent berry counts. Berry size was determined at each harvest for treatments of each cultivar. Twelve samples of 50 ripe berries were randomly taken from the harvested fruit for each treatment and were weighed immediately. Yields were obtained using a commercial mechanical harvester made available to the UGA Blueberry Research Farm by B.E.I., Inc. The 10 to 12 plants of each treatment in a replication were harvested together, and the total weight of fruit obtained was divided by the number of plants. There were three harvests for 'Tifblue' and 'Bluebelle', and two harvests for 'Climax'.

Results

CPPU significantly increased fruit set of 'Bluebelle' and 'Climax', but not 'Tifblue' (Table 1). The high degree of fruit set for the 'Tifblue' control treatment overall (52.8%), is far greater than growers experience in most years. 'Tifblue' fruit set can be as low as 10% when relying only on pollination (Lyrene and Crocker, 1983; Lyrene and Goldy, 1983; NeSmith and Krewer, 1997a; NeSmith et al., 1999). The GA₃ treatment resulted in greater fruit set than the control and the CPPU treatment for 'Bluebelle'; however, no increase in 'Tifblue' fruit set occurred for the GA₃ treatment, which is in contrast to previous work (NeSmith et al., 1995; NeSmith et al., 1999; NeSmith and Krewer, 1992, 1997a, 1997b, 1999). Again, perhaps the better-than-average 'Tifblue' fruit set of control treatments is part of the reason.

Berry size of 'Bluebelle' and 'Climax' treated with CPPU was increased by 5 to 15% over that of control treatments at the first harvest (Table 1). Berry size overall harvests was increased by 8 to 12% for CPPU treated 'Bluebelle' and 'Climax' compared to controls. CPPU had no effect on 'Tifblue' berry size, but the overall size of 'Tifblue' control berries was greater than typically occurs for the cultivar. Again, perhaps better-than-average pollination occurred for 'Tifblue', which would tend to increase berry size. The GA₃ treatment resulted in decreased berry size for both 'Bluebelle' and 'Tifblue' as compared to the control and CPPU treatments. This response to GA₃ has been noted previously (NeSmith and Krewer, 1999). In fact, the occurrence of small berries is a *primary* reason growers are reluctant to use GA₃ on some cultivars.

Yield of 'Bluebelle' was increased by nearly 32% for CPPU treated plants as compared to control plants across all harvests (Table 1). This yield increase occurred without a penalty of delayed maturity or lower yields at the first harvest. There was a slight increase in total yield of 'Climax' with CPPU, and no effect of CPPU on 'Tifblue' yields. Again, CPPU did not result in lessened yields of these cultivars at the first harvest. GA₃ treated 'Bluebelle' plants had total yields equal to the control, but yield at the first harvest was less when GA₃ was applied. GA₃ resulted in lower yield at the first harvest and overall for 'Tifblue', likely due to the small fruit size (discussed previously).

Summary

These data suggest that CPPU could be beneficial in rabbiteye blueberry production for increasing fruit set and berry size, which can result in substantial yield increases for some cultivars. When pollination is poor, the benefits of CPPU would be greater than when pollination is favorable. CPPU appears to be more desirable than GA₃ for rabbiteye blueberries, because even though GA₃ can increase fruit set, the result is often small berry size. There was no apparent delay of maturity or lessened early harvest with CPPU for the mechanically harvested fruit in this study. Additional research is needed to determine the benefits of CPPU in different years and locations.

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Sugiyama, N. and Y.T. Yamaki. 1995. Effects of CPPU on fruit set and fruit growth in Japanese persimmon. *Scientia Horticulturae* 60: 337-343.

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Table 1. Fruit set, berry size, and yield of three rabbiteye blueberry cultivars in response to field applications of CPPU and GA₃ at the University of Georgia Blueberry Research Farm near Alapaha, Ga. during 2001. Yields were for mechanically harvested bushes. CPPU was applied with an airblast sprayer.

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Treatment	Fruit set (%)	Berry size (g/50 berries)		Yield (lbs/bush)	
		First harvest	Overall harvests	First harvest	Total all harvests
<i>Bluebelle</i> ^{VI}					
Control	48.4 ± 3.7	84.9 ± 1.2	65.2 ± 1.9	8.5 ± 1.5	12.2 ± 2.3
CPPU only	67.1 ± 3.6	97.7 ± 1.7	70.3 ± 2.6	9.6 ± 0.4	16.1 ± 1.3
GA ₃ only	85.0 ± 2.3	74.5 ± 0.9	56.3 ± 1.8	6.3 ± 0.4	13.1 ± 1.2
<i>Tifblue</i> ^{VI}					
Control	52.8 ± 3.4	70.0 ± 0.4	62.0 ± 0.9	10.5 ± 0.4	20.3 ± 1.9
CPPU only	52.6 ± 3.1	71.8 ± 1.0	64.1 ± 1.0	10.6 ± 0.5	21.3 ± 2.6
GA ₃ only	48.0 ± 4.5	64.5 ± 0.7	57.7 ± 0.8	8.7 ± 0.2	17.2 ± 1.4
<i>Climax</i> ^{VI}					
Control	29.2 ± 3.2	57.7 ± 0.6	52.3 ± 1.2	4.1 ± 0.4	5.5 ± 0.6
CPPU only	42.0 ± 4.3	60.8 ± 0.6	58.9 ± 0.6	4.3 ± 0.3	6.2 ± 0.5

^{VI} Values are means ± standard error with n=12, except for yield which was for n=3 for 'Bluebelle' and 'Tifblue', and n=2 for 'Climax'.

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On-Farm CPPU Trials with 'Climax' and 'Tifblue' Rabbiteye Blueberries

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Research efforts in Georgia over the past ten years with the growth regulator gibberellic acid (GA_3) have overcome some of the fruit set problems with rabbiteye blueberry and have led to significant yield increases (NeSmith et al., 1995; NeSmith et al., 1999; NeSmith and Krewer, 1992, 1997a, 1997b, 1999). Even though research has shown positive benefits from using GA_3 in many instances, there are still some problems with small, late ripening fruit when using the growth regulator (NeSmith and Krewer, 1999). The cytokinin compound N-(2-chloro-4-pyridyl)-N'-phenylurea (CPPU) has shown some positive results in increasing fruit size and fruit set in a number of fruit crops including table grapes, kiwifruit, apples, table olives, and Japanese persimmon (Antognozzi et al., 1993a and 1993b; Greene, 1989 and 1993; Looney, 1993; Reynolds et al., 1992; Sugiyama and Yamaki, 1995). Recently, preliminary research at The University of Georgia has shown that CPPU may also be beneficial in rabbiteye blueberry production (NeSmith, 1999). The objective of this research was to test the usage of CPPU, along with GA_3 , in a number of commercial rabbiteye blueberry fields during the 2001 growing season for potential benefits of increased fruit set and berry size.

Materials and Methods

The cultivars Tifblue and Climax represent the two most widely grown rabbiteye blueberry cultivars in the state of Georgia, therefore, these were selected for the CPPU trials. Treatments at each site consisted of controls (no CPPU and no GA_3), CPPU only, GA_3 only, and GA_3 plus CPPU. When GA_3 was used, growers applied the product using their own established method. Generally, this consisted of 1 to 3 applications (10 to 14 days apart) of 16 to 24 g/acre of GA_3 , applied with an airblast sprayer beginning at 25 to 50% bloom. All CPPU applications utilized a solution containing 10 mg/L of CPPU and X-77 surfactant at 0.25%. CPPU was applied either using a back-pack sprayer, or using a commercial airblast sprayer. A total of five commercial farms were involved in this test. Table 1 summarizes dates of bloom and CPPU application details for each farm. A brief characterization of each farm site follows:

Scarborough:

Farm 1: This farm, located in southwestern Appling Co., consists of more than 280 acres of blueberries. Bushes used in this experiment were from 5 to 8 years old, and were considered very vigorous. Generally, row width at the farm was 10 to 12 ft, and plant spacing was 4 ft. The farm had raised beds, used a herbicide strip, had cultivated middles, and had overhead irrigation. The

'Climax' and 'Tifblue' blueberries utilized at this farm were in separate plantings, and each was grown with the variety Premier. Control and CPPU only treatments were applied at this farm. A freeze on March 8 caused little or no damage to flowers at this farm.

Wade:

Farm 2: This farm, located in eastern Bacon Co., consists of more than 25 acres of blueberries. Bushes used in this experiment were more than 10 years old, and were considered moderately vigorous. Generally, row width at the farm was 12 ft, and plant spacing was 6 ft. The farm had raised beds, used a herbicide strip, had grass middles, and had no irrigation. The 'Climax' and 'Tifblue' blueberries utilized at this farm were in the same planting. Control and CPPU only treatments were applied at this farm. A freeze on March 8 caused slight to moderate flower damage at this farm.

Morris:

Farm 3: This farm, located in central Appling Co., consists of more than 60 acres of blueberries. Bushes used in this experiment were more than 12 years old, and had a low degree of vigor. Generally, row width at the farm was 12 ft, and plant spacing was 6 ft. The farm had raised beds, used a herbicide strip, had cultivated middles, and had subsurface irrigation. The 'Climax' and 'Tifblue' blueberries utilized at this farm were in the same planting. Control, CPPU only, GA₃ only, and GA₃ plus CPPU treatments were applied at this farm. A freeze on March 8 caused only slight flower damage at this farm.

Bell

Farm 4: This farm, located in northern Pierce Co., consists of more than 100 acres of blueberries. Bushes used in this experiment were more than 14 years old, and were considered moderately vigorous. Generally, row width at the farm was 12 ft, and plant spacing was 5 ft. The farm had flat beds, used a herbicide strip, had cultivated middles, and had subsurface irrigation. The 'Climax' and 'Tifblue' blueberries utilized at this farm were in the same planting. Control, CPPU only, GA₃ only, and GA₃ plus CPPU treatments were applied at this farm. A freeze on March 8 caused severe flower damage at this farm.

Standland

Farm 5: This farm, located in central Bacon Co., consists of more than 10 acres of blueberries. Bushes used in this experiment were more than 12 years old, and had a low to moderate degree of vigor. Generally, row width at the farm was 12 ft, and plant spacing was 6 ft. The farm had raised beds, had cultivated middles, and had no irrigation. There were no 'Climax' at this farm, and 'Tifblue' blueberries utilized were planted with 'Woodard'. Control, CPPU only, GA₃ only, and GA₃ plus CPPU treatments were applied at this farm. A freeze on March 8 caused slight flower damage at this farm.

Data taken from treatments at all farms consisted of fruit set and berry size. Prior to treatments, branches were tagged and flower bud numbers were determined. The average number of flowers per bud was also determined for 100 buds for each treatment at each farm. A total of 12 to 15 plants was tagged for each treatment at each site. Fruit set was calculated from the flower bud counts and subsequent berry counts. Berry size was determined at the beginning of commercial harvest for each cultivar at each site. Samples of 50 ripe berries were randomly taken from each of the tagged bushes and were weighed.

Results

CPPU increased fruit set of 'Climax' at all commercial farms as compared to the control treatment (Table 2). In fact, across all farms, CPPU more than doubled fruit set (27.7% for control versus 60.0% for CPPU alone). Fruit set for the GA₃ only treatment was also better than fruit set of controls at the two farms where the treatment was present. The combination of GA₃ and CPPU did not result in greater fruit set than CPPU alone, except at Farm 4. Control fruit set of 'Climax' varied across the 4 farms from a low of 15.5%, to a high of 40.1%. Some of the control variability could be attributed to the degree of freeze damage flowers at each farm experienced as discussed in the Materials & Methods section. The greatest response to CPPU was on Farm 2, where the resulting fruit set was nearly 82%, compared to only 21% for the control.

In general, CPPU applications increased berry size of 'Climax' slightly as compared to control plants (Table 2). The exception was berry size at Farm 1. 'Climax' at this farm was interplanted with 'Premier', which is a better pollinizer for 'Climax' because of more similar bloom times. 'Tifblue' was the pollinizer at the other 3 farms. The increase in berry size overall due to applications of CPPU was around 10%. The greatest increase in berry size of 'Climax' was on Farm 3. Control plants on this farm had very small fruit, likely due to poor pollination. 'Tifblue' and 'Climax' bloom times were separated by a longer period of days at this site than at any other (Table 1). As expected, GA₃ only did not increase berry size as compared to the control; however, GA₃ plus CPPU did result in increased berry size.

For 'Tifblue', CPPU increased fruit set to a degree on 4 of 5 farms (Table 3). The increases of the CPPU only treatment over the control were less than that observed for 'Climax'. There was a slight decrease in fruit set at Farm 1 caused by CPPU. Again, one of the most pronounced increases of 'Tifblue' fruit set due to CPPU was on Farm 3, where the bloom time separation between the cultivars was the greatest. 'Tifblue' at Farm 4 had extremely poor fruit set due to severe freeze damage. The high degree of fruit set for the 'Tifblue' control treatment overall (33%), is greater than growers experience in many years. 'Tifblue' fruit set can be as low as 10% when relying only on pollination, especially in a 'Tifblue/Climax' mix (Lyrene and Crocker, 1983; Lyrene and Goldy, 1983; NeSmith and Krewer, 1997a; NeSmith et al., 1999). GA₃ treatments resulted in little increase in 'Tifblue' fruit set, which is in contrast to previous work (NeSmith et al., 1995; NeSmith et al., 1999; NeSmith and Krewer, 1992, 1997a, 1997b, 1999). Again, perhaps the better-than-average 'Tifblue' fruit set of control treatments is part of the reason. Interestingly, the combination of GA₃ and CPPU resulted in a considerable increase in fruit set at Farm 5.

There was essentially no difference in berry size among treatments for 'Tifblue' (Table 3). This is in contrast to results from previous work with CPPU, in which 'Tifblue' fruit size was significantly increased by CPPU application (NeSmith, 1999). As with fruit set, perhaps better-than-average pollination of 'Tifblue' during 2001 resulted in a nominal fruit size for all plants.

Summary

In general, the data from these field trials indicate that CPPU enhances fruit set of rabbiteye blueberries considerably. The effects were dramatic with 'Climax', and lesser so with 'Tifblue'. The usage of CPPU and GA₃ in combination does not seem to be beneficial for fruit set. CPPU would likely be the better growth regulator to use, because there is a tendency for increasing fruit size under some circumstances as well. Also, CPPU worked well on 'Climax', which has been a troublesome cultivar for using GA₃. Additional, large-scale field trials are needed for further evaluating the benefits of CPPU in rabbiteye blueberry production.

References

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Sugiyama, N. and Y.T. Yamaki. 1995. Effects of CPPU on fruit set and fruit growth in Japanese persimmon. *Scientia Horticulturae* 60: 337-343.

Table 1. Dates of 50% bloom, dates of CPPU application, method of CPPU application, and bush fraction treated with CPPU for five commercial farms in south Georgia during 2001.

Farm	Estimated date of 50% bloom	Date of CPPU application	CPPU application method	Bush fraction treated with CPPU
<i>Climax</i>				
Farm 1	March 2	March 14	back-pack	whole plants
Farm 2	March 3	March 13	back-pack	one side of bush
Farm 3	February 26	March 13	back-pack	whole plants
Farm 4	March 1	March 14	airblast	whole plants
<i>Tifblue</i>				
Farm 1	March 12	March 27	back-pack	one side of bush
Farm 2	March 10	March 26	back-pack	one side of bush
Farm 3	March 11	March 27	back-pack	whole plants
Farm 4	March 12	March 27	airblast	whole plants
Farm 5	March 14	March 26	airblast	whole plants

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Table 2. CPPU and GA₃ effects on fruit set and berry size of 'Climax' rabbiteye blueberry on four commercial farms in Georgia during 2001.

Treatment	Commercial Farms ^z				Average of all farms
	Farm 1	Farm 2	Farm 3	Farm 4	
	<i>Fruit set (%) ^w</i>				
Control	40.1 ± 3.9	20.9 ± 3.6	34.1 ± 6.4	15.5 ± 2.3	27.7
CPPU only	60.2 ± 5.9	81.7 ± 2.2	66.5 ± 6.6	31.6 ± 5.6	60.0
GA ₃ only	---	---	67.0 ± 6.8	26.0 ± 3.9	46.5
CPPU/GA ₃	---	---	73.6 ± 8.5	52.0 ± 4.8	62.8
	<i>Berry size (g/50 berries) ^w</i>				
Control	53.2 ± 1.8	65.6 ± 1.2	49.8 ± 0.8	69.4 ± 2.3	59.5
CPPU only	53.2 ± 1.9	76.8 ± 1.9	60.6 ± 0.8	71.2 ± 1.4	65.5
GA ₃ only	---	---	57.0 ± 1.3	68.4 ± 1.6	62.7
CPPU/GA ₃	---	---	67.4 ± 2.2	77.8 ± 1.4	72.6

^z Commercial farms were scattered over a three county area in south Georgia.

^w Values are means ± standard error with n=12.

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Table 3. CPPU and GA₃ effects on fruit set and berry size of 'Tifblue' rabbiteye blueberry on five commercial farms in Georgia during 2001.

Treatment	Commercial Farms ²¹					Average of all farms
	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	
	<i>Fruit set (%)²¹</i>					
Control	58.0 ± 6.1	32.4 ± 2.9	33.5 ± 8.1	7.8 ± 1.3	34.0 ± 4.9	33.1
CPPU only	41.1 ± 2.3	46.8 ± 5.7	51.6 ± 9.2	16.9 ± 4.2	43.9 ± 4.8	40.1
GA ₃ only	---	---	40.6 ± 3.5	14.4 ± 4.5	46.8 ± 3.9	33.9
CPPU/GA ₃	---	---	45.3 ± 8.2	15.1 ± 2.1	61.3 ± 5.3	40.6
	<i>Berry size (g/50 berries)²¹</i>					
Control	77.2 ± 1.9	69.6 ± 1.4	66.8 ± 2.2	67.8 ± 2.7	66.0 ± 2.6	69.5
CPPU only	75.8 ± 1.3	66.2 ± 1.4	62.8 ± 1.3	64.4 ± 2.1	68.2 ± 1.7	67.5
GA ₃ only	---	---	70.0 ± 2.0	64.0 ± 1.6	71.2 ± 1.2	68.4
CPPU/GA ₃	---	---	60.8 ± 2.3	65.2 ± 1.1	68.8 ± 3.5	64.9

²¹ Commercial farms were scattered over a three county area in south Georgia.

²¹ Values are means ± standard error with n=12.