

## ABSTRACT

OH, JIYOUNG. Growth regulator Effects on Watermelon Chilling Resistance, Flowering, and Fruiting. (Under the direction of Dr. Todd C. Wehner.)

The watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] is classified as a tender warm season crop and is native to the Old World tropics and subtropics (Bates and Robinson, 1995). Watermelon seedlings may be exposed to temperatures between chilling and optimal for weeks before temperatures stabilize. An experiment was conducted to determine the abscisic acid effect on the watermelon chilling resistance in nine different cultivars that examined whether they were resistant or susceptible. Eighty, 160, and 320 mg/kg ABA applied 12, 24, 36 or 72 hours before chilling provided protection from chilling damage.

Hybrid watermelon seeds are produced by hand pollination using alternating rows in a field for the two parental inbred lines. In some cases, a male sterile has been used to make hybrid seed production easier. However, since the male sterile is the genic type, it is necessary to check each plant to make sure it is male sterile and must be removed before pollination begins. To avoid the labor involved in removing the male flowers, we conducted a study to identify growth regulators that would convert monoecious into gynoecious. We evaluated ten different growth regulators for their effect on staminate: pistillate flower ratio in more gynoecious and androecious watermelon cultivars. AVG (aminoethylvinylglycine) with 100 mg/kg of etrel appears to increase the percentage of pistillate nodes. However, a single treatment of AVG without etrel had no effect on sex expression. None of the other treatments induced higher gynoecious sex expression compared with the control.

Watermelon plants have fewer pistillate flowers than other cucurbit crops and affected by different environmental conditions. We conducted experiments with four different environmental conditions and evaluated watermelon sex expression. Tests were run with four different growth chambers (32/24°C/8h, 32/24°C /16h, 24/16°C/8h, and 24/16°C/16h). Low temperature and long-day induced more pistillate flowers than staminate flowers while day length and fertilization with high temperature did not result in any difference in the sex expression. Fertilization did not effect sex expression, but at low temperature, it had an effect on vine length.

We evaluated growth regulators for parthenocarpic fruit set on four different diploid watermelons with CPPU (2-chloro-4-pyridyl-N-phenylurea), NAA (1-naphthaleneacetic acid), and 2,4-D (2,4-dichlorophenoxyacetic acid) combinations. Pistillate flowers were applied with CPPU 50 mg/kg, NAA 100 mg/kg, and 2,4-D 50 mg/kg in various combination induce parthenocarpic fruit set and increase yield.

The growth regulators applied to the pistillate flowers planted in isolation (no diploid pollen available) will induce fruit set in triploid watermelon. The most effective growth regulators from previous studies were tested. These included NAA, 2,4-D and CPPU. CPPU was the most effective in setting fruit of seedless watermelon. However, when CPPU and NAA were used in combination, the highest yield was obtained with 200 mg/kg CPPU plus 100 mg/kg NAA. When the whole plant was sprayed using a backpack sprayer, the plants were injured or killed. In order to increase fruit set and yield, we applied growth regulators in combination without CPPU (100 mg/kg NAA and 50 mg/kg 2,4-D) or with CPPU (50 mg/kg CPPU, 100 mg/kg NAA, and 50 mg/kg 2,4-D) for 1, 2, 3, and 4 weeks (2 times/week). We used a backpack sprayer to simulate tractor spraying. The combination

without CPPU (100 mg/kg NAA, and 50 mg/kg 2,4-D) gave the most fruit set. After two weeks (2 times/week) of spraying, there was damage evident on the whole plant with CPPU.

Growth regulator Effects on Watermelon Chilling Resistance, Flowering, and Fruiting

by  
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She married Jeongmin Lee on January 11<sup>th</sup>, 1998 and has two daughters, Chaeun (Jiah) and Yaeun (Linda).

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## **Absciscic acid treatment improves chilling resistance of watermelon seedlings**

## Abstract

An experiment was conducted to determine the effect of abscisic acid on watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] chilling resistance in nine watermelon cultivars that were examined whether resistance or susceptible in 2005, 2006, and 2007. 'Orangeglo' and 'Dixielee' were most chilling resistant, and 'Sugar Baby' and 'Navajo Sweet' were most susceptible. In order to determine the optimum concentration and application time of ABA to induce resistance, we tested nine rates (ABA 0, 10, 20, 40, 80, 160, 320, 640, and 1280 mg/kg) and six application times (0, 12, 24, 48 or 72 hours before chilling and 12 hours after chilling). Nine cultivars and plant introduction accessions (hereafter collectively referred to as cultigens) were treated. The cultigens were 'Dixielee', 'Navajo Sweet', 'Sunshade', 'Chubby Gray', PI 255137, 'Charleston Gray', 'Sugar Baby', 'Orangeglo' and 'Golden Honey'. ABA treatment of watermelons before chilling provided protection from foliar damage. ABA treatment provided more protection for susceptible than resistant cultivars. Plants treated with ABA after chilling were not significantly different from untreated plants. After chilling exposure, untreated plants had significant injury. However, 80, 160, or 320 mg/kg ABA applied 12, 24, 36 or 72 hours before chilling provided protection from damage in watermelon seedlings. The most effective treatment was a concentration of 160 mg/kg ABA applied before chilling, since higher concentrations increased the danger of stunting or chlorosis of the plants.

## **Introduction**

Tropical and subtropical plants exhibit a marked physiological dysfunction when exposed to low or nonfreezing temperatures below 10°C. Lyons suggested that this physiological harm should be referred to as "chilling injury" to differentiate it from freezing damage [7]. Such injury to susceptible plants has also been referred to as "low temperature injury" or "cold injury" and in apples as "low-temperature breakdown". Chilling injury appears to be the preferable term [12], because it is not easily confused with freezing injury or with phenomena related to cold or winter hardiness [6, 19].

Chilling injury is the physical and/or physiological changes induced by exposure to low temperatures, together with the subsequent expression of characteristic symptoms, are commonly combined. Often the symptoms that develop are merely exaggerations of the effects of physiological injury or other physiological stresses. For example, chilling often increases the occurrence of senescence and decay after chilling and the rate of water loss both during and after chilling. Similar symptoms can be induced by factors other than chilling temperatures. However, several commonly occurring symptoms are often used as indication of the severity of chilling injury are cellular changes, altered metabolism, reduced plant growth and death, surface lesions, water-soaking of the tissue, internal discoloration, accelerated senescence, increased susceptibility to decay, failure to ripen normally and loss of vigor. Many investigations have focused on treatments or procedures designed to increase tolerance to chilling injury or to reduce the severity of the resulting symptoms.

The watermelon is classified as a very tender warm season crop and is native to the Old World tropics and subtropics (tropical and southern Africa) [1]. Watermelon seeds germinate

at a minimum soil temperature of 15°C and the optimum air temperature for plant growth ranges from 20°C to 32°C. Once the seedlings have been planted in the field, they may be exposed to temperatures cycling between chilling and optimal for weeks before temperatures stabilize. Exposure of seedlings to low temperatures retards growth, delays flowering, reduces total yields and quality, and even kills the plants [5].

Chilling injury occurs on watermelon plants in the field since they are planted early in the spring. Chilling injury occurs when the temperature gets below 4°C. Early harvested watermelons get the best price in the market, so growers usually start a little too early in the spring when temperatures can get below 4°C. Growth regulators may provide protection from frost, and might be applied by growers when there is a forecast for temperatures to go below 4°C.

A number of chemicals have been shown to be effective in reducing chilling injury in plants. The effect of calcium on chilling injury has been investigated extensively. The resistance to low-temperature stress, including chilling and freezing injury, is related to the balance of growth inhibitors and growth promoters. Growth regulators often affect many metabolic processes which, in turn, may also influence the susceptibility of crops to chilling injury. Applications of benzyladenine, GA and 2,4-dichlorophenoxyacetic acid significantly altered the susceptibility of grapefruit to chilling injury [2]. Ethylene treatment reduces chilling injury in some crops but increases chilling injury in others. Ethylene may alter the maturity and physiology of fruit and indirectly change the sensitivity of these crops to chilling. Whether the effect is beneficial or detrimental depends upon the stage of development and the kind of fruit involved.

The relationship between polyamines and chilling injury has been receiving increasing attention. The apparent involvement of membrane damage in chilling injury and the ability of polyamines to stabilize membranes have generated the hypothesis that polyamines may play a role in reducing chilling injury. Correlations between increased resistance to chilling injury and increased polyamines levels have been reported in several plant species. Polyamine levels increased markedly upon chilling in bean (*Phaseolus vulgaris*) plants and cucumber (*Cucumis sativus* L.) seedlings [17]. The beneficial effect of ABA treatment in reducing chilling injury has been shown in grapefruit (*Citrus paradise*) [3], tomato (*Lycopersicon lycopersicum*, syn. *Solanum lycopersicum*) seedlings [4], cotton (*Gossypium* spp.) seedlings [11], cucumber cotyledons and plants [14] and a red-pigmented cultivar of coleus [15]. High endogenous levels of ABA are related to increase chilling tolerance. The protection of rice seedlings and corn leaves against chilling injury by mefluidide was found to be mediated through its effect on ABA levels [21].

Mefluidide was capable of triggering an increase in endogenous ABA content in maize leaves when the plants were grown in a nonchilling environment with sufficient water supply. It was suggested that this increase in endogenous ABA before chilling could be an essential step in activating a protecting mechanism against chilling injury during low-temperature exposure [15]. The mechanism through which ABA reduces chilling injury is not fully understood. It is known that high ABA levels induce stomatal closure [16] and reduce water loss [8]. Exogenous ABA treatment has been employed to induce stomatal closure before exposure to low temperatures and, thus, avoid dehydration and chilling injury in seedlings of cotton, cucumber, and bean [10, 11]. Markhart and co-workers suggested that ABA might

directly affect the membrane that limits water flow through the root. The protective effect of ABA during the chilling of soybean seedlings was independent of its effect on stomata, because stomatal resistances were lower and leaf water potential were higher in the ABA-treated plants than in the non-treated plants when roots were at 10°C and shoot 25°C [20]. It was suggested that the mechanism of action of ABA in the response of plants to chilling stress involves membrane alterations. Rikin et al. reported that depolymerization of the microtubular network was involved in the development of chilling injury in cotton seedlings and that ABA decreased chilling injury by stabilizing the microtubular network [10, 11]. ABA has also has been reported to suppress chilling-induced ion leakage and to prevent the loss of reduced glutathione and membrane phospholipids.

The objective of this study was to determine whether ABA would protect watermelon seedlings from chilling injury, and to develop methods for protecting watermelon from a predicted chilling event in the field.

## **Materials and Methods**

### **ABA Phytotoxicity Study**

The watermelon cultigens used in this study were ‘Dixielee’, ‘Navajo Sweet’, ‘Sunshade’, ‘Chubby Gray’, PI 255137, ‘Charleston Gray’, ‘Sugar Baby’, ‘Orangeglo’, and ‘Golden Honey’. A preliminary study was conducted to determine whether there were differences in chilling resistance among the cultivars (Kozik, 1996). ‘Navajo Sweet’, ‘Sunshade’, ‘Chubby Gray’, PI 255137, ‘Dixielee’, and ‘Charleston Gray’ were resistant and ‘Golden Honey’, ‘Orangeglo’, and ‘Sugar Baby’ were susceptible.

### **ABA Chilling Protection**

Chilling experiments were conducted under controlled environment conditions in the growth chambers of the Southeastern Plant Environment Laboratory at North Carolina State University [13]. Seeds were sown in peat pots (57 mm square, 100 ml volume) filled with a standard substrate of gravel and peat in a 1:1 ratio and placed in flats. Two seeds were sown in each pot, with 54 pots contained in each flat. After seeding the plants, the flats were placed in growth chambers set at 26/22°C (day/night) under long days, consisting of 12 hours of combined fluorescent and incandescent light (from 8 am to 8 pm). Light intensity (PPFD) was 650 and 44  $\mu\text{mol m}^{-2}\text{s}^{-1}$ , respectively. Plants were watered with the standard phytotron nutrient solution [13].

In order to secure the phytotoxicity and determine the optimal concentration of ABA on the watermelon plant, we tested four cultigens (‘Sugar Baby’, ‘Calhoun Gray’, PI 244018, and PI 595203) with five ABA concentrations (100, 200, 400, 800, 1600 mg/kg) in the greenhouse. Disease chamber used to test watermelon plant differing for phytotoxicity of

ABA. The plant growth regulators were applied over the foliage with a hand sprayer to spray runoff. The plants were treated when seedlings were get cotyledons for four times by weekly. After one week of application, plants were rated the damage of phytotoxicity. The scale was 0 to 9: 0 = none, 1-2 = chlorosis of leaf edge, 3-4 = slight chlorosis or stunt, 5-6 = moderate necrosis or stunt, 7-8 = majority of leaves necrotic or stunt, 9 = plant dead (Wehner and Zohair, 1994).

The effect of ABA on chilling resistance was studied in a series of experiments. All were randomized complete block designs with 4 replications. Experimental plots consisted of 16 flats that have 18 plots per flat and each plot has three plants. In 2005, plants were treated with ABA at the cotyledon stage, either 12 hours before or 12 hours after chilling. Treatments were seven concentrations (0, 10, 20, 40, 80, 160, and 320 mg/kg) for run 1 and a higher set of seven concentrations (0, 40, 80, 160, 320, 640 and 1280 mg/kg) for run 2. After treating with ABA, plants were moved from the main growth chamber to the chilling chamber for treatment at 4°C under a light intensity of 500  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  PPFD for duration of 36 hrs. After the chilling treatment, they were returned to the main growth chamber and placed under the same light and temperature regime as before. Plants were rated 1, 3, and 7 days after chilling, rating the damage on the cotyledon, true leaf, and growth point. The scale was 0 to 9: 0 = none, 1=black little spot on one leaf, 2=leaf edge necrotic, 3=<50% of more than 1 leaf, 4=>50% of 1 leaf necrotic, 5=>50% of more than 1 leaf, 6=>90% of 1 leaf necrotic, 7=>90% of more than 1 leaf, 8=>90% of all leaves necrotic, 9=plant dead.

In 2006, plants were treated with ABA either 12 or 24 hours before chilling. Plants were treated with four concentrations (0, 80, 160, and 320 mg/kg). In 2007, plants were treated at



four growth stages (1, 2, 3, and 4 true leaves) at 0, 24, 48, or 72 hours before chilling with ABA at 160 mg/kg.

Individual ratings were summarized as mean or maximum over replications. Data were analyzed using the MEANS, ANOVA and GLM procedures of the SAS statistical package (SAS Institute, Cary, NC).

## **Results and Discussion**

In the phytotoxicity test, ABA had only a small effect on plants from lower concentrations to higher. Leaf damage ratings were near 4 (slight damage) at 100, 200, and 400 mg/kg ABA, indicating that there was slight phytotoxicity from ABA at our higher treatment rates (Table 1). We assumed that phytotoxicity on watermelon seedlings would be higher at rates above 400 mg/kg ABA.

After exposure to chilling for 36 hours, the plants not treated with ABA showed typical symptoms of chilling injury, including surface lesions and water-soaking of the cotyledon tissue. To determine the best treatment for protection from chilling with ABA, plants were treated with 0 to 1280 mg/kg ABA. However, 160 mg/kg ABA was the most effect on chilling resistance both resistance cultivar and susceptible cultivar (Table 2). Untreated plants lost a large area of their foliage, as indicated by higher damage ratings, compared to plants treated with ABA 12 or 24 hours before chilling. However, plants treated with ABA after chilling were not significantly different from untreated plants (Table 3 and 4). Three and seven days after chilling exposure, treated and untreated plants continued to suffer and injury rating values increased.

Plants had the least damage from chilling when they were treated before chilling with 80, 160, or 320 mg/kg of ABA. However, 320 mg/kg of ABA caused slight chlorosis of the foliage (Table 5). Of the 9 cultigens tested, ‘Dixielee’ was the most chilling resistant, and ‘Sugar Baby’ was the most chilling susceptible (Tables 5 and 6). ABA treatment was more beneficial for susceptible cultivars than on resistant ones, since they had the greatest improvement in leaf damage ratings.

When plants at four growth stages (1, 2, 3, or 4 true leaves) were treated with 160 mg/kg of ABA at 24, 48, or 72 hours before chilling, there was significant protection from the chilling injury compared with untreated plants (Table 6 and Fig. 1A, B).

Chilling injury occurs in watermelon plants when the temperature drops below 4°C. Chilling protection is important for watermelon planted in the spring, especially when expensive triploid seedless transplants are used. In that situation, ABA may provide protection from chilling, and can be applied by growers when there is a forecast for chilling temperatures. In that case, protection is provided using 160 mg/kg of ABA applied to the crop 12 to 72 hours before the chilling event. Future research is needed to determine whether it is possible to obtain protection when ABA is applied more than 72 hours before the chilling event, and how low a temperature ABA will protect against.

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Table 1. Phytotoxicity of ABA ratings for watermelon seedlings.<sup>z</sup>

ABA (mg/kg)	<u>Damage rating on plants</u>				
	Mean	Max	week 1	Week 2	week 3
0	2.1	3.3	2.4	1.5	2.5
100	3.0	4.0	3.4	3.3	2.3
200	3.2	4.1	2.8	2.9	3.9
400	3.4	3.9	3.1	3.9	3.3
800	4.5	5.3	3.5	4.8	5.3
1600	5.2	6.6	3.9	5.3	6.4
LSD (5%)	0.2	0.3	0.3	0.2	0.3

<sup>z</sup> Damage rated 0-9 (0=no damage, 9=plant dead), data are means of 2 replications and 4 cultivars.

Table 2. Chilling damage ratings for resistance and susceptible watermelons treated with ABA (0 to 1280 ppm) 12 hours before a chilling treatment.<sup>z</sup>

Cultivar	0	Tw20	10	20	40	80	160	320	
<b>Cotyledon rating (run 1)</b>									
Charleston Gray			2.9	1.9	2.3	2.3	2.5	2.3	1.7 1.9
Sugar Baby	6.8		7.1	6.0	5.1	6.3	5.2	3.7	4.9
LSD (5%)					2.2				
<b>Cotyledon rating (run 2)</b>									
Cultivar	unchilled	Tw20	40	80	160	320	640	1280	
Charleston Gray		0.0	4.1	2.9	3.9	2.9	3.0	2.8	3.0
Sugar Baby	0.0	6.7	5.4	5.9	4.9	5.3	4.7	5.1	
LSD (5%)			2.0						

<sup>z</sup> Damage rated 0-9 (0=no damage, 9=plant dead). ABA treatment 0 in run 1 was not sprayed, but chilled; in run 2 it was not sprayed and not chilled.



Table 3. Chilling damage ratings for resistant and susceptible watermelons treated with 0 and 160 mg/kg ABA 12 hours before and after chilling treatment in Run 1 (2005).<sup>z</sup>

ABA	Cultigen	<u>Damage rating on plants</u>			
(mg/kg)		Mean	1 day	3 days	7 days
<b>Spray after chilling</b>					
0	Charleston Gray	1.7	1.3	2.0	1.7
	Orangeglo	1.8	2.3	1.2	2.0
	Dixielee	3.3	3.3	2.7	4.0
	PI 255137	3.2	2.7	3.3	3.7
	Sunshade	3.8	3.7	3.7	4.0
	ChubbyGray	4.6	4.3	4.7	4.7
	Golden Honey	6.0	6.0	5.3	6.7
	Navajo Sweet	6.1	6.0	7.7	4.7
	Sugar Baby	7.7	7.7	7.7	7.7
160	Charleston Gray	3.0	3.7	2.7	2.7
	Orangeglo	1.0	3.0	0.0	0.0
	Dixielee	1.1	1.7	1.0	0.7
	PI 255137	2.0	1.0	1.0	4.0
	Sunshade	2.0	2.0	2.0	2.0
	Chubby Gray	3.7	3.0	3.7	4.3

Table 3 (continued)

	Golden Honey	3.4	3.0	3.3	4.0
	Navajo Sweet	3.8	3.0	4.0	4.3
	Sugar Baby	4.6	4.0	4.7	5.0
<b>Spray before chilling</b>					
0	Charleston Gray	2.2	2.0	2.5	2.0
	Orangeglo	0.7	1.0	1.0	0.0
	Dixielee	1.3	0.7	1.8	1.3
	PI 255137	3.9	3.2	4.0	4.7
	Sunshade	4.4	3.3	5.3	4.7
	Chubby Gray	2.8	3.0	2.0	3.3
	Golden Honey	6.7	6.7	7.3	6.0
	Navajo Sweet	6.3	6.0	8.0	5.0
	Sugar Baby	6.4	5.8	6.5	7.0
160	Charleston Gray	0.3	1.0	0.0	0.0
	Orangeglo	1.0	0.0	0.0	3.0
	Dixielee	0.6	0.0	0.3	1.3
	PI 255137	0.2	0.0	0.5	0.0
	Sunshade	2.9	2.3	2.7	3.7
	Chubby Gray	1.2	0.0	1.0	2.7
	Golden Honey	2.6	1.3	2.3	4.0

Table 3 (continued)

Navajo Sweet	2.9	2.0	3.3	3.3
Sugar Baby	2.8	2.0	2.8	3.5
LSD (5%)	2.2	2.4	2.6	2.7

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z Damage rated 0-9 on cotyledon (0=no damage, 9=plant dead). Data are means of 2 replications, and 3 ratings.

Table 4. Chilling damage ratings for resistant and susceptible watermelons treated with 0 and 160 mg/kg ABA 12 hours before and after chilling treatment in Run 2 (2005).<sup>z</sup>

ABA	Cultigen	<u>Damage rating on plants</u>			
(mg/kg)		Mean	1 day	3 days	7 days
<b>Spray after chilling</b>					
0	Charleston Gray	3.2	3.0	3.0	3.5
	Orangeglo	1.0	0.0	2.0	1.0
	Dixielee	1.5	0.5	2.0	2.0
	PI 255137	4.7	4.0	6.0	4.0
	Sunshade	3.6	3.3	3.7	3.7
	Chubby Gray	3.9	3.7	4.0	4.0
	Golden Honey	5.8	5.8	5.8	5.8
	Navajo Sweet	3.8	3.3	4.0	4.0
	Sugar Baby	5.8	5.3	6.0	6.0
160	Cahrleston Gray	4.4	3.7	4.7	5.0
	Orangeglo	0.2	0.7	0.0	0.0
	Dixielee	1.1	0.3	1.0	2.0
	PI 255137	1.5	1.5	1.5	1.5
	Sunshade	4.9	4.3	5.3	5.0
	Chubby Gray	4.3	4.7	4.0	4.3

Table 4 (continued)

	Golden Honey	7.1	7.3	7.7	6.3
	Navajo Sweet	7.0	6.7	7.7	6.7
	Sugar Baby	7.0	7.0	7.0	7.0
<b>Spray before chilling</b>					
0	Charleston Gray	5.1	4.7	6.3	4.3
	Orangeglo	2.0	2.0	0.0	2.0
	Dixielee	2.2	2.0	2.3	2.3
	PI 255137	3.7	4.0	3.7	4.0
	Sunshade	5.0	4.0	6.0	5.0
	Chubby Gray	3.9	4.3	2.7	4.7
	Golden Honey	7.6	8.0	7.3	7.3
	Navajo Sweet	6.3	6.7	5.3	7.0
	Sugar Baby	7.6	7.3	8.0	7.3
160	Charleston Gray	1.3	0.5	2.0	1.5
	Orangeglo	0.3	0.0	1.0	0.0
	Dixielee	1.7	0.0	4.0	1.0
	PI 255137	1.7	0.0	2.3	1.7
	Sunshade	1.6	1.3	0.7	2.7
	Chubby Gray	2.3	1.0	2.3	3.7
	Golden Honey	1.3	2.6	2.3	4.0

Table 4 (continued)

Navajo Sweet	3.1	2.5	3.0	3.7
Sugar Baby	2.8	2.3	1.7	4.3
LSD (5%)	2.0	2.4	2.6	2.7

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<sup>z</sup> Damage rated 0-9 on cotyledon (0=no damage, 9=plant dead). Data are means of 2 replications and 3 ratings.

Table 5. Damage ratings 1, 3, and 7 days after chilling for watermelons treated with ABA (0, 80, 160, and 320 mg/kg) before (12 or 24 hours) chilling treatment applied at the cotyledon stage (2006).<sup>z</sup>

ABA (mg/kg)	Cultivar	<u>Damage rating after chilling</u>		
		1 day	3 days	7 days
0	Dixielee		0.2	0.2
	Sugar Baby		2.9	3.3
80	Dixielee		0.0	0.0
	Sugar Baby		0.1	0.1
160	Dixielee		0.0	0.0
	Sugar Baby		0.0	0.0
320	Dixielee		0.0	0.0
	Sugar Baby		0.0	0.0
LSD (5%)			0.6	0.8

<sup>z</sup> Damage rated 0-9 on cotyledon stage (0=no damage, 9=plant dead). Data are means of 2 replications and 3 ratings. Data for only 2 of 9 cultigens are shown.

Table 6. Damage ratings 7 and 14 days after chilling for watermelons treated with ABA (0 and 160 mg/kg) 0, 24, 48, or 72 hours before chilling treatment applied at the true leaf stage (2007).<sup>z</sup>

ABA (mg/kg)	Time (hour)	Cultivar	<u>Damage rating after chilling</u>		
			Mean	7 days	14 days
0	0	Dixielee	4.9	4.8	5.0
		Orangeglo	6.0	5.8	6.1
		Navajo Sweet	5.8	5.6	5.9
		Sugar Baby	6.3	6.2	6.5
24		Dixielee	4.2	4.1	4.4
		Orangeglo	5.6	5.4	5.8
		Navajo Sweet	5.3	5.0	5.6
		Sugar Baby	6.3	6.2	6.3
48		Dixielee	4.6	4.5	4.6
		Orangeglo	5.9	5.8	6.1
		Navajo Sweet	5.7	5.6	5.9
		Sugar Baby	6.1	6.0	6.3
72		Dixielee	3.6	3.4	3.8
		Orangeglo	5.5	5.3	5.8
		Navajo Sweet	5.6	5.4	5.9



Table 6 (continued)

		Sugar Baby	5.9	5.8	6.0
160	0	Dixielee	3.5	3.2	3.8
		Orangeglo	5.3	5.2	5.5
		Navajo Sweet	4.9	4.8	5.0
		Sugar Baby	5.1	4.9	5.3
	24	Dixielee	1.2	0.9	1.4
		Orangeglo	1.9	1.7	2.0
		Navajo Sweet	1.7	1.5	1.9
		Sugar Baby	1.8	1.8	1.9
	48	Dixielee	0.8	0.5	1.0
		Orangeglo	0.3	0.2	0.4
		Navajo Sweet	0.8	0.5	1.0
		Sugar Baby	1.1	1.0	1.1
	72	Dixielee	1.1	1.1	1.2
		Orangeglo	0.6	0.4	0.7
		Navajo Sweet	0.9	0.8	1.0
		Sugar Baby	0.9	1.1	0.8
LSD (5%)			1.2	1.3	1.3

Table 6 (continued)

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z Damage rated 0-9 on true leaves (0=no damage, 9=plant dead) after 7, 14 days after chilling.

Data are means of 2 replications and 2 ratings.

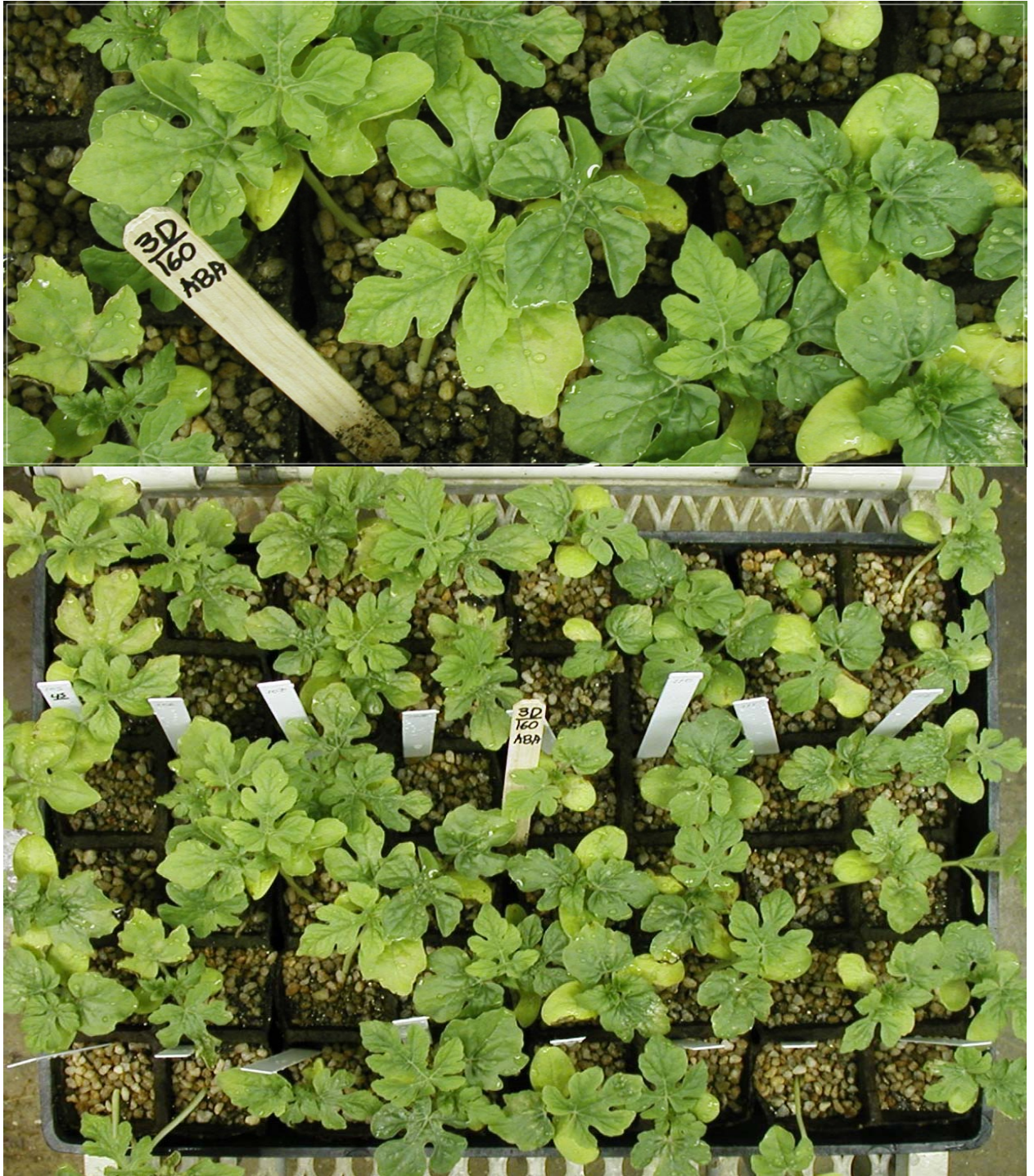


Figure 1A. Watermelon plants treated 72 hours before chilling with 160 ppm ABA provides protection from chilling damage.





Figure 1B. Watermelon plants not treated with ABA with chilling damage.

**Photoperiod, growth temperature, and fertilization effect on sex expression in  
watermelon**

### **Abstract**

An experiment was conducted to determine the environmental conditions effect on sex expression in watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai]. Watermelon plants have few pistillate flowers. Under normal environmental conditions, watermelon plants have few pistillate flowers. Tests were run with four growth chambers set (day/night) at 32/24°C (8 hr photoperiod), 32/24°C (16 hr photoperiod), 24/16°C (8 hr photoperiod), and 24/16°C (16 hr photoperiod) in the phytotron. Fertility treatments consisted of fertilizing once a week for low nutrition and three times per week for high nutrition. Plants were rated for days to first flower, number of pistillate and staminate flowers, and vine length to determine the photoperiod and temperature effect on the sex expression. Low temperature and long-day induced more pistillate flowers than staminate flowers in watermelon plants, while daylength and fertilization with high temperature had no significant effect on sex expression. Fertilization did not effect sex expression, but at low temperature, it had an effect on vine length.

## Introduction

Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] is a major vegetable crop in the U.S., and is increasing in popularity. Production increased from 1.1 million Mg•year<sup>-1</sup> in 1980 to 1.9 million Mg•year<sup>-1</sup> in 1995 [6]. Watermelon consumption in the U.S. is 6.4 kg per capita [12].

Hybrid watermelon seeds are produced by hand pollination using alternating rows in a field for the two parental inbred lines. In some cases, a male sterile has been used to make hybrid seed production easier. However, since the male sterile is the genetic type, it is necessary to check each plant in the female parent row to make sure it is male sterile, since about half the plants are male fertile, and must be removed from the field before pollination begins.

There is a diversity of sex expression and sex ratio in the Cucurbitaceae, making it of interest in plant breeding. In this taxonomic family, most types of sex expression are represented, from androecious to gynoecious. Monoecy is common; androecy and gynoecy are next; and hermaphroditism is rare. Most watermelon cultivars are monoecious, and the ratio of staminate to pistillate flower can be modified by climatic and environmental factors [1, 8-9]. Although sex expression in cucumber is determined genetically, it also can be modified by environment factors. High nitrogen fertility, short daylength, low light intensity, and low night temperature are among the factors that favor gynoecy. The reverse conditions tend to cause androecy [4].

The percentage of pistillate flowers and staminate flowers in watermelon was higher at 8 hour than at 16 hour daylength, and higher at 27°C than at 22°C or 32 °C [8]. Buttrose and

Sedgley reported that higher temperature caused increased ovary size. However, low light intensity and reduced daylength resulted in smaller ovaries in watermelon [1]. Heyer worked on sex ratio in cucumber and pumpkin (*Cucurbita* spp.), and found that the proportion of staminate to pistillate flowers could be changed by environmental conditions [5]. Low light intensity or short daylength resulted in increased gynoecey, and low temperature intensified this effect in *Cucurbita pepo* L. cv. Acorn, *Cucumis sativus* L. cv. Boston Pickling and *Cucumis anguria* L. gherkin. The modification of sex expression in cucurbits due to mineral nutrition has been reported by Tiedjens, Hall, Nitsch et al., and others [3, 7, 10]. The marked influence of light in changing the sex expression in cucurbits has been reported by Tiedjens, Whitaker, Currence and Nitsch et al. [2, 10, 13].

The objective of this experiment was to determine whether photoperiod and temperature affect sex expression in watermelon, and whether they affect the percentage and number of pistillate nodes, days to first flower, and vine length.



## Materials and Methods

Experiments were conducted under controlled environment conditions in the growth chambers of the Southeastern Plant Environment Laboratory (phytotron) at North Carolina State University [11]. The experiment was a randomized complete block design with four replications. Each of growth chamber consisted of 16 plots and each plot has two plants. There were two temperatures, two photoperiods, two fertility levels, and two cultivars. Four growth chambers were used to provide the different environmental conditions. The chambers were set (day/night) at 32/24°C (8 hr photoperiod), 32/24°C (16 hr photoperiod), 24/16°C (8 hr photoperiod), and 24/16°C (16 hr photoperiod). Fertility treatments consisted of fertilizing once a week for low nutrition and three times per week for high nutrition. ‘Cream Of Saskatchewan’ and ‘Congo’ were used to represent genetically diverse backgrounds for plant characteristics.

Seeds were sown in polyethylene bags (3 gallon) filled with a standard substrate of gravel and peat in a 1:1 ratio and placed in carts. Two seeds were sown in each bag, with 2 bags on each cart. After seeding the plants, the carts were placed in one of four growth chambers. Plants were fertilized with the standard phytotron nutrient solution [11] containing the following: 106.23 ppm  $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ,  $\text{NH}_4\text{NO}_3$ , and  $\text{KNO}_3$ , 10.41 ppm  $\text{KH}_2\text{PO}_4$  and  $\text{K}_2\text{HPO}_4$ , 111.03 ppm  $\text{KH}_2\text{PO}_4$ ,  $\text{K}_2\text{HPO}_4$ ,  $\text{K}_2\text{SO}_4$ , and  $\text{KNO}_3$  54.40 ppm  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ , 12.40 ppm  $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ , 5 ppm Sequestrene 330, 13.19 ppm  $\text{K}_2\text{SO}_4$  and  $\text{Na}_2\text{SO}_4$ , 0.113  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ , 0.24 ppm  $\text{H}_3\text{BO}_3$ , 0.013 ppm  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.005 ppm  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.00003 ppm  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ , 0.005 ppm  $\text{MoO}_3 \cdot 2\text{H}_2\text{O}$ , and 11.04 ppm  $\text{Na}_2\text{SO}_4$ . After plants were get the first flower, plants were rated number of flowers for

four weeks. Vine length was measured from soil line to shoot apex after four weeks ratings for number of flowers.

Data taken in the experiment included days to first flower, number of pistillate nodes, and vine length. Individual ratings were summarized as mean and maximum over replication and cultivars. Data were analyzed using the MEANS, ANOVA and GLM procedures of the SAS statistical package (SAS Institute, Cary, NC).

## Results and Discussion

The percentage of pistillate nodes remained fairly constant over the four weeks of measurement. Percentage of pistillate nodes on the first week was significantly affected by all three environmental factors: temperature, photoperiod, and fertility (Table 1). The percentage of pistillate nodes was higher with high fertilizer (3 times/week) than low fertilizer (1 time/week) at low temperature (24/16°C). Sedgley and Buttrose suggested temperature is the only environmental variable which altered the sex expression and the laterals, where present, had a higher staminate to pistillate ratio than the main shoot in most cases [1]. However, low temperature (24/16°C) and long-day (16 hr photoperiod) induced the most pistillate nodes in watermelon plants. However, photoperiod and fertilization with high temperature were not significantly difference effect on the watermelon sex expression (Table 1).

Although 'Cream Of Saskatchewan' and 'Congo' represented genetically diverse backgrounds, they were similar for days to first flower, as well as for number and percentage of pistillate nodes. However, days to first flower was affected by all three environmental factors (temperature, photoperiod, and fertilization). Days to first flower was reduced at high temperature, however short-day increase the days to first flower at low temperature. Both of cultivars have the shortest days to first flower at high temperature and long-day condition (Table 2). Therefore, we assumed early flowering is induced by high temperature and long-day condition in watermelon.

Measurement of vine length was significantly difference with fertilization at low temperature (24/16°C) condition. High fertilization increase vine length regardless of

photoperiod at low temperature, however fertilizer was not effect on vine length at high temperature condition (32/24°C) (Table 2). Hideyuki et al. found the growth of plants is greatly reduced under long-day in cucumber [7]. Similarly, the vine length and the percentage of pistillate nodes was reversely affected from photoperiod in watermelon. Short-day (8 hr photoperiod) increase the vine length at both of temperature conditions, however decrease the percentage of pistillate nodes (Table 2 and Figure 1, 2).

In conclusion, temperature was the main effect on the sex expression and days to first flower, while fertilizer was the main effect on the vine length. However, photoperiod had a synergy effect with other environmental factors rather than direct effect on the sex expression in watermelon.

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Table 1. Percentage and number of pistillate nodes for watermelons grown under four different environmental conditions (temperature and daylength).

Temp. Day (°C) length	Fertilizer (times/week)	<u>% of pistillate nodes</u>				<u>No. of pistillate nodes</u>			
		Wk1	Wk2	Wk3	Wk4	Wk1	Wk2	Wk3	Wk4
32/24 16	High	11	17	17	18	2	5	6	8
	Low	12	13	17	16	2	3	4	6
	8 High	9	15	17	18	1	3	4	6
	Low	17	18	18	18	2	3	5	6
24/16 16	High	16	19	19	19	3	4	5	7
	Low	23	23	22	24	2	3	4	6
	8 High	6	9	13	14	1	1	3	4
	Low	14	18	19	20	1	2	3	4
LSD (5 %)		11	7.5	6.5	5.3	1.4	1.4	1.5	1.6

<sup>z</sup> Temperatures were (day/night) at 24/16°C (16 hr), and 24/16°C (8 hr) 32/24°C (16 hr), 32/24°C (8 hr); high fertilizer was 3 times per week and low fertilizer was 1 time per week. Data are means of 4 replications and 2 cultivars. Plants were rated beginning at first flower, and continuing for four weeks.

Table 2. Days to first flower, percentage pistillate nodes, number of pistillate nodes, and vine length for watermelons grown under four different environmental conditions (temperature and daylength).<sup>z</sup>

Temp.	Day	Fertilizer	Days to	Vine	<u>Pistillate nodes (week 4)</u>	
(°C)	length	(times/week)	1st flower	length(m)	%	Number
32/24	16	High	26	1.4	18	8
		Low	28	1.2	16	6
	8	High	30	3.4	18	6
		Low	29	2.3	18	6
24/16	16	High	34	2.1	19	7
		Low	38	1.0	24	6
	8	High	51	2.4	14	4
		Low	61	1.3	20	4
LSD (5%)			4.5	0.7	5.3	1.6

<sup>z</sup> Temperatures were (day/night) at 24/16°C (16 hr), and 24/16°C (8 hr) 32/24°C (16 hr), 32/24°C (8 hr); high fertilizer was 3 times per week and low fertilizer was 1 time per week. Data are means of 4 replications and 2 cultivars. Vine length was measured from soil to shoot apex at week 4. Data shown for pistillate nodes was rating 4 (week 4).





Figure 1. Watermelon grown at 32/24°C/8h (B3 chamber), and 32/24°C/16h (B4 chamber).



Figure 2. Watermelon grown at 24/16°C/8h (B7 chamber), and 24/16°C/16h (B8 chamber).

## **Growth regulator-induced parthenocarpic fruit set in triploid watermelon**

## Abstract

Previous research has shown that parthenocarpic fruit set is stimulated by certain growth regulators applied to the pistillate flowers of triploid seedless watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] planted in isolation from diploid pollen. The most effective growth regulators from previous studies included naphthalene acetic acid (NAA), 2,4-dichlorophenoxyacetic acid (2,4-D), and 2-chloro-4-pyridyl-N-phenylurea (CPPU). When CPPU was applied to pistillate flowers, fruit were set. However, when the whole plant was treated using a backpack sprayer in 2005, the plants were injured or killed. Therefore in 2006, we applied three growth regulators (CPPU, NAA, and 2,4-D) in combination and used a low concentration (50 mg/kg) of each. The combination of CPPU, NAA, and 2,4-D 50 mg/kg was most effective on watermelon fruit set when applied using a hand sprayer compared with other combinations or the control. In order to increase fruit set and yield in 2007, we applied growth regulators in combination with and without CPPU for 1, 2, 3, and 4 weeks (2 times/week). We used a backpack sprayer to simulate tractor spraying. The combination without CPPU (0 mg/kg CPPU, 100 mg/kg NAA, and 50 mg/kg 2,4-D) gave the most watermelon fruit set. Other traits of watermelon fruit were not affected uniformly. After two weeks (2 times/week) of spraying, there was damage evident on the whole plant. Treatments with CPPU had more severe damage than those without CPPU. In conclusion, the combination of CPPU, NAA, and 2,4-D 50 mg/kg is the best application for watermelon fruit set. However, more than one week of treatment causes plant damage.



## Introduction

Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] is a major vegetable crop in the U.S., and is increasing in popularity. Production increased from 1.1 million Mg•year<sup>-1</sup> in 1980 to 1.9 million Mg•year<sup>-1</sup> in 1995 [15]. Watermelon consumption in the U.S. is 6.4 kg per capita [24].

There is a great deal of interest in hybrid seedless watermelons in the U.S. because of improved quality for consumers. In order to produce seedless cultivars, watermelon breeders have to make a tetraploid from the female diploid inbred. The process takes several years and results in low seed production and high seed costs. In order to make hybrids, the two inbred lines have to be hand pollinated, further increasing seed costs. An alternative to production of seedless cultivars using triploids (tetraploid x diploid crosses) would be parthenocarpy, the method used in cucumber (*Cucumis sativus* L.). Cucumber is a close relative of watermelon in the *Cucurbitaceae* family, so it may be possible to use parthenocarpy in watermelon.

Generally, field production of seedless watermelon requires that one diploid seeded watermelon should be planted for every four to five triploid seedless watermelon [11]. It is recommended that one row of pollinizer should be planted for every two or four rows of seedless watermelon to provide adequate pollen for yield [14, 18-20]. Additionally, bees are important pollinators of fruit set to triploid seedless watermelon production. However, fruit set in watermelon is unstable at low temperatures and under cloudy or rainy weather, as the activity of flower-visiting insects is sluggish and dehiscence of anthers is hindered [23].

Parthenocarpy is used to produce seedless fruit in banana, citrus, vinifera grape, Chinese persimmon, and greenhouse cucumber. Induced or stimulated parthenocarpy in many species and varieties has been observed to occur as a result of certain internal and external influences. Parthenocarpic fruits, induced by means of hormones in lanolin paste, have been obtained by Gustafson in tomato, pepper, summer squash, hubbard squash, eggplant and some ornamental plants [7]. Several attempts were made to induce fruit set in watermelon and pumpkins, but it was failed. Garner and Marth obtained parthenocarpic fruits in 'American holly' and strawberry by spraying the pistillate flowers with different concentrations of hormones [4]. Wang found that indoleacetic and indol-propionic acid stimulated the formation of orange fruit, although parthenocarpy did exist in both seedless and seeded cultivars of oranges in Florida [25]. Wang conducted an experiment with 'National Pickling' cucumber in an attempt to produce seedless fruit by means of growth-promoting substances. It was found that naphthalene acetic acid (NAA) caused parthenocarpic fruits either when applied in lanolin paste of 1 to 5 % concentrations or as a 0.05 % aqueous solution. The percentage of fruit set in hormone-treated flowers was higher than from self pollination [26].

With watermelon, both indolebutyric acid (BA) and NAA have been used. Hormone-treated watermelons were seedless but varied in fruit shape. In general, the hormone-treated fruit were triangular in shape. Some, however, were normal in shape and size. The fruit were solid and firm. No differences in flavor could be detected from normally-pollinated fruits. Cantliffe studied hormones to promote fruit set on watermelon in the presence or absence of pollination with growth regulator sprays [3]. Fruit set was induced in non-

pollinated ovaries of 'Sugar Baby' by application of IAA and NAA and a combination of these auxins with chlorflurenol. Chlorflurenol alone did not induce fruit set. Size and weight of the parthenocarpic fruit were similar to pollinated controls. However, fruit shape was irregular unless chlorflurenol was used. Without pollination, fruit set was increased by growth regulators over pollinated controls. Pak tested plant growth regulators individually or as mixtures of two or three hormones to assess capability for production of seedless watermelon [17]. A mixture of 50 % tomatotone plus GA<sub>3</sub> at 2000 mg/kg and a mixture of 50 % tomatotone plus BA at 1000 mg/kg were applied to the ovary and peduncle. Both of these treatments were found to be the best methods among all treatments throughout the experiment for producing good quality parthenocarpic fruits.

The plant growth regulator CPPU (2-chloro-4-pyridyl-N-phenylurea), a newly developed urea-derivative cytokinin, promotes grape berry growth [16], prevents grape berry shatter [22], prevents pear thickening [2], prevents kiwifruit thickening [9], and increases fruit set in melon [6, 8]. Recently, it was reported that CPPU could induce parthenocarpy in melon [6, 22]. Hayata and Niimi reported that CPPU treatment (20 and 200 mg/kg) reduced the proportion of normal seeds in pollinated fruit, and increased the percentage of fruit set in pollinated and non-pollinated fruit [8]. CPPU was applied to the fruits at anthesis [10].

The objective of this study was to identify a method for using growth regulators to induce parthenocarpic fruit set in triploid seedless watermelon without using pollenizers or bees.

## Materials and Methods

Field tests were run to evaluate plant growth regulators for parthenocarpic fruit setting ability in triploid watermelon. Tests were run at the Horticultural Crops Research Station at Clinton, North Carolina. Transplants were used to plant in the field since triploid watermelons are difficult to germinate. The experiment was conducted in the spring of 2005, 2006 and 2007. The design was a randomized complete block with four replications. Rows were covered with black polyethylene mulch and drip irrigated. The experiment was conducted using recommended practices [21] .

Watermelon seedlings were grown in the greenhouse. Seeds were sown in the trays (11" x 21.5") filled with a soil mix (Metro-Mix 300) and placed in a germination mat that kept the soil temperature at 85°F for proper emergence. Seeds were not watered until seedlings emerged to prevent waterlogging of the soil. Greenhouse temperature was set at 85/65°F day/night. After four to six weeks seedlings were moved to cold frames to harden them and prepare them for transplanting to the field.

In order to evaluate phytotoxicity of CPPU, NAA, and 2,4-dichlorophenoxyacetic acid (2,4-D) on watermelons, we tested four cultigens ('Sugar Baby', 'Calhoun Gray', 'PI 244018', and 'PI 595203') with five concentrations (100, 200, 400, 800, and 1600 mg/kg) in the greenhouse. The plants were treated three times starting at the cotyledon stage. One week after applying growth regulator treatments, shoot damage was rated for phytotoxicity on a scale of 0 to 9: 0 = no damage, 1-2 = trace of damage, 3-4 = slight damage, 5-6 = moderate damage, 7-8 = advanced damage, 9 = plant dead.



In 2005, growth regulators were applied to four triploid cultivars ('Tri-X-313', 'Millionaire', 'SS 5244', and 'Petiteperfection') 4, 5, and 6 weeks after transplanting. Pistillate flowers were treated with three growth regulators (CPPU, 2,4-D, and NAA) and six concentrations (12, 25, 50 mg/kg with 50 mg/kg NAA). Three seasons (spring isolation, spring with row covers, summer isolation) and two replications were conducted at Clinton research station. Spray methods were hand sprayer (pistillate flower treatment) and backpack sprayer (whole plant treatments) (Fig. 1 and 2).

In 2006, we applied to four triploid cultivars ('Tri-X-313', 'Millionaire', 'SS 5244', and 'Petiteperfection') with three growth regulators (CPPU, NAA, and 2,4-D) in combination at 50 mg/kg each. We used backpack sprayer and hand sprayer as application methods.

In 2007, we applied to two triploid cultivars ('Tri-X-313' and 'Petiteperfection') with growth regulators in combination without CPPU (0 mg/kg CPPU, 100 mg/kg NAA, and 50 mg/kg 2,4-D) or with CPPU (50 mg/kg CPPU, 100 mg/kg NAA, and 50 mg/kg 2,4-D) to increase fruit set and yield. Growth regulators were applied 1, 2, 3, and 4 weeks (2 times/week). We used a backpack sprayer to simulate tractor spraying.

Data was collected by plot for fruit weight, fruit number, weight per fruit, and fruit defect traits (percentage of seeded, tetraploid, and hollow fruit) using one or two harvests. To measure the defect traits, every fruits were cut and look inside fruit of seeded and hollow. We determine whether triploid or tetraploid by fruit rind color, pattern and size. Individual ratings were summarized as mean and maximum over

replications and harvests. Data were analyzed using the MEANS, ANOVA and GLM procedures of the SAS statistical package (SAS Institute, Cary, NC).

## Results and Discussion

The growth regulators 2,4-D and CPPU were most damaging to watermelon seedlings, with plants killed by the time of rating 3. NAA was moderately damaging to the plants with lower concentrations of 25 to 50 mg/kg (Table 1). Using backpack sprayer, CPPU 400 mg/kg cause severe damage (plants dead) on watermelon plants with four weeks treatment (Figure 3). The plant growth regulators in combination without CPPU (0 mg/kg CPPU, 100 mg/kg NAA, and 50 mg/kg 2,4-D) or combination with CPPU (50 mg/kg CPPU, 100 mg/kg NAA, and 50 mg/kg 2,4) was damage to watermelon plants with four weeks of treatment (Table 9).

The three growth regulators (NAA, 2,4-D, and CPPU) were effective to fruit set in 2005. CPPU with hand sprayer was the most effective in setting fruit of seedless watermelon in spring season (Table 2). The highest yield was obtained with 200 mg/kg CPPU and 100 mg/kg NAA combination by using hand sprayer in summer season (Table 3). When growth regulators were applied to the pistillate flowers, fruit were set with no phytotoxicity evident. However, when the whole plant was treated using a backpack sprayer to simulate tractor spraying, the plants were injured or killed (Table 3 and Figure 3). The control treatment (without growth regulators) was damaged with rating 3 or 4 that caused by natural environment conditions (wind, rain, storm etc.) or physical condition (harvest labor) (Table 3). The percentage of culls were reduced with CPPU and NAA combination treatments, however, single treatment of CPPU, 2,4-D, or NAA increase the percentage of culls (Table 2 and 3).

In 2006, fruit defect traits (percentage of seeded, tetraploid, and hollow fruit) were not significantly affected by growth regulator treatment (Table 4). As in 2005, the hand sprayer was more effective than the backpack sprayer for watermelon fruit set. Fruit set was greatest using the backpack sprayer with the combination of NAA at 50 mg/kg and 2,4-D at 50 mg/kg in spring (Table 5) and summer (Table 6) seasons. However, the percentage of culls was increased by that treatment in the spring (Table 5) but not the summer (Table 6) season. The combination of CPPU, NAA, and 2,4-D at 50 mg/kg each was most effective on watermelon fruit set when applied using a hand sprayer compared with other combinations or the control (Table 5 and 6). Treatment with CPPU at 50 mg/kg alone was also effective for fruit set, but yield was lower than with the combination of CPPU, NAA, and 2,4-D at 50 mg/kg each. The percentage of culls was reduced slightly, but not significantly, when using CPPU at 50 mg/kg alone compared to the three-growth regulator combination in the spring (Table 5) and summer (Table 6) seasons. Using the backpack sprayer on field-grown plants, the highest concentration of CPPU caused severe damage after four weeks of treatment (Fig. 3).

As in 2006, the treatment without CPPU was most effective. Similarly, the combination without CPPU (0 mg/kg CPPU, 100 mg/kg NAA, and 50 mg/kg 2,4-D) induced the most watermelon fruit set. However, yield in 2007 was lower than in 2006 (Table 7). Other traits of watermelon fruit were not affected uniformly (Table 8). Vine damage was rated 2 and 4 weeks after treatment of the plants with growth regulators. Two weeks after growth regulator treatment (2 times/week), plant damage was evident. Treatments with CPPU had more severe damage than those without. In the second rating, both growth

regulator treatments (with or without CPPU) caused significant injury to the watermelon plants (Table 9). CPPU was the most effective in setting fruit on the watermelon plants. However, the highest yield was obtained with 200 mg/kg CPPU combined with 100 mg/kg NAA. When growth regulators were applied to the pistillate flowers, fruit were set. However, when the whole plant was treated using a backpack sprayer to simulate tractor spraying, the plants were injured or killed.

Therefore, the combination of CPPU, NAA, and 2,4-D 50 mg/kg was most effective on watermelon fruit set when applied using a hand sprayer compared with other combinations or the control. Treatment with CPPU at 50 mg/kg by itself was also effective on fruit set, but yield was lower than with the combination of CPPU, NAA, and 2,4-D at 50 mg/kg each. More than one week of treatment causes damage to the plants. In conclusion, the combination of CPPU, NAA, and 2,4-D at 50 mg/kg each was the most effective treatment for watermelon fruit set.

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Table 1. Phytotoxicity for watermelon seedlings (six true-leaf stage) grown in the greenhouse and treated with 2,4-D, CPPU, and NAA.<sup>z</sup>

Growth regulator	Treatment (mg/kg)	Phytotoxicity rating				
		Mean	Maximum	Rating 1	Rating 2	Rating 3
2,4-D	25	6.1	7.8	4.3	6.3	7.8
	50	6.4	8.3	4.4	6.5	8.3
	100	6.9	9.0	4.8	7.0	9.0
	200	7.0	8.8	5.4	6.9	8.8
	400	7.3	9.0	5.6	7.3	9.0
CPPU	25	4.9	6.3	3.6	4.9	6.1
	50	6.0	7.9	4.4	5.8	7.9
	100	6.8	8.6	4.3	7.5	8.6
	200	6.9	9.0	4.0	7.8	9.0
	400	7.8	9.0	6.8	7.8	9.0
NAA	25	4.8	5.6	3.8	5.0	5.6
	50	5.1	6.3	3.9	5.1	6.3
	100	5.7	7.3	4.5	5.4	7.3
	200	5.6	6.8	4.8	5.4	6.6
	400	6.3	8.3	4.5	6.3	8.3
Check	0	2.1	3.3	2.4	1.5	2.5

Table 1 (continued)

LSD (5%)	0.2	0.3	0.3	0.2	0.3
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<sup>z</sup> Data are means of 2 replications and 4 cultivars. Leaf damage rated 0-9 (0=no damage, 9=plant dead).

Table 2. Marketable and total yield (fruit weight and number), percentage culls, percentage seeded fruit, percentage tetraploid fruit, and weight per fruit of parthenocarpic watermelons treated with CPPU, NAA, and 2,4-D growth regulators using a hand sprayer for spring season (No-cover) in 2005.<sup>Z</sup>

Treatment	Mark.	Total	Mark.	Total	Cull	Seeded	4X	Fruit wt.
(mg/kg)	(Mg/ha)	(Mg/ha)	(Th/ha)	(Th/ha)	(%)	(%)	(%)	(kg/fruit)
<b>Hand spray (ovary only)</b>								
Check 0	0.7	1.2	0.2	0.3	13	4	0	3.2
Check Tw20	0.0	0.7	0.0	0.1	13	7	0	-
NAA 12	0.0	0.5	0.0	0.1	13	0	0	-
25	3.6	7.6	0.6	1.2	41	0	0	6.4
50	5.5	15.2	1.0	2.6	62	3	0	5.3
100	7.5	16.0	0.7	1.7	33	1	0	9.7
200	3.0	12.5	0.4	2.0	63	3	2	6.5
400	0.0	11.2	0.0	1.5	63	0	0	-
2,4 D 12	3.0	10.5	0.7	1.7	48	4	0	5.2
25	1.7	14.4	0.4	2.2	48	2	0	4.0
50	7.3	34.7	1.5	3.3	58	1	0	6.5
100	19.3	47.2	3.5	5.6	35	0	0	6.0
200	8.2	24.6	1.8	4.6	54	3	1	5.8
400	15.5	25.3	2.7	4.7	38	1	0	6.1

Table 2 (continued)

CPPU 12	3.4	9.2	0.3	1.0	56	2	0	10.2
25	3.3	13.2	0.6	1.6	48	2	0	5.9
50	14.7	42.2	2.3	4.2	63	2	0	6.3
100	16.6	38.2	2.6	5.6	54	4	1	7.0
200	15.0	41.9	1.7	4.6	65	3	0	8.9
400	26.4	56.9	3.3	5.9	39	3	0	8.3
LSD (5%)	18	46	2.1	3.8	88	5	3	1.5

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<sup>z</sup> Data are means of 2 replications and 4 cultivars.

Table 3. Yield of parthenocarpic watermelons treated with CPPU and NAA treatments applied by backpack and hand sprayer for summer season (Isolation) in 2005.<sup>z</sup>

Treatment (mg/kg)		Mark.	Mark.	Cull	Fruit wt.	Damage rating	
CPPU	NAA	(Mg/ha)	(Th/ha)	(%)	(kg/fruit)	Harv.1	Harv.2
<b>Hand spray (ovary only)</b>							
0	0	0.0	0.0	6	-	0.0	3.1
	50	5.2	1.3	2	4.2	1.0	3.3
	100	4.1	1.3	16	3.5	2.0	2.9
100	0	7.4	2.2	13	3.6	1.0	4.3
	50	8.5	2.9	23	3.0	1.1	6.3
	100	6.9	2.9	18	2.6	2.0	3.9
200	0	5.1	1.7	28	3.5	1.0	6.6
	50	8.8	2.7	22	3.5	1.8	4.8
	100	9.4	3.5	12	2.8	1.1	5.5
400	0	8.3	3.1	22	2.8	1.0	7.4
<b>Backpack spray (whole plant)</b>							
0	0	0.4	0.1	0	3.6	1.8	4.3
	50	1.6	0.8	18	2.2	2.0	3.9
	100	1.0	0.4	16	2.3	1.5	4.3
100	0	0.0	0.0	0	-	6.0	9.0
	50	0.0	0.0	0	-	6.0	9.0

Table 3 (continued)

	100	0.0	0.0	0	-	6.0	9.0
200	0	0.0	0.0	0	-	6.9	9.0
	50	0.0	0.0	0	-	7.0	9.0
	100	0.0	0.0	0	-	7.9	9.0
400	0	0.0	0.0	0	-	7.3	9.0
LSD (5%)		7.2	2.6	33	1.6	1.9	2.6

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<sup>z</sup> Data are means of 2 replications and 4 cultivars. Plants were rated using a 0 to 9 scale (0=no damage, 1-2=trace, 3-4=slight, 5-6=moderate, 7-8=advanced, 9=dead).

Table 4. Cull, seeded, tetraploid and hollow fruit from parthenocarpic watermelons treated with combinations of CPPU, NAA, and 2,4-D growth regulators using a backpack sprayer or hand sprayer in the spring season at Clinton, NC (2006).<sup>z</sup>

Treatment (mg/kg)			Seeded	4X	Hollow	Fruit wt.
CPPU	NAA	2,4 D	(%)	(%)	(%)	(kg/fruit)
<b>Backpack sprayer</b>						
0	0	0	2	1	1	3.5
		50	3	1	1	2.3
	50	0	0	0	0	4.0
		50	1	0	0	3.1
50	0	0	2	0	0	2.4
		50	2	0	0	2.2
	50	0	3	0	0	2.6
		50	2	1	1	2.9
<b>Hand sprayer</b>						
0	0	0	3	0	0	3.8
		50	1	0	0	4.8
	50	0	0	1	1	3.7
		50	0	0	0	4.3
50	0	0	4	1	1	5.8
		50	3	1	1	4.8

Table 4 (continued)

50	0	2	0	0	4.2
	50	4	0	0	4.3
LSD (5%)		5	2	2	1.5

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z Weight per fruit, percentage seeded, tetraploid and hollowheart rating (rated over 4 harvests).



Table 5. Total, marketable and cull yield and weight per fruit of parthenocarpic watermelons treated with combinations of CPPU, NAA, and 2,4-D growth regulators using a backpack sprayer or hand sprayer in the spring season at Clinton, NC (2006).<sup>z</sup>

Treatment (mg/kg)			Total	Mark.	Mark.	Culls	Fruit wt.
CPPU	NAA	2,4-D	(Mg/ha)	(Mg/ha)	(Th/ha)	(%)	kg/fruit
<b>Backpack sprayer</b>							
0	0	0	8.4	6.5	1.5	1	3.5
		50	6.8	6.1	2.8	10	2.3
	50	0	14.8	14.4	3.6	0	4.0
		50	20.5	19.0	5.8	17	3.1
50	0	0	12.9	12.2	4.2	2	2.4
		50	4.0	3.4	1.6	5	2.2
	50	0	10.7	10.6	3.9	0	2.6
		50	13.9	12.9	4.2	9	2.9
<b>Hand sprayer</b>							
0	0	0	4.1	3.8	1.1	3	3.8
		50	26.4	25.4	5.5	7	4.8
	50	0	7.8	6.4	1.9	2	3.7
		50	26.7	25.1	5.8	10	4.3
50	0	0	46.4	46.3	8.3	3	5.8
		50	37.4	34.4	7.5	18	4.8

Table 5 (continued)

50	0	40.3	38.2	9.4	6	4.2
	50	63.8	62.0	14.8	12	4.3
LSD (5%)		20	20	5.0	17	1.5

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z Weight per fruit, percentage seeded, tetraploid and hollowheart rating (rated over 4 harvests).

Table 6. Yield of parthenocarpic watermelons treated with combinations of CPPU, NAA, and 2,4-D growth regulators using a backpack sprayer or hand sprayer in the summer season at Clinton, NC (2006).<sup>z</sup>

Hormone (mg/kg)			Mark.	Mark.	Cull	Fruit wt.
CPPU	NAA	2,4-D	(Mg/ha)	(th/ha)	(%)	(kg/fruit)
<b>Backpack sprayer</b>						
0	0	0	0.0	0.0	0	.
		50	2.9	2.2	31	1.6
	50	0	0.5	0.6	0	0.8
		50	2.1	1.4	15	1.3
50	0	0	0.2	0.2	20	0.9
		50	1.5	1.5	0	1.0
	50	0	0.0	0.0	0	.
		50	0.0	0.0	0	.
<b>Hand sprayer</b>						
0	0	0	0.0	0.0	0	.
		50	0.8	0.9	20	0.9
	50	0	0.0	0.0	0	.
		50	0.1	0.3	38	0.5
50	0	0	5.2	2.4	27	2.4
		50	2.2	0.8	52	2.7

Table 6 (continued)

50	0	2.9	1.6	0	1.6
	50	5.7	6.0	30	1.1
LSD (5%)		5.6	3.6	62	1.9

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<sup>z</sup> Yield, percentage culls (one harvest).

Table 7. Yield of parthenocarpic watermelons treated with combinations of CPPU, NAA, and 2,4-D growth regulators using a backpack sprayer in the spring season at Clinton, NC (2007).<sup>z</sup>

Growth regulator (mg/kg)	Weeks of trt	Total (Mg/ha)	Mark. (Mg/ha)	Mark. (Th/ha)	Total (Th/ha)	Fruit wt. (kg/fruit)
<b>Check (Tween 20)</b>	0	8.3	8.0	1.9	1.9	4.1
<b>Without CPPU (0)</b>						
NAA100 2,4 D 50	1	13.3	13.1	3.3	3.3	3.9
	2	8.0	7.5	2.2	2.3	3.3
	3	2.1	1.9	0.8	0.8	2.4
	4	1.3	1.1	0.5	0.5	2.0
<b>With CPPU (50)</b>						
NAA100 2,4 D 50	1	7.5	7.2	2.3	2.3	3.2
	2	3.3	3.2	1.5	1.5	2.4
	3	1.7	1.4	0.8	0.8	1.6
	4	0.2	0.1	0.2	0.2	0.7
LSD (5%)		7.0	6.9	1.8	2.0	2.7

<sup>z</sup> Yield (summed over 2 harvests). Data means over 4 cultivars and 4 replications.

Table 8. Cull, seeded, tetraploid and hollow fruit from parthenocarpic watermelons treated with combinations of CPPU, NAA, and 2,4-D growth regulators using a backpack sprayer in the spring season at Clinton, NC (2007).<sup>z</sup>

Growth regulator (mg/kg)	Weeks of trt	Cull (%)	Seeded (%)	4X (%)	Hollow (%)
<b>Check (Tween 20)</b>	0	5	0	1	1
<b>Without CPPU (0)</b>					
NAA100 2,4 D50	1	2	0	0	0
	2	6	1	0	0
	3	10	1	0	0
	4	9	0	0	0
<b>With CPPU (50)</b>					
NAA100 2,4 D50	1	6	0	1	1
	2	1	0	1	1
	3	7	6	0	0
	4	9	0	0	0
LSD (5%)		20	8	4	4

<sup>z</sup> Percentage cull, seeded, tetraploid and hollowheart rating (means of 2 harvests).

Table 9. Damage of watermelons treated with combinations of CPPU, NAA, and 2,4-D growth regulators using a backpack in the spring season at Clinton, NC (2007).<sup>z</sup>

Growth regulator (mg/kg)	Weeks of trt	Mark. (Mg/ha)	Mark. (Th/ha)	Cull (%)	<u>Damage rating</u> DM DM1 DM2		
<b>Check (Tween 20)</b>	0	8.0	1.9	5	0.0	0.0	0.0
<b>Without CPPU (0)</b>							
NAA100 2,4 D50	1	13.1	3.3	2	2.8	1.4	4.1
	2	7.5	2.3	6	3.4	1.6	5.3
	3	1.9	0.8	10	3.7	1.3	6.1
	4	1.1	0.5	9	4.0	1.3	6.7
<b>With CPPU (50)</b>							
NAA100 2,4 D50	1	7.2	2.3	6	4.0	4.1	3.9
	2	3.2	1.5	1	4.3	3.8	4.9
	3	1.4	0.8	7	4.9	4.0	5.7
	4	0.1	0.2	9	4.8	3.8	5.7
LSD (5%)		6.9	1.8	20	0.7	0.7	1.3

<sup>z</sup> Damage rated 0-9 on true leaves (0=no damage, 9=plant dead), with ratings made 2 and 4 weeks (after growth regulator treatment was begun).



Figure 1. Using hand sprayer on pistillate flowers in watermelon plants.





Figure 2. Using backpack sprayer on whole watermelon plants.



Figure 3. Using backpack sprayer with the highest concentration of CPPU caused severe damage (plants dead) on watermelon plants.

## **Production of seedless watermelons using plant growth regulators in diploid cultivars**

### **Abstract**

Seedlessness is an important breeding objective for watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai]. This study was conducted to determine whether parthenocarpic fruit set could be induced in diploid watermelon using plant growth regulators. The experiment involved four cultivars ('Mickylee', 'Sugar Baby', 'Crimson Sweet', and 'Charleston Gray') treated with combinations of CPPU, NAA, and 2,4-D. Pistillate flowers were treated with the combination of CPPU at 50 mg/kg, NAA at 100 mg/kg, and 2,4-D at 50 mg/kg in the absence of bee pollination.

## Introduction

Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] is a major vegetable crop in the U.S., and is increasing in popularity. Production increased from 1.1 million Mg•year<sup>-1</sup> in 1980 to 1.9 million Mg•year<sup>-1</sup> in 1995 [12]. Watermelon consumption in the U.S. is 6.4 kg per capita [16].

Parthenocarpy is used to produce seedless fruit in banana, citrus, vinifera grape, Chinese persimmon, and greenhouse cucumber. Induced or stimulated parthenocarpy in many species and varieties has been observed to occur as a result of certain internal and external influences. Parthenocarpic fruits, induced by means of hormones in lanolin paste, have been obtained by Gustafson in tomato, pepper, summer squash, hubbard squash, eggplant and some ornamental plants. Several attempts were made to induce fruit set in watermelon and pumpkins, but failed [6,7].

Fruit set in watermelon is unstable at low temperatures and under cloudy or rainy weather, as the activity of flower-visiting insects is sluggish and dehiscence of anthers is hindered [15]. The percentage of fruit set in hormone-treated flowers was higher than from self pollination. Cantliffe studied hormones to promote fruit set on watermelon in the presence or absence of pollination with growth regulator sprays [3]. Pak tested plant growth regulators individually or as mixtures of two or three hormones to assess capability for production of seedless watermelon. A mixture of 50% tomatotone plus GA<sub>3</sub> at 2000 mg/kg and a mixture of 50% tomatotone plus BA at 1000 mg/kg were applied to the ovary and peduncle. Both of these treatments were found to be the best

methods among all treatments throughout the experiment for producing good quality parthenocarpic fruits [14] .

The plant growth regulator CPPU (2-chloro-4-pyridyl-N-phenylurea), a newly developed urea-derivative cytokinin, promotes grape berry growth [13], prevents grape berry shatter [16], prevents pear thickening [2], prevents kiwifruit thickening [9], and increases fruit set in melon [5, 8]. Recently, it was reported that CPPU could induce parthenocarpy in melon [5, 10]. Hayata and Niimi reported that CPPU treatment (20 and 200 mg/kg) reduced the proportion of normal seeds in pollinated fruit, and increased the percentage of fruit set in pollinated and non-pollinated fruit [8].



## Materials and Methods

Field tests were run to evaluate plant growth regulators for parthenocarpic fruit setting ability in diploid watermelon. Tests were run in spring, 2007 at the Horticultural Crops Research Station at Clinton, North Carolina. The experiment was a factorial in a randomized complete block design with 4 replications, 4 cultivars, and 2 growth regulator treatments. Cultivars were 'Mickylee', 'Sugar Baby', 'Crimson Sweet', and 'Charleston Gray'. Growth regulator treatment consisted of a water control vs. a mixture, consisting of CPPU at 50 mg/kg, NAA at 100 mg/kg, and 2,4-D at 50 mg/kg.

Recommended cultural practices were used [15] that 10' plots, 6' alleys, and 6 hills of 1 plant each (stake 6-hills 2-blanks). 3 seeds per hill were direct-seeded on raised beds covered with black polyethylene mulch with drip irrigation.

After four weeks of seeded, plant growth regulators were applied directly to the pistillate flowers 2 times per week for 4 weeks. Plant growth regulators were applied to the ovaries of pistillate flowers using a hand-pumped sprayer. In order to prevent pollination by honeybees and other insects, the watermelons were covered with polyester spunbonded floating row covers before they began to flower.

Individual ratings were summarized as mean and maximum after summarizing over replication and harvest. Data were analyzed using the MEANS, ANOVA and GLM procedures of the SAS statistical package (SAS Institute, Cary, NC).

## **Results and Discussion**

Fruit set in diploid watermelons, plant growth regulators applied to the pistillate flowers of diploid watermelon planted in isolation (no diploid pollen available) induced fruit set. In previous studies, CPPU was the most effective in setting fruit of seedless triploid watermelon cultivars. However, when CPPU and NAA were used in combination, the highest yield was obtained. When the plant growth regulators were applied to the pistillate flowers, fruit were set. The most effective plant growth regulator treatment from previous studies for setting triploid watermelon fruit was used in this study. It consisted of a mixture of CPPU at 50 mg/kg, NAA at 100 mg/kg, and 2,4-D at 50 mg/kg.

In order to increase parthenocarpic fruit set and yield in 2007, we applied plant growth regulators in combination (50 mg/kg CPPU, 100 mg/kg NAA, and 50 mg/kg 2,4-D) with control (water+tween-20) for 4 weeks (2 times/week). We used a hand sprayer to spray on the pistillate flowers in diploid watermelon cultivars. Compared with the control treatment (water+tween-20), the mixture of plant growth regulators increased parthenocarpic fruit set of diploid watermelon cultivars when isolated from pollen (Table 1 and Figure 1). Fruit set was poor in the first harvest, probably because we did not begin applying growth regulators soon enough. Therefore, data are presented only for fruit set in the second harvest.

Preliminary results suggest that the mixture of plant growth regulators not only might be substitution for pollination with bees in diploid watermelon production but induce seedless diploid watermelon. More research is needed to determine the diploid



parthenocarpic fruit set and increase yield with plant growth regulators without bee pollination.

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Table 1. Marketable fruit weight, marketable fruit number, percentage culls, percentage seeded fruit, and weight per fruit from watermelons isolated from bees and treated with a combination of CPPU, NAA, and 2,4-D growth regulators.<sup>z</sup>

Growth regulator (mg/kg)	Watermelon cultivar	Mark. (Mg/ha)	Mark. (Th/ha)	Cull (%)	Seeded (%)	Fruit wt. (kg/fruit)
CPU00N000D00	Charleston Gray	7.3	1.1	0	7	6.7
	Crimson Sweet	3.4	1.1	0	13	3.6
	Mickylee	1.5	1.1	0	33	1.4
	Sugar Baby	6.5	3.0	0	21	2.2
CPU50N100D50	Charleston Gray	17.0	3.8	6	6	4.5
	Crimson Sweet	6.8	2.7	0	6	2.4
	Mickylee	20.5	10.8	13	3	1.9
	Sugar Baby	15.0	10.0	7	3	1.5
LSD (5%)		13.4	3.8	18	6	2.5

<sup>z</sup> Percentage cull, seeded, and hollowheart rating (second harvest only). Growth regulator treatment consisted of 50 mg/kg CPPU, 100 mg/kg NAA and 50 mg/kg 2,4D applied directly to the pistillate flowers 2 times per week for 4 weeks; the control treatment received no growth regulators.



Figure 1. Parthenocarpic fruit set in diploid watermelon 'Sugar Baby' resulting from treatment with a combination of growth regulators (CPPU at 50 mg/kg, NAA at 100 mg/kg, and 2,4-D at 50 mg/kg).

## **Plant growth regulators and their effect on sex expression in watermelon**

## Abstract

This study was to identify treatment with plant growth regulators that would convert monoecious watermelons [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] to gynoecious phenotype. We evaluate ten different plant growth regulators (AVG, Ethrel, NAA, B9, Cyclinilide, Chlomaquat, Uniconazol, Paclobutrazol, Brassinolide, and GA4/7) for effect on changing the staminate to pistillate flower ratio in two cultivars, 'Charleston Gray' and 'Jubilee'. Ethrel had no effect on sex expression in watermelon in the range we tested (0 to 1600 mg/kg). In combination with amino vinylglycine (AVG) at 100 mg/kg, ethrel appears to increase the percentage of pistillate nodes. Treatment of plants with amino vinylglycine (AVG) without ethrel had no effect on watermelon sex expression. In later experiments, we tested eight plant growth regulators, but none of the treatments produced higher gynoecious sex expression compared with the controls. Further studies are needed to identify plant growth regulators or combinations that increase the percentage pistillate nodes from the current level of approximately 9% pistillate nodes to a useable level of 99% for watermelon hybrid production.



## Introduction

Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai var. *lanatus*] is a major vegetable crop in the United States. Total production in 1999 to 2002 was 1.67 million Mg•year<sup>-1</sup> of marketable fruit, and the consumption per capita was 6.6 kg of fresh fruit [18-21]. In the same period, the major center of production was Florida (ca. 380,000 Mg•year<sup>-1</sup>), even though the state with the largest cultivated area was Texas (ca. 18,000 ha•year<sup>-1</sup>) [21,22].

Hybrid watermelon seeds are produced by hand pollination using alternating rows in a field for the two parental inbred lines. In some cases, a male sterile has been used to make hybrid seed production easier. However, since the male sterile is the genic type, it is necessary to check each plant in the female parent row to make sure it is male sterile, since about half the plants are male fertile, and must be removed from the field before pollination begins.

In cucumber, it is possible to make a hybrid between two monoecious inbred lines by planting them in an isolation block and supplying bees to pollinate the flowers. At the cotyledon stage and one week later, the female parent rows are treated with etherl [1, 7]. It may also be useful to treat the male parent rows with silver thiosulfate. The female parent plants then become phenotypically gynoecious, and the male parent plants become phenotypically androecious. Any fruit harvested from the female parent would contain hybrid seeds since the female parent has no staminate flowers for self pollination [10, 24].

Growth regulators might be used to change sex expression in watermelon for hybrid production in the same way as for cucumbers. Previous research indicates that watermelon may respond differently to growth regulators than cucumber [13]. However, it appears that the situation for cucumber is reversed in watermelon, with silver thiosulfate making more gynoecious, and ethephel making it more staminate. None of the treatments tested so far have converted watermelon to a completely gynoecious type, and it is probably not necessary to make the male parent inbreds more androecious for hybrid seed production. Additional work is needed to identify treatments that might convert plants from monoecious to gynoecious.

The growth regulators abscisic acid (ABA), 6-benzylamino purine (BA), (2-chloroethyl), phosphonic acid (ethephon), aminoethylvinylglycine (AVG), and silver nitrate were applied in weekly biweekly foliar sprays to watermelon [4]. They investigated that both BA and ABA inhibited stem elongation, had no effect on flowering patterns. AVG at 100 and 200 mg/kg and 500 mg/kg AgNO<sub>3</sub> reduced the number of staminate flowers and promoted hermaphroditic flowering. In watermelon, contrary to other cucurbits, applied ethylene appears to suppress rather than promote ovary development during flower bud differentiation. McArdle reported that silver nitrate treatments increased gynoecy and average of 138 % over control [9].

The objectives of this study were to evaluate plant growth regulators for effect on the percentage of pistillate and staminate nodes, and if possible, to identify plant growth regulator treatments that would make monoecious watermelons become phenotypically gynoecious.

## Materials and Methods

The experiment was conducted in 2005, 2006 and 2007 at the Horticultural Crops Research Station at Clinton, North Carolina, and at the Cunningham Research Station at Kinston, North Carolina. The experiment was a randomized complete block with four replications. Rows were covered with black polyethylene mulch and drip irrigated. The experiment was conducted using recommended cultural practices [12].

The growth regulators treatments were AVG, Ethrel, and AVG plus Ethrel in 2005 and Cyclanilide, NAA, B9, Chlomaquat, Uniconazol, Paclobutrazol, and Ethrel in 2006. The plants were direct seeded and applied plant growth regulators 3, 4, 5 and 6 weeks after seeding by hand sprayer. In 2005, the concentrations of plant growth regulators were 0, 100, 200, 400, 800, 1600 mg/kg. In 2005, cultivars tested were 'Minilee', 'Charleston Gray', 'Sugar Baby', and 'Jubilee'. In 2006, the concentrations of plant growth regulators were 0, 1, 10, 100, 1000 mg/kg for Cyclanilide, B9, Chlomaquat, and Ethrel, and 0.5, 5, 50, 500 mg/kg for Uniconazol, Paclobutrazol. In 2006, cultivars tested were 'Charleston Gray' (with a more pistillate flower ratio) and 'Jubilee' (with a more staminate flower ratio). In 2007, the concentrations of plant growth regulators were 0.5, 1, 10, 20 uM for Brassinolide, 2.5, 5, 7.5, 10 mg/kg for Paclobutrazol, 1, 10, 100, 1000 mg/kg for GA4/7, and 5, 10, 15, 20 mg/kg for Cyclanilide plus 2 controls (water, and water+tween-20).

Plants were rated four weeks after seeding. For each plant, pistillate and staminate nodes were counted, and vine size was rated (1=small 9=large). Individual ratings were summarized as mean and maximum over replication and harvest. Data were analyzed

using the MEANS, ANOVA and GLM procedures of the SAS statistical package (SAS Institute, Cary, NC).

## Results and Discussion

In cucumber, ethrel treatment of plants at the seedling stage increases gynoecious sex expression at the flowering stage. In watermelon, ethrel treatment at the seedling stage appears to have no effect on sex expression at the flowering stage. However, it does cause stunting of the watermelon plants as well as phytotoxicity at high concentrations (Table 1 and Fig. 1). AVG induces androecious sex expression in cucumber, but appears to increase the percentage of pistillate nodes in watermelon. AVG treatment without ethylene was not significantly different from untreated plants for the percentage of pistillate nodes and for vine size (Table 1). AVG increases the percentage of staminate nodes in cucumber, but appears to increase the percentage of pistillate nodes in watermelon. A single treatment of AVG without ethrel had no effect on watermelon sex expression in 2005. None of the treatments produced higher gynoecious sex expression compared with the untreated controls (Table 1).

In 2006, seven different plant growth regulators (BNine, chlomaquat, cyclanilide, ethrel, NAA, paclobutrazol, and uniconazol) were tested to determine if any would increase the percentage pistillate nodes. None of the treatments produced higher gynoecious sex expression compared with the controls (Table 2). However, higher concentrations of cyclanilide caused stunting of the watermelon plants compared with the untreated controls (Fig. 2 and 3).

In 2007, four plant growth regulators (Brassinolide, Paclobutrazol, GA4/7, and Cyclanilide) were tested for their effect on increasing the percentage pistillate nodes. None of the growth regulators had an effect on sex expression in watermelon (Table 3).

In summary, none of the growth regulators tested alone or in combination was useful in altering the percentage of pistillate nodes in watermelon. Further studies are needed to determine methods for altering the percentage of pistillate nodes from the current level of 9% to a useable level for hybrid production of 99%.

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Table 1. Percentage pistillate nodes for watermelons treated with AVG and ethrel at Clinton and Kinston, NC in 2005.<sup>z</sup>

AVG	Ethrel	Pistillate	Vine size
(mg/kg)	(mg/kg)	(%)	(rating)
0	0	7.6	8.0
0	100	4.6	7.1
0	200	5.3	6.7
0	400	5.2	5.4
0	800	4.6	4.2
0	1600	5.7	2.6
100	0	9.5	7.6
100	100	6.1	6.7
100	200	7.6	6.9
100	400	7.0	6.3
100	800	6.2	5.7
100	1600	2.7	4.4
200	0	7.4	7.1
200	100	9.4	7.3
200	200	6.6	7.1

Table 1 (continued)

200	400	5.5	6.4
200	800	5.4	6.1
200	1600	5.0	4.3
400	0	6.4	7.3
400	100	8.8	7.7
400	200	8.0	7.1
400	400	7.1	6.8
400	800	5.5	5.3
400	1600	5.2	4.4
800	0	7.9	7.5
800	100	10.0	7.2
800	200	7.9	7.2
800	400	6.3	6.7
800	800	4.6	5.5
800	1600	4.7	3.6
1600	0	8.9	7.4
1600	100	8.0	7.2
1600	200	4.6	6.9

Table 1 (continued)

1600	400	4.9	5.9
1600	800	5.2	5.4
1600	1600	4.9	3.7
LSD (5%)		5.2	0.8

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<sup>z</sup> Percentage of pistillate nodes and vine size (rated 1-9, 1=small, 9=large).

Table 2. Percentage pistillate and staminate nodes for watermelons treated with 7 different growth regulators at Clinton and Kinston, NC in 2006.<sup>z</sup>

Growth regulator	Conc. (mg/kg)	<u>Main stem sex expression</u>	
		% pistillate	<u>% staminate</u>
Tween 20	0	9	91
Water	0	11	89
BNine	1	8	92
	10	12	88
	100	11	89
	1000	7	93
Chlomaquat	1	9	91
	10	9	91
	100	10	90
	1000	8	92
Cyclanilide	1	9	91
	10	15	85
	100	-	-
	1000	-	-
Ethrel	1	7	93
	10	4	96

Table 2 (continued)

	100	0	100
	1000	-	-
NAA	1	6	94
	10	12	88
	100	8	92
	1000	5	95
Paclobutrazol	0.5	12	88
	5	14	86
	50	10	90
	500	12	88
Uniconazol	0.5	12	88
	5	11	89
	50	5	95
	500	50*	50
LSD (5%)		5.5	5.5

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<sup>z</sup> Percentage of female and male nodes. Data are means of 2 locations, 2 replications and 2 cultivars

\* plants severely stunted.

- indicates plants killed by treatment.

Table 3. Percentage pistillate and staminate nodes for watermelons treated with 4 different growth regulators at Clinton and Kinston, NC in 2007.<sup>z</sup>

Growth regulator	Conc. (mg/kg)	<u>Main stem sex expression</u>	
		% pistillate	% staminate
Tween 20	0	17	83
Water	0	17	83
Brassinolide	0.5	13	87
	1	20	80
	10	16	84
	20	23	77
Cyclanilide	5	9	91
	10	16	84
	15	16	84
	20	20	80
GA4/7		1	24
	10	15	85
	100	14	86
	1000	16	84
Paclobutrazol	2.5	14	86
	5	15	85
	7.5	27	73

Table 3 (continued)

	10	17	83
LSD (5%)		13	13

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<sup>z</sup> Data are means of 2 locations, 2 replications and 2 cultivars.





Figure 1. Ethrel 1000 mg/kg cause stunt on watermelon plants.



Figure 2. Cyclanilide 100 mg/kg cause stunt and shrink on watermelon plants.





Figure 3. Control treatment with Water on watermelon plants.