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Effect of Hydrogen Cyanamide on Bud Break, Fruit Yield and Quality of Highbush Blueberry in Greenhouse Production

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Abstract: Highbush blueberries need sufficient chilling exposure to induce bud break and flowering, which limits their cultivation in warm areas as well as the profitability of protected cultivation in greenhouses. Hydrogen cyanamide (H_2CN_2, HC), gibberellic acid (GA_3), ethephon (CE), mineral oil (MO), and potassium nitrate (KNO_3) are often applied to deciduous fruit trees to advance bud break and fruit set. In this study, experiments were conducted in northeast China to determine the effects of different concentrations of HC or HC in combination with GA_3, CE, MO, and KNO_3 on bud break, fruit quality, and fruit yield in greenhouse-grown highbush blueberry (*Vaccinium corymbosum* L.). The results showed that all of the treatment agents could advance bud break by at least 15 days and fruit ripening by 16 days compared to the control. In addition, all treatments could promote the development of flowers and fruits and shorten the flowering and harvest periods. Compared with the control, 0.5% HC or 0.67% HC treatment increased the fruit yield of 5-year-old and 7-year-old bushes, especially early yield. Experiments carried out over two consecutive years in two different varieties, namely 'M7' and 'Brigitta', further confirmed the positive effect of 0.67% HC application on fruit yield. The results also showed that 0.67% HC had no negative effects on fruit quality. These findings may encourage growers to consider spraying HC in greenhouses to increase fruit yield, especially early yield, in order to schedule the harvest time for a more lucrative marketing period.

Keywords: highbush blueberry; hydrogen cyanamide; buds break; fruit

Citation: Wang, H.; Xia, X.; An, L. Effect of Hydrogen Cyanamide on Bud Break, Fruit Yield and Quality of Highbush Blueberry in Greenhouse Production. *Agriculture* **2021**, *11*, 439. https://doi.org/10.3390/agriculture11050439

Academic Editor: Sally Bound

Received: 16 April 2021
Accepted: 10 May 2021
Published: 12 May 2021

1. Introduction

To survive winter temperatures, deciduous perennials in temperate zones cease growing and enter a physiological phase known as dormancy [1]. Dormancy release and the development of floral and vegetative buds require a certain amount of low temperatures (0 °C to 7 °C) followed by warm temperatures, which come in spring [2]. Insufficient chilling results in erratic bud break and often leads to prolonged flowering as well as reduced fruit production [3]. Budbreak and flowering regulation are very important for the survival and productivity of perennial plants.

Some chemical products, such as gibberellins (GA) [4], cytokinins [5], mineral oil (MO) [6], and potassium nitrate (KNO_3) [7] have been shown to exert a positive effect on the release of dormancy and thus on bud break when applied at the right concentration to the buds of many fruit crops. Hydrogen cyanamide (HC) is the most effective of a range of chemicals used to advance and synchronize bud break, promote early ripening, and increase fruit productivity in deciduous fruit trees [8–10]. Hydrogen cyanamide can also be used in combination with mineral oil [6], abscisic acid [11], gibberellins [12], and garlic extracts [13].

Highbush blueberry (*Vaccinium corymbosum* L.) is an important perennial deciduous fruit shrub that belongs to the Ericaceae family [14]. Blueberry has become a popular and highly priced small fruit crop because of its high nutraceutical and potential pharmaceutical values [15]. Commercial blueberry production has expanded rapidly worldwide in recent years. The environmental conditions in the greenhouse, such as temperature, humidity,

light and CO_2 concentration, can be controlled artificially. The climate inside greenhouses has been shown to accelerate flowering, expedite fruit ripening, increase yields, improve berry quality, extend the harvest season, and decrease berry losses to rain and frost [16]. Greenhouse production can bring greater economic benefits to growers, so it has been widely practiced in northeast China and is also growing rapidly worldwide [17].

It is economically advantageous to control the timing of bud break so that the fruit can be harvested at a more favorable marketing period. The earlier the blueberries are harvested, the more economic benefits can be obtained. Advance harvest would be the best scenario for blueberry growers to capture a higher financial return in greenhouse production. Therefore, highbush blueberries need sufficient chilling exposure to induce bud break and bloom [18]. Due to insufficient chilling requirements in the greenhouse, delayed and irregular bud breaking and flowering often occur, resulting in a decrease in blueberry yield or even no harvest. A large number of studies have demonstrated that dormancy breaking agents such as HC can substitute part of the chilling requirements, advance and synchronize bud breaking and blooming, and hasten fruit maturity [19–22]. The application of dormancy breaking agents will be an important strategy to promote the early ripening of fruits in blueberry greenhouse production. Currently, dormancy breaking agents have been successfully used by growers in the greenhouse production of many plants, including peach (*Prunus persica* L.) [16], tree peony (*Paeonia suffruticosa* Andr.) [23], and grape (*Vitis vinifera* L.) [24].

The application concentration of the dormancy breaking agents is the key to successful dormancy breaking. It has been confirmed in many practical applications of fruits that low-concentration dormancy breaking agents cannot release plants from dormancy, and high-concentration can cause serious phytotoxicity to plants [6]. The optimal concentration range varies with treatment time, plant species, tree vigor and production system, and the appropriate concentration under specific conditions needs to be determined through experiments.

Preliminary work has demonstrated that HC can advance vegetative bud break, increase leaf to fruit ratio, shorten the flowering period, and hasten fruit maturity for the southern highbush [25] and rabbiteye blueberry varieties [26]. Compared with other deciduous fruit trees, the knowledge and experience of budbreak and flowering regulation is limited in highbush blueberry. As far as we know, few studies have examined the effect of exogenous HC on blueberry fruit quality or the effect of HC combined with other dormant release agents on phenology and fruit yield in highbush blueberry under greenhouse production.

The aim of this study was to examine the effect of different concentrations of HC and HC combined with other agents on budbreak, flowering, fruit ripening and fruit yield in highbush blueberry in greenhouse production. The effect of HC on blueberry fruit quality was also examined.

2. Materials and Methods

2.1. Plant Material and Treatment

Experiments were carried out in 2015, 2016, 2017, and 2018, in greenhouses located in Chengzitan, Dalian, Liaoning Province, China (39.53° N, 122.41° E). Highbush blueberry (*Vaccinium corymbosum* L.), variety 'M7' and 'Brigitta', were used. Blueberry bushes were planted in rows, with each row separated from the adjacent row by 2.2 m, and within-row spacing was maintained at 1 m. The soil was sandy loam with 8.7% organic matter content and 4.7 pH value. Drip irrigation was adopted for irrigation. Appropriate amounts of N, P, and K fertilizer were applied with drip irrigation every 20–30 days. The annual fertilization management on the entire orchard plantation was 120 kg N ha^{-1}, 120 kg P_2O_5 ha^{-1} and 120 kg K_2O ha^{-1}. The temperature in the greenhouse during the dormant period was kept below 10 °C. After chemical treatment, the temperature in the greenhouse was maintained at 25~28 °C. during the day and 10~15 °C. at night. The air humidity was

60~80%. Biological methods or environmentally friendly pesticides were used for pest and disease control.

Uniform 5-year-old and 7-year-old bushes of 'M7' grown in the greenhouse were sprayed with different concentrations of HC or 0.67% HC in combination with GA_3, CE, MO or KNO_3 as indicated in Table 1 in the 2015–2016 growing season. Five-year-old bushes of the two varieties ('M7' and 'Brigitta') were sprayed with 0.5% and 0.67% (v/v) HC in the 2016–2017 season, and with 0.67% (v/v) HC in the 2017–2018 season (Table 1). The spray applications were conducted at the beginning of December every year. According to our previous experiences, at this time more than two-thirds of the chilling requirement had been met and the buds were still dormant. A randomized complete block design with eight replications per treatment was used during 2015–2016. In the 2016–2017 and 2017–2018 seasons, 18 bushes were used in each treatment. Each plant was sprayed with a 10 L sprayer until run-off. Triton X-100 surfactant was included in each spray.

Table 1. Description of agent treatments on blueberry plants in different growing seasons.

Growing Season	Treatment	Treatment Description
2015–2016	Control (CK)	Tap water
	HC1	0.5% (v/v) HC
	HC2	0.67% (v/v) HC
	HC3	0.83% (v/v) HC
	HC2/GA_3	0.67% (v/v) HC + 100 mg/L GA_3
	HC2/CE	0.67% (v/v) HC + 200 mg/L CE
	HC2/MO/KNO_3	0.67% (v/v) HC + 2% (v/v) MO + 3% (m/v) KNO_3
2016–2017	Control (CK)	Tap water
	HC1	0.5% (v/v) HC
	HC2	0.67% (v/v) HC
2017–2018	Control (CK)	Tap water
	HC2	0.67% (v/v) HC

2.2. Agronomic Survey

During the 2015–2016 season, the onset of budbreak, flowering, and fruit ripening was recorded for all plants in each treatment. Budbreak was defined as the appearance of green tissue emerging from a bud, flowering as the stage when a flower was fully open, and fruit ripening as when the color of the entire fruit was blue. The flowering period is the length of time from the beginning of flowering to the end of flowering. Harvesting period is defined as the length of time from the beginning of harvesting to the end of harvesting.

2.3. Yield Measurement

Beginning at the first ripe fruit of each year, all ripe fruits were hand-picked and weighed at 3–6 d intervals. We classified the harvests into early-season yield (harvested before 1st May for 'M7'; harvested before 10th May for 'Brigitta'), mid-season yield (harvested from the 2nd to the 10th of May for 'M7'; harvested from the 11th to the 25th of May for 'Brigitta'), and late-season yield (harvested after 11th of May for 'M7'; harvested after 26th of May for 'Brigitta').

2.4. Determination of Soluble Solid Concentration (SSC) in Blueberry Fruit and Fruit Weight

At each harvest, the total number and weight of fruits from bushes treated by different agents were recorded, and the average berry weight was determined. Additionally, 18 berries per treatment were selected to measure SSC (°Brix) using a handheld temperature compensated digital refractometer (pocket pal-1, Atago Co., Tokyo, Japan). The refractometer was calibrated using deionized water.

2.5. Fruit Quality Parameters Measurements

At the early harvest season in 2018, twenty ripe fruits were randomly sampled from each treatment (five fruits in each replicate plant) of 'M7' and 'Brigitta', and then imme-

diately transferred to the laboratory for quality determination. The content of vitamin C (mg/100g FW) in fruits was determined according to the method described by Chanwitheesuk et al. [27]. With reference to the description of Petridis et al., the total anthocyanin content in fruits was determined by the pH differential method, and the total phenol content was determined by the Folin–Ciocalteu assay [14]. Total flavonoid content was calculated as rutin equivalents and determined according to the method of Wang et al. [28]. Optical density (OD) was measured using a Varioskan Flash (Thermo Fisher Scientific Waltham, MA, USA). Titratable acidity (TA) was determined by the colorimetric technique described by López-Vargas et al. [29]. Fruit firmness was determined with a manual penetrometer fitted with a 2 mm diameter flat probe (Model GY-2, Hangzhou Scientific Instruments, Hangzhou, China).

2.6. Statistical Analysis

Statistical analysis was carried out using the software package SPSS v17.0 (SPSS Inc., Chicago, IL, USA). Comparisons among the averages of each treatments were based on the analysis of variance (one-way ANOVA) according to Duncan's multiple range test and t-test. Statistical significance was considered at the $p < 0.05$ or $p < 0.01$ level.

3. Results

3.1. Effect of Agent Treatments on the Phenological Stages of Blueberry

Application of different concentrations of HC or HC combined with GA_3, CE, MO, and KNO_3 advanced the time of budbreak, flowering and fruit ripening, and shortened the flowering and harvest period (Table 2). Compared with the control, agent treatments advanced budbreak, flowering, and fruit ripening in 5-year-old bushes by 17–19, 2–3, and 6–7 days, respectively, and 7-year-old bushes by 15–17, 1–3, and 7–8 days, respectively (Table 2). The flowering period and harvesting period were shortened by agent treatments by 4–5 days and 3–6 days, respectively, in 5-year-old bushes, and by 5–6 days and 4–5 days, respectively, in 7-year-old bushes, compared with the respective controls (Table 2). We noticed that there was no clear difference in advancing early budbreak and fruit ripening, or in shortening the flowering and harvesting period among the agent treatments.

Table 2. Onset of budbreak, flowering, and fruit ripening, and flowering and harvesting period of 'M7' blueberry bushes of different ages affected by agent treatments in the 2015–2016 season.

Treatments	Days after Treatment (d)						Flowering Period (d)		Harvesting Period (d)	
	Budbreak		Flowering		Fruit Ripening					
	5 Years	7 Years	5 Years	7 Years	5 Years	7 Years	5 Years	7 Years	5 Years	7 Years
CK	42	37	64	63	142	145	40	40	63	62
HC1	23	20	62	61	126	128	35	34	58	57
HC2	23	20	61	60	125	127	35	34	57	57
HC3	23	20	61	60	125	127	36	34	58	58
$HC2/GA_3$	25	22	62	61	126	127	35	35	57	58
HC2/CE	25	20	61	62	126	127	35	34	60	57
$HC2/MO/KNO_3$	23	20	62	61	126	127	36	35	57	58

CK, tap water; HC1, 0.5% HC; HC2, 0.67% HC; HC3, 0.83% HC; $HC2/GA_3$, 0.67% HC + 100 mg/L GA_3; HC2/CE, 0.67% HC + 200 mg/L CE; $HC2/MO/KNO_3$, 0.67% HC + 2% MO + 3% KNO_3.

3.2. Effect of Agent Treatments on Fruit Yield

For 5-year-old bushes, compared with the control, HC1, HC2, and HC2/CE treatment significantly increased the total fruit yield, while HC3, $HC2/GA_3$, and $HC2/MO/KNO_3$ treatment significantly decreased the total fruit yield (Figure 1A). During the early harvest period, all the agent treatments significantly increased fruit yield compared with the control (Figure 1B). For 7-year-old bushes, HC1, HC2, HC3, and HC2/CE treatment significantly increased the total fruit yield compared with the control (Figure 1A). During the early harvest period, all the agent treatments significantly increased fruit yield compared with the control, except for the $HC2/GA_3$ treated group (Figure 1C).

As demonstrated in Figure 1, all the agent treatments significantly increased the early season yields compared with the control in 5-year-old bushes except for the HC2/GA_3 treated group for 7-year-old bushes.

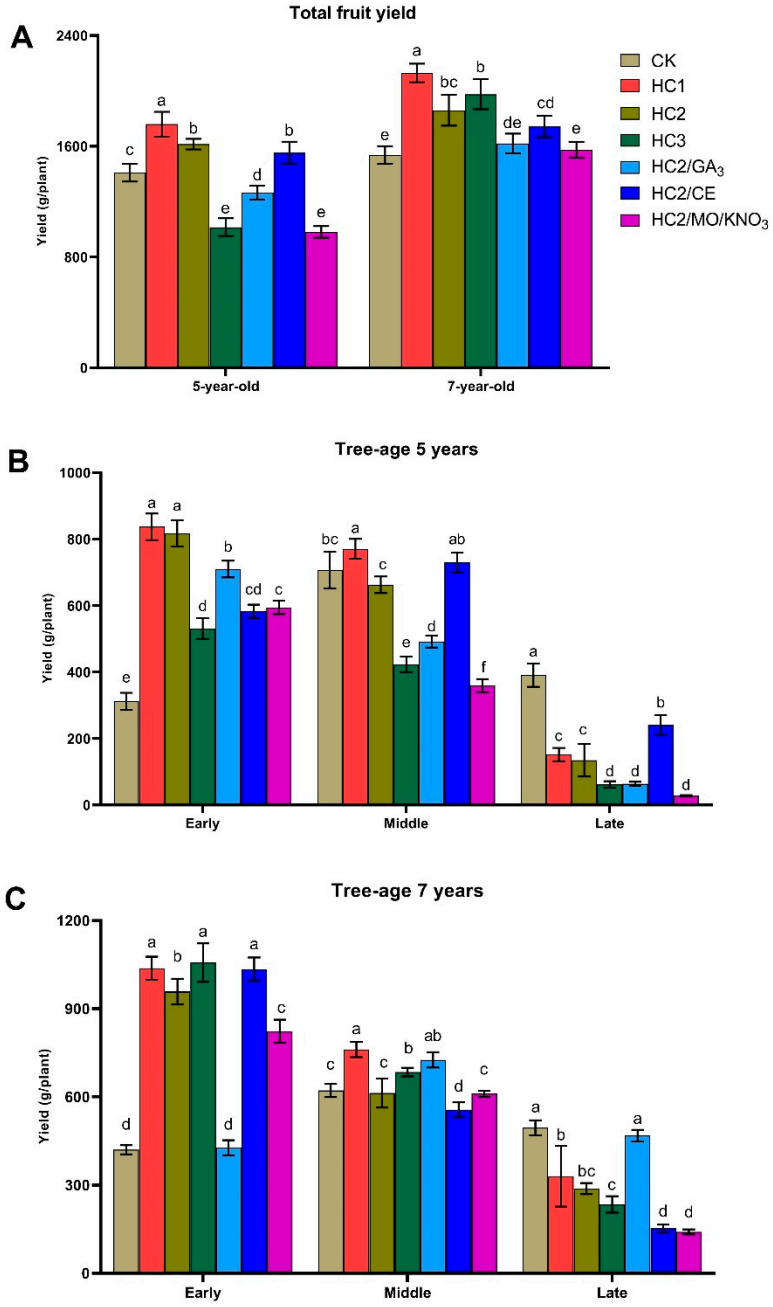

Figure 1. Effects of treatment with agents on the fruit yield of 'M7' blueberry bushes of two ages in 2016. (**A**) Total yield of 5-year-old and 7-year-old bushes; (**B**) Stage yield of 5-year-old bushes; (**C**) Stage yield of 7-year-old bushes. Mean values with the same letter within a group are not significantly different according to Duncan's multiple range test at $p < 0.05$. Data are the mean ± SE ($n = 8$). CK, tap water; HC1, 0.5% HC; HC2, 0.67% HC; HC3, 0.83% HC; HC2/GA_3, 0.67% HC + 100 mg/L GA_3; HC2/CE, 0.67% HC + 200 mg/L CE; HC2/MO/KNO_3, 0.67% HC + 2% MO + 3% KNO_3.

3.3. Effect of Agent Treatments on Soluble Solids Content (SSC) in Fruits

The SSC data are presented in Table 3. For 5-year-old bushes, SSC in fruits of the HC2, HC3, and HC2/GA_3 treatment groups were significantly higher than that of the control group in the three harvest seasons. In addition, SSC in mid-season-harvest fruits

was increased by HC1 and HC2/MO/KNO_3 treatments. HC2/MO/KNO_3 treatment and HC2/CE treatment increased SSC in early-season-harvest and late-season-harvest fruits, respectively. For 7-year-old bushes, HC2 treatment significantly increased SSC in fruits of mid-season and late-season. Except for HC2/GA_3 treatments, all other agent treatments significantly increased SSC in fruits of late-season. In general, HC2 treatment improved SSC in fruits regardless of the age of bushes.

Table 3. Effects of agent treatments on the SSC (°Brix) in fruits of 'M7' blueberry at different bush ages.

Treatments	Early-Season Harvest		Mid-Season Harvest		Late-Season Harvest	
	5-Year-Old	7-Year-Old	5-Year-Old	7-Year-Old	5-Year-Old	7-Year-Old
CK	10.03 ± 0.31 b	10.73 ± 0.75 b	9.77 ± 0.32 c	9.27 ± 0.25 b	9.80 ± 0.40 c	10.00 ± 0.17 c
HC1	10.03 ± 0.06 b	10.47 ± 0.29 b	11.30 ± 0.46 b	9.07 ± 0.45 b	9.80 ± 0.40 c	11.77 ± 0.55 ab
HC2	10.90 ± 0.50 a	10.20 ± 0.53 b	11.33 ± 0.29 b	10.50 ± 0.75 a	12.93 ± 0.45 a	11.17 ± 0.55 b
HC3	11.23 ± 0.06 a	10.73 ± 0.51 b	10.70 ± 0.40 b	9.53 ± 0.38 ab	11.57 ± 0.71 b	12.03 ± 0.95 a
HC2/GA_3	11.27 ± 0.25 a	9.87 ± 0.15 b	11.53 ± 0.42 ab	9.27 ± 0.64 b	12.07 ± 0.67 ab	9.20 ± 0.10 c
HC2/CE	10.67 ± 0.46 ab	10.63 ± 0.38 b	10.60 ± 0.46 c	9.89 ± 0.75 ab	11.53 ± 0.65 b	11.87 ± 0.15 ab
HC2/MO/KNO_3	11.27 ± 0.45 a	12.03 ± 0.67 a	12.30 ± 0.87 a	10.03 ± 0.47 ab	10.47 ± 0.71 c	11.63 ± 0.12 ab

Mean values with the same letter within a column are not significantly different according to Duncan's multiple range test at $p < 0.05$. Data are the mean ± SE (n = 18) for SSC. Fruit was harvested on 1st May (Early), 10th May (Mid), and 22nd May (Late) in 2016. CK, tap water; HC1, 0.5% HC; HC2, 0.67% HC; HC3, 0.83% HC; HC2/GA_3, 0.67% HC + 100 mg/L GA_3; HC2/CE, 0.67% HC + 200 mg/L CE; HC2/MO/KNO_3, 0.67% HC + 2% MO + 3% KNO_3.

3.4. Effect of Agent Treatments on the Fruit Weight

The individual fruit weight data for fruits from different treatment bushes are presented in Table 4. Although HC3 and HC2/KNO_3/MO treatment significantly decreased the weight of fruits harvested from 7-year-old bushes at the early harvest stage, and HC2/GA_3 and HC2/KNO_3/MO treatments significantly increased the weight of fruits harvested from 5-year-old bushes at the mid harvest stage, all the other treatments did not affect the individual fruit weight when compared with the control. In general, application of HC alone or HC in combination with other agents had basically no negative effect on the individual fruit weight of blueberry.

Table 4. Effect of agent treatments on the individual fruit weight (g) of 'M7' blueberry at different bush ages.

Treatments	Early-Season Harvest		Mid-Season Harvest		Late-Season Harvest	
	5-Year-Old	7-Year-Old	5-Year-Old	7-Year-Old	5-Year-Old	7-Year-Old
CK	2.15 ± 0.49 a	2.53 ± 0.22 a	1.59 ± 0.41 b	1.83 ± 0.56 a	1.70 ± 0.69 a	1.73 ± 0.35 a
HC1	2.50 ± 0.24 a	2.41 ± 0.58 ab	1.77 ± 0.12 b	2.34 ± 0.28 a	1.95 ± 0.61 a	1.97 ± 0.60 a
HC2	2.43 ± 0.14 a	2.29 ± 0.33 ab	2.18 ± 0.41 ab	2.14 ± 0.45 a	1.83 ± 0.14 a	2.04 ± 0.61 a
HC3	2.31 ± 0.41 a	1.84 ± 0.31 b	2.08 ± 0.19 ab	2.05 ± 0.58 a	1.47 ± 0.33 a	2.04 ± 0.21 a
HC2/GA_3	2.56 ± 0.29 a	2.30 ± 0.36 ab	2.36 ± 0.18 a	2.09 ± 0.37 a	1.94 ± 0.26 a	1.99 ± 0.48 a
HC2/CE	2.51 ± 0.26 a	2.43 ± 0.50 ab	2.20 ± 0.43 ab	2.23 ± 0.46 a	1.78 ± 0.03 a	2.12 ± 0.33 a
HC2/MO/KNO_3	2.25 ± 0.36 a	1.84 ± 0.20 b	2.25 ± 0.25 a	1.96 ± 0.44 a	1.66 ± 0.44 a	1.78 ± 0.22 a

Mean values with the same letter within a column are not significantly different according to Duncan's multiple range test at $p < 0.05$. Data are the mean ± SE (n = 30). Fruit was harvested on 1st May (Early-season), 10th May (Mid-season), and 22nd May (Late-season) in 2016. CK, tap water; HC1, 0.5% HC; HC2, 0.67% HC; HC3, 0.83% HC; HC2/GA_3, 0.67% HC + 100 mg/L GA_3; HC2/CE, 0.67% HC + 200 mg/L CE; HC2/MO/KNO_3, 0.67% HC + 2% MO + 3% KNO_3.

3.5. Effect of HC Treatment Alone on Fruit Yield of Different Blueberry Variety

We noticed that the fruit yield of bushes treated with 0.5% HC differed greatly among individual bushes, which is undesirable for obtaining stable yields in blueberry production practices (Figure S1). Thus, 0.67% HC was selected to further study the effect of HC on the yield of blueberry for two consecutive years. The results showed that the total yield of HC-treated bushes was increased by 34.55% (Figure 2A) and 21.62% (Figure 2C) in 'M7', and by 14.48% (Figure 2B) and 41.89% (Figure 2D) in 'Brigitta' in 2017 and 2018, respectively, when

compared with the control bushes. Moreover, 0.67% HC treatment significantly improved the early-season yield of these two varieties for two consecutive years, increasing the yield by 84.38% (Figure 2A) and 42.86% (Figure 2C) with 'M7' and 163.33% (Figure 2B) and 128.57% (Figure 2D) with 'Brigitta', respectively, as compared with the control. Although the late-season yield of 'Brigitta' in 2017 was decreased by application of 0.67% HC, the other mid- and late-season yields were not affected by HC application. The above results indicated that the application of 0.67% HC can steadily increase the fruit yield of highbush blueberry 'M7' and 'Brigitta', especially the early-season yield.

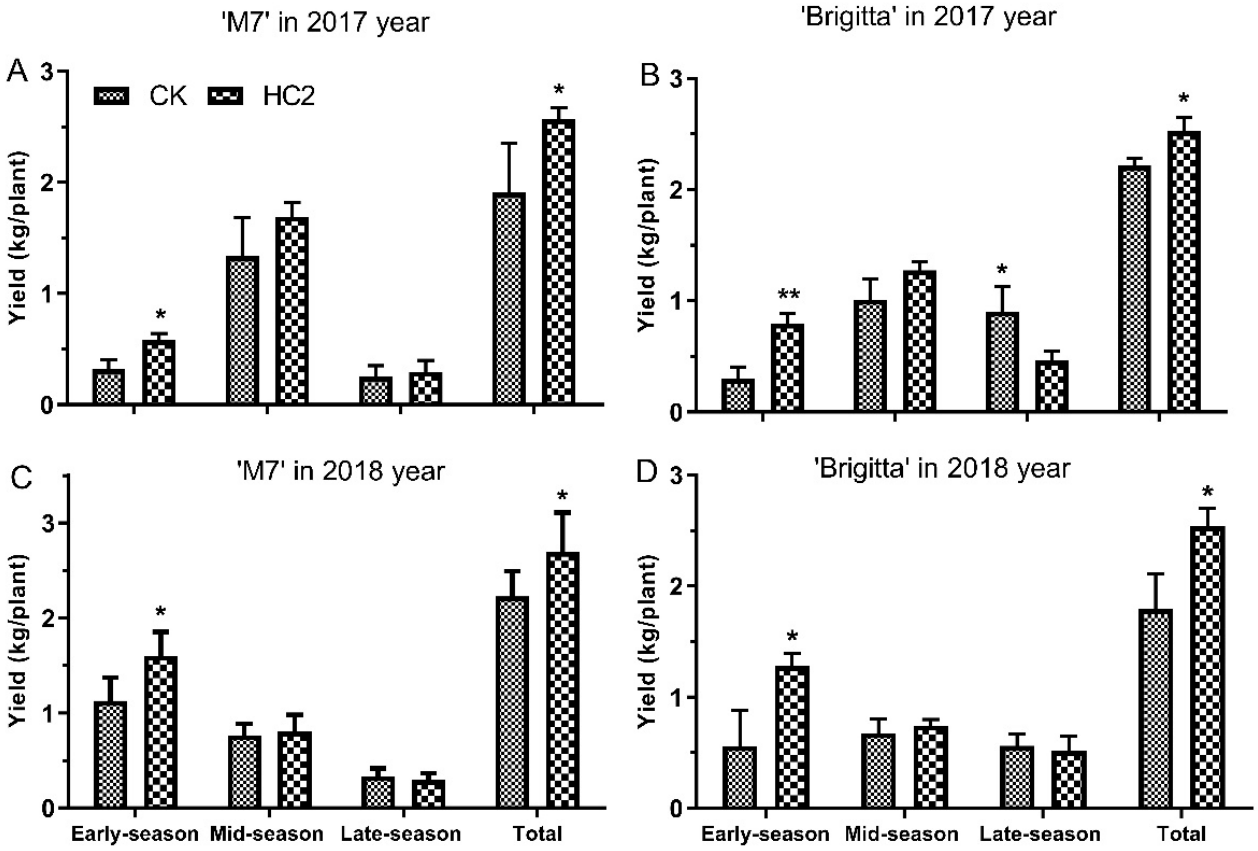

Figure 2. The effect of HC application on the fruit yield of 'M7' and 'Brigitta' for two consecutive years (2017 and 2018). (**A**) Yield of 'M7' in 2017; (**B**) Yield of 'Brigitta' in 2017; (**C**) Yield of 'M7' in 2018; (**D**) Yield of 'Brigitta' in 2018. Data are the mean ± SE (n = 18). "*" and "**" indicate significantly different from the CK at the $p < 0.05$ and $p < 0.01$ levels. CK, tap water; HC2, 0.67% HC.

3.6. Effect of HC Treatment Alone on Fruit Quality

Concerning fruit quality, no significant effect of HC was observed on the content of vitamin C, anthocyanins, polyphenols, flavonoids and titratable acidity in fruits of 'M7' and 'Brigitta' (Figure 3). In addition, no significant difference in the fruit firmness was found between the HC treatment group and the control group. These results indicated that 0.67% HC had no negative effect on the fruit quality of blueberry.

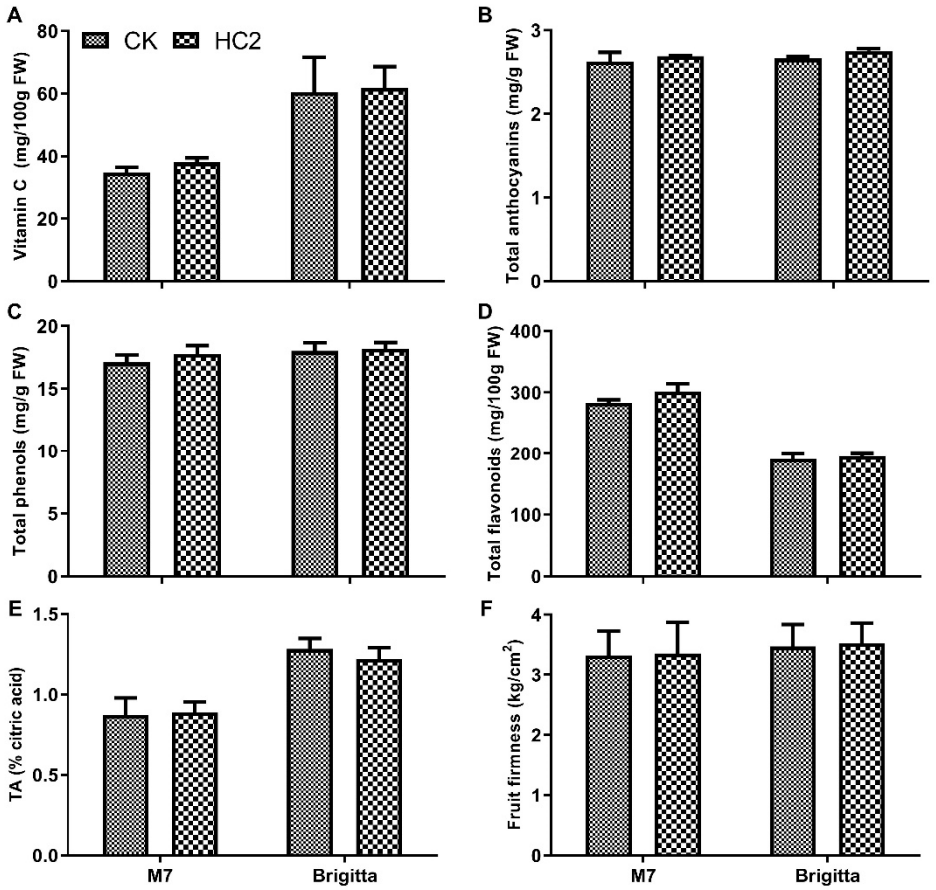

Figure 3. Effect of 0.67% HC treatment on the content of (**A**) vitamin C, (**B**) total anthocyanin, (**C**) total polyphenols, (**D**) total flavonoids in fruits (**E**) titratable acidity and (**F**) fruit firmness. Data are the mean ± SE (n = 3). CK, tap water; HC2, 0.67% HC.

4. Discussion

The blueberry harvesting window in northeast China is uniquely early, resulting in high fruit quality and the yielding of high profits in the fresh fruit market. However, the period of high profits is relatively short since, after May, the blueberry price and quality drop rapidly. Therefore, developing methods that could promote fruit maturity and increase early fruit yield is crucial for the fresh blueberry market. Growing blueberries in a greenhouse can enable plants to resist cold and promote their growth and early flowering and fruiting [30] and is widely practiced in northeast China. Hydrogen cyanamide has been effectively used to advance bud breaking [19] and promote early ripening in deciduous fruit trees [31]. It has also been reported that HC application can increase yield in 'Perlette' grape (*Vitis vinifera* L.) [32] and pistachio (*Pistacia vera* L.) [22]. In the present study, the application of HC in appropriate concentrations (0.5–0.67%) to greenhouse grown blueberries for three consecutive years revealed positive impacts on the fruit yield especially the early-season yield. This is consistent with the results of previous studies on blueberries under field conditions. The application of HC increased the yield of 'O'Neal' blueberries, and the application of 1% HC increased the yield of blueberries harvested in the first 10 days for two consecutive years [33]. HC treatment also shortened the time taken for the fruits to mature (Table 1 and Figure 1). Combining HC treatments with protected greenhouse cultivation in northeast China has the potential to allow growers to harvest fruits during a more profitable marketing period, thereby providing greater economic benefits to blueberry growers. The use of HC as a bud-breaker and yield enhancer for greenhouse highbush blueberries has practical commercial value.

Hydrogen cyanamide can be used in combination with other dormancy-breaking agents. Campoy et al. (2011) observed advanced blooming and harvest times for 'Early

Maycrest' peach plants when they were treated with HC in combination with mineral oil [34]. Similarly, the application of 0.6% HC in combination with 4% mineral oil to 'Eva' apple trees can also effectively promote earlier vegetative and reproductive bud break [6]. In our study, application of HC alone or in combination with GA_3, mineral oil, and KNO_3, as well as ethephon promoted budbreak and fruit ripening in highbush blueberry (Table 2), and there is no clear difference in the effect on advancing budbreak and fruit ripening among different treatments. In addition, the fruit yield of bushes treated with 0.67% HC alone was higher than that of bushes treated with 0.67% HC in combination with other agents (Figure 1). We consider that the effect of HC combined with other agents on promoting budbreak may mainly be caused by HC, and there is no interaction between HC and GA_3, CE, MO, and KNO_3 in promoting bud break and fruit production of highbush blueberry. Similarly, Zheng et al. (2018) reported that grapevine buds treated with a combination of HC and GA were found to have improved bud break compared with those treated with GA alone [12]. While HC spray alone had the best effect, HC combined with GA significantly attenuated the enhancing effect of HC on bud break. Whether HC interacts with other agents to produce a synergistic effect, the existing results are not consistent. The results of Theron et al. [35] showed that 2% HC and 2% mineral oil combined decreased bud break in two cultivars but was effective on the other cultivar, and thidiazuron and HC combined increased the number of fruit in two cultivars but decreased fruit size of another cultivar. The results on pistachios indicated that the combination of HC and mineral oil was effective in increasing the bud break rate and yield per branch, but there was no statistical difference when compared with the application of HC or mineral oil alone [36]. However, Mahawer et al. (2017) found that the combination of ABA/ethephon and HC had a synergistic effect in advancing ripening in grape [11]. We believe that these inconsistent results may be due to differences in the application concentration and time of dormancy agents, as well as difference in plant species. In this study, we only used one concentration of GA_3, CE, MO, and KNO_3. Therefore, in order to clarify the interaction between HC and other dormancy release agents, more concentration combinations need to be used in future studies.

The balance of source–sink relationships between vegetative growth and reproductive growth is important for obtaining high production in fruit trees [37,38]. Excessive induction of vegetative growth might have a negative effect on fruit set because of sink competition [39]. On the other hand, for most deciduous crops, floral buds are more sensitive to the phytotoxic effects of dormancy-breaking chemicals compared with vegetative buds [40]. We noticed that compared with 0.67% HC application alone, 0.67% HC application combined with GA_3, CE, and MO/KNO_3 reduced fruit yield (Figure 1). Few studies have compared the effects of application HC alone and HC combined with other agents on fruit yield. Mahawer et al. (2017) reported that application of 2% HC in combination with 400 ppm ethephon reduced yield in 'Flame Seedless' grapes compared with an untreated control [11], but the author did not compare it with HC application alone. We speculate that the additional application of GA_3 and CE may exert a phytotoxic effect on the reproductive buds because of their flower thinning effect [5,41], thereby reducing the fruit yield. The additional application of KNO_3 may promote the development of vegetative branches due to its nutritional effects [7], so that the developing floral buds and fruits are forced to compete for reserves with the prolific new leaf development, which ultimately leads to a decline in fruit production. The appropriate concentration of HC can regulate vegetative growth and reproductive growth, and maintain source–sink balance, thereby increasing fruit yield.

HC has been used as a dormancy-breaking agent to promote budbreak and to advance the harvest time of fruit; the concentration of HC that yields the optimal effect tends to depend on the species, variety, and age of the plants. For example, the application of 4% HC seems to be an effective treatment that can significantly improve the yield and quality of pistachio trees [22] and 'Ain Shemer' apple trees [42], while 1.5% HC is a suitable concentration for promoting vegetative growth and high yield of fig in the 'Zidi'

cultivar [9]. For rabbiteye and southern highbush blueberry varieties, treatment with 1.5% to 2.0% (v/v) HC was found to result in significant flower bud injury and reduced total fruit yield compared with the control [26]. Williamson and Maust (2001) also reported that the application of 2% HC to southern highbush blueberry varieties 'Climax' and 'Misty' often leads to damage in reproductive buds [43], while lowering the concentration of HC to 0.75% can induce an earlier harvest, shorter harvest season, and higher total yield [25]. In the present study, the application of 0.5% or 0.67% (v/v) HC led to an increase in fruit yield, while 0.83% (v/v) HC led to decrease in fruit yield of 5-year-old bushes (Figure 1). However, all three concentrations of HC, including 0.83%, increased the yield of fruit of 7-year-old bushes. We think that high concentrations of HC cause toxicity to productive buds. We suggest that the suitable application concentration of HC depends on the age and vigor of the bushes.

Hydrogen cyanamide is typically used to control the ripening of fruits, but little attention has been paid to its effect on fruit quality. Arora et al. (2011) indicated that the application of HC improved the quality of the fruits in grape by increasing the total soluble solids content and decreasing the acidity [32], 1.5% HC significantly increased the dry matter content in fruits of fig (*Ficus carica* L.) [9]. In addition, studies in 'Fuji' apple [44] and peach [45] proved that HC treatment had no detrimental effect on fruit quality. In this study, 0.67% HC application increased SSC (Table 3) but did not affect the content of vitamin C, anthocyanin, polyphenols and flavonoids in fruits of blueberry (Figure 3). The metabolic pathways of vitamin C, anthocyanins, polyphenols and flavonoids are complex, and affected by many factors [46]. We speculate that HC has little effect on these metabolic pathways under our experimental conditions. In addition, we only sampled the fruits harvested in the early season, and the measurement was performed immediately after the harvest. Therefore, there were no significant differences in the content of vitamin C, anthocyanins, polyphenols and flavonoids in fruits between the HC-treated bushes and the untreated bushes.

In summary, our results show that spraying 0.67% (v/v) HC on greenhouse grown highbush blueberries in early December (when more than two-thirds of the chilling requirement for blueberries has been met) can effectively promote bud break and fruit maturity, increase fruit yield, especially early season fruit yield, and can maintain fruit quality. This strategy can be recommended for greenhouse production of highbush blueberries in northeast China and similar regions.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agriculture11050439/s1, Figure S1: The effect of HC application on the fruit yield of 'M7' (A) and 'Brigitta' (B) in 2017.

Author Contributions: H.W., X.X. and L.A. conceived and designed the research. H.W. performed the research. H.W. interpreted the experimental data and drafted the manuscript. H.W. and X.X. revised the paper and approved the final version. L.A. and X.X. provided the financial support for this study. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors would like to thank all of the individuals that were involved in this study.

Conflicts of Interest: The authors declare no conflict of interest.

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