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To cite this article: Lei Wang, Caixi Zhang, Jiancheng Huang, Lina Zhu, Xiuming Yu, Jiefa Li, Yusui Lou, Wenping Xu, Shiping Wang & Chao Ma (2017) Hydrogen cyanamide improves endodormancy release and blooming associated with endogenous hormones in ‘Summit’ sweet cherry trees, New Zealand Journal of Crop and Horticultural Science, 45:1, 14-28, DOI: 10.1080/01140671.2016.1229344

To link to this article: http://dx.doi.org/10.1080/01140671.2016.1229344

Published online: 27 Sep 2016.

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Hydrogen cyanamide improves endodormancy release and blooming associated with endogenous hormones in ‘Summit’ sweet cherry trees

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ABSTRACT
A trial was conducted to verify the effect of hydrogen cyanamide (HC) application on endodormancy release and blooming of ‘Summit’ sweet cherry trees, as well as on endogenous gibberellic acids (GAs) and abscisic acid (ABA) levels which were identified and quantified by gas chromatography/mass spectrometry in branches with spurs. Results showed that HC efficiently hastened endodormancy release and budbreak, shortened blooming duration and improved fruit set. Furthermore, bioactive GAs play different roles in the process of endodormancy release and blooming. GA₃ was recognised as the highest isomer of bioactive GA and was closely related with endodormancy release improved by HC treatment as well as GA₄. GA₇ displayed a rapid increase when buds went into the burst stage and associated with promoted budburst and blooming by HC treatment. GA₁ showed irregular changes during this process. A higher GAs:ABA ratio was observed in HC-treated ‘Summit’ branches from endodormancy release until full bloom. However, a reciprocal pattern occurred thereafter due to senescence of the flowers.

ARTICLE HISTORY
Received 20 January 2016
Accepted 6 August 2016

KEYWORDS
Abscisic acid; blooming; endodormancy release; gibberellins; hydrogen cyanamide; sweet cherry

Introduction
Fruit trees from temperate or colder regions have specific genetic winter chilling requirements to break endodormancy and for economic production (Saure 1985; Erez 1995). Sufficient winter chill along with favourable spring temperature conditions guarantee full and uniform development of foliage and flowering in deciduous fruit trees such as peach (Chaar & Astorga 2012), apricot (Ruiz et al. 2007), apple (Hauagge & Cummins 1991) and sweet cherry (Alburquerque et al. 2008). Otherwise, the vegetative and reproductive behaviour of the cultivars will be affected negatively (Samish 1954).

Sweet cherries (Prunus avium L.) grown in temperate climates are commercially cultivated in more than 40 countries worldwide, mainly between 35°N and 55°S latitudes (Chadha 2003). In China, sweet cherry cultivation has expanded quickly beyond the
traditional temperate regions surrounding Bohai Bay to higher latitude areas in the southwest due to increasing consumer demand and the high profit for growers (Han et al. 2008; Zhang et al. 2013; Wang et al. 2014). The ability to cultivate sweet cherries around Shanghai, a southern warm winter region with a low number of chill hours, has been evaluated in recent years (Li et al. 2010; Zhang et al. 2013). One of the major limitations of cultivating sweet cherries successfully in this region is satisfying the chilling requirements, which are generally from 600 to 1400 h by a 7.2 °C model (Baldochi & Wong 2008). Symptoms of delayed foliation, deformed flowers or abortion of flowers and subsequent poor fruit set i.e. a reduction in potential yield, have been widely observed (Wang et al. 2004; Li et al. 2010).

Warm winters impair the breaking of endodormancy, possibly due to inadequate chilling accumulation (Yamamoto et al. 2010). Various methods have been used to supplement chilling. Hydrogen cyanamide (HC), an effective dormancy-breaking agent, has been used widely in grape (Vitis vinifera L.) (Dokoozlian et al. 1995), kiwifruit (Actinidia delicosa L.) (Mcpherson et al. 2001), apple (Malus domestica L.) (Krisanapook et al. 1995), peach (Prunus persica [L.] Batsch.) (Siller-Cepeda et al. 1992), pear (Pyrus spp.) (Singh & Mann 2002) and sweet cherry (Godini et al. 2005). Previous studies revealed that applying HC to fruit trees 2–5 weeks before field budburst resulted in early and uniform budbreak, increased flower and fruit numbers, faster flowering and fruiting, and improved fruit quality (Shuck & Petri 1995; Pramanik et al. 2009). Recent studies in grapevines have shown that the application of HC increased H2O2 levels and inhibited catalase activity, along with up-regulation of a series of transcriptions, including grape dormancy-breaking related protein kinase (GDBRPK), a sucrose non-fermenting protein kinase (SNF-like protein), pyruvate decarboxylase (PDC), alcohol dehydrogenase (ADH), thioredoxin h (Trxh), glutathione S-transferase (GST), ascorbate peroxidase (APX), glutathione reductase (GR) and sucrose synthase (SuSy) (Or et al. 2000; Neill et al. 2002; Pérez & Lira 2005; Keilin et al. 2007; Halaly et al. 2008; Pérez et al. 2008). However, the biological mechanism that underlies its dormancy-breaking effect has not been fully described. In pea seedlings and Malus sylvestris Mill. ‘Anna’ trees, the responses to HC are usually associated with the action of plant hormones (Guevara et al. 2008; Seif El-Yazal et al. 2014).

Plant hormones play pivotal roles in plant growth, development, and response to biotic and abiotic cues (Davies 1995, 2004). Endogenous hormone changes in the process of bud dormancy induction and release have been studied (Fernie & Willmitzer 2001; Yamazaki et al. 2002; Suttle 2004a, 2004b; Qin et al. 2009). Previous studies showed that gibberellic acids (GAs) often release endodormancy, whereas abscisic acid (ABA) is associated with endodormancy induction and maintenance. However, there is little information about the relationships between HC and endogenous phytohormones during endodormancy release and blooming (flowering) in sweet cherries. Therefore, the objectives of this study are to explain how changes in GAs and ABA levels in the branches of sweet cherry are associated with the regulation of endodormancy and blooming following HC application in ‘Summit’ sweet cherry trees, which are classified as a moderate-high chill variety with an 800 chilling hour requirement. These trees exhibit developmental defects in flowers, fruit sets and yields in the Shanghai region (Li 2011; Zhang 2011). In addition, the effect of HC on the release of bud dormancy under mild winters in Shanghai was investigated.
Materials and methods

Plant materials and treatments

The research was carried out on 6-year-old trees of ‘Summit’ grafted on Daqingye (*Prunus pseudocerasus* L.) rootstock by using *P. avium* L. ‘Lapins’ and *P. avium* L. ‘Tieton’ as pollinisers in a sweet cherry commercial orchard located in Shanghai (31°14′N, 121°29′E) in 2012. Trees were trained to a spindle system, spaced 5 × 6 m and regularly irrigated and fertilised.

On 8 February, nine sweet cherry trees were selected completely randomly and treated with a spray application of HC, which was approximately 30 days before the natural onset of budbreak in the Shanghai region. Each group of three trees was designated as one replicate. Each treatment was repeated in triplicate. Spray applications of HC were conducted once as follows: each tree was divided into two spray areas with polyethylene barriers. One side was sprayed to the point of run-off (3 L tree⁻¹) with 2% (v/v) hydrogen cyanamide (Ningxia Darong Chemicals & Metallurgy Co) using a 16 L knapsack sprayer. Another side received the equivalent volume of distilled water as a control. The applications were performed early in the morning on non-windy days to avoid significant drift, and no precipitation was recorded in the following 48 h.

To identify the effect of HC on dormancy release and blooming, the preliminary studies were conducted in both 2010 and 2011. HC was applied once or twice at different rates. A single application of HC at a concentration of 2% (v/v) was found to be the most effective treatment for later bud growth in ‘Summit’ sweet cherry trees (data not shown).

Phenophase observation, sampling and dormancy status testing

Five fruiting branches were randomly selected from the outer canopy approximately 1.5–1.8 m above the ground of nine trees in each treatment (HC and control). The bud phenophase of ‘Summit’ trees was recorded every 3 days until post bloom (senescence of the flowers). The floral bud phenophase of the sweet cherries was assessed and categorised into eight stages: first swelling, side green, green tip, tight cluster, first white, first bloom, full bloom and post bloom, according to the stages described by Washington State University (http://cahns-cms.wsu.edu/StoneFruit/research/Pages/GenesSpringFloralBuds.aspx) with minor modifications. The period of phenological growth of the whole branches was defined as the time when 50% buds or flowers on the branches reached the classified stages, except for the ‘first bloom’ period, during which approximately 5% of flowers opened. In addition, the dates at which approximately 75% and 100% of flowers opened were recorded in each treatment. Endodormancy release means the buds of temperate trees can burst in a suitable environment after a certain period of chilling. Budbreak was defined as the time when the green tips break through the brown scale and are just visible. Blooming duration indicated the days from ‘first bloom’ to ‘post bloom’. The final fruit set was calculated 4 weeks after the full bloom stage as the number of persistent fruits per 100 flowers under open pollination by bees.

To assess biochemical changes, two 2-year-old fruiting branches uniformly distributed over the periphery canopy at 1.5–1.8 m in each tree (HC and control) were chosen and tagged. Approximately 5 cm of each cleaned branch was sampled at 0, 14, 21, 28, 32, 36, 40, 44, 48, 52, 56 and 60 days after HC treatment. The buds, flowers and leaves of
each sampled branch were removed to avoid the changes of bud status after budburst and the confounding effects of their density variation on the endogenous hormone concentration. These branches were immediately frozen in liquid nitrogen and stored at −80 °C for further analysis.

To test the dormancy status of ‘Summit’ trees on each sampling date, nine 2-year-old branches with spurs in similar growth vigour were collected and put into vials containing distilled water after being cut, and were cultivated under controlled growth chamber conditions with a light intensity of 40 µmol m⁻² s⁻¹. The temperature ranged from 25 °C during the light period (16 h) to 18 °C in the dark, and the relative humidity was 80%–90%. The water was renewed every 2 days. After 4 weeks of cultivation, the budburst rate of floral buds was investigated, and a budburst rate greater than 50% indicated that endodormancy had been released.

In addition, to evaluate the behaviour of sweet cherry under Shanghai climatic conditions, macroclimate data including monthly average temperatures and total rainfall during shedding, dormancy and blooming were obtained from the local weather stations.

Branch water content

Branch water content was determined by drying a part of each sample in a convection oven at 80 °C for 96 h until constant weight was attained.

Gibberellic acids and abscisic acid extraction and purification

The branch samples were ground by a cryo-grinder (Freezer Mill 6870, SPEX SamplePrep) in liquid nitrogen. Hormone extraction and purification were based on the method developed by Zhang (2007) with minor modifications. The powder (approximately 2 g fresh weight [FW]) was extracted at 4 °C in the dark overnight in 10 mL 80% cold aqueous methanol containing 0.2 g L⁻¹ butylated hydroxytoluene (BHT), which served as an antioxidant. At this point, 100 ng deuterated GAs ([17-²H₂]GA₁, [17-²H₂]GA₃, [17-²H₂]GA₄ and [17-²H₂]GA₇) and 100 ng [²H₆]ABA were added to the extract, serving as internal standards for estimating the recovery after purification. The standards were purchased from OlChemIm. The solution was then centrifuged and the residue re-extracted twice with 5 mL of cold extraction solvent to ensure the complete extraction of the chemical and bioactive compounds. After centrifuging, the combined extracts were concentrated by a rotary evaporator under reduced pressure at 42 °C to remove organic solvent. The aqueous solution was adjusted to pH 6–7 and partitioned against hexane three times. The pH of the combined aqueous phases was acidified to 2.5 with 1 M HCl and partitioned against ethyl acetate. The combined ethyl acetate fraction was partitioned against 0.5 M phosphate buffer (pH 8.3). Then insoluble PVPP was added to the combined buffered phases and filtered. The filtered aqueous solution was adjusted to pH 2.5 by adding 6 M HCl and partitioned against ethyl acetate again. After drying with Na₂SO₄ and filtering, the combined ethyl acetate solution was evaporated in vacuo. The residue was dissolved in a small amount of 80% aqueous methanol and passed through a C18 Sep-Pak cartridge (Waters). The cartridge was pre-wetted with methanol, water and 80% water, and followed by drying under a stream of nitrogen. The residue was redissolved in a small amount of 45% methanol-water containing 0.1% acetic acid and was further
loaded on to a Bondesil DEA column (diethylaminopropyl, 40 µm, Agilent Technologies). After loading the sample, the column was eluted with distilled water, followed by methanol. The eluent was reduced to dryness under a nitrogen stream and stored at −20 °C before further purification by high-performance liquid chromatography (HPLC).

**High-performance liquid chromatography analysis**

The crude GAs and ABA were redissolved in methanol and subjected to a reversed-phase Senshu-Pak ODS-4253-D HPLC column (10 mm i.d. × 250 mm, Senshu Scientific). They were eluted with 0.1% acetic acid in 30% aqueous methanol (solvent A) and 100% aqueous methanol (solvent B) at 40 °C as follows: 0–3 min, elution with solvent A; 3–30 min, linear gradient of 0% to 100% solvent B; 30–50 min, elution with 100% solvent B. The flow rate of the solvent was 3 mL min⁻¹ and eluate fractions were collected every minute. The retention times of ABA, GA1, GA3, GA4 and GA7 were 22, 17, 15–16, 29 and 28 min, respectively, and these times were identified by running authentic standards under the same conditions.

**Gas chromatography/mass spectrometry-selected ion monitoring**

The bioactive GA-like fractions were dissolved in 50 µL methanol, then methylated with 2 µL of (trimethylsilyl) diazomethane (2 M solution in hexane, Alfa Aesar China [Tianjin] Co) at room temperature for 30 min followed by trimethylsilylation with 50 µL of Sylon BFT (BSTFA + TMCS, 99:1, Supelco) at 70 °C for 1 h in a sealed glass vial, and they were then used for gas chromatography/mass spectrometry (GC-MS) (7890A/5975C, Agilent Technologies) analysis. 1 µL of each silylated sample was injected into a DB-1 capillary column (0.25 mm i.d. × 30 m; 0.25 µm film thickness; J&W Scientific). The oven temperature for GAs was held at 80 °C for 3 min and then programmed to increase from 15 °C per minute to 300 °C, followed by 5 min at 300 °C. Helium was used as a carrier gas at a linear flow of 1 mL min⁻¹. The GC was directly interfaced to a mass-selective detector with an interface and source temperature of 280 °C, an electron energy of 70 eV and a dwell time of 100 ms.

A portion of the ABA equivalent fraction from ODS-HPLC was subjected to GC/SIM analysis after esterifying with 2 µL of 2 M trimethylsilyl-diazomethane solution in n-hexane. The temperature programme was set as follows: 3 min held at 60 °C, followed by an increase at a rate of 20 °C per minute to 290 °C and 5 min of constant temperature.

**Quantification of biologically active endogenous GAs and ABA**

To identify the eluted GAs using their retention times, diagnostic ions of endogenous GAs and deuterated GAs were monitored. The levels of endogenous GAs were determined by measuring the abundance of the following ion pairs: m/e 506/508 for GA1; m/e 504/506 for GA3; both m/e 418/420 and 284/286 for GA4; and m/e 416/418 and 222/224 for GA7. In the GC/SIM analysis of ABA, the characteristic ions m/z 190/194 were monitored.

The endogenous levels of the detected hormones were calculated by multiplying the peak area ratios of the endogenous hormones by their standard quantities and dividing by the tissue fresh weight (mg). Each treatment was repeated in triplicate.
**Statistical analysis**

Pairs of values were compared by Student’s t-test using SAS 9.1.3 software (SAS Institute). Differences were considered significant at the $P = 0.05$ level.

**Results**

**Effect of HC on dormancy release, floral budbreak and fruit set**

Shanghai has a humid subtropical monsoon climate. The monthly average temperature and precipitation during shedding, dormancy and blooming are presented in **Table 1**.

When release from endodormancy occurs, the buds of fruit trees can burst in a suitable environment. However, if the environmental temperature is low, buds cannot burst, a situation referred to as ecodormancy. In the Shanghai region, the budburst rate of ‘Summit’ sweet cherry was approximately 30% on 8 February (**Table 2**), indicating that ‘Summit’ was still in the endodormancy stage at 0 days after HC treatment. Subsequently, the budburst rate increased to 72% and 96% under HC treatment, and 42% and 68% under the control treatment on 22 February and 7 March, respectively. Therefore, the release date of bud endodormancy under HC treatment was mid-February, whereas it was early March in the control group, which was approximately half a month later than the HC treatment.

In addition, the spray application of HC to endodormant ‘Summit’ sweet cherry trees was found to advance the phenological development compared with the controls, in which parts of the trees were sprayed with distilled water (**Table 3, Figure 1**). Application of HC induced budbreak initiation 11 days earlier than the control. Application of this agent also significantly induced early blooming compared with the control. Blooming occurred 5 days earlier in the treated trees compared with the controls. HC was found to be effective in causing full bloom to occur earlier. The blooming duration was also shortened to 8 days compared with 10 days in the controls. Furthermore, HC significantly increased the fruit set by 359.6% compared with the controls.

**Effect of HC on branch water content**

Water is one of the basic determining factors for bud development because it is quantitatively the major component of plant tissues (Zeuthen 2001). Dormancy of woody plants is considered to be closely related to changes in water movement (Welling & Palva 2006). Branch water content changes are shown in **Figure 2**. Similar trend of water content changes were found in HC-treated branches and in the controls, except that the increased water content induced by application of HC was significantly higher than the reduced water content in the controls at the beginning. However, there is a rapid increased after budbreak. Additionally, branch water content was consistently higher in HC treatment than in the controls.

**Table 1.** Monthly average temperature (°C) and precipitation (mm) during shedding, dormancy and blooming in Shanghai, China.

<table>
<thead>
<tr>
<th></th>
<th>2011</th>
<th>2012</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>October</td>
<td>November</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>19.1</td>
<td>12.8</td>
</tr>
<tr>
<td>Precipitation (mm)</td>
<td>63.3</td>
<td>54.1</td>
</tr>
</tbody>
</table>
Changes in endogenous biologically active gibberellic acids and abscisic acid

Levels of GAs isomers in branches of ‘Summit’ sweet cherry tree showed different patterns during the dormancy release and blooming, as shown in Figure 3. Four bioactive gibberellic acids (GA1, GA3, GA4 and GA7) were detected in branches during this process. Generally, GA3 (Figure 3B) was the most abundant gibberellin, followed by GA7 (Figure 3D), then GA4 (Figure 3C), and the concentration of GA1 (Figure 3A) was the lowest. From endodormancy release to blooming, levels of GA1, GA3 and GA4 tended to decline in both HC-treated and the control branches, and GA7 increased rapidly after buds burst. During endodormancy release and the initial phase of blooming, the level of GA3 in HC-treated branches was always higher than that in the controls. However, this difference diminished gradually with budburst. GA3 levels were even lower than in the controls after full bloom. For GA1 and GA4, there were significant differences between HC-treated and control branches sometimes, but there was no regular change during the processes of endodormancy release and blooming. Before budburst, no significant difference was observed in GA7 levels between HC treatment and controls. However, GA7 levels rapidly increased in HC-treated branches and were significantly higher compared with controls from the burst stage to full bloom. After full bloom, the levels of GA1, GA3, GA4 and GA7 in HC-treated branches were lower than those in the controls in most cases. As for total amount of bioactive GAs (Figure 3E), it displayed a similar curve to GA3, and HC treatment had higher total GAs levels than the controls before full bloom.

Changes in ABA levels in branches during the dormancy release and blooming are shown in Figure 4. The ABA levels declined rapidly, and no significant difference was found between HC treatment and the controls at the early stages of endodormancy release (41 days before blooming). However, the ABA concentrations in HC treatments were lower than those in the controls from 31 days before the start of blooming until full bloom. Then, they were higher than the controls as the flowers became senescent.

In addition, the ratio of GAs:ABA (Figure 5) in natural conditions was relatively low, with little fluctuation during endodormancy release and blooming. However, the GA:ABA ratio in HC-forcing conditions increased continuously before the ‘green tip’ stage, and it was higher than that in the control before ‘first bloom’.

Table 2. Rates of budburst in HC-treated and control branches of ‘Summit’ sweet cherry trees, sampled in 2012.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>8 February</th>
<th>22 February</th>
<th>7 March</th>
<th>14 March</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>34%</td>
<td>42%</td>
<td>68%</td>
<td>90%</td>
</tr>
<tr>
<td>HC</td>
<td>30%</td>
<td>72%</td>
<td>96%</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3. Effect of spray-applied HC on floral budbreak, blooming and fruit set of ‘Summit’ sweet cherry trees in 2012.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Date of floral budbreak</th>
<th>Date of blooming</th>
<th>Blooming duration (days)</th>
<th>Fruit set (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14 March</td>
<td>8 April</td>
<td>9 April</td>
<td>10</td>
</tr>
<tr>
<td>HC</td>
<td>3 March</td>
<td>3 April</td>
<td>5 April</td>
<td>8</td>
</tr>
</tbody>
</table>

All values are means ± SEM (n = 5).

*Significant differences at P = 0.05 by a standard t-test.
Discussion

In temperate woody perennial plants, endodormancy release by exposure to low temperatures involves complicated mechanisms that are regulated by many factors. Dormancy

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Phenophase</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC</td>
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</tr>
<tr>
<td>Control</td>
<td><img src="Control_8Feb.png" alt="Image" /></td>
</tr>
<tr>
<td></td>
<td>26 Mar.</td>
</tr>
<tr>
<td>HC</td>
<td><img src="HC_26Mar.png" alt="Image" /></td>
</tr>
<tr>
<td>Control</td>
<td><img src="Control_26Mar.png" alt="Image" /></td>
</tr>
</tbody>
</table>

* and ** indicate significant differences at $P = 0.05$ and $P = 0.01$ by a standard t-test, respectively. FW, fresh weight.

**Figure 1.** Spray application of HC on blooming of ‘Summit’ sweet cherry trees.

**Figure 2.** Effect of spray-applied HC on branch water content of ‘Summit’ sweet cherry trees in 2012. The stages of floral budbreak are indicated by arrows (solid, HC-treated; dotted, control); full bloom stages are indicated by lines (solid, HC-treated; dotted, control). All values are means ± SEM ($n = 3$). * and ** indicate significant differences at $P = 0.05$ and $P = 0.01$ by a standard t-test, respectively. FW, fresh weight.
Figure 3. Effect of spray-applied HC on levels of: A, endogenous gibberellic acid 1 (GA1); B, GA3; C, GA4; D, GA7; E, total bioactive GAs, in branches of ‘Summit’ sweet cherry trees. The stages of floral budbreak are indicated by arrows (solid, HC-treated; dotted, control); full bloom stages are indicated by lines (solid, HC-treated; dotted, control). All values are means ± SEM (n = 3). * and ** indicate significant differences at P = 0.05 and P = 0.01 by a standard t-test, respectively. FW, fresh weight.

Figure 4. Effect of spray-applied HC on endogenous abscisic acid (ABA) in branches of ‘Summit’ sweet cherry trees. The stages of floral budbreak are indicated by arrows (solid, HC-treated; dotted, control); full bloom stages are indicated by lines (solid, HC-treated; dotted, control). All values are means ± SEM (n = 3). * and ** indicate significant differences at P = 0.05 and P = 0.01 by a standard t-test, respectively. FW, fresh weight.
status testing and observation of phenological stages in this study showed that 2% (v/v) of the aqueous solution of HC was able to advance endodormancy release, budbreak and blooming of 'Summit' sweet cherry trees (Figure 1; Tables 2 and 3). Moreover, HC also shortened the blooming duration of 'Summit' under the natural field conditions of Shanghai. However, the blooming advancement induced by HC was lower than the endodormancy release and bud-break advancement. This may indicate that HC sapped its effect in inducing endodormancy release, whereas climatic factors began to influence the blooming processes from bud-break onward, generally decreasing the initial advancement (Godini et al. 2005).

In previous works, HC was found to play a role in inducing enzyme activity, promoting the retranslocation of stored reserves and increasing the uptake of nitrogen (Yang et al. 1990; Seif El-Yazal & Rady 2012). Catalase activities were increased by applying HC at an appropriate dose (Bartolini et al. 1997), which results in the detoxification of H$_2$O$_2$ in plant tissues by a chain of reactions that link to the pentose phosphate pathway, leading to increased turnover of the pathway and increased bud-break (Bichler 1999). Although no visible phenological differences were observed between HC treatment and the controls early in the experiment, significant differences were found in branch water content. Furthermore, significantly higher water content was found under HC-forcing conditions in most of the phenological phase (Figure 2). Similar results were obtained by Trejo-Martinez et al. (2009), indicating that the changes were favoured by HC sprays.

Although there is no generally accepted mechanism to explain the endodormancy results in perennial plants, hormones have been regarded as one of the key factors associated with dormancy based on concentration measurements and expression analysis of hormone biosynthesis and hormone-responsive genes (Rinne et al. 2011). It has been suggested that GAs may function in the timing of dormancy establishment and chilling-induced release (Hazebroek et al. 1993; Zanewich et al. 1995; Yamazaki et al. 2002; Suttle 2004a, 2004b). In contrast, ABA was found to be correlated with the inhibition of bud growth and dormancy induction and maintenance (Yamazaki et al. 2002; Suttle

**Figure 5.** Effect of spray-applied HC on the ratio of endogenous total bioactive GAs and abscisic acid (ABA) levels in branches of 'Summit' sweet cherry trees. The stages of floral budbreak are indicated by arrows (solid, HC-treated; dotted, control); full bloom stages are indicated by lines (solid, HC-treated; dotted, control).
A high ABA level was found in dormant buds. However, ABA declines at the end of the dormancy stage (Fernie & Willmitzer 2001; Yamazaki et al. 2002). In the current study, both GAs and ABA in the branches of ‘Summit’ declined during the endodormancy release stage, but they increased slightly before budbreak and increased continuously during blooming under natural climate conditions (Figures 3 and 4), which indicated that a reduced ABA level is not always associated with growth resumption. The results of the present study are consistent with the findings of Yamazaki et al. (2002), which indicate that endogenous ABA levels increase concomitantly with bud elongation after dormancy release.

The major isoform of endogenous bioactive GA varies among plant species. GA1 is considered to be the main GA involved in stem elongation, with low flowering activity, whereas GA3 has higher florigenic activity (Evans et al. 1993). In addition, GA3 can partially or totally substitute for the action of low temperature in beech nuts (Fagus sylvatica L.) (Bonnet-Masimbert & Muller 1976) and it hastens floral bud development and shortens the time to anthesis at the late flower bud developmental stages during winter dormancy in peach (Reinoso et al. 2002). However, GA4 is thought to be the main GA involved in shoot elongation in hybrid aspen (Israelesson et al. 2004) and it is able to reverse the obstructed transport paths in the symplasm and cell walls. It also induces canoical budburst and development in a concentration-dependent way, whereas GA3 fails to induce the same response (Rinne et al. 2011). GA7 has often been associated with blooming, especially when applied exogenously in conifers (Ross 1992; Cecich et al. 1994). Our analysis of GA isomers showed that there were remarkable differences in the patterns of endogenous bioactive GA concentrations (GA1, GA3, GA4 and GA7) during the endodormancy release and blooming stages (Figure 3). In ‘Summit’ sweet cherry, GA3 was found to be the most abundant isomer of bioactive GA, followed by GA7 and GA4. The concentration of GA1 was lowest during the endodormancy release and blooming stages. This suggests that GAs may have selective effects on endodormancy release and blooming, and GA3 may play a major role in endodormancy release and blooming in ‘Summit’. In addition, a sharp and transient increase in both GA7 and total GAs in branches of ‘Summit’ were observed during blooming (Figure 3D–E). This suggests the involvement of gibberellin in induction of earlier blooming in sweet cherry.

Regarding the effects of HC on endogenous phytohormone concentration, Luna et al. (1993) indicated that the favourable effects of HC (Dormex) on the date of floral bud opening may be due to the stimulatory effects on natural gibberellins. HC may release buds from dormancy by decreasing the ABA concentration (Nashaat 1996). Our results showed that there were higher concentrations of gibberellins and lower concentrations of ABA in branches that were sprayed with HC than in control branches (Figure 3). This finding agrees with the report of Mohamed et al. (2014), who found that HC (Dormex) is rapidly metabolised in the plant and increases the synthesis of GAs, which promotes the activity and biosynthesis of α-amylase, whose products are either a necessary substrate or a signal for accelerated growth that contributes to budbreak and the metabolism of ABA (Goggin et al. 2011; Rentzsch et al. 2012).

Plant dormancy is controlled by numerous integrated plant structures and functions (Simpson 1990; Crabbe 1994) and is induced and released by changes in the balance between inhibiting and stimulating endogenous substances. It is reported that GAs often release dormancy and stimulate bud growth (Fernie & Willmitzer 2001). In contrast, ABA
is considered to be antagonistic with GAs in their roles during the processes of dormancy initiation in plants (Anderson et al. 2001; Jacobsen et al. 2002; Seo et al. 2006). The ratio of the endogenous hormones GA and ABA is considered to be a relevant factor regulating endodormancy. In contrast to the changes in GA and ABA levels, the ratio of GA:ABA in branches increased during endodormancy release and in the budbreak stage, but decreased after full bloom due to senescence of the flowers. This suggests that the relatively higher GA concentrations and lower ABA concentrations were needed for endodormancy release in ‘Summit’ sweet cherry. Qin et al. (2009) reported that the ratio of GA₃:ABA in apple buds decreased in dormant buds, then increased in opening buds. In the current study, hydrogen cyanamide effectively reduced ABA levels but slowed the decline of bioactive GA levels, which resulted in a sustained increase in the GA:ABA ratio during the process of endodormancy release.

**Conclusion**

In this study, spray application of HC on ‘Summit’ sweet cherry trees hastened bud release from endodormancy, shortened blooming duration and improved bud growth and fruit set. The current study showed that GAs might have selective effects on endodormancy release and blooming. GA₃ was the most abundant GA isomer and it actively responded to the application of HC as well as GA₄, which might be closely related to endodormancy release, which is improved by HC application. GA₇ displayed a rapid increase when buds went into the budburst stage until full bloom, and it also responded positively to HC and was associated with budburst and blooming improvement induced by HC application. The GA₁ level had no regular changes during the process of endodormancy release and blooming. Furthermore, a higher GA:ABA ratio was observed in HC-treated ‘Summit’ branches from the phase of endodormancy release until full bloom. However, a reciprocal pattern occurred afterwards due to senescence of the flowers. Therefore, if it is economically feasible, hydrogen cyanamide could be an option for the production of sweet cherries with higher chilling requirements, including ‘Summit’ in low winter chill regions.

**Acknowledgements**

Authors wish to thank the anonymous reviewers for their helpful comments.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Funding**

The authors would like to thank the Foundation of ‘948’ Project of Ministry of Agriculture of China (grant number 2013-Z23) for financial support.

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