SCIENTIFIC NOTE

CHILEANJAR
CHILEAN JOURNAL of AGRICULTURAL RESEARCH

EFFECTS OF HYDROGEN CYANAMIDE ON THE FLORAL MORPHOGENESIS OF KIWIFRUIT BUDS

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ABSTRACT

The influence of hydrogen cyanamide (HC) on the flower bud development of kiwifruit (*Actinidia deliciosa* (A. Chev.) C.F. Liang & A.R. Ferguson). 'Hayward' was studied. The bud samples were taken every 5-10 d starting from dormant season (March) and fixed in FAA (10% formalin, 50% ethanol, 5% glacial acetic acid). Flower bud development was compared in three HC concentrations and the control. 1%, 2%, and 3% of HC was applied 35 d before the expected natural bud break. During the onset of bud break, only 57.6% of control buds had sepal primordia developed. On the other hand, HC treated buds had almost completed their stamen formation and started stigma primordia. When the control vines were in advanced bud break, gynoecial plateau already began to form in the vines treated with 2 and 3% HC. Vines treated with 1% HC lagged a little behind and had not started developing the gynoecial plateau. As the bud developed from the open cluster to the tight bud stage, the differences between the control and HC treated plants were more distinct. However, there were no differences between HC treatments as the ovule initiation took place in the buds.

Key words: *Actinidia deliciosa*, ovule initiation, floral formation, flower primordia.

INTRODUCTION

Flower bud initiation is of great importance in the fruit growing because with development, flowers become the fruit. Information on floral ontogeny in plant species is essential for the establishment of breeding programs and for the understanding of the evolutionary processes involved in the development of the floral organs. Flower bud initiation occurs through a biochemical signal. This biochemical signal makes it possible for the tissue to change from vegetative to reproductive state in a programmed way (Faust, 1989). Flower bud initiation and differentiation are affected by multiple factors. There is sufficient data regarding the effect of the position of the buds in the vine (*Actinidia deliciosa* (A. Chev.) C.F. Liang & A.R. Ferguson) (Lebon *et al*., 2004), the effect of high temperatures (Rhee, 1977; Walton and Fowke, 1993), and water availability (Engin, 2006) during flower bud formation.

Also, in many woody species, many authors have observed a relationship between flower sexuality and plant growth regulators (Chailakhyan and Khrianin, 1987; Sedgley and Griffin, 1989). Hydrogen cyanamide is a growth regulator which promotes the breaking of dormancy in fruit trees. It has been used for a number of years on fruit crops to replace the lack of winter chilling and to induce uniform bud break and yield increase (Henzell and Briscoe, 1986; Linsley-Noakes, 1989; Costa *et al*., 1997) in countries such as New Zealand, Chile, Australia, Israel and Turkey on several fruit crops including grape (*Vitis vinifera* L.), kiwifruit, apple (*Malus communis* L.), pear (*Pyrus communis* L.), peach (*Prunus persica* L.), plum (*Prunus domestica* L.) and cherry (*Prunus avium* [L.] L.) It also stimulates shoot growth, earliness in blooming, enhances flowering and fruiting and provides other desirable changes in plant performance. Dormex, a commercial product (SKW Trostberg AG, Trostberg, Germany) containing hydrogen cyanamide as an active ingredient, was found effective in replacing lack of chilling in kiwifruit to significantly increase flowering and fruiting (Powell *et al*., 2000) and in synchronizing bud break (Walton, 1996). Therefore, Dormex application might be expected cause a variation in the progression of flower differentiation.

Flower bud development has been described for *Actinidia kolomikta* (Maxim. & Rupr.) Maxim (Kolbasina, 1969) and *Actinidia chinensis* Planch.

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Received: 14 September 2009.
Accepted: 20 February 2010.

(Brundell, 1975a). Floral differentiation in kiwifruit occurs after the dormant period. Sepals, petals, stamens, stigma and ovule differentiate sequentially (Brundell, 1975a). The knowledge about the timing of the different stages of flower bud initiation and developmental changes in kiwifruit is important for developing management strategies to enhance flowering and ultimately, to regulate fruit crop load.

Determining the time of floral bud initiation and the developmental changes occurring during kiwifruit development, may help in choosing the appropriate time for cultural practices. The major objectives of this research were to determine the time of floral initiation and differentiation and to better understand the morphological changes occurring at the apex of an axillary bud of kiwifruit during flower formation and when vines were exposed to hydrogen cyanamide.

MATERIAL AND METHODS

An 11-yr old commercial block of kiwifruit 'Hayward' vines located near the University of Çanakkale (40°24'54'' N; 26°24'24'' E), Turkey, was used for this study. The staminate kiwifruit cultivar was Matua. Kiwifruit vines were managed using the standard procedures used in Turkey that is trained on T-bars. Daily temperatures were hourly recorded by a datalogger and chill units accumulated were calculated by the 7.22 °C and under model (Darrel, 1993) from at the beginning of November 2006 till the end of March 2007 (Table 1).

Hydrogen cyanamide at 1, 2 and 3% (490 g L^{-1} active ingredient, SKW Trostberg AG, Trostberg, Germany) was sprayed on the vines at a rate of 980 L ha^{-1}, 35 d before natural bud break in the control vines that took place around 2 April 2007. Pure water was sprayed on the control. A small sprayer was used to apply the treatments and the vines were sprayed to the dripping point.

Between 10 and 15 buds from the replacement canes of 1-yr old uniform in size and vigor were collected arbitrarily every 5-10 d. Bud samples were taken starting from March 2007 during the dormant season. Attention was paid during collection of the buds to take those facing upwards and originated in vines of similar diameter. Buds were fixed and stored in a solution of formalin, 70% ethanol, and glacial acetic acid (10:50:5, by volume) (McLaughlin and Greene, 1991). At least 10 buds from each sample were dissected by removing each bract or leaf using a scalpel and a stereo zoom microscope (SZ61, Olympus, Southend-on-Sea, Essex, UK). Each axillary structure within each bud was examined and identified. Morphological changes in each sample were recorded using a camera (Olympus C-7070) mounted on the microscope. The morphological changes in flower primordia were observed and the developmental stage of the flower buds was classified as described by Brundell (1975a) for kiwifruit. For each collecting time, the percentage of flowers at a distinct stage was registered (Table 2). The number of buds observed varied depending on availability and necrotic or aborted flowers were neglected.

Effects of the hydrogen cyanamide applications in each stage during the progression of flower bud development were tested with z-proportion test (comparing two proportions) after data normalization. The number of observations was 12.

RESULTS

The use of stereomicroscope revealed the developmental stages of the flower buds in 'Hayward' kiwifruit (Figure 1). When flower bud was almost invisible to the human eye in the leaf axil, reproductive meristem was dome shaped (Figure 1A). As the flower bud enlarges, still very hard to see, two bracts flanking the meristem become visible (Figure 1B). When the flower bud was as big as half of a pin head, sepal primordia begin to differentiate (Figure 1C). From this point forward, flower bud was visible to the naked eye and primordia of petal (Figure 1D) and stamen

Table 1. Temperatures and accumulated chill units at the experiment location for kiwifruit 'Hayward'.

	Mean maximum temperature	Mean minimum temperature	Mean temperature	Accumulated chill units
	ºC			
November, 2006	14.9	7.2	10.10	242
December, 2006	9.8	4.1	5.90	478
January, 2007	8.8	3.7	4.20	540
February, 2007	10.1	3.8	6.70	410
March, 2007	16.3	11.2	13.59	72
Total chill units				1742

[1]Soil C emissions were assumed to come from the whole-soil mass under a surface section and homogeneous bulk density in the profile each 10 cm depth.

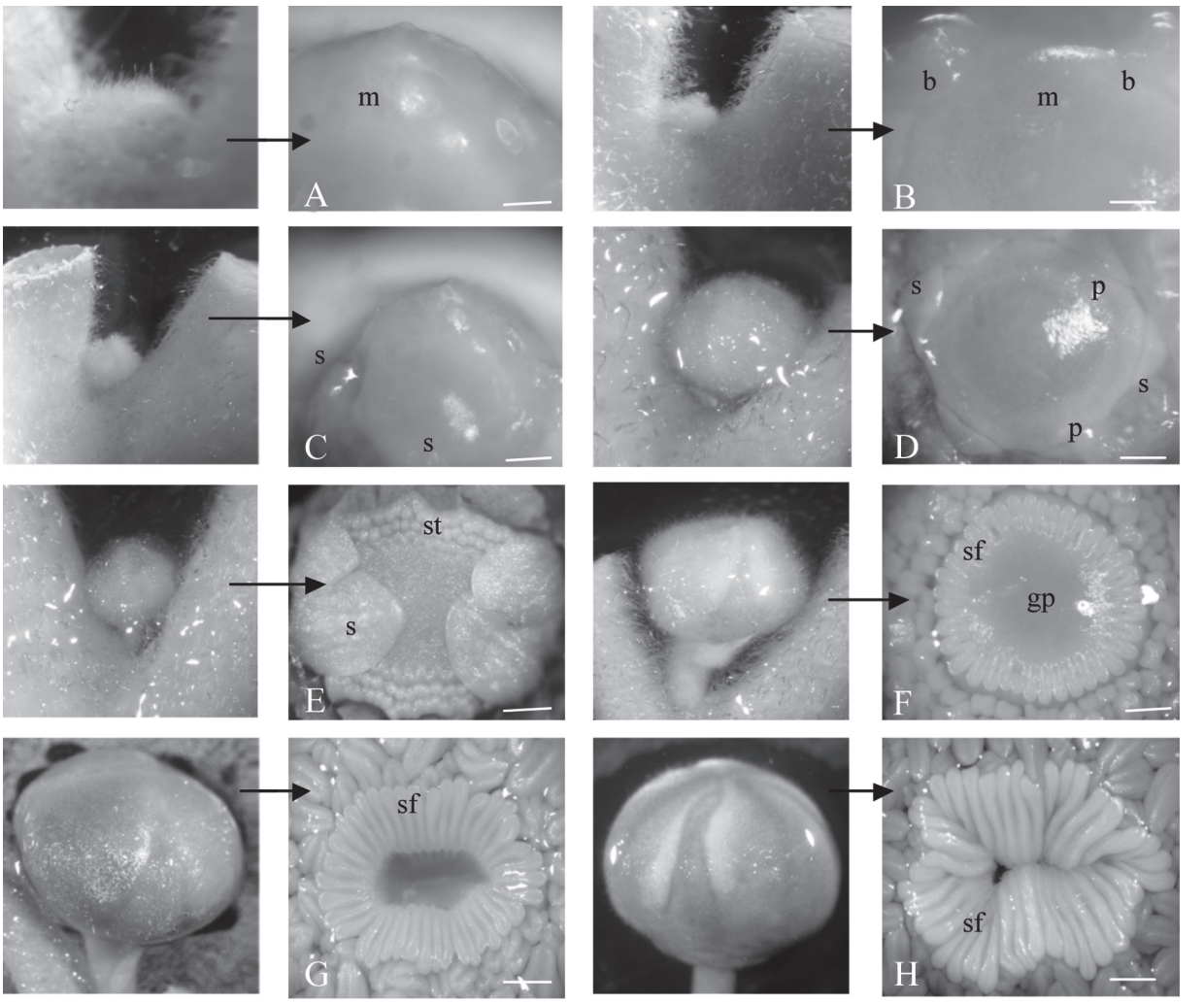
Table 2. Influence of hydrogen cyanamide on the progression of flower bud formation in kiwifruit 'Hayward'.

Sampling time	Hydrogen cyanamide treatments	Flower phenological stage[1]							
		Flower primordia	Bract primordia	Sepal primordia	Petal primordia	Stamen primordia	Stigma primordia	Gynoecial plateau	Ovule initiation
		%							
Dormant	0%								
(14 Mar.)	1%	100.0a							
	2%	100.0a							
	3%	91.6a	8.4						
Dormant	0%								
(19 Mar.)	1%	36.7a	63.3a						
	2%	28.5a	71.5a						
	3%	11.4a	82.8a	5.8					
Bud swell	0%	95.7a	4.3b						
(24 Mar.)	1%	4.4b	66.8a	28.8b					
	2%		49.7a	50.3ab					
	3%		6.7b	79.8a	13.5				
Advanced	0%	22.5	72.0a	5.5b					
bud swell	1%		6.3b	49.9a	43.8b				
(27 Mar.)	2%			27.1ab	72.9ab				
	3%			7.7b	82.4a	9.9			
Bud break	0%	4.6	37.8	57.6a					
(1 April)	1%			23.1a	39.4a	37.5b			
	2%				22.6a	71.8ab	5.6a		
	3%				10.3a	77.8a	11.9a		
Advanced	0%		10.2	45.8	44.0a				
bud break	1%				27.2a	33.8a	39.0b		
(6 April)	2%					12.3a	80.5a	7.2a	
	3%					17.4a	66.2ab	16.4a	
Open cluster	0%			15.2	20.1	64.7a			
(13 April)	1%					8.4b	51.5a	40.1b	
	2%						27.4ab	72.6ab	
	3%						9.4b	90.6a	
Advanced open	0%				5.2	22.1	62.5a	10.2ab	
cluster	1%						12.4b	36.2a	51.4b
(26 April)	2%							3.2b	96.8a
	3%								100.0a
Tight bud	0%						23.6	23.8	52.6b
(7 May)	1%								100.0a
	2%								100.0a
	3%								100.0a

[1]Phenological stages were determined according to the control (0%) vines.

(Figure 1E) are distinguished. Stalk also became easily seen. As the flower bud developed, pistil enlarged (Figure 1F) and stigma and gynoecial plateau start to develop (Figure 1G). In the following days, stigma developed more (Figure 1H) and floral morphogenesis completed.

Early signs of floral differentiation were already visible on the vines exposed to HC treatment at the first collecting date (Table 2). However, the vines were still dormant until the end of March and reproductive meristems remained uninitiated as explained by Brundell (1975a). Flower bud differentiation occurred almost 2 wk before bud break (1 April) in the treated vines.



A) Initial phase of change from vegetative to reproductive stage, showing rounded meristem (m) and swollen flower primordia, bar = 50 μm; B) flower (f) primordia enlarged and rounded; and initiation of bracts (b), bar = 50 μm; C) sepal (s) primordia initiation, bar = 50 μm; D) petal (p) primordia initiation in pentagonal whorl, bar = 100 μm; E) stamen (st) primordia, bar = 150 μm; F) stigma formation (sf) and gynoecial plateau (gp), bar = 200 μm; G) pistil enlargement and differentiation, bar = 250 μm; and H) pistil continuing to develop, stigma developing, and ovule formation, bar = 250 μm.

Figure 1. External (left) and internal (right) developmental stages of flower bud of kiwifruit 'Hayward'.

During bud swelling in the control vines, flower primordia had already differentiated by 95.7% in the control buds and differences between HC treatments began to surface. Flower primordia in 1 and 2% HC treated vines were completely formed while 3% HC progressed well into forming sepal primordia. Concurrently, 3% HC treated vines were already developing petal primordia. Vines treated with 1% HC started to develop sepal (28.8%), while 2% had sepal primordia (50.3%) and 3% had formed sepals (79.8%) and been into forming petal primordia (13.5%).

At advanced bud swell stage, control buds had only small amount of sepal formation (5.5%), while half of the 1% HC treated buds completed their sepal and petal formations. The highest dose of HC was one stage ahead of other treatments and had completed 9.9% of its stamen primordia development.

During the onset of bud break in the control vines, only 57.6% of control buds had sepal primordia developed. On the other hand, HC treated buds almost completed their stamen formation and started stigma primordia. When the control vines were in advanced bud

break, gynoecial plateau already began to form in the vines treated with 2 and 3% HC. Vines treated with 1% HC lagged a little behind and had not started developing it. As the bud developed from open cluster to tight bud stage, the differences between the control vines and HC treated vines were more distinct. However, there were no differences between the HC treatments as the ovule initiation took place in the buds. The bud break of the HC treated vines were approximately 10 d before control vines.

The chilling requirement of 'Hayward' ranges from 650 to 1200 chill units (Brundell, 1976; Guerriero *et al*., 1992). Temperatures and the chill units accumulated at the experiment location showed that 1742 chill units accumulated from the beginning of November to the end of March (Table 1), which was enough to satisfy the chilling requirement of this species for uniform bud break.

DISCUSSION

In kiwifruit, growth and flowering occur in a 2-yr cycle, with the current season's shoots originating from axillary meristems on the previous season's growth (Snowball, 1997; Walton *et al*., 2001). In the spring of the second year, the active bud begins to swell and develops into an open cluster containing few leaves (Brundell, 1975b). Flowers are borne at the base of an emerging shoot in leaf axils. Brundell (1975a) stated that flower primordia are not present in the buds during dormancy, but begin to differentiate at bud swell, approximately 10-15 d prior to bud break.

This study indicates the timing of the progression of kiwifruit flower bud formation. For 'Hayward' in western Turkey, differentiation occurred at the end of March. This was in agreement with the findings of Linsley-Noakes and Allan (1987) and Snowball and Walton (1992). Differentiation occurs 15 d later in Japan (Watanabe and Takahashi, 1984). They reported in kiwifruit (*A. chinensis*) that the meristem initiated flower primordia by the mid-March, and the initial formation of stamens was observed in the beginning of April. Caldwell (1989) reported the occurrence of differentiation in South Carolina at the beginning March, and Brundell (1975a) in New Zealand in mid-late September.

Schuck and Petri (1995) and Linsley-Noakes (1989) sprayed HC to anticipate bud break and improve bud phenological synchrony. Hydrogen cyanamide has been shown to promote early and more uniform bud break of kiwifruit. Bud break advancement varied with concentration, application time and thermic conditions before bud break (Schuck and Petri, 1995). In our study, HC (especially 3% and 2%) was observed to promote uniform bud development and increased and advanced bud burst compared to the controls at least 10 d. These results also indicated that floral differentiation may be under hormonal regulation. The kiwifruit flower bud exposed to HC reached more advanced floral stages sooner than buds from control (0%) vines, agreeing with McPherson *et al*. (1997), who found that HC advanced the date of bud break and increased bud break and the total number of flowers. Our results clearly show that HC application during dormant period markedly affected the progression of floral differentiation in 'Hayward' kiwifruit, suggesting that increasing HC concentration accelerated the progression of flower differentiation. This pattern supports the comment made by Linsley-Noakes (1989) that HC produced a more compact bud break period. These findings also agree with Powell *et al*. (2000), who reported 4% after mild winters Dormex application rate increased the number of flowers produced. Veloso *et al*. (2003) showed that 4% Dormex application was the most efficient in increasing marketable yield due to increase of flower bud formation. The effects of HC application in different New Zealand kiwifruit ('Hayward') growing areas were studied by McPherson *et al*. (2001). They found that HC applications tended to advance bud break and flowering. Hydrogen cyanamide had greater effect at the warmest sites (Costa *et al*., 1997).

Winter temperatures seem to alter the response of vines to HC. Richardson *et al*. (1995) found that the response of vines to HC varied from year to year and that the absolute numbers of flowers per winter bud were much higher in the year with the highest natural chilling. Costa *et al*. (1997) stated that the greater the number of chilling units at the time of HC application, the greater was the advance in the date of bud break. The experiment site had more chill units than the cv. Hayward required, possibly leading to earlier bud break in the vines, beyond the effect of the HC treatment.

We hope that, with the results presented here, kiwifruit researchers can design and interpret experiments with a clearer understanding of the stages of flower bud development and differentiation that may be affected by their experimental manipulations.

CONCLUSION

In general, HC advanced flower bud initiation, differentiation and development, the dose and application time heavily determine on the outcome in terms of flowering and fruit set. However, earlier bud break and flowering might pose a risk of frost in the areas with higher chill unit accumulation. Hydrogen cyanamide treatment causes a compressed flowering period.

RESUMEN

Efectos de la cianamida de hidrógeno sobre la morfogénesis floral de kiwi. El presente estudio evalúa la influencia de la aplicación de cianamida de hidrogeno (HC) sobre el desarrollo de las yemas florales de kiwi (*Actinidia deliciosa* (A. Chev.) C.F. Liang & A.R. Ferguson) cv. Hayward. Las muestras de yemas se tomaron cada 5-10 días comenzando en la época de dormancia en marzo y se fijaron en FAA (10% formaldehido, 50% etanol, 5% ácido acético glacial). Se comparó el desarrollo de las yemas florales en tres concentraciones de HC y el control. Se aplicó HC al 1%, 2% y 3% 35 días antes del brote natural de las yemas. En el momento de la apertura de las yemas, sólo el 57,6% de las yemas de control habían desarrollado los primordios de los sépalos. Por el contrario, las yemas tratadas con HC casi habían completado la formación de estambres y habían empezado el desarrollo de primordios del estigma. Cuando las plantas de control estaban en un estado avanzado de apertura de las yemas, la meseta del gineceo ya había empezado a formarse en las plantas tratadas con HC al 2% y al 3%. Las plantas tratadas con HC al 1% quedaron un poco retrasadas y no habían comenzado a desarrollarlo. A medida que las yemas se fueron desarrollando desde el estado de racimo abierto hasta el de yema apretada, las diferencias entre las plantas control y las tratadas con HC fueron más evidentes. Sin embargo, en el momento de la iniciación de los óvulos en las yemas no se observaron diferencias entre los diferentes tratamientos con HC.

Palabras clave: *Actinidia deliciosa,* iniciación ovular, formación floral, primordios florales.

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