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EFFECT OF TIME OF PRUNING AND HYDROGEN CYANAMIDE APPLICATION ON HARVESTING TIME AND FRUIT QUALITY IN GRAPES (*Vitis vinifera* L.)

N K Arora[1]*, Rachna Arora[1], Gagandeep Kaur[1] and M I S Gill[2]

[1]*Department of Fruit Science,* [2]*Dean, College of Horticulture and Forestry, Punjab Agricultural University, Ludhiana -141004, Punjab*

ABSTRACT

An experiment was conducted to ascertain the effect of pruning time and hydrogen cyanamide application on bud burst, maturity and fruit quality of Flame Seedless grapes. The plants were pruned at six different intervals and foliar application of hydrogen cyanamide @ 2 % was given to the vines immediately after each pruning interval. These were compared with the vines kept untreated at each pruning interval. The study revealed that bud burst was 12-23 days earlier in vines treated with hydrogen cyanamide as compared to untreated vines. Amongst the hydrogen cyanamide treated vines, bud burst was the earliest when pruning was done in last week of December. In case of untreated vines, bud burst occurred during the month of March. The application of hydrogen cyanamide immediately after pruning advanced the time of flowering and ripening by 5-9 days and 4-5 days, respectively, as compared to untreated vines. The pruning time of first week of January registered more yield (31.4 kg/vine), while the overall yield (28.6 and 28.8 kg/vine) and bunch weight (378.1 and 378.4 g) were at par among the for treated and untreated vines, respectively. The fruit quality in terms of higher soluble solids content (18.8 °Brix), total sugar, (15.62 %) and lower acidity (0.5 %) was also significantly achieved in treated vines. The study provides useful insight into improving the fruit quality and enhancing the marketability in grapes through advancement of budburst and ripening with the application of hydrogen cyanamide.

Keywords: Bud burst, Flowering, Fruit quality, Pruning, Ripening

Grape (*Vitis vinifera* L.) is an important temperate fruit crop grown in tropical, sub-tropical and temperate regions across the world. It is the fourth most widely cultivated fruit in the world after citrus, banana and apple. The current world production of grapes was 77.14 million tonnes from an area of 6.93 million ha and in India, it occupies 0.14 million ha area accounting for about 3.04 million tonnes production in the country (FAOSTAT, 2019). In India grapes are commercially grown in tropical region of southern states viz. Maharashtra, Karnataka, Andhra Pradesh and Tamil Nadu. Moreover, the subtropical climate of north-western regions comprising of Punjab, Haryana, Uttar Pradesh, Rajasthan and Madhya Pradesh also holds the potential for successfully growing short duration, good quality grapes. These are basically the vines of temperate region having origin in the region of Caspian Sea. Therefore, dormancy or rest period is a genetic necessity in cropping cycle of grape vines. Climate is the chief factor controlling the productivity and quality of grapes. The proclamation of vegetative and floral buds from endodormancy to an active state requires certain chilling hours which are sufficiently met in colder regions all across the globe (Muhtaseb and Ghnaim, 2008). In sub-tropical regions like India, there are mild winters and winter chilling hours are insufficient to break the dormancy of the vines. That's why the bud break is non-uniform or delayed (Liang *et al*., 2019). This insufficient accumulation of chilling results in non-uniform flowering, reduction in number of shoots and clusters per vine, poor fruit set percentage, non-uniformity of fruit development, resulting in reduced fruit yield and poor quality in table grapes (Arora *et al*., 2011 and Mujahid *et al*., 2020). The cultivar Flame Seedless selected for the study has crimson red coloured, seedless berries, relatively higher yield, more anthocyanins and better SSC/acid ratio than the most popular variety Perlette grown in the region. However, this variety under subtropical region of north India does not attain the desired intensity of red color, probably due to high temperature at the time of ripening, which inhibit the accumulation of anthocyanins, therefore the advance ripening with hydrogen cyanamide could be helpful for attaining uniform ripening/colour in this cultivar as reported earlier for coloured table grape varieties (El-Boray *et al*., 2019). Many plant growth regulators and chemicals have been used worldwide for stimulation of bud break in grapes. The chemical agent hydrogen cyanamide (H_2CN_2) has been used for dormancy release in various fruit crops commercially such as apple (Krasuska *et al*., 2014) and grapes (Liang *et al*., 2019). In grapes, the early pruning and use of rest breaking chemical

*Corresponding author : naresh_arora@pau.edu
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hydrogen cyanamide has been proven to advance the bud burst and ripening in different agro-climatic regions across the globe (Lombard *et al*., 2006; Muhtaseb and Ghnaim, 2008 and Liang *et al.*, 2019). Pruning results in enhanced levels of cytokinin in the vines which hastens the formation of floral primordia and ultimately the early maturity (Lombard *et al*., 2006). In addition, the time of pruning of grapevines directly influences the bud break and flowering time, shoot characteristics, fruit set, yield components and berry composition (Buesa *et al*., 2020). Time of grapevine pruning in *cv.* Flame Seedless needs to be standardized in combination with hydrogen cyanamide for getting the early bud burst, alleviating fruit yield and improving fruit quality in grape cv. Flame Seedless. Therefore, present study was conducted to know the optimum time of pruning in combination with foliar application of hydrogen cyanamide and its effect on fruit ripening and quality of grape *cv.* Flame Seedless.

MATERIALS AND METHODS

The investigation was carried out for three consecutive years at Fruit Research Farm, Department of Fruit Science, PAU, Ludhiana on 13-year-old own rooted vines of Flame Seedless grapes trained and planted at 3 m x 3 m distance on bower system. The vines having uniform trunk diameter were selected for the experiment and were provided with recommended dose of manures, fertilizers and others cultural practices during the study (Anonymous, 2019). The pruning of vines was performed at 4-buds level starting from last week of December to first week of February at weekly intervals. T_1- Last week of December; T_2 First week of January; T_3 Second week of January; T_4 Third week of January; T_5 Last week of January; T_6 First week of February.

At each of six intervals, 24 vines were pruned which were further divided into two sets of 12 vines each. Immediately after pruning, one set of 12 vines per interval was applied with hydrogen cyanamide (H_2CN_2) @ 2.0% as foliar application and other set was kept unsprayed for comparison between effect of treated and untreated vines at each pruning interval. The experiment was laid out in Randomized Block Design. Twenty-five canes were tagged randomly on each vine for recording observations on per cent bud burst, time of bud burst, time of flowering, time of ripening, yield and quality attributes.

The commencement of bud burst was considered when green tissue from the one-year-old shoot (cane) became visible. The time of bud burst and flowering was considered when more than 75 per cent buds and flowers opened, respectively. Likewise, time of ripening was considered when more than 75 per cent bunches attained the desired soluble solids content. The yield (kg/vine) and bunch weight were calculated for all treatments separately. For analysis of chemical attributes, 50 berries from each replication were squeezed and juice obtained was filtered through cheese cloth. The soluble solids (SSC) content of juice was recorded with a temperature compensated digital refractometer (Atago PAL^{-1}, model 3810, Japan). The titratable acidity was determined by titrating the known volume of juice with 0.1 N NaOH, using phenolphthalein as indicator. The total sugars of different samples were analyzed by using standard procedure as mentioned in AOAC, (2019).

Peel colour of berries was recorded in terms of L^* (L^* = 0 represents blackness and L^* = 100 represents whiteness), a^* ($-a^*$ represents greenness and $+a^*$ represents redness) and b^* ($-b^*$ represents blueness and $+ b^*$ represents yellowness), with the help of Hunter colour laboratory meter (ColourFlex® EZ, USA). CIRG index was calculated from these values after calculating h^* (hue angle) = arc tan (b^*/a^*) and C^* (Chroma) = C^* = sqrt ($a^{*2} + b^{*2}$), representing the colour intensity of the berries. CIRG index of the berries was computed as: (180 - h^*)/ $L^* + C^*$).

The experimental data for three years was pooled and subjected to statistical analysis as per the SAS 9.3 package. The interactions were subjected to mean comparison using LSD ($p \leq 0.05$), where found significant.

RESULTS AND DISCUSSION

The data pertaining to time of bud burst and flowering (Table 1) revealed that the vines treated with hydrogen cyanamide immediately after pruning advanced the time of bud burst and flowering at all pruning times. The commencement of bud burst was 12-23 days earlier in vines treated with hydrogen cyanamide after pruning as compared to untreated vines. Amongst the various treatments, bud burst was the earliest (10th Feb) in T_1 in which hydrogen cyanamide was applied after pruning during last week of December. The vines in which pruning was done during first week of February and hydrogen cyanamide was applied, time of bud burst was recorded to be on 22nd February, which was 12 days earlier than treatment in which only pruning was executed at the same time. The unsprayed vines had bud burst on 5th and 6th of March. Similar trend was observed on initiation of flowering in the vines under study. It was observed that the vines pruned earlier recorded significant advancement in flower initiation irrespective of hydrogen cyanamide treatment. However, the vines applied foliarly with hydrogen cyanamide registered the earliest flower initiation (18th to 26th March) as compared to the unsprayed vines

Table 1. Effect of time of pruning and hydrogen cyanamide on time of bud burst of Flame Seedless grapes.

Treatment	Time of Pruning	Time of bud burst		Time of flowering		Time of ripening	
		*Hydrogen cyanamide	Unsprayed	*Hydrogen cyanamide	Unsprayed	*Hydrogen cyanamide	Unsprayed
T_1	Last week of December	10th Feb	5th March	18th March	27th March	6th June	11th June
T_2	First week of January	12th Feb	5th March	18th March	27th March	6th June	11th June
T_3	Second week of January	17nd Feb	5th March	20th March	29th March	8th June	12th June
T_4	Third week of January	21th Feb	6th March	26th March	31stMarch	8th June	12th June
T_5	Last week of January	20th Feb	6th March	26th March	31stMarch	8th June	12th June
T_6	First week of February	22thFeb	6th March	26th March	31stMarch	8th June	12th June

*foliar application @ 2.0 %

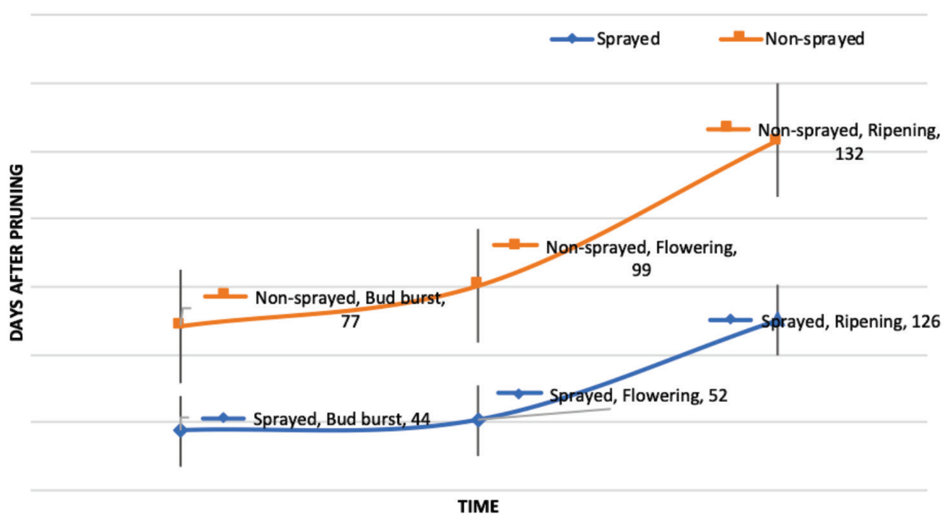

Fig. 1. Effect of early pruning (T1- last week of December) and hydrogen cyanamide on time of bud burst, flowering and ripening of Flame Seedless grapes.

(27th to 31st March). In Flame Seedless vines subjected to early pruning along with applications of hydrogen cyanamide immediately after pruning, the time of bud burst, flowering and ripening days were recorded to be 44, 52 and 126 days after pruning, respectively (Fig. 1). Whereas, vines in which only pruning was conducted at recommended time, the time of bud burst, flowering and ripening was 77, 99 and 132 days after pruning, respectively. The delayed pruning was responsible for the late bud break in grape vines as reported by Çelik *et al*., (2020). The application of hydrogen cyanamide brought about the uniformity of budburst, increased the bud burst percentage, number of flowers and fruits and ultimately the productivity of grapevines (Muhtaseb and Ghnaim, 2008). There are several dormancy related proteins located in chloroplasts and cytoplasm whose expression patterns are altered when the vines are treated with hydrogen cyanamide. These proteins are associated with hormonal signaling and are stimulated by the application of hydrogen cyanamide which in turn leads to alteration of the grapevine buds from dormancy to active state (Khalil-ul-Rehman *et al*., 2020). The application of hydrogen cyanamide is responsible for inactivating the catalase enzyme which leads to hydrogen peroxide accumulation and development of oxidative stress in buds (Perez and Lira, 2005) and it ultimately results in the increased concentration of IAA and GA_3 and decrease in ABA, thereby promoting the bud burst in grapes (El-Boray *et al*., 2019).

It is evident from data presented (Table 1) that the fruit harvested from vines with foliar application of hydrogen cyanamide had 4-6 days advancement of ripening as compared to the untreated vines. Likewise, the fruit harvested from the earlier pruned vines recorded an advancement in ripening by 1 and 2 days in untreated and treated vines, respectively. The vines treated with hydrogen cyanamide immediately after pruning during last week of December and first week of January had time of ripening as 6th June. However, the date of ripening was recorded to be 11th June in vines which were unsprayed but pruned at the same time of the year (last week of December and first week of January). These results are in agreement with several research workers who noticed that fruits of hydrogen cyanamide treated vines reached harvest maturity earlier than those of untreated vines pruned on the same day thereby advancing the harvesting time in grapes (Arora *et al*., 2011 and El-Boray *et al*., 2019). The advancement in ripening of grape berries may be attributed to the reduction in crop load on the vines. The heavy crop load causes reduced accumulation of dry matter content and also depletion of starch reserves in roots which ultimately leads to the delay in fruit ripening (Inglese *et al*., 2002).

The data presented (Table 2) regarding effect of time of pruning and hydrogen cyanamide revealed that the yield per vine was higher in vines pruned earlier as compared to vines pruned late. In vines treated after pruning during first week of January, significantly higher yield was registered as compared to vines pruned late i.e last week of January with an improvement of 24.03 % over control. Likewise, in case of unsprayed vines, the ones pruned during first week of January recorded maximum yield, followed by last week of December. The untreated vines pruned in second fortnight of January lead to the minimum yield per vine. The overall yield of sprayed and unsprayed grape vines registered non-significant differences. The time of pruning and application of hydrogen cyanamide showed non-significant effect among the treatments on bunch weight (Table 2). The bunch weight was observed highest in treatment in which vines were pruned and treated with hydrogen cyanamide during last week of December while the effect within the treatments was found to be non-significant. Similar results were registered for unsprayed treatments. Muhtaseb and Ghnaim (2008) who found that hydrogen cyanamide treated plants had higher fruit fresh weight than the control as the fruits from early opening flowers have faster initial growth rate and they get more time for their development than late opening flowers. The combined treatment of pruning and hydrogen cyanamide application increases the levels of cytokinin Zeatin Riboside (ZR) in grapevine buds which may have positive effect on better bunch development and fruit yield (Lombard *et al*., 2006).

With advancement of maturity, the anthocyanins accumulation occurs in Flame Seedless berries and the berry colour changes from green to yellow and eventually to red colour. L^* values registered significant improvement in hydrogen cyanamide treated fruits than the unsprayed ones recording overall increment to the tune of 12.2 % (Table 3). Pruning in first week of January and subsequent treatment with hydrogen cyanamide enhanced the L^* value by 15.7 % over average L^* values in unsprayed vines. The L^* values for different pruning times and their interactions with application of hydrogen cyanamide or unsprayed were at par with one another. The surface colour of the grape berries indicated more enrichment of red colour (a^* value) in HCN treated berries when compared with untreated ones at all pruning intervals. The mean a^* value of treated vines registered an enhancement of approx. 6.8 % over unsprayed vines irrespective of the pruning intervals. However, the b^* values obtained for

Table 2. Effect of time of pruning and hydrogen cyanamide on yield and bunch weight of Flame Seedless grapes.

Treatment	Time of Pruning	Yield (kg/vine)		Mean	Bunch weight (g)		Mean
		*Hydrogen cyanamide	Unsprayed		*Hydrogen cyanamide	Unsprayed	
T_1	Last week of December	30.4^{a}	30.4^{abc}	30.4^{a}	384.7	380.3	382.5
T_2	First week of January	32.0^{a}	30.7^{ab}	31.4^{a}	381.3	379.6	380.5
T_3	Second week of January	30.4^{abc}	29.7^{abcd}	30.1^{a}	379.5	381.3	380.4
T_4	Third week of January	26.7^{e}	26.8^{de}	26.8^{b}	370.6	377.3	374.0
T_5	Last week of January	25.8^{e}	27.6^{cde}	26.7^{b}	374.3	378	376.2
T_6	First week of February	26.5^{e}	28.1^{bcde}	27.3^{b}	378.0	374.0	376.0
Mean	-	28.6	28.8		378.1	378.4	
LSD (P<0.05)	-	Application (A) = NS Pruning time (B) = 1.77 A X B = NS			Application (A) = NS Pruning time (B) = NS A X B = NS		

* foliar application @ 2.0 %

unsprayed vines were significantly higher than treated vines overall, without taking into account the time of pruning. The *b** values did not differ significantly with the pruning intervals. Similarly, the interactions were found to be at par with one another. Colour index of red grapes (CIRG) was affected by time of pruning and hydrogen cyanimide application however the results were non consistent as expressed in CIE lab parameters (Table 3). Among HC sprayed vines harvest, the higher (4.54) colour index of red grapes (CIRG) was observed in vines where pruning was done in third week of January or in first week of February. Among the unsprayed vines the highest CIRG was recorded when pruning was delayed till third week of January. The enhancement of berry colour and related parameters with hydrogen cyanamide might be due to efficient carbohydrate metabolism and signaling of plant hormones, resulting in upregulation of red colour in berries (Liang *et al*., 2019).

The effect of time of pruning and hydrogen cyanamide application on fruit quality of Flame Seedless grapes was recorded and presented in Table 4. The statistically highest SSC were found in treatments in which vines were applied with hydrogen cyanamide @ 2.0 % as compared to pruning only. However, varying times of pruning irrespective of whether they were sprayed or unsprayed, indicated non-significant differences SSC of the fruits. In treated vines, SSC ranged from 19.2 °Brix (in T_1 and T_3) to 18.1 °Brix in T_5. The acidity values of the fruits obtained from sprayed and unsprayed vines as well as those pruned at six different intervals were obtained to be statistically at par. The values ranged from 0.52 per cent to 0.56 per cent in treated and untreated pruning treatments. Arora *et al*. (2011) observed that hydrogen cyanamide treatment improved the soluble solids content in grape berries. Hydrogen cyanamide being the strong inhibitor of catalase (due to the reaction of nitrile group with the thiols and haematin of the enzyme), consequently results in increasing the hydrogen peroxide concentration and increase in chemical constituents of buds (Amberger, 2013).

The SSC:TA ratio was significantly influenced by time of pruning and hydrogen cyanamide application (Table 5). The results revealed that the maximum average SSC:TA ratio registered was 34.73 in the vines pruned during last week of December, followed by second, third and first week of January, recording 33.13, 33.08 and 32.86 and were at par with one another. Average SSC:TA ratio was significantly higher with hydrogen cyanamide application (34.43) when compared to unsprayed (31.70). The interactions between pruning time and application of hydrogen cyanamide were found significant. The SSC:TA ratio was increased due to significant improvement in SSC content in treated fruits as reported by Arora *et*

Table 3. Effect of time of pruning and hydrogen cyanamide on fruit colour of Flame Seedless grapes.

Treatment	Time of Pruning	*Hydrogen cyanamide				Unsprayed				Mean			
		*L**	*a**	*b**	CIRG**	*L**	*a**	*b**	CIRG	*L**	*a**	*b**	CIRG**
T_1	Last week of December	27.10	7.37	3.00	4.50	24.90	6.50	4.10	4.53	26.00	6.94	3.55	4.52
T_2	First week of January	28.20	7.30	2.89	4.39	24.38	6.88	4.22	4.58	26.29	7.09	3.56	4.49
T_3	Second week of January	27.30	7.14	3.00	4.49	24.20	7.10	3.98	4.66	25.75	7.12	3.49	4.58
T_4	Third week of January	27.00	7.00	2.95	4.54	24.40	7.00	3.78	4.69	25.70	7.00	3.36	4.62
T_5	Last week of January	26.90	7.50	3.20	4.48	23.98	6.98	4.00	4.69	25.44	7.24	3.60	4.58
T_6	First week of February	27.0	7.30	2.90	4.54	23.80	6.40	4.12	4.69	25.40	6.85	3.15	4.61
Mean		27.25 ±0.18[a]	7.27 ±0.07[a]	2.99 ±0.03[b]	4.49 ±0.02[b]	24.28 ±0.18[b]	6.81 ±0.08[b]	4.03 ±0.06[a]	4.64 ±0.03[a]				
LSD (P<0.05)		For L: Application (A) = 0.53 Pruning time (B) = NS A X B = NS		For a: Application (A) = 0.21 Pruning time (B) = NS A X B = NS			For b: Application (A) = 0.14 Pruning time (B) = NS A X B = NS			For CIRG: Application (A) = 0.08 Pruning time (B) = NS A X B = NS			

* foliar application @ 2.0 %: CIRG**- Colour Index of Red Grapes

Table 4. Effect of time of pruning and hydrogen cyanamide on soluble solid content and acidity of Flame Seedless grapes.

Treatment	Time of Pruning	SSC (°Brix)		Mean	Titratable Acidity (%)		Mean
		*Hydrogen cyanamide	Unsprayed		*Hydrogen cyanamide	Unsprayed	
T_1	Last week of December	19.2[a]	17.9[ab]	18.6	0.52	0.55	0.53
T_2	First week of January	19.0[ab]	17.8[ab]	18.4	0.56	0.56	0.56
T_3	Second week of January	19.2[a]	17.2[ab]	18.2	0.54	0.56	0.55
T_4	Third week of January	18.8[ab]	17.6[ab]	18.2	0.56	0.54	0.55
T_5	Last week of January	18.1[ab]	17.4[ab]	17.8	0.54	0.55	0.54
T_6	First week of February	18.2[ab]	17.0[b]	17.6	0.55	0.55	0.55
Mean		18.8±0.49[a]	17.5±0.15[b]		0.5	0.6	
LSD (P<0.05)		Application (A) = 0.49 Pruning time (B) = NS A X B = 2.1			Application (A) = NS Pruning time (B) = NS A X B = NS		

* foliar application @ 2.0 %

Table 5. Effect of time of pruning and hydrogen cyanamide on SSC: TA ratio and total sugars of Flame Seedless grapes.

Treatment	Time of Pruning	SSC: TA ratio		Mean	Total Sugars (%)		Mean
		*Hydrogen cyanamide	Unsprayed		*Hydrogen cyanamide	Unsprayed	
T_1	Last week of December	36.92[a]	32.55[e]	34.73±0.98[a]	16.3	14.9	15.6
T_2	First week of January	33.93[c]	31.79[f]	32.86±0.37[b]	15.8	14.5	15.2
T_3	Second week of January	35.56[b]	30.71[g]	33.13±0.51[b]	16.0	14.7	15.4
T_4	Third week of January	33.57[cd]	32.59[e]	33.08±0.37[b]	15.4	14.5	15.0
T_5	Last week of January	33.52[cd]	31.64[f]	32.57±0.30[c]	15.0	14.5	14.8
T_6	First week of February	33.09[de]	30.91[g]	32.00±0.36[d]	15.2	14.7	15.0
Mean		34.43±0.33[a]	31.70±0.18[b]		15.62±0.19[a]	14.63±0.15[b]	
LSD (P<0.05)		Application (A) = 0.15 Pruning time (B) = 0.39 A X B = 0.64			Application (A) = 0.52 Pruning time (B) = NS A X B = NS		

* foliar application @ 2.0 %

al.(2011). In the pruned vines, the competition for metabolites becomes less and consequently the plant vigour improves and physiological activities increase, resulting in more SSC and SSC:TA ratio (Porika *et al.,* 2015). The scrutiny of data pertaining to total sugars indicated significantly highest value in vines treated with hydrogen cyanamide (15.62 %) when compared with unsprayed (14.63 %). While the time of pruning did not have any significant effect on total sugar content in grape *cv.* Flame Seedless. The interaction between pruning time and application of hydrogen cyanamide were non-significant. The increase in sugars with hydrogen cyanamide application is in corroboration with the findings of Chandel and Devi (2010).

The results of present experiment suggest that the time of ripening in Flame Seedless grapes may be advanced by one week by performing early pruning (last week of December/first week of January) with application of hydrogen cyanamide @ 2.0% (a.i.).

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Authors' contribution

Conceptualization of research work and designing of experiments (NKA, MIS); Execution of field/lab experiments and data collection (RA, GK); Analysis of data and interpretation (GK, RA); Preparation of manuscript (NKA, RA, GK)

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