

Induction of Budbreak in Apple Trees that Received Insufficient Chilling by Hydrogen Cyanamide

Krisana Krisanapook¹, Suranant Subhadrabandhu¹, and Ryosuke Ogata²

ABSTRACT

Various concentrations of hydrogen cyanamide were applied to 'Anna' apple to induce budbreak at the time when the trees were in dormant stage. The optimum concentration of hydrogen cyanamide to induce good budbreak was 2.5%. High concentrations of hydrogen cyanamide were noticed to be toxic by dried dead shoots.

'Tsugaru' apple trees at different dormant stages kept in the heated glasshouse (minimum temperature was 15°C) were treated with 2.5% hydrogen cyanamide plus 50% 'Merit', a foliar fertilizer. It was found that the mixer of hydrogen cyanamide and 'Merit' could relatively induce budbreak even when buds were in the deepest dormant stage, when compared with the buds in lesser dormant stage. Buds from 'Tsugaru' apple trees treated or not treated with 2.5% hydrogen cyanamide were analyzed for abscisic acid (ABA) content. It was found that three days after treatment, ABA content in buds treated with hydrogen cyanamide was two times lower than that in the untreated buds. This result suggested that hydrogen cyanamide may induce budbreak by decreasing ABA content.

Key words: ABA, bud dormancy, hydrogen cyanamide, *Malus prunifolia*

INTRODUCTION

Poor budbreak, especially in the lateral buds is the great obstacle that prevents the success in growing temperate fruit trees including apple trees in the tropical and sub-tropical areas which are under insufficient chilling. In Thailand, apple trees have been introduced to grow on high mountains in the northern part for nearly twenty years. At present, the cultivars accepted to be suitable are the low-chilling requirement ones such as 'Anna' and

'Ein Shemer' from Israel. However, it is always found that these trees have few growing points due to the failure of breaking lateral buds. Besides, these buds sporadically burst all year around caused by insufficient chilling of the growing areas. To induce budbreak, bending the branch and leaf stripping was reported to be successful for apple trees in Indonesia (Edwards and Notodimedjo, 1987; Janick, 1974), horizontally bending of branches of 'Anna' and 'Ein Shemer' apple trees in this experiment were done, however, very few budbreak occurred.

¹ Department of Horticulture, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand.

² Department of Horticulture, Faculty of Agriculture, Utsunomiya University, Tochigi 321, Japan.

Leaf stripping which successfully induced terminal budbreak in tip bearing cultivar, 'Rome Beauty' were unpracticed for 'Anna' and 'Ein Shemer'.

Several kinds of chemicals have been widely used to induce budbreak. Many new budbreak inducing chemicals have been found and the efficacy of the newer chemicals seem to be higher than the former ones. Cyanamide has been found to be effectively stimulate budbreak in many temperate fruit trees. At the beginning, calcium cyanamide which was used as fertilizer, but sometimes, as herbicide and defoliant was successfully used to induce budbreak in many fruit species (Fuchigami and Nee, 1987; Morimoto and Kumashiro, 1978). However, this chemical is in paste-like form, which is difficult to handle and needs high concentrations to induce budbreak (Erez, 1987). Recently, hydrogen cyanamide or acid cyanamide which is in liquid form, has been substituted to calcium cyanamide and widely used to break dormancy of temperate fruit species grown in warm areas (Erez, 1985; George and Nissen; 1988; Shulman *et al.*, 1983). Hydrogen cyanamide is more effective than calcium cyanamide since hydrogen cyanamide is directly active to plant tissue (Shulman *et al.*, 1983). However, the mechanisms of these chemicals in inducing budbreak are unclear. In this study hydrogen cyanamide was selected to use for breaking dormancy of apple trees grown under warm condition. Optimum concentration of this chemical was previously examined. Effects of this chemical when applied to dormant buds at different stages were also determined in order to find the optimum time of chemical application. Since abscisic acid (ABA) has been reported to be involved in bud dormancy, endogenous ABA in the buds treated and not treated with hydrogen cyanamide were determined concerning possible involvement of this plant hormone to the action mechanisms of hydrogen cyanamide

MATERIALS AND METHODS

Experiment 1 : Optimum concentration of hydrogen cyanamide used to induce budbreak;

This experiment was conducted at the Royal Angkhang Station located on the high mountain (Elevation ca. 1400 m) in Amphur Fang district, Chiangmai Province, Thailand. Planting materials were five-year-old 'Anna' apple trees grafted on MM 106 rootstock. One-year-old shoots of similar size were cut to 30 cm in length. Each shoot had 6 to 10 uniform buds. These buds were approximately divided into three equal parts as upper, middle, and lower parts. Each bud was painted with 0, 0.5, 1, 2.5, 5 or 10% hydrogen cyanamide (Dormex; Trostberg AG. Germany). This experiment was carried out on December 19, 1988 when apple trees were in the dormant period and randomized complete block design was applied. Each treatment had 3 replications, and each replication had 10 shoots. Numbers of bursted buds, their positions on shoots, growth after bursting and toxicity of hydrogen cyanamide were recorded on April 24, 1989

Experiment 2 : Effects of hydrogen cyanamide on budbreak at different stages of dormancy.

This experiment was conducted at Faculty of Agriculture, Tohoku University, Japan. Eight pots of four-year-old 'Tsugaru' apple trees grafted onto *Malus prunifolia* kept in the field were transferred at every month interval into the glasshouse minimum temperature was 15°C from September, 1990 to March 1991. These trees had 3 to 5 one-year-old shoots of similar size. After transferring into the glasshouse these shoots were cut to remain 30 cm. in length. Four pots of the trees were left as control. The other four was painted with 2.5% hydrogen cyanamide plus 50% Merit (7-5-3 formula foliar fertilizer). Number of bursted buds and their positions on the shoot were recorded after six weeks.

Experiment 3: Effects of hydrogen cyanamide on changing of endogenous ABA

This experiment was conducted at Faculty of Agriculture, Tohoku University, Japan on June 24, 1991, Twenty four pots of four-year-old 'Tsugaru' apple trees grafted onto *Malus prunifolia* kept under the glasshouse at minimum temperature of 15°C were used. The trees were divided into two groups, each had 60 one-year-old shoots, about 60 cm in length. At this period of time, the trees showed no growth. One-year-old shoots from one group were painted with 2.5% hydrogen cyanamide and the other group was left as control. Three days after the treatment, shoots from both groups were cut off. Apical and lateral buds were separately divided and each kinds of the buds was divided into 3 replications. These buds were weighted and immediately soaked into liquid nitrogen and stored at -30°C until analyzed.

Analysis of ABA

1) Extraction, partition and purification of samples

The brief scheme for extraction, partition and determination of ABA from samples was shown in Fig1. The frozen buds mentioned previously were homogenized by mortar and pestle three times with fresh 80% methanol until became fine pieces. The supernatant was partitioned with 3 steps of ethyl acetate, sodium bicarbonate and ethyl acetate, simultaneously. After that, it was loaded onto a C₁₈ Sep-Pak cartridge (Water Ltd., U.S.A.). The extraction was injected to an ODS preparative column equipped to a liquid chromatography, model UVILOG (Oyo Bunko, Japan), and be eluted with 40% methanol at a flow rate of 2 ml/min. The eluant of which retention time between 13 and 18 minutes was collected as ABA fraction. The ABA fraction was dried by freeze dryer.

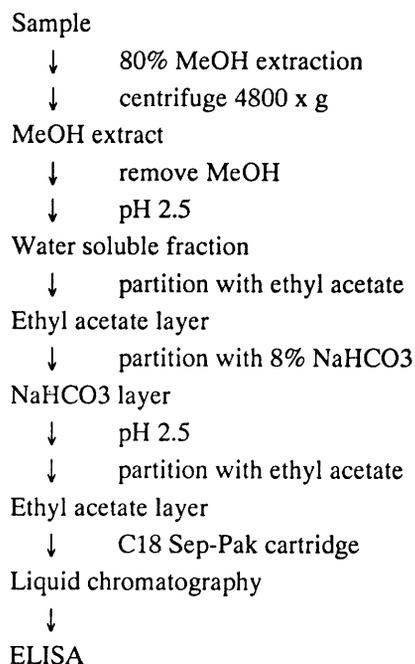


Figure 1 Brief scheme of ABA extraction partition and determination

2) Enzyme linked immunosorbent assay (ELISA)

The method of ELISA was used to analyzed ABA by using Phytodetek ABA kit (Idetek Co., U.S.A.). The ABA fractions collected from LC was dissolved in 100 μ l of 25 mM Tris buffered saline (pH 7.5) and put in wells coated with 2-cis-(s)-ABA monoclonal antibody. (\pm) ABA at the serial concentrations which was used as a standard. Then, 100 μ l of alkaliphosphatase labeled ABA was added to the wells of sample and standard ABA. The reaction mixtures were incubated at 4°C for 3 hours. After the incubation, solutions were discarded and the wells were washed three times by 200 μ l of wash reagent. Then, the wells were filled with 200 μ l of p-nitrophenyl phosphate as a substrate for the alkali phosphatase. The reaction mixtures were again, incubated at 37°C for 1 hour. The reaction was stopped by adding one drop of stop reagent, and

left for 5 minutes. Absorption at 405 nm was measured by a microplate photometer (Corona-MTP-12).

RESULTS AND DISCUSSION

Experiment 1

Hydrogen cyanamide could promoted budbreak of 'Anna' apple trees at the concentration of 1% up to 5% (Table 1). The higher percentage of

budbreak was found at the higher concentration. Sixty eight percents of shoots dried at 10% concentration. Budbreak were found in all parts of shoots, approx. 87-97%. In the untreated trees there were approx. 59% of bursted buds at the upper part, too. Elongation of new shoots which developed from the bursted buds in the middle and lower parts of shoots grew better with the trees that treated with hydrogen cyanamide at 2.5 and 5% (Table 2). The results indicated that hydrogen cyanamide pro-

Table 1 Effect of hydrogen cyanamide on burst to lateral buds in 'Anna' apple trees.

Concentration (%)	Budbreak (%)	% Budbreak in			Dried shoots (%)
		Upper part	Middle part	Lower part	
0	26.8c1/	58.9	8.3	12.6	0
0.5	31.3bc	66.4	20.8	0.1	1.8
1	46.7b	97.2	21.1	51.8	0.9
2.5	67.2a	94.2	69.6	38.2	0.9
5	69.2a	86.5	63.1	58.7	7.0
10	10.8a	18.6	9.2	4.4	68.3

1/ Mean separation within the column by Duncan's multiple range test at 5% level

Table 2 Elongation of shoots developed from the bursted buds in 'Anna' apple trees which were treated with hydrogen cyanamide as in Table 1.

Concentration %	Length (cm)		
	Shoots from upper part	Shoots from middle part	Shoots from lower part
0	36.8 + 6.61/	1.5 + 0.9	2.2 + 1.3
0.5	17.1 + 2.9	6.3 + 4.0	1.1 + 0.5
1	21.0 + 3.3	2.6 + 1.5	2.9 + 2.1
2.5	23.0 + 3.6	10.8 + 4.0	9.5 + 4.6
5	39.1 + 5.0	13.7 + 4.3	8.8 + 3.2
10	18.1 + 8.6	12.3 + 11.3	0.4 + 0.1

1/ mean + SE

moted dormancy breaking in 'Anna' apple trees. This result agreed with many works on several fruit tree species in the warm areas (Erez, 1985, 1987; Kaewpaluek, 1986; Kuroi, 1974; Shulman et al., 1986; Yang *et al.*, 1982). Besides, it suggested that the optimum concentration of hydrogen cyanamide to induce budbreak was 2.5% and the later experiments were carried out using this concentration. However, very high concentration of hydrogen cyanamide (10%) caused the damage of shoots as well as that happened in peach (Erez, 1987).

Experiment 2

The effect of hydrogen cyanamide plus Merit on budbreak of the trees early transferred into glasshouse, was less than those transferred at the

later time (Table 3). This indicated that the dormancy of the buds in the former trees were deeper than that of the later ones. The deepest dormancy periods were during November and December. At this time, the effect of hydrogen cyanamide was markedly decreased when compared with those treated at the other dates (approx. 58% in Nov. and 37% in Dec.). However, the different numbers of budbreak between untreated and treated trees was significant. This indicated that hydrogen cyanamide could induce budbreak even when the buds were in the deep dormancy. Budbreak in the hydrogen cyanamide untreated trees transferred in February was approx. 70% indicated that at this time these trees had received sufficient chilling from outside. However budbreak in the treated trees was still

Table 3 Effect of hydrogen cyanamide plus Merit applied at different days on budbreak in 'Tsugaru' apple trees. The trees were transferred from natural condition to glasshouse and treated with a mixture of 2.5% hydrogen cyanamide and 50% Merit on the day indicated. Budbreak was scored 6 weeks after the treatment.

Date of treatment	Hydrogen cyanamide plus Merit	% Budbreak	% Budbreak in		
			Upper part	Middle part	Lower part
Sep.3,1990	-	12.3	33.4	0	0
	+	77.5	90.6	74	62.7
Oct.3,1990	-	13.4	35.6	0	0
	+	75.7	83.2	62.1	62.8
Nov.3,1990	-	1.5	3.6	0	0
	+	57.5	78.6	68.9	32.3
Dec.3,1990	-	0.9	2.3	0	0
	+	37.1	62.9	21.4	24.0
Jan.3,1990	-	17.8	33.2	11.9	4.2
	+	76.4	86.6	71.8	70.3
Feb.3,1990	-	69.6	87.3	63.9	52.5
	+	92.8	92.2	93.7	82.2
Mar.3,1990	-	79.3	96.4	84.5	59.6
	+	91.0	97.7	91.0	84.9

higher than that in the untreated ones. From this result, application time seems to be very important since it showed big difference in budbreak inducing. To achieve the best result, the practical application time was suggested to beat the time when the buds are going to be released from dormancy which in this experiment was February. However, this may be different in other cultivars due to their chilling requirement. The effect of hydrogen cyanamide seen especially with buds on the middle and lower parts of the shoots since higher budbreak was found on these two parts of the treated trees than the untreated trees. Improvement of budbreak on the middle and lower parts of the shoot is the most important for apple growing under warm areas because budbreak on these parts caused the increasing of numbers of leaves and fruit yields per tree. The effect of hydrogen cyanamide was enhanced when used together with Merit especially to induce lateral budbreak at the basal part of the shoot (data not shown). Merit has been reported to enhance the effect of calcium cyanamide on budbreak in grapevines (Kaewpaluek, 1986; Yang *et al.*, 1982). Action mechanisms of Merit is not clear, but it may support plant growth as a fertilizer.

Experiment 3

Three days after the treatment with hydrogen cyanamide in 'Tsugaru', ABA content in the treated trees was approximately half of that in the untreated trees in both apical and lateral buds

(Table 4). The mechanism of hydrogen cyanamide in inducing budbreak is not clear. Shulman *et al.* (1986) suggested that hydrogen cyanamide breaks dormancy by involving in the conjugation of thiol group which is assumed to be involved in breaking dormancy. The present result found that hydrogen cyanamide decreased ABA content in the buds before budbreak. It has been postulated by many evidences that completion of the bud dormancy is related to a decrease of endogenous ABA (Harrison and Saunders, 1975; Webber *et al.*, 1979; Wright, 1975). Therefore, this suggested that hydrogen cyanamide may help breaking dormancy by decreasing the ABA content.

CONCLUSION

From this study, it showed that hydrogen cyanamide can induce budbreak in apple trees grown under insufficient chilling areas. This will improve the performances of apple trees in these areas at the present time. Time of chemical application seems to be very important to induce good budbreak. The mechanism of hydrogen cyanamide in inducing budbreak may involved many processes. One possibility is that it involves in decreasing the ABA content, a hormone concerning with dormancy. Further study is necessary to reveal the mechanism of dormancy in bud and the mechanism of dormancy breaking by hydrogen cyanamide.

Table 4 ABA Content in 'Tsugaru' apple trees after treated with hydrogen cyanamide.

Treatment	ABA content (ng/g FW eq.)	
	In apical buds	In lateral buds
Untreated	74.2 + 3.3 ^{1/}	96.4 + 18.6
2.5% Hydrogen cyanamide	32.6 + 9.0	40.7 + 3.2

1/ mean + SE

LITERATURE CITED

- Edwards, G.R. and S. Notodimedjo. 1987. Defoliation, bending and tip pruning of apple under tropical conditions. *Acta Hort.* 199:125-127.
- Erez, A. 1985. Chemical defoliation of deciduous fruit trees with magnesium chlorate and cyanamide. *HortScience.* 20:452-453.
- Erez, A. 1987. Chemical control of budbreak. *HortScience.* 22:1240-1243.
- Fuchigami, L.H. and C. C. Nee. 1987. Degree growth stage model and rest breaking mechanism in temperate woody perennials. *HortScience.* 22:836-845.
- George, A.P. and R.J. Nissen. 1988. Effect of hydrogen cyanamide on dormancy release, growth and yield of table grapes in subtropical Australia. *Aus. J. of Exp. Agr.* 28:425-429.
- Harrison, M.A. and P.F. Saunders, 1975. The abscisic acid content of dormant birch buds. *Planta.* 123:291-298.
- Janick, J. 1974. The apple in Java. *HortScience* 9:13-15.
- Kaewpaluek, I. 1986. Effect of some chemicals on bud burst of 'White Malaga' grapevine in winter. M.S. Thesis, Kasetsart University, Bangkok.
- Morimoto, F.R. and K. Kumashiro. 1978. Studies on the rest-breaking of buds of deciduous fruit trees by chemical treatment. *J. Faculty Agr. Shinshu Univ.* 15 : 1-17.
- Shulman, Y., G. Nir, L. Fanberstein, and S. Lavee. 1983. The effect of cyanamide on the release from dormancy of grapevine buds. *Sci. Hort.* 19:97-104.
- Shulman, Y., G. Nir, and S. Lavee, 1986. Oxidative processes in bud dormancy and the use of hydrogen cyanamide in breaking dormancy. *Acta. Hort.* 179:141-148.
- Webber, J.E., M.L. Laver, J.B. Zaerr, and D.P. Lavender. 1979. Seasonal variation of abscisic acid in the dormant shoots of Douglas-fir. *Can. J. Bot.* 57:534-538.
- Wright, S.T.C. 1975. Seasonal changes in the levels of free and bound abscisic acid in blackcurrant (*Ribes nigrum*) buds and beech (*Fagus sylvatica*) buds. *J. of Eup. Bot.* 26:161-174.