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Effect of Temperature on 'Pione' Grapevine Budbreaking at Different Stages of Dormancy

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The effects of temperature on budbreak of cuttings obtained at different stages of dormancy from 'Pione' grapevines (*Vitis labrusca* × *V. vinifera*) grown in open field were investigated. Cuttings were collected at monthly intervals from July to March. Judging from the number of days to initial and 60% budbreak after treatment, indicating promotion and the uniformity of budbreak, respectively, 30°C was the most effective in budbreak, followed by 25 and 20°C in that order in all treatment times. However, the effect of temperature on budbreak was markedly affected by treatment time. The number of days to initial budbreak (NDIB) increased gradually from July to October, peaked in December and thereafter decreased gradually towards March. The periods from July to September, from October to December, and from January to March were assumed to correspond to paradormancy, endodormancy, and ecodormancy of 'Pione' grapevines, respectively. Final percentage of budbreak was less than 100% until endodormancy for all temperatures. It was below 60% at 20°C treatments of July to September. On the other hand, a uniform budbreak was observed in the treatments after the middle of endodormancy for all temperatures, resulting in almost 100% of final percentage of budbreak. There was a significant negative correlation between NDIB and cumulative chilling hour (CCH) of exposure to below 7.2°C in the treatments after November, and also between NDIB and cumulative temperature (CT, °C·h), a summation of temperature and hours of exposure to above 0°C from November 1 to each treatment time and hours of exposure to 20, 25, or 30°C from start of treatment to budbreak in each plot. The results suggest that besides CCH, CT can also be used to estimate the completion of dormancy in 'Pione' grapevine bud.

Key words : breaking of grapevine bud, cumulative chilling hour (CCH), cumulative temperature (CT), different dormant stages, temperature

Introduction

Many studies have been done on bud dormancy of temperate fruit trees, including grapevine, with focused on issues such as physiology of dormancy[3,4,6], artificial termination of dormancy[9,12,16,19,22,27,33] among other things. It is well known that the grapevine buds need to be exposed to low temperature such as below 7.2℃ during winter for some period of time to terminate their rest (endodormancy), so called chilling requirement[6,13,32]. However, chilling requirement varies markedly among fruit tree species, growing areas, years among other factors[34], and it has been reported to range from 200 to 3,500 hours for grapevine[15]. Chill-unit, a model for estimating the completion of endodormancy, was proposed for peach[2], and its applicability was investigated for various fruit tree species[18,24,30]. An accurate estimation of the completion of bud dormancy is very important for fruit production, especially for grapevines, as grape growing in glass or plastic houses is widespread in Japan[26]. At present, however, it is still difficult to estimate the exact completion of bud dormancy in grapevines.

In recent years, there has been a growing concern about the effects of global warming on fruit tree growth and development parameters such as dormancy and flowering behavior by Japanese researchers[5,28,29]. Sugiura and Yokozawa[29] have reported that global warming will greatly affect the cultivation environment of apples and satsuma mandarin in Japan by the middle of the 21st century due to changes in average temperature. Honjo[5] has pointed out that decision of forcing time for protected cultivation of Japanese pear in temperate regions throughout winter is difficult, because of its imperfect completion of endodormancy. Under circumstances mentioned above, development of fruit tree cultivars with less chilling requirement has been a subject for breeding in many parts of the world[7,14,25].

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Therefore, a detailed study on the relationship between temperature and breaking bud dormancy in grapevine is necessary. However, there is little information about response of grapevine to temperature for budbreaking during dormancy induction, maintenance and release.

The purpose of this study is to investigate the effects of temperature on the budbreak of 'Pione' grapevine cuttings obtained at monthly intervals throughout the dormant period, from July to March.

Materials and Methods

Grapevines and treatment

Four mature 'Pione' grapevines (*Vitis labrusca* × *V. vinifera*) grown at the Research Farm of the Faculty of Agriculture, Okayama University were used for the experiment. Nine canes were collected from each grapevine at monthly intervals from July 18, 2003 to March 20, 2004. Cuttings with a single bud (6cm in length) were prepared from the 4th to the 11th nodes on cane, and mounted on a plastic foam plate, floated in a water bath. They were put into growth chambers (EYELA, FLI-301N, Tokyo, Japan) maintained at 20, 25, or 30℃ with 14-hour day length, and the number of cuttings broken was monitored at 2-day intervals for 60 days after treatment. Buds were regarded as broken when the buds tips became green. Four sets of 8 cuttings were allocated to each treatment. Throughout the experiment water in the bath was renewed weekly to prevent decomposition.

Cumulative chilling hours (CCH) and cumulative temperature (CT)

Temperature was recorded at the field where 'Pione' grapevines were grown throughout the experiment. Cumulative chilling hours (CCH) of exposure to below 7.2℃ were calculated for each treatment time. The CCHs in the July 18, August 20, September 22, October 21, November 20, December 24, January 22, February 24, and March 20 of cane collecting dates, were 0, 0, 0, 0, 14, 325, 839, 1376, and 1652, respectively. Cumulative temperatures (CT, ℃・h), a summation of temperature and hours of exposure to above 0℃, were also calculated in each month, but only CTs after November were used to determine the relationship between number of days to initial budbreak (NDIB) and CT, because CCH was first recorded in November. The CTs on November 20, December 24, January 22, February 24, and March 20 were 7294, 14267, 15999, 21051, and 26334, respectively.

Statistical analysis

An analysis of variance was conducted to test the differences among temperatures and treatment times. Mean separation was done with LSD and/or *t*-test. Relationships between NDIB in each plot and CCH or CT were evaluated based on data obtained in the treatments after November. The CT was calculated by summation of temperature and hours of exposure to above 0℃ from November 1 to each treatment time and of hours of exposure to 20, 25, or 30℃ from start of treatment to budbreak in each plot.

Results and Discussion

The effect of temperature on budbreak in grapevines is evaluated on the basis of: 1) the number of days to initial budbreak after the treatment, indicating promotion of budbreak and 2) the rate of budbreak, that is, the uniformity of budbreak[10]. Based on changes in the percentage of budbreak in each treatment (Fig. 1), the number of days to initial and 60% budbreak was compared among the treatments (Table 1). Judging from the number of days to initial and 60% budbreak in

Table 1 Effect of temperature on number of days to initial and 60% budbreak of cuttings from 'Pione' grapevines at different stages of dormancy

Treatment time	No. of days to initial budbreak			No. of days to 60% budbreak		
	20℃	25℃	30℃	20℃	25℃	30℃
July	25.5c[a]	10.7b	6.2a	–[b]	21.3b	13.0a
August	25.0b	11.2a	11.0a	–	20.5ns	16.5ns
September	28.2b	14.7a	13.2a	–	26.7ns	19.2ns
October	41.7c	23.7b	17.0a	51.5c	30.5b	23.0a
November	26.0c	20.0a	17.7a	29.7c	22.2b	20.0a
December	25.0c	19.7b	17.5a	28.7c	22.0b	19.7a
January	23.7b	17.0a	15.5a	25.2b	19.2a	17.0a
February	19.7c	16.7b	13.7a	22.0b	17.5a	17.5a
March	19.7b	16.0a	13.7a	22.7b	20.5b	16.0a

[a] Means within each row of initial and 60% budbreak followed by different letters are significantly different based on LSD or *t*-test ($P \leq 0.05$). ns: not significant.

[b] Percentage was less than 60% within examination.

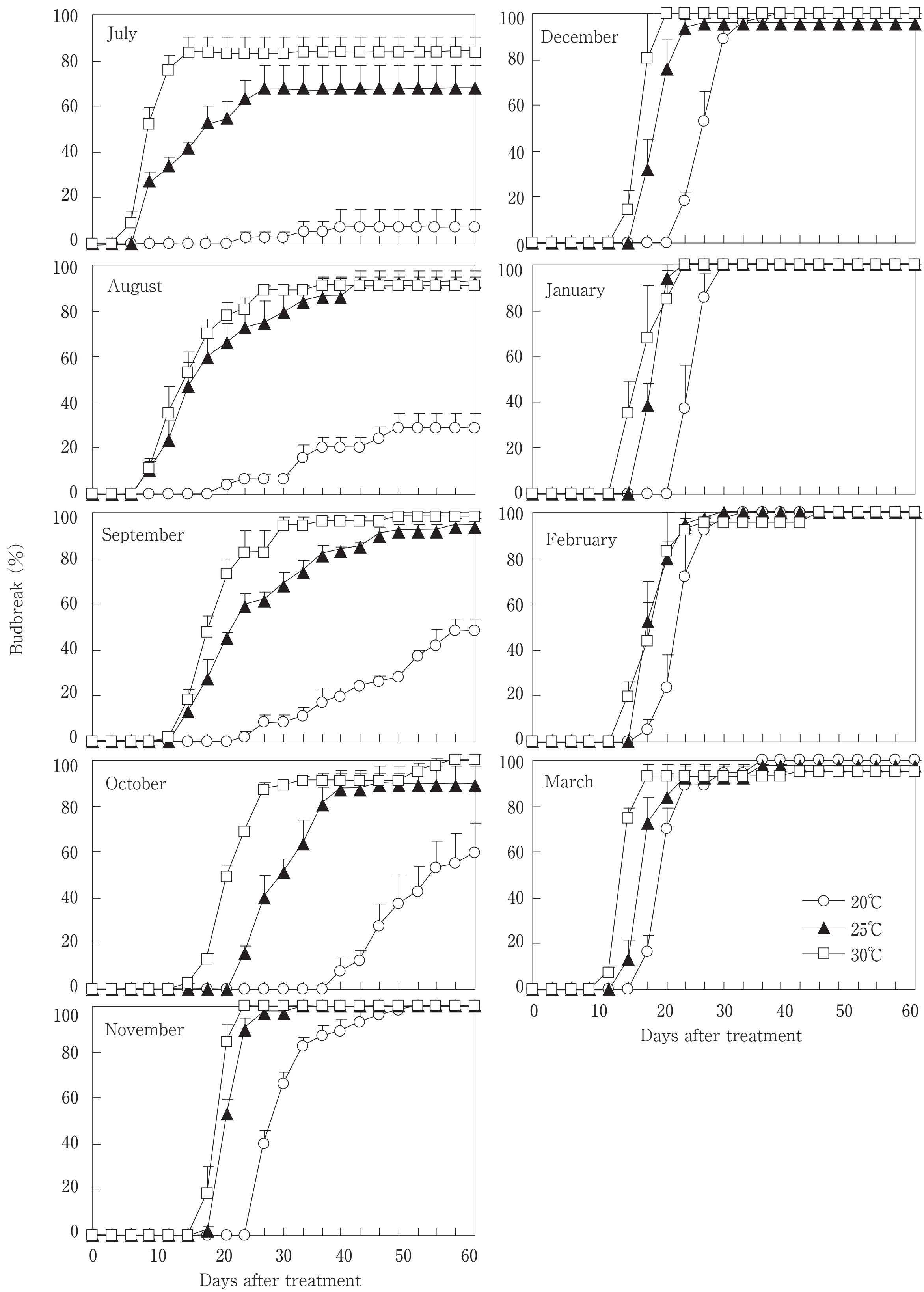

Fig. 1 Effect of temperature on budbreak of single bud cuttings of 'Pione' grapevines at different stages of dormancy. Vertical bars indicate the SE (n=4).

'Pione' cuttings, 30℃ was the most effective, followed by 25 and 20℃ in that order regardless of treatment time. In the 30℃ treatment, budbreak was significantly accelerated after treatment and the percent budbreak thereafter also increased steadily, resulting in the least number of days to initial budbreak and in higher rate of final budbreak percentage. Similar results were obtained at 25℃, but the efficiency of budbreak was slightly lower than at 30℃. On the other hand, treatment at 20℃ showed inferior budbreak, resulting in many days required for initial budbreak and an uneven budbreak thereafter. However, response of grapevine to temperature for budbreaking varied markely among the treatment times, that is, the different stages of dormancy (Table 1, Fig. 2). Irrespective of temperature treatment, the number of days to initial budbreak after treatment was little in July and increased with the treatment time, reaching a peak in October or November treatments depending on temperature, and thereafter gradually decreasing in February to March treatments (Fig. 2). Similar observations were made in the number of days to 60% budbreak, although an uneven rate of budbreak was observed at 20℃ from July to October, especially in earlier treatments such as July, August and September, resulting in less than 60% of final budbreak (Table 1). But there was no significant difference in the number of days to 60% budbreak between 25 and 30℃ in August and September treatments. Also, treatments after December showed more uniform budbreak regardless of temperature, and the later treatment at 25 or 30℃ resulted in higher percentage of budbreak (Fig. 1). On the other hand, at 20℃, the earliest treatment had the lowest final percentage of budbreak. The reasons for the differences in response of grapevine bud to temperature among the different stages of dormancy were not clearly understood. Late treatments such as January or later resulted in slight differences among temperatures in the initiation and the uniformity of budbreak. Slight differences were observed in the number of days to initial budbreak (NDIB) among treatment times. There was a large difference from September to November for 20 ℃ and from October to December for 25 and 30℃. The reasons for early induction of dormancy at 20℃ than at 25 and 30℃ were also unclear.

Overall, higher temperatures significantly reduced the number of days to initial and 60% budbreak (Table 2). Average number of days to initial or 60% budbreak at 30, 25, and 20℃ was 13.9, 16.7, and 26.0, or 18.8, 22.0, and 30.0, respectively. Also, the average number of days to initial budbreak was least in July of 14.0 and increased with treatment time, and peaking in October to December, corresponding to deep dormant stages. Thereafter, the number of days to initial budbreak gradually decreased to February to March. Similar results were obtained in the number of days to 60% budbreak, although final percentage of budbreak in July to September treatments was less than 60%. The maximum number of days to 60% budbreak was observed in September, but its number gradually decreased to February to March. These results suggest that each period of July to September, October to December, and January to March could correspond to paradormancy, endodormancy, and ecodormancy,

Table 2 Comparison of number of days to initial and 60% budbreak of 'Pione' cuttings among temperatures and treatment times

Variables	No. of days to initial budbreak	No. of days to 60% budbreak
Temperature		
20℃	26.0c[a]	30.0c
25℃	16.6b	22.0b
30℃	13.9a	18.8a
Treatment time		
July	14.0a	—[b]
August	15.7ab	—
September	18.7cd	—
October	27.5f	35.0c
November	21.2e	24.0b
December	20.7de	23.5b
January	18.7cd	20.5a
February	16.7bc	19.0a
March	16.5bc	19.7a

[a] Means within initial and 60% budbreak in each variable followed by different letters are significantly different based on LSD test ($P \leq 0.05$).

[b] Percentage was less than 60%.

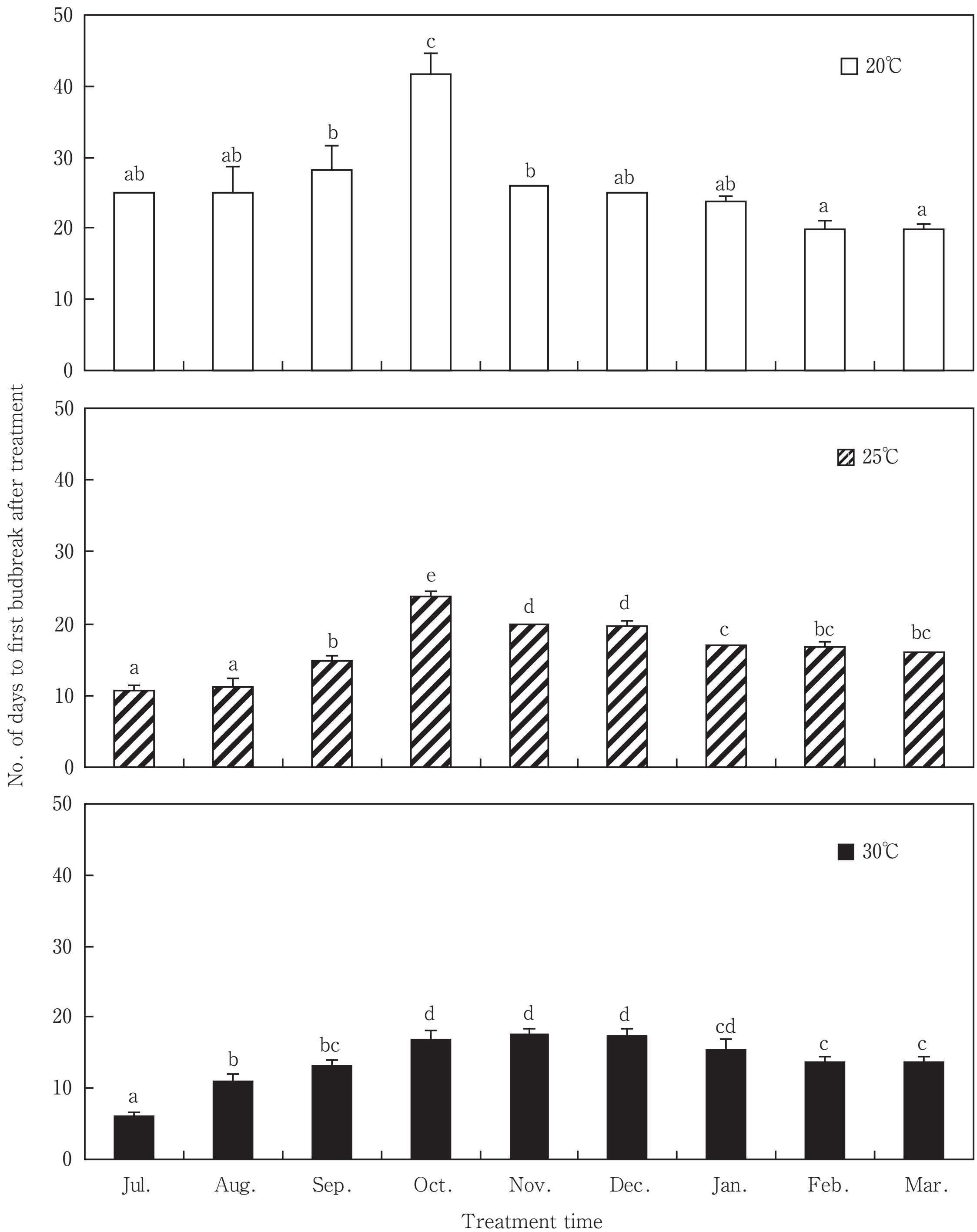

Fig. 2 The number of days to initial budbreak of 'Pione' grapevine cuttings as affected by different stages of dormancy in each temperature. Different letters within each temperature are significantly different based on LSD test ($P \leq 0.05$). Vertical bars indicate the SE (n=4).

respectively[3]. The results also agree with those of Horiuchi et al.[6] that bud dormancy of 'Delaware' grapevines was deep at the beginning of autumn, but its intensity gradually decreased from late autumn to early winter. Faust et al.[3] divided dormancy of perennial fruit trees into three phases, such as induction, maintenance and release based on physiological aspects. Available evidence on the factors and mechanisms involved in these phases has been reviewed by many researchers[1,4,21]. Reviews of Dennis[1] and Powell[21] have focused on the physiological involvement of plant hormones.

In the treatments after the last stage of endodormancy, from December to March, only slight differences in budbreak were observed among temperatures. Almost all the buds broke, even at 20℃, although initiation of budbreak was significantly later than at 25 and 30℃. However, aspects of budbreak were markedly different among temperatures in the treatments before endodormancy, from July to October. Treatments at 30 and 25℃ in July to October resulted in significantly fewer days required to initial budbreak compared to other treatment times. The uniformity was remarkably inferior and 100% budbreak was not obtained within the 60 days of examination. When grapevines are grown in tropical and subtropical areas, such as Thailand, they continue to grow throughout the year, having evergreen trees. Intensive grape production such as double cropping, that is, production of two crops per year has been widely conducted in those regions[35]. Our results indicate that high temperatures such as 30℃ are not sufficient for breaking of grapevine buds, but can support shoot growth. Therefore, artificial methods such as application of dormancy breaking chemicals are necessary to obtain uniform budbreak for grape growing in tropical regions[8,35]. These findings are useful to study the physiology of dormancy in grapevine, although the causes of large variation in response of buds to temperature during the period of paradormancy remain to be elucidated. On the other hand, 20℃ showed significantly inferior budbreak compared to 25 and 30℃, especially for the uniformity of budbreak, resulting in less than 60% of final budbreak in treatments from July to September. The results may suggest that temperature conditions such as 20℃ or lower are strongly related to induction of dormancy. Tohbe et al.[31] has reported previously that bud dormancy of Delaware grapevines is induced by 20/15℃ (day/night), and glutathione, compound with SH group, is strongly related to its control. It is well known that not only low temperature but also shorter day conditions induce dormancy of temperate-zone woody plants, including grapevines[6,15,34].

Breaking of bud dormancy by low temperature such as below 7.2℃ was recognized centuries ago, with a 'chilling requirement' being a widely accepted concept in the dormancy physiology of temperate-zone woody plants. The chilling requirement of the grapevine bud has been thought to be less than other deciduous fruit tree species[15,34], although the duration of the deepest stage of dormancy is prolonged by warm temperatures in winter[23]. However, the precise chilling hours required for budbreak in grapevines, especially for tetraploid cultivars such as 'Pione', have not been established yet. We previously reported that response of grapevine to temperature for budbreaking varied largely among tetraploid cultivars[20]. Nishimoto et al.[17] also reported that the chilling requirement of 'Kyoho' grapevine, the most popular tetraploid cultivar in Japan, is about 360 to 390 hours. In this experiment, correlations between the number of days to initial budbreak (NDIB) and cumulative chilling hours (CCH) and cumulative temperature (CT) were compared. The CCH was calculated using temperature data obtained after November, corresponding to the middle stage of endodormancy, because it was the first month of exposure to below 7.2℃ after start of the experiment. The CT was calculated as summation of temperature and hours of exposure to above 0℃ from November 1 to each treatment time and hours of exposure to 20, 25, or 30℃ from start of treatment to budbreak in each plot. There was a significant negative correlation between NDIB and both CCH and CT (Table 3). This fact may suggest that estimating the completion of dormancy in grapevine bud based on CT is possible in addition to the traditional method using CCH, although the results did not agree with those of our previous report[11] that were obtained in 'Kyoho' and 'Pione' grapevines grown in 7 vineyards with different temperature conditions. The CT is an important factor for selection of suitable growing area of fruit trees and production of fruit with excellent quality among other things[15,32,34]. Generally, the CT is calculated by summing up hours of temperature exposure to above 10℃, because almost all fruit tree species begin their growth at 10℃ or higher. In this study, however, we focused on release from dormancy in autumn and winter seasons, therefore CT was calculated by summation of

Table 3 Correlation coefficient between cumulative chilling hour (CCH) or cumulative temperature (CT) and number of days to initial budbreak of 'Pione' cuttings obtained from November to March

Temperature	CCH[a]	CT[b]
20℃	−0.9753**[c]	−0.9258*
25℃	−0.9586**	−0.9057*
30℃	−0.9814**	−0.9049*

[a] Cumulative chilling hours of exposure to below 7.2℃ from November 1 to start of each treatment.
[b] Summation of temperature and hours of exposure to above 0℃ from November 1 to each treatment time and hours of exposure to 20, 25, or 30℃ from start of treatment to budbreak in each plot.
[c] ** and * indicate significance at $P \leq 0.01$ and 0.05.

hours of exposure to above 0℃ after November, because exposure to below 7.2℃ was observed in the November treatment and thereafter. The relationship between the completion of dormancy in grapevine bud and CT has not been sufficiently established before, although Takagi and Tamura[30] suggested possibility for prediction of the sprouting date in a forcing culture of 'Muscat of Alexandria' grapevines by CT. Our finding, that estimation of the completion of dormancy based on CT is possible, is a significant step for studying the physiology of dormancy. However, further investigation is needed to confirm its usefulness for other grape cultivars.

Based on the above observations, we conclude that the response of 'Pione' grapevine to temperature for budbreaking varies largely among the treatment times throughout the dormant period from July to March. This shows a significant effect of temperature in the early treatments before endodormancy, based on the number of days to initial and 60% budbreak after treatment. Treatment at 20℃ had inferior budbreak compared to 25 and 30℃ during the period of paradormancy such as July to September. A significant negative correlation between the number of days to initial budbreak and both cumulative chilling hours (CCH) and cumulative temperature (CT) may suggest that estimating the completion of dormancy in grapevine bud based on CT is possible in addition to the traditional method using CCH. Further investigation is necessary to confirm its usefulness for other grape cultivars.

References

1) Dennis, Jr., F. G.: Dormancy—what we know (and don't know). HortScience, **29**, 1249–1255 (1994)
2) Erez, A., S. Lavee and R. M. Samish: Improved methods to control rest in the peach and other deciduous fruit species. J. Amer. Soc. Hort. Sci., **96**, 519–522 (1971)
3) Faust, M., A. Erez, L. J. Rowland, S. Y. Wang and H. A. Norman: Bud dormancy in perennial fruit trees: Physiological basis for dormancy induction, maintenance, and release. HortScience, **32**, 623–629 (1997)
4) Fuchigami, L. H. and C. C. Nee: Degree growth stage model and rest-breaking mechanisms in temperate woody perennials. HortScience, **22**, 836–845 (1987)
5) Honjo, H.: Effects of global warming on dormancy and flowering behavior of temperate fruit crops in Japan. Hort. Res. (Japan)., **6**, 1–5 (2007) (In Japanese)
6) Horiuchi, S., S. Nakagawa and A. Kato: General characteristics of bud dormancy in the vine. J. Japan. Soc. Hort. Sci., **50**, 176–184 (1981) (In Japanese with English Summary)
7) Kovacs, L. G., P. L. Byers, M. L. Kaps and J. Saenz: Dormancy, cold hardiness, and spring frost hazard in *Vitis amurensis* hybrids under continental climatic conditions. Amer. J. Enol. Vitic., **54**, 8–14 (2003)
8) Kubota, N., M. A. Matthews, T. Takahagi and W. M. Kliewer: Budbreak with garlic preparations: Effects of garlic preparations and of calcium and hydrogen cyanamides on budbreak of grapevines grown in greenhouses. Amer. J. Enol. Vitic., **51**, 409–414 (2000)
9) Kubota, N. and M. Miyamuki: Breaking bud dormancy in grapevines with garlic paste. J. Amer. Soc. Hort. Sci., **117**, 898–901 (1992)
10) Kubota, N., M. Miyamuki, Y. Yamane, A. Kobayashi and F. Mizutani: Breaking bud dormancy in grapevine cuttings with garlic volatiles. J. Japan. Soc. Hort. Sci., **68**, 927–931 (1999)
11) Kubota, N., C. Potjanapimon, F. Fukuda, Y. Fujii, T. Ono, Y. Kurafuji, A. Ogoro, Y. Kunugi, K. Kobayashi, I. Shigehara, H. Yamashita and H. Fujishima: Estimation of completion of dormancy in grapevines bud based on cumulative temperature. Sci. Rep. Fac. Agr. Okayama Univ., **98**, 9–16 (2009) (In Japanese with English Summary)
12) Kuroi, I., Y. Shiraishi and S. Imano: Studies on breaking the dormancy of grape vines (I) Effect of lime nitrogen treatment for shortening the rest period of glasshouse-grown grape vines. J. Japan. Soc. Hort. Sci., **32**, 175–180 (1963) (In Japanese with English Summary)
13) Magoon, C. A. and I. W. Dix: Observations on the response of grape vines to winter temperatures as related to their dormancy requirements. Proc. Amer. Soc. Hort. Sci., **42**, 407–412 (1943)
14) Maneethon, S., N. Kozai, K. Beppu and I. Kataoka: Rootstock effect on budburst of 'Premier' low-chill peach cultivar. Sci. Hort., **111**, 406–408 (2007)
15) Nakagawa, S.: Principles of Pomology. pp. 442–466, Yokendo, Tokyo (1978) (In Japanese)
16) Nir, G., I. Klein, S. Lavee, G. Spieler and U. Barak: Improving grapevine budbreak and yields by evaporative cooling. J. Amer. Soc. Hort. Sci., **113**, 512–517 (1988)
17) Nishimoto, N., K. Kizaki, O. Kumamoto and K. Sano: Chilling requirement for rest completion of grapevine. J. Japan. Soc. Hort. Sci., **67**(Suppl. 2), 227 (1998) (In Japanese)
18) Norvell, D. J. and J. N. Moore: An evaluation of chilling models for estimating rest requirements of highbush blueberries (*Vaccinium corymbosum* L.) J. Amer. Soc. Hort. Sci., **107**, 54–56 (1982)
19) Potjanapimon, C., F. Fukuda and N. Kubota: Effects of chilling exposures and chemicals on breaking bud dormancy in seven tetraploid grape cultivars. Hort. Res. (Japan)., **7**, 261–268 (2008) (In Japanese with English Summary)
20) Potjanapimon, C., Y. Ikuta, N. Kubota, F. Fukuda and T. Ono: Differences in chilling requirement for bud break among tetraploid grape cultivars. ACIAR Technical Reports, **61**, 54–60 (2005)
21) Powell, L. E.: Hormonal aspects of bud and seed dormancy in temperate-zone woody plants. HortScience, **22**, 92–109 (1987)
22) Samish, R. M.: Dormancy in woody plants. Ann. Rev. Plant Physiol., **5**, 183–204 (1954)
23) Saure, M. C.: Dormancy release in deciduous fruit trees. Hort. Rev., **7**, 239–300 (1985)
24) Shaltout, A. D. and C. R. Unrath: Rest completion prediction model for 'Starkrimson Delicious' apples. J. Amer. Soc. Hort. Sci., **108**, 957–961 (1983)

25) Sherman, W. B. and J. Rodriguez : Breeding of low-chill peach and nectarine for mild winters. HortScience, **22**, 1233-1236 (1987)
26) Shiraishi, M. : Grape. *In* Horticulture in Japan 2006 (The Japanese Society for Horticultural Science eds.), pp. 42-50, Nakanishi Printing, Kyoto (2006)
27) Shulman, Y., G. Nir, L. Fanberstein and S. Lavee : The effect of cyanamide on the release from dormancy of grapevine buds. Sci. Hort., **19**, 97-104 (1983)
28) Sugiura, T., H. Kuroda and H. Sugiura : Influence of the current state of global warming on fruit tree growth in Japan. Hort. Res. (Japan)., **6**, 257-263 (2007) (In Japanese with English Summary)
29) Sugiura, T. and M. Yokozawa : Impact of global warming on environments for apple and satsuma mandarin production estimated from changes of the annual mean temperature. J. Japan. Soc. Hort. Sci., **73**, 72-78 (2004) (In Japanese with English Summary)
30) Takagi, N. and F. Tamura : Evaluations of chill unit and accumulated temperature for prediction of sprouting date of 'Muscat of Alexandria' grapevines. J. Japan. Soc. Hort. Sci., **56**, 24-30 (1987) (In Japanese with English Summary)
31) Thobe, M., R. Mochioka, S. Horiuchi, T. Ogata, S. Shiozaki and H. Kurooka : The role of glutathione on the onset of endodormancy of grape buds. J. Japan. Soc. Hort. Sci., **67**, 912-916 (1998) (In Japanese with English Summary)
32) Weaver, R. J. : Grape Growing. pp. 43-59, John Wiley & Sons, New York (1976)
33) Weaver, R. J., L. Manivel and F. L. Jensen : The effects of growth regulators, temperature and drying on *Vitis vinifera* buds. Vitis, **13**, 23-29 (1974)
34) Westwood, M. N. : Temperate Zone Pomology. pp. 299-332, W. H. Freeman and Company, San Francisco (1978)
35) Yaacob, O. and S. Subhadrabandhu : The production of economic fruits in south-east asia. pp. 269-274, Oxford University Press, Oxford (1995)

休眠程度の異なるブドウ‘ピオーネ’の発芽に及ぼす温度の影響

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露地栽培されているブドウ‘ピオーネ’について，休眠の深さが異なる7月から翌年3月まで約1か月間隔で枝を採取し，1芽を有す挿し穂を調整した後，20，25および30℃に制御したインキュベーター（いずれも14時間日長）に入れ，経時的に発芽を調査した．発芽の早さを示す発芽所要日数と発芽の揃いを示す60％発芽所要日数から発芽に及ぼす温度の影響を評価した．実験期間中の温度を測定し，休眠完了と温度との関係を考察した．いずれの処理時期においても30℃の発芽が最も優れ，次いで25℃，20℃の順であった．しかし，発芽に及ぼす温度の影響は処理時期によって大きく異なった．すなわち，発芽所要日数は7月から10月までは徐々に増加し，11月に最大に達した後，3月に向けて少しずつ減少した．このことから，‘ピオーネ’では7月から9月が条件的休眠期，10月から12月が自発休眠期，1月から3月が他発休眠期と推察された．自発休眠期までの最終発芽率はいずれの温度も100％未満であり，また7月～9月の20℃処理では60％未満の発芽率であった．一方，自発休眠期の中期以降の処理ではいずれの温度とも均一な発芽を示し，最終発芽率はほぼ100％であった．11月以降の処理において，発芽所要日数と7.2℃以下の温度に遭遇した時間数（CCH）との間に有意な負の相関があった．また，11月1日から各処理時期までの0℃以上の温度に遭遇した時間数と20，25または30℃で処理を始めた日から各処理区の発芽までの時間数との積算（CT，℃・h）との間にも有意な負の相関が認められた．以上のことから，‘ピオーネ’の芽の休眠完了の予測には低温遭遇量だけでなく，0℃以上の積算温度による方法も有効と考えられた．