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*(Course of Applied Plant Science)*

ブドウの芽の休眠打破に及ぼす化学物質の種類と濃度の影響

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## Effects of Various Chemicals and their Concentrations on Breaking Bud Dormancy in Grapevines

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The effects of various chemicals and their concentrations on budbreak of 'Pione' grapevine (*Vitis labrusca* L. x *V. vinifera* L.) were studied by using single-bud cuttings obtained in endodormancy. When seven chemicals were applied to the upper half of cuttings, including bud, 2% hydrogen cyanamide ( $\text{H}_2\text{CN}_2$ ) was most effective in budbreak, judging from acceleration and uniformity of budbreak. However, neither 10% suspension of calcium cyanamide ( $\text{CaCN}_2$ ) nor 5% diallyl disulfide ( $\text{C}_6\text{H}_{10}\text{S}_2$ ) had any effect in breaking bud dormancy of 'Pione' cuttings. Budbreak in cuttings treated with 10% hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) was inhibited slightly compared to the control cuttings. No effect of 2% potassium chlorate ( $\text{KClO}_3$ ), 2% sodium chlorate ( $\text{NaClO}_3$ ) or 2% paclobutrazol (PBZ) on breaking bud dormancy in 'Pione' cuttings was observed. The effects of  $\text{CaCN}_2$ ,  $\text{H}_2\text{CN}_2$  and  $\text{C}_6\text{H}_{10}\text{S}_2$  on breaking bud dormancy in 'Pione' cuttings were compared at three to four concentrations. With  $\text{CaCN}_2$ , a 20% suspension significantly promoted budbreak, but a 5% suspension resulted in no effect. Both 5% and 2% of  $\text{H}_2\text{CN}_2$  accelerated budbreak significantly and resulted in uniform budbreak, especially at 5%, whereas at 0.5%  $\text{H}_2\text{CN}_2$  no effect was observed. Of three concentration of  $\text{C}_6\text{H}_{10}\text{S}_2$ , only a 10% solution showed any effectiveness in budbreak. The results indicated that  $\text{H}_2\text{CN}_2$  is most effective in breaking bud dormancy of 'Pione' grapevine cuttings, followed by  $\text{CaCN}_2$  and  $\text{C}_6\text{H}_{10}\text{S}_2$  in that order, although their effectiveness varied largely according to the concentrations for all chemicals.

**Key words :** grapevine, breaking bud dormancy, chemicals, concentration, cutting

### Introduction

The forcing of grapevines growing under protective structures is a common practice in Japan. Because grapevine buds are usually dormant when forcing starts, it is very important to break dormancy efficiently. Many investigations have been conducted for artificial terminations of dormancy in woody plants, including grapevines, by applying chemicals such as mineral oil and dinitro-*o*-cresol (DNOC)<sup>15)</sup>, calcium cyanamide ( $\text{CaCN}_2$ )<sup>3,9,10)</sup>, hydrogen cyanamide ( $\text{H}_2\text{CN}_2$ )<sup>1,13,14,16,17,21-24)</sup>, and growth regulators<sup>3,20)</sup>.

Kubota and Miyamuki<sup>6)</sup> previously reported that freshly grated garlic (*Allium sativum* L.) to the cross-sectional cut surface of the cane immediately after pruning to stimulate budbreak in several dormant grape cultivars. Kubota et al.<sup>7,8)</sup> further reported that the active substances in garlic, responsible for breaking bud dormancy in grapevines, are volatile sulfur-containing compounds with an allyl group ( $\text{CH}_2\text{CHCH}_2$ ), particularly diallyl disulfide ( $\text{C}_6\text{H}_{10}\text{S}_2$ ). In addition, Kuroda et al.<sup>11,12)</sup> reported that hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) is effective

in breaking endodormancy in flower buds of the Japanese pear (*Pyrus pyrifolia* Nakai). On the other hand, in tropical regions, especially in Thailand<sup>14,18)</sup>, several chemicals such as potassium chlorate ( $\text{KClO}_3$ ), sodium chlorate ( $\text{NaClO}_3$ ) and paclobutrazol (PBZ) have been applied to induce the flower buds of longan (*Dimocarpus longan* Lour.) and mango (*Mangifera indica* L.) for their off-season production. However, we have little information on the effectiveness of these chemicals and their suitable concentrations for breaking bud dormancy in grapevines.

The objective of this study was to examine in detail the effects of various chemicals and their concentrations on breaking bud dormancy in 'Pione' grapevines by using single-bud cuttings collected in endodormancy.

### Materials and Methods

All vine materials were obtained from the mature 'Pione' grapevines (*Vitis labrusca* L. x *V. vinifera* L.) grown at the Research Farm of the Faculty of

Agriculture, Okayama University, in endodormancy.

#### ***Effects of painting with various chemicals on budbreak of 'Pione' cuttings***

The canes of 'Pione' grapevines were pruned on October 27, 2004, corresponding to endodormancy, then cuttings (6 cm in length) with a single-bud were prepared. The upper half of the cuttings, including a bud, were treated with the supernatant of a 10% suspension of calcium cyanamide ( $\text{CaCN}_2$ ) (Nihon Carbide Industry Co. Ltd., Tokyo), 2% hydrogen cyanamide ( $\text{H}_2\text{CN}_2$ ) (Nihon Carbide), 10% hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) (Nakalai Tesque Co. Ltd., Kyoto) or 5% diallyl disulfide ( $\text{C}_6\text{H}_{10}\text{S}_2$ ) (Tokyo Kasei Industry Co. Ltd., Tokyo) which is an active substance in garlic, responsible for breaking bud dormancy in grapevines<sup>8</sup>. Control cuttings were treated with distilled water.

For another treatment, canes were obtained from endodormant 'Pione' grapevines on November 11, 2004, then cuttings with a single-bud were prepared as described above. The upper half of the cuttings were treated with 2% potassium chlorate ( $\text{KClO}_3$ ) (Nakalai Tesque), 2% sodium chlorate ( $\text{NaClO}_3$ ) (Nakalai Tesque), and 2% paclobutrazol (PBZ) (Zeneca Co. Ltd., Tokyo). The control cuttings were treated with distilled water.

Immediately after treatment, the cuttings were mounted on a plastic foam plate, floated in a water bath, then placed in a growth chamber maintained at 25°C. Each treatment consisted of four replications of seven to nine cuttings. Budbreak in each cutting was surveyed at two days interval for 60 days after treatment. Budbreak was regarded as the date when a green tinge was seen beneath the bud scales.

#### ***Effects of painting with different concentrations of $\text{CaCN}_2$ , $\text{H}_2\text{CN}_2$ or $\text{C}_6\text{H}_{10}\text{S}_2$ on budbreak of 'Pione' cuttings***

Cuttings with a single-bud were prepared from endodormant 'Pione' grapevines on November 11, 2004. The upper half of the cuttings, including a bud, were treated with a supernatant of 20, 10 or 5% suspensions of  $\text{CaCN}_2$ , 5, 2, 1 or 0.5%  $\text{H}_2\text{CN}_2$ , and 10, 5 or 2%  $\text{C}_6\text{H}_{10}\text{S}_2$ . The control cuttings were treated with distilled water. Immediately after treatment, cuttings were mounted on a plastic foam plate floating in water and placed in a growth chamber kept at 25°C. Four replications of seven to nine cuttings were done for each treatment. Budbreak in each cutting was surveyed at two days interval for 60 days after treatment as mentioned above.

#### ***Statistical analysis***

An analysis of variance was applied to the results of the determinations to test for significant differences

among the chemicals or concentrations. Statistical methods employed were ANOVA and the LSD test.

### **Results and Discussion**

In practice,  $\text{H}_2\text{CN}_2$  is the most popular chemical for breaking bud dormancy of fruit trees throughout the world because of its higher effectiveness in budbreak. However,  $\text{H}_2\text{CN}_2$  is a dangerous substance, suggesting some kind of bad effect on growers' health, although it depends on the treatment conditions. Therefore, many grape growers hope that someday the chemicals which do not injure their health will be developed. In this experiment, the effects of various chemicals and their concentrations on budbreak of grapevines were compared. In Japan, supernatants of  $\text{CaCN}_2$  suspensions have been widely used for stimulation of budbreak in various grapevine cultivars since a report by Kuroi *et al.*<sup>9</sup>, but in many other countries, including the US, the use of  $\text{H}_2\text{CN}_2$  to enhance budbreak is common<sup>14,16,22,24</sup>. In general, the supernatant of a 20% suspension of  $\text{CaCN}_2$  is painted on buds of canes<sup>6,9,10</sup>, but  $\text{H}_2\text{CN}_2$  is sprayed onto the canes at a concentration of 2% or less<sup>14,22,24</sup>. On the other hand, fresh garlic paste, which promotes budbreak of 'Muscat of Alexandria' vines being forced, is painted on the cut surface of the cane<sup>6</sup>. In this experiment with 'Pione' grapevine, all substances tested, including  $\text{C}_6\text{H}_{10}\text{S}_2$  which is the most important substance in garlic responsible for breaking bud dormancy in grapevines<sup>5,8</sup>, were painted on the upper half of cuttings, including a bud.

#### ***Effects of painting with various chemicals on budbreak in dormant 'Pione' cuttings***

The effectiveness of the chemicals on budbreak in dormant grapevines is evaluated on the following basis: 1) a fewer 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.

When the effects of a 10% suspension of  $\text{CaCN}_2$ , 2%  $\text{H}_2\text{CN}_2$ , 5%  $\text{C}_6\text{H}_{10}\text{S}_2$  and 10%  $\text{H}_2\text{O}_2$  on budbreak of endodormant 'Pione' cuttings were compared, painting with 2%  $\text{H}_2\text{CN}_2$  significantly promoted onset of budbreak and also increased the rate of budbreak thereafter (Fig. 1, Table 1). That is, budbreak in cuttings treated with a 2%  $\text{H}_2\text{CN}_2$  was initiated in only 12 days, compared to the control in which the first budbreak appeared 22.5 days after treatment with water (Table 1). This is in agreement with previous results that showed effectiveness of  $\text{H}_2\text{CN}_2$  for breaking bud dormancy in grapevines<sup>14,16,22,24</sup>. However, a 10% suspension of  $\text{CaCN}_2$  and 5%  $\text{C}_6\text{H}_{10}\text{S}_2$  had no effect on budbreak of 'Pione' cuttings, although many results that showed

the effectiveness of these substances in breaking bud dormancy of grapevines irrespective of exposing to chilling<sup>3,5,6,9,10</sup>. Different results of budbreak among the reports might be caused by lower or higher concentration of  $\text{CaCN}_2$  or  $\text{C}_6\text{H}_{10}\text{S}_2$ , respectively. Kuroda et al.<sup>11,12</sup> indicate that  $\text{H}_2\text{O}_2$  is an effective compound for breaking endodormancy in flower buds of the Japanese pear, but budbreak in 'Pione' cuttings treated with a 10%  $\text{H}_2\text{O}_2$  was somehow inhibited slightly compared to the controls. The reasons for different responses by buds of different fruit trees to  $\text{H}_2\text{O}_2$  are not known. Gibberellic acid, a plant growth regulator, increases percent budbreak in peaches (*Prunus persica* Batsch)<sup>3</sup>, but it decreases the emergence rate in grapes<sup>19</sup>.

In recent years, a technology of artificial induction of flower buds in longan and mango by application of  $\text{KClO}_3$ ,  $\text{NaClO}_3$ , or paclobutrazol (PBZ) has been developed in southeast Asia, especially Thailand, for their off-season production<sup>4,18</sup>. When dormant 'Pione' grapevine cuttings were treated with  $\text{KClO}_3$ ,  $\text{NaClO}_3$ , or PBZ at 2% in each substance, there was no effect on breaking

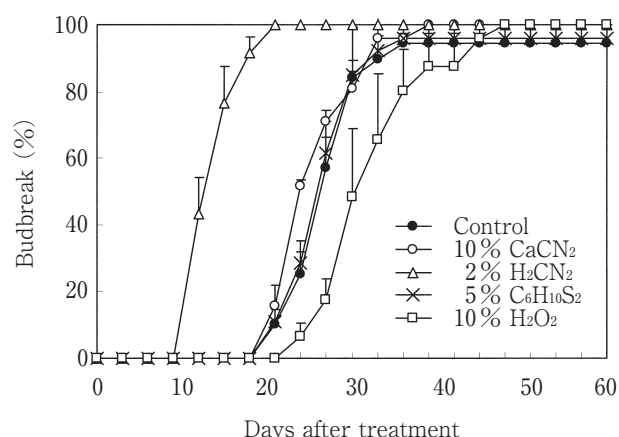


Fig. 1 Effects of painting with 10% suspension of  $\text{CaCN}_2$ , 2%  $\text{H}_2\text{CN}_2$ , 5% diallyl disulfide ( $\text{C}_6\text{H}_{10}\text{S}_2$ ) and 10%  $\text{H}_2\text{O}_2$  on budbreak of single-bud cuttings of dormant 'Pione' grapevines (treated on October 27, 2004).

Vertical bars are the SE ( $n=4$ ).

Table 1 Number of days required for first and 80% budbreak in each treatment

Chemicals	Days after treatment to first budbreak	Days after treatment to 80% budbreak
Control	22.5b	29.0b
10% $\text{CaCN}_2$	21.8b	30.8b
2% $\text{H}_2\text{CN}_2$	12.0a	16.5a
5% $\text{C}_6\text{H}_{10}\text{S}_2$	21.8b	30.0b
10% $\text{H}_2\text{O}_2$	26.3c	36.8c

Means with the different letter within each column are significant ( $P=0.01$ ) as indicated by one-way ANOVA followed by LSD test.

bud dormancy for any of the chemicals tested (Fig. 2). Another of our experiments showed that 0.2% solutions of these chemicals also were ineffective in budbreak of 'Pione' cuttings (unpublished data). Judging from these facts, we consider that the effects of  $\text{KClO}_3$ ,  $\text{NaClO}_3$  and PBZ on breaking bud dormancy in grapevine are little or small.

#### Effects of painting with different concentrations of $\text{CaCN}_2$ , $\text{H}_2\text{CN}_2$ , or $\text{C}_6\text{H}_{10}\text{S}_2$ on budbreak in dormant 'Pione' cuttings

Effects of painting with different concentrations of  $\text{CaCN}_2$ ,  $\text{H}_2\text{CN}_2$  and  $\text{C}_6\text{H}_{10}\text{S}_2$  on breaking bud dormancy in 'Pione' grapevine cuttings were shown in Fig. 3 and Table 2.

When cuttings were treated with supernatants of 5, 10 and 20% suspension of  $\text{CaCN}_2$ , a 20% suspension significantly promoted budbreak (Fig. 3-A, Table 2). In Japan, the supernatant of a 20% suspension of  $\text{CaCN}_2$  is used practically for breaking bud dormancy in grapevines irrespective of cultivars<sup>3,5,9,10</sup>. A 10% suspension did not accelerate budbreak although it showed uniform budbreak. Treatment with a 5% suspension resulted in no effect in breaking bud dormancy. It can therefore be considered that 10% or less suspensions of  $\text{CaCN}_2$  are too low for stimulating budbreak of dormant grapevines.

$\text{H}_2\text{CN}_2$  promoted budbreak and increased consistently the rate of budbreak of 'Pione' cuttings thereafter for all concentrations tested, and the higher concentration resulted in fewer days required to first budbreak (Fig. 3-B, Table 2). That is, budbreak in cuttings treated with 5, 2, 1 and 0.5% of  $\text{H}_2\text{CN}_2$  was initiated 11.3, 12.0, 13.5 and 18 days, respectively, whereas the first bud-

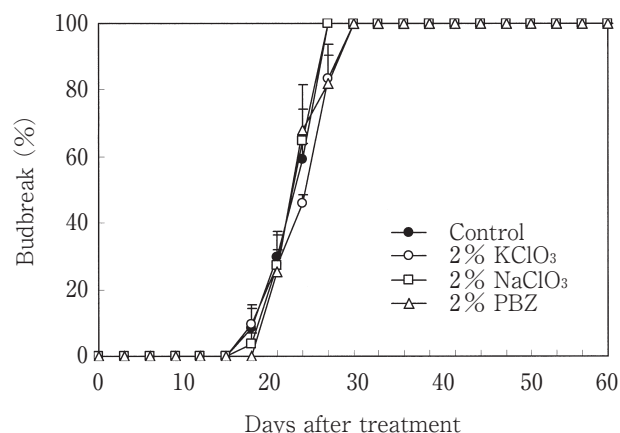


Fig. 2 Effects of painting with 2%  $\text{KClO}_3$ , 2%  $\text{NaClO}_3$  and 2% PBZ (paclobutrazol) on budbreak of single-bud cuttings of dormant 'Pione' grapevines (treated on November 11, 2004).

Vertical bars are the SE ( $n=4$ ).

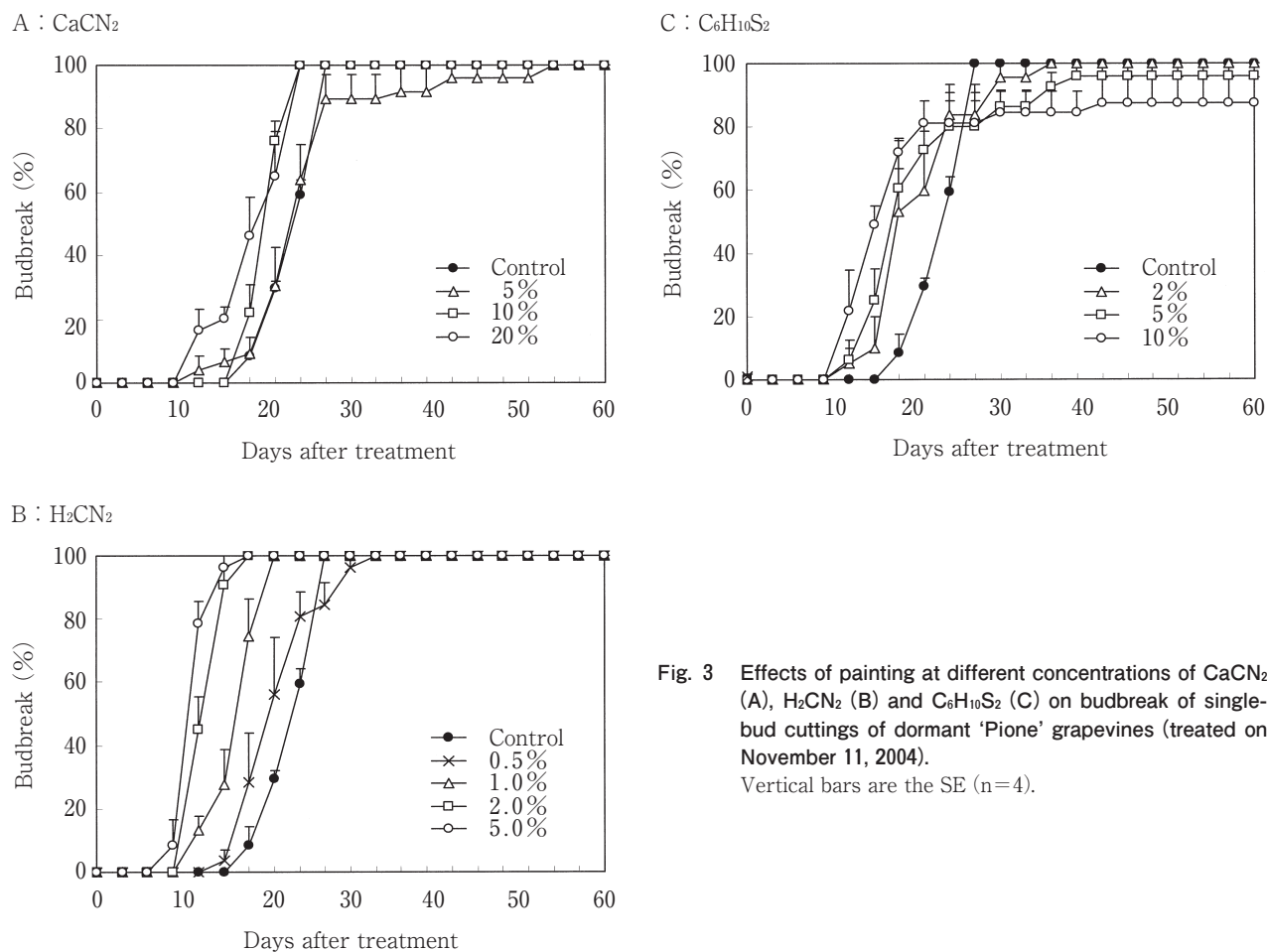


Fig. 3 Effects of painting at different concentrations of  $\text{CaCN}_2$  (A),  $\text{H}_2\text{CN}_2$  (B) and  $\text{C}_6\text{H}_{10}\text{S}_2$  (C) on budbreak of single-bud cuttings of dormant 'Pione' grapevines (treated on November 11, 2004). Vertical bars are the SE ( $n=4$ ).

Table 2 Number of days required for first and 80% budbreak in each treatment

Chemicals	Concentration (%)	Days after treatment to first budbreak	Days after treatment to 80% budbreak
Control		19.5d	27.0c
$\text{CaCN}_2$	5.0	18.0cd	30.0c
	10.0	18.8cd	21.8bc
	20.0	12.8ab	23.5bc
$\text{H}_2\text{CN}_2$	0.5	18.0cd	27.0c
	1.0	13.5ab	19.5ab
	2.0	12.0a	15.8a
	5.0	11.3a	15.0a
$\text{C}_6\text{H}_{10}\text{S}_2$	2.0	16.5bcd	24.0bc
	5.0	15.0abc	25.5c
	10.0	13.5ab	22.6bc

Means with the different letter within each column are significant ( $P=0.01$ ) as indicated by one-way ANOVA followed by LSD test.

break occurred 19.5 days after treatment in the control (Table 2). Most uniform budbreak was also observed at 5%, followed by 2 and 1%. Judging from the initiation and the uniformity of budbreak, a 5%  $\text{H}_2\text{CN}_2$  was most

effective in budbreak, although  $\text{H}_2\text{CN}_2$  is applied in practice for stimulating budbreak of dormant grapevines at less than 3%<sup>5,14,16,22</sup>. The result obtained here indicates that a 0.5%  $\text{H}_2\text{CN}_2$  is too low for breaking bud dor-

mancy in grapevines. It is well known that the effects of  $H_2CN_2$  on budbreak in fruit trees, including grapevines, varied according to the depth of dormancy<sup>1,6,17</sup>.

In 'Pione' cuttings treated with  $C_6H_{10}S_2$ , budbreak was promoted for all concentrations tested, particularly at 10%, comparing with the control cuttings (Fig. 3-C, Table 2). However, the uniformity of budbreak in cuttings treated with  $C_6H_{10}S_2$  was markedly inferior than in those of  $H_2CN_2$  irrespective of concentration. Kubota et al.<sup>5,8</sup> have reported that painting or exposing to 30% or more of  $C_6H_{10}S_2$  is effective in breaking bud dormancy in grapevines, including 'Pione'. These results suggest that the higher the concentration of  $C_6H_{10}S_2$  the more pronounced the effect on budbreak.

Based on the results mentioned above, we conclude that  $H_2CN_2$ , now commonly used for the breaking of bud dormancy in table grapes in the world, is most effective in promotion and uniformity of budbreak, although details of the effects differed depending on the chemicals and their concentrations. As for  $C_6H_{10}S_2$ , further investigation is needed to establish the suitable concentrations and methods for its application.

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## ブドウの芽の休眠打破に及ぼす化学物質の種類と濃度の影響

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自発休眠期に採取したブドウ‘ピオーネ’の挿し穂を用い、芽の休眠打破に及ぼす化学物質の種類と濃度の影響を調査した。7種類の化学物質について休眠打破の効果を比較したところ、発芽の促進と揃いからみて、2%シアナミド( $\text{H}_2\text{CN}_2$ )の効果が最も大きかった。一方、10%石灰窒素( $\text{CaCN}_2$ )と5%2硫化ジアリル( $\text{C}_6\text{H}_{10}\text{S}_2$ )には休眠打破の効果は認められなかった。10%過酸化水素( $\text{H}_2\text{O}_2$ )では発芽がやや抑制された。2%の塩素酸カリウム( $\text{KClO}_3$ )、塩素酸ナトリウム( $\text{NaClO}_3$ )およびパクロブトラゾール(PBZ)はいずれも休眠打破の効果を示さなかった。石灰窒素、シアナミドおよび2硫化ジアリルの濃度を3~4段階に変え、‘ピオーネ’挿し穂の休眠打破に及ぼす影響を調査した。石灰窒素では、20%区の発芽が著しく促進されたが、5%区では発芽の揃いがよくなかった。シアナミドでは、5%区と2%区の休眠打破効果が著しかったのに対し、0.5%区では休眠打破の効果は認められなかった。2硫化ジアリルでは、10%区で休眠打破効果がみられただけであった。以上の結果から、ブドウの芽の休眠打破に及ぼす化学物質の効果はシアナミドで最も大きく、次いで石灰窒素、2硫化ジアリルの順であったが、効果の程度は濃度によって異なった。