Breaking Bud Dormancy in Grape Cuttings with Non-volatile and Volatile Compounds of Several *Allium* Species

Naohiro Kubota*, Yasushi Yamane and Koji Toriu

*Faculty of Agriculture, Okayama University, Tsushima, Okayama 700 - 8530*

**Summary**

The effect of non-volatile (as a paste) and volatile compounds from five *Allium* species on the bud dormancy of grapevine cuttings ('Muscat of Alexandria' and 'Kyoho') were examined. Painting freshly grated paste from the five, garlic, Chinese chive, rakkyo, onion and Welsh onion on the upper cut surface of single-bud cuttings of dormant grapevines did not accelerate budbreak, whereas volatile compounds from these plants hastened it. The effect was greatest with volatile compounds from etiolated Chinese chive, followed by non-etiolated Chinese chive, rakkyo, garlic, and onion. The advancement of budbreak by these compounds was less than that by diallyl disulfide and trisulfide substances in garlic. Volatile compounds from garlic, etiolated Chinese chive, and rakkyo were less effective when the preparations were boiled. A 12 hr - or 24 hr-exposure to volatile compounds of garlic, etiolated Chinese chive, and rakkyo promoted budbreak to the same extent as did commercial garlic oil. Temperature had no effect on budbreak when cuttings were exposed to volatiles from non-etiolated Chinese chive for 24 hr, although high temperature induced budbreak in cuttings treated with garlic volatiles. Chinese chive and rakkyo promoted budbreak in grapevines; the substances were supposed to be volatiles similar to those in garlic.

**Key Words:** *Allium* species, budbreak, cuttings, grapevines, volatiles.

**Introduction**

Artificially inducing budbreak on grapevines, growing under protected cultivation, is common in Japan. The aim is to obtain an efficient, uniform budbreak. Many chemicals, such as mineral oil (Samish, 1954), dinitro-o-cresol (DNOC) (Samish, 1954), calcium cyanamide (CaCN₂) (Iwasaki, 1960; Kuroi, 1985), hydrogen cyanamide (H₂CN₂) (Lin and Wang, 1985; Nir et al., 1988; Shulman et al., 1983; Zelleke and Kliewer, 1989), and other growth regulators (Donoho and Walker, 1957; Iwasaki, 1980; Weaver, 1959; Weaver et al., 1974), have been used to terminate bud dormancy in woody plants, including grapevines.

In Japan, supernatants of CaCN₂ suspensions have been used for stimulating budbreak in various grape cultivars. A paste of fresh garlic (*Allium sativum* L.) applied to cross-sectional cut surfaces of canes immediately after pruning stimulated budbreak on 'Muscat of Alexandria' grapevines in November or December, (Kubota et al., 1987). We reported previously that freshly grated garlic paste is as effective as the supernatant of a CaCN₂ suspension and H₂CN₂ in promoting budbreak in several grape cultivars, including 'Muscat of Alexandria' (Kubota and Miyamuki, 1992; Kubota et al., 2000). We also observed that an application of boiled grated garlic on 'Muscat of Alexandria' cuttings had little effect on budbreak (Kubota et al., 1999a). Moreover, we found that the substances in garlic that broke bud dormancy in the grapevines are volatile compounds containing sulfur with an allyl group (CH₂CH₂S), particularly diallyl disulfide (Kubota et al., 1999b).

Many *Allium* plant species, including garlic, have a characteristic odor arising from sulfide compounds. Different species contain different sulfides (Freeman and Whenham, 1975; Kojima, 1982; Obata, 1961; Saghiri et al., 1964; Yu et al., 1989), suggesting that the pastes of *Allium* species other than garlic or their volatile compounds also may promote budbreak in grapevines. The purpose of this study was to examine the effects of paste and volatile compounds of several *Allium* species on breaking bud dormancy in grapevines.

**Materials and Methods**

*Grapevines and general procedures.*

Single-bud cuttings (10 cm in length), including the 5th to the 9th nodes of canes, were obtained from dormant grapevines (*Vitis vinifera* L. 'Muscat of Alex-
andria' and *V. labruscana* Bailey × *V. vinifera* L., 'Kyoho') at the research farm of the Faculty of Agriculture, Okayama University, Japan. Cumulative chilling hours (CCH) to which grapevines were exposed at 7.2 °C or lower ranged from 0 to 354 hr depending on the experiment. Immediately after treatment with the appropriate *Allium* preparation, sulfides, or the control treatment, cuttings were mounted on a plastic foam plate, floated in a water bath, and placed in a growth chamber at 25 °C. Each treatment was replicated thrice, using 10 cuttings per replicate except in Exp. 2, in which there were two replications. Buds were regarded as no longer dormant when green tissue appeared through the bud scales.

**Effects of freshly grated *Allium* plants and their volatile compounds (Exp. 1).**

Eighteen sets of 30 cuttings, comprising three treatment replications with 10 cuttings for each, were prepared from 'Muscat of Alexandria' grapevines in mid-December (316CCH). The five *Allium* species: garlic (*Allium sativum* L.), Chinese chive (*A. tuberosum* Rottler), rakkyo (a kind of scallion; *A. chinense* G. Don), onion (*A. cepa* L.), and Welsh onion (*A. fistulosum* L.) were used. Scales of garlic, leaf sheaths of rakkyo, onion, and Welsh onion ('Senju-aigara'), etiolated and non-etiolated leaves of Chinese chive, and leaves of Welsh onion ('Senju-aigara' and 'Kujo-buto') were grated into a paste. The upper cut surfaces of grape cuttings were dabbed with pastes of the following: 1) garlic; 2) etiolated Chinese chive; 3) non-etiolated Chinese chive; 4) rakkyo; 5) onion; 6) 'Senju-aigara' leaf sheaths; 7) 'Senju-aigara' leaves; or 8) 'Kujo-buto' leaves, and 9) distilled water for the control. Comparable sets of cuttings were exposed to volatile compounds by placing them in a 3-liter desiccator containing 30 g of a grated plant material or 30 ml of distilled water (control) for 24 hr.

**Effects of three grated *Allium* plants, their volatiles, or sulfide compounds (Exp. 2).**

Twelve sets of replicated 10 cuttings were prepared from 'Kyoho' grapevines in mid-October (OCCH). The upper cut surfaces of six sets of cuttings were painted with freshly grated plant materials or sulfide compounds of the following: 1) garlic; 2) etiolated Chinese chive; 3) rakkyo; 4) 30% diallyl disulfide (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan); or 5) 30% diallyl trisulfide (Riken Chemical Industry Co., Ltd., Kyoto, Japan); and 6) distilled water (control). Both sulfides are active substances in garlic and have a potential to break bud dormancy in grapevines (Kubota et al., 2000). The other six sets were placed in a 3-liter desiccator containing 30 g of a grated *Allium* plant material, 5 ml of a sulfide compound, or 30 ml of distilled water for 24 hr.

**Effects of boiling grated garlic, Chinese chive, and rakkyo on budbreak (Exp. 3).**

Scales of garlic, leaves of etiolated Chinese chive, and leaf sheaths of rakkyo were grated into a paste. Half of each portion of grated material was boiled for one hr. Cuttings from 'Muscat of Alexandria' prepared in late October (OCCH) were placed in a 3-liter desiccator containing 30 g of boiled or unboiled grated material for 24 hr. Control cuttings were placed for 24 hr in a desiccator containing 30 ml of distilled water.

**Effects of duration of exposure to volatile compounds from grated garlic, Chinese chive, rakkyo, and garlic oil (Exp. 4).**

Cuttings from 'Kyoho' made in mid-December (284CCH) were placed in a 3-liter desiccator containing 30 g of grated garlic, etiolated Chinese chive, rakkyo, or 5 ml of commercial garlic oil (Riken Chemical Industry Co., Ltd., Kyoto, Japan), for 12 or 24 hr. Volatiles from garlic oil were effective in breaking of grapevine buds (Kubota et al., 1999a). Control cuttings were placed for respective hours in a desiccator containing 30 ml of distilled water.

**Effects of temperatures on cutting response to volatile compounds of grated garlic and Chinese chive (Exp. 5).**

Twelve sets of 30 cuttings from 'Kyoho' taken in late December (354CCH), were placed in a 3-liter desiccator containing 30 g of grated garlic scales or non-etiolated Chinese chive leaves, or 30 ml of distilled water (control), and kept in a growth chamber at 5, 15, or 25 °C for 24 hr.

**Results**

**Exp. 1.**

Painting 'Muscat of Alexandria' cuttings with grated *Allium* materials had little effect on the budbreak except non-etiolated Chinese chive, rakkyo, and onionm, which gave a uniform budbreak (Fig. 1, top). Cuttings exposed to volatile compounds from etiolated or non-etiolated Chinese chives, rakkyo, and garlic significantly promoted early budbreak (Fig. 1, bottom), compared with the control. Budbreak on cuttings exposed to volatile compounds of etiolated Chinese chive or rakkyo was more uniform than those exposed to extracts of onion and garlic, although the number of days required for 50% budbreak was shorter for all *Allium* materials tested than the control (Table 1). The volatile compounds from Welsh onion had no effect on budbreak.

**Exp. 2.**

All plant materials and sulfide compounds accelerated the budbreak of 'Kyoho' cuttings, whether the cuttings were painted or exposed to volatile compounds (Fig. 2). Budbreak in cuttings exposed to volatiles from diallyl
Fig. 1. Budbreak of single-bud cuttings of 'Muscat of Alexandria' grapevines after painting (top) with grated *Allium* plant species and exposure (bottom) to volatile components from the plants for 24 hr. Left: control (×), garlic scales (○), etiolated Chinese chive leaves (△), non-etiolated Chinese chive leaves (▲), rakkyo leaf sheaths (□). Right: control (×), onion leaf sheaths (○), Welsh onion ('Senju-agara') leaf sheaths (△), Welsh onion ('Senju-agara') leaves (▲), Welsh onion ('Kujo-buto') leaves (■). Vertical bars are the SE (three replications with 10 cuttings per treatment).

Fig. 2. Budbreak of single-bud cuttings of 'Kyoho' grapevines after painting (top) with grated garlic scales (○), etiolated Chinese chive leaves (△), rakkyo leaf sheaths (□), 30% diallyl di- (●) or trisulfide (▲), and distilled water for the control (×), and exposure (bottom) to their volatile components for 24 hr.

| Table 1. The average number of days to 50% budbreak per treatment in Exp. 1, Exp. 2, Exp. 3, and Exp. 4. |
|--------------------------------------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| Exp. 1                                           | Days             | Exp. 2                                           | Days             | Exp. 3                                           | Days             | Exp. 4                                           | Days             |
| Treatment                                        | Painting         | Exposure                                      | Treatment        | Painting         | Exposure                                      | Treatment        | Painting         | Exposure     |
| Control                                          | 35.6a 7          | 53.3a                                        | Control          | >61.0            | 61.0                                         | Control          | 25.6a 7          | 25.0a        |
| Garlic                                           | 35.6a 7          | 41.6b                                        | Garlic           | 41.5             | 46.0                                         | Garlic           | 21.3b 7          | 17.0c        |
| Etiolated Chinese chive                          | 35.0a            | 25.3c                                        | Etiolated Chinese chive | 46.0            | 28.5                                         | Etiolated Chinese chive | 18.6c          | 16.3c        |
| Rakkyo                                           | 33.3a            | 33.0bc                                       | Rakkyo           | 41.5             | 46.5                                         | Rakkyo           | 21.0b 7          | 19.6b        |
| Onion                                            | 33.6a            | 39.3b                                        | Diallyl disulfide | 24.5             | 24.0                                         | Diallyl disulfide | 18.3c 7          | 18.3b        |

7 Exp. 1, Exp. 2, Exp. 3, and Exp. 4 correspond to Fig. 1, Fig. 2, Fig. 3, and Fig. 4, respectively.

7 Mean separation within each column by Duncan's multiple range test (P>0.05). In Exp. 2, Duncan's multiple range test was not applied, because of two replications for each treatment.

NII-Electronic Library Service
trisulfide, diallyl disulfide, etiolated Chinese chive, or rakkyo occurred at 16, 18, 24, and 27 days, respectively, whereas the first budbreak occurred 30 days after treatment in the control. Cuttings painted with diallyl trisulfide, diallyl disulfide, etiolated Chinese chive, or rakkyo started budbreak 16, 17, 26, and 28 days after treatment, respectively. Etiolated Chinese chive was as effective as both sulfides in promoting budbreak, regardless of the method of treatment. Budbreak was more uniform in cuttings exposed to volatiles than those painted with extracts (Table 1).

Exp. 3.

Volatile compounds from boiled grated garlic, rakkyo, and particularly the etiolated Chinese chive were less effective on promoting uniform budbreak in 'Muscot of Alexandria' cuttings than the non-boiled extracts (Fig. 3 and Table 1).

Exp. 4.

Budbreak of 'Kyoho' was promoted by exposure to volatiles from grated garlic, etiolated Chinese chive, rakkyo, and garlic oil, whether the exposure was for 12 or 24 hr (Fig. 4), the etiolated Chinese chive and garlic oil induced a more uniform budbreak than did grated garlic and rakkyo. In cuttings exposed to volatiles from garlic, etiolated Chinese chive, and rakkyo, the number of days required for 50% budbreak was shorter at 24 hr than at a 12 hr exposure (Table 1).

Exp. 5.

Budbreak in 'Kyoho' cuttings exposed to volatile compounds of grated garlic, was accelerated more at 25 °C than at 15 °C; it was unaffected at 5 °C (Fig. 5, top), compared to the control. Budbreak in cuttings exposed to volatile compounds of non- etiolated Chinese chive at 5 and 15 °C was delayed compared to the controls (Fig. 5, bottom). No difference in the onset of budbreak was found between cuttings treated with extracts of Chinese chive and control at 25 °C .

Discussion

When freshly grated paste of five Allium species was painted to the upper cut surface of dormant 'Muscot of Alexandria' cuttings, budbreak was not accelerated (Fig. 1). Exposure to volatile compounds from Chinese chive, rakkyo and garlic, however, promoted budbreak, especially the Chinese chive. Also volatile extracts of these three species shortened the number of days to 50% budbreak on cuttings compared to the control (Table 1). Etiolated Chinese chive was more effective than non- etiolated Chinese chive, so light may affect the synthesis or activation of the active substance(s) supposedly involved in breaking bud dormancy. Freshly grated Chinese chive and rakkyo and their volatile compounds were almost as effective as garlic in promoting budbreak in grapevines (Kubota et al., 1987; Kubota and Miyamuki, 1992; Kubota et al., 1999a). The active substances in garlic that have the potential to break bud dormancy in grapevines are volatile sulfide compounds
with two allyl groups: diallyl disulfide and possibly diallyl trisulfide (Kubota et al., 1999b). Volatile components from *Allium* plants include diallyl mono- and disulfides, dimethyl mono- and disulfides, and mercaptans, among others (Freeman and Whenham, 1975; Saghir et al., 1964; Yu et al., 1989). Hosoki et al. (1986) reported that sulfide compounds in garlic and Japanese horseradish (*Wasabia japonica* L.) break bud dormancy in some corms and ornamental trees.

The responses of buds in cuttings, painted with granted etiolated Chinese chive and rakkyo, were different in 'Muscad of Alexandria' and 'Kyoho' cultivars. Painting with extracts of etiolated Chinese chive and rakkyo did not accelerate budbreak in 'Muscad of Alexandria' (Fig. 1), whereas both treatments promoted budbreak in 'Kyoho' cuttings, although the effects were less than those of diallyl di- and trisulfide (Fig. 2). Cumulative chilling hours to which 'Muscad of Alexandria' and 'Kyoho' grapevines were exposed to 7.2°C or lower were 316 and 0, respectively. The reasons for different responses by the two grape cultivars are unknown. We previously reported that the responses of grapevine buds to garlic paste significantly varied among the cultivars (Kubota and Miyamuki, 1992).

Exposure of dormant 'Kyoho' cuttings to volatile compounds of garlic, etiolated Chinese chive, rakkyo, and garlic oil promoted budbreak regardless of the duration of exposure (Fig. 4). In 'Kyoho' cuttings, exposed to volatiles from garlic, budbreak was accelerated more at 25°C than at 15 and 5°C, but the temperature had little effect on budbreak in cuttings exposed to volatile compounds of non- etiolated Chinese chive (Fig. 5). We do not know why budbreak is unaffected by volatile compounds from non- etiolated Chinese chive at different temperatures.

We tried but failed to extract essential oil from the leaves of etiolated and non- etiolated Chinese chive and the leaf sheaths of rakkyo by steam - distillation (unpublished), although the same method produced essential oil from garlic scales (Kubota et al., 1999b). Budbreak in 'Muscad of Alexandria' cuttings, exposed to volatile compounds from boiled etiolated Chinese chive, and rakkyo, was delayed, compared to exposure to unboiled preparations (Fig. 3), probably because the boiling inactivated the substances. Saghir et al. (1964) reported that more than 90% of volatile components in Chinese chive and rakkyo are substances with methyl groups, but in garlic the most abundant volatile components are substances with allyl groups. This difference may account for the variations in responses among Chinese chive, rakkyo and garlic.

Chinese chive and rakkyo may be useful to promote budbreak of dormant grapevines, as well as garlic which has been used for forcing culture of 'Muscad of Alexandria' (Kubota et al., 1987; Kubota and Miyamuki, 1992), because their volatile compound(s) are active although their mode of action is still unknown.

Based on the above observations, we conclude that painting freshly grated paste from five *Allium* plant species on the upper cut surface of dormant grape cuttings had little effect on the onset of budbreak. However, volatile compounds from Chinese chive and rakkyo as well as garlic, hastened the onset of budbreak, especially for etiolated Chinese chive. Hence, they may be useful as replacement for calcium and hydrogen cyanamides and garlic paste in promoting budbreak of grapevines.

**Acknowledgment**

We thank Mr. S. Nishimura and Dr. Y. Takeyama, both of Riken Chemical Industry Co., Ltd., Kyoto, Japan, for providing garlic oil and a sulfide compound and for valuable suggestions.

**Literature Cited**


ブドウ挙し穂の休眠打破に及ぼす数種アリウム属植物のペーストおよび揮発性物質の効果

久保田尚浩・山根康史・鳥生幸司

岡山大学農学部 700-8530 岡山市津島中1-1-1

摘要

ブドウ‘マスカット・オブ・アレキサンドリア’および‘巨峰’の1芽に調整した挙し穂を用いて、休眠打破に及ぼす数種アリウム属植物のペーストおよび揮発性物質の影響を調査した。5種のアリウム属植物（ニンニク、ニラ、ラッキョウ、タマネギ、ネギ）のペーストを挙し穂の切り口に塗布したところ、発芽は促進されなかった。しかし、揮発性物質の気温処理では発芽が促進され、その程度は黄ニラで最も大きく、次いでニラ、ラッキョウ、ニンニクおよびタマネギの順であつた。これら植物の揮発性物質の休眠打破効果は、ニンニク中の休眠打破有効成分である2硫化ジアリルや3硫化ジアリルよりも小さかった。ニンニク、黄ニラおよびラッキョウを煮沸し、それらの揮発性物質で気温処理したところ、発芽は促進されなかった。挙し穂をニンニク、黄ニラおよびラッキョウの揮発性物質で12時間あるいは24時間気温処理したところ、市販のガーリックオイルの揮発性物質と同程度に発芽が促進された。挙し穂をニンニクおよび黄ニラの揮発性物質で、温度を変えて24時間気温処理したところ、ニンニクでは高温条件で発芽が促進されたが、黄ニラでは温度の影響は認められなかった。以上のことから、ニラとラッキョウはブドウの芽の休眠打破に有効であること、休眠打破に有効な物質はニンニクと同じ揮発性であることが明らかとなった。