# Timing of hydrogen cyanamide application to grapevine buds

by

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S u m m a r y : One major factor limiting the application of hydrogen cyanamide  $(H_2CN_2)$  is the difficulty in deciding when to apply, since mistiming may lead to bud and crop damage. Since an effective method for monitoring the developmental stage of dormant buds is not yet available, minimizing such a risk involves the regional evaluation of application timing. For three successive years, several  $H_2CN_2$  application dates were evaluated in cv. Perlette vineyards in the Jordan valley in Israel. The level and uniformity of bud break did not differ significantly among the application dates tested. However, major effects of the application date on cluster number, cluster size and yield were found. The sensitivity of the reproductive meristem to  $H_2CN_2$  is discussed.

K e y w o r d s : grapevine, bud dormancy, hydrogen cyanamide, fruit set.

# Introduction

Prolonged dormancy is considered to be the major obstacle to economic production of temperate fruits in warm winter regions. In these regions, the need for artificial means to compensate for lack of natural chilling becomes a dominant factor for maintaining economic production (SAMISH 1954; SHULMAN *et al.* 1983; SAURE 1985; GEORGE *et al.* 1986; EREZ 1987, 1995).

Hydrogen cyanamide ( $H_2CN_2$ ) has been found to be the most useful dormancy breaking agent for grape (SHULMAN *et al.* 1983) and many other deciduous fruit crops (EREZ 1987, 1995; HENZEL *et al.* 1991).

In most fruit trees, the strongest effect of  $H_2CN_2$  application was observed some weeks before natural bud break and after chilling (EREZ 1987, 1995; PONTIKIS 1989). Too early application will have no or a negative effect on the uniformity of bud opening. Late application may be harmful, as the resistance to the chemical declines rapidly upon release from endodormancy (SHULMAN *et al.* 1983; FUCHIGAMI and NEE 1987; GEORGE and NISSEN 1988; SNIR 1988; KLINAC *et al.* 1991; SILLER-CEPADA *et al.* 1992; EREZ 1995).

 $H_2CN_2$  is extremely effective for breaking dormancy of vegetative buds. Floral buds, however, generally require less exposure to chilling and are, therefore, less resistant to the toxic effects of  $H_2CN_2$  at late application. It appeared that the more protected buds, e.g. compound buds of grape and kiwi, are not damaged, while less protected, simple flower buds are more sensitive (SHULMAN *et al.* 1983; SNIR 1983; GEORGE and NISSEN 1990, 1993; EREZ 1995).

In the more resistant species,  $H_2CN_2$  application at optimal timing may have a major impact on yield and crop economy. In grape, bunch production of  $H_2CN_2$ -treated vines increased with each year of application. This increase has been related to a higher and more uniform level of bud break, that led to increased spur and shoot production and to a wider choice for bud selection (GEORGE *et al.* 1988; ZELLEKE and KLIEWER 1989; GEORGE and NISSEN 1990; PIRES *et al.* 1995).

Under warm subtropical conditions, early application of  $H_2CN_2$  advanced fruit maturity but decreased yield. The yield decrease was related to a reduced number of shoots, caused, in turn, by a reduction in the budburst percentage, as well as non-uniform and delayed bud break (LAVEE *et al.* 1984; GEORGE *et al.* 1988; MARASCHIN *et al.* 1992).

In the absence of a reliable means to predict the time of natural bud break,  $H_2CN_2$  is applied at a fixed date each year, and this practice entails the risk of serious bud damage if the application subsequently proves to have been mistimed. Toward reducing this risk, we have evaluated several  $H_2CN_2$  application dates in cv. Perlette vineyards in the Jordan valley, during three successive years.

## **Materials and Methods**

The experiments were conducted from 1995 to 1998 in commercial vineyards in the central Jordan Valley of Israel, located 300 m below sea level. Mature, cordon- trained grapevines (*Vitis vinifera* cv. Perlette, grafted on 140 Ru) were used for this study. All plants were subjected to the cultural practices commonly used in this region.

Changes in the depth of dormancy of Perlette vines during fall and winter were determined by monitoring changes in bud break percentage and timing of single-node cuttings. Canes were collected at 2-week intervals from September to January. Ten groups of 10 single-node cuttings were prepared on each collecting date and then forced for

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40 d at 23 °C and 12  $h \cdot d^{-1}$  light. The bud break percentages after 14, 21 and 40 d were used to express the dormancy depth of the vines.

Dormancy breaking treatments were applied for three years on three application dates: mid-December, beginning of January and mid-January. These dates were chosen to represent the time range used for application of  $H_2CN_2$  in the commercial vineyards in the Jordan valley. The experiments were designed as randomized blocks with three treatments, each treatment in three replications. Each replication consisted of 6 grapevines. After pruning to 3-node spurs, 4 vines in each group were sprayed with  $H_2CN_2$  and two vines with water control. Dormex (SKW, Trostberg, Germany), a commercial formulation containing 49 %  $H_2CN_2$ , was applied at a concentration of 5 % (v/v) after addition of 0.02 % Triton-X 100 wetting agent. The vines were sprayed at full volume till runoff with a hand-operated Knapsack sprayer.

At each application date in 1997, three additional vines were pruned as described above. These vines, from three pruning dates, were sprayed with  $H_2CN_2$  only in mid-January.

The total number of the buds left on each vine after pruning was counted. The number of bursting buds was monitored weekly for the first 6 weeks after  $H_2CN_2$  application, and the bud break percentages were calculated. Bud break was defined as the stage when green tissue was visible beneath the bud scales.

The number of clusters per vine was counted prior to berry thinning. The fruit was harvested at approx. 15 °Brix. The yield of each vine as well as cluster weight and berry size were determined. Ten clusters from each vine were taken for cluster weight and 10 berries from each of the 10 clusters for berry weight determination.

# Results

Bud break response of single-bud cuttings under forcing conditions is the common indicator used to describe dormancy depth of grapevines (SHULMAN et al. 1983). Our findings (Fig. 1) indicate that in mid-September the buds were still active and showed about 80 % bud break after 21 d of forcing. Thereafter, dormancy developed to a maximum level between early November and early December. In mid-December buds exhibited overcoming of dormancy, as indicated by increased bud break. The dormancy curve based on data taken after 14 d of forcing accentuates the transition between dormant and active growth stages. After 40 d of forcing most of the buds had overcome dormancy, and the differences between active and dormant populations become minor. However, the pattern of the dormancy curve was retained and the deep dormancy stage of buds collected during November is stressed.

The dormancy curve had similar patterns over the three years of experiments. Minor differences were observed in the timing of transitions between stages and in the duration of the deep dormancy stage. From Fig. 1 it can be concluded that in mid-December the bud population is close to the

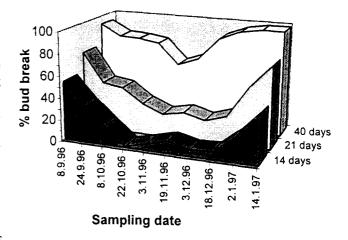


Fig. 1: Dormancy depth of buds during fall and winter of 1996-97. Dormancy depth is expressed as bud break percentage. Values are averages of 10 repetitions per harvest date. Bud break (%) was calculated after 14, 21 and 40 d.

transition point between the endodormancy and ecodormancy stages.

Bud burst started about 4 weeks after  $H_2CN_2$  application and continued for about two weeks, independently of application date (Fig. 2). Buds that had not burst during these two weeks did not burst at all. Vines that were pruned and sprayed in mid-December showed 50 % bud burst after 4 weeks and an additional 20 % within the following 2 weeks. The bud break level of vines that were treated at the beginning of January and in mid-January did not differ significantly from that of mid-December-treated vines. However, during the three years, a non-significant tendency was noticed for up to 10 % increase in bud break when the  $H_2CN_2$ was applied at the late application date, compared with the early one. Bud break rates were similar for vines from all application dates.

As opposed to the small and non-significant influence of  $H_2CN_2$  application date on bud break level and uniformity, we found a major and significant effect of the application

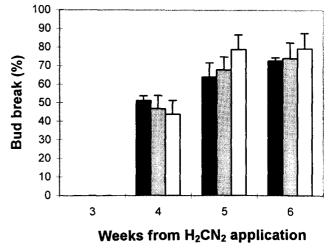


Fig. 2: Influence of the  $H_2CN_2$  application date on the level and uniformity of bud break. Bud break (%) of vines treated with  $H_2CN_2$  at three different dates: 18 December 1996 (black columns); 2 January 1997 (grey); 14 January 1997 (white). Mean values of three repetitions on each application date. Bars represent standard deviation.

date on the number of clusters, cluster size and, therefore, the yield per vine.

Vines pruned and sprayed with  $H_2CN_2$  in mid-December produced significantly less clusters than vines pruned and treated in mid-January (Fig. 3).

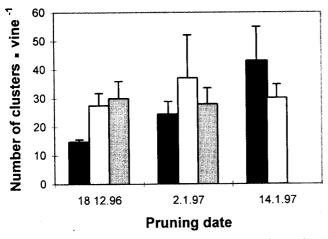


Fig. 3: The influence of the H<sub>2</sub>CN<sub>2</sub> application date on the number of clusters per vine. Black columns: immediate application; grey: postponed application; white: control. For details: Fig. 2.

Pruning date had no effect on the number of clusters per vine as can be seen from the control vines which were sprayed with water at each application date. At all application dates they carried 30-35 clusters per vine, similar to the number of clusters carried by vines treated with  $H_2CN_2$  at the late application date. This was more than twice the number of clusters carried on vines that were treated early with  $H_2CN_2$  (Fig. 3).

Late application of  $H_2CN_2$  to early pruned vines prevented the decrease in cluster number observed for vines that were pruned at the early application date and sprayed immediately thereafter (Fig. 3).

Apart from the influence of early application of  $H_2CN_2$ on reducing the number of clusters, it also affected cluster weight (Fig. 4). The clusters harvested from vines which were exposed early to  $H_2CN_2$ , were significantly smaller than those from the late treated ones. Clusters from vines that were treated in mid-December weighed about 125 g, while those from vines treated in mid-January had an average weight of 275 g.

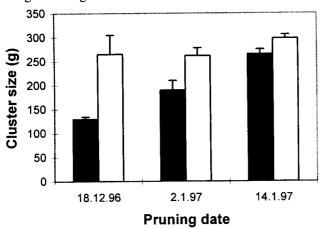
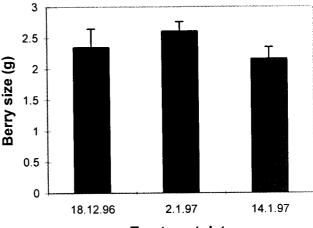


Fig. 4: Influence of the  $H_2CN_2$  application date on cluster size. Black columns: treated vines; white: control. For details: Fig. 2.

Cluster weight of the control vines at harvest was not affected by the different pruning dates, and was similar to that of  $H_2CN_2$ -treated vines from the late application date. Thus, clusters from the control vines pruned and treated in mid-December weighed twice as much as clusters from vines treated at the same date.

Berry weight did not differ significantly among vines treated at different application dates, although a tendency for weight decrease was noticed after the late  $H_2CN_2$  application (Fig. 5).

The average number of berries per cluster, which was calculated from the cluster weight and berry weight data, (Fig. 6) was about 110 in the control treatments, with no significant difference related to the application date.



**Treatment date** 

Fig. 5: Influence of the  $H_2CN_2$  application date on berry weight. For details: Fig. 2.

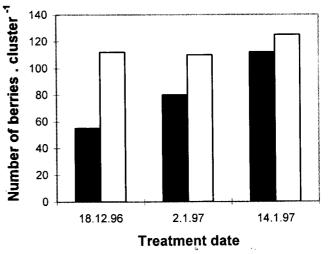


Fig. 6: Influence of the  $H_2CN_2$  application date on berry number per cluster. Black columns: treated vines; white: control. For details: Fig. 2.

In the  $H_2CN_2$  treatments the average number of berries per cluster decreased when  $H_2CN_2$  was applied early. The number of berries in the mid-January treatment was about 115, similar to the number of berries in the controls, and within the range considered optimal for high-quality Perlette clusters. On vines from the early  $H_2CN_2$  treatment, the number of berries was about 50, less than half the number on the late-treated or control vines. The average yields per vine for two consecutive seasons show a significant decrease in yield following early application of  $H_2CN_2$  (Fig. 7). The average yield of vines treated in mid-December was 2.5 kg in the first season and 6.1 kg in the second season while the average yield for vines treated in mid-January was about 13 kg in both seasons. The average yield of a control vine, from all pruning dates, was 10-12 kg, similar to that of the vines treated with  $H_2CN_2$ in mid-January. Vines treated at the early application date produced only 15-50 % of the yield of the control vines.

Vines treated in mid-December resumed growth 28 d earlier than vines treated in mid-January, yet these vines were harvested only 11 d prior to those treated one month later (Table). The number of days from  $H_2CN_2$  application to harvest indicated that vines which were treated earlier needed a longer growth period to reach maturity.

#### Discussion

It is generally accepted that the effect of chemicals used to break dormancy is both dose and timing dependent. According to this concept, the higher the dosage and the later the treatment, the stronger is the effect obtained (SHULMAN *et al.* 1983; PONTIKIS 1989; KLINAC *et al.* 1991; SILLER-CEPADA *et al.* 1992; EREZ 1995).

Bud break data that have been collected in the Jordan valley for the last three years do not fit into the above concept, as there was no significant difference in bud break level or uniformity between vines that were treated with  $H_2CN_2$  a month apart. Dormancy-breaking treatments were applied from mid-December to mid-January. According to the dormancy curve it appears that in mid-December the buds were close to the transition point from endodormancy to ecodormancy. Single-bud cuttings sampled at that time showed about 40 % bud break under forcing conditions, while those sampled in mid-January reached about 75 % bud break under the same conditions. However, application of H<sub>2</sub>CN<sub>2</sub> on either date led to a similar bud break level, indicating that the dormant bud population, treated in mid-December, had the ability to express full bud break potential in response to H<sub>2</sub>CN<sub>2</sub>. This may indicate that at that time the bud population is fairly uniform in its dormancy status, and that dormancy depth of most of the buds is shallow enough to allow the strong signal, transduced by  $H_2CN_2$ , to overcome bud break barriers.

In contrast to the small and non-significant influence of the application date on bud break level and uniformity, strong and significant effects were detected on the number and size of clusters and, therefore, on the yield per vine.

The influence of  $H_2CN_2$  on grapevine yield has usually been attributed to its effect on the level of bud break: a high level of bud break would lead to an increased shoot number and, hence, to high yield and low yields were usually related to low bud break rates. Low bud break has been related either to phytotoxic effects, caused by too late or too concentrated  $H_2CN_2$  applications, or to too early application that failed to overcome deep endodormancy (SHULMAN *et al.* 1983; GEORGE *et al.* 1988; GEORGE and NISSEN 1990; MARASCHIN *et al.* 1992; PIRES *et al.* 1995). Within the compound bud the reproductive meristem was considered to be well protected and was not suspected to be more sensitive than the vegetative meristem at any stage (EREZ 1987, 1995).

The yield loss associated with early  $H_2CN_2$  application cannot be explained by lower bud break, since the bud break levels were similar for early and late applications. Thus, the results indicate a specific negative influence of the early application of  $H_2CN_2$  on the reproductive part of the bud.

The full reproductive potential of the bud population that was pruned in mid-December was demonstrated by the number and size of clusters, and by the yield of control vines which were pruned at the same time. These results

### Table

Effect of H<sub>2</sub>CN<sub>2</sub> application date on the time span between application and harvest

H <sub>2</sub> CN <sub>2</sub> application date	Harvest date	Days from H <sub>2</sub> CN <sub>2</sub> application to harvest
18/12/1996	03/06/1997	168
02/01/1997	08/06/1997	156
14/01/1997	14/06/1997	152

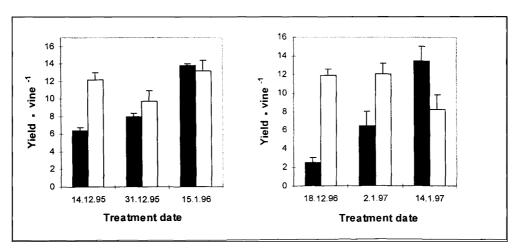


Fig. 7: Influence of the H<sub>2</sub>CN<sub>2</sub> application date on the yield per vine in two seasons. Black columns: treated vines; white: control. For details: Fig. 2.

indicate that early pruning *per se* had no negative influence on the reproductive meristem; therefore we conclude that it was the early application of  $H_2CN_2$  that led to an actual loss of clusters that would otherwise have developed well.

There are several possible ways to explain this effect on the reproductive organs:

1.  $H_2CN_2$  might act indirectly, by inducing early bud break, so that the reproductive meristem, that might be only partially developed at that time, would be forced to emerge. The result would be abortion of floral primordia or an outburst of clusters that were not fully developed. We have no data as yet to support or rebut this hypothesis, but anatomical comparisons of buds are currently planned to clarify this issue. Indirect support to this concept can stem from the finding that late application of  $H_2CN_2$  to early pruned vines led to bud break a month later, which was not accompanied by damage of the reproductive meristems, as indicated from the number and size of clusters.

2. By inducing early bud break,  $H_2CN_2$  may expose the floral organ to temperatures that are unfavorable to its subsequent development, thus leading to cluster abscission or poor development.

Low yields associated with early pruning followed by immediate  $H_2CN_2$  application have been related to low temperatures during flowering for several cultivars, such as Muscat of Hamburg, Sultana and Cardinal, grown in a rather cool climate (McColl 1986; GEORGE *et al.* 1988). Low temperatures during flowering were claimed to affect pollination and seed development adversely. However, cv. Muscat grown in a warmer region was not affected by such phenomena (GEORGE *et al.* 1988). Cool conditions have also been claimed to enhance the phytotoxic effect of  $H_2CN_2$  (EREZ 1995). However, temperature data for the Jordan valley during the years of the present experiment do not support this hypothesis. During the winter season of 1995/96, for example, the maximum day temperature ranged from19 to 21°C and the minimum night temperature was 9-10°C.

3. At the early application date there might have been a specific phytotoxic effect of H2CN2 on the reproductive meristem. For most deciduous crops, floral buds have lower chilling requirements than vegetative buds, therefore, they are more sensitive to the phytotoxic effects of H<sub>2</sub>CN<sub>2</sub> than vegetative buds at the same time (SNIR 1983, 1988; GEORGE and NISSEN 1988, 1993; KLINAC et al. 1991; SILLER-CEPADA et al. 1992; EREZ 1995). For this reason stone fruits, with simple floral buds, are highly sensitive, and even marginal toxicity affects their yields (GEORGE and NISSEN 1988, 1993; SILLER-CEPADA et al. 1992, EREZ 1995). Treating pome fruit, having compound buds, with H2CN2 elicited strong enhancement of vegetative appearance, accompanied by low yield (EREZ 1995). This differential effect of H<sub>2</sub>CN<sub>2</sub> on vegetative and reproductive meristems within the same mixed bud supports the hypothesis that H2CN2 might specifically damage the reproductive meristem within the compound grape bud; this is in contrast to the assumption that the grape reproductive meristem is well protected within the compound bud (EREZ 1995).

In most fruit crops it has been shown that the later the application, the greater is the damage to the floral buds. Our present results indicate a different situation, in which the reproductive meristem is more sensitive to the phytotoxic effect early in the season than late in the season. It might be that the vegetative part of the bud is not fully developed early in the season, and therefore the sensitive reproductive meristem is not protected by the vegetative meristem, whereas later in the season, the vegetative part has grown and physically covers the reproductive part, thus protecting it. Currently there are no data to support this hypothesis.

4. The compound bud of grape comprises three partially developed shoots; the middle one is the primary shoot and only if it is damaged, the secondary bud develops. Fruit buds are usually in the primary position in the compound bud. If early application of  $H_2CN_2$  induced secondary buds to break instead of primary buds, for some unknown reason, it would explain the loss of yield, since these buds form small clusters or no clusters at all (WINKLER *et al.* 1974).

At present, we can not rule out any of the above options, apart from the thermal effect on flowering, and each of the remaining three might be involved in the yield reduction effect of early  $H_2CN_2$  application.

Natural bud break occurred in the second half of February. Thus, the early application was given 9-10 weeks before natural bud break and the late application 5-6 weeks before natural bud break. According to the harvest dates, application of  $H_2CN_2$  5-6 weeks before natural bud break had no advancing effect, whereas its application 9-10 weeks before natural bud break advanced maturation by 12 d. Similar findings have been reported for Muscat of Hamburg (GEORGE *et al.* 1988). High prices received for early-maturing fruit may sometimes compensate for the loss of yield associated with early application of  $H_2CN_2$ , depending on the extent of yield loss and the prices. Since these two variables are liable to fluctuate unexpectedly, early application should be considered as carrying a high economic risk.

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