Daskalakis I *et al.* (2025)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 53, Issue 2, Article number 14555
DOI:10.15835/nbha53214555

Effect of four formulations on the dormancy release of dormant buds and the subsequent development of shoots under typical vineyard conditions

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Abstract

One of the key challenges of sustainable viticulture under climate change is the insufficient chilling necessary for dormancy release, leading to uneven budbreak and reduced productivity. This study examined the effect of four formulations: Dormex® (hydrogen cyanamide), Theocopper, Erger (and Active Erger), and garlic extract on budbreak in two early-maturity, seedless table grape varieties: 'Prime Seedless' and 'Ralli Seedless'. The research aimed to promote earlier budbreak, control apical dominance, and advance grape ripening. In addition to evaluating the chemical composition and concentration of each treatment, the study also examined the importance of application timing. Field measurements were taken from the grapevine shoots during four stages (one per application), across three consecutive years. The data revealed that both the year and timing of application significantly influenced budbreak and the intensity of acrotony. Different formulations had varying effects on breaking dormancy and modifying growth patterns. Regulating bud dormancy is viticulturally important, as it promotes uniform budbreak, consistent flowering, and earlier, more reliable yields. Effective dormancy management improves grape quality, reduces losses due to irregular budbreak, and mitigates apical dominance, contributing to balanced vegetative growth. This is especially crucial in regions with mild winters, where traditional chilling requirements may not be met. The study supports the strategic use of chemical agents to adapt grapevine cultivation to changing climatic conditions.

Keywords: budbreak; dormancy; dormant buds; grapevine; physiology

Introduction

The annual vegetative cycle of grapevine plants in the temperate zone includes the dormancy period of dormant buds, a physiological state that reduces the bud's ability to break in a reversible manner. It is mainly referred to as "dormancy". Dormancy is distinguished into three, main stages: pre-dormancy, main dormancy, and post-dormancy (Davidis, 1982; Lang *et al.*, 1987; Lavee and May, 1997; Stavrakakis, 2019). During the main dormancy stage, which coincides with winter in the northern hemisphere, the temporary inhibition of dormant bud sprouting during that dormancy stage is owed to factors found within the dormant bud. It is

Received: 30 Apr 2025. Received in revised form: 24 Jun 2025. Accepted: 24 Jun 2025. Published online: 26 Jun 2025.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

worth noting that the dormant buds' sprouting ability is restored after exposure to low temperatures. Thus, for dormant buds to regain their vegetative capacity, they must be exposed to low temperatures for a certain period of time that is determined based on genetic factors. Depending on the variety, the temperatures in question differ (Balandier *et al.*, 1993; Egea *et al.*, 2003). Due to climate change, the environmental conditions in winter no longer suffice in meeting that requirement and result in prolonged dormancy, which further triggers problems in flowering and subsequent grape production (Erez, 1995). To resolve that problem, various chemicals have been and are being applied to break the dormancy of dormant buds. To date, hydrogen cyanamide (HC) is the substance yielding the best results in breaking the dormancy of grapevine dormant buds (Dokoozlian *et al.*, 1995).

Although growth is inhibited during dormancy, the buds are physiologically and biochemically active, with numerous physiological and biochemical changes occurring (Saure, 1985), such as changes in the respiration rate, phytohormone concentration, carbohydrate metabolism, water content, and other compounds believed to be involved in breaking the dormancy of dormant buds (Arora *et al.*, 2003; Trejo-Martínez *et al.*, 2009). Therefore, although several explanations have been proposed as effective in breaking the dormancy of dormant buds, none can be said to have been satisfactory. Some suggest that dormancy is related to changes in oxidative processes and stress responses within the cell (Pacey-Miller *et al.*, 2003). The modes of action of chemicals that break dormancy, such as hydrogen cyanamide (HC), have been associated with stress that leads to dormancy breaking (Fuchigami and Nee, 1987). Or *et al.* (2002), and Pérez and Lira (2005) have demonstrated that dormancy breaking is closely related to oxidative processes within a grapevine's dormant buds. HC inhibits the expression and activity of catalase, leading to a transient increase in hydrogen peroxide (H_2O_2) concentration (Pérez and Lira, 2005; Halaly *et al.*, 2008). In view of the above, it appears that HC application triggers oxidative stress that may be part of the mechanism leading to dormancy breaking. The present study examines the hypothesis that the oxidative stress caused by applications of HC, Theocopper, Erger, and garlic extract, applied at different concentrations and times over three years, promotes the breaking of endodormancy.

Materials and Methods

Plant material and experimental vineyard

The grapevine varieties (*Vitis vinifera* L.) studied are the white 'Prime Seedless' and the red 'Ralli Seedless'. These are two super-early maturity table grape varieties, with particular production and of commercial interest. The experimental vineyard (Figure 1) is located in Corinth, northeastern Peloponnese (latitude: 37°.900204; longitude: 22°.817040; an altitude: 10 meters).

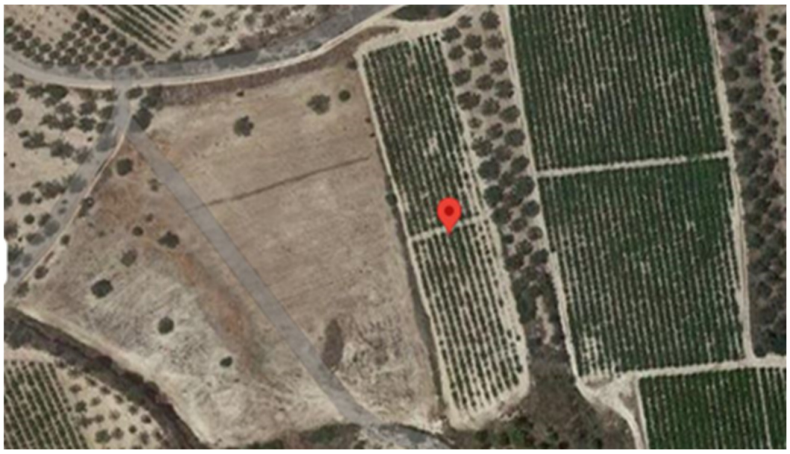

Figure 1. Location of the experimental vineyard

The selected vines of the two varieties are grafted into rootstock 1103 Paulsen with a planting distance of 2.2 meters (between rows) x 1.2 meters (between vines in the row) (Figure 2), they are bilaterally cordon-trained (bilateral Casenave) and mixed fruit pruning applied (spur-pruned to 2-nodes per arm and cane-pruned to 10-node per arm). The vineyard is irrigated via the drip irrigation system installed. Other annual cultural practices include fertilization (dosage: 250 g per vine with fertilizer type: 11-15-15 NPK); green pruning (shoot thinning, topping, and leaf removal); plant protection; irrigation (at appropriate time periods), and weed control (usually with the help of light hoeing).

Figure 2. Experimental vineyard

The climatic parameters that prevailed during the years of the experiment are presented in Figure 3. The lowest winter temperatures (on average) were recorded in year 2016, compared to years 2017 and 2018. During December of 2015 until February 2016, the highest precipitation values were recorded.

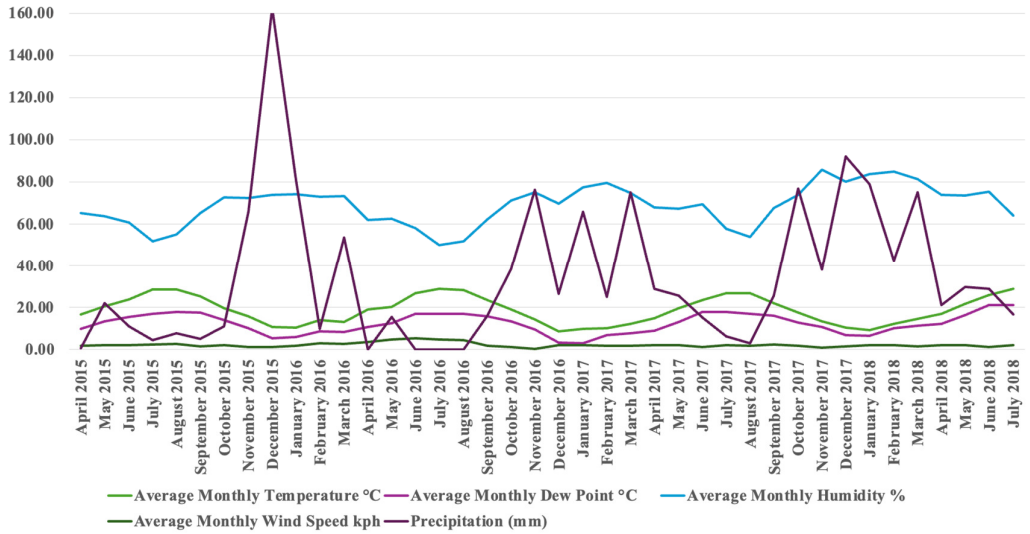

Figure 3. Climatic parameters during the years of the experiment (April 2015 to June 2018)

Experimental design – treatments

In this study, four formulations (Dormex®, Erger, Theocopper and garlic extract) were used for bud dormancy release according to Daskalakis *et al.* (2021). The information related to the formulations and their active substances are presented in Table 1.

Table 1. Treatments, formulations and their active substances applied in the experiment

Treatment	Product	Formulation composition	Spray liquid concentration	Final dosage / Experimental group
Control	Control	Purified water	100%	1 L
DA	Dormex®	H_2CN_2 490 g L^{-1} (Basf Co.)	2.5% v/v	25 mL Dormex®, 975 mL H_2O
DB	Dormex®	H_2CN_2 490 g L^{-1} (Basf Co.)	5% v/v	50 mL Dormex®, 950 mL H_2O
TA	Theocopper	10% sugars, 10% amino acids, 12% urea, 1.4% potassium in organic form, 12% nitrogen in organic form and 3.5% organic matter	1% v/v	10 mL Theocopper 1 g Theocalcium 990 mL H_2O
	Theocalcium	30% calcium, 35% organic matter pH: 7.1	0.1% w/v	
TB	Theocopper	10% sugars, 10% amino acids, 12% urea, 1.4% potassium in organic form, 12% nitrogen in organic form and 3.5% organic matter	2% v/v	20 mL Theocopper, 2 g Theocalcium, 980 mL H_2O
	Theocalcium	30% calcium, 35% organic matter pH: 7.1	0.2% w/v	
EA	Erger	Total Nitrogen (N) 15.0%, Nitric Nitrogen (N) 5.8%, Ammonium Nitrogen (N) 3.1%, Uric Nitrogen (N) (poor in diurea) 6.1%, Water-soluble calcium oxide (CaO) 4.7%	3.5% v/v	35 mL Erger, 80 mL Active Erger, 885 mL H_2O
	Active Erger	Total Nitrogen (N) 15.0%, Nitric Nitrogen (N) 9%, Ammonium Nitrogen (N) 6%, Water-soluble calcium oxide (CaO) 6.5%	8% v/v	
EB	Erger	Total Nitrogen (N) 15.0%, Nitric Nitrogen (N) 5.8%, Ammonium Nitrogen (N) 3.1%, Uric Nitrogen (N) (poor in diurea) 6.1%, Water-soluble calcium oxide (CaO) 4.7%	7% v/v	70 mL Erger, 160 mL Active Erger, 770 mL H_2O
	Active Erger	Total Nitrogen (N) 15.0%, Nitric Nitrogen (N) 9%, Ammonium Nitrogen (N) 6%, Water-soluble calcium oxide (CaO) 6.5%	16% v/v	
Garlic Extract	Garlic Extract		15% v/v	150 mL Garlic Extract, 850 mL H_2O

In the experiment for bud dormancy release conducted in the vineyard during the years 2016, 2017, 2018, the Randomized Complete Block Design was followed, with three repetitions of ten (10) vines per treatment. The groups of ten vines were selected based on phenotypic and morphological characteristics, as well as the phytosanitary condition of each variety in the experiment. There were eight (8) treatments per variety, including one control per variety, per spraying time every year for three years. Eight (8) applications were carried out three (3) times a year for each of the two varieties and, namely, on December 15, January 15, and February 15, as follows:

- 10 mL Theocopper and 1 g Theocal per Liter [TA];
- 20 mL Theocopper and 1 g Theocal per Liter [TB];
- 25 mL Dormex® per Liter [DA];

- 50 mL Dormex® per Liter [DB];
- 35 mL Erger and 80 mL Active Erger per Liter [EA];
- 70 mL Erger and 160 mL Active Erger per Liter [EB];
- extract from 300 g (F.W.-fresh weight) garlic evaporated and then diluted in 1 L water [GA]; and
- distilled water (control treatment) [Control].

Overall, during the three years of the experiment, there were 144 treatments applied.

Recording - phenological stages parameterization

In the experimental vineyard, during the budburst period and shoot development, all phenological stages (A-H) of the two varieties, were studied and recorded according to the Baillod and Baggiolini scale (1993). Specifically, at the phenological stages of budburst, 253.145 buds were measured and recorded for all productive units of each vine, each replication of each treatment on four consecutive dates, every fifteen (15) days. The procedure remained the same for all three years of the experiment.

Measurements - determinations

The percentage of budburst for dormant buds and the apical dominance of canes (acrotony) were determined and measured according to Daskalakis and Biniari (2019).

Statistical analysis

The statistical analysis of the experiment's results was performed using the Statgraphics Centurion statistical package (Statgraphics Technologies, Inc., version 17). The significance of the results was checked by analyzing the variance. The comparison of means was conducted using Tukey's HSD method at a significance level of $P<0.05$.

Depending on each individual experimental procedure and the factors examined, single-factorial, two-factorial, and multi-factorial experiments were designed and as mentioned, the Randomized Complete Block Design was carried out. For the multifactorial part of the analysis, the analysis of variance using the Multi Factor, ANOVA (Analysis of Variance) method was followed, with factors: the variety ('Prime' and 'Ralli'), the three years in which the experiment was conducted, the three dates on which the treatments were carried out (15/12, 15/01, 15/02) and the eight treatments with Dormex®, Erger, Theocopper (low and high concentration), garlic extract and control. The comparison of the means was made at the level of significance: $P< 0.05$, $P<0.01$ and $P<0.001$. In the presented results, means followed by different letters of the Latin alphabet indicate statistically significant differences. The results are presented and described in detail in the present study's tables and diagrams.

Results and Discussion

Effect of variability factors on dormancy break, budburst of dormant buds, and shoot growth in the Varieties 'Prime' and 'Ralli'

The effect of application timing

The highest value of the average percentage of budburst for both varieties ('Prime' and 'Ralli') was observed in the treatments carried out on December 15 across all three years (2016, 2017, 2018), with statistically significant differences compared to the other two treatments (Table 2). In this experiment, the highest budburst percentage was observed sixty (60) days after the treatments were compared to the control vine. Additionally, the results indicate that the response of the buds to the applied substances and their effectiveness in breaking dormancy and budburst depend on the timing of substance application (George *et al.*, 1988; Lavee and May, 1997). Last, the late application of substances on February 15 did not appear to have

negative effects on budburst or on the physiology of the buds, contrary to the findings of George *et al.* (1988) and Lavee and May (1997) who reported that late application of Dormex® with high concentrations could cause significant damage to the buds and delay their budburst. In this experiment, the last treatment on February 15 appeared to have minimal or no effect on budburst on both varieties (Table 2). The application of calcium cyanamide (containing 1.5% of HC) on the buds of Cabernet Sauvignon grafts that had not undergone chilling induced budburst in 80% of the buds after thirty-five (35) days of application compared to the controls (Botelho *et al.*, 2007).

Table 2. Average percentage of budburst (%) per variety in the treatments carried out on the three dates of application for three years of experiment

Time of application	Variety	
	'Prime'	'Ralli'
December 15	57.57 a	57.85 a
January 15	54.51 b	55.54 b
February 15	50.17 c	52.15 c
Standard Error Mean	0.037	0.183

Values are the means of triplicates. Values on the same column carrying a different letter (a–c) are significantly different at significance level $p \leq 0.05$, according to Tukey's test

The analysis of the measurements made after the treatments that were conducted on the dormant buds of the canes on December, January, and February 15 (years: 2016, 2017, 2018) are presented in Table 3. It appears that the shoots from the canes subjected to the chronologically earliest treatment on December 15 were at a more advanced stage of shoot development, with a statistically significant difference separating them from the canes subjected to the other two treatments. In their study, Or *et al.* (1999) report that delayed application of HC may prove detrimental, as the resistance to the toxicity of the chemical substance by the buds is reduced after the main dormancy period. However, those results were not confirmed by the present study's last chronological treatment with Dormex® for all three years of the experiment.

Table 3. Average stage of shoot development per variety in the treatments carried out on the three dates of application for three years of experiment; the stage of shoot development was calculated as described in Daskalakis and Biniari (2019)

Time of application	Variety	
	'Prime'	'Ralli'
December 15	4.642 a	4.170 a
January 15	4.306 b	4.007 b
February 15	3.891 c	3.715 c
Standard Error Mean	0.037	0.018

Values are the means of triplicates. Values on the same column carrying a different letter (a–c) are significantly different at significance level $p \leq 0.05$, according to Tukey's test

In Table 4, from the analysis of the measurements, for the apical dominance of the canes (acrotony), after the treatments conducted on the dormant buds of the experiment's canes on December, January, and February 15 for the years 2016, 2017, and 2018, it appears that none of the three treatments showed any statistically significant differences in their effect on the average acrotony displayed by the canes.

Table 4. Average acrotony of canes for both varieties in the treatments carried out on the three dates of application for three years of experiment; the acrotony was calculated as described in Daskalakis and Biniari (2019)

December 15	January 15	February 15	Standard Error Mean
0.432 a	0.435 a	0.43 a	0.001

Values are the means of triplicates. Values on the same row carrying a different letter (a) are significantly different at significance level $p \leq 0.05$. according to Tukey's test

The effect of observation-recording time

In Table 5, there is a significant variation in the mean percentage of budburst between the time intervals that elapsed between the observations (recordings) for both varieties over the course of three years. Moreover, it was observed that 'Prime', when compared to 'Ralli', exhibits earlier budburst: measurements taken on February 20 of each year of the experiment indicated that 'Prime' had an average budburst percentage of 16.57%, while 'Ralli' registered a mere 4.44%.

Table 5. Average percentage of budburst (%) per variety in the treatments carried out on the three dates of application as per time of observation for three years of experiment

Time of observation	Variety	
	'Prime'	'Ralli'
February 20	16.57 d	4.44 d
February 27	43.66 c	58.40 c
March 13	73.10 b	73.28 b
April 2	83.00 a	84.58 a
Standard Error Mean	0.440	0.312

Values are the means of triplicates. Values on the same column carrying a different letter (a–d) are significantly different at significance level $p \leq 0.05$, according to Tukey's test

The statistical analysis of the observations and recordings carried out after the treatments showed that there was a significant variation in the average stage of shoot development between the time intervals from one recording to the next. It should be noted that during the first observation-recording, conducted on February 20 of every year and throughout the three years of the experiment, the average stage of shoot development was 2.058 for 'Prime', and 1.435 for 'Ralli'. It thus appears that, when compared to the 'Ralli' variety, 'Prime' undergoes an earlier shoot development stage (Table 6).

Table 6. Average stage of shoot development per variety in the treatments carried out on the three dates of application as per time of observation for three years of experiment; the stage of shoot development was calculated as described in Daskalakis and Biniari (2019)

Time of observation	Variety	
	'Prime'	'Ralli'
February 20	2.058 d	1.435 d
February 27	3.432 c	3.717 c
March 13	5.213 b	4.868 b
April 2	6.415 a	5.836 a
Standard Error Mean	0.043	0.02

Values are the means of triplicates. Values on the same column carrying a different letter (a–d) are significantly different at significance level $p \leq 0.05$, according to Tukey's test

During the experiment, acrotony was calculated per variety and per treatment with chemical substances and per replication for each one of the three years of the experiment. Statistical analysis of the data (Table 7) revealed that all treatments with chemical dormancy-breaking substances reduced the intensity of the acrotony

with both varieties registering statistically significant differences when compared to the control, for all three years of the experiment. Further, the treatment with garlic extract (GA) reduced the mean shoot tip tension for both varieties where it was applied, with statistically significant differences when compared to the controls. Last, in the 'Prime' variety, the lowest acrotony was observed after treatment with the highest concentration of Erger (EB). However, with regard to the 'Ralli' variety, the lowest acrotony was observed after the vines were treated with high concentrations of not only Erger (EB) but Theocopper (TB) as well without any statistically significant differences between the two treatments. The treatments mentioned above, besides inducing earlier budbreak in both varieties, increased the uniformity of shoot development along the vine by reducing the intensity of acrotony. Similar results are reported by Mattheou *et al.* (1996) with the application of hydrogen cyanamide, in concentrations of 1% and 2.5%, on the 'Razaki', 'Perlette', and 'Victoria' varieties, six weeks before budbreak, under Greek vineyard conditions, i.e., early dormancy release and uniform bud break.

Table 7. Average acrotony of canes per variety after treatment with chemical dormancy-breaking formulations for three years of experiment; the acrotony was calculated as described in Daskalakis and Biniari (2019)

Treatment	Variety	
	'Prime'	'Ralli'
Control	0.584 e	0.53 f
DA	0.475 d	0.47 d
DB	0.428 c	0.364 b
TA	0.437 c	0.506 e
TB	0.392 b	0.324 a
EA	0.445 c	0.435 c
EB	0.317 a	0.334 a
GA	0.444 c	0.437 c
Standard Error Mean	0.005	0.003

Values are the means of triplicates. Values on the same column carrying a different letter (a–f) are significantly different at significance level $p \leq 0.05$, according to Tukey's test

The effect of the chemical compounds Dormex®, Theocopper, Erger, and garlic extract

The observation from Table 8 indicates that dormancy-breaking chemicals had a consistent positive effect on shoot development across both varieties and all three years of the experiment. The differences registered were statistically significant when compared to the control. Regarding the 'Prime' variety, the highest stage of shoot development was recorded after its treatment with a high Erger (EB) concentration. In the case of the 'Ralli' variety, the high stage of shoot development occurred after treatments with high concentrations of Erger (EB), Theocopper (TB), and Dormex® (DB), with no statistically significant differences between them. Over the three years of the experiment, treatment of either variety with garlic extract not only increased the average stage of shoot development but also registered statistically significant differences when the two varieties were compared to the control.

What is more, the results indicated that treatments with garlic extract did not show statistically significant differences when compared to treatments with low-concentration hydrogen cyanamide over the three years of the experiment. Given the high toxicity of hydrogen cyanamide, it is evident that garlic extract, which is non-toxic, has a clear advantage over hydrogen cyanamide. A study by Kubota and Miyamuki (1992) showed that the garlic paste treatment applied to the Muscat of Alexandria variety proved, on the one hand, more effective than calcium cyanamide but, one the other, less effective than hydrogen cyanamide. Satisfactory results were also obtained via the application of garlic oil instead of paste in the cases of the Pione hybrid grapevine and the 'Thomson Seedless' variety (Kubota *et al.*, 2000). It is worth noting that the use of garlic paste at a concentration of 3% proved less effective and even somewhat detrimental as it caused 70% bud

damage, but only when the buds had been exposed to low temperatures for a considerable period of time. Those studies indicate that the present experiment's results are clearly moving in the right direction.

Table 8. Average bud break percentage (%) per variety and per treatment with chemical dormancy-breaking formulations for three years of experiment. The bud break percentage was calculated as described in Daskalakis and Biniari (2019)

Treatment	Variety	
	'Prime'	'Ralli'
Control	45.47 f	47.86 e
DA	53.04 cd	54.00 c
DB	58.69 b	57.94 b
TA	50.76 c	54.28 c
TB	57.88 b	60.77 a
EA	54.52 c	54.87 c
EB	61.36 a	60.60 a
GA	50.96 de	51.09 d
Standard Error Mean	0.509	0.3

Values are the means of triplicates. Values on the same column carrying a different letter (a–f) are significantly different at significance level $p \leq 0.05$, according to Tukey's test

Effect and interaction between variability factors

Four variability factors were taken into consideration: time of treatment, treatment, variety, and year. It should be noted that there were statistically significant effects of all the main factors and their interactions on budbreak percentage, the intensity of the apical dominance of the canes (acrotony), and the developmental stage of the dormant buds, confirming the findings of the study (Table 9).

Table 9. Effect of variability factors on average development stage, on the apical dominance of the cane (acrotony) and percentage of budbreak; interaction of these factors for three years of experiment

Factor	Average development stage	Apical dominance of cane (acrotony)	Percentage of budbreak
Factors of variability	**Significance level**		
A: Time of treatment	***	***	***
B: Treatment	***	***	***
C: Variety	***	***	***
D: year	***	***	***
Interactions			
AB	***	***	***
AC	***	***	***
AD	***	***	***
BC	***	***	***
BD	***	***	***
CD	***	***	***
ABC	***	***	***
ABD	***	***	***
ACD	***	***	***
BCD	***	***	***
ABCD	***	***	***

Single-factorial, two-factorial, and multiparametric analysis of mean values
(*), (**), (***): significant at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$ ns: non-significant

According to Table 9, the effect of application time and concentration of the formulations was decisive, as the intervention with high doses of Dormex®, Erger and Theocopper on December 15 brought about the most significant results, highlighting the importance of the appropriate combination of factor-variety-year for the optimization of the desired results depending on the variety.

Conclusions

It is a well-known fact that, in warm viticultural areas, failure to meet the needs a vine's dormant buds have during cold spells may lead to prolonged dormancy and result in delayed flowering and, ultimately, reduced production. Successful grape production in such areas, especially production of table grapes, depends on the chemical substances which, compensating for the lack of ambient cold, are used in helping to break dormancy. Be that as it may, the effective compounds available today are expensive and carry the risk of bud damage due to their phytotoxicity.

Regarding the formulations used, Dormex® (hydrogen cyanamide) has been extensively studied and thus was used as the study's control since its effectiveness on the dormancy release mechanism is well known. Additional formulations included Theocopper, Erger, and garlic extract. The study aimed at developing a new as well as effective dormancy-breaking strategy that would not involve phytotoxic effects.

According to the results of all the measurements carried out, the two varieties appear to be affected to different extents, with the 'Prime' variety appearing to reach an earlier budburst than the 'Ralli' variety. The two table grape varieties under study are early-sprouting ones. The entrance of dormant buds into main dormancy temporally coincides with the cessation of the shoot growth rate and the onset of the buds' differentiation into canes, which occurs approximately during the first fortnight of July. The degree to which dormant buds respond to the chemical substances applied and break their dormancy depends on the grape variety, the stage of dormancy, the substances' concentration, and the timing of substance application.

Despite the need for further studies so as to safeguard the accuracy of the present study's conclusions during the timeframe selected, the study did demonstrate that desired control of the dormancy breaking phenomenon can be achieved without phytotoxic effects through knowledge of the biochemical processes induced by chemical treatments as well as through timely treatment with chemical formulations on dormant buds with specific compositions and concentrations. In conclusion, apart from the interest they present for scientists and researchers, the results of the present study do have a direct application to viticulture through the use of chemical formulations for dormancy breaking.

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