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Effects of Early-Season Leaf Removal on Shoot Development and the Physiology and Viability of Winter Buds in the Karaerik (*Vitis vinifera* L.) Grape Cultivar

Tevhit GEÇİM[1✉], Özkan KAYA[2], Nalan Nazan KALKAN[3], Abdurrahim BOZKURT[4], Birol KARADOĞAN[5], Serkan CANDAR[6], İsmail ESMEK[7]

[1,2,3,4]Horticultural Research Institute Directorate, Erzincan, Türkiye, [5]General Directorate of Agricultural Research and Policies, Ankara, Türkiye, [6]Çanakkale Onsekiz Mart University, Faculty of Agriculture, Horticulture Department, Çanakkale, Türkiye, [4]Elmalı District Directorate of Provincial Agriculture and Forestry, Antalya, Türkiye

[1]https://orcid.org/0000-0003-2406-9929, [2]https://orcid.org/0000-0002-1679-6125, [3]https://orcid.org/0000-0002-9204-7281, [4]https://orcid.org/0000-0001-7315-202X, [5]https://orcid.org/0000-0002-1526-1751, [6]https://orcid.org/0000-0002-2608-8691, [7]https://orcid.org/0009-0002-3883-0862

✉: dr.tgecim@gmail.com

ABSTRACT

This study evaluated the effects of leaf removal applied at different phenological stages on phenological development, shoot characteristics, and the physiological status of winter buds in the Karaerik grape cultivar (*Vitis vinifera* L.). The experiment was conducted over three consecutive growing seasons (2019–2021). Leaf removal was applied at the pre-flowering (PF), full flowering (FF), and fruit set (FS) stages, while untreated vines served as the control. Based on three-year mean observations, leaf removal treatments tended to be associated with earlier phenological development compared with the control vines. Veraison occurred between July 28 and August 1 in the PF and FF treatments, whereas it was delayed until August 5–8 in the control vines. The budburst ratio was not significantly affected by the treatments and ranged from 89.25% to 90.74%. In contrast, the total number of inflorescences increased under leaf removal, reaching 27.44, 27.33, and 27.22 in the PF, FF, and FS treatments, respectively, compared to 26.22 in the control. Winter bud soluble carbohydrate content was significantly enhanced by leaf removal, with the highest concentration observed in the PF treatment (10.10 mg g^{-1}), whereas the control vines exhibited markedly lower levels (4.87 mg g^{-1}). Primary bud necrosis ranged from 3.00% to 4.11% in treated vines but increased to 9.11% in the control. The lowest cold injury rate was recorded in the FS treatment (4.67%), which also produced the highest bud fresh (3.05 g) and dry weights (2.10 g). Overall, leaf removal, particularly when applied at the pre-flowering and fruit set stages, was shown to be an effective cultural practice for advancing phenological development and improving the physiological quality and cold tolerance of winter buds in the Karaerik grape cultivar.

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Karaerik (*Vitis vinifera* L.) Üzüm Çeşidinde Erken Dönemde Yaprak Almanın Sürgün Gelişimi ile Kış Gözlerinin Fizyolojisi ve Canlılığı Üzerine Etkileri

ÖZET

Bu çalışma, Karaerik üzüm çeşidinde (*Vitis vinifera* L.) farklı fenolojik dönemlerde uygulanan yaprak alma işlemlerinin asmanın fenolojik gelişimi, sürgün özellikleri ve kış gözlerinin fizyolojik durumu üzerindeki etkilerini belirlemek amacıyla yürütülmüştür. Araştırma 2019–2021 yılları arasında gerçekleştirilmiş; yaprak alma uygulamaları çiçeklenme öncesi (PF), tam çiçeklenme (FF) ve meyve tutumu (FS) dönemlerinde yapılmış, uygulama yapılmayan asmalar kontrol olarak değerlendirilmiştir. Üç yıllık ortalama gözlemlere göre, yaprak alma uygulamaları kontrol asmalarına kıyasla daha erken fenolojik gelişim eğilimi ile ilişkilendirilmiştir. Ben düşme dönemi PF ve FF uygulamalarında 28 Temmuz–1 Ağustos tarihleri arasında

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gerçekleşirken, kontrol asmalarında bu dönem 5–8 Ağustos'ta gözlenmiştir. Yaprak alma uygulamalarının uyanma oranı (%89.25–90.74) üzerinde etkisi olmamıştır. Buna karşın toplam çiçek salkımı sayısı PF, FF ve FS uygulamalarında sırasıyla 27.44, 27.33 ve 27.22 adet olarak belirlenmiş, kontrol uygulamasında bu değer 26.22 adet olarak saptanmıştır. Kış gözlerinde çözünebilir karbonhidrat içeriği bakımından en yüksek değer PF uygulamasında 10.10 mg g^{-1} iken, kontrol asmalarında bu değer 4.87 mg g^{-1} olmuştur. Primer göz nekrozu oranı yaprak alma uygulamalarında %3.00–4.11 arasında değişmiş, kontrol uygulamasında ise %9.11'e ulaşmıştır. En düşük soğuk zararı oranı FS uygulamasında %4.67 olarak belirlenmiştir. Ayrıca FS uygulaması göz taze (3.05 g) ve kuru ağırlığı (2.10 g) bakımından öne çıkmıştır. Sonuç olarak, Karaerik üzüm yetiştiriciliğinde özellikle çiçeklenme öncesi ve meyve tutumu dönemlerinde uygulanan yaprak almanın erkencilik sağladığı ve kış gözlerinin fizyolojik dayanıklılığını artırdığı belirlenmiştir.

Soğuğa dayanım

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INTRODUCTION

Turkey is among the world's leading grape-producing countries, and viticulture represents an important agricultural activity both economically and culturally. However, climatic variabilitg across different ecological regions and the increasing intensity of environmental strectors necessitate the re-evaluation of cultivation strategies to ensure the sustainability of grape production. This situation becomes more pronounced in the cultivation of grapevine (Vitis vinifera L.), which, despite its wide ecological adaptability, exhibits high sensitivity to environmental conditions. Grapevine has a broad global distribution and is considered one of the temperate climate crops particularly vulnerable to climate change (Fennell, 2004). In many regions, environmental stress factors limit yield and quality, thereby threatening the sustainability of economic viticulture (Mickelbert et al., 2006). The inability to directly control climatic factors under field conditions has led growers to optimize various cultural practices, including adjustments of row orientation and spacing, soil cultivation, winter and summer pruning, and canopy density management (Cincotta et al., 2022).

In viticulture, leaf area, canopy density, and the position of leaves within the canopy are among the fundamental factors that determine the light regime and the associated microclimate (Anonymous, 2011; Geçim et al 2026). The main objective of current canopy management approaches is to utilize solar radiation as efficiently as possible, increase photosynthetic capacity, and create a suitable microclimate in the fruit zone (Lan, 2025). It has been reported that this approach serves four main goals: providing sufficient leaf area for fruit development and ripening, exposing clusters to light at different phenological stages, promoting bud development that determines the yield potential of the following year, and reducing disease risk by increasing air circulation within the canopy (Hunter, 2000).

In this context, green pruning practices (such as shoot thinning, leaf removal, topping, etc.) are among the fundamental cultural practices that regulate the balance between vegetative and generative growth of the vine, thereby determining both the yield characteristics of the current year and bud fertility in subsequent years (Poni et al., 2006; Keller, 2020). Leaf removal practices, in particular, are widely used to increase light penetration and air circulation in the cluster zone, reduce disease pressure, improve fruit quality, and regulate carbohydrate distribution (Intrieri et al., 2008; Geçim et al 2026).

Mutual shading of leaves reduces the amount of photosynthetic products; therefore, the timing, severity, and shoot position of leaf removal are decisive for vine growth status, crop load, and tolerance to stress conditions (Dokoozlian & Kliewer, 1996; Phelps, 1999). Excessive crop load not only delays ripening but also reduces carbohydrate reserves in shoots, thereby decreasing cold hardiness, preventing balanced vegetative growth, and leading to a reduction in vine size and quality losses (Bravdo et al., 1984; Edson et al., 1995; Dami et al., 2005; Geçim et al 2026). This

situation makes leaf removal a critical canopy management tool that balances crop load and supports shoot maturation.

The timing of leaf removal is particularly important for physiological outcomes. Leaf removal applied before and during flowering creates a temporary source limitation, directing carbohydrates toward developing inflorescences; this reduces fruit set, leads to the formation of looser clusters, and contributes to a more balanced yield continuity in the long term (Pastore et al., 2013; Lopes et al., 2020; Anić et al., 2021; Geçim et al 2026). In contrast, late or excessively severe leaf removal may reduce bud reserves, increasing the incidence of primary bud necrosis and the number of non-bursting buds (Eltom et al., 2015; Frioni et al., 2018).

Microclimatic conditions altered by leaf removal practices (light, temperature, and air movement) affect not only photosynthetic activity but also the qualitative development of buds through carbon/nitrogen balance and hormonal signaling pathways (Cataldo et al., 2021). The development and viability of latent buds largely depend on carbohydrate accumulation of the vine; adequate light exposure and appropriate carbohydrate distribution are critical for budburst rate and bud fertility (Vasconcelos et al., 2009). Carbohydrate deficiency, low light intensity, and stress conditions are considered among the main causes of primary bud necrosis (Fennell, 2004).

In addition, carbohydrate reserves in wood and buds are among the primary determinants of the vine's ability to withstand low temperatures during winter dormancy due to their cryoprotective properties (Keller, 2020). Previous studies have shown that leaf removal applied at different phenological stages can alter carbohydrate content in buds and thus affect sensitivity to cold injury (Keller & Tarara, 2010).

The Karaerik grape cultivar, which is widely grown in the Erzincan region of Türkiye, has significant economic and cultural value due to its unique flavor profile and adaptation to local ecological conditions; however, it has been the subject of a limited number of scientific studies. In this context, elucidating the effects of leaf removal practices on shoot growth, canopy structure, bud physiology, and cold hardiness in the Karaerik cultivar is important for the development of sustainable viticulture practices.

This study aims to determine, the effects of removing the first five leaves from the basal part of the shoot during the flowering period on crop load balance, carbohydrate accumulation in shoots, shoot maturation, physiological characteristics of buds (budburst rate, fertility, primary bud necrosis, carbohydrate content, and cold hardiness), as well as canopy development and phenological processes, of the Karaerik grape cultivar grown under the 54 ecological conditions of Erzincan. The findingd are intended to provide a scientific basis for developing optimized canopy management strategies in Karaerik viticulture.

MATERIAL and METHOD

This study was conducted in a vineyard located within the boundaries of Bahçeliköy village in Erzincan Province, Türkiye (39.75131 N, 39.36012 E), at an altitude of 1375 m above sea level. The vineyard was established in 2002. All vines were grown on their own roots under uniform management and standard cultural practices. The vines used in the study were at full production age and were trained to a bilateral cordon system established on a double T trellis, with a trunk height of 1 m. Planting distances were arranged as 2.0 m between rows and 2.0 m within rows.

The experiment was designed according to a randomized block design with three replications, each replication consisting of three vines. During winter pruning, a total of 30 buds were retained on each vine. Fourteen days after budburst, shoot and inflorescence numbers were counted and standardized to 24 each. Throughout the vegetation period, shoot thinning, removal of water sprouts, and topping were carried out uniformly on all vines (including the control). Topping was performed immediately after shoot lengths were measured at the berry-softening stage. Leaf removal treatments were applied by following the phenological scale of Lorenz et al. (1995) and consisted of manually removing the first five leaves from the basal part of the shoot at three stages: pre-flowering (PF), full flowering (FF), and fruit set (FS) (Figure 1 and Figure 2). The study was conducted between 2019 and 2022, and the designated observations were systematically recorded each year.

Phenological Development Stages

Phenological observations were carried out throughout the vegetation period according to the methods jointly accepted by the OIV in the 2nd Edition of the OIV Descriptor List for Grape Varieties and *Vitis* Species (OIV, 2009). Accordingly, the dates of budburst, full flowering, veraison, and harvest of the vines were recorded.

Measurements Related to Shoot Growth

Number of sprouted buds: The number of buds that normally burst and formed shoots during the vegetation period.

Number of non-sprouted buds: The number of buds that did not burst, dried out, or lost viability due to cold injury.

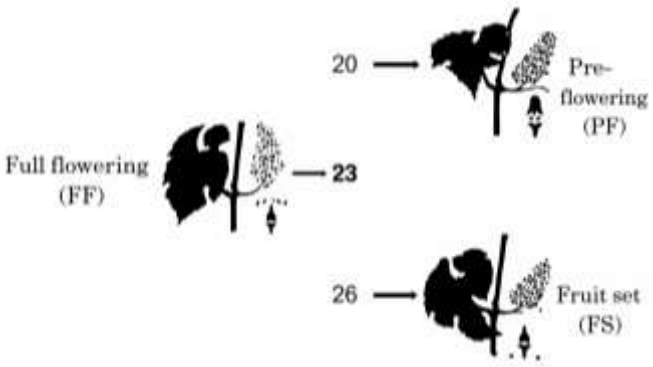

Figure 1. Leaf removal periods according to the phenological scale stages (Lorenz et al., 1995)
Şekil 1. Fenolojik gelişim evrelerine göre yaprak alma dönemleri (Lorenz ve ark., 1995)

Figure 2. Grapevine without leaf removal (A) and with leaf removal (B)
Şekil 2. Yaprak alma uygulanmamış asma (A) ve yaprak alma uygulanmış asma (B)

Bud break rate (%): The ratio of the number of sprouted buds on the vine to the number of buds left on the vine at pruning time in spring.

Number of inflorescences: The total number of inflorescences on each summer shoot when shoot length reaches approximately 30–40 cm.

Number of inflorescences per shoot: The number of inflorescences per shoot.

Pruning weight (kg vine^{-1}): Calculated by weighing the pruning residues consisting of one-year-old canes during pruning.

Measurements Performed on Winter Buds

Soluble carbohydrate analysis (mg g^{-1}): To determine the level of carbohydrate accumulation during the winter period, the first four fruitful winter buds from ten one-year-old cuttings collected from each vine in December were used as samples. The amount of soluble carbohydrates in the buds was analyzed using a modified version of the anthrone method developed by Yemm and Wills (1954).

Primary bud necrosis (PBN) (%): Immediately after leaf fall, ten one-year-old cuttings were collected from each vine, and the first four fruitful winter buds on these cuttings were brought to the laboratory. As a result of microscopic examinations, the incidence of necrosis in the primary buds was determined and expressed as a percentage (%).

Primary bud cold injury (%): In order to determine the effects of leaf removal treatments applied to the vines on the frost tolerance of winter buds, approximately 15–20 days before spring pruning, ten cuttings containing four buds each were collected from each vine. The cuttings were transferred to the laboratory, and frost damage

occurring in the winter buds was evaluated by microscopic examinations. Based on the obtained data, frost tolerance of winter buds was calculated as a percentage (%).

Fresh and dry weight of buds (g): Immediately after leaf fall, ten one-year-old cuttings were collected from each vine, and the first four fruitful winter buds on these cuttings were used as sample material. The collected buds were brought to the laboratory, and their fresh weights were determined using a precision balance. Subsequently, the buds were dried at a constant temperature (e.g., at 65 °C until constant weight) to determine their dry weights.

Shoot length and diameter: Shoot length measurements were carried out on ten randomly selected shoots immediately before topping in the second week of july. Measurements were taken with a measuring tape from the point of shoot emergence to the apical meristem and recorded in centimeters (cm). Shoot diameter measurements were performed immediately after leaf fall on ten randomly selected shoots from each vine. Measurements were taken from the middle internode of each shoot using a digital caliper, and shoot diameter was expressed in millimeters (mm) as the average of the recorded values.

Statistical Analyses

Statistical analyses were performed using JMP® Pro 17.0.0 software (JMP Statistical Discovery LLC, United States). The experiment was designed according to a factorial arrangement within a randomized complete block design. The data were analyzed using a two-way ANOVA including year, leaf removal treatments, and their interaction (Year × Treatment). Blocks were included in the statistical model; however, block effects were not statistically significant and therefore are not presented in the tables. Mean comparisons were performed using the LSD (least significant difference) test due to its higher sensitivity in detecting differences among treatments. Statistical significance was evaluated at the $p<0.05$ and $p<0.01$ levels.

RESULTS and DISCUSSION

In this study, the Karaerik grape cultivar was investigated between 2019 and 2021. Leaf removal treatments were determined according to the phenological scale of Lorenz et al. (1995), and leaf removal at the pre-flowering stage (PF) was carried out on 10 June 2019, at full flowering (FB) on 16 June 2019, and at the fruit set stage (FS) on 19 June 2019 (Table 1). The effects of leaf removal treatments applied at different times on the phenological development stages, canopy development, and winter buds of the vine are presented under the following headings.

Table 1. Dates of Leaf Removal Periods
Çizelge 1. Yaprak alma dönemlerinin tarihleri

Year \ Treatment	Pre-flowering (PF)	Full flowering (FF)	Fruit set (FS)
2019	10. June	16. June	19. June
2020	15. June	18. June	22. June
2021	07. June	10. June	15. June

Effects of Leaf Removal on Phenological Stages

In the first year of the study, 50% budburst occurred between May 6 and May 8, and full flowering occurred between June 13 and June 17. Leaf removal treatments were applied on June 10 at the pre-flowering stage, on June 16 at the full flowering stage, and on June 19 at the fruit-set stage. Veraison occurred on August 1 in vines subjected to leaf removal at the pre-flowering and flowering onset stages, on August 4 in vines subjected to leaf removal at the fruit-set stage, and on August 8 in control vines. When the total soluble solids content of the berries reached an average of 17 °Brix, harvest was carried out on September 16, 2019, in all treatments.

In the second year, budburst in vines subjected to leaf removal in the previous year occurred between May 9 and May 11, whereas in control vines this period was delayed until May 18. Full flowering was observed on June 18–19 in leaf removal treatments and on June 22 in the control vines. Veraison occurred on August 14 in the pre-flowering and flowering onset treatments, on August 16 in the fruit-set treatment, and on August 21 in control vines. Ripening and harvest were completed on October 4, 2020.

In the third year, phenological development showed similar patterns to those observed in the second year. Budburst occurred between April 26 and April 29 in leaf removal treatments and on May 3 in control vines. Full flowering was recorded on June 9 in the pre-flowering and fruit-set treatments, on June 8 in the flowering onset treatment, and on June 16 in control vines. Veraison occurred between July 28 and August 1 in vines subjected to leaf removal treatments, whereas it took place on August 5 in control vines. Harvest was carried out on September 15, 2021 (Table 2).

Table 2. Phenological development stages and dates
Çizelge 2. Fenolojik gelişim dönemleri ve tarihleri

Year	Treatment	Budburst Date	Full Flowering Date	Veraison Date	Harvest Date
2019	PF	06 May	13 June	01 August	16 September
	FF	08 May	16 June	01 August	
	FS	06 May	18 June	04 August	
	C	08 May	17 June	08 August	
2020	PF	09 May	18 June	14 August	04 October
	FF	10 May	19 June	14 August	
	FS	11 May	19 June	16 August	
	C	18 May	22 June	21 August	
2021	PF	27 April	09 June	28 July	15 September
	FF	29 April	08 June	28 July	
	FS	26 April	09 June	01 August	
	C	03 May	16 June	05 August	

PF: Pre-flowering, FF: Full flowering, FS: Fruit set, C: Control

Overall evaluation of the three-year observations indicated that leaf removal treatments tended to be associated with an advancement of approximately 4–7 days in several phenological stages compared with control vines. Earlier veraison and ripening dates were generally observed in vines subjected to leaf removal during the pre-flowering and full-flowering periods. However, because leaf removal applications in 2019 were conducted after early phenological stages such as bud burst and full flowering, these observations should be considered descriptive rather than definitive evidence of treatment effects. Moreover, as no statistical analysis was performed for phenological dates, the reported differences represent observed trends. The earlier phenological development may be related to improved cluster light exposure and modified canopy microclimate resulting from reduced canopy density. These findings are generally consistent with previous reports (Haselgrove et al., 2000; Tarara et al., 2008; Verdenal et al., 2019).

Effects of Leaf Removal on Shoot Development

The microclimatic characteristics of the grapevine canopy consist of several interacting factors, including solar radiation, temperature, wind speed, humidity, and evaporation (Goldammer, 2018; Candar, 2018). Among these factors, the light environment within the canopy is considered a key determinant influencing grape composition, productivity, ripening dynamics, and the cold hardiness of buds and shoots (Smart & Robinson, 1991; Dokoozlian & Kliewer, 1995; Howell, 2001). When the effects of leaf removal practices on shoot development were examined, no significant differences were observed among treatments with respect to the number of sprouted buds, non-sprouted buds, or budburst ratio ($p > .05$). Based on the three-year mean values, the number of sprouted buds was recorded as 27.22, 27.11, and 27.11 in the PF, FF, and FS treatments, respectively, compared with 26.77 in the control. Similarly, the budburst ratio ranged from 89.25% to 90.74%, and the period main effect (PME) was not significant (Table 3). These results indicate that budburst capacity is largely determined by genetic characteristics and winter environmental conditions rather than by the timing of leaf removal. Consistent with these findings, Anić et al. (2021) reported that although leaf removal increased ultraviolet radiation within the canopy and cluster zone, it did not affect berry sugar concentration, bud fruitfulness, or yield performance.

In contrast, both the year main effect × period main effect (YME × PME) interaction and the PME were significant for the number of inflorescences ($p < .01$). According to the three-year mean values, the PF, FF, and FS treatments were grouped together (Group A), with inflorescence numbers of 27.44, 27.33, and 27.22, respectively, whereas the control treatment showed a significantly lower value (26.22) and formed a separate group (Group B) (Table 3). This finding suggests that leaf removal positively influences photosynthetic efficiency and assimilate allocation. Early leaf removal is known to enhance inflorescence development by improving the nutritional status of generative organs. Conversely, the lower inflorescence number observed in the control treatment may be attributed to excessive canopy density, which likely created shading conditions that limited floral bud differentiation. Reduced canopy density facilitates greater light penetration, thereby enhancing cluster formation, bud differentiation, and overall photosynthetic activity (Smithyman et al., 1997).

With respect to the number of inflorescences per shoot, neither the main effect of period (PME) nor the main effect of year (YME) was found to be significant ($p > .01$). Across the three-year mean, values ranged from 0.99 to 1.01, with no meaningful differences among treatments (Table 3).

Table 3. Effects of different leaf removal periods on budburst, inflorescence formation, and pruning weight
Çizelge 3. Farklı yaprak alma dönemlerinin göz uyanması, somak oluşumu ve budama ağırlığı üzerine etkileri

Observations	Years	Periods				
		PF	FF	FS	C	YME
Number of Sprouted Buds	2019	27.00±0.82 ^{ns}	27.00±1.41	27.00±0.82	27.00±1.41	27.00±1.14 ^{ns}
	2020	27.33±0.47	26.33±1.25	27.00±0.82	27.33±0.47	27.00±0.91
	2021	27.33±0.94	28.00±1.41	27.33±1.70	26.00±0.82	27.61±1.46
	PME	27.22±0.79 ^{ns}	27.11±1.52	27.11±1.20	26.77±1.13	
Number of Non sprouted Buds	2019	3.00±0.82 ^{ns}	3.00±1.41	3.00±0.82	3.00±1.41	3.00±1.14 ^{ns}
	2020	2.67±0.47	3.67±1.25	3.00±0.82	2.67±0.47	3.00±0.91
	2021	2.67±0.94	2.00±1.41	2.67±1.70	4.00±0.82	2.83±1.46
	PME	2.77±0.79 ^{ns}	2.88±1.52	2.88±1.20	3.22±1.13	
Bud Break Rate (%)	2019	90.00±2.72 ^{ns}	90.00±4.71	90.00±2.72	90.00±4.71	90.00±3.85 ^{ns}
	2020	91.11±1.57	87.78±4.16	90.00±2.72	91.11±1.57	90.00±3.04
	2021	91.11±3.14	93.33±4.71	91.11±5.67	86.67±2.72	90.55±4.87
	PME	90.74±2.61 ^{ns}	90.37±5.08	90.37±3.99	89.25±3.78	
Number of inflorescence	2019	28.00±1.63 ab *	27.00±0.82 c	27.00±0.82 c	26.67±0.47 cd	27.16±1.14 ^{ns}
	2020	27.33±0.94 bc	26.67±0.94 cd	27.33±0.47 bc	26.00±0.82 d	26.83±0.97
	2021	27.00±0.82 c	28.33±1.70 a	27.33±1.25 bc	26.00±1.41 d	27.16±1.57
	PME	27.44±1.26 A *	27.33±1.41 A	27.22±0.92 A	26.22±1.03 B	
Number of inflorescences per shoot	2019	1.04±0.03 ^{ns}	1.00±0.03	1.00±0.03	0.99±0.04	1.01±0.04 ^{ns}
	2020	1.00±0.03	1.01±0.02	1.01±0.02	0.95±0.03	0.99±0.04
	2021	0.99±0.02	1.01±0.02	1.00±0.05	1.00±0.06	1.00±0.04
	PME	1.01±0.04 ^{ns}	1.01±0.02	1.00±0.03	0.98±0.05	
Pruning Weight (kg vine^{-1})	2019	2.62±0.05 cd *	2.44±0.06 e	2.48±0.10 e	2.62±0.11 cd	2.54±0.11 C *
	2020	2.79±0.09 a	2.74±0.10 b	2.81±0.15 a	2.78±0.13 a	2.78±0.12 A
	2021	2.71±0.10 b	2.59±0.12 d	2.65±0.14 c	2.66±0.16 c	2.65±0.14 B
	PME	2.71±0.11 A *	2.59±0.15 C	2.65±0.19 B	2.69±0.16 A	

Values are presented as mean ± standard deviation (M±SD).
PF: Pre-flowering, FF: Full flowering, FS: Fruit set, C: Control; PME: Period Main Effect, YME: Year Main Effect.
Different uppercase letters in rows (PME) and columns (YME), and different lowercase letters within the same row and column indicate statistically significant differences among the respective main effect means according to the LSD test (*p<.01). ns: indicates non-significant differences (p>.05).

This result demonstrates that although leaf removal practices increased the total number of inflorescences, they did not alter the number of inflorescences per shoot. Despite the increase in total inflorescence number, the absence of change in inflorescences per shoot suggests that leaf removal treatments may have enhanced the formation of secondary inflorescences or reduced the proportion of non-productive shoots. Therefore, the observed increase in total inflorescence number likely originated not from increased shoot number but from improved bud fertility and secondary cluster differentiation within individual shoots, contributing to an overall increase in yield potential. These findings support the view that leaf removal does not directly affect flowering intensity, but rather indirectly influences the overall physiological balance of the vine.

Pruning weight was significantly affected by the YME, PME, and their interaction (p<.01). Based on three-year mean values, the PF and control treatments exhibited the highest pruning weights (2.71 and 2.69 kg vine^{-1}, respectively), whereas the FF treatment showed the lowest value (2.59 kg vine^{-1}). On an annual basis, the highest mean pruning weight was recorded in 2020 (2.78 kg vine^{-1}), followed by 2021 (2.65 kg vine^{-1}) and 2019 (2.54 kg vine^{-1}) (Table 3). The higher pruning weights observed in the PF and control treatments indicate that early leaf removal may stimulate vegetative growth. The elevated pruning weight recorded in 2020 suggests that favorable climatic conditions, particularly temperature and precipitation, supported vegetative development during that season. Conversely, leaf removal applied at full flowering appeared to partially restrict vegetative growth. These results are consistent with those reported by Dami et al. (2006), Kurtural et al. (2006), and Verdenal et al. (2019), who observed only minor increases in pruning weight following pre-flowering leaf removal in several *Vitis vinifera* cultivars. On the other hand, although the PF treatment promoted vegetative growth as reflected by higher pruning weight, the FS treatment exhibited similar pruning weight values; in addition, it resulted in higher bud fresh weight and lower cold injury rates (Table 4). This finding indicates that leaf removal applied at the fruit set stage not only increased vegetative growth but also improved shoot maturation and the allocation of assimilates

toward bud development. Therefore, a distinction should be made between the intensity of vegetative growth and the physiological maturity of the shoot, and the FS treatment is considered to contribute to the formation of a more balanced vegetative–generative equilibrium.

Effects of Leaf Removal on Winter Buds

Statistically significant differences were observed among leaf removal periods with respect to soluble carbohydrate content in winter buds ($p < .01$). Based on the three-year mean values, the highest soluble carbohydrate content was recorded in the PF treatment (10.10 mg g^{-1}), which was classified in statistical group A. This was followed by the FS (9.28 mg g^{-1}) and FF (9.26 mg g^{-1}) treatments, whereas the control vines exhibited the lowest value (4.87 mg g^{-1}) and were placed in group C. When evaluated annually, the highest mean carbohydrate content was observed in 2020 (9.84 mg g^{-1}), while the lowest was recorded in 2019 (6.59 mg g^{-1}) (Table 4). The elevated soluble carbohydrate concentration detected in the PF treatment indicated that early leaf removal promoted assimilate accumulation in winter buds. This result is consistent with previous studies reporting that leaf removal enhances carbohydrate reserves in basal buds (Smith & Centinari, 2019; Kaya, 2020). Moreover, studies conducted on different grape cultivars have demonstrated that increased carbohydrate storage in buds is closely associated with improved cold hardiness (Rende et al., 2018; Smith & Centinari, 2019). Similarly, Geçim et al. (2026) reported that early leaf removal applied at full flowering (FF) and pea-size berry (PS) stages significantly increased soluble carbohydrate accumulation in winter buds compared with veraison and untreated vines. The authors concluded that improved canopy openness enhanced photosynthetic efficiency and promoted reserve carbohydrate storage before dormancy, thereby improving winter bud physiology. Although the highest carbohydrate content in the present study was obtained from the PF treatment, both studies consistently demonstrate that early leaf removal favors carbohydrate accumulation in dormant buds.

The period main effect (PME) was statistically significant for the primary bud necrosis rate ($p <.01$). According to the three-year mean data, necrosis rates were determined as 3.00%, 4.11%, and 3.44% in the PF, FF, and FS treatments, respectively, and these treatments were included in the same statistical group (B). In contrast, the control treatment exhibited a significantly higher necrosis rate (9.11%) and was classified in group A. Across years, primary bud necrosis rates ranged from 4.58% to 5.25%, and the year main effect (YME) was not statistically significant ($p >.05$) (Table 4). The markedly lower necrosis rates observed in leaf removal treatments suggested that these practices positively affected bud health. These findings are in agreement with those reported by Chalfant and Dami (2021), who found that leaf removal during the dormant period increased bud survival. Furthermore, reports indicating that early leaf removal reduces bud necrosis and enhances winter bud cold hardiness support the present results (Smith & Centinari, 2019). The approximately threefold higher necrosis rate observed in the control treatment suggested that excessive canopy density, through increased shading and moisture retention, may have led to physiological deterioration of bud tissues. This interpretation is supported by Geçim et al. (2026), who likewise observed significantly lower primary bud necrosis following early leaf removal, particularly at the full flowering stage, while untreated vines exhibited nearly threefold higher necrosis rates. The authors suggested that increased light penetration and improved tissue maturation resulting from early canopy opening contributed to enhanced bud viability before dormancy.

Significant differences were also detected among treatments with respect to the primary bud cold injury rate ($p <.01$). Based on the three-year mean values, the lowest cold injury rate was recorded in the FS treatment (4.67%), while the PF treatment (5.11%) was included in the same statistical group. The FF treatment exhibited a moderate injury level (6.66%), whereas the control treatment showed the highest cold injury rate (8.22%) and was assigned to a separate statistical group (A) (Table 4). The lower cold injury rates observed in the FS and PF treatments indicated that leaf removal applied during these periods allowed winter buds to enter dormancy with a more balanced physiological status. Increased shoot exposure to solar radiation was associated with improved wood maturation and enhanced cold acclimation, mechanisms widely reported in the literature (Howell & Shaulis, 1980; Fennell & Hoover, 1991; Zabadal et al., 2007). In addition, the inverse relationship between high carbohydrate content and low cold injury rates was consistent with the "high carbohydrate–high cold hardiness" relationship described in previous studies (Rende et al., 2018; Smith & Centinari, 2019).

The FS treatment was statistically superior in terms of bud fresh and dry weights ($p <.01$). Based on the three-year mean values, bud fresh weight and dry weight in the FS treatment were determined as 3.05 g and 2.10 g, respectively, and were higher than those recorded in all other treatments (Table 4). This result indicated that leaf removal performed at the fruit set stage positively affected bud development and tissue integrity. Increased bud weight is generally considered an indicator of stronger and healthier shoot formation. In contrast, no statistically significant differences were detected among treatments with respect to shoot length ($p >.05$). However, shoot diameter was significantly lower in the control treatment (6.24 mm; $p <.01$). These findings demonstrated that

although leaf removal had a limited effect on shoot elongation, it contributed to improvements in shoot mechanical strength and physiological quality.

Table 4. Effects of different leaf removal periods on winter buds
Çizelge 4. Farklı yaprak alma dönemlerinin kış gözleri üzerine etkileri

Observations	Years	Periods				
		PF	FF	FS	C	YME
Soluble Carbohydrate Content (mg g^{-1})	2019	8.31±0.14 d*	7.00±0.17 e	6.83±0.32 e	4.24±0.24 g	6.59±1.49 C*
	2020	12.02±0.23 a	10.72±0.38 b	11.29±0.24 b	5.35±0.26 f	9.84±2.65 A
	2021	9.98±0.36 c	10.07±0.16 c	9.72±0.61 c	5.03±0.38 f	8.69±2.16 B
	PME	10.10±1.54 A*	9.26±1.64 B	9.28±1.90 B	4.87±0.55 C	
Primary Bud Necrosis (%)	2019	3.33±0.47 *ns*	5.00±1.63	4.33±1.25	7.00±1.63	4.92±1.89 *ns*
	2020	2.00±0.82	4.00±1.63	3.00±0.82	9.33±1.25	4.58±3.07
	2021	3.67±1.25	3.33±1.25	3.00±1.41	11.00±2.16	5.25±3.68
	PME	3.00±1.15 B*	4.11±1.66 B	3.44±1.34 B	9.11±2.38 A	
Primary Bud Cold Injury (%)	2019	6.00±0.82 *ns*	6.33±0.94	4.67±0.74	8.33±1.25	6.33±1.60 *ns*
	2020	5.00±1.70	6.33±2.49	4.67±0.37	8.00±1.63	6.00±1.91
	2021	4.33±2.49	7.33±0.82	4.67±0.44	8.33±1.70	6.17±1.99
	PME	5.11±1.29 C*	6.66±1.25 B	4.67±0.47 C	8.22±1.55 A	
Bud Fresh Weight (g)	2019	2.55±0.36 *ns*	2.36±0.54	3.18±0.25	2.52±0.49	2.65±0.53 *ns*
	2020	2.47±0.15	2.30±0.23	2.91±0.27	2.33±0.36	2.50±0.36
	2021	2.51±0.11	2.33±0.33	3.05±0.13	2.42±0.21	2.58±0.35
	PME	2.51±0.24 B*	2.33±0.39 B	3.05±0.25 A	2.42±0.38 B	
Bud Dry Weight (g)	2019	1.75±0.22 *ns*	1.61±0.32	2.25±0.25	1.63±0.32	1.81±0.39 *ns*
	2020	1.70±0.04	1.58±0.17	2.05±0.27	1.48±0.20	1.70±0.29
	2021	1.73±0.09	1.60±0.21	1.99±0.13	1.55±0.15	1.72±0.23
	PME	1.73±0.14 B*	1.60±0.24 B	2.10±0.25 A	1.55±0.24 B	
Shoot Length (mm)	2019	310.67±7.32 *ns*	314.00±3.27	312.33±10.34	323.67±14.08	315.17±10.85 *ns*
	2020	311.67±15.84	319.33±9.29	321.67±12.28	322.67±7.93	318.83±12.50
	2021	311.17±7.35	316.67±3.09	317.00±7.08	323.17±5.95	317.00±7.44
	PME	311.17±10.94 *ns*	316.67±6.34	317.00±10.82	323.17±9.95	
Shoot Diameter (mm)	2019	6.97±0.91 *ns*	6.56±0.68	7.36±0.29	6.37±0.04	6.81±0.70 *ns*
	2020	6.82±0.06	6.95±0.67	6.58±0.16	6.12±0.19	6.62±0.48
	2021	6.89±0.44	6.75±0.50	6.97±0.22	6.24±0.09	6.71±0.45
	PME	6.89±0.59 A*	6.75±0.64 A	6.97±0.39 A	6.24±0.16 B	

Values are presented as mean ± standard deviation (M±SD).
PF: Pre-flowering, FF: Full flowering, FS: Fruit set, C: Control; PME: Period Main Effect, YME: Year Main Effect.
Different Different uppercase letters in rows (PME) and columns (YME), and different lowercase letters within the same row and column indicate statistically significant differences among the respective main effect means according to the LSD test (*p<.01). ns: indicates non-significant differences (p >.05).

CONCLUSION

This study was conducted between 2019 and 2021 to elucidate the effects of leaf removal practices applied at different phenological stages on phenological development, shoot characteristics, and the physiological status of winter buds in the Karaerik grape cultivar (*Vitis vinifera* L.). The results clearly demonstrated that leaf removal practices, particularly with respect to application timing, exerted decisive effects on vine developmental processes.

When the three-year observations were evaluated collectively, leaf removal treatments tended to be associated with an advancement of approximately 4–7 days in several phenological stages compared with the control vines. In particular, leaf removal applied during the pre-flowering (PF) and full flowering (FF) periods significantly accelerated véraison and ripening. This effect was attributed to increased light exposure and improved microclimatic conditions within the cluster zone resulting from reduced canopy density following leaf removal. Accordingly, the primary hypothesis of the study—that the timing of leaf removal influences phenological development and physiological performance of the vine—was confirmed.

With respect to shoot development, leaf removal practices did not exert a marked effect on budburst rate or the number of clusters per shoot; however, they increased the total number of inflorescences. This finding indicated that leaf removal exerted an indirect effect by enhancing photosynthetic efficiency and assimilate distribution rather than directly altering the generative capacity of the vine. Pruning weight responses varied depending on leaf removal timing and year, revealing that leaf removal performed during the flowering period partially restricted vegetative growth.

Findings on winter buds indicated that leaf removal practices enhanced thvine's e physiological resilience. Leaf removal applied during the pre-flowering period resulted in the highest soluble carbohydrate content in winter buds. Correspondingly, primary bud necrosis and cold injury rates were significantly lower in the leaf removal treatments than in the control. Notably, leaf removal conducted during the fruit set (FS) period produced the most favorable results in terms of bud fresh and dry weights. These outcomes demonstrated that leaf removal contributed to winter buds entering dormancy with a more balanced and robust physiological condition.

In conclusion, leaf removal practices in the Karaerik grape cultivar were found to promote phenological earliness and to exert positive effects on carbohydrate accumulation, bud viability, and cold hardiness. In particular, leaf removal applied during the pre-flowering and fruit set periods emerged as the most advantageous in terms of earliness, bud health, and physiological balance. Based on these findings, it was recommended that leaf removal practices in Karaerik grape production be carefully scheduled at appropriate phenological stages.

Since the primary focus of this study was winter bud physiology, the detailed evaluation of marketable yield as well as cluster and berry quality parameters, which were not included within the scope of the research, together with the implementation of additional biochemical analyses, emerges as an important requirement for future studies. Such integrative approaches will contribute to a more comprehensive and multidimensional understanding of the agronomic and physiological effects of leaf removal practices. Furthermore, investigating leaf removal applications in the Karaerik grape cultivar under different ecological conditions will elucidate environmental × treatment interactions and strengthen the generalizability of the findings.

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The authors declare that they have contributed equally to the article.

Conflict of Interest

The authors declare that there are no conflicts of interest among them.

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