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Interactions between Automated Cooling Irrigation and Basal Leaf Removal in cv. Barbera Grapevines.

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Abstract: Over a span of two years, a study on Barbera explored the interactive effects of localized cluster nebulization compared to uncooled vines, alongside the presence or absence of late basal leaf removal, using a full factorial design. In this design, basal leaf removal was implemented in both cooled and uncooled vines. The cooling system employed foggers that delivered a water flow of 11.2 L/h, automatically activating when air temperature exceeded 33 °C and relative humidity dropped below 55%. Throughout both seasons, assessments were made on leaf gas exchange, water status, yield components, and grape composition at harvest. In 2025, additional measurements included cluster temperature, soil water potential, and the evaluation of nebulized water dripping onto the soil. While the presence or absence of basal leaf removal had a minor effect on the gas exchange of the immediately upper leaves, leaf function was positively influenced by cooling in both seasons. In 2025, a direct correlation between cooling and non-limiting pre-dawn water potential was established. On average, the presence of basal leaves around the cluster reduced their temperature by 3-4 °C, while nebulization achieved up to 12 °C of cluster cooling. Late basal defoliation had minimal impact on must composition. Conversely, cooling did not affect yield per vine but resulted in higher malic acid at harvest and significantly reduced the concentration and content of total anthocyanins and phenolics compared to uncooled vines. In the absence of cooling, maintaining leaves around the cluster is crucial for controlling sunburn. This study highlights two main and novel findings: i) the impact on final grape composition can vary depending on how cluster cooling is achieved (e.g., through simple shading or berry wetting), particularly concerning berry pigmentation; ii) under the chosen activation conditions, under the row soil management and weather and soil characteristics, the irrigation system effectively serves a dual function: providing the expected cooling and enhancing soil moisture with consequent benefits for leaf physiology.

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Keywords: *Vitis vinifera L.*, cluster temperature, gas exchange, yield, grape composition, berry transpiration, total anthocyanins

1. Introduction

In a recent review on the concept of "vineyard naturalness"[1], a fundamental aspect of sustainable vineyard management is the utilization of natural resources that are freely available to farmers. This list is rather limited, primarily including radiation and precipitation. However, a crucial distinction exists: while rainfall is seasonally restricted, solar radiation is inherently unlimited, offering substantial potential for dry matter production through photosynthesis. As a result, there is a consensus that a vineyard is deemed efficient when its design and management optimize the following objectives: i) effective total light interception by the canopies, coupled with efficient light distribution near and within the canopy, and ii) efficient allocation of photo-assimilates to the clusters. Among these, the pattern of light distribution around the canopy, especially at the cluster level, exhibits the closest interactions with changes induced by global warming. There is ample evidence that, in temperate-warm climates, any fruiting area exposed to excessive light and thermal load may face higher risks of: i) earlier basal leaf senescence, which can lead to excessive K^+ accumulation in clusters and consequently higher pH in wine[2–6]; ii) poor coloration in red varieties and excessive depletion of malic acid in white varieties[7,8]; iii) increased incidence of berry shrivel, sunburn, and necrosis[9,10]; iv) deviation from the expected aroma profile, with a predominance of fruity, overripe, and cooked flavors, which in some varieties may appear quite atypical[11,12].

Among the various summer pruning techniques commonly used in vineyards, traditional leaf removal undoubtedly has the most direct and intense effect on the cluster microclimate[13]. In warmer regions, medium to late-season basal leaf removal is now approached with greater caution, if not abandoned altogether, due to substantial evidence linking sunburn damage to berry exposure to high skin temperatures[14,15]. However, variability exists depending on the cultivar, cluster and berry morphology, and the timing of berry exposure during the season[16,17]. It has also been established that earlier leaf removal not only increases the likelihood of new leaf cover developing but also reduces the risk of berry sunburn. Joanna Gambetta et al.[18] discovered that in Chardonnay grapes, cluster exposure resulting from pre-veraison leaf removal enhances

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the synthesis of photoprotective compounds, such as flavonoids, carotenoids, and chlorophylls. This synthesis is significantly reduced when defoliation occurs later, such as post-veraison.

While the leaves surrounding the cluster can offer some shading and help regulate berry skin temperature, implementing a cooling irrigation system seems to be a more effective solution. However, beyond the obvious requirement for blue water availability on the farm, this approach must take into account social and technical issues, as well as the economic impact. Socially, especially in traditional European grape-producing countries, the use of irrigation water is sometimes perceived as a yield-enhancing practice that could potentially compromise the final grape quality[19]. Additionally, within the appealing framework of regenerative agriculture, irrigation is not considered a regenerative practice because it introduces an external input into the system, counteracting the reliance on natural rainfall[20]. From a technical standpoint, the growing awareness of the need for water-saving protocols[21] has raised concerns about traditional over-canopy cooling using high-volume sprinklers[22,23], which can result in poor water distribution and efficiency, prolonged wetting of leaves and clusters, and limited usage flexibility.

In recent years, there has been a noticeable increase in the use of localized cooling systems beneath or within canopies[24–28]. This trend is driven by several potential advantages: i) efficient cooling with reduced water consumption and improved water use efficiency; ii) relatively simple programming and automation of water delivery based on environmental conditions; iii) only clusters and basal leaves are moistened, keeping the rest of the canopy mostly dry; iv) nebulized water, typically provided in an on/off intermittent manner, ensures rapid evaporative cooling and minimizes the duration of canopy wetness.

It is logical to assume that maintaining a leaf cover for cluster cooling could be a viable alternative to an automated cooling system, which is not only more expensive but also requires specific skills from the grower. Exploring the interaction between these two methods could illuminate the following: i) what additional benefits does thermoregulation through water sprinkling offer compared to merely avoiding leaf removal? ii) assuming both systems effectively control fruit temperature and reduce sunburn incidence, what is the impact of temperature control involving berry wetting versus keeping berries completely dry in non-irrigated plots? iii) when cooling is

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applied to either defoliated or non-defoliated vines, does the presence of leaves significantly reduce the effectiveness of lowering cluster temperature?

The primary objectives of this two-year study were: i) to explore the interaction between cooling and leaf removal concerning leaf gas exchange, soil and leaf water status, cluster temperature, yield components, and grape composition; ii) to determine whether the benefits of the cooling system are confined to the canopy and target organs or if the water reaching the ground during nebulization cycles could also enhance soil moisture levels and, consequently, improve vine performance.

2. Materials and Methods

2.1. Plant material and experimental layout

The study was conducted from 2024 to 2025 in a 16-year-old vineyard of the Barbera cultivar (clone 102R4 grafted on SO4 rootstock), situated at Colle del Podio Farm Estate (Ancarano di Rivergaro, Colli Piacentini wine district, 42° 50' 00" North, 13° 06' 00" East, 194 m a.s.l.). The vineyard spans approximately one hectare, with rows-oriented North-South and a negligible longitudinal slope. Physico-chemical soil analyses for the 0-60 cm depth indicate a texture of 31% loam, 10% sand, and 58% clay, classifying it as Silty Clay Loam. Hydrological constants, calculated according to Saxton et al.[29], are as follows: wilting point = 19.1% vol., field capacity = 36.6% vol., resulting in an available water reserve of 0.170 cm/cm.

The vines were spaced 0.9 m apart within rows and 2.5 m between rows, resulting in a maximum vine density of 4,444 per hectare. They are trained to a spur-pruned, vertically shoot positioned (VSP) cordon system. The main wire is set 90 cm above the ground, topped by three foliage wires arranged as a pair, a single, and another single from bottom to top, creating a total canopy height of no more than 2.3-2.4 m above ground. During winter pruning, typically 5-6 two-node spurs per vine are retained, resulting in a count bud load of about 10-12. No shoot or cluster thinning was applied during each season. The early 2024 season was fairly wet, with 269 mm of rainfall over April and May, which stimulated vigorous shoot growth. Consequently, shoot trimming was performed twice, on June 26 and August 19, to prevent procumbent shoots and facilitate vineyard operations. In contrast, the early 2025 season was quite dry and warm, with only 104 mm of rainfall over April and May, which quickly checked shoot growth, eliminating the need for

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shoot trimming. The vineyard employs dry farming techniques, managing the soil through repeated shallow tillage beneath the rows to control weeds, while maintaining native vegetation between the rows with periodic mechanical mowing.

In 2024, three adjacent uniform rows, each approximately 100 meters long, were selected and equipped with a multi function irrigation system aimed at providing anti-frost, cooling and ordinary irrigation actions. As shown in Figure S1, each function was served by separate water pipelines. Over the two years, the anti-frost mode was never used as no critical temperatures were registered, whereas the ordinary irrigation function was not activated to avoid interference with cooling system as discussed in greater detail in Bonini et al.[27].

In these rows, sixteen healthy vines were randomly selected to represent the cooling treatment (CL). For comparison, an equal number of vines (n = 16) were randomly chosen from three adjacent rows without the cooling system, serving as the control treatment (C). At the onset of veraison, full basal leaf removal (LR) was applied to half of each batch of C and CL vines, as detailed further below. Each vine was then used for comprehensive agronomic and physiological measurements conducted throughout the season. In 2025, the same vines were maintained for each treatment to allow for the estimation of carryover effects from the applied treatments.

In both seasons, the onset of veraison, marked by berry softening and translucency, was estimated to occur around July 22 (DOY 203). The following day, full basal defoliation (D) was manually performed on half of the vines designated for Control (C) and cooling (CL) treatments. This process involved removing all main leaves from node 1 up to one node above any second cluster along a shoot, as well as any lateral shoots, if present, to maximize cluster light exposure and make it permanent (Fig. S1). The remaining vines were left non-defoliated (ND). The four resulting treatment combinations were: C-ND (control – non-defoliated); C-D (control – defoliated); CL-ND (cooled – non-defoliated); and CL-D (cooled – defoliated).

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2.2. Cooling system: description and setup.

The cooling system was installed on the three selected Barbera rows by mounting Super Fogger (SF) Micro Sprinklers (Naandanjain Italy, Pomezia, Rome). Each sprinkler, equipped with two outlets providing opposing water flows parallel to the row axis, operates at a medium pressure of 3 bars (green cover) to deliver a maximum flow rate of 11.2 L/h. Each unit includes a built-in

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leakage prevention device and a unique filter for added protection. The Super Foggers (SF) were positioned at intervals matching the vine spacing within the row (90 cm), ensuring each sprinkler was centered at mid cordon (Fig. S1). Each SF was connected to the dripper pipeline using 3/8" connection threads and extended about 10 cm above the cordon. The selected medium pressure SF ensures an average droplet size of approximately 69 microns, aiming to favor homogeneous foliage and cluster wetting during pulsed operation. Since SFs were not installed in cases of missing or dead vines, the total number of operational sprinklers was 297.

The intermittent cooling system was configured to run in cycles of 1 minute on and 2 minutes off. Its automation and programming were controlled via the central irrigation system, SAPIR 2 (Talgil Computing and Control LTD, Kiryat Motzkin, Israel). The program uses a "start by condition" trigger to activate the system whenever the air temperature (T) exceeds 33°C and relative humidity (RH) drops below 55%, while a "stop by condition" trigger deactivates it when the air T falls below 31°C. T and RH are continuously monitored by two built-in analog sensors, which record data every 10 seconds. In 2025, to estimate the amount of water reaching the ground during cooling activation—whether from direct fogger leakage or, more likely, from canopy drainage—a dedicated collection protocol was implemented on two representative dates (DOY 223 and 228). On each date, three plastic collection baskets (60 cm in length, 26.4 L in total volume) were randomly placed on the soil surface beneath the vines in each of the three cooled rows, totaling nine baskets per date (n = 9). Each basket was aligned parallel to the row axis and centered beneath the cordon to capture water dripping from the canopy and the fogger unit above. Collection was conducted over exactly one hour of system operation, during which the cooling system ran under its standard intermittent cycle (1 min on/2 min off). At the end of each collection period, the water volume accumulated in each basket was measured individually using a graduated cylinder.

2.3. Thermal canopy assessment

In 2025, thermal images of clusters and/or leaves were captured using a FLIR E8 Pro infrared camera (FLIR Systems, Inc., Wilsonville, OR, USA), which operates within the 7.5-13 µm wavelength range and offers a spatial resolution of 320 x 240 pixels. For the D treatments, individual clusters were measured, while in the ND treatments, the images captured included an

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exposed cluster along with portions of the surrounding leaves. Readings (two per vine) were collected both in the morning (10:00-12:00) and afternoon (14:00-16:00) on DOY 204, 221, 223, and 228. Morning and afternoon readings were taken while standing in front of the east- and west-facing sides of the row, respectively. Measurements were taken at a focus distance of approximately 0.5 meters, using a wet white paper sheet as a backdrop to capture the full silhouette of the cluster. Each image underwent manual processing to isolate the largest inset areas for clusters, while leaf pixels were excluded in the ND treatments. Subsequently, the images were analyzed using FLIR QuikReport software to determine the average and maximum temperatures of the clusters.

2.4. Leaf gas exchange and water status

In 2024, gas exchange variables—specifically, leaf assimilation rate (A), leaf transpiration rate (E), and stomatal conductance (g_s)—were measured on DOY 211, 233, and 240 between 11:00 and 13:00 using an LCi T Pro (ADC Bioscientific Ltd., Hoddesdon, Herts., UK). Standing in front of the east-facing side of the row, two mature mid-shoot leaves (located at nodes 7 to 10) were sampled at ambient CO_2 and relative humidity without altering their natural position, ensuring they received saturating light (PAR >1,400 μmol m^{-2} s^{-1}). Using the same methodology, a more extensive gas exchange screening was conducted in 2025, with readings taken on seven dates throughout the season. Three of these (DOY 181, 189, and 203) occurred before system activation to assess any carryover effect from the previous year's cooling and establish a pre-cooling baseline, while the remaining four were conducted on DOY 220, 231, 238, and 246. In both seasons, the same leaves used for gas-exchange measurements were subsequently processed for midday leaf water potential (Ψ_{MD}). They were swiftly enclosed in a transparent bag to minimize transpiration, cut at the petiole with a razor blade, and quickly inserted into the pressure chamber with a well-protruding petiole stub. Pressurization ceased at the first sign of xylem sap appearing at the cut surface. In 2025, to account for seasonal variability in soil water potential, the predawn leaf water potential (Ψ_{PD}) was measured at each date before sunrise on two basal leaves per test vine, following the chamber pressure method described above.

2.5. Yield components and grape composition at harvest

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On September 11 (DOY 255) in 2024 and September 8 (DOY 251) in 2025, the harvest was conducted by hand. Each vine was picked separately, with the total number of clusters per vine recorded and the average weight of clusters calculated. At the same time, three clusters from each vine, usually from the basal, middle, and top sections of the cordon, were taken to the lab. These clusters were weighed, and a subsample of 50 berries was randomly chosen by carefully cutting each berry at the pedicel with small, sharp scissors. The remaining berries were crushed, and the musts were immediately analyzed for total soluble solids concentration (TSS, expressed in °Brix), pH, and titratable acidity (TA as g L^{-1}). TSS concentration was measured using a temperature-compensating refractometer (RX-5000 Atago U.S.A., Bellevue, WA). The pH of the must was determined with a digital PHM82 pH-meter (Radiometer Analytical s.s.s, Villeurbanne Cedex, France), and TA was evaluated by titration with 0.1 N NaOH (pH 8.2 endpoint) and expressed as g L^{-1} of tartaric acid equivalents.

The concentrations of total anthocyanins and phenolics were assessed following the method described by Iland[30]. In summary, each subsample of 50 berries, kept frozen, was homogenized at a speed of 10,000 rpm for one minute using the Ultra-Turrax T25 homogenizer (Rose Scientific, Edmonton, AB, Canada). Subsequently, 2 g of the homogenized sample was placed into a pre-weighed centrifuge tube, combined with 10 mL of 50% aqueous ethanol (pH 5.0), sealed, and intermittently mixed for an hour before being centrifuged at 959 g for five minutes. A 0.5 mL portion of the extract was then mixed with 10 mL of 1 mol/L HCl, allowed to sit for three hours, and the absorbance was measured at 520 and 280 nm using a Jasco V-530 UV spectrophotometer (Jasco Analytical Instruments, Easton, MD, USA). The total anthocyanins and phenolic content were reported as mg/g and mg/berry, respectively.

To assess the levels of tartaric and malic acids in all samples gathered at harvest, a portion of the must was diluted to four times its original volume, filtered through a 0.22 µm polypropylene syringe for high-performance liquid chromatography (HPLC) analysis, and then placed into auto-sampler vials. All solvents utilized were of HPLC grade. Milli-Q quality water, acetonitrile, and methanol were procured from VWR. Standards for L-(+)-tartaric acid and L-(-)-malic acid were sourced from Sigma-Aldrich. The chromatographic procedure was developed using an Agilent 1260 Infinity Quaternary LC (Agilent Technology), which featured a G1311B/C quaternary pump

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with an inline degassing unit, a G1329B autosampler, a G1330B thermostat, a G1316B thermostated column compartment, and a G4212B diode array detector (DAD) equipped with a 10 mm path, 1 µL volume Max-Light cartridge flow cell. The instrument was managed using Agilent Chemstation software version A.01.05. The analysis of organic acids was conducted with an Allure Organic Acid Column, 300 × 4.6 mm, 5 µm (Restek). Separation occurred under isocratic conditions using water, with the pH adjusted to 2.5 using ortho-phosphoric acid, at a flow rate of 0.8 mL/min. The column temperature was maintained at 30 ± 0.1 °C, and 15 µL of the sample was injected. Elution was observed at wavelengths ranging from 200 to 700 nm and detected by UV–Vis absorption with DAD at 210 nm. Organic acids were identified using authentic standards, and quantification was based on peak areas, performed through external calibration with standards.

2.6. Statistical analysis

The variance analysis for gas exchange and vine performance data was performed using the ANOVA routine in version 2025.1.3 of XLSTAT software (Addinsoft, Paris, France). The normal distribution of each variable population was assessed using the Shapiro-Wilk test at P = 0.05. A normal distribution was confirmed for all variables except for tartaric acid, whose values were subjected to square root transformation before further analysis.

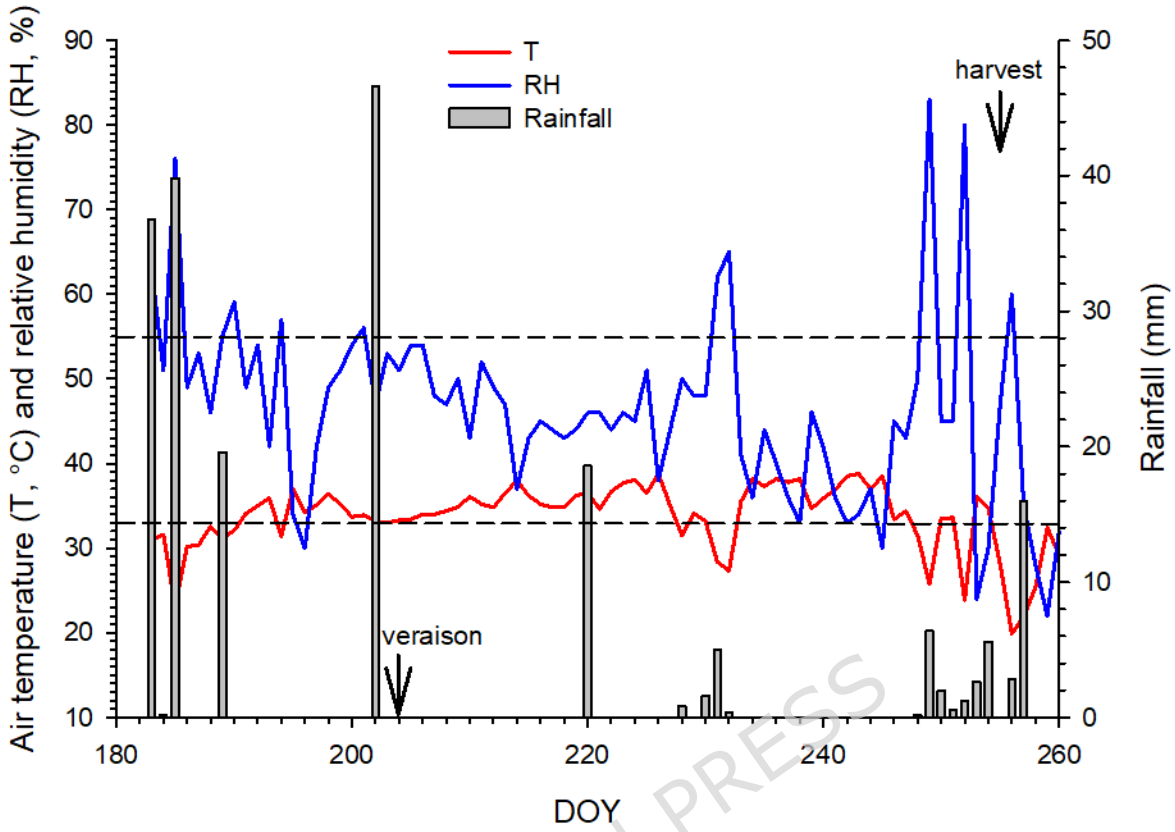
The primary factors considered were year (Y), irrigation (I), and leaf removal (LR), each with two levels. When significant differences were found within the main effects (i.e., C vs. CL and ND vs. D), mean separation was determined using the t-test at $p \leq 0.05$ under protected F conditions. First-order interactions, such as Y x I, Y x LR, and I x LR, and second-order interactions (Y x I x LR, if present) were analyzed only if the Fisher test indicated significance ($p \leq 0.05$), with interactive means separated using the Tukey HSD at the same probability level.

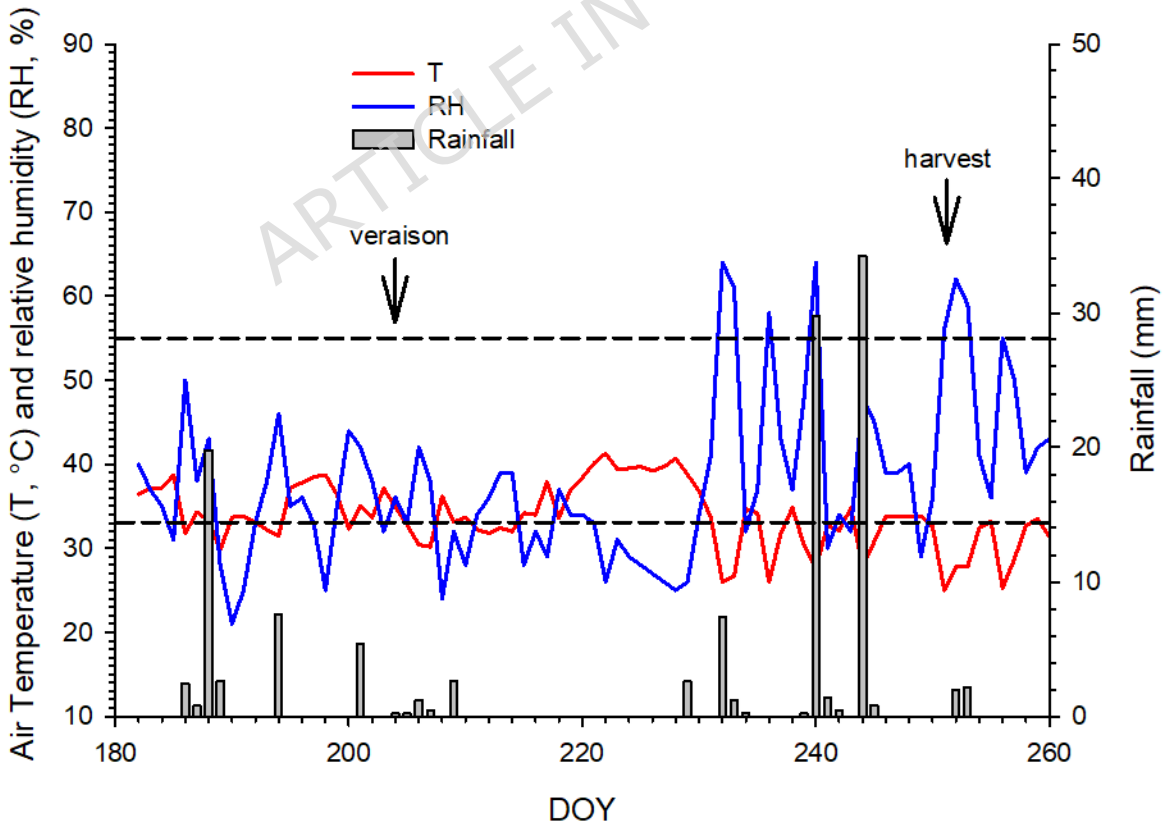
3. Results

3.1. Weather trends, frequency and amount of water supply

Daily values for 2024 (top) and 2025 (bottom) from DOY 180 (29 June) to DOY 260 (17 September) for maximum air temperature (T_{max}, °C), relative humidity (RH, %), and daily rainfall (mm) are presented in Fig. 1.

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Fig. 1. Daily maximum air temperature (Tmax as °C), air relative humidity (RH as %) and precipitation (mm) recorded at the experimental site by the SAPIR-2 and Netsens weather stations in 2024 (top) and 2025 (bottom) for the period comprised between DOY 183-274. Horizontal dotted lines identify climatic thresholds chosen for foggers activation (i.e. T max ≥ 33 °C and RH ≤ 55%).

This period covers the lag-phase of berry growth through to the immediate post-harvest stage. Veraison was estimated to begin on average on DOY 203 in both years, with the cooling system activated on DOY 204. Depending on the weather conditions during harvest, cooling ceased on DOY 254 in 2024 (one day after harvest) and on DOY 251 in 2025 (the day of harvest). Out of a potential activation period of 51 days in 2024 and 48 days in 2025 (Table 1), the conditions required to trigger the cooling system were met on 44 days in 2024 (86.3% of potential activation events) and on 33 days in 2025 (75% of potential activation events). Despite fewer activation days in 2025, the cooling system delivered a total water volume of 2299 m^3/ha, surpassing the 1830 m^3/ha supplied in 2024. This was primarily due to an extremely hot and dry spell from DOY 219 to DOY 231, during which the system operated continuously within the set intermittent cooling cycle of 60 seconds "on" and 120 seconds "off," reaching up to 408 minutes on DOY 222 (equivalent to 6h 48'), whereas the longest event in 2024 occurred on DOY 243, lasting 291 minutes (4h 51'). The calculated mean daily water supply was 4.1 mm/day in 2024 and 6.96 mm/day in 2025. Measurements taken on two dates (DOY 223 and 228) to estimate water leakage onto the ground (n = 9 on each day) showed a leakage of 38.91 m^3/ha, corresponding to 25.9% of the total volume applied (150.39 m^3/ha over the two days).

Table 1. Water volume daily supplied in 2024 and 2025 to cooled Barbera grapevines and expressed on a hectare basis. Calculations refer to an intermittent supply (60sec "on" and 120sec "off" "of" and "off" cycles). The used fogger type delivers 11.2 L/hours. To scale up parcel water supply to a hectare basis, one fogger per vine was considered (i.e. 4444/hectare).

2024				**2025**			
DOY	Total water supply (m^3/ha)	Time length of foggers "on" (mins)*	Total time length for each "on-off" cycle (mins)	DOY	Total water supply (m^3/ha)	Time length of foggers "on" (mins)*	Total time length for each "on-off" cycle (mins)
204	23.94	20	60	204	118.37	87	261
205	19.00	16	49	205	18.11	13	39
206	33.67	28	84	206	0.00	-	-

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207	29.03	26	79	207	0.00	-	-
208	40.10	34	103	208	92.63	63	188
209	50.13	46	137	209	13.17	10	31
210	74.37	68	203	210	41.60	28	85
211	48.93	43	130	211	7.63	3	8
212	36.21	31	93	212	22.90	11	34
213	45.64	48	144	213	10.77	8	23
214	16.16	15	45	214	0.00	-	-
215	45.19	79	236	215	16.76	12	36
216	79.46	70	209	216	22.00	15	46
217	71.97	61	183	217	134.53	98	294
218	82.15	72	216	218	2.24	2	5
219	20.50	24	71	219	61.50	44	131
220	21.10	23	69	220	135.58	96	289
221	59.86	50	149	221	144.56	104	313
222	12.57	10	30	222	189.30	136	408
223	17.36	13	40	223	152.94	117	352
224	15.86	12	35	224	162.07	115	344
225	7.18	5	15	225	154.13	107	321
226	57.31	42	125	226	120.76	87	260
227	48.48	33	100	227	160.12	123	369
228	0.00	-	-	228	147.85	109	326
229	7.03	5	15	229	121.96	94	282
230	0.00	-	-	230	80.66	57	170
231	0.00	-	-	231	16.91	12	35
232	0.00	-	-	232	0.00	-	-
233	27.08	18	55	233	0.00	-	-
234	82.15	72	216	234	41.00	29	86
235	5.98	5	15	235	11.07	8	24
236	59.05	59	177	236	0.00	-	-
237	90.08	75	225	237	0.00	-	-

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238	83.20	69	207	238	43.10	31	93
239	28.73	21	64	239	0.00	-	-
240	31.42	25	74	240	0.00	-	-
241	59.40	51	152	241	0.00	-	-
242	45.49	28	85	242	0.00	-	-
243	134.08	97	291	243	27.09	20	59
244	79.91	60	179	244	0.00	-	-
245	116.57	88	263	245	0.00	-	-
246	2.09	1	2	246	7.18	4	13
247	41.30	31	92	247	8.53	6	17
248	0.00	-	-	248	7.03	5	14
249	0.00	-	-	249	4.94	3	8
250	1.79	2	5	250	0.00	-	-
251	6.43	4	11	251	0.00	-	-
252	0.00	-	-	-	-	-	-
253	21.54	16	49	-	-	-	-
254	10.02	6	18	-	-	-	-
Total	1829.65	1601	4803	Total	2298.99	1654	4962

3.2. Cluster temperature

Table 2 displays the cluster T_{max} and T_{mean} recorded during the morning and afternoon on four different dates throughout the 2025 season. The first sampling day (DOY 204) was warm and dry, with T_{air} at 33.7°C and RH at 43%, leading to a daily leaf-to-air VPD range of 2.99 to 3.42 kPa. The following sampling dates were characterized by a very high evaporative demand, as T_{max} reached 38-39°C and the leaf-to-air VPD varied from 3.38 kPa (morning readings on DOY 233) to 4.84 kPa (afternoon readings on DOY 228).

According to the morning assessment (Table 2), no significant I x LR interactions were observed for either T_{max} or T_{mean}. This was due to the readings taken in the morning (10-12 AM) when the conditions for system activation were never met, resulting in similar surface cluster temperatures for both C and CL treatments. In contrast, the cluster T_{max} and T_{mean} for ND and D vines differed;

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over the four sampling dates, the cluster T_{mean} increased by an average of 2.65 °C in D vines compared to ND. The same calculation for T_{max} showed a 3.32 °C increase in cluster heating due to leaf removal.

Thermal data collected in the afternoon, typically at the end of the cooling period, presented a different scenario. While basal leaf maintenance (ND) proved effective in significantly cooling down clusters (-2.95°C for T_{mean} and -4.05°C for T_{max}), the cooling effect was most pronounced in CL vs. C (-14.1°C for T_{mean} and -12.8°C for T_{max}). However, on six out of the eight measurement dates, a significant I x LR interaction occurred, as shown in Fig. 2, illustrating the variation among the four treatment combinations. T_{max} data indicate that the I x LR interaction reached significance on two dates (DOY 223 and 228), highlighting two responses: i) a cooling gradient exists from the warmest (C-D) to the coolest treatment combination (CL-ND), and within each water regime (i.e., C vs. CL), no leaf removal achieved significant cooling. On dates DOY 204 and DOY 221, the interaction did not reach significance, although the tendencies described above were confirmed. This behavior is further emphasized when T_{mean} data are plotted: on any sampling date, the I x LR interaction was significant; however, while in the non-irrigated treatment, the presence of basal leaves led to significant cluster cooling, in the cooled treatment, the presence or absence of leaves surrounding clusters did not affect cluster heating, except on the initial sampling date.

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Table 2. Cluster mean (T_{mean}) and maximum (T_{max}) temperature recorded in the morning (10-12) and afternoon (14-16) of 23 July, 9 August, 11 August, and 16 August 2025 for the two levels of each factor. C = non-irrigated control; CL = cooled; ND = non defoliated; D = defoliated. Probability in bold indicates $p < 0.05$.

Means represent data taken on one cluster per vine (n = 8) in the morning hours (10-12) and in the afternoon (14-16), before and after the end of the cooling respectively. In case of significance of the F, within column and within factor separation was performed by t-test, p = 0.05.

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MORNING	23 July		9 August		11 August	
	T_{mean}	T_{max}	T_{mean}	T_{max}	T_{mean}	T_{max}
Irrigation (I)						
C	38.2 ± 0.57	43.2 ± 0.93	43.3 ± 0.48	48.2 ± 0.66	42.5 ± 0.60	47.8 ± 0.54
CL	38.9 ± 0.64	44.8 ± 1.07	42.7 ± 0.60	48.6 ± 0.55	41.1 ± 0.56	46.3 ± 0.60
Pr > F	0.293	0.178	0.227	0.625	0.069	**0.040**
Leaf removal (LR)						
ND	37.0 ± 0.47	41.7 ± 0.90	41.4 ± 0.41	46.7 ± 0.45	40.7 ± 0.40	45.7 ± 0.54
D	40.0 ± 0.50	46.3 ± 0.76	44.6 ± 0.32	50.1 ± 0.41	42.9 ± 0.66	48.3± 0.46
Pr > F	**0.000**	**0.000**	**0.000**	**0.000**	**0.007**	**0.001**
I x LR (Pr > F)	0.590	0.294	0.500	0.769	0.257	0.978
AFTERNOON	T_{mean}	T_{max}	T_{mean}	T_{max}	T_{mean}	T_{max}
Irrigation (I)						
C	38.2 ± 0.48	41.6 ± 0.76	46.7 ± 0.66	51.1 ± 0.77	47.0 ± 0.72	50.5 ± 0.53
CL	28.4 ± 0.70	31.9 ± 0.68	30.6 ± 0.48	36.4 ± 0.93	30.5 ± 0.33	36.2 ± 0.72
Pr (>F)	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
Leaf removal (LR)						
ND	31.5 ± 1.48	34.6 ± 1.37	37.8 ± 1.90	42.4 ± 1.94	37.1 ± 1.93	41.0 ± 1.94
D	35.2 ± 1.14	38.8 ± 1.21	39.5 ± 2.35	45.0 ± 2.16	40.3 ± 2.37	45.7 ± 1.78
Pr > F	**0.000**	**0.000**	**0.019**	**0.027**	**0.000**	**0.000**
I x LR (Pr > F)	**0.006**	0.069	**0.021**	0.314	**0.001**	**0.015**

3.3. Gas exchange and water status

In 2024, gas exchange assessments were conducted on three occasions following the cooling cycles (Table 3). On July 30, measurements generally indicated good leaf function, although notable differences in T_{leaf}, g_s, and Ψ_{MD} between C and CL levels suggested a mild stomatal limitation in the uncooled vines. On the same day, no differences were detected due to leaf removal. On August 21 and 28, two particularly hot days with leaf temperatures exceeding 44 °C, the limitation in leaf function for the uncooled vines was more pronounced. Specifically, the C treatment showed a fractional reduction in g_s and A of 59.5% and 61.5% on August 21, and 67.1% and 51.9% on August 28. Importantly, these significant differences occurred at similar Ψ_{MD} values.

In terms of LR effects, significant differences were observed only on the final measurement date (28 August), when g_s and A recorded in D were 39.6% and 24.2% higher than those measured in ND. Similar trends were noted a week later, although they did not reach significance. At no point was there a significant I x LR interaction for any of the measured variables (Table 3).

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Table 3. Leaf function and water status assessed in 2024 on three summer dates as leaf stomatal conductance (gs), leaf assimilation (A), intrinsic water use efficiency (A/g_s) and midday leaf water potential (Ψ_{MD}) in Barbera grapevines assigned to two irrigation (I) treatments (C = uncooled control and CL = cooled) and two leaf removal (LR) treatments (ND = non defoliated and D = defoliated). For each treatment mean (main effects) n = 16. Pr > F values in bold indicate significance at p = 0.05.

	30 July (DOY 211)					**21 August (DOY 233)**					**28 August (DOY 240)**				
Irrigation (I)	T_{LEAF} (°C)	g_s (mmol $m^{-2}s^{-1}$)	A (μmol $m^{-2}s^{-1}$)	A/g_s (mmol/mol)	Ψ_{MD} (-MPa)	T_{LEAF} (°C)	g_s (mmol $m^{-2}s^{-1}$)	A (μmol $m^{-2}s^{-1}$)	A/g_s (mmol/mol)	Ψ_{MD} (-MPa)	T_{LEAF} (°C)	g_s (mmol $m^{-2}s^{-1}$)	A (μmol $m^{-2}s^{-1}$)	A/g_s (mmol/mol)	Ψ_{MD} (-MPa)
C	40.1	0.224	10.1	45.1	1.08	46.6	0.064	3.7	53-0	1.27	44.5	0.049	5.2	106.1	1.32
CL	38.4	0.331	11.4	34.4	0.88	44.3	0.158	9.6	60.7	1.24	40.1	0.149	10.8	72.5	1.35
Pr > F	**0.000**	**0.020**	0.087	0.292	**0.001**	**0.000**	**0.000**	**0.000**	0.737	0.468	**0.001**	**0.000**	**0.000**	**0.001**	0.599
Leaf removal (LR)															
ND	39.5	0.263	10.8	41.1	1.02	46.0	0.099	5.88	59.4	1.25	42.8	0.075	6.9	92.0	1.34
D	38.9	0.293	10.8	36.9	0.94	44.9	0.124	7.42	59.8	1.26	41.8	0.124	9.1	73.4	1.34
Pr > F	0.094	0.491	0.986	0.570	0.147	0.07	0.244	0.057	0.241	0.779	0.168	**0.007**	**0.017**	0.05	0.891
I x LR															
Pr > F	0.644	0.318	0.714	0.765	0.930	0.65	0.445	0.863	0.960	0.595	0.933	0.240	0.867	0.55	0.553

In case of significance of the F test, within-column and within factor mean separation was performed by t-test, p = 0.05.

The gas exchange campaign conducted in 2025 included a total of seven seasonal readings, with three taken before treatment assignment and four afterward (Figs. 3A, B, C, D, E, F)). On any given date, no significant I x LR interaction was found for any measured variable, allowing us to focus on the main effects. While T_{leaf}, leaf gas exchange, and leaf and soil water status never differed between ND and D vines, cooling had a pronounced impact on leaf function and water status, and notably, on soil water potential.

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While occasional differences were observed in the pre-treatment measurement dates for all considered variables, it is noteworthy that moderate water stress was recorded on DOY 182 and 204 before the cooling began, with Ψ_{PD} reaching -0.5 to -0.6 MPa, resulting in significant limitations in leaf A and g_s across the main factors. The initiation of cooling (from DOY 204 onward) had a profound impact on leaf function, particularly on soil Ψ_{PD} (Fig. 3F). The three readings taken between DOY 220 and 240 under high VPD conditions showed that CL leaves were 4-5°C cooler than those sampled in C (Fig. 3A). The most responsive parameters to these conditions were leaf A and leaf g_s, which were severely limited in C, nearly leading to full stomatal closure (Figs. 3B, C). The related changes in leaf A and g_s recorded over these three dates resulted in significantly higher intrinsic WUE in the C treatment (Fig. 3D). Notably, midday (Ψ_{MD}) and pre-dawn leaf water potential (Ψ_{PD}) exhibited somewhat different responses over the three dates under consideration: except for readings on DOY 232, when Ψ_{MD} was more negative in C as expected, Ψ_{MD} did not differ on the two remaining dates; conversely, the CL plots showed a much less negative Ψ_{MD} —ranging between -0.2 and -0.4 MPa—compared to the values recorded in the C plots (around -0.9 to -1.0 MPa).

The heavy rainfall recorded before the last reading significantly replenished leaf function and soil water status in the C treatment (Fig. 3). However, this treatment still exhibited slightly lower A and g_s rates compared to the CL treatment.

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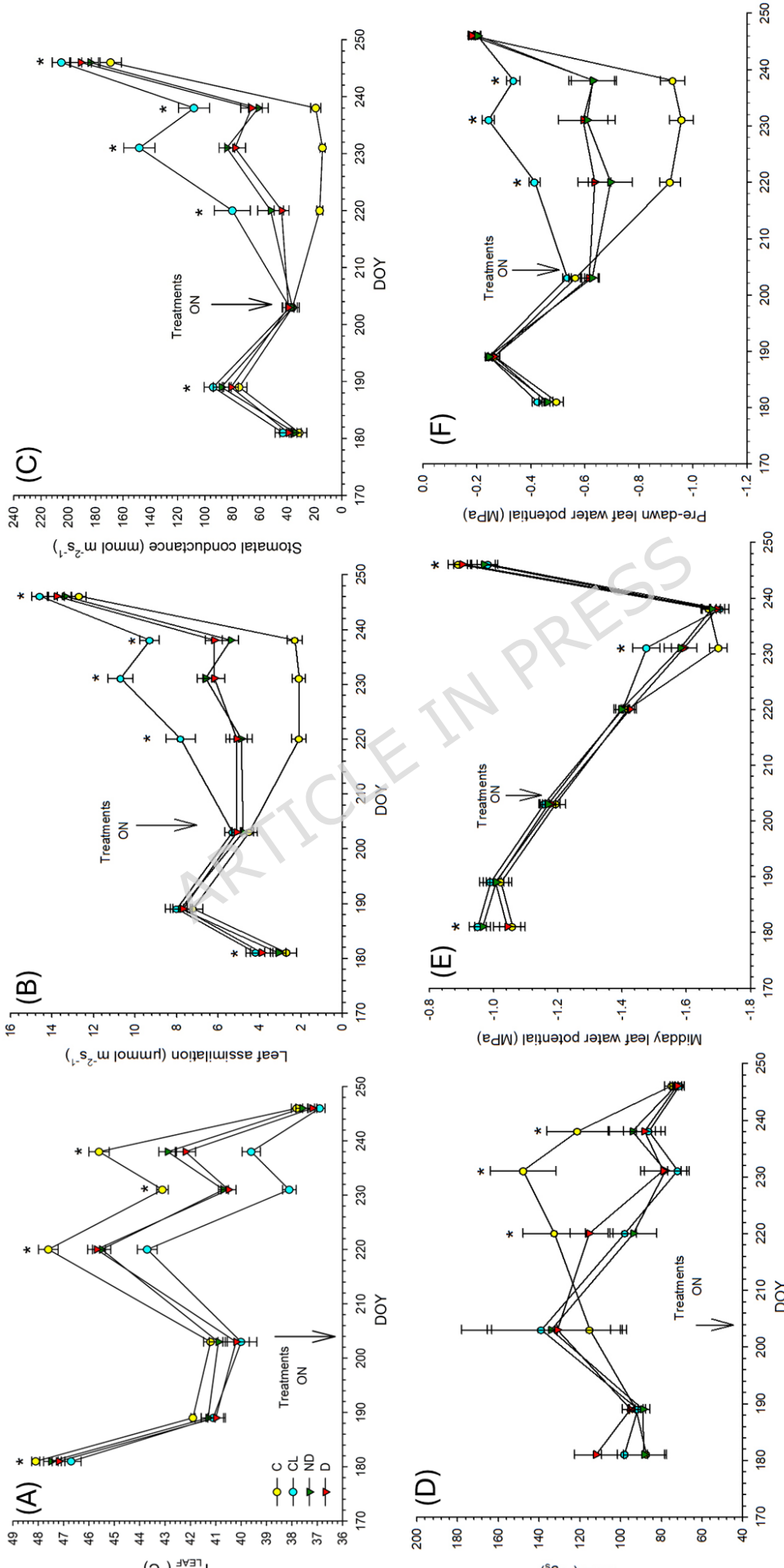


Fig. 3. Seasonal variation of TLEAF, (A) leaf assimilation (B), leaf stomatal conductance (C), intrinsic water use efficiency (D), midday leaf water potential (E) and pre-dawn leaf water potential (F) recorded over seven dates in 2025 in uncooled control (C), cooled (CL) non defoliated (ND) and defoliated (D) main treatments. Arrow indicates activation of the cooling system. Across different dates and variables, ND and D never differed; asterisk indicates at which dates C and CL differed according to t-test ($p = 0.05$).

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3.4. Yield components and grape composition at harvest

Table 4 presents the effects of leaf removal and irrigation levels over the two-year study period, including first-order interactions and probability levels. Cooling had a significant impact on several yield components and grape composition parameters. Although the differences in yield per vine were not statistically significant, the CL treatment resulted in heavier clusters and larger berries compared to the uncooled vines. While TSS and TA were unaffected and reached typical maturity levels for the Barbera variety, cooling reduced tartaric acid concentration at harvest while maintaining a higher amount of malic acid. Regardless of whether measured by concentration or content, total anthocyanins and phenolics were higher in the uncooled vines. The incidence of berry sunburn was four times lower (5.6%) in the cooled vines compared to the control treatment (24.7%).

Main effects stemming from the presence or absence of basal leaf removal at the onset of veraison were less pronounced in terms of the final must composition. In contrast, the smaller clusters observed in the D treatment resulted in a significantly lower yield per vine, while maintaining cluster leaf cover (ND) led to a threefold reduction in berry sunburn incidence compared to the defoliated vines.

Table 4. Yield components, grape composition and cluster sunburn incidence determined in 2024-2025 on Barbera grapevines assigned to two irrigation (I) treatments (C = non irrigated control and CL = cooled) and two leaf removal (LR) treatments (ND = non defoliated and D = defoliated). For each treatments mean (main effects) n = 16. Pr > F values in bold indicate significance at p = 0.05.

	Clusters/ vine	**Cluster weight (g)**	**Berry weight (g)**	**Yield/ vine (kg)**	**TSS (°Brix)**	**pH**	**TA (g/L)**	**Tartaric (g/L)**	**Malic (g/L)**	**Total anthocyanins (mg/g)**	**(mg/berry)**	**Total phenolics (mg/g)**	**(mg/berry)**	**Berry sunburn incide**
Year														
2024	20.6	83.3	2.82	1.64	24.9	3.43	10.6	7.82	2.72	0.76	2.16	1.73	4.59	10.9
2025	35.3	98.0	2.04	3.43	24.1	3.12	9.3	8.52	2.09	1.13	2.23	2.51	5.06	8.5
Pr > F	**0.001**	**0.031**	**0.001**	**0.001**	0.090	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	0.434	**0.001**	0.554	0.204
Irrigation (I)														
C	29.6	74.0	2.26	2.36	24.3	3.24	10.1	8.76	2.09	1.10	2.37	2.39	5.15	24.7
CL	26.3	93.0	2.60	2.71	24.7	3.31	9.8	7.58	2.72	0.80	2.03	1.85	4.70	5.6

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Pr > F	0.065	**0.001**	**0.001**	0.110	0.376	**0.004**	0.550	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.006**	**0.001**
Leaf removal														
ND	28.1	106.0	2.50	2.89	24.8	3.28	10.0	7.97	2.48	0.81	2.30	2.09	5.02	4.3
D	27.8	75.5	2.36	2.19	24.2	3.27	9.9	8.37	2.33	0.78	2.10	2.15	4.82	15.1
Pr > F	0.864	**0.001**	0.052	**0.002**	0.186	0.841	0.849	**0.001**	0.071	0.791	**0.037**	0.305	0.221	**0.001**
Y x I	0.775	0.530	**0.013**	0.120	0.082	0.849	0.236	**0.001**	0.083	**0.015**	0.192	**0.002**	0.205	0.882
Y x LR	0.377	0.102	0.870	0.589	**0.026**	0.934	0.214	0.837	0.209	**0.040**	**0.009**	**0.011**	**0.024**	**0.016**
I x LR	0.324	0.489	0.652	0.104	0.379	0.071	0.935	0.309	0.523	0.590	0.973	0.517	0.913	**0.001**
Y x I x LR	0.501	0.536	0.915	0.348	0.059	0.482	0.056	0.062	0.260	0.061	0.160	0.055	0.162	0.281

In case of significance of the F test, within-column and within factor mean separation was performed by t-test, p = 0.05.

This scenario is further complicated by several significant Y x I (Fig. S2), Y x LR (Fig. S3), and I x LR (Fig. S4) interactions. Regarding the significant Y x I interactions, all of them (Fig. S2A-D) were ordinal, indicating that the effect of one independent variable change in magnitude across levels of another, while the direction of the effect remains consistent.

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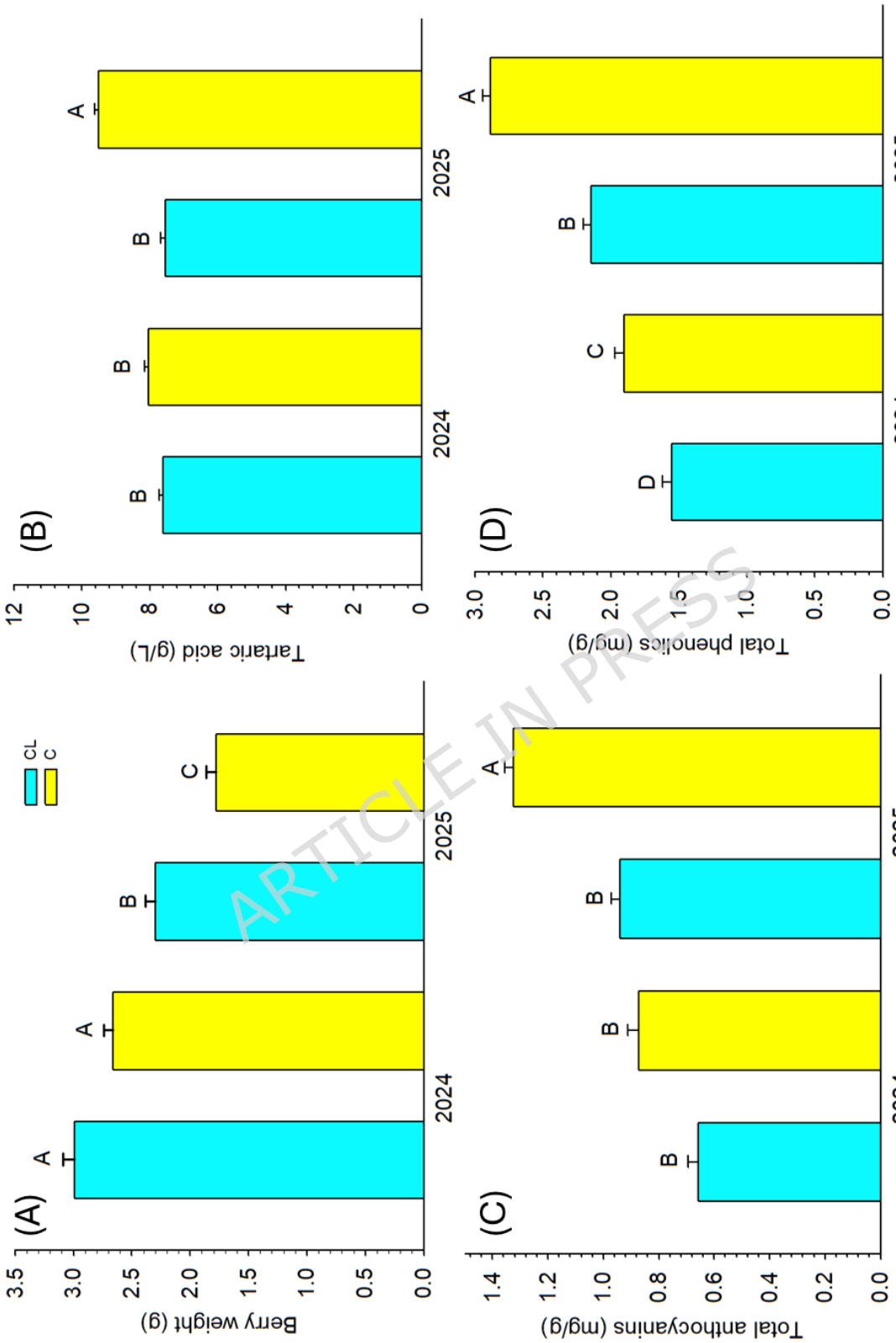


Fig. S2. Partitioning of the significant Y x I interaction detected at harvest for berry weight (A), tartaric acid concentration (B), total anthocyanins concentration (C) and total phenolics concentration (D). C = control; CL = cooled. Vertical bars indicate interactive standard errors (SE) Means separation among the four treatment combinations was performed by Tukey (HSD) post hoc test (n = 16, p = 0.05).

The nature of the Y x LR interaction was more variable: in only one instance (Fig. S3B) was the ordinal interaction confirmed, as leaf removal, regardless of the season, consistently increased

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the incidence of berry sunburn. The Y x LR interactions for TSS (Fig. S3A) and total phenolics concentration (Fig. S3D) were semi-ordinal, with a significant difference between the two independent variables in a single year. Lastly, the Y x LR interaction for total anthocyanins concentration (Fig. S3C) was dis-ordinal or cross-over, meaning that one independent variable reverses direction depending on the level of the other.

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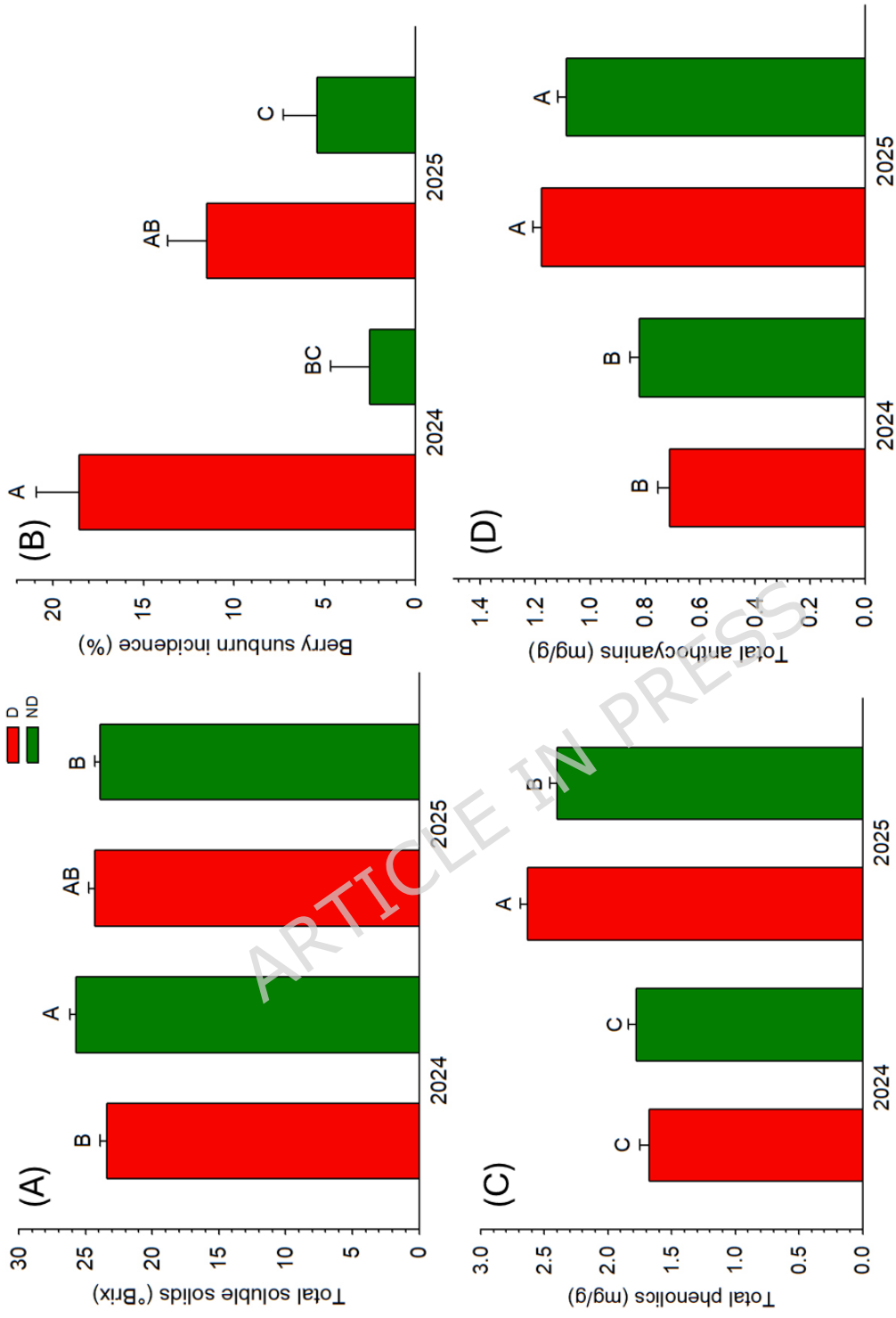


Fig. S3. Partitioning of the significant Y x LR interaction detected at harvest for total soluble solids (A), berry sunburn incidence (B), total anthocyanins concentration (C) and total phenolics concentration (D). D = defoliated; ND = non defoliated. Vertical bars indicate interactive standard errors (SE) Means separation among the four treatment combinations was performed by Tukey (HSD) post hoc test (n = 16, p = 0.05).

When cooling was applied, the incidence of berry sunburn remained consistent at 5.5%, regardless of leaf removal. In contrast, without cooling, having a basal leaf cover was crucial to significantly reduce berry sunburn compared to the highest sunburn level, which reached nearly 25% under the C-D treatment (Fig. S4).

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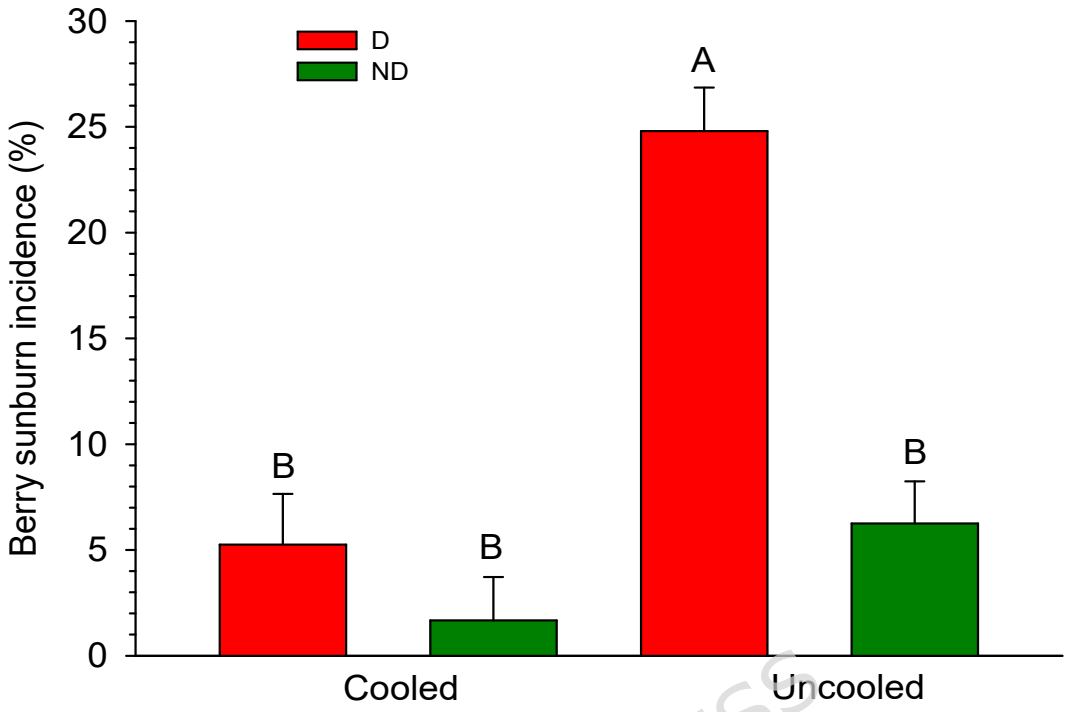


Fig S4. Partitioning of the significant I x LR interaction detected at harvest for berry sunburn incidence. C = control; CL = cooled; D = defoliated; ND = non defoliated. Vertical bars indicate interactive standard errors (SE) Means separation among the four treatment combinations was performed by Tukey (HSD) post hoc test (n = 16, p = 0.05).

4. Discussion

The discussion of the results obtained in this two-year study will begin by evaluating the benefits of maintaining a full leaf cover around clusters from the onset of veraison until harvest, at the main effects level. Subsequently, the additional advantages of a localized cooling system, both with and without basal defoliation, will be addressed.

A recent review paper[13] discusses the need to reconsider summer pruning practices in light of climate change. In warm-to-hot climates, the extensive removal of basal leaves, particularly when done late in the season when canopy regrowth is unlikely and changes in cluster microclimate may become permanent, is now viewed more conservatively. Additionally, extensive research indicates that early defoliation (at the end of flowering) in Chardonnay, as opposed to late defoliation (at veraison), leads to significantly less sunburn damage[9,18] due to higher concentrations of flavonoids in the berries, which serve as sunscreen compounds. However, in

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our study, we intentionally opted for a late and complete basal leaf removal to create the most challenging scenario compared to retaining all leaves.

Regarding cluster microclimate and berry sunburn susceptibility, a significant I x LR interaction (Fig. S4) reveals key findings: i) without cooling, D led to the highest sunburn fraction (24.5%), proving quite detrimental, and ii) preserving basal leaves was crucial in significantly reducing the sunburn fraction. Notably, the presence of basal leaves lowered cluster T by 3.0°C and 3.5°C on average during morning and afternoon readings, respectively, indicating that this level of cooling was sufficient to limit sunburn under the study's conditions. This conclusion is further supported by the ordinal I x LR interactions (Fig. 2), where, in all six cases representing T_{mean} or T_{max} taken in the afternoon, T cluster in ND is consistently lower than in D. In a similar two-year experiment on Pignoletto white grapes in the Po Valley (Northern Italy), basal leaf removal at veraison caused cluster heating of +2.65°C compared to non-defoliated vines[28].

The impact of basal leaf removal on yield components and final grape quality was primarily limited to a reduced final yield, which could also be attributed to increased berry sunburn. In D, berry weight was nearly statistically significant ($P = 0.052$). Theoretically, the smaller berry size might be due to a post-veraison restriction on berry expansion, linked to the source limitation caused by leaf removal. Although the removed leaves are the oldest in the canopy and thus have sub-optimal photosynthetic rates[31], it is important to note that these basal leaves are also the largest. Consequently, when data is expressed on a per-leaf basis, it suggests that canopy photosynthesis can be significantly reduced[32], thereby imposing some limitations on residual post-veraison berry growth.

However, the gas exchange data and final ripening observed in both D and ND vines suggest a limited vine reactivity to late-season leaf removal. Firstly, considering the gas exchange readings collected in 2024 and 2025 (a total of ten dates), only on one occasion (28 August 2024) did the leaf assimilation in the D vines surpass the rates recorded at similar leaf positions in ND. This indicates a modest effect of photosynthetic compensation, which in grapevines is often triggered by the removal of a portion of the leaf area, as extensively documented in previous studies[32,33]. Secondly, it is noteworthy that at no point during both seasons were significant I x LA interactions

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found for gas exchange variables; this suggests that cooling had a predominant effect on vine physiology, while late basal leaf removal was a less significant factor.

The higher concentration of tartaric acid observed at harvest in D vines is likely due to less dilution compared to the ND treatment. However, the truly intriguing finding concerns the response of total anthocyanins and phenolics over the two-year period. The significant Y x LR interaction found for these parameters suggests that the effect of defoliation on pigment accumulation was rather inconsistent, as indicated by the cross-over interaction types (Fig. S3C, D). This behavior contradicts the abundant evidence suggesting that high temperatures, when combined with high light, rather than high light alone, are primarily responsible for reduced pigmentation in grape berries, which might result from either inhibited synthesis or degradation of already formed pigments[7,8,34]. The cultivars covered in previous work were Merlot and Cabernet Sauvignon; however, another study[16] examined the effect of leaf removal on anthocyanin and flavonol composition at harvest in four red Vitis vinifera L. varieties: Cabernet Sauvignon, Nero d'Avola, Raboso Piave, and Sangiovese, and found that the concentration of total anthocyanins in berries did not vary among control and defoliated vines in all varieties across both vintages. The conclusion is that the phenolics response to high light and temperature conditions is strongly genotype-dependent, and Barbera should definitely be included in the low-responding category. It can therefore be concluded that, although the seasonal cluster maximum temperature recorded in the morning in D under no cooling was 45.7°C, and the same calculation made for afternoon readings right after cooling led to 40.4°C, such values were, in fact, not limiting for berry pigmentation.

The primary reason for conducting a two-factor study was to assess any marginal benefits from using a cooling system compared to the more cost-effective approach of simply retaining basal leaves around the clusters. We anticipated that cooling might have a significant effect under conditions of leaf removal, while its impact would be less pronounced when leaves were retained. Statistically, such a pattern would be indicated by a high number of I x LR interactions. However, these interactions were significant in only two instances: the incidence of berry sunburn (Table 4) and several cluster T readings, as illustrated in Fig. 2. The latter showed an ideal response; when vines were left undefoliated, the presence or absence of a cooling system did

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not affect sunburn incidence, which remained below 6% (Fig. S4). In contrast, under D conditions, cooling was crucial in significantly reducing sunburn incidence.

The effectiveness of the cooling system is best demonstrated by the I x LR interaction for cluster heating (Fig. 2). At no point, whether in the morning or afternoon, did the CL-ND treatment combination result in a higher cluster temperature than the CL-D combination. This indicates that the presence of leaves around the cluster did not impede the cooling effect. In fact, in some instances, the cluster temperature in CL-ND was even lower than that in CL-D, likely because the initial warming of the fully exposed clusters reached or exceeded the 50°C threshold. When cooling efficacy is calculated as the temperature difference between CL and C across the two defoliation levels, it results in a reduction of 12.3°C. This cooling potential is significantly greater than the 4.65°C reduction observed in a recent similar study on Pignoletto[28].

Apart from the aforementioned aspects, gas exchange, water relations, yield components, and final grape composition did not exhibit any significant I x LR interaction, indicating that cooling operated independently of leaf removal. Therefore, the discussion should focus on its primary effects. The most noteworthy and original finding related to gas exchange readings over the two seasons is that, under decreasing leaf Ψ_{MD} (2024 and 2025) and leaf Ψ_{PD} (2025), cooling was able to maintain both A and g_s rates (FigS. 3B, C). Since no soil moisture assessment was conducted in 2024, the hypothesis was that, regardless of whether basal leaves were removed, continuous canopy refreshing might have preserved the function of the upper leaves (approximately nodes 7 to 10), mitigating the impact of atmospheric water stress. This effect also helps explain why, on August 28, 2024, A and g_s measured in D were higher than the values recorded for ND (Table 3). However, in 2025, the inclusion of Ψ_{PD} readings added complexity to the scenario. Indeed, on the three measuring dates immediately following cooling activation, the overall medium-to-high A rates recorded in CL corresponded well with moderate soil water stress. Conversely, on the same dates, A and g_s in the C treatment were almost shut down, and Ψ_{PD}, which dropped to nearly -1.0 MPa, played a role, suggesting a severe stress level. The conclusion is both surprising and pleasing: there is no doubt that the total amount of water delivered in 2025 with the cooling system (230 mm), with 25.9% of it reaching the ground, implies that about 60 mm of water reached the soil and potentially served an irrigation function. Supporting this hypothesis, the

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following should be considered: i) the under-the-row strip was mostly weed-free, and the frequent wetting might have favored water infiltration; ii) vines grafted on SO4 rootstock typically display a fairly shallow root system, which, under the circumstances of this study, is favored by a Silty-Clay-Loam soil with a water holding capacity estimated at around 170 mm/m; iii) it is well known that Ψ_{PD} tends to equilibrate with any wet part of the root system, even if this represents a smaller fraction of the total root volume[35,36]. Despite the large differences found in Ψ_{PD}, A, and g_s between CL and C, leaf Ψ_{MD} was rather unresponsive; it is hypothesized that cultivar-dependent stomatal sensitivity to increased VPD is a primary controlling factor. Alongside behavior resembling iso-hydric adjustment, VPD-driven stomatal closure might also explain why the C treatment maintained a Ψ_{MD} similar to the CL treatment. Our data from 2025 offer unique evidence suggesting that the cooling system could serve as a notable source of soil water supply under certain environmental and cultural conditions. We are unaware of any prior contributions on this topic; field studies on canopy cooling have either ignored this aspect[25,37] or, as in the case of data collected on Semillon[38], concluded that the fine mist, when in direct contact with the vegetative surface, evaporates quickly without reaching the soil.

Nonetheless, it appears premature to label the cooling system as a dual-function strategy beforehand. The experimental design used does not include a treatment solely for soil irrigation, nor does it evaluate the interaction between cooling and soil irrigation. As a result, it is not possible to identify the specific effects of each individual factor without introducing bias.

The marginal gain from using a cooling system compared to a simple natural leaf cover requires a careful examination of its effects on yield components and must composition (Table 4). Overall, the effects of cooling fell short of expectations: although berry and cluster weight increased in line with other studies[24,28], the yield per vine did not show a statistically significant difference between CL and C. Similarly, technological maturity was comparable, although CL retained higher levels of malic acid, which could be advantageous when cultivating for sparkling wine in warm areas. However, this is not the case for Barbera, which is notably adept at maintaining high TA levels even during hot seasons[39].

Unexpectedly, the CL main treatment resulted in lower levels of color and phenolics compared to the C treatment. This outcome might be partially attributed to the larger berry size observed

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in CL, which likely results in a less favorable skin-to-flesh ratio. However, the significant cooling effect induced by the foggers was anticipated to enhance berry pigmentation. In Sangiovese and Montepulciano cultivars, Valentini et al.[26] found that cluster fogging combined with a 50% water supply (WS) based on vine water use significantly increased anthocyanin concentration over two seasons compared to a treatment with WS alone. In contrast, our study with Barbera presents a different conclusion; if cooling is achieved through leaf maintenance without wetting, color might be slightly affected. Conversely, if cooling is present, pigmentation is limited regardless of the presence or absence of basal leaves.

The literature on this topic, specifically regarding cooling applied to red-grape cultivars, is not comprehensive. An intra-canopy sprinkler system tested at the cluster zone on Cabernet Sauvignon vines[24] showed similar anthocyanin concentrations in both cooled and uncooled control treatments over two years. However, in 2015, the cooled treatment exhibited a higher content due to increased berry size. Similarly, in a study on Carignan, Kliewer and Schultz[22] found little difference in anthocyanin concentration between sprinkled and control treatments.

In our study, the inability of the cooled treatments to enhance anthocyanin concentration, despite a notable decrease in surface cluster temperature, redirects attention to other influencing factors. The most likely factor is the extended wetting condition that clusters experienced during the daily cooling event (Table 1). It is well-documented that the air VPD surrounding the cluster is the primary driver of berry transpiration[40,41]. In grape berries, as in other fleshy fruits, transpiration contributes to fruit weight loss[42] and affects fruit ripening and solute accumulation[43]. A recent experiment by Cabodevilla et al.[44] examined Tempranillo grapevine cuttings exposed to varying VPDs, including two treatments with day/night conditions set at 28°C/18°C and 43%/58%, with or without the application of the anti-transpirant di-1-p-menthene. When the chemical was applied, berry transpiration was significantly reduced, and total anthocyanins decreased from 521 g L^{-1} (in the absence of anti-transpirants and with high transpiration) to 364 g L^{-1}. Further consistency with these results was recently provided by Martinez-Lüscher et al.[45], who worked on Malbec and reduced berry transpiration using heavy shading nets and an anti-transpirant. Consequently, these two treatments resulted in reduced anthocyanin and flavonol content in the berries. In our study, prolonged berry wetting acted as

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the operative factor, likely causing a VPD drop and, in turn, reduced berry transpiration. Overall, several findings align with the foundational work of Zhang and Keller[40], which demonstrated that berry ripening—characterized by solute accumulation and color change—was significantly impaired in Syrah, Merlot, and Concord grapes when berry transpiration was partially inhibited by immersing clusters in an anti-transpirant solution. These findings further support the notion that the accumulation of sugar and color in ripening grape berries requires the removal of excess phloem-derived water, either through transpiration across the berry skin or via xylem backflow.

A third factor that, along with the already covered dilution/concentration and metabolic mechanisms, might have contributed to the flavonoid response of CL and C treatments is that, according to the literature, light to moderate water stress, especially when occurring pre-veraison, can stimulate the overexpression of genes encoding for flavonoid compound synthesis such as anthocyanins, terpenes, and isoprenoids[46]. In our study in 2025, pre-veraison variation of leaf function and soil water stress among treatments was minimal (Figure 3), shifting attention to the post-veraison time frame, for which the response of anthocyanins to water stress becomes much more variable and less clear-cut. For instance, Sangiovese vines were overall unresponsive in terms of gene regulation to a post-veraison severe water deficit, and no improvement in berry color was observed compared to a well-watered treatment[47,48]. Additionally, an experiment conducted on the Tempranillo grape in Spain[49] suggested that severe late water stress, which significantly impairs the photosynthetic function of leaves, can lead to a reduction in berry sugar and color accumulation at harvest. Data collected in the same direction on Barbera grown in the Piacenza area clearly show that the sugar and color levels reached in 2003—which was a historic year for record thermal and radiation levels—were significantly reduced compared to those achieved in the relatively cooler 2002 and 2024 seasons[39].

The final topic of discussion is whether adopting a localized cooling system offers significant advantages compared to the conservative approach of a dry-farmed vineyard without leaf removal. Our two-year study on Barbera suggests that the use of an automated cooling system is questionable for several reasons: i) the total yield per vine did not increase in the irrigated vines, whereas it did in the non-defoliated plots compared to the D plots; ii) TSS and TA at harvest were unaffected by either of the two experimental factors; iii) ND was only slightly less effective

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than cooling in reducing the incidence of berry sunburn; iv) most importantly, while the presence or absence of late-season basal defoliation had a neutral effect on phenolics, cooling led to a reduction in berry pigmentation, which could be problematic for a variety like Barbera, known for its poor color accumulation. Overall, the only distinctive effect on ripening attributable to cooling is the higher retention of malic acid at harvest, which might be desirable in the context of sparkling wine production.

The total amount of water delivered by the system in a season clearly depends on several parameters, including the number and flow rate of emitters, whether applications are above or within the canopy, the climate settings for automated system activation, and the duration of the chosen intermittency, among others. In this study, activating the T and RH thresholds and setting the intermittency to a 60sec/sec120 on/off cycle resulted in a notably generous water supply (4.1 mm/day in 2024 and 6.96 mm/day in 2025) compared to the delivery rates reported in similar studies[25,28,37]. Given the significant cooling effect observed on clusters (-12.4 °C compared to uncooled clusters), it is quite plausible that a 60sec/240sec on/off cycle could have been equally effective while conserving half of the water supply.

5. Conclusions

A two-year study conducted on Barbera to explore the interactions between different levels of basal leaf removal and the presence or absence of a nebulized cooling system indicated that each factor operated quite independently. However, the responses varied significantly when considering physiological data versus vine performance data.

Notably, in both seasons, the cooling system effectively preserved leaf assimilation rates compared to the uncooled vines. Even more significant was the fact that, in 2025, a significant portion of water supplied by the foggers reached the ground, enhancing soil moisture in the rooting volume, as clearly demonstrated by the substantial differences measured in the pre-dawn leaf water potential. This effect is also crucial from an operational perspective, as the cooling system itself fulfills the role of "ordinary irrigation"—a task that poses challenges in countries lacking a true irrigation culture, such as France and many districts in Italy. Indeed, growers may find some relief upon realizing that a well-designed and properly programmed nebulized cooling system can also serve as a tool for standard irrigation. However, this dual

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function should not be taken for granted, as it clearly depends on the total amount of nebulized water, which in turn is influenced by weather conditions, threshold activation, and likely, soil type and under-the-row soil management. To better understand the benefits of reusing water from the nebulization system, future research should incorporate distinct treatments for cooling and soil irrigation. This approach will allow for the independent evaluation of their effects and enable a thorough investigation of any statistical interactions.

The positive aspects of the cooling system diminish when evaluating its impact on vine performance. There was no significant variation in yield, TSS, and TA, and, even though leaf function remained consistent from post-veraison to harvest, the concentration of anthocyanins and phenolics was lower compared to the uncooled vines. In the study site, this does not seem to justify the use of a cooling approach.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Figure Captions

Figure 1. Daily maximum air temperature (T_{max} as °C), air relative humidity (RH as %) and precipitation (mm) recorded at the experimental site by the SAPIR-2 and Netsense weather stations in 2024 (top) and 2025 (bottom) for the period comprised between DOY 183-274. Horizontal dotted lines identify climatic thresholds chosen for foggers activation (i.e. T max ≥ 33 °C and RH ≤ 55%).

Figure 2. Partitioning of the significant I x LR interaction detected for cluster T_{max} and T_{mean} at different dates during afternoon 2025 measurements. D = defoliated; ND = non defoliated. C = control; CL = cooled. Means separation among the four treatment combinations was performed by SNK test (n = 12, p = 0.05).

Figure 3. Seasonal variation of T_{LEAF}, (A) leaf assimilation (B), leaf stomatal conductance (C), intrinsic water use efficiency (D), midday leaf water potential (E) and pre-dawn leaf water potential (F) recorded over seven dates in 2025 in uncooled control (C), cooled (CL) non defoliated (ND) and defoliated (D) main treatments. Arrow indicates activation of the cooling system. Across different dates and variables, ND and D never differed; asterisk indicates at which dates C and CL differed according to t-test (p = 0.05).

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Supplementary figure captions

Figure S1. Front view of a non-defoliated (left) and a defoliated (right) Barbera canopy both equipped with a fogger which can be spotted just above the cordon.

Figure S2. Partitioning of the significant Y x I interaction detected at harvest for berry weight (A), tartaric acid concentration (B), total anthocyanins concentration (C) and total phenolics concentration (D). C = control; CL = cooled. Vertical bars indicate interactive standard errors (SE) Means separation among the four treatment combinations was performed by Tukey (HSD) post hoc test (n = 16, p = 0.05).

Figure S3. Partitioning of the significant Y x LR interaction detected at harvest for total soluble solids (A), berry sunburn incidence (B), total anthocyanins concentration (C) and total phenolics concentration (D). D = defoliated; ND = non defoliated. Vertical bars indicate interactive standard errors (SE) Means separation among the four treatment combinations was performed by Tukey (HSD) post hoc test (n = 16, p = 0.05).

Figure S4. Partitioning of the significant I x LR interaction detected at harvest for berry sunburn incidence. C = control; CL = cooled; D = defoliated; ND = non defoliated. Vertical bars indicate interactive standard errors (SE) Means separation among the four treatment combinations was performed by Tukey (HSD) post hoc test (n = 16, p = 0.05).

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Contributions

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P.B. and S.P. conceived the work, P.B., F.T., H.T. took in field measurements, S.P. and P.B. run the statistical analysis, S.P. and P.B. wrote the manuscript. All authors reviewed the manuscript.

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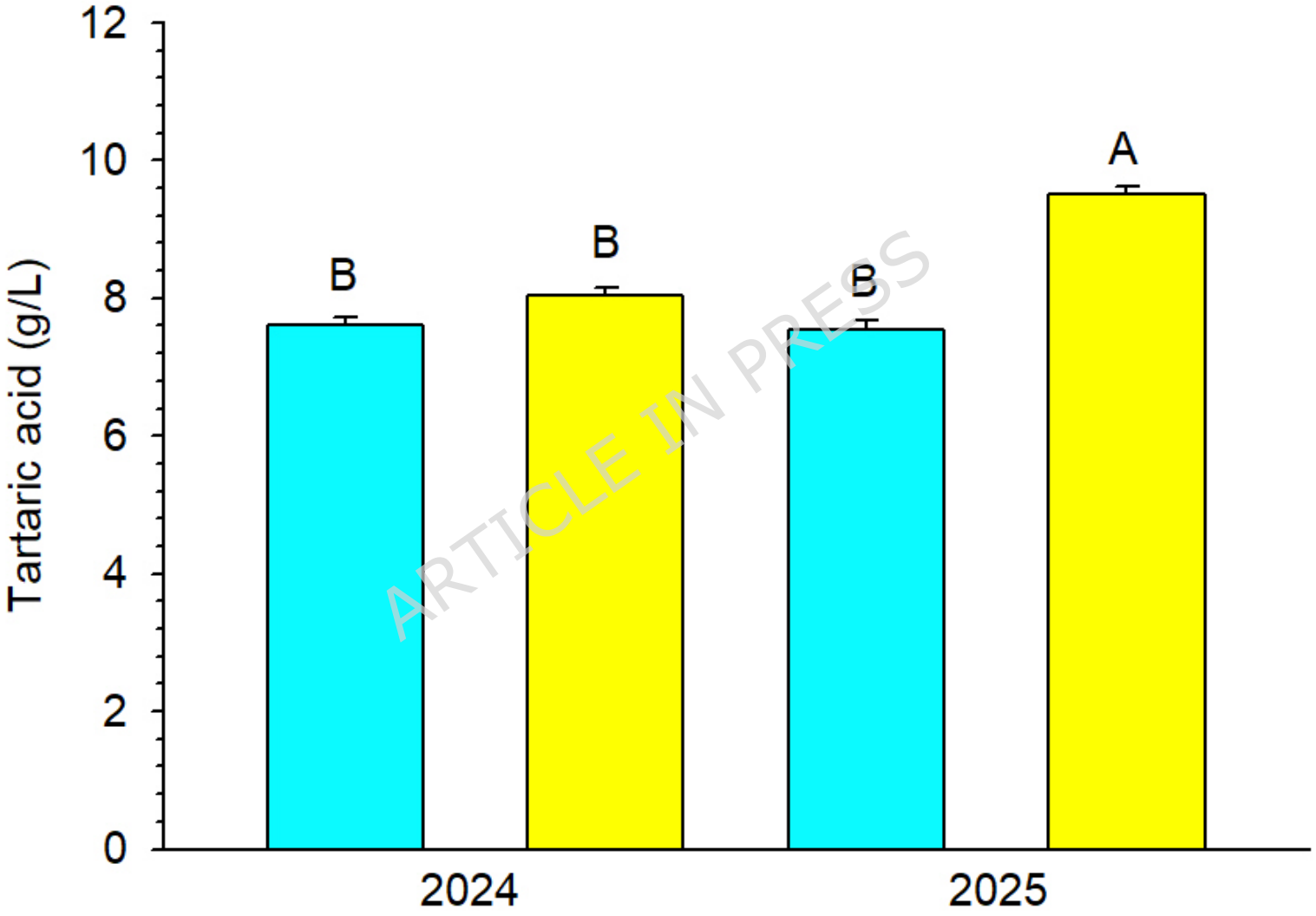
Ethics declarations

Competing interests

The authors declare no competing interests.

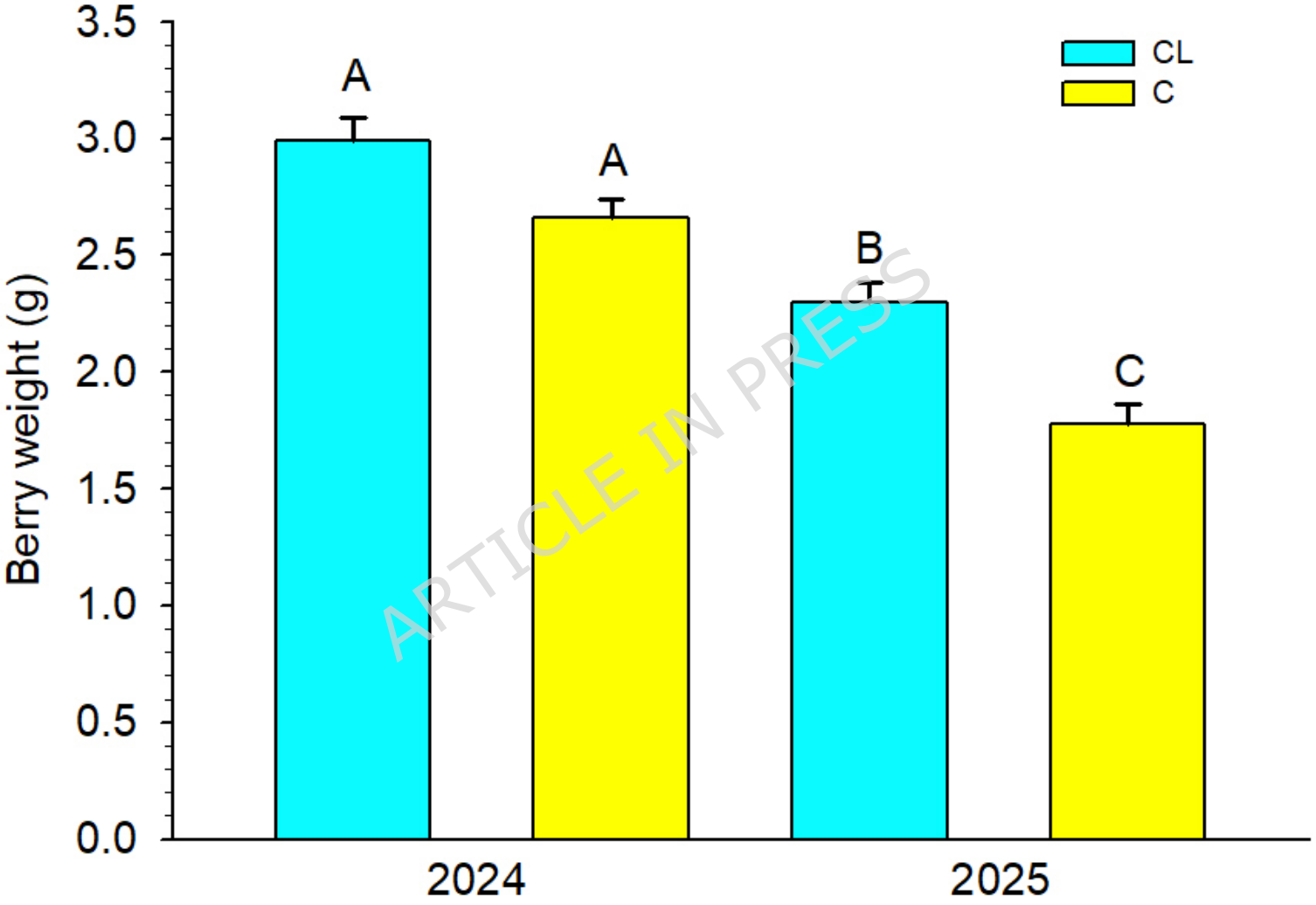
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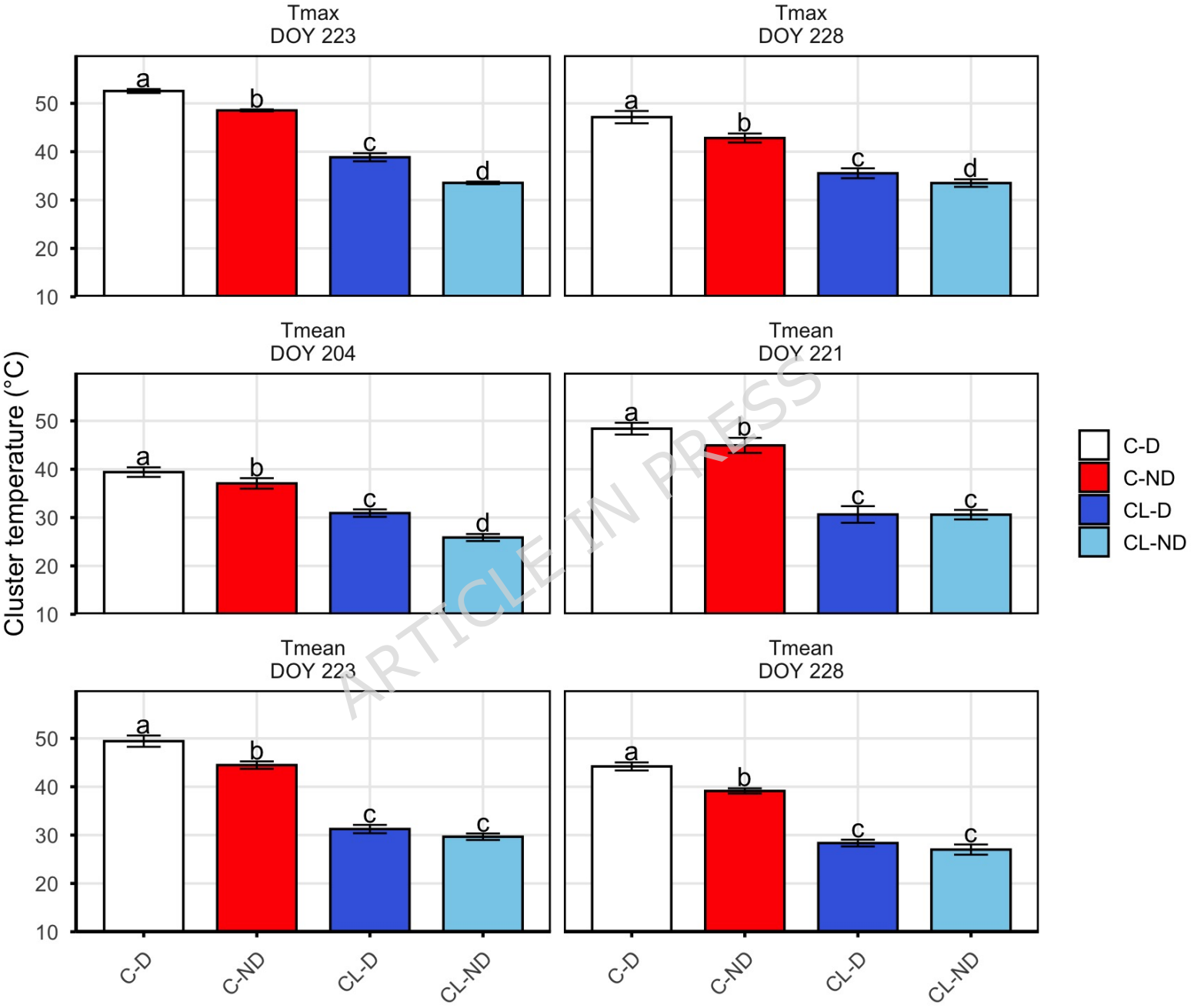
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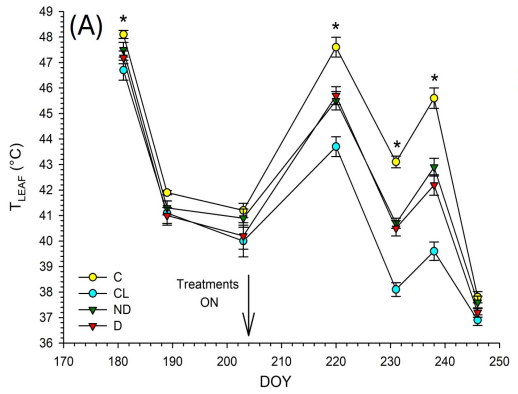
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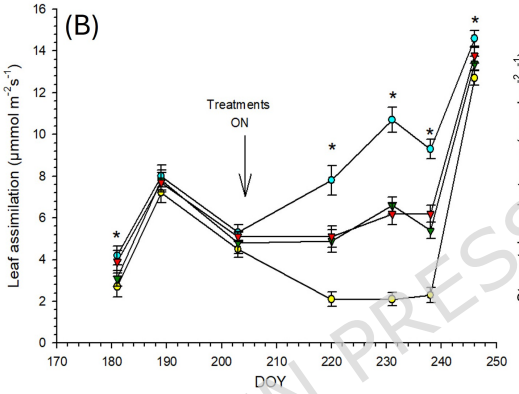
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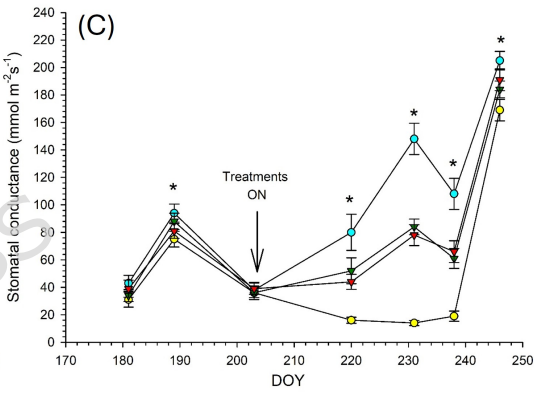


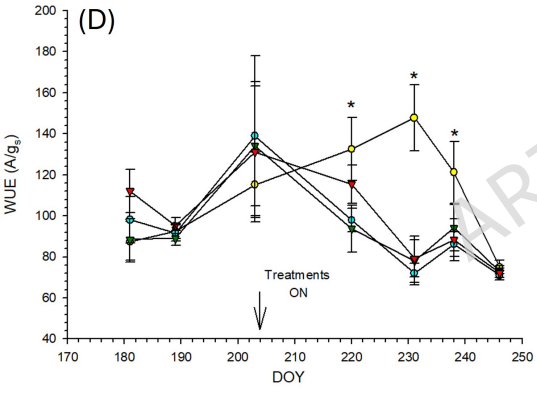
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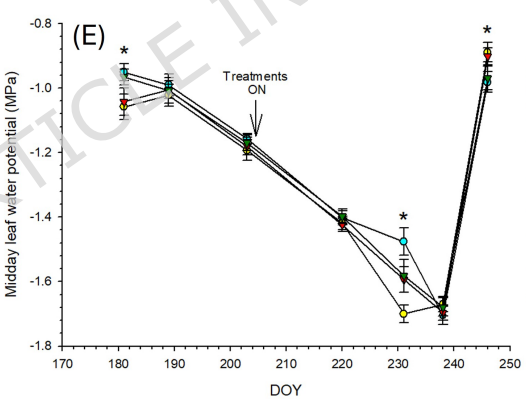
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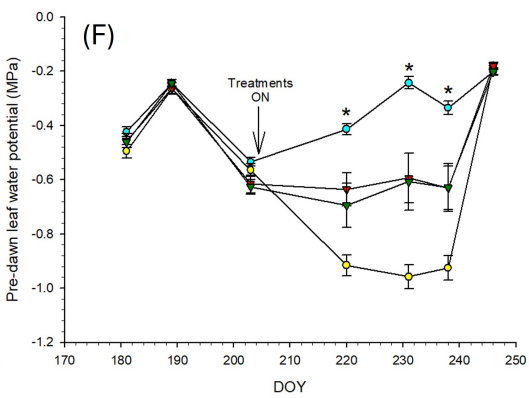




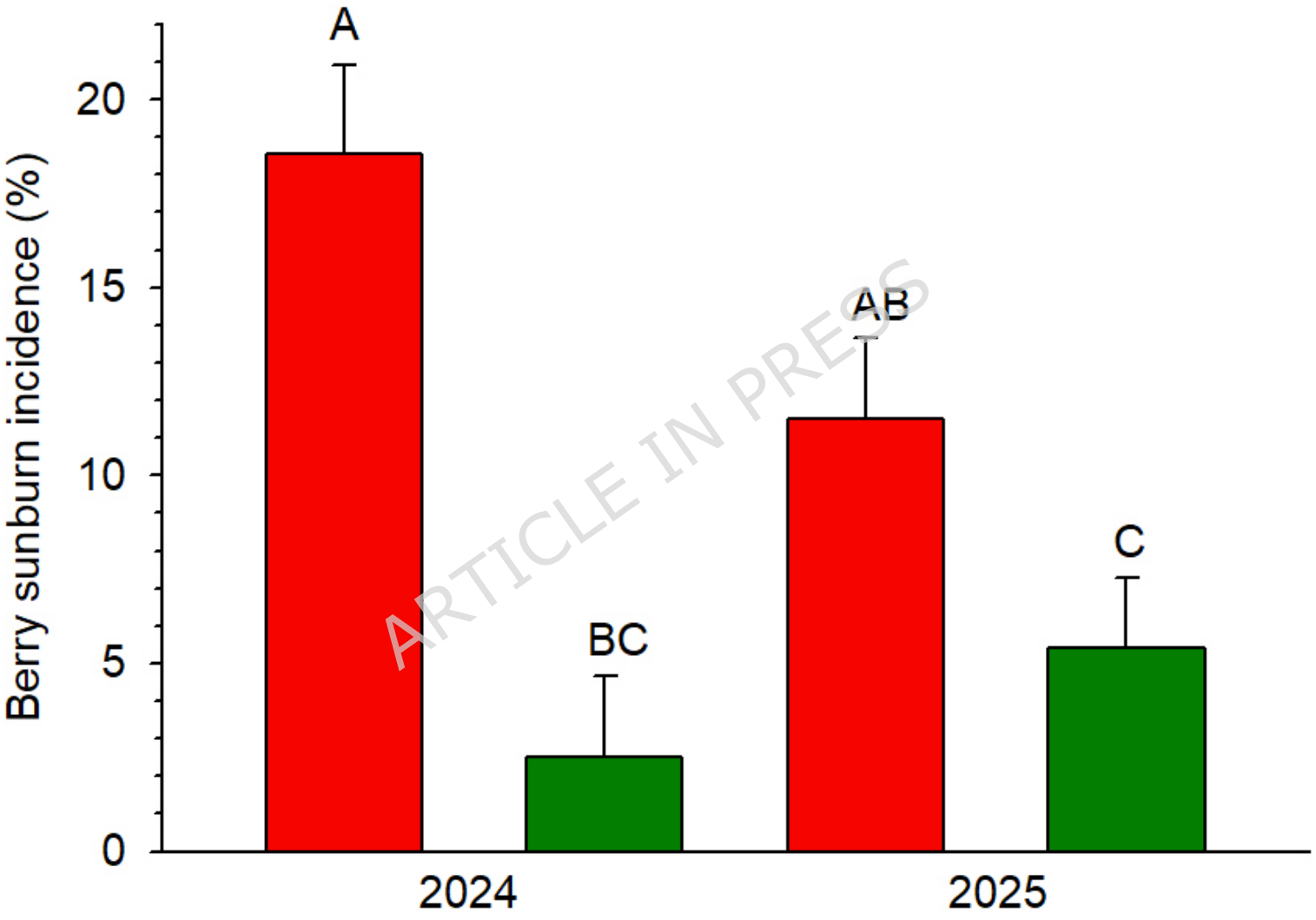






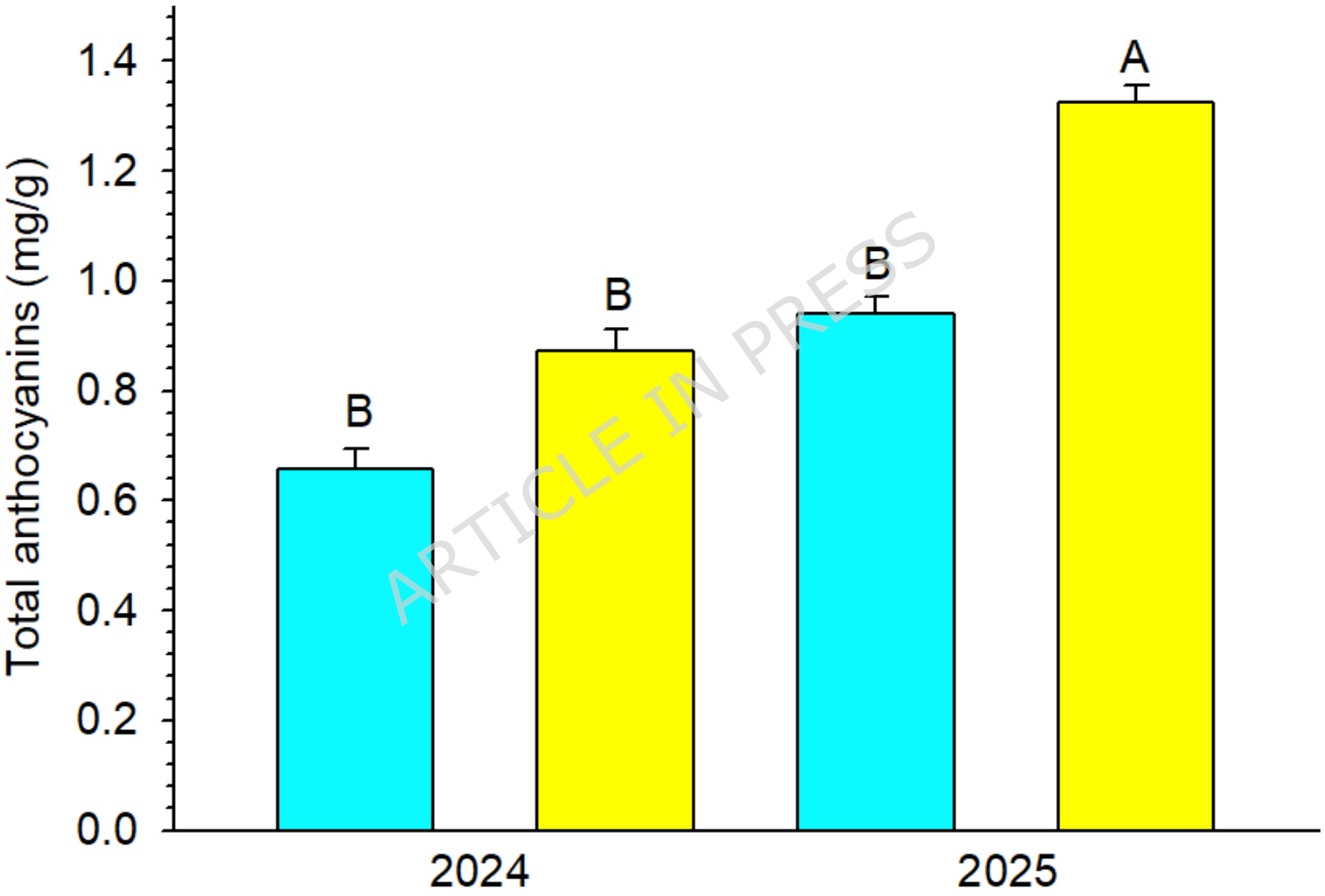


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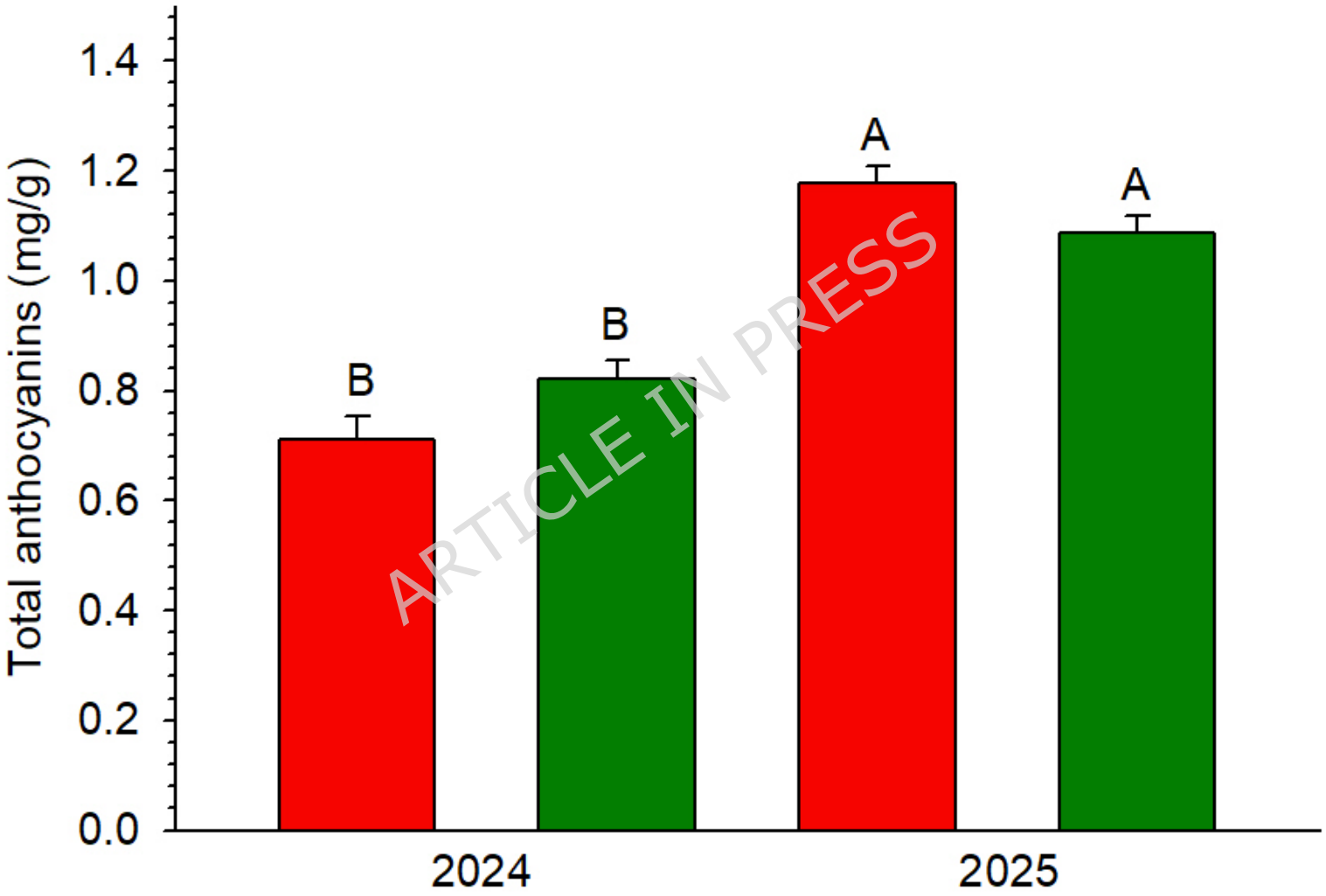
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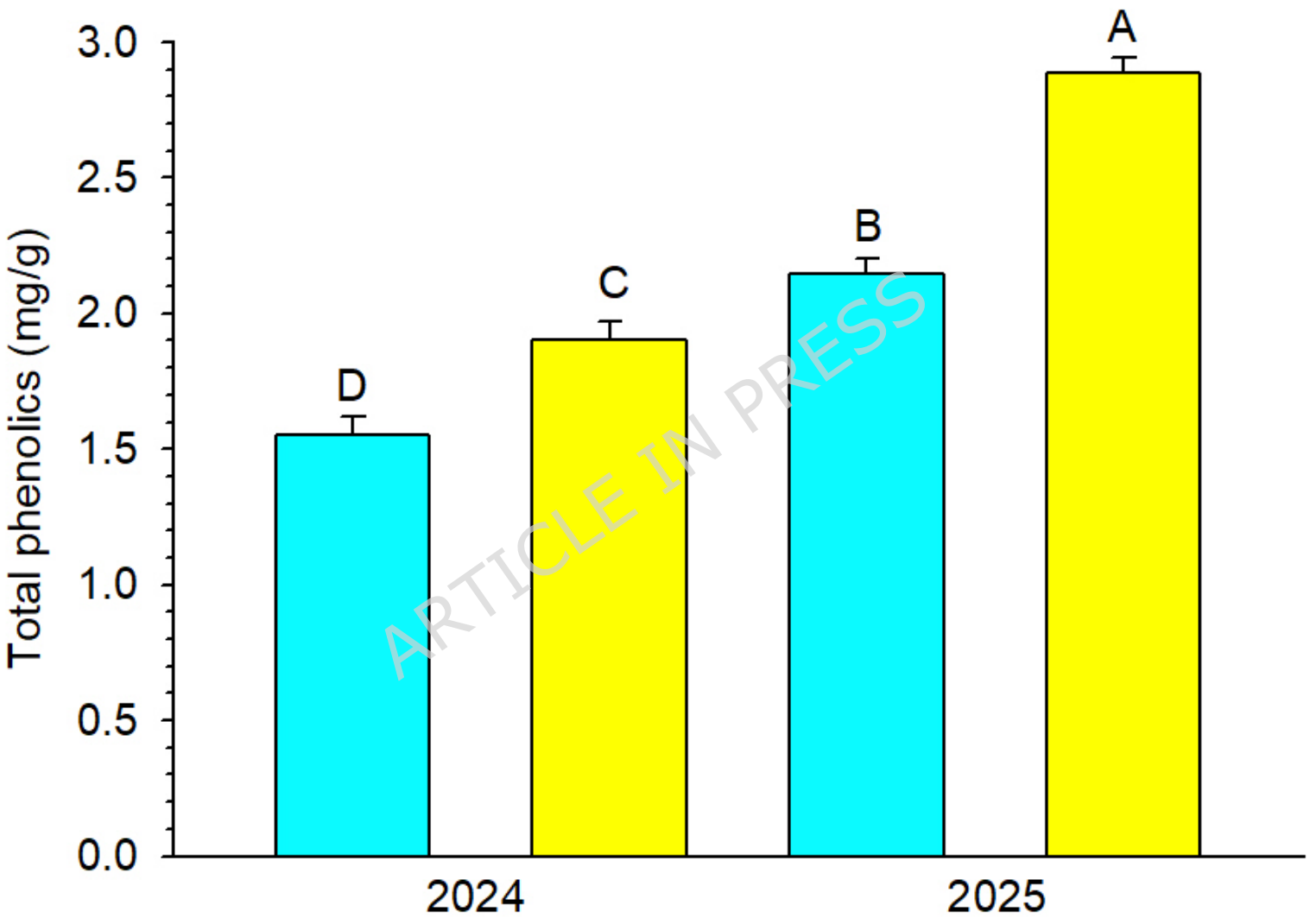
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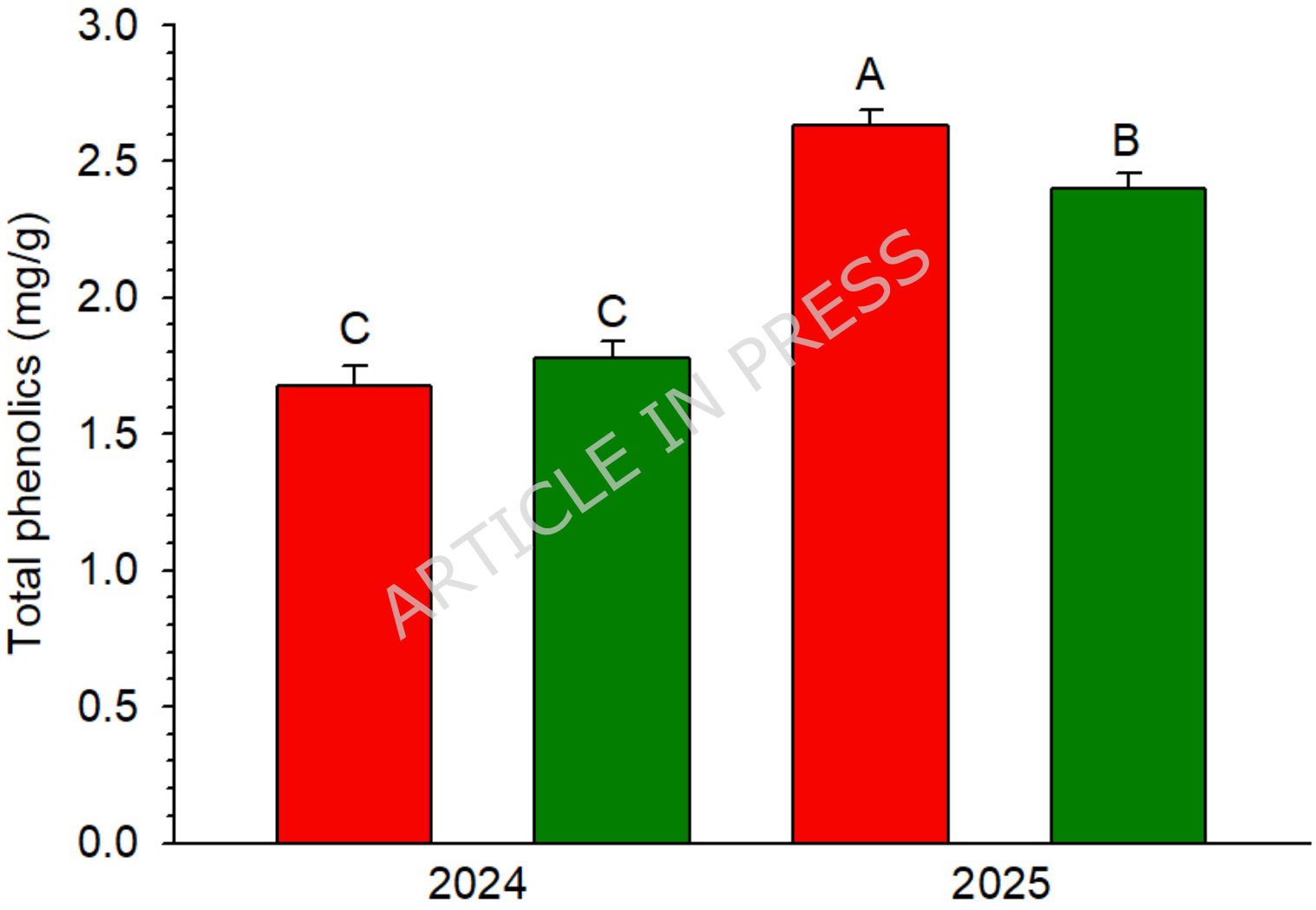
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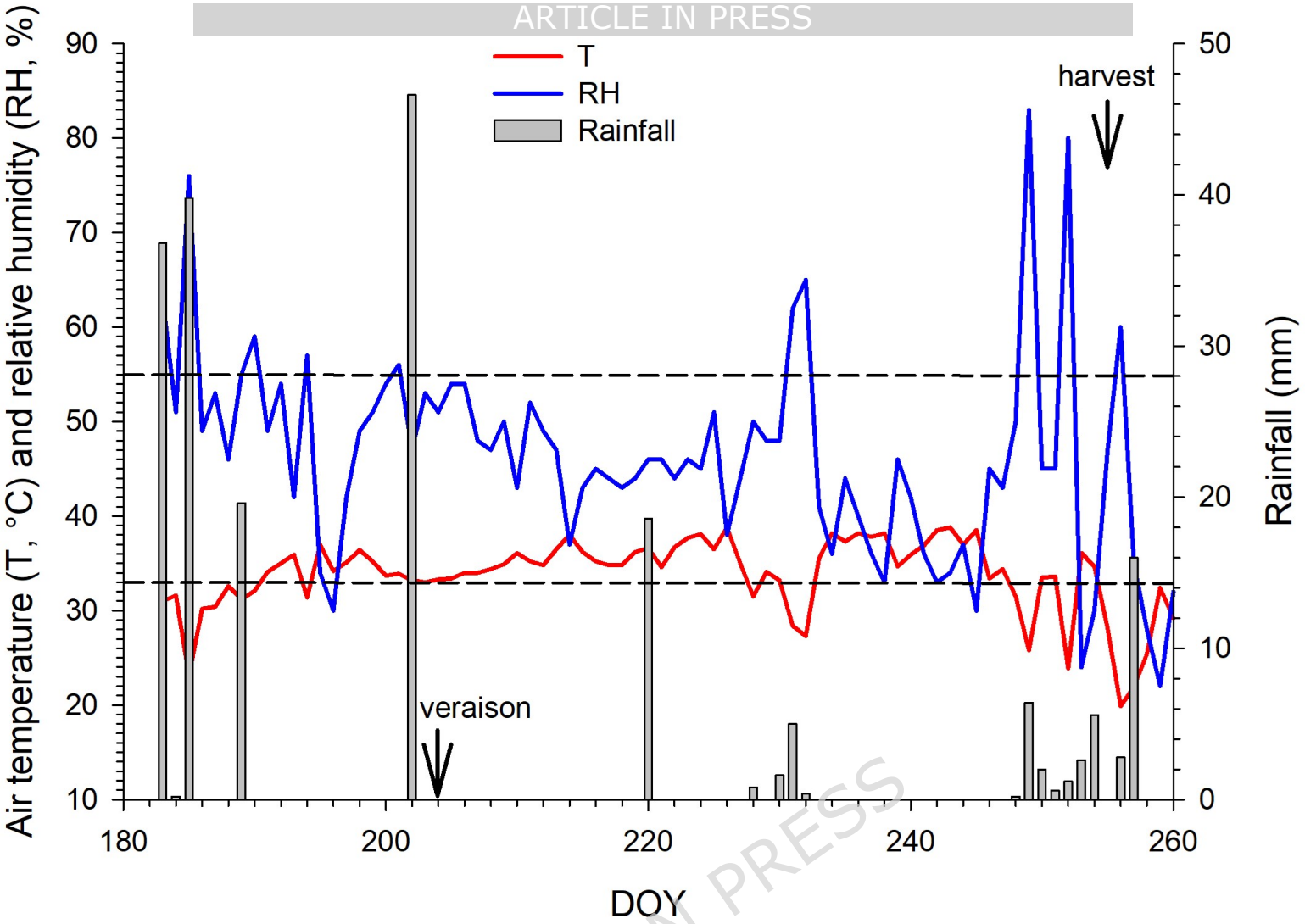
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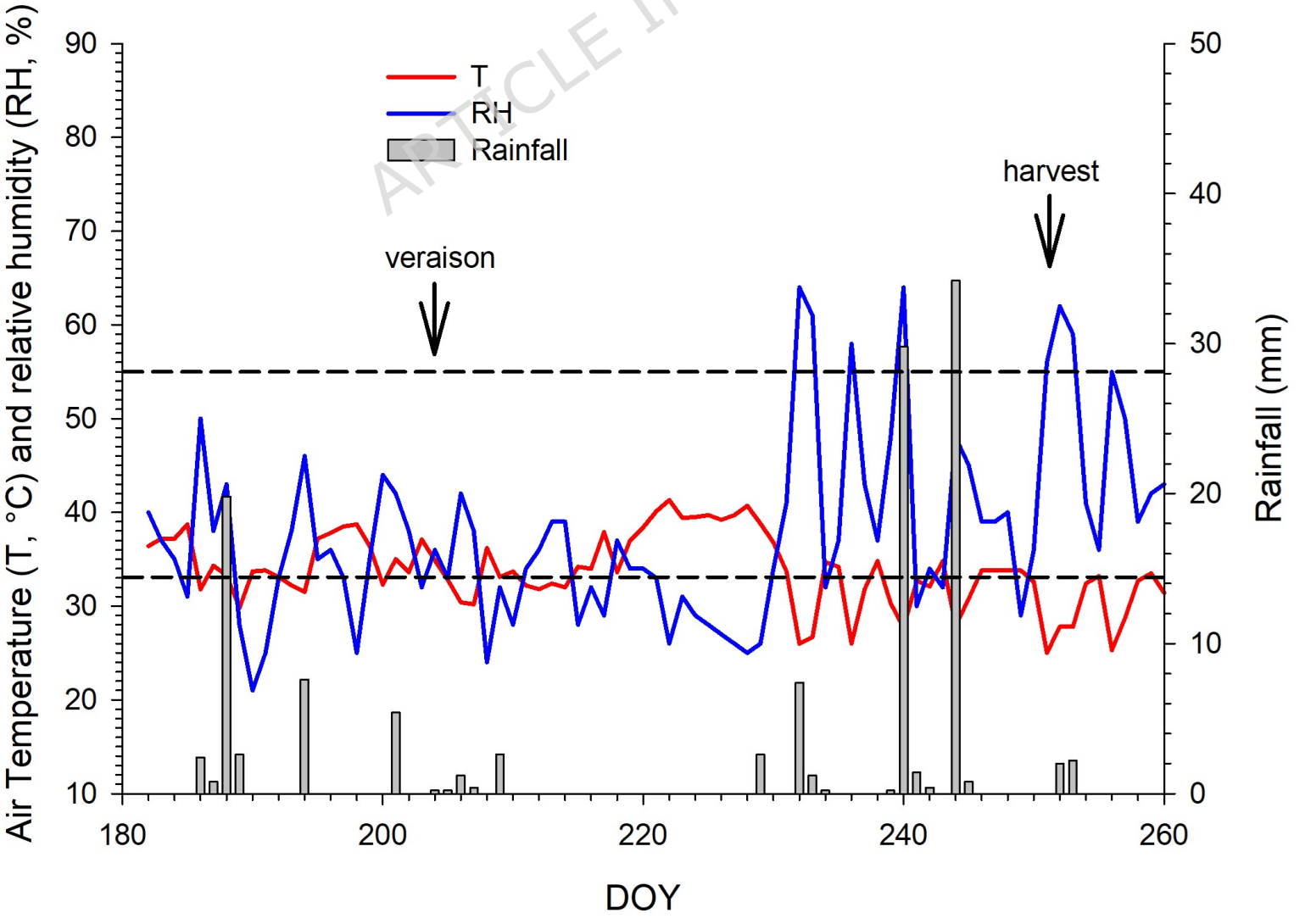


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Table 1. Water volume daily supplied in 2024 and 2025 to cooled Barbera grapevines and expressed on a hectare basis. Calculations refer to an intermittent supply (60sec "on" and 120sec "off" "of" and "off" cycles). The used fogger type delivers 11.2 L/hours. To scale up parcel water supply to a hectare basis, one fogger per vine was considered (i.e. 4444/hectare).

2024				2025			
DOY	Total water supply (m^3/ha)	Time length of foggers "on" (mins)*	Total time length for each "on-off" cycle (mins)	DOY	Total water supply (m^3/ha)	Time length of foggers "on" (mins)*	Total time length for each "on-off" cycle (mins)
204	23.94	20	60	204	118.37	87	261
205	19.00	16	49	205	18.11	13	39
206	33.67	28	84	206	0.00	-	-
207	29.03	26	79	207	0.00	-	-
208	40.10	34	103	208	92.63	63	188
209	50.13	46	137	209	13.17	10	31
210	74.37	68	203	210	41.60	28	85
211	48.93	43	130	211	7.63	3	8
212	36.21	31	93	212	22.90	11	34
213	45.64	48	144	213	10.77	8	23
214	16.16	15	45	214	0.00	-	-
215	45.19	79	236	215	16.76	12	36
216	79.46	70	209	216	22.00	15	46
217	71.97	61	183	217	134.53	98	294
218	82.15	72	216	218	2.24	2	5
219	20.50	24	71	219	61.50	44	131
220	21.10	23	69	220	135.58	96	289
221	59.86	50	149	221	144.56	104	313
222	12.57	10	30	222	189.30	136	408
223	17.36	13	40	223	152.94	117	352
224	15.86	12	35	224	162.07	115	344
225	7.18	5	15	225	154.13	107	321
226	57.31	42	125	226	120.76	87	260
227	48.48	33	100	227	160.12	123	369
228	0.00	-	-	228	147.85	109	326
229	7.03	5	15	229	121.96	94	282
230	0.00	-	-	230	80.66	57	170
231	0.00	-	-	231	16.91	12	35
232	0.00	-	-	232	0.00	-	-
233	27.08	18	55	233	0.00	-	-
234	82.15	72	216	234	41.00	29	86
235	5.98	5	15	235	11.07	8	24

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236	59.05	59	177	236	0.00	-	-
237	90.08	75	225	237	0.00	-	-
238	83.20	69	207	238	43.10	31	93
239	28.73	21	64	239	0.00	-	-
240	31.42	25	74	240	0.00	-	-
241	59.40	51	152	241	0.00	-	-
242	45.49	28	85	242	0.00	-	-
243	134.08	97	291	243	27.09	20	59
244	79.91	60	179	244	0.00	-	-
245	116.57	88	263	245	0.00	-	-
246	2.09	1	2	246	7.18	4	13
247	41.30	31	92	247	8.53	6	17
248	0.00	-	-	248	7.03	5	14
249	0.00	-	-	249	4.94	3	8
250	1.79	2	5	250	0.00	-	-
251	6.43	4	11	251	0.00	-	-
252	0.00	-	-	-	-	-	-
253	21.54	16	49	-	-	-	-
254	10.02	6	18	-	-	-	-
Total	1829.65	1601	4803	Total	2298.99	1654	4962

Table 2. Cluster mean (T_{mean}) and maximum (T_{max}) temperature recorded in the morning (10-12) and afternoon (14-16) of 23 July, 9 August, 11 August, and 16 August 2025 for the two levels of each factor. C = non-irrigated control; CL = cooled; ND = non defoliated; D = defoliated. Probability in bold indicates $p < 0.05$.

MORNING	23 July		9 August		11 August		16 August	
	T_{mean}	T_{max}	T_{mean}	T_{max}	T_{mean}	T_{max}	T_{mean}	T_{max}
Irrigation (I)								
C	38.2 ± 0.57	43.2 ± 0.93	43.3 ± 0.48	48.2 ± 0.66	42.5 ± 0.60	47.8 ± 0.54	43.2 ± 0.68	48.1 ± 0.96
CL	38.9 ± 0.64	44.8 ± 1.07	42.7 ± 0.60	48.6 ± 0.55	41.1 ± 0.56	46.3 ± 0.60	42.2 ± 0.66	48.6 ± 0.70
Pr > F	0.293	0.178	0.227	0.625	0.069	**0.040**	0.259	0.689
Leaf removal (LR)								
ND	37.0 ± 0.47	41.7 ± 0.90	41.4 ± 0.41	46.7 ± 0.45	40.7 ± 0.40	45.7 ± 0.54	41.6 ± 0.67	47.0 ± 0.91
D	40.0 ± 0.50	46.3 ± 0.76	44.6 ± 0.32	50.1 ± 0.41	42.9 ± 0.66	48.3± 0.46	43.8 ± 0.56	49.7 ± 0.57
Pr > F	**0.000**	**0.000**	**0.000**	**0.000**	**0.007**	**0.001**	**0.019**	**0.014**
I x LR (Pr > F)	0.590	0.294	0.500	0.769	0.257	0.978	0.364	0.089

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AFTERNOON	T_{mean}	T_{max}	T_{mean}	T_{max}	T_{mean}	T_{max}	T_{mean}	T_{max}
Irrigation (I)								
C	38.2 ± 0.48	41.6 ± 0.76	46.7 ± 0.66	51.1 ± 0.77	47.0 ± 0.72	50.5 ± 0.53	41.7 ± 0.70	45.0 ± 0.68
CL	28.4 ± 0.70	31.9 ± 0.68	30.6 ± 0.48	36.4 ± 0.93	30.5 ± 0.33	36.2 ± 0.72	27.7 ± 0.35	34.5 ± 0.41
Pr (>F)	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
Leaf removal (LR)								
ND	31.5 ± 1.48	34.6 ± 1.37	37.8 ± 1.90	42.4 ± 1.94	37.1 ± 1.93	41.0 ± 1.94	33.1 ± 1.59	38.2 ± 1.24
D	35.2 ± 1.14	38.8 ± 1.21	39.5 ± 2.35	45.0 ± 2.16	40.3 ± 2.37	45.7 ± 1.78	36.3 ± 2.06	41.3 ± 1.55
Pr > F	**0.000**	**0.000**	**0.019**	**0.027**	**0.000**	**0.000**	**0.000**	**0.000**
I x LR (Pr > F)	**0.006**	0.069	**0.021**	0.314	**0.001**	**0.015**	**0.000**	**0.032**

Means represent data taken on one cluster per vine (n = 8) in the morning hours (10-12) and in the afternoon (14-16), before and after the end of the cooling respectively. In case of significance of the F, within column and within factor separation was performed by t-test, p = 0.05.

Table 3. Leaf function and water status assessed in 2024 on three summer dates as leaf stomatal conductance (gs), leaf assimilation (A), intrinsic water use efficiency (A/g_s) and midday leaf water potential (Ψ_{MD}) in Barbera grapevines assigned to two irrigation (I) treatments (C = uncooled control and CL = cooled) and two leaf removal (LR) treatments (ND = non defoliated and D = defoliated). For each treatment mean (main effects) n = 16. Pr > F values in bold indicate significance at p = 0.05.

	30 July (DOY 211)					21 August (DOY 233)					28 August (DOY 240)				
Irrigation (I)	T_{LEAF} (°C)	g_s (mmol $m^{-2}s^{-1}$)	A (µmol $m^{-2}s^{-1}$)	A/g_s (mmol/mol)	Ψ_{MD} (-MPa)	T_{LEAF} (°C)	g_s (mmol $m^{-2}s^{-1}$)	A (µmol $m^{-2}s^{-1}$)	A/g_s (mmol/mol)	Ψ_{MD} (-MPa)	T_{LEAF} (°C)	g_s (mmol $m^{-2}s^{-1}$)	A (µmol $m^{-2}s^{-1}$)	A/g_s (mmol/mol)	Ψ_{MD} (-MPa)
C	40.1	0.224	10.1	45.1	1.08	46.6	0.064	3.7	53-0	1.27	44.5	0.049	5.2	106.1	1.32
CL	38.4	0.331	11.4	34.4	0.88	44.3	0.158	9.6	60.7	1.24	40.1	0.149	10.8	72.5	1.35
Pr > F	**0.000**	**0.020**	0.087	0.292	**0.001**	**0.000**	**0.000**	**0.000**	0.737	0.468	**0.001**	**0.000**	**0.000**	**0.001**	0.599
Leaf removal (LR)															

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ND	39.5	0.263	10.8	41.1	1.02	46.0	0.099	5.88	59.4	1.25	42.8	0.075	6.9	92.0	1.34
D	38.9	0.293	10.8	36.9	0.94	44.9	0.124	7.42	59.8	1.26	41.8	0.124	9.1	73.4	1.34
Pr > F	0.094	0.491	0.986	0.570	0.147	0.07	0.244	0.057	0.24	0.779	0.168	**0.007**	**0.017**	0.05	0.891
I x LR															
Pr > F	0.644	0.318	0.714	0.765	0.930	0.65	0.445	0.863	0.96	0.595	0.933	0.240	0.867	0.55	0.553

In case of significance of the F test, within-column and within factor mean separation was performed by t-test, p = 0.05.

Table 4. Yield components, grape composition and cluster sunburn incidence determined in 2024-2025 on Barbera grapevines assigned to two irrigation (I) treatments (C = non irrigated control and CL = cooled) and two leaf removal (LR) treatments (ND = non defoliated and D = defoliated). For each treatments mean (main effects) n = 16. Pr > F values in bold indicate significance at p = 0.05.

	Clusters/ vine	Cluster weight (g)	Berry weight (g)	Yield/ vine (kg)	TSS (°Brix)	pH	TA (g/L)	Tartaric (g/L)	Malic (g/L)	Total anthocyanins (mg/g)	(mg/berry)	Total phenolics (mg/g)	(mg/berry)	Berry sunburn incide
Year														
2024	20.6	83.3	2.82	1.64	24.9	3.43	10.6	7.82	2.72	0.76	2.16	1.73	4.59	10.9
2025	35.3	98.0	2.04	3.43	24.1	3.12	9.3	8.52	2.09	1.13	2.23	2.51	5.06	8.5
Pr > F	**0.001**	**0.031**	**0.001**	**0.001**	0.090	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	0.434	**0.001**	0.554	0.204
Irrigation (I)														
C	29.6	74.0	2.26	2.36	24.3	3.24	10.1	8.76	2.09	1.10	2.37	2.39	5.15	24.7
CL	26.3	93.0	2.60	2.71	24.7	3.31	9.8	7.58	2.72	0.80	2.03	1.85	4.70	5.6
Pr > F	0.065	**0.001**	**0.001**	0.110	0.376	**0.004**	0.550	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.006**	**0.001**
Leaf removal (LR)														
ND	28.1	106.0	2.50	2.89	24.8	3.28	10.0	7.97	2.48	0.81	2.30	2.09	5.02	4.3
D	27.8	75.5	2.36	2.19	24.2	3.27	9.9	8.37	2.33	0.78	2.10	2.15	4.82	15.1
Pr > F	0.864	**0.001**	0.052	**0.002**	0.186	0.841	0.849	**0.001**	0.071	0.791	**0.037**	0.305	0.221	**0.001**
Y x I	0.775	0.530	**0.013**	0.120	0.082	0.849	0.236	**0.001**	0.083	**0.015**	0.192	**0.002**	0.205	0.882
Y x LR	0.377	0.102	0.870	0.589	**0.026**	0.934	0.214	0.837	0.209	**0.040**	**0.009**	**0.011**	**0.024**	**0.016**
I x LR	0.324	0.489	0.652	0.104	0.379	0.071	0.935	0.309	0.523	0.590	0.973	0.517	0.913	**0.001**
Y x I x LR	0.501	0.536	0.915	0.348	0.059	0.482	0.056	0.062	0.260	0.061	0.160	0.055	0.162	0.281

In case of significance of the F test, within-column and within factor mean separation was performed by t-test, p = 0.05.