Southern Region Small Fruit Consortium

Final Report Research

Title: Determining chilling requirements for blackberry cultivars using long-cane plants and forced cuttings

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Public Abstract

Lack of winter chill accumulation is a major limiting factor for floricane-fruiting blackberry production in regions with mild or absent winters, such as the Southeastern US. For blackberry buds to overcome endodormancy they must first undergo exposure temperatures between 0°C and 7.2°C for a genotype-specific period of time. Insufficient chill in blackberries results in incomplete bud development, irregular budbreak, extended flowering periods, nonsynchronous fruit set, and lower fruit yields. The University of Arkansas Division of Agriculture (UADA) Fruit Research Station, located in Clarksville, Arkansas, experiences >1000 hours of annual chill each year, clouding phenotypic identification of potentially low-chill blackberry germplasm that would perform well in regions with mild or absent winters. The current study compares two methodologies for determining chilling requirements in six blackberry cultivars (Von, Natchez, Navaho, Ouachita, Sweet-Ark® Ponca, and A-2491T). Long-cane, potted blackberry plants were exposed to controlled artificial chilling in a cooler, with removal at six weekly intervals from 0 to 840 hours, followed by exposure to forcing conditions in a heated greenhouse. Concurrently, stem cuttings from the same cultivars were collected from field-grown plots, subjected to natural field chill accumulation, and subsequently forced under a mist bed in a heated greenhouse. The number of buds broken, reproductive laterals, and open flowers were recorded on a weekly basis for both studies. Results indicated that both methods effectively differentiated chilling requirements between cultivars, with budbreak progressing more rapidly and synchronously once plants reached their cultivar-specific chilling requirement.

Introduction

Blackberries (*Rubus* subgenus *Rubus*) are economically valuable perennial fruit crops grown predominantly in temperate and subtropical regions. Blackberry, like many temperate perennial crops, exhibits a state of dormancy during the winter to protect frost-sensitive tissues until favorable conditions return in spring. Winter dormancy includes two distinct phases: endodormancy (internal physiological blocks prevent bud outgrowth) and ecodormancy (unfavorable environmental conditions prevent budbreak). Endodormancy is released following exposure to adequate chilling temperatures (typically between 0 and 7 °C). Once the chilling requirement is fulfilled, buds enter ecodormancy, a state in which growth remains inhibited by low winter temperatures until sufficient heat accumulation enables budbreak (Figure 1) (Guillamón et al., 2022; Beauvieux et al., 2018; Melke, 2015; Atkinson et al., 2013).

Therefore, in regions with mild or absent winters, including parts of the Southeastern US, insufficient chill accumulation poses a major limitation of production to fruit growers. Insufficient chill fails to satisfy the requirements for endodormancy release of axillary buds, leading to irregular budbreak, asynchronous flowering, prolonged fruit ripening; ultimately lowering yields (Clark 2005; Clark and Finn 2011; Atkinson et al. 2013; Lin and Agehara 2020). To compensate for inadequate chill accumulation, fruit growers in low-chill environments often depend on chemical dormancy-substitution agents. Commonly applied compounds include hydrogen cyanamide (Dormex®) and thidiazuron (TDZ), increasing production costs and raising concerns for potential health risks (Schep et al. 2009; Bernasconi et al. 2023; Martínez et al. 2021; Lin and Agehara 2021).

Phenotyping chilling requirement is essential for breeding cultivars adapted to low-chill–environments, but this task is complicated by the consistently high winter chill (>1000 chill units) at the UADA Fruit Research Station in Clarksville, AR (35°31′5″N, 93°24′12″W, USDA zone 7b) (Drake and Clark, 2000; Yazzetti and Clark, 2001). UADA fruit breeders commonly rely on early field budbreak and flowering as a proxy to low chilling requirement, under the assumption that genotypes satisfying endodormancy earlier will transition more quickly into key developmental stages such as budbreak and anthesis.

Previous UADA work from 20–25 years ago provides important context: earlier studies placed Arkansas cultivars into broad chilling requirement groups, with some cultivars (e.g., Kiowa, Ouachita, Prime-Jim) showing relatively low chilling needs, others (e.g., Arapaho, Choctaw, Shawnee) requiring moderate exposure, and still others (e.g., Chickasaw, Navaho, Apache) classified as high-chill types. Subsequent work by Warmund and Krumme (2005) confirmed this, identifying Kiowa and Arapaho as among the lowest-chill cultivars and Apache and Darrow as among the highest-chill. However, no comparable evaluations have been conducted for the more recent Arkansas cultivars released since Ouachita's release, leaving a major gap in understanding how modern UADA germplasm responds to chilling.

Traditional field phenotyping is further confounded by cold injury, which reduces the accuracy of genotype differentiation. Low-chill germplasm may satisfy endodormancy early and begin heat accumulation prematurely. Once chilling is fulfilled, buds enter ecodormancy and become metabolically active, often before the final spring frost. Newly emerged tissues are highly vulnerable to freeze damage at this stage, meaning that low-chill cultivars may appear weak or dead during spring evaluations. This bias can cause breeders to inadvertently discard selections that would otherwise perform well in milder regions.

The current study aims to develop a standardized phenotyping protocol for determining chilling requirement in blackberry germplasm adapted to Arkansas. Utilizing both long-cane plants and field cuttings, the project quantifies the chilling effects on budbreak and reproductive development across modern blackberry cultivars selected for wide variation in anticipated chilling requirement.

Building on the work of Drake et al. (2000) and Yazzetti and Clark (2001), who showed both the potential and limitations of estimating chilling requirement from field-dug plants and naturally chilled field cuttings, this study addresses their key conclusion that high ambient chill and winter injury can mask true genotype differences, and underscore the need for a repeatable phenotyping protocol capable of resolving genotype-specific chilling responses with greater accuracy and stability across years. Ultimately, these findings will support the UADA blackberry breeding pipeline by facilitating the selection and advancement of cultivars suitable for production within low-chill environments.

Materials and Methods

Long-Cane Study

Six blackberry cultivars were selected for this study selected for high variability in estimated chilling requirements. These include Von (Fernandez et al. 2013), Natchez, Navaho, Ouachita, Sweet-Ark® Ponca, and A-2491T. Chilling requirement estimates were derived from breeder field-earliness observations and grower feedback on cultivar performance across breeding test sites positioned at different latitudes across diverse environmental conditions. Cultivar chilling responses ranged from low-chill A-2491T to Navaho, estimated to have a chilling requirement of >700 hours (Drake and Clark 2000).

A total of 300 plants (50 per genotype) were received as plug trays and transplanted into 2 gal nursery pots (9’’ diameter × 10’’ depth). The substrate was ProMix BX supplemented with biofungicide and mycorrhizae, amended with 1 cup Osmocote 14-14-14 controlled-release fertilizer and surface-applied with 0.25 cup Micromax micronutrients per pot.

Long-cane plants were grown outdoors at the University of Arkansas System Division of Agriculture Fruit Research Station (FRS, Clarksville, AR; 35°31′5″N, 93°24′12″W) on a three-row trellis (88 ft length, T-posts spaced every 22 ft with six wires per post) in a complete randomized block design over landscape fabric. Weekly fertigation with 17-4-17 neutral pH water-soluble fertilizer plus micronutrients (applied at 600 ppm N once per week) began June 15, 2024 and ceased September 1, 2024 to allow plants to terminate growth and begin hardening-off at the onset of endodormancy.

On November 20, 2024, irrigation was ceased and plants were removed from trellises, pruned, defoliated, and tied to bamboo cane stakes using twine. Each plant was pruned to contain 60-100 viable buds, with final bud counts recorded for each plant individually. Thirty-six plants per genotype (approximately 216 total), selected for vigor and upright stature to facilitate handling and reduce cane damage, were randomized within onsite walk-in coolers five days after first chill accumulation per the 32°F to 45°F (0°C to 7.2°C) chilling model with chilling inception occurring at the first instance of -2.2 °C (28 °F) (North Carolina State Climate Office, 2025).

Plants underwent six artificial chilling treatments (0, 168, 336, 504, 672, and 840 hours at 0°C to 7.2°C) in walk-in coolers maintained at 90-95% relative humidity on site at the FRS in Clarksville, AR. Cooler temperatures fluctuated parallel to outdoor conditions but stayed within effective chilling ranges per the 32°F to 45°F (0°C to 7.2°C) model. Weekly, 36 plants (six long-cane, potted plants per genotype) were removed and transferred to a greenhouse (~25 °C) for forcing. On November 25, 2024, a non-chilled control subset (0 h) was directly introduced to the greenhouse 48 hours following the first -2.2 °C (28 °F) incidence, simulating forcing conditions (Figure 3).

Field Cutting Study

Six-node cuttings were collected biweekly beginning on November 25, 2024, from dormant field-grown plants of the same six genotypes used in the long-cane study (Natchez, Navaho, Ouachita, Sweet-Ark® Ponca, Von, and A-2491T) at the University of Arkansas Fruit Research Station in Clarksville, AR (USDA Zone 7a; Linker fine sandy loam soil). Six replicate cuttings per genotype were collected at each sampling date. Following collection, the field cuttings were planted within plastic lugs containing a 60:40 perlite:ProMix BX potting mix amended with biofungicide and mycorrhizae, without added fertilizer. Each lug represented one chilling treatment, and field cuttings were randomized by position within lug. Field cuttings were exposed to seven natural field chill intervals/treatments (44, 226, 353, 479, 596, 712, 853 hours at 0°C to 7.2°C) (Figure 4), distinct from the artificially-chilled long-cane study. Subsequent forcing was under mist benches (~25 °C, 12-hour photoperiod, high humidity). On November 25, 2024 one subset of field cuttings were immediately introduced into the greenhouse, 48 hours following the first -2.2 °C (28 °F) incidence, simulating forcing conditions.

Combined Data Collection

Traits Evaluated

Phenological traits were scored and recorded weekly in both studies: 10 weeks for the long-cane study and 6 weeks for the field cutting study (Figures 2a–c). Traits included:

- Budbreak: Recorded when leaf tips emerged above bud scales. Expressed as % of viable buds.

- Floral laterals: Axillary shoots showing reproductive primordia. Expressed as % of viable buds.
- Open flowers: Buds at anthesis with fully reflexed petals.

Budbreak and floral lateral percentages were calculated as:

$$\%\ \text{Trait} = \frac{\text{Count of buds expressing trait}}{\text{Total viable buds}} \times 100$$

Area Under the Progress Curve (AUPC)

Budbreak progression, floral lateral development, and open flower counts were summarized across observation weeks using the Area Under the Progress Curve (AUPC) (Shaner & Finney, 1977). Trait values (Y; expressed as percent or count) were integrated across observation days (t) to generate a single cumulative measure capturing both the rate and extent of developmental progression.

$$\text{AUPC} = \sum_{i=1}^{n-1} \frac{Y_i + Y_{i+1}}{2} (t_{i+1} - t_i)$$

Two-way ANOVA tested effects of Genotype, Chill Treatment, and their interaction on AUPC for each trait. Tukey's HSD ($\alpha = 0.05$) determined pairwise differences. Results are summarized in Table 1 (long-cane study) and Table 2 (field cuttings). Boxplots with compact letter displays are shown in Figures 6 and 9.

Results and Discussion

Long-Cane Study

Two-way ANOVA detected highly significant effects of Genotype ($P < 0.001$), Chill Duration ($P < 0.001$), and their interaction ($P < 0.001$) between long-cane budbreak AUPC (Table 1). Figure 6 presents final budbreak AUPC for each chilling treatment, with separate boxplots showing how chill treatment x genotype interaction influenced cumulative budbreak response. Phenotype appearance of long-cane plants post-forcing is illustrated in Figure 3.

Under the lowest chilling treatment interval (0 h), all genotypes exhibited limited budbreak (Tukey mean separation groups h-m), confirming that chilling exposure is necessary for endodormancy release and subsequent outgrowth of axillary buds in blackberry. Budbreak progression curves (Figure 5) show a clear acceleration in budbreak rate as hours under chilling exposure increased. The AUPC post-hoc results (Figure 6; Table 1) and final percent budbreak heatmap (Figure 7) demonstrates a notable increase in average final budbreak at chilling durations ≥504 h. The highest mean-separation groupings occurred in A-2491T and Ouachita, aligning with their classification as anticipated lower-chill genotypes (Figure 6; Table 1). In contrast, Navaho and Von exhibited only limited improvement even at 840 h and consistently fell within the lowest Tukey groupings (f–m and c–h, respectively) (Figure 6; Table 1). This observation aligns with prior expectations, as field observations and grower feedback consistently identify Navaho and Von as high-chill cultivars that perform poorly under low-chill conditions.

Despite the highly significant positive influence of chilling on budbreak ($P < 0.001$), overall budbreak in the long-cane study did not exceed 50%, even under the maximum chill treatments (672 h and 840 h) (Figures 6 and 7; Table 1). However, The AUPC post-hoc results (Figure 6) clearly demonstrate pronounced within-genotype gains across chilling durations ≥504 h. The reduced observation of budbreak likely reflects the limitations of artificial chilling, which may not fully reproduce key physiological-signaling environmental cues present under naturally occurring field conditions. They potentially include diurnal temperature fluctuations, seasonally increasing photoperiod, and ambient field humidity.

Field Cutting Study

ANOVA indicated significant effects of Genotype ($P < 0.001$), Chill Duration ($P < 0.001$), and Genotype × Chill ($P < 0.001$) (Figure 9; Table 2). Phenotype appearance of field cuttings post-forcing is illustrated in Figure 4, with budbreak progression displayed in Figure 8.

Compared to the long-cane study, the field cutting study exhibited greater variability and less stable genotype ranking, reflecting the same challenges previously noted by Yazzetti and Clark (2001). Nevertheless, field cuttings frequently reached >80% final budbreak under natural chill accumulation (Figures 8 and 10), indicating that natural field chilling is highly effective at promoting dormancy release prior to greenhouse forcing of cuttings. The field cuttings one-way ANOVA post-hoc results for the effects of chill on budbreak (Table 2) demonstrate sharp weekly increases of budbreak withinin several genotypes at ≥712 h of chill exposure, reflecting rapid dormancy release under high natural field chill. Although field cuttings responded rapidly once forced, their responses were inconsistent across replicates and chill intervals, limiting the confidence and precision of chilling-requirement estimation with field cuttings.

It is critical to consider that most field cuttings assigned to the 596 h chill treatment desiccated during forcing conditions, resulting in poor physiological development of the three traits analyzed. Diagnostic evaluation by the UADA Arkansas Plant Health Clinic confirmed that this mortality was due to abiotic dehydrative stress, rather than disease. The loss of the 596 h treatment group during weekly observations reduced interpretability for that interval (Table 2), and resulted in comparatively lower mean separation (Tukey mean separation groups b-k) at the 596 h treatment.

Conclusions

The long-cane study provided a clear separation of blackberry genotypes response to chill, with far less variability than the complementary cutting forcing study. However, despite its stability, the long-cane system produced relatively low final budbreak percentages across all genotypes, even under the highest chilling durations. This limited budbreak response likely reflects the absence of key environmental cues in the artificial chilling environment that contribute to endodormancy release under naturally occurring field conditions. These findings suggest that artificial cooler conditions may not fully replicate the physiological requirements for the release of internal physiological blocks via blackberry endodormancy.

In contrast, the cutting study produced much higher overall budbreak percentages, often exceeding 80% in several treatments/genotypes, which indicates that naturally accumulated field chill may be more effective at overcoming the physiological barriers to budbreak than artificial chilling. However, cuttings also showed substantial variability and inconsistent genotype rankings across chill treatments. This instability, combined with abiotic stress sensitivity (including severe desiccation in the 596h treatment), aligns with the concerns raised by Yazzetti and Clark (2001), who reported that field-collected cuttings can yield unreliable and variable budbreak responses.

From a practical standpoint, the long-cane system is more controlled and accurate, but is also considerably less feasible for large-scale, routine phenotyping in the UADA breeding pipeline. The requirement for hundreds of whole potted plants, extensive space in coolers and greenhouses, and intensive labor makes repetition across years or integration into the annual UADA phenotyping pipeline less feasible than phenotyping via field-cutting forcing. The cutting-based approach is far more logistically manageable and space-efficient, making it attractive for routine screening despite its high variability. With additional refinement, such as improved simulation of field environmental conditions (humidity management, diurnal temperature fluctuations, ideal temperature control, nutrient management of cuttings) the field cutting method may remain a viable and practical option for phenotypic assessment despite its relatively lower precision.

Overall, these findings emphasize a compromise between accuracy (long-cane system) and scalability (cutting system). Further optimization of the cutting protocol, along with continued validation through the ongoing second-year trial, will help determine whether a refined field cutting-based methodology can reliably characterize differing chilling requirements within UADA blackberry germplasm.

Description of Outreach Activity

A repeat of the complementary cutting-forcing trial is currently underway to validate the initial findings reported here. Repeating the field cutting trial will allow us to determine whether the variability observed in year one was due to abiotic stress unique to that season or whether it is a consistent limitation of cutting-based phenotyping. Due to the scale of the experiment, limited personnel, and greenhouse space constraints, the long-cane study will not be repeated in 2025.

Preliminary results from the first year of data collection were presented at the ASHS 2025 Annual Conference and published in the conference proceedings (Brock et al., 2025). This project informs the development of a standardized chilling-requirement phenotyping protocol for UADA's blackberry breeding program.

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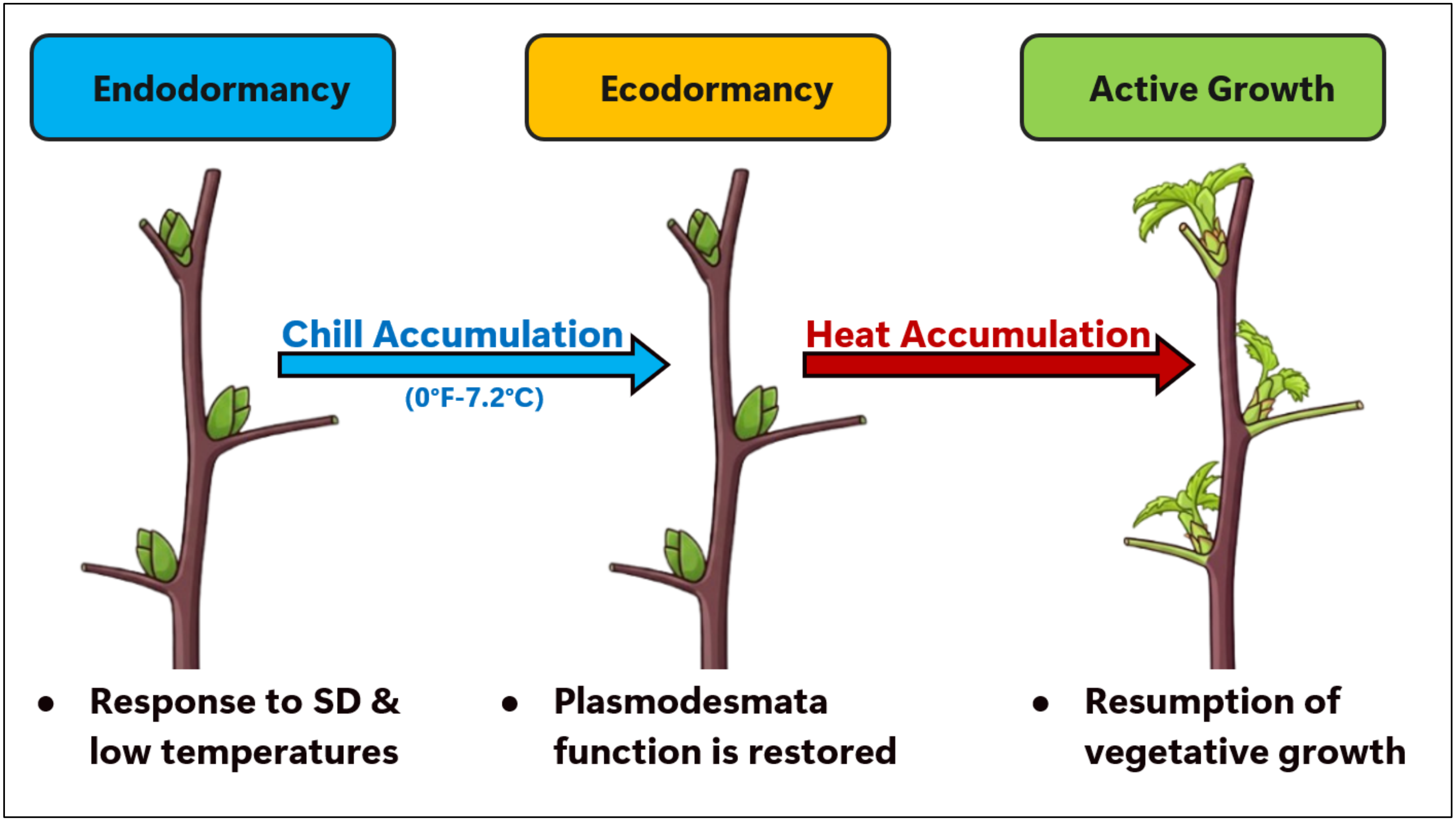

Figure 1. Diagram illustrating the dormancy cycle in blackberry axillary buds. Endodormancy (left) is induced by decreasing daylength (SD) and low temperatures, during which buds cannot resume growth even under favorable conditions. Chill accumulation between 0°C and 7.2°C transitions buds to ecodormancy (center), where chilling satisfaction lifts internal physiological blocks, restoring plasmodesmata function. Subsequent heat accumulation promotes the solubilization of stored carbohydrates and translocates phytohormones conducive to budbreak, initiating the onset of budbreak and active vegetative growth (right). Visual elements were generated using the Google Gemini, with photographic inputs serving as the basis for stylized enhancement and modification.

Figure 2. Weekly visual assessment of traits in both studies: A) buds broken, B) floral laterals, and C) open flowers.

Figure 3. Long-cane blackberry genotypes exhibiting budbreak after chilling treatment and 70 days under greenhouse forcing conditions. A) A-2491T, B) Navaho, C) Natchez, D) Ouachita, E) Sweet-Ark® Ponca, F) Von.

Figure 4. Six-node cuttings of blackberry genotypes exhibiting budbreak after chilling treatment and 42 days under greenhouse forcing conditions. Genotypes shown: A) A-2491T, B) Sweet-Ark® Ponca, C) Natchez, D) Ouachita, E) Von, F) Navaho.

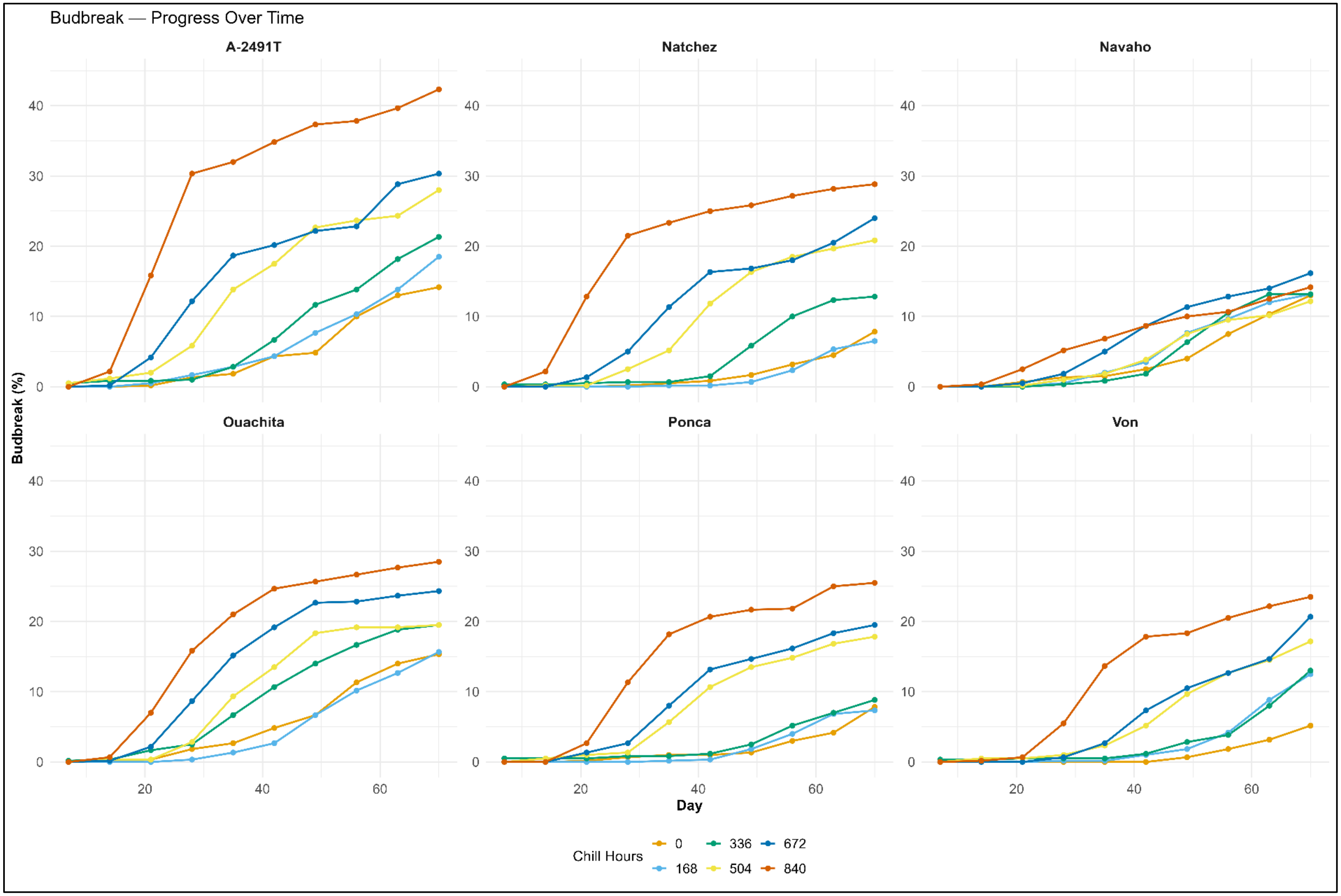

Figure 5. Weekly budbreak progression across chilling treatments in the long-cane study. Increased chilling treatment reduced time-to-budbreak onset and steepened early curve shape, demonstrating that chilling affected both earliness and intensity of budbreak.

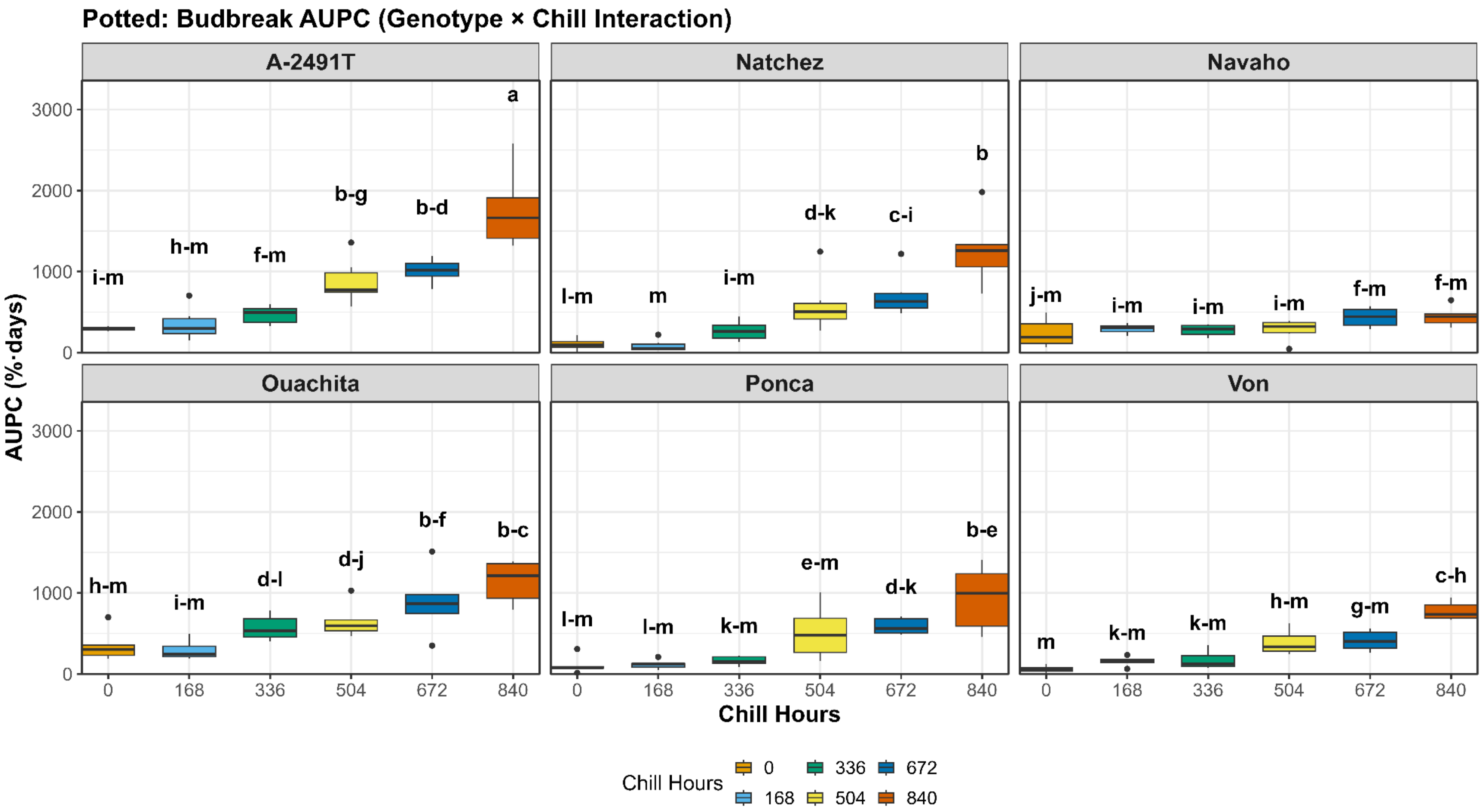

Figure 6. Two-way ANOVA of budbreak area under the progress curve (AUPC; %·days) for the long-cane study, illustrating main effects of Genotype and Chill interaction. For each Genotype × Chill combination, boxplots summarize the distribution of replicate AUPC values across chill treatments (0–840 h), with compact letter displays plotted above each box to denote Tukey post-hoc mean separation for final average AUPC. “a” denotes the highest mean, and descending letters indicate decreasing average AUPC. Letter ranges (e.g., b-f) represent groups of treatments with statistically insignificant difference of means.

Genotype	0	168	336	504	672	840
Von	5.2%	12.5%	13.0%	17.2%	20.7%	23.5%
Ponca	7.8%	7.3%	8.8%	17.8%	19.5%	25.5%
Ouachita	15.3%	15.7%	19.5%	19.5%	24.3%	28.5%
Navaho	13.0%	13.2%	13.2%	12.2%	16.2%	14.2%
Natchez	7.8%	6.5%	12.8%	20.8%	24.0%	28.8%
A-2491T	14.2%	18.5%	21.3%	28.0%	30.3%	42.3%

Figure 7. Final average budbreak (%) heatmap at Day 70 for the long-cane study. Even under higher chilling treatments (≥504 h), no treatments/genotypes achieved >50% budbreak.

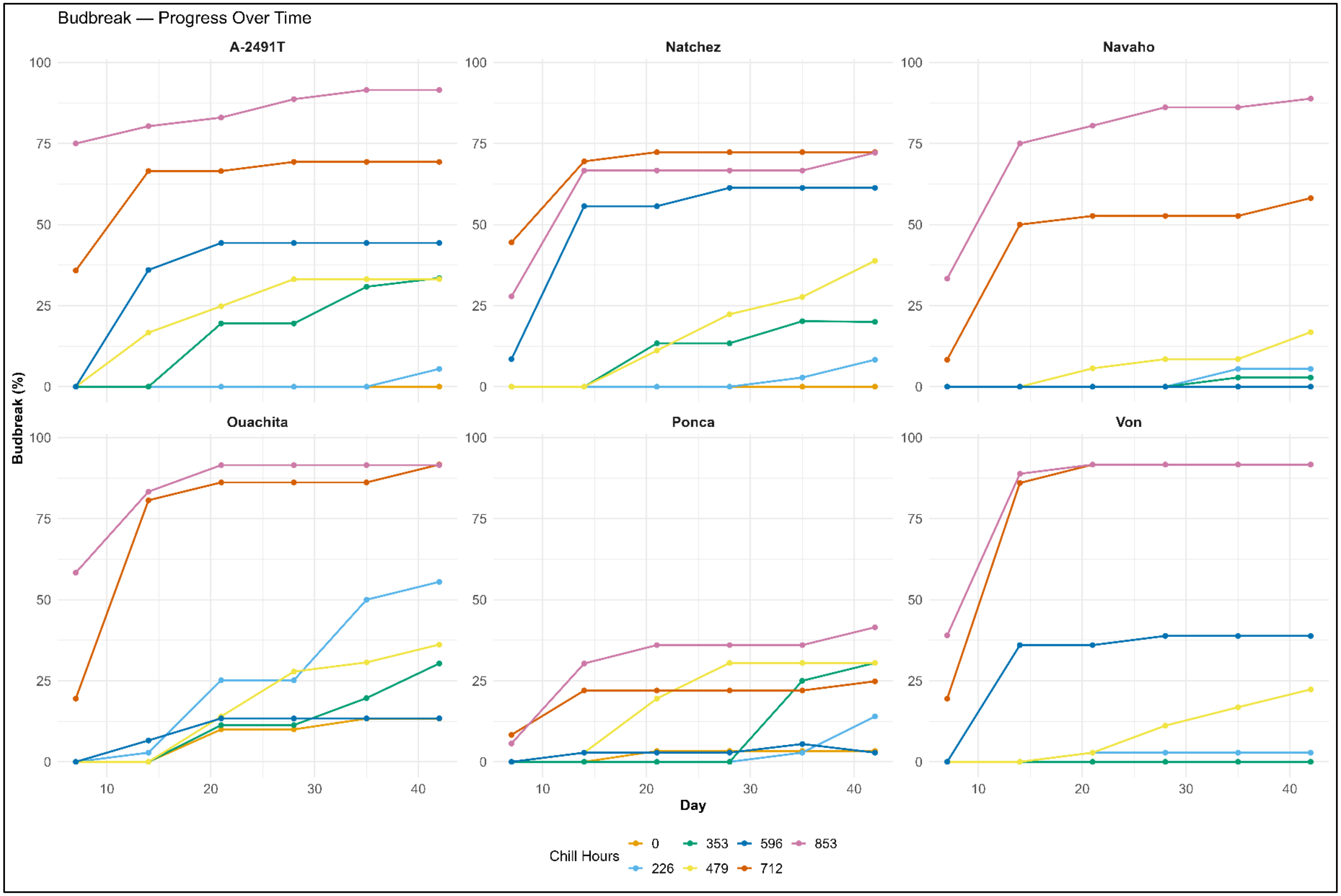

Figure 8. Weekly budbreak progression across chilling treatments in the cutting study. Increased chilling treatment reduced time-to-budbreak onset and steepened early curve shape, demonstrating that chilling affected both earliness and intensity of budbreak.

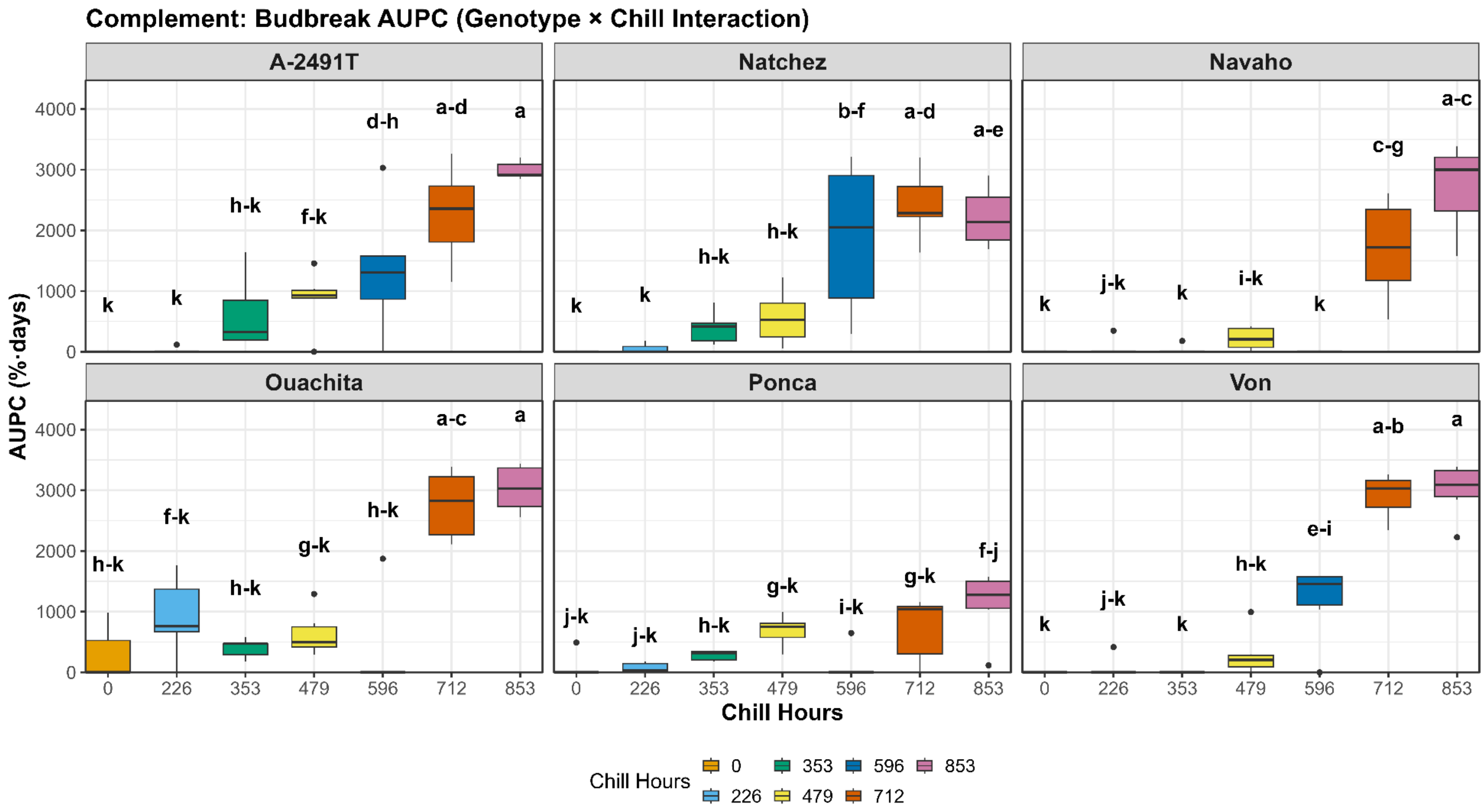

Figure 9. Two-way ANOVA of budbreak area under the progress curve (AUPC; %·days) for the field cutting-forcing study, illustrating main effects of Genotype and Chill and their interaction. For each Genotype × Chill combination, boxplots summarize the distribution of replicate AUPC values across mapped chill treatments (0–853 h), with compact letter displays plotted above each box to denote Tukey post-hoc mean separation for final average AUPC. “a” denotes the highest mean, and descending letters represent lower average AUPC values. Letter ranges (e.g., j-k) indicate treatment groups that do not differ significantly in mean AUPC following multiplicity-adjusted pairwise comparisons. Variation in chill response within genotypes was greater than that observed in the long-cane study.

Figure 10. Final average budbreak (%) heatmap for field cuttings. High chill durations (≥712 h) produced multiple instances of final budbreak >90% in Von, Ouachita, and A-2491T. Notably, certain early chill intervals (Ouachita at 226 h) also demonstrated unexpectedly high budbreak (>50%), outperforming larger chilling treatments.

Table 1.

Two-way ANOVA results summarizing the main effects of Genotype and Chill, and their interaction, on final budbreak area under the progress curve (AUPC; %·days) in the artificially chilled long-cane study. For each Genotype × Chill combination, mean AUPC ± standard error (SE) is reported alongside compact mean separation letter displays for: (i) the genotype main effect, (ii) the chill main effect, and (iii) the genotype × chill interaction. "a" indicates the highest mean separation group and descending letters denote decreasing means. Letter ranges (e.g., b–g) represent groups with statistically indistinguishable mean AUPC values.

Long-Cane

Genotype	Chill Treatment	AUPC	Genotype mean-separation group	Chill mean-separation group	Genotype x Chill mean-separation group
A-2491T	0	298.08± 9.28	a	c	ijklm
	168	352.92± 81.27	a	c	hijklm
	336	467.25± 46.57	a	c	fghijklm
	504	876.75± 115.50	a	b	bcdefg
	672	1010.33± 58.43	a	b	bcd
	840	1758.17± 193.87	a	a	a
Natchez	0	103.25± 28.56	c	c	lm
	168	83.42± 30.42	c	c	m
	336	268.92± 49.06	c	c	ijklm
	504	593.25± 140.03	c	b	defghijk
	672	709.33± 109.16	c	b	cdefghi
	840	1262.92± 170.70	c	a	b
Navaho	0	240.33± 69.99	d	c	jklm
	168	294.58± 24.26	d	c	ijklm
	336	277.08± 29.33	d	c	ijklm
	504	281.75± 53.07	d	b	ijklm
	672	435.75± 48.47	d	b	fghijklm
	840	446.25± 48.60	d	a	fghijklm
Ouachita	0	345.33± 75.93	b	c	hijklm
	168	291.67± 48.00	b	c	ijklm
	336	568.17± 62.48	b	c	defghijkl
	504	649.25± 82.02	b	b	defghij
	672	886.67± 155.54	b	b	bcdef
	840	1143.92± 107.80	b	a	bc
Ponca	0	106.75± 41.79	cd	c	lm
	168	117.83± 22.91	cd	c	lm
	336	162.17± 23.21	cd	c	klm
	504	512.75± 133.32	cd	b	efghijklm
	672	588.58± 41.17	cd	b	defghijk
	840	938.58± 163.96	cd	a	bcde
	0	57.75± 17.45	d	c	m
	168	156.92± 23.14	d	c	klm

Von	336	170.33± 45.23	d	c	klm
	504	384.42± 60.87	d	b	hijklm
	672	411.83± 50.15	d	b	ghijklm
	840	774.08± 46.34	d	a	cdefgh

Table 2.

Two-way ANOVA results summarizing the main effects of Genotype and Chill, and their interaction, on final budbreak area under the progress curve (AUPC; %·days) in the field-cutting forcing study. For each Genotype × Chill combination, mean AUPC ± standard error (SE) is reported alongside compact mean separation letter displays for: (i) the genotype main effect, (ii) the chill main effect, and (iii) the genotype × chill interaction. “a” indicates the highest mean separation group and descending letters denote decreasing means. Letter ranges (e.g., d–g) represent groups with statistically indistinguishable mean AUPC values under multiplicity-adjusted pairwise comparisons.

Field Cutting

Genotype	Chill Treatment	AUPC ± SE	Genotype mean-separation group	Chill mean-separation group	Genotype x Chill mean-separation group
A-2491T	0	0.00± 0.00	a	e	k
	168	19.25± 19.25	a	e	k
	336	606.08± 242.00	a	d-e	h-k
	504	870.92± 194.56	a	c-d	f-k
	672	1338.17± 413.86	a	c	d-h
	840	2269.75± 316.62	a	b	a-d
Natchez	0	2987.25± 60.41	a	a	a
	168	0.00± 0.00	a	e	k
	336	49.00± 32.04	a	e	k
	504	399.00± 122.56	a	d-e	h-k
	672	564.08± 177.91	a	c-d	h-k
	840	1882.42± 509.22	a	c	b-f
Navaho	0	2414.42± 223.57	a	b	a-d
	168	2216.67± 198.97	a	a	a-e
	336	0.00± 0.00	b	e	k
	504	57.75± 57.75	b	e	j-k
	672	29.75± 29.75	b	d-e	k
	840	217.58± 74.89	b	c-d	i-k
Ouachita	0	0.00± 0.00	b	c	k
	168	1688.75± 332.41	b	b	c-g
	336	2722.42± 293.57	b	a	a-c
	504	280.00± 180.74	a	e	h-k
	672	916.42± 264.81	a	e	f-k
	840	402.50± 64.86	a	d-e	h-k
Ponca	0	634.08± 150.13	a	c-d	g-k
	168	374.50± 374.50	a	c	h-k
	336	2763.25± 228.62	a	b	a-c
	504	3029.25± 154.24	a	a	a
	672	81.67± 81.67	b	e	j-k
	840	68.83± 35.40	b	e	j-k
Von	0	281.75± 33.69	b	d-e	h-k
	168	690.08± 99.74	b	c-d	g-k
	336	107.92± 107.92	b	c	i-k

	504	732.08± 222.92	b	b	g-k
	672	1133.42± 222.95	b	a	f-j
	840	0.00± 0.00	a	e	k