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Chilling Affects Budbreak of *Ginkgo biloba* L.¹

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Abstract

The effects of chilling and heat on budbreak of *Ginkgo biloba* L. seedlings were evaluated in a study initiated on October 1, 1999, and terminated on April 8, 2002. Chilling was a determinate factor in foliar budbreak for *G. biloba*. Thirteen levels of chilling were applied to trees in increments of 100 hours, with twelve replications per treatment. Once the chilling requirement had been satisfied, increased chilling led to a decreased heat unit requirement, resulting in accelerated foliar budbreak and higher budbreak percentage. Trees receiving the same treatment for three consecutive years required a minimum of 100 chill hours plus 936 greenhouse hours to initiate budbreak, and a minimum of 500 chill hours plus 1168 greenhouse hours to reach 50 percent budbreak. In years one and two, trees receiving less than 100 chill hours failed to break bud and eventually died. In year three, trees receiving less than 100 chill hours broke an average of 0.05 percent buds per tree, but no subsequent growth occurred.

Index words: chilling requirement, cold storage, dormancy, endodormancy, heat, ornamental.

Species used in this study: *Ginkgo biloba* (*Ginkgo biloba* L.).

Significance to the Nursery Industry

Growers often must make decisions about which species best fit into their production cycle. Many aspects influence the product decision, most notably whether a species can be produced in a given region in a timely manner. For *Ginkgo biloba* L., satisfying dormancy requirements is important in the production process. This study reveals chilling to be a determinate factor in foliar budbreak of *G. biloba*. Optimal chilling for all treatment levels and groups appeared to begin in the 700–800 chill hour range. Increasing chill hours leads to a decreased need for heat to initiate budbreak. For growers using greenhouse production methods, adjusting environmental conditions to allow chilling to occur can accelerate and lead to more efficient production of *Ginkgo*. With equal hours inside the greenhouse, percent budbreak levels increased with increasing chill hours. Chilling impact became evident the longer trees were held in the greenhouse. For trees receiving 200 chill hours, this impact became evident after six weeks inside the greenhouse. For trees receiving 400 to 800 chill hours, this became evident after four weeks inside the greenhouse. For trees receiving 900 to 1200 chill hours, this became evident after only three weeks inside the greenhouse. It is reasonable to predict that tissue cultured plantlets and rooted cuttings may be produced at a faster rate by alternating cold storage with greenhouse growing conditions. For growers using field production methods for *Ginkgo*, modifying lifting and transplanting schedules based upon chilling accumulation can lead to improved performance for the customer, providing more rapid foliar emergence. While *G. biloba* is a suitable landscape choice based upon the amount of chilling received for much of the United States, this study indicates that field production may be bet-

ter suited to areas receiving 700 or more hours of chilling. This work provides a basis for future evaluation of chilling response in *G. biloba* cultivars.

Introduction

Ginkgo biloba L., often referred to as a living fossil, is a valuable (5) and interesting ornamental tree in the green industry. The only species within a single genus, *G. biloba* is native to China. In North America, *Ginkgo* is grown from Southern Canada to Central Florida, and from the East Coast to the West Coast (USDA Hardiness Zones 3–9) (7). *G. biloba* can reach a mature height of 24.4 m (80 ft) and a width of 12.2 m (40 ft), with a naturally slow to medium growth habit. Trees are often pyramidal when young and become wide spreading when older. *G. biloba* is adaptable, but performs best in full to partial sun and moist, well-drained soils. Being known as resistant to insects and diseases and with about forty available cultivars in the industry, *G. biloba* is an excellent choice for a landscape tree (4).

Dormancy in plants has been described as a state in which visible growth is temporarily suspended (12), a phase in a plant's development allowing it to survive winter conditions (13), and a state in which deciduous plants are not in leaf or lacking visible growth (16).

Endodormancy release from within plant parts as controlled by chilling temperatures is a major factor in determining a plant's performance in a given climate or hardiness zone (16). Temperate zone plants must be exposed to a certain period of chilling temperatures above freezing (16), or a minimum number of hours below 7°C (45°F) (13), in order for dormancy release to occur. This is referred to as the chilling requirement. Dormancy release is of particular interest to the nursery industry, for in some cases early budbreak can not only lead to a longer growing season and accelerated production, but in other cases can result in cold and frost damage (8).

Much work has been reported on fruit species with respect to dormancy and chilling requirements. In a study with peaches (*Prunus persica* (L.) Batsch), once rest was satisfied, prolonged chilling was shown to enhance leafing over blooming (2). There were also cultivar differences in heat requirement for bloom. In a study with several fruits, once

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rest was satisfied, prolonged chilling led to a decreased need for heat (referred to as Grower Degree Hours) to reach budbreak (3).

Some work has been conducted with dormancy and chilling requirements for ornamental species. Increased chill hours in dogwood (*Cornus florida* L.) led to fewer days to budbreak, indicating early lifting and transplanting increases survival rates (10). Ashby et al. (1), reported terminal buds required more chilling than lateral buds in silver maple (*Acer saccharinum* L.). Optimal chilling requirements for two red maple (*Acer rubrum* L.) cultivars and one freeman red maple (*Acer ×freemanii* E. Murray) cultivar have been determined (18, 14). Increasing the duration of chilling accelerated the rate of foliar budbreak and reduced the number of heat units required to initiate foliar budbreak in linden (*Tilia spp.*) cultivars (17, 14).

Most research on *G. biloba* has been focused on medicinal uses (5, 6), with little attention given to cultural and environmental concerns. No reports have addressed dormancy requirements for *G. biloba*. Therefore, the objectives of this work were to: 1) establish whether chilling is a determinate factor in foliar budbreak; 2) evaluate foliar budbreak response to different chilling levels; and 3) establish the optimum chilling requirement, for *G. biloba*.

Materials and Methods

Two hundred, 0.46 m (18 in) liners of *Ginkgo biloba* L. seedlings were purchased from Musser Forests, Inc. (Indiana, PA) from a Mississippi seed source, in winter of 1999. Trees were initially potted into 3.8 liter (#1) containers using a 6:1 pinebark:sand substrate amended with 2.0 kg/cu m (3.3 lb/cu yd) dolomitic limestone, 0.6 kg/cu m (1.0 lb/cu yd) Micromax (The Scotts Co., Marysville OH), and 4.2 kg/cu m (7.3 lb/cu yd) 18N–6P–12K Osmocote (The Scotts Co.) for year one of the study. Following final bud count in the spring, trees were shifted to 7.9 liter (#2) and 13.5 liter (#3) containers for years two and three, respectively. Trees were grown outdoors with overhead irrigation at the Paterson Greenhouse Complex, Auburn, AL (32° 36'N × 85° 20'W, USDA Hardiness Zone 8a).

The three-year study included 13 treatment levels of chilling applied in increments of 100 hours (0–1200). Chilling hours were calculated using the Old 45 Chilling Model (9), which counts hours below 7C (45F) as chilling hours, beginning October 1 each year. Each treatment was applied to four single tree replications in year one (1999–2000) for a total of 52 trees. Trees not selected for treatment in year one received ambient chilling (998 hours for the season), while remaining on the container pad area. In year two (2000–2001), four single tree replications were added to each treatment for a total of 104 trees. Trees not selected for treatment in year two received ambient chilling (1487 hours for the season), while remaining on the container pad area. In year three (2001–2002), four single tree replications were added to each treatment for a total of 156 trees. Treatment levels were applied to the same replicates each year. Therefore, four replications received the exact same treatment for three years (3 yr group); four replications received the same treatment for two years (2 yr group); and four replications received the same treatment for one year (1 yr group).

Each September prior to receiving any chill hours, four trees were randomly selected to receive 0 chill hours and placed into a heated greenhouse maintained at a minimum of

22.2C (72F). In the first year, upon accumulation of 100 and 200 natural, ambient chill hours, four trees were randomly selected to receive 100 and 200 chill hours, respectively, and were placed into the greenhouse. After receiving 200 ambient chill hours, the 40 trees to receive 300–1200 chill hours were placed into a thermostatically controlled cooling unit (Funchess Hall, Auburn University, AL) maintained at 3C (38F) in the dark until desired chill hours were accumulated. Trees were removed in 100-hour increments and placed into the greenhouse. Trees were arranged in a completely randomized design (CRD), and hand-weeded and watered as needed. The same pattern of treatment management was used the following two years.

After placement in greenhouse, trees were monitored twice weekly for foliar budbreak until April of each year. Total number of buds was counted to calculate total percent foliar budbreak. Budbreak was considered to be the point where overlapping bud scales began to separate, revealing leaf tips. Dormancy end was considered to be the point when 50 percent of buds were broken. Therefore, the number of chilling hours required to reach 50 percent budbreak was defined as the chilling requirement and represented the best point of comparison between treatments. Data were analyzed with regression analysis using orthogonal contrast statements and the SAS GLM procedure (11).

Results and Discussion

This report presents results for all three years evaluated. Foliar budbreak rates were accelerated with increased chilling levels. Number of greenhouse hours required for budbreak at all chilling treatment levels was determined (Tables 1–2). Chilling level exposure was inversely related to heat unit accumulation. By extending chilling duration, fewer heat units were required to produce budbreak across treatment levels (Fig. 1). Increased chilling lead to higher mean percentage budbreak results over the course of the experiment (Table 3). Treatments were separated into three groups, where the 3 yr group represented trees placed in chilling treatments in the first year of the study, the 2 yr group represented trees initiated in the second year of the study, and the 1 yr group represented trees initiated in the third year of the study.

3 Year Group

Year 1. *Ginkgo biloba* L. seedlings required a minimum of 100 chill hours to initiate budbreak (data not shown). Trees receiving 400 chill hours did not initiate budbreak until 1518 greenhouse hours were applied, as compared to 1140 greenhouse hours for trees receiving 800 chill hours and 1584 greenhouse hours for trees receiving 1200 chill hours. The fastest level to initiate budbreak, calculating greenhouse hours plus chill hours, was obtained from trees receiving 400 chill hours and 1518 greenhouse hours, for a total of 1918 hours.

Trees required a minimum of 400 chill hours to reach a level of 50% foliar budbreak (Table 1). Trees receiving 400 chill hours did not reach 50% budbreak until 1854 greenhouse hours were applied, as compared to 1260 greenhouse hours for trees receiving 800 chill hours and 1680 greenhouse hours for trees receiving 1200 chill hours. The fastest level to reach 50% budbreak, calculating greenhouse hours plus chill hours, was obtained from trees receiving 800 chill hours and 1260 greenhouse hours, for a total of 2060 hours (Table 1). Budbreak percentage began to level off after the eighteenth count date (March 20), which was eight weeks

Table 1. Number of hours in greenhouse² to reach 50 percent budbreak, in which a minimum of 22C was constantly maintained, following incremental chilling on *Ginkgo biloba* L.

Chill hours	3 yr group			2 yr group		1 yr group
	Year 1 ^y	Year 2 ^y	Year 3 ^y	Year 2 ^y	Year 3 ^y	Year 3 ^y
100	— ^x	2592 ± 0	—	3232 ± 148	—	—
200	—	3054 ± 80	—	2760 ± 97	—	—
300	—	2496 ± 69	—	2556 ± 169	—	—
400	1854 ± 148	2232 ± 97	—	2400 ± 69	—	—
500	1782 ± 62	2094 ± 80	1168 ± 352	2248 ± 112	—	—
600	1662 ± 55	1956 ± 49	816 ± 0	—	—	960 ± 144
700	1368 ± 74	1902 ± 42	1016 ± 115	1944 ± 97	768 ± 46	—
800	1260 ± 85	1680 ± 69	936 ± 209	1736 ± 56	720 ± 46	792 ± 147
900	1278 ± 41	1560 ± 0	920 ± 273	1560 ± 0	696 ± 0	696 ± 96
1000	1206 ± 57	1548 ± 49	696 ± 68	1506 ± 42	792 ± 192	600 ± 0
1100	1014 ± 80	1410 ± 42	600 ± 0	1494 ± 42	632 ± 32	632 ± 32
1200	1680 ± 48	1440 ± 0	624 ± 28	—	—	—
	L***w	L***Q***	NS	L***Q*	NS	L**
LSD ^v	275	170	655	341	NS	430

²One greenhouse hour = one hour at a minimum of 22C (72F) inside the greenhouse.

^yStandard error of the mean (±) performed by PROC MEANS, SAS.

^xTrees in these treatments did not reach 50% budbreak.

^wNS, L, and Q represent non-significant, linear, and quadratic response to chill hours, respectively.

^vLeast significant difference within a column using Fisher's Protected LSD ($\alpha = 0.05$).

*, **, *** represent significance at the 0.05, 0.01, and 0.001 level, respectively.

after the last batch of trees were placed into the heated greenhouse (Fig. 1).

Following the last observation date (April 7), total percent budbreak was calculated. Foliar budbreak results were accelerated with increasing chill levels. Trees receiving 400 chill hours produced 62% budbreak, as compared to 79% for trees receiving 800 chill hours and 55% for trees receiving 1200 chill hours (Table 2). The maximum budbreak (89%)

for year one was obtained by trees receiving 900 chill hours. The lowest budbreak (23%) was obtained by trees receiving 100 chill hours.

Overall visual differences were apparent among the treatments. As chill levels were increased, plant height and width increased. There seemed to be more foliage per tree and overall appearance seemed to improve with increasing chill hours. Optimum chilling appeared to begin at 700 hours of chilling.

Table 2. Maximum percent budbreak² achieved following chilling on *Ginkgo biloba* L.

Chill hours	3 yr group			2 yr group		1 yr group
	Year 1 ^y	Year 2 ^y	Year 3 ^y	Year 2 ^y	Year 3 ^y	Year 3 ^y
100	23 ± 7	57 ± 17	16 ± 4	65 ± 10	34 ± 7	23 ± 10
200	33 ± 9	75 ± 8	36 ± 12	67 ± 12	38 ± 9	24 ± 5
300	25 ± 6	72 ± 8	42 ± 7	73 ± 5	39 ± 2	25 ± 7
400	62 ± 6	64 ± 10	39 ± 8	82 ± 8	38 ± 11	37 ± 4
500	76 ± 6	85 ± 6	60 ± 7	50 ± 5	34 ± 6	33 ± 12
600	80 ± 8	81 ± 7	52 ± 16	32 ± 13	45 ± 1	51 ± 5
700	81 ± 1	72 ± 5	51 ± 3	76 ± 7	62 ± 3	34 ± 5
800	79 ± 6	85 ± 6	65 ± 5	79 ± 12	79 ± 6	65 ± 10
900	89 ± 2	73 ± 5	54 ± 7	52 ± 15	46 ± 4	57 ± 10
1000	77 ± 2	79 ± 8	61 ± 4	89 ± 5	68 ± 6	63 ± 10
1100	78 ± 3	82 ± 3	68 ± 6	75 ± 8	53 ± 4	55 ± 11
1200	55 ± 18	63 ± 8	60 ± 3	51 ± 7	52 ± 6	48 ± 3
	L***Q****x	Q*	L***Q**	NS	L***Q*	L***
LSD ^w	21	NS	20	27	17	23

²Obtained between placement into greenhouse and mid-April each year, in which a minimum of 22C (72F) was maintained.

^yStandard error of the mean (±) performed by PROC MEANS, SAS.

^xNS, L, and Q represent non-significant, linear, and quadratic response to chill hours, respectively.

^wLeast significant difference within a column using Fisher's Protected LSD ($\alpha = 0.05$).

*, **, *** represent significance at the 0.05, 0.01, and 0.001 level, respectively.

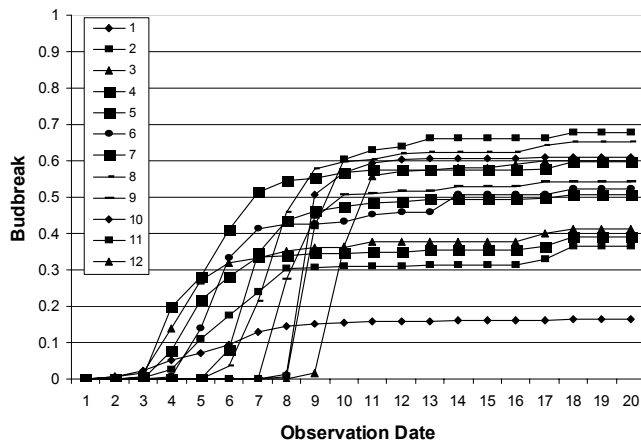


Fig. 1. *Ginkgo biloba* L. foliar budbreak percent over time in response to chilling following three years of forced chilling accumulation (3 yr Group). Observation date based on interval schedule of 4 days.

Year 2. In year two, *G. biloba* seedlings required a minimum of 100 chill hours to initiate budbreak (data not shown). Trees receiving 400 chill hours did not initiate budbreak until 2106 greenhouse hours were applied, as compared to 1596 greenhouse hours for trees receiving 800 chill hours and 1314 greenhouse hours for trees receiving 1200 chill hours. The fastest level to initiate budbreak, calculating greenhouse hours plus chill hours, was obtained from trees receiving 800 chill hours and 1596 greenhouse hours, for a total of 2396 hours.

Trees receiving 100 chill hours reached a level of 50% foliar budbreak (Table 1). Trees receiving 400 chill hours did not reach 50% budbreak until 2232 greenhouse hours were applied, as compared to 1680 greenhouse hours for trees receiving 800 chill hours and 1440 greenhouse hours for trees receiving 1200 chill hours. The fastest level to reach 50% budbreak, calculating greenhouse hours plus chill hours, was obtained from trees receiving 900 chill hours and 1560 greenhouse hours, for a total of 2460 hours (Table 1). Budbreak percentage began to level off after the twelfth count date (April 5), which was thirteen weeks after the last batch of trees were placed into the heated greenhouse (Fig. 1).

Following the last observation date (April 12), total percent budbreak was calculated. Foliar budbreak results were accelerated with increasing chill levels. Trees receiving 400 chill hours produced 64% budbreak (Table 2), as compared to 85% for trees receiving 800 chill hours and 63% for trees receiving 1200 chill hours. The maximum budbreak (85%) for year two was obtained by trees receiving 800 and 500 chill hours. The lowest budbreak (57%) was obtained by trees receiving 100 chill hours (Table 2).

Overall visual differences were again apparent among the treatments. Plants responded basically the same as in year one. With increasing chill hours, trees appeared to have more branching and foliage. As chill levels were increased, heat units required to initiate budbreak and reach 50% budbreak decreased. Optimum chilling appeared to begin at 800 hours of chilling.

Year 3. In year three, *G. biloba* seedlings required a minimum of 100 chill hours to initiate budbreak (data not shown). Trees receiving 400 chill hours did not initiate budbreak until 720 greenhouse hours were applied, as compared to 552

greenhouse hours for trees receiving 800 chill hours and 480 greenhouse hours for trees receiving 1200 chill hours. The fastest level to initiate budbreak, calculating greenhouse hours plus chill hours, was obtained from trees receiving 100 chill hours and 936 greenhouse hours, for a total of 1036 hours.

Trees required a minimum of 500 chill hours to reach a level of 50% foliar budbreak (Table 1). Trees receiving 500 chill hours did not reach 50% budbreak until 1168 greenhouse hours were applied, as compared to 936 greenhouse hours for trees receiving 800 chill hours and 624 greenhouse hours for trees receiving 1200 chill hours. The fastest level to reach 50% budbreak, calculating greenhouse hours plus chill hours, was obtained from trees receiving 600 chill hours and 816 greenhouse hours, for a total of 1410 hours (Table 1). Budbreak percentage began to level off after the eleventh count date (March 3), which was four weeks after the last batch of trees were placed into the heated greenhouse (Fig. 1).

Following the last observation date (April 8), total percent budbreak was calculated. Foliar budbreak results were accelerated with increasing chill levels. Trees receiving 400 chill hours produced 39% budbreak, as compared to 65% for trees receiving 800 chill hours and 60% for trees receiving 1200 chill hours (Table 2). The maximum budbreak (68%) for year three was obtained by trees receiving 1100 chill hours. The lowest budbreak (16%) was obtained by trees receiving 100 chill hours (Table 2).

Overall visual differences were again apparent among the treatments. As chill levels increased, plant height and width increased also. Trees appeared to have more branching, foliage, larger caliper trunks and greater overall appearance. The data suggest that the optimal chilling range (for the 3 yr group) appeared to begin in the range of 700 hours of chilling.

Table 3. Percent budbreak obtained after receiving a minimum of 1248 greenhouse hours^a following three years of forced chilling on *Ginkgo biloba* L.

Chill hours	% Budbreak ^b
100	16
200	36
300	41
400	39
500	60
600	52
700	61
800	55
900	64
1000	61
1100	68
1200	60
L***Q**	
LSD ^w	2

^aOne greenhouse hour = one hour at a minimum of 22C (72F) inside the greenhouse.

^bPerformed by PROC GLM, SAS.

^cNS, L, and Q represent non-significant, linear, and quadratic response to chill hours, respectively.

^wLeast significant difference within a column using Fisher's Protected LSD ($\alpha = 0.05$).

*, **, *** represent significance at the 0.05, 0.01, and 0.001 level, respectively.

2 Year Group

Year 2. Trees within the 2 yr group received ambient chilling for the first season of growth (998 hours), but were then placed into the forced chilling regime for years two and three. Therefore the age of the trees was the same as the 3 yr group, but treatments were only applied to the 2 yr group in Year 2 and Year 3 of the study. All treatments initiated budbreak after receiving a minimum of 100 chill hours and 2952 greenhouse hours (data not shown). All treatments reached 50% budbreak after receiving 100 chill hours and 3232 greenhouse hours (Table 1). The fastest budbreak initiation was obtained from trees receiving 900 chill hours and 1476 greenhouse hours, for a total of 2376 hours. The fastest 50% budbreak was also obtained from trees receiving 900 chill hours and 1560 greenhouse hours, for a total of 2460 hours (Table 1). The maximum budbreak (89%) was obtained from trees receiving 1000 chill hours (Table 2). Optimum chilling appeared to begin at 700 chill hours.

Year 3. All treatments again initiated budbreak after receiving only 100 chill hours (data not shown). The fastest trees to initiate budbreak received 200 chill hours and 816 greenhouse hours, for a total of 1016 hours. Trees within this group and year required a minimum of 700 chill hours to reach 50% budbreak (Table 1). The fastest to reach 50% budbreak were trees receiving 700 chill hours and 768 greenhouse hours, for a total of 1468 hours (Table 1). The maximum budbreak (79%) was obtained from trees receiving 800 chill hours (Table 2). Optimum chilling (for the 2 yr group) appeared to begin at 700 chill hours (Fig. 1).

1 Year Group

Year 3. Trees within the 1 yr group received ambient chilling for the first two seasons (998 and 1487 hours, respectively), before being placed into the chilling regime for year three. All treatments once again initiated budbreak after receiving only 100 chill hours (data not shown), but required a minimum of 600 chill hours to reach 50% budbreak (Table 1). The fastest treatment to initiate budbreak was obtained from trees receiving 400 chill hours and 672 greenhouse hours, for a total of 1072 hours (data not shown). The fastest treatment to reach 50% budbreak was obtained from trees receiving 600 chill hours and 960 greenhouse hours, for a total of 1560 hours (Table 1). The maximum budbreak (65%) was obtained from trees receiving 800 chill hours (Table 2). Optimal chilling (for the 1 yr group) appeared to begin at 800 hours of chilling.

Results shown for the 2 yr group and the 1 yr group support results from the 3 yr group. Optimal chilling for all treatment levels and groups appeared to begin in the 700–800 chill hour range. Results indicate chilling to be a determinate factor in foliar budbreak for *G. biloba*. Increasing the level of chilling led to higher budbreak numbers and percentages. Budbreak rate was accelerated by increasing chilling, therefore resulting in a reduced heat requirement. With equal hours inside the greenhouse, percent budbreak levels increased with increasing chill hours (Table 3). Chilling impact became evident the longer trees were held in the greenhouse. For trees receiving 200 chill hours, this impact became evident after six weeks inside the greenhouse. For trees receiving 400 to 800 chill hours, this became evident after four weeks inside the greenhouse. For trees receiving 900 to 1200 chill hours, this became evident after only three weeks

inside the greenhouse. Growers who use greenhouse production methods can accelerate production of *Ginkgo* by adjusting greenhouse conditions. It is reasonable to predict that tissue cultured plantlets and rooted cuttings may be produced at a faster rate by alternating cold storage with greenhouse growing conditions (15, 19). While *G. biloba* is a suitable landscape choice based upon the amount of chilling received for much of the United States, this study indicates that field production may be better suited to areas receiving 700 or more hours of chilling.

To develop regional planting models, more research would be of benefit. Many interacting factors lead to the onset of plant dormancy and subsequent budbreak (light, temperature, chronological age of plant, provenance, hormones, environmental conditions, etc.). These factors must be studied further to present a more accurate view of specific chilling requirements in *G. biloba* cultivars. The study presented here assumed ambient temperatures below 7C (45F) and a constant 3C (38F) when applied in a cooler as adequate to accomplish chilling, and that maintaining the greenhouse environment above 22C (72F) was ideal to allow for foliar emergence. Lower or higher temperatures could be considered more effective for breaking dormancy. Also, differences between constant versus fluctuating temperatures in a natural or simulated environment merit additional study.

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