Impact of Chilling on *Ginkgo biloba*©

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***Ginkgo biloba* L. seedlings were evaluated in studies initiated on 1 Oct. 1999 and terminated on 20 Sept. 2002. Forty-six-centimeter (18 inch) tall liners were potted in 3.8-liter (1-gal) containers using a standard nursery medium. Thirteen levels of chilling were applied to trees in increments of 100 h, with 12 replications per treatment. Measurements were taken on total percent budbreak, new shoot extension, number of limbs, average length of each limb, and total height. Total percent budbreak increased linearly and quadratically with increasing chill hours. All other measurements increased linearly with increasing chill hours. Optimum chilling was determined to begin at 800 chill hours. Determining optimum chilling range for *G. biloba* could potentially decrease production costs and time, supplying a product for market more efficiently and more affordably.**

INTRODUCTION

Ginkgo biloba L. is an ornamental tree of prominence and value in the green industry. Often referred to as a "living fossil", *G. biloba* grows well in U.S.D.A. Hardiness Zones 3 through 9 (Kwant, 2002), reaching a mature size of up to 24 m (80 ft) tall and 12 m (40 ft) wide. With a slow to medium growth habit, trees are often pyramidal when young and become wide spreading when older.

Ginkgo biloba is very adaptable, but prefers full to partial sun and moist, well-drained soils. Known to be insect and disease resistant and while the only species in the *Ginkgo* Genus, there are over 40 cultivars available (Dirr, 1998), it is an excellent choice for a landscape tree. Most studies with *G. biloba* have been for medicinal purposes (Juretzek, 1997), with little attention given to its culture and production. Though much work has been done with fruit species with respect to dormancy and chilling requirements (Citadin, et al, 2001; Couvillon and Erez, 1985), and with limited work on ornamental species (Ashby, et al., 1991; Ruter, et al., 1994; Sibley, et al., 2001; Wilson, et al., 2002), no work has reported the chilling requirements for *G. biloba*. Chilling units are generally based on the number of chilling hours accumulated below 7°C (45°F), referred to as the Old 45 Model (Powell, et al., 1999). The objectives of this study were to establish whether chilling is a determinate factor in foliar budbreak of *G. biloba*, to evaluate foliar budbreak response to different chilling levels, to establish the optimal chilling requirement for this species, and to evaluate overall plant growth and performance for different chilling levels.

MATERIALS AND METHODS

Forty-six-centimeter (18-inch) tall liners of *G. biloba* seedlings were obtained from Musser Forest, Inc. (Indiana, PA) in 1999. All trees were initially potted into full 1-gal (3.8-liter) containers and shifted to 2-gal (7.9 liter), and 3-gal (13.5 liter) nursery containers in Years 2 and 3. A standard pine bark and sand medium (6 : 1, v/v) amended with dolomitic limestone, Micromax (The Scotts Co., Marysville,

Ohio), and 18N-6P-12K Osmocote (The Scotts Co.) was used. The study was set up with 13 treatments in 100-chill hour increments. Treatments 0 to 12 represent 0 to 1200 total chilling hours accumulated. There were four single tree replications per treatment in Year 1 (1999 – 2000), for a total of 52 trees. The remaining 104 trees received ambient chilling (998 h for the season), while remaining on container pad area. In Year 2 (2000 – 2001), four single tree replications were added to each treatment, for a total of 104 trees. The remaining 52 trees received ambient chilling (1487 h for the season), while remaining on container pad area. In Year 3 (2001 – 2002), four single tree replications were again added to each treatment bringing the total to 156 trees treated in the various chilling regimes. Each year, Treatment 0 was placed into a greenhouse maintained at a minimum of 22°C (72°F) before accumulating any chill hours. Treatment 1 was placed into a greenhouse upon receiving 100 ambient chill hours (21 Dec. 2001), which allowed trees to defoliate naturally. Upon receiving 200 ambient chill hours (28 Dec. 2001), Treatment 2 was placed into the greenhouse. Trees receiving 300 to 1200 total chill hours were then placed into a cooler maintained at 3°C (38°F), until desired forced chilling was accumulated. At appropriate times, trees were removed from the cooler and placed into the greenhouse. Plants were monitored twice weekly for foliar budbreak for approximately 17 weeks. All trees were measured for total percent budbreak, new shoot extension, number of limbs, average length of each limb, and total plant height. All data were analyzed with regression analysis, using orthogonal contrast statements and the SAS GLM procedure (SAS, 1999). All work was conducted at the Paterson Greenhouse Complex in Auburn, Alabama (32° 36'N × 85° 29'W, USDA Hardiness Zone 8a).

RESULTS AND DISCUSSION

Percent Budbreak. This report covers details of results for the 2001 to 2002 year. Increasing the number of chill hours of *G. biloba.* led to a decreased amount of time required inside the greenhouse to reach 50% foliar budbreak. In most cases, increased chilling produced a higher percent budbreak. Over the period of the study, trees receiving a minimum of 800 chill hours were the first to reach the point of 50% budbreak. Trees receiving less than 300 total chill hours failed to reach a point of 50% budbreak in 2 of 3 years evaluated. Chilling not only increased percent budbreak overall, but also allowed the trees to do so at a faster rate (Fig. 1). After the eleventh date for bud counts (3 March 2002), budbreak percentages for all treatments leveled off (Fig. 1), after which budbreak percent responded quadratically with increasing chill hours (Fig. 2). That is, budbreak percent increased with increasing chill hours up to around 1000 h. Trees that were in the cooler for an extended period of time were able to reach the point of 50% budbreak with less accumulated time inside the greenhouse. This was evident with trees receiving 800 to 1100 chilling hours when compared to all other treatments. This work shows chilling to be a determinate factor in foliar budbreak of *G. biloba.* Increased chilling led to a decreased heat requirement to initiate budbreak.

Plant Growth. Overall plant development was affected by amount of chilling applied to trees. Results showed that different treatment levels affected total length of limbs (p value = 0.0063). Total length of limbs was calculated by adding length of all limbs, which were measured from trunk to tip of each limb. As chilling lev-

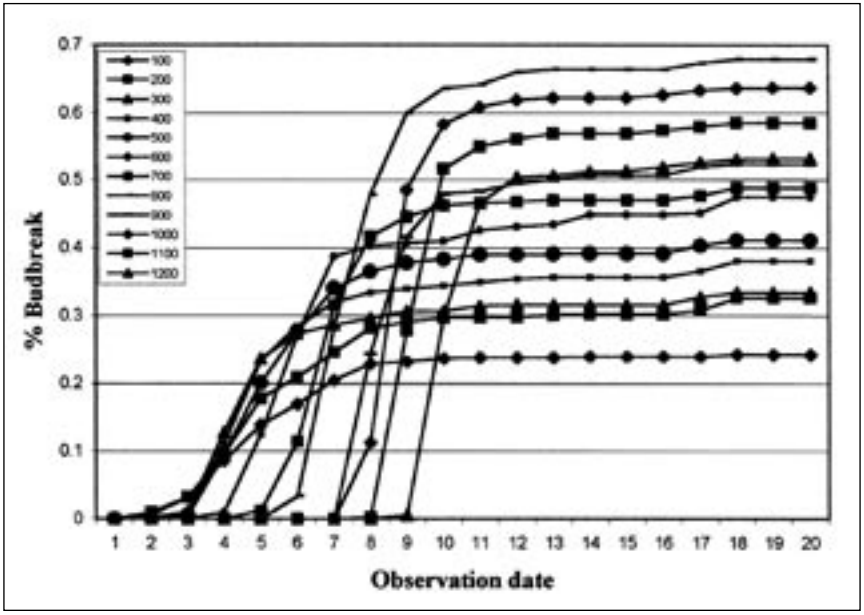

Figure 1. *Ginkgo biloba* L. budbreak percentage over time in response to incremental (0 to 1200 hours) chilling following three years of forced chilling.

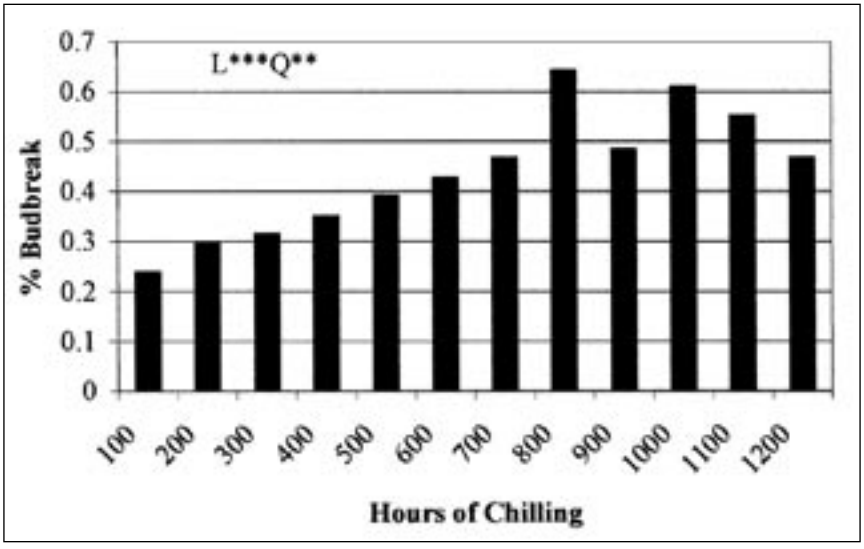

Figure 2. *Ginkgo biloba* L. budbreak response to chilling where trees were placed incrementally into heated greenhouse following each 100 h of forced chilling, with heat accumulation greater for trees receiving less chilling on any given observation date.

Table 1. Growth of *Ginkgo biloba* exposed to different chilling regimes, following 3 years of forced chilling at thirteen levels.

Chill hours	Total shoot extension[z]	Total limb length[z]
0	---[y]	---[y]
100	46	217
200	56	243
300	50	181
400	52	246
500	48	201
600	56	362
700	62	216
800	59	307
900	58	339
1000	52	288
1100	71	404
1200	62	335

L**L***
L** = p value<.01
L*** = p value<.001
[z]Values represent means of twelve replications per treatment.
[y]Values represent measures in cm.
--- = all trees for these treatments failed to break bud and eventually died.

els were increased, total length of limbs increased linearly (Table 1). Results also showed different treatment levels affected total new shoot extension (p value = 0.0698). Total new shoot extension was measured from point where terminal bud broke from previous seasons growth to highest point on tree. As chilling levels were increased, total new shoot extension increased linearly (Table 1).

For growers producing *G. biloba* liners in greenhouses, adjusting environmental conditions to allow chilling can accelerate and lead to more efficient production. As for field or outdoor production, while it is possible to produce *G. biloba* in USDA Hardiness Zones 3 through 9, our work indicates trees may reach a profitable size more rapidly in regions that accumulate greater than 800 chilling hours.

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Initial Shoot Growth and Development of Micropropagated Blueberry Plants Following Inoculation with an Ericoid Mycorrhizal Isolate©

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INTRODUCTION

Hymenoscyphus ericae (Read) Korf and Kernan is a widespread ericoid mycorrhizal fungus found on North American and European continents. *Hymenoscyphus ericae* has demonstrated mycorrhizal associations with many ericaceous species including *Vaccinium angustifolium* Ait. (lowbush blueberry) and *V. corymbosum* L. (highbush blueberry).

Inoculation of species of *Vaccinium* with isolates of *H. ericae* have resulted in mixed responses. In several studies, in vitro inoculation of *V. corymbosum,* with *H. ericae* resulted in positive effects on shoot growth. However, in some instances, mycorrhizal colonization has had a negative impact on shoot growth.

In the field, seasonal conditions can dramatically effect the intensity of root colonization. To avoid the influence of seasonal fluctuations on mycorrhizal activity in the host plant, this study used controlled environmental conditions in a greenhouse to investigate the effects of isolates of *H. ericae* on shoot growth of *V. corymbosum* 'Bluecrop' grown in a commercially available growing medium. Although several field studies involving ericoid mycorrhizal inoculation have used the highbush blueberry cultivar 'Bluecrop' as the host, there have been no apparent studies conducted using this selection in greenhouse conditions.

MATERIALS AND METHODS

Microshoots of blueberry (*V. corymbosum* 'Bluecrop') were rooted directly in autoclaved Jiffy-7® Peat Pellets containing established cultures of one of five different isolates of the ericoid mycorrhizal fungus, *H. ericae* or remained non-inoculated. Microshoots were grown for 8 weeks under aseptic conditions in a growth chamber and then transferred to a greenhouse for 16 weeks. In the greenhouse, light was provided by filtered natural sunlight and supplemented by 400-W sodium-vapor high-intensity discharge (HID) lamps which provided an average daily maximum PPF of 220 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for a 16-h day. The greenhouse was air conditioned and therefore