Dikegulac Sodium and Benzyladenine Effects on Six Landscape Tree Species during Container Production[1]

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Abstract

A study was conducted to evaluate dikegulac sodium (dikegulac) and benzyladenine (BA) as branching agents on landscape trees during production. Common among the six species in this two-year study was an increase in new shoot development following the application of dikegulac. Relative to shoot counts of nontreated plants, trees treated with a single foliar application of 800 to 3200 ppm of dikegulac had an increase in shoot numbers of 29 to 107% in Japanese maple, 75 to 158% in red maple, 67% in redbud, 50 to 65% in bald cypress, and 56 to 103% in black gum. Nontreated plants of green ash formed only one or two lateral shoots in 2011, whereas dikegulac-treated green ash had 10 to 12 new shoots. In 2012, green ash treated with 200 to 800 ppm of dikegulac developed 100 to 150% more new shoots than nontreated green ash. Foliage of all species, except Japanese maple, was injured to varying degrees by dikegulac, but the injury dissipated over the growing season. BA promoted increased shoot development only in bald cypress, and canopies of that species were visually fuller and more compact than those of bald cypress treated with dikegulac.

Index words: nursery production, plant growth regulator, branching agent.

Chemicals used in this study: dikegulac sodium (Augeo) [sodium salt of 2,3:4,6-bis-O-(1-methylethylidene)-a-L-xylo-2-hexulofuranosonic acid]; benzyladenine (BA) (BAP-10) [6-N-(phenylmethyl)-1H-purine-6-amine].

Species used in this study: bald cypress (*Taxodium distichum* L. Rich.); black gum (*Nyssa sylvatica* Marsh.); green ash (*Fraxinus pennsylvanica* Marsh.); Japanese maple (*Acer palmatum* Thunb.); redbud (*Cercis canadensis* L.); and red maple (*Acer rubrum* L.).

Significance to the Horticulture Industry

Landscape trees often require repeated pruning of lateral and terminal shoots during nursery production to develop dense, well-branched canopies. When the central leader of trees is removed, a new leader must usually be reestablished from a lateral shoot to promote height growth, and its attachment to the original shoot may not be as strong, occasionally leading to failure in the landscape. Mechanical pruning is costly and time-consuming, especially when tree canopies must be worked from lifts. The plant growth regulator dikegulac offers growers an additional tool for promoting branching during production of at least the six tree species evaluated in this study without pruning the central leaders, although some mechanical pruning will still be required to ensure branches are spaced optimally along the trunks. Benzyladenine (BA) was not effective on five of the six species evaluated. However, bald cypress treated with BA was well-branched with dense canopies without additional mechanical pruning.

Introduction

Trees are the most dominant and visible element of most landscapes, providing numerous environmental, economic, social and aesthetic benefits to a setting (Freedman and Keith 1996, Gold 1977, Tyrväinen 1997). The high-end market for landscape trees is driven by landscape architects and discriminating clients who demand a product with straight trunks and symmetrical canopies much denser than most species develop naturally. Growers develop high-quality shade trees through a combination of pruning of lateral and terminal shoots and a reestablishment of a central leader, a process that has to be repeated multiple times during a production cycle. This process is labor intensive and is exacerbated when canopies cannot be worked from the ground (Bold Spring Nursery, Hawkinsville, GA, personal communication).

Apical dominance, the control that terminal buds exert over lateral buds on a plant stem (Cline 1997), is regulated by auxin, which diffuses basipetally from terminal buds and inhibits the outgrowth of lateral buds (Tamas 1995), often resulting in sparsely branched plants. Pruning of shoot tips removes the source of apical dominance, increases the balance of cytokinin to auxin, and stimulates lateral bud development (Cline 1997, Tamas 1995). Plant growth regulators (PGRs), many of which have cytokinin activity, have been evaluated as branching agents on numerous woody and herbaceous plants with mixed results (Banko and Stefani 1995, Bruner et al. 2002, Cochran and Fulcher 2013a and 2013b, Fain et al. 2001, Hester et al. 2013, Holland et al. 2007, Jacyna 1994). Dikegulac sodium is the only PGR labelled for use as a branching agent and growth retardant on woody landscape plants, sold commercially as Atrimmec, and recently as Augeo for use on woody and herbaceous plants during production. In previous studies, dikegulac suppressed shoot growth of cleyera (*Cleyera* spp.), yaupon holly (*Ilex vomitoria* Sol. ex Aiton), and thorny elaeagnus (*Elaeagnus pungens* Thunb.), but also increased new shoots in yaupon holly (Banko and Stefani 1996). Dikegulac also increased branching in cotoneaster (*Cotoneaster* spp.), abelia (*Abelia* spp.), and foster holly (*Ilex ×attenuata* 'Fosteri') (Banko and Stefani 1995) and Little Lime™ hydrangea (*Hydrangea paniculata* Siebold) (Cochran et al. 2013a). Augeo currently has only three tree species on the label: crape myrtle (*Lagerstroemia* spp.), green ash and red oak (*Quercus rubra* L.) (OHP product label, Anonymous 2010). The cytokinin 6-benzyladenine (BA) has been effective in promoting new shoots in both woody and herbaceous ornamentals (Garner et al. 1998, Hester et al. 2013, Keever and Foster 1990), but it is only labelled for use on three herbaceous species (Fine Americas product label, Anonymous 2012). Because of

[1]Received for publication July 15, 2014; in revised form November 3, 2014. This research was supported, in part, by the Alabama Agricultural Experiment Station, OHP, the J. Frank Schmidt Family Charitable Foundation, and the Alabama Forestry Commission.

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the prior activity of these two PGRs on woody plants, the objective of this study was to evaluate dikegulac and BA as branching agents on landscape trees during container production.

Materials and Methods

Liners of green ash, Japanese maple, red maple, and redbud were potted into 12.4 L (3.3 gal) containers (EconoGrip, GL 1400, Nursery Supplies, Inc., Chambersburg, PA) between February 2 and February 6, 2011. The 7:1 pine bark:sand substrate was amended per m^3 (yd^3) with 7.3 kg (16 lb) of 17N-2P-9K (PolyOn 17-5-11, Pursell Industries, Sylacauga, AL), 0.7 kg (1.5 lb) Micromax (Everris NA, Dublin, OH) and 2.3 kg (5 lb) dolomitic limestone. Plants were placed outdoors in full sun under twice-daily irrigation, receiving approximately 2.6 cm (1 in) of water per day. Plants were re-spaced as they grew.

On June 5, 2011, trees were selected for uniformity and staked, and shoots from the lower 46 cm (18 in) of green ash and the lower 30.5 cm (12 in) of Japanese maple, red maple, and redbud trunks were removed. Tree height, trunk caliper, and shoot counts were recorded on June 6. A single foliar application of 800, 1600, or 3200 ppm of dikegulac (Augeo, OHP Inc., Mainland, PA) or 2500 ppm of BA (BAP10, Plantwise Biostimulant Co., Louisville, KY) was applied to all trees on June 6, 2011, at which time foliage from the first flush of growth was soft but fully developed. Buffer X (Kalo Agr. Chemicals, Overland, KS), a non-ionic surfactant, was added to the spray solution of BA at 0.2% (v/v). A nontreated control was also included as a treatment. For each treatment, green ash was replicated with seven plants, and redbud, Japanese maple and red maple were replicated with eight plants. Foliar treatments were applied using a CO_2 sprayer with a flat spray nozzle (TeeJet 8004VS, TeeJet Technologies, Wheaton, IL) at 1.4 $kg{\cdot}cm^{-2}$ (20 psi) by making four passes over each plant, resulting in complete coverage with minimal runoff. Foliar applications were made between 3 p.m. and 5 p.m. in shade to prolong drying times. Treated plants were hand watered and foliage was allowed to dry overnight before trees were returned to the irrigated growing area. Dry bulb temperature and relative humidity ranged from 33 to 35C (91 to 95F) and from 48 to 54%, respectively.

Observations of phytotoxicity were made multiple times following treatment. New shoots were counted and plant height and trunk caliper measurements were recorded on Japanese maple, redbud, and red maple on July 15, 2011, 39 days after treatment (DAT). Green ash formed tight clusters of shoots in response to dikegulac application that were slow to elongate, delaying the collection of shoot counts and plant height and trunk caliper measurements until September 27, 2011 (65 DAT).

A second experiment was conducted in 2012 using similar methodology unless otherwise noted. Liners of green ash, bald cypress, and black gum were potted between January 7 and January 11, 2012. A single foliar application of 200, 400, and 800 ppm of dikegulac or 2500 ppm of BA was applied to green ash on May 25, 2012, after foliage from the first flush of growth was fully mature. Concentrations applied to green ash were lowered in 2012 due to the formation of tight clusters of shoots in 2011, many of which subsequently dried and aborted. Dikegulac and BA treatments to bald cypress and black gum were the same as in 2011 and were applied on May 24, 2012. Applications were made in the morning under shade to maximize drying times. Dry bulb temperatures ranged from 24 to 26C (76 to 78F) and relative humidity from 82 to 88%. Treatments were replicated with seven green ash, eight black gum and ten bald cypress plants each. New shoots were counted and trunk caliper and height measured on October 3, 2012 (132 DAT).

Analysis of variance was performed on all responses using PROC GLIMMIX in SAS version 9.3 (SAS Institute, Cary, NC). Each species was analyzed as a separate experiment in a completely randomized design. Pre-treatment shoot count, plant height, or trunk caliper was included in the model as a covariate when a significant linear trend was found between the covariate and the corresponding response. Where residual plots and a significant covariance test for homogeneity indicated heterogeneous variance, a RANDOM statement with the GROUP option was used in the analysis. Shoot counts were analyzed using the normal, Poisson, and negative binomial probability distributions, and the model that gave the Pearson Chi-square / degrees of freedom value closest to 1 was chosen. Single degree of freedom orthogonal contrasts were used to test linear and quadratic trends over dikegulac concentrations. Differences in least squares means between the BA treatment and the dikegulac treatments were determined using the Shaffer Simulated Method. All significances were at $\alpha = 0.05$.

Results and Discussion

Japanese maple. Japanese maple exhibited no visible adverse effect from any treatment during the study, in contrast to what was observed following dikegulac application to all other species in this study and to what has been frequently reported by others (Arzee et al. 1977, Cochran et al. 2013b, Hester et al. 2013, Banko and Stefani 1995 and 1996), although Japanese maple was not included in any of the cited studies. New shoot counts in Japanese maple increased linearly in response to increasing dikegulac concentration and were 29 (800 ppm), 50 (1600 ppm), and 107% (3200 ppm) higher than those of nontreated plants by mid-July (Table 1). Plants treated with 1600 ppm and 3200 ppm of dikegulac developed 40 and 93%, respectively, more new shoots than plants treated with BA, which formed similar numbers of new shoots as nontreated plants. Increased branching from dikegulac application agrees with previous results by others (Banko and Stefani 1995, Bruner et al. 2002, Jacyna et al. 1994). Final plant height was not affected by any treatment, while final plant caliper changed quadratically in response to increasing dikegulac concentration. Trunk caliper of plants treated with 800 ppm and 1600 ppm of dikegulac was 23 and 14% greater, respectively, than that of nontreated plants, while caliper of plants treated with 3200 ppm of dikegulac was 24% smaller than that of the nontreated plants. This inhibition in caliper growth in trees treated with 3200 ppm of dikegulac could reduce their market value since most trees are graded and priced by trunk caliper. Trunk caliper measurements of BA-treated plants were similar to those of plants in all other treatments (Table 1).

Red maple. By 2 weeks after treatment (WAT), red maples treated with 1600 ppm and 3200 ppm of dikegulac had developed necrotic shoot terminals and bronzed to chlorotic immature leaves, while plants treated with 800 ppm of dikegulac or BA were unaffected. By 5 WAT, plants in all dikegulac treatments had developed numerous new lateral

Table 1. Effects of dikegulac and benzyladenine on growth of Japanese maple, red maple, redbud, and green ash in 2011.

Growth regulator	Rate (ppm)	New shoot counts[z]	Plant height (cm)[y]	Caliper (mm)[x]
	Japanese maple			
Dikegulac	0	14	101.4	8.4
	800	18	113.8	10.3
	1600	21*[w]	100.4	9.6
	3200	29*	105.3	6.4
	Significance[v]	L***	NS	Q*
Benzyladenine	2500	15	111.8	9.9
	Red maple			
Dikegulac	0	12	132.4	13.7
	800	12	126.6	14.6
	1600	21*	109.2*	13.8
	3200	31*	103.1*	12.4
	Significance	L***	L**	L*
Benzyladenine	2500	9	137.2	14.4
	Redbud			
Dikegulac	0	6	146.6	14.4
	800	9	140.7	15.9
	1600	10*	145.1	15.6
	3200	10*	141.2	14.3
	Significance	L*	NS	Q*
Benzyladenine	2500	6	135.2	15.0
	Green ash			
Dikegulac	0	2	153.4	18.2
	800	10*	128.3	17.1
	1600	12*	131.8	17.0
	3200	11*	108.9*	14.9*
	Significance	Q***	L**	L***
Benzyladenine	2500	1	159.9	18.2

[z]Existing lateral shoots were removed from the lower 30.5 cm (12 in) of the trunk, except on green ash, which were removed on the lower 45.7 cm (18 in), before treatment on June 6 and June 7, 2011. Data were collected on July 15, 2011, and September 27, 2011 (green ash only).

[y]Plant height was measured from soil line to the uppermost growing point on the plant.

[x]Trunk caliper was measured 15.3 cm (6 in) above the base of the plants.

[w]Least squares means comparisons of benzyladenine to dikegulac sodium rates using the Shaffer Simulated Method at $\alpha = 0.05$ (*).

[v]Nonsignificant (NS) or significant linear (L) or quadratic (Q) trends using orthogonal polynomials at $\alpha = 0.05$ (*), 0.01 (**) or 0.001 (***).

shoots that were elongating rapidly, and shoots appeared to increase in number with increasing concentration. Nontreated plants and plants treated with BA appeared similar and lacked the vigorous branching of dikegulac-treated plants. As with Japanese maple, red maple increased new shoot counts linearly in response to increasing dikegulac concentration (Table 1). Plants treated with 1600 ppm and 3200 ppm of dikegulac developed 75 and 158%, respectively, more new shoots than nontreated plants and 133 and 244% more new shoots than plants treated with BA. Plant height decreased linearly, 4 to 22%, with increasing concentrations of dikegulac. The suppression in height growth appeared due to reduced growth of the terminal as numerous new shoots were developing, and not due to dikegulac killing the terminal. Treatment with 800 ppm of dikegulac appeared to increase trunk caliper, while 1600 ppm had no effect and 3200 ppm reduced caliper by up to 9%. These changes in trunk caliper were probably not of horticultural significance. BA had no effect on either height or caliper when compared to plants in all other treatments.

Redbud. Redbud treated with the highest dikegulac concentration showed slight interveinal chlorosis by 2 WAT that dissipated over the following 2 weeks (data not shown). Redbud increased new shoot counts linearly in response to increasing dikegulac concentration (Table 1). Plants treated with 1600 ppm and 3200 ppm of dikegulac developed 67% more new shoots than nontreated plants and plants treated with BA. Final plant height was not affected by any treatment, while final trunk caliper was minimally affected by dikegulac.

Green ash. Of the four species in the 2011 study, green ash was the most sensitive to dikegulac canopy sprays. By 2 WAT upper leaves were chlorotic, necrotic, and curled, and the severity increased with increasing dikegulac concentration (data not shown). By 4 WAT, axillary buds on dikegulac-treated plants began to elongate and injury diminish. By 5 WAT, tight clusters of compressed shoots developed on dikegulac-treated plants, and this phenomenon increased with treatment concentration. Plants treated with dikegulac also were noticeably shorter than nontreated and BA-treated plants. Although shoots elongated on most plants, on a few plants, new shoots darkened and died, but remained on the plants at the end of the season. Green ash was not injured by BA application. Lateral shoot development was most significant on dikegulac-treated green ash compared to BA- and nontreated plants at the end of the growing season (10 to 12 vs 1 and 2, respectively, Table 1). Plant height and trunk caliper decreased linearly by up to 29 and 18%, respectively, with increasing dikegulac concentration. Plants were shorter with smaller caliper when treated with 3200 ppm of dikegulac compared to those treated with BA. These results suggest that, in an attempt to minimize injury, concentrations of 800 ppm of dikegulac or lower may be more appropriate to promote branching in green ash. Injury was unacceptable, and shoot and caliper growth was significantly reduced at rates above 800 ppm.

In 2012, dikegulac concentrations of 0, 200, 400, and 800 ppm were applied to green ash. Green ash developed mild interveinal leaf chlorosis on plants in all dikegulac treatments within 2 weeks, but chlorosis was minimal compared to that observed in 2011 (data not shown). A few green ash plants in all dikegulac treatments also developed excessively branched, but stunted, clusters of terminal shoots that never elongated. These clusters remained on the plants but dried over time and were hidden by new shoots that developed from axillary buds. New shoot counts changed quadratically in response to increasing dikegulac concentrations, with increases of 117, 150, and 100% from application of 200, 400, and 800 ppm of dikegulac, respectively, relative to those on nontreated plants (Table 2). The dikegulac label for nursery use (Augeo specimen label 2010) lists three tree species, including green ash. Based on our results, the labeled concentration of 400 to 800 ppm of dikegulac is above the optimal of 200 to 400 ppm for promoting branching, which also minimized foliar injury and stunted clusters of buds in green ash. Plant height decreased quadratically in response to increasing dikegulac concentration. Height growth was

Table 2. Effects of dikegulac and benzyladenine on growth of green ash, bald cypress, and black gum in 2012.

Growth regulator	Rate (ppm)	New shoot counts[z]	Plant height (cm)[y]	Caliper (mm)[x]
		Green ash		
Dikegulac	0	6	194.4	21.3
	200	13*[w]	155.1*	21.1
	400	15*	159.3*	21.7
	800	12*	169.5*	21.7
	Significance[v]	Q**	Q*	NS
Benzyladenine	2500	4	219.0	22.5
		Bald cypress		
Dikegulac	0	46*	136.4	23.7
	800	69	161.3	25.1*
	1600	76	151.9	23.9
	3200	71	147.6	22.9
	Significance	Q***	Q**	NS
Benzyladenine	2500	80	150.7	22.0
		Black gum		
Dikegulac	0	32	160.9*	19.9*
	800	50	172.0	23.8
	1600	65*	168.2	21.2
	3200	65*	165.2	21.0
	Significance	L***	NS	Q*
Benzyladenine	2500	39	191.3	23.7

[z]Existing lateral shoots were removed from the lower 30.5 cm (12 in) of the trunk, except on green ash, which were removed on the lower 45.7 cm (18 in), before treatment on May 24 and May 25, 2012. Data were collected on October 3, 2012.

[y]Plant height was measured from soil line to the uppermost growing point on the plant.

[x]Trunk caliper was measured 15.3 cm (6 in) above the base of the plants.

[w]Least squares means comparisons of benzyladenine to the dikegulac sodium rates using the Shaffer Simulated Method at $\alpha = 0.05$ (*).

[v]Nonsignificant (NS) or significant linear (L) or quadratic (Q) trends using orthogonal polynomials at $\alpha = 0.05$ (*), 0.01 (**) or 0.001 (***).

suppressed 13 to 20% by dikegulac, which was less than the 29% suppression from 3200 ppm of dikegulac in 2011 (Table 1) but similar to the 14 and 16% suppression from 800 ppm and 1600 ppm of dikegulac in 2011. In contrast to the decrease in trunk caliper with increasing concentrations of dikegulac recorded in 2011, caliper was not affected by the lower concentrations of dikegulac applied in 2012. BA did not injure plants and had no effect on shoot counts, plant height and trunk caliper.

Bald cypress. Bald cypress exhibited temporary bronzing of the foliage and necrosis of the shoot tips when treated with dikegulac, regardless of concentration, and 2500 ppm of BA. However, injury was transient and disappeared by mid-season. New shoots responded quadratically to increasing dikegulac concentration (Table 2). Relative to shoots on nontreated plants, numbers increased by 50 to 65% in response to dikegulac application, with the greatest increase from the intermediate dikegulac concentration. In contrast to the lack of response in other species to BA in 2011 or 2012, bald cypress developed 74% more new shoots than nontreated plants following BA application. In addition, new shoots formed along the entire trunk, as opposed to only above the lower 30 cm (12 in) of trunk stripped of shoots prior to treatment application, which was the case in other species following dikegulac application. This response to BA would be beneficial to growers seeking dense plants that are full to the ground, but treated trees could require additional labor to remove these shoots if a clear trunk is desired. It is unknown if BA would cause a similar response in older trees that do not typically develop lateral shoots from wood several years old. There was a quadratic increase in height with increasing dikegulac concentration. Plants treated with dikegulac were 8 (3200 ppm) to 18% (800 ppm) taller than nontreated plants, while trunk calipers were similar. Plants treated with BA were similar in height and caliper to nontreated plants and those treated with dikegulac, except for a smaller caliper compared to plants treated with 800 ppm of dikegulac.

Black gum. The youngest shoot growth on black gum exhibited minor chlorosis within 2 weeks of dikegulac application, and foliage of plants treated with 3200 ppm of dikegulac was slightly curled (data not shown). In addition, plant height was visually suppressed following dikegulac application (data not shown), but these effects dissipated over the subsequent 3 to 4 weeks. Numerous new shoots developed on dikegulac-treated plants between 3 and 5 WAT. At the end of the growing season, new shoot counts in black gum increased linearly with increasing dikegulac concentration and were 56 (800 ppm), 103 (1600 ppm) and 103 (3200 ppm) higher than those of nontreated plants (Table 2). Trunk caliper growth at the end of the growing season increased quadratically with increasing dikegulac concentration and was 6 to 20% greater in dikegulac-treated plants than in nontreated plants; however, plant height was unaffected. Dikegulac visually suppressed height growth in the flush that immediately followed treatment, but over time the original terminal shoot and several of the new shoots on each plant gained apical dominance and elongated vigorously, which accounted for the lack of dikegulac effect on plant height in October. However, the upper canopies formed by these vigorous shoots lacked the visual density seen earlier in the growing season and sought by growers. This species would have possibly benefitted from a second dikegulac application mid-season to suppress shoot elongation and further increase shoot counts. Black gum was not visually affected by BA application, nor was shoot counts or plant height, while plant caliper was up to 19% greater than that of nontreated plants.

Common among all species in this two-year study was an increase in shoot development following the application of dikegulac. BA promoted new shoot development in only bald cypress; however, plant canopies in bald cypress were visually fuller and more compact than those of plants treated with dikegulac. In promoting new axillary shoot development in all species, dikegulac broke apical dominance and canopies were fuller, a goal in the production of nursery trees. In nursery production of shade trees where the shoot terminal is often removed to promote branching and a fuller canopy, a practice referred to as heading back, a single shoot would have to be selected and reestablished as the central leader to facilitate height growth and allow future removal of lower branches. However, the developing canopy formed following heading back consists of many acutely angled branches which, while not a problem during production, create potential failure points while in the landscape due to the development of included bark, which is a weak fusion of tissues (Chalker-Scott 2010). Therefore, heading back the central leader has been one of the most criticized practices in tree production. If new shoots can be promoted by branching PGRs, heading back can be avoided. Reestablishing a central leader after treatment with dikegulac did not appear necessary for Japanese maple or redbud that are grown as small multi-branched ornamental trees. Plant height varied with species and either increased (bald cypress), decreased (red maple and green ash) or was not affected (Japanese maple, redbud, and black gum) by dikegulac application. To a degree; we expected a suppression in plant height in species that developed new shoots following dikegulac application, at least in the short term. Dikegulac is a growth retardant, as well as a branching agent (Banko and Stefani 1995 and 1996, Bruner et al. 2002, Jacyna et al. 1994); in addition, branching agents often suppress height growth by distributing plant photosynthates in numerous developing shoots, as opposed to concentrating them in one or more dominant shoots (Holland et al. 2007). Trunk caliper likewise varied among the species evaluated. BA had minimal effects on plant height and trunk caliper, except in black gum, both of which increased in response to BA application. Dikegulac appears useful for canopy development of the species evaluated in this study. While manual pruning is not expected to be eliminated, dikegulac has the potential to be used in combination with less manual pruning to develop well-branched canopies during production without heading back the central leader. BA was very effective in promoting branching and a full canopy of bald cypress, but not other species, and has the potential to be used as a substitute for pruning of this species.

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