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BA Application Frequency and Concentration Effects on Two Indian Hawthorn Cultivars[1]

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Abstract

Benzyladenine (BA) is a synthetic cytokinin that promotes lateral shoot development in herbaceous and woody ornamentals and may be a viable alternative to mechanical pruning in nursery production. A study was conducted to determine the effects of BA application number, interval and concentration on two Indian hawthorn [*Rhaphiolepis* Lindl. Cor. Poir. *indica* (L.) Lindl. (*Crataegus indica* L.)] cultivars. In spring 2002, actively growing 'Olivia' and 'Eleanor Taber' received three applications of 2500 or 5000 ppm BA at a one-, two-, or three-week interval. Data collected four weeks after initial treatment (WAT) reflected the effects of one, two, or three BA applications at each concentration. At this time new shoot counts in both cultivars and foliar injury in 'Olivia', but not in 'Eleanor Taber', increased with increasing number of applications and BA concentration. Data collected 12 WAT reflected the effects of BA application interval and concentration. At this time new shoot counts increased with decreasing application interval in 'Olivia', while 'Eleanor Taber' formed the most new shoots when BA was applied at a two-week interval. All treated plants sustained minimal to moderate injury to immature foliage. The experiment was repeated in 2003 using 1750 ppm and 3500 ppm BA on 'Olivia' Indian hawthorn. Applications were halted at the first sign of foliar injury resulting in plants treated at one, two, and three-week intervals receiving three, two, and two applications, respectively. New shoot counts on 'Olivia' treated at the three application intervals were similar, but greater than those on untreated plants. Control plants had a foliar injury rating similar to that of plants treated at a two or three-week interval, but less than that of plants treated at a one-week interval. Growth index was not affected by treatment in either year.

Index words: lateral branching, cytokinin, plant growth regulator, application frequency.

Species used in this study: 'Olivia' and 'Eleanor Taber' Indian hawthorn [*Rhaphiolepis* Lindl. Cor. Poir. *indica* (L.) Lindl. (*Crataegus indica* L.)].

Chemical used in this study: N^6-benzyladenine (benzylaminopurine; BA; BAP-10).

Significance to the Nursery Industry

Several concentrations of benzyladenine (BA) applied multiple times at different intervals were effective in promoting branching of Indian hawthorn. In general, branching of both 'Olivia' and 'Eleanor Taber' was most pronounced when three applications of 2500 to 5000 ppm BA were made at one- or two-week intervals. However, foliar injury to immature foliage of 'Olivia', and to a lesser extent to 'Eleanor Taber', increased as application number and concentration increased, and, relative to untreated plants, foliar injury to both cultivars was greater when treated with BA, regardless of application interval. While multiple BA applications applied at different intervals promoted new shoot formation in two Indian hawthorn cultivars and may be a viable substitute to mechanical pruning, foliar injury to developing foliage is a possibility. Although this injury was transient, it may make plants unmarketable during the season of application.

Introduction

Indian hawthorns are dense, mound forming evergreen shrubs that grow 1 to 2 m (3 to 6 ft) tall and form dark glossy green leaves. White to pink fragrant flowers are borne in dense upright tomentose racemes or panicles from mid April to early May in Alabama. Hardy in USDA Cold Hardiness Zones 7b to 10, Indian hawthorns are widely utilized for textural effect in containers, groupings, and in mass plantings (3).

Without pruning, Indian hawthorns are sparsely branched, mis-shapened, and unmarketable. Plants in 3.8 liter (#1) containers require at least one pruning and up to 20 months to produce marketable plants from liners, while those in 11.4 liter (#3) containers require two additional prunings and up to an additional 12 months of production time (Tom Dodd Nurseries, Semmes, AL, pers. comm). A minimum of three weeks of active growing time is lost after each pruning (13). Vegetative growth in Indian hawthorn is most pronounced in early spring, prior to flowering, and is greatly reduced thereafter. Pruning to remove fruit and to stimulate new growth is labor intensive and time consuming, but considered necessary to efficiently produce marketable Indian hawthorn.

Benzyladenine (BA), a synthetic cytokinin, has been employed on whole plants to induce lateral bud growth, but its effectiveness is species specific. Single BA applications of up to 3750 ppm increased the number of new shoots in numerous *Hosta* cultivars (4, 5, 6, 7), *Nandina domestica*, *Ilex crenata*, *Ilex vomitoria*, and *Rhododendron* x 'Formosa', but not in Indian hawthorn (8). A minimum of two weekly BA applications were required to stimulate new shoot production in *Rhaphiolepis indica* 'Olivia' and 'Eleanor Taber' (9). Foliar injury occurred with multiple weekly applications of 1250 to 5000 ppm BA (9), but not with a single application of 1000 ppm BA (8). The short interval between applications may have resulted in insufficient time for full manifestation of foliar injury before subsequent applications were made, thus exacerbating symptoms. No research on the efficacy of longer BA application intervals has been published.

Our objective was to explore the interactions between BA application number, interval, and concentration on Indian hawthorn. Through this research we hoped to identify an optimal BA concentration, application number, and applica-

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tion interval for promoting new shoot development of 'Olivia' and 'Eleanor Taber' Indian hawthorn, while minimizing plant injury.

Materials and Methods

2002. Unbranched liners of 'Olivia' and 'Eleanor Taber' Indian hawthorn in 32-cell flats were potted in the fall of 2001 into 3.8 liter (#1) containers of a pine bark:sand (7:1 by vol) medium amended per m^3 with 3 kg (5 lb/yd^3) dolomitic limestone, 0.9 kg (1.5 lb/yd^3) Micromax (The Scotts Co., Marysville, OH), and 3.6 kg (6 lb/yd^3) 17N–3.1P–10K (Osmocote 17–7–12, The Scotts Co.) and placed outdoors in full sun. In early April 2002, plants were topdressed with 18 g (1 tbsp) 17N–3.1P–10K (Osmocote 17–7–12) each. Overhead irrigation was applied twice daily during the growing season at a rate of approximately 1.3 cm (0.5 in) per irrigation. Beginning May 20, 2002, both cultivars received three foliar applications of either 2500 or 5000 ppm BA (BAP-10, Plant-Wise Biostimulant Co., Louisville, KY) applied at a one-, two-, or three-week interval. Foliar sprays included 0.2% (by vol) Buffer X (Kalo Agr. Chemicals, Overland, KS), a nonionic surfactant, and were applied at 0.2 liter/m^2 (2 qt/100 ft^2) with a compressed CO_2 sprayer equipped with a flat spray nozzle (Tee Jet 8001VS, Bellspray, Inc., Opeloussa, LA) at 138 kPa (20 psi). At the time of initial application, plants were about 13 cm (5 in) tall, actively growing and had immature foliage. Temperature and relative humidity ranged from 20 to 25C (68 to 77F) and from 60 to 82% during May applications, and from 27 to 32C (80 to 90F) and 57 to 74% during June applications.

Data were collected prior to BA application on June 10, three weeks after initial application (WAT), and again on August 1, 12 WAT. New shoots longer than 2.5 cm (1 in) per plant were counted and an injury rating (1 = healthy; 2 = minimal discoloration/distortion; 3 = moderate discoloration/distortion/minimal necrosis; 4 = moderate discoloration/distortion/ necrosis; 5 = highly necrotic) of immature foliage was made by the same person. Also, a growth index [GI = (height + widest width + width perpendicular to widest width) / 3] was determined for each plant. On June 10, plants had received zero, one, two, or three BA applications, while on August 1 plants had received three BA applications at a one, two, or three-week interval. Treatments in this 2 × 3 factorial experiment (concentration × application number/interval) plus a control were completely randomized within cultivar and replicated with 10 single plants per cultivar.

2003. The experiment was repeated in 2003 with the following changes. 'Olivia' liners were potted into 3.8 liter (#1) containers of a pine bark-sand (7:1 by vol) medium amended per m^3 with 3 kg (5 lb/yd^3) dolomitic limestone, 0.9 kg (1.5 lb/yd^3) Micromax, and 7.2 kg (12 lb/yd^3) Osmocote 17N–3.1P–10K (17–7–12) on March 18, 2003, and placed in full sun under overhead irrigation. Beginning May 8, plants received up to three applications of either 1750 or 3500 ppm BA with one, two, or three weeks between applications. BA concentrations were lowered in an attempt to reduce foliar injury. At the time of initial application, plants were about 10 cm (4 in) tall, actively growing and immature foliage was present. An untreated control and a pruned treatment were included for comparison. One-third of the new growth on plants in the pruned treatment was removed on the day of initial BA application. Plants received BA applications inside a PVC frame covered with shade cloth and were allowed to dry before being returned to full sun. BA applications were terminated at the first sign of foliar injury to plants in any treatment. Plants treated weekly received three BA applications. Plants treated every two or three weeks received two BA applications because of the appearance of foliar injury on plants in both treatments prior to the third application. Treatments were completely randomized and replicated with 9 single plants. Temperature and relative humidity ranged from 30 to 34C (86 to 92F) and from 47 to 67%, respectively, during BA applications. Data were collected prior to BA application on May 29, three WAT, and on June 29, eight WAT. New shoot counts and growth index on all plants were recorded, and immature foliage was rated for injury. At the time of the second data collection plants had received three weekly applications and two applications at a two- or three-week interval.

In both experiments, the significance of main effects and interactions was determined using analysis of variance (ANOVA) (11). Orthogonal polynomials were used to test the significance of BA application number, application interval, and concentration, and single degree of freedom contrasts were used to compare the control to each application interval treatment, and the pruned treatment to each other treatment (11).

Results and Discussion

All interactions were non-significant, hence main effects only are reported.

2002. On June 10, 3 WAT, a quadratic increase in new shoot counts occurred with increasing application number to 'Olivia' (Table 1). Plants receiving one, two, or three BA

Table 1. Response of 'Olivia' and 'Eleanor Taber' Indian hawthorn to BA application number and concentration, 2002[z].

	'Olivia'		'Eleanor Taber'
Application number	**New shoot number[y]**	**Injury rating[x]**	**New shoot number**
0	2.5	1.0	7.0
1	5.1	1.0	18.4
2	7.6	1.4	18.4
3	12.6	1.6	30.6
Significance[w]	Q**	Q*	L**
BA concentration (ppm)			
0	2.5	1.0	7.0
2500	8.2	1.3	19.9
5000	8.3	1.3	25.3
Significance	Q**	L*	L***

[z]Concentration × application number was not significant for any measured attribute. Data were collected June 10, 2002.

[y]New shoots measured at least 2.5 cm (1 in) in length with first leaves unfurling.

[x]Injury rating for immature foliage: 1 = healthy; 2 = minimal discoloration/distortion; 3 = moderate discoloration/distortion/minimal necrosis; 4 = moderate discoloration/distortion/ necrosis; 5 = highly necrotic.

[w]Response linear (L) or quadratic (Q) at P = 0.05 (*), 0.01 (**), or 0.001 (***).

Table 2. Response of 'Olivia' and 'Eleanor Taber' Indian hawthorn to multiple applications of BA at different intervals, 2002[z].

	'Olivia'		'Eleanor Taber'	
Application interval (weeks)	**New shoot number[y]**	**Injury rating[x]**	**New shoot number**	**Injury rating**
1	11.3*[w]	2.5*	10.7*	1.5*
2	10.4*	2.7*	16.8*	1.7*
3	6.7*	2.3*	12.7*	1.7*
Significance[v]	L**	NS	Q**	NS
BA concentration (ppm)				
0	1.3	1.1	2.6	1.0
2500	9.8	2.3	11.6	1.3
5000	8.9	2.7	15.4	2.0
Significance	Q***	Q*	L***	L***

[z]Concentration × application interval was not significant for any measured attribute, and data were collected August 1, 2002.

[y]New shoots measured at least 2.5 cm (1 in) in length with first leaves unfurling.

[x]Injury rating for immature foliage: 1 = healthy; 2 = minimal discoloration/distortion; 3 = moderate discoloration/distortion/minimal necrosis; 4 = moderate discoloration/distortion/ necrosis; 5 = highly necrotic.

[w]Means followed by an asterisk significantly differ from the control (0 ppm BA) at $P = 0.05$ based on a single degree of freedom contrast.

[v]Response non-significant (NS), linear (L) or quadratic (Q) at $P = 0.05$ (*), 0.01 (**), or 0.001 (***).

applications developed two, three, or five times as many new shoots, respectively, as controls. These results contrast with earlier results (8) on the lack of effectiveness of a single BA application of 1000 ppm on Indian hawthorn. However, differences may be due to the higher concentrations used in this study. The positive effect of BA on branching supports findings of Oates et al. (9) on the effectiveness of multiple BA applications of 1250 to 5000 ppm BA on Indian hawthorn. New shoot counts in 'Eleanor Taber', generally two to three times those of 'Olivia', increased linearly as application number increased, from 163% with one application to 337% with three applications (Table 1). These results contrast with earlier studies with Indian hawthorn on the ineffectiveness of a single BA application (8), but support the findings of Oates et al. (9) on the effectiveness of multiple BA applications.

Foliar injury to 'Olivia' changed quadratically with increasing application number (Table 1), whereas 'Eleanor Taber' was not injured by treatment (data not shown). 'Olivia' receiving a single BA application were not injured, whereas immature foliage on plants that received two or three applications exhibited minimal discoloration and distortion, supporting previous results of a positive correlation between application number and foliar injury and cultivar differences in sensitivity to BA (9).

'Olivia' showed a quadratic increase in new shoot formation in response to increasing BA concentration (Table 1). Treated plants formed more than three times as many new shoots as untreated controls. New shoot counts in 'Eleanor Taber' increased linearly with increasing BA concentration. Plants treated with 2500 and 5000 ppm BA formed 184 and 261%, respectively, more new shoots than controls, supporting the positive effects of BA over a concentration range of 1250 ppm to 5000 ppm (9), and contrasting with the results of a single application of up to 1000 ppm BA on Indian hawthorn (8).

Foliar injury increased linearly in 'Olivia' as concentration increased (Table 1), as previously reported (9). Symptoms were also similar to those previously reported, and included minimal discoloration and distortion of immature foliage. Foliar injury to 'Eleanor Taber' was not affected by BA concentration (data not shown), supporting a previous report of greater tolerance of 'Eleanor Taber' to BA than 'Olivia' (9).

By August 1, new shoot formation in both cultivars was affected by BA application interval. 'Olivia' responded to decreasing BA application interval with a linear increase in new shoots (Table 2). Plants treated at a two or one-week interval formed about 62% more new shoots than plants treated at a three-week interval. When BA was applied at three-, two-, and one-week intervals, plants formed five, eight, and nine times as many new shoots, respectively, as untreated plants. 'Eleanor Taber' responded to decreasing application interval with a quadratic change in new shoot numbers (Table 2). A two-week BA application interval induced the most new shoots, with plants forming 32 and 57% more new shoots than those treated at one- and three-week intervals, respectively. Plants treated at one-, two-, and three-week intervals formed four, six, and five times as many new shoots, respectively, as untreated controls.

Foliar injury rating of neither cultivar was affected by application interval (Table 2). However, ratings of all treated plants were higher than those of untreated plants. Maturing foliage and new immature foliage on treated plants of both cultivars showed varying degrees of discoloration, distortion, and necrosis. On average, injury was minimal to moderate, and although not compared statistically, injury to 'Olivia' appeared greater than that to 'Eleanor Taber'. Growth index was unaffected by BA treatments in 'Olivia' ($\bar{x}$ = 26.7, SE = 0.56) or 'Eleanor Taber' ($\bar{x}$ = 26.4, SE = 0.36).

By August 1, 'Olivia' responded to BA concentration with a quadratic increase in new shoot numbers (Table 2). Plants treated with 2500 and 5000 ppm BA formed 654 and 585% more new shoots, respectively, than untreated plants. New shoot counts on 'Eleanor Taber' increased linearly in response to BA concentration, with plants receiving 2500 ppm and 5000 ppm BA forming 646 and 492% more new shoots, respectively, than untreated plants.

BA concentration affected injury to maturing treated foliage and new immature foliage not present on June 10 in both cultivars at 12 WAT (Table 2). 'Olivia' treated with 2500 ppm BA responded with minimal to moderate discoloration, distortion, and necrosis. With 5000 ppm BA, in addition to the injury observed at 2500 ppm, foliage exhibited more extensive necrosis, with the most extreme cases showing moderate necrosis on the majority of the foliage formed after treatment. Foliar injury rating for 'Eleanor Taber' treated with 2500 ppm BA reflected minimal discoloration and distortion of immature foliage on some of the treated plants. All plants treated with 5000 ppm BA showed some signs of discoloration and distortion, and some showed signs of necrosis. Treatments had no effect on growth index of either 'Olivia' ($\bar{x}$ = 26.7, SE = 0.56) or 'Eleanor Taber' ($\bar{x}$ = 26.4, SE = 0.36).

Results of this study showed cultivar-specific sensitivity in Indian hawthorn to BA application number, application interval, and concentration. New shoot formation increased in both cultivars after one, two, or three applications, with

Table 3. Response of 'Olivia' Indian hawthorn to BA application number and concentration, 2003[z].

Application number	New shoot number[y]	Injury rating[x]
0	2.0	1.0
1	1.8	1.1
2	1.3	1.2
3	2.3	1.3
Significance[w]	Q*	L**
BA concentration (ppm)		
0	2.0	1.0
1750	1.4	1.1
3500	2.3	1.2
Significance	Q*	L*
Pruned[v]	2.1	1.0

[z]Data were collected May 29, 2003, three weeks after initial application; concentration × application number was not significant for either measured attribute.

[y]New shoots measured at least 2.5 cm (1 in) in length with first leaves unfurling.

[x]Injury rating for immature foliage: 1 = healthy; 2 = minimal discoloration/distortion; 3 = moderate discoloration/distortion/minimal necrosis; 4 = moderate discoloration/distortion/ necrosis; 5 = highly necrotic.

[w]Response linear (L) or quadratic (Q) at $P = 0.05$ (*) or $P = 0.01$ (**).

[v]One-third of the new growth was removed on May 8, the day of initial BA application.

three applications inducing the most new shoots in both cultivars, as previously reported (9). 'Olivia' was not injured by single BA applications. However, immature foliage of 'Olivia' showed a minimal level of discoloration and distortion with two applications that increased with a third application, as previously reported (9). 'Olivia' formed the most new shoots when BA was applied at a one-week interval, however injury was moderate to immature foliage. New shoot counts on 'Olivia' treated with either 2500 or 5000 ppm BA were similar, and immature foliage on plants in both treatments showed minimal discoloration and distortion, contrasting with previous reports which showed that BA at 5000 ppm BA caused more foliar injury to 'Olivia' than at 2500 ppm BA (9). 'Eleanor Taber' treated at a two-week interval with either 2500 or 5000 ppm BA formed the most new shoots with minimal injury to new foliage.

2003. At three WAT, new shoot formation changed quadratically with increasing application number (Table 3); however, the range across all treatments was only one new shoot per plant and was not considered of horticultural significance. Foliar injury rating increased linearly with increasing applications number, but the injury was minimal. A similar minimal response to BA concentration was evident at three WAT. Growth index was not affected by BA application number or concentration ($\bar{x} = 10.6$, SE = 0.23). Neither were new shoot counts on pruned plants significantly different from those of untreated or BA treated plants (Table 4).

BA application interval had no effect on shoot formation, and all treated plants formed two to three times as many new shoots as controls (Table 4). Pruned plants averaged 2.8 new shoots, which was similar to those of BA treated plants and greater than those of untreated controls. Foliar injury was minimally affected by application interval. Only when BA was applied three times at a one-week interval was the injury rating greater than that of the control, and the difference was not considered horticulturally important. Growth index was not affected by treatment ($\bar{x} = 12.8$, SE = 0.28).

By eight WAT new shoot formation increased linearly with increasing BA concentration (Table 4). When plants were treated with 1750 ppm or 3500 ppm, new shoot formation increased 127 and 173%, respectively, compared to controls. New shoot counts on pruned plants were similar to those on plants treated with 1750 ppm, but less than those on plants treated with 3500 ppm BA. Foliar injury was not affected by BA concentrations.

'Olivia' Indian hawthorn responded differently to BA application number, interval, and concentration in the two years of this study. For example, plants had formed 3.5 times as many new shoots at the first data collection following treatment with 2500 ppm BA in 2002 as with 3500 ppm BA in 2003. Also, in 2002, new shoots increased with a single BA application and each additional application, whereas in 2003, a minimum of three BA applications was required to induce lateral shoot development, and no significant injury to foliage was observed. Foliar injury was minimal to moderate in all treatments in both years at the time of the first data collection. By the second data collection in 2002, 'Olivia' had

Table 4. Response of 'Olivia' Indian hawthorn to multiple BA applications at weekly intervals, 2003[z].

Application interval (weeks)	New shoot number[y]	Injury rating[x]
1	2.8*[w]	1.2*
2	3.2*	1.0
3	2.3*	1.1
Significance[v]	NS	Q*
BA concentration (ppm)		
0	1.1	1.0
1750	2.5+[u]	1.1
3500	3.0+	1.1
Significance	L***	NS
Pruned[t]	2.8	1.0

[z]Data collected June 29, 2003, eight weeks after initial application. Plants treated weekly received 3 applications and plants treated every two or three weeks received two applications. Concentration × application interval was not significant for either measured attribute.

[y]New shoots measured at least 2.5 cm (1 in) in length with first leaves unfurling.

[x]Injury rating for immature foliage: 1 = healthy; 2 = minimal discoloration/distortion; 3 = moderate discoloration/distortion/minimal necrosis; 4 = moderate discoloration/distortion/ necrosis; 5 = highly necrotic.

[w]Means followed by an asterisk were significantly differ from the control (0 ppm BA) at $P = 0.05$ based on single degree of freedom contrasts.

[v]Non-significant (NS), linear (L) or quadratic (Q) response at $P = 0.05$ (*) or $P = 0.001$ (***).

[u]Means followed by a + were significantly different from the pruned treatment, $P = 0.05$.

[t]One-third of the new growth was removed on May 8, the day of initial BA application.

formed the most new shoots after three weekly applications, whereas, in 2003, plants treated twice at a two-week interval had formed the most new shoots with two applications.

Variations in a number of factors in the two experiments may help explain the different responses. Establishment time and plant size may have influenced the number of new shoots that formed in response to BA treatment. In 2002, liners were potted in the fall and treated about seven months later. In 2003, liners were potted in March and treated about seven weeks later. At 4 WAT plants tested in 2002 were visibly larger than those tested in 2003, and by the second data collection, growth index of plants treated in 2002 was double that of plants treated in 2003, 26.7 cm (10.5 in) and 12.7 cm (5.0 in), respectively. The larger plants had more surface area to react with BA and larger root masses to supply plants with nutrients and water for new shoot production. Second, the BA concentrations used in 2002 were much more effective in new shoot induction than the concentrations used in 2003. This may be due, in part, to the difference in plant sizes and a cultivar-specific BA concentration threshold, as 'Eleanor Taber' was much more tolerant of and responsive to 5000 ppm BA than 'Olivia'. Third, over the time period of the two studies, in addition to overhead irrigation, 2003 plants received over twice as much precipitation as 2002 plants, and lower solar radiation (1).

Limiting factors that antagonize exogenous PGR applications include limited available leaf area and sensitivity of plant tissue to burn damage caused by applied compounds and wetting of the target area by precipitation (10). Also, irrigation may leach applied substances off the leaves, promote photo-oxidation, or allow surface catalyzed decomposition of sensitive organic compounds, like amino-purines (BA), to occur in solution prior to cuticular penetration of plants (10).

Effectiveness of BA is determined, in part, by the physiological age of the plant and the environmental conditions during application and the following 24 hours (2). The primary environmental conditions that increase BA activity are increasing temperature and precipitation (2, 14). Activities of other plant growth regulators are affected by humidity and water quality (12), but effects of these environmental factors on BA activity have not been reported. If foliar injury is driven, in part, by BA concentration, water relations, solar radiation, and plant size (since only immature leaves were visibly affected), then it may be best to apply BA after new tissue has hardened or after a plant has reached a minimum size to reduce plant stress.

In spite of these differences, BA consistently promoted new shoot formation in 'Olivia' in both years and in 'Eleanor Taber' in 2002. Foliar injury to 'Eleanor Taber' was minimal. However, foliar injury to 'Olivia' was present in all BA treatments, regardless of application interval or concentration in 2002, demonstrating a cultivar-specific sensitivity to BA. BA was most effective in promoting new shoot formation in 'Olivia' when applied at a one- or two-week interval and in 'Eleanor Tabor' when applied at a two-week interval. However, injury to developing foliage, especially in 'Olivia', may result in plants being unmarketable in the season of application.

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