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Timing Cyclanilide and Cytokinin Applications in the Nursery to Obtain Desired Lateral Branch Height in Apple and Sweet Cherry Trees

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***Abstract*. The height above the bud union at which induced feathers develop on fruit trees in the nursery is an important determinant of tree quality for an intended market. The bioregulators cyclanilide (CYC; Bayer Environmental Science, Research Triangle Park, NC) and a proprietary formulation of 6-benzyladenine and gibberellins A_4 and A_7 (Promalin [PR]; Valent BioSciences, Walnut Creek, CA) affected the final height above the union of the lowest induced sylleptic shoot (feather) differently in apple and sweet cherry trees in the nursery. In apple, both products resulted in the lowest induced feather developing at approximately 4 to 20 cm *below* the height of the central leader shoot tip at the time of bioregulator application. In sweet cherry, the lowest induced feather typically originated starting approximately 2 to 20 cm *above* the central leader shoot tip height at the time of bioregulator application. Nursery tree height can serve as a suitable criterion for timing bioregulator applications to obtain feathers starting within a specific range of height above the bud union as long as species-specific feathering response characteristics are taken into account. Chemical names used: 1-(2,4-dichlorophenylaminocarbonyl)-cyclopropane carboxylic acid (Cyclanilide), N-(phenylmethyl)-1H-purine-6-amine + gibberellins A_4A_7 (Promalin), polyoxyethylenepolypropoxypropanol, dihydroxypropane, 2-butoxyethanol (Regulaid).**

A new bioregulator, cyclanilide (CYC; Bayer Environmental Science, Research Triangle Park, NC), is an effective product for induction of sylleptic shoot (feather) development in nursery apple and sweet cherry trees (Elfving and Visser, 2005, 2006). Application of the cytokinin 6-benzyladenine (BA) with or without gibberellic acid isomers GA_4 and GA_7 (GA_{4+7}) also improves feather formation in apple and sweet cherry trees in the nursery (Cody et al., 1985a, 1985b; Elfving and Visser, 2005, 2006; Hrotkó et al., 1999; Neri et al., 2003; Plich and Basak, 1978; Wustenberghs and Keulemans, 1999), although no information has been found that describes how feather height above the bud union relates to date of bioregulator application or height of the nursery fruit trees at the time of treatment. The preferred final height for feather development in nursery fruit trees differs depending on the intended market for the trees but is critical for each market. A simple criterion is needed to help nursery producers to determine when to apply a feathering agent such as CYC or a proprietary cytokinin-containing product to obtain induced feathers at the desired height above the bud union.

Because growth of young nursery trees is largely temperature-dependent, a physiological application timing parameter such as tree height seems preferable to a calendar date as a criterion for scheduling the application of feathering treatments. However, information is needed about the location of feather development on the stem in relation to the height of the shoot tip when nursery trees are treated with products such as CYC or a proprietary cytokinin-containing product such as Promalin (PR; Valent BioSciences, Walnut Creek, CA). We know of no reports comparing the feathering response of apple and sweet cherry nursery trees with tree height at time of treatment. The research described here was undertaken in apple and sweet cherry to relate the final height above the bud union of the lowest feather induced on the central leader by CYC or PR to the actual height of the nursery trees on the date of bioregulator treatment.

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Materials and Methods

Five experiments were conducted between 2003 and 2004. All trials used randomized complete-block designs with 4 replications distributed down a row of the appropriate scion/rootstock combination. Proprietary formulations of CYC and BA plus GA_{4+7} (PR) were used in the trials. All bioregulator treatments were supplemented with 0.1% v/v Regulaid (Kalo, Inc., Overland Park, KS) and applied with a manually operated Solo backpack sprayer fitted with 2 spray heads that applied a dilute spray to the upper half of each nursery tree.

At the time of treatment, a piece of small-diameter plastic pipe 33 cm in length was used to locate the point to apply a white latex paint mark on each treated tree 33 cm below the position of the shoot tip on the day of treatment. This mark was used to determine the height of the shoot tip at the time of treatment when detailed growth measurements were made after the trees completed their development.

Spontaneous formation of feathers in low numbers occurs frequently in the nursery. In these trials, feathers that formed well below the approximate height of the shoot tip and that were in active growth at the time of treatment were ignored. All bioregulator concentrations are expressed in terms of the a.i.(s).

Expt. 1, 2003, Quincy, Washington

CYC (100 $mg \cdot L^{-1}$) with or without PR (500 $mg \cdot L^{-1}$) as a tank-mix was applied to 15 tree plots of nursery trees of 'Bing' and 'Skeena'/Mazzard sweet cherry trees in 4 randomized blocks on 16 June, when the central leader shoot tips were approximately 60 cm above the bud unions. Another group of 15 trees in each block was treated with PR (500 $mg \cdot L^{-1}$) on the same date. Control trees were unsprayed. The trees were removed from the nursery in November, 2003, bundled by replicate, and stored for further evaluation. Ten trees representative of the block in caliper, height, and branching were selected from each replicate for the following growth measurements: 1) the distance from the bud union to the white mark applied at the time of treatment, 2) the total length of the central leader shoot from bud union to tip, and 3) the distance from the bud union to the first induced feather or the first spontaneously formed feather on untreated control trees. Feathers were defined as sylleptic shoots 15 cm or more in length. All feathers meeting this criterion were measured and mean feather length calculated.

Expt. 2, 2003, Quincy, Washington

CYC (50 or 100 $mg \cdot L^{-1}$) with or without PR (500 $mg \cdot L^{-1}$) as a tank-mix was applied to 15 tree plots of nursery trees of 'Lapins'/Mazzard sweet cherry trees in 4 randomized blocks on 16 June, when the central leader shoot tips were approximately 90 cm above the bud unions. Another group of 15 trees in each block was treated with PR (500 $mg \cdot L^{-1}$)

Table 1. Effects of cyclanilide (CYC) and/or Promalin (PR) applications on height of lowest feather above bud union in relation to shoot tip height at time of treatment in 'Bing'/Mazzard and 'Skeena'/Mazzard sweet cherry trees in the nursery (experiment 1, 2003, Quincy, WA).

	'Bing'/Mazzard			'Skeena'/Mazzard		
Growth regulator (mg/L^{-1})[z,y]	A. Distance from bud union to shoot tip at treatment (cm)	B. Distance from bud union to lowest induced feather (cm)	Final height of lowest induced feather *above* tip ht at treatment (cm) (B minus A)	A. Distance from bud union to shoot tip at treatment (cm)	B. Distance from bud union to lowest induced feather (cm)	Final height of lowest induced feather *above* tip height at treatment (cm) (B minus A)
CYC						
0	66 a[x]	96 a	29 a	60 a	78 a	17 a
100	68 a	82 b	14 b	62 a	72 b	10 b
PR						
0	68 a	91 a	24 a	59 b	72 a	13 a
500	67 a	87 a	20 a	63 a	77 a	15 a

[z]Dilute applications with backpack sprayer 16 June 2004.
[y]Mean feather number/length for treated 'Bing' trees = 8.1/45 cm; for treated 'Skeena' trees = 7.6/43 cm.
[x]Mean separation in columns within treatments by F test ($P \leq 0.05$). No significant CYC*PR interactive effects.

on the same date. Control trees were unsprayed. The trees were removed from the nursery in November, 2003, bundled by replicate, and stored for further evaluation. Measurements were carried out as described for experiment 1.

Expt. 3, 2003, Quincy, Washington

CYC (100 $mg \cdot L^{-1}$) with or without PR (250 $mg \cdot L^{-1}$) as a tank-mix was applied to 15 tree plots of nursery trees of 'Cameo'/M.26 and 'Scarletspur Delicious'/MM.111 apple trees in 4 randomized blocks on 16 June, when the central leader shoot tips were approximately 60 cm and 50 cm above the bud unions, respectively. Another group of 15 trees in each block was treated with PR (250 $mg \cdot L^{-1}$) on the same date. Control trees were unsprayed. The trees were removed from the nursery in November, 2003, bundled by replicate, and stored for further evaluation. Measurements were carried out as described for expt. 1.

Expt. 4, 2004, Quincy, Washington

CYC (100 $mg \cdot L^{-1}$) or PR (500 $mg \cdot L^{-1}$) was applied either once or twice 1.5 weeks apart to separate 15 tree plots of nursery trees of 'Bing' and 'Skeena'/Mazzard sweet cherry trees in 4 randomized blocks on 28 May only or on 28 May and again on 8 June. On the first application date, the central leader shoot tips of both cultivars were approximately 45 cm above the bud unions. Another 2 groups of 15 tree plots in each block were treated on the first date with either CYC or PR and 1.5 weeks later with the opposite product. Control trees were unsprayed. The trees were removed from the nursery in November, 2004, bundled by replicate, and stored for further evaluation. Measurements were carried out as described for expt. 1, except feather lengths were not measured.

Expt. 5, 2004, Quincy, Washington

CYC (100 $mg \cdot L^{-1}$) or PR (500 $mg \cdot L^{-1}$) was applied either once or twice 1 week apart to separate 15 tree plots of nursery trees of 'Cameo'/M.7 apple trees in 4 randomized blocks on 4 June only or on 4 June and again on 11 June. On the first application date, the central leader shoot tips were approximately 50 cm above the bud unions. Another 2 groups of 15 tree plots in each block were treated on the first date with either CYC or PR and 1 week later with the opposite product. Control trees were unsprayed. The trees were removed from the nursery in November, 2004, bundled by replicate, and stored for further evaluation. Measurements were carried out as described for expt. 1, except feather lengths were not measured.

Analyses of variance and regression were used to assess the significance of treatments and the relation of response to bioregulator concentration. Mean values were separated with analysis of variance or the Waller-Duncan Bayesian k-ratio test ($P \leq 0.05$). Analyses of regression assessed the presence of significant linear and curvilinear effects of bioregulator concentrations (Elfving, 1990; Snedecor and Cochran, 1980). Statistical analyses were performed using the General Linear Models procedure of the Statistical Analysis System program package (SAS Institute, Cary, NC).

Results

Expt. 1

Tree height at the time of bioregulator application did not differ among treatments for either 'Bing' or 'Skeena' trees, except for a small difference in PR-treated 'Skeena' trees (Table 1, Fig. 1). CYC induced new lateral (sylleptic) shoots (feathers, 15 cm or greater in length) starting at a height above the bud union lower than the few spontaneously formed feathers that developed on untreated trees. PR did not affect the final height of the lowest induced feather. The final height above the bud union of the lowest CYC- and PR-induced feathers in both sweet cherry cultivars in 2003 was approximately 10 to 25 cm above the height of the shoot tip at the time of treatment. Mean feather number and

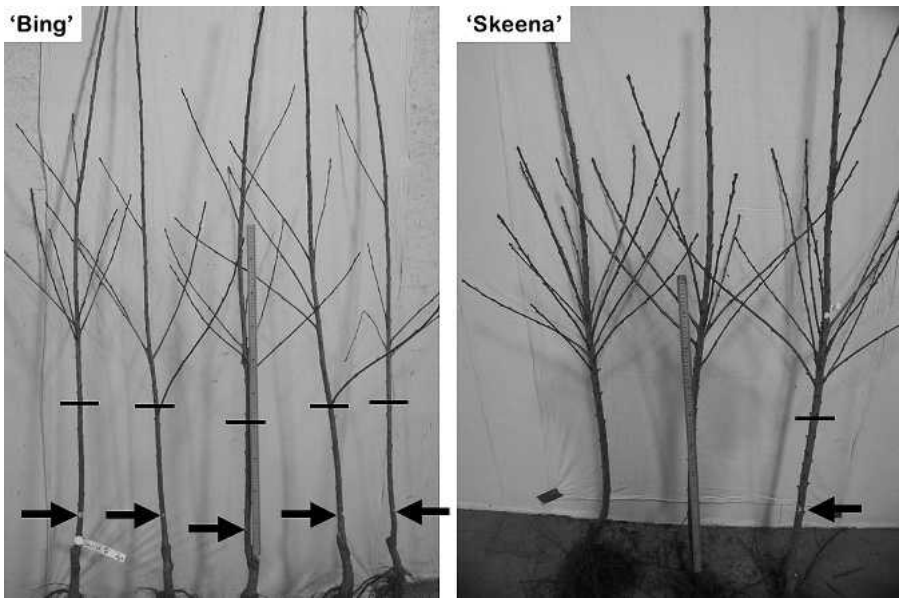

Fig. 1. 'Bing' and 'Skeena' sweet cherry trees treated with cyclanilide (expt. 1). Black arrows indicate visible paint mark locations; black bars 33 cm above paint marks indicate location of shoot tip at time of cyclanilide application.

length of cyclanilide-treated trees for both cultivars averaged approximately 8 feathers per tree with mean feather lengths greater than 40 cm (Table 1).

Expt. 2

There were no differences in tree height at the time of treatment in 'Lapins' trees in 2003 (Table 2). CYC produced feathers starting at a progressively lower height above the bud union on the central leader as concentration increased. In contrast, PR did not affect the final height of the lowest induced feather. Again, final height above the bud union of the lowest induced feather ranged from approximately 15 to 20 cm above the height of the shoot tip at the time of bioregulator treatment. Mean feather number and length of cyclanilide-treated trees averaged approximately 9 feathers per tree with mean feather length greater than 40 cm (Table 2).

Expt. 3

Tree height at the time of treatment was uniform in each apple cultivar treated in 2003 (Table 3, Fig. 2 for 'Scarletspur Delicious' only). The height above the bud union of the lowest induced feather was not influenced by bioregulators in 'Cameo' but was lower for CYC on 'Scarletspur Delicious'. In both cultivars, the final height of the lowest induced feather ranged from 2 to 17 cm below the height of the shoot tip at the time of treatment. Mean feather number and length of cyclanilide-treated trees for both cultivars averaged approximately 5 feathers per tree for 'Cameo' and 5.6 per tree for 'Scarletspur Delicious' with mean feather lengths equal to 48 cm (Table 3).

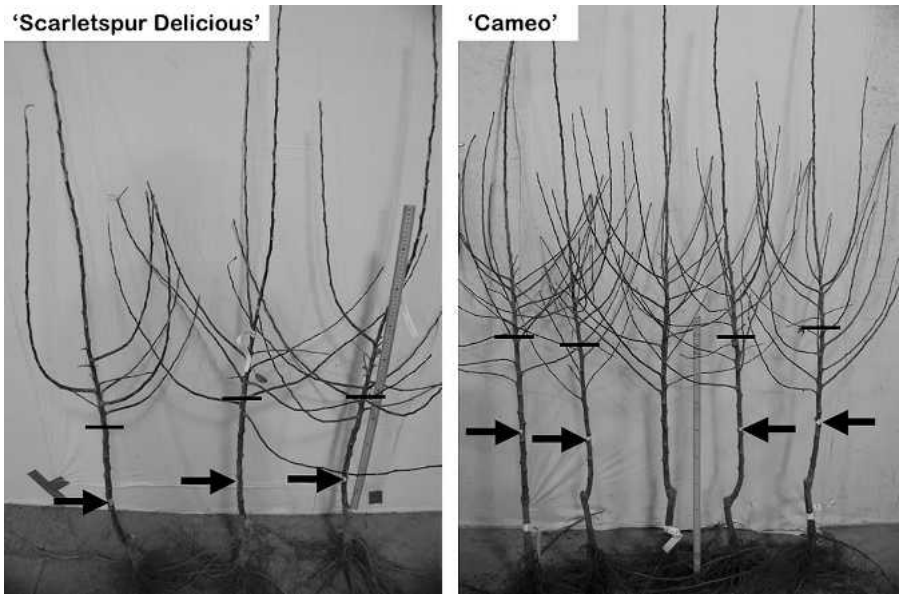

Fig. 2. 'Scarletspur Delicious' (expt. 3) and 'Cameo' (expt. 5) apple trees treated with cyclanilide. Black arrows indicate visible paint mark locations; black bars 33 cm above paint marks indicate location of shoot tip at time of cyclanilide application.

Table 2. Effects of cyclanilide (CYC) and/or Promalin (PR) applications on height of lowest feather above bud union in relation to shoot tip height at time of treatment in 'Lapins'/Mazzard sweet cherry trees in the nursery (experiment 2, 2003, Quincy, WA).

Growth regulator ($mg \cdot L^{-1}$)[z,y]	A. Distance from bud union to shoot tip at treatment (cm)	B. Distance from bud union to lowest induced feather (cm)	Final height of lowest induced feather *above* tip height at treatment (cm) (B minus A)
CYC			
0	95	119	24
50	96	114	18
100	93	107	14
Significance			
CYC linear	NS	***	***
CYC quadratic	NS	NS	NS
PR			
0	95 a[x]	111 a	16 a
500	95 a	115 a	20 a
Interaction CYC X PR	NS	NS	NS

[z]Dilute applications with backpack sprayer 16 June, 2003.
[y]Mean feather number/length for treated "Lapins" trees = 9.1/42 cm.
[x]Mean separation in columns by F test ($P \leq 0.05$).
NS,***Nonsignificant or significant analysis of regression at $P \leq 0.001$.

Expt. 4

In 2004, there were minor differences in tree height at the time of bioregulator treatment in 'Bing' but not in 'Skeena' sweet cherry (Table 4). Large differences in the height above the bud union of the lowest induced feather reflected the difference

Table 3. Effects of cyclanilide (CYC) and/or Promalin (PR) applications on height of lowest feather above bud union in relation to shoot tip height at time of treatment in 'Cameo'/M.26 and 'Scarletspur Delicious'/MM.111 apple trees in the nursery (expt. 3, 2003, Quincy, WA).

	'Cameo'/M.26				'Scarletspur Delicious'/MM.111		
Growth regulator ($mg \cdot L^{-1}$)[z,y]	A. Distance from bud union to shoot tip at treatment (cm)	B. Distance from bud union to lowest induced feather (cm)	Treatment ($mg \cdot L^{-1}$)	Final height of lowest induced feather *below* tip ht at treatment (cm) (B minus A)	A. Distance from bud union to shoot tip at treatment (cm)	B. Distance from bud union to lowest induced feather (cm)	Final height of lowest induced feather *below* tip height at treatment (cm) (B minus A)
CYC							
0	59 a[x]	50 a	Control	–5 a	51 a	52 a	0 a
100	61 a	47 a	CYC 100	–17 b	52 a	45 b	–7 b
PR							
0	60 a	48 a	PR 250	–11 ab	52 a	48 a	–5 a
250	60 a	49 a	CYC + PR	–10 ab	51 a	49 a	–2 a
Interaction CYC X PR	NS	NS	*		NS	NS	NS

[z]Dilute applications with backpack sprayer 16 June, 2003.
[y]Mean feather number/length for treated 'Cameo' trees = 4.9/48 cm; for treated 'Scarletspur Delicious' trees = 5.6/48 cm.
[x]Mean separation in columns within treatments by F test or by Waller-Duncan Bayesian k-ratio test following significant F test ($P \leq 0.05$).
NS,*Nonsignificant, or significant at $P \leq 0.05$.

Table 4. Effects of single, double, and combination applications of cyclanilide (CYC) and/or Promalin (PR) on height of lowest feather above bud union in relation to shoot tip height at time of treatment in 'Bing'/Mazzard and 'Skeena'/Mazzard sweet cherry trees in the nursery (expt. 4, 2004, Quincy, WA).

	'Bing'/Mazzard			'Skeena'/Mazzard		
Growth regulator (mg·L^{-1})[z]	A. Distance from bud union to shoot tip at treatment (cm)	B. Distance from bud union to lowest induced feather (cm)	Final height of lowest induced feather *above* tip height at treatment (cm) (B minus A)	A. Distance from bud union to shoot tip at treatment (cm)	B. Distance from bud union to lowest induced feather (cm)	Final height of lowest induced feather *above* tip height at treatment (cm) (B minus A)
Control	46 a[y]	98 a	52 a	41 a	81 b	40 b
CYC 100, 1 application	45 ab	69 bc	24 bc	44 a	49 cd	6 d
CYC 100, 2 applications	45 ab	59 c	14 c	44 a	46 d	2 d
PR 500, 1 application	44 bc	79 ab	36 ab	41 a	103 a	63 a
PR 500, 2 applications	43 c	61 bc	18 bc	46 a	71 b	25 bc
CYC 100, then PR 500	45 ab	71 bc	26 bc	43 a	51 cd	9 cd
PR 500, then CYC 100	44 bc	63 bc	19 bc	42 a	66 bc	24 bc

[z]Dilute applications with backpack sprayer 28 May, 2004. Second applications 8 June, 2004.
[y]Mean separation in columns by Waller-Duncan Bayesian k-ratio test following significant F test ($P \leq 0.05$).

Table 5. Effects of single, double, and combination applications of cyclanilide (CYC) and/or Promalin (PR) on height of lowest feather above bud union in relation to shoot tip height at time of treatment in 'Cameo'/M.7 apple trees in the nursery (expt. 5, 2004, Quincy, WA).

	'Cameo'/M.7		
Growth regulator (mg·L^{-1})[z]	A. Distance from bud union to shoot tip at treatment (cm)	B. Distance from bud union to lowest induced feather (cm)	Final height of lowest induced feather *above* or *below* tip height at treatment (cm) (B minus A)
Control	52 a[y]	80 a	28 a
CYC 100, one application	56 a	39 c	–17 cd
CYC 100, 2 applications	54 a	38 c	–16 cd
PR 500, one application	55 a	52 b	–4 b
PR 500, 2 applications	56 a	42 bc	–13 cd
CYC 100, then PR 500	56 a	37 c	–19 d
PR 500, then CYC 100	54 a	45 bc	–10 bc

[z]Dilute applications with backpack sprayer 4 June, 2004. Second applications 11 June, 2004.
[y]Mean separation in columns by Waller-Duncan Bayesian k-ratio test following significant F test ($P \leq 0.05$).

in behavior between trees producing only spontaneous feathering or those treated with PR versus those receiving at least one CYC application. The magnitude of the difference in final height of the lowest feather compared with shoot tip height at the time of treatment varied considerably. However, this value in both cultivars was consistently positive, indicating that the lowest feather was located at a final height greater then the height of the shoot tip at the time of treatment and within the same approximate range for most treatments as was found for sweet cherry the previous year. Treatments receiving 2 applications did not appear to differ in the height of the lowest induced feather from those receiving only one application of either product.

Expt. 5

In 2004, 'Cameo' nursery trees did not differ in height at the time the bioregulator treatment program was initiated in early June (Table 5, Fig. 2 for 'Cameo' only). Unlike 2003, both CYC and PR treatments produced feathering at a substantially lower height than the spontaneous feathers that developed on untreated trees. When the height above the bud union of the lowest induced feather was compared with the shoot tip height at the time of treatment, the lowest feather again appeared below the shoot tip height at the time of treatment, ranging from 4 to approximately 20 cm below that tip height, very comparable to the observations for apple in 2003. Treatments receiving 2 applications did not appear to differ in the height of the lowest induced feather from those receiving only one application of either product.

Discussion

There is a cell elongation zone of approximately 10 to 15 cm in length behind the shoot tip in an actively growing nursery tree. Therefore, the final height above the bud union of any feather originating from a newly formed bud near the shoot tip will depend on whether that activated bud is associated with an internode that has not begun to elongate or one that has already partially completed the elongation process. In these trials, the lowest induced feathers on all sweet cherry cultivars tested consistently appeared at final heights *greater than* where the shoot tip was located at the time of treatment, whereas similar feathers on the apple cultivars appeared at final heights *lower than* that shoot tip height. These observations suggest that the mechanism by which both CYC and PR interact with apical dominance and thus influence which buds are induced to grow must differ between apple and sweet cherry, although the result, formation of sylleptic shoots, appears the same. Both CYC and PR are most likely active in sweet cherry in the distal-most portion of the shoot tip; the internodes associated with those activated buds then undergo their normal elongation, resulting in feathers that appear higher in the tree than where the tip was located at the time of treatment. In apple, these same products must activate newly formed buds on internodes that have already begun, or nearly completed, elongation. Because CYC appears to be an auxin transport and action inhibitor (Pederson et al., 1997), whereas PR contains the cytokinin growth promoter 6-benzyladenine (Sachs and Thimann, 1967), it seems likely that the mode of action of these 2 products should differ. Yet their overall effect on final tree structure appears to be dictated more by species characteristics than by any inherent differences between bioregulator products in physiological effects on apical dominance.

The principal practical consequence of these observations is that nursery producers interested in establishing feathers starting at a particular height on apple or sweet cherry trees need to adjust their application program based on the desired height of branching and the characteristics of the feathering response in apple versus sweet cherry. The consistent feathering response in apple and the consistent but different response in sweet cherry over 2 years in these trials suggest that nursery tree height appears to be a good criterion for timing CYC or PR applications as long as the characteristic behavior of each species to feathering bioregulator products is taken into account.

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