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Cyclanilide Foliar Applications Induce Greater Lateral Branching than Pruning in Container-Grown Whips[1]

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Abstract

A major goal in the production of ornamental whips is to produce small well-branched liners with a crown form similar to that of a mature tree. Pruning is used to induce lateral branching and regulate height. Pruning may result in poor tree quality, reduced whip growth and is labor intensive. This study was done to determine the effects of foliar Cyclanilide (CYC) applications with and without pruning on branching in container grown whips. Four taxa were used: *Diospyros virginiana* L., *Eucommia ulmoides* Oliv., *Malus* × 'Prairie Fire' and *Tilia cordata* 'Greenspire'. In a two factorial experiment, CYC concentrations of 0, 50 and 100 ppm were applied to *Diospyros* and *Eucommia* (2005) and 0, 56 and 112 ppm to *Malus* and *Tilia* (2006), with half of the whips of each species receiving terminal shoot pruning, the remaining whips were left intact. Pruning reduced whip height in two out of four species tested. Cyclanilide sprays significantly increased branching and reduced height in *Malus* and *Tilia* whips. An interaction between CYC sprays and terminal shoot pruning occurred with branching in *Tilia*. Here, the combination of CYC spray and terminal shoot pruning significantly reduced the number of new lateral branches compared to CYC sprays alone. Overall CYC was more successful in inducing branching than terminal shoot pruning, but failed to induce branching in the strongly decurrent species *Eucommia*. Cyclanilide sprays are a more effective method for inducing branching and reducing height than terminal shoot pruning, but the combination of both, as done in this experiment, is not recommended.

Index words: plant growth regulator, terminal shoot pruning

Species used in this study: Eucommia (*Eucommia ulmoides* Oliv.), Persimmon (*Diospyros virginiana* L.), Crapapple (*Malus* × 'Prairiefire'), Littleleaf Linden (*Tilia cordata* 'Greenspire').

Chemicals used in this study: 1-[(2,4-dichlorophenylamino-carbonyl)-cyclopropane carboxylic acid] (Cyclanilide), Mixture of modified phthalic glycerol alkyd resin and butyl alkcohol (Latron B-1956).

Significance to the Nursery Industry

Container-grown whips are becoming a popular alternative to bare-root whips. Growth of container-grown whips can be excessive, often more than 3 m (10 ft). Demand for smaller sized branched whips is increasing; however many species do not branch during the first year of production. To produce smaller, branched whips, producers prune the terminal shoot to induce branching and reduce height. Cyclanilide, (Bayer Environmental Science, Montvale, NJ) is a growth regulator that induces lateral branching in container-grown whips without damaging the terminal shoot apex. Cyclanilide foliar sprays to actively elongating shoots increased lateral branch development and reduced height growth resulting in one-year branched whips. Cyclanilide spray produced more lateral branching than terminal shoot pruning. Cyclanilide sprays are a more effective method for inducing branching and reducing height than terminal shoot pruning, but the combination of both, as done in this experiment, is not recommended.

Introduction

In whip production the goal is a 1.8 to 2.0 m (5.9 to 6.6 ft) tall branched whip with a crown form similar to that of a mature tree (13). Many deciduous trees display little branching during their first year of nursery production. Ideally, branching would begin at approximately 180 cm (6 ft) height. To reach this goal producers use mechanical pruning to regulate height, set lower branch height and induce lateral branching. Terminal shoot pruning is necessary because in intensive production systems (irrigation, high fertilizer, pest control) whips can grow too tall in both field and container production systems (15).

Excurrent species require only little pruning since they naturally develop a strong central leader and their lateral buds elongate during the year of initiation, forming a conical shaped crown (5, 8). In contrast lateral buds of decurrent species do not elongate until the year following their initiation, and when the lateral buds elongate they often outgrow the terminal shoot resulting in a broad spreading crown. Decurrent species require regular pruning to establish and maintain a central leader (6, 9).

Auxin is thought to be the main inhibitor of lateral bud outgrowth, but the exact dormancy mechanism is unknown (3, 16) and several hormones interact in dormancy control and release. It is likely that auxin indirectly inhibits lateral bud outgrowth (1, 2, 13). Far more important than the auxin concentration is the auxin-to-cytokinin ratio, since both hormones are involved in lateral bud outgrowth (11, 12). Cline (2) divided apical dominance and its release into four stages, based on differential hormone response. In stage one, cytokinin promotes lateral bud formation. In stage two, apical dominance is imposed by auxin, produced in more terminal buds and shoots. If buds enter stage three (resulting from compromised apical meristem due to injury or removal) apical dominance is released and cytokinin initiates lateral bud elongation. In stage four, auxin and gibberellin promote the further elongation of the lateral bud into a branch.

A new plant growth regulator, Cyclanilide (CYC, Bayer Environmental Science, Montvale, NJ) inhibits auxin synthesis or transport and temporarily interrupts apical dominance.

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In a previous study, CYC foliar sprays at concentration of 56, 112 and 223 ppm applied to one-year-old *Malus*, *Cercis* and *Tilia* whips successfully induced lateral branching at all concentrations, with 112 ppm being most effective (14). Cyclanilide sprays significantly reduced shoot elongation after treatment in *Amelanchier* and *Malus* whips (14). The response to CYC was species dependent, but it appears that excurrent or slightly decurrent species respond better to CYC sprays than strongly decurrent species (14).

In oaks, intercluster buds usually do not elongate until the growing season following their initiation (10). In a preliminary study, northern red oak (*Quercus rubra*) trees received foliar CYC applications of 50 to 200 ppm, but little branching occurred. When the CYC-treated plants were pruned, intercluster buds elongated suggesting that CYC sprays combined with pruning might be an effective method of inducing lateral branch development in decurrent species. Similar results were seen in a preliminary experiment with greenhouse grown Honeylocust (*Gleditsia triacanthos* L.) treated with CYC. Here, pruned lateral branches (induced by the first CYC application) produced secondary lateral branches following the second application.

These observations lead to the hypothesis that removal of apical dominance by pruning combined with CYC treatment would increase lateral branching in species that do not usually respond to pruning or CYC treatments alone. Two experiments were conducted to determine the effects of a factorial combination of foliar CYC sprays and terminal shoot pruning on lateral branch development in container grown whips

Material and Methods

Experiment 1 (2005): Two species, *Eucommia ulmoides* Oliv. (hardy rubber tree) and *Diospyros virginiana* L. (persimmon) were used. *Diospyros* was selected because it is strongly decurrent when old but when young it can express an excurrent growth habit (Sternberg, personal observation). In contrast *Eucommia* was chosen because of its strong decurrrent growth habit even when young.

The plants for this experiment were grown from seeds under greenhouse conditions in spring 2005 (conditions: ±21C (70F) temperature, ±48% relative humidity). Germinated seeds were planted in black plastic pots (13 × 13 × 15 cm, 1.95 liter vol, Classic 250, Nursery Supplies, Chambersburg, PA) in February 2005 and grown in the greenhouse where they received an eight-nine month controlled-release fertilizer (19N:2.2P:6.6K Osmocote Pro plus minors, The Scotts Miracle-Gro Company, Marysville, OH), which was surface applied in 5 g per pot.

In May, the plants were transplanted into No. 3 containers (28 dia. × 24 cm deep (11 × 9.4 in), 11.4 liters (12 qt), Classic 1200, Nursery Supplies, Chambersburg, PA) and placed on 0.3 m (1 ft) within row and 1.2 m (3.9 ft) between row spacing on a nursery pad in Columbus, OH. Each plant received 13.8 liters (14.6 qt) per day of irrigation, split into two equal 30 minute applications. Plants were fertigated with 100 mg N/liter (21N:3.1P:5.7K Plantex 20–10–20 High Nitrate, water soluble fertilizer) applied with a DI-16 Dosatron (Dosatron, Clearwater, FL). At least 100 seedling of each species were potted. Central leaders were trained to 2 m bamboo stakes as described in Sternberg and Struve (14).

Thirty individual *Diospyros* plants were selected for their decurrent habit (little lateral branching). Before spraying, half the plants were randomly selected and pruned by removing the apical 45 cm (1.5 ft) of the terminal shoot (approximately eight fully expanded leaves). For *Diospyros* all lateral branches longer than 15 cm (0.5 ft) were pruned to 15 cm length. Branches <15 cm long were not pruned. None of the *Eucommia* plants had lateral branches and thus only the terminals were pruned. After pruning applications of CYC were made on August 2, 2005 at concentrations of 0, 100 and 200 ppm to which a surfactant (Tween 20 ml, polyoxyethylen sorbitan monolaurate, 0.1 ml/liter) was added. The plants were sprayed until run-off with 60 ml solution per plant using a hand-sprayer (Model 30161, Hudson Capacity Eliminator® Multi-Purpose Poly Sprayer, H.D. Hudson Manufacturing Company, Chicago, IL), applying approximately 3.0 and 6.0 mg a.i., per plant. The spray was applied between 1100 to 1200 hours, on a cloudless day when temperatures where less than 20C (68F), relative humidity was 58% and wind speed was 16 km/h (9.9 m/h).

Before spraying, plant height and trunk caliper were measured and the number of existing pruned and not pruned branches counted on *Diospyros* plants. The plants were destructively harvested at the end of September. Plant height and trunk caliper, number of branches on the terminal shoot (first order lateral branches), as well as the number of second order branches formed from extant first order branches were counted. Leaf area was determined with a leaf area meter (LI-3100, LiCor Corp., Lincoln, NE). Roots were washed to remove all substrate. Leaves, shoots and roots were separated and placed in individual paper bags, dried for one week at 55C (131F) (Blue M Electric, Williamsport, VA) and dry weights recorded.

Terminal growth after treatment was calculated by subtracting August height from September height. Trunk caliper increase was calculated similarly. The specific leaf area was obtained by dividing the leaf area by the leaf dry weight. The total number of branches reported was the combined first and second order branches that developed after CYC application. The number of second order shoots was converted to average number of second order shoots per branch by dividing the total number of second order shoots by the number of first order branches. Leaf area ratio was calculated by dividing total leaf area by total plant dry weight. Shoot-root ratio was calculated by dividing the combination of leaf and shoot dry weight by the root dry weight.

Experiment 2 (2006): Two taxa *Malus* × 'Prairiefire' and *Tilia cordata* 'Greenspire' were used for this experiment. All plants were propagated through tissue culture (Microplant Nurseries, Inc., Gervais, OR) and received as rooted plantlets between November 23, 2005, and February 2, 2006. The experiment in 2006 was similar to 2005, except as modified below.

All plants were potted into 24 cell HIKO™ trays (V150SS, Stuewe & Sons, Inc. Corvallis, OR) using Farard 3B. *Malus* were covered with clear foil and placed in a growth chamber (conditions: ±23C (72F) temperature, ±35% relative humidity and ±49 W/m PAR). *Tilia* were placed into an intermittent mist room (mist/minute) under shade cloth (% shading). After three weeks all plants were placed on a greenhouse bench covered by shade cloth for one week. Plants received 50 mg N/liter water soluble fertilizer (Plantex 20–10–20 high nitrate, 21N:3.1P:7K). Seven weeks later plants were transplanted into 150 XL containers (Nursery Supplies,

Table 1. Increase in growth and branching of *Diospyros virginiana* whips following a factorial treatment combination of foliar Cyclanilide spray and terminal shoot pruning.

Cyclanilide concentration (ppm)[z]	Pruning[y]	Increase in shoot elongation (cm)	Increase in caliper (mm)	branch number	Total branch number	Dry weight leaves (g)	Dry weight shoots (g)	Dry weight roots (g)	Shoot-root ratio	Leaf area ratio (cm^2/g)	Specific leaf area (cm^2/g)
0	No	74[x]	4.6	0	1	89	165	164	1.6	26	123
	Yes	51	3.7	1	5	75	176	168	1.5	24	137
50	No	40	3.1	0	5	86	157	136	1.8	27	119
	Yes	41	2.8	2	8	84	152	141	1.7	32	142
100	No	36	3.5	1	6	84	165	170	1.5	28	138
	Yes	46	2.8	1	6	73	143	145	1.5	29	145
Cyclanilide concentration		*[w]	NS	NS	NS	NS	NS	NS	NS	NS	NS
Pruning		NS	NS	NS	NS	NS	NS	NS	NS	NS	*
Cyc × pruning		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[z]Foliar CYC sprays were applied to whips during active shoot elongation. An average of 60 ml solution was applied per plant, 3.0 or 6.0 mg active ingredient for the 50 and 100 ppm treatments respectively.

[y]Terminal shoot was pruned by approximately 45 cm, before CYC application.

[x]Each value is the mean of five single plant replication

[w]*, **, and *** indicate significance at 0.05, 0.01 and 0.001 level using ANOVA. NS indicates no statistical difference at $\alpha = 0.05$.

Chambersburg, PA) and placed pot-to-pot on a greenhouse bench. Plants were top dressed with 3 g per container of a 2–3 month controlled release fertilizer (Top dress special, 12N:2.2K:7.4P; Scotts, Marysville, OH).

On August 10, 2006, thirty plants were selected for lack of lateral branching; half of the plants of each species were pruned. On *Malus*, the terminal was reduced by six fully extended leaves, on *Tilia* by two. Following pruning CYC was applied at concentrations of 0, 56 and 112 ppm. Latron B-1956 (Loveland Industries Ltd., Greeley, CO) was added to the spray solutions at 0.06% (by vol). Plants were sprayed until run-off using a hand-sprayer (Model 30161, Hudson 1-Gallon Capacity Eliminator® Multi-Purpose Poly Sprayer, H.D. Hudson Manufacturing Company, Chicago, IL). Plants were sprayed between 1100 and 1200 hours. An average of 52 ml solution was applied per plant, 2.9 or 5.8 mg active ingredient for the 56 and 112 ml/liter treatments respectively. At the time of CYC application the temperature was 21C (70F), relative humidity 74% and the wind speed was 6 km/h (4 m/h). Initial data taken before CYC treatment included height, caliper and number of buds on the terminal shoot.

At harvest on September 28, 2006, the following data were taken: height, caliper, number of branches (>5 cm (2 in) long), and branch length. Branch length was measured only for *Tilia*, for *Malus* whips the number of branches >5 cm (2 in) but <15 cm (5.9 in), and those >15 cm were counted. Increase in height and caliper after CYC application were calculated as in experiment one.

For both experiments, the effects of CYC treatment and pruning were analyzed using a two-factorial ANOVA with five single plant replications. When there were no significant interactions, the main effect means were separated using a one-way ANOVA and means declared significant at $\alpha = 0.05$ level of significance using the Student-Neuman-Keuls test.

Table 2. Increase in growth and branching of *Eucommia ulmoides* whips following a factorial treatment combination of foliar Cyclanilide spray and terminal shoot pruning.

Cyclanilide concentration (ppm)[z]	Pruning[y]	Increase in shoot elongation (cm)	Increase in caliper (mm)	branch number	Dry weight leaves (g)	Dry weight shoots (g)	Dry weight roots (g)	Shoot-root ratio	Leaf area ratio (cm^2/g)	Specific leaf area (cm^2/g)
0	No	74[x]	3.8	0	53	111	49	3.4	21.7	86
	Yes	91	4.0	1	54	113	45	3.8	27.4	107
50	No	75	2.4	0	62	111	50	3.5	21.8	78
	Yes	90	1.5	1	43	107	43	3.5	23.6	106
100	No	62	2.0	0	45	92	34	4.0	21.3	80
	Yes	63	1.7	1	40	79	36	3.3	24.5	92
Cyclanilide concentration		NS[w]	***	NS	NS	*	***	NS	NS	*
Pruning		NS	NS	***	NS	NS	NS	NS	*	***
Cyc × pruning		NS	NS	NS	NS	NS	NS	NS	NS	NS

[z]Foliar CYC sprays were applied to whips during active shoot elongation. An average of 60 ml solution was applied per plant, 3.0 or 6.0 mg active ingredient for the 50 and 100 ppm treatments respectively.

[y]Terminal shoot was pruned by approximately 45 cm, before CYC application.

[x]Each value is the mean of five single plant replication

[w]*, **, and *** indicate significance at 0.05, 0.01 and 0.001 level using ANOVA. NS indicates no statistical difference at $\alpha = 0.05$.

Table 3. Increase in growth and branching of *Malus* × 'Prairie Fire' whips following a factorial treatment combination of foliar Cyclanilide spray and terminal shoot pruning.

Cyclanilide concentration (ppm)[z]	Pruning[y]	Increase in shoot elongation (cm)	Increase in caliper (mm)	branch number	Extant buds elongating <5 cm (%)	Total branch length (cm)	Average branch length (cm)	Distance of branching (cm)
0	No	70[x]	3.3	5	8	285	6	27
	Yes	37	3.8	5	8	252	8	22
56	No	34	2.7	14	20	308	6	59
	Yes	26	1.9	8	12	234	6	30
112	No	39	2.3	14	32	294	6	68
	Yes	12	2.4	10	19	241	6	43
Cyclanilide concentration		***[w]	*	*	***	NS	NS	NS
Pruning		***	NS	NS	*	NS	NS	NS
Cyc × pruning		NS	NS	NS	NS	NS	NS	NS

[z]Foliar CYC sprays were applied to whips during active shoot elongation. An average of 52 ml solution was applied per plant, 2.9 or 5.8 mg active ingredient for the 56 and 112 ppm treatments respectively

[y]Terminal shoot was removed by 6 fully extended leaves.

[x]Each value is the mean of five single plant replication

[w]*, **, and *** indicate significance at 0.05, 0.01 and 0.001 level using ANOVA. NS indicates no statistical difference at $\alpha = 0.05$.

Those means and their level of significance are presented in the text.

Results and Discussion

Diospyros virginiana. There were no significant interactions between CYC sprays and pruning (Table 1). As main effect CYC sprays significantly decreased terminal shoot elongation (approximately 22 cm (8.7 in)) and pruning significantly increased specific leaf area by 14 cm^2/g (data not shown). There were no other significant main effects.

Eucommia ulmoides. There were no significant interactions between CYC sprays and pruning (Table 2). Main effect statistical differences occurred: Cyclanilide sprays decreased caliper (2 mm), shoot and root dry weight (15 g and 6 g, respectively), and increased leaf area ratio by 8 cm^2/g (data not shown). Pruning increased branch number by one per plant, and increased both leaf area ratio (3.6 cm^2/g) and specific leaf area (21 cm^2/g, data not shown).

Malus × 'Prairie Fire'. There were no CYC by pruning interactions (Table 3). The one-way ANOVA showed the following main effects: Cyclanilide sprays decreased shoot elongation and trunk caliper 26 cm (10 in) and 1.3 mm, respectively and increased the branch number (3.3) and the percentage of extant buds elongating (4.6, data not shown). Pruning decreased terminal shoot elongation (21 cm (8.3 in), data not shown).

Tilia cordata 'Greenspire'. There were significant CYC by pruning interactions for number of branches, total and average branch length and the distance of branching on the terminal shoot (Table 4). For branch number, when *Tilia* whips were sprayed, but not pruned, the number of branches

Table 4. Increase in growth and branching of *Tilia cordata* 'Greenspire' whips following a factorial treatment combination of foliar Cyclanilide spray and terminal shoot pruning.

Cyclanilide concentration (ppm)[z]	Pruning[y]	Increase in shoot elongation (cm)	Increase in caliper (mm)	branch number	Extant buds elongating <5 cm (%)	Total branch length (cm)	Average branch length (cm)	Distance of branching (cm)
0	No	118[x]	5.4	0	5	0	0	0
	Yes	85	4.4	1	5	91	78	2
56	No	88	3.2	7	13	198	29	27
	Yes	60	2.2	2	8	96	51	5
112	No	82	3.6	5	22	152	26	17
	Yes	65	2.7	1	12	62	40	6
Cyclanilide concentration		*[w]	***	**	**	**	NS	**
Pruning		**	*	**	*	NS	***	**
Cyc × pruning		NS	NS	*	NS	**	***	*

[z]Foliar CYC sprays were applied to whips during active shoot elongation. An average of 52 ml solution was applied per plant, 2.9 or 5.8 mg active ingredient for the 56 and 112 ppm treatments respectively

[y]Terminal shoot was removed by two fully extended leaves.

[x]Each value is the mean of five single plant replication

[w]*, **, and *** indicate significance at 0.05, 0.01 and 0.001 level using ANOVA. NS indicates no statistical difference at $\alpha = 0.05$.

produced (5 and 7, for 56 and 112 ppm, respectively) was greater than when whips were sprayed and pruned (2 and 1, for 56 and 112 ppm, respectively) (Table 4). A similar pattern was found for total branch length and distance of branching on the terminal shoot (spraying without pruning resulted in more total branch length and distance in branching). In contrast, the average branch length was increased by spraying and pruning.

Deciduous shade tree whips with a decurrent growth habit do not readily branch in the first growing season. Removal of the terminal shoot apex can induce branching, but also leads to an undesirable tree structure if a central leader is not reestablished and vigorously growing lateral buds are not pruned (9, 17). Lateral shoot elongation is an important adaptive feature since it allows the plant to regenerate a central leader (7, 12).

Eucommia is a strongly decurrent species. Terminal shoot pruning induced only one new shoot per plant which replaced the lost central leader, resulting no increase in branching. Cyclanilide sprays, terminal shoot pruning or a combination, applied to this strongly decurrent species did not result in increased lateral branching.

For most species foliar sprays of CYC had little effect on plant growth other than increasing lateral branch number and height. Shoot and root dry weights and shoot:root dry weight ratio were not affected by CYC applications except for Eucommia shoot and root dry weights at the highest CYC rate.

In general, the species with an excurrent or slightly decurrent growth habit (*Malus*, *Tilia*, *Diospyros*) responded to CYC sprays by increasing lateral. Of the species tested, *Tilia* responded best to CYC sprays. Cyclanilide sprays, averaged over pruning treatments, induced four lateral branches to form while only one branch formed when the terminal shoot was pruned. Non-pruned plants produced no lateral branches. It was expected that a combination of CYC sprays and pruning would increase the number of lateral branches developing; however, the opposite occurred. The combination of CYC sprays and pruning significantly reduced the number of lateral branches formed compared to CYC sprays alone.

In *Malus*, CYC sprays induced significantly more branches than terminal shoot pruning (Fig. 1). The number of lateral branches induced, as well as the percentage of extant buds that elongated was reduced by the combination of CYC sprays and pruning when compared to not pruned but CYC treated whips.

One possible explanation for this result might be found in Cline's concept of (2) stages of apical dominance. Apical dominance is imposed by auxin produced in the terminal shoot apex, in stage three, after removal of the terminal shoot apex, cytokinin induced bud elongation. However, in stage four, auxin is needed to promote the elongation of the lateral bud into a branch (4). It is possible that the immediate removal of the auxin source (terminal bud) and the longer lasting auxin inhibiting effect of CYC resulted in auxin levels so low, that an ineffective auxin concentration was available in stage four for the buds to elongate into branches although.

Although the response to CYC is strongly species dependent (14); for the species in this study, a combination of pruning and CYC sprays can not be recommended. Further

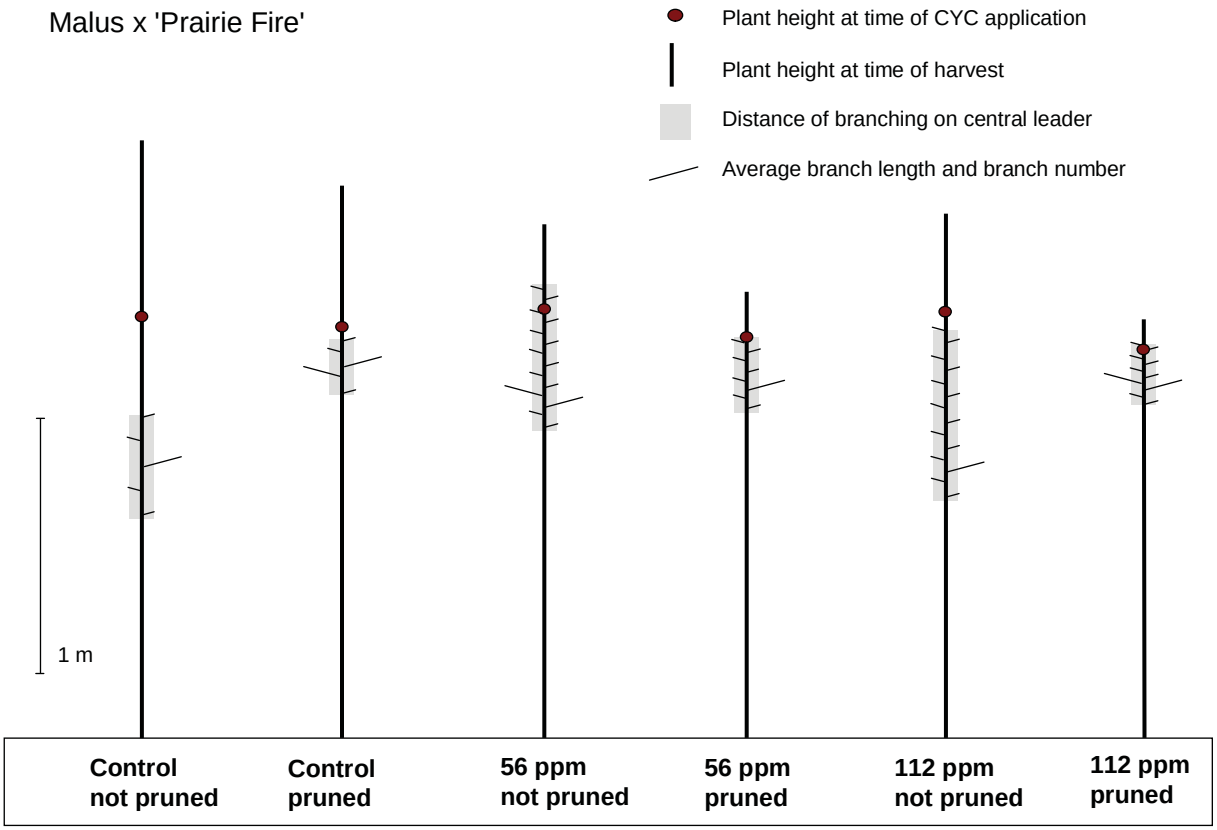

Fig. 1. Schematic diagram (drawn to scale) of *Malus* × 'Prairie Fire' whips on September 28 following foliar application of Cyclanilide at concentrations of 0, 56 or 112 ppm + 0.06% Latron B-1956 (v/v) and/or terminal shoot pruning on August 10. Control treatments included unsprayed and not pruned whips. Each whip is drawn to scale and represents the average of five single whip replications.

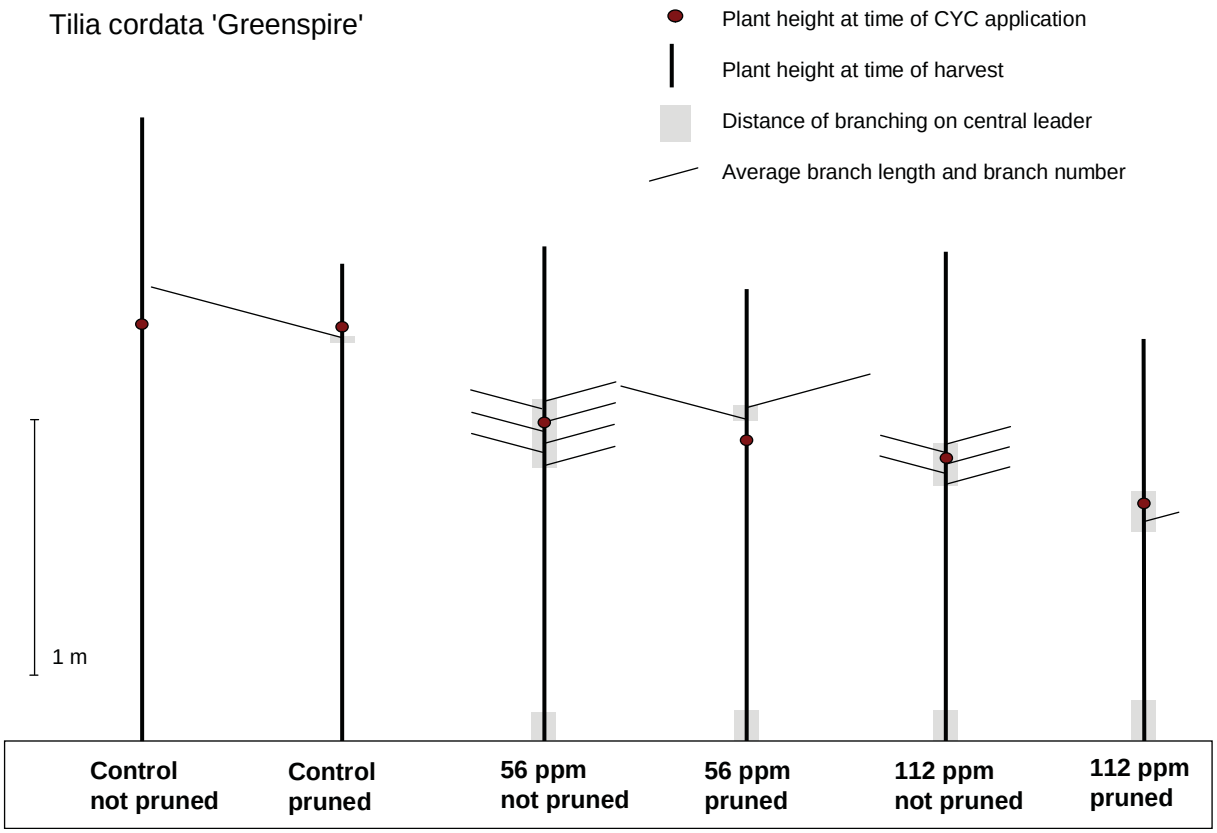

Fig. 2. Schematic diagram (drawn to scale) of *Tilia cordata* 'Greenspire' whips on September 28 following foliar application of Cyclanilide at concentrations of 0, 56 or 112 ppm + 0.06% Latron B-1956 (v/v) and/or terminal shoot pruning on August 10. Control treatments included unsprayed and not pruned whips. Each whip is drawn to scale and represents the average of five single whip replications.

investigation is also needed to investigate if lower concentrations of CYC in combination with pruning lead to better results.

In general, CYC application is superior to terminal shoot pruning in increasing lateral branching in container-grown whips used in this study. It induces lateral branching in excurrent to slightly decurrent species without damaging the central leader. Terminal shoot elongation in *Amelanchier*, *Malus* (Fig. 1) and *Tilia* (Fig. 2) was significantly reduced by CYC foliar application. This reduction in height is desirable, since CYC treated whips are shorter and more highly branched, pruning to induce branching is unnecessary, resulting in labor savings and reduced rotation times (15). Also CYC spray did not alter the height:caliper ratio.

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