Attachment C

Christmas Tree Promotion Board

CTPB Project Number: 19-03-MSU

Project Title: Managing cone formation in *Abies* Christmas tree species

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Final Report

1. Introduction
   Early cone production of Fraser fir trees is a major concern for Christmas tree growers in the Upper Midwest and North Carolina. Individual plantation-grown Fraser fir trees can produce hundreds of cones, and growers have reported over 1,000 cones on large trees. Fir cones disintegrate in the fall leaving unsightly cone stalks that reduce the salability of trees (Photo 1). Moreover, developing cones compete for photosynthate reserves and reduce shoot and needle growth if they are not removed. Presently, growers remove cones using picking crews, which has become a major labor expense. In plantations with large trees, pickers use ladders, which creates potential worker safety concerns. Ironically, most true firs (*Abies spp*.) do not produce cones until trees are 15 years old and usually produce infrequent cone crops. In fact, lack of consistent cone production is a bottleneck in tree improvement efforts for Christmas trees. In this research project, we focus on four objectives related to cone formation in *Abies spp.*

   - Objective 1 Determine the effectiveness of plant growth regulators (PGRs) in reducing cone formation in Fraser fir Christmas trees

   - Objective 2 Determine the effectiveness of post-emergent herbicides to stop development of Fraser fir cones once they have emerged

   - Objective 3 Identify Fraser fir genotypes for delayed coning (i.e., trees that will not cone until after typical harvest age)

   - Objective 4 Initiate a program of studies to identify environmental factors controlling coning in *Abies spp*.

   Here, we report Methods and Results under Objective 1 (use of PGRs to control coning). For Objective 2 (post-emergent cone control) we have identified products and rates that can kill 90% or more of developing cones without causing injury to trees (Cregg et al., 2018). In 2020, we planned to work with grower-cooperators to initiate operational-scale trials using tractor-mounted sprayers, but were unable to do so due to Covid-19 travel restrictions. We will resume this work in 2021. For Objective 3 (delayed coning seed orchard) we have several dozen seedlings of four open-pollenated families from the first seed collection from our delayed-coning seed orchard that are currently in transplant beds in a cooperating nursery (Peterson's Riverview Nursery). The seedlings will be lifted, out-planted, and compared with the nursery-run stock for coning, growth, and needle retention and other traits. In fall 2019, we made a second collection of cones from 20 mother trees in the seed orchard. The seeds were cleaned and processed and sown in individual 20' bed sections at Peterson's Nursery. Germination has been good for all but two of the 20 families. The seedlings will be grown on for 2 years and then lifted and planted in transplant beds before out-planting and testing. Under Objective 4 (environmental control of coning), we are currently growing 100 trees of each of Fraser fir, Korean fir, concolor fir, and Nordmann fir (400 trees total) in #3 containers in our Pot-in-Pot research nursery. In the summer of 2020, we monitored shoot water potential and photosynthetic gas exchange in order to develop baseline information on tree responses to irrigation rate. In 2021, we will initiate a trial to evaluate the relationship between tree water stress in *Abies spp.* and cone formation.

Objective 1 Determine the effectiveness of plant growth regulators (PGRs) in reducing cone formation in Fraser fir Christmas trees

Background/rationale

Reproductive development in conifers is controlled by a series of factors including environmental conditions, particularly temperature and tree water stress, and genetic predisposition (Crain and Cregg, 2018). Both environmental and genetic control are mediated through hormonal signaling within trees. In particular, gibberellic acid (GA) levels are a primary driver of coning in conifers and seed orchard managers often apply GA to induce coning. For example, trunk-injection of GA4/7 combined with fertilizer, girdling, and tenting resulted in a 30-fold increase in cone production in Pacific silver fir (*Abies amabilis* [Douglas ex Loudon] Douglas ex Forbes) (Owens et al., 2001). Likewise, endogenous GAs accumulate at the meristem from neighboring tissues immediately prior to strobilus initiation, and regulate development of reproductive structures (Pharis and Kuo, 1977). Based on the stimulatory effect of GA on coning, we hypothesized that application of GA inhibitors to Fraser fir trees may reduce coning. GA inhibitors are used as plant growth regulators (PGRs) to control shoot growth in a range of horticultural applications. Previously, we applied a GA inhibitor (paclobutrazol) to Fraser fir trees that had coned previously and were able to reduce coning density (number of cones per tree) by 33% to 54% (Crain and Cregg, 2017).

Methods

Soil-applied PGR trials

We initiated two series of field trials in 2016 and 2017 to investigate the effect of paclobutrazol on coning in Fraser fir. We installed field plots at four cooperating farms in Michigan (Badger Evergreen Nursery, Allegan, MI; Korson's Tree Farm, Sidney, MI; Gwinn's Tree Farm, Horton, MI; and Dutchman Tree Farm, Manton, MI). At each farm we applied paclobutrazol (tradename: Cambistat®) at three rates (100, 200, or 300 ml of ready-to-use product per tree) using a soil injection system (Table 1; Photo 2). Ready-to-use product was prepared from concentrated product using an 11:1 dilution (v:v; water:concentrate) as directed by the product label. We also applied paclobutrazol as a foliar spray (tradename: Trimtect®) using 4:1 dilution (v:v; water:concentrate). Treatments were applied to six 10-tree row plots (i.e., n = 60 for each treatment at each farm). Soil treatments were applied once (spring 2016 or spring 2017), and the foliar spray treatments were applied annually each spring. We evaluated coning and leader growth at each farm in the 2016 series of trials at all farms in 2017, 2018, and 2019, and at two farms (Gwinn's and Dutchman) in 2020 (Photo 3). The 2017 series of plots were evaluated in 2018, 2019, and 2020.

Foliar-applied PGR trial

We initiated trials at four farms in Michigan in 2018 to evaluate the effectiveness of foliar-applied PGRs in reducing coning. We selected three PGRs (Citadel®, Concise®, and Dazide®) for the trials along with paclobutrazol (Trimtect®) (Table 1). Each compound was applied to the upper one-third of the tree crowns using a backpack sprayer in early summer. For each compound, the treatments were applied once or applied twice, one week apart (2X). Each PGR x application combination was applied to five 5-tree row plots (i.e., n=25 trees) per farm. We repeated all of the foliar PGR treatments in summer 2019. The first foliar application for each season was applied when current year's terminal shoots had reached approximately 50% of their total growth based on the MSU Fraser fir shoot growth phenology model. We evaluated coning and leader growth of trees on each plot in 2019 and 2020.

Table 1. Products used for Michigan State University Plant Growth Regulator (PGR) trials

Product/manufacturer	Active ingredient	Application
Cambistat®/ Rainbow Treecare Scientific Advancements	Paclobutrazol	Soil Injection
Trimtect®/ Rainbow Treecare Scientific Advancements	Paclobutrazol	Foliar
Citiadel®/ Fine Americas, Inc	Chlormequat Chloride	Foliar
Concise®/ Fine Americas, Inc	Uniconazole-p	Foliar
Dazide®/ Fine Americas, Inc	Daminozide	Foliar

Results

Soil-applied PGR trials

Soil application of paclobutrazol significantly ($p<0.05$) reduced cumulative cone production in both the 2016 trial and the 2017 trial (Table 2 and Table 3). Foliar application of paclobutrazol also reduced cumulative cone production (Table 2 and Table 3). In the 2016 trial, application of 300 ml of paclobutrazol reduced the cumulative number of cones per tree by 38%. Foliar application of paclobutrazol reduced coning by 28% in the 2016 trial. In the 2017 trial, all soil applied treatments and foliar application of paclobutrazol reduced cumulative coning by 32% or more. The effectiveness of paclobutrazol in controlling cones varied among farms in each trial. In the 2016 studies, maximum effectiveness (greatest % reduction in coning relative to the untreated control) of soil applied treatments ranged from 30% at Badger to 67% at Korson’s. In the 2017 trials, maximum effectiveness of soil-applied treatments ranged from 35% at Badger to 55% at Gwinn’s. In both trials, control of coning from soil-application of paclobutrazol lasted three years or longer. Soil application of paclobutrazol in 2016 reduced coning in 2019 (3 years after treatment) by up to 36% across all farms, compared to untreated control trees. Soil application in 2017 reduced coning in 2020 across all farms by 47%. For the two farms in which we were able to evaluate a fourth year response in the 2016 trial, soil application of paclobutrazol reduced coning by up to 47% at Dutchman and by up to 59% at Korson’s.

Soil application of paclobutrazol reduced terminal leader lengths in both the 2016 and 2017 trials (Figure 1). Leader growth control in response to paclobutrazol application generally followed a dose-rate response, with growth reduction increasing with application rate. Maximum reductions in annual leader growth ranged from 2.1 cm in 2018 following 2017 application to 8.1 cm in 2018 for the

2016 application. Foliar application of paclobutrazol significantly reduced leader growth in 4 out of 6 evaluations but did not reduce coning in 2018 in either trial (Figure 1).

Foliar-applied PGR trial

Foliar-applied PGRs reduced coning across farms, however repeating applications did not affect coning. Therefore, we combined the single and repeated application data for analysis and present means for each product. Overall, all of the PGRs reduced cumulative coning (2019 cones + 2020 cones) relative to trees in the untreated control (Table 4). Trimtect® reduced cumulative coning by 45% compared to control trees. Efficacy of the products varied among farms. All products significantly reduced coning at Badger relative to the control. Trimtect® reduced coning at Dutchman in 2020. PGR-treated trees had fewer cones than untreated trees at Korson's but the difference was not significant. Likewise, PGR treatment did not affect coning at Gwinn's in 2019 (Table 4).

Leader growth control with foliar applied PGRs varied among farms, however Trimtect® generally provided the greatest growth control and there was a slight, but consistent, additive effect of repeating the applications (Figure 2).

Discussion

As in our earlier trials, soil and foliar applied PGRs reduced coning and shoot elongation. Soil applications of paclobutrazol are particularly promising as a single application may reduce coning for up to four years. The principal limitation of using PGRs to reduce coning is inconsistency of responses among farms and years. For example, in the 2016 trial, trees on the farm with the greatest amount of cones (Badger) had the smallest proportionate response to PGR application. The site had a very sandy soil and it is likely trees were under significant water stress. Nonetheless, the high rate of soil applied PGR reduced cumulative coning by 67 cones per tree, or approximately 80,000 cones per acre, assuming a 6' tree spacing.

Foliar-applied PGRs were effective in controlling cones but results were also variable among farms and years. The variation in response to foliar applied PGRs may be due variation in timing and tree phenology. We attempted to time applications based on our growth phenology model, in order to apply the GA-inhibitors when shoot growth was 50% complete. This point of shoot development corresponds to the typical start of bud differentiation in firs (Owens and Molder, 1977; Owens and Singh, 1982). However, weather conditions after this time could affect persistence of the compounds on the shoots and foliage. Logistically, foliar applications may be more limiting than soil application as they need to be repeated each year and spraying is constrained by tree phenology and weather conditions.

From this and our earlier studies we conclude that GA-inhibitors, especially paclobutrazol, can reduce coning and shoot growth and have good residual activity when soil-applied. Proportionately, paclobutrazol is more effective when cone density is low. We hypothesize that the compound is effectively delaying tree maturation and that applying at younger ages (i.e., before trees begin to cone) may be an effective strategy to reduce coning. Based on this, in 2020 we initiated a trial on three farms in Michigan with plantations with relatively young trees (approx. 1 m tall or less) that have not yet coned. We installed the current trial as a factorial design with soil and foliar paclobutrazol applications so that we can evaluate the potential of synergistic responses of both foliar and soil applications.

Table 2. Mean cone density (cones per tree) of Fraser fir trees treated with soil injected or foliar applied plant growth regulator (paclobutrazol). Soil treatments (Cambistat) were applied in 2016. Foliar treatments (Trimtect) were applied annually 2016-2018.

		Farm				
	Treatment	Badger	Dutchman	Gwinn	Korson	**Overall[1]**
2017	Control	47.9	4.1	10.0	3.6	**16.4a**
	Soil 100 ml	43.3	2.8	10.6	2.9	**14.9ab**
	Soil 200 ml	46.8	2.6	10.9	4.3	**16.2a**
	Soil 300 ml	32.2	1.7	7.4	1.8	**10.8ab**
	Trimtect	27.8	0.8	8.3	1.1	**9.5b**
2018	Control	84.2	14.6	12.7	19.1	**32.6a**
	Soil 100 ml	76.5	12.9	10.1	19.1	**29.7ab**
	Soil 200 ml	72.2	4.8	8.7	12.2	**24.5abc**
	Soil 300 ml	54.2	6.2	6.3	4.4	**17.8c**
	Trimtect	50.6	7.9	8.2	14.2	**20.2bc**
2019	Control	87.6	62.9	42.3	42.7	**58.8a**
	Soil 100 ml	80.5	44.5	29.6	43.2	**49.7ab**
	Soil 200 ml	83.8	33.5	24.7	27.2	**42.3b**
	Soil 300 ml	66.7	51.4	18.0	15.4	**37.9b**
	Trimtect	70.7	35.8	28.6	51.9	**46.7ab**
2020	Control	NA	46.7	58.8	NA	**NA**
	Soil 100 ml		36.9	34.1		
	Soil 200 ml		24.7	23.9		
	Soil 300 ml		52.4	26.7		
	Trimtect		58.6	31.3		
Cumulative[2]	Control	219.7	77.3	64.7	65.3	**106.8a**
	Soil 100 ml	200.8	60.1	50.2	65.3	**94.1ab**
	Soil 200 ml	202.8	40.8	42.5	43.7	**82.5abc**
	Soil 300 ml	153.1	59.3	31.7	21.6	**66.4c**
	Trimtect	149.1	44.5	45.6	67.5	**76.4bc**

NA = plots were harvested in 2019

[1] means followed by the same letter at not different at $p<0.05$

[2] sum of 2017-2019 cone counts

Table 3. Mean cone density (cones per tree) of Fraser fir trees treated with soil injected or foliar applied plant growth regulator (paclobutrazol). Soil treatments (Cambistat) were applied in 2017. Foliar treatments (Trimtect) were applied annually 2017-2019.

		Farm				
	Treatment	Badger	Dutchman	Gwinn	Korson	**Overall[1]**
2018	Control	2.2	20.8	4.6	17.1	**11.2**
	Soil 100 ml	4.5	21.5	2.2	15.6	**10.9**
	Soil 200 ml	6.6	23.6	3.5	8.7	**10.6**
	Soil 300 ml	7.0	11.8	3.1	15.0	**9.2**
	Trimtect	1.1	21.0	3.3	12.8	**9.5**
2019	Control	12.8	NA	16.6	56.8	**28.8**
	Soil 100 ml	10.7		10.1	36.2	**19.0**
	Soil 200 ml	18.5		8.4	29.7	**19.0**
	Soil 300 ml	13.1		7.6	37.5	**19.4**
	Trimtect	6.3		7.5	40.0	**17.9**
2020	Control	26.6	NA	20.8	53.7	**33.7a**
	Soil 100 ml	11.4		11.6	29.9	**17.7b**
	Soil 200 ml	25.5		9.3	35.1	**23.4ab**
	Soil 300 ml	15.8		8.4	31.5	**18.5b**
	Trimtect	11.1		11.9	34.5	**19.2b**
Cumulative	Control	41.0	NA	42.0	127.6	**70.2a**
	Soil 100 ml	26.6		23.9	77.8	**42.7b**
	Soil 200 ml	50.6		21.3	70.1	**47.3ab**
	Soil 300 ml	35.6		19.1	82.6	**45.8ab**
	Trimtect	18.2		22.7	88.2	**43.0b**

NA = cones were picked before evaluation

[1] means followed by the same letter at not different at $p<0.05$

Table 4. Mean cone density (cones per tree) of Fraser fir trees treated with four foliar applied plant growth regulators or not treated (control). Treatments were applied annually 2018-2019.

		Cones per tree		
Farm	Treatment	Cones 2019	Cones 2020	Cumulative
Badger	Control	122.5a	122.0a	244.4a
	Citadel	79.4b	71.8b	151.2b
	Concise	63.1b	75.1ab	138.2b
	Dazide	66.9b	67.5b	134.4b
	Trimtect	64.9b	60.9b	125.6b
Korson's	Control	49.8	46.0	95.8
	Citadel	30.1	44.2	73.5
	Concise	23.5	43.1	66.1
	Dazide	42.3	33.8	76.1
	Trimtect	34.9	28.9	65.3
Dutchman	Control	NA	89.3a	
	Citadel		63.9ab	
	Concise		75.7ab	
	Dazide		67.0ab	
	Trimtect		28.9b	
Gwinn's	Control	38.6	NA	
	Citadel	31.2		
	Concise	40.2		
	Dazide	52.2		
	Trimtect	39.9		
All farms*	**Control**	**70.8a**	**86.8a**	**118.4a**
	Citadel	**46.9b**	**60.0ab**	**80.15b**
	Concise	**42.4b**	**65.1bc**	**80.3b**
	Dazide	**53.8ab**	**56.1bc**	**82.4b**
	Trimtect	**46.5b**	**39.7c**	**64.6b**

* 2019 + 2020 cones for Badger and Korson's, 2020 for Dutchman, 2019 for Gwinn's

Means within a column for a given farm that are followed by the same letter are not different at $p<0.05$.

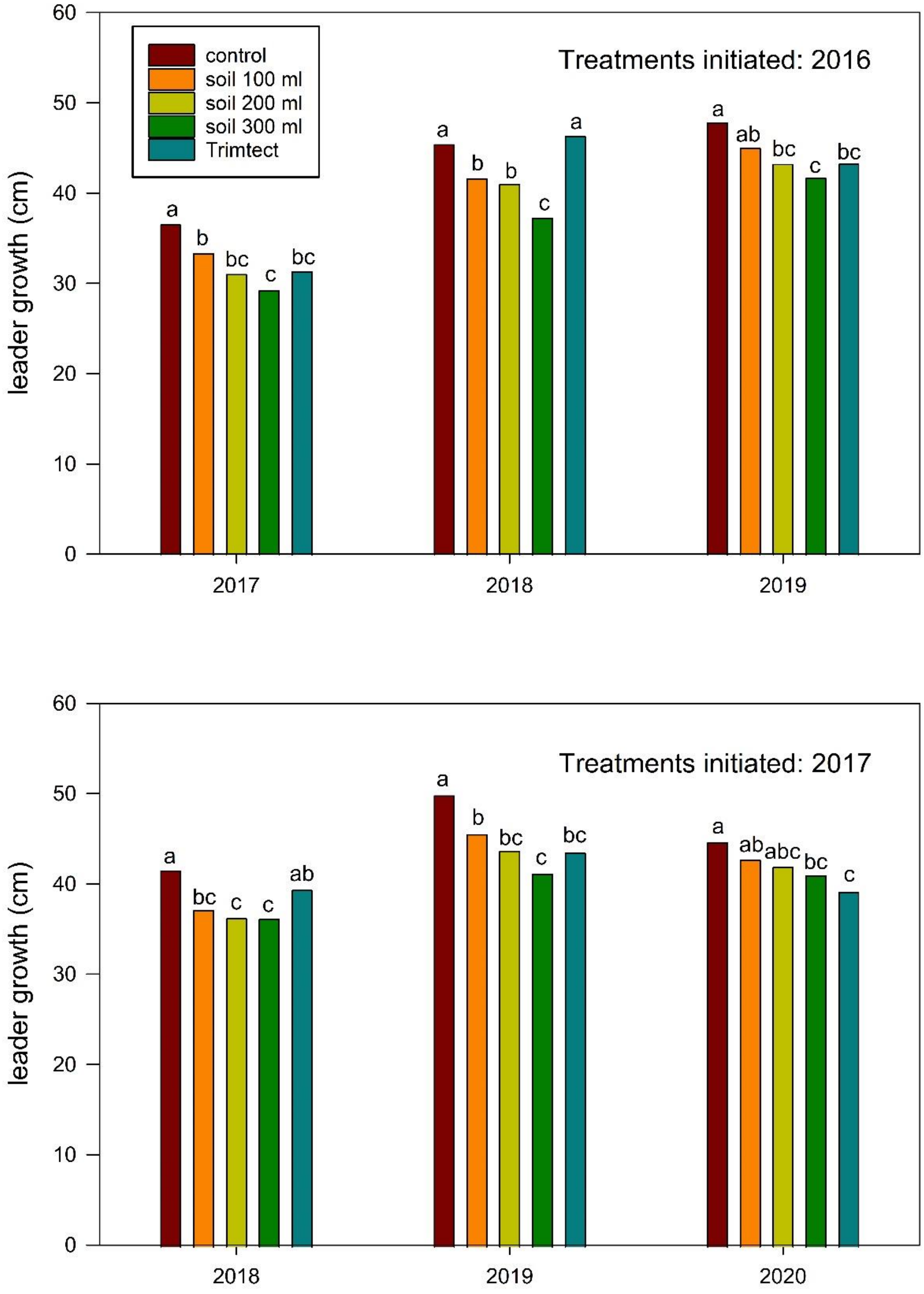

Figure 1. Mean annual leader growth of Fraser fir trees treated with three rates soil applied paclobutrazol (Cambistat) or foliar applied paclobutrazol (Trimtect). Top: trees treated with soil application in 2016 or treated annually 2016-2018. Bottom: trees treated with soil application in 2017 or treated annually 2017-2019. Means within a year indicated by the same letter are not different at P<0.05.

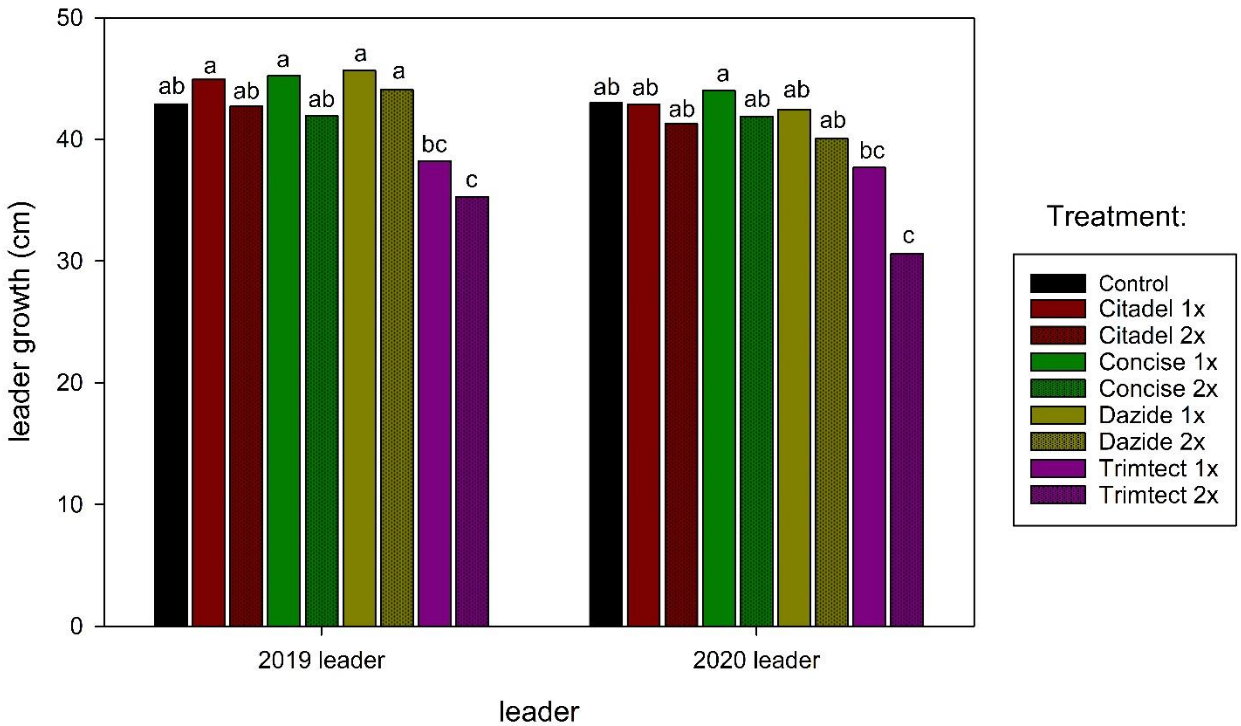

Figure 2. Mean annual leader growth of Fraser fir trees treated with four plant growth regulators or left untreated. Each PGR was applied once (1x) or applied a second time approximately 1 week later (2x). Foliar treatments were applied annually in 2018 and 2019. Means within a year indicated by the same letter are not different at P<0.05.

References

Crain, B. A., & Cregg, B. M. (2017). Gibberellic acid inhibitors control height growth and cone production in *Abies fraseri*. *Scandinavian Journal of Forest Research, 32(5), 391-396.*

Crain, B. A., & Cregg, B. M. (2018). Regulation and management of cone induction in temperate conifers. *Forest Science, 64(1), 82-101.*

Cregg, B., Ellison, D., & O'Donnell, J. (2018). Post-emergent control of nuisance cones in Fraser fir Christmas tree plantations. *Forests, 9(5), 233.*

Owens, J. N., Chandler, L. M., Bennett, J. S., & Crowder, T. J. (2001). Cone enhancement in *Abies amabilis* using GA4/7, fertilizer, girdling and tenting. *Forest Ecology and Management, 154(1-2), 227-236.*

Owens, J. N., & Molder, M. (1977). Vegetative bud development and cone differentiation in *Abies amabilis. Canadian Journal of Botany, 55(8), 992-1008.*

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Photo 1. Fraser fir trees produce cones that disintegrate in the fall, leaving unsightly stalks on the tree.

Photo 2. A plant growth regulator (paclobutrazol) is soil-injected in a Fraser fir plantation using a back-pack injection system.

Photo 3. Michigan State University research assistants assess coning on PGR plots at Dutchman Tree Farm in Manton, MI

2. Summary of Research Report for Public Release by CTPB- Summary should be suitable for non-scientific audience and should not exceed one page.

   Early cone production is a major concern for Christmas tree growers that produce Fraser fir. Trees often produce heavy amounts of cones that must be removed by hand, resulting in significant labor costs. In this project, we hypothesized that plant growth regulators that inhibit GA synthesis or translocation would reduce cone formation in Fraser fir. In a series of trials at four farms in Michigan, we applied paclobutrazol as a one-time soil injection (Tradename: Cambistat®) and as an annual foliar spray (Tradename: Trimtect®). We evaluated coning for 3 years (4 years on 2 sites) and found that the high rate of soil injected paclobutrazol reduced coning across all site by approximately one-third, and reduced coning by up 67%. Paclobutrazol also reduced leader growth by up to 8 cm. Effects of soil application of paclobutrazol persisted three to four years. In a second series of trials we evaluated four different GA-inhibitors (Citadel®, Concise®, Dazide®, and Trimtect®), which were applied annually as foliar sprays. Responses varied among sites, but paclobutrazol reduced coning by up to 70% on one site. The results indicate that GA inhibitors can disrupt cone formation in Fraser fir. Depending on the level of coning on a given farm, the cost of application could compare favorably with manual cone removal. Work is on-going and additional trials will compare the potential of additive effect of combining soil and foliar applications

3. List of all Publications related to this Research Grant

Cregg, B., D. Ellison, and J. O'Donnell. 2019. Controlling coning in Fraser fir with plant growth regulators. 14th International Christmas Tree Research and Extension Conference, Quebec, Canada, August 26-30, 2019.

Cregg, B., D. Ellison-Smith, J. O'Donnell, and R. Rouse. 2021. Controlling coning and shoot growth of Fraser fir with plant growth regulators. In preparation for Forests (anticipated submission date February 2021).