

## Effects of gibberellin A<sub>4/7</sub>, 6-benzylaminopurine and chlormequat chloride on the number of male and female strobili and immature cones in Chinese Pine (*Pinus tabulaeformis*) with foliar sprays

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**Abstract:** Three kinds of plant growth regulators, gibberellinA<sub>4/7</sub> (GA<sub>4/7</sub>), 6-benzylaminopurine (BA), and chlormequat chloride (CCC), were evaluated for their ability to promote strobilus and cone production in a Chinese pine (*Pinus tabulaeformis* Carr.) clonal seed orchard. Treatments (0, 250, 500, or 1000 mg·L<sup>-1</sup>) were applied during three periods (June–July, July–August and August–September) in 2005. Of the three plant growth regulators, GA<sub>4/7</sub> was the best for promoting flower and cone production. Trees sprayed with GA<sub>4/7</sub> (500 mg·L<sup>-1</sup>) from June to September had significantly more female strobili and immature cones than controls and other treatments ( $p \leq 0.0001$ ). The best time to apply GA<sub>4/7</sub> was in June. BA at 500 mg·L<sup>-1</sup> significantly increased female flower and immature cone production, compared to other treatments and controls ( $p \leq 0.0001$ ), while promotion of male strobili was unchanged. Chlormequat chloride at 1000 mg·L<sup>-1</sup> significantly increased the number of male strobili, compared to the other treatments ( $p \leq 0.0001$ ). Spraying CCC at

500 mg·L<sup>-1</sup> significantly increased the number of female strobili, compared to controls ( $p < 0.05$ ), but it had no effect on immature cone production ( $p > 0.05$ ). These results are important for improving seed production and seed orchard management of Chinese pine.

**Keywords:** flower promotion; conifer seed production; breeding; *Pinus tabulaeformis* Carr.

### Introduction

Chinese pine (*Pinus tabulaeformis* Carr.), sometimes called Chinese red pine, Chinese oil pine, or southern Chinese pine, is one of the most important tree species for afforestation from northeast to southwest China (Xu 1993). It is ecologically and economically valuable because of its fast growth on dry and barren sites. Previous research focused on genetic improvement of Chinese pine for increased seed yield because there are significant differences among clones for numbers of female and male strobili (Zhao et al. 2007). Unfortunately, low and untenable seed production from seed orchards remains a problem (Xu 1993; Yang et al. 2006) because of unmet demand for seeds for afforestation and breeding. Good cone crops occur irregularly, and rarely in consecutive years (Zhang 2000).

Several treatments are effective for inducing or enhancing flower and cone production in *Pinaceae*, including pruning, fertilizing, girdling, drought treatments, and application of plant growth regulators (Ross et al. 1976). Many of the commonly applied treatments are intended to enhance flowering, but can be interpreted as affecting root growth (Bonnet-Masimbert 1982, 1987). The most important hormone for flower induction in conifers is gibberellin. Gibberellins (GAs) are plant hormones with many different chemical forms (Takahashi et al. 1991). GAs regulate plant growth and developmental processes, including stem elongation, seed germination, dormancy, flowering, and fruit senescence (Hans et al. 1997). Kato et al. (1958) first re-

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ported that GA improved flowering in *Cryptomeria fortunei*. Since then, promotion of flowering and seed yield following GA application has been shown in numerous *Pinaceae* species (Ho et al. 1992), (Table 1). The most commonly used mixture for exogenous GA application on conifer species is a polar mixture of

GA<sub>4</sub> and GA<sub>7</sub> (GA<sub>4/7</sub>) (Cecich 1981; Brockerhoff and Ho 1997; Eriksson et al. 1998), (Table 1). It is believed that GA and cytokinin not only influence cell division and cell differentiation (Hans et al. 1997), but also flower development and young cone growth (Owens et al. 1972; Owens 1985).

**Table 1. Effect of plant growth regulators on flowering and cone production in conifers**

Plant growth regulator	Concentration (mg·L <sup>-1</sup> )	Application time	Application method	Species	Result	Reference
GA <sub>4/7</sub>	200	Jul-Aug	Foliar spray	Longleaf Pine ( <i>Pinus palustris</i> )	Increased male flowering	Hare 1984
GA <sub>4/7</sub>	200	Jul-Aug	Foliar spray	Slash Pine ( <i>Pinus elliotii</i> )	Increased male flowering	Hare 1984
GA <sub>4/7</sub>	200	Jul-Aug	Foliar spray	Loblolly Pine ( <i>Pinus taeda</i> )	Increased female flowering and cones	Hare 1984
GA <sub>4/7</sub>	200	May-Jun	Foliar spray	Sitka Spruce ( <i>Picea sitchensis</i> )	Increased female flowering	Almqvist 2003
GA <sub>4/7</sub>	--- <sup>b</sup>	May-Jun	Stem injection	Western Hemlock ( <i>Tsuga heterophylla</i> )	Increased female and male flowering	Harrison et al. 1992
GA <sub>4/7</sub>	3	July	Stem injection	Black Spruce ( <i>Picea mariana</i> )	Increased female flowering and cones	Smith et al. 1995
GA <sub>4/7</sub>	---	Mar-Apr	Bud base injection	Caribbean Pine ( <i>Pinus caribaea</i> )	Increased male and female flowering	Harrison et al. 1991
GA <sub>4/7</sub>	---	May-Jun	Foliar spray	Chinese pine ( <i>Pinus tabuliformis</i> )	Increased male flowering and shoot growth	Sheng et al. 1990
GA <sub>4/7</sub>	500	May-Jul	Foliar spray	Eastern White Pine ( <i>Pinus strobus</i> )	Increased female and male flowering	Pijut 2002
GA <sub>4/7</sub>	500	Jul-Aug	Foliar spray	Eastern White Pine ( <i>Pinus strobus</i> )	Increased cones	Ho et al. 1995
GA <sub>4/7</sub>	500	Aug-Sept	Stem injection	Eastern White Pine ( <i>Pinus strobus</i> )	No effects	Ho et al. 1995
GA <sub>4/7</sub>	100	May-Jun	Stem injection	European Larch ( <i>Larix decidua</i> )	Increased male and female flowering	Bonnet-Masimbert 1982
GA <sub>4/7</sub>	100	May-Jun	Stem injection	Japanese Larch ( <i>Larix kaempferi</i> )	Increased male and female flowering	Bonnet-Masimbert 1982
GA <sub>4/7</sub>	600	May-Jul	Foliar spray	Jack Pine ( <i>Pinus banksiana</i> )	Increased cones	Ho et al. 1994
GA <sub>4/7</sub>	1.53	Jul-Aug	Stem injection	Jack Pine ( <i>Pinus banksiana</i> )	Increased male flowering	Fogal et al. 1996
GA <sub>4/7</sub>	---	May-Aug	Foliar spray	Jack Pine ( <i>Pinus banksiana</i> )	Increased female flowering	Cecich 1981
GA <sub>4/7</sub>	0.25	Jul-Aug	Stem injection	Scots Pine ( <i>Pinus sylvestris</i> )	Increased flowering and cones	Eriksson et al. 1998
GA <sub>4/7</sub>	1.53	June	Stem injection	White Spruce ( <i>Picea glauca</i> )	Increased female flowering	Fogal et al. 1996
BA	3 or 7	July	Stem injection	Black Spruce ( <i>Picea mariana</i> )	Reduced flowering	Smith et al. 1995
BA	2000	Early Sept	Paste on branch/Stem injection	Japanese Red Pine ( <i>Pinus densiflora</i> )	Increased female flowering and cones	Wakushima 2004
BA	2000	Early Sept	Paste on branch/Stem injection	Japanese Black Pine ( <i>Pinus thunbergii</i> )	Increased female flowering and mature cones	Wakushima 2004
BA	400	May-June	Stem injection	Chinese pine ( <i>Pinus tabuliformis</i> )	Increased vegetative bud; decreased floral bud	Sheng et al. 1990
GA+BA	---	---	---	Douglas fir ( <i>Pseudotsuga menziesii</i> )	Increased male and female flowering	Ross et al. 1976
GA+BA	400	May-June	Stem injection	Chinese pine ( <i>Pinus tabuliformis</i> )	Decreased cones	Sheng et al. 1990
GA+BA	---	---	Bud application	Sitka Spruce ( <i>Picea sitchensis</i> )	Increased male and female flowering	Tompsett 1977
GA <sub>3</sub> ,GA <sub>5</sub> ,GA <sub>7</sub>	---	---	Drops on buds	Sitka Spruce ( <i>Picea sitchensis</i> )	Increased female, male flowering and cones	Tompsett, 1977
GA+CKs	---	July	Stem injection	Black Spruce ( <i>Picea mariana</i> )	Reduced flowering	Smith et al. 1995

**Notes:** <sup>a</sup> GA<sub>4/7</sub> (gibberellinA<sub>4/7</sub>), BA (6-benzylaminopurine), and CCC (Chlormequat chloride). <sup>b</sup>“---” indicates no data reported.

Sheng and Wang (1990) determined that the percentage of shoots with male flowers was significantly increased by spraying with GA<sub>4/7</sub> in May and June in Chinese pine. The percentage of shoots with female flowers and the number of flowers per shoot were increased by spraying with 2000-mg·L<sup>-1</sup> GA<sub>4/7</sub> in the second half of July in Japanese red pine (*Pinus densiflora*) (Wakushima 2004). Benzylaminopurine (BA) is a synthetic cytokinin with a

structure resembling adenine (Kong et al. 2004). Benzylaminopurine treatments were shown to increase flower and cone production in Japanese red pine and Japanese black pine (*Pinus thunbergii*) (Wakushima et al. 1996, 1997; Wakushima 2004).

Chlormequat chloride (CCC) inhibits endogenous gibberellin biosynthesis and promotes an increase in cytokinin content

(Mobli et al. 2008). Application of an appropriate concentration of CCC can inhibit stem elongation and promote tillering and reproductive growth (El-Fouly et al. 1977). CCC is used to promote flowering and fruiting in many crops, including wheat, cotton and rice (El-Fouly et al. 1977; Sliman et al. 1992), but the effect of CCC treatment on flower and cone production of pine trees is not well tested (Kong et al. 2004).

It was our goal to determine if spray treatment with the growth regulators 6-benzylaminopurine, chlormequat chloride, or gibberellinA<sub>4/7</sub> could be used to increase the number of male and female flowers and immature cones produced by Chinese pine. We also sought to determine the optimal concentration and time of application of these plant growth regulators to improve seed production in a clonal Chinese pine seed orchard.

## Materials and methods

Treatments were applied to 26-year-old trees in a clonal Chinese pine seed orchard in 2005. Treated trees had a mean height of 6.2 m and mean diameter (dbh) of 12.0 cm at 1.5 m height. The 33.3-ha seed orchard, which is located at Luonan Gucheng, Shaanxi Province, China (33°55'N, 110°22'W; elevation 950 m; slope 28°), included 326 Chinese pine clones selected from natural forests of Shaanxi Province. The annual mean temperature in the seed orchard site is 11.1°C and the annual mean rainfall is 772.3 mm. The frost-free period is 215 days. Treatments were applied to 864 branches across eight clones. Three plant growth regulators, GA<sub>4/7</sub> (Ruigu Biotechnology, Shanghai, China), BA (Biaojia Biotechnology, Guangzhou, China), and CCC (Nongda Biochemical, Zhengzhou, China) were tested to determine their effect on flower and cone production. For the application of treatments, healthy trees were chosen arbitrarily in two blocks, but not on the edge of the seed orchard. These trees were randomly assigned to one of three growth regulator treatments; all treatments were applied to branches of similar size (1.5 to 2.0 m in length). Branches were sprayed with plant growth regulator treatments three times each month: June (June 1, June 15 and June 29); July (July 1, July 15 and July 29); August (August 1, August 15 and August 29), for a total of nine applications from June to September. For each application, twelve branches of each clone were treated with one of three concentrations (250 mg·L<sup>-1</sup>, 500 mg·L<sup>-1</sup>, or 1000 mg·L<sup>-1</sup>) of one of three plant growth regulators (GA<sub>4/7</sub>, BA, and CCC) or doubly distilled water (ddH<sub>2</sub>O) as a control (Table 2). Thus, at each of nine application times, four similar branches on one tree of each clone were identified and treated with either water or 250 mg·L<sup>-1</sup>, 500 mg·L<sup>-1</sup>, or 1000 mg·L<sup>-1</sup> of one of the three plant growth regulators, and not treated again. The timing of application was based on Chinese pine floral morphogenesis (Zhang 2000). We used GA<sub>4/7</sub> concentrations being comparable to those used by Philipson (1985) and Fogal et al. (1996). GA<sub>4/7</sub> was dissolved in 100% ethanol, BA was dissolved in 0.1-mol·L<sup>-1</sup> HCL (pH=7.0), and CCC was dissolved in water. The resulting stock solutions were diluted with ddH<sub>2</sub>O to the desired treatment concentration. Using a hand spray bottle, each branch was sprayed until the solution dripped

off the treated branch. In the spring of the year following treatment, data were collected when pollen cones (male strobili) had emerged from the bud scales.

The number of female and male flowers on treated branches was counted from 28 April to 10 May 2006 and the number of immature seed cones on treated branches was counted from 15 to 30 September, 2006 (Fig. 1). The number of male flowers was compared within plant growth regulators among treatments (concentration and time of application) using PROC mixed of SAS software version 9.1 (SAS Institute, Cary, NC). Clones were considered random effects for ANOVA. We used a Wilcoxon test (Wilcoxon 1945) and Van der Waerden test (Van der Waerden 1952) in Proc NPAR1WAY to analyze the number of female strobili and immature (four-month-old) cones because the data were not normally distributed. A Wilcoxon test was used to evaluate differences between treatment effects and controls.



**Fig. 1** Immature cones (upper), female flowers (middle), and male flowers (lower) of Chinese pine (*Pinus tabulaeformis*).

## Results

We observed large and significant differences among treatments for numbers of male and female strobili and numbers of immature cones (Table 2). The mean number of male strobili per branch ranged from 15.1 to 71.9. The mean number of female strobili per branch ranged from 0.42 to 4.42, and the mean number of immature cones ranged from 0.22 to 2.92 per branch (Table 2).

**Table 2. Number of male strobili, female strobili, and immature cones per branch following treatment with GA<sub>4/7</sub>, BA and CCC in Chinese pine (*Pinus tabulaeformis*)**

Plant growth regulator	Concentration (mg·L <sup>-1</sup> )	Time	Male strobili		Female strobili		Cones		Clones (N) <sup>d</sup>
			Mean/Median <sup>a</sup>	Std Dev	Mean/Median <sup>a</sup>	Std Dev	Mean/Median <sup>a</sup>	Std Dev	
BA (6-benzylaminopurine)	0	August	21.0/19	11.8	0.50/0	0.72	0.46/0	0.59	4
		July	22.5/20	15.3	0.58/0	0.83	0.35/0	0.49	1
		June	22.3/18.5	14.5	0.67/1	0.70	0.54/1	0.51	4
		Mean	21.9	13.9	0.58	0.75	0.45	0.53	2.3
	250	August	39.6/22	38.0	0.88/1	1.26	0.50/0	0.88	3
		July	30.3/21	27.1	0.96/1	1.00	0.71/1	0.62	4
		June	49.8/28.5	57.8	1.42/1	1.35	0.92/1	0.97	5
		Mean	39.9 ns <sup>b</sup> /ns <sup>c</sup>	40.9	1.08 ns/**	1.20	0.71 ns/ns	0.83	4.0
	500	August	33.0/20	29.9	1.21/1	0.98	0.71/1	0.69	5
		July	28.4/22	21.2	1.33/1	1.37	1.04/1	1.16	6
		June	25.8/12	28.2	1.83/1	1.90	1.25/1	1.26	5
		Mean	29.1 ns/ns	26.5	1.46***/***	1.42	1.00**/**	1.04	5.3
	1000	August	31.4/20	34.7	0.75/0	1.11	0.50/0	0.72	2
		July	48.8/22.5	51.7	0.79/1	0.78	0.78/1	0.67	5
		June	43.2/17	80.2	0.88/1	1.08	0.58/0	0.72	3
		Mean	41.1 ns/ns	55.5	0.81 ns/ns	0.99	0.62 ns/ns	0.70	3.3
GA <sub>4/7</sub> (gibberellinA <sub>4/7</sub> )	0	August	23.0/20	13.3	0.75/1	0.68	0.58/1	0.58	4
		July	23.6/21	13.4	0.92/1	0.65	0.63/1	0.49	5
		June	22.0/16	15.0	0.92/1	0.5	0.67/1	0.56	5
		Mean	22.9	13.9	0.86	0.61	0.63	0.55	4.7
	250	August	52.6/32.5	51.3	2.04/2	1.00	1.63/2	0.77	8
		July	41.0/19	46.1	2.46/2	1.06	1.67/1	0.87	8
		June	53.2/25	55.4	2.08/2	1.21	1.46/2	0.78	8
		Mean	48.9 ns/*	50.9	2.20 ns/**	1.09	1.58 ns/**	0.81	8.0
	500	August	43.6/24	51.8	3.46/3.5	1.50	2.38/2	1.13	8
		July	45.7/22	56.7	2.67/2	1.71	1.79/2	1.25	8
		June	44.50/24	49.0	4.42/4	2.39	2.92/2.5	1.79	8
		Mean	44.6 ns/*	52.5	3.52***/***	1.87	2.36***/***	1.39	8.0
	1000	August	50.4/35	42.0	1.54/1	1.41	1.13/1	0.95	7
		July	50.5/21	63.0	1.79/1	1.86	1.17/1	1.05	6
		June	65.6/27	88.2	1.83/2	1.40	1.17/1	1.09	6
		Mean	55.5 ns/*	64.4	1.72 ns/**	1.56	1.15 ns/**	1.03	6.3
CCC (Chlormequat chloride)	0	August	19.5/19	10.0	0.54/0	0.66	0.43/0	0.51	3
		July	15.1/14.5	6.45	0.58/0	0.72	0.42/0	0.58	3
		June	17.8/13.5	13.2	0.42/0	0.50	0.39/0	0.58	2
		Mean	17.5	9.89	0.51	0.63	0.41	0.56	2.7
	250	August	28.3/21.5	19.8	0.96/1	0.91	0.58/0.5	0.65	3
		July	31.1/21	28.6	0.75/0.5	0.99	0.38/0	0.71	2
		June	31.0/20	30.2	0.92/0	1.38	0.54/0	0.83	2
		Mean	30.1 ns/**	26.2	0.88 ns/ns	1.09	0.50 ns/ns	0.73	2.3
	500	August	42.0/25	42.2	1.08/0.5	1.41	0.79/0	1.22	4
		July	40.5/26	40.5	0.79/1	0.72	0.42/0	0.65	2
		June	32.2/15	37.6	1.17/1	1.49	0.71/1	0.95	5
		Mean	38.2 ns/*	40.1	1.01 ns/**	1.21	0.64 ns/ns	0.94	3.7
	1000	August	51.5/42	31.7	1.00/0.5	1.22	0.65/0	0.88	4
		July	69.8/40	70.2	0.54/0	0.78	0.22/0	0.42	2
		June	71.9/39	68.0	0.96/1	0.86	0.50/0	0.72	2
		Mean	64.4***/***	56.6	0.83 ns/ns	0.95	0.46 ns/ns	0.68	2.7

**Notes:** <sup>a</sup> The mean and median number of male strobili, female strobili and immature cones ( $\pm$  std dev.). When observations for a treatment included zeros (0), means and medians deviated considerably, and standard deviations were correspondingly large. <sup>b</sup> Comparison among treatments (three concentrations). <sup>c</sup> Comparison of control with treatments. <sup>d</sup> The number of clones out of eight with immature cones. \* indicates  $p < 0.05$ , \*\* indicates  $p < 0.01$ , \*\*\* indicates  $p < 0.0001$ .

The effect of GA<sub>4/7</sub> treatment on flower and cone production in Chinese pine

Compared to controls, spraying with GA<sub>4/7</sub> significantly increased the number of male strobili ( $p \leq 0.05$ ; Table 2). ANOVA showed that for GA<sub>4/7</sub>, neither concentration nor treatment time significantly influenced the promotion of male strobili (Table 3). The order of effectiveness of GA<sub>4/7</sub> treatments in promoting male strobili ranked as 1000 mg·L<sup>-1</sup> > 250 mg·L<sup>-1</sup> > 500 mg·L<sup>-1</sup> (Table 2). The treatment of 1000-mg·L<sup>-1</sup> GA<sub>4/7</sub> in June resulted in most male strobili ( $65.6 \pm 88.2$ ), and significantly more than the number of male strobili on control branches ( $22.0 \pm 14.9$ ), (Table 2). There were no significant effects for male strobili production at treatments with time×concentration interaction (Table 3). GA<sub>4/7</sub> treatments also resulted in a significant increase in the number of female strobili and immature cones compared to controls (Table 2). GA<sub>4/7</sub> at 500 mg·L<sup>-1</sup> stimulated production of significantly more female strobili and immature cones than did other concentrations (Table 2,  $p \leq 0.0001$ ). Moreover, GA<sub>4/7</sub> at 500 mg·L<sup>-1</sup> applied in June resulted in significantly more female strobili and immature cones than GA<sub>4/7</sub> 500 mg·L<sup>-1</sup> applied in July or August ( $p \leq 0.05$ ). When GA<sub>4/7</sub> (500 mg·L<sup>-1</sup>) was applied in June, the mean number of female flowers was 4.8 times more than controls (Table 2). Treatment with 500 mg·L<sup>-1</sup> of GA<sub>4/7</sub> resulted in the greatest mean number of immature cones in September, 2006 ( $2.36 \pm 1.39$ ), significantly more than controls ( $0.63 \pm 0.55$ ), and about one cone per branch more than the other two treatment concentrations (Table 2).

The effect of BA treatment on flower and cone production

BA treatment did not significantly increase the number of male strobili, compared to controls (Table 2) ( $F=1.56$ ,  $p > 0.21$ ; Table 3). BA treatments did not result in any significant effects for number of male strobili for treatments with time×concentration interaction (Table 3). Numbers of female strobili increased significantly on branches treated with 500-mg·L<sup>-1</sup> BA ( $p \leq 0.0001$ ) and 250 mg·L<sup>-1</sup> ( $p \leq 0.01$ ) (Table 2). The number of immature cones increased significantly with the application of 500 mg·L<sup>-1</sup> BA ( $p \leq 0.01$ ) compared to controls and the two other BA concentrations (Table 2). Time of treatment with BA did not significantly affect the number of female strobili, male strobili, or immature cones ( $p > 0.05$ ).

The effect of CCC treatment on flower and cone production

The concentration of applied CCC significantly affected the number of male strobili in 2006 ( $p \leq 0.0001$ ; Table 3). Although all three tested concentrations of CCC produced more male strobili than controls (Table 2), application at the highest concentration (1000 mg·L<sup>-1</sup>) resulted in the greatest number of male flowers. CCC treatments did not result in any significant effects for the number of male strobili at treatment with time × concentration interaction (Table 3), although for the 1000-mg·L<sup>-1</sup> CCC treatment there was a clear trend of decreasing numbers of male strobili when early treatment (June) was compared to late treat-

ment (August), (Table 2). The number of female strobili was significantly increased compared to controls after treatment with CCC 500 mg·L<sup>-1</sup> ( $p \leq 0.01$ ), (Table 2), but the number of immature cones did not increase significantly (Table 2). The number of clones that contribute to the seed crop in any year is important because it influences the genetic diversity or status number of the seed orchard. We observed that all eight clones that were treated with GA<sub>4/7</sub> and BA formed immature cones, but only four or five clones bore cones in the control group (Table 2).

## Discussion

GA treatment probably increases cone production by regulating flower development (Guttridge 1962). Bonnet-Masimbert (1987) reported that the differentiation of floral primordia of male strobili continues from early July to early September in conifers. Differentiation of female strobili continues from mid-September to mid-October. At present, there is one report that *Pinus tabulaeformis* female flowers originate in September and male flowers originate in June (Zhang 2000). In this study, we treated branches from June 1 to August 29, and found that time of application affected the number of male strobili (earlier was better for both GA<sub>4/7</sub> and CCC). The early (June) treatment with 500 mg·L<sup>-1</sup> GA<sub>4/7</sub> significantly increased the number of female strobili and immature cones ( $p < 0.05$ ), but later treatments did not (July to September). Our results were similar to those of Pijut (2002), who applied a foliar spray of GA<sub>4/7</sub> onto Eastern white pine from May to June. On the other hand, we observed that August treatment with GA<sub>4/7</sub> significantly increased the number of male strobili over controls, but treatment in June or July did not. A possible reason that our results did not agree with Zhang (2000) with respect to the best time to apply GA<sub>4/7</sub>, is that Zhang introduced plant growth regulators into the xylem of trees using stem injection, whereas our treatments were foliar applications. We conclude that in our experiment 500 mg·L<sup>-1</sup> of GA<sub>4/7</sub> was the most effective concentration for stimulating flower and cone production in Chinese pine. This result was in agreement with the result reported by Pijut (2002) for Eastern white pine. In general, stem injection of GA<sub>4/7</sub> is effective at promoting flowering at much lower concentrations, compared to our study, at least in some species (Table 1). The relative merits of foliar sprays versus stem injection for stimulation of flowering in conifers are discussed in Bonnet-Masimbert and Zaerr (1987). In general, foliar sprays are more convenient and injure trees less than stem injection. The consequences of repeated hormone treatments on the long-term health of trees and the seed orchard environment are not well studied.

We report here for the first time that CCC treatment of Chinese pine increased the number of both male and female strobili, but it did not significantly increase the number of immature cones. CCC (1000 mg·L<sup>-1</sup>) applied in June significantly increased the number of female strobili ( $p < 0.05$ ). It is likely that environmental factors such as insects, weather, or herbivores destroyed some young, developing cones, and for this reason some treatments increased the number of female strobili but did not in-

crease the number of immature cones. The mechanism of action for CCC was not obvious, but other anti-GAs, such as paclobutrazol, were shown to elicit flowering responses (Griffin et al. 1993). CCC mainly promoted the formation of male strobili, but this effect was of limited utility because pollen production generally does not limit Chinese pine breeding (Zhao et al. 2007).

Some clones in seed orchards produce no flowers or no cones in some years. We applied treatments to ramets of eight different clones, and observed that treatments that increased overall flower production also increased the number of different clones that produced immature cones compared to controls (Table 2). This result was important because it implies that plant growth regulator treatment might increase the genetic diversity of the seed crop. Our research method did not permit us to evaluate whether Chinese pine clones differed significantly in their responses to CCC, BA and GA<sub>4/7</sub>. If clones differ in their response to hormone

treatment, then it may be that a single hormone treatment delivered at one time would cause uneven flowering in the seed orchard, and the result might be seed crops with sub-optimal genetic diversity, despite the application of flower-inducing treatments. Tests of combinations of treatments (for example BA+GA<sub>4/7</sub>) could produce additional gains in Chinese pine seed production, and horticultural manipulation (for example, the timing of fertilization, pruning and irrigation) might also present opportunities for increasing seed yield (Bonnet-Masimbert et al. 1995). For this reason, the use of plant growth regulator treatments to augment the productivity of seed orchards should be integrated with other management considerations. In the years when high flower production occurs naturally, the number of cones and seeds may be limited by physiological factors. So the application of plant growth regulators to increase flowering may not result in larger cone crops.

**Table 3.** ANOVA for effect of plant growth regulator concentration and time of application on promotion of male strobili production in Chinese pine (*Pinus tabulaeformis*)

Plant growth regulator	Source <sup>a</sup>	Df	Type III SS	Mean square	F Value	Pr > F
BA (6-Benzylaminopurine)	Time	2	948.56	474.28	0.24	0.7893
	Concentration	2	6230.32	3115.16	1.56	0.2136
	Concentration×Time	4	7949.23	1987.31	0.99	0.4127
CCC (Chlormequat Chloride)	Time	2	1621.98	810.99	0.42	0.6608
	Concentration	2	45947.61	22973.80	11.76	<0.0001
	Concentration×Time	4	5849.91	1462.48	0.75	0.5599
GA <sub>4/7</sub> (Gibberellin <sub>A<sub>4/7</sub></sub> )	Time	2	2799.34	1399.67	0.43	0.6542
	Concentration	2	4330.70	2165.35	0.66	0.5190
	Concentration×Time	4	3197.74	799.44	0.24	0.9137

**Notes:** <sup>a</sup> Comparisons were among plant growth regulator treatments only, comparisons with controls were performed using Wilcoxon test, (see Table 2).

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