Journal of Forestry Research (2011) 22(3): 353–359
DOI 10.1007/s11676-011-0179-3

ORIGINAL PAPER

Effects of gibberellin $A_{4/7}$, 6-benzylaminopurine and chlormequat chloride on the number of male and female strobili and immature cones in Chinese Pine (*Pinus tabuliformis*) with foliar sprays

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Received: 2010-07-18 Accepted: 2010-12-08

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

Foundation project: Partial funding for this research was provided by The State Forestry Administration of China (No. 2006-85).
The online version is available at http:// www.springerlink.com

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Responsible editor: Zhu Hong

Introduction

Chinese pine (*Pinus tabuliformis* 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 reported 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_4 and GA_7 ($GA_{4/7}$) (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^{-1})	Application time	Application method	Species	Result	Reference
$GA_{4/7}$	200	Jul-Aug	Foliar spray	Longleaf Pine (*Pinus palustris*)	Increased male flowering	Hare 1984
$GA_{4/7}$	200	Jul-Aug	Foliar spray	Slash Pine (*Pinus elliottii*)	Increased male flowering	Hare 1984
$GA_{4/7}$	200	Jul-Aug	Foliar spray	Loblolly Pine (*Pinus taeda*)	Increased female flowering and cones	Hare 1984
$GA_{4/7}$	200	May-Jun	Foliar spray	Sitka Spruce (*Picea sitchensis*)	Increased female flowering	Almqvist 2003
$GA_{4/7}$	---[b]	May-Jun	Stem injection	Western Hemlock (*Tsuga heterophylla*)	Increased female and male flowering	Harrison et al. 1992
$GA_{4/7}$	3	July	Stem injection	Black Spruce (*Picea mariana*)	Increased female flowering and cones	Smith et al. 1995
$GA_{4/7}$	---	Mar-Apr	Bud base injection	Caribbean Pine (*Pinus caribaea*)	Increased male and female flowering	Harrison et al. 1991
$GA_{4/7}$	---	May-Jun	Foliar spray	Chinese pine (*Pinus tabuliformis*)	Increased male flowering and shoot growth	Sheng et al. 1990
$GA_{4/7}$	500	May-Jul	Foliar spray	Eastern White Pine (*Pinus strobus*)	Increased female and male flowering	Pijut 2002
$GA_{4/7}$	500	Jul-Aug	Foliar spray	Eastern White Pine (*Pinus strobus*)	Increased cones	Ho et al. 1995
$GA_{4/7}$	500	Aug-Sept	Stem injection	Eastern White Pine (*Pinus strobus*)	No effects	Ho et al.1995
$GA_{4/7}$	100	May-Jun	Stem injection	European Larch (*Larix decidua*)	Increased male and female flowering	Bonnet-Masimbert 1982
$GA_{4/7}$	100	May-Jun	Stem injection	Japanese Larch (*Larix kaempferi*)	Increased male and female flowering	Bonnet-Masimbert 1982
$GA_{4/7}$	600	May-Jul	Foliar spray	Jack Pine (*Pinus banksiana*)	Increased cones	Ho et al. 1994
$GA_{4/7}$	1.53	Jul-Aug	Stem injection	Jack Pine (*Pinus banksiana*)	Increased male flowering	Fogal et al. 1996
$GA_{4/7}$	---	May-Aug	Foliar spray	Jack Pine (*Pinus banksiana*)	Increased female flowering	Cecich 1981
$GA_{4/7}$	0.25	Jul-Aug	Stem injection	Scots Pine (*Pinus sylvestris*)	Increased flowering and cones	Eriksson et al. 1998
$GA_{4/7}$	1.53	June	Stem injection	White Spruce (*Picea glauca*)	Increased female flowering	Fogal et al. 1996
BA	3 or 7	July	Stem injection	Black Spruce (*Picea mariana*)	Reduced flowering	Smith et al. 1995
BA	2000	Early Sept	Paste on branch/Stem injection	Japanese Red Pine (*Pinus densiflora*)	Increased female flowering and cones	Wakushima 2004
BA	2000	Early Sept	Paste on branch/Stem injection	Japanese Black Pine (*Pinus thunbergii*)	Increased female flowering and mature cones	Wakushima 2004
BA	400	May-June	Stem injection	Chinese pine (*Pinus tabuliformis*)	Increased vegetative bud; decreased floral bud	Sheng et al. 1990
GA+BA	---	---	---	Douglas fir (*Pseudotsuga menziesi*)	Increased male and female flowering	Ross et al. 1976
GA+BA	400	May-June	Stem injection	Chinese pine (*Pinus tabuliformis*)	Decreased cones	Sheng et al. 1990
GA+BA	---	---	Bud application	Sitka Spruce (*Picea sitchensis*)	Increased male and male flowering	Tompsett 1977
GA_3,GA_5,GA_7	---	---	Drops on buds	Sitka Spruce (*Picea sitchensis*)	Increased female, male flowering and cones	Tompsett, 1977
GA+CKs	---	July	Stem injection	Black Spruce (*Picea mariana*)	Reduced flowering	Smith et al. 1995

Notes: [a] $GA_{4/7}$ (gibberellin$A_{4/7}$), BA (6-benzylaminopurine), and CCC (Chlormequat chloride). [b] "---" indicates no data reported.

Sheng and Wang (1990) determined that the percentage of shoots with male flowers was significantly increased by spraying with $GA_{4/7}$ 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^{-1} $GA_{4/7}$ in the second half of July in Japanese red pine (*Pinus densiflora*) (Wakushima 2004). Benzlaminopurine (BA) is a synthetic cytokinin with a structure resembling adenine (Kong et al. 2004). Benzlaminopurine 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 gibberellin$A_{4/7}$ 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, $GA_{4/7}$ (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 $mg \cdot L^{-1}$, 500 $mg \cdot L^{-1}$, or 1000 $mg \cdot L^{-1}$) of one of three plant growth regulators ($GA_{4/7}$, BA, and CCC) or doubly distilled water (ddH_2O) 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 $mg \cdot L^{-1}$, 500 $mg \cdot L^{-1}$, or 1000 $mg \cdot L^{-1}$ 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 $GA_{4/7}$ concentrations being comparable to those used by Philipson (1985) and Fogal et al. (1996). $GA_{4/7}$ was dissolved in 100% ethanol, BA was dissolved in 0.1-$mol \cdot L^{-1}$ HCL (pH=7.0), and CCC was dissolved in water. The resulting stock solutions were diluted with ddH_2O 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 tabuliformis*).

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 $GA_{4/7}$, BA and CCC in Chinese pine (*Pinus tabuliformis*)

Plant growth regulator	Concentration ($mg \cdot L^{-1}$)	Time	Male strobili Mean/Median[a]	Male strobili Std Dev	Female strobili Mean/Median[a]	Female strobili Std Dev	Cones Mean/Median[a]	Cones Std Dev	Clones (N)[d]
	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
BA		Mean	39.9 ns[b]/ns[c]	40.9	1.08 ns/**	1.20	0.71 ns/ns	0.83	4.0
(6-benzylaminopurine)	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
	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
$GA_{4/7}$		Mean	48.9 ns/*	50.9	2.20 ns/***	1.09	1.58 ns/***	0.81	8.0
(gibberellin$A_{4/7,}$)	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	55.5ns/*	64.4	1.72 ns/**	1.56	1.15 ns/**	1.03	6.3
	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
CCC		Mean	30.1 ns/**	26.2	0.88 ns/ns	1.09	0.50 ns/ns	0.73	2.3
(Chlormequat chloride)	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	1.01ns/**	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:. [a] The mean and median number of male strobili, female strobili and immature cones (± std dev.). When observations for a treatment included zeros (0), means and medians deviated considerably, and standard deviations were correspondingly large. [b] Comparison among treatments (three concentrations). [c] Comparison of control with treatments. [d] 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 $GA_{4/7}$ treatment on flower and cone production in Chinese pine

Compared to controls, spraying with $GA_{4/7}$ significantly increased the number of male strobili ($p\leq0.05$; Table 2). ANOVA showed that for $GA_{4/7}$, neither concentration nor treatment time significantly influenced the promotion of male strobili (Table 3). The order of effectiveness of $GA_{4/7}$ treatments in promoting male strobili ranked as 1000 mg·L^{-1}>250 mg·L^{-1}>500 mg·L^{-1} (Table 2). The treatment of 1000-mg·L^{-1} $GA_{4/7}$ in June resulted in most male strobili (65.6 ± 88.2), and significantly more than the number of male strobili on control branches (22.0 ±14.9), (Table 2). There were no significant effects for male strobili production at treatments with time×concentration interaction (Table 3). $GA_{4/7}$ treatments also resulted in a significant increase in the number of female strobili and immature cones compared to controls (Table 2). $GA_{4/7}$ at 500 mg·L^{-1} stimulated production of significantly more female strobili and immature cones than did other concentrations (Table 2, $p\leq0.0001$). Moreover, $GA_{4/7}$ at 500 mg·L^{-1} applied in June resulted in significantly more female strobili and immature cones than $GA_{4/7}$ 500 mg·L^{-1} applied in July or August ($p\leq0.05$). When $GA_{4/7}$ (500 mg·L^{-1}) was applied in June, the mean number of female flowers was 4.8 times more than controls (Table 2). Treatment with 500 mg·L^{-1} of $GA_{4/7}$ resulted in the greatest mean number of immature cones in September, 2006 (2.36±1.39), significantly more than controls (0.63±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^{-1} BA ($p\leq0.0001$) and 250 mg·L^{-1} ($p\leq0.01$) (Table 2). The number of immature cones increased significantly with the application of 500 mg·L^{-1} BA ($p\leq0.01$) 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 ($p\leq0.0001$; 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^{-1}) 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^{-1} CCC treatment there was a clear trend of decreasing numbers of male strobili when early treatment (June) was compared to late treatment (August), (Table 2). The number of female strobili was significantly increased compared to controls after treatment with CCC 500 mg·L^{-1} ($p\leq0.01$), (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 $GA_{4/7}$ 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 tabuliformis* 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 $GA_{4/7}$ and CCC). The early (June) treatment with 500 mg·L^{-1} $GA_{4/7}$ 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 $GA_{4/7}$ onto Eastern white pine from May to June. On the other hand, we observed that August treatment with $GA_{4/7}$ 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 $GA_{4/7}$, 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^{-1} of $GA_{4/7}$ 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 $GA_{4/7}$ 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^{-1}) 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 increase 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 $GA_{4/7}$. 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+$GA_{4/7}$) 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 tabuliformis*)

Plant growth regulator	Source[a]	D*f*	Type III SS	Mean square	*F* Value	Pr > F
	Time	2	948.56	474.28	0.24	0.7893
BA (6-Benzylaminopurine)	Concentration	2	6230.32	3115.16	1.56	0.2136
	Concentration×Time	4	7949.23	1987.31	0.99	0.4127
	Time	2	1621.98	810.99	0.42	0.6608
CCC (Chlormequat Chloride)	Concentration	2	45947.61	22973.80	11.76	<0.0001
	Concentration×Time	4	5849.91	1462.48	0.75	0.5599
	Time	2	2799.34	1399.67	0.43	0.6542
GA4/7 (Gibberellin$A_{4/7,}$)	Concentration	2	4330.70	2165.35	0.66	0.5190
	Concentration×Time	4	3197.74	799.44	0.24	0.9137

Notes: [a] Comparisons were among plant growth regulator treatments only, comparisons with controls were performed using Wilcoxon test, (see Table 2).

Acknowledgements

Thanks to Peihua Yang, Yonghong Liu for their helpful comments during the preparation of the manuscript. We thank two anonymous reviewers for their insightful reviews of the manuscript. The use of trade names is for the information and convenience of the reader and does not imply official endorsement or approval by the United States Department of Agriculture or the Forest Service of any product to the exclusion of others that may be suitable. The authors also thank Fei Cheng, Liang He, Ximin Yu, Junfeng Xie for their assistance in data collection. Partial funding for this research was provided by The State Forestry Administration of China (No. 2006-85).

References

Almqvist C. 2003. Timing of GA4/7 application and the flowering of *Pinus sylvestris* grafts in the greenhouse. *Tree Physiology*, **23**: 413–418.

Bonnet-Masimbert M. 1982. Effect of growth regulators, girdling, and mulching on flowering of young European and Japanese larches under field conditions. *Canadian Journal of Forest Research*, **12**: 270–279.

Bonnet-Masimbert M. 1987. Floral induction in conifers: a review of available techniques. *Forest Ecology and Management*, **19**: 135–146.

Bonnet-Masimbert M, Webber JE. 1995. From flower induction to seed production in forest tree orchards. *Tree Physiology*, **15**: 419–426.

Bonnet-Masimbert M, Zaerr J B. 1987. The role of plant growth regulators in promotion of flowering. *Plant Growth Regulation*, **6**: 13–35

Brockerhoff EG, Ho RH. 1997. Effects of gibberellin $A_{4/7}$ stem injection on seed cone production in mature black spruce. *Canadian Journal of Forest Research*, **27**: 1325–1328.

Cecich RA. 1981. Applied gibberellin $A_{4/7}$ increases ovulate strobili production in accelerated growth Jack pine seedlings. *Canadian Journal of Forest Research*, **11:** 580–585.

El-Fouly MM, Mohamed BR, Fawzi AFA. 1977. Chlormequat (CCC) induced enhancement of flowering in carnation in relation to changes in carbohydrate metabolism. *Scientia Horticulturae*, **6**: 241–249.

Eriksson U, Jansson G, Almqvist C. 1998. Seed and pollen production after stem injections of gibberellin $A_{4/7}$ in field-grown seed orchards of *Pinus sylvestris*. *Canadian Journal of Forest Research*, **28**: 340–346.

Fan J, Yang P, Guo S. 2006. Progress in genetics and improvement of *Pinus tabuliformis* Carr. in Shaanxi province. *Journal of Northwest A&F University*, **34**: 45– 50. (In Chinese).

Fogal WH, Jobin G, Schooley HO, Coleman SJ, Wolynetz MS. 1996. Stem incorporation of gibberellins to promote sexual development of white spruce, Norway spruce and jack pine. *Canadian Journal of Forest Research*, **26**: 186–195.

Griffin AR, Whiteman P, Rudge T, Burgess IP, Moncur M. 1993. Effect of paclobutrazol on flower-bud production and vegetative growth in two species of Eucalyptus. *Canadian Journal of Forest Research*, **23**: 640–647.

Guttridge CG. 1962. Inhibition of fruit-bud formation in apples with gibberellic acid. *Nature*, **196**: 1008.

Hans KH, Zeevaart JAD. 1997. The five "classical" plant hormones. *Plant Cell*, **9:** 197–121.

Hare RC. 1984. Application method and timing of gibberellin $A_{4/7}$ treatments for increasing pollen conebud production in southern pines. *Canadian Journal of Forest Research*, **14**: 128–131.

Harrison DLS, Slee MU. 1991. Gibberellin $A_{4/7}$ enhanced flowering in *Pinus caribaea* var. *hondurensis*. *Canadian Journal of Forest Research*, **21**: 788–793.

Harrison DLS, Owens JN. 1992. Gibberellins $A_{4/7}$ enhanced cone production in *Tsuga heterophylla*: the influence of gibberellins $A_{4/7}$ on seed and pollen-cone production. *International Journal of Plant Science*, **153**: 171–177.

Ho RH, Eng K. 1995. Promotion of cone production on field-grown eastern white pine grafts by gibberellin $A_{4/7}$ application. *Forest Ecology and Management*, **75**: 11–16.

Ho RH, Hak O. 1994. Optimum timing of gibberellin $A_{4/7}$ sprays to promote cone production in jack pine seedlings. *New Forest*, **8**: 61–69.

Ho RH, Schnekenburger F. 1992. Gibberellin $A_{4/7}$ promotes cone production on potted grafts of eastern white pine. *Tree physiology*, **11**: 197–203.

Kato Y, Miyake I, Ishikawa H. 1958. Initiation of flower bud by gibberellin in *Cryptomeria japonica*. *Journal of Japan Forestry Society*, **40**: 35–36.

Kong L, Aderkas P. 2004. Plant growth regulators and cone induction in Pinaceae. Centre for Forest Biology, Department of Biology, University of Victoria,3800 Finnerty Rd., Victoria. Available at http://www.fgcouncil.bc.ca/Pinaceae-cone-ind-lit-rev.pdf.

Mobli M, Baninasab B. 2008. Effects of plant growth regulators on growth and carbohydrate accumulation in shoots and roots of two almond rootstock seedlings. *Fruits*, **63**: 363–370.

Owens JN, Pharis RP. 1972. Initiation and development of western red cedar cones in response to gibberellin induction, and under natural conditions. *Canadian Journal of Botany*, **49**: 1165–1175.

Owens JN. 1985. Interaction between gibberellin $A_{4/7}$ and root-pruning on the reproductive and vegetative processes in Douglas-fir. III. Effects on anatomy of shoot elongation and terminal bud development. *Canadian Journal of Forest Research*, **15**: 354–364.

Philipson JJ. 1985. The promotion of flowering in large field-grown Sitka spruce by girdling and stem injections of gibberellin $A_{4/7}$. *Canadian Journal of Forest Research*, **15**: 166–170.

Pijut PM. 2002. Eastern white pine flowering in response to spray application of gibberellin $A_{4,7}$ or procone^{TM}. *Northern Journal of Applied Forestry*, **19**: 68–72.

Ross SD, Pharis RP. 1976. Promotion of flowering in the Pinaceae by gibberellins. I. Sexually mature non-flowering grafts of Douglas-fir. *Physiologia Plantarum*, **36**: 182–186.

Sheng C, Wang S. 1990. Effect of applied growth regulators and cultural treatments on flowering and shoot growth of *Pinus tabuliformis*. *Canadian Journal of Forest Research*, **20**: 679–685.

Sliman ZT, Ghandorah MO. 1992. Response of two wheat cultivars to chlormequat (CCC) application. *Journal King Saud University Agricultural Science*, **4**: 57–65.

Smith R, Greenwood M. 1995. Effects of gibberellin $A_{4/7}$, root pruning and cytokinins on seed and pollen cone production in black spruce (*Picea mariana*). *Tree Physiology*, **15**: 457–465.

Takahashi N, Phinney BO, MacMillan J. 1991. *Gibberellins*. New York: Springer-Verlag, p. 426.

Tompsett PB. 1977. Studies of growth and flowering in *Piceae sitchensis* (Bong) Carr. I. Effect of growth regulator application to mature scions on seedling rootstocks. *Annals of Botany*, **41**: 1171–1178.

Van der Waerden BL. 1952. Order tests for the two-sample problem and their power. *Indagationes Mathematicae*, **14**: 453–458.

Wakushima S. 2004. Promotion of female strobili flowering and seed production in two Japanese pine species by 6-benzylaminopurine (BAP) paste application in a field seed orchard. *Journal Plant Grow Regulation*, **23**: 135–145.

Wakushima S, Yoshioka H, Sakurai N. 1996. Lateral female strobili production in a Japanese red pine (*Pinus densiflora* Sieb. Et Zucc.) clone by exogenous cytokinin application. *Japanese Forestry Society*, **1**: 143–148.

Wakushima S, Yoshioka H, Sakurai N. 1997. Promotion of lateral female strobili production in *Pinus densiflora* by cytokinin application at a special stage. *Japanese Forestry Society*, **2**: 51–57.

Wilcoxon F. 1945. Individual comparisons by ranking methods. *Biometrics Bulletin*, **1**: 80–83.

Xu Hua-cheng. 1993. *Pinus tabuliformis*. Beijing: China Forestry Press, pp1–20. (In Chinese)

Zhang Hua-xin. 2000. Study of reproduction in a Chinese pine (*Pinus tabuliformis*) Seed Orchard. Beijing: Chinese Forestry Press, pp.35–60. (In Chinese)

Zhao Peng, Fan Jun-feng, Liu Yong-hong, Yang Pei-hua, Han Chuang-ju, Xie Jun-feng, Yu Xi-min, Yang Wen-xue. 2007. Analysis of the variation in female and male strobilus and cone production in clonal seed orchards of Chinese red pine (*Pinus tabuliformis Carr.*) *Journal of Anhui Agricultural Science*, **35**: 3847–3849, 3876. (In Chinese)