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Lateral Female Strobili Production in a Japanese Red Pine (*Pinus densiflora* Sieb. et Zucc.) Clone by Exogenous Cytokinin Application[1]

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Application of N^6-benzylaminopurine (BAP) and gibberellin (GA_3) to a grafted Japanese red pine (*Pinus densiflora* Sieb. et Zucc.) clone promoted flowering. BAP (250 mg/*l*) was sprayed five times to the top of branches in July or September 1992. GA_3 (100 or 500 mg/*l*) was applied five times to the whole plants by spray treatment during the above period, or GA_3 solution of the same concentration was injected once into a small incision made in the main stem of clone trees at the beginning of July or the middle of September 1992. In May 1993, no apical female strobili were observed at the top of the new shoots in any treatment. However, lateral female and bisexual strobili were produced in the lower part of new shoots by BAP application in September, regardless of GA_3 application. BAP applied in September without GA_3 application was sufficient to produce lateral female and bisexual strobili. In such treatment, production of female and bisexual strobili was 47 and 25, and they occurred in 16 out of 68 new shoots, 11 out of 15 treated branches and 5 out of 5 treated trees. The average number of female and bisexual strobili per new shoot was 1.06. BAP treatment either in July or in September significantly decreased ($p < 0.05$) the production of male strobili. These results indicate that BAP application induces the development of lateral female strobili in Japanese red pine when applied during the period of floral differentiation.

Key words: benzylaminopurine, flowering, gibberellin, lateral female strobili, *Pinus densiflora*.

Many attempts have been made to promote flowering in conifers with applications of plant growth regulators, since 1950's. Successful initiation of strobilus by gibberellin (GA) application has been shown in numerous conifer species of *Cupressaceae* and *Taxodiaceae* by many workers (Bonnet-Masimbert,1987; Hashizume, 1959, 1960, 1966, 1968; Kato *et al.*, 1958, 1959; Pharis and Kuo, 1973, 1977; Pharis *et al.*, 1987; Shidei *et al.*, 1959, 1960). GA_3 application increases the number of strobili and leads to high yields of seeds in breeding programs of *Cryptomeria japonica* and *Chamaecyparis obtusa* (Hashizume, 1973; Kanekawa, 1984; Katsuta, 1977).

Several GAs have been used to promote flowering in members of the *Pinaceae*, cf. Pharis and Kuo (1977), Pharis *et al.* (1987) and Bonnet-Masimbert (1987), showed that foliar-spray application or stem injection of less polar GAs, such as GA_4 or $GA_{4/7}$, was effective in enhancing strobili production.

Spray application of GA_3 to Japanese red pine (*Pinus densiflora* Sieb. et Zucc.) was reported to have only a slight and occasional promotive effect on strobilus production (Hashizume, 1966, 1968; Katsuta, 1977; Kawamura *et al.*, 1978). Recently, it has been reported that application of GA_3, GA_4 or $GA_{4/7}$ either as a carboxymethyl cellulose paste or by direct injection of an 80% acetone solution is more effective in promoting strobilus production than spray application (Hashizume, 1985; Kanekawa and Katsuta, 1982; Kanekawa, 1984). However, the total number of female strobili produced was comparatively low, about 0.1 to 0.4 per new shoot. Effectiveness of these GAs on the production of female strobili depended on the individuals as well as the age of the red pine clones used; some individuals treated with GA did not produce any female strobili (Hashizume, 1985). A stable and effective application method of plant growth regulators is needed to stimulate more female strobili and thus high seed yields in red pine.

In nature, a few female strobili are produced by Japanese red pine trees at the top of their shoot apicies. Many lateral male strobili develop on the lower part of the shoot. When the terminal bud or shoot of red pine is injured, lateral female strobili as well as bisexual strobili that have both female and male organs in each strobilus, are occasionally observed at the lower part of the shoot (Fujii, 1895; Matsuda, 1892). Saito (1957) tried to control sex differentiation in Japanese red pine and in black pine (*Pinus thunbergii*) by artificial methods such as auxin sprays. He succeeded in inducing lateral female strobili on the shoots of red and black pine by spray applications of NAA (α-naphthaleneacetic acid, 10 mg/*l*), but the lateral female strobili so produced were confined to only one shoot of a limited number of trees. Saito also tried physical methods such as pinching the top 1/3 of the shoot. He succeeded frequently in inducing development of strobili to female and bisexual forms. He concluded that pinching was more effective than hormone application. Since then, control of sex differentiation in Japanese red pine by hormone applications has been a neglected field. $GA_{4/7}$ injection with aqueous ethanol to *Pinus caribaea* induced the interspersed production of bisexual strobili (hermaphroditic cone) amongst male strobili (pollen cone) in some clones (Harrison and Slee, 1991); bisexual strobili occurred in 33 to 50% of GA-treated buds.

Not only auxin and GAs, but also cytokinin is seemingly involved in sex conversion and floral development in several plant species. In *Vitis vinifera*, the male organ was converted

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to the female one by cytokinin application (Negi and Olmo, 1972). Also cytokinin application caused conversion of the male flower to the female in *Luffa acutangula* (Bose and Nitch, 1970) and *Mercurialis annua* (Kahlem *et al.*, 1975). Cytokinin was also tried on conifer plants to promote the differentiation of strobili (Bonnet-Masimbert, 1987). These experiments, however, did not aim to control sex conversion. Therefore there are no reports of consistent sex conversion in pine trees following cytokinin treatment. BAP application in combination with GAs was able to enhance slightly GA-induced flowering in *Pseudotsuga menziesii* (Ross and Pharis, 1976) and in *Picea sitchensis* (Tompsett, 1977). Also Imbault *et al.* (1988) reported a successful enhancement of female flowering with exogenous application of isopentenyladenine in *Pseudotsuga menziesii*. Conversely, BAP injection significantly decreased both male and female strobili production in *Pinus tabulaeformis* (Sheng and Wang, 1989). In Japanese red pine, promotion of flowering has not been observed with BAP application (Odani, 1977; Wakushima *et al.*, 1992; Wakushima and Yoshioka, 1993). These conflicting results indicate that the role of cytokinins in the flowering of conifers is still unclear.

In the present study, performed with a grafted clone of Japanese red pine, we applied BAP and GA_3 in various combinations at different seasonal times corresponding to the period of flower differentiation (July or September). The results were then interpreted to clarify the role of BAP and GA_3 in the differentiation and sex determination of strobili.

Materials and Methods

All experiments were carried out at the Hiroshima Prefectural Forestry Experiment Station, Miyoshi, Hiroshima, Japan (34°47′N, 132°51′E). Forty-eight trees of a Japanese red pine clone (named Miyajima 54), which were grafted to two-year-old, seed-propagated *P. thunbergii* in 1988, were grown for three years in the field and then transferred to the green house in 1991. At the time of hormonal treatment in 1992, the heights of these grafted clone trees ranged from 1.5 to 2 m. Since frequent watering of the whole crown of the trees weakened the effect of BAP spray treatments (Wakushima *et al.*, 1992), water at weekly intervals was supplied only to the base of the trees to avoid dilution and run-off of the applied hormones. Fifteen or sixteen branches were randomly selected across four or five different trees for each treatment (Table 1).

One gram of BAP (N^6-benzylaminopurine, Wako Pure Chemical Industries, Ltd., Tokyo, Japan) was dissolved in 15 ml of 1.0 M KOH. GA_3 (Gibberellin A_3, Wako Pure Chemical Industries, Ltd.) was dissolved in 70% ethanol. Stock concentrations of BAP and GA_3 were adjusted to 1,000 mg/*l* with distilled water and stored at 3°C in the dark. The concentration of the BAP applied was 250 mg/*l*. Two concentrations of GA_3 (100 and 500 mg/*l*) were used. A surfactant (Tween 20, Wako Pure Chemical Industries, Ltd.) was added at the final concentration of 0.1% (v/v) to the solution before treatment.

A 3.0 ml volume of BAP solution (250 mg/*l*) was applied five times, at weekly intervals, to terminal buds of the selected branches using a handy sprayer (Hisprayer, Canyon Co., Ltd., Tokyo, Japan). Also 40 ml of GA_3 solution (100 and 500 mg/*l*) was sprayed five times, at weekly intervals, to the whole crown of the trees. The dates of spray treatments were July 1, 7, 14, 21 and 31 (July treatment) or Sept. 7, 19, 28, Oct. 5 and 14 (September treatment), 1992. A 0.2 ml volume of GA_3 solution (100 or 500 mg/*l*) was injected into a small piece of absorbent cotton that was inserted into a small incision (2 × 2 cm) of the main stem. The incision was then sealed with "Parafilm" (American National Can, Greenwich, CT, USA) and adhesive tape. The injection treatment was carried out on July 1 (July treatment) or Sept. 7 (September treatment), 1992. Control branches were without hormone treatment. The number of new shoots and strobili that emerged from the treated branches were counted in early May 1993, when the elongation of new shoots was completed and flowers were in bloom.

Results

1 Number of new shoots

The number of new shoots was significantly ($p < 0.05$)

Table 1 Number of trees, branches and new shoots used for hormonal treatments.

Treatment		July			September		
		Number of			Number of		
BAP	GA_3(mg/*l*)	Trees	Branches	New shoots	Trees	Branches	New shoots
–	–	13	39	173	–	–	–
–	100(spray)	5	15	59	5	15	59
–	500(spray)	5	15 (14)	51	5	15	52
–	100(injection)	4	16	57	4	16	60
–	500(injection)	4	16	59	4	16	59
+	–	5	15	189	5	15	68
+	100(spray)	5	15 (14)	132	5	15	66
+	500(spray)	5	15 (6)	32	5	15	67
+	100(injection)	4	16	292	4	16	86
+	500(injection)	4	16	242	4	16	67

Figures in parentheses are numbers of live branches after treatment. The apical parts of the branches were sprayed with BAP (250 mg/*l*) solution.

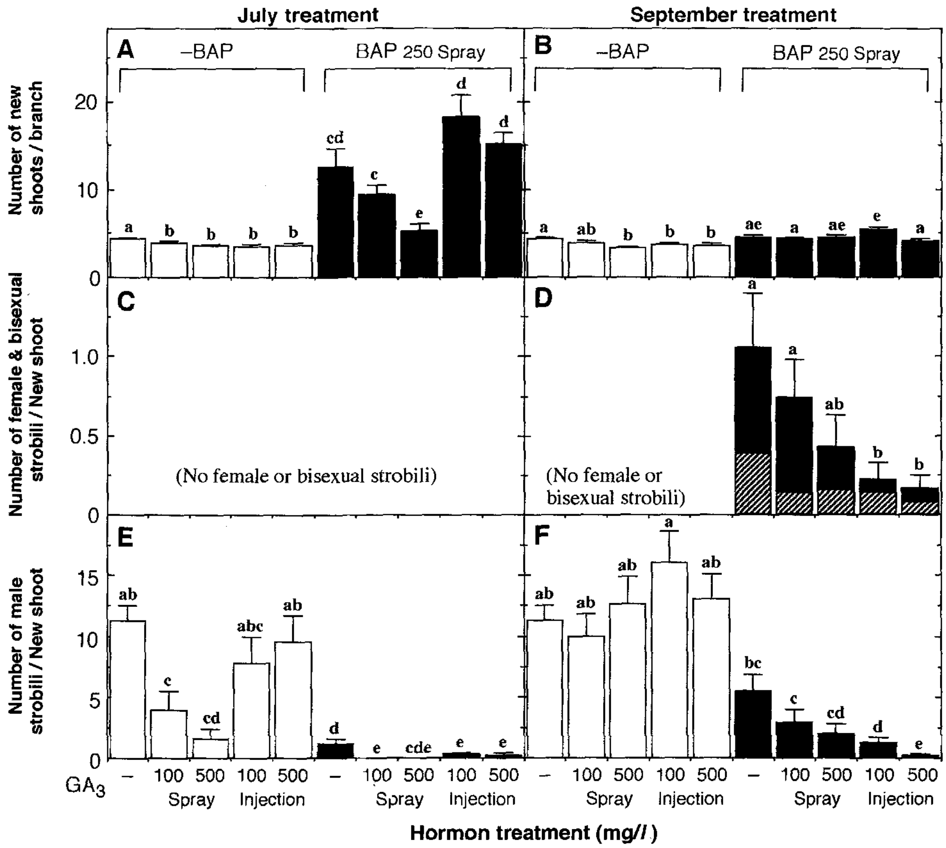

Fig. 1 A and B: Number of new shoots per treated branch. Means of shoot number per treated branch are shown by open (without BAP treatment) and solid (with BAP treatment) columns. Vertical bars indicate the SE of the mean. Different characters (a, b, c, d, e) indicate differences significant at the 5%. C and D: Number of lateral female and bisexual strobili per new shoot. Means of lateral female and bisexual strobili per new shoot are shown by solid (female) and slashed (bisexual) columns, respectively. Vertical bars indicate the SE of the mean. Different characters (a, b) indicate differences significant at the 1%. Lateral female and bisexual strobili were observed only at the September BAP treatment. E and F: Number of male strobili per new shoot. Means of number of male strobili per new shoot are shown by open (without BAP treatment) and solid (with BAP treatment) columns. Vertical bars indicate the SE of the mean. Different characters (a, b, c, d, e) indicate differences significant at the 5%. No male strobili were observed for the July BAP treatment with GA_3 (100 and 500 mg/*l*) spray treatment.

increased by BAP treatments in July (Fig. 1, A). In many members of the *Pinaceae*, exogenous application of cytokinin releases the lateral apex enclosed in needle leaves, from dormancy (Cohen and Shanks, 1975; Stiff and Boe, 1985; Stiff *et al.*, 1989; Wakushima *et al.*, 1992, Wakushima and Yoshioka, 1993). Although the number of new shoots of trees treated with BAP in September was almost similar to that of the control (Fig. 1 B), growth of needle leaves on the lower part of the new shoots was sometimes inhibited by BAP (data not shown). These shoots showed a "cow tailed" shape, since the growth of the needle leaves was restricted to the top of shoots. Leaf primordia in the lower part of the shoots treated with BAP formed small axillary buds, but these did not grow further. Spray or injection of GA_3 alone in both seasons slightly decreased the number of new shoots (Fig. 1 A).

2 Strobili production

Under the natural condition, a few female strobili of Japanese red pine, if any, appear on the top of shoot axes, but under the present experimental conditions, no apical female strobilus was observed in any of the treatments. This is possibly due to the characteristics of the Miyajima 54 clone that usually yields a few seed cones in a seed orchard. BAP application in September induced lateral female strobili at the lower part of new shoots (Fig. 2 A and B). Some lateral bisexual strobili, which had both female (upper part) and male (lower part) organs in each strobilus, were also observed at the lower position (Fig. 2 C). Maximum production of the lateral female and bisexual strobili was observed by BAP treatment in September without GA_3 (Fig. 1 D). Sixty-eight new shoots developed from 15 treated branches on five trees. Forty-seven female and 25 bisexual strobili were induced by BAP on 16 new shoots of 11 treated branches of all five trees (Table 2). The maximum number of female and bisexual strobili per new shoot was 1.06 only by BAP in September (Fig. 1 D). GA_3 rather seems to inhibit the production of female and bisexual strobili. BAP had no effect on strobili production when applied in July (Fig. 1 C).

The ratio of female to male form in each bisexual strobilus was not consistent. Bisexual strobili with high proportions of male form naturally dried out and fell from the shoots as do the normal male strobili. Conversely, bisexual strobili with high ratios of female form remained on the shoots and developed into seed cones as do female strobili (Fig. 2 D).

BAP application in both July and September and spray

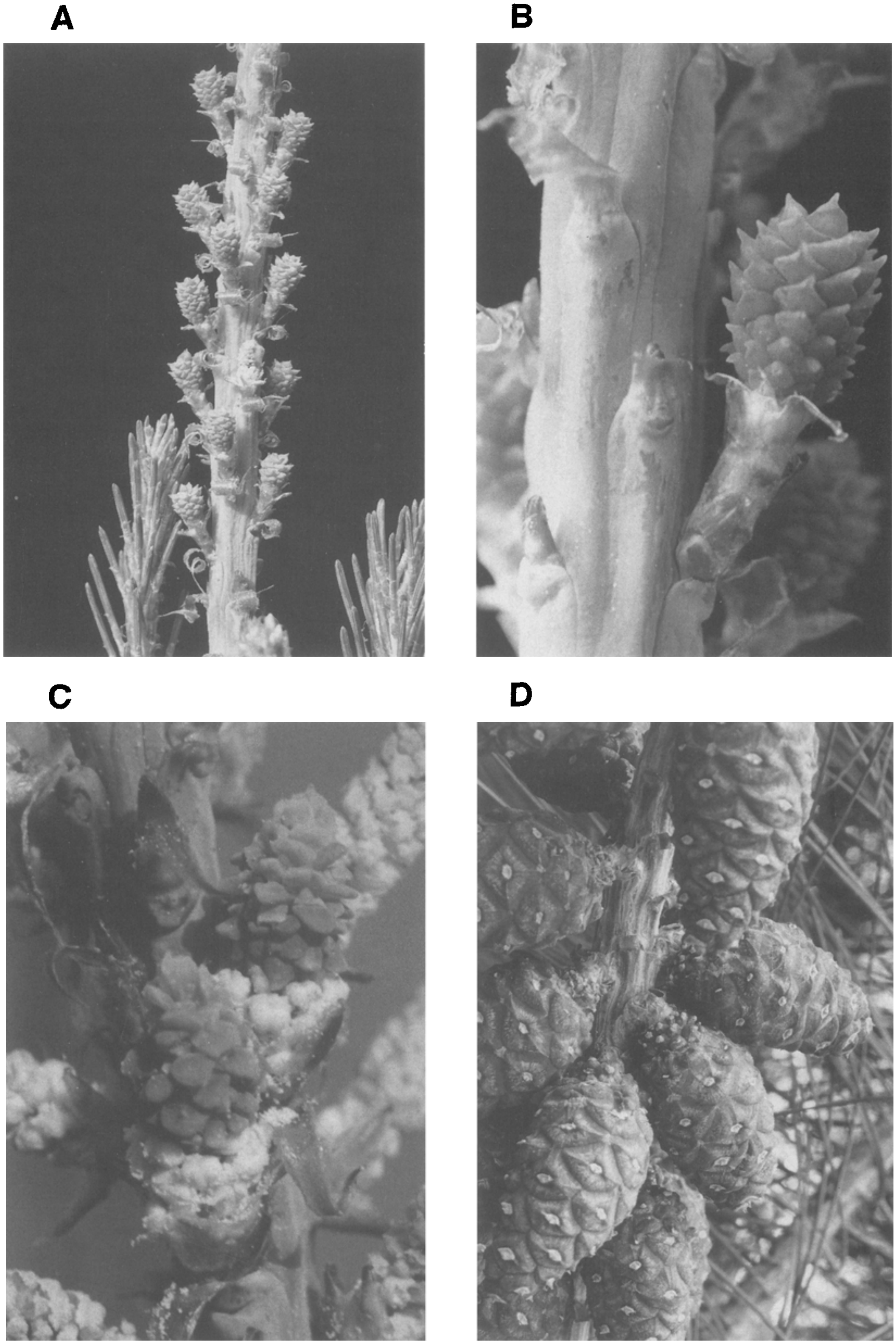

Fig. 2 A and B: Lateral female strobili occurred at the lower part of new shoot (photographed at middle May, 1993). BAP was applied to the top of the branch in September, 1992 and new shoots elongated from treated branches in April, 1993. C: Lateral bisexual strobili occurred with female strobili at the lower part of new shoot (photographed at middle May, 1993). These strobili had both female (upper part) and male (lower part) characteristics in each strobilus. D: Seed cones developed from the lateral female and bisexual strobili. Traces of male organs were observed at the base of seed cones (photographed at middle August, 1994).

Table 2 Number of female and bisexual strobili and of trees, branches and shoots with female or bisexual strobili.

Treatment		Production of strobili		Occurence of female and bisexual strobili		
BAP	GA_3 (mg/*l*)	Female	Bisexual	Trees	Branches	Shoots
−	−	0	0	0/13 (0)	0/39 (0)	0/173 (0)
+	−	47	25	5/5 (100)	11/15 (73)	16/68 (24)
+	100 (spray)	40	9	5/5 (100)	10/15 (67)	14/66 (21)
+	500 (spray)	18	11	4/5 (80)	8/15 (53)	9/67 (13)
+	100 (injection)	7	12	3/4 (75)	4/16 (25)	5/86 (6)
+	500 (injection)	7	4	3/4 (75)	4/16 (25)	5/67 (7)

Figures in parentheses are percentages. BAP was applied to the tops of branches at 250 mg/*l* in September.

application of GA_3 in July inhibited the development of male strobili (Fig. 1 E and F).

Discussion

According to Hashizume (1973), in Japanese red pine in Tottori Prefecture, which is near the Hiroshima Prefecture, differentiation of floral primordia of male strobili (lateral strobili) continues from early July to early September; then, floral differentiation of both the male and the female strobili continues from the middle of September to mid-October. In our experiments, the period of BAP treatment in July overlapped with the period of differentiation of floral primordia; this treatment did not show any promotive effects on strobili production, but rather decreased the number of male strobili (Fig. 1 E). In *Pinus tabulaeformis*, Sheng and Wang (1989) obtained a similar result wherein biweekly BAP injections during the growing period from May to June inhibited male and female flowering. The period of BAP treatment in September overlapped with the period of the differentiation of strobili; such treatment induced the development of lateral female strobili. These results suggest that exogenous cytokinin application stimulates female strobili development in Japanese red pine only when applied during the period of flower differentiation.

In our experiments, BAP application was more effective than GA application in promoting female flowering of red pine. Kanekawa and Katsuta (1982) and Hashizume (1985) reported that GA application promoted the production of apical strobili in Japanese red pine. Their procedure enhanced the production of female strobili 2−6 fold (Kanekawa and Katsuta, 1982) or 2−10 fold (Hashizume, 1985) as compared with control trees. However, the total number of female strobili was comparatively low, on average only 0.1 to 0.4 strobili per new shoot under the optimum conditions. On the other hand, our experiments indicated that BAP spray application in September induced the development of lateral female strobili, and 1.06 female and bisexual strobili per new shoot were induced under the optimal conditions (Fig. 1 D). This value is 3−10 times that of the GA application method of Kanekawa and Katsuta (1982) and Hashizume (1985).

With other species of *Pinaceae* such as *Pinus banksiana* (Cecich, 1981, 1983), *P. sylvestris* (Chalupka, 1984, 1987), *P. elliottii, P. palustris* (Hare, 1984), *P. radiata* (Ross *et al.*, 1984), *P. taeda* (Greenwood, 1982; Ross and Greenwood, 1979) and *P. caribaea* (Harrison and Slee, 1991), GA_4 or $GA_{4/7}$ application showed positive effects on strobili formation. These GA application methods cannot be compared directly with the present BAP application method, since the experiments differed in the tree species used, their size and age. Earlier GA application methods showed only low efficiency in the production of female strobili; the number of female strobili per new shoot generally did not exceed 1.0. The present BAP application method is equal to or more effective in promoting the formation of female strobili than GA application.

Hashizume (1985) has described that GA application was less effective in young than in aged, over five or six years, seedlings and graftings. Also according to Ross and Greenwood (1979), "certain degree of sexual maturity" is needed before *Pseudotsuga menziesii* seedlings will respond to GA treatment. BAP induced lateral female strobili even in four-year-old seedlings of Japanese red pine (unpublished data), suggesting that the effect is independent of age.

GA_3 spray or injection did not show any positive effect on the induction of either male or female strobili (Fig. 1 C−F). In contrast, negative effects, such as early leaf fall, death of branches, and even death of new shoots, were observed in some treatments, especially in the July GA_3 (500 mg/*l*) spray treatment. These results suggest that spray treatment of red pine in July with high concentrations of GA_3 is inadvisable. On the other hand, spray treatment with BAP caused no evident damage.

Considerable damage has been caused to pine stands of Japan recently by a pine wood nematode (*Bursaphelenchus xylophilus*), with losses in tree volume of more than one million m^3 per year (Fujimoto *et al.*, 1989). Trees of red and black pine resistant to this nematode, including the Miyajima 54 clone, have been selected from pine forests in order to propagate them for the reforestation of damaged areas. The method we report here to induce lateral female strobili by cytokinin, has a great potential to obtain high yields of seeds and seedlings. Further work is in progress to confirm the fertility of the seeds obtained from induced lateral female strobili, and to optimize conditions of BAP application with regard to environmental factors such as season, and hormonal factors like the duration, frequency and concentration, in order to develop a practical method for application in seed orchards.

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Literature cited

Bonnet-Masimbert, M. (1987) Floral induction in conifers: a review of available techniques. For. Ecol. Manage. 19:135-146.

Bose, T.K. and Nitch, J.P. (1970) Chemical alteration of sex expression in *Luffa acutangula*. Physiol. Plant. 23:1206-1211.

Cecich, R.A. (1981) Applied gibberellin $A_{4/7}$ increases ovulate strobili production in accelerated growth Jack pine seedlings. Can. J. For. Res. 11: 580-585.

Cecich, R.A. (1983) Flowering in a Jack pine seedling seed orchard increased by spraying with gibberellin $A_{4/7}$. Can. J. For. Res. 13: 1056-1062.

Chalupka, W. (1984) Time of $GA_{4/7}$ application may affect the sex of Scots pine flowers initiated. Silvae Genet. 33: 4-5.

Chalupka, W. (1987) Stimulation of flowering in Scots pine (*Pinus sylvestris*) grafts by gibberellin injection. For. Ecol. Manage. 19: 177-181.

Cohen, M.A. and Shanks, J. (1975) Effect of N^6-BA, GA_3 and removal of terminal buds on dwarf shoot development in *Pinus ponderosa*. J. Am. Soc. Hortic. Sci. 100: 404-406.

Fujii, K. (1895) Physiological researches on the sexuality of the flowers of *Pinus desiflora* Sieb. et Zucc. Bot. Mag. Tokyo 9: 271-275.

Fujimoto, Y., Toda, T., Nishimura, K., Yamate, H., and Fuyuno, S. (1989) Breeding project on resistance to the pine-wood nematode — An outline of the research and the achievement of the project for ten years —. Bull. For. Tree Breed. Inst. Jpn. 7:1-84. (in Japanese with English summary)

Greenwood, M.S. (1982) Rate, timing, and mode of gibberellin application for female strobilus production by grafted loblolly pine. Can. J. For. Res. 12: 998-1002.

Hare, R.C. (1984) Application method and timing of gibberellin $A_{4/7}$ treatments for increasing pollen conebud production in southern pines. Can. J. For. Res. 14: 128-131.

Harrison, D. L. S. and Slee, M. U. (1991) Gibberellin $A_{4/7}$ enhanced flowering in *Pinus caribaea* var. *hondurensis*. Can. J. For. Res. 21: 788-793.

Hashizume, H. (1959) The effect of gibberellin upon flower formation in *Cryptomeria japonica*. J. Jpn. For. Soc. 41:375-381. (in Japanese with English summary)

Hashizume, H. (1960) The effect of gibberellin upon sex differentiation in *Cryptomeria japonica* strobilus. J. Jpn. For. Soc. 42:176-180. (in Japanese with English summary)

Hashizume, H. (1966) Chemical regulation of flower-bud formation and flower-sex differentiation in conifers. Chem. Regul. Plants 1:148-156. (in Japanese)

Hashizume, H. (1968) Chemical regulation of flower-bud formation in conifers. J. Jpn. For. Soc. 50:14-16. (in Japanese)

Hashizume, H. (1973) Studies on flower bud formation, flower sex differentiation and their control in conifers. Bull. Tottori Univ. For. 7:1-139. (in Japanese with English summary)

Hashizume, H. (1985) Effect of gibberellins on the promotion of flowering in *Pinaceae* species. Bull. Fac. Agric., Tottori Univ. 37: 80-87. (in Japanese with English summary)

Imbault, N., Tardieu, I., Joseph, C., Zearr, J.B., and Bonnet-Massimbert, M. (1988) Possible role of isopentenyladenine and isopentenyladenosine in flowering of *Pseudotsuga menziesii*: endogenous variations and exogenous applications. Plant Physiol. Biochem. 26: 289-295.

Kahlem, G., Champault, A., Louis, J. P., Bazin, M., Chabin, A., Delaigue, M., Dauphin, B., Durand, R., and Durand, B. (1975) Détermination génétique et régulation hormonale de la differénciation sexulle chez *Mercurialis annua* L. Physiol. Vég. 13: 763-779.

Kanekawa, T. (1984) Promotion of strobilus production in *Cryptomeria japonica, Chamaecyparis obtusa, Pinus thunbergii* and *P. densiflora* seed orchard by gibberellins. Bull. Ibaraki Pref. For. Exp. Stat. 14: 3-30. (in Japanese with English summary)

Kanekawa, T. and Katsuta, M. (1982) Promotion of strobilus production in *Pinus thunbergii* Parl. and *P. densiflora* Sieb. et Zucc. by gibberellin. J. Jpn. For. Soc. 64:101-106.

Kato, Y., Miyake, I., and Ishikawa, H. (1958) Stimulation of differentiation of flower buds in Sugi (*Cryptomeria japonica*) by gibberellin. J. Jpn. For. Soc. 40: 35-36. (in Japanese)

Kato, Y., Fukuhara, N., and Kobayashi, R. (1959) Stimulation of differentiation of flower bud in conifer by gibberellin (I). J. Jpn. For. Soc. 41: 309-311. (in Japanese)

Katsuta, M. (1977) Promotion of flowering in the *Pinaceae* by gibberellins. Rinbokunoikusyu 105:1-4. (in Japanese)

Kawamura, K., Uetsuki, Y., and Ida, S. (1978) Promotion of flowering in pines by gibberellins. Ann. Rep. Kansai For. Tree Breed. Inst. 14: 39-42. (in Japanese)

Matsuda, S. (1892) Bisexual cone of *Pinus densiflora*. Bot. Mag. Tokyo 6: 238-239. (in Japanese)

Negi, S. S. and Olmo, H. P. (1972) Certain embryological and biochemical aspects of cytokinin SD 8339 in converting sex of a male *Vitis vinifera* (sylvestris). Am. J. Bot. 59: 851-857.

Odani, K. (1977) Lammas shoot growth of *Pinus densiflora* Sieb. et Zucc. mediated by the exogenous cytokinin. J. Jpn. For. Soc. 59: 22-23.

Pharis, R.P. and Kuo, G.C. (1973) Physiology of gibberellins in conifers. Chem. Regul. Plants 8: 63-71.

Pharis, R.P. and Kuo, G.C. (1977) Physiology of gibberellins in conifers. Can. J. For. Res. 7: 299-325

Pharis, R.P., Webber, J.E., and Ross, S.D. (1987) The promotion of flowering in forest trees by Gibberellin $A_{4/7}$ and cultural treatments: A review of the possible mechanisms. For. Ecol. Manage. 19: 65-84.

Ross, S.D. and Pharis, R.P. (1976) Promotion of flowering in the Pinaceae by gibberellins. I. Sexually mature, non-flowering grafts of Douglas-fir. Physiol. Plant. 36: 182-186.

Ross, S.D. and Greenwood, M.S. (1979) Promotion of flowering in the Pinaceae by gibberellins. II. Grafts of sexually mature and immature *Pinus taeda*. Physiol. Plant. 45: 207-210.

Ross, S.D., Bollman, M.P., Pharis, R.P., and Sweet, G.B. (1984) Gibberellin $A_{4/7}$ and the promotion of flowering in *Pinus radiata*: Effects on partitioning of photoassimilate within the bud during primordia differentiation. Plant Physiol. 76: 326-330.

Saito, Y. (1957) Artificial control of sex differentiation in Japanese red pine and black pine strobilus. J. Fac. Agric., Tottori Univ. 3: 1-29.

Sheng, C. and Wang, S. (1989) Effect of applied growth regulators and cultural treatments on flowering and shoot growth of *Pinus tabulaeformis*. Can. J. For. Res. 20: 679-685.

Shidei, T., Akai, T., and Ichikawa, S. (1959) Flower buds formation on sugi (*Cryptomeria japonica*) and metasequoia (*M. glyptostroboides*) by gibberellic acid treatment (I). J. Jpn. For. Soc. 41: 312-315. (in Japanese)

Shidei, T., Ichikawa, S., and Kihira, Y. (1960) Flower-buds formation in sugi (*Cryptomeria japonica*) and metasequoia (*M. glyptostroboides*) by gibberellic acid treatment (II). J. Jpn. For. Soc. 42: 363-368. (in Japanese)

Stiff, C.M. and Boe, A.A. (1985) Effects of foliar-applied benzylaminopurine on fascicular bud development in Mugo pine. Hortscience 20 (2): 285-287.

Stiff, C.M., Wenny, D.L., Dumroese, R. K., Roberts, L.W., and Stiff, C. T. (1989) Establishment of western white pine shoots *in vitro* using needle fascicles. Can. J. For. Res. 19: 1330-1333.

Tompsett, P. B. (1977) Studies of growth and flowering in *Picea sitchensis* (Bong.) Carr. I. Effect of growth regulator applications to mature scions on seedling rootstocks. Ann. Bot. 41: 1171-1178.

Wakushima, S. and Yoshioka, Y. (1993) Propagation of *Pinus densiflora* (Japanese red pine) clone resistant to pinewood nematode. — Effects of BAP application frequency and period to the number of shoot induction and form of shoots —. Bull. Hiroshima Pref. For. Exp. Stat. 27: 95-100. (in Japanese with English summary)

Wakushima, S., Yoshioka, H., and Masuhara, K. (1992) Propagation of *Pinus densiflora* (Japanese red pine) clone resistant to pinewood nematode. — Induction of shoot development with BAP application —. Bull. Hiroshima Pref. For. Exp. Stat. 26: 15-22. (in Japanese with English summary)

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