PROPAGATION AND REGULATION OF PHASE CHANGE IN SOME NEW ZEALAND HETEROBLASTIC SPECIES

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Abstract. Various heteroblastic species endemic to New Zealand were investigated. Seeds of most species germinated in four to five months when they had been overwintered outside, although some required scarification and others failed to germinate even after two periods of overwintering. The best rooting of cuttings was obtained from material collected in summer and placed under intermittent mist. Juvenile cuttings rooted much better than cuttings from adult plants.

When gibberellic acid (0.4 mg per plant) was applied to rooted cuttings of adult plants it produced a transient elongation of stems of all species investigated and induced a juvenile-like habit and leaf form in *Carpodetus serratus* and *Pennantia corymbosa*, a juvenile growth form with transitional leaf-form in *Parsonsia heterophylla*, but no such changes in *Elaeocarpus hookerianus*, whereas treated adult rooted cuttings of *Pseudopanax crassifolius* died Attempts to manipulate the internal gibberellin levels of juvenile rooted cuttings by the application of gibberellic acid (GA_3) and paclobutrazol (PP333) failed to induce any adult characteristics

INTRODUCTION

The occurrence of distinct juvenile and adult stages in the life cycle of many woody plants is one of the most distinctive features of the New Zealand flora. The phenomenon has been recognised for many years and has been referred to as "heteroblasty", "phase change", or "maturation". Phase change in New Zealand species is frequently observed as a change in both form and leaf shape (11). The "classic" example of heteroblasty is the common ivy, *Hedera helix*, while the phenomenon in the New Zealand species is exemplified by *Pseudopanax crassifolius* (10).

Research on phase change has a practical application as plant propagators frequently are interested in prolonging the juvenile phase or rejuvenating mature tissue in order to obtain a supply of material that is easy to root or micropropagate, whereas plant breeders may wish to hasten the onset of the adult phase to obtain early flowering and seed set.

Although little is known of the nature of the physiological and biochemical factors associated with maturation and phase change, the gibberellins promote rejuvenation in ivy and may be involved in the maintenance of the juvenile condition (12). However, attempts to promote maturation by reducing the effective endogenous gibberellin levels by use of gibberellin antagonists or inhibitors have failed to promote the mature form of ivy (2, 5).

This paper reports on germination and rooting procedures needed to produce seedlings and rooted cuttings of juvenile and mature forms, the effect of gibberellic acid (GA_3) on rejuvenation, and of paclobutrazol (PP333), a potent inhibitor of gibberellin biosynthesis (3) on maturation of several heteroblastic species endemic to New Zealand. Ivy was used as a model system and the details of experiments with ivy are reported elsewhere (4, 5).

MATERIALS AND METHODS

Asexual propagation. Cuttings for propagation were collected in the early morning and kept cool and moist. Approximately ⅓ to ½ of the foliage was removed from each cutting and in large-leaved plants the remaining leaves were reduced in size to minimize transpiration losses. The base of each cutting was dipped into a commercial rooting powder (Seredix No. 2) containing 0.3% B-indolebutyric acid. The cuttings were planted directly into either coarse river sand with a layer of peat at the bottom of the propagating box (when placed under an intermittent mist system (with or without heat), or in a peat:sand mix (1:3 v/v) (when placed under a cold frame or on a hot bed). Once rooted, cuttings were transferred into a 1:1:1 mixture of soil, peat, and sand, with added fertiliser, then potted into 500cm³ planta bags, and repotted into 2 litre planta bags prior to experimentation.

Sexual propagation. Both seeds and cuttings were collected from the same adult trees of *Carpodetus serratus* J.R. & G. Forst., *Elaeocarpus hookerianus* Raoul, *Pseudopanax ferox* Kirk, and *Pennantia corymbosa* J.R. & G. Forst., whereas seeds only were collected from other plants of *Pseudopanax crassifolius* (A. Cunn.) C. Koch, *P. simplex* (Forst. f.) Philipson and *Parsonsia heterophylla* A. Cunn.. All plants were located close to Dunedin, South Island. Seeds were sown either fresh or stratified. Seeds with very hard or impervious seed coats were chipped with a knife. The fleshy outer coat was removed from a portion of the *Pseudopanax* species' seeds.

Seeds were sown onto a modified John Innes seedling mix (4). The seed boxes were covered initially with wet newspaper, then glass, and left in a heated glasshouse (25 °C) for six weeks. Seed boxes containing ungerminated seeds were then placed outside for winter stratification (June to mid-August 1985), and then back into a glasshouse (25 °C) with a 16-hr photoperiod.

Germinated seedlings were ''pricked out'' into trays containing equal parts of soil, peat, and sand and maintained in the same glasshouse. Seedlings were potted out after six to eight weeks, initially into size ¾ planta bags and then six weeks prior to experimentation into size 3 planta bags using the same soil mixture used for pricking out. During repotting matted roots were loosened.

Boxes which still had ungerminated seeds were placed outside for a second winter.

Experimental plants were maintained in a glasshouse under a 16-hr photoperiod at 25 °C and investigated between July and December, 1986.

Application of plant growth regulators. Procedures are described by Horrell (4) and Horrell *et al.* (5, 6, 7). Briefly, rooted cuttings or seedlings were supplied with gibberellic acid (GA_3) *via* a wick threaded through the first internode above soil level (8). Each end of the wick was immersed in an Eppendorf tube supplying an aqueous solution of GA_3, which was applied weekly for four weeks as 1 ml aliquots containing 0.1 mg GA_3 per plant. Paclobutrazol (PP333) was applied directly on to the soil near the base of the plant as 5 ml of an aqueous suspension of "Cultar" (9), containing 1.25 mg PP333, daily for 20 days. In some instances GA_3 and PP333 were supplied simultaneously.

RESULTS

Germination. Seeds of most species germinated after four or five months and required a chilling period. However, seeds of *Elaeocarpus hookerianus* required scarification and two overwintering periods before any seeds germinated, whereas germination occurred within four weeks when the fleshy outer layer was removed from seeds of *Pseudopanax crassifolius*. In contrast, no germination occurred even after two overwintering periods when the flesh was removed from seeds of *P. ferox* and *P. simplex*. However, some germination occurred when seeds of *P. crassifolius* and *P. ferox* were sown with the flesh attached, but only after overwintering, whereas no *P. simplex* seeds germinated even after two overwintering periods. High germination occurred in seeds of *Carpodetus serratus* and *Pennantia corymbosa*, although seeds of *Pennantia* required scarification, whereas seeds of *Elaeocarpus hookerianus* and *Parsonsia heterophylla* showed only low germination.

Rooting of cuttings. Attempts were made to root cuttings of 12 heteroblastic species. Juvenile cuttings rooted much more readily than adult ones (Table 1). No juvenile or adult cuttings rooted in the cold frame, although this may be due to their collection in autumn. The best rooting occurred in cuttings collected in summer (January or February) and placed under intermittent mist (with or without heat). However, mature cuttings proved extremely difficult to root so that only five species were available for experimentation.

Table 1. Percentage of rooted cuttings of juvenile and adult forms of various heteroblastic New Zealand species (The propagation structure and month of propagation are given and the figures in the brackets indicate the total initial number of cuttings taken)[a]

	Growth phase	Percentage rooting		
		Hot bed	Intermittent Mist	
		(spring)	Heat (summer)	No heat (summer)
Carpodetus serratus	juvenile	—[b]	87 (100)[c]	90 (100)
	adult	—	0 (50)[c]	10 (70)
Pennantia corymbosa	juvenile	0 (50)	70 (50)	98 (200)
	adult	0 (50)	7 (70)	26 (100)
Parsonsia heterophylla	juvenile	70 (100)	—	98 (200)
	adult	8 (100)	—	32 (170)
Elaeocarpus hookerianus	adult	0 (70)	0 (100)	10 (100)
Pseudopanax crassifolius	adult	0 (100)	0 (100)	12 (100)
P ferox	adult	0 (100)	0 (100)	—
P simplex	adult	—	0 (200)	—
Weinmannia racemosa	adult	16 (50)	—	—
W silvicola	juvenile	—	—	—
	adult	0 (50)	—	—

[a] Additionally, cuttings from adult plants of *Plagianthus betulinus*, *Pseudopanax edgerleyi* and *Hoheria angustifolia*, as well as those listed in the table were taken in winter and placed in the cold frame No rooting occurred

[b] no cuttings propagated

[c] cuttings taken in spring.

Responses of adult plants to gibberellic acid. GA_3 promoted juvenile-like habits and leaf forms in *Carpodetus serratus* and *Pennantia corymbosa*. There was a statistically significant increase in internode angle in *Carpodetus serratus* which indicated reversion to the divaricate juvenile habit whilst plants of *Pennantia corymbosa* developed the reflexed laterals characteristic of the juvenile form. Plants of *Parsonsia heterophylla* produced a vine-like growth with a transitional type of foliage while *Elaeocarpus hookerianus* remained "adult" in appearance. *Pseudopanax crassifolius* exhibited a rapid but transient elongation, as did all the previously listed species, but then died. Further details are given in Horrell et al. (7).

Responses of juvenile plants to paclobutrazol and GA_3. PP333 caused almost complete inhibition of stem elongation in all five native species, although the application of GA_3 completely overrode this inhibition and often caused stimulation relative to the control (Table 2). For example, GA_3 alone or in combination with PP333, promoted elongation growth and caused hyponasty in *P. crassifolius*, whereas PP333 alone severely reduced elongation growth (6).

PP333 did not induce the formation of any mature characteristics in the New Zealand native species.

Table 2. Effects, relative to untreated controls, of PP333[a] and GA_3[b] on the extension of the main shoots of juvenile forms of various New Zealand heteroblastic species[c]

	Carpodetus serratus	*Elaeocarpus hookerianus*	*Parsonsia heterophylla*	*Pennantia corymbosa*	*Pseudopanax crassifolius*
PP333	depression	died	depression	depression	depression
GA_3	stimulation	stimulation	stimulation	stimulation	stimulation
GA_3 + PP333	no effect	died	stimulation	stimulation	stimulation

[a] PP333 Paclobutrazol was applied directly to the soil surface as 5 ml of an aqueous suspension of "Cultar" (9) containing 1 25 mg PP333, daily for 20 days

[b] GA_3 was applied weekly for four weeks as 1 ml aliquots containing 0 1 mg GA_3 per plant

[c] Except for *Parsonsia heterophylla*, where there were insufficient surviving plants for statistical analysis, all differences are significantly different from the controls ($P < 0\ 05$)

DISCUSSION

The application of GA_3 to adult native plants caused some reversion to juvenility, particularly in *Carpodetus serratus*, *Pennantia corymbosa*, and *Parsonsia heterophylla* (7). There are other unpublished reports of partial reversion following the application of GA_3 for *Elaeocarpus hookerianus* (13) and *Parsonsia heterophylla* (E Beuzenberg, personal communication). Our own studies of ivy have also reconfirmed that at least partial rejuvenation can be induced in adult ivy plants by the application of gibberellic acid (GA_3) (4).

However, no mature characteristics were induced by attempts to reduce levels of endogenous gibberellins. The fact that Cultar is a mixture of two enantiomers of PP333 (14), (ICI personal communication 1988), one of which is an inhibitor of sterol biosynthesis in plants (1), might account for the severe inhibition

that occurred in treated native plants, although GA_3 completely overcame the inhibitory effect of PP333 in *Pseudopanax crassifolius*, *Parsonsia heterophylla* and *Pennantia corymbosa*, suggesting that the predominant effect of PP333 had been to reduce the endogenous levels of gibberellin (6, 7) (Table 2).

In conclusion it seems likely that gibberellic acid could be supplied to mature tissues of at least some New Zealand native plants to promote juvenile-like growth for use in propagation. Promotion of novel forms such as the hyponastic response of the juvenile *P. crassifolius* following GA_3 application (6) may also have commercial relevance. However, any reduction in endogenous gibberellins caused by the inhibition of their biosynthesis by PP333 appeared unable to promote maturation, at least at the concentrations of PP333 used in our experiments.

Acknowledgements. We are pleased to acknowledge that this study was funded by the Botany Division of the Department of Scientific and Industrial Research. We are grateful to ICI New Zealand for the supply of paclobutrazol (as "Cultar"), and to Newman's Nursery, Gore, and to Dunedin Botanical Gardens for the use of their propagating equipment

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