Seed Germination of Endangered South Australian Plants

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INTRODUCTION

As part of ongoing studies on the conservation biology of endangered plants of South Australia, experiments were conducted to establish protocols for their effective propagation. The species studied were all endemic to South Australia and were classified as nationally endangered (Briggs and Leigh, 1988).

This paper describes experiments on propagation of five endangered species from seed.

MATERIALS AND METHODS

Seeds were collected from known wild provences and stored dry at 15C until required. Seeds were generally less than 1 year old at the time of testing.

Seed for germination testing was routinely spread on filter paper overlying dry vermiculite in a petri dish, each component having been previously sterilised by autoclaving. Unless otherwise stated, seed was irrigated with distilled water, incubated at 20C in the dark, and each treatment (20 seed/rep) was replicated three times. Germinations were recorded daily.

Moist heat was applied by immersing seed in boiling water for 30 sec or by soaking seed in 200-ml just-boiled water and allowing it to cool overnight. Dry heat was applied by exposing seeds for up to 30 min to temperatures up to 150C in an oven. Microwave treatments involved placing seed in a microwave oven operating at full power (500W) for 1 or 2 min with or without a water load (50 or 100 ml). Seeds soaked in concentrated sulphuric acid (H_2SO_4) or sodium hypochlorite (NaOCl) for up to 40 min were rinsed three times in distilled water following treatment. Manual scarification was performed by nicking seeds individually using a sharp blade. To study the effect of gibberellin (GA) on germination, seeds were irrigated for the total incubation period with 1, 10, 100, or 1000 mg litre^{-1} GA_3 or $GA_{4/7}$. Some seeds were infused with the hormone under reduced pressure for 30 min using a tap-mounted Venturi vacuum pump to evacuate a flask containing seed and 20-ml treatment solution. Again, seeds were irrigated with GA treatments during the incubation period.

Stratification of *Prostanthera eurybioides*. Untreated seeds were placed between two layers of filter paper overlying moist vermiculite in a petri dish, moistened with 1 g litre^{-1} Benlate solution, sealed with parafilm, and incubated at 4C for 1, 2, or 3 months prior to germination testing.

Incubation Temperature and Germination of *Acacia pinguifolia*. Nicked seed was incubated at 15, 20, 25, 30, or 35C. A Zankel thermogradient incubator was used to achieve temperature control for each dish individually, and each temperature was replicated five times.

Storage of Pretreated *Dodonaea subglandulifera* Seed. Seeds were soaked in just-boiled water for 30 sec, air-dried, and then stored at 15C in darkness for

periods of 0, 2, 4, 6, 8, 10, or 12 weeks. Seeds were then sown directly into seedling trays to simulate in situ conditions. Boiling water pre-treatments were staggered in time, to allow seed from all treatments to be sown on the same day. Treatments were replicated four times.

RESULTS

Acacia pinguifolia. Nicking the seed coat produced optimal germination (Table 1). Boiling seeds for 30 sec or soaking seeds in concentrated sulphuric acid H_2SO_4 for 30 min also yielded high germination rates. All treatments tested increased germination significantly above the control.

Table 1. Germination of *Acacia pinguifolia* seed incubated at 20C for 54 days.

Seed pre-treatment	Germination (%)
Nick seed coat with sharp blade	91
Soak in conc. H_2SO_4 for 30 min	77
Boil for 30 sec	88
Soak in 200 ml just-boiled water overnight	60
Heat in microwave oven for 2 min (50 ml water load)	26
Heat in microwave oven for 2 min (no water load)	22
Heat in 95C oven for 30 min	23
Control	3
LSD (P=0.05)	8

Germination increased with decreasing incubation temperatures (Table 2). Optimal germination (97%) occurred following incubation at 20C.

Table 2. Influence of incubation temperature on the germination of *Acacia pinguifolia* seed after 28 days incubation. Seed coats were nicked before incubation.

	Incubation temperature (C)	Germination (%)
	15	92
	20	97
	25	80
	30	11
	35	2
Significance		
Linear		***
Quadratic		***

***Significant at $P \leq 0.001$.

Acacia cretacea. Nicking the seed coat produced optimal germination (Table 3). Boiling seed in water for 30 sec or soaking in 200-ml just-boiled water overnight yielded high germination rates (89% and 75%, respectively). Dry heating seed was not successful as a pre-treatment for germination of this species. A short soak (10 min) in H_2SO_4 yielded 9% germination and it is possible that extending this treatment may lead to further increases in germination.

Table 3. Germination of *Acacia cretacea* seed incubated at 20C for 38 days.

Seed pre-treatment	Germination (%)
Nick seed coat with sharp blade	99
Soak in conc. H_2SO_4 for 10 min	9
Boil for 30 sec	89
Soak in 200-ml just-boiled water overnight	75
Heat in microwave oven for 1 min (100-ml waterload)	5
Heat in microwave oven for 2 min (100-ml water load)	5
Heat in microwave oven for 1 min (no water load)	1
Heat in microwave oven for 2 min (no water load)	0
Heat in 95C oven for 30 min	0
Control	0
LSD (P=0.05)	7

Table 4. Effect of soakage time in 100 ml just-boiled water on *Dodonaea subglandulifera* germination after 30 days.

Soakage time (sec)	Germination (%)
0	7
5	33
10	47
30	50
60	43
120	40
Significance : Linear[1]	**

[1]Linear regression was fitted following $\log_{10}$ transformation of both germination and soakage time.
**Significant at P<0.01

Dodonaea subglandulifera. Soaking seeds in just-boiled water significantly increased germination to a maximum of 50% (Table 4). While soakage times up to 120 sec significantly improved germination according to a double logarithmic relationship, in practice germination rate plateaued rapidly beyond 10 sec soakage.

Table 5. Effects of boiling water and NaOCl (100%) on the germination of *Dodonaea subglandulifera* seed.

Treatment time (sec)		Germination(%)
Boiling water	NaOCl	
0	0	7
	30	13
	45	12
	60	8
30	0	70
	30	80
	45	48
	60	52
Significance:		
NaOCl		*
Boiling water		***
Interaction		*

*,*** Significant at $P < 0.05$ or $P < 0.001$, respectively.

Seeds were treated with 100% NaOCl for up to 60 sec following a 30 sec boiling water treatment. A slight NaOCl-induced stimulation in germination was observed following 30 sec treatment (Table 5). However, boiling water was the more effective germination stimulant and the added effect of NaOCl was insufficient to warrant practical usefulness. Neither $GA_{4/7}$ nor GA_3 stimulated germination significantly at any concentration studied.

Seed treatment with concentrated H_2SO_4 increased germination to over 50% (Table 6). The effect of acid was time-dependant following a double logarithmic relationship.

Seed air-dried and stored at 15C for varying periods following a 30 sec soak in just-boiled water was found to retain its viability for at least 12 weeks. Final germination percentage was proportional to the square of storage time, with optimum germination observed after 6 weeks storage at 15C (Fig. 1).

Prostanthera eurybioides. Pre-treatment of seed with dry heat (150C for up to 30 min), just-boiled water (left to cool overnight), concentrated H_2SO_4 (for up to 40 min), and low temperature (stratification for up to 3 months) applied as treatments in isolation failed to stimulate germination. Irrigation of seed with GA_3 produced some stimulation in germination (4%, 1%, 38%, and 30% germination with 1, 10, 100, and 1000 mg litre^{-1} GA_3, respectively). However, following gibberellin infusion into the seed under reduced pressure, 100 and 1000 mg litre^{-1} GA_3 resulted in 80.3% and 83.3% germination respectively, and 10 and 100 mg litre^{-1} $GA_{4/7}$ in 66.7% and

50% germination, respectively, after 70 days incubation. A further experiment was set up to examine the effect of H_2SO_4 pre-treatment on subsequent response to GA. Seeds were pretreated with concentrated H_2SO_4 for 0, 15, or 30 min, and then incubated with GA. Optimal germination (after 50 days) followed 1000 mg litre^{-1} GA_3 or 1 to 10 mg litre^{-1} $GA_{4/7}$ treatment of seed pretreated with H_2SO_4 for 15 min. Treatment of seed with H_2SO_4 for 30 min or $GA_{4/7}$ at concentrations of 100 mg litre^{-1} or greater proved superoptimal.

Table 6. Germination of *Dodonaea* subglandulifera seed pre-soaked in concentrated H_2SO_4 and recorded after 41 days.

Soakage time (min)	Germination (%)
0	8
30	53
60	50
90	57
120	58
Significance: Linear[1]	***

[1]Linear regression was fitted following $\log_{10}$ transformation of both germination and soakage time.
*** Significant at $P < 0.001$.

Pultenaea trichophylla. Over 95% of seed germinated within 63 days following a 40-min presoak in H_2SO_4 (Fig. 2). Final germination percentage was proportional to the logarithm of H_2SO_4 presoaking time. As well as enhancing the germination percentage, H_2SO_4 also increased the rate of germination as presoaking time increased. The combined effects of H_2SO_4 and GA_3 were also tested, but GA_3 failed to stimulate any further germinations.

DISCUSSION

The results highlight the importance of hard seed coats as a survival mechanism. All five species showed some germination response to manual (nicking), heat (moist or dry), or chemical (H_2SO_4 or NaOCl) scarification. Similar responses have been observed in other Australian species (Aveyard, 1968; Burrows, 1991; Clemens et. al., 1977).

Dry heat was significantly less active than moist heat for acacias, although the response to dry heat varied between the two species, with *A. pinguifolia* being far more responsive to radiated and microwave heat than *A. cretacea*. Tran (1981a) tested the effects of microwave energy on seed of 15 *Acacia* species and results ranged from 0 to 84% germination. He found that the percentage swelling and germination were inversely proportional to seed coat thickness (Tran, 1981b) and that microwaves acted by fracturing the strophiole and seed coat allowing them to become permeable to water (Tran, 1979). Microscopic observations indicated that the seed coat of *A. cretacea* was nearly twice the thickness of that of *A. pinguifolia*

(unpublished data) possibly accounting for their disparate response to dry heat.

Acacia pinguifolia germinated optimally at 20C, and germination declined as temperature increased. Similar results were reported for *A. pulchella* (Bellairs and Bell, 1990), while germination of *A. blakelyi* showed little change over the range 15 to 30C, indicating intra-specific variation in temperature response of *Acacia* seed. Germination rate of *D. subglandulifera* was improved following storage at 15C when compared with seeds germinated immediately after boiling water treatment (data not shown). This suggests that there may be distinct advantages in storing seed for 4 to 6 weeks following dormancy-breaking treatments, resulting in enhanced germination response following subsequent sowing. Both treatment with H_2SO_4 and treatment with just-boiled water yielded similar time-dependant double logarithmic relationships for germination. It is likely, therefore, that both treatments are producing their effect via a common mechanism, that of progressive weakening of the seed coat.

While many *Prostanthera* species will germinate after sowing fresh seed directly onto potting soil (Bonney, 1994), *P. eurybioides* did not germinate without scarification and hormonal pretreatment, and such recalcitrance may be an important factor in the extremely limited regeneration of this species seen in the wild. The improved germination in response to GA suggests that dormancy in this seed may have a physiological as well as a physical component. The physical component could be overcome by treatment with acid, or by infusion of the GA solution into the seed under reduced pressure. This technique of vacuum infiltration has been used successfully on a range of other native species to overcome the effects of mechanical constraints of the seed coat (Loveys and Jusaitis, 1994).

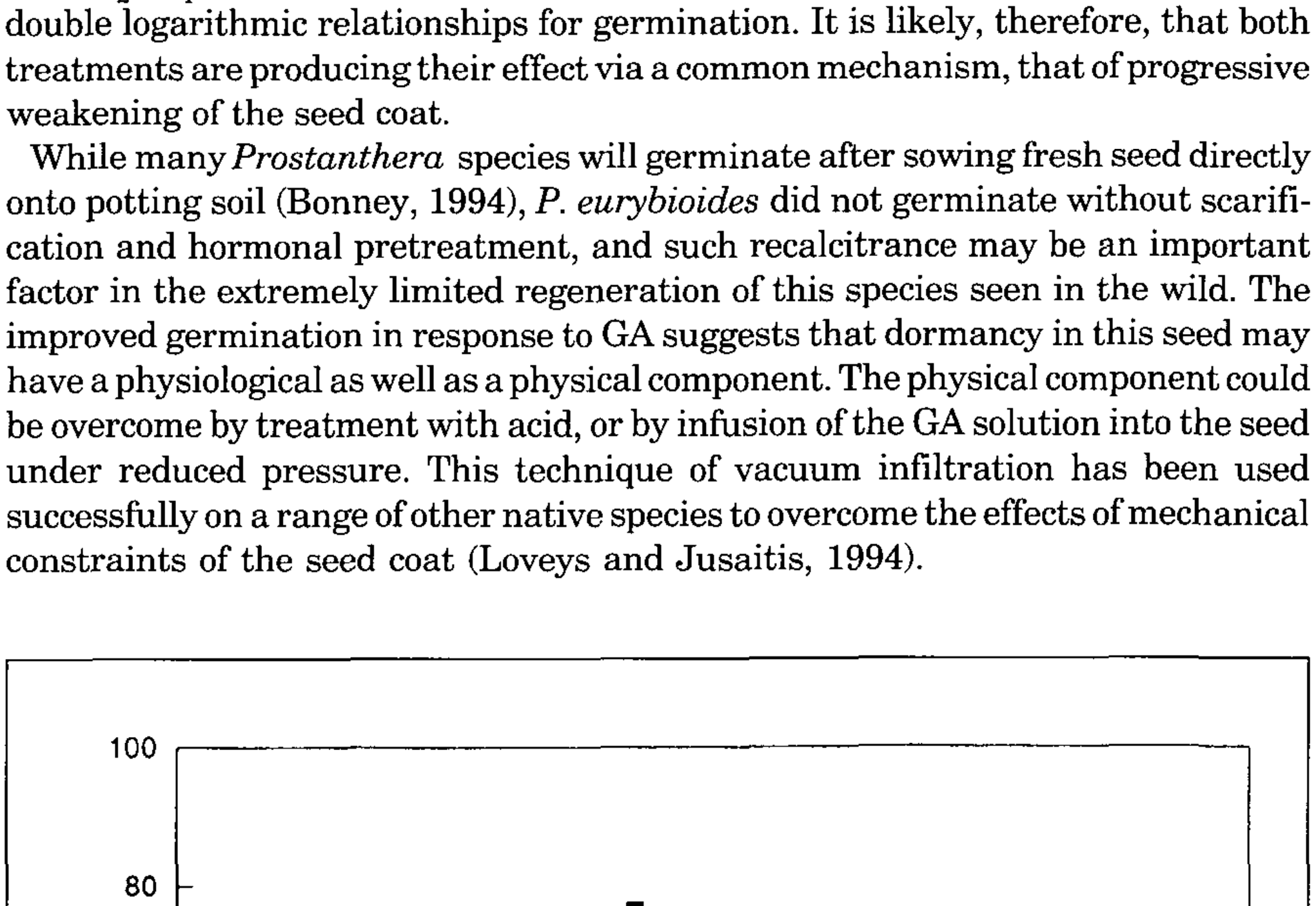

Figure 1. Final percentage germination of pretreated *Dodonaea subglandulifera* seed following storage at 15C for up to 12 weeks.

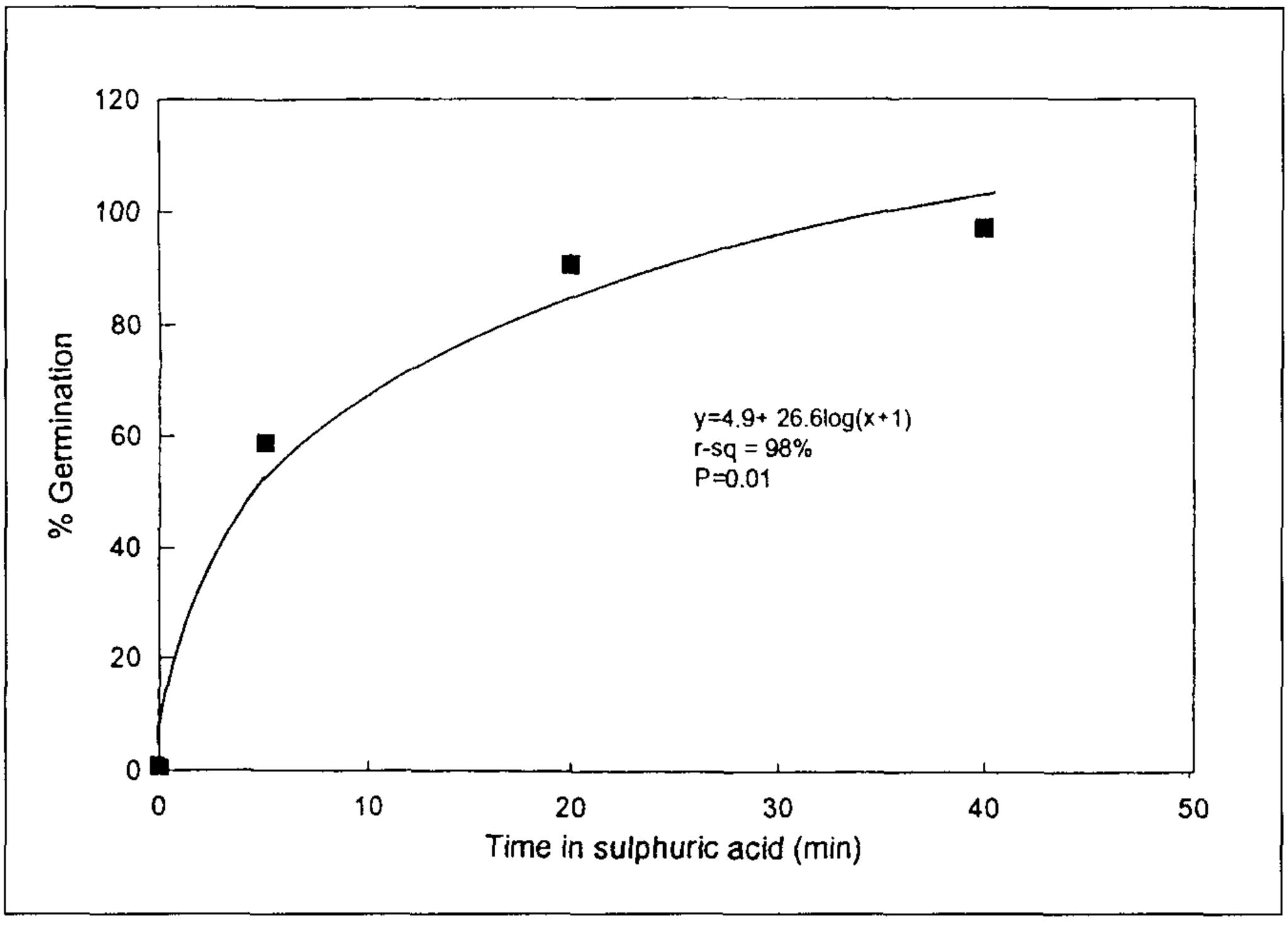

Figure 2. Final percentage germination (after 63 days) of *Pultenaea trichophylla* seed pretreated with concentrated H_2SO_4 for up to 40 min.

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