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The germination requirements of *Hemigenia exilis* (Lamiaceae) – seed plug removal and gibberellic acid as a successful technique to break dormancy in an arid zone shrub from Western Australia

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ABSTRACT

Hemigenia exilis S. Moore (Lamiaceae) is a previously presumed extinct species known only from the eastern goldfields of Western Australia. A number of populations of this species are under threat from mining. Seed was collected with the objective of developing a suitable protocol for the germination of *H. exilis* with the ultimate aim of developing a rehabilitation program for the species should mining proceed. A reduced surface tension flotation test indicated that less than one third of the seed produced at any one time was potentially viable. A range of treatments were used to stimulate germination including varying concentrations of the growth hormone gibberellic acid (GA_3)(10 mg L^{-1} and 25 mg L^{-1}), scarification of the seed coat, heat and/or cold stratification, the application of aqueous smoke solution and the removal of the seed plug (located on the ventral surface of the seed) under three different temperature regimes (constant 15° C, alternating 25°/10° C and 30°/5° C). These treatments were used singly or in combination. Seed commenced germination within 6 days and continued for up to 120 days, with germination ranging from 0 per cent to 87 per cent. The results of these germination trials suggest that this species possesses some form of dormancy which can be partially broken by the application of the growth hormone gibberellic acid (38 per cent germination) and in full by removal of the seed plug in combination with gibberellic acid application (87 per cent germination). This research has significant implications for the conservation and rehabilitation of wild populations of this species.

INTRODUCTION

Hemigenia exilis S. Moore is an upright multi-stemmed shrub with divaricate branches to 1.5 m high, with sessile leaves and purple flowers. The fruits of *Hemigenia* contain

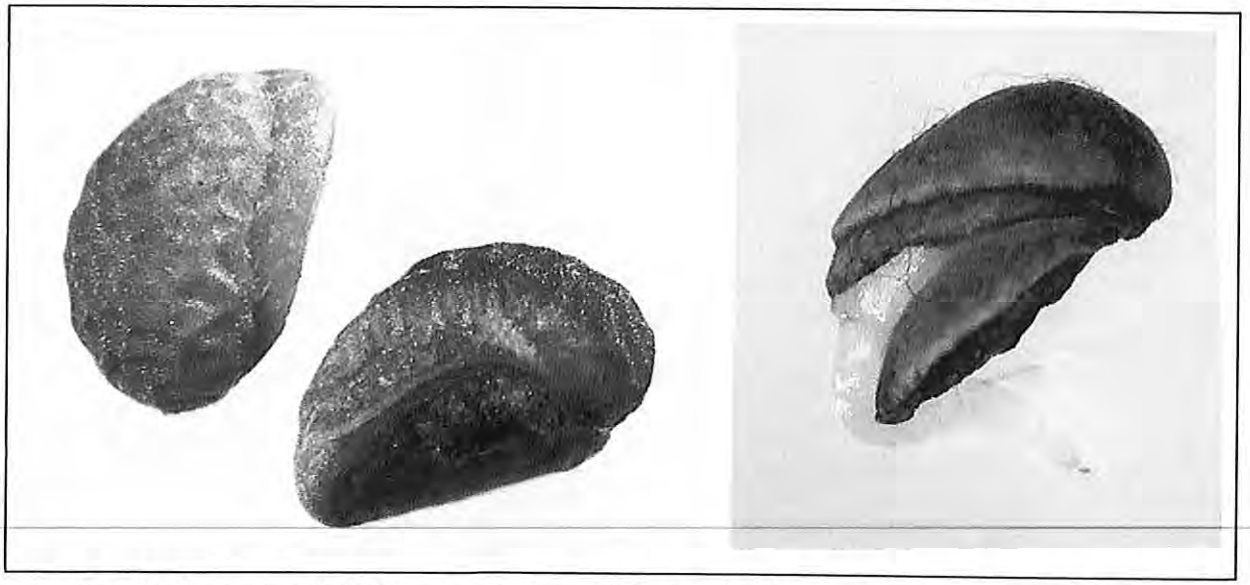

Figure 1. Seed of Hemigenia exilis (x25) *and germinating seed showing plug-like structure (epistomium) on the ventral surface.*

4 nutlets which are reticulate or rugose and those of the species *H. exilis* ripen in early summer. The seed of *H. exilis* is surrounded by a moderately hard pericarp with a plug-like structure on the ventral surface (Fig. 1). In the apparent absence of a suitable term to describe that portion of the seed, the plug, we have used the term *epistomium* (from the Greek *epi* – upon and *stoma* – mouth).

H. exilis appears to be root-suckering with few seedlings evident around mature plants (Bennett 1996). Despite the large quantities of seed produced by individual plants, their root-suckering ability indicates that the species may not be an obligate seeder. The species response to fire or cutting is as yet unknown, although Bennett (1996) noted that plants damaged by vehicular disturbance had the ability to produce regrowth.

After its initial discovery in 1895, *H. exilis* was not seen again until 1995 when the species was rediscovered on a mining lease east of Leonora in the Western Australian goldfields (lat. 28° 56' 48" long. 121° 47' 30"). The species had been gazetted by the Western Australian government as Declared Rare Flora, Presumed Extinct. This classification is for 'taxa which have not been collected, or otherwise verified, over the past 50 years despite thorough searching, or of which all known wild populations have been destroyed more recently, and have been gazetted as such...' (Department of Conservation and Land Management 1997). Most populations of the species occur on shallow soils on laterite ridges, although a number of smaller populations occur on eroded slopes and gullies adjacent to the laterite hills. Several populations occur in areas proposed for mining. Because of the rare nature of the species specific strategies were developed by the mining company, Anaconda Nickel NL, to ensure environmental protection for this species. These included research into the biology and ecology of the species with the aim of establishing a plan of management and a rehabilitation program in the event of mining. In particular, a good knowledge of the seed biology and germination ecology of the species would help ensure the successful rehabilitation of the species after disturbance.

The Australian flora has evolved a wide range of germination responses to ensure survival (Bell *et al.* 1993; Dixon *et al.* 1995; Roche *et al.* 1997; Bell in press), and environmental cues for germination and ecological conditions that occur in the habitat of the plant and seeds is often strongly correlated (Mayer and Poljakoff-Mayber 1989). In Western Australia (WA), germination response in a wide range of species is largely unknown. There is limited published information on Australian seed germination, with the exception of those species used in mine-site rehabilitation and those with horticultural potential (see Kullman 1981; Langkamp 1987; Bell and Bellairs 1992; Bell *et al.* 1993; Dixon *et al.* 1995). The large size of the flora (some 12 000 taxa) has also rendered the comprehensive study of native seed germination very difficult. In particular, very little has been reported in the scientific literature on the germination of seed of *Hemigenia* species. Elliot and Jones (1990) report that few species of *Hemigenia* are propagated from seeds, but those that are do not require any pre-sowing treatment and should be sown fresh as viability decreases quickly with age. Previous studies on *H. exilis* by Cochrane *et al.* (1996) revealed that fresh seed of *H. exilis* required applications of the growth hormone gibberellic acid for the stimulation of germination.

A major limitation to the germination of native species is the mechanism of seed dormancy. Although dormancy is considered biologically advantageous (Villiers 1972) and assists in seed survival, it creates problems for research into seed biology.

Dormancy takes many forms and includes both external (coat-imposed) and internal (embryo or innate) controls on germination (Bewley and Black 1994). Both types of dormancy can occur singularly or together to inhibit germination (Bewley and Black 1994). Coat imposed dormancy occurs when the seed is unable to germinate owing to a restriction of the seed coat. The seed coat may exert a physical barrier preventing embryo development (Bradbeer 1988). The embryo may require a certain force to break the barrier, or the barrier must degrade the restraining tissues by enzyme activity (Bewley and Black 1994). Such barriers may prevent water or gas exchange. The impermeability of seed coats to water is widespread in the families Leguminosae and Mimosaceae, but may also occur in other families (Richards and Beardsell 1987). Embryo dormancy is more complicated but may also be caused by inhibitory substances, or alternatively, by the underdevelopment of the embryo and a metabolic deficiency (Bradbeer 1988). Naturally occurring inhibitors may prevent the seed from germinating in a hostile environment. These will dissipate in time owing to weathering by wind, water and soil abrasion in the environment. The manual removal of the pericarp of fresh seed has been found to enhance germination in a range of Western Australian species (for example Bell *et al.* 1993; Plummer *et al.* 1995; Schatral 1996).

Lack of specific hormones at seed shed may contribute to dormancy. These hormones include gibberellins, cytokinins, ethylene and abscisic acid. They can act singly or in complex interactions to enhance or inhibit germination. In some cases, a period of after-ripening may also be required before dormancy is broken. Mott and Groves (1981) report that gibberellins are effective in breaking dormancy in seeds which after-ripen in dry storage, as well as those requiring chilling and light (Baskin and Baskin 1971; Bell in press). The use of smoke in either aqueous or aerosol form has also been found to break dormancy in a range of species from WA (Dixon *et al.* 1995). Roche *et al.* (1997) have indicated that the genus *Hemigenia* responds to smoke under field conditions, although Cochrane *et al.* (1996) report that the application of aqueous smoke did not enhance germination in *H. exilis* under controlled laboratory conditions.

Seed in the natural environment, especially in the semi-arid and arid zones of WA, may be subjected to high summer temperatures before germinating. It is thought that heat may increase the ability of some seed to synthesize endogenous gibberellins (Choengsaat *et al.* 1997). In addition, it has been reported that high temperature storage

of seed, in conjunction with applications of gibberellins, can lead to germination in otherwise dormant seed (Mott and Groves 1981; Choengsaat *et al.* 1997). Conversely, low temperatures have also been found to reduce the duration of after-ripening (Richards and Beardsell 1987) and chilling can delay germination until conditions are more favourable for germination and successful establishment.

Germination may also be prevented by unsuitable conditions immediately after seed shed (for example, insufficient moisture, inappropriate temperature or inadequate light) and thus postponement of germination by some mechanism of imposed dormancy may be required to give the seed maximal chance of survival.

This paper describes treatments that were used to stimulate germination and confirms that dormancy exists in seed of *H. exilis*. Treatments used include a variety of temperature regimes, the application of the growth hormone gibberellic acid (GA_3), scarification, smoked water extract, heat shock, cold and heat stratification and removal of part of the seed coat (the seed plug or epistomium). The objective of these trials was to develop a useful protocol for the germination of seed of *H. exilis*. These data will contribute to the development of a successful rehabilitation program and will assist with efficient use of resources for seed collection and propagation.

MATERIALS AND METHODS

Seed was collected over three years (1995 to 1997) from seven populations of *Hemigenia exilis* located on Anaconda Nickel's Murrin Murrin mining lease east of Leonora, WA. Viability testing and storage of the seed took place in the laboratory of CALM's Threatened Flora Seed Centre (TFSC).

A reduced surface tension flotation method was used to distinguish filled seed (seed that sank) from empty seed (seed that floated). Results were verified using a cut test (Table 1). Processing of seed was identical for all years and was conducted prior to any seed germination trials to ensure that 100 per cent potentially viable seed was used in those trials. The seed was germinated in glass Petrie dishes on a 0.75 per cent agar solution, in temperature and light controlled incubation cabinets, using a 12-hour photoperiod. A 0.75 per cent agar solution was used after extensive germination trials in the laboratory of the TFSC confirmed that this concentration was successful for moisture uptake and radicle penetration for a wide range of seed from taxa from the south-west of WA. Temperature control was varied for a range of treatments and included a constant 15° C, an alternating 25° C day/10° C night regime, and an alternating 30° C day/5° C night regime. A total of 48 treatments were used (two of which were repeated in year 1 and year 2) over the three years. All populations were treated individually each year owing to possible differences in seed viability between populations and between years. Each treatment in year 1 and 2 contained three replicates of 25 seeds. In year 3, four replicates of 25 seeds were used per treatment, except where there was insufficient seed and in these treatments fewer seeds were used (minimum number of seeds used per treatment in year 3 was 84). All seed was surface sterilized prior to sowing with a 5 per cent solution of 40 g L^{-1} sodium hypochlorite for 10 minutes, with the exception of seed that was either scarified or had the epistomium removed.

Treatments

Table 2 shows all treatments for all years.

Year 1

Year 1 treatments consisted of a control (no treatment) incubated at 25° C day/10° C night, a treatment incubated at a constant 15° C and treatments using the growth hormone gibberellic acid at varying concentrations

TABLE 1
Results of flotation and cut-tests for populations of *Hemigenia exilis* (1995-1997).

	% HEALTHY SEED			% OF FLOATING SEED WITH SHRIVELLED ENDOSPERM			% OF SUNKEN SEED WITH HEALTHY ENDOSPERM		
POPULATION	1995/96	1996/97	1997/98	1995/96	1996/97	1997/98	1995/96	1996/97	1997/98
1	24	30	32	94	95	92	100	95	96
2	24	32	33	97	93	99	90	93	97
3	24	29	38	93	90	95	100	90	97
4	29	34	30	98	94	90	90	94	97
5	NC	42	NC	NC	100	NC	NC	100	NC
6	NC	NC	32	NC	NC	93	NC	NC	100
7	NC	NC	32	NC	NC	91	NC	NC	100
ANNUAL MEAN	25	33	33	95	93	93	95	94	98
OVERALL MEAN		30			94			96	

NB: 'NC' denotes no collection.

TABLE 2
Percentage of germination, mean percentage of germination and mean days to first and last germination by treatment for 7 populations of *Hemigenia exilis*. (RT = room temperature)

TREATMENT (all incubated at 25° / 10° C unless otherwise specified)	POPULATION 1	POPULATION 2	POPULATION 3	POPULATION 4	POPULATION 5	POPULATION 6	POPULATION 7	MEAN DAYS TO FIRST GERMINATION	MEAN DAYS TO MAXIMUM GERMINATION	MEAN % GERMINATION	SE
YEAR 1											
Control	1%	3%	0%	0%	3%	-	-	56	106	1%	0.67
GA_3 25 mg L^{-1}	44%	45%	32%	43%	27%	-	-	15	109	38%	3.68
GA_3 10 mg L^{-1}	53%	44%	20%	8%	24%	-	-	16	100	30%	8.24
Smoke water	-	-	9%	-	-	-	-	31	122	9%	0
15°C	-	-	0%	-	-	-	-	-	-	0%	0
GA_3 10 mg L^{-1} @ 15°C	-	-	40%	-	-	-	-	13	90	40%	0
GA_3 25 mg L^{-1} @ 15°C	-	-	36%	-	-	-	-	20	83	36%	0
Smoke water @ 15° C	-	-	8%	-	-	-	-	27	31	8%	0
YEAR 2											
Control	0%	0%	0%	0%	-	-	-	-	-	0%	0
GA_3 25 mg L^{-1}	40%	39%	32%	13%	-	-	-	20	117	31%	6.26
Scarify	0%	0%	4%	0%	-	-	-	53	53	<1%	1
Scarify + GA_3 25 mg L^{-1}	45%	45%	39%	21%	-	-	-	16	106	37%	5.68
RT storage 2 month	0%	-	-	-	-	-	-	-	-	0%	0
RT storage 4 month	0%	-	-	-	-	-	-	-	-	0%	0
RT storage 6 month	0%	-	-	-	-	-	-	-	-	0%	0
70° C storage 2 month	0%	0%	0%	0%	-	-	-	-	-	0%	0
70° C storage 4 month	0%	0%	0%	0%	-	-	-	-	-	0%	0
70° C storage 6 month	0%	0%	0%	0%	-	-	-	-	-	0%	0
RT storage 2 month + scarify	0%	-	-	-	-	-	-	-	-	0%	0
RT storage 4 month + scarify	0%	-	-	-	-	-	-	-	-	0%	0
RT storage 6 month + scarify	0%	-	-	-	-	-	-	-	-	0%	0
70° C storage 2 month + scarify	0%	0%	0%	0%	-	-	-	-	-	0%	0
70° C storage 4 month + scarify	0%	0%	0%	0%	-	-	-	-	-	0%	0
70° C storage 6 month + scarify	0%	0%	0%	0%	-	-	-	-	-	0%	0
RT storage 2 month + GA_3 25 mg L^{-1}	29%	-	-	-	-	-	-	25	119	29%	0
RT storage 4 month + GA_3 25 mg L^{-1}	19%	-	-	-	-	-	-	20	143	19%	0
RT storage 6 month + GA_3 25 mg L^{-1}	23%	-	-	-	-	-	-	25	120	23%	0
70° C storage 2 month + GA_3 25 mg L^{-1}	8%	12%	4%	0%	-	-	-	29	85	6%	2.58
70° C storage 4 month + GA_3 25 mg L^{-1}	0%	0%	0%	0%	-	-	-	-	-	0%	0
70°C storage 6month + GA_3 25 mg L^{-1}	0%	0%	0%	0%	-	-	-	-	-	0%	0
RT storage 2 month + scarify + GA_3 25 mg L^{-1}	16%	-	-	-	-	-	-	19	60	16%	0
RT storage 4 month + scarify + GA_3 25 mg L^{-1}	13%	-	-	-	-	-	-	41	143	13%	0
RT storage 6 month + scarify + GA_3 25 mg L^{-1}	24%	-	-	-	-	-	-	25	120	24%	0
70° C storage 2 month + scarify + GA_3 25 mg L^{-1}	28%	15%	10%	0%	-	-	-	30	94	11%	5.82
70° C storage 4 month + scarify + GA_3 25 mg L^{-1}	0%	0%	0%	0%	-	-	-	-	-	0%	0
70° C storage 6 month + scarify + GA_3 25 mg L^{-1}	0%	0%	0%	0%	-	-	-	-	-	0%	0
YEAR 3											
GA_3 25 mg L^{-1} @ 30/5° C	32%	20%	15%	-	-	11%	28%	18	63	21%	3.92
GA_3 25 mg L^{-1} + scarify @ 30° /5° C	4%	5%	6%	-	-	0%	0%	12	32	3%	1.26
4° C for 1 week	4%	-	6%	-	-	-	-	49	64	5%	0.99
4° C for 1 week + GA_3 25 mg L^{-1}	63%	-	51%	-	-	-	-	20	73	57%	6
Heat 80° C for 9 days	4%	-	7%	-	-	-	-	38	66	5%	1.5
Heat 80° C for 9 days + GA_3 25 mg L^{-1}	12%	-	28%	-	-	-	-	16	66	20%	7.99
Heat 80° C for 9 days + 4°C for 1 week	5%	-	4%	-	-	-	-	35	58	4%	0.5
Heat 80° C for 9 days + 4° C for 1 week + GA_3 25 mg L^{-1}	6%	-	8%	-	-	-	-	33	47	7%	0.99
Epistomium remove	41%	10%	13%	-	-	16%	38%	8	55	24%	6.58
Epistomium remove + GA_3 25 mg L^{-1}	80%	87%	88%	-	-	96%	82%	6	42	87%	2.79
Epistomium remove @ 30° /5° C	13%	12%	23%	-	-	17%	45%	11	72	22%	6.06
Epistonium remove + GA_3 25 mg L^{-1} @ 30° C	56%	51%	55%	-	-	71%	49%	8	45	56%	3.87

(GA_3 at 10 mg L^{-1}, 25 mg L^{-1}) and at both 15° C and 25° C day/10° C night. A 24 hour pre-soaking treatment in a 100 per cent concentration of aqueous smoke solution was used for both constant (15° C) and alternating (25° C day/ 10° C night) temperature regimes in one population only.

Year 2

In year 2 the application of growth hormones was confined to the alternating temperature regime (25° C day/10° C night) and at the higher concentration of gibberellic acid (25 mg L^{-1}). Treatments involving scarification and scarification combined with the use of gibberellic acid at 25 mg L^{-1} were trialed. Seed was individually scarified using a metal emery board until the seed coat had been filed away and the endosperm exposed to allow imbibition. Methods of storage used by Mott (1972) in studies of various arid zone plants were used in year 2 and some seed was stored at 70° C (simulating possible maximum summer soil temperatures) and at room temperature (20–25° C) for 2, 4 and 6 months prior to germination testing to allow for any after-ripening requirements in the seed.

Year 3

Additional treatments involving the removal of the seed plug or epistomium and heat and cold stratification were trialed in year 3. The epistomium was removed from each seed by easing plugs out with a scalpel blade under a dissecting microscope. Following on from the lack of response to treatments involving months of prolonged storage at high temperatures in year 2, year 3 testing involved shorter bursts of heat (11 days at 70° C). Similarly, the minimum temperatures that the seed of *H. exilis* would be subjected to prior to germination in autumn may well reach below 0° C (Bureau of Meteorology 1998), therefore a treatment involving cold stratification at 4° C for seven days, alone or following heat stratification, was chosen to simulate climatic conditions that the seed may be subjected to in its natural environment. Seed subjected to heat treatment was placed on mesh above water for 48 hours to allow seeds to rehydrate before placing on agar. This prevented possible imbibition damage to the seed following heat treatment.

As little to no germination (0–1 per cent) occurred in controls in year 1 and 2, it was considered unnecessary to include a control in year 3.

There were no comparisons between years for populations for the majority of treatments, other than the control (no treatment) and the application of growth hormone gibberellic acid at 25 mg L^{-1} at 25° C day/10° C night between years 1 and 2. Insufficient seed was collected from all populations over the three years to conduct comprehensive comparative germination trials. Seed from populations 1, 3 and 4 were tested each of the three years, whereas population 5 was tested only in year 1 and seed from populations 6 and 7 was tested only in year 3. Seed from population 2 was tested in both years 1 and 2. The number of germinants was recorded twice weekly. The time to first germination refers to the period of time from exposure of the seed to moisture until emergence of the embryonic radicle through the testa.

RESULTS

The percentage of potentially viable seed with healthy endosperm varied between populations and between years of collection. Over the three years of seed collection, the mean proportion of filled to empty seed over all populations tested ranged from 25 per cent in 1995 to 33 per cent in 1996 and 1997. Of the total seed collected over the three years 30 per cent had healthy endosperms (Table 1).

Germination trials determined the total percentage germination for *H. exilis* in the 48 treatments (Table 2).

The control and the treatment at a constant 15° C gave less than 10 per cent germination. Treatments involving seed storage at either room temperature or at 70° C for periods of 2, 4 and 6 months produced no germination. Scarification treatments incubated at 25°/10° C and tested after 0, 2, 4 and 6 months storage at room temperature and at 70° C for 2, 4 and 6 months also gave no germination. In addition, four further treatments (GA_3 at 25 mg L^{-1}, and scarification in conjunction with GA_3 at 25 mg L^{-1} and stored at 70° C for 4 and 6 months) produced no germination. The soaking of seed in a 100 per cent solution of smoked water extract for 24 hours prior to incubation at temperatures of 15° C and 25°/10° C produced less than 10 per cent germination. Treatments involving short periods at high temperatures (80° C) produced very low germination (< 20 per cent) as did a chilling treatment (1 week at 4° C) (5 per cent).

All treatments with germination results over 10 per cent required the application of gibberellic acid either alone, or in combination with some other treatment (scarification, heat, chill, storage or plug removal). The exceptions to this were treatments involving plug removal at both alternating temperatures.

A range of treatments will produce between 11 per cent and 40 per cent germination with the application of gibberellic acid, although plug removal on its own will produce 22–24 per cent germination (Fig. 2). By itself, gibberellic acid will give 30–40 per cent germination. To effect more than 50 per cent germination, either plug removal or a one week chilling at 4° C in combination with gibberellic acid is required. The maximum germination shown for any year for any treatment was 87 per cent, this being achieved by the removal of the plug and incubation at 25°/10° C on gibberellic acid 25 mg L^{-1}.

In the treatment using plug removal in combination with gibberellic acid application germination commenced between day 6 and day 8, and maximum germination had virtually occurred by day 20. First germination occurred between 10 and 17 days after incubation for other treatments (Fig. 3). Germination continued to occur for up to 120 days in most of these treatments.

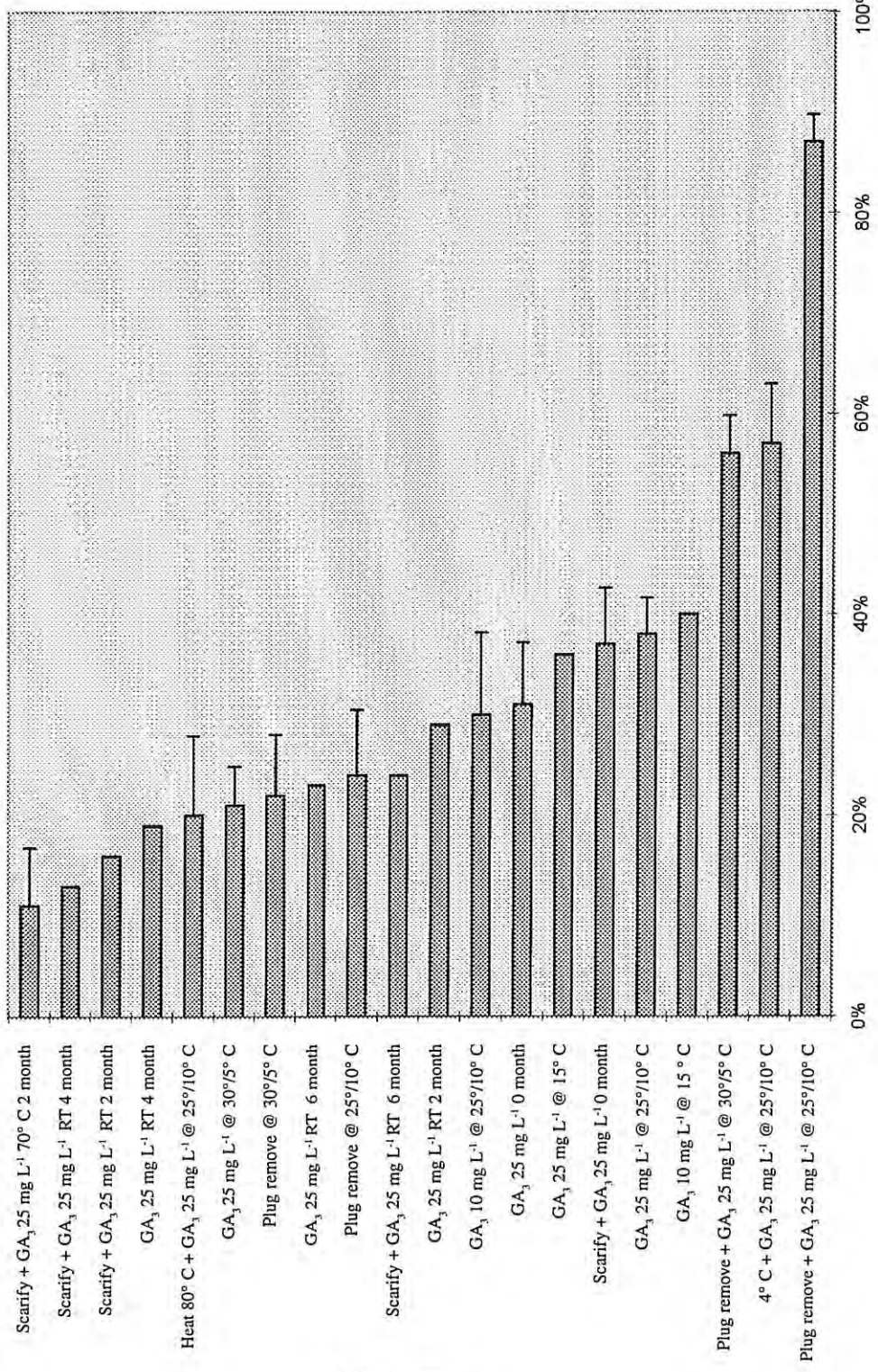

Figure 2. Percentage germination (mean ± SE) for populations of Hemigenia exilis *in 20 treatments (only mean germination over 10% is displayed; no SE indicated that results for that treatment are based on a single population; RT = room temperature).*

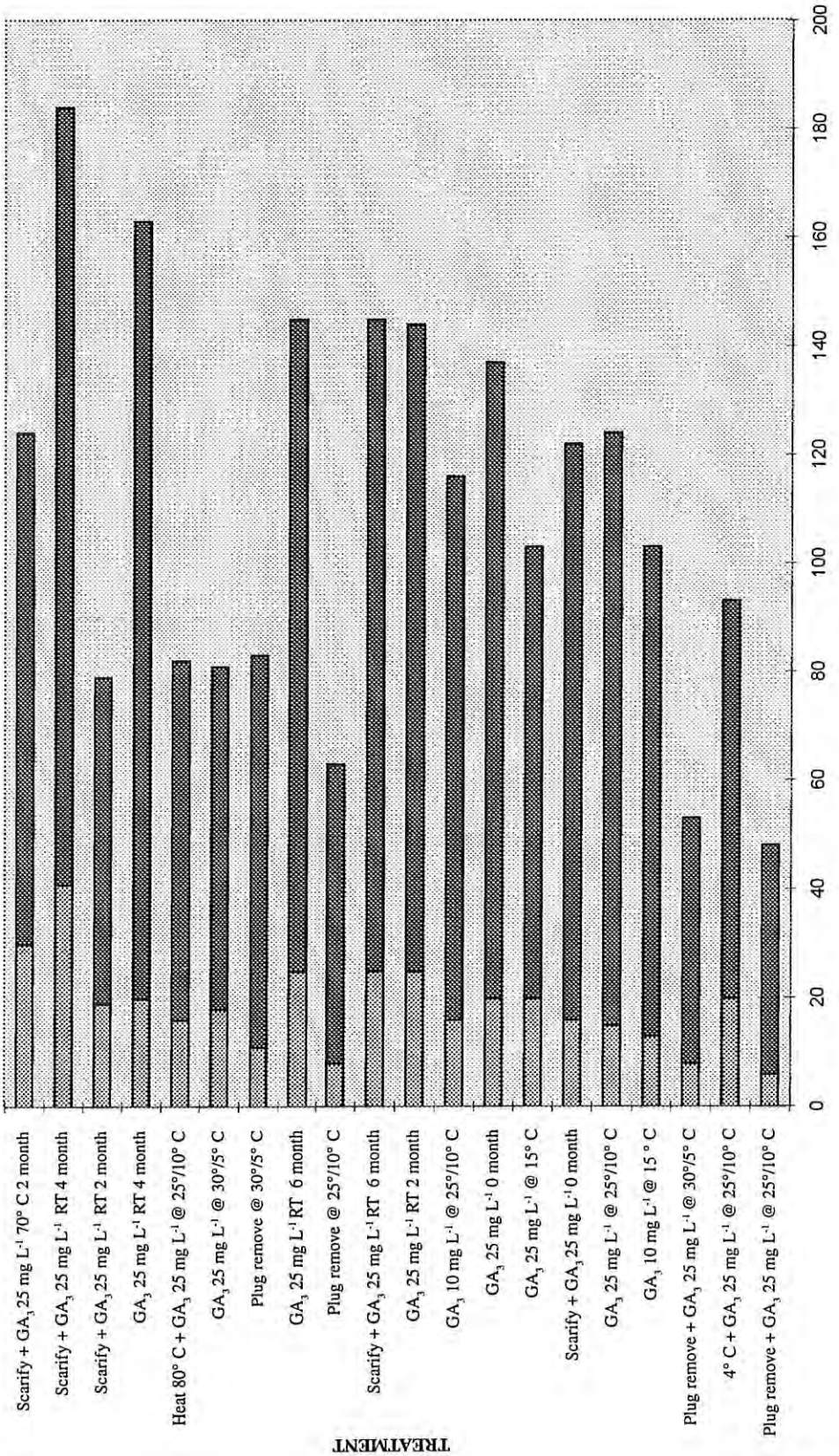

Figure 3. Mean minimum and maximum days to germination for populations of Hemigenia exilis *in 20 treatments (only mean germination over 10% is displayed; RT = room temperature).*

DISCUSSION

Seed quality is an important factor in the germinability of a species and the quality of a seed batch will determine the numbers of germinable seed produced (Dixon 1997). In these trials, seed flotation experiments showed a good correlation between seed buoyancy and endosperm presence. They indicated that less than one third of *H. exilis* seed collected over the three years contained embryos or endosperm (Table 1). Seeds and seed coats developed and seeds appeared full but over two-thirds were empty or with degenerated 'seeds'. Ovule abortion can be caused by genetic factors, or a lack of resources including pollen, pollinators and nutrient and water availability (Stephenson 1981; Bawa and Webb 1984). Wiens *et al.* (1987) suggest that temperature changes, seed-eating insects, disease, and developmental phenomena can also contribute to ovule abortion. In addition, *H. exilis* appears to be a resprouter. Bennett (1996) reported that this species is capable of resprouting from damaged rootstock and few seedlings were located at the various sites. Bell *et al.* (1993) report that, in general, resprouter species set lower numbers of viable seed compared with obligate reseeders. It is possible that the reproductive strategy of *H. exilis* is contributing to the low production of viable seed.

In addition to low viable seed production, the results of these germination trials indicate that there is a dormancy mechanism preventing the full germination of 'good' fresh and laboratory stored seed of *H. exilis*. Little (<10 per cent) or no germination occurred in 28 of the treatments used in these trials (control, storage at 70° C for longer than 2 months, scarification, aqueous smoke application, chilling and short heat burst) (Table 2). Our results show that dormancy in *H. exilis* cannot be broken by these treatments alone. Holding seed of this species at high temperatures (i.e. 70° C) for longer than 2 months appears to be detrimental to seed germinability. Storage for even short periods at high temperature produces very low germination, in contrast to the results reported by Choengsaat *et al.* (1997) for the Western Australian annual, *Schoenia filifolia* (Turcz.) Wilson ssp. *subulifolia* (F. Muell.) Wilson (Asteraceae) and by Mott (1972) for a range of arid zone annuals. Studies have also shown that a continuous cycle of wetting and drying of seed of *H. exilis* does not elicit germination indicating that chemical inhibition owing to seed coat inhibitors may not be preventing germination in this species (Cochrane unpublished data). However, by removing the epistomium successful germination was achieved, indicating that the barrier to germination may be owing to the inability of the radicle to develop and elongate. This appears to be specifically related to the epistomium as scarification of the seed coat in general was unable to cue germination. If prevention of water or gas exchange are factors in the inability of the seed to germinate it would be expected that scarification of the seed coat would stimulate germination. Although moderate germination (30–40 per cent) was achieved with the application of gibberellic acid alone, a substantial increase in percentage and rate of germination was obtained by removal of the plug in combination with gibberellic acid.

Factors that could facilitate the loss of the epistomium in the natural environment could include movement of seed due to weathering by wind and water, microbial attack, passage through the digestive tract of an animal or exposure to alternating high and low temperatures which may rupture the seed coat (Mayer and Poljakoff-Mayber, 1989). Berg (1975) indicates that myrmecochory (dispersal by ants owing to ant attracting structures or eliaosomes) may occur in the genus *Hemigenia*. The eliaosome-like structure surrounding the epistomium in *H. exilis* may be targeted by ants as a food source. With the removal of the elaiosome, the epistomium may be dislodged and allow germination should suitable conditions of moisture, temperature and light be available. According to Bennett (1996) ants have been implicated with this species and extra-flora nectaries have been observed on the stem of fruiting material below the leaf junction.

Treatments involving epistomium removal alone gave 22–24 per cent germination, and epistomium removal in combination with the application of gibberellic acid gave 56–87 per cent. It therefore appears that seed of *H. exilis* requires hormonal balancing as well as the removal of a physical barrier for maximum germination to occur. Growth hormones, such as gibberellins, can overcome inhibitors, stimulate the weakening of surrounding tissues, promote the germination of immature embryos (Bell *et al.* 1993) and can overcome the requirement for light or cold stratification (Bachelard 1967). Gibberellic acid can shorten the period of after-ripening required for successful germination in a range of species (Bell in press). All treatments producing over 11 per cent germination included the application of gibberellic acid.

Cold temperature storage *per se* has little effect on dormancy breaking, although cold storage for a period of one week in combination with the application of gibberellic acid will increase germination greatly, despite the actual rate of germination being reduced. Baskin and Baskin (1971) report that chilling can overcome the mechanical restriction of the seed coat in *Ruellia humilis* Nutt. (Acanthaceae) by increasing the growth potential of the embryo. Moist chilling of the seed may also assist the removal of the epistomium perhaps owing to a softening of the seed coat structure. *H. exilis* seed germinated in the laboratory in sterile soil after moist chilling with gibberellic acid showed signs of fungal attack at the point of the epistomium attachment to the remainder of the pericarp (Cochrane unpublished data). The presence of these micro-organisms, and/or the effect of chilling at 4° C, appear to have assisted the release of the epistomium from the body of the pericarp. These observations support the results of chilling trials in combination with the application of gibberellic acid of this present study.

This research indicates that combined or sequential environmental stimuli are required to maximize germination in *H. exilis* rather than a single trigger which is consistent with findings for a range of other Western Australian species (Bell *et al.* 1993; Bell *et al.* 1995).

Knowledge concerning germination response is vital to the mining industry for the successful rehabilitation of mined sites and timing of direct seeding. Given that epistomium removal is a time consuming process, the use of moist chilling in combination with gibberellic acid application is possibly the most practical method to stimulate germination in this species on a large scale.

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