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The combined effects of osmotic priming with plant growth regulator and fungicide soaks on the seed quality of five bedding plant species

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Summary

The effect of fungicide and plant growth regulator (PGR) soaks and osmotic priming, both alone and in combination, on the germination of five bedding plant species are reported. The species were *Primula acaulis*, *Impatiens wallerana*, *Salvia splendens*, *Verbena X hybrida* and *Petunia X hybrida*. Germination results are reported from seeds air dried after treatment. In general priming treatments reduced mean germination time of all species. PGRs applied in a 48 h soak at 5 °C increased percentage germination of *Verbena* seeds in the light and *Primula* and *Impatiens* seeds in the dark. These same PGRs enhanced the benefits of osmotic priming to these species when added to priming solutions. In each of these cases the most effective of the range of treatments that were tested is identified. With the exception of *Salvia* seeds which could not be dried following treatment without damage, significant benefits were shown with all species for practical seed treatments that have commercial potential.

Introduction

For the efficient and profitable production of direct sown bedding plants in cellular trays it is essential to have seeds of high quality, however, for many bedding plant species such seeds are difficult to obtain reliably in adequate quantities. A survey of UK growers was used to identify six species that had major establishment problems in practice. In a previous study the germination responses of seeds of these species to environmental conditions were investigated (Finch-Savage, Gray and Dickson, 1991). It was shown that for several of these species germination performance, even under optimum conditions, was inadequate for direct seeding into cellular trays. Clearly improvement of seed quality in commerce will result from improved seed production techniques, from treatment of seeds or a mixture of both approaches. Many seed treatments have been tested and developed to improve seed performance of a wide range of species (Heydecker and Coolbear, 1977) and the effects of some of these treatments on flower seed germination have been reviewed recently by Alderson (1987). However, few studies have been made with seeds of bedding plant species. The purpose of this present study was to investigate the potential for seed technology

techniques now becoming widely used on vegetable seeds (Gray, 1989) to improve seed quality in bedding plant species.

Materials and methods

General experimental details

Five bedding plant species were studied; *Primula acaulis* cv. Improved Biedermeier Strain, *Impatiens wallerana* cv. Dwarf Baby Mixed, *Salvia splendens* cv. Blaze of Fire, *Verbena X hybrida* cv. Olympia Mixed and *Petunia X hybrida* cv. Red Star. In all experiments before drying of the seed after treatment or before testing untreated seeds, they were washed under running tap water for 0.5 mins and then rinsed 3 times in distilled water. When seeds were dried they were placed in a flow of air at 15 °C and 45 ± 5% rh for 48 h and this was found to be sufficient to allow the seeds to reach an equilibrium moisture content. Comparisons between treated and control seeds were made in germination tests carried out in 80 × 120 × 20 mm transparent polystyrene boxes containing fifty seeds on two layers of moistened absorbent paper (Whatman grade 181). Germination (radicle visible) was recorded daily initially, then at longer time intervals on three replicates per treatment. In treatments where germination response in the dark was measured seeds were placed into germination boxes under green light and then wrapped in aluminium foil to exclude light. When germination in the light (*c.* 100–170 μmol m^{-2} s^{-1}) was complete a single germination count was made on seeds kept in the dark. When germination counts were complete seedlings were evaluated according to International Seed Testing Association (ISTA) rules (Anon, 1985). Percentage data were angularly transformed and spreads in time to germination (Orchard, 1977) were log transformed before all measured parameters were subjected to analyses of variance.

Preliminary experiments

Different ratios of water to absorbent paper were evaluated in preliminary germination tests to develop optimum seed test conditions. The optimum quantity of distilled water for germination of *Verbena* was 7.5 ml per box. All other species performed best with 10 ml per box which was sufficient to wet the paper fully. These quantities of water were then used under test conditions. For priming studies using the same boxes and paper, 15 ml of polyethylene glycol (PEG) was found to be most effective.

In order to develop a simple prophylactic treatment against seed borne fungal proliferation in test conditions, seeds of all species were imbibed for 3 and 6 h in two concentrations of Iprodione (0.1 and 0.3% a.i. as Rovral, 50% a.i. wettable powder). Treated seeds gave visibly cleaner seedlings. Treatments that had no phytotoxic effect were then adopted as standard pre-treatments in all experiments unless otherwise stated. These pre-treatments were 3 h imbibition in 0.1% a.i. Iprodione for *Verbena* and *Impatiens* and 0.3% for the other species. All seeds not treated with Iprodione were soaked in water for the same period. Seeds were subsequently dried before entering priming treatments, but were not dried before entering PGR soak treatments.

Osmotic priming
Seeds of all five species were primed on solutions of 232, 273 and 342 g PEG '6000' Kg^{-1} water giving nominal osmotic potentials of –0.75, –1.00 and –1.50 MPa respectively (Michel & Kaufmann, 1973). All treatments were placed at 15 °C for three durations (7, 10 and 14 days). Following treatment seeds were placed in germination tests at 20 °C either after drying to equilibrium, after partial drying for 8 h or without drying. Treatments were compared to a double replicated untreated control.

Plant growth regulator soaks
In a series of experiments, seeds of *Primula, Impatiens* and *Verbena* were soaked in plant growth regulator (PGR) solutions for 48 h at 5 °C before drying and were then compared with controls in germination tests. The controls included untreated seeds and seeds soaked in water and in buffer. Comparisons were made at 20 °C for *Impatiens* and at 15 °C for *Primula* and *Verbena*. Germination of *Primula* and *Impatiens* PGR soaked seeds in the dark was compared to that from the controls in the light and in the dark. Comparisons of the germination of PGR soaked *Verbena* seeds with control seeds were made in the light only. *Verbena* seeds were not iprodione pre-treated.

The PGR treatment included solutions of gibberellins A_4 and A_7 ($GA_{4/7}$), benzyladenine (N-6-Benzyl-aminopurine, BA), or daminozide (N-dimethylaminosuccinamic acid) used individually and as $GA_{4/7}$ + BA and $GA_{4/7}$ + daminozide combinations. All PGRs were dissolved in 0.013 M phosphate buffer at pH 6.3. $GA_{4/7}$ was used at concentrations of 10^{-3}–10^{-5} M, BA at 10^{-4}–10^{-6} M and daminozide at 10^{-2}–10^{-4} M.

Early seedling growth from treated and untreated seeds was compared using a slope test technique described by Gray and Steckel (1983).

Combined treatments
Primula seeds were primed for 10 days at 15 °C on a PEG solution at a nominal osmotic potential of –1.50 MPa, *Impatiens* for 14 days at –0.75 MPa and *Verbena* for 14 days at –1.50 MPa. Seeds were also primed under the same conditions following PGR soaks in the most effective concentrations of $GA_{4/7}$ alone and combined with BA and with daminozide which were identified in previous experiments. Seeds were dried following the PGR soak prior to priming. In a further set of treatments seeds were primed on PEG solutions containing the same concentrations of PGRs used for soaking seed. All treatments were compared with untreated controls after drying.

Germination test comparisons were made in both the light and dark with *Primula* and *Impatiens* at 15 °C and 20 °C respectively. *Verbena* seed treatments were compared in the light only at 15 °C.

Results and discussion

The purpose of the experiments presented was to determine what improvements in seed quality might be achieved by seed treatments. As seed drying is a necessary pre-

Table 1. The effect of osmotic priming at 15 °C on the germination of 5 bedding plant species. *Primula* (–1.5 MPa, 10 days), *Impatiens* (–0.75 MPa, 14 days), *Salvia* (–1.5 MPa, 14 days), *Verbena* (–1.5 MPa, 14 days), *Petunia* (–1.0 MPa, 14 days), S.E.1, for control means, S.E.2, for other treatment means (59 DF). Angular transformations of percentages are in parentheses.

Species	Treatment	Mean germination time (days)	Spread in time to germination (in variance days)	Percentage germination
Primula	Control	12.2	2.14	82 (65)
	Primed	17.6	2.69	69 (56)
	Primed & dried	17.5	2.60	66 (54)
	S.E.1,2	1.14, 1.60	0.085, 0.121	(1.9, 2.7)
Impatiens	Control	7.9	0.72	92 (74)
	Primed	5.0	0.62	89 (71)
	Primed & dried	5.9	0.37	89 (71)
	S.E.1,2	0.13, 0.19	0.063, 0.089	(1.7, 2.4)
Salvia	Control	8.9	1.55	66 (54)
	Primed	4.0	1.19	76 (61)
	Primed & dried	8.5	1.73	64 (53)
	S.E.1,2	0.39, 0.55	0.083, 0.118	(1.8, 2.6)
Verbena	Control	7.7	1.14	69 (56)
	Primed	4.0	0.92	68 (56)
	Primed & dried	3.8	0.97	67 (55)
	S.E.1,2	0.24, 0.34	0.098, 0.139	(1.8, 2,5)
Petunia	Control	5.6	0.04	94 (76)
	Primed	2.0	0.15	94 (76)
	Primed & dried	2.3	0.28	94 (76)
	S.E.1,2	0.07, 0.10	0.099, 0.140	(1.2, 1.7)

requisite for storage and handling under commercial conditions the data selected for presentation were from treatments that gave the best response from seeds air dried after treatment. These treatments could therefore form the basis of practically useful techniques.

Osmotic priming

Priming without subsequent drying reduced mean germination time compared to that from untreated seeds of all species except *Primula* (table 1). Germination began earlier from primed *Primula* seeds, but as a result of a greater spread in time to germination mean germination time was delayed compared to untreated seeds. There was relatively little effect of priming on the spread in time to germination of other species. Priming increased percentage germination of *Salvia* seeds, but reduced percentage germination of *Primula* seeds. The general effects of priming on *Impatiens* seeds are in agreement with those published elsewhere (Simmonds, 1980; Frett and Pill, 1989), but optimum treatments cannot be compared as different ranges of treatments were used in each study.

The effect of drying seeds following priming was small except with *Salvia* where

Table 2. The effect of PGR soak treatments on percentage germination (angular transformation in parentheses) of *Primula* and *Impatiens* seeds in the dark and subsequent growth of *Impatiens* seedlings on slope tests in the light.

Treatment	*Impatiens*					*Primula*
	Percentage germination	Shoot length		Root length		Percentage germination
		mm	cv(%)	mm	cv(%)	
Control	99 (85)	4.5	29	51.7	46	64 (53)
Control in the dark	79 (64)					41 (40)
$GA_{4/7}$ 10^{-4}M	89 (71)	4.7	33	51.7	39	55 (48)
$GA_{4/7}$ 10^{-3}M	90 (72)	7.5	42	41.5	56	53 (47)
$GA_{4/7}$ 10^{-4}M + BA 10^{-6}M	93 (77)	5.0	33	48.1	47	48 (44)
$GA_{4/7}$ 10^{-3}M + BA 10^{-5}M	92 (74)	7.0	44	31.4	71	50 (45)
$GA_{4/7}$ 10^{-4}M + D 10^{-4}M	90 (72)	5.0	37	49.0	48	51 (46)
$GA_{4/7}$ 10^{-3}M + D 10^{-3}M	95 (79)	8.4	33	47.6	41	57 (49)
S.E. (DF = 46)	(3.2)	0.17	2.1	2.03	2.9	(2.5)

germination was reduced and delayed compared to that from undried primed seeds. In this species even the partial drying treatment damaged seeds. Drying damage was also reported following low temperature imbibition treatments to *Salvia* seeds (Carpenter, 1989). Here drying damage also occurred in *Salvia* seeds primed for shorter durations and in seeds primed in a further experiment using solutions with more negative osmotic potentials (–2.0 MPa) to prime seeds. This suggests that damage is not the result of 'over priming'. The results of Carpenter (1989) show a progressive reduction in percentage germination as solutions with increasingly negative osmotic potentials were used for priming *Salvia* seeds. In these experiments no such trend was shown.

Plant growth regulator soaks

In a previous paper (Finch-Savage, Gray and Dickson, 1991) it was shown that germinating *Primula* and *Impatiens* seeds on $GA_{4/7}$ or $GA_{4/7}$ + BA solutions, respectively, promoted germination in the absence of light. Such experimental observations in celery have been used to develop commercially effective seed soak treatments (Thomas and Whitlock, 1980). This soak treatment technique was tested here with *Primula*, *Impatiens* and *Verbena* seeds. High $GA_{4/7}$ concentrations may be required for an effective soak treatment which could result in residual etiolation effects. In an attempt to reduce the concentration of $GA_{4/7}$ required it was used in combination with daminozide and with BA which have promoted $GA_{4/7}$ effects on germination in other species (Thomas *et al.*, 1975; Biddington, Thomas and Dearman, 1980). The results of seed soak treatments with *Primula* and *Impatiens* are summarised in Table 2. Germination in the dark was increased by soaking seeds in solutions containing $GA_{4/7}$ both with and without the addition of BA and of daminozide. The most effective treatment with both species involved the highest concentrations of $GA_{4/7}$ and daminozide. However, early

seedling growth measurements on *Impatiens* show significant etiolation and reduced root growth when $GA_{4/7}$ was used at 10^{-3} M (table 2). Seedling growth from seeds soaked in $GA_{4/7}$ at 10^{-4} M was not significantly different from the control, but a small though significant increase in shoot length occurred with the further addition of BA or daminozide. There was little effect of BA and daminozide on *Impatiens* root growth, but with *Primula* BA at 10^{-5} M significantly increased the number of abnormal roots in seedling evaluations. PGR seed soaks can therefore be effective, but the concentration used is critical if adverse effects are to be avoided.

PGR soaks also increased percentage germination of *Verbena* seeds (Figure 1) in contrast to earlier reported results with the same cultivar germinated on PGR solutions (Finch-Savage, Gray and Dickson, 1991). Mean germination time of *Verbena* was reduced by soaking seeds in $GA_{4/7}$ at 10^{-3}M (7.0 days) compared to that in the untreated control (8.4 days, SE 0.41, DF 18). However, soaking seeds in $GA_{4/7}$ at 10^{-4}M did not significantly reduce mean germination time either alone (8.7 days) or with the addition of BA (8.6 days) or daminozide (8.3 days).

The percentage germination of *Verbena* seeds can be variable even under the same test conditions and this is largely due to substrate moisture levels (Heit, 1963). Preliminary experiments showed the need for reduced substrate moisture levels in seed tests with *Verbena* compared to other species. These results are in agreement with those of Heit (1963) and of Ely (1979). It is suggested that the higher substrate moisture levels used in the previous study (Finch-Savage, Gray and Dickson, 1991) masked the beneficial effects of PGRs that were demonstrated here. In related experiments not reported here a clear relationship was shown between *Verbena* seed colour and germination performance. Seed quality progressively improved from green through light brown to dark brown seeds. Green seeds presumably were less mature at harvest.

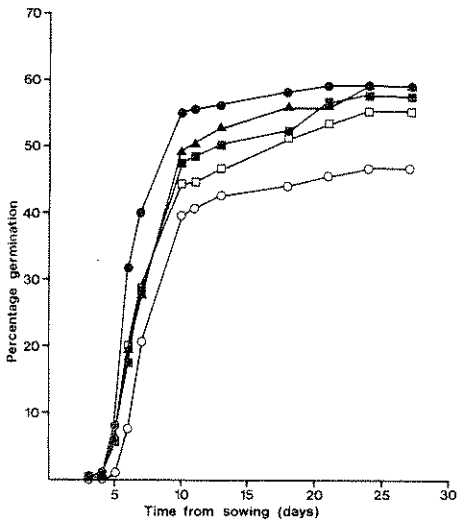

Figure 1. Germination response of *Verbena* seeds to PGR soak treatments. ○, Control; ●, $GA_{4/7}$ 10^{-3}M; □, $GA_{4/7}$ 10^{-4}M; ■, $GA_{4/7}$ 10^{-4}M + BA 10^{-5}M; ▲, $GA_{4/7}$ 10^{-4} + daminozide 10^{-3}M.

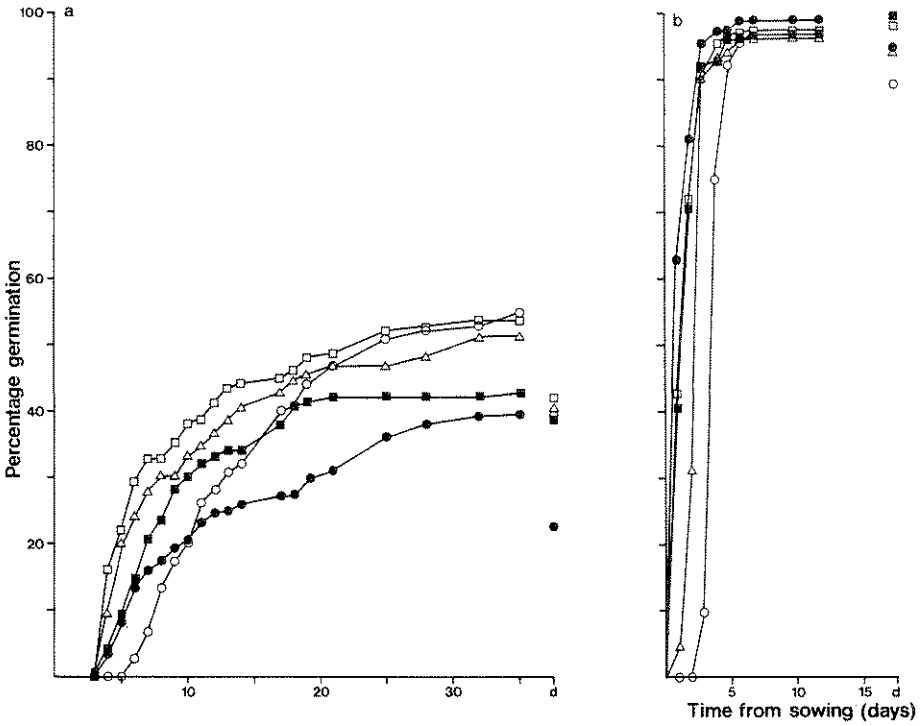

Figure 2a. Germination response of a, *Primula* and b, *Impatiens* seeds to priming in solutions containing PGRs. ○, Control; ●, Primed; □, Primed with $GA_{4/7}$ 10^{-4}M; ■, Primed with $GA_{4/7}$ 10^{-4}M + BA 10^{-6}M (*Primula* and *Impatiens*) or $GA_{4/7}$ 10^{-4}M + BA 10^{-5}M (*Verbena*); △, Primed with $GA_{4/7}$ 10^{-3}M + daminozide 10^{-3}M (*Primula* and *Impatiens*) or $GA_{4/7}$ 10^{-4}M + D 10^{-3}M (*Verbena*). Germination percentages in the dark for *Primula* and *Impatiens* are shown at d, on the x axis.

The potential therefore exists to further increase *Verbena* seed quality by colour sorting.

Combined treatments

There are few reports in the literature on the response of seeds to combinations of treatments. Here several combinations of treatments were tested including: a combined fungicide and PGR soak followed by priming; a fungicide soak followed by priming in solutions containing PGRs; and a fungicide soak followed by priming without PGRs. A fungicide soak was used here as a more accurate method of application than dusting with a powder, especially on an experimental scale. Fungicides applied by soaking are also less likely to interact with subsequent treatments. In general, there was no significant difference in germination response between the two methods of combining priming and PGR treatments in any species. Results are therefore presented from treatments that had PGRs added to the priming solutions (Figure 2a and 2b). Seeds from all priming treatments germinated earlier than untreated seeds. For the untreated seeds and most effective priming treatments, mean germination time for

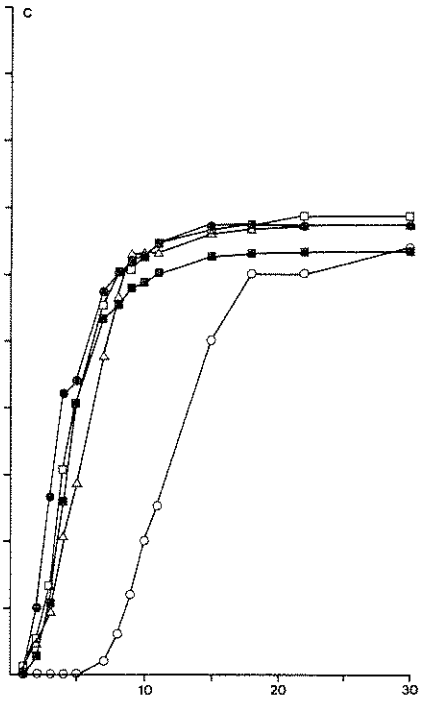

Figure 2b. Germination response of c, *Verbena* seeds to priming in solutions containing PGRs. ○, Control; ●, Primed; □, Primed with $GA_{4/7}$ 10^{-4}M; ■, Primed with $GA_{4/7}$ 10^{-4}M + BA 10^{-6}M (*Primula* and *Impatiens*) or $GA_{4/7}$ 10^{-4}M + BA 10^{-5}M (*Verbena*); △, Primed with $GA_{4/7}$ 10^{-3}M + daminozide 10^{-3}M (*Primula* and *Impatiens*) or $GA_{4/7}$ 10^{-4}M + D 10^{-3}M (*Verbena*). Germination percentages in the dark for *Primula* and *Impatiens* are shown at d, on the x axis.

Primula was 13.8 and 8.7 days (SE 0.18, DF 18), respectively, corresponding figures for *Impatiens* were 3.7 and 1.5 days (SE 0.08, DF 18), and for *Verbena* 12.7 and 4.5 days (SE 0.46, DF 38). With *Primula*, priming reduced percentage germination as it did in earlier experiments, however when $GA_{4/7}$ was added to the priming solution percentage germination was not significantly different to that from untreated seed. The reason for this reduction in germination percentage following priming is not understood and requires further investigation. Percentage germination of *Impatiens* and *Verbena* seeds in all priming treatments was not significantly different from that in the untreated control. In the dark, percentage germination of both *Primula* and *Impatiens* was increased by priming seeds in solutions containing PGRs. The most effective treatments were priming with $GA_{4/7}$ and $GA_{4/7}$ with BA for *Primula* and *Impatiens* respectively. These were also the most effective PGR treatments for these species when not combined with priming (Finch-Savage, Gray and Dickson, 1991). The addition of BA or daminozide to $GA_{4/7}$ in the priming solution had no additional benefit with *Primula* or *Verbena* seeds.

Seed treatments have been shown to have beneficial effects on all the species studied in the relatively unstressfull conditions of the seed test. However, with *Salvia* the potential value of practical seed treatments is limited by seed damage during subsequent drying. More work is required to develop and optimise the treatments outlined in these experiments. In a further study (Finch-Savage, 1991) the potential to 'scale up' these techniques and their effect on seedling production in cellular trays from seeds

of the same species was investigated.

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