Microbial enhancement of seed germination in *Rosa corymbifera* 'Laxa'

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Abstract

Germination of native tree and shrub species from seed can be unpredictable. Germination of *Rosa corymbifera* 'Laxa' was 2% under normal commercial conditions. This was obtained in the presence of the natural microflora found on the seeds. The microflora originated on the hips and the seeds become inoculated during extraction. Exclusion of microbes from such pretreatments resulted in no germination. Inoculation of surface sterilized seeds with members of the natural microflora resulted in 3% germination. The addition of Garotta™, a commercial compost activator, to the commercial pretreatment increased germination to 95%. This high germination percentage was sustained over a 5 year period using seeds from the same stock bushes. Addition of the compost activator resulted in a 20-fold increase of microbial activity in the pretreatment mixture, indicating that enhanced microbial growth resulted in higher and more predictable germination percentages.

Keywords: commercial compost activator, *Rosa corymbifera*, microbial enhancement of germination, microflora

Introduction

Owing to the variation encountered with tree and shrub seed germination from year to year within a species (Rolston, 1978), researchers have devoted much time and effort over the decades to increasing germination percentages and speed of emergence. Most studies have been of a physiological nature, with particular reference to hormones [see Jackson (1968) for a review with respect to *Rosa*], afterripening and germination [Amen (1968), with early work by Tincker and Wisley (1935) on rose seeds].

Although much has been published regarding tree and shrub seed germination, there is no definitive work explaining exactly how and why a seed may be dormant, and how this dormancy can be overcome. Many papers report altered levels of endogenous hormones during seed pretreatment, e.g. a decline in abscisic acid in *Rosa* (Tillberg, 1983; Yambe *et al.*, 1992), *Crataegus* (Qrunfleh, 1991) and *Acer* (Webb and Wareing, 1972; Pinfield and Davies, 1978). Evidence for the relationship between hormones and dormancy is often circumstantial, with a rise or fall of a particular hormone coinciding with alleviation of dormancy or increase in germination.

Most seeds of native tree and shrub species are generally viable, but to obtain commercially acceptable germination percentages, pretreatment prior to sowing is required. Dormancy is a natural mechanism that has evolved in tree and shrub species, resulting in germination over a considerable time period (Harper, 1977). This mechanism is more likely to ensure survival of a species than would 100% germinability as soon as the seeds mature. Dormant seeds are unacceptable from a commercial point of view; a high yielding, uniform population is desired (Bewley and Black, 1994). Pretreatment must overcome inherent dormancy characteristics, which may be exogenous or endogenous in nature (Nikolaeva, 1977; Baskin and Baskin, 1998).

Seeds of most tree and shrub species that are dormant require a warm (20–30°C) and cold (1–5°C) pretreatment of approximately 24 weeks, although time spans vary considerably. The ratio of recommended warm to cold periods varies from 2 weeks warm followed by 18 weeks cold (e.g. *Prunus* spp.) to a recommended 40 weeks warm and 24 weeks cold for *Ilex* spp. (Gordon and Rowe, 1982). Nikolaeva (1977) classified dormancy into two distinct forms: endogenous (morphological or physiological) or exogenous (physical or mechanical). It is usually a combination of both forms that contribute to dormancy in tree and shrub seeds. Morphological dormancy is caused by underdeveloped embryos, although a period of warmth allows embryos to fully develop (Schopmeyer, 1974; Nikolaeva, 1977). Physiological dormancy requires exposure of fully imbibed seeds to

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cold conditions (1–5°C). The time required under such conditions to overcome physiological dormancy depends upon the depth of dormancy and is species specific. This form of dormancy release is not fully understood, but appears to affect the balance and subsequent effect of growth regulators (a rise in endogenous stimulators and a reduction in inhibitors) and mobilization of storage products (Lewak and Rudnicki, 1977).

Physical dormancy, or hardseededness (the impermeability of the seed coat to water) is removed gradually under natural conditions by biological, thermal and other influences (Nikolaeva, 1977) and may take many years. Scarification is often used to break physical dormancy (Nikolaeva, 1977). Mechanical dormancy is the prevention of embryo growth by a tough outer covering (Nikolaeva, 1977). Such coverings are often thick and fibrous but permeable to water with prolonged soaking (Schopmeyer, 1974). Under natural conditions the best methods for overcoming mechanical constraint are the biological processes stimulated by warm conditions (i.e. decay caused by microorganisms, for example in the leaf litter where the seeds normally fall). Such processes are speeded up commercially by controlling the environment, thus ensuring warmth, moisture and aeration. As with other tree and shrub species, *Rosa corymbifera* 'Laxa' seeds have a structural weakness (the suture) which, once split, exposes the embryo to effective leaching of inhibitors (Nikolaeva, 1977). A few authors mention seed coat decay in passing, but without citation or evidence (Trumble, 1937; Jackson and Blundell, 1963; Campbell, 1985; Bradbeer, 1988; Mayer and Poljakoff-Mayber, 1989; Bewley and Black, 1994). However, Pfeiffer (1934), from studies on snowberry [*Symphoricarpos racemosus*: now *S. albus* (L.) S.F. Blake (Stace, 1991)] found that fungi or another agent capable of fibre decomposition was necessary for germination. She found that seeds would not germinate in the absence of fungi and concluded it was entirely possible that fungi play a role in the disintegration of hard layers of other seeds or fruits, especially indehiscent forms, similar to *S. racemosus*.

This paper aims to show the essential role of natural microflora in the pretreatment of *Rosa corymbifera* 'Laxa', the rootstock of the British Rose industry (closely related to *Rosa canina* 'Inermis', which is extensively used in Dutch rootstock production). The subsequent high and consistent level in germination achieved when these microflora are allowed to flourish with the addition of Garotta™ (a commercial compost activator) is also shown. The results were obtained over a 5 year study period (Morpeth, 1998).

Materials and methods

Plant material and pretreatment

Rosa corymbifera 'Laxa' hips were harvested from stock bushes at Writtle College, Chelmsford, UK and from stock plants at Wheatcroft Nurseries, Nottingham, UK. The hips (pseudocarps typical of the genus *Rosa* containing achenes, subsequently referred to as seeds) were picked when red and firm, and manually crushed (to aid seed extraction) prior to soaking in sterile tap water (1 kg hips/2 l water). After 3 d, the seeds were recovered from the softened fruit by sieving. Water from the extraction process was retained when required for inoculation experiments. Seeds were washed in sterile tap water and then air dried. Seeds were mixed with vermiculite, an inorganic medium with good water retention properties, since it is essential to keep the seeds moist during temperature pretreatment (Gordon and Rowe, 1982; Gosling and Aldhous, 1994). Vermiculite contains no nutrients and was sterilized (autoclaved) before use.

Seed samples were subjected to different pretreatment conditions (Fig. 1). The commercial (control) treatment was based on moist vermiculite and seed (10:1 w/w vermiculite/seed). The enhanced treatment was achieved by the addition of Garotta™ (J. Arthur Bower's, Lincoln, UK), a commercial compost activator (10% w/w Garotta™/seed). This activator is a widely available sterile product consisting of a mixture of inorganic mineral nutrients such as calcium carbonate, ammonium salts and phosphates (Morpeth, 1998). Any visual changes in the physical properties of the seeds were recorded on a weekly basis. The control and enhanced treatments were applied to fresh collections of seeds, from the same stock bushes, every year from 1992 to 1996. Detailed data are provided for the 1996 experiments.

Surface sterilization of seeds

Both control and enhanced treatments were applied to surface-sterilized seeds in 1996. Surface sterilization of seeds was achieved by taking pre-soaked seeds and agitating them for 20 min in 2% v/v detergent (Fairy Liquid, Proctor and Gamble, Newcastle, UK). The seeds were then rinsed with sterile distilled water and agitated for 5 min in excess 70% ethanol. Rinsing was repeated, and excess 2% w/v sodium chloride solution was added to the seeds with agitation for 20 min, followed by a final rinse with sterile distilled water. This surface sterilization procedure was adopted after several less rigorous combinations failed to retain a sterile environment beyond 2 weeks.

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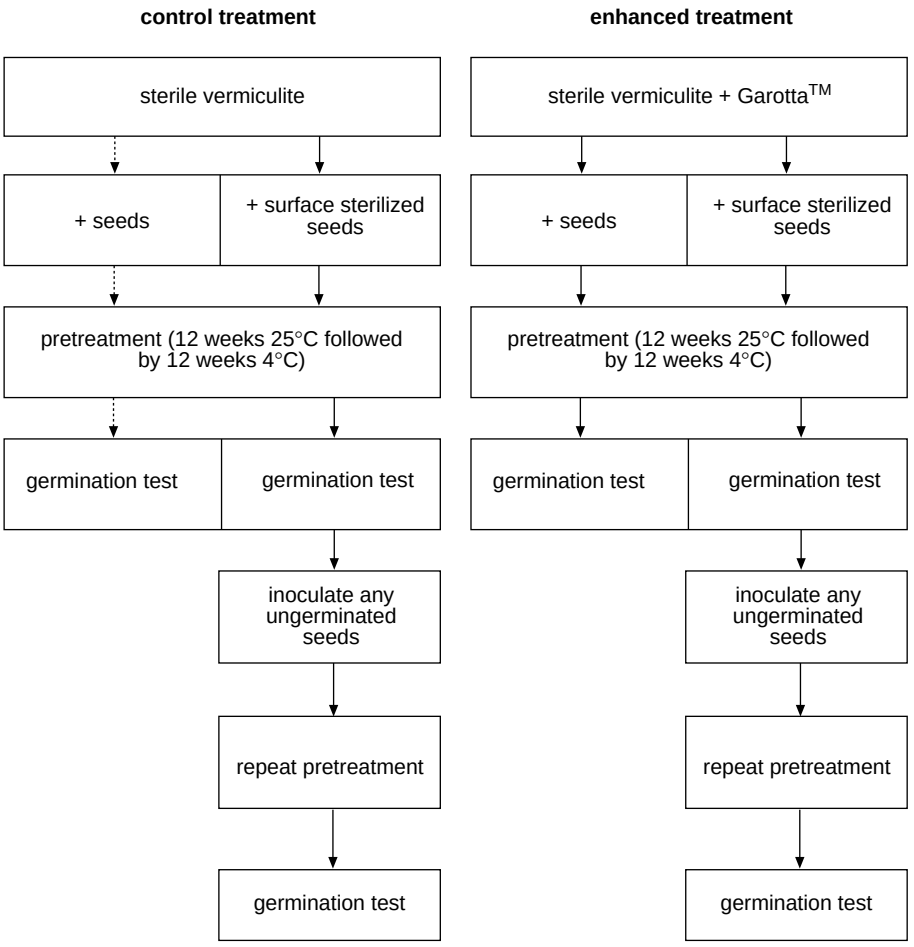

Figure 1. Summary of the different treatments used to pretreat *Rosa corymbifera* 'Laxa' seeds. Lighter arrows indicate the commercial treatment.

To show that the viable, ungerminated, surface-sterilized seeds following both treatments required microbes to germinate, new experiments were conducted in 1997 and 1998, in which they were inoculated with the water in which *Rosa corymbifera* 'Laxa' seeds had been extracted. A second pretreatment of 24 weeks (subsequently referred to as 'inoculated' treatments) was then carried out on these seeds.

Germination test

Germination tests with six replicates of 100 seeds for each treatment were carried out at 20°C on moist filter paper (Whatman No. 1, 90 mm; Whatman, Maidstone, UK) in 9 cm Petri dishes. After final germination counts, viability of the remaining ungerminated seeds was determined using the excised embryo technique (cutting the seed coat and visually assessing the intact embryo). Firm, white embryos were scored as viable.

Microbial counts

Ten randomly selected ripe hips were individually harvested aseptically from the parent bushes into 10 ml sterile tap water by cutting the flower stalk at the base of the hip with a sterile scalpel and allowing the hip to drop into a 20 ml sterilized flask. Microbial spread plates (Dhingra and Sinclair, 1995) were made from this water (following agitation) and from seeds removed under aseptic conditions from the hips. Sampling of seeds also occurred during their extraction from the hips.

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Statistical analysis

An analysis of variance was performed on transformed data to evaluate the differences in the percentage germination of seeds following the pretreatments.

Results

The presence of microorganisms during the pretreatment of *Rosa corymbifera* 'Laxa' seeds was essential for germination. Germination following pretreatment under control conditions was only 2% and variable (SE ± 0.6) (Table 1), while that of the enhanced treatment was 95.2% and consistent (SE ± 0.4) (Table 1). When these treatments were conducted using surface-sterilized seeds, no germination was recorded in either case. After only 2–3 weeks into the warm period of the enhanced treatment, seeds split open (Tables 1 and 2) and turned dark brown (Morpeth, 1998). However, seeds in the control and surface-sterilized treatments did not split open.

The experiments were repeated to include seeds that had been surface-sterilized and had already undergone one pretreatment cycle but failed to germinate after treatment whilst remaining viable (Table 2). These seeds were then inoculated with the microflora found on unsterilized seed and subjected to a further 24 week pretreatment period. After the enhanced treatment, germination percentages were elevated compared with the control, although overall percentages were reduced (Table 2).

There was a significant difference ($P < 0.001$) between the control and enhanced treatments, and this was also the case when surface-sterilized seeds were inoculated with microbes and pretreated for a second time. There was no significant difference between the enhanced and inoculated enhanced treatments.

Microbial loading (fungal and bacterial colony forming units, cfus) was determined for seeds during extraction as well as for the constituents of the pretreatment. The results (Fig. 2) clearly show that the natural microbial loading required for germination enters the pretreatment mix from the seed. This loading originates on the hips (Fig. 2) and is transferred to the previously sterile seeds during extraction, which is effectively a fermentation process. The highest microbial numbers were recorded 72 h into the extraction process, the stage at which the hip material had softened to allow extraction of the seed. The other constituents of the pretreatment (i.e. vermiculite and Garotta™) were sterile.

Discussion

Germination was very low (2%) following the control treatment (commercial conditions) when seeds had been extracted from rose hips and pretreated in vermiculite. A natural microbial loading was found in the pretreatment that originated on the rose hips of the parent bushes. However, these pretreatment conditions are unnatural and essentially provide starvation conditions for the microbes as vermiculite is totally lacking in nutrients. The addition of a sterile compost activator (a source of inorganic nutrients) to the vermiculite and seed mix resulted in greatly increased germination (95.2%).

The presence of the natural microbial loading was essential for germination of *Rosa corymbifera* 'Laxa'. When pretreatment studies were repeated using a subsequent seed harvest, low per cent germination with the control treatment and high per cent germination with the enhanced treatment was again observed (Table 2). When these treatments utilized surface-sterilized seeds to eliminate any microbes during pretreatment, no germination resulted in either the nutrient-starved (control) or nutrient-rich (enhanced) treatments (Table 2). These seeds remained viable. When these seeds were subsequently inoculated with the equivalent natural microflora that had previously been removed by sterilization, increased germination (approx. 50%) was observed for

Table 1. Percentage germination (mean ± SE) and viability of *Rosa corymbifera* 'Laxa' seeds following control and enhanced pretreatments. Germination percentages not followed by the same letter were significantly different from each other at the 0.001 probability level. S, seeds split open after 2–3 weeks at 25°C; US, seeds remained unsplit throughout pretreatment

	Germination (%)	Viability (%)	Germination (%) 5 year average
Control^{US}	2.0 ± 0.6^{a}	100	26
Control (surface-sterilized seeds)^{US}	0 ± 0^{a}	100	–
Enhanced^{S}	95.2 ± 0.4^{b}	100	89
Enhanced (surface-sterilized seeds)^{US}	0 ± 0^{a}	100	–

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Table 2. Percentage germination (mean ± SE) and viability of *Rosa corymbifera* 'Laxa' seeds following control and enhanced treatments, control and enhanced treatments with surface-sterilized seeds and inoculated treatments (following surface-sterilized seed treatment). Germination percentages not followed by the same letter were significantly different from each other at the 0.001 probability level. S, seeds split open after 2–3 weeks at 25°C; US, seed remained unsplit throughout pretreatment

	Germination (%)	Viability (%)
Control^{US}	1.8 ± 1.1^{a}	100
Control (surface-sterilized seeds)^{US}	0 ± 0^{a}	100
Control (surface-sterilized seeds) inoculated^{US}	2.8 ± 0.8^{a}	100
Enhanced^{S}	52.7 ± 3.7^{b}	100
Enhanced (surface-sterilized seeds)^{US}	0 ± 0^{a}	100
Enhanced (surface-sterilized seeds) inoculated^{S}	55.0 ± 2.1^{b}	100

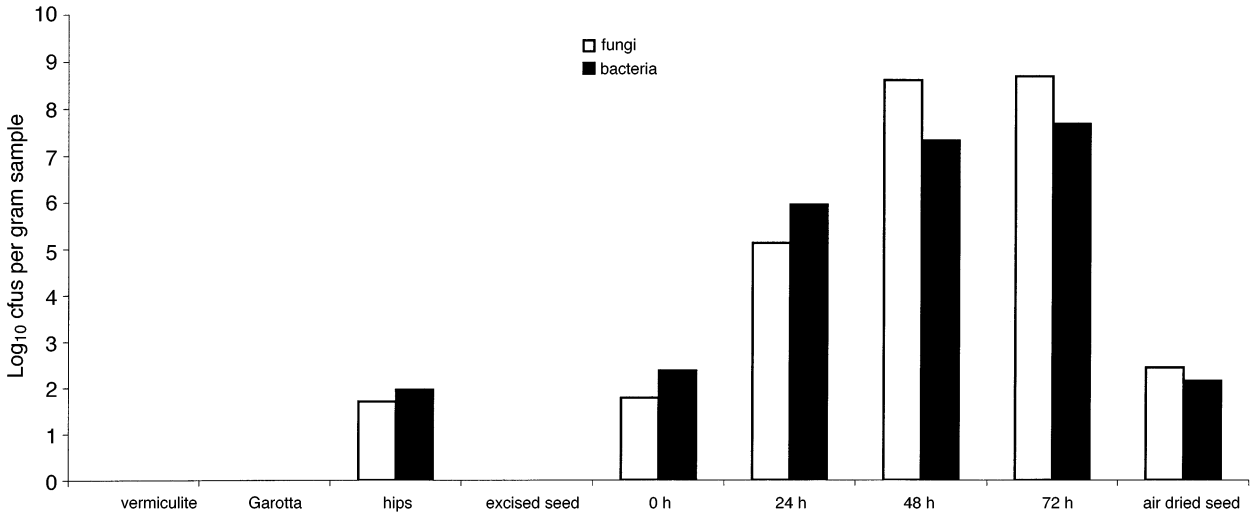

Figure 2. Microbial loading of the constituents of the pretreatment and of the seeds during extraction (hours after crushing) of *Rosa corymbifera* 'Laxa'.

the enhanced treatment (Table 2), showing that it is the presence of microbes which breaks mechanical dormancy in *Rosa corymbifera* 'Laxa' seed.

These results compare well with the results of a 5 year study (Morpeth, 1998, Table 1). The increased activity of the natural microflora caused by the compost activator promoted germination levels to a consistently high level. This is achieved by facilitating the splitting of the seed at the suture during the warm period of pretreatment. The splitting of the seed may render the embryo accessible to increased circulation of water during the cold period of pretreatment, allowing leaching of germination inhibitors (Nikolaeva, 1977). Total absence of microbes resulted in zero germination, although the control treatment showed that just their presence was sufficient for low levels of germination. Under natural conditions in the soil, microbes would grow much better than in the control treatment due to the presence of nutrients, so dormancy break and thus germination could be expected to be greater than the control. However, it is unlikely that the levels of germination found in the enhanced treatment would occur in soil, as nutrient levels and microbial activity would be lower.

Enhanced microbial activity and microbial numbers resulted in seeds turning dark brown and splitting (Tables 1 and 2) after only 2–3 weeks (Morpeth, 1998). Seeds in the control pretreatment remained light brown and failed to split. This splitting was a clear indication that the mechanical dormancy of *Rosa corymbifera* 'Laxa' had been broken. No surface-sterilized seeds germinated in any of the pretreatments although viability remained at 100%. Thus, the mechanism by which dormancy was

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overcome under control conditions and the mechanism by which germination was enhanced by the addition of Garotta™ had been eliminated by surface sterilizing the seed. Therefore, the microbial loading and subsequent growth of microorganisms during the pretreatment facilitated seed germination. This contrasts markedly with the current practice whereby a commercial grower may introduce fungicides to combat potentially harmful microbes (Morpeth *et al*., 1997).

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