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Project number:	**HNS 51**
Project leader:	**F. J. Cullum** **Writtle College** **Chelmsford** **Essex CM1 3RR**
Location:	**Writtle College, University of Hertfordshire and Oakover Nurseries, Kent**
Project Co-ordinator	**Mr Tom Wood**
Date commenced:	**1 October 1992**
Date completed:	**March 1995**
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PRACTICAL SECTION FOR GROWERS

Objectives and background

Seed germination can be a limiting factor in the production of native trees. Both emergence and percentage germination are highly variable. A more predictable seed batch at sowing is required by the commercial producer to reduce unnecessary expenditure particularly when estimating how much seed to harvest, treat and how much seed bed to prepare. Supply can also be an associated problem, in that if germination rates are low and unpredictable, sufficient quantities of native seed may not be available - resulting in imports of foreign seed.

Mature seeds of most native tree and shrub species require pretreatment prior to sowing. This is because with few exceptions tree seed will not germinate immediately after maturation and dispersal from the mother plant. This failure to germinate is caused by dormancy, which is common to most plant species in one form or other, and ensures germination occurs under the most conducive conditions possible.

It is this dormancy which presents the most problems to the hardy nursery stock producer, as it can manifest itself in several ways including low percentage germination, sporadic emergence and poor yield of 'useable' seedlings. Such a plethora of factors can cause and influence the depth of dormancy that the task of predicting germination rates is almost impossible. Seed of the same species harvested at different geographical locations will have different germination rates, and prevailing weather conditions during fruit set can greatly influence dormancy levels.

Under natural conditions, seed is shed in the autumn and dispersed by various mechanisms. In the majority of cases native tree species have a hard woody seed coat which has to be breached before germination can occur. This physical dormancy is overcome naturally by breakdown of the seed coat over a varying length of time depending upon species. Decay occurs under the influence of microorganisms occurring naturally in the soil and leaf litter. However, as the seed is shed at the end of the year and the onset of winter, little decay occurs due to low temperatures which do not facilitate microbial growth. As temperatures rise in the spring and summer, microbial growth increases rapidly and will break down the seed coat. Therefore, not until the following spring will the majority of the seed germinate, as winter chilling is required for the removal of endogenous inhibitors.

This type of scenario has resulted in such seeds being classed as "two year seeds", requiring a warm period in the soil to breakdown the coat, followed by a cold period to overcome physiological dormancy. The grower overcomes these requirements by a process which is commonly referred to as stratification. This is an artificial warm period followed by a cold period using controlled conditions, to give both treatments before the first spring after harvest. However, germination can still be low and inconsistent, as what is an 18 month spell in the natural environment is condensed into 6 months (at most) artificially or pretreatment cannot start until the spring following harvest if a longer period of treatment is necessary.

It was with these ideas in mind that the role of microbial organisms in seed pretreatments have been looked at from a grower and scientific point of view. Field

and laboratory trials were conducted on several species representing a cross section of native trees and shrubs. However, the majority of work focused on one species, *Rosa corymbifera* 'Laxa', traditionally a rootstock for roses which preliminary trials had shown to be responsive to the pretreatment with a compost activator. The results obtained from these specific studies would appear to be of importance in the pretreatment of all similar structured native tree and shrub species.

Summary of Results

The fundamental hypothesis to this work is that microbes are beneficial during the warm period of pretreatment of seed and will enhance germination following the cold pretreatment. It was consistently found that under a commercial pretreatment where microbes were present but not encouraged, germination was low and unsatisfactory. However, when a compost activator (Garotta) was added to the pretreatment, germination of *Rosa corymbifera* 'Laxa' was greatly increased. It was also found that the year to year variation (or range) of germination was much more consistent than for the commercial pretreatment (figure 1).

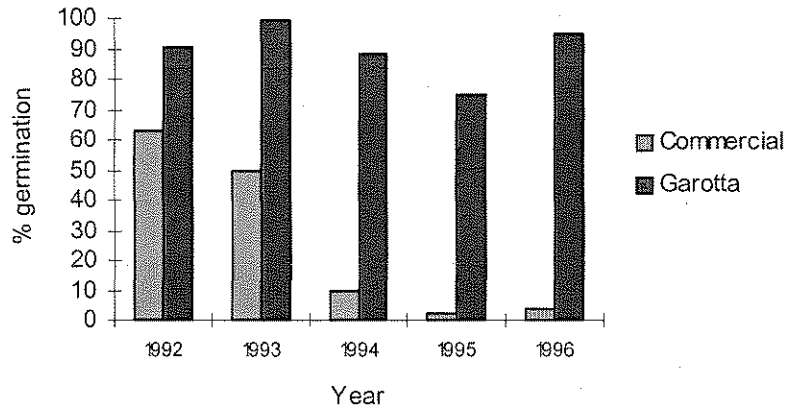

Figure 1 Germination (after 14 days) following pretreatment of *Rosa corymbifera* 'Laxa' under commercial conditions and with the addition of Garotta. Both pretreatments were subjected to the same pretreatment conditions (12 weeks at 25°C followed by 12 weeks at 4°C).

When the microbial levels were compared between the two pretreatments, numbers and activity were greatly enhanced in the presence of the compost activator over the commercial pretreatment. Trials were conducted using surface sterilised seed and pretreatment conditions, resulting in no seed germination. Thus the presence and levels of microbes enhanced the germination seen in figure 1.

Studies showed that in the presence of Garotta seed chitted within 3-4 weeks in the warm (ambient ≈ 25°C) period of pretreatment, and this served as the indicator as to when to transfer the seed into the cold (4°C). This also resulted in a total pretreatment of only 18 weeks, which is considerably shorter than the more usual and recommended time for this species.

Field trial data were equally convincing, showing that this technique could easily be used to produce *Rosa corymbifera* 'Laxa' in the field. Other species (e.g. *Crateagus monogyna*, *Prunus spinosa*, *Tilia platyphyllos*, *Rosa canina* and *Acer campestre*) were also pretreated in a similar way, however for the requirements of the work the same pretreatment temperatures and lengths were used as for *Rosa corymbifera* 'Laxa'. This resulted in encouraging results, which would have shown further enhancement if they had been given more exact requirements to their needs. The exact temperature and duration of pretreatments for these need to be tailored for each species.

Action points for growers

- The presence of naturally occurring microbes was found to be essential for the successful pretreatment of *Rosa corymbifera* 'Laxa'.

- Aeration, particularly during the warm period of pretreatment, is essential to retain viability of seed lots - elevated CO_2 levels inhibit subsequent germination. However a balance must be found between aeration and water loss. Aeration is also essential for beneficial microbes, whereas poor aeration results in detrimental microbes causing damage.

- Increasing the numbers and therefore subsequent activity of the microbes using a compost activator greatly enhanced germination over a non enhanced commercial equivalent pretreatment.

- The occasional commercial practice of incorporating fungicides and seed surface sterilisation into pretreatments would inhibit microbes. This helps control the detrimental microbes, but at the same time will prevent the beneficial microbes from having their effect.

- This pretreatment technique does not eliminate the requirements of a warm and cold period, however the length of the warm period can be reduced.

- The extraction of the seed by softening fruits in water (a type of fermentation) lends itself extremely well in providing a microbial 'loading' for the seed.

Practical and financial benefits from study

The addition of a small amount of compost activator at the start of pretreatment of *Rosa corymbifera* 'Laxa' greatly increased percentage germination, produced a more rapid and synchronous emergence and a consistent range of germination over the trial period. This was also found with other species, albeit not on such a marked level. However, in the course of trialling over 18 species, none showed a detrimental response to being treated with a compost activator, and the majority showed a significant enhancement in germination.

Practically this means the grower can be more confident in predicting germination rates, which can be elevated over those normally found. This reduces either the amount of seed harvested or purchased, and so reduces the costs associated with production thereafter. Included in this would be more exact field rates, and thus reduced amounts of land required to produce the same quantity of trees. All cultural aspects would be reduced because of this, including irrigation, spraying and weeding.

As the emergence is more synchronous, there is less grade out wastage. Added to these factors the compost activator used is a safe household product with no harmful effects to the user or the environment. The technique can be used to replace acid scarification, eliminating the use of noxious chemicals, the cost involved, and of course the associated COSHH and disposal problems.

Several growers have tried this technique, and have reported very encouraging results. It is applicable to a wide range of hard coated seeds, particularly within the *Rosaceae* family.

CURRENT PLANT SCIENCE AND BIOTECHNOLOGY
IN AGRICULTURE

Basic and Applied Aspects of Seed Biology

R.H. Ellis
M. Black
A.J. Murdoch
T.D. Hong
editors

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Basic and Applied Aspects of Seed Biology

Proceedings of the Fifth International Workshop on Seeds, Reading, 1995

Editors

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29. The Involvement of Microbes and Enzymes in the Pretreatment of Woody Seeds to Overcome Dormancy

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Abstract

The addition of a compost activator, 'Garotta', to an otherwise 'normal' commercial stratification of *Rosa corymbifera* 'Laxa' has been found to greatly enhance the percentage germination at the end of this period. Germination in this study was seen to rise from 21 to 81% in the field and more markedly from 10 to 88% in the laboratory. Commercial stratification occurs over 24 weeks for this species, consisting of 12 weeks at 25°C followed by 12 weeks at 4°C. Studies have found that whilst the cold period is critical, i.e. it cannot be shortened, the warm period can be reduced by at least 6 weeks. During this warm period microbes are encouraged by providing near ideal conditions for their growth, warmth, moisture and a food source ('Garotta'). It is suspected that the combination of these conditions produces the enhanced germination.

Introduction

Woody tree seed often exhibits dormancy which usually prevents synchronous germination at the wrong time of year. This obviously has advantages for survival, but from a commercial point of view is undesirable. When seed is dispersed naturally, the hard seed coat is exposed to microbial decay in the soil, causing the seed coat to be weakened and any inhibitors can then be degraded or leached from the seed (Trumble, 1937; Campbell, 1985; Bewley and Black, 1994; Mayer and Poljakoff-Mayber, 1975; Bradbeer, 1988). Seeds usually shed in the autumn may not start decaying until the following spring and summer when temperatures rise to stimulate microbial growth (Jackson and Blundell, 1963) and will then germinate in the second spring following a cold period over the winter (Crocker, 1948).

Traditional stratification attempts to mimic the conditions experienced in nature; however this can often lead to poor germination both in quantity and quality. Such commercial stratification is based on guidelines for individual species (Lines, 1987), and it is therefore not surprising to find great variation in germination from year to year when such parameters as prevailing weather conditions influence the state of dormancy within an individual tree (Rolston, 1978).

This paper reports a novel pretreatment for woody seeds using a proprietary compost activator, 'Garotta', and discusses the probable mechanisms involved.

R.H. Ellis, M. Black, A.J. Murdoch, T.D. Hong (eds.), Basic and Applied Aspects of Seed Biology, pp. 261-277.

The aim of this research is to identify the process leading to enhanced germination with *Rosa corymbifera* 'Laxa' and translate this into a significant contribution to the industry.

Materials and Methods

Seed Supply

Rosa corymbifera 'Laxa' hips were harvested from the stock bushes at Writtle College, Chelmsford, Essex and from stock plants at Wheatcroft Nurseries, Nottingham. Hips were picked when red and firm and manually crushed prior to soaking in tap water. After three days the achenes were recovered from the softened fruit through a series of sieves. Achenes were washed in running tap water before being allowed to air dry on the bench.

Stratification Procedure

A standard ratio of seed, 'Garotta' and moist vermiculite (water added to give a water holding capacity of 70%) was adopted based on initial work by Cullum *et al.* (1990). 10 g moist seed (soaked for 24 h in sterile tap water) was mixed with 25 g moist vermiculite. 1g of 'Garotta' was added for each 10g moist seed to give the 'Garotta' treatment whilst the control had no such addition (i.e. the current 'commercial' procedure).

Material used for laboratory experiments was set up in rigid 2-litre plastic containers, whilst that destined for field trials was stratified in plastic bags of similar volume (like the commercial growers). All treatments were given 12 consecutive weeks at 25°C followed by a further 12 weeks at 4°C unless otherwise stated. During the warm period the contents of the bags/containers were shaken or stirred to allow aeration.

After the stratification period was completed four replicates of 100 seeds were taken for germination tests. Laboratory testing was carried out in Petri dishes whilst the field trials were conducted in pre-prepared seed beds at Oakover Nurseries, Kent.

Cotton Strip Assay

Burial Test Fabric (BS 2576) was purchased from Shirley Dyeing and Finishing Limited, Cheshire in 10 metre bolts. Strips were cut and frayed to give a final length of 20 cm and width of 25 mm. Strips were inserted into the stratification media (2-litre containers) using a modified method of Latter and Howson (1977).

Five strips were placed in each stratification treatment and incubated for seven days. These strips were then removed and loose media shaken off. If appropriate 5 fresh strips were inserted into the containers and stratification

continued. Removed strips were frozen in aluminium foil until sufficient numbers were available to test.

Tensile strength of the cotton strips was measured on a tensometer (Hounslow, 600 N beam). Strips were soaked with water and wrung to remove excess prior to testing.

Fluorescein Diacetate (FDA) Hydrolysis

Samples (1 g) from the stratification mix of each treatment were processed according to the following protocol. 1 g of well mixed sample (referred to in the text as '1 g sample') was transferred to a 50 ml conical flask and 10 ml of sodium phosphate buffer (pH 7.6) was added to each. FDA hydrolysis was initiated by the addition of 200 µl of a 1 mg/ml FDA solution to give a final FDA concentration of 20 µg/ml. The flask was capped with parafilm and incubated at 37°C for 30 min in a shaking water bath (approx. 180 strokes/min).

The sample was removed and 10 ml of acetone added to terminate the reaction. 1.5 ml of fluid sample was centrifuged at 13 000 rpm for 30 s before the absorbance was read at 490 nm using a spectrophotometer.

After seven weeks small pieces of cotton (2 cm^2) were also inserted into the stratification mix to allow FDA analysis. This was to avoid the measurement of esterases from the seed which the 1 g samples would contain (FDA hydrolysis has been used for viability testing (Pritchard, 1985)).

Microbial Counts

A serial dilution series was used with an initial 1 g sample taken from the stratification mix. Plates were made using nutrient agar (NA) and Potato Dextrose Agar (PDA) to select for bacteria and fungi respectively. Once set the plates were incubated at 22°C for 48 h before colonies were counted.

Results

Stratification Procedure

In all stratification protocols with *Rosa corymbifera* 'Laxa' the addition of the activator greatly enhanced the germination of the seed batch from less than 21% to over 80% in both the field and the laboratory (Table 1).

Other observations during the stratification found characteristic changes in the appearance of the seed. After 6 weeks of the warm period of stratification the seed treated with the activator darkened considerably compared to the control which remained the light brown of fresh seed. It was also observed that control seed remained firmly intact around the suture, whilst the majority of seed from the activator treatment had split. This splitting has become an indicator for successful germination.

Table 1. Germination after 14 days of control and 'Garotta' treated *Rosa corymbifera* 'Laxa' achenes following stratification

		% Germination	Standard deviation
Laboratory[a]	Control	10.17	3.49
	Garotta	87.67	9.61
Field[b]	Control	20.81	5.77
	Garotta	80.56	10.34

[a]Average of six replicates of 100 seeds
[b]Average of four replicates of 400 seeds

Cotton Strip Assay

Tensile strength is rapidly lost in the treatment containing the compost activator, reaching a peak of nearly 50% loss after only three weeks (Fig.1). The strips in the control treatments show little in the way of loss in strength, a maximum loss of only 15% was recorded after 4 weeks.

Once the stratification was continued in the cold (4°C), the loss in tensile strength in both activator and control treatments was negligible (see Fig. 1).

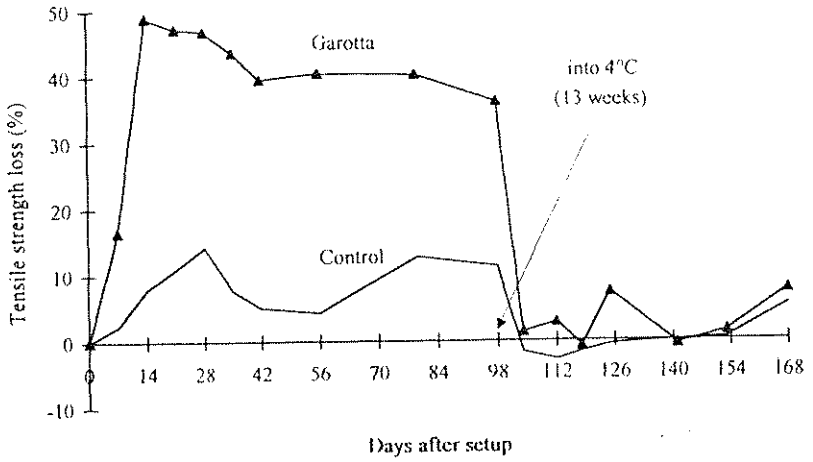

Figure 1. Loss in tensile strength of cotton strips during stratification *of Rosa corymbifera* 'Laxa'. Each point is the combination of three replicates (i.e. mean of 15 strips), exposed for 7 days to the medium

Fluorescein Diacetate (FDA) Hydrolysis

The hydrolysis of FDA is brought about by the cleaving of the diacetate by esterases. Thus esterases produced within the system by microbes will result in such a reaction, and this can be seen in Figure 2.

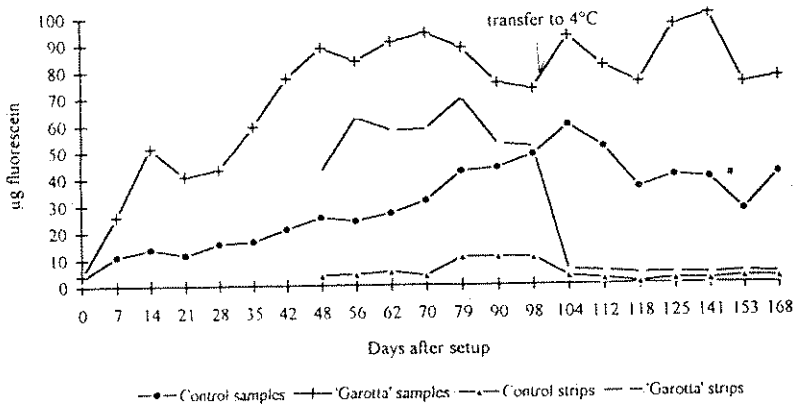

Figure 2. FDA results for the 1g samples and cotton strips during the stratification of *Rosa corymbifera* 'Laxa'. Results are shown for 1g samples of stratification medium and after 7 weeks for small cotton strips in the media

During the course of this experiment it was also found that esterases produced by the seeds could influence the result; in fact the procedure has been used as an orchid seed viability test (Pritchard, 1985). This led to the addition of small pieces of cotton being introduced to the stratification media for FDA testing. These results are also shown in Figure 2.

The graph shows that the treatment with the activator consistently has a much higher metabolic activity than the control, both for the 1 g samples and cotton strips. However a difference is seen once the treatment enters the cold period of the stratification. The 1 g samples continue to maintain their FDA activity, whilst that for the cotton squares dropped to a level where no activity was measured.

Microbial Counts

Microbial counts during the stratification of *Rosa corymbifera* 'Laxa' show an increase in both fungi and bacteria during the first four weeks of the warm period (Figure 3). This is enhanced in the presence of the activator.

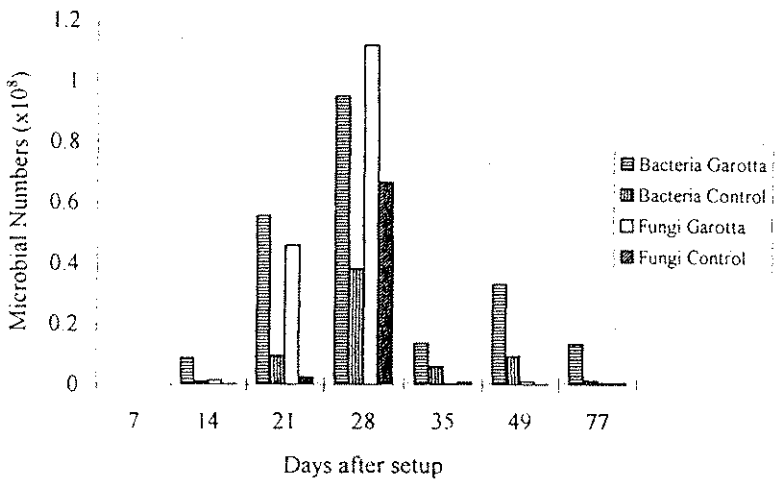

Figure 3. Fungal and bacterial counts during the warm period of stratification of *Rosa corymbifera* 'Laxa'

Discussion

Germination of *Rosa corymbifera* 'Laxa' is greatly enhanced when the commercial stratification protocol has an addition of a compost activator. It was thought that perhaps this was due to the involvement of microorganisms and their associated extracellular enzymes. This was suspected due to the darkening of the seed coat during the procedure, coupled with the splitting of the seed coat and enhanced microbial numbers in the presence of the activator.

During commercial stratification a grower may introduce fungicides to combat potentially harmful microbes. Interestingly, there has been no observed detrimental effects from encouraging the microbes in this system; on the contrary very recent results have shown a decrease in germination if the seed is surface sterilised prior to stratification. However work so far has not conclusively shown it to be purely the microbial action causing the enhanced germination.

Cotton strips lose half of their strength when placed in the activator treatment, but less than 15% in the control. This is either due to enzymatic action on the cellulose fibres, or chemical action from the activator. Recent work has found that the activator alone will cause weakening of the strips. Enzymatic and chemical studies are currently being carried out to investigate the nature of this loss in tensile strength.

Measuring metabolic activity in the stratification mix using the FDA assay showed a much higher activity in the presence of the activator over control, both when measured on samples of the mix and indirectly using cotton. Whilst it is likely that such activity is partially due to the activator and possibly the seed, it

has also been found that FDA can also be cleaved by a suspension of the activator. Further work will elucidate the exact contribution made by each of these components.

Microbial numbers increase with time during the stratification period. These microorganisms are introduced to the system on the seed; seed is sterile in the hip, but gains a microbial loading when extracted. Stratification with surface sterilised seed in the presence of the activator still shows a marked increase in germination over control, although such seed does not attain the same percent germination as the non sterile seed. It has proved very difficult to keep such a system totally sterile for the entire stratification, partly due to the irregular surface of the seed coat. Prolonged exposure to sterilant could cause damage to the embryo, as *Rosa corymbifera* 'Laxa' is permeable to water.

It is evident that a novel stratification procedure has been successfully introduced for certain woody tree seed, but what remains uncertain is the exact mechanism involved. Current research is looking at the mechanism and once sufficient understanding is obtained then the protocol will be adapted to cover many more species.

Acknowledgements

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Seed Symposium 12-14th June 1995.

The Influence of enhanced microbial activity on woody seed germination

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Seed dormancy is common in many temperate tree and shrub species. There are obvious evolutionary advantages in possessing dormant seeds, such as avoiding synchronous germination at the wrong time of year. When such seeds are dispersed naturally, the hard seed coat is exposed to microbial decay in the soil, causing the seed coat to be weakened and any inhibitors can then be degraded or leached from the seed. Seeds are usually shed in the autumn and may not start decaying until the following summer when temperatures rise to stimulate microbial growth. Following the second winter's chilling the seeds may then emerge erratically during the second spring.

Traditional commercial stratification attempts to mimic conditions experienced in nature. However this natural process can result in seeds not germinating synchronously and thus produces a very varied quality of seedlings. A more effective seed pretreatment for hard coated tree and shrub species than already exists would be welcomed by the industry, especially if it were economic and easy to implement.

Over some years researchers at Writtle College have developed a novel pretreatment for woody seeds. The effect of a commercially available compost activator on the rose rootstock *Rosa corymbifera* 'Laxa' has been investigated. This activator, 'Garotta', has been used successfully to germinate the rose achenes in the first spring. The pretreatment consists of 10g moist achenes (seeds), 25g moist vermiculite and 1g 'Garotta', followed by storage at 25°C for 12 weeks and then at 4°C for 12 weeks. Results consistently show increased germination rates with the addition of 'Garotta' compared with achenes which have only received the warm and cold treatments (23 to 79% in the field and 10 to 87% in the laboratory).

This pretreatment is based on the 'natural' stratification previously described, but in addition provides ideal conditions and nutrient status for microbial growth. The *Rosa corymbifera* 'Laxa' achenes are extracted from the hips by fermentation. This provides the seeds with a microbial loading which they would not otherwise have if they had been physically extracted. The promotion of this microbial growth, both in terms of numbers of microorganisms and activity, results in the increased germination of the seed batch at the

end of the 24 week process. One of the immediate benefits of this length of treatment is that it can be timetabled to only one season i.e. seed harvested in the autumn can be sown in the following spring with a high yield in terms of germination. Study has also shown that 'Garotta' treated seed emerges more synchronously (within 14 days) and provides a uniform stand of seedlings.

Microbial numbers were measured using the dilution plate method. Samples were taken during stratification in the presence and absence of 'Garotta', and dilution series made to quantify the numbers of microorganisms present. Potato dextrose agar (PDA) and Nutrient agar (NA) were used to count fungal and bacterial numbers respectively. It was found that whilst numbers of bacteria and fungi increased during normal stratification, the increase was much greater in the presence of 'Garotta'.

Coinciding with this increase in microbial numbers, microbial activity was also shown to increase, again to a much greater extent in the presence of 'Garotta'. The activity was measured using the cotton strip assay (BS 2576). This is an assay which quantifies degradation of cloth by loss in tensile strength. Strips of cotton of exact warp (i.e. the same number of threads in width) were placed in the stratification treatments for 7 days, removed and snapped on a tensometer. This gives a measure in newtons of the force required to break the strips. Activity of the microbes is therefore measured indirectly by measuring their ability to produce cellulases.

Following stratification in the presence and absence of 'Garotta' there is an obvious colour difference between the seeds of the two treatments. The seed from the 'Garotta' treatment is much darker than the control seed. It is also found that at the end of the 12 week warm period the seeds treated with 'Garotta' have split along the suture, whereas the majority from the normal treatment have not. Scanning electron microscopy (SEM) and light microscopy have not revealed any dramatic changes to the physical appearance of the seed during stratification, either in the presence or absence of 'Garotta'. It is thus a more subtle mechanism having effect on the suture of the seed, than an 'eroding' of the whole seed coat. This splitting acts as the indicator to the grower that the seed will germinate successfully.

Recent experiments have found that the 12 week warm period of stratification can be reduced to 6 weeks with no loss in germination. This would allow a longer 'window' of time for the commercial producer to stratify the seed harvested in the autumn to sow in the spring. It is also anticipated that this process will be transferred to many other species of woody tree seed.

The authors are grateful to the Horticulture Development Council (HDC), UK, for supporting this project.

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The Influence of Enhanced Microbial Activity on Woody Seed Germination

Awarded to: *D.R. MORPETH, A.M. HALL & F.J. CULLUM*

By: D. J. Scott, President, ISTA

At: 24th ISTA Congress, Copenhagen, Denmark

Date: June 14, 1995

Signature: *D J Scott*

Conception to germination; the *Rosa* story

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Under natural conditions, the *Rosa* flower is fertilised and seeds develop inside the hip, which then matures and ripens to the familiar red hip of the English autumn. These hips then either fall to the ground and decay, or else they are eaten by birds and the seeds survive passage through the gut and are then dispersed. This seed will then germinate sporadically over many seasons.

Commercial growers however cannot wait this long for seeds to germinate and so, in order to regulate germination the seeds are put through a stratification process. The paper presented at this meeting last year described the effect of 'Garotta' and enhanced microbial numbers on the stratification process and the effects of this on germination time and rate. This year's paper follows the progress from flowering to germination in *Rosa corymbifera* 'Laxa' and describes the conditions under which the seed is stratified resulting in higher germination with the addition of 'Garotta'.

The paper also describes the anatomical changes which take place as the seed germinates, and highlights the visual differences between seed which has germinated under commercial stratification and seed which has been stratified in the presence of 'Garotta'. Additional data on enhanced microbial activity will also be presented.

This work was supported by the Horticultural Development Council (HDC).

Dave Mapeth

PROGRAMME,
ABSTRACTS
AND
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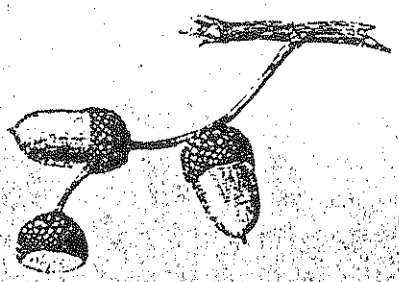

Whittle College

The influence of enhanced microbial activity on woody seed germination

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Abstract

Low germination and sporadic emergence are frequently encountered with tree seed, and are often believed to be associated with hard seed coat imposed dormancy. Traditional stratification in a moist media such as vermiculite or sand attempts to overcome this by chillling seed over winter to induce seed after-ripening, physical fracture and possibly inhibitor leaching. Mechanical or chemical scarification are other techniques used to erode hard seed coats, but often require constant supervision (especially acid scarification).

A technique developed at Writtle College using a patented compost activator added to the vermiculite has shown encouraging results with *Rosa corymbifera* 'Laxa', a rootstock in the rose industry. Germination in the field increased from 32 to 47%, whilst laboratory tests showed a more marked difference (2 to 99%). *Acer campestre* has also shown increased germination over control. A 'quality' difference between treatments also appears to exist, where treated seedlings are more vigorous than their control counterparts.

The mechanism by which the activator enhances germination during stratification is unclear. Observations during stratification (12 weeks warm (22°C) followed by 12 weeks chilling (4°C)) with the addition of the activator suggested possible enhancement of natural microbial activity. Experiments have shown that there is indeed an increase in microbial numbers with addition of the activator, resulting in enhanced levels of microbial activity.

Both fungal and bacterial numbers increased during the warm period of stratification, as found by washings taken from the vermiculite. Initial microbial loading was found to originate from the seed, and not from any of the constituents of the pretreatment mix. Metabolic activity was shown to increase during the pretreatment using the Fluorescein Diacetate (FDA) assay, especially with addition of the activator. The cotton strip assay indicated an increase in cellulase activity over time, as well as an increase with the additive.

Although results strongly indicate an increase in microbial activity in the presence of the activator, the exact nature of the action is unknown. Whether the increase in germination is due to the enhanced levels of microbial activity (by increases in pectolytic, cellulases or other enzymes for example) or a chemical effect, further work will elucidate. Organic acids affecting the seed coat and/or production of secondary metabolites (possibly plant growth hormones) could be a direct result of enhanced microbial action.

Light and Scanning Electron Microscopy have allowed some detailed study of the seed coat during the stratification process. If microbial action proves not to be responsible, then the biochemical and genetic regulation of the apparent break in dormancy will be investigated.

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Seed Biology

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