

Figure 1A, B. Eight and six celled colonies of *Actinastrum hantzschii* Lagerheim var. *intermedium* Teiling.

The present specimens are similar to those from Pakistan in all characters except in having greater length and lesser breadth, the latter measuring  $14-16.5 \times 4.5 \mu\text{m}$ .

One of the authors (LJ) thanks UGC, New Delhi for financial assistance.

13 August 1987; Revised 24 September 1987

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#### INVERSE RELATIONSHIP BETWEEN ENDOGENOUS PHENOLS AND ALPHA AMYLASE ACTIVITY DURING CHILLING OF *ROSA MACROPHYLLA* LINDL. SEEDS

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SEEDS of a number of plant species require exposure to low temperature of variable periods before germination<sup>1</sup>. Best known examples of such cases are found among the members of Rosaceae and Conifers. It is logical to assume that during exposure to low temperature, some biochemical changes occur in seeds enabling them to respond positively to favourable conditions of germination. However, a direct relationship between these changes and the termination of dormancy need not always exist<sup>2</sup>.

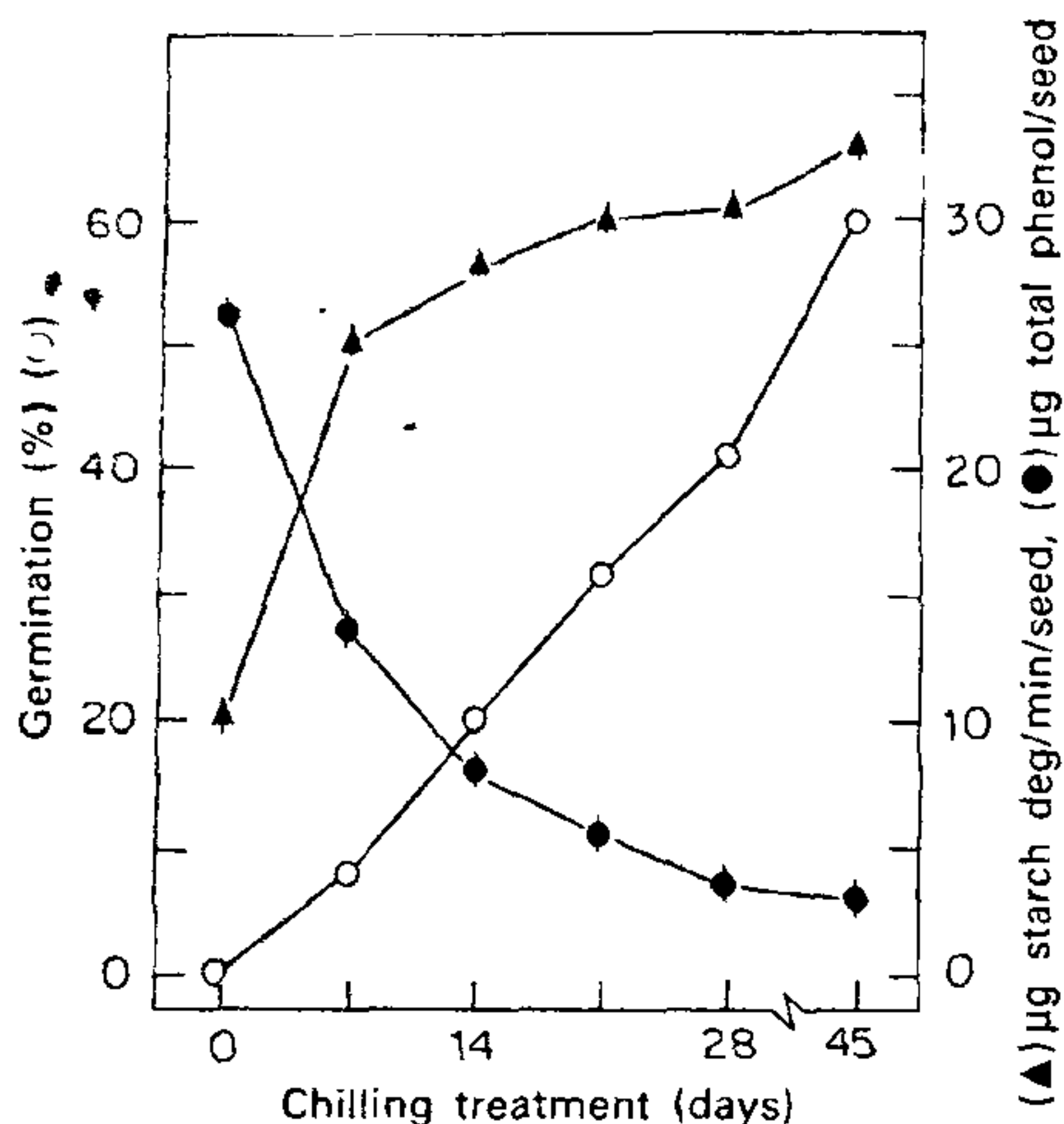
In the seeds of *Rosa* spp. although the mechanical resistance offered by the seed coat contributes to dormancy, the role of inhibitors has been shown to be more important<sup>3</sup>. Abscisic acid is present in

achenes and flesh of *Rosa* spp. in relatively high concentrations<sup>4</sup>, the levels of which have been demonstrated to be lowered during breaking of dormancy, phenolic compounds, categorized as growth-inhibitors<sup>5</sup>, have also been implicated in seed dormancy<sup>6</sup>. In the present investigation, the overcoming of seed dormancy in *Rosa macrophylla* by chilling is reported. This is accompanied by changes in the phenolic content of seeds. Since  $\alpha$ -amylase plays an important hydrolytic role during germination and is responsible for the availability of mobilizable carbohydrates through starch degradation, we have also monitored the changes in its activity.

Seeds of *R. macrophylla* Lindl. were collected from Narkanda and adjacent areas (W. Himalayas, altitude ca 2600m) during September and October, which, at the time of harvest exhibited total dormancy. Seeds were selected for uniformity of size and surface-sterilized with 0.1%  $\text{HgCl}_2$ . The seeds were then imbibed for 24h in distilled water and kept on a wet substratum at  $5 \pm 1^\circ\text{C}$  in a freezer for 45 days. At definite intervals during the chill treatment, the seeds were checked for percentage germination<sup>7</sup>, phenol content<sup>8</sup> and  $\alpha$ -amylase activity<sup>9</sup>.

Chill treatment to dormant *R. macrophylla* seeds helped them recover from dormancy. After incubation at low temperature for 7 days the seeds exhibited ca 10% germination which improved gradually in proportion to the length of the low temperature treatment leading to more than 50% germination after 45 days of chilling (figure 1). Changes in the total phenolic level in seeds monitored during breaking of dormancy revealed that the chill-induced alleviation of seed dormancy in *R. macrophylla* is paralleled by a gradual decline in the total phenolic content of the seeds. During the first week of exposure to low temperature, seeds lost 50% of their initial phenol content which was further lowered to 13% of the control levels after 45 days treatment (figure 1). Further, the removal of seed dormancy by chilling was accompanied by a simultaneous rise in  $\alpha$ -amylase activity (figure 1). A 2.5 fold increase in  $\alpha$ -amylase activity over that of the controls was observed in seeds chilled for 7 days.  $\alpha$ -amylase activity was further stimulated (3.3 fold) after 45 days of low temperature treatment.

That low temperature is effective in removal of dormancy in many plant species is well known. In *Rosa* spp. low temperature treatment results in germination of dormant seeds. Barton and Crocker<sup>10</sup> showed that *Rosa multiflora* necessarily



**Figure 1.** Removal of seed dormancy in *Rosa macrophylla* by low temperature treatment of seeds exhibiting the relative phenolic content and  $\alpha$ -amylase activity as a function of time. Vertical bars represent S.D.

requires low temperature treatment (5–10°C) for 2 months for dormancy removal, whereas *Rosa rubiginosa* requires 6 months stratification. Regarding the biochemical changes occurring in the seeds during removal of chill-induced dormancy, most important perhaps is the induction of gibberellin in *Corylus avellana* seeds<sup>11</sup> and a decline in levels of ABA in the kernels of *Juglans regia*<sup>12</sup>.

Our experiments reveal that breaking of seed dormancy in *R. macrophylla* is induced by a low temperature treatment, and is paralleled by a gradual decline in the total phenolic component levels of the seeds. The inhibitory effect of phenolics on germination of *Enonymus europaeus* is attributed to the presence of *p*-coumaric acid during stratification<sup>13</sup>. Enhancement in the activity of hydrolytic enzymes in the seeds during chilling has already been demonstrated<sup>14–16</sup>. It seems likely that phenolics have a repressive effect on  $\alpha$ -amylase activity and lowering of the phenolics results in a significant induction of  $\alpha$ -amylase activity during germination of *R. macrophylla* seeds. That phenolic compounds have a regulatory role through interaction with ABA on  $\alpha$ -amylase activity has already been shown by us<sup>17</sup>.

9 October 1987

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#### HAEMOLYMPH RESPONSE TO THE DEVELOPMENT OF MICROFILARIAE OF WUCHERERIA BANCROFTI IN CULEX QUINQUEFASCIATUS

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THE haemolymph of mosquito is a rich and varied fluid and its study has provided many surprises. Changes in haemolymph during infection may reflect the nature of the pathological and defensive