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## The Improvement of Germinability of the Immature Rhododendron Seeds by Cold Treatment

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### Introduction

In the research for the heat resistant gene sources of rhododendrons conducted in 1985<sup>1)</sup>, the capsules of *Rhododendron simiarum* were collected on October 2-3, in Ngau-ngak-shan of Maon-shan in Hong Kong. They were accepted at Kagoshima airport on October 5, but the seeds included were found to be immature, *i. e.*, their endosperm was still soft and jelly like, although their embryo seemed to have attained almost their full length when compared with the fully matured seeds of other rhododendrons.

These situations might occasionally arise in such a case as the present research in overseas, in which the various restraints should be frequently met with. Therefore, it might be of consequence to devise a proper measure to improve the germinability of such immature seeds. The present investigation was conducted to find out some proper measure to solve these problems.

### Materials and Methods

Of 23 *R. simiarum* capsule lots collected separately from the different mother plants, 8 capsule lots in which relatively large amount of seeds seemed to be contained, were selected and used in the present experiment. Immediately after the arrival of capsules, they were divided into two groups and were subjected to the procedures shown in Fig. 1, namely (1) the respective half of the 8 capsule lots were either dehisced immediately after their arrival or stored intact for 60 days and then dehisced, and (2) the resultant seeds stored at 4°C in dry condition were subjected further to the cold treatments for 0, 25, 50 or 75 days under moist and dark condition.

In this experiment the fully matured seeds of 5 different lots of *R. pseudochrysanthum* were also included as control, at the time of their collection in Taiwan from early to middle October in 1985, some early capsules having been dehiscent. They were stored at 4°C in dry condition from late October (the left hand procedure in Fig. 1) till the start of moist, cold treatment.

The number of seeds per experimental plot in *R. simiarum* was from the minimum 21 to the maximum 84 because of the limited availability of seeds, whereas those in *R. pseudochrysanthum* were all 150. In 75 days' cold treatment, 7 experimental plots instead of 8 ones were also due to

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the insufficiency of the number of seeds available. The germination of seeds was traced every 5 days for 230 days from April 1, to November 16, 1986.

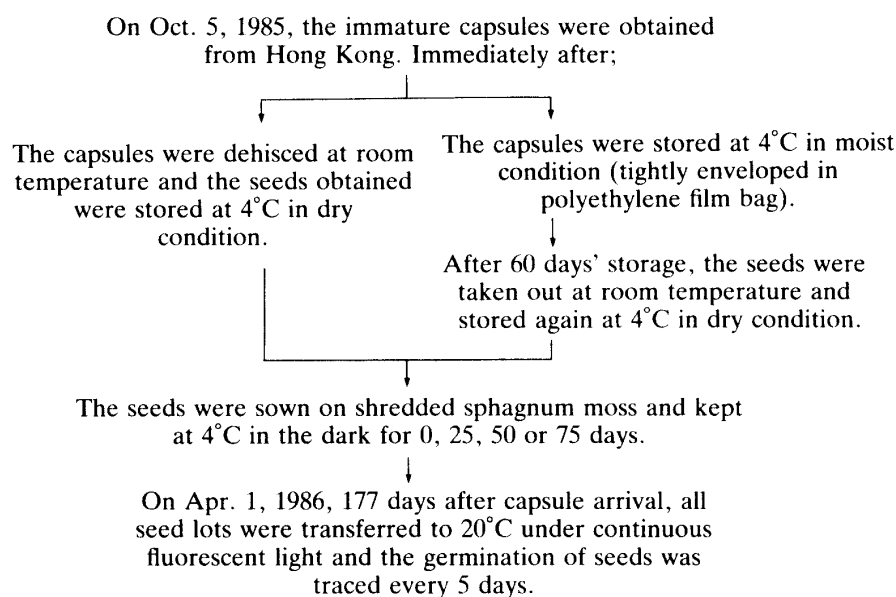


Fig. 1. The scheme of preparation, storage and germination of the immature seeds of *R. simiarum*.

### Results and Discussion

The final germination percentage 230 days after the transferring to 20°C under continuous illumination was given for *R. simiarum* in Fig. 2 and for *R. pseudochrysanthum* in Fig. 3.

As seen in these figures, a considerable variation was encountered with the difference of

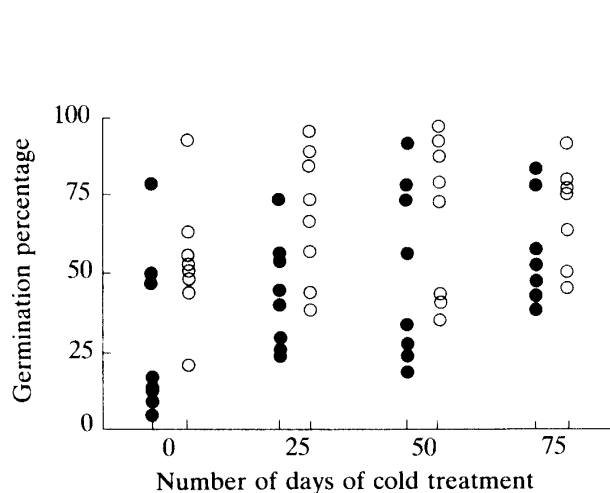


Fig. 2. The effect of cold treatment on the germination of immature seeds of *R. simiarum*. Seed lots from 8 different mother plants were examined. Closed circle; capsules dehisced immediately. Open circle; capsules stored for 60 days. For details see Fig. 1.

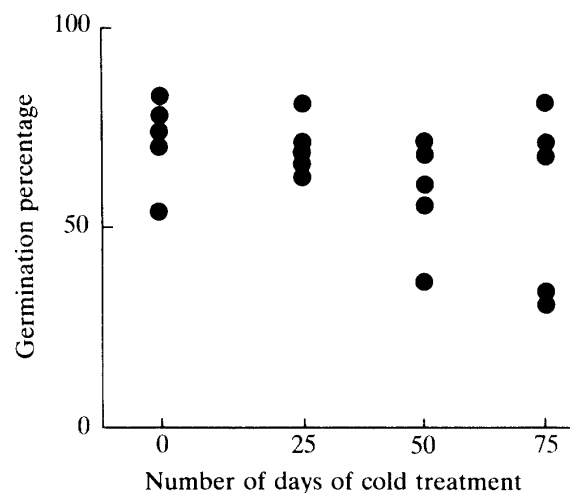


Fig. 3. The effect of cold treatment on the germination of mature seeds of *R. pseudochrysanthum*. Seed lots from 5 different mother plants were examined. The procedures for preparation, storage and germination were the same as those of the left hand in *R. simiarum* in Fig. 1.

different seed sources concerned. There was, however, a general trend that the seed lots with high germination percentage in one treatment showed high germinability in others, and *vice versa*, but a closer examination on the data revealed also some distortions inconsistent with above mentioned generalism. This was interpreted, on one aspect, to indicate the genetic difference for seed fertility of mother plants, from which the seeds were collected, and on another aspect, to show the unavoidable experimental error due mainly to the limited number of seeds available, thus being unable to replicate the experimental plot.

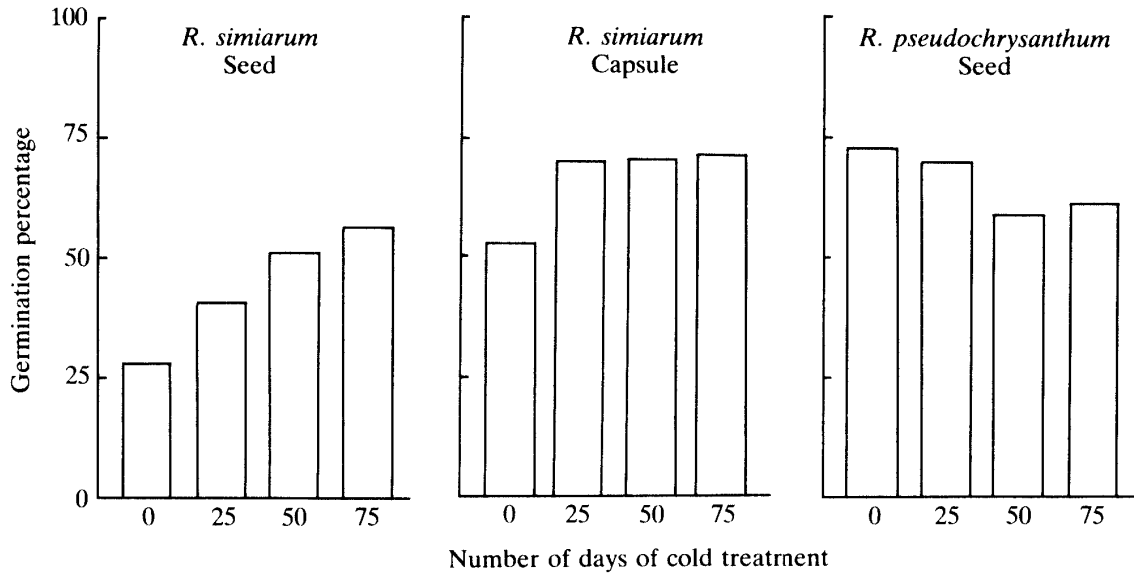


Fig. 4. The effect of cold treatment on the germination of the seeds of *R. simiarum* and *R. pseudochrysanthum*. Mean germination percentages were presented.

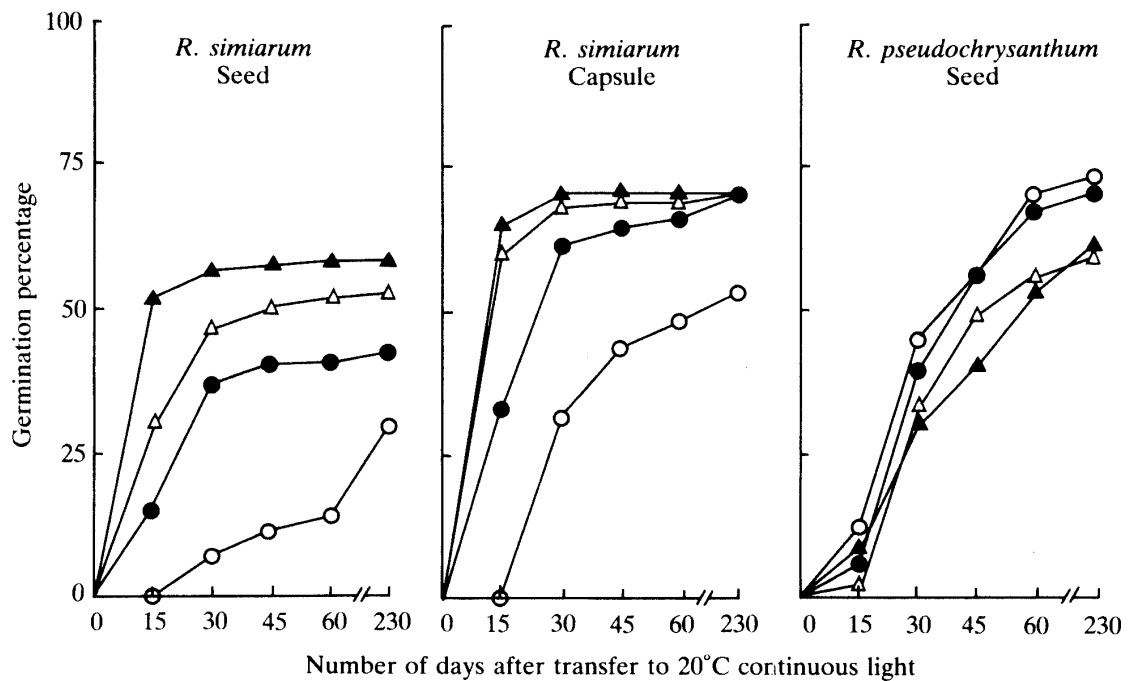


Fig. 5. The effect of cold treatment on the germinating rate of the seeds of *R. simiarum* and *R. pseudochrysanthum*. Open circle, closed circle, open triangle and closed triangle represent 0, 25, 50 and 75 days' cold treatment, respectively.

The summarized, final germination percentage of both species was presented in Fig. 4. and the consecutive change of germinating rate was presented in Fig. 5. As seen in these figures, any effect of cold treatment after sowing on the germination of the mature seeds of *R. pseudochrysanthum* could not be found, whereas the germination of the immature seeds of *R. simiarum* was largely enhanced by the cold treatment of seeds in moist condition. It was evident that the longer was the duration of cold treatment, the higher was the germinating rate. Furthermore, the cold storage of capsules carried out prior to the enforced dehiscence accelerated the maturing of immature seeds of *R. simiarum*.

According to the germinative characteristics in the seeds of trees and shrubs, Hartmann and Kester<sup>3)</sup> classified the seeds of rhododendron into their 'group 4', in which the seeds germinate in 14 to 28 days if exposed to the artificial light and to the warm alternating temperatures of 20° to 30°C. In some instances, the seeds of 'group 4' may also respond to the moist-chilling.

It has been well known that the moist-chilling, the stratification, breaks the dormancy of many seeds, especially that of the plant species of temperate zone. In the present experiment, the cold treatment enhanced the germination of the immature seeds of *R. simiarum*, although it was unbelievable that this enhancement was directly related to the breaking of seed dormancy. On the other hand, the same cold treatment did not exert any effect on the mature seeds of *R. pseudochrysanthum*. It might be reasonable, therefore, to assume that the action of the moist, low temperature on the immature rhododendron seeds might lie in the acceleration of their maturation, and not in the breaking of dormancy.

Furthermore, in the present experiment the cold storage of capsules enhanced the subsequent germination. Gotoh and Yasumatsu<sup>2)</sup> studied the aftereffect of cold storage of capsules on the seed germination of some azaleas and rhododendrons, and revealed that, if combined with the cold storage (2–5°C) in the triply enveloped polyethylene bag till late October, the maturation of seeds was to be accelerated and accordingly, the earlier harvest of capsules came to be feasible; in July for *R. japonicum*, *R. metternichii* and *R. macronulatum*, from August to September for *R. weyrichii*, and in September for *R. decandrum* and *R. scabrum*. The present investigation on the cold storage of capsule wholly substantiated those results.

Okada and Yasuoka<sup>5)</sup> studied the effect of gibberellin applied in foliar spray or by capsule soaking, on the later germination of some light sensitive herbaceous plant seeds, and revealed that the previous treatment of gibberellin on the stock plant or capsule enhanced the germination of the resultant seeds. Moreover, in *Primula malacoides* and *Nicotiana tabacum* the improvement of seed germination was greater in the treatment of immature seeds than in that of mature seeds, and in the former species the effect of gibberellin treatment on the germinability of seeds was maintained for at least 15 months' storage. From these findings they concluded that the gibberellin applied to leaves or capsules was translocated into the seeds and improved their later germinations, although they failed to make clear whether the gibberellin was translocated *per se* or after-transformed.

According to Nakamura<sup>4)</sup>, the two most effective agents in breaking seed dormancy, in other words, those having the widest action spectrum, are the moist, low temperature treatment and the gibberellin, either endogenous or exogenous. He further stated that in many cases the seed dormancy is determined by the balance between the endogenous germination promoter and inhibitor, and reviewed many instances in which the amount of gibberellin was increased by the moist, cold treatment and/or with the decrease of dormancy level.

Therefore, it might be reasonable to assume that in the present experiment the low

temperature applied to capsules and/or seeds enhanced the maturation of seeds themselves, together with the production (or the internal setup ready for the production) of the endogenous gibberellin in the immature seeds of *R. simiarum*, both of which favoured eventually the seed germination.

Anyways, the effect of cold storage of capsules and that of the moist, cold treatment of the resultant seeds were revealed, in the present experiment, to be synergistic. Although it was not clear how much the actual germinating rate of naturally matured seeds of *R. simiarum* might be, the average germination percentage measuring over 70%, which was attained by the duplicated cold treatments to both capsules and seeds, might be considered to be sufficient, from the practical point of view.

### Summary

The germinability of immature seeds of *Rhododendron simiarum* was improved by the cold treatment applied to either capsules or seeds, and when applied jointly, the effects of these treatments were revealed to be synergistic, whereas no improvement was found in the cold treatment to the mature seeds of *R. pseudochrysanthum*. The average germination percentage measuring over 70%, which was attained in the joint treatment, was considered to be sufficient enough, from the practical point of view.

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