# EFFECT OF COLLECTION DATE ON CONE AND SEED CHARACTERISTICS IN HIMALAYAN CYPRESS (*CUPRESSUS TORULOSA*)

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**PANDIT, A. & RAM, J. 2004. Effect of collection date on cone and seed characteristics in Himalayan cypress (*Cupressus torulosa*).** The failure of plantation programmes may be due to the collection of immature and non-viable seeds. *Cupressus torulosa* is an important tree species in the central and western Himalaya and is distributed between 1700 and 2700 m asl. Change in colour of cone from grey to dark brown at two collection sites in this study indicated that the seeds were sufficiently mature for collection. Changes in colour are much easier to determine than other characteristics which require laboratory facilities. Cone moisture content reached 44.5 to 47.2% and seed moisture content 14.9 to 17.3% towards maturity at both the sites. Size and weight of cone were significantly lower, but weight of seed was higher ($p < 0.01$) at site 2 compared with site 1. Cone and seed moisture contents were negatively correlated with seed germination. Electrical conductivity of seeds was positively correlated ($r = 0.74$) with germination but negatively correlated ($r = - 0.70$) with seed moisture content. Seed germination significantly increased from 4.8 to 41.8% towards maturity at both sites. Low germination occurred during the initial stages of seed collection, which may be due to empty seeds and high indistinct embryo percentage (immature seed). Seed maturity period was between late March and mid April.

*Key words: Cone colour – Himalaya – maturation – moisture content*

**PANDIT, A. & RAM, J. 2004. Kesan tarikh kutipan terhadap ciri kon dan biji benih sipres Himalaya (*Cupressus torulosa*).** Kegagalan program hutan ladang mungkin disebabkan oleh penggunaan biji benih yang tidak matang serta tidak berdaya hidup. *Cupressus torulosa* merupakan spesies pokok yang penting di Himalaya tengah dan barat. Taburannya adalah antara 1700 m hingga 2700 m di atas aras laut. Perubahan warna kon daripada kelabu ke perang tua di dua tapak kajian menunjukkan biji benih tersebut cukup matang untuk dikutip. Perubahan warna lebih mudah ditentukan berbanding ciri lain yang memerlukan kemudahan makmal. Menjelang kematangan, kandungan kelembapan kon di kedua-dua tapak mencapai 44.5% hingga 47.2% sementara kelembapan biji benih, 14.9% hingga 17.3%. Saiz serta berat kon lebih kecil dengan bererti di tapak 2 berbanding tapak 1 tetapi berat biji benih adalah sebaliknya. Kandungan kelembapan kon serta biji benih berkorelasi secara negatif dengan percambahan biji benih. Konduktiviti elektrik biji benih pula berkorelasi secara positif ($r = 0.74$) dengan percambahan biji benih tetapi berkorelasi secara negatif ($r = - 0.70$) dengan kandungan kelembapan biji benih. Percambahan biji benih di kedua-dua tapak meningkat secara bererti daripada 4.8% hingga 41.8% menjelang kematangan. Percambahan adalah rendah pada peringkat awal kutipan biji benih. Ini mungkin akibat biji benih kosong serta peratusan tinggi embrio yang tak nyata (biji benih tak matang). Tempoh kematangan biji benih adalah antara lewat Mac dan pertengahan April.

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## Introduction

Himalayan cypress (*Cupressus torulosa*) is distributed in the outer ranges of central and western Himalaya between 1700 to 2700 m elevations. Troup (1921) described its local distribution on the slopes of Naina hill at Nainital, in Jaunsar on limestone cliffs of Moila and Lokhandi below Karamba peak, in the Shimla catchment area on shale, and in various localities in Chamba, Kullu and other parts of central and western Himalaya. *Cupressus torulosa* is a large evergreen conifer tree that grows on limestone-rich and eroded soils. The Himalayan mountains is susceptible to landslides and soil erosion and has reached an advanced stage of degradation. Forest regeneration either by natural or artificial means, has been proven to ameliorate the effects of severe ecological disturbances and improve denuded areas.

Forest seeds play a vital role in the regeneration of tree species. Collection of forest fruits and seeds is usually based on reliable guidelines of maturity that allow the earliest possible collection (Bonner 1988). It is useful to know the physical and chemical changes that occur during maturation as such information may determine the best time of collection, storage procedure and testing methods for the species. These aspects have been studied in several tree species (e.g. Farrant *et al.* 1992, Bonner 1996, Uniyal & Nautiyal 1996). However, there is little or no information on seed characteristics of Himalayan trees. Thus, the present study deals with periodic cone collection of *C. torulosa* to observe the appropriate period of seed collection and to avoid the collection of immature and non-viable seeds for future plantation programmes.

## Materials and methods

Cone and seed maturation studies were conducted at two sites. Naina peak (site 1) is located at 29° 24' N and 79° 28' E along an altitudinal transact of 2100 to 2300 m. Major associated trees include *Quercus leucotrichophora, Myrica esculenta, Cedrus deodara* and *Fraxinus micrantha.* Kalika (site 2) lies at 29° 40' N and 79° 28' E along an altitudinal transact of 1700 to 1800 m. Dominant trees at this site are *M. esculenta, Aesculus indica, Q. leucotrichophora* and *Rhododenderon arboreum.*

Ten average size, healthy trees were flagged at a distance of about 100 m. A total of 50 cones were first collected from each tree in the last week of December 1999 from site 1 and in the first week of January 2000 from site 2 and from then on, at 15 day intervals until some of the cones started to open. Physical characteristics of cones/seeds, namely, cone colour, cone size, weight of 100 cones, and density of cones were recorded. Cone size was the mean diameter of two axes (at right angle) of the cone measured using a digital vernier. Cone density was the value of mass divided by the volume of the cone. Cones were spread on a cement floor in a glass house and left to open ( 5–7 days) and the seeds were extracted by gentle threshing. Seed characteristics, namely, seed colour, seed size and weight of 100 seeds were recorded. Initial moisture contents of seed and cone were expressed on fresh weight basis for each collection date using three replications of 1.5 g seed and 15.0 g cones (ISTA 1981). Initial test of viability was determined by cutting the seeds and rating them as sound, empty, or seeds with indistinct embryo. Four replications of 100 seeds at each collection were used. Seed size was measured with a digital vernier and weight of 100 seeds, with an electronic balance. Electrical conductivity of seed leachates was recorded with a conductivity tester (TD Scan). Three replications of 2 g of seed for each site and collection date were carried out.

Germination tests were carried out (on four replications of 100 seeds) at 25 ± 1 °C in a dual-chambered seed germinator that provided 8 hours of light and 16 hours of darkness, with humidity at the top of the filter paper controlled at approximately 90%. Germinants were counted when protrusion of the radicle exceeded 1 mm. Germination was monitored for 28 days and throughout the time, water was added as required. At the end of the test ungerminated seeds were classified as sound or empty seeds. Results were expressed as germination capacity (GC), which was calculated following Paul (1972):

$$GC\% = \frac{\text{total germinated seeds + total ungerminated sound seeds}}{\text{Total seeds tested}} \times 100$$

Results were also expressed as germination value (GV) which combined germination speed (represented by peak value, PV) and completeness (mean daily germination, MDG) into a single index (Czabator 1962):

$$GV = MDG \times PV$$

where

MDG = accumulated number of germinants at the end of the test divided by the number of days in the test, and

PV = maximal quotient obtained by dividing the accumulated number of germinants by the corresponding number of days.

Data were analysed using two-way analysis of variance (ANOVA) (Snedecor & Cochran 1967) to determine differences in site, various cone and seed characteristics, leachate conductivity, moisture content and germination. Interactions between collection dates and sites were also determined. Student-Newman-Keuls' (SNK) test (Newman 1939) was used to compare differences in seed germination and moisture content on different collection dates. Simple correlation was also developed between different parameters.

## Results

### *Cone characteristics*

The colour of cone changed from grey to dark brown from initial to final collection dates at both sites. Cone size at the sites varied from 14.6 to 16.3 mm (Table 1). ANOVA showed that cone size was significantly different ($p < 0.01$) at different collection dates and sites (Table 2). Cone weight of 100 cones varied significantly

**Table 1** Effect of collection dates on cone and seed characteristics of Himalayan cypress from two collection sites

| | | Cone characteristics | | | | Seed characteristics | |
|---|---|---|---|---|---|---|---|
| Site | Collection date* | Cone colour | Mean cone size (mm) | Mean cone weight/100 cones (g) | Mean density of cones g $cm^{-3}$ | Seed colour | Mean seed weight/100 seeds (g) |
| Nainital | Jan 1 | Grey | 16.3 ± 0.3 | 243 ± 14.1 | 1.00 ± 0.01 | Whitish yellow | 0.44 ± 0.01 |
| | Jan 16 | Grey | 15.8 ± 0.3 | 241 ± 14.9 | 0.96 ± 0.01 | Whitish yellow | 0.43 ± 0.01 |
| | Jan 31 | Greyish brown | 15.8 ± 0.3 | 245 ± 14.6 | 0.91 ± 0.02 | Whitish yellow | 0.43 ± 0.01 |
| | Feb 15 | Greyish brown | 15.7 ± 0.3 | 237 ± 14.6 | 1.12 ± 0.07 | Whitish yellow | 0.42 ± 0.01 |
| | Mar 1 | Greyish brown | 15.8 ± 0.3 | 236 ± 13.7 | 1.01 ± 0.02 | Light brown | 0.38 ± 0.01 |
| | Mar 16 | Light brown | 15.9 ± 0.2 | 229 ± 12.9 | 0.97 ± 0.03 | Light brown | 0.35 ± 0.01 |
| | Mar 31 | Light brown | 15.9 ± 0.2 | 224 ± 11.7 | 0.99 ± 0.01 | Light brown | 0.34 ± 0.01 |
| | Apr 15 | Dark brown | 16.0 ± 0.3 | 230 ± 13.3 | 1.02 ± 0.03 | Dark brown | 0.33 ± 0.01 |
| | May 1 | Dark brown | 16.1 ± 0.3 | 221 ± 12.3 | 0.99 ± 0.01 | Dark brown | 0.33 ± 0.01 |
| | May 16 | Dark brown | 16.2 ± 0.3 | 218 ± 12.7 | 1.00 ± 0.01 | Dark brown | 0.32 ± 0.01 |
| Kalika | Dec 28 | Grey | 15.0 ± 0.4 | 187 ± 9.0 | 0.96 ± 0.02 | Whitish yellow | 0.47 ± 0.005 |
| | Jan 12 | Grayish brown | 15.3 ± 0.3 | 179 ± 11.9 | 0.96 ± 0.01 | Whitish yellow | 0.46 ± 0.005 |
| | Jan 27 | Light brown | 14.6 ± 0.4 | 178 ± 11.7 | 0.87 ± 0.02 | Whitish yellow | 0.45 ± 0.006 |
| | Feb 11 | Light brown | 14.9 ± 0.3 | 178 ± 8.4 | 1.00 ± 0.01 | Whitish yellow | 0.46 ± 0.007 |
| | Feb 26 | Light brown | 15.5 ± 0.1 | 185 ± 9.3 | 1.05 ± 0.01 | Light brown | 0.46 ± 0.006 |
| | Mar 12 | Light brown | 15.2 ± 0.2 | 184 ± 9.0 | 1.01 ± 0.01 | Light brown | 0.41 ± 0.01 |
| | Mar 27 | Dark brown | 15.5 ± 0.2 | 181 ± 9.6 | 1.05 ± 0.01 | Light brown | 0.41 ± 0.01 |
| | Apr 11 | Dark brown | 15.2 ± 0.3 | 184 ± 7.0 | 1.00 ± 0.01 | Dark Tan | 0.40 ± 0.01 |
| | Apr 26 | Dark brown | 15.5 ± 0.3 | 178 ± 7.3 | 1.05 ± 0.01 | Dark Tan | 0.40 ± 0.01 |
| | May 11 | Dark brown | 15.7 ± 0.2 | 171 ± 7.1 | 1.02 ± 0.01 | Dark Tan | 0.39 ± 0.01 |

* All dates in 2000 except December 28 for Kalika which was in 1999

($p < 0.01$) from 171 to 245 g at both sites at different collection dates. The interaction between sites and collection dates was also significantly different ($p < 0.01$). Cone density varied significantly ($p < 0.01$) from 0.87 to 1.12 g $cm^{-3}$ at both sites. The cone size and weight were significantly lower at site 2 compared with site 1.

## *Seed characteristics*

Seed size at both the sites varied significantly ($p < 0.01$) from 10.0 to 14.0 $mm^2$ (results not shown). Mean seed weight of 100 seeds ranged between 0.32 and 0.47 g at both the sites (Table 1). The values varied significantly ($p < 0.01$) at different collection dates (Table 2). The seed weight was higher at site 2 compared with site 1 and it decreased towards maturity. Seed colour changed from whitish yellow to dark brown/tan from initial to final collection dates at both sites (Table 1). Variation in seed size and germination were not significant between the sites ($r = - 0.28$, df = 18).

## *Cutting test*

Results of the cutting test indicated that sound seeds at both sides ranged between 25 and 51%, empty seeds between 21 and 50%, whereas seeds with indistinct embryo between 3 and 47%.

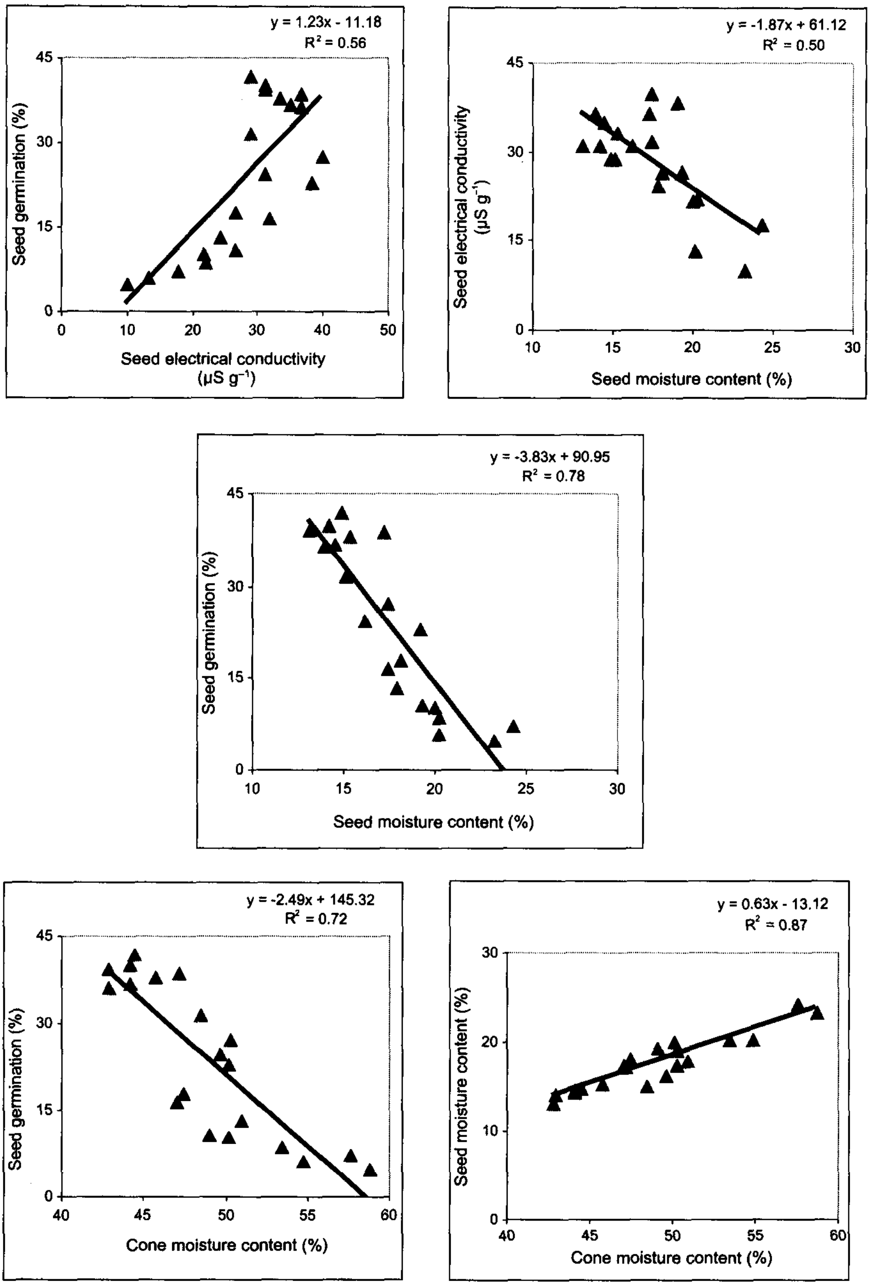



**Figure 2** Relationships between germination and other cone and seed parameters of *Cupressus torulosa*

## *Electrical conductivity*

Mean electrical conductivity of seeds varied significantly from 10.0 to 40.0 µS $g^{-1}$ across both sites at all collection dates. Electrical conductivity of seeds was positively correlated (r = 0. 75) with seed germination and negatively correlated (r = - 0.71) with seed moisture content (Figure 2).

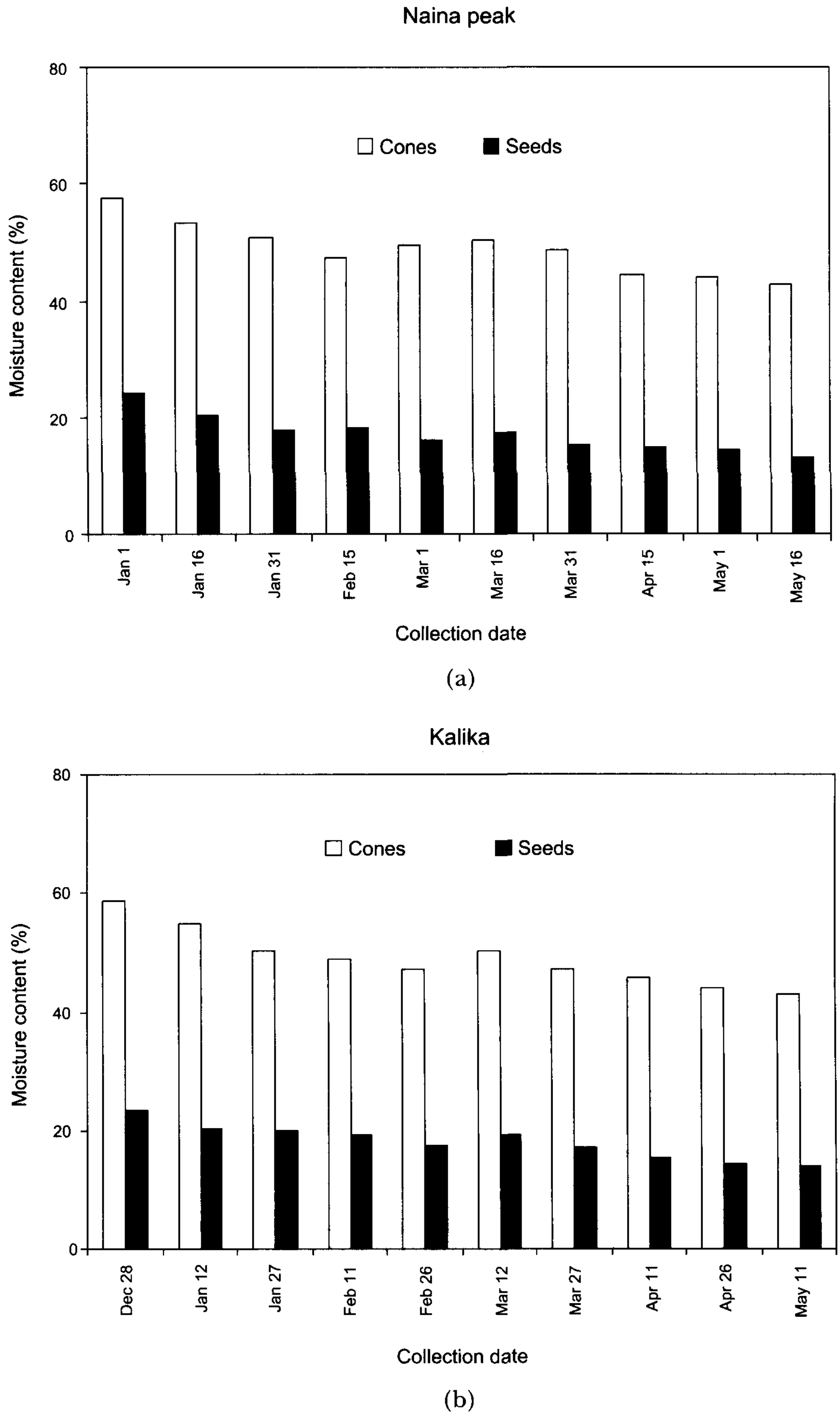
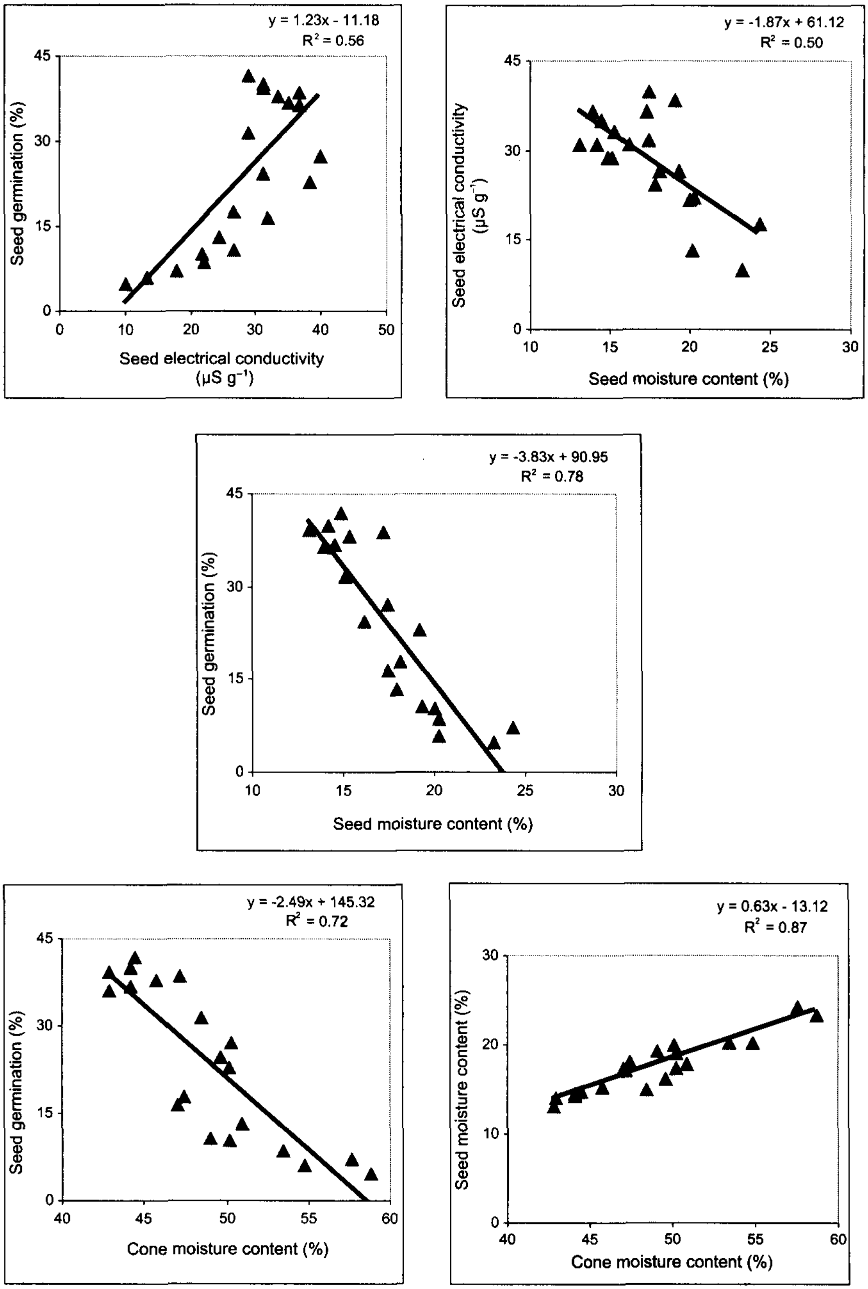



**Figure 1** Effect of collection date on cone and seed moisture contents of *Cupressus torulosa*

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### *Germination*

Germination at site 1 varied significantly ($p < 0.01$) between 7.2 and 41.8%. At this site, germination capacity ranged between 35.0 and 62.0%, daily germination between 0.25 and 1.49%, and germination value between 0.06 and 2.26 at different collection dates. Germination at site 2 varied significantly ($p < 0.01$) between 4.8 and 38.8% for different collection dates. At this site, germination capacity ranged between 34.0 and 60.5%, daily germination between 0.16 and 1.38%, and germination value between 0.03 and 1.91 at different collection dates. S-N-K test indicated that germination was not significantly different between any two successive collection dates at both study sites. Germination per cent was significantly lower on first collection date than later dates. Collections made after 31 March at site 1 ($W_p$ = 11.18; df 9, 30) and 27 March at site 2 ($W_p$ = 11.46; df 9, 30) were not significantly different. Germination per cent was negatively correlated with seed moisture content ($r = -0.88$).

## Discussion

The success or failure of any forest plantation depends primarily on the quality of seeds used to produce seedlings and, more importantly, on the time of collection of fruits/seeds in order to avoid immature or non-viable seeds. With an increasing demand for large quantities of seeds to meet annual reforestation and afforestation programmes, it is necessary to collect the maximum quantity of seeds within the allowable ripening period (Wang 1991). Seed maturity period of *C. torulosa* is between late March and mid April and it is earlier at lower elevation (site 2) compared with higher elevation (site 1). Cone colour of *C. torulosa* changed from greyish to dark brown towards maturity and seed colour from whitish yellow to dark brown/tan. In some conifers, colour changes in cones indicate seed maturity (Tewari *et al.* 2001). Colour changes are much easier to determine than cone and seed moisture contents and other parameters which require laboratory facilities. Thus, colour change can be used as indicator to determine when seeds are sufficiently mature for collection. Arisman and Powell (1986) reported that seeds from brown cones of *Pinus merkusii* recorded higher germination than those from green cones. Opening of cones is also an indicator of seed maturity. Pandit *et al.* (2002) reported that *Populus ciliata* seeds were best collected when the capsules turn dark green, which synchronised with the opening of the majority of the capsules. The high percentage of immature embryos in *C. torulosa* during the earlier collection dates of this study may be due to the underdeveloped embryos. Seeds with abnormal embryos (milky, decayed or shrivelled) and abortive seeds that have no embryo are considered non-viable. Earlier studies on *Cupresaceae* indicated that low germination of normal shape seeds is largely a consequence of low number of filled seed (Owens *et al.* 1990, El-Kassaby *et al.* 1993). As soon as the colour of the seed coat became light brown, the percentage of indistinct embryo decreased and emptiness increased. Emptiness may be due to abortive, decayed and shrivelled embryo. Cone size and weight were significantly lower and seed weight was higher ($p < 0.01$) at site 2 compared with site 1. Variation in seed size and germination were not significant. This indicated that early seed maturation at low elevation site retained higher amount of moisture in seeds and may be the reason for higher seed weight. Cone and seed moisture contents were negatively correlated with seed germination. Higher seed germination was recorded at lower cone and seed moisture contents for *C. torulosa*. According to Pandit *et al.* (2002) capsule moisture content of *P. ciliata* dropped from 80 to 60% during maturation, which coincided with maximum germination of the seeds. Seed germination increased with increase in electrical conductivity of seeds. Loss of moisture damages cellular membrane and increase concentrations of solutes in seed leachates. Higher conductivity towards maturity may be due to the accumulation of seed leachates. Another reason may be the increase in permeability of seed coat for leachates. Thus, germination capacity may be predicted on the basis of electrical conductance of seed leachates.

## Conclusions

A change in cone colour from greyish to dark brown towards maturity at the time of opening of cones is an indicator of mature and viable seeds in *C. torulosa*. Other parameters studied, namely, seed colour, cone and seed moisture contents, electrical conductivity, and seed germination, can also be used as maturity indicators for seed collection. The collection of immature and non-viable seeds should be avoided in plantation programmes.

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## References

ARISMAN, H. & POWELL, G. R. 1986. Effects of cone colour and seed extraction methods on yield and quality of seeds of *Pinus merkusii* in Indonesia. *Seed Science and Technology* 14: 177–190.

BONNER, F. T. 1988. Seeds of woody plants. *Advqnces in Research and Technology of Seeds* 11: 81–112.

BONNER, F. T. 1996. Pp. 139–142 in Trino, T. D. & Luis F. J. N (Eds.) *Responses to Drying of Recalcitrant Tropical Forest Tree Seeds.* Series documentation No. 18. Bogota, Columbia.

CZABATOR, F. J. 1962. Germination value: an idea combining speed and completeness of pine seed germination. *Forest Science* 8: 386–396.

EL-KASSABY, Y. A., CHAISURISRI, K., EDWARDS, D. G. W. & TAYLOR, D. W. 1993. Genetic control of germination parameters of Douglas fir, Sitka spruce, western red cedar and yellow cedar and its impact on container nursery production. Pp. 37–42 in Edwards, D. G. W (Ed.) *Proceedings on an International Symposium of IUFRO: Dormancy and Barriers to Germination.* Project Group P2. 04-00 (Seed problems). Victoria Press, Columbia.

FARRANT, J. M., PAMMENTER, N. W & BERJAK, P. 1992. Development of recalcitrant (homoihydrous) seeds of *Aviceannia marina*: anatomical, ultrastructural and biochemical events associated with development from histo differentiation maturation. *Annals of Botany* 70: 75–86.

ISTA (INTERNATIONAL SEED TESTING ASSOCIATION). 1981. Moisture content and equipment working group.

Report of the Forest Tree Seed Committee 1977–1980. 19th International Seed Testing Congress 1980. *Seed Science and Technology* 9: 187–195.

NEWMAN, D. 1939. The distribution of range in samples from a normal population, expressed in terms of an independent estimate of standard deviation. *Biometrika* 31: 20–30.

OWENS, J. N., COLANGELI, A. M. & MORRIS, S. J. 1990. The effect of self-cross and no pollination on ovule, embryo, seed and cone development in western red cedar (*Thuja plicata*). *Canadian Journal of Forest Research* 20: 66–75.

PANDIT, A., PANT, K. & RAM, J. 2002. Effect of collection date on capsule moisture content and germination of *Populus ciliata* Wall. ex Royle from central Himalaya. *New Forests* 23: 121–130.

PAUL, D. K. 1972. *A Handbook of Nursery Practice for Pinus caribaea hondurensis and other Conifers in West Malaysia.* Working Paper No. 19, FO: SF/MA 1 12, UNDP/FAO, Kuala Lumpur.

SNEDECOR, G. W. & COCHRAN, W. G. 1967. *Statistical Methods.* Sixth edition. Oxford & IBH Publishing Co. Pvt. Ltd. New Delhi.

TEWARI, P., TEWARI, A. & RAM, J. 2001. Seed characteristics and germination behaviour of some important coniferous trees of central Himalaya, India. *Journal of Tropical Forest Science* 13: 140–147.

TROUP, R. S. 1921. *The Silviculture of Indian Trees.* Vol I–III, Oxford University Press, London.

UNIYAL, R. C. & NAUTIYAL, A. R. 1996. Physiology of seed development in *Aesculus indica*, a recalcitrant seed. *Seed Science and Technology* 24: 419–424.

WANG, B. S. P. 1991. Tree and shrub seed. Pp. 34–43 in Bradnock, W. T (Ed.) *Advances in Research and Technology of Seed.* International Seed Testing Association, Zurich.