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Seed maturation timing in *Quercus leucotrichophora* A. camus along an altitudinal gradient in Uttarakhand Himalaya

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Abstract

Quercus leucotrichophora **A. Camus is a dominant forest forming species in Central Himalayan region between 1600 and 2100 m elevation. The species is under severe anthropogenic pressure and failing to regenerate in its natural environment. Climatic irregularities may further aggravate the problem as acorn ripening time may shift. Acorns of *Q. leucotrichophora* were collected from three sites located along the altitudinal limits of the species to determine the acorn maturation time. Across the sites the mean weight of acorns between the first and final collection ranged from 9.3±2.2 gm to 21.6±2.1. Inspite of variation in moisture content initial germination commenced at the same time across the sites. Maximum acorn germination was between 56.7±1.2 and 59.6±0.6 %. The mean acorn size was larger at the lower elevation site than higher elevation site across the collection dates. The change in acorn colour from green to dark brown, the removal of acorns cap with ease and acorn moisture content between 36.4±0.8 – 37.6±1.2 % at the time of maximum germination appear to be reliable indication of maturity. Comparison with an earlier study indicates that acorns are now maturing earlier which can be severely affects its regeneration potential.**

Key Word: Climate Change, Germination, Himalaya, Moisture, Quercus leucotrichophora, Seed Maturity

Introduction

Climate affects the distribution of plants, their regeneration (Adler & Lambers, 2008) and emergence of seedlings (Woodward & Williams, 1987). Climatic variations alone influence ecological dynamics which ultimately influence plant survival and growth (Fitch et al., 2007; Walck & Dixon, 2009; Baeten et al., 2010). Young seedlings are more sensitive than adults to climatic irregularities. Climate change influences seedling dynamics of species by influencing seed germination and longevity of seeds in the soil banks. Global climate change can lead to variation in reproductive phenology of trees which can consequently affect the germination and stand development.The Himalayan region is highly vulnerable to Global warming (Singh *et al.*, 2010). Himalayan ecosystems are degrading and regeneration of such ecosystems is difficult due to their physical instability and environmental features of the area (Tewari *et al.*, 2016). In life cycle of forest trees seed ripening is an important part. Studying variations in maturation time of seeds is a recommended way to study the effect of climatic variations on forest tree species (Bhatt and Ram, 2015). Change in seed maturity period can influence regeneration, development and hence impact stand composition and structure. Determing exact time of seed maturation is essential for the collection and regeneration. Physical attributes of seeds have been related to seed maturation in several species (Negi and Todaria, 1995 and Tewari *et al.*, 2016). The oak forests of the region are broadleaved with approximately one year leaf life span and concentrated leaf drop during spring/ summer (Singh & Singh,1987).These forest have been placed under low to mid-montane hemi-sclerophyllous broadleaf forests (Champion and Seth,1968). The local people generally relate oaks with water and soil conservation and in sustaining rural livelihoods (fodder and fuel wood needs) (Purohit *et al.*, 2009). Himalayan Oak forests are under chronic anthropogenic disturbances (Singh, 1998; Silori 2001) due to indiscriminate harvesting (Purohit *et al.*, 2009)and are facing the danger of extinction. *Quercus leucotrichophora, A. Camusis*

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the dominant Oak species of central Himalaya between 1400-2200 m elevations. Local communities generally relate oak forests with water and dry sites with *P. roxburghii* (Chir-pine). The pressure on these oak forests is relentless and trees are lopped bare for fodder. The species is failing to regenerate in its natural environment for which climatic irregularities and anthropogenic pressure have been highlighted as the biggest culprits. Maturation time of seeds/acorns might shift due to global warming further aggreviating the regeneration issue. We have tried to determine the maturation time of acorns across an elevation transect and relate it with earlier studies to assess the shifts in time of acorn maturation.

Material and Methods

The study sites are located between $29^0$22'-29^0 24' N Latitude and $79^0$25'-79^0 27' E Longitude along an elevational transact of 1730 to 2160 m in Kumaun Himalaya, India. After a thorough survey, three sites i. e. 1730 m(site I), 1950 m(site II) and 2160 m(site III) spread over 0.5 ha area each were selected during study year 2015-2016. Snowfall is quite frequent at higher elevations (above 2000m) during winter months. Average annual precipitation is close to 220 cm of which two third occurs during rainy season (Tewari *et al.*, 2015). The difference in temperature between the low elevation site I and high elevation site III was pronounced and average difference across all collection dates was close to 1.4°C, being lower at higher site III (Figure 1).

Ten phenotypically mature superior trees with well developed crown of *Q. leucotrichophora* were randomly selected and marked for seed collection at each site. Height and CBH of each selected tree were measured with a Ravi multimeter (patent no.A 000191, India) and measuring tapes respectively (1.37m).

Seed Collection and germination

Collection of acorns of *Q. leucotrichophora* commenced from first week of August up to the availability of acorns in last week of January from all the sites. Seeds were collected from the selected trees from each site and mixed to form a composite sample. For maturity indices seeds collection was made at an interval of 1 week till the seeds were available on marked tree. Five replicates of 25 acorns each, from the seed lot were used for determination the seed physical parameters. Seed size was taken as the product of length and width expressed as mm^2 and measured with digital vernier caliper (Model No. CD-6"CS, accuracy + 0.02mm Mitutoyo, Co.). Digital balance (Dolfinmake) was used for estimating weight of acorns. The moisture content of acorns was expressed on the basis of fresh weight by drying at 103 ± 2^0C for 16 ± 1 hr (ISTA, 1993; Shah et al., 2006, Tewari et al., 2015) and then reweighted. Acorns were subjected to float test followed by surface sterilization with 0.1% $HgCl_2$ prior to the germination test. Five replicates of 25 seeds each were used to determine germination percentage. Acorns collected at each date were germinated on top of the paper in a dual door seed germinatorat 25±1^0C. 2 mm radical emergence in seeds was the criteria followed for considering the acorns as germinated. Water was added as required during the experiment. Germination percent was calculated as the total number of germinated seeds out of tested seeds (125 acorns) for each site and each collection date within the test period. At the end of germination test un-germinated seeds were classified as sound seeds and unsound seeds. Results were expressed as germination capacity (GC), the percentage of seeds that had germinated at the end of the test, calculated following Paul (1972). Temperature and humidity at each site oneach collection date was estimated using a thermo-hygrometer.

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

The results were expressed following (Pandit *et al.*, 2002, Tewari *et al.*, 2011, Bhatt and Ram, 2007).

Data analysis

Blocking factor ANOVA model (Newman et al., 1997) was used to study the effect of date of collection (DOC) on different fruits/seed variables viz. size, weight, moisture and germination. In this model collection dates and elevation were considered as fixed factor. The experimental design was fully factorial. Paired t- test was also carried out for seeds characteristics between the elevation to observe difference more precisely. The data was analyzed using SPSS software (16.0 versions). Simple correlations used between seed germination and seed dry weight, fruit/seed moisture and seed germination using Microsoft Excel Program.

Results and Discussion

Tree Characteristics

Across the three elevations (sites) the diameter of the selected *Q. leucotrichophora* trees measured at 1.37m varied from 23.4± 0.60 to 28.3 ± 1.34 cm and height of trees from 17.2 ± 0.98 to 22.4 ± 0.48 m (Table 1).

Table 1. Average diameter and height of *Q. leucotrichophora* trees at different elevations selected for the study

Site	Elevation(m)	Tree Diameter (cm)	Tree Height (m)
I	1730 (low elevation)	28.3±1.34	22.4±0.48
II	1950 (mid elevation)	26.8±1.33	19.6±1.52
III	2060(high elevation)	23.4±0.60	17.2±0.98

Acorns Characteristics

Across the sites the mean weight of acorns between initial and final collection varied between 9.3 ± 2.2 g and 21.6±2.1g. The change in acorns weight was maximum at low elevation site and minimum at the high elevation site between the I[st] collection date and final collection (11.8±1.2g at low elevation site and 8.4±1.5 at the high elevation site). Weight of acorns varied significantly across elevation and collection dates ($p<0.05$) (Table 2). The mean acorns number / 10 g declined with increasing time from initial to final collection dates and varied significantly across sites ($p<0.05$) (Table 2). At site 1 the mean acorns size of *Q. leucotrichophora* was 128.1±1.5 mm^2 at the initial collection in first week of August and at final collection in fourth week of January it was 304.2±1.4 mm^2, the changes in acorns size across this period was 176.1±2.3 mm^2. At site II the mean acorns size was 132.5±2.1 mm^2 in first week of August and 298.2±0.8 mm^2 in fourth week of January, from initial to final collection the change in acorns size was 165.7±1.4 mm^2 and at site III the mean size was 118.8±1.5 mm^2 at initial collection and 284.5±2.6 mm^2 at final collection, the acorns size change in this period was 165.7±0.6. ANOVA showed that acorns size varied significantly across sites ($p<0.05$) and collection dates ($p<0.01$) (Table 2).

Table 2. Effect of collection dates on acorns characteristics of *Q. leucotrichophora* at different elevations.W1A signifies 1st week August; W3 A-3rd week August; W1 S-1st week September; W3 S-3rd week September; W1 O-1st week October; W3 O-3rd week October; W1 N-1st week November; W3 N-3rd week November; W1 D-1st week December; W3 D-3rd week December; W1 J-1st week January; W3 J-3rd week January; W4 J-4th week January.

Date of collection	Mean wt. of 10 acorns (g)			Mean no. of acorns/10g			Mean acorn size (mm^2)		
	Site I	Site II	Site III	Site I	Site II	Site III	Site I	Site II	Site III
W1 A	9.8±1.5	9.3±2.2	9.4±2.1	14.2±1.7	13.6±1.5	13.5±2.0	128.1±1.5	132.5±2.1	118.8±1.5
W3 A	10.4±0.2	9.7±2.4	9.8±1.5	13.6±2.5	12.2±2.4	12.6±2.1	133.5±2.4	134.2±2.0	127.8±1.4
W1 S	13.5±2.1	11.7±1.3	10.5±0.4	12.8±1.4	12.5±1.1	11.5±1.5	148.7±1.6	144.3±2.1	139.8±1.3
W3 S	14.6±1.5	12.5±0.8	11.8±2.2	12.4±0.8	11.9±2.4	11.1±1.7	155.0±1.7	148.9±0.0	141.5±0.1
W1 O	15.4±2.1	14.3±2.6	12.5±2.4	11.8±0.5	9.6±0.6	9.7±2.6	178.4±0.9	164.3±2.1	156.1±0.8
W3 O	16.8±0.8	14.8±1.4	13.9±1.3	11.2±1.4	9.5±1.7	8.5±1.4	188.4±0.9	193.2±1.5	178.6±0.6
W1 N	17.2±0.6	15.7±2.1	15.3±0.1	10.8±2.2	8.4±0.5	8.2±1.5	198.4±2.4	198.9±1.1	182.7±0.2
W3 N	18.7±1.4	16.5±2.2	15.8±1.	10.1±1.4	8.2±1.4	7.6±0.8	212.4±1.5	209.6±2.2	195.9±2.1
W1 D	19.5±0.8	16.8±0.5	15.9±0.0	8.9±2.6	7.0±1.5	7.3±1.4	236.4±0.9	221.1±0.4	218.6±1.0
W3 D	19.9±1.4	17.8±1.7	16.5±1.4	7.4±1.8	5.9±2.2	6.5±0.6	245.6±1.1	253.2±1.2	227.8±2.0
W1 J	20.8±0.5	18.0±0.4	16.8±1.5	5.6±1.5	5.1±2.4	6.1±1.4	271.0±1.5	265.1±0.8	258.9±1.5
W3 J	21.3±0.9	18.7±1.5	17.4±1.8	4.6±2.9	4.5±1.5	4.4±2.0	298.5±1.7	295.4±2.0	279.8±2.8
W4 J	21.6±2.1	19.7±1.6	17.8±2.1	4.2±0.4	3.9±1.8	3.6±1.6	304.2±1.4	298.2±0.8	284.5±2.6
Site F value	*	*	*	*	*	*	*	*	*
Date F value	*	*	*	NS	NS	NS	**	**	**

NS = Non significant, ** = Significant at 0.5% and * = Significant at 0.1%

Table 3. Effect of moisture content on seed germination of *Q. leucotrichophora* across the collection dates.W1 A signifies 1st week August; W3 A-3rd week August; W1 S-1st week September; W3 S-3rd week September; W1 O-1st week October; W3 O-3rd week October; W1 N-1st week November; W3 N-3rd week November; W1 D-1st week December; W3 D-3rd week December; W1 J-1st week January; W3 J-3rd week January; W4 J-4th week January.

Date of collection	Mean moisture content (%)			Mean germination (%)			Mean germination capacity (%)		
	Site I	Site II	Site III	Site I	Site II	Site III	Site I	Site II	Site III
W1 A	45.6±0.7	53.5±1.5	56.7±2.4	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
W3 A	44.7±2.3	48.4±0.4	55.3±1.5	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
W1 S	42.9±0.5	46.5±1.4	54.3±1.6	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
W3 S	40.9±1.2	43.5±0.2	50.4±2.2	8.6±0.4	8.8±0.6	8.3±1.4	75.4±1.1	73.7±0.9	74.2±2.1
W1 O	40.1±1.3	39.4±1.2	47.4±1.1	12.6±0.8	11.8±1.1	10.8±1.1	69.8±1.2	70.4±2.1	68.3±2.0
W3 O	39.6±2.0	39.4±2.5	44.2±0.7	17.8±0.6	18.7±2.0	15.9±2.5	68.7±0.5	65.8±2.2	66.5±1.5
W1 N	38.5±1.2	36.4±1.7	43.6±0.8	54.3±1.1	38.6±1.1	37.6±2.2	66.4±2.6	64.7±1.4	66.4±2.1
W3 N	37.0±2.8	36.4±0.8	38.4±1.0	59.6±0.6	56.7±1.1	43.8±1.8	65.8±2.0	64.2±0.5	66.1±1.6
W1 D	36.0±1.1	35.2±0.6	38.1±0.8	55.7±1.8	49.7±0.5	46.4±0.6	62.6±0.5	63.5±0.9	60.4±0.9
W3 D	34.0±0.8	29.8±0.5	37.6±1.2	50.5±1.5	50.8±0.6	57.5±1.2	60.8±2.4	61.5±2.8	58.5±1.1
W1 J	30.0±1.3	29.4±0.9	30.3±0.5	48.7±0.5	45.8±1.4	51.6±0.4	54.8±1.6	57.5±1.5	53.2±0.5
W3 J	28.0±0.5	31.2±1.5	32.5±0.9	46.8±1.0	52.8±2.0	55.6±0.6	54.2±0.7	53.6±1.3	52.8±2.1
W4 J	29.8±0.9	33.5±1.4	34.4±0.7	42.6±1.1	52.6±1.4	53.6±1.0	52.7±1.2	53.1±0.7	52.2±1.5
SiteF value	*	*	*	*	NS	NS	NS	NS	NS
DateF Value	*	*	*	**	*	*	*	*	*

± = Standard Error, NS = Non significant, ** = Significant at 0.5% and * = Significant at 0.1%

Moisture Content and Germination

Irrespective of altitudinal variations in selected sites the germination in acorns commenced from the third week of September, however the related range of moisture content of acorns at the time was highly variable being 40.9 ± 1.2 %at lower elevation site I and 50.4 ± 2.2 % at high elevation site III. Maximum acorn germination however varied in percent and week of germination. The maximum germination varied between 56.7 ± 1.1 % and 59.6 ± 0.6 % on site I and II in third week of November when moisture content was between 36.4 ±0.8 and 37.0 ± 2.8 percent. At site III the high elevation site the maximum germination was 57.5 ± 1.2 in third week of December when moisture content of acorns was 37.6 ± 1.2 (Table 3). Mean germination capacity decline from the time of initial germination to final germination. ANOVA showed that moisture content of seed varied significantly across sites and collection dates ($p<0.01$) and germination varied significantly only across collection dates ($p<0.01$).

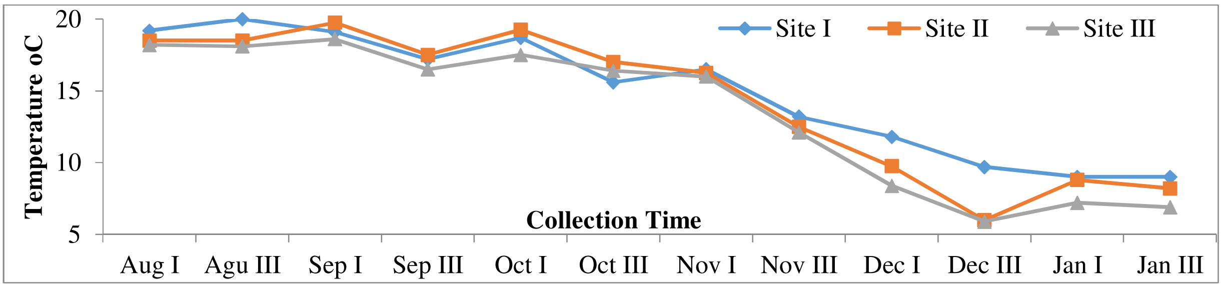

Fig 1. Variations in temperature across the collection dates at site I, II and site III during mid-day (12.00-1.00 P.M) Aug I signifies 1st week August; Aug III-3rd week August; Sep I-1st week September; Sep III-3rd week September; Oct I-1st week October; Oct III-3rd week October; Nov I-1st week November; Nov III-3rd week November; Dec I-1st week December; Dec III-3rd week December; Jan I-1st week January; Jan III-3rd week January.

Significance difference in physical acorns characteristics and germination of seeds collected from different elevations was observed on same collection dates. Such differences have also been reported when comparisons were made between seeds for 10 separate populations in west Timor for *Santalum album* (Brand *et al*., 1994). Various researchers have proposed seed colour as indicator of seed maturity. The colour of oak acorns changed from light green to dark brown at maturity. Colour change in seed is a simple indicator of maturity than other seed parameters which require laboratory facilities (Pandit *et al*., 2002). Acorn maturity was best indicated by the ease with which the acorns could be separated from the acorn cap with the commencement of browning of acorns. The acorns could be easily separated from the acorn cap from November Istweek onwards at site I and II (lower elevation site) and from December Ist week on site III (high elevation site). The weight of acorns were significantly higher at lower elevation site (I & II) compared to high elevation site III. Significantly higher seed weight at lower elevation sites of oak than high elevation site has also been reported by other workers (Bhatt and Ram, 2005). The mean acorns size was also larger at lower elevation site I compared to higher elevation site III. These variations may be due to environmental condition operating at the time of seed development (Harper, 1977). At lower elevation site I, II the temperature were higher by 1.4° C compare to higher elevation site III.

Across the sites moisture content of seeds varied from 56.7 ± 2.4% to 28.0±0.5 % during thirteen collection dates and declined with each collection.Initial moisture content of acorns was significantly high at high elevation site compared to mid and low elevations. The maximum germination occurred when moisture content across all sites ranged between 36.4 - 37.0. Best seed germination between 39.1±0.8 and 41.7±3.5 percent moisture content has also previously been reported (Bhatt and Ram, 2005). Moisture content of oak acorns has been proposed a useful indicator of viability retention and germination (Juhnel, 1957; Griffin, 1971; Schopmeyer, 1974). The seed germination started after 46 days of start of acorn collection and was almost complete after 135 days. At site I and site II located at lower elevations the maturation time was in third week of November as evident from peak germination. However at site III the high elevation site the maturation was in third week of December. In an earlier study (Rao, 1984) conducted 32 years ago indicate that peak seed fall in *Q. leucotrichophora* at a site located at 1818m elevation commenced from January and continued upto March – April. The maximum germination under laboratory conditions at 25°C and 30°C ranged between 80% - 100 %. The seed size was 21.29 x 12.80 mm and seed weight 2034.5 mg (fresh) and 1463.2 (dry) mg seed^{-1}. In the present study the maximum germination was much lower (30-40%) under similar conditions and time of commencement of natural seed fall earlier by 45 days starting from November 2nd week and attaining a peak by December end/January 1st week. The acorns had attained a size and weight relatively similar to the values reported by (Rao, 1984) at the time of maximum germination in the present study by November 3rd week at lower elevations sites and December 3rd week at high elevation site. Present study indicated that acorns of *Q. leucotrichophora* matured and germinated earlier at lower elevation sites I and site II compared to higher elevation site III. The mean germination capacity did not show significant variation across the elevation. Seed moisture content and seed germination were negatively correlated (r^2= 0.586, 0.797 and at 0.5%) across site I, II and III respectively. It is evident from the study that the colour change of acorns from green to light brown/brown ease of separation of acorns from the cap, acorn size and moisture content range between 36.4 ± 0.8 – 37.6 ± 1.2 appear to be reliable indicators of maturity in *Q. leucotrichophora* along its elevational range.

Conclusion

It appears that time of seed maturation in *Q. leucotrichophora* has become earlier when compared with earlier studies and germination percent has declined. This could be a yearly variation impacted by climatic irregularity and needs to be authenticated through studies spread over several years and more sites. We conclude that shifts in timing of seed maturation can have major repercussions on seed germination and recruitment of this species under natural conditions. The data generated in this study can be used as a reliable indicator for assessing the acorn maturation time which can be use by various departments involved in plantation drives.

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