

PHENOTYPIC PLASTICITY IN WESTERN REDCEDAR¹

Yousry A. El-Kassaby

PRT Management Inc., 4 - 1028 Fort Street, Victoria, British Columbia, Canada V8V 3K4 &
 Department of Forest Sciences, Faculty of Forestry, The University of British Columbia, Vancouver, British Columbia,
 Canada V6T 1Z4

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ABSTRACT

Compared to its associates, western redcedar (*Thuja plicata* Donn) has been recognized to harbour the lowest level of genetic variability. Genetically depauperate species occupying a wide range resort to various strategies for their survival and adaptation. Phenotypic plasticity, the ability of an individual to alter its morphology/physiology in response to changes in the environment, was identified as the mechanism by which western redcedar secured its survival. Events in the species' history, coupled with its unique reproductive biology, have allowed the perpetuation of its low genetic variability through inbreeding. Examples of phenotypic plasticity in western redcedar will be provided and a novel method for exploiting the species' phenotypic plasticity for enhancing commercial production of seedlings will be presented.

Key words: *Thuja plicata*, Western redcedar, phenotypic plasticity

INTRODUCTION

Western redcedar (*Thuja plicata* Donn) is a widespread native species of mesic forests in both coastal and montane areas of western North America (Figure 1). It grows from coastal southern Alaska to the coastal ranges of British Columbia, western Washington and Oregon, to northern California. It seldom occurs in pure natural stands, and usually grows with a wide variety of associated species that vary in composition according to where it is found (FOWELLS 1965). Western redcedar is considered to be genetically depauperate based on biochemical and/or molecular gene markers. Low genic heterozygosity for allozyme loci and nuclear RFLP (COPES 1981; YEH 1988; EL-KASSABY *et al.* 1994; GLAUBITZ *et al.* 1998) and low intra- and inter-population diversity in leaf oil terpene composition (VON RUDLOFF & LAPP 1979; VON RUDLOFF *et al.* 1987) constitute the basis for the reported genetic uniformity in this species. Paleobotanical records suggest that western redcedar experienced a severe population bottleneck during the Vashon stage of the most recent ice age (MATHEWES 1973; CRITCHFIELD 1984; HEBDA & MATHEWES 1984; WAINMAN & MATHEWES 1987). Fossil pollen records indicated that "cedar" pollen was absent from its contemporary range and that the species has spread along the glaciated coast of British Columbia (B.C.), invading the Fraser lowland of B.C. during

the early Holocene (6,000–10,000 BP). These findings were considered by population geneticists as evidence to support the hypothesis of its genetic uniformity (YEH 1988; VON RODLOFF *et al.* 1987; EL-KASSABY *et al.* 1994; GLAUBITZ *et al.* 1998). However, the debate

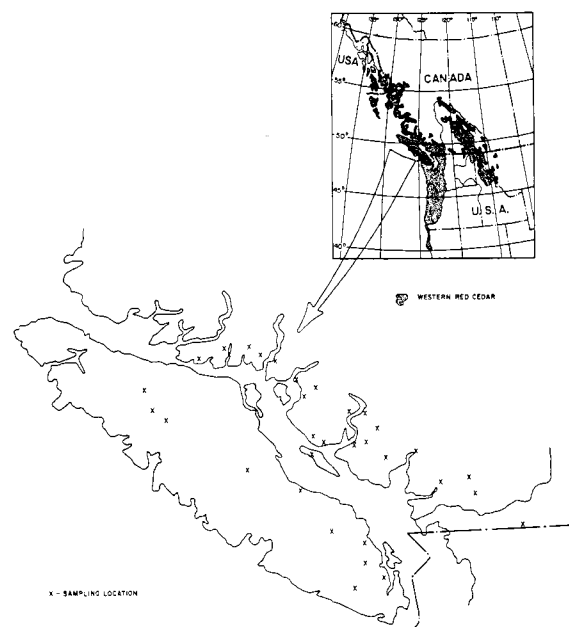


Figure 1. Distribution of *Thuja plicata* and sampling locations of the 100 clones studied.

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about the utility/validity of these “neutral or nearly neutral” markers in demonstrating the “actual” level(s) of genetic diversity in quantitative and adaptive attributes, doomed the genetic uniformity concept of western redcedar to be suspect in spite of the paleobotanical records that corroborated the population genetic findings. Recent studies demonstrated lack of differences in western redcedar early growth traits (*i.e.*, quantitative) (BOWER & DUNSWORTH 1988; REHFELDT 1994, but see CHERRY 1995) and provided evidence on the lack of inbreeding depression in its reproductive biology traits (OWENS *et al.* 1990). EL-KASSABY *et al.* (1994) substantiated the results of OWENS *et al.* (1990) on the lack of inbreeding depression in western redcedar's reproductive biology traits and reported the lowest ever outcrossing rate in any conifer studied ($t = 32\%$). However, the limited number of populations studied by BOWER and DUNSWORTH (1988) and the low number of individuals investigated by OWENS *et al.* (1990) weaken these conclusions, and the question of western redcedar's genetic uniformity remains unanswered.

REPRODUCTIVE PHENOLOGY

EL-KASSABY and WORRALL (unpublished) observed uniformity of reproductive phenology (the time and duration of pollen shedding and seed-cone receptivity among individuals during a pollination season) among 100 western redcedar clones growing in a seed orchard (Figure 1). They also presented an argument attempting to interpret the observed uniformity as a by-product of the species' phenotypic plasticity. In their study, the reproductive phenology of each clone was monitored throughout the 1989 and 1991 pollination seasons to determine the time and duration of seed-cone receptivity and pollen-shed. The 1989 pollination season covered a 20-day period while the 1991 was shorter and only covered 11 days (Figure 2). This major difference in pollination season was caused by the drastically different weather conditions of the winter and spring

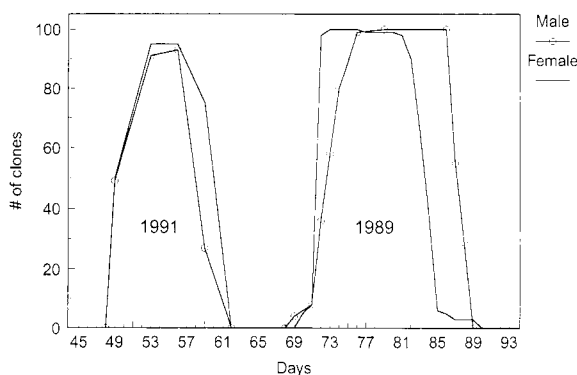


Figure 2. Reproductive phenology profile of the 100-clonal experimental population.

prior to reproductive bud burst. The winter and spring of 1989 were typical to that of the seed orchard location, however, the 1991 winter was unusually cooler and was suddenly interrupted by a short warm period. The duration of clonal receptivity and pollen-shedding of both years resulted in the participation of most or all of the orchard's clones in pollination during the same time. This pattern produced unprecedented uniformity that has not been observed for any coniferous tree species seed orchard. Substantial reproductive phenological differences were observed for all investigated seed orchards of many conifers (*Picea abies* Karst., ERICSSON *et al.* 1973; *Pinus sylvestris* L., JONSSON *et al.* 1976; *Picea mariana* (Mill.) B.S.P., O'REILLY *et al.* 1983; *Pseudotsuga menziesii* (Mirb.) Franco, EL-KASSABY *et al.* 1984, FASHLER & EL-KASSABY 1987; COPES & SNEZKO 1991; *Pinus taeda* L., ASKEW 1986; *Picea sitchensis* (Bong.) Carr., EL-KASSABY & REYNOLDS 1990). Reproductive phenology bud burst is an adaptive attribute known to be very sensitive to environmental conditions and is well demonstrated to be under strong genetic control in many conifer species (see BOYER 1972; WORRALL 1983; EL-KASSABY *et al.* 1984; BLUSH *et al.* 1993 for reviews). It has been demonstrated for several coniferous species that both time and temperature (summarized as heat sums) have a significant effect on flowering time (BOYER 1972; EL-KASSABY *et al.* 1984; WORRALL 1993). Therefore, climatic variability in origin of the seed orchard's clones is expected to produce variation in reproductive phenology development when they are placed in one location. The near-to-perfect synchrony in flowering phenology within and among clones indicates that the seed orchard's 100 clones are not differentially sensitive to environmental cues and that a lack of genetic variation in flowering timing might exist in this species. The observed reproductive phenology uniformity in the seed orchard population provided further evidence supporting the notion that the species lacks genetic variability. However, EL-KASSABY and WORRALL provided an example demonstrating that different genotypes with different heat sums and threshold requirements could reach reproductive bud flushing at the same time, thus producing the illusion of genetic similarity. Thus, it is most conceivable that western redcedar, as a species, requires low heat sum for bud burst. This could be a characteristic associated with its indeterminate growth habit. EL-KASSABY and WORRALL considered the low heat sums and/or threshold requirement is an essential characteristic of western redcedar survival as a species and it represents a form of phenotypic plasticity.

SEED GERMINATION

The observed high level of inbreeding in western red cedar seed orchard and in particular its observed wide variation among clones (EL-KASSABY *et al.* 1984) provided a challenge in identifying the outcrossed seeds from the inbred ones without destructive sampling (*i.e.*, biochemical finger printing). Thus, the concept of seed germination under sub-optimal environmental condition was adopted (ANONYMOUS 1992). This method, (the Cambridge Vigour Test), was developed at the Official Seed Testing Station (OSTS) for England and Wales for testing wheat and barley. The main concept of this test is to germinate seeds under sub-optimal environmental conditions, thus challenging them to germinate. Successful germination under these conditions was interpreted as a true test of seed vigour. The optimal laboratory environmental conditions for western red cedar seed germination under the International Seed Testing Association's (ISTA) (1985) rules was used to germinate seed from 22 seed orchard clones. Unstratified seeds were set into germination boxes, then placed into the germination cabinet. Temperature was maintained alternately at 30 °C for eight hours and 20 °C for 16 hours, with light at approximately 1000 lux, using cool-white fluorescent tubes, being provided during the higher temperature period. Germinants were counted every second day throughout the 28-day test period. Germinants were removed when the radicle had reached twice the length of the seed coat (EDWARDS 1982). Germination counts were summarized to estimate germination capacity (GC), the number of germinants expressed as a percentage of filled seeds, at the end of the test.

Figure 3 represents the course of germination for the 22 clones tested. Germination commenced for the majority of the clones after nine days and substantial variation was observed for GC (range: 58 to 98%) (Figure 3). The vigour test was conducted under identical conditions with the exception of using temperature regime that alternated between 15 °C for eight hours and 5 °C for 16 hours. Under these sub-optimal environmental conditions, no germination was observed for 70 days. After this period (*i.e.*, 70 days), the temperature regime in the germination cabinet was changed to follow that recommended by the ISTA and germination commenced after five days for the majority of the clones (*i.e.*, four days sooner than that observed for the previous test). It should be emphasized that all clones responded after the change, however, the range in GC was greater than observed under the ideal test (range: 40 to 94%) (Figure 4). The same vigour test was repeated for 90 days with similar results (*i.e.*, no germination under the sub-optimal environmental

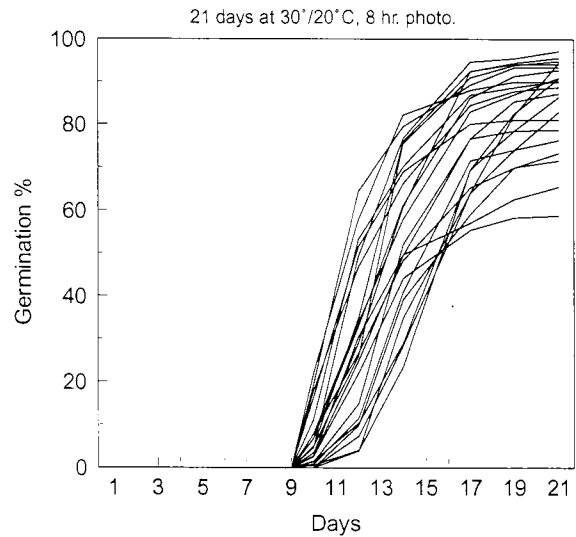


Figure 3. Germination course of 21 western redcedar trees germinated at 30°/ 20 °C and 8 hours photoperiod.

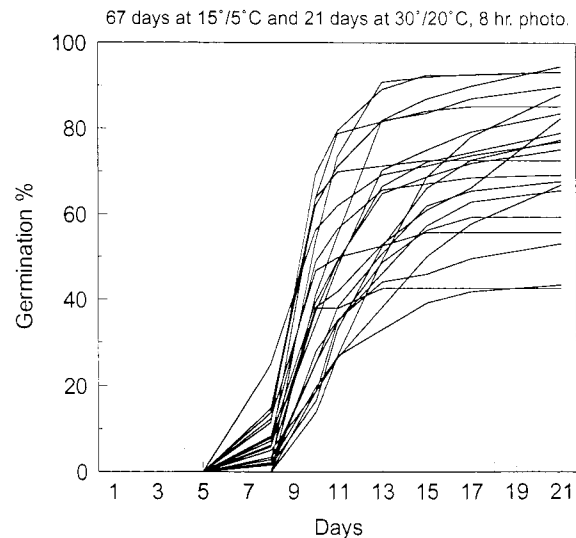


Figure 4. Germination course of 21 western redcedar trees incubated at 15°/ 5 °C and 8 hours photoperiod for 70 days and then germinated at 30°/ 20 °C and 8 hours photoperiod..

conditions) and germination started after changing the temperature to that recommended by ISTA. However, germination started after four days and the variation in GC was much greater than that observed for the previous two tests (range: 18 to 90%) (Figure 5). Significant difference was observed among the three treatments when the germination data were analysed using ANOVA and two significant groups were produced by the Duncan's Multiple Range Test. The ISTA test formed one group with a mean GC of 85.4 while the two "vigour" tests formed another group with means of 73.1 and 72.1 for the 70 and 90 days treatments, respectively. The ISTA standard germination test and

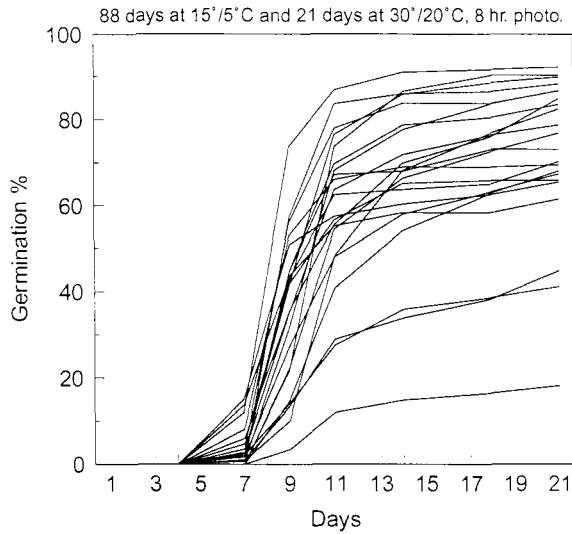


Figure 5. Germination course of 21 western redcedar trees incubated at 15°/ 5 °C and 8 hours photoperiod for 90 days and then germinated at 30°/ 20 °C and 8 hours photoperiod..

Cambridge Vigour Tests provided insight on how western redcedar responds to unfavourable environmental conditions. Western redcedar as a species has the ability to react according to the environmental conditions. If the environmental conditions are favourable, the species takes advantage of it and its response is immediate. However, if the environmental conditions are not favourable, the species will “turn itself off” waiting for changes to occur. These are the attributes of an opportunist. Western redcedar as a species is suited for this due to its indeterminate growth strategy. It should be stated that this test did not provided conclusive answer on which seed was inbred or outcrossed, however, we concluded that the increase in GC variance (represented by the three germination tests ranges) is indicative of some loss in germination that might be caused by inbreeding. It is noteworthy to mention that the response of western redcedar seeds to the vigour tests represented evidence of the species’ phenotypic plasticity.

SEEDLING PRODUCTION

The common method of producing western redcedar seedlings in container nurseries is to sow the seed in the spring and then provide the proper culture conditions (heat, fertilizer, and supplemental lighting) necessary for the seedlings to reach their target size (height, caliper and root mass).

Top pruning is usually required when the seedlings exceed their target height before target caliper is attained. A study was conducted to investigate the effect of fall sowing (September) on western redcedar.

The objective was to produce quality seedlings that could be available for the following year’s late-summer planting. Additional benefits would include: 1) the production of sufficient root mass to maintain a plug at harvest, 2) producing seedlings which met designated stock specifications through cultural practices which avoid pruning, and 3) cost saving realized by producing seedlings during a period which would not require heat and/or supplemental lighting. The study was conducted to compare fall sowing date and the commonly practiced spring sowing (control). One western redcedar seedlot was used and seeds were sown into styro-blocks™ (type PSB 313b, 160 cavities / block) and placed in a commercial container nursery. Nursery culture regime used was the same for both sowing times. Heat to prevent freezing and cold below 10 °C was used for the fall-sowing treatment while 20 °C / 10 °C (day/night) was used for the spring (control). Stock specification used to determine minimal levels of acceptability for comparison were those set by the British Columbia Ministry of Forests. These were 15 cm (min. height) and 2.0 mm (min. caliper) for the

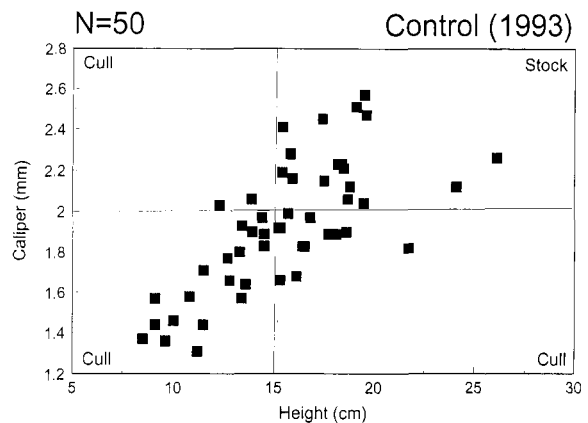


Figure 6. Height and caliper distributions for 50 western redcedar seedlings grown under standard Spring sowing (control).

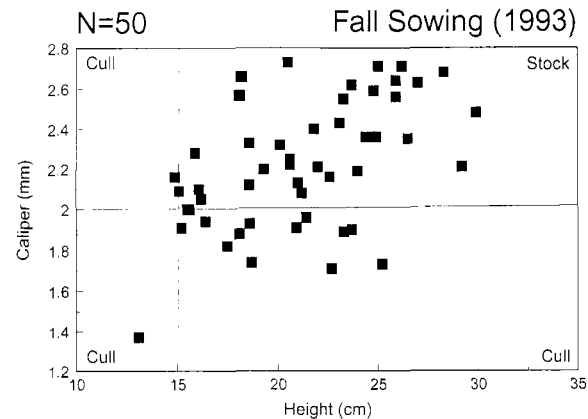


Figure 7. Height and caliper distributions for 50 western redcedar seedlings grown under Fall sowing.

stock type used. A random sample of 50 seedlings was collected prior to harvest and height and caliper were determined for each sample. An anticipated recovery of 98 and 34% for the fall and control sowing, respectively, was expected (Figures 6 and 7). Additionally, clustering of fall seedlings was observed as opposed to the wide distribution of the control ones (Figures 6 and 7). ANOVA of the two attributes produced highly significant differences between the two sowing times and Duncan's Multiple Range Test results produced significant differences for height (22.4 vs. 15.4 cm) and caliper (2.4 vs. 1.8 mm). This experiment demonstrated that the phenotypic plasticity of western redcedar allowed the species to take advantage of the favourable growing environment during September and October. During this period three-crop development stages were attained. These are 1) complete seed germination (the temperature during this period was warm enough to attain complete germination), 2) good root development, and 3) seedling shoot development did not exceed the cotyledon stage. After this period the temperature dropped and the crop went into a resting stage (*i.e.*, the species 'turned itself off') over the winter. During the following spring this crop had fully-developed root and cotyledons and the crop developed at a faster rate than those sown during the spring.

CONCLUSION

Phenotypic plasticity, the ability of a single genotype to produce a diversity of responses to varying environmental conditions, is considered a genetic mechanism that acts as a buffer against fluctuating spatial or temporal habitat conditions (SCHLICHTING 1986; WEST-EBERHARD 1989). This mechanism is fundamental for the survival of plants due to their sessile life-style. The three previously mentioned studies (reproductive phenology, germination and seedling production) have demonstrated a high degree of phenotypic plasticity in western red cedar. Further evidence of the species' phenotypic plasticity is frequently observed in many experiments (ADAMS & MAHONEY 1991; KRASOWSKI & OWENS 1991; SILIM & LAVENDER 1994; REHFELDT 1994; MAJOR *et al.* 1994; FOLK *et al.* 1994). Although these experiments were not designed to investigate the phenotypic plasticity of western red cedar, the results could be easily explained by phenotypic plasticity. The species, lacking vegetative buds and shoot dormancy (*i.e.*, indeterminate growth), is a shoot-growth opportunist. Shoot growth is markedly responsive to unseasonably warm temperatures during the colder months. Unlike other bud-forming conifers (*i.e.*, determinant), where the timing of growth cessation is determined by heat sum/photoperiod requirements (WORRAL 1983),

termination of shoot growth in this species is controlled by temperature alone (SILIM & LAVENDER 1994). This may help to explain how a species with this low level of genetic variation, at least at the isozymes, terpenes, and RFLP levels, can have such wide ecological amplitude and latitudinal range. As suggested by LEWONTIN (1957), homeostasis for the fitness of a genotype in the face of environmental variation (*e.g.* temporal or spatial variation in the length of the growing season) may be achieved by phenotypic plasticity (*e.g.* of shoot growth in response to temperature). Hence, adaptive phenotypic plasticity can be viewed as an alternative strategy to genetic diversity to deal with environmental heterogeneity (LEWONTIN 1957). It has been hypothesized that a species or population with abundant adaptive phenotypic plasticity may have less need for genetic variation and thus may be more likely to lose it (LEVINS 1963; MARSHALL & JAIN 1968). However, a consistent inverse relationship between genetic diversity and plasticity of species or populations has failed to materialize in plants (SCHLICHTING 1986). It seems likely that these two alternative adaptive strategies are not mutually exclusive but may coexist in varying degrees within a species or population (SCHLICHTING 1986), as was originally suggested by LEWONTIN (1957).

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