Research note

Seed Dormancy and Germination in *Neolitsea acuminatissima* (Lauraceae)

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【Summary】

Fresh seeds of *Neolitsea acuminatissima* germinated slowly at 30/20℃ in light (with a 12-h daily photoperiod) and required > 20 wk to complete germination. Seeds cold-stratified at 4℃ for 9 mo or for 1 yr not only retained their original viability, but the germination rate significantly increased. Fresh seeds have a fully-developed embryo and a water-permeable seed coat and endocarp, and they require > 4 wk to germinate in a warm temperature regime. Thus, we concluded that seeds of *N. acuminatissima* have nondeep physiological dormancy.

Key words: cold stratification, *Neolitsea acuminatissima*, physiological dormancy, seed germination.
Chen SY, Kuo SR, Baskin JM, Baskin CC, Chien CT. 2006. Seed dormancy and germination in *Neolitsea acuminatissima* (Lauraceae). Taiwan J For Sci 21(1):125-9.

研究簡報

高山新木薑子種子的休眠與發芽

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摘 要

新鮮採收的高山新木薑子種子在變溫30/20℃和每天12時光照下發芽緩慢，需要20週以上的時間才能全部發芽完畢。種子以4℃低溫層積9個月或1年，不但能保持與原來新鮮種子一樣的發芽活力，而且發芽速率顯著地增加。新鮮種子具有發育完全的胚，以及水分可通透的種皮和內果皮，但它們卻需要4週以上時間才開始發芽。因此，我們推論高山新木薑子種子具有非深度的生理休眠。

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Received December 2005, Accepted February 2006. 2005年12月送審 2006年2月通過。

關鍵詞：低溫層積作用、高山新木薑子、生理的休眠、種子發芽。
陳舜英、郭幸榮、Baskin JM、Baskin CC、簡慶德。2006。高山新木薑子種子的休眠與發芽。台灣林業科學21(1):125-9。

The genus *Neolitsea* of the tribe Laureae and the family Lauraceae (Van der Werff and Richter 1996) consists of about 100 species of trees and shrubs distributed in Indomalaysia, East Asia and Australia (Rohwer 1993, Mabberley 1997). Nine species occur in Taiwan (Liao 1996), and one of them, *Neolitsea acuminatissima* (Hayata) Kanehira & Sasaki, is a small evergreen tree endemic to Taiwan, where it occurs in natural broadleavf evergreen forests throughout the island at elevations from 1,500 to 2,500 m. The seed dormancy in *Neolitsea* species has not been characterized in much detail. Neither Nikolaeva et al. (1985) nor Baskin and Baskin (1998) included any species of *Neolitsea* in their compendia of the kinds of seed dormancy among several thousand species. However, the small amount of information on storage and germination indicates that seeds of this Old World tropical/subtropical genus do exhibit some dormancy (discussed below). The purpose of this study was to characterize seed dormancy in the Taiwan endemic, *N. acuminatissima*.

Mature dark-red fruits of *N. acuminatissima* were harvested from a natural subtropical montane evergreen forest at Peitungyenshan (24°05'N, 121°07'E) on 27 November 2002, Nantou County, Taiwan. Fruits were macerated by hand in water, and the clean, sunken seeds were allowed to dry on newspapers for several hours at room temperature, after which they were stored temporarily in a closed container at 4℃ before treatments began. The moisture content (MC) was determined with 4 replications of 15 seeds each and was calculated on a fresh-weight basis (fwb) after oven drying for 17 h at 103℃ (International Seed Testing Association 1999).

Fresh seeds were cold-stratified at 4℃ in moist sphagnum in sealed polyethylene (PE) bags (0.04 mm in thickness) for 0, 1, 2, 3, 6, 9, and 12 mo prior to testing them for germination. The sphagnum used for treatments was cut into small pieces, and then wetted to a MC of about 400% of its dry mass.

For germination tests, fresh and cold moist-stratified seeds were mixed with moist sphagnum in sealed PE bags and incubated at 30/20℃ with 12 h of fluorescent light (80~100 μmol $m^{-2}s^{-1}$, 400~700 nm) at the higher temperature. Each treatment consisted of 3 replications of 35 seeds each. Germination, i.e., a radicle at least 5 mm long, was recorded weekly. Results are expressed as the percent germination and as the mean germination time (MGT) in days (Naylor 1981) after 12 wk of incubation. $MGT = (\Sigma n_i t_i)/N$, where n_i is the number of seeds germinated in t_i days from the beginning of the test, and N is the total number of germinated seeds at the end of the test. MGT is a measure of the rate of germination and of the sharpness of the germination peak. Means of germination percentages and of MGTs were compared by analysis of variance (ANOVA) and by the least significant difference (LSD) test at a 5% level of significance (SAS 2004).

The MC of freshly harvested seeds (seed + thin endocarp, hereafter referred to as seeds) of *N. acuminatissima* was 29.2%, and mean number of seeds per liter was 5,183 seeds. The seed is 6~9 mm long×4~6 mm wide and consists of a thin endocarp, a seed coat, and a large dicotyledonous embryo; no

endosperm is present (Rohwer 1993, Fig. 1). Using fresh seeds, 79.3% germinated during a 20-wk incubation at 30/20℃, and the MGT was 93.8 d. No fresh seeds germinated during the first 4 wk of incubation, and germination continued throughout the remainder of the 20-wk incubation period (Fig. 2). Cold stratification at 4℃ significantly increased the germination rate but not the germination percentage after 20 wk. Germination percentages of seeds cold-stratified for 0 (fresh), 3, 9, and 12 mo were 79.3, 72.4, 71.4, and 74.3%, respectively ($p > 0.05$), whereas MGTs were 93.8, 93.3, 53.8, and 47.6 d, respectively ($p < 0.05$) (Table 1). No germination occurred during cold stratification prior to incubation.

A dormant seed is considered one that does not germinate in about 4 wk when incubated under conditions that otherwise are favorable for its germination after dormancy is broken (Baskin and Baskin 1998). No freshly harvested seeds of *N. acuminatissima* had germinated after 4 wk of incubation. Cold stratification for 6, 9, or 12 mo caused an increase in the germination rate, e.g., 40% of seeds cold-stratified at 4℃ for 12 mo germinated after 4 wk of incubation compared to 0% of those that were nonstratified (Fig. 2). Thus, since the MGT of fresh seeds was nearly twice as high as those of seeds cold-stratified (at 4℃) for 9 or 12 mo (Table 1), it is clear that the embryo is fully developed (Rohwer 1993, Fig. 1), and the seed coat is water-permeable, i.e., members of the Lauraceae do not have water-impermeable seed

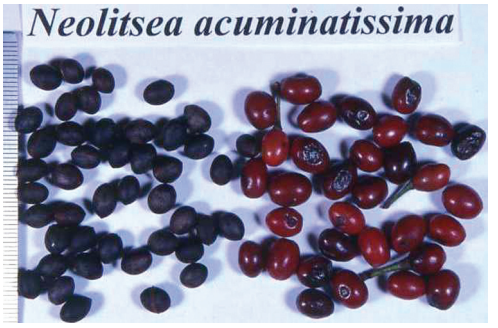

Fig. 1. *Neolitsea acuminatissima*: fruits (right) and seeds (left), scale is in mm.

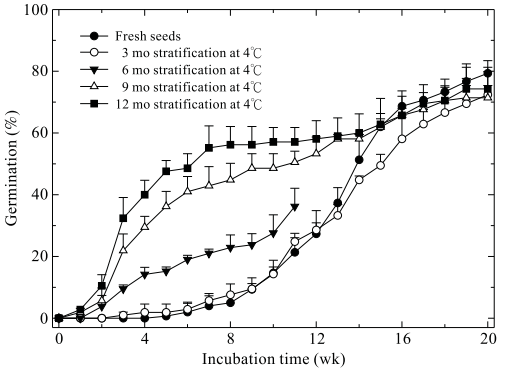

Fig. 2. Cumulative germination curves (mean±SE) for freshly harvested and cold-stratified seeds of *Neolitsea acuminatissima* incubated at 30/20℃ for 20 wk. Data from 6 mo cold-stratified seeds were recorded for only 11 wk.

Table 1. Effect of cold moist (4℃) stratification on the percentage and rate (MGT) of germination of *Neolitsea acuminatissima* seeds. Germination percentages are for seeds incubated at 30/20℃ in light for 20 wk. Means ($n = 3$) in each column with the same letter do not significantly differ (LSD, $p = 0.05$). MGT, mean germination time in days. Values in germination represent the mean±standard error. "—" data missing

Length of stratification (mo)	Germination (%)	MGT (d)
0	$79.3^{a}\pm4.1$	93.8^{a}
1	$65.7^{b}\pm7.0$	97.9^{a}
2	$71.4^{ab}\pm2.3$	100.1^{a}
3	$72.4^{ab}\pm7.5$	93.3^{a}
6	—	—
9	$71.4^{ab}\pm4.0$	53.8^{b}
12	$74.3^{ab}\pm7.0$	47.6^{b}

(or fruit) coats (Baskin et al. 2000). Baskin and Baskin (2004) summarized that seeds with nondeep physiological dormancy require cold (0~10℃) or warm (≥ 15℃) stratification to break dormancy, whereas seeds with deep physiological dormancy require 3~4 months of cold stratification to germinate. We concluded that seeds of *N. acuminatissima* exhibit nondeep physiological dormancy.

Seeds of the few other species of *Neolitsea* whose germination requirements have been investigated also appear to have nondeep physiological dormancy, or to be nondormant. It respectively took 46 and 119 d for 50% of the seeds of *N. fuscata* and *N. involucrata* collected in a tropical wet evergreen forest in Sri Lanka and sown in garden trials to germinate (based on the total number that germinated), indicating that most fresh seeds are physiologically dormant (Holmes 1954). Cold stratification of seeds of *N. aciculata* and of *N. sericea* at 5℃ for 1 mo caused increases in germination percentages at 25℃ compared to untreated seeds of from 36 to 69.5% in *N. aciculate* and from 54 to 84% in *N. sericea* (Goo 1976). Fresh seeds of *N. parvigemma* collected from subtropical montane evergreen forests in Taiwan were dormant. It took 15 wk for them to complete germination at 22℃ in light, and their dormancy was overcome by cold-moist storage at 4℃ for 6 mo (Lin 1996). Cold stratification enhanced seed germination in *N. ariabillima*, and a period of afterripening in dry storage caused an increase in germination percentage of seeds of *N. acuminatissima* (reported in Smith et al. (2002) as personal communication in 1994 with TP Lin). In a recent study by SY Chen et al. (unpublished), seeds of *N. aciculata* var. *variabillima* also exhibited slow germination, and cold stratification increased the germination rate, indicating that they exhibit physiological dormancy.

Cold stratification not only increased the germination rate (i.e., MGT decreased) of *N. acuminatissima*, but the seeds retained their original viability for at least 1 yr. Seeds of several other lauraceous species in Taiwan also retain their viability in moist sphagnum at 4℃ for 1 or 2 yr (Lin 1996, Chien et al. 2004). Thus, this method seems to be good for storing seeds of the Lauraceae in Taiwan and other countries.

ACKNOWLEDGEMENTS

The authors thank Chiung-Mei Lee, Kuan-Hung Liu, Allen Wu, and Chiou-Jin Wu for their technical assistance. This study was supported by a grant from the Council of Agriculture, Executive Yuan, Taiwan.

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