

Delayed Opening of *Pinus yunnanensis* Cones and Its Canopy Seed Bank Postprint

Authors: Su Wenhua, Cui Fengtao, Zhao Yuanjiao, Zhou Rui, Zhang Guangfei, Cao Jianxin

Date: 2017-02-09T00:00:00+00:00

Abstract

The retention of mature fruits in the canopy with delayed scale opening for seed release is a common adaptive trait of plants in fire-prone habitats. Taking *Pinus yunnanensis* distributed in the western suburbs of Kunming as the research object, we investigated the number of cones retained in the canopy, the open/closed status of scales, the age distribution of cones, the germination rate of seeds within cones, and the response of scale opening in serotinous cones to high temperature and fire. The results showed that: in the canopy of *P. yunnanensis*, besides current-year mature cones, there were also scale-closed cones that matured from 1 to 7 years previously, and scale-opened cones that matured from 1 to 8 years previously. Serotinous closed cones contained germinable seeds, and the germination rate of seeds decreased with prolonged cone retention time; the average germination rate of seeds in 9-year-old retained cones was only 2.9%. The canopy of each 100 m² forest plot stored approximately 105 viable seeds, which is twice the average seed production of the recent three years. During the retention period, some cones from each retained year cohort opened their scales and released seeds annually; generally, all cones gradually opened and released seeds within 8 years after maturation. Temperatures above 40°C could induce cone scale opening, and the time required for cone opening decreased with increasing baking temperature. The quantity of seeds released by *P. yunnanensis* after fire was approximately 2.6 times the average annual seed release in unburned areas, and the germination rate of seeds released in burned areas was (69.8±22.8)%. The research results indicate that *P. yunnanensis* possesses a non-strict canopy seed bank; ground fire can induce scale opening in closed cones in the canopy to release seeds; the maximum retention time and delayed opening time of cones in the canopy are basically consistent with the seed survival time; seeds released naturally each year and those released after fire are both composed of seeds matured over multiple years; the delayed opening of *P. yunnanensis* cones may be related to the fire-prone nature of the habitat.

Full Text

Canopy Seed Bank and Serotinous Cones of *Pinus yunnanensis* Forests

Su Wenhua¹, Cui Fengtao¹, Zhao Yuanjiao¹, Zhou Rui¹, Zhang Guangfei¹, Cao Jianxin²

¹Institute of Ecology and Geobotany, Yunnan University, Kunming 650091, China

²Yunnan Academy of Forestry, Kunming 650201, China

Abstract

Serotiny and canopy seed banks represent prominent adaptations in fire-prone ecosystems. This study investigated *Pinus yunnanensis* forests in the western suburbs of Kunming to quantify canopy seed bank dynamics, cone age structure, and seed viability. We recorded the number of open and closed cones in the canopy, observed cone ripening years, and examined how closed cones respond to high temperatures and forest fires. Closed cones could be retained in the canopy for seven years, while open cones persisted for nine years. Seed germination rates from closed cones decreased significantly with increased serotiny time, with seeds from cones retained in the canopy for nine years showing only 2.9% germination. The canopy seed bank contained approximately 100,000 viable seeds per 100-m² plot, which was twice the annual seed production. From every cohort, some cones would release seeds annually, with all seeds being released within eight years after maturation. The serotinous cones of *P. yunnanensis* could be induced to open at high temperatures (over 40°C), with opening time decreasing as temperature increased. Post-fire plots released 2.6-fold more seeds than unburned plots, and the germination rate of released seeds in post-fire plots was (69.8±22.8)%. These results suggest that *P. yunnanensis* is a weakly serotinous species. Fire significantly increased seed release from the canopy seed bank, whereas the serotiny time was similar to seed longevity in closed cones. Furthermore, seeds released in both post-fire and unburned plots originated from cones that ripened in different years. In sum, the serotinous cones of *P. yunnanensis* represent an adaptation to fire in fire-prone habitats.

Keywords: pine; cone; serotiny; canopy seed bank; fire ecology

Introduction

Most plants release seeds from the canopy immediately upon fruit maturation, creating a seed rain that falls to the ground where seeds either germinate promptly or persist in the soil seed bank within litter and soil layers. However, a minority of plant species retain mature fruits and seeds in the canopy rather than releasing them immediately. When fruits persist in the canopy until the next fruiting season, this phenomenon is termed canopy seed storage or delayed opening (serotiny), forming a canopy seed bank. Research on canopy

seed banks dates back to early ecological studies, with investigations covering adaptation to fire and drought, classification systems, seed release mechanisms, seed viability within banks, genetic characteristics of accumulated seeds, relationships between seed banks and parent plants, and the costs associated with delayed opening.

The ecological significance of canopy seed banks is generally considered to include four main aspects: (1) augmenting seed supply after fire to maximize seed sources for seedling recruitment; (2) buffering inter-annual fluctuations in seed production to ensure adequate seeds for seedling establishment following fire; (3) utilizing fire or rainfall cues to time seed release for optimal germination and seedling establishment conditions; and (4) minimizing seed losses to predation, pathogens, and decay. Serotinous plants typically inhabit two types of regions: fire-prone areas and arid zones. Over 500 serotinous plant species have been identified in fire-prone regions, while approximately 100 species occur in arid areas. In fire-prone habitats, delayed-opening fruits generally release seeds in response to fire or high temperatures, with fire-induced serotiny considered a primary adaptation to fire. In contrast, arid-zone plants rely on moisture-induced splitting to release seeds.

Yunnan Province represents a high-risk area for forest fires, with a wildfire history traceable to the late Permian period. Despite China being a global center of distribution for Pinaceae, with numerous pine species, no serotinous tree species had been reported prior to this study. *Pinus yunnanensis*, endemic to southwestern China, dominates the forests of the central Yunnan Plateau, which is considered the species' center of origin and distribution. Field observations have noted persistent cones on *P. yunnanensis*, but quantitative data on persistence duration and seed viability in retained cones remain lacking. This study investigates canopy seed bank characteristics in burned and unburned *P. yunnanensis* forests through field surveys of open and closed cones, germination testing of retained seeds, and experimental examination of temperature effects on cone scale opening to determine whether *P. yunnanensis* is a fire-induced serotinous species.

1. Study Area

The study was conducted in Kunming, located in the central Yunnan Plateau of southwestern China. The region experiences a subtropical climate with distinct wet and dry seasons due to southwest monsoon influence, differing from the humid subtropical climate of eastern China. Mean annual precipitation is approximately 1,035 mm, with about 124 mm falling during the dry season (November-April). The zonal vegetation is semi-humid evergreen broadleaf forest, though the most common forest type is secondary *Pinus yunnanensis* forest that establishes after disturbance of the primary broadleaf forest.

The canopy seed bank study plots were established in a *P. yunnanensis* forest near Qiongzhu Temple in western Kunming at 1,950-2,200 m elevation. The

forest had a canopy density of $(7.1\pm 1.3)\%$, with *P. yunnanensis* trees averaging (10.5 ± 1.8) m in height and (37.2 ± 8.4) cm diameter at breast height. The tree layer included occasional *Pinus armandii*, while common understory shrubs included *Rhododendron microphyton*, *R. sphaeroblastum*, and *Lyonia ovalifolia*. The burned plot was located in a *P. yunnanensis* forest southeast of Qiongzhū Temple, where a surface fire occurred on March 19, 2010. Field investigation in July 2010 revealed that herbs and shrubs had largely recovered, with *P. yunnanensis* seedlings emerging in the herbaceous layer, though most burned trees gradually died back.

2. Methods

2.1 Canopy Seed Bank Investigation in Unburned Areas

Pinus yunnanensis seed development spans two years, with cones maturing in October of the year following fertilization. The main stem and branch tips produce distinct annual nodes where cones develop. Cone age can be determined by counting internodes from the branch tip to the node bearing the cone. In October 2009, we established five $10\text{ m} \times 10\text{ m}$ plots near Qiongzhū Temple. Two investigators recorded the number of open and closed cones of each age class per tree. For each tree, five closed cones were collected from each retention year, with cones from the same retention year pooled across all trees in a plot. From these, three cones were randomly selected for seed counting, germination testing, and temperature response experiments. The mean values from three cones represented the seed count per cone for each retention year. The proportion of open cones was calculated as the percentage of open cones within the total of open and closed cones for each retention year. Annual seed production per tree was calculated by summing the products of closed and open cone numbers multiplied by mean seed count from sampled cones of the same year. Plot-level annual seed production was the sum across all trees.

2.2 Post-Fire Canopy Seed Bank Investigation

In July 2010 (four months post-fire), we established five $10\text{ m} \times 10\text{ m}$ plots in the burned area that had experienced surface fire but not crown fire. We recorded the number of open and closed cones of each age class remaining on branches and collected newly fallen seeds from the forest floor for germination experiments. On the same day, we established five additional $10\text{ m} \times 10\text{ m}$ plots in unburned forest 10-50 m from the burned area, conducting identical canopy seed bank assessments.

2.3 Seed Viability and Germination Testing

For each plot and retention year, we collected five closed cones. Additional closed cones retained for nine years were collected outside the plots. Cones were oven-dried at 76°C until all scales opened completely. After counting seeds per

cone, all seeds from cones of the same retention year were pooled for germination experiments using the filter paper method in culture bottles, with radicle emergence as the germination criterion. Each retention year had three replicates of 50 seeds each. Germination rate was calculated as the number of germinated seeds divided by total experimental seeds.

2.4 Temperature Effects on Cone Opening

For each plot and retention year, we collected five closed cones. After pooling cones from each retention year, we randomly selected 15 cones and divided them into three groups of five for temperature treatments. Cones were placed in ovens at 40°C, 60°C, 80°C, 100°C, and 120°C, with opening time recorded for each cone.

2.5 Data Analysis

Differences in the number of open and closed cones, seed germination rates, and percentages of open cones among different retention years were tested using F-tests. Differences between burned and unburned plots in the proportion of open cones for the same retention year, as well as differences in cone opening time at the same temperature among retention years and at different temperatures for the same retention time, were also analyzed with F-tests. Differences between annual seed production and canopy seed bank size were tested using t-tests. Pearson correlation coefficients were used to analyze relationships between total/viable seed accumulation and serotiny time. All analyses were performed using SPSS 13.0.

3. Results

3.1 Age Structure of Closed and Open Cones in the Canopy

Pinus yunnanensis cones persisted in the canopy for varying durations, with retained cones spanning up to nine years and closed cones retained for up to seven years. Among the five study plots, cone retention time differed significantly, with maximum retention reaching nine years. All current-year cones were closed. The proportion of closed cones declined annually with retention age, with closed and open cone numbers becoming approximately equal after five years. By seven years, open cones exceeded closed cones, and no cones remained closed after nine years. No trees retained cones beyond nine years. Across all retained cones in the study plots, $(50.4 \pm 21.1)\%$ were closed cones [Figure 1: see original paper].

3.2 Seed Germination Rates from Cones of Different Retention Ages

Seeds from current-year cones exhibited low germination under conventional conditions. Germination rates peaked in cones that had been retained for two to three years, then declined progressively with increased retention time. Seeds

retained for less than seven years achieved average germination rates of 73.9%. Germination declined sharply thereafter, with seeds from eight-year-old cones showing only 40.2% germination. Seeds from nine-year-old cones had completely lost viability. Germination rates differed significantly among retention years ($F = 75.122$, $P < 0.001$), though the trend of declining germination was not significant among years with viable seeds [Figure 2: see original paper].

3.3 Viable Seed Accumulation and Release in the Canopy Seed Bank

Due to delayed cone opening, seeds from different maturation years accumulated in the canopy seed bank. The number of accumulated seeds did not differ significantly from current-year seed production ($t = 1.25$, $P = 0.306$). However, the number of viable seeds showed a significant positive correlation with serotiny time ($r = 0.394$, $P = 0.014$), with both total and viable seed quantities increasing with retention duration. Viable seeds from current-year cones comprised only 9.8% of total viable seeds in the canopy. The viable seed accumulation from closed cones was approximately twice that of current-year viable seeds, accounting for 98% of the canopy seed bank. Across the five plots, the canopy seed bank averaged $(46,231 \pm 33,150)$ seeds per 100 m², with $(31,491 \pm 24,661)$ viable seeds, substantially exceeding the annual seed production of $(24,820 \pm 16,373)$ seeds per 100 m² [FIGURE:3, FIGURE:4].

In unburned *P. yunnanensis* forests, previously matured cones included both closed and open forms, with the proportion of open cones increasing with retention age. Approximately 16.6% of cones opened one year after maturation, with all cones opening by eight years. Seed release began in November, peaking in December-January. Retained cones gradually released seeds over eight years, with annual seed release from delayed-opening cones averaging $(3,149 \pm 2,466)$ seeds per 100 m², exceeding current-year production [Figure 5: see original paper].

3.4 Comparison of Cone Opening and Seed Release Between Burned and Unburned Areas

Fire significantly promoted seed release from the canopy seed bank. Following surface fire, retained cones opened extensively, with approximately 50% of cones less than 0.5 years old opening and nearly 100% of older cones releasing seeds. Post-fire seed release from the canopy seed bank reached $(46,231 \pm 33,150)$ seeds per 100 m², approximately 2.6 times that of unburned plots. The germination rate of seeds released after fire was $(69.8 \pm 22.75)\%$ [Figure 6: see original paper].

3.5 Temperature Effects on Cone Scale Opening

Current-year *P. yunnanensis* cones required approximately 1,760 minutes to open at 40°C. Opening time decreased to 400 minutes at 60°C, 30 minutes at 80°C, 26 minutes at 100°C, and 10 minutes at 120°C. For cones retained for two years, opening times at 40°C, 60°C, 80°C, 100°C, and 120°C were 466

minutes, 54 minutes, 22 minutes, 10 minutes, and 10 minutes, respectively. At each temperature, opening time decreased with increased retention duration, and for each retention age, opening time decreased significantly with increasing temperature [Figure 7: see original paper].

4. Discussion

Pinus yunnanensis cones mature and persist in the canopy rather than immediately releasing seeds, a characteristic consistent with serotinous species from Australia and the Mediterranean. Our study demonstrates that *P. yunnanensis* possesses a canopy seed bank and is a serotinous species, expanding the known global distribution of delayed-opening species previously documented in Europe, America, and Australia. Based on seed release patterns, canopy seed banks are classified as either strict (strongly serotinous), where seeds release only following fire, or non-strict (weakly serotinous), where some seeds release during fire-free periods. The presence of open cones among retained cones and the gradual increase in open cones with retention age indicate that *P. yunnanensis* exhibits non-strict serotiny, releasing some seeds even without fire.

The adaptive significance of non-strict serotiny remains poorly understood, though it may benefit species in environments with multiple disturbance types. As a pioneer species in central Yunnan, *P. yunnanensis* experiences disturbances beyond fire, including drought and landslides. Non-strict serotiny facilitates regeneration during fire-free intervals, helping maintain the species' dominance. The maximum delay time for cone opening aligns with seed longevity, with seed viability declining sharply after seven years and cones showing no retention beyond nine years. This synchrony between serotiny duration and seed lifespan represents an optimal strategy for balancing seed protection and timely release.

Fire temperatures exceeding 40°C effectively induced cone opening in *P. yunnanensis*, with opening time inversely related to temperature. This response mirrors that of serotinous species in fire-prone ecosystems of Australia and the Mediterranean, where delayed opening represents a classic fire-adaptation trait in pines. The central Yunnan Plateau experiences frequent forest fires with a history extending to the late Permian, coinciding with the rise of gymnosperms. As a species originating and centered in this region, *P. yunnanensis* exhibits fire-adaptive traits including canopy seed storage and temperature-enhanced germination, suggesting that fire has been an important evolutionary pressure and ecosystem driver in this region. The presence of fire-adapted traits in Chinese pines indicates that fire may have played a significant role in the evolution and diversification of *Pinus* species, potentially characterizing the central Yunnan Plateau ecosystems as fire-shaped systems.

Acknowledgments

We thank Chang Yunxia, Wang Lingling, and Zhao Guanhua for their assistance with fieldwork.

References

- [1] Roberts H A. Seed banks in soils // Coaker T H, ed. *Advances in Applied Biology*. London: Academic Press, 1981: 1-55. [2] Le Maitre D C. Current interpretations of the term serotiny. *South African Journal of Science*, 1984, 81: 284-290. [3] Lamont B B. Canopy seed storage and release: what's in a name?. *Oikos*, 1991, 60(2): 266-268. [4] Lamont B B, Le Maitre D C, Cowling R M, Enright N J. Canopy seed storage in woody plants. *The Botanical Review*, 1991, 57(4): 277-317. [5] Tapias R, Gil L, Fuentes-Utrilla P, Pardos J A. Canopy seed banks in Mediterranean pines of south-eastern Spain: a comparison between *Pinus halepensis* Mill., *P. pinaster* Ait., *P. nigra* Arn. and *P. pinea* L. *Journal of Ecology*, 2001, 89(4): 629-638. [6] Gutterman Y, Ginott S. Long-term protected "seed bank" in dry inflorescences of *Asteriscus pygmaeus*; achene dispersal mechanism and germination. *Journal of Arid Environments*, 1994, 26(2): 149-163. [7] Ayre D, O' Brien E, Ottewell K, Whelan R. The accumulation of genetic diversity within a canopy-stored seed bank. *Molecular Ecology*, 2010, 19(13): 2640-2650. [8] Barrett L G, He T, Lamont B B, Krauss S L. Temporal patterns of genetic variation across a 9-year-old aerial seed bank of the shrub *Banksia hookeriana* (Proteaceae). *Molecular Ecology*, 2005, 14(13): 4169-4179. [9] Günster A. Seed bank dynamics-longevity, viability and predation of seeds of serotinous plants in the central Namib Desert. *Journal of Arid Environments*, 1994, 28(3): 195-205. [10] Enright N J, Mosner E, Miller B P, Johnson N, Lamont B B. Soil vs. canopy seed storage and plant species coexistence in species-rich Australian shrublands. *Ecology*, 2007, 88(9): 2292-2304. [11] Goubitz S, Nathan R, Roitemberg R, Shmida A, Ne' eman G. Canopy seed bank structure in relation to: fire, tree size and density. *Plant Ecology*, 2004, 173(2): 191-201. [12] Midgley J J. What are the relative costs, limits and correlates of increased degree of serotiny?. *Austral Ecology*, 2000, 25(1): 65-68. [13] Benkman C W, Siepielski A M. A keystone selective agent? Pine squirrels and the frequency of serotiny in lodgepole pine. *Ecology*, 2004, 85(8): 2082-2087. [14] Ma J L, Liu Z M, Zeng D H, Liu B. Aerial seed bank in *Artemisia* species: how it responds to sand mobility. *Trees*, 2010, 24(3): 435-441. [15] Zedler P H. Closed-cone conifers of the chaparral. *Fremontia*, 1986, 14(3): 14-17. [16] Keeley J E, Pausas J G, Rundel P W, Bond W J, Bradstock R A. Fire as an evolutionary pressure shaping plant traits. *Trends in Plant Science*, 2011, 16(8): 406-411. [17] Pausas J G, Bradstock R A, Keith D A, Keeley J E. Plant functional traits in relation to fire in crown-fire ecosystems. *Ecology*, 2004, 85(4): 1085-1100. [18] Bond W J, Woodward F I, Midgley G F. The global distribution of ecosystems in a world without fire. *New Phytologist*, 2005, 165(2): 525-538. [19] Shao L Y, Wang H, Yu X H, Lu J, Zhang M Q. 1957-2007 forest fire risk changes in Yunnan Province. *Chinese Journal of Ecology*, 2009, 28(11):

2333-2338. [20] Shao L Y, Wang H, Yu X H, Lu J, Zhang M Q. Paleo-fires and atmospheric oxygen levels in the latest Permian: evidence from maceral compositions of coals in Eastern Yunnan, Southern China. *Acta Geologica Sinica: English Edition*, 2012, 86(4): 949-962. [21] Wu Z Y. *Pinus yunnanensis* // Flora of China Editorial Committee. *Flora of China: Gymnosperms*. Beijing: Science Press, 1978: 204-204. [22] Enright N J, Lamont B B, Marsula R. Canopy seed bank dynamics and optimum fire regime for the highly serotinous shrub, *Banksia hookeriana*. *Journal of Ecology*, 1996, 84(1): 9-17. [23] He T H, Pausas J G, Belcher C M, Schwilk D W, Lamont B B. Fire-adapted traits of *Pinus* arose in the fiery Cretaceous. *New Phytologist*, 2012, 194(3): 751-759. [24] Clarke P J, Knox K E J, Butler D. Fire, soil fertility and delayed seed release: a community analysis of the degree of serotiny. *Evolutionary Ecology*, 2013, 27(2): 429-443. [25] Pausas J G. Evolutionary fire ecology: lessons learned from pines. *Trends in Plant Science*, 2015, 20(5): 318-324. [26] Holmes P M, Newton R J. Patterns of seed persistence in South African fynbos. *Plant Ecology*, 2004, 172(1): 143-158. [27] Cramer M D, Midgley J J. Maintenance costs of serotiny do not explain weak serotiny. *Austral Ecology*, 2009, 34(6): 653-662. [28] He T H, Pausas J G, Belcher C M, Schwilk D W, Lamont B B. Fire-adapted traits of *Pinus* arose in the fiery Cretaceous. *New Phytologist*, 2012, 194(3): 751-759. [29] Verdú M, Pausas J G. Fire drives phylogenetic clustering in Mediterranean basin woody plant communities. *Journal of Ecology*, 2007, 95(6): 1316-1323. [30] Li Y H, Wang Y J. Effects of short-term high temperature treatment on *Pinus yunnanensis* seed germination. *Journal of Southwest Forestry University*, 2014, 34(2): 19-24. [31] Flora of China Editorial Committee. *Flora of China: Gymnosperms*. Beijing: Science Press, 1978: 204-204. [32] Lamont B B, Enright N J. Adaptive advantages of aerial seed banks. *Plant Species Biology*, 2000, 15(2): 157-166. [33] Tapias R, Climent J, Pardos J A, Gil L. Life histories of Mediterranean pines. *Plant Ecology*, 2004, 171(1/2): 53-68. [34] Naveh Z. The evolutionary significance of fire in the Mediterranean region. *Vegetatio*, 1975, 29(3): 199-208. [35] Yunnan Provincial Forestry Department. Forest fire cause analysis and management strategies in Yunnan Province. *Yunnan Forestry*, 2000, (2): 10-11. [36] Li Y H, Wang Y J. Origin, evolution and dispersal of *Pinus*. *Journal of Northwest Forestry University*, 1999, 14(3): 1-5. [37] Bowman D M J S, Balch J K, Artaxo P, Bond W J, Carlson J M, Cochrane M A, D'Antonio C M, DeFries R S, Doyle J C, Harrison S P, Johnston F H, Keeley J E, Krawchuk M A, Kull C A, Marston J B, Moritz M A, Prentice I C, Roos C I, Scott A C, Swetnam T W, van der Werf G R, Pyne S J. Fire in the earth system. *Science*, 2009, 324(5926): 481-484. [38] Pausas J G, Keeley J E. A burning story: the role of fire in the history of life. *Bioscience*, 2009, 59(7): 593-601. [39] Don Bradshaw S, Dixon K W, Hopper S D, Lambers H, Turner S R. Little evidence for fire-adapted plant traits in Mediterranean climate regions. *Trends in Plant Science*, 2011, 16(2): 69-76.

Note: Figure translations are in progress. See original paper for figures.

Source: ChinaXiv – Machine translation. Verify with original.