PRODUCTION RATE OF *MORUS ALBA* POLLEN GRAINS IN AN ABANDONED *M.ALBA* PLANTATION

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Abstract As a basic study for pollen analysis, the production rate of *Morus alba* (white mulberry) pollen grains in an abandoned *M. alba* plantation was determined over a three-year period from 1997 to 1999. The number of pollen grains per male catkin was assessed and the fall rate of male catkins was measured using five litter traps. The mean production rate was 2.6×10^{12} grains $ha^{-1}yr^{-1}$ (range = $2.2\text{-}3.4 \times 10^{12}$ grains $ha^{-1}yr^{-1}$). This is near the lower end of the range of pollen production rates that have been obtained from many other tree species across several forests.

Resumo en Esperanto Kiel fundamenta studado por polen-analizo, polenera produktaĵo por jaro de morsarbo, *Morus alba* en forlasita *M. alba* plantejo estis determinita dum tri-jara periodo de 1997 ĝis 1999. La nombro de poleneroj por vira infloresko estis esplorita, kai la defalonombro por jaro de vira infloreskoj estis mezrita, uzante kvin rubajn kaptilojn, t.e. sakojn por ricevi defalintajn infloreskojn. La meza produktaĵ-rilatumo de poleneroj estis 2.6×10^{12} eroj $hektaro^{-1}$ $jaro^{-1}$ (amplekso estis $2.2\text{-}3.4 \times 10^{12}$ eroj $hektaro^{-1}$ $jaro^{-1}$). Ĉi tiu estas apud malalta fino de amplekso de polena produktaĵo, kio estis akirita por multaj aliaj arbospecioj el kelkaj arbaroj.

Key words: Pollen grains, production rate, *Morus alba*, forest stand, abandoned plantation

1. Introduction

The results of pollen analysis are usually expressed as the percentage of fossil pollen of each taxon at each horizon of a sediment section. Some pollen-analytical results are expressed as pollen influx or the number of pollen grains per cm^3 of sample sediments. However, neither of these approaches directly indicates the relative dominance of particular pollen-resource plant taxon, because there is great variation in the production and dispersal patterns of pollen grains across taxa (Erdtman 1969; Faegri *et al.* 1989; Moore *et al.* 1991). Thus numerical studies on pollen production and dispersal are required to reconstruct paleovegetation. The studies on the production rate of pollen grains for many species are positioned as a part of these studies.

In Japan, pollen production rates in forests have been investigated since the 1980s in order to assess the productivity of forest ecosystems (e.g. Saito and Takeoka 1983, 1985; Saito *et al.* 1991; Saito *et al.* 2007). The previous studies determined pollen production as the production rate of pollen grains, i.e. the number of pollen grains produced per unit area per year by dominant in the

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forest stand. These studies provide important data that may be used as a basis for pollen analysis studies. Accordingly, some palynologists have studied the production rates of pollen grains in forest stands since the 1990s (e.g. Kiyonaga 1991; Miyake *et al.* 1999; Kiyonaga 2002).

The present study aimed to obtain a preliminary measure of the production rate of *Morus alba* L. (white mulberry) pollen grains in an old *M. alba* plantation that has a forest physiognomy of this species, as a basic study for pollen analysis. *M. alba* belongs to Moraceae, whose fossil pollen are occasionally found at remarkably high percentages in sediments of the late prehistoric age and historic times in central Japan (e.g. Tsuji *et al.* 1992; Kiyonaga 1999; Yoshikawa 2004).

2. Method

The production rate of *M. alba* pollen grains (P) was determined by multiplying the number of pollen grains per male catkin of *M. alba* (P_C) by the production rate of male catkins of *M. alba* in a *M. alba*-dominated forest stand (M) using equation (1).

$$P = Pc \cdot M \qquad (1)$$

Number of pollen grains per male catkin

Branches bearing male catkins were collected from a *M. alba* sample tree (DBH: Diameter at breast height = 21 cm, height = 6 m) that grew near the study stand. Branches were taken just before pollen release in mid-April in 1997, 1998, and 1999.

Male catkins of *M. alba* hold many male flowers, therefore, the number of male flowers per male catkin was first counted for all male catkins on the collected branches. The number of anthers per male flower was then counted for flowers randomly sampled from all male catkins counted. For above two-step counting a stereoscopic microscope was used. Finally, the number of pollen grains per anther was counted for all anthers on male flowers chosen randomly from all male flowers counted. For this procedure, the anther was torn open using a needle and the pollen grains in the anther were washed in a single drop of water on a glass slide. This water was then evaporated in a desiccator, and the pollen grains remaining on the slide were covered with glycerin and overlaid with a cover glass. The total number of *M. alba* pollen grains on the slide was counted under × 100 magnification using a biological microscope with a mechanical stage and an ocular micrometer.

The mean number of male flowers per male catkin (f), mean number of anthers per male flower (a) and mean number of pollen grains per anther (p) were determined for the sample tree for each year. These values were then used to calculate the number of pollen grains per male catkin (P_C) using equation (2).

$$P_C = f \cdot a \cdot p \qquad (2)$$

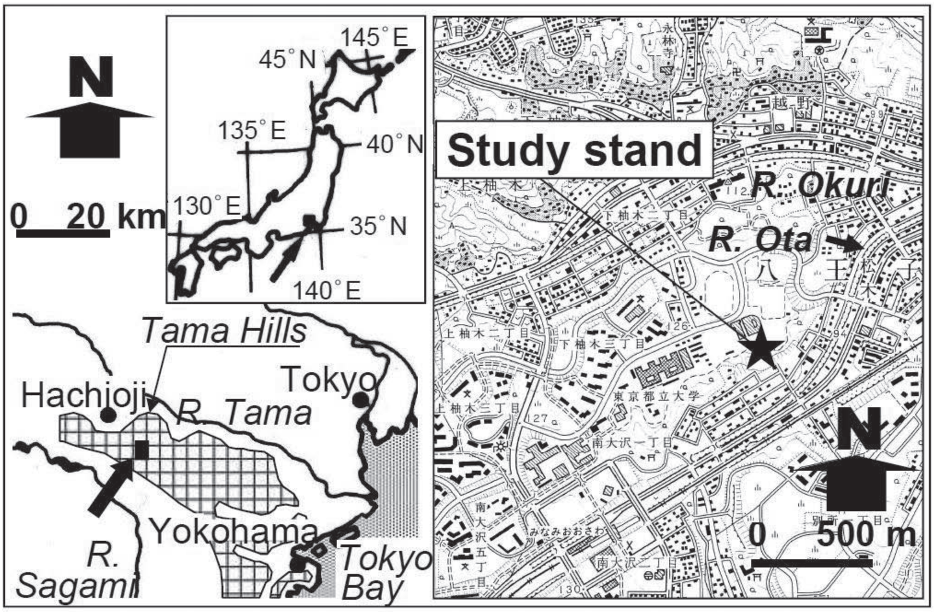

Fig.1 Map showing the study stand, according to the 1:25,000 topographic map "Musashi-Fuchu" published in 2000 by Geospatial Information Authority of Japan.

Production rate of male catkins

The production rate of male catkins of *M. alba* was measured by counting the number of fallen male catkins in the study stand, which was dominated by *M. alba* trees.

Because the male catkins of *M. alba* fall after flowering without coming apart, the number of fallen male catkins within a unit area during the flowering season must be equal to their production rate (*M*). Consequently, *M* can be determined by counting the number of male catkins in the litter traps set beneath the canopy of the stand.

The study stand is a forest in Tokyo Metropolitan University, approximately 40 km west of the center of Tokyo City. It is situated on a gentle side slope facing south in the northwestern part of Tama Hills (Fig. 1). The area of the study stand is ca. 0.01 ha, and the forest canopy was 13 m high.

The stand was composed of three vertical layers: a tree layer, a shrub layer and a herbaceous layer. The tree layer was strongly dominated by *M. alba*, with only this species present in the center of the stand, but a few trees of *Quercus serrata*, *Carpinus laxiflora* and *Prunus jamasakura* are also present toward the periphery. The shrub layer was strongly dominated by *Arundinaria chino* with a small number of additional species such as *Magnolia kobus*, *Neolitsea sericea*, *Cornus controversa* and *Ligustrum japonicum*. Because of the dense growth of *A. chino*, the herbaceous layer contained very few other plants, with only a few *Liriope platyphylla*, *N. sericea*, *Rubus palmatus*, *Aralia elata*, *Aucuba japonica* and *L. japonicum* present. This stand is considered to be an abandoned mulberry plantation because of the dense distribution of *M. alba* trees and thick growth of *A. chino*. Supporting this estimation, the base map for a 1:10,000 vegetation map drawn in 1974 (Miyawaki and Tohma 1975) shows the land use of the area adjacent to this stand

Table 1 General description of the study stand

Arboreal species in the quadrat (Tree height ≥ 2 m)	Number of trees	DBH [cm] Mean (Range)	Height [m] Mean (Range)
Morus alba	5	23.6 (19-33)	11.6 (10-13)
Area of the quadrat	45 m^2		
Altitude	120 m		
Slope gradient and exposure	10°, S20°E		
Soil type	Ando soil (Kuroboku soil)		
Annual mean temperature	14.1°C *		
Warmth Index**	112.9°C・month *		
Coldness Index**	-3.1°C・month *		
Annual precipitation	1,572 mm *		

* Based on climatic data at Hachioji Met. Sta., 8 km northwest from the study stand (Japan Meteorological Agency 2008)
** Kira (1977)

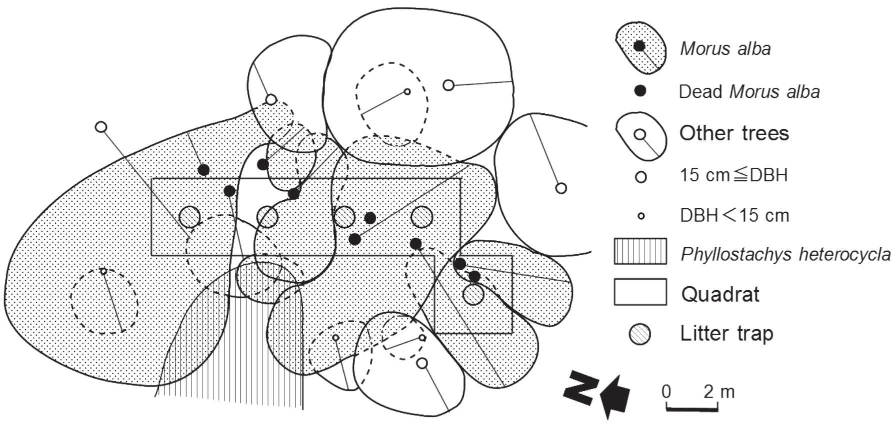

Fig. 2 Crown projection diagram showing the coverage of the quadrat and litter traps in the study stand.

as mulberry plantation. Although the age of the stand is not clear, it is estimated that the mulberry plantation was abandoned 10–20 years before, as the vegetation in this area is shown as not mulberry plantation but broadleaved forest on a 1:2,500 topographic map drawn in 1990 that was published by Tokyo Metropolitan Government.

A 45 m^2 quadrat (Table 1 and Fig. 2) was situated in the study stand. Five litter traps were placed in this quadrat for one or one and half months each spring during the flowering period: April 20–May 18, 1997; April 18–May 23, 1998; April 17–May 30, 1999.

The litter traps used in this study were made of cotton cloth bags attached to 80 cm diameter wire frames. The mouth of each litter trap had an area of 0.5 m^2 and was 90 cm above the ground.

Production rate of pollen grains

Based on the results obtained in the above procedure, the production rate of pollen grains in each year was determined using equation (1).

Table 2 Results of counting male flowers per male catkin, anthers per male flower and pollen grains per anther, obtained from a sample tree whose DBH and height were 21 cm and 6 m, and numbers of pollen grains per male catkin calculated from these parameters in *Morus alba*

Year	Mean number of male flower per male catkin (f)	Mean number of anthers per male flower (a)	Mean number of pollen grains per anther (p)	Mean number of pollen grains per male catkin ($P_C=f \cdot a \cdot p$)
1997	37.3 (7; 28-45; 7.9)	3.9 (9; 3-4; 0.3)	14674.5 (8; 10198-18547; 2551.2)	2.13×10^6
1998	39.9 (20; 13-60; 11.7)	3.9 (27; 2-4; 0.4)	14244.1 (20; 6333-20996; 4075.5)	2.22×10^6
1999	55.8 (12; 38-64; 8.2)	3.7 (12; 2-4; 0.8)	13974.7 (12; 10401-19030; 2688.5)	2.89×10^6

Figures in a parenthesis show the number of samples, range and standard deviation in order.

Table 3 Annual fall rates of male catkins for *Morus alba*, measured with five litter traps in the study stand, and estimated annual production rates for the number of *Morus alba* pollen grains in the study stand

Year	Number of pollen grains per male catkin (P_C) [$\times 10^6$ grains]	Fall rate of male catkins* (M) [$\times 10^6$ grains $ha^{-1}yr^{-1}$]	Production rate of pollen grains ($P=P_C \cdot M$) [$\times 10^{12}$ grains $ha^{-1}yr^{-1}$]
1997	2.13	1.596±0.810	3.4
1998	2.22	0.996±0.673	2.2
1999	2.89	0.750±0.344	2.2
Mean	2.41	1.114	2.6

* Mean±standard deviation for five litter traps (0.5 m^2 each)

3. Results and Discussion

The values of *f*, *a*, *p* and P_C determined in this study are shown in Table 2. There was little variation in the values of *a* and *p*, with a ratio of maximum to minimum (max:min) ratio of approximately 1.0. In contrast, there was slightly significant variation in the values of *f*, resulting in a slightly significant variation in the values of P_C (max:min ratio = 1.5 and 1.4, respectively).

The values of *Pc*, *M* and *P* are shown in Table 3. The value of *M* decreased year by year, leading to a significant max:min ratio of 2.1. However, the year-to-year fluctuation in P_C and *M* showed different patterns, resulting in not so large variation in *P* (2.2-3.4 × 10^{12} grains $ha^{-1}yr^{-1}$; max:min ratio = 1.6). The mean value of *P* across the three years was 2.6 × 10^{12} grains $ha^{-1}yr^{-1}$.

This value is similar to, but near the lower end of, the pollen production rates that have been obtained for other species (10^{11}-10^{13} grains $ha^{-1}yr^{-1}$). Similar pollen production values have been obtained for a giant tree forest of *Abies firma* (3.0 × 10^{12} grains $ha^{-1}yr^{-1}$; Saito *et al.* 2007) , a young *Cryptomeria japonica* forest (2.8 × 10^{12} grains $ha^{-1}yr^{-1}$; Hashizume and Sakamoto 1992) , an old *Juglans ailanthifolia* forest (2.0 × 10^{12} grains $ha^{-1}yr^{-1}$; Saito 1986), a young *Castanea crenata* and *Q. serrata* forest (2.0 × 10^{12} grains $ha^{-1}yr^{-1}$; Kiyonaga 1995), a mature *Quercus myrsinaefolia* forest (2.1 × 10^{12} grains $ha^{-1}yr^{-1}$; Kiyonaga 2003) and a young *Quercus acutissima* forest (2.1 × 10^{12} grains $ha^{-1}yr^{-1}$; Kiyonaga 2009).

It should be noted that the pollen production rate of *M. alba* was obtained from only one stand in the present study. Therefore, additional measurements from various other stands of this species are required to improve our understanding of the production rates of this species. Additionally, assessments of pollen production rates of other Moraceae species are also required as basic study for pollen analysis.

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