REPRODUCTIVE AND POLLINATION BIOLOGY OF *SORBUS ALNIFOLIA,* AN ORNAMENTAL SPECIES

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Abstract

Sorbus alnifolia (Rosaceae) is grown as an ornamental and as a fruit tree, but has low fruit set rate. Wood from *S. alnifolia* is used in making equipment, vehicles, and models. Knowledge of its pollination biology may prove useful in solving the problem of low fruit set, but is still lacking. The present study investigated the pollination biology of *Sorbus alnifolia* in temperate montane forests of eastern China, including aspects of morphology, floral biology, the reproductive system, flower-visitor observations, and an analysis of the pollen ovule ratio. The flowers are sequentially hermaphrodite and protogynous. Pollen and stigmas have a dynamic overlapping period of fertility of at least 3 d. Flowers of this species can set fruit via natural pollination (23.5%), artificial self-pollination (8.63%), and artificial cross-pollination (27.41%) and apomixis has not been observed (0%). The main pollinators were insects of the families Nymphalidae, Apidae and Tenthredinidae. Although *S. alnifolia* is adapted to insect pollination, it readily sets fruit via autogamy when no insect visits occur; this can be supported by artificial self-pollination as an adaptive strategy for reproduction under unfavorable environmental conditions. In rainy days, both pollen viability and insect activity decreased significantly. Hand pollination is recommended and could significantly increase fruit set thereby solving the problem of low fruit set in *S. alnifolia*. The information gained from this study should provide a useful reference for implementing long-term conservation and management strategies for this species.

Key words: Anthesis, Breeding system, *Sorbus alnifolia*, Stigma receptivity, Protogyny.

Introduction

Reproduction lies at the core of the process of evolution and has important effects on the genetic structure of plants (Rech *et al*., 2016). The study of the reproductive process has become an important consideration due to the problems relating to crop production, reproduction, evolution, or climate change (Waser *et al*., 1996; Meffe, 1998; Boreux *et al*., 2013). Plant reproductive success often depends on pollination biology, including the frequency and identity of floral visitors, capability of a species to have autonomous self-pollination, and/or the magnitude of pollen limitation (Banks, 1980; Mehrhoff, 1983; Burd, 1994; Knight *et al*., 2005; Lavergne *et al*., 2005; Rymer *et al*., 2005; Aizen &Harder, 2007). In addition, the complex process of pollination it also serves as the main and nearly exclusive system that controls gene flow between individuals within populations of plants, and thus affects genetic diversity, population structure, and the fitness of the species for survival (Loveless & Hamrick 1984; Hamrick & Godt, 1996).

Due to lack of adequate pollination mechanism the ability of many plants to set seed is limited resulting in low reproductive rates, and even endangering the existence of some species (Kearns *et al*., 1998; Richards, 2001). The process of pollination begins with the shedding of pollen from mature anthers; male gametes carry two nuclear pollen grains and become exposed to dry conditions. However, to ensure fertilization occurs smoothly the pollen must arrive at a receptive stigma at an appropriate time. Several factors, from pollen viability to the receptivity of the stigma to pollen, may all have a profound effect on the pollination in some species. For example, the pollination rate varies during different stages of plant development (Petanidou, 2001; Wilcock & Neiland, 2002). The relative importance of pollinators and the mutual interaction of the stamen and pistil contribute to successful pollination as does the competition of different pollen genotypes (Faegri & Van der Pijl, 2013). Therefore, pollen viability and longevity as well as stigmatic receptivity are often used in pollination biology to elucidate the reproductive success of a population. In addition, interactions between plants and pollinators form an important ecological relationship in ecosystems because they are fundamental to the reproductive success, regeneration, and persistence of plants (Hamrick & Godt, 1996; Kearns *et al*., 1998; Arias-Cóyotl *et al*., 2006).

Sorbus alnifolia (Rosaceae) is an underutilized forest tree species with a fine green color. The horticulture industry could promote the cultivation of this species and it could be used in unique ways to offset the cold and drab winter colors of cities. The conical crown and scarlet leaves in fall make it a beautiful ornamental tree with attractive, large clusters of white flowers, and most individuals bear colorful fruits. The hard, heavy, and fine-grained wood is suited for making furniture or small, carved items. The fruits are used for making jam, marmalade, various drinks, wine, vinegar, etc. (Lu *et al*., 2003). However, the fruit set is generally low and currently poorly understood, which greatly impedes the exploitation of the plants. A complete knowledge of its pollination biology may prove useful in solving the problem of low fruit set. The primary aim of present study was to determine the phenology, pollination biology, flower visitors, and breeding system of this species, in order to analyze the reason of low fruit set as it relates to its cultivation and management.

Material and Methods

Study species: *Sorbus alnifolia*, a small deciduous tree, may grow as tall as 20 m. It occurs widely in eastern China growing on slopes, gullies, mixed forests, and thickets at elevations between 500–2300 m elevations and also occurs in Japan and Korea. The dark reddish brown young branchlets change into a dark grayish brown when mature. The white flowers are arranged in compound corymbs at the terminal ends of the branchlets. Flowering occurs from April to May and fruiting from August to September.

Study sites: The study site (36°12'26.66"N, 118°36'44.38"E) lies in Yishan, Shandong Province at an elevation of 1032 m. This typical warm temperate region had a semi-humid continental monsoon climate, with a hot and rainy summer and a cold and dry winter. The information on climate is available in "Flora of YiShan" (Zhao & Song, 2012). The annual average temperature is 10.8°C, with a maximum and minimum daily average temperature of 31°C and −8.2°C in July and January, respectively; the extreme maximum and minimum temperatures are 35°C and −19.5°C, respectively. The area receives about 845.9 mm of rainfall annually, with 525.1 mm (62% of total precipitation) of that concentrated in summer from June to August.

Floral morphology and phenology: Flower phenology was recorded from three individual *S. alnifolia* plants. The number of flowers per inflorescence was counted from 20 corymbs. Lengths of pistil, style, and anther were measure by vernier caliper. Anthers were collected from 20 randomly selected flowers, placed into 2 ml centrifuge tubes, and water was added to make a volume of 2 ml. After shaking, 0.1 ml of the solution was removed by pipette and then diluted to 10 ml with water and a volume of 20 μl was taken for observation on slides from the 10 ml solutions; this procedure was repeated three times. The ovary was removed from each flower after the anther was removed and a scalpel was used carefully to cut the carpel; a dissecting microscope (Nikon 80i, Nikon Corp., Tokyo, Japan) was used to determine the number of ovules in each carpel. For each flower, the number of pollen grains and ovules was observed, and pollen/ovule ratio (P/O) was calculated.

Determining pollen viability: Pollen viability, an important parameter for evaluating the quality of pollen, was measured in both dry and moist conditions at room temperature after different storage times. Pollen from individual anthers was collected from 20 randomly selected flowers and stored for 0–7 d under dry and moist conditions; then, the pollen was used to estimate pollen viability. Pollen was incubated on two layers of Whatman No. 1 filter paper moistened with distilled water in 5-cm-diameter Petri dishes. We used 2,3,5-triphenyl tetrazolium chloride to determine pollen viability and longevity. Each pollen sample was spread on a glass slide; a droplet containing 10% sucrose solution with 2,3,5-triphenyl tetrazolium chloride was applied and the slide was rapidly covered with a cover slip; the slide was then placed on moist filter paper in a petri dish and the dish placed in the

dark for 2 h at 35°C. We counted the proportion of red pollen in five fields of view on the slide.

Stigma receptivity: Stigma receptivity was determined using hydrogen peroxide with benzidine. During flowering, 20 flowers were collected daily and stigmas of flowers were immersed in a reaction solution containing 1% benzidine, 3% hydrogen peroxide, and water at a ratio of 4:11:22, V/V/V) in the concave surface of a glass slide. If the stigma was receptive to pollen it exhibited peroxidase activity; i. e. the reaction mixture surrounding the stigma appeared blue with large number of bubbles.

Pollinators and their behavior: In *S. alnifolia*, flowering occurs continuously from 7:00–19:00 on sunny days. Individual flowers were tracked and observed. The number of insects visiting the flower was recorded. Finally, some insects were randomly netted for identification. The foraging rate at the flower for each insect was calculated.

Breeding system: To determine the breeding system of *S. alnifolia*, pollination experiments were performed on randomly chosen pollen from five trees. On each plant, 4 to 10 inflorescences were randomly tagged and analyzed using several processing as follows: (1) Control (CK), no emasculation, not bagged, natural pollination for the studying the pollination under natural conditions; (2) Autogamy, using bagging to check for self-pollination; (3) emasculation, artificial cross-pollination and bagging; (4) no emasculation, bagging, to detect natural self-pollination (5) emasculation, bagging, no pollination, to detect apomixis.

Data analysis

All analyses were performed with SPSS Version 18.0 (SPSS Inc., Chicago, IL, USA). Percentage data were arcsine transformed prior to statistical analysis to ensure homogeneity of variance. Paired sample t-tests and analysis of variance were used to analyze floral morphology, pollen viability, breeding system and stigma receptivity. If analysis of variance indicated significant differences in the data, Tukey's HSD test was used to determine the differences between treatments ($p < 0.05$).

Results

Floral morphology and phenology: *S. alnifolia*, has complex corymbs with 15.43 ± 3.15 flowers per corymb; corymb diameter was 70.83 ± 5.83 mm; pedicels 8.33 ± 0.84 mm long; flowers white, diameter 13.91 ± 0.98 mm; calyx bell-shaped, 2.79 ± 0.21 mm long, 2.23 ± 0.15 mm in width or triangular oval-shaped, the inner surface with dense white trichomes; petals obovate, 6.71 ± 0.69 mm long, 5.69 ± 0.90 mm wide; pistil matured before stamens, stamens 20, length 5.42 ± 0.38 mm, anther length 1.42 ± 0.10 mm; styles 2, 4.42 ± 0.18 mm long, base or the middle of the styles connate, glabrous, shorter than stamens. A single flower of *S. alnifolia* had $29,652 \pm 7224$ pollen grains, 4 ovules, and the P/O ratio is 7413 ± 1806.

Determination of pollen viability: With either in dry ($F=1130.5$, $p<0.05$) or moist ($F=2126.9$, $p<0.05$) conditions, pollen viability decreased significantly with increased storage time. The percentage of viable pollen

decreased significantly and more rapidly under moist than under dry conditions (Fig. 1). Pollen in a moist environment lost its viability completely after only 2 d; however, in dry conditions the pollen remained viable longer. Pollen viability was 94.25 ± 3.65% at anther dehiscence when the flower was open; however, after storage for 1 d pollen viability decreased slightly to 87.34 ± 2.16%. Under dry conditions, at 2nd day after flower opening, the decline in viability was not significant compared with freshly opened flowers; however, viability decreased significantly at 3rd day of flowering, and the viability of pollen was nearly 0% after 6th day of flowering.

Stigma receptivity: From the day of anthesis (0 DAA) to 3 DAA, the stigma was pale yellow, and the stigma was most receptive to pollen (more than 90%, Table 1). After the receptivity of the stigma gradually decreased. By 5th day after flowering the stigma becomes dark yellow and at 6 d after flowering, the stigma became dark yellow or brown, and gradually dried and became unreceptive to pollen. After successful fertilization, the ovary became visibly enlarged, and an immature fruit began to form.

Pollinators and their behavior: The flowers of *S. alnifolia* were typically wind-pollinated, but insects might also be involved in the process of pollination. During the observations conducted for this study, we recorded 8insect species visiting *S. alnifolia* flowers. The main pollinators were members of the Nymphalidae, Apidae, and Tenthredinidae. Butterflies (Nymphalidae) spent more time on the flowers than other insects. Members of the Apidae and Tenthredinidae mainly visited flowers to forage for nectar. Members of these two families often slowly visited the opened flowers of the same inflorescence repeatedly to forage for nectar while members of the Apidae visited flowers before they were fully open. When bees forage for nectar and pollen often became attached to their bodies. Our field reservations revealed that the members of the Apidae were the most effective pollinators of *S. alnifolia.*

Pollination of *S. alnifolia* was rarely observed before 08:30 when the temperature was low, dew was heavy, and humidity was high. After 08:30 or later, as the temperature began to rise and dew evaporated, pollinators emerged and visitation to flowers gradually increased. A wide variety of pollinators appeared frequently when temperatures peaked from 11:00 to 14:00 when the air was relatively dry. After 15:00, when the temperature began to fall, the number of pollinator visits declined rapidly. The peak frequency of flower-visits by insect pollinators occurred at 10: 00–14: 00, when the light intensity and temperature had risen substantially and peaked. Pollinators were less active on rainy days, so that rainy days influenced the efficiency of pollinators (Fig. 2).

Breeding system: Five different treatments resulted in significantly different pollination and fruit-setting rates (Table 2). No fruit set was observed in the self-pollination and apomixes treatments; at the end of the experiment, all flowers had aborted, indicating that populations of *S. alnifolia* did not exhibit spontaneous self-pollination or apomixis. The fruit setting rate of artificial cross-pollination reached 27.41%, which was significantly higher than the natural pollination (control) 23.5% and artificial self-pollination (8.63%).

Table 1. Stigma receptivity of *Sorbus alnifolia*.

Time of collecting stigmata	Percentage of receptive stigmata	Stigma color
0 DAA	100	Pale yellow
1 DAA	98.85	Pale yellow
2 DAA	93.27	Pale yellow
3 DAA	91.85	Pale yellow
4 DAA	78.37	Pale yellow
5 DAA	76.64	Dark yellow
6 DAA	56.26	Dark yellow, brown
7 DAA	25.27	Dark yellow, brown
8 DAA	0.00	Withered yellow

Note: DAA, day of anthesis

Table 2. Effects of different pollination treatments on the rate of fruit set in *Sorbus alnifolia*.

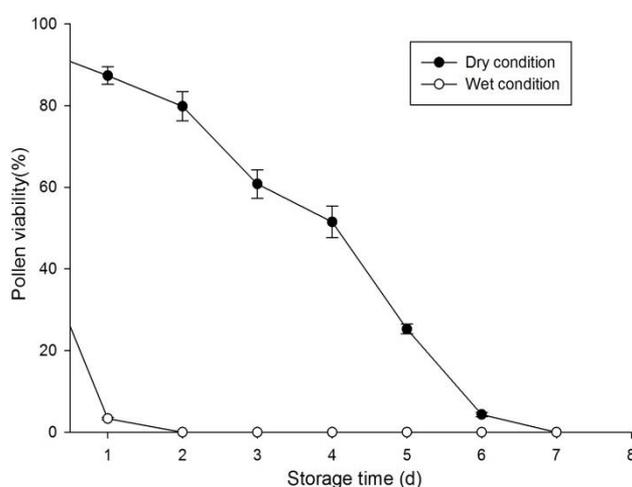| Treatments	Fruit setting (%)	Fruit abortion (%)
Natural pollination	23.5	76.5
Artificial self-pollination	8.63	91.37
Artificial cross-pollination	27.41	72.59
Self-pollination	0	100
Apomixis	0	100

Fig. 1. Pollen viability of *Sorbus alnifolia* at room temperature under dry and wet conditions.
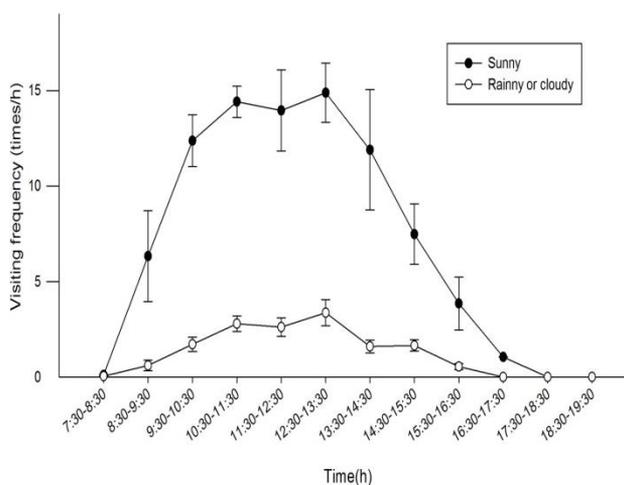

Fig. 2. Effects of frequency of insect visitation on pollination.

Discussion

Studies in reproductive biology provide useful information in support of planning conservation strategies and for developing suitable measures to guide cultivation (Quillet *et al*., 1995). The more flowers, the larger diameter of the flower, the more pollen to attract the pollinators, which leads to excellent seed setting and pollen yield (Brantjes, 1983; Clutton-Brock, 1988; Rieseberg *et al*., 1999). The flowers of *S. alnifolia* are borne on corymbs; this may attract pollinators to visit flowers and increase pollination opportunities. Flowers of *S. alnifolia* are sequentially hermaphroditic, opening from the proximal to the distal part of the florescence. After the distal flowers open, each flower remains open and viable for about two days. Pollinators migrate upward in the inflorescence and carry pollen from one flower to another; in addition, the upward movement of insects from the proximal to distal parts of the inflorescence generates the movement of a large amount of pollen.

More than 50% of pollen of *S. alnifolia* that were stored in dry conditions at room temperature for 1–3 d remained viable, indicating that no serious problems related to pollen viability existed, and that pollen quality might not be the main cause of seed abortion in *S. alnifolia*. Pollination by visitors should be accomplished in 3 DAA. Environmental conditions, especially humidity, may be the main factors causing low pollen quality in *S. alnifolia*. High temperatures, drought, or excessive rain can undermine the viability of pollen, and may adversely affect secretions of the stigma; therefore, inclement weather conditions occurring during flowering will limit successful pollination (Stiles, 1978; Hamilton *et al*., 1990; Javorek *et al*., 2002; Gan *et al*., 2013).

For successful fertilization, the pollen must be viable and the stigma should be receptive. Stigmatic secretions may anchor pollen that lands on the stigma, resulting in successful pollination. Stigma receptivity varies between species (Edlund *et al*., 2004; Sigrist & Sazima, 2004). In *S. alnifolia*, stigmata are receptive from 2 d before flowering until 4 d after flowering. However, although pistils mature first, pollen and stigmas have a dynamic overlapping period of viability/receptivity for at least a 3 d period of fertility. The time during which both the pollen were viable and the stigma was receptive, occurs when pollination in *S. alnifolia* was most successful, so this were not the main reason the plants might fail to reproduce. Although pollen remained viable for 3 d after flowering in the laboratory, in the nature habitat, most pollen were dispersed within 1–2 d after flowering. If continuous damp, rainy weather occured on the day and/or 1–2 d after flowering, pollen viability declined sharply; therefore, adverse weather conditions may strongly limit pollination in *S. alnifolia*.

Fruit set of *S. alnifolia* from artificial cross-pollination was higher than that from the control and self-pollination under natural conditions, indicating pollinator limitation occured in the natural population. Fruit set of artificial self-pollination was 8.63%, indicating *S. alnifolia* was self-compatible. Sometimes needs a pollination vector, and can be pollinated by wind or

insects (Syrphid flies and bees), which is in accordance with data from the out-crossing index and the P/O ratio. According to Dafni (1992), the out-crossing index is four, indicating *S. alnifolia* is out-crossing partially self-compatible, and requires pollination. The P/O ratio (7413 ± 1806) fell in the range of 2108.0–195,525.0 corresponding to the values reported for obligate outcrossing (Cruden, 1977). Therefore, the breeding system of *S. alnifolia* suggested that outcrossing predominated in the breeding system of *S. alnifolia*. Our results indicated that the breeding system of *S. alnifolia* involved both selfing and outcrossing simultaneously. However, outcrossing is predominated, and self-pollination only played a supporting role to assure pollination success, this is especially ture when conditions for outcrossing are unfavorable, such as unfavorable environment conditions, or a lack of pollinators (Huang *et al*., 2006; Jordan & Harder, 2006).

The number and behavior of pollinators and their frequency of visits also affects the success of pollination. Unfavorable weather (wind, rain, cold, etc.) is likely to affect the normal process of pollination, especially when plants are in full bloom (Webb & Littleton, 1987; Vicens & Bosch, 2000). If *S. alnifolia* flowers open on rainy days, this seriously affect insect activity, making availability of pollen extremely limited. For plants pollinated by insects, the rate of seed set is typically determined by the number of insect species visiting a flower and the frequency of those visits. In addition, because animal pollinators are not reliable, this may result in a decline in the rate of outcrossing (Boyle–Makowski *et al*., 1985).

For wind-pollinated plant populations, pollination efficiency is critical for effective reproduction (Friedman & Barrett, 2009). After the pollen were dispersed, some pollen must reach a receptive stigma and this process is affected by climatic conditions, i.e. temperature, humidity, light, wind direction, wind speed, and plant density (Gan *et al*., 2013). *S. alnifolia* plants are distributed in a scattered pattern in the natural environment. Therefore, if the distance is short between plants, pollination is more easily accomplished. However, longer gap between plants successful pollination is difficult. In addition, different degrees of reproductive isolation may occur between populations and this has disadvantages in genetic exchange between sub-regions and between populations distributed in isolation. In contrast, a human controlled planting environment can artificially improve the effectiveness of pollination success, thereby increasing fruit set.

In adverse weather conditions (high winds, continuous dampness, rainy weather, low temperature, etc.) pollen viability decline sharply on the day or 1–2 days after flowering and fewer insects belonging to few species visit the plants, resulting in low foraging frequencies, which create strong constraints on fertilization in *S. alnifolia*. Thus, if the source of pollen is greatly restricted by inclement weather, this will affect the normal pollination process, and further affect the breeding process plants. Such harsh weather conditions and a lack of pollinators are two important reasons for this kind of population distribution and reproductive success.

Acknowledgements

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