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Title	Leaf herbivory causes abortion of selfed seeds in <i>Rhododendron kaempferi</i> var. <i>kaempferi</i>
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Citation	Plant Species Biology, 40(2), 123-135 https://doi.org/10.1111/1442-1984.12492
Issue Date	2024-10-19
Doc URL	https://hdl.handle.net/2115/96100
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Type	journal article
File Information	Takahashi&Kudo(PSB)_HUSCAP.pdf



Original Article

Leaf herbivory causes abortion of selfed seeds in *Rhododendron kaempferi* var. *kaempferi*

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Abstract

Plant reproduction is often inhibited by herbivorous leaf damage, which affects the resource pool allocated for reproduction. *Rhododendron kaempferi* var. *kaempferi* is a semi-evergreen shrub widely distributed in Japan, and its leaves are occasionally damaged by sawfly larvae (*Arge similis*). This species is partially self-compatible, but its selfed seedlings suffer from inbreeding depression. Our study aimed to clarify (1) the magnitude of herbivorous damage under natural conditions, (2) the effects of foliage damage on seed number and mass, and (3) the possibility of selective abortion of selfed seeds to maintain outcrossed seed production under resource limitation. In the 2022 survey, 2 of the 20 ramets observed were collectively parasitized by sawfly larvae, and the most damaged ramet had a 59.6% foliage loss. In contrast, the herbivorous damage in 2023 was consistently low throughout the growing season, averaging a 3.2% loss. Leaf excision reduced the seed number per fruit (i.e., low seed-set rate) but did not affect the individual seed mass. The outcrossing rate of the excision treatment (81.7%) was significantly higher than that of the control (72.0%). The values of seed-set rate $\times$ outcrossing rate were 0.172 for the excision treatment and 0.171 for the control, nearly equal. Our results indicated that (1) sawfly larvae damage was concentrated on a few specific plants, (2) intense foliage damage reduced seed number but not seed mass, and (3) *R. kaempferi* mitigated the decline in outcrossed seed production by the abortion of selfed seeds when leaves were intensively damaged.

Keywords defoliation, resource limitation, *Rhododendron*, seed, selective abortion

Introduction

Herbivorous leaf damage often inhibits plant reproduction, but the response patterns of plants vary depending on the intensity and timing of herbivory (Boege, 2005; García & Ehrlén, 2002; Imai & Ogawa, 2009). Herbivorous damage can cause serious impacts on seed production when the photosynthetic products of the current year are used for reproduction. Some deciduous trees, such as spicebush (*Lindera benzoin*) and white oak (*Quercus alba*), display fruit abortion under intensive foliar damage (Hochwender et al., 2003; Niesenbaum, 1996). In evergreen and semi-evergreen species, herbivorous damage of overwintering leaves can negatively impact the subsequent reproduction if those leaves store resources for reproduction. Overwintering leaves in evergreen plants of cold regions often store carbon; thus, herbivory depletes the carbon pool, affecting growth and reproduction (Jonasson, 1989).

Defoliation negatively affects seed mass and/or quality when the resources stored in leaves are used for seed production. Theoretically, three response patterns to severe resource limitation are predictable in seed production. First, only a certain number of embryos can develop into seeds, while the rest is aborted. Seed abortion is common in plants even when stigmas receive sufficient amounts of pollen, especially under resource limitation (Bawa & Webb, 1984). In the perennial legume, *Centrosema virginianum*, seed abortion increased with the defoliation intensity (Cardel & Koptur, 2022). Second, the individual seed mass is reduced while the seed number remains stable (Thalmann et al., 2003). The production of lighter seeds may decrease the fitness because seed size is often related to germination activity and seedling survival (Bonfil, 1998; Cevallos et al., 2022; Lamichhane et al., 2018). Third, both seed number and mass decrease (Koptur et al., 1996). These responses depend on the intensity of resource limitation and are specific to individual species; however, little is known about the physiological mechanisms.

A previous study revealed that *Rhododendron* species (Ericaceae) with overwintering leaves store carbon resources in their leaves, and defoliation negatively impacts the subsequent shoot growth, suggesting the importance of leaves as a carbon resource pool (Jonasson, 1995). However, the effects of defoliation on reproductive success remain unknown. The present study aims to evaluate the impact of defoliation on seed production in a semi-evergreen *Rhododendron kaempferi* Planch. var. *kaempferi*. Generally, *Rhododendron* species have hundreds of ovules within a single ovary (Jain et al., 2000; Ono et al., 2008), and the number of seeds per fruit is highly variable due to pollen limitation and seed abortion. *Rhododendron*

kaempferi leaves are eaten by sawfly larvae (*Arge similis*, Argidae), a specialist of *Rhododendron* species, including *R. indicum*, *R. japonicum*, *R. macrosepalum*, and *R. kaempferi*. Their feeding scars are easy to identify; only mesophyll tissues are eaten, leaving the central veins (Figure 1). In *R. kaempferi*, the period between flowering and seed dispersal lasts 4–5 months, and intensive leaf loss during the fruiting period may negatively affect the reproduction in the current year. Thus, seed production is expected to be influenced by one of the three response patterns described above.

Moreover, herbivorous damage can cause selective abortion due to resource limitation, thus affecting plant reproduction (Stephenson, 1981). Selective seed (embryo) abortion is explained as a result of pollen-tube competition among pollen grains and the subsequent control of the mother plant (Casper, 1988). Developing seeds within ovaries often compete for resources supplied by the mother plant, and active embryos fertilized earlier have a higher probability of seed maturation (microgametophytic competition hypothesis; Marshall & Ellstrand, 1986). Self-incompatible species prevent the growth of self-pollen tube or self-fertilization, but partial self-compatibility allows the production of some self-fertilized seeds (Stephenson et al., 2003). The outcrossing rate per fruit may be enhanced if a partially self-compatible species undergoes severe resource limitation. In *R. kaempferi*, 89% of self-pollinated seedlings die within one year due to inbreeding depression (Takahashi & Itino, 2022). Thus, selective abortion of selfed seeds would be advantageous to maintain the resource investment in outcrossed seeds.

This study aimed to clarify: (1) How does herbivorous damage to *R. kaempferi* leaves occur during the growing season? (2) Does foliage damage negatively affect seed production in the current year? If so, does it reduce seed number or mass? (3) If seed abortion occurs under resource limitation caused by defoliation, is it to maintain the outcrossed seed production at the cost of selfed seed production?

Materials and Methods

Study Site

The field survey was conducted near the Mt. Apoi Geopark Visitor Center in Samani Town, Hokkaido (42°06'N, 142°59'E; 80 m a.s.l.). The study site is about 1 km inland from the Pacific coastline and at the foot of Mt. Apoi (810 m a.s.l.). The annual mean temperature is 8.2°C (average of 1991–2020), ranging from –2.4°C (January) to 19.9°C (August). The average annual precipitation is 1,118.3 mm, with little snowfall during the winter (Japan Meteorological

Agency, 2022).

About 40 *R. kaempferi* individuals were observed within a 50 m × 50 m area at forest edges leading to open grassland. The surrounding area comprised a mixed forest with conifers, such as *Pinus parviflora* var. *pentaphylla* and *Picea glehnii*, and broad-leaved trees, such as *Acer japonicum* and *Alnus japonica*. Dwarf bamboo (*Sasa nipponica*) dominated on the forest floor. Wild deer (*Cervus nippon yessoensis*) inhabit the study area but do not feed on *R. kaempferi* leaves.

Plant Material

Rhododendron kaempferi (Ericaceae) is a semi-evergreen shrub that commonly grows in mixed forests below 1,600 m throughout Japan, except in Okinawa Prefecture (Yamazaki, 1996). The study site was near the northern limit of its distribution range; therefore, flowering occurred in late May, as opposed to mid-April in the southern areas. *Rhododendron kaempferi* produces 1–3 red flowers per inflorescence, and the longevity of each flower is about seven days. The shoots are composed of non-overwintering (i.e., deciduous) leaves (2–7 cm long, 1.5–4 cm wide) and overwintering leaves (1–2 cm long, 0.5–1 cm wide; Appendix Figure A1). Non-overwintering leaves simultaneously appear in the spring, with some additional emergence in the summer, and die off by late autumn. Overwintering leaves emerge around winter buds (flower and spring leaf buds) in late summer and are retained until after the next flowering period. Insects mostly damage non-overwintering leaves. Mature fruits dehisce from October to November, and tiny seeds (1 mm long, 0.4 mm wide; Yamazaki, 1996) are dispersed by wind and gravity. In addition to sexual reproduction, clonal growth by rhizome has been reported in this species (Aizawa et al., 2016). Thus, plants with a single stem on the ground are referred to as ramets in this study.

Survey of Pollination Environment

Fruit-set success was measured in *R. kaempferi* to evaluate the pollination conditions at the study site. Moreover, flower-visiting insects were recorded arbitrarily and identified from the photographs to overview the available pollinators in the study area. On July 1, 2022, one branch was randomly selected from each of the 20 ramets, and the number of original flowers and developing fruits was recorded. On July 2, 2023, similar data were obtained from 40 ramets. The fruit-set rate per ramet was calculated based on the number of flowers and fruits. All

statistical analyses were conducted using R software version 4.3.1 and the package *lme4* (Bates et al., 2015; R core team, 2023). A generalized linear mixed model (GLMM) postulating a binomial distribution (logit-link) was run with the year of measurement as the explanatory variable, fruit set as the response variable, and individual ID as the random effect.

Investigation of Herbivory Damage

The extent of herbivorous damage on *R. kaempferi* leaves was determined by classifying the degree of herbivory using the method of Nakamura et al. (2014): 0, no damage; 1, 1%–10%; 2, 11%–25%; 3, 26%–50%; 4, 51%–75%; and 5, 76%–100%. The measurements were conducted for non-overwintering (deciduous) leaves because almost all overwintering leaves had been shed by early July. The degree of herbivorous damages was recorded for randomly selected, with up to 100 leaves in each ramet, and they were converted to the median values for each category as follows: 0 = 0%, 1 = 5.5%, 2 = 18%, 3 = 38%, 4 = 63%, and 5 = 88%. The herbivory rate in each ramet was calculated by averaging the individual leaf values.

In 2022, measurements were conducted twice: on August 1 (mid- season of fruit development) and October 9 (before seed dispersal). *Arge similis* larvae and their feeding scars were also observed on August 1 (Figure 1). Twenty ramets with five or more fertile fruits were selected and marked with identification tags, and the first and second measurements were conducted on the same ramets. Because the inter-ramet distance was greater than 1 m, all individuals were considered genetically different.

In 2023, we increased the frequency of the measurements because the herbivory pattern was heterogeneous in 2022. Eight measurements were conducted at three-week intervals from May to October to describe seasonal pattern of herbivory damage. On May 20, 40 ramets were tagged. A subsequent DNA analysis revealed that they comprised 23 genetically different individuals. Measurements were made on the 40 ramets from May 20 to July 2. After that, 14 of the ramets were excluded from the measurements because they were used for the leaf excision described later. Herbivorous insects feeding on leaves were photographed and identified where possible.

Fruit and Seed Information

On October 9, 2022, four fruits were collected from each of the 20 ramets examined for herbivory rate. Fruit length and width were measured using a digital caliper (CD-20C, Mitutoyo

Corporation, Kanagawa, Japan). Each fruit was preserved in a paper bag at room temperature. After the pericarp was dehiscent, the contents were removed and placed on one Petri dish per fruit. Developed seeds, aborted seeds, and ovules were then counted with the naked eye using a magnifying glass. Aborted seeds and unfertilized ovules were treated as the same group, as they were sometimes difficult to distinguish. The seed-set rate per fruit was calculated based on the number of ovules and seeds. Seeds from each fruit were weighed on an electronic balance (MS204S, Mettler-Toledo International Inc., Switzerland). The effects of herbivory damage on seed production, the effects of seed production and herbivory damage on seed mass, and the effects of seed number and herbivory damage on fruit length were tested using GLMMs. These analyses were conducted to determine whether seed and fruit development are influenced by intensive leaf herbivory. In the GLMM for seed production, seed-set rate was the response variable, herbivory rate was the explanatory variable, and binomial error distribution (with logit-link function) was applied. In the GLMM of seed mass, individual seed mass was the response variable, seed-set rate and herbivory rate were the explanatory variables, and gamma error distribution (with log-link function) was applied. In the GLMM for fruit length, fruit length was the response variable, seed number and herbivory rate were the explanatory variables, and gamma error distribution (with log-link function) was applied. For each GLMM, individual ID was set as the random effect. Herbivory rates for the first and second measurements were treated separately. The same analyses were conducted in 2023 using fruits in the natural state. Herbivory rates from the fourth (July 23) and eighth (October 14) measurements were used to align the timing with the 2022 data.

Leaf Excision Experiment

A leaf excision experiment was conducted to more precisely evaluate the effect of leaf damage on seed development. On July 2, 2023, sawfly larvae were found on *R. kaempferi* leaves. On July 23, 14 ramets producing fruits were chosen for the excision treatment. All non-overwintering leaves within 20 cm distance from the infructescence on the branch were cut with scissors (up to 100 leaves per ramet). This was done because infructescence and leaves are often clustered at the terminal branches within 20 cm length. Non-overwintering leaves were not distinguished between spring and summer foliation, although the majority were from spring. The leaf cut imitated the foraging behaviour of sawfly larvae, i.e., removal of leaf blade while leaving the central vein intact (Figure 2). The herbivory rate of individual ramets after the

excision treatment was roughly to 8%–45%, based on the assumption that the leaf damage category of excised leaves was 5 (88%). Furthermore, 26 ramets were placed in their natural state as a control. On October 14, 1–6 fruits were collected from the 12 excised and 14 control ramets. In the remaining ramets, the fruits were either not fully fertile or deformed by disease. The herbivory rates of the 14 control ramets were consistently lower than 7% throughout the measurements. Thus, the effects of natural herbivory on this experiment might be negligible. Fruit and seed data were recorded using the same procedures as in 2022. Fruit length, fruit width, ovule number, seed number, seed set, and seed mass were compared between treatments using GLMM. In all analyses, treatment (excision or control) was the explanatory variable, and individual ID was set as the random effect. The error distribution of individual GLMMs was determined based on the response variable: gamma distribution (log-link) for the continuous variable (fruit length, fruit width, and seed mass), Poisson distribution (log-link) for the discrete variable (ovule number and seed number), and binomial distribution (logit-link) for the ratio (seed set).

Outcrossing Rate Estimation Using Microsatellite Markers

Seeds were genetically analyzed using microsatellite markers to determine the outcrossing rate per fruit. One leaf was taken from each of the 40 ramets used in the leaf excision experiment and genotyped to determine the mother plants. These data were also used to identify individual ramets. After excluding fruits with fewer than 24 seeds, 20 fruits were selected from each experimental group (excision and control). DNA data were obtained for 12–24 seeds per fruit.

DNA was extracted using the cetyltrimethylammonium bromide extraction procedure (CTAB method; Stewart & Via, 1993). The following five microsatellite markers were used: RM3D2, RM9D6 (Naito et al., 1998), AZA003, AZA009 (Dendauw et al., 2001), and N25 (Tan et al., 2009). The extracted DNA products were amplified by Two-step multiplex PCR using a TaKaRa PCR Thermal Cycler Dice Gradient (Takara Bio Inc., Shiga, Japan). In the first step, 0.4 µl of each forward tailed and reverse pigtail primers was added to 196 µl of distilled water to create a 200 µl primer set. The first step of PCR was performed with 1 µl of primer set, 1 µl of DNA extract, 2.5 µl of 2 × Type-it Multiplex PCR Master Mix (QIAGEN, Hilden, Germany), and 0.5 µl of distilled water (total: 5.0 µl). The thermocycling steps were as follows: initial denaturation at 95.0°C for 5 min, followed by 22 cycles at 95.0°C for 30 s, annealing at 52.0°C for 90 s, and extension at 72.0°C for 30 s. In the second step, 4.8 µl each of four fluorescent

dye-labeled universal primers (Blacket et al., 2012) and five reverse pigtail primers were added to 56.8 μ l of distilled water to prepare a 100 μ l R + D set. 0.5 μ l of R + D set and 2 $\times$ Type-it Multiplex PCR Master Mix were added to each PCR product and subjected to a second PCR step (total: 6.0 μ l): 18 denaturation cycles at 95°C for 30 s, followed by 52.0°C for 90 s, extension at 72.0°C for 30 s, and a final extension at 60.0°C for 30 min. The final PCR products were analyzed using Applied Biosystems 3730 DNA Analyzer with a GeneScan 500 LIZ Size Standard (Thermo Fisher Scientific Inc., Waltham, MA, USA). Samples were genotyped using Peak Scanner Software version 2.0 (Thermo Fisher Scientific Inc.).

Genetic diversity in seeds was assessed by calculating the number of alleles per locus (A), observed heterozygosity (H_o), expected heterozygosity (H_e), and Wright's inbreeding coefficient (F_{is}) using FSTAT version 2.9.4 (Goudet, 1995, 2003). Furthermore, we tested whether the F_{is} for each locus differed from the expected value under the Hardy–Weinberg equilibrium using 1,000 randomizations. The outcrossing rate per fruit (multilocus genotypes, t_m) was determined using the software MLTR version 3.2 (Ritland, 2002; Ritland & Jain, 1981). Standard errors (SE) for the parameters were estimated from 1,000 bootstraps. GLMMs were conducted to compare the outcrossing rate (postulating a binomial error distribution with logit-link) and the value of seed-set rate $\times$ outcrossing rate, an index of outcrossed seed production (postulating a gamma error distribution with log-link) between the treatments. In these analyses, treatment was set as the explanatory variable, and individual ID as the random effect.

Results

Fruit Set and Flower Visitors

The fruit-set rates under natural conditions were higher in 2022 (0.52 ± 0.05 SE, $N = 20$, range: 0.20–0.89) than in 2023 (0.32 ± 0.04 , $N = 40$, range: 0.00–0.79, $P = 0.0009$). Swallowtails (*Papilio maackii*) and bumblebees (queens of *Bombus schrencki albidopleuralis*) were identified as major flower visitors (Appendix Figure A2). The worker bees of *B. ardens sakagamii* were also flying around the area and were considered potential pollinators, while visits from dipteran insects were not observed.

Herbivory Pattern and Effect of Chewing Herbivory Rate on Seed Production

In 2022, 2 of the 20 ramets were collectively parasitized by sawfly larvae (*A. similis*), indicating a large variation in the herbivory rate among plants (Figure 1). The herbivory rate during the

middle of the fruiting season (August 1) was $8.2\% \pm 3.1\%$ ($N = 20$, range: 1.1%–59.6%), which significantly affected the seed-set rate (Figure 3, Table 1). The herbivory rate right before seed dispersal (October 9) was $4.6\% \pm 0.8\%$ ($N = 20$, 1.5%–15.1%), and it did not affect the seed-set rate (Table 1). Individual seed mass was not affected by either seed-set or herbivory rates (Table 1).

Herbivory rates in 2023 were consistently low, around 3% throughout the entire season (Figure 3). *Arge similis* adults were observed on June 11, and larvae were seen on July 2 and September 3 (Figure 4). Leaf damage peaks were recorded on July 23 and October 14 (Figure 3). The first peak occurred after the activity period of sawfly larvae. In August, the herbivory rate decreased temporarily due to the additional emergence of non-overwintering leaves. At the second peak, the abscission of non-overwintering leaves had already begun, and 11%–25% of damaged leaves were noticeable. The seed-set rate was independent of the herbivory rates during the middle fruiting season (July 23) and right before seed dispersal (October 14) in this year (Table 1). Seed mass was not affected by the seed-set and herbivory rates, similar to the findings in 2022 (Table 1).

Moth larvae (*Lymantria dispar*, Lymantriinae and Geometridae) and adults of leaf beetles (Chrysomelidae) were also observed as herbivores (Figure 4). They were solitary insects, and the feeding scars on leaves were smaller than those of *A. similis*.

Fruit length was significantly related to the seed number (Table 1). The effect of leaf damage on fruit length was not detected in 2022, but a significant difference was observed during the middle fruiting season in 2023 (Table 1).

Effects of Artificial Leaf Excision on Fruit and Seed Development

This analysis was performed only for 2023. Fruit length, seed number, ovule number, and seed-set rate were significantly lower in the excision treatment (Tables 2 and S1). Fruit width and seed mass did not differ between the excision and control groups (Tables 2 and S1). Fruits of the control group contained, on average, about 100 seeds, while the number of seeds in the excision group decreased about three-quarters. Eight fruits in the excision group contained fewer than 20 seeds (Figure 5). Naturally or artificially damaged leaves remained on the branches until October.

Outcrossing Rate

The mean F_{is} displayed negative values in both the excision and control groups, indicating the dominance of outcrossing in this population (Table 3). The average t_m in the excision group (0.82 ± 0.05 , $N = 20$, range: 0.30–1.00) was significantly higher than that in the control group (0.72 ± 0.06 , $N = 20$, range: 0.04–1.00; Figure 6 and Table S1). The values of seed-set rate $\times$ outcrossing rate (i.e., the index of outcrossed seed production) were 0.172 ± 0.022 ($N = 20$, range: 0.022–0.388) in the excision group and 0.171 ± 0.022 ($N = 20$, range: 0.007–0.398) in the control group, indicating no difference between the two groups (Figure 6 and Table S1). This means that the production of outcrossed seeds was retained in the excision group, even under lower seed-set rates.

Discussion

Leaf Herbivores of *R. kaempferi*

Herbivorous damage by sawfly larvae was less severe than expected during the research period (2022 and 2023) in the *R. kaempferi* population. Only two ramets were parasitized by sawfly larvae in 2022. Based on the herbivore pattern in 2023 (Figure 3), *A. similis* is expected to be bivoltine in Hokkaido. Secondary foliation of non-overwintering leaves occurred in August, and the oviposition of sawflies might be synchronized with this event. As the abscission of non-overwintering leaves began in early October, the feeding period of sawfly larvae was completed by October. Overwintering leaves might be insufficient as a food resource for sawfly larvae due to their smaller number and size.

Leaf damage caused by sawfly larvae has been reported in some tree species. For instance, intensive defoliation by sawflies (*Fagineura crenativora*, Tenthredinidae) was reported in beech trees (*Fagus crenata*) in the Tanzawa Mountains of eastern Japan (Shinohara et al., 2000). Although broad-leaved trees are rarely killed by defoliation caused by herbivorous insects in Japan (Kamata, 2002), many beech trees died after intensive herbivory by sawfly larvae during the outbreak year (Taniwaki & Watanabe, 2012). The elm zigzag sawfly (*Aproceros leucopoda*, Argidae), originally native to East Asia, has expanded in Europe and Canada in the 21st century as an invasive alien species. It feeds on *Ulmus* tree leaves, causing serious damage (Martel et al., 2022; Vetek et al., 2017). Although herbivory damage caused by *A. similis* larvae was sporadic and occasional in our study, continuous monitoring is needed to clarify the ecological impacts of sawfly herbivory under natural conditions.

Defoliation Effects on Reproductive Success

A previous study reported that current photosynthetic products from adjacent shoots are an important resource pool for fruit production in woody plants (e.g., Miyazaki et al., 2007). The present study revealed that non-overwintering leaves are an essential resource pool for current seed production in *R. kaempferi*. The major implications of this study are that (1) seed maturation progresses slowly during the summer season and (2) partial seed abortion occurs to maintain individual seed mass when resource supply from the nearby leaves is restricted due to intensive defoliation. Perennial plants often prioritize seed mass over seed number under resource limitation because of the importance of vigorous offspring production (Cardel & Koptur, 2022).

Although the photosynthetic products of adjacent spring leaves might be used for seed production, the contribution of summer leaves to seed production was limited. Considering the timing of secondary foliation, photosynthetic products of summer leaves may be stored and used for shoot growth and/or flower production in the following spring. In the wintergreen dwarf shrub, *Rhododendron lapponicum*, the overwintering leaves are responsible for the storage of nutrients and carbon acquisition in the following year (Jonasson, 1995). If the overwintering leaves have a storage function in *R. kaempferi*, carbon products from summer leaves may be transported to the overwintering leaves before senescence in autumn.

The fruit length of *R. kaempferi* was related to the seed number, as reported in other *Rhododendron* species (Padgett et al., 2019; Stout, 2007), while the effect of herbivory rate on fruit size was inconsistent between years (Table 1). This is because (1) the intensity of herbivorous damage varies greatly among plants and between years, and (2) actual seed number per fruit is affected by pollination success under natural conditions. In contrast, seed abortion caused by the excision treatment consistently suppressed fruit elongation, suggesting the importance of photosynthetic products from current leaves as a resource pool for seed production.

The ovule number in the excision group was significantly smaller than in the control, probably due to stable accuracy in counting ovules. Undeveloped ovules within small fruits were often difficult to distinguish from other fragmentary tissues. If ovules were miscounted in undeveloped or small fruits, the ovule number in the excision treatment might be underestimated.

Selective Abortion under Resource Limitation

The outcrossing rate was estimated to be 72.0% under natural conditions, while the excision group showed 10% higher outcrossing rate than the control group. This suggests the possibility of selective abortion of selfed seeds under resource limitation. When available resources are limited, full development of embryos into seeds might be difficult, resulting in a decreased seed number due to embryo abortion (Cardel & Koptur, 2022). Seed (embryo) abortion is caused by the competition among embryos within fruits for resources provided by the mother plant (Ganeshaiah & Shaanker, 1988), where variation in pollen-tube growth among pollen grains is a key factor (Carney et al., 1996) because ovules fertilized earlier have higher advantage of resource acquisition. Our previous study revealed that fruit-set rate of self-pollinated flowers was less than half of outcrossed flowers in *R. kaempferi* (Takahashi & Itino, 2022). Thus, the partial self-compatibility in this species may be because of the unstable ability of pollen-tube growth in self-pollen. Selfed embryos that begin to develop later may be disadvantage in resource competition, resulting in a higher risk of abortion. Because our measurements were conducted under natural pollination, it is difficult to conclude the mechanism of selective abortion. To test this possibility, a comprehensive study with a mixed pollination experiment is necessary.

The values of seed-set rate $\times$ outcrossing rate were nearly equal in the excision and control groups, suggesting that selective abortion assures outcrossed seed production. In response to the decreased seed number caused by sawfly larvae herbivory, mother plants might mitigate the reduced reproductive output by aborting selfed seeds that potentially have lower survivorship. Our findings indicate that outcrossing rate may change as an adaptive response to fluctuations in available photosynthetic resources. This means that mating system (the proportion of selfed vs. outcrossed seeds) is influenced not only by plant–pollinator interactions but also by plant–herbivore interactions. However, the advantages of selective seed abortion remain to be further clarified. For instance, abortion of selfed seeds might simply be caused by early-acting inbreeding depression during the fruit development stage, as reported in *Rhododendron brachycarpum* and *R. aureum* (Hirao, 2010; Kudo et al., 2011). Partial self-compatibility and inbreeding depression in *R. kaempferi* remain to be rigorously measured. Moreover, because leaf excision was conducted under natural pollination in our study, the quality and quantity of pollen deposited on stigmas were not uniform, thus, it is difficult to assess the effects of resource limitation. Finally, the resource pool for seed production (e.g., storage starch content in non-

overwintering leaves; Miyazaki et al., 2007) was not assessed, although defoliation negatively affected seed production. Further studies are necessary to clarify the reproductive strategy of this species under fluctuating pollination and herbivory conditions.

Acknowledgments

We thank Y. Mizunaga, curator of Mt. Apo UNESCO Global Geopark, for her assistance in the fieldwork; Y. Shiotani, A. Shibata, and A. Wakui for technical advice on microsatellite marker analysis; and Y. Ito for valuable comments on the investigation of herbivory rate. This work was supported by JSPS KAKENHI Grant Number JP22J20087 and JP22KJ0100.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Table 1. Results of generalized linear mixed model (GLMM) analyses for *Rhododendron kaempferi* reproductive performance in the natural state.

Response variable	Year	<i>N</i>	Explanatory variable	Estimate	Standard error	Statistic value (<i>z</i> , <i>t</i>)	<i>p</i> level
Seed-set rate	2022	76	Intercept	−0.798	0.188	−4.24	<0.0001
			Herbivory rate 1st	−0.018	0.008	<i>z</i> −2.36	0.018
			Herbivory rate 2nd	−0.010	0.029	−0.35	0.726
Seed mass	2022	76	Intercept	−2.176	0.104	−20.84	<0.0001
			Seed set	−0.109	0.193	<i>t</i> −0.57	0.571
			Herbivory rate 1st	−0.002	0.004	−0.63	0.530
			Herbivory rate 2nd	−0.002	0.014	−0.16	0.876
Fruit length	2022	76	Intercept	1.875	0.055	33.80	<0.0001
			Seed number	0.144	0.010	<i>t</i> 13.94	<0.0001
			Herbivory rate 1st	0.001	0.002	0.30	0.768
			Herbivory rate 2nd	0.009	0.008	1.09	0.274
Seed-set rate	2023	50	Intercept	−1.336	0.310	−4.30	<0.0001
			Herbivory rate 4th	0.020	0.120	<i>z</i> 0.17	0.867
			Herbivory rate 8th	−0.033	0.083	−0.39	0.695
Seed mass	2023	50	Intercept	2.419	0.141	17.16	<0.0001
			Seed set	−0.031	0.188	<i>t</i> −0.16	0.870
			Herbivory rate 4th	0.041	0.052	0.80	0.425
			Herbivory rate 8th	−0.038	0.037	−1.04	0.296
Fruit length	2023	50	Intercept	2.068	0.046	44.78	<0.0001
			Seed number	0.165	0.017	<i>t</i> 9.99	<0.0001
			Herbivory rate 4th	−0.070	0.018	−3.98	<0.0001
			Herbivory rate 8th	0.021	0.013	1.66	0.098

Table 2. Fruit length, fruit width, seed number, ovule number, seed-set rate, and individual seed mass of *Rhododendron kaempferi* in 2022 and 2023.

Year	Treatment	<i>N</i>	Fruit length (mm)	Fruit width (mm)	Seed number	Ovule number	Seed-set rate	Individual seed mass (mg)
2022	Natural	76	8.46 ± 0.20	4.18 ± 0.10	132.6 ± 14.0	468.7 ± 26.6	0.242 ± 0.016	0.109 ± 0.003
2023	Natural	50	8.50 ± 0.21	4.08 ± 0.11	100.5 ± 12.5	463.9 ± 28.2	0.192 ± 0.013	0.115 ± 0.002
2023	Excision	50	8.06 ± 0.23	4.00 ± 0.10	76.1 ± 9.9	429.5 ± 28.3	0.166 ± 0.014	0.116 ± 0.003

Note: Refer to Table S1 for statistical analyses of comparisons between treatments.

Table 3. Characteristics of the five microsatellite markers used for the genetic analysis of *Rhododendron kaempferi*.

Locus	Control (20 fruits, 403 seeds)				Excision (20 fruits, 405 seeds)			
	<i>A</i>	<i>H_o</i>	<i>H_e</i>	<i>F_{is}</i>	<i>A</i>	<i>H_o</i>	<i>H_e</i>	<i>F_{is}</i>
AZA003	10	0.832	0.676	−0.231	10	0.785	0.641	−0.225
AZA009	7	0.222	0.201	−0.107	9	0.314	0.286	−0.098
N25	7	0.648	0.578	−0.122	8	0.603	0.553	−0.090
RM3D2	6	0.573	0.494	−0.161	9	0.634	0.569	−0.114
RM9D6	13	0.618	0.543	−0.138	14	0.575	0.506	−0.138
Mean	8.6	0.579	0.498	−0.152	10.0	0.582	0.511	−0.133

Note: *A*: number of alleles; *H_o*: observed heterozygosity; *H_e*: expected heterozygosity; *F_{is}*: Wright's inbreeding coefficient. *F_{is}* per locus did not differ significantly from the expected values under Hardy–Weinberg equilibrium ($p > 0.05$, 1,000 randomizations).

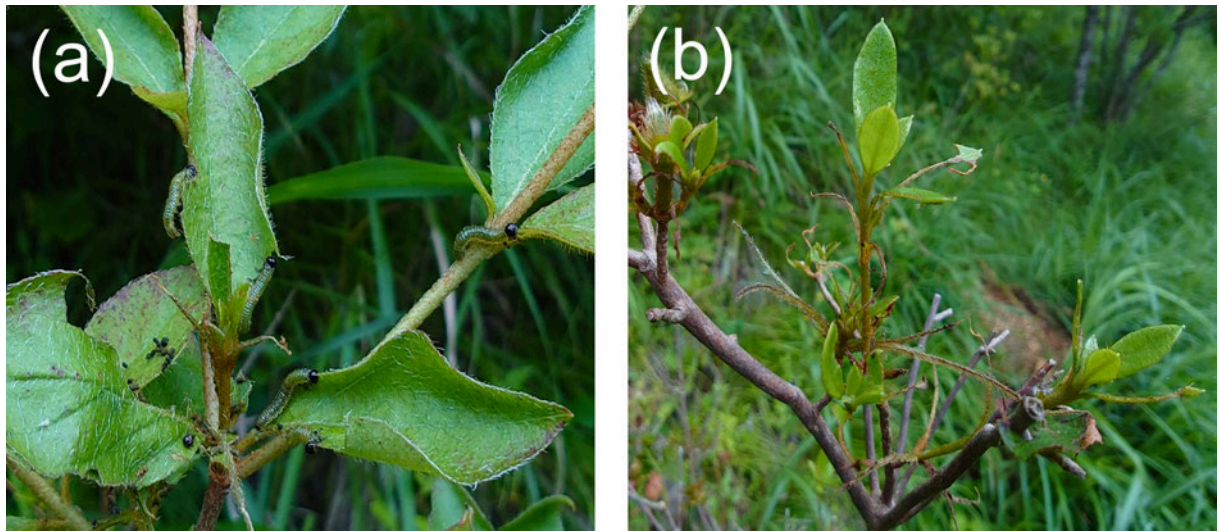


Figure 1. The reality of leaf feeding damage on *Rhododendron kaempferi*. (a) Sawfly larvae (*Arge similis*) and (b) damaged leaves. Photos taken by K. Takahashi on August 1, 2022.

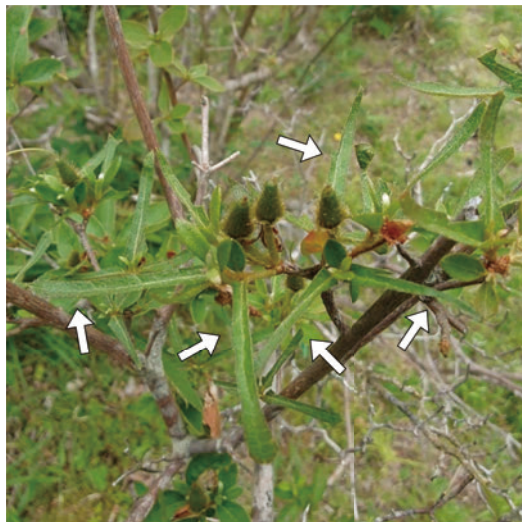


Figure 2. Fruits and excised leaves of *Rhododendron kaempferi*. White arrows indicate some of the excised leaves. Photo taken by K. Takahashi on July 23, 2023.

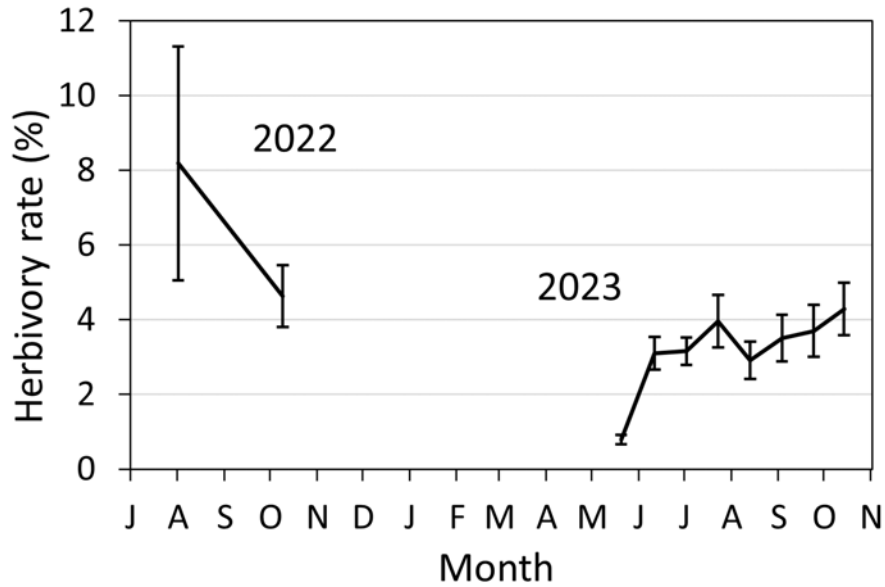


Figure 3. Transition of herbivory rate (%) on *Rhododendron kaempferi* leaves in 2022 and 2023 (mean $\pm$ SE). The mean herbivory rate was calculated by dividing the sum of the herbivory rates in each ramet by the number of ramets.

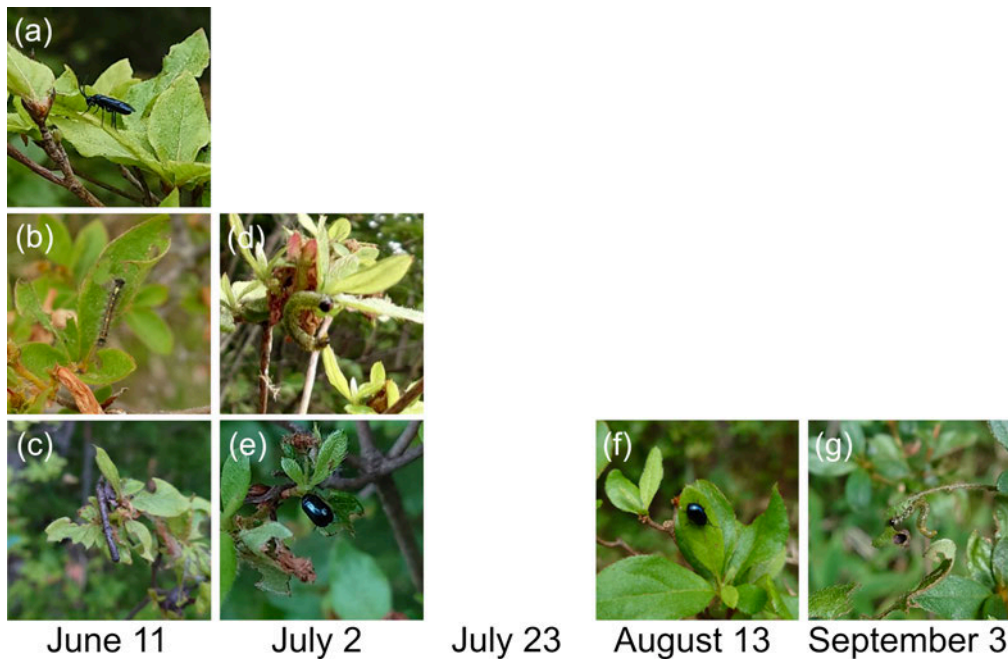


Figure 4. Herbivorous insects observed on *Rhododendron kaempferi* leaves. (a) *Arge similis* (adult), (b) *Lymantria dispar* (larva), and (c) Geometridae sp. (larva) on June 11; (d) *A. similis* (larva) and (e) *Agelastica coerulea* (adult) on July 2; (f) *A. coerulea* (adult) on August 13; (g) *A. similis* (larva) on September 3. No insects were recorded on July 23.

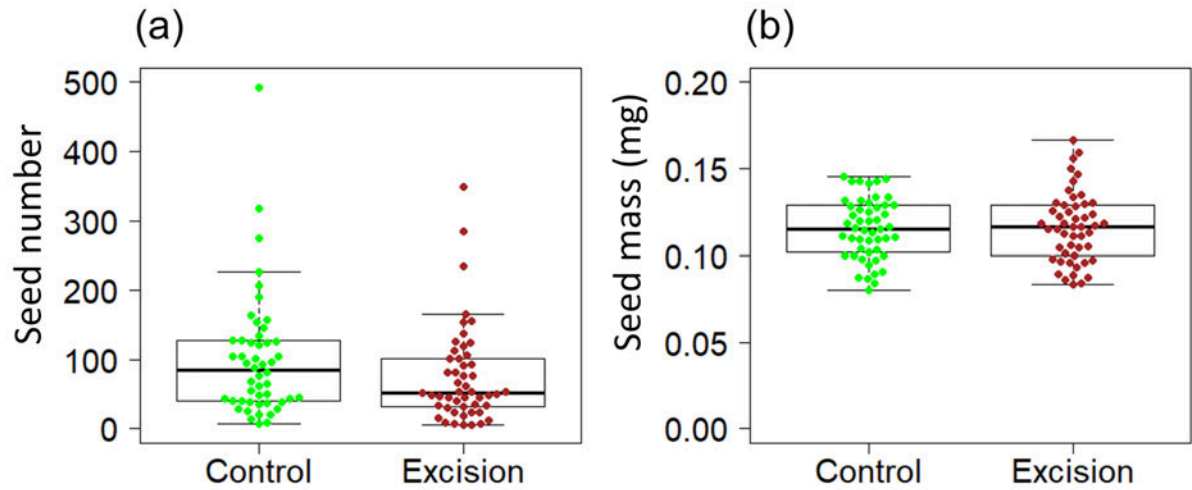


Figure 5. Seed traits of *Rhododendron kaempferi* in the excision and control groups. (a) Seed number and (b) individual seed mass per fruit.

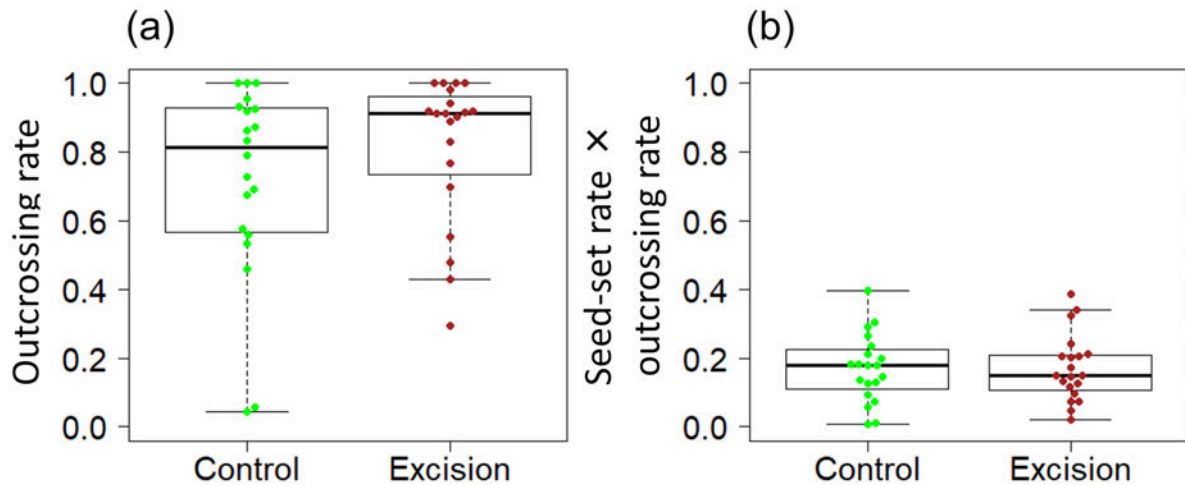


Figure 6. Outcrossed seed production of *Rhododendron kaempferi* in the excision and control groups. (a) Outcrossing rate and (b) the value of seed-set rate $\times$ outcrossing rate.

Appendix (Takahashi & Kudo)



Figure A1. Overwintering and non-overwintering leaves of *Rhododendron kaempferi*. Orange arrows and white arrows indicate non-overwintering leaves and overwintering leaves. Photo taken by K. Takahashi on May 4, 2024.



Figure A2. Flower visitors of *Rhododendron kaempferi*. (a) *Papilio maackii* and (b) a queen of *Bombus schrencki albidopleuralis*. Photos taken by K. Takahashi on June 11, 2023.

Table S1. Results of generalized linear mixed model (GLMM) analyses for *Rhododendron kaempferi* reproductive performance after leaf excision

Response variable	<i>N</i>	Explanatory variable*	Estimate	Standard error	Statistic value (<i>z</i> , <i>t</i>)		<i>p</i> level
Fruit length	100	Intercept	2.157	0.041	<i>t</i>	52.11	<0.0001
		Treatment	−0.077	0.036		−2.10	0.035
Fruit width	100	Intercept	1.395	0.042	<i>t</i>	33.54	<0.0001
		Treatment	−0.036	0.035		−1.01	0.314
Seed number	100	Intercept	4.525	0.126	<i>z</i>	35.78	<0.0001
		Treatment	−0.481	0.025		−19.55	<0.0001
Ovule number	100	Intercept	6.086	0.074	<i>z</i>	82.06	<0.0001
		Treatment	−0.173	0.011		−16.23	<0.0001
Seed-set rate	100	Intercept	−1.313	0.091	<i>z</i>	−14.40	<0.0001
		Treatment	−0.380	0.028		−13.79	<0.0001
Seed mass	100	Intercept	−2.188	0.049	<i>t</i>	−44.56	<0.0001
		Treatment	0.0003	0.027		0.01	0.992
Outcrossing rate	40	Intercept	1.877	0.344	<i>z</i>	5.46	<0.0001
		Treatment	0.519	0.105		4.96	<0.0001
Seed-set rate × outcrossing rate	40	Intercept	−1.812	0.173	<i>t</i>	−10.50	<0.0001
		Treatment	0.027	0.210		0.13	0.899

* Intercept, control; Treatment, leaf excision