



Title	Explosive wind-pollination in a monoecious plant, <i>Laportea bulbifera</i> (Urticaceae)
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1 **Title:**

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3 **Explosive wind-pollination in a monoecious plant, *Laportea bulbifera* (Urticaceae)**

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24 **Abstract**

25 *Laportea bulbifera* (Urticaceae) is a monoecious plant that has a unique sexual  
26 expression: female flowers form on the upper part and male flowers on the lower part  
27 on an individual shoot. Therefore, for the seed reproduction, pollen needs to be  
28 transferred from the lower (male) to the upper (female) flowers. Our observations of  
29 male flowers confirmed that pollen was dispersed upward by "explosive wind-  
30 pollination". A male flower has five stamens, and when the petals are open, the stamens  
31 were caught in a pistillode. With the growth of the stamens, they were released from the  
32 pistillode, and then straightened with a spring-like movement of the filament. At the  
33 same time, the anthers dehisced, and the pollen was dispersed. The explosive release of  
34 pollen from the anthers and light wind in the habitat (forest edge or gap) contributes to  
35 wind-pollination in *L. bulbifera*.

36

37 Keywords: anemophily, geitonogamy, monoecy, pistillode, self-pollination

38

## 39 INTRODUCTION

40 Most species of flowering plants are hermaphroditic. Among angiosperm species, only  
41 5% of monoecious plants have separate sex flowers on the same individual (Barrett,  
42 2002; Torices et al., 2011). Hermaphroditic flowers are more economical because the  
43 costs of the non-sexual parts of the flower, such as nectar, petals, and sepals, are shared  
44 by male and female functions. On the other hand, it has been argued that monoecy  
45 favors outcrossing, reduces pollen–stigma interference, allows a more flexible  
46 allocation of gender in a variable environment, and allows a more exact sex allocation  
47 in a constant environment (Chalesworth & Chalesworth, 1979; Lloyd, 1979). In a  
48 monoecious plant, male and female flowers are physically separated within an  
49 individual rather than having hermaphrodite flowers. Thus, a mechanism for pollen  
50 transfer between the separated flowers is required even in the case of geitonogamy.

51 *Laportea bulbifera* (Urticaceae) is a monoecious perennial herb growing on mesic  
52 forest edges. This plant forms female flowers on the upper part of the shoot and  
53 develops inflorescences of male flowers from the lower axils of the shoot (Fig. 1).  
54 Therefore, it is considered that for the seed reproduction, this plant should have a  
55 mechanism for pollen dispersal that allows pollen to move from the lower to upper part  
56 of the plant. High-speed pollen release from male flowers found in Moraceae can  
57 achieve such upward wind pollination (Taylor et al. 2006). Pederoli et al. (2019)  
58 clarified anatomical mechanisms of the explosive pollen release in the urticalean rosids  
59 (Cannabaceae, Moraceae, and Urticaceae), including a congeneric species, *L. aestuans*.  
60 The purpose of this study is to elucidate the mechanism that enables such pollen  
61 movement in *L. bulbifera* by careful observations and pollination experiments in the  
62 field

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## 65 **MATERIAL AND METHODS**

### 66 **Study species and study site**

67 *Laportea bulbifera* (Siebold et Zucc.) Wedd. (Urticaceae) is a perennial herb that grows  
68 on the edge of forests. In Japan, this species is widely distributed from Hokkaido to  
69 Kyushu (Ohashi et al., 2016). This plant is monoecious and forms female flowers on the  
70 upper part (Fig. 1a, d) and male flowers in the lower part of the shoot (Fig. 1 b, e).

71 Female flowers form on the shoot apex and have one ovule per stigma. Male flowers  
72 form in the axils with hundreds to thousands of small flowers with a diameter of 1 to 3  
73 mm per shoot (Ohashi et al., 2016). The flowering period is from late July to late  
74 September, and seed formation occurs from late August to mid-October. In addition to  
75 seed production via female flowers, this plant forms bulbils, a vegetative propagation  
76 organ, on the axils (Fig. 1c).

77 Field studies (observations and experiments) were conducted on a population of *L.*  
78 *bulbifera* in Nopporo Forest Park (43°06'N, 141°51'E), Ebetsu City (vicinity of Sapporo  
79 City), Hokkaido.

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81

### 82 **Field observations and pollination experiments**

83 To elucidate the mechanism of pollen dispersal, we made careful observations of the  
84 morphology of male flowers including anther dehiscence in the field. In addition, the  
85 dynamics of male flowers were recorded by using a camera, OLYMPUS STYLUS TG-  
86 2 Tough (Olympus Corporation).

87 We conducted emasculation, i.e., male flowers were removed from the shoot before  
88 flowering, to investigate the possibility of pollen transfer to female flowers from each  
89 shoot. If the female flowers of the emasculated shoot produced seeds, they must have  
90 received the pollen from other male flowers. We randomly selected 33 plants and  
91 carried out emasculation in 2021. We also randomly marked 55 plants with both male  
92 and female flowers as controls. At fruiting (September), we counted the total number of  
93 seeds and undeveloped female flowers. Female flowers have one ovule per flower, and  
94 the presence or absence of seed can be judged from the appearance. We calculated seed-  
95 setting rates (ratio of total number of seeds to total number of female flowers) for each  
96 shoot and compared between the two treatments.

97 In addition, we applied two artificial pollination treatments to evaluate the degree  
98 of self- compatibility, as follows: (1) self-pollination: female flowers were pollinated  
99 with using pollen of male flowers on the same shoot, (2) cross-pollination: female  
100 flowers were pollinated with pollen from male flowers on a different shoot (at least 100  
101 m apart). After treatment, the flowers were covered by cellophane bags to prevent  
102 unintentional pollination. We used 20 plants randomly selected in the field for both self-  
103 pollination and cross-pollination experiments. At fruiting (September), we calculated  
104 seed-setting rates of each shoot and compared between the two treatments. A  
105 generalized linear model (GLM) was used to evaluate the effect of treatment on seed-  
106 setting rates (binomial distribution). The response variable was seed-setting rates, and  
107 the explanatory variable was the pollination treatment. GLM was performed with R  
108 version 4.0.2, using package “stats” (R Development Core Team, 2020).

109

## 110 **RESULTS**

111 **Observation of male flowers**

112 Figure 2 shows sequential photographs of the male flowers within 0.1 second. It can be  
113 readily recognized that pollen was released upwards from the male flowers like white  
114 dust. Additional detailed observation of male flowers clarified that each flower had five  
115 stamens, and when the petals were open, the stamens were caught in a pistillode (Fig.  
116 3a, c-left). With the growth of the stamens, the stamens were released from the  
117 pistillode and straightened with a spring-like movement of the filament (Fig. 3b). At the  
118 same time, the anthers dehisced, and the pollen was released (Fig. 3c-right).

119

120 **Pollination experiments**

121 Figure 4 shows the results of pollination experiments. Of the individuals marked and  
122 treated for the experiments, some were damaged by insect feeding. Figure 4a illustrates  
123 the seed-setting rates of the emasculated shoots and controls. The average seed-setting  
124 rate of the emasculated shoots was  $64.3 \pm 1.6\%$  (SD) ( $n = 21$ ), and that of the control  
125 was  $61.8 \pm 2.2\%$  ( $n = 44$ ). A GLM showed no significant difference between the two  
126 treatments ( $P = 0.77$ ). Therefore, it was confirmed that pollen transfer to other shoots  
127 can occur sufficiently.

128 Figure 4b illustrates the seed-setting rates of “self-pollination” and “cross-  
129 pollination”. The average seed-setting rate of self-pollination was  $67.5 \pm 5.5\%$  ( $n = 10$ ),  
130 and that of cross-pollination was  $69.5 \pm 4.9\%$  ( $n = 13$ ). A GLM showed no significant  
131 difference between the two treatments ( $P = 0.49$ ), indicating that there was no  
132 difference in seed formation ability between self-fertilization and cross-fertilization.  
133 Therefore, high self-compatibility of this plant was confirmed.

134

135 **DISCUSSION**

136 Based on careful field observations of male flowers of *L. bulbifera*, it was clarified that  
137 the stamens were caught in a pistillode, and pollen was dispersed upward with a spring-  
138 like movement of the filament (Figs. 2 & 3). This pattern of pollen dispersal is called  
139 "Explosive wind-pollination" and has also been reported in several anemophilous  
140 herbaceous plants such as *Nanocnide japonica* Blume, *Boehmeria silvestrii* (Pamp.)  
141 W.T.Wang, and *Acalypha australis* L. (Knuth, 1906; Tanaka, 2000). Pedersoli et al.  
142 (2019) anatomically clarified the unusual synorganization of the staminate flower in  
143 wind-pollinated utricalean rosids including *Laportea aestuans* by using light and  
144 scanning electron microscopy. They detailed that the the pistillodes, stamens, and sepals  
145 form a floral apparatus that explosively release pollen to be carried by wind. The  
146 anthers dehisce when the stamens are still inflexed on the floral bud and an enveloped  
147 by the sepals and supported by an inflated pistillodes. The present study also confirmed  
148 that the same mechanisms operate in *L. bulbifera*.

149 In *L. bulbifera*, explosive wind-pollination functions well to enhance the impulse of  
150 pollen and successfully transfer pollen from lower (male) flowers to upper (female)  
151 flowers and to produce the seeds (Fig. 4). As shown in Fig. 4b, *L. bulbifera* has high  
152 self-compatibility, suggesting that seed formation is possible by geitonogamy within its  
153 own shoot. Further investigation is needed to elucidate how self-fertilized seed  
154 formation contributes to population maintenance in this plant. In addition, as the species  
155 name '*bulbifera*' indicates, *L. bulbifera* produces bulbils on the axils (asexual  
156 propagules) that are much larger in size than the seeds (Fig. 1c). Thus, *L. bulbifera* is a  
157 species suitable for evaluating the contribution of sexual and asexual reproductive  
158 options to population dynamics (Tsujimoto et al., in preparation).

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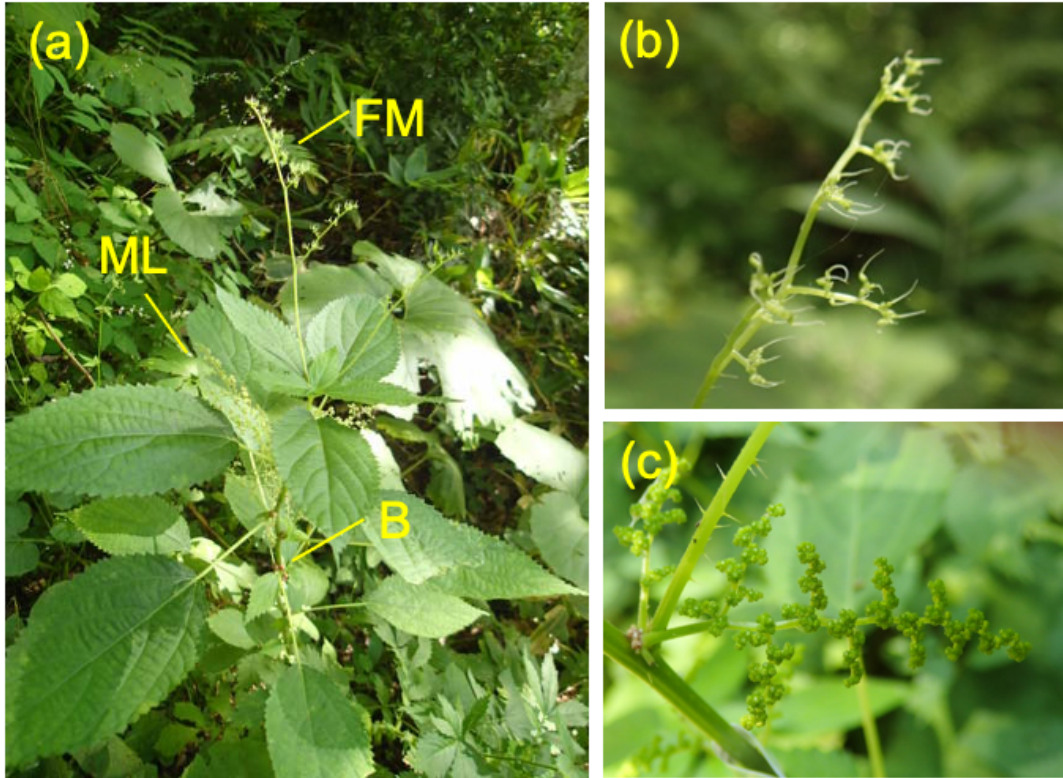
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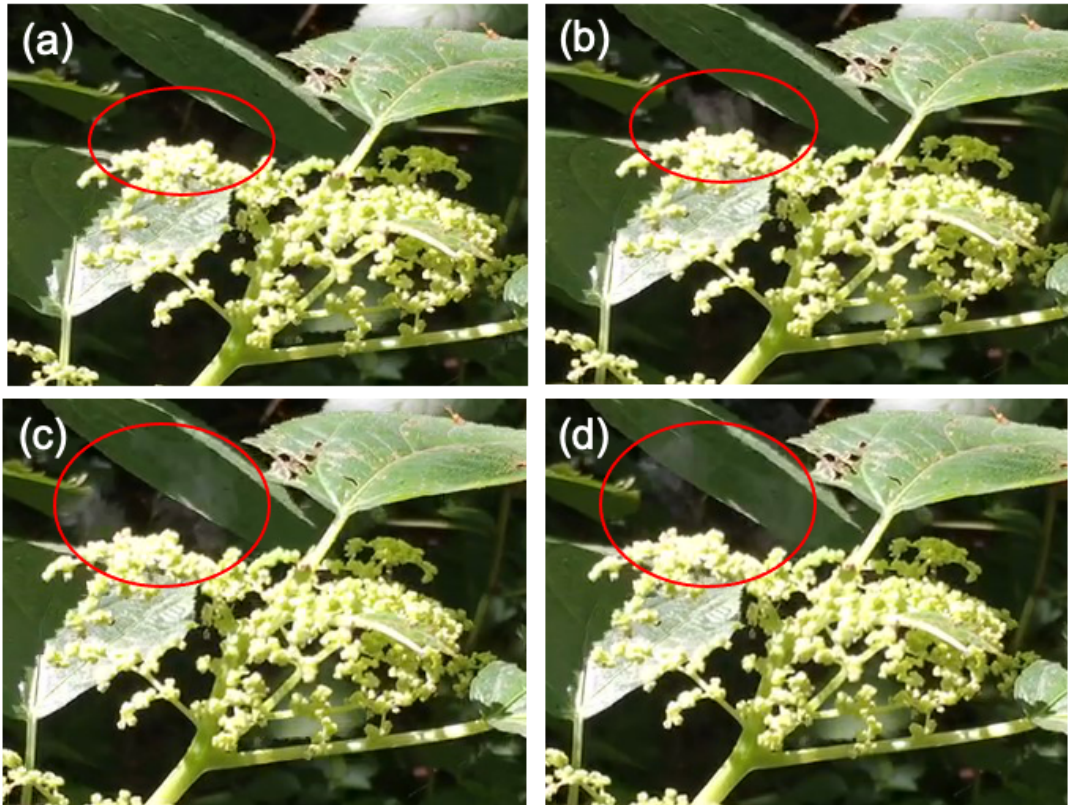
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194 **Figure Legends**



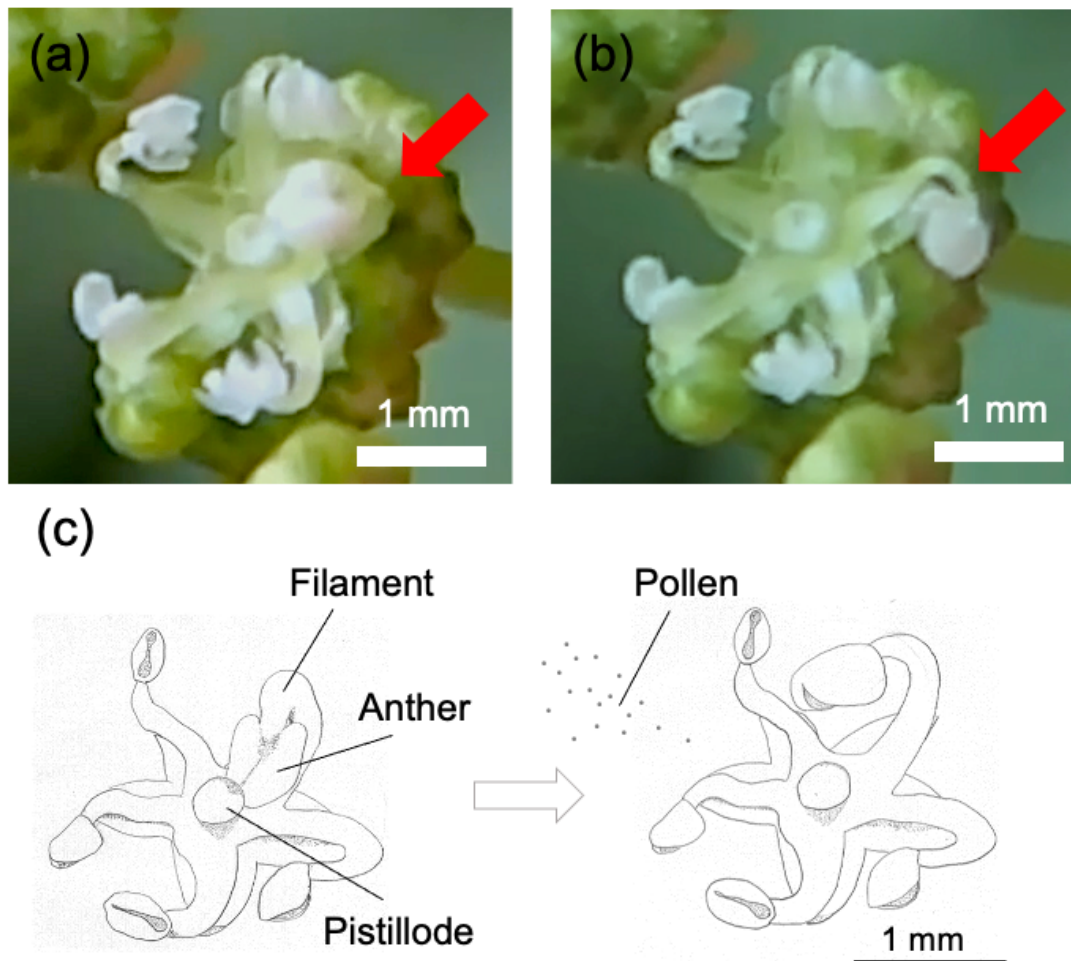
195 Figure 1: Flowering individual of *Laportea bulbifera*. (a) FM, female flowers; ML,  
196 male flowers; B, bulbils, (b) enlarged photo of female flowers, (c) buds of male  
197 flowers.

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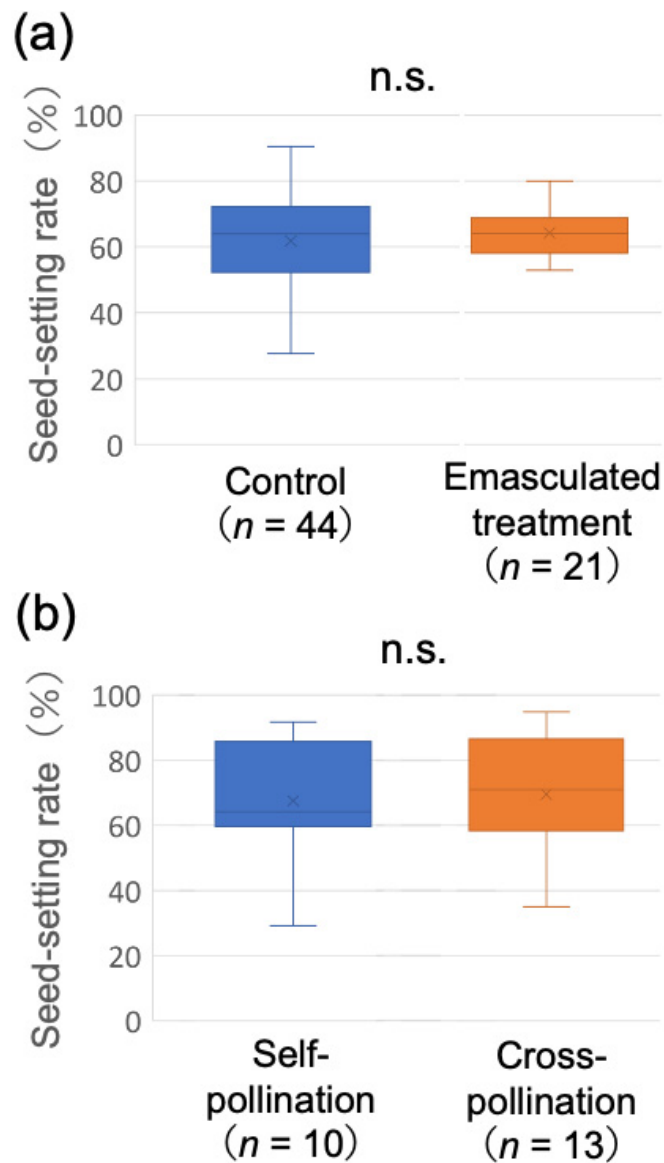
206 Figure 2: High-speed consecutive photographs of pollen dispersal from male flowers.  
207 Photos taken by a digital camera: OLYMPUS STYLUS TG-2 Tough (Olympus  
208 Corporation). Photos shown at (a) 0.00 second, (b) 0.04 s, (c) 0.09 s, (d) 0.10 s. The  
209 red color ellipses show the diffusion states at each timing.

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218 Figure 3: Mechanisms of pollen dispersal of a male flower. (a) inflexed stamen (red  
 219 allow), (b) dehiscence of anthers with a spring-like movement of filament, (c)  
 220 illustrations showing the mechanisms of pollen dispersal.

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227 Figure 4: (a) Comparison of seed setting rates between control and emasculated shoots.  
 228 (GLM,  $P = 0.77$ ). (b) Comparison of seed setting rates between self-pollination and  
 229 cross-pollination. (GLM; Generalized Linear Model,  $P = 0.49$ ). The numbers in the  
 230 parentheses are sample sizes. “n.s” means that there are no significant differences  
 231 between the treatments.