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SHORT COMMUNICATION

Incidence of Germinable Seeds of *Ludwigia grandiflora* subsp. *hexapetala* (Onagraceae) in the Feces of Lake Biwa's Waterfowl

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Endozoochorous dispersal of the invasive alien aquatic plant *Ludwigia grandiflora* subsp. *hexapetala* through waterfowl in Lake Biwa, Japan, is reported. Germination of seeds collected from the feces of waterfowl in Yabase, southern Lake Biwa proved them to be viable. It is necessary to be vigilant against the sudden appearance of *L. grandiflora* subsp. *hexapetala* in water systems where it is absent and to monitor its dispersal by waterfowl.

Keywords: aquatic macrophytes, freshwater, invasive alien species, Onagraceae, seed dispersal

Ludwigia grandiflora (Michx.) Greuter & Burdet subsp. *hexapetala* (Hook. & Arn.) G. L. Nesom & Kartesz, native to South America (Grewell *et al.* 2016, Nesom & Kartesz 2000), is an invasive alien aquatic plant. Its spread and extensive growth are a matter of concern in Lake Biwa (Hieda *et al.* 2020, Nakai 2020). It has been regarded as an invasive alien in Europe (*e.g.*, Armitage *et al.* 2013, Haury *et al.* 2014, Kamigawara *et al.* 2020) and in the U.S.A. (Grewell *et al.* 2016). Propagation of *L. grandiflora* subsp. *hexapetala* is mainly vegetative (Okada *et al.* 2009), and stems and leaves of *L. grandiflora* have the ability to regenerate (Hussner 2009). Gillard *et al.* (2017) noted that sexual reproduction in *L. grandiflora* subsp. *hexapetala* also contributes to its invasiveness.

Zoochory by waterfowl is considered an important vector for the distribution of aquatic plants (*e.g.*, Figuerola & Green 2002). In southern France, the seeds of *L. peploides* (Kunth) P. H. Raven, a species similar to *L. grandiflora* in the same section *Jussiaea* (L.) Hoch, W. L. Wagner & P. H. Raven (Hoch *et al.* 2015), were recorded from the rectum of a teal in Camargue, but the seeds were unviable (Brochet *et al.* 2010). García-Álvarez *et al.* (2015) experimentally fed seeds of *L. grandiflora* to *Anser anser* and to *Anas platyrhynchos* and reported a germination rate of the digested seeds to be about 37% and 15%, respectively, but lower than in a control group of seeds not fed to waterfowl (about 60%). Waterfowl and humans are the suspected vectors because newly invaded populations in the Netherlands and Germany are far from the known invasion sites in France (García-Álvarez *et al.* 2015). Accordingly, there is a high possibility of germinable seeds of *L. grandiflora* subsp. *hexapetala* in waterfowl feces. In this study, we attempted to germinate seeds of *L. grandiflora* subsp. *hexapetala* obtained from waterfowl feces collected in Lake Biwa to determine if waterfowl served as an endozoochorous vector.

Materials and Methods

Between October and December 2018, we collected fecal samples of waterfowl along the coast of Lake Biwa, the largest lake in Japan. The samples were wet, fibrous, cylindrical in shape and contained smaller proportions of urine than in non-waterfowl bird feces. The collection sites were: Yabase (35°00′24.9″N, 135°55′09.7″E to 35.00′05.5″N, 135°54′37.6″E in Yabase-cho and Shinhama-cho, Kusatsu City) and Kitayamada (35°01′45.3″N, 135°54′49.6″E to 35°01′38.1″N, 135°54′59.7″E in Kitayamada-cho, Kusatsu City) on east shore of the southern part of Lake Biwa, and Nagasawa (35°20′50.9″N, 135°16′38.9″E to 35°20′45.5″N, 136°16′37.6″E in Nagasawa, Maibara City) and Kurimishinden (35°12′11.6″N, 136°06′20.8″E to 35°12′05.8″N, 136°06′20.7″E in Kurimishinden-cho, Higashiomi City) on the east shore of the northern part of Lake Biwa.

We sieved the fecal samples using a 0.25 mm fine mesh while washing with tap water. The fecal samples were examined with a digital microscope (VHX-100, Keyence Corporation, Osaka, Japan). Seeds from the samples were confirmed to be *L. grandiflora* subsp. *hexapetala* based on shape by referring to our previous seed germination study. In this study, the seeds of *L. grandiflora* subsp. *hexapetala* from waterfowl feces were stored in the dark at 4 °C.

For seed pre-treatment, a plastic cup (8 cm diam. at bottom, 10 cm diam. at top and 4.4 cm tall) was half filled with leaf mold. A non-woven fabric bag containing the seeds was placed on the leaf mold. The cup was then filled with leaf mold until full. Tap water was added until the cup overflowed. The cup was closed with a lid with air vents on top and was covered with a double thickness of aluminum foil (under dark conditions) and stored at 25 °C for 40 days (from 2 August 2019 to 11 September 2019).

To test for germination, the pre-treated seeds were placed on paper towels wetted with tap water in petri dishes (9 cm diam. at bottom and 1.5cm tall) and covered with a lid. The dishes were then placed in an incubator (NK system LH-30-8CT; Nippon Medical & Chemical Instruments, Osaka, Japan) for 12 h at 25 °C under light and 12 h at 15 °C under dark conditions [light condition: 49.8 ± 21.8 microeinsteins $sec^{-1}m^2$ (average ± SD), as measured by an irradiance meter (QSL-100 BOX; Biospherical Instruments, San Diego, CA, U.S.A.)] for 35 days (from 11 September 2019 to 16 October 2019).

Results

Among the seeds of *Ludwigia grandiflora* subsp. *hexapetala* obtained from waterfowl feces collected in the southern part of Lake Biwa (Yabase and Kitayamada), four (Yabase) germinated (Fig. 1). The four germinated seeds varied in appearance. Seed A nearly maintained its original shape, whereas seeds C and D exhibited damage. Seed B largely maintained its original shape with minor damage. Its condition was intermediate between seed A and seeds C and D in the shoot angle shown in Fig. 1.

Discussion

Seeds that disperse by exozoochory have hooks, barbs, bristles, spines or glue for attaching to animals (Willson 1983). Seeds that disperse by endozoochory are enclosed in or attached to edible portions, which serve as a reward for animals (Willson 1983). Waterfowl are important vectors for dispersal, but have been overlooked as vectors for alien and invasive aquatic plants (Reynolds *et al.* 2015), as the aquatic seeds lack obvious morphological adaptations for zoochory by birds (*e.g.*, Brochet *et al.* 2009, Reynolds *et al.* 2015). Endozoochory by ducks is considered much more important for the spread of aquatic plants than exozoochory (Brochet *et al.* 2010). In the case of submerged plants, Clausen *et al.* (2002) reported that the most obvious seed dispersal candidates either take seeds directly from the plant body or through filter-feeding of bottom substrates. Further, unintentional ingestion of seeds or fruits occurs through ingestion of the plant body (Clausen *et al.* 2002, Tanaka 2012).

The seed structure of species of section *Jussiaea* may affect the efficiency of endozoochory in waterfowl. In a feeding experiment using seeds of *Ludwigia grandiflora*, García-Álvarez *et al.* (2015) noted that large, hard seeds had the longest

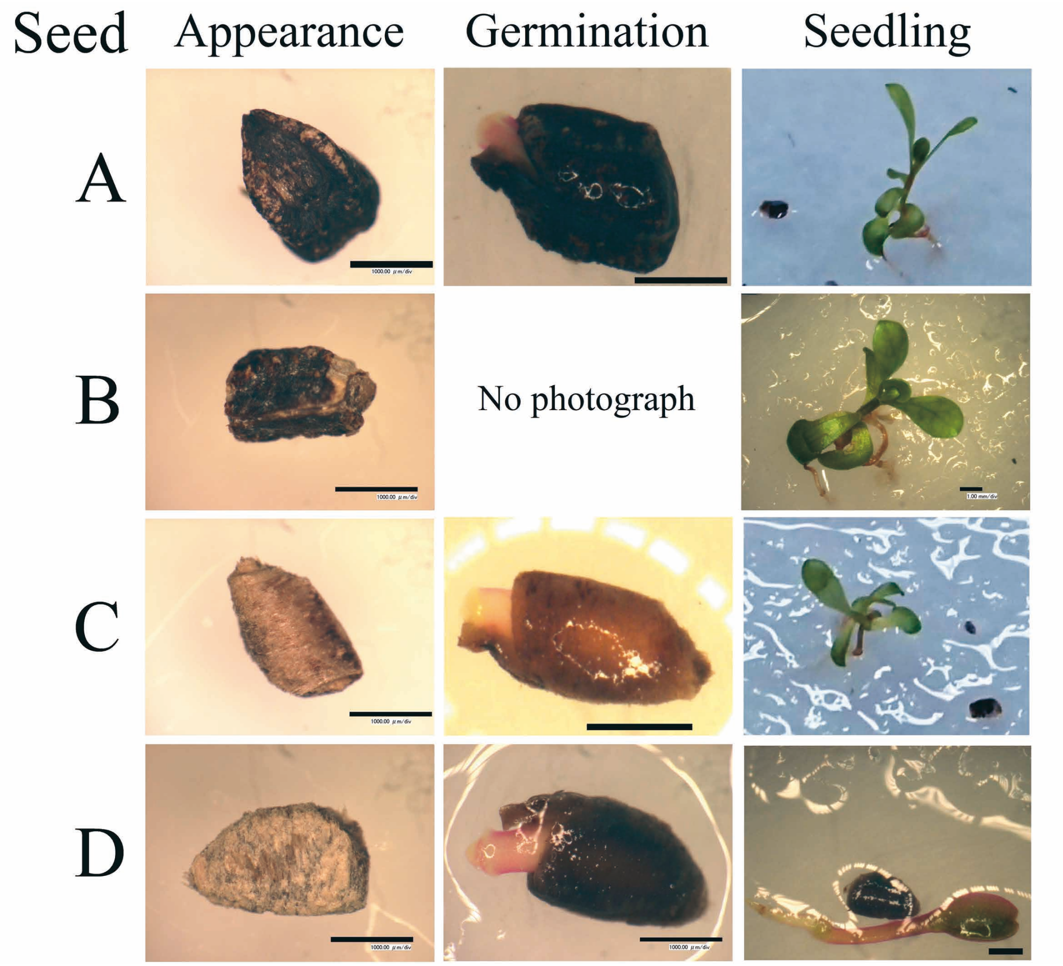

FIG. 1. Four germinable seeds of *Ludwigia grandiflora* subsp. *hexapetala* obtained from waterfowl feces collected in the Yabase on the east shore of the southern part of Lake Biwa, Japan. From top to bottom: germinable seeds (**A**, **B**, **C** & **D**); from left to right: seed appearance, seed germination, and seedling. We could not obtain a photograph depicting seed B germination. Bar: 1 mm, except for seedlings of seeds A and C.

retention time. Seeds and/or fruit structure of *Ludwigia peploides* (a species similar to *L. grandiflora* in the same section *Jussiaea*) (Hoch *et al.* 2015), such as the endocarp fibers, isodiametric endocarp cells (Eyde 1978), and exceptionally lignified and thick-walled cells of the exotesta (Tobe & Raven 1986), may contribute to seed survivability and germinability despite passage through the digestive tract. While the terminology for the structures differs between endocarp (Eyde 1978) and exotesta (Tobe & Raven 1986), both studies suggest that the structure on the seeds and/or fruits of *L. peploides* is tough.

New populations of *L. grandiflora* subsp. *hexapetala* have been recorded in Lake Biwa and its vicinity. The processes of invasion are not always accounted for by hydrochory, as satellite lakes are not freely connected to Lake Biwa, inland waterways and isolated ponds in the vicinity of Lake Biwa (*e.g.*, Council for the Management of Invasive Alien Aquatic Plants in Lake Biwa 2019, 2020, Nakai 2021). The results of our study suggest that invasions may have been derived by endozoochory via waterfowl. We have not yet identified which species of waterfowl is the dispersal vector through endozoochory. In Lake Biwa and surrounding areas, waterfowl contribute to the dispersal of various plant species through endozoochory (*e.g.*, Yabuuchi & Hamabata 2016). It is necessary to be vigilant and identify sudden invasions of *Ludwigia grandiflora* subsp. *hexapetala* in non-invaded areas and waterfowls that could be dispersal vectors.

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