

Laundry washing increases dispersal efficiency of cloth-dispersed propagules

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Academic editor: G. Karrer | Received 28 April 2020 | Accepted 17 August 2020 | Published 2 October 2020

Citation: Valkó O, Lukács K, Deák B, Kiss R, Migléc T, Tóth K, Tóth Á, Godó L, Radócz S, Sonkoly J, Kelemen A, Tóthmérész B (2020) Laundry washing increases dispersal efficiency of cloth-dispersed propagules. *NeoBiota* 61: 1–16. <https://doi.org/10.3897/neobiota.61.53730>

Abstract

Due to increased human mobility, cloth-dispersed propagules can be transported over long distances, which would not have been bridged otherwise. We studied a potentially important component of human-mediated seed dispersal by assessing the effects of laundry washing on the dispersed propagules. We studied the germination of 18 species, which have morphological adaptations for epizoochory and are commonly dispersed by people. We tested six treatments (washing with water, soap nut or detergent, at 30 °C or 60 °C) compared to an untreated control. Washing intensity was the most significant factor affecting germination. Washing at 30 °C was neutral for 14 species, suppressed one species and supported three species. Washing at 60 °C decreased seedling numbers of half of the studied species. The intensive washing treatments at 60 °C significantly decreased the synchrony of germination. We showed that people are not purely transporting propagules from one location to another, but via the laundry cycle, we can also influence the fate of the transported propagules by affecting germination potential, seedling fitness and germination dynamics. These results have new implications for understanding the early stages of biological invasions and call for improved biosecurity measures in nature reserves subjected to a growing pressure of tourism.

Keywords

epizoochory, germination, human-mediated plant dispersal, invasive species, seed dispersal, seedling establishment, seed retention, synchrony

Introduction

Increasing human population, mobility and globalisation make humans a highly effective dispersal vector of plant propagules (Bullock et al. 2019). Human-mediated seed dispersal is amongst the most important ways of long-distance plant dispersal in modern times (Nathan 2006; Ansong and Pickering 2014). People play an increasingly important role as dispersal vectors by unintentionally transporting propagules on vehicles (Pickering and Mount 2010; Ansong and Pickering 2013a) and clothes (Ansong and Pickering 2014). Propagules can travel extremely long distances, up to hundreds of kilometres while attached to cars (Taylor et al. 2012). The potential dispersal distances are supposed to be shorter in case of clothing-dispersal (couple of kilometres; Auffret and Cousins 2013; Ansong et al. 2015). However, if clothing-dispersal is combined with transport by vehicles, propagules can travel considerably longer distances. Clothing-dispersal has been documented for approximately 450 plant species so far and, presumably with future studies, this number is certainly going to increase strikingly (Ansong and Pickering 2014).

Morphological adaptations for epizoochory, such as hooks, awns, hairs or glabrous surface (Römermann et al. 2005; Hintze et al. 2013) make propagules especially capable for clothing-dispersal, a modern analogue of the classical epizoochory on mammals' fur. The most striking ecological consequence of clothing-dispersal compared to epizoochory, is that it can connect habitats with completely different species pools, such as isolated mountain ranges, islands with the mainland or biogeographical regions which otherwise would not have any biological connections. This implies that some of the propagules arrive at an environment which is unsuitable for their establishment, but others can establish in novel habitats which can be the first step of biological invasions. Suitability of novel habitats depends on a range of factors. For instance, climatic changes and the heat island effect in urban habitats (Shochat et al. 2006) make cities in the temperate region especially suitable for the establishment of thermophilous species (Rysiak and Czarnecka 2018). This process is further aggravated by the increasing global mobility of people, especially for those living in cities (Glaesser et al. 2017). The vast majority of clothing-dispersed propagules belong to species considered as weeds and it is plausible that clothing-dispersal played an important role in the transport of 43% of the invasive species in the United States (Ansong and Pickering 2014). Clothing-dispersal is of an increasing concern in nature reserves which are receiving growing pressures from tourism (Pickering et al. 2011), as tourists can be very effective dispersal vectors of propagules of alien species (Ansong and Pickering 2013b).

There are many open questions regarding the fate of human-dispersed propagules, especially regarding their establishment prospects. It is still a question how the mechanical and chemical effects to which propagules are exposed during human-mediated dispersal, affect their germination potential and establishment. One of the most drastic events that can happen to a clothing-dispersed propagule is laundry washing. Everyday observations of field biologists, hikers and people participating in outdoor sports show that propagules attached to clothing often end up in washing machine. In a questionnaire survey, Ansong and Pickering (2013) found that approximately 15% of people visiting an Australian nature reserve put their clothes in the laundry without removing the propagules. Even though many people are willing to remove seeds and fruits from their clothes, there are some small propagules, especially in safe microsites, such as pockets, shirt-sleeves or inside socks which are not noticed and hence not removed before washing. Huiskes et al. (2014) studied propagules on the clothes of people visiting Antarctica. They found a considerable amount of propagules on the clothes and the interviewed people were using their clothes in other ecosystems before visiting Antarctica. This is an indirect evidence for the possibility of washed propagules to be introduced into new locations.

Lefcort and Lefcort (2014) reported on the effect of laundry washing on cheatgrass (*Bromus tectorum*). They hypothesised that laundry washing affects the water potential of the propagules which might result in altered germination and establishment rates. They observed no effect of washing on the seedling number and seedling height of the washed seeds; however, they found that addition of bleach significantly decreased seedling heights.

To study the effect of laundry washing on a large set of species, here we tested the germination potential (seedling number), seedling fitness (approximated by seedling biomass) and dynamics (germination time, start of germination, synchrony) of the propagules washed in laundry compared to unwashed seeds. We applied two washing intensities and three types of washing medium to test whether these circumstances have an effect of the germination rates. Washing intensity can have different mechanical and heat effects on the propagules and the presence/absence of toxic compounds (surfactants, brighteners) in detergents can affect their survival (see also Lefcort and Lefcort 2014). Previous studies have shown the potential of bleaching for breaking the dormancy of seeds of grass (Hsiao 1979) and legume species (Okonkwo and Nwoke 1975). Besides studying seedling numbers, we also used seedling biomass as a proxy for seedling fitness (Sonkoly et al. 2020). We used three variables, i.e. germination time, start of germination and germination synchrony to describe germination dynamics, since all of them can be relevant in the establishment of plants in a novel environment; therefore, they can be important factors in an invasion process. Rapid and synchronised germination might be a good strategy to mitigate the effects of interspecific competition (Fenesi et al. 2014), but can be risky in a novel and unpredictable environment (Gioria and Pyšek 2016). The review of Gioria and Pyšek (2016) showed that early germination is more widespread in invasive plants compared to their non-invasive congeners and they assume that the strategy of early occupation of empty niches can

be highly effective in a novel environment. Early germination is a major advantage as it increases seedling growth and fecundity (Verdú and Traveset 2005).

Methods

Studied species

We selected 18 species for the experiments, all species having the ability for epizoochorous and clothing-dispersal and are widespread in Central-Europe (Table 1). The native and non-native ranges of the species are indicated in Table 1. Fourteen of the studied species have already been introduced to continents outside their native range. The propagules were collected in Hungary in 2017 from at least 30 plant individuals per species. The propagules of the tested species were different types of fruits; we call them for convenience ‘propagules’ hereafter. In Table 1, the morphological units used for the germination experiments and experiments on retention potential are given for each species.

Germination after laundry washing

During the experiments, we applied combinations of washing intensity (30 °C or 60 °C) and washing medium (water, soap nuts or detergent) as follows: (i) unwashed control and washing with (ii) water at 30 °C, (iii) soap nuts at 30 °C, (iv) detergent at 30 °C, (v) water at 60 °C, (vi) soap nuts at 60 °C, (vii) detergent at 60 °C. Washing intensity had two levels: the ‘extensive washing’ treatment was at 30 °C and lasted for 40 minutes; the ‘intensive washing’ temperature was 60 °C and the treatment lasted for 185 minutes. Washing medium had three levels: water, soap nuts (four nuts of *Sapindus mukorossi*, representing an eco-friendly alternative) and detergent (66 ml of Ariel Colour fluid detergent).

We tested germination of five replicates of 25 propagules per treatment for 17 species and three replicates of 25 propagules for *Hordeum murinum*. We put the sets of 25 propagules in small fabric sacks (Suppl. material 1: Fig. S1), sewed each sack and appended them with a string to prevent propagules escaping. In total, we had 528 sacks. Each treatment (88 sacks) was washed in a separate laundry cycle in September 2017.

After washing, propagules were germinated in an unheated greenhouse under natural light conditions. We put the propagules from each sack (25 seeds), as well as the unwashed control propagules in pots (8 cm × 8 cm × 12 cm) filled with potting soil. We watered the pots daily with 5 ml tap water. We counted all the emerged seedlings on every fourth day (monitoring days). We terminated the germination for each species when more than 95% of the propagules germinated in at least one treatment or when we did not detect new seedlings for more than 5 monitoring days. When terminating the germination, we removed all individuals and measured the total dry aboveground biomass (referred to as ‘seedling biomass’) and recorded the total number of germinated individuals per pot.

Table 1. Characteristics of the studied 18 species. Native and non-native ranges are given based on the CABI Invasive Species Compendium (<https://www.cabi.org/isc/>) and the EPPO Global Database (<https://www.gd.eppo.in>).

| Species | Life form | Morphological adaptation for epizoochory | Morphological unit tested | | Native range | Non-native range |
|-------------------------------|-----------------|---|---------------------------|-------------------------------|----------------------|-------------------------------|
| | | | germination experiment | experiments on retention rate | | |
| <i>Agrimonia eupatoria</i> | perennial forb | fruit surface hairy with hooks | fruit | fruit | Eurasia | N-America, Oceania |
| <i>Arctium lappa</i> | perennial forb | involucrum with many hooks | fruit | flower head at fruiting stage | Eurasia | N-America, Oceania |
| <i>Bromus sterilis</i> | annual grass | awned lemma with backward hairs as part of the dispersal unit | fruit | fruit | Eurasia | N-Africa, N-America, Oceania |
| <i>Bromus tectorum</i> | annual grass | awned lemma with backward hairs as part of the dispersal unit | fruit | fruit | Eurasia | N-America, S-America, Oceania |
| <i>Cenchrus spinifex</i> | annual grass | spiny bracts of infructescences | fruit | fruit | N-America, S-America | Europe, Oceania |
| <i>Chaerophyllum temulum</i> | biennial forb | fruit surface with smooth hairs | fruit | fruit | Eurasia, N-Africa | |
| <i>Cruciata pedemontana</i> | annual forb | hooked hairs on the stem, hooked pedicels | seed | – | Eurasia, N-Africa | N-America |
| <i>Cynoglossum officinale</i> | biennial forb | prickly-surfaced fruit | fruit | fruit | Eurasia | N-America |
| <i>Daucus carota</i> | biennial forb | hooked bristles on fruit | fruit | fruit | Eurasia | N-America, Oceania |
| <i>Geum urbanum</i> | perennial forb | fruit with one long hooky attachment | fruit | fruit | Eurasia | N-America, Oceania |
| <i>Hordeum hystrix</i> | annual grass | awned lemma | fruit | fruit | Eurasia | N-America, S-America, Oceania |
| <i>Hordeum murinum</i> | annual grass | awned lemma | fruit | fruit | Eurasia | N-America, S-America, Oceania |
| <i>Melica transilvanica</i> | perennial grass | hairy diaspore | fruit | fruit | Eurasia | |
| <i>Physocaulis nodosus</i> | annual forb | fruit surface covered with very low hooks | fruit | fruit | Eurasia | |
| <i>Secale sylvestris</i> | annual grass | awned lemma | fruit | fruit | Eurasia | |
| <i>Setaria verticillata</i> | annual grass | bristles with backward barbs on panicle | fruit | fruit | Eurasia | N-America, S-America, Oceania |
| <i>Torilis arvensis</i> | annual forb | fruit surface covered with fine short hooks | fruit | fruit | Eurasia | Africa, N-America, S-America |
| <i>Tragus racemosus</i> | annual grass | upper glume with hooked spiny bristles | fruit | fruit | Eurasia, Africa | N-America, S-America |

The fate of propagules before and after washing

We tested the likelihood of (i) human-dispersed propagules entering the laundry cycle and (ii) that washed propagules are detached during clothes drying. For these experiments, we used three cloth/fabric types typically worn during outdoor activities: polar fleece sweater (fleece), jeans (denim) and cotton socks (cotton).

For estimating the likelihood of human-dispersed propagules entering the laundry cycle, five people put sets of dry propagules of 17 of the studied species (except for *Cruciata pedemontana*) on their clothing (sweater, jeans, socks) at 09:00 h. All persons put 25 prop-

agules per species on each cloth type; each species was tested on a separate day in the autumn 2017. All persons continued their normal daily activities at the university including mainly indoor, but also several outdoor activities (short walks between buildings). We counted the number of propagules on the three fabric types in every hour until 17:00 h.

We tested the fate of the propagules after washing at 30 °C and 60 °C. We used five replicates of 25 propagules per species and fabric type (fleece, denim, cotton). We cut 6 cm × 6 cm pieces from the three fabrics and attached 25 propagules of one species on one piece (90 pieces per fabric type and washing temperature, in total 540 pieces). In total, we had six separate laundry cycles (three fabric types at 30 °C and 60 °C). Directly after washing, propagules were counted on each fabric piece. We determined the proportion of propagules that (i) remained attached to the original fabric piece, (ii) became attached to another fabric piece and (iii) lost during the laundry washing (remained in the washing machine or passed to the sewerage system; Suppl. material 1: Table S1). After counting the propagules that remained attached on the fabrics after washing (i+ii), fabrics were hung on an indoor washing line (Suppl. material 1: Fig. S1). Fabrics were left for drying for 8 hours. We modelled outdoor drying conditions (e.g. wind) by using a fan. Finally, we counted the propagules that remained attached on the fabrics after drying. In the analyses, we used the ratio of propagules that have been retained on the fabric pieces after drying to the propagules that have been retained on the fabrics after washing.

Statistical analyses

In the analyses, we used dependent variables related to the fitness (seedling number and biomass) and phenology (mean germination time, start of germination, synchrony) of the germinated seedlings. Seedling number was the number of germinated seedlings per pot. Biomass referred to the dry biomass of the germinated seedlings per pot and was used as a proxy of seedling fitness (Sonkoly et al. 2020). Mean germination time (days) was calculated for the seedlings of each pot. Start of germination referred to the first day when a seed germinated in a pot. Germination synchrony was expressed by the Shannon diversity of germination dates of seeds per pot. Zero refers to completely synchronised germination (all seeds germinated at the same date); higher values refer to less synchronised germination.

We tested the effect of ‘Species identity’, ‘Washing intensity’, ‘Washing medium’ and their interactions (fixed factors) on the Relative Response Index (RRI, Armas et al. 2004), calculated for each of the above-listed dependent variables with generalised linear models (GLMs) in SPSS 22.0. RRI shows the effects of the washing treatments compared to the unwashed control and was calculated as follows:

$$\text{RRI} = (\text{DV}_w - \text{DV}_c) / (\text{DV}_w + \text{DV}_c),$$

where DV_w and DV_c are the scores of a dependent variable (DV) in a particular washing treatment (DV_w) and in the control (DV_c), respectively. RRI ranges between -1 and $+1$, zero means that the control and the treatment are not different. In the GLM

models, we accounted for normal distribution. The values of the dependent variables, i.e. RRI, calculated for seedling number, seedling biomass, germination time, germination synchrony and start of germination, were log-transformed to approximate them to normal distribution.

We used GLMs for testing which factors influence the retention rate of dry and washed propagules. We tested the effect of 'Species identity', 'Cloth type' and their interaction (fixed factors) on the retention rate of dry propagules after 8 hours (dependent variables). In the analysis of the retention rate of the washed propagules, fixed factors were 'Species identity', 'Cloth type', 'Washing intensity' and their interactions.

Results

Gentle washing at 30 °C did not affect the germination potential of fourteen species, suppressed one and was beneficial for three species (Table 2, Fig. 1, Suppl. material 1: Fig. S2). Intense washing at 60 °C decreased the seedling number of nine and the biomass of ten species (Table 2, Fig. 1, Suppl. material 1: Fig. S2). Washing medium had no effect on germination potential (Table 2).

When exploring the temporal dynamics, we found that the start of germination, mean germination time (MGT) and synchrony were all affected by washing intensity

Table 2. The results of generalised linear models (GLM) fitted on (A) the relative response index (RRI) calculated for the germination characteristics after washing, (B) the attachment rates of propagules on dry clothes and (C) the attachment rate of washed propagules on washed and dried clothes. Significant effects are marked with boldface.

| <i>(A) Seed germination characteristics after washing</i> | | | | | | | | | | | | |
|---|--------------|--------------|--------------|--------------|----------------------|----------|------------------------|--------------|------------------------|----------|---------------------------|----------|
| | Species | | W. intensity | | W. medium | | Species × W. intensity | | Species × W. medium | | W. intensity × W. medium | |
| | F | <i>p</i> | F | <i>p</i> | F | <i>p</i> | F | <i>p</i> | F | <i>p</i> | F | <i>p</i> |
| Seedling number | 3.63 | 0.000 | 32.66 | 0.000 | 0.38 | 0.682 | 8.35 | 0.000 | 0.82 | 0.000 | 0.53 | 0.592 |
| Seedling biomass | 3.39 | 0.000 | 60.31 | 0.000 | 0.27 | 0.765 | 8.47 | 0.000 | 0.90 | 0.610 | 0.62 | 0.541 |
| Mean germination time | 2.66 | 0.002 | 3.64 | 0.057 | 0.13 | 0.880 | 5.30 | 0.000 | 0.33 | 0.999 | 0.18 | 0.839 |
| Germination synchrony | 1.99 | 0.024 | 2.31 | 0.129 | 0.14 | 0.869 | 3.14 | 0.000 | 0.65 | 0.900 | 0.01 | 0.997 |
| Start of germination | 13.60 | 0.000 | 8.84 | 0.003 | 0.29 | 0.747 | 4.53 | 0.000 | 1.22 | 0.189 | 0.01 | 0.994 |
| <i>(B) Attachment rate on dry clothes – Possibility for entering the laundry cycle</i> | | | | | | | | | | | | |
| | Species | | Cloth type | | Species × Cloth type | | | | | | | |
| | F | <i>p</i> | F | <i>p</i> | F | <i>p</i> | | | | | | |
| Seed attachment rate | 17.79 | 0.000 | 10.97 | 0.000 | 1.21 | 0.223 | | | | | | |
| <i>(C) Attachment rates on washed and dried clothes – Possibility for dispersal after washing</i> | | | | | | | | | | | | |
| | Species | | Cloth type | | W. intensity | | Species × Cloth type | | Species × W. intensity | | W. intensity × Cloth type | |
| | F | <i>p</i> | F | <i>p</i> | F | <i>p</i> | F | <i>p</i> | F | <i>p</i> | F | <i>p</i> |
| Seed attachment rate | 41.20 | 0.000 | 50.58 | 0.000 | 0.02 | 0.882 | 3.39 | 0.000 | 0.97 | 0.476 | 0.12 | 0.890 |

In part A, fixed factors were 'Species' (DF₁ = 12; DF₂ = 336), 'Washing intensity' (DF₁ = 1; DF₂ = 336), 'Washing medium' (DF₁ = 2; DF₂ = 336), the interaction of 'Species × Washing intensity' (DF₁ = 12; DF₂ = 336), the interaction of 'Species × Washing medium' (DF₁ = 24; DF₂ = 336) and the interaction of 'Washing intensity × Washing medium' (DF₁ = 2; DF₂ = 336). In part B, fixed factors were 'Species' (DF₁ = 12; DF₂ = 336), 'Cloth type' (DF₁ = 2; DF₂ = 144) and the interaction of 'Species × Cloth type' (DF₁ = 22; DF₂ = 144). In part C, fixed factors were 'Species' (DF₁ = 12; DF₂ = 620), 'Cloth type' (DF₁ = 2; DF₂ = 620), 'Washing intensity' (DF₁ = 1; DF₂ = 620), the interaction of 'Species × Cloth type' (DF₁ = 24; DF₂ = 620), the interaction of 'Species × Washing intensity' (DF₁ = 12; DF₂ = 620) and the interaction of 'Washing intensity × Cloth type' (DF₁ = 12; DF₂ = 336).

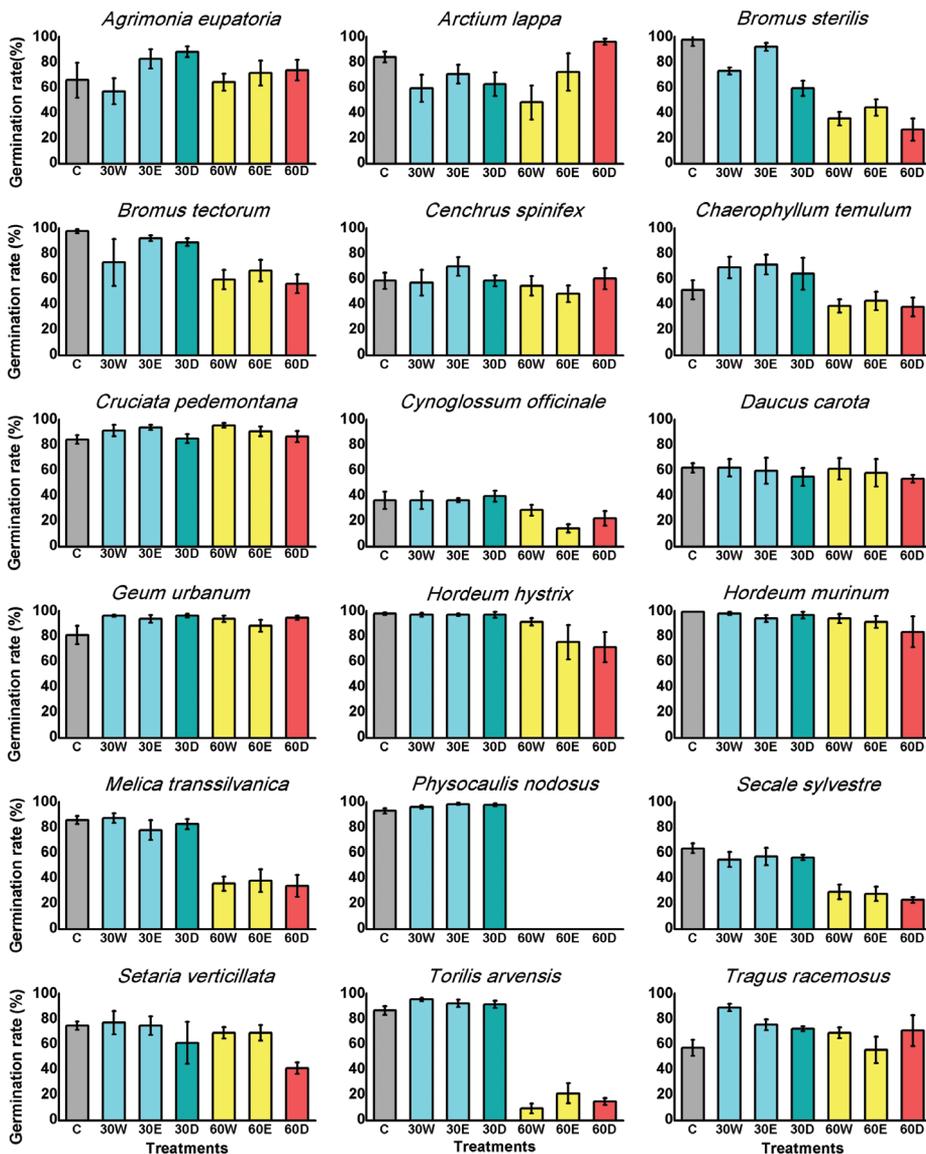


Figure 1. Germination rate (% mean \pm SE) in the control and the six washing treatments. Notations: grey column, C – control; blue columns: gentle washing at 30 °C (30W – washing with water at 30 °C, 30E – washing with eco-friendly soap nut at 30 °C and 30D – washing with detergent at 30 °C); yellow and orange columns – intense washing at 60 °C (60W – washing with water at 60 °C, 60E – washing with eco-friendly soap nut at 60 °C and 60D – washing with detergent at 60 °C). Germination rate is expressed as the percentage of sown propagules that germinated in a treatment (25 propagules were sown in five replicates per treatment except for *H. murinum*, where 25 propagules were sown in three replicates per treatment).

and species identity (Table 2). Washing at 60 °C significantly increased MGT of eight species (Suppl. material 1: Fig. S3) and desynchronised the germination of eight species (Fig. 2). The intensive washing treatments induced earlier germination for three species and later germination for four species (Suppl. material 1: Fig. S4).

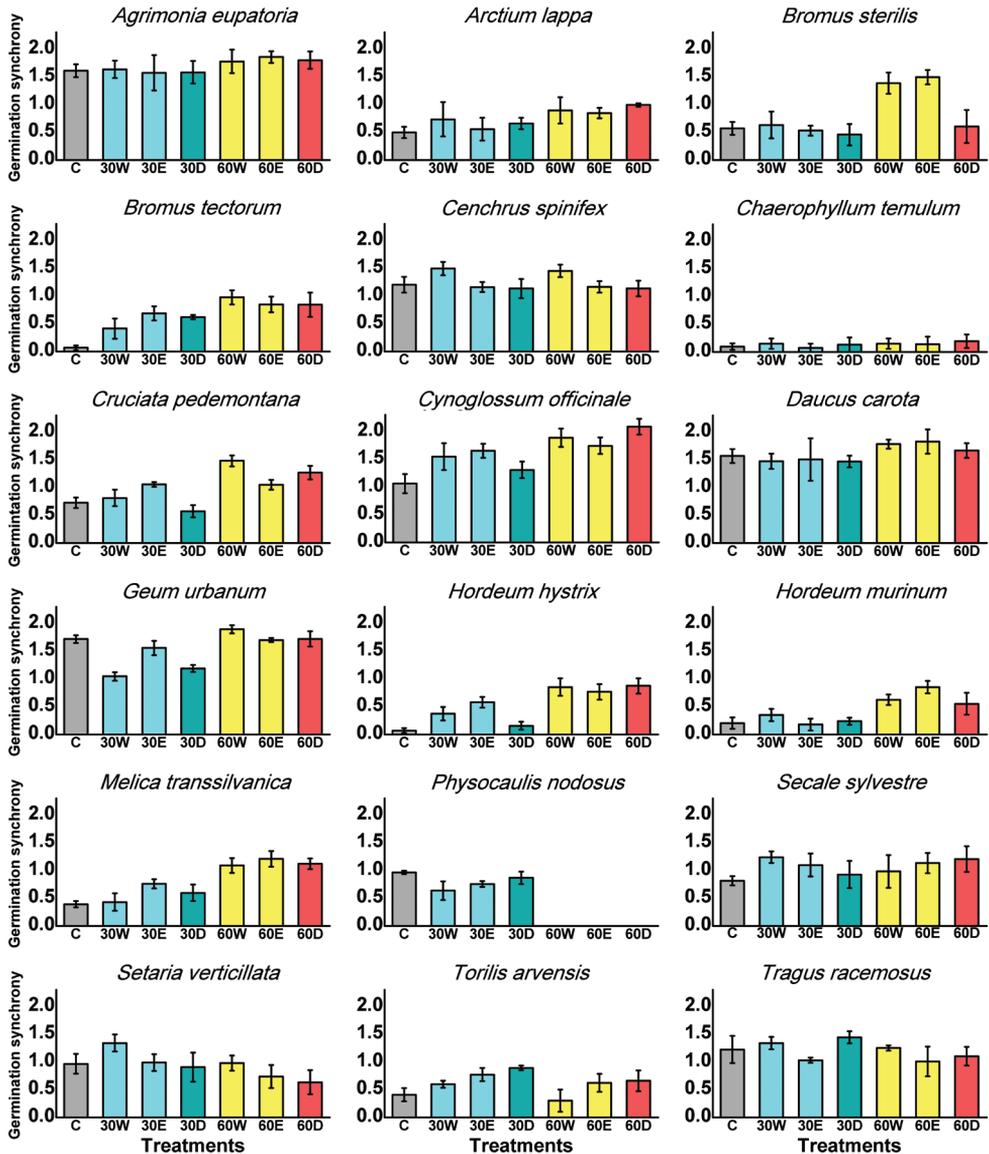


Figure 2. Germination synchrony, expressed as the Shannon diversity of the number of seedlings germinated in certain observation dates (mean \pm SE) in the control and in the six washing treatments (5 replicates of 25 seeds were sown per species and treatment). Notations: grey column, C – control; blue columns: gentle washing at 30 °C (30W – washing with water at 30 °C, 30E – washing with eco-friendly soap nut at 30 °C and 30D – washing with detergent at 30 °C); yellow and orange columns – intense washing at 60 °C (60W – washing with water at 60 °C, 60E – washing with eco-friendly soap nut at 60 °C and 60D – washing with detergent at 60 °C).

After attaching dry propagules on clothes, we found that the lowest proportion of propagules (32.4%) remained attached on jeans (Suppl. material 1: Fig. S5). Average retention rates were 42.6% on the fleece sweater and 47.5% on cotton socks (Suppl. material 1: Fig. S5). We found that approximately one third of the propagules

remained attached on clothes after washing and the others were lost in the washing machine and might have entered the sewerage system (Suppl. material 1: Table S1). After drying, out of the fraction of propagules that remained attached on fabrics after washing, on average 95.2% remained attached on fleece, 54.2% on denim and 72.4% on cotton (Suppl. material 1: Fig. S6).

Discussion

We showed that laundry washing, by affecting seedling fitness, germination dynamics and potential dispersal distances, can enhance the dispersal of species outside their native range. We revealed that gentle washing at 30 °C was neutral or even favourable for the germination of the majority of the studied species. Intense washing at 60 °C was detrimental for half of the species. The most important factors mediating germination are probably related to the intensity of washing, i.e. the duration of water-logging, mechanical effects and heat effects. For separating the effects of the components of washing intensity (water-logging, mechanical effects and heat effects), further experiments, focussing on particular parameters of washing cycles would be needed. Our results suggest that, in general, the new trend for using lower washing temperatures to reduce energy consumption (Morgan et al. 2018) probably increases the ratio of viable propagules that leave the laundry cycle. We found that the washing medium had no effect on germination potential. In future studies, it would be interesting to test detergents with different enzymatic activities, which might affect the germination of washed propagules differentially.

We showed that intensive washing desynchronises the germination. Compared to the classical case of epizoochory on mammal's fur, here the dispersal process itself has direct effects on germination dynamics. These effects of laundry washing on germination dynamics have important consequences for establishment: elongated and desynchronised germination is especially advantageous in unstable environments characterised by frequent and unpredictable disturbances (Sales et al. 2013), although it is disadvantageous for establishment in stable or harsh environments (Giménez-Benavides and Milla 2013). If germination is desynchronised, there is a higher chance that at least some seeds will germinate under the most suitable conditions in a new environment (Verdú and Traveset 2005).

In our experiments, we tested the most typical scenario, when propagules are attached on clothes at the time the seeds are ripened (typically early autumn) and the clothes are washed right after that. To model the fate of propagules, we monitored the germination from early autumn until late spring, which includes the main germination period for Central-European plants. For twelve species already germinated in the autumn, washing did not have an effect on the start of the germination (see Suppl. material 1: Fig. S4). There were six species whose control seeds germinated only or mostly in spring. For *Daucus carota* and *Tragus racemosus*, we found that the washed propagules germinated significantly earlier than the control, which implies probably that the mechanical and chemical effects during washing could break the dormancy of these seeds (Hsiao 1979; Okonkwo and Nwoke 1975). However, understanding the physiological background behind the effect of washing treatments on dormancy needs further experimental testing.

We found that a considerable amount of propagules has the potential to enter the laundry cycle, especially in the case of cotton and fleece clothing. We found that washed propagules had even higher retention rates compared to dry ones; thus, laundry washing increases potential dispersal distances for a fraction of the propagules that remain attached even after washing. The retention rates of dry and washed propagules were influenced by species identity, being the longest for species with the most developed appendages. Species with the highest potential for zoochory are amongst the most successful invasives (Moravcová et al. 2015). Additionally, these are the ones that enter the laundry cycle with the highest chance and have the longest potential dispersal distances.

Our results suggest that there are two main directions of post-washing dispersal. (i) Propagules that are detached during drying of the clothes probably get into rural or urban environments or some of them do not get outside of the houses. As urban habitats often provide suitable conditions for the establishment of alien species, it is possible that some of the seeds will germinate and establish in urban habitats and it is also possible that some might become urban invaders (Richardson et al. 2000; Wichmann et al. 2009; Arredondo et al. 2018). Altered germination dynamics after washing can support the establishment of clothing-dispersed propagules in urban areas, which are often starting points of invasions to the peri-urban natural habitats (Chytrý et al. 2008). (ii) Those propagules that remain attached on clothes after drying have the potential for post-laundry long-distance dispersal. We showed that, after laundry washing and drying, there is a fraction of the washed propagules that attach better to the clothes than the dry ones (Suppl. material 1: Figs S5, S6). The transport of washed propagules on the clothes to natural ecosystems is a realistic threat if we consider that people wear their outdoor clothes primarily during their outdoor activities and therefore propagules have a high chance to be dispersed outdoors.

Globally, the largest mass invasion events are connected with transport by vehicles, construction of roads and buildings, international trade and agriculture (Liu et al. 2019); all these processes move a considerable amount of soil, plants and animals over large distances and contain a large number of viable propagules. Clothing-dispersal can also transport a large number of propagules from native ranges to new areas, if we consider the increasing size and mobility of the human population. However, the most important feature of clothing-dispersal is that it can also affect the relatively undisturbed nature reserves which are not exposed to the above-mentioned mass invasions caused by vehicles, construction works, trade or agriculture. Long-distance dispersal after laundry washing might be a major source of plant invasions in such reserves, hiking areas and other remote locations having a unique flora (see also Pickering and Hill 2007; Pickering and Mount 2010; Pickering et al. 2011). Mountains and islands harbour a considerable amount of the protected areas worldwide, but they are also under an increasing pressure by tourism (Pauchard et al. 2009). Geographical isolation and the harsh environmental conditions were able to prevent the spread of invasive species in these areas in the past, but due to the increased human pressure and climatic changes, these areas have recently become increasingly threatened by plant invasions (Pauchard et al. 2009). The dispersal mode described in our study can further aggravate this process and increase the vulnerability of these ecosystems to invasions.

We found that washing of clothing-dispersed propagules might increase the dispersal distances and also affect germination dynamics. In this way, laundry washing can support alien species in a new environment to overcome both propagule and establishment limitations, those factors that controlled their establishment in the past.

Visitors to nature reserves can be the most important dispersal vectors of propagules of non-native species which would otherwise have little chance for being transported there (Pickering et al. 2011). We draw attention to the fact that not only the plants growing along the visitors' actual routes represent a potential source of invasion, but also the whole 'trekking history' of visitors should be considered. This process is already demonstrated for footwear: Ware et al. (2012) estimated that tourists coming from arctic and alpine regions introduce approximately 270,000 propagules yearly into Svalbard on their shoes and the majority of the propagules belong to non-native species. In another study, they found that golf players can carry propagules of non-native species on their shoes to alpine environments in New-Zealand (McNeill et al. 2011). These all emphasise the importance of trekking history in human-mediated seed dispersal and our results suggest that biosecurity protocols should target not only footwear, but also tourists' clothing items. In island countries that face a huge risk of unintended introduction of plants and animals by passengers, such as in Australia (<https://www.agriculture.gov.au/biosecurity/avm/military/adf/adf-cleaning-instructions>) and New Zealand (<https://www.mpi.govt.nz/travel-and-recreation/arriving-in-new-zealand/items-to-declare/#types>), regulations on entering the country include the clearance from seeds of the worn clothes. However, these regulations usually do not apply to the clothes carried in the luggage and, in many countries, such biosecurity regulations have not yet been established. Additionally, these rules apply only to the crossing of national borders or other administrative boundaries, such as the Schengen zone of the European Union, implying that, within large countries or within the EU, seeds might be dispersed on passengers' clothes between distant biogeographical regions. For protecting the flora of nature reserves, the establishment of biosecurity regulations would also be necessary at the site level. Our study highlights the importance of personal responsibility for introducing exotic species to areas with high conservation value. Wearing clothes made of fabrics with low seed retention potential (e.g. linen or denim, Ansong and Pickering 2016) in nature reserves can effectively decrease retention rates and can be a good mitigation measure. It is also crucial not picking and leaving the attached propagules on natural sites.

Acknowledgements

The study was supported by NKFI FK 124404 grant. The support of NKFI KH 126476 (OV), NKFI K 116639 (BT), NKFI KH 126477 (BT), NKFI KH 130338 (BD), NKFI PD 124548 (TM) and NKFI PD 128302 (KT) is greatly acknowledged. OV, BD and AK were supported by the Bolyai János Research Scholarship of the Hungarian Academy of Sciences. OV, BD, KL, LG and AK were supported by the New National Excellence Program of the Hungarian Ministry of Human Capacities.

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Supplementary material I

Figs S1–S6 and Table S1

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Explanation note: **Fig. S1.** Photos about the experiments. **Fig. S2.** Seedling dry mass (g, mean \pm SE) in the control and the six washing treatments (5 replicates of 25 propagules were sown per species and treatment, except for *H. murinum*, where 3 replicates of 25 propagules were used). Notations: grey column, C – control; blue columns: gentle washing at 30 °C (30W – washing with water at 30 °C, 30E – washing with eco-friendly soap nut at 30 °C and 30D – washing with detergent at 30 °C); yellow and orange columns – intense washing at 60 °C (60W – washing with water at 60 °C, 60E – washing with eco-friendly soap nut at 60 °C and 60D – washing with detergent at 60 °C). **Fig. S3.** Mean germination time (day, mean \pm SE) in the control and the six washing treatments (5 replicates of 25 propagules were sown per species and treatment, except for *H. murinum*, where 3 replicates of 25 propagules were used). Notations: grey column, C – control; blue columns: gentle washing at 30 °C (30W – washing with water at 30 °C, 30E – washing with eco-friendly soap nut at 30 °C and 30D – washing with detergent at 30 °C); yel-

low and orange columns – intense washing at 60 °C (60W – washing with water at 60 °C, 60E – washing with eco-friendly soap nut at 60 °C and 60D – washing with detergent at 60 °C). **Fig. S4.** Start of germination (days after sowing, mean \pm SE) in the control and the six washing treatments (5 replicates of 25 propagules were sown per species and treatment, except for *H. murinum*, where 3 replicates of 25 propagules were used). Notations: grey column, C – control; blue columns: gentle washing at 30 °C (30W – washing with water at 30 °C, 30E – washing with eco-friendly soap nut at 30 °C and 30D – washing with detergent at 30 °C); yellow and orange columns – intense washing at 60 °C (60W – washing with water at 60 °C, 60E – washing with eco-friendly soap nut at 60 °C and 60D – washing with detergent at 60 °C). **Fig. S5.** Retention rate (% , mean \pm SE) of dry propagules of the studied species on three types of fabrics (blue jeans, cotton socks and fleece sweater) during a period of 8 hours. Notations: blue symbols – blue jeans, grey symbols – cotton socks, orange symbols – fleece sweater. **Fig. S6.** Retention rate (% , mean \pm SE) of propagules of the studied species washed at 30 °C and 60 °C on washed and dried fabrics of three types (denim, cotton and fleece). Species are listed in a decreasing order of mean retention rate. Notations: blue symbols – denim, grey symbols – cotton, orange symbols – fleece. Species names are abbreviated using the first letters of their genus and species names. Retention rate is calculated as the percentage of the propagules that remained attached on the fabrics after drying in relation to the propagules that remained attached after washing. **Table S1.** Fate of the propagules right after washing at 30 °C, on three fabric types, given as the proportion of propagules (%) remaining on the same fabric where it was originally attached ('same fabric'), moved to other fabric piece ('other fabric') and lost in the washing machine or into the sewerage system ('lost').

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Link: <https://doi.org/10.3897/neobiota.61.53730.suppl1>