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Large-scale assessment of artificially coated seeds for forest regeneration across Sweden

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Abstract

We report the results of two years' field performance of Scots pine (*Pinus sylvestris*) seedlings regenerated using artificially coated seeds. The coated seeds were used for regeneration on 12 clearcut sites, covering a 1000 km latitudinal gradient across Sweden. The coating was either combined with arginine-phosphate fertilizer (10 mg N per seed) or had no additions. Interactions with environmental variables associated with sites were also assessed. Coated seeds were deployed in May–June 2017 and surveyed in August–September of 2018 and 2019. After two years, the mean establishment rate of seedlings from coated seeds was 56 ± 4% across the 12 sites. The fertilizer addition did not affect survival, and the biomass response to fertilizer varied significantly between sites. Maximum precipitation and wind speed during the first six weeks after deployment were correlated with seedling survival, regardless of fertilization treatment. Establishment increased with increasing precipitation and decreased with increasing wind speed. This highlights the importance of initial weather conditions for the seeds' establishment. Our data suggest that Scots pine regeneration using coated seeds can be practiced in boreal forests, but also that the method is sensitive to the weather conditions at the time of deployment of the seeds.

Keywords Scots pine · Coated seeds · Forest regeneration · Seeding · SeedPAD · Boreal forest

Introduction

In boreal forests, new forest stands have long been artificially regenerated after harvest using nursery-grown conifer seedlings (Nilsson et al. 2010). Currently in Sweden, approximately 80% of the clearcut area, i.e. c. 160,000 hectares per year, is regenerated by this method (Bergquist et al. 2016). This method normally results in 70–80% survival

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of the planted seedlings over the first three years in the field, although it varies greatly with sites, tree species, methods of mechanical site preparation and protection against pests e.g., pine weevil (*Hylobius abietis*) (von Sydow 1997; Holmström et al. 2019; Sikström et al. 2020). However, the use of nursery-grown seedlings is very expensive due to high production costs, as well as the need for planting by hand in a standardized manner that has proved challenging to mechanize (Larsson and Danell 2001; Mattsson and Bergsten 2003; Karlsson and Nilsson 2005).

The development of alternative methods using seeds reduces cost and simplifies mechanization, but their use has been limited due to the generally low establishment success of bare seeds on clearcuts, combined with limited availability of seeds from orchards (Grossnickle and Ivetić 2017). It has, therefore, been of a great interest to forest managers to find ways to enhance the survival and establishment of seedlings following direct seeding. Microsite preparation methods like the use of pyramidal indentations (Winsa and Bergsten 1994; Wennström et al. 1999), covering seeds in substrate (Nilson and Hjältén 2003) and seedbed preparation (Oleskog and Sahlén 2000), have all been shown to increase seedling establishment rates following direct seeding. Such methods may, however, increase silvicultural costs as they require additional investments in site preparation. As an alternative, different techniques have been developed over the years that focus on better protection of seeds from external damage by herbivores, for example (Bellot et al. 2002; Barton et al. 2015; Ceccon et al. 2016) and to improve the germination conditions for seeds (Brofas and Varelides 2000; Becker 2001; Oliveira et al. 2012). A recent product that was developed specifically for use in Nordic boreal forests, LandPuck, features conifer seeds embedded in compressed peat (Wennström 2014). However, so far, large scale field trials to assess the outcomes of forest regeneration have been lacking.

Artificially coating seeds for the delivery of nutrients, protectants, and to ensure growth is a well-developed practice for agricultural crops and horticultural species (Pedrini et al. 2017; Rocha et al. 2019). Such coatings have recently been adapted to the needs of Scots pine (*Pinus sylvestris*) seeds, in particular for boreal forest regeneration. This involves coating seeds with a thin layer of vermiculite wrapped in polysaccharide foil. The foil, made from biodegradable sugars, dissolves quickly when in contact with water, thus attaching and immobilizing the coated seed. The vermiculite forms a cap that traps capillary water transported from the ground, keeping the seed within moist. Previous studies have highlighted the importance of water availability when establishing seedlings from coated seeds, as the success rate largely depends on sufficient moisture retention for the seed to germinate (Winsa 2016). One of the main benefits of this method is the reduced cost because it avoids expensive nursery pre-growing; in addition, deployment can be very speedy (4000–8000 coated seeds/day/planter, compared to 2000–3000 nursery seedlings/day/planter), which offers economic as well as logistic benefits.

One of the major goals in a coated seed-based forest regeneration system is to show a success rate comparable to that of nursery grown seedlings, due to the restricted supply of seeds from orchards. Key factors that determine seedlings' field performance are nutrition and root-shoot ratio of seedlings (Grossnickle 2012). Application of conventional fertilizer (ammonium-nitrate based) enhances the nutrition status of seedlings, but it can also reduce the root-to-shoot ratio (Axelsson and Axelsson 1986). Alternatively, the application of nitrogen fertilizer based on the amino acid arginine has been found to enhance the nutrition of seedlings, promote root development and increase subsequent seedling growth (Cambui et al. 2011; Gruffman et al. 2012; Lim et al. 2021). Thus, applying such a fertilizer directly to the coating may facilitate seedling establishment as well as enhancing growth rates.

In this study, we assess the performance of seeds with a coating including arginine-phosphate fertilizer on 12 clearcuts situated along a south-to-north gradient ranging from latitudes 59°N to 67°N in Sweden. For two consecutive years, we monitored establishment rates and the growth of seedlings grown from coated seeds at the 12 sites. We further examine the effects of site-associated variables to identify environmental factors influencing establishment success of the seedlings. We also tested the effects of some of these variables in a controlled laboratory setting.

Our aim was to examine geographically large-scale field performance of seedlings regenerated from coated seeds and assess whether the performance can be augmented by addition of organic nitrogen fertilizer. We hypothesized that (1) addition of the fertilizer enhances both establishment and early growth of seedlings from coated seeds, and (2) environmental factors affect the seedling establishment.

Materials and methods

The coated seeds were produced by Arevo and SweTree Technologies under the name SeedPAD (afterwards SP). SP consists of a single seed of Scots pine (*Pinus sylvestris*) covered in vermiculite and wrapped in polysaccharide foil with a diameter of 35 mm and thickness of 3.5 mm (Fig. 1). In contact with water, the polysaccharide dissolves, attaching the vermiculite to the underlying mineral layer, thus immobilizing and protecting the seed. The SPs used in the following experiment were either fertilized or unfertilized. Fertilized SPs included the commercial fertilizer arGrow in the coating of vermiculite material, i.e., the fertilizer adds 10 mg nitrogen in the chemical form of the amino acid arginine and 5.5 mg phosphorus as phosphate to each SP.

Both fertilized and unfertilized SPs were deployed in May–June of 2017 to 12 clearcut forest sites across Sweden between the latitudes of 59°N and 67°N (Fig. 2, Table 1). The sites were chosen because of their dry characteristics, locations where Scots pine is commonly planted. Considering the large latitudinal gradient, deployment time had to be adapted to local growing conditions. Therefore, southern sites were planted during May and northern sites during June after the snow melted and at the onset of growing season. Before deployment, mechanical site preparation was performed on all sites in the form of disc trenching. This is a standard scarification process which involves making long furrows and exposing the mineral soil underneath. The SPs were deployed on the exposed mineral soil and marked with sticks next to the deployed pads. Deployment, scarification and marking were performed by forest landowners, i.e., forest companies or private forest owners who were members of a private forest owners' association. Orchard seeds used in the SPs

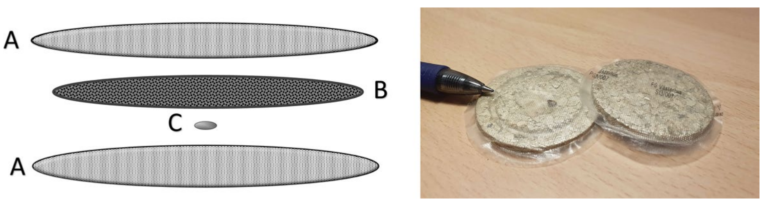

Fig. 1 Left**:** diagram of SeedPAD composition: A—polysaccharide foil, B—layer of vermiculite, C—*P. sylvestris* seed. Right: two unfertilized SeedPADs version 5.0, used since 2016. The pads are deployed with the seed underneath

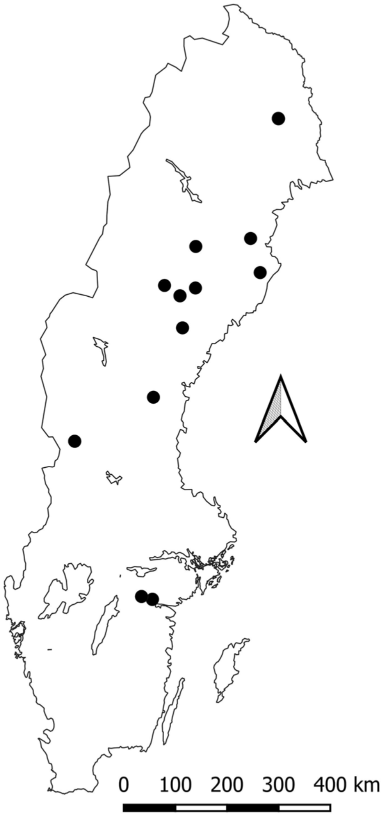

Fig. 2 Map of the 12 study sites between 59°N and 67°N in Sweden

were proprietary to each company and had an average of 98% germination capacity percentage. Non-research parts of clearcuts were regenerated with nursery-grown seedlings.

On each of the 12 regenerated clearcut sites, survey plots were established in the form of circles or rectangles (both 100 m^2) with the location completely randomized (Table 1). Plots were sufficiently away from clearcut edges (> ~ 20 m) to avoid edge effects and thus did not include a buffer zone. Within each of the circular survey plots either 35 fertilized or 35 unfertilized SPs were placed directly on the mineral soil that had been cleared of entire organic dominated layer by the process of soil scarification. The rectangular plots consisted of two parallel 50 m rows, one with 50 fertilized SPs and one with 50 unfertilized SPs deployed directly on the mineral soil. At the sites where both types of plots were established, we had enough buffer area between circular and rectangular plots, and therefore, we used both plot types as similar replicates at each site in the subsequent analyses.

Establishment rate and growth of the seedlings were recorded for each of the two regeneration methods in late August and September of 2018 and 2019. Seedlings were recorded as surviving when there was a live and vigorous seedling next to a marker stick. Growth was measured as distance from the ground to the top of the shoot. Scots pine seedlings growing within a radius of 10 cm around marker sticks were counted as originating from our SPs to account for the possible slight movement of seeds due to precipitation. Marker sticks with either dead (characterized as dried out with brown needles) or missing plants

Table 1 Description of the 12 experimental forest clearcut sites listed from north to south. Mean annual precipitation across the sites was in the range 500–700 mm (2010–2019). Sites with plot type "both" contained plots of both circular and rectangular type

Site name	Location (lat/long)	Elevation [m; a.s.l]	Growing season [days]	Topsoil composition	Plot type	No. of replicated plots	
						SeedPAD unfertilized	SeedPAD fertilized
Rissavägen	66.99/22.13	241	139	Silty till	Both	1	3
Nyback	64.95/20.44	143	170	Coarse till	Both	6	2
Bergsvik	64.88/18.17	268	149	Coarse till	Circular	2	
Backmyran	64.35/20.71	175	170	Coarse till	Both	5	7*
Varpsjovägen	64.22/16.84	364	145	Coarse till	Rectangular	4	4
Tallsjö	64.14/17.92	319	149	Moist till	Rectangular	5	3
Stormon	64.03/17.44	297	145	Till	Rectangular	6	2
Svanatjarn	63.47/17.49	319	146	Till	Both	4	2
Storulvsjö	62.28/16.3	418	146	Moist till	Rectangular	3	2
Nyhult	61.51/13.4	570	146	Sandy till	Circular		2
Lilla Malthult	58.8/15.77	69	194	Sandy till	Both	6*	2
Hostdagskär-ret	58.75/16.13	86	194	Podzol	Rectangular	6	2

*Due to a sampling error in the 2018 survey, three fertilized plots at Backmyran and two unfertilized plots at Lilla Malthut had to be excluded from the analysis

within the 10 cm radius were recoded as non-surviving. The establishment rate was estimated at the plot scale, as the number of live seedlings per the total number deployed SeedPADs. In addition, in 2019 five seedlings from each treatment plot within each clearcut were carefully excavated and taken for biomass estimation. These samples were dried for 24 h at 60 °C (to a constant weight) cut at the stem base and then the root and shoot parts weighed separately. Based on the harvested samples, we developed allometric equations for roots and shoots using height as a predict variable for each treatment at each site. In contrast to establishment rate, height was recorded on randomly chosen 20 seedlings across all plots for each treatment in each site. Thus, the fertilization effect within a site was not assessed on height or biomass due to non-plot-replication, therefore we only assessed the effect of fertilization across sites.

To assess the cross-site response of establishment rate to fertilization, we recorded environmental factors for each of the 12 sites; type of vegetation present on the clearcut according to National Inventory of Landscapes in Sweden—NILS (Ståhl et al. 2011) and topsoil composition, using visual assessment. Climate data were downloaded from the open database of the Swedish Meteorological and Hydrological Institute. We used data on daily precipitation, temperature, wind, topsoil type, and dates of the onset and end of the vegetation period (a daily mean air temperature $\geq$ 5 °C) from the nearest available climate monitoring station for each site (mean distance between sampling site and climate station was 32 km and the maximum distance was 46 km). Because we considered that weather conditions around the time of seed deployment and during the growing season after germination, are important factors, we extracted weather data for the first six weeks after deployment in 2017, and during the growing season in 2018 and 2019.

For all the statistical analyses we used R-Studio software (R Core Team 2019). We analyzed response of establishment rate, separate from height and biomass, because the sampling unit of the former is a replicated plot while the latter has no replication at a plot scale. For establishment rate, we assessed the response using a generalized linear model following binomial distribution with logit-link function. After finding no significant effect of the plot shape (circular or rectangular) or its interaction with site, we set fertilizer-treatment, site and their interaction as independent variables, and establishment rate as response variables. For this analysis we used the 10 sites with both fertilizer-treated plots and control plots at each site. Due to an unbalanced dataset (unequal numbers of observations for each treatment), we employed a type III ANOVA model using the car package in R, investigating the effect of fertilizer addition while considering the interaction with site. After concluding that there was no interaction, we employed type II ANOVA. Next, we examined the effects of site-specific weather variables on seedling survival in control plots and fertilized plots, using a generalized linear model across all 12 sites. In order to examine the causality, stepwise selection procedures were employed, based at a level of $\alpha < 0.05$, and the new model was checked with Akaike Information Criteria (AIC) against the previous one. When values of AIC differed by less than 2, the model with fewer degrees of freedom was selected. After several steps this results in a final model including only significant variables. For the response of height and biomass, we performed a type II ANOVA model, without interaction term between fertilization and site. The normality of all models' final residuals were checked visually by plotting them against predictions.

Since precipitation during the first six weeks following deployment of the SPs showed a significant positive effect on seedling establishment, we performed a follow up experiment in a controlled laboratory environment to investigate the relationship between water addition and SP attachment to the ground. The aim was to examine how much water was required for dissolution of the coating and to attach SPs to the ground. We used 135 SPs in total, separated into three different water addition rates combined with three different soil types. The soil used was collected in February 2020 from a forest clearcut site in mid-Sweden and dried in the lab, then sifted to give three different grain sizes. Water addition rates were determined based on local precipitation data averaged for all sites. The grain sizes were set to 1.7, 5.6 and 10.0 mm diameter for fine, medium, and coarse soil, respectively. SPs were placed on a pile of each soil type and room temperature water was dripped onto them using a pipette. At each step, 0.5 ml of water was dripped directly onto the SPs every 1.5, 3 and 5 min for fast, medium, and slow water addition rates, respectively. Dissolution was considered successful when the SP was firmly attached to the ground and could not easily be pushed sideways from the soil without breaking apart.

Results

Two years after forest regeneration, on average 54% of the deployed fertilized SeedPADs (SPs) and 58% of the unfertilized SPs had developed into established seedlings (Table 2). Notably, some seedlings had also established between the first and the second year following the SP deployment, i.e., the establishment rates had increased between the two survey years (Table 2). There was no significant difference in seedling establishment and survival between fertilized and unfertilized SPs for any of the years (Table 3). The effect of site on seedling establishment rate was significant in both years, i.e., it ranged between 87% (at Varpsjövägen) and 22% (at Bäckmyran) (Table 3).

Table 2 Average establishment rate, height and biomass of seedlings established from the SeedPADs. Surveys were performed in August–September 2018 and 2019. Estimated marginal mean values ± SE

	Years after deployment	SeedPAD unfertilized	SeedPAD fertilized
Establishment rate	1	53.4 ± 4.4	46.7 ± 4.9
Establishment rate	2	57.8 ± 4.3	53.7 ± 4.7
Height [cm]	1	6.5 ± 0.3	6.8 ± 0.3
Height [cm]	2	14.6 ± 0.9	14.6 ± 0.9
Total biomass [g]	2	6.5 ± 0.9	5.6 ± 0.9
Shoot biomass [g]	2	5.6 ± 0.8	4.9 ± 0.8
Root biomass [g]	2	0.9 ± 0.1	0.8 ± 0.1
Root: shoot ratio	2	0.2 ± 0.0	0.2 ± 0.0

Table 3 Results (F- and *p*-values) from model II ANOVA examining the effect of fertilization treatment (AP), site and their interaction on SeedPAD establishment rate, height and biomass. Significant effects ($p < 0.05$) are highlighted in bold

	Years after deployment	Treatment	Site	Interaction
Establishment rate	1	$F(1,46) = 2.085$, $p = 0.16$	$F(9,46) = 4.505$, $\boldsymbol{p < 0.001}$	$F(9,46) = 0.823$, $p = 0.60$
Establishment rate	2	$F(1,51) = 0.089$, $p = 0.77$	$F(9,51) = 4.155$, $\boldsymbol{p < 0.001}$	$F(9,51) = 1.169$, $p = 0.33$
Height	1	$F(1,9) = 0.295$, $p = 0.59$	$F(9,9) = 13.570$, $\boldsymbol{p < 0.001}$	NA
Height	2	$F(1,9) = 0.0001$, $p = 0.99$	$F(9,9) = 8.470$, $\boldsymbol{p = 0.002}$	NA
Total biomass	2	$F(1,9) = 0.429$, $p = 0.52$	$F(11,9) = 36.578$, $\boldsymbol{p < 0.001}$	NA
Shoot biomass	2	$F(1, 9) = 0.405$, $p = 0.54$	$F(11, 9) = 37.368$, $\boldsymbol{p < 0.001}$	NA
Root biomass	2	$F(1, 9) = 0.574$, $p = 0.46$	$F(11, 9) = 31.621$, $\boldsymbol{p < 0.001}$	NA

Analyzing the seedlings' growth across the 12 sites, the addition of fertilizer had no significant overall effect on seedling height, biomass or root-to-shoot ratio (Tables 2 and 3) whereas these growth variables varied significantly between the sites (Fig. S1).

To elucidate the factors responsible for the large spatial variation across sites, we considered site-specific weather variables, and found that maximum wind speed and maximum precipitation within the first six weeks after SP deployment had significant effects on the seedling establishment (Fig. 3). High windspeeds following SP deployment had a negative effect on seedling survival recorded at the time of our survey one year later (Fig. 3a). Large precipitation events following deployment, on the other hand, had a positive effect (Fig. 3b).

Our SP dissolution experiment, designed to test water requirements of SPs in a controlled laboratory setting, further highlighted the key role of precipitation for successful forest regeneration with SPs. The results showed two clear trends: increasing the speed

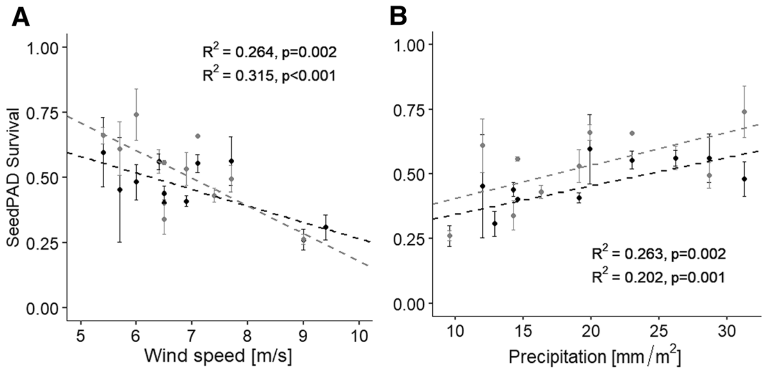

Fig. 3 Effect of maximum wind speed (**A**) and maximum precipitation on a single day (**B**) within six weeks of SeedPAD (SP) deployment in early summer 2017 in 12 clearcut forest sites on establishment of seedlings from unfertilized and fertilized SPs in late summer 2018. Black lines and circles denote fertilized SPs, while gray lines and circles denote unfertilized ones

of water addition resulted in dissolution with less water, while for soils with smaller grain size a smaller volume of water was sufficient for SP dissolution (Fig. 4). On average, SPs on coarse grained soil required 34% more water for dissolution than on fine grained soil. Similarly, the difference between water requirements of fast and slow water application within each soil treatment increased by 27%, 38% and 26% for fine, medium, and coarse soil, respectively.

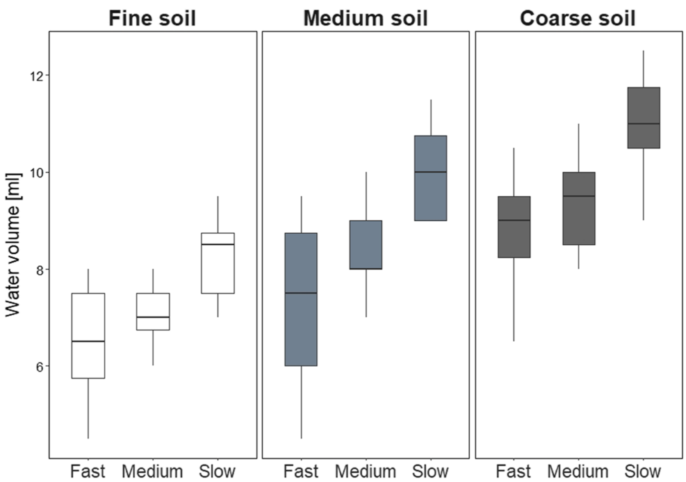

Fig. 4 Water volume required for dissolution of SeedPADs (SP), compared across three different soil textures and speeds of water addition. For soil treatments, grain sizes were set to 1.7, 5.6 and 10.0 mm diameter for fine, medium, and coarse soil, respectively. For speed of treatments, SPs had water dripped onto them every 1.5, 3 and 5 min for fast, medium, and slow treatments, respectively

Discussion

One regeneration goal when using the SeedPADs (SPs) is to achieve comparable results to planting nursery-grown seedlings. The average establishment rate of seedlings from SPs across sites was c. 56 ± 4.5%. Direct comparisons to other regeneration methods were not undertaken in this study, but other studies report establishment rates of conventional direct seeding at c. 20% (Grossnickle and Ivetić 2017), while establishment rates of nursery grown seedlings are reported at c. 76% (Holmström et al. 2019; Sikström et al. 2020). The SP method dramatically increased the seedling establishment rate compared to direct seeding, yet it was c. 35% lower than when planting nursery-grown seedlings. To overcome this deficit, one possible solution could be to deploy a higher number of SeedPADs per hectare. However, increasing seedling density to compensate for lower survival can lead to overstocking, as shown by Ilintsev et al. (2021), which may require additional early tending. Regarding SPs, this remains to be tested over a longer period e.g., following up on SP seedling performance several years into the future, which was beyond the scope of this study. However, it is reasonable to assume that SPs may be exposed to a higher risk of mortality over a longer period than nursery grown seedlings on a clear-cut, as their smaller size makes them more susceptible to damage (Johansson et al. 2015).

The variation in establishment rate in our study was largely explained by site-specific factors, i.e., precipitation and wind speed six weeks after SP deployment, indicating the importance of soil moisture for adhesion of SPs to soils. Indeed, our controlled lab experiment confirmed the importance of the timing and speed of water supply for SP adhesion (Fig. 4). Other factors that may have influenced establishment rates include damage by pine weevil, browsing damage by ungulates and seed predation by rodents and birds, as previously reported in other forest regeneration trials (Heikkilä and Härkönen 1996; Castro et al. 1999; Nilson and Hjältén 2003; Huggard and Arsenault 2009; Bergqvist et al. 2012). Visual inspection of the study plants during two survey seasons revealed no effect of pine weevil or browsing damage, but this may be due to the small size of the study plants. Seed predation, on the other hand, may have occurred prior to the first survey year and seedlings were thus recorded as non-surviving. However, the vermiculite layer may offer protection against seed predation since the seed is completely covered. This suggestion is supported by Nilsson and Hjältén (2003) who showed that covering seeds in substrate following direct seeding reduced seed predation, especially by bank voles (*Clethrionomys glareolus*).

The positive effect of precipitation on SP establishment indicates that initial local weather conditions after SP deployment are crucial for seedling establishment and further development. While it is recommended that *P. sylvestris* seedlings be planted in early summer (Luoranen and Rikala 2013), during this time, precipitation in northern Sweden is relatively low (average 53.1 mm in May–June, 2010–2019) (SMHI 2019). Therefore, at least in this area, we can expect the majority of seedlings from SPs to struggle with early germination. The importance of good contact between roots and available soil water is well documented for the first growing season (Burdett 1990; Grossnickle 2005), and a late start can lead to reduced growth and uneven stand development. To further validate this result, we conducted the dissolution experiment. Here we showed that SPs do, indeed, require a considerable amount of water to properly dissolve, attach and maintain moist conditions close to the seed. We found that water requirements for dissolution were lower on fine soils, owing to a closer contact between the pad with the soil. Further, faster water addition also reduced the total water requirement for dissolution, which confirms the benefit of intense precipitation, as shown by our analysis of field deployed SPs (Fig. 3). Note that our short-term laboratory experiment did not allow for comparable evaporation from the soil media as under clearcut conditions in the field and, therefore, SPs in situ may have required even more water to attach properly, due to evaporation. In relation to this, our model did, indeed, show wind speed as having a negative impact on establishment. The highest recorded wind speed was relatively low (9.6 m/s = 34.6 km/h) thus a reasonable explanation would be the drying effect of wind, as sites with the highest wind speeds were also driest, based on topsoil composition. In this sense, high wind speeds would slow down the process of attachment and germination through removal of moisture from the pads.

Fertilization of SeedPADs with arginine-phosphate had no significant effects on seedling establishment rates. This is in contrast to a recent study by Castro et al. 2021, performed at one of the sites included in the present study (Svanatjarn), who found a 50% increase in establishment rate after one growing season as a result of adding nitrogen-based fertilizer, either in the form of arginine phosphate or mineral ammonium nitrate. However, at this particular site we also recorded increased seedling establishment of arginine fertilized SPs, amounting to 14% after two growing seasons. The apparent site dependency of the arginine-phosphate effect on the seedlings from SPs highlights the interaction between fertilizer application and other environmental variables. Further, while nutrient availability is important for seedling growth, it may not be as important during the early germination process, when water availability is a more important factor. Other studies on the effects of arginine-phosphate additions on survival of nursery grown seedlings in the field have demonstrated both positive effects (Häggström et al. 2021) and no effects (Gruffman et al. 2012).

In comparison to other similar methods of alternative direct seeding (see review by Grossnickle and Ivetić 2017), SPs achieved comparable results to seed shelter methods, where authors report field survival of 54–70% in several species of chestnut four years after planting (Barton et al. 2015). However, a notable downside to seed shelters is the required involvement on planting, which can diminish the cost advantage of direct seeding over nursery seedlings. In this way, simply placing the SPs directly on the mineral soil may avoid such costly involvement while retaining the increased survival benefits.

In conclusion, using SeedPADs significantly augments the establishment of seeds and may have the potential to be used as an alternative method to nursery grown seedlings for forest regeneration, especially in areas with heavy precipitation events in early summer or when combined with manual watering directly after deployment. The method may benefit from further development in relation to SP dissolution and attachment to the ground, to ensure sufficiently moist conditions for successful seed germination and seedling establishment.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11056-022-09920-2.

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Author contributions AN, JÖ and MD contributed to the study conception and design. Material preparation and data collection were performed by MD and BH, analysis was undertaken by MD, BH and HL. The manuscript was written by MD with contributions from all other authors. All authors read and approved the final version of the manuscript.

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Availability of data and material The data that support the findings of this study are available from the corresponding author upon request.

Code availability Not applicable.

Declarations

Conflicts of interest The authors declare no conflict of interest.

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