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Cover Photo: Lodgepole pine in Steinadalur SE-Iceland
Photograper Ólafur Eggertsson

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CONTENTS

Editorial

The 2022 IAS issue includes six contributions on diverse topics ranging from forestry, plant diseases, and parasitology to organic fertilizers in horticulture.

Of these articles, three relate to forestry. The first is on individual-tree growth models for Sitka spruce developed in order to support multifunctional forest management and planning. The second is a short communication on a hot topic regarding natural regeneration of Lodgepole pine in Iceland. The results presented here suggests that with time, in addition to an increase in the average density of plants, the expansion of pine will continue.
The third, also a short communication, is on the pathogenicity of the blue stain fungus in Scots pine seedlings which does not seem to seriously stress the tree seedlings, when kept in favorable conditions.

Two articles relate to the animal sciences. These are firstly an article about absorption of colostral immunoglobulin G by Icelandic dairy calves. Compared to published data on foreign breeds, relatively low IgG serum concentration in Icelandic calves seems to be sufficient for the infection pressure in their environment. The second article on animal science is on how parasite composition of a raccoon, that was accidentally transported to Iceland, was used to prove the racoon´s American origin.

Finally, the issue contains an article on the interactions between nitrogen release from organic fertilizers and organic horticultural soils. This is an important topic in the search for useful nitrogen sources in organic horticulture in Iceland that have been missing.

Many important research topics lie ahead in many aspects of applied life sciences that are relevant under boreal, alpine, arctic or subarctic conditions which defines the scope of our journal. Hopefully, many scientists will see the benefit in sending their manuscripts to IAS, an open access journal that does not require payment for the publication of articles.

Björn Thorsteinsson
Editor in Chief

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Individual-tree growth models for Sitka spruce (*Picea sitchensis*) in Iceland

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ABSTRACT

Sitka spruce (*Picea sitchensis*) is one of the main tree species used in forestry in Iceland, growing well in all parts of the country. In view of the importance of Sitka spruce in Iceland, there is need for a reliable growth model that will support multifunctional forest management and planning. This study developed growth models for Sitka spruce using data from permanent sample plots established by the Icelandic Forest Service between 1970 and 2020. The measurement interval on the plots varied from 3 to 16 years. To deal with irregular measurement intervals, an optimization approach was used to recover models for annual diameter increment and survival rate. The aim was to find parameters for diameter increment and survival models that would minimize the difference between the measured and simulated diameter distributions of the plots at the end of the measurement interval (when the simulation begins from the diameter distribution at the beginning of the interval). To enable the simulation of stand development, models for dominant height development and tree height were also developed.

Keywords: Diameter increment, height model, individual-tree model, site index model, stand dynamics

YFIRLIT

Jöfnur sem lýsa vexti sitkagrenis (Picea sitchensis) á Íslandi

Sitkagreni (*Picea sitchensis*) er ein af þeim trjátegundum sem mest hafa verið notaðar í Íslenskri skógrækt enda vex tegundin vel víða um land. Vegna mikilvægi tegundarinnar er þörf á að aðlaga jöfnur sem lýsa vexti hennar svo hægt sé að áætla lotulengd og hvaða umhirðuaðgerðir skila mestum arði til skógareiganda. Vaxtarjöfnur voru aðlagaðar fyrir sitkagreni með gögnum frá föstum mæliflötum sem Skógræktin stofnaði til á árunum 1970 til 2013. Tíðni endurmælinga á mæliflötunum var mismunandi eða frá 3 og upp í 16 ár. Vegna óreglulegrar tíðni endurmælinga þurfti að beita bestunar nálgun (optimization approach) til að endurskapa eins árs þvermálsvöxt og sjálfgrisjun skóga. Markmiðið var að finna fasta fyrir þvermáls- og sjálfgrisjunar jöfnurnar sem lágmarka munin á milli mældrar og útreiknaðar þvermálsdreifingar trjáa í enda mælingartímabilsins. Auk þvermáls- og sjálfgrisjunarjafna var aðlöguð yfirhæðarjafna sem lýsa frjósemi viðkomandi skógar og jafna sem lýsir hæðarvexti stakra trjáa.

INTRODUCTION

Sitka spruce (*Picea sitchensis* (Bong.) Carr.) is one of the main tree species used in forestry in Iceland, growing well in all parts of the country and covering an area of around 5,000 hectares (Snorrason 2014). No growth models exist for Sitka spruce in Iceland, and scientific knowledge regarding its growth, yield and management is scant. The main reason for this is the young age of most Icelandic plantations. The oldest Sitka spruce forests were planted between 1940 and 1950. The size of these first plantations was usually smaller than one hectare. The small size of Icelandic plantations with a consequent edge effect and the small size of sample plots are common features of Icelandic tree growth data. Because of the young age of the forests, the longest measurement series is only 50 years and the oldest stands with permanent sample plots are less than 70 years old.

Forest growth models assist forest researchers and managers in many ways due to their ability to predict future yields in alternative management schedules. The importance of growth models is demonstrated by the large number and variability of models published and used (e.g., Hartig 1795, Wiedemann 1942, Clutter 1963, Newnham 1964, Pukkala 1987, Trasobares et al. 2004). The complexity of these approaches has varied from simple regression equations, expressing yield per hectare, to detailed equations that simulate the growth of single trees in a stand (Clutter et al. 1983).

Growth models can broadly be classified as stand-level or tree-level models. Stand-level models use stand variables (e.g. age, site index, basal area and number of trees per hectare) as predictors while at least some of the predictor variables in a tree-level model are individual tree characteristics (Clutter et al. 1983, Palahí et al. 2003, Weiskittel 2011). Stand-level models provide rather limited information about the forest stand, in some cases only stand volume (Vanclay 1994); they may also project the values of other stand variables such as basal area, mean diameter, height and number of trees per hectare (Mabvurira & Miina 2002). Tree-level models are further classified as distance-dependent (spatial) or distance-independent (non-spatial) models.

The first growth model for Iceland was developed by Heidarsson and Pukkala (2012) for Siberian larch. The model was a distance-independent individual-tree model. Distance-independent models do not use spatial information to express competition. Instead, they use predictors that describe stand density (for example, stand basal area) and thus express the overall competition in the stand (Vanclay 1994, Van Laar & Akca 1997).

Distance-dependent models include spatial competition indices as predictors. The competition index may differ from model to model, but it is usually a function of both the size and location of the subject tree in relation to the size and location of its competitors (Bella 1971, Ek & Monserud 1974, Pukkala 1988, 1989, Alegria & Tomé 2013). Distance-dependent models may provide more accurate information about individual tree growth than distance-independent models; however, they are more difficult to use because they require a map of the stand, which is often too costly in a routine forest management context (Munro 1974, Wimberly & Bare 1996). Moreover, distance-dependent models have sometimes shown little or no improvement over distance-independent models, especially in plantation forests (Munro 1974, Vanclay 1994, Dong et al. 2021).

When individual-tree information for a stand is available, tree-level models can be developed since they provide more detailed information on the stand structure and its dynamics than stand-level models (Mabvurira & Miina 2002, Palahí et al. 2003, Juma et al. 2014). The ideal data for the development of individual-tree models is repeated measurements of permanent sample plots, in which all trees have been numbered and measured at regular intervals for diameter and survival (Juma et al. 2014). However, this last requirement is not met in the permanent Sitka spruce sample plots in Iceland, where the measurement interval has varied between 3 and 16 years. The impact of irregular measurement intervals on modelling has received some attention in earlier research (Cao 2000, 2004, Nord-Larsen 2006, Crecente-Campo et al. 2010). Assuming a constant growth rate between measurements can lead to under- or over-estimation of tree growth when the growth dynamics are clearly nonlinear (Clutter 1963, McDill & Amateis 1992).

To deal with irregular measurement intervals in this research, an optimization approach originally suggested by Pukkala et al. (2011) and later used by de-Miguel et al. (2014), Juma et al.

(2014) and Jin et al. (2019) among others, was used to fit the diameter increment and survival models. This method seems to overcome some of the problems related to other methods and has been shown to produce similar models and model parameters to regression analysis (Pukkala et al. 2011).

The modelling approach used to fit diameter increment and survival models requires only the diameter distribution of plots at the beginning and end of the measurement interval. The method uses non-linear optimization to derive model parameters. Optimization seeks models than would minimize the difference between measured and simulated diameter distribution of the plot at the end of the measurement interval. The simulation begins from the measured diameter distribution at the beginning of the interval (Pukkala et al. 2011).

In view of the importance of Sitka spruce in Iceland, there is a need for a reliable system of growth models that would allow managers to predict harvests and future stand development in alternative treatment schedules, thus providing valuable support for silvicultural decision making. The plantations may also be aimed at carbon sequestration, in which knowledge of maximum stand volumes and long-term stand development is essential. Because of the young age of Sitka plantations in Iceland and considering that Sitka spruce can live 700–800 years and reach 80 m height (Savill 1991), it is not reasonable to postpone growth modelling until the data cover the whole life span of trees and stands. On fertile soils in coastal areas of western Norway, the yield of Sitka spruce will peak between the age of 70–115 years (Öyen 2005).

The aim of this study was to develop a system of models that allow managers to simulate the development of Icelandic Sitka spruce stands over a few decades. The system consists of site index and dominant height models and individual-tree models for diameter increment, tree height and tree survival.

MATERIALS

Two different datasets were used to develop the model: the dataset from permanent sample plots (PSP data) and data from the National Forest inventory (NFI data). The PSP data consists of measurements in 50 stands from seven locations, established in even-aged plantations (Figure 1). The NFI datasets were collected in 31 permanent sample plots from 27 locations (Figure 1). These 81 permanent sample plots were established in even-aged Sitka spruce stands by the Icelandic Forest Service between 1970 and 2013. They include a total of 197 measurement intervals. The stands are located in southern (37), western (29), northern (5) and eastern (13) Iceland and include both heavily thinned and unthinned sample plots. The plots cover a wide range of different site types and growth conditions. All the locations have an oceanic climate with annual precipitation of 700–1200 mm and a mean annual temperature of 3.2–4.5˚C (1964–1990) (Vedurstofa Islands 2017). The mean

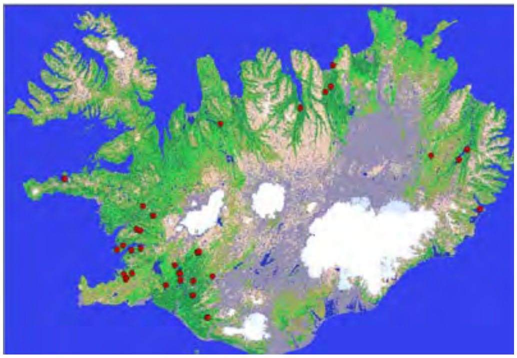

Figure 1. Geographical location of the study sites.

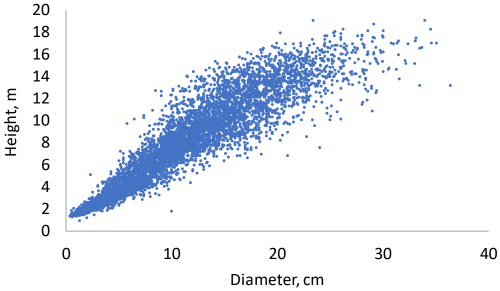

Figure 2. Observed height and diameter at breast height in the Sitka spruce plots.

Table 1. Mean, standard deviation (SD) and range of the main characteristics in the empirical data of the study material. N: number of observations; Dbh: diameter at breast height; G: stand basal area; Age: stand age; Hdom: dominant height.

Variable	N	Mean	SD	Maximum	Minimum
Dbh (cm)	7276	9.46	7.17	36.4	0.0
Height	8432	5.92	4.62	19.0	0.28
G (m2ha-1)	197	18.25	15.89	79.38	0.0
Age (years)	197	36.6	15.7	63.0	6.0
Hdom (m)	197	8,65	5.1	18.0	0.38
Growth periods	197	5.48	1.62	16.0	3.0
Stems per hectare	197	2222	1414	5026	458

maximum daytime temperature in June, July and August was 12.9–13.6 ˚C during the period 1964–1990 (Vedurstofa Islands 2017). The range in elevation is between 60 and 180 m a.s.l.

The sample plots were either circular or rectangular. The plot size varied between 0.01 and 0.053 ha and the measurement interval was 3 to 16 years with an average of 5.5 years. There was mortality only among small trees in plots with a basal area up to 80 m^2/ha. On every measurement occasion, two measurements of diameter at breast height (1.3 m) at right angels were made using callipers, and the arithmetic mean of the two measurements was calculated. The total tree height was measured on standing trees using a height measuring pole or Vertex Laser VL5, and felled trees were measured on the ground using a tape measure. The measured heights and diameters of the trees are shown in Figure 2.

METHODS

Site index and dominant height modelling

The first equation required in the model set was the site index model, which was used to calculate the site index of the inventory plots. The index age was taken as 80 years, which has been previously used in Iceland (Heiðarsson & Pukkala 2012); site index is defined to be the dominant height of the stand at 80 years. Besides calculating site index, the site index model can be used to predict dominant height development. Based on experiences from previous studies in plantation forests (Palahí et al. 2004; Guzmán et al. 2012a, 2012b; Heiðarsson & Pukkala 2012, de-Miguel et al. 2013), the following equations were tested: Lundkvist & Korf (Korf 1939), Chapman and Richards (Richards 1959), McDill & Amateis (1992), and Schumacher (1939). Alternative models were compared based on the mean of squared errors and Akaike Information Criterion (AIC).

Of the tested models, the formula of McDill and Amateis was selected for predicting site index and dominant height development. When fitting the model, only the first and last measurement of each plot were used, which removed the need to include random plot factors:

$$H_{Last} = \frac{a_0}{1-\left(1-\frac{a_0}{H_{First}}\right)\times\left(\frac{T_{First}}{T_{Last}}\right)^{a_1}} + \varepsilon \qquad (1)$$

where H_{First} and T_{First} are, respectively, dominant height and stand age at the first measurement, and H_{Last} and T_{Last} are the same variables at the last measurement of the plot.

The model is used to calculate the site index as follows:

$$\hat{SI} = \frac{a_0}{1-\left(1-\frac{a_0}{H}\right)\times\left(\frac{T}{80}\right)^{a_1}} \qquad (2)$$

where $\hat{SI}$ is site index (dominant height at 80 years, m), H is dominant height (m) and T is stand age. When the model is used in simulations to calculate the dominant height for certain site index and age, H is replaced by SI, stand age T is replaced by 80 (index age), and 80 is replaced by stand age.

Tree height modelling

The model for individual-tree height was based on the model of Schumacher (1937). Two alternative models analysed by Mehtätalo et al. (2015), namely the models of Näslund (1937) and Curtis (1967) were also tested, but the model of Schumacher was selected based on the square root of the mean squared error (RMSE) and the Akaike Information Criterion. The models were fitted as mixed-effects models, by adding random plot factors to the fixed parameters. The best combination of random plot factors was found by comparing all possible combinations.

The parameters of the height curve were modelled as a function of stand characteristics, which allowed the diameter-height curve to change when the stand developed. Two versions of the height model were fitted. The first used dominant height as a predictor, in addition to diameter at breast height. Since this model is not suitable for simulations where the dominant height could decrease as a consequence of thinning from above, another model version was fitted where dominant height was replaced by site index and stand height. The two model versions were as follows:

Model 1

$$\hat{h} = 1.3 + [a_0 + (a_1 + a_{1k})H] \times exp\left[\frac{-\{(b_0+b_{0k})+(b_1+b_{1k})H\}}{d}\right] \quad (3)$$

where a_0, a_1, b_0 and b_1 are fixed parameters and a_{1k}, b_{0k} and b_{1k} are random parameters for plot *k*.

Model 2

$$\hat{h} = 1.3 + [a_0 + (a_1 + a_{1k})T + (a_2 + a_{2k})SI] \times exp\left[\frac{-(b_0+b_{0k})}{d}\right] \quad (4)$$

where a_0, a_1, a_2 and b_0 are fixed parameters and a_{1k}, a_{2k} and b_{0k} are random parameters for plot *k*.

Diameter increment and survival modelling

The diameter increment and survival data had the problem that the interval between measurements varied from 3 to 16 years. Since the linear growth rate or the constant survival rate during the whole interval cannot be assumed, the optimization approach originally suggested by Pukkala et al. (2011) and later used by de-Miguel et al. (2014), Juma et al. (2014) and Dong et al. (2021) was used to fit the diameter increment and survival models. The aim of the method is to find parameters for the diameter increment and survival models that minimize the difference between the measured and simulated diameter distributions of the plots at the end of the measurement interval, in cases when the simulation starts from the diameter distribution at the beginning of the interval. Diameter increment and survival were simulated in one-year time steps.

As the first step in modelling, predictors and model forms were selected based on previous literature, preliminary regression analyses and preliminary optimizations. The following model forms were selected:

$$\hat{i}_d = exp\left[a_0 + a_1 lnd + a_2 d + a_3\sqrt{G} + \frac{a_4 BAL}{ln(d+1)} + a_5 SI\right] \quad (5)$$

$$\hat{s} = \frac{1}{1+exp\left[-\left(b_0+b_1 lnd+b_2\sqrt{G}+b_3 BAL/ln(d+1)\right)\right]} \quad (6)$$

where $\hat{i}_d$ is diameter increment (cm/year), $\hat{s}$ is annual survival rate, *d* is diameter at breast height (cm), *G* is stand basal area (m^2/ha), *BAL* is basal area of trees larger than the subject tree (m^2/ha), *SI* is site index (m) and $a_0,\ldots, a_5, b_0,\ldots, b_3$ are regression coefficients. The subject tree is the tree for which the diameter increment or survival is predicted.

The minimized loss function was:

$$\underset{\theta}{argmin} \sum_{k=1}^{K}\left[\sum_{j=1}^{J_k} w_{jk} \sum_{i=1}^{I_j} v_G\left|G_{ijk}^m - G_{ijk}^s(\theta)\right|^{1.5} + v_F\left|F_{ijk}^m - F_{ijk}^s(\theta)\right|^{1.5}\right] \quad (7)$$

where Θ is the set of coefficients (parameters $a_0,\ldots a_5$, $b_0,\ldots b_3$ of Equations 5 and 6), *K* is the number of plots, J_k is the number of measurement intervals of plot *k*, I_j is the number of 3-cm diameter classes in measurement interval *j* of plot *k*, G_{ijk}^{m} and $G_{ijk}^{s}(\Theta)$ are, respectively, measured and simulated cumulative basal area (m^{2}ha^{-1}) of diameter class *i* at the end of measurement interval *j* of plot *k*, and F_{ijk}^{m} and $F_{ijk}^{s}(\Theta)$ are, respectively, the measured and simulated cumulative number of trees per hectare of diameter class *i* at the end of measurement interval *j* of plot *k* (see e.g. de-

Miguel et al. 2014 for details). The symbol w_{jk} is the weight of measurement interval j of plot k. The models were fitted with three alternative weighting schemes: (1) without weight (w_{jk} = 1 for all j and k), (2) using the area of plot k as the weight and (3) using the number of trees in plot k in the beginning on period j as the weight. Symbols v_G and v_F are the weights of the deviations of the basal area (in m^2ha^{-1}) and the number of trees per hectare. They also remove the effect of different units of basal area (G) and number of trees (F). They were as follows $v_G = 1$, $v_F = 0.005$.

The models were also fitted by adding a plot and measurement factor to both sub-models (diameter increment and survival probability). Since the 81 study plots had altogether 191 measurement intervals, the number of additional parameters in this model fitting was 382.

The optimization simultaneously produced the coefficients for the annual diameter increment model and the annual survival model. The method of Nelder and Mead (1965) was used in parameter optimization.

Since the optimization did not provide any statistics about the significance of the predictors, bootstrapping was used to assess the statistical significance of the regression coefficients. Fifty samples of 197 measurement intervals were selected using random sampling with replacement, and the same model fitting procedure as described above was performed with every sample (without plot and measurement factors, using number of trees as the weight).

RESULTS

Site index model

The parameters of the selected site index and dominant height model (Equation 1) were: a_0 (asymptote) 131.03, and a_1 1.3847. The RMSE of the model was 1.315 m. Figure 3 shows that the model depicts the dominant height development of the plots reasonably well. No signs of decreased dominant height growth rate with increasing age can be seen in the data or the model. The asymptote, i.e., the maximum possible dominant height, of the model is 131 m.

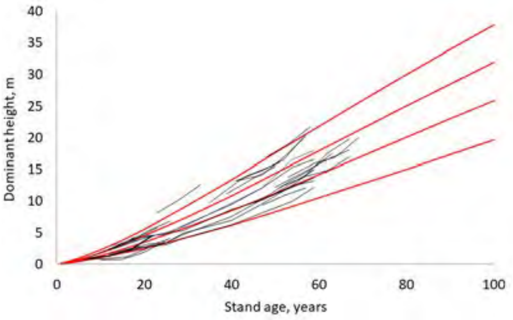

Figure 3. Dominant height development in site indices 15, 20, 25 and 30 m at 80 years according to the site index model (thick curves) and the measured dominant height development in some of the plots used as modelling data (thin black lines).

According to Harris (2022) Sitka spruce may live 700–800 years and reach a height of 60–90 m.

Height model

The parameters of the two versions of the individual-tree height model are shown in Tables 2 and 3. The RMSEs of the models were 0.752 m (Equation 3) and 0.765 m (Equation 4). Both models predict that the height curve of a stand rises when the stand develops and its dominant height or age becomes larger (Fig. 4)

Table 2. Parameters of the individual-tree height model where the predictors are diameter at breast height and dominant height (Equation 3).

Parameter	Value	t value
a_0	-1.1427	-4.2583
a_1	1.3357	55.8218
b_0	2.0137	5.4161
b_1	0.4334	13.8027
Random parameters	Standard deviation	
a_{1k}	0.7480	
b_{0k}	1.7725	
b_{1k}	0.1144	

Correlations: a_{1k}–b_{0k} 0.760; a_{1k}–b_{1k} -0.356; b_{0k}–b_{1k} -0.769

Table 3. Parameters of the individual-tree height model where the predictors are diameter at breast height, stand age and site index (Equation 4).

Parameter	Value	t value
a_0	-8.8135	18.2905
a_1	0.4229	16.3186
a_2	0.2849	31.2452
b_0	6.4704	28.3320
Random parameters	Standard deviation	
a_{1k}	0.0984	
a_{2k}	0.0548	
b_{0k}	1.6966	

Correlations: a_{1k}–a_{2k} -0.827; a_{1k}–b_{0k} 0.127; a_{2k}–b_{0k} 0.362

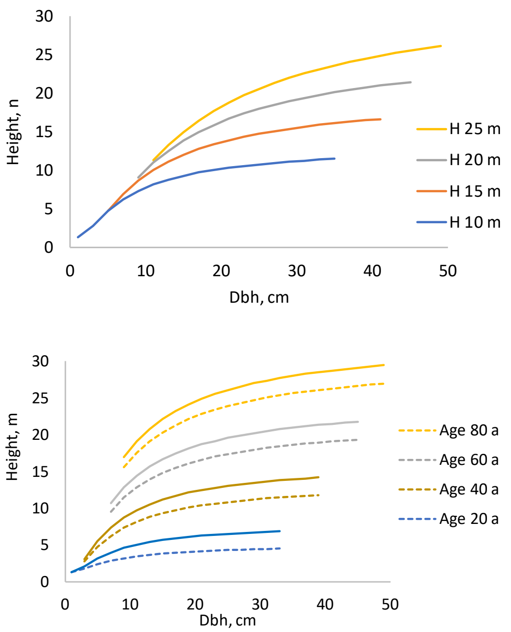

Figure 4. Relationship between diameter at breast height (Dbh) and tree height at different dominant heights (H) and stand ages according to the two versions of the height model (Top: Equation 3; Bottom: Equation 4). In the lower diagram, continuous lines show the tree height in site index of 25 m and dashed lines for site index of 15 m at 80 years.

Diameter increment and survival models

When the diameter increment and survival models were fitted using different weights in the loss function (w_k in Equation 7), or by adding plot and measurement factors to both models, the diameter increment models behaved slightly differently, as shown in Figure 5. The model versions where the plot area or the number of trees were used as the weight (w_k in Equation 7) were very close to each other. Since the loss functions of the fitting were not the same in different weighting schemes, the model versions cannot be compared based on the loss function value. Therefore, the models were compared by calculating the measured and predicted mean annual basal area increment (periodical increment divided by the length of the period) for each measurement interval of each plot. Then the square root of the area-weighted mean of the squared errors in annual basal area increment was calculated. According to this analysis, the

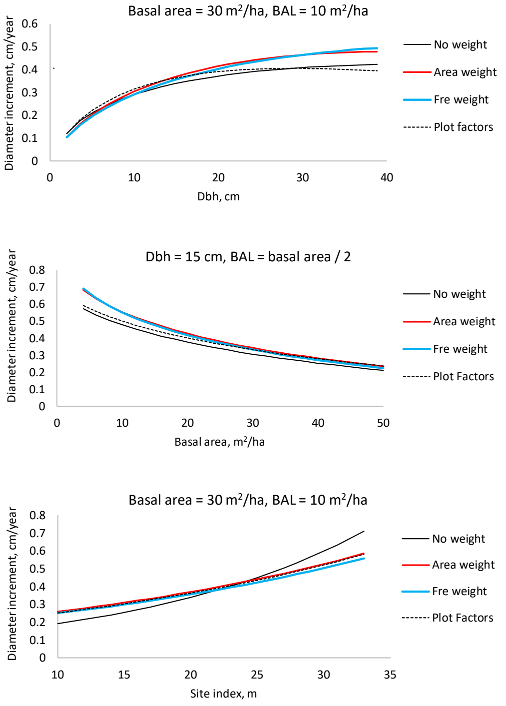

Figure 5. Dependence of diameter increment on diameter, stand basal area and site index according to four different versions of the diameter increment model (see text for explanation). "Fre weight" means that the number of trees within the plot was used as the weigh variable in the loss function (Equation 7).

use of number of trees as the weight resulted in the best model (area-weighted RMSE 0.444 $m^2ha^{-1}a^{-1}$) and a model fitted without any weight was the worst (RMSE 0.449 $m^2ha^{-1}a^{-1}$). Therefore, the model where the number of trees was used as the weight was selected for further analyses. The bias of the selected model, in terms of mean annual basal area increment, was -0.00086 $m^2ha^{-1}a^{-1}$ (0.064%). The model version that included additional plot and measurement factors was fitted using the number of trees as the weight.

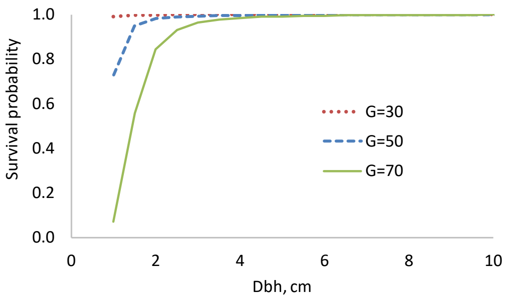

Figure 6. Dependence of one-year survival probability on diameter at breast height (Dbh) and stand basal area (G, m^2/ha) according to Equation 6.

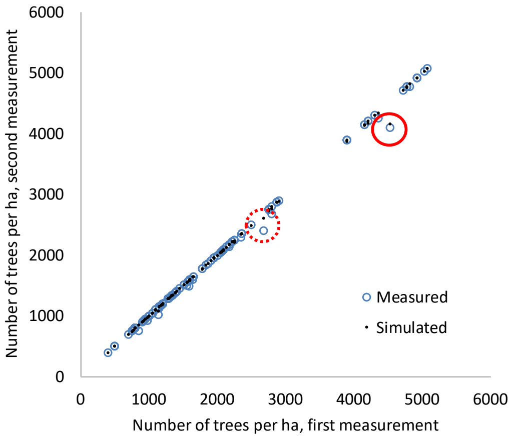

Figure 7. The measured and predicted number of trees per hectare in the beginning and at the end of the measurement interval (3–16 years). In most plots, there was no mortality between the two successive measurements. The two dots inside the circle drawn with the continuous red line show a case where the model successfully predicted the mortality. The dashed circle is a case in which the model failed to accurately predict mortality.

Table 4. Parameter estimates and bootstrap results for the optimization-based diameter increment and survival models. SD stands for standard deviation.

		Parameter	Bootstrap results				
Predictor	Parameter	estimate[1]	Min	Mean	Max	SD	SD/Mean
Diameter increment model							
Constant	a_0	-2.4548	-2.866	-2.419	-2.051	0.184	-13.110
ln(*d*)	a_1	0.6321	0.415	0.642	0.826	0.097	6.608
d	a_2	-0.0088	-0.040	-0.014	-0.006	0.007	-1.931
$\sqrt{G}$	a_3	-0.1895	-0.245	-0.163	-0.065	0.050	-3.281
BAL/ln(*d*+1)	a_4	-0.0353	-0.055	-0.036	-0.021	0.008	-4.438
SI	a_5	0.0505	0.027	0.046	0.076	0.010	4.630
Survival model							
Constant	b_0	14.3240	7.745	15.656	19.491	2.943	5.320
ln(*d*)	b_1	0.3552	-0.948	0.277	0.943	0.471	0.588
$\sqrt{G}$	b_2	-0.3392	-0.948	-0.424	-0.015	0.281	-1.506
BAL/ln(*d*+1)	b_3	-0.2424	-0.380	-0.205	-0.018	0.087	-2.359

[1] Number of trees within the plot was used as the weight variable in the loss function (Equation 7).

The survival model that was fitted simultaneously with the diameter increment model predicted mortality only among small trees with a very high stand basal area, which is in line with the data (Fig. 6). The model predicted significant mortality only in very few plots, which is also in line with the data (Fig. 6). Figure 7 shows that of the two cases in which there was significant mortality between the two measurements (i.e., more than one tree died), one case was predicted well (red circle drawn with continuous line in Fig. 7) and the other case was less successful (red circle drawn with dashed line).

The results of the bootstrapping (Table 4) indicated that the regression coefficients of the diameter increment model were significant whereas two of the coefficients of the survival model, particularly the coefficient for ln(*d*), had lower significance. However, the practical significance of this shortcoming is small, since the mortality rate of the Icelandic Sitka spruce plantations was very low and the model also predicted very low mortality except for heavily suppressed small trees.

The model set was used to simulate the development of plots 1 and 2 of the modelling dataset (Figs. 8 and 9) using the measured diameters of the trees at the age of 21 years as the starting point. Mortality was simulated by multiplying the frequencies of the trees by their predicted survival probability. In plot 1, the trees that were removed in light pre-commercial thinning in year 24 were removed also in the

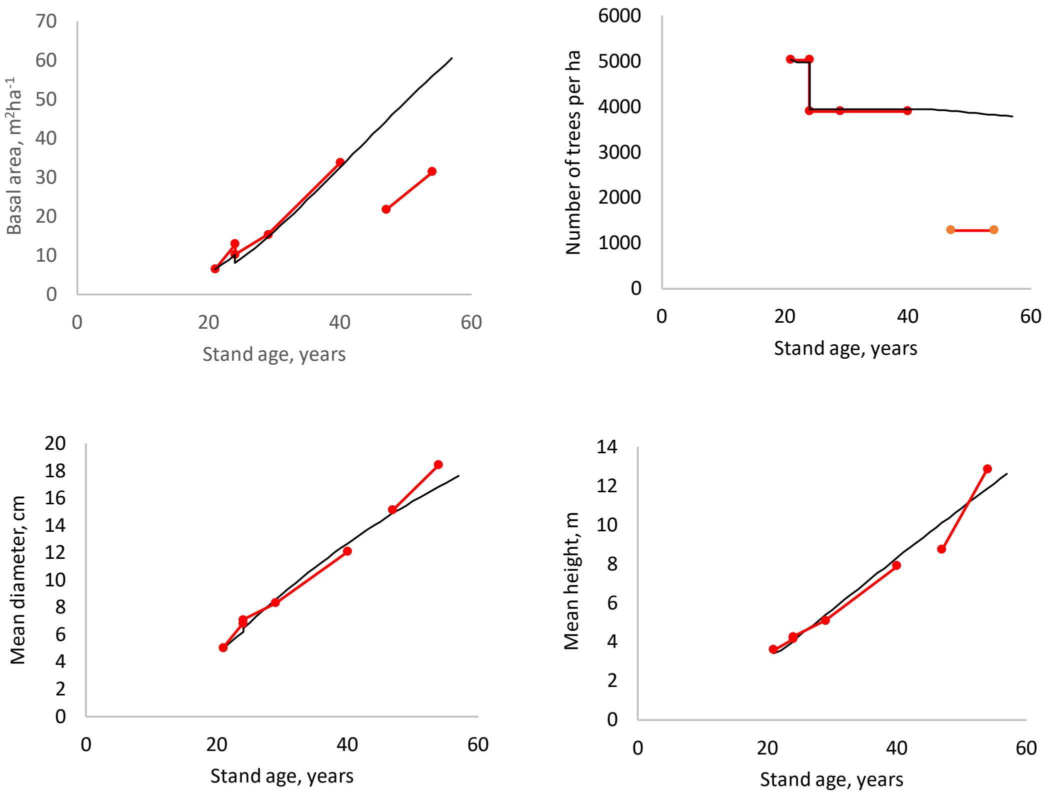

Figure 8. Development of plot 1 according to the measurements (red dots indicate measurements) and simulation (black line). The same trees that were removed in the light thinning at 24 years were also removed in the simulation. The thinning treatment between stand ages 40 and 47 was not simulated (the simulation is for non-thinned stand and the measurement at 47 and 57 years are for thinned stand).

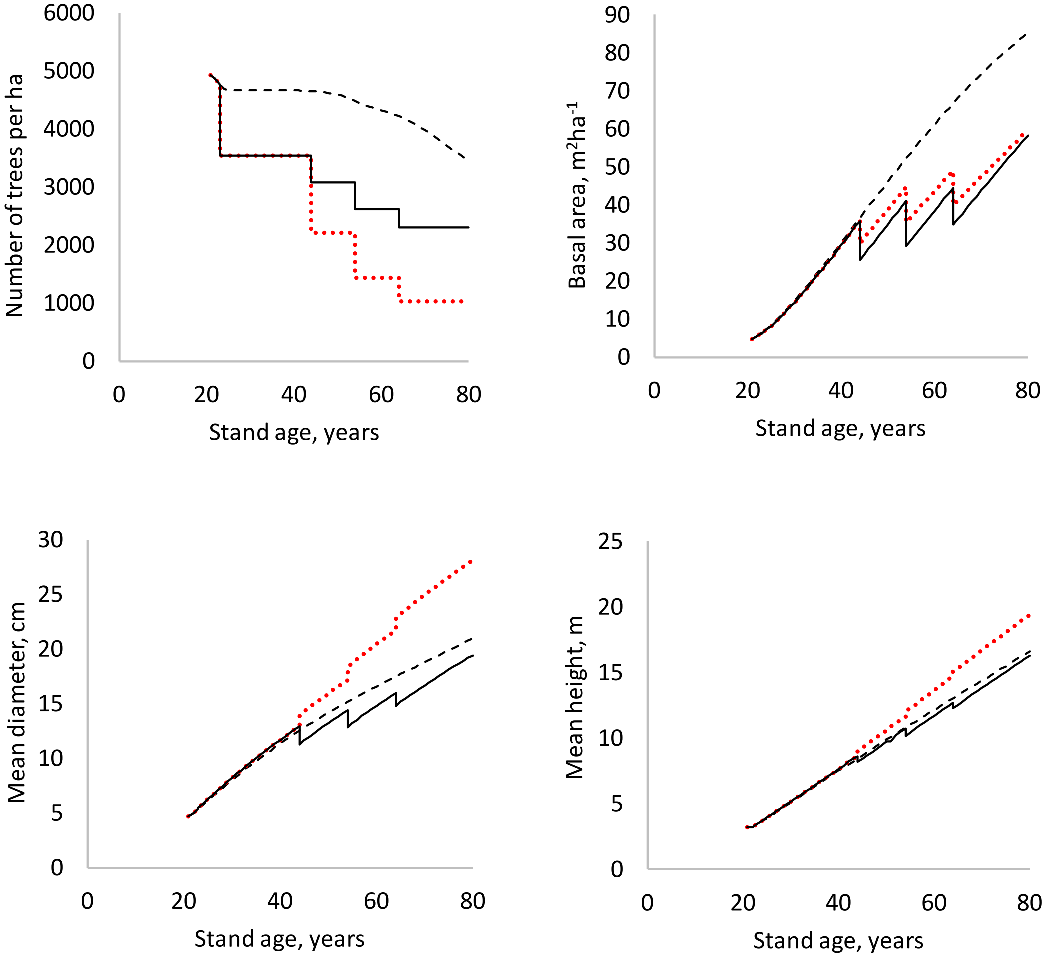

Figure 9. Simulated development of plot 2 without cuttings (black dashed line), and when the treatment schedule includes three thinnings from below (red dotted line) or three thinnings from above (black continuous line). In both thinning schedules, a pre-commercial thinning was simulated at 23 years, where the smallest trees were removed.

simulation. Plot 1 has been thinned also between 40 and 47 years but because it was not known which of the original trees were removed, the second thinning was not simulated. The simulated stand development was very close to the measured development until stand age 40 years, after which the plot was thinned (Fig. 8). When the simulation was continued beyond 40 years, without simulating the thinning treatment, the stand basal area reached 60.5 m^2ha^{-1} at 57 years. This is a logical basal area as there were a few plots in the dataset where the measured stand basal area exceeds 78 m^2ha^{-1} at stand ages ranging from 47 to 58 years (Table 1).

In plot 2, a pre-commercial thinning was simulated at 23 years where the smallest trees of the plot were removed. Then, the stand development was simulated until the stand age reached 80 years. The simulation was done: a) without any cuttings, b) with three thinnings from below and c) with three thinnings from above. The results (Fig. 9) do not reveal any anomalies in the simulated stand development. The simulations conducted for plots 1 and 2 (Figs. 8 and 9) suggest that the models may be used without problems to simulate the future development of Icelandic Sitka spruce stands until stand ages of about 80 to 100 years.

DISCUSSION

The article presents the first growth and yield models for Sitka spruce in Iceland. The models are distance-independent individual-tree models. The developed models include all the components required to simulate stand dynamics in the context of forest management planning. The model set of this study does not include a taper model, which would allow the prediction of the total and merchantable volumes for different definitions of industrial wood. Taper models for Icelandic Sitka spruce plantations should be developed in future studies. Before that, models developed in other countries (e.g., Fonweban et al. 2011) or models developed in Iceland for other conifers (Heiðarsson & Pukkala 2011) may be used.

According to the site index model, the dominant height growth rate starts to decrease after a stand age of 50 years. The model predicts that the dominant height of a stand with site index of 25 m would reach 31.5 m at 100 years and 45.9 m at 150 years. Although these predictions are not illogical (Savil 1991), other factors such as wind and snow may prevent Sitka spruce forests from reaching these heights in many parts of Iceland. Special attention was paid in this study to obtaining models that behave logically in extrapolations. Therefore, we are confident that the presented models can be used to analyse the growth and yield of Icelandic Sitka spruce in stands younger than 90 years old. However, since the modelling data set used in this study contained little data on mature stands, the models need to be updated as Sitka spruce plantations get older.

The weakest part of the model set is probably the survival model, but little mortality was measured on the sample plots, even at very high stand basal areas (Table 1). The model predicts density-related mortality, but mortality begins at very high stand density. A study from Alaska in natural mixed stands with Sitka spruce and Western hemlock showed much higher basal areas than this study, suggesting that Sitka spruce is able to grow and survive in very dense stands (Taylor 1934). In future growth studies, emphasis should also be given to collecting more data from unthinned stands and data from the northwest and southeast parts of the country, which were not included in the dataset of this study (Figure 1).

The method that was used to fit the diameter increment and survival models (Pukkala 2009) was able to deal with irregular measurement intervals. The method also works with datasets containing tree identification errors (de-Miquel et al. 2014), which means that tree numbering and labelling are not required. Previous studies have shown that with regular data (a constant measurement interval, no tree identification errors), the method produces very similar models to those obtained with regression analysis (Pukkala et al. 2011). When the measurement interval varies greatly, optimization has been reported to produce more logical models than obtained from regression analyses that assume a constant growth rate between two consecutive measurement occasions (Juma et al. 2014). When several models are fitted simultaneously, there is the possibility of having mutual errors that cancel out each other. For example, an overestimated diameter increment may be compensated for by overestimated mortality. However, the objective function used in the current study included the diameter distribution of both the stand basal area and number of trees, with the consequence that the possibility of this kind of error was small. If ingrowth models were estimated simultaneously with survival models, which is also possible (Pukkala 2009), the risk of mutual errors would be higher.

The developed growth models will be used in the forest management planning system at the Icelandic Forest Service. The planning system makes it possible to optimize the thinning schedules and rotation length to maximize the net present value, wood production, or carbon sequestration. The new models will be an important component of the management planning system used in Iceland because the stand structure and the amount of wood in Sitka spruce forests can now be better evaluated, and forest resources can be used more efficiently. Good knowledge about the yield and suitable rotation lengths of Sitka spruce plantations also provides investors with a better basis for making informed decisions.

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Absorption of colostral immunoglobulin G by Icelandic dairy calves

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ABSTRACT

Serum samples were taken from 11 Icelandic dairy calves, at birth and 24 hours later, and 11 colostrum samples were taken from their mothers with the concentration of IgG measured by ELISA. The amount of colostrum ingested by each calf was also measured. The results showed that the mean concentration of IgG in cow colostrum at birth was 11.54 mg ml^{-1}. The mean concentration in the serum of calves that had ingested known quantities of colostrum was 8.02 mg ml^{-1} 24 hours postpartum. Both values were lower than described in other cattle breeds. Significant correlation existed between the amount of colostral IgG consumed and serum concentration of IgG in the calves 24 hours postpartum. The mean apparent efficiency of absorption (AEA) was 43%, which is high compared to other studies. Although the study group was small, the results give an indication of the ability of Icelandic calves to absorb colostral IgG.

Keywords: Bovine colostrum, immunoglobulin G (IgG), passive transfer of immunity, apparent efficiency of absorption (AEA).

YFIRLIT

Upptaka íslenskra kálfa á ónæmisglóbúlíni G úr broddi

Sermissýnum var safnað úr 11 kálfum við burð og sólarhring síðar, auk þess sem broddsýni voru tekin úr mæðrum þeirra. Styrkur IgG var mældur í öllum þessum sýnum með elísuaðferð, auk þess að magn brodds sem hver kálfur drakk var skráð. Meðalstyrkur IgG í broddi var 11,54 mg IgG ml^{-1} og í sermi sólarhringsgamalla kálfa 8,02 mg IgG ml^{-1}, hvort tveggja undir viðmiðum sem notuð eru fyrir erlend ræktunarkyn. Fylgni fannst milli heildarmagns IgG í broddinum sem kálfarnir drukku og styrks IgG í sermi þeirra sólarhring eftir burð. Meðalgildi áætlaðs frásogs á IgG var 43% sem er hátt miðað við erlendar rannsóknir. Þó rannsóknin sé gerð á fáum einstaklingum gefa niðurstöðurnar nokkra hugmynd um getu íslenskra kálfa til þess að taka upp IgG úr broddi.

INTRODUCTION

Immunoglobulin G (IgG) can be found in most body fluids and is important when fighting bacterial or viral infections. In cattle, IgG cannot penetrate through the placenta, so maternal IgG must be passed via colostrum and is absorbed through the calf's intestinal wall (Simister 2003; Pross & Grattendick 2007). This absorption is called **passive transfer of immunity** and gives the offspring its first protection against infectious diseases (Furman-Fratczak et al. 2011). Calves that fail to absorb sufficient levels of IgG are more susceptible to disease and mortality than those with higher levels of IgG in serum (Rajala & Castrén 1995). Adequate passive transfer has taken place when a calf's IgG level in serum has reached at least 10 mg ml^{-1} 24-48 hours after birth (Gay 1983). Even though passive transfer of immunity is important for the calf and its survival, calves with low IgG concentrations have survived infections and diseases, indicating that other factors are also involved in protection against some diseases (Dewell et al. 2006). For the calf to obtain a serum IgG concentration of 10 mg IgG ml^{-1} from colostrum, a minimum reference value of 50 g L^{-1} in colostrum has been calculated (Halleran et al. 2017).

The Icelandic cattle breed is the only dairy breed in Iceland and is most related to the northern Scandinavian breeds (Gautason et al. 2019; Lien et al. 1999). In previous studies, colostral Ig concentration has been shown to vary between cattle breeds (Muller & Ellinger 1981), so it is relevant to study passive immunity of Icelandic calves. Preliminary studies have already implied that the IgG level in colostrum of Icelandic cattle is lower than the published reference value for good quality colostrum (Jóna Kristín Vagnsdóttir, 2018). Young calves in Iceland are prone to bacterial and coccidial diarrhoea, however viral diarrhoea and respiratory diseases are very rare in Icelandic calves, in contrast to calves in many other countries (Virtala et al. 1999). Therefore, although Icelandic bovine colostrum may be of suboptimal IgG concentration, Icelandic calves seem to receive sufficient levels of IgG to fend off existing pathogens in their environment.

The aim of this study was to measure the concentration of IgG in calf serum at birth and at 24 hours postpartum, to determine whether calves receive sufficient IgG after birth for immunological protection, as defined by studies on other breeds. The aim was also to measure the IgG in the colostrum of mothers to determine whether there is a correlation between the concentrations of IgG in colostrum and calf serum at 24 hours.

MATERIALS AND METHODS

Animals and ethical approval

All samples were collected from Icelandic cattle at Hvanneyri dairy farm, Agricultural University of Iceland. Paired serum samples were taken from 11 calves at birth and approximately 24 hours later. In addition, 11 colostrum samples from their mothers were collected immediately after birth. The samples were taken during the period of 23 January until 13 March 2019.

Calves were taken away from their dams at birth. They were kept on dry hay with other calve in a pen of approx. 43 m^2 (ca 8.5m x 5m). Calves were given 1.5-2.9 L of colostrum from their mothers at each feeding, 2-3 times during the first 24 hours, totalling on average 5.3 L. The first colostrum feeding, averaging 1.7 L, was given within one hour of birth. Eight of the calves were weighed at birth.

The study was carried out under licence no. 2018-11-02 according to law no. 55/2013 on animal welfare and regulation 460/2017 on the protection of animals used for scientific purposes.

Serum samples

Blood samples were taken from calves by jugular vein puncture at birth (0 hours) and again 24 hours later, using 9 ml BD Vacutainer tubes containing serum clot activator. Samples were tilted a few times then kept upright at 4°C until centrifuged within 24 hours at 1932 x g for 10 minutes at 4°C; 1.5 ml of serum was taken from the top and kept frozen at -70°C until analysed.

Colostrum samples

Colostrum samples from the first milking postpartum were taken. Samples of 45-50 ml were stored in falcon tubes at -20°C until they were moved to the laboratory, where they were thawed in a hotbox, their IgG concentration measured with ELISA and two small samples (1.9 ml) were stored at -70°C.

ELISA

Bovine IgG ELISA DuoSet (R&D Systems, Bio-Techne) was used for all IgG measurements according to the manufacturer's instructions. All wells on a 96-well Maxisorp plate (Nunc) were incubated overnight at room temperature with 150 µl of goat anti-bovine IgG, at 0.2 µg ml^{-1} in phosphate buffered saline (PBS, pH 7.4). After incubation, the plate was washed four times with washing solution (PBS with 0.05% Tween 20) and then the plate was incubated with 300 µl of PBS containing 5% Tween-20 (Elisa buffer) for one hour at room temperature, followed by a wash as before. Recombinant bovine IgG, serially diluted in Elisa buffer, was used as standard in the range of 93.8 to 6000 pg ml^{-1}. The serum samples were measured in four two-fold serial dilutions, starting at 1/5,000 for serum samples and 1/800,000 for samples taken 24 h after birth. The colostrum samples were measured in eight two-fold dilutions starting at 1/8,000,000. Samples and standard were measured in duplicate, with 50 µl per well. Biotinylated goat anti-bovine IgG was diluted 1:2,000 in Elisa buffer and 100 µl added immediately into all wells and the plate was put on a shaker at 50 rpm for two hours at room temperature. Following a wash as before, Streptavidin-HRP was diluted 1:40 in Elisa buffer and 150 µl pipetted into all wells and incubated for 20 minutes in the dark at room temperature, followed by a further wash as before. Then 150 µl of substrate (TMB One, Kem-En-Tec) was pipetted into all wells and incubated in the dark at room temperature, until a dark blue colour had developed in the highest standard when 50 µl of stop solution (0.18 mM H_2SO_4) were added. The absorbance (OD) of the wells was read at 450 nm with an Original Multiskan EX reader (Thermo Electron). A seven-point standard curve was constructed and the concentration of IgG in the samples read therefrom.

Apparent efficiency of absorption

The apparent efficiency of absorption (AEA) of IgG can be calculated using the following formula (Conneely et al. 2014):

$$AEA = ((\text{Serum IgG } (g\ L^{-1}) * \text{Plasma volume } (L)) / \text{IgG intake } (g) * 100$$

This requires the knowledge of the plasma volume of the calf, which is not known for Icelandic calves. The plasma volume of Jersey and Holstein calves has been defined as 9-10% of the birth weight of the calf (Quigley et al. 1998). In this study, 9% of birth weight was used.

Statistics and processing

The mean ± standard deviation (SD) was calculated for the samples and statistical significance was set at $p < 0.05$. The graphs and simple linear regression were done with GraphPad Prism 2019.

RESULTS

Calves and cows

Seven bull-calves and four heifer-calves were included in the study group (Table 1). Calves were fed on an average 5.3 litres of colostrum during the first 24 hours, and the average weight of the eight calves that were weighed was 35.8 kg. Parity of dams ranged from first to sixth calf.

IgG concentration

On average, IgG concentration in calf serum went from 0.009 to 8.02 mg ml^{-1} during the first 24 hours (Table 2). Colostrum fed to the calves contained on average 11.54 mg ml^{-1} IgG, with a range from 4.65 to 18.67 mg ml^{-1}. Figure 1 shows the correlation between the IgG concentration in the colostrum consumed and in the serum of calves 24 hours after first colostrum feeding ($R^2 = 0.3930$, $p = 0.039$). During the first 24 hours

Table 1. Data on the 11 calves included in the study, their date of birth, gender, birthweight, parity of dam and total amount of colostrum fed during the first 24 hours.

Sample no.	Date of birth	Calf gender	Weight at birth (kg)	Parity of dam	Amount of colostrum fed (L)
001	28.01.19	male	-	2	5.0
002	29.01.19	female	37.0	3	5.0
003	31.01.19	male	-	1	4.0
004	08.02.19	female	31.0	1	6.0
005	11.02.19	male	42.0	2	6.3
006	12.02.19	female	26.0	1	5.5
007	15.02.19	male	32.0	3	6.0
008	23.02.19	female	39.0	6	5.1
009	04.03.19	male	-	5	5.6
010	12.03.19	male	35.5	5	5.0
011	13.03.19	male	36.0	1	4.5
		Average	35.8	Average	5.3

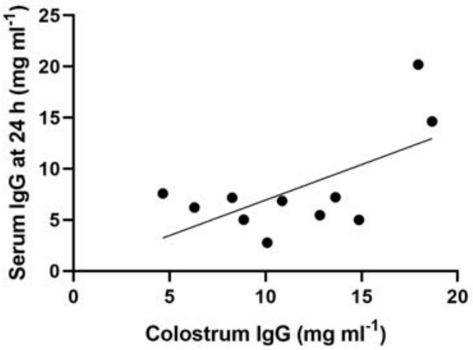

Figure 1. Correlation between IgG concentration in colostrum consumed by Icelandic calves, and IgG concentration in their serum 24 hours postpartum ($R^2 = 0.3930$, $p = 0.039$).

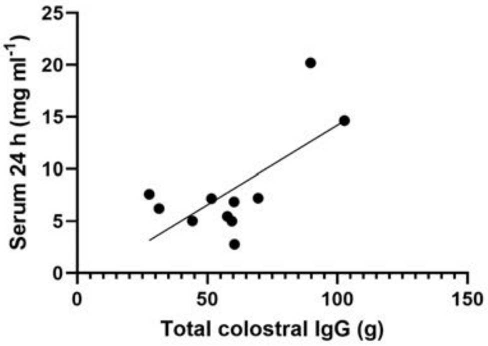

Figure 2. Correlation between the total amount of colostral IgG consumed by Icelandic calves and IgG concentration in their serum 24 hours postpartum ($R^2 = 0.4645$, $p = 0.021$).

postpartum, calves received in total 27.7-102.7 g IgG, corresponding to 0.09-0.39% of their birthweight (for the eight calves weighed). Figure 2 shows the correlation between the total amount of IgG received and the resulting IgG concentration in serum ($R^2 = 0.465$, $p = 0.021$). Figure 3 shows the correlation between the ratio of IgG amount to birthweight and the resulting IgG concentration in serum ($R^2 = 0.500$, $p = 0.0498$). No significant correlation was found between IgG concentration in serum and either the ratio of total of colostrum fed per kg birthweight for the eight calves weighed ($R^2 = 0.2294$, $p = 0.230$) or the birthweight for the eight calves ($R^2 = 0.2102$, $p = 0.253$).

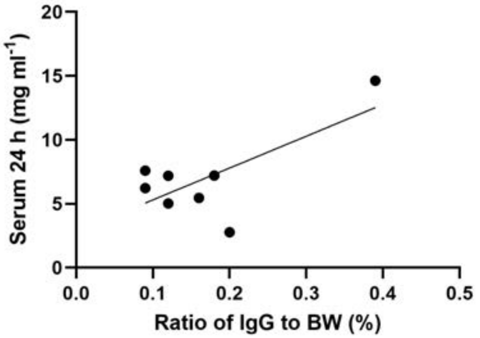

Figure 3. Correlation between the ratio of IgG consumed and birthweight of Icelandic calves and their serum concentration of IgG 24 hours postpartum (R^2 = 0.500, p = 0.0498). Birthweight was only registered for eight calves.

Table 2. Concentration of IgG in serum samples taken from calves at birth (0h) and 24 hours after first colostrum feeding, as well as in the colostrum they were fed.

	Serum IgG		Colostrum IgG
Sample no.	0h (mg ml^{-1})	24h (mg ml^{-1})	(mg ml^{-1})
001	0.007	20.20	17.95
002	0.011	6.22	6.29
003	0.008	5.02	14.86
004	0.013	2.78	10.08
005	0.009	7.19	8.26
006	0.008	14.63	18.67
007	0.012	7.59	4.65
008	0.005	7.22	13.64
009	0.007	6.87	10.86
010	0.014	5.03	8.86
011	0.009	5.46	12.83
Mean	0.009	8.02	11.54
SD	0.003	5.00	4.52

Apparent efficiency of absorption

The eight calves for which birthweight was available had a mean estimated blood volume of 3.1 litres and consumed on average 55.7 g of IgG via colostrum, leading to a mean AEA of 43% (Table 3).

Table 3. Apparent efficiency of absorption (AEA) of IgG in eight Icelandic dairy calves, as calculated using serum IgG 24 hours after birth, estimated blood volume and total colostral IgG consumed.

Sample no.	Serum IgG 24h (mg ml^{-1})	Estimated blood volume (L)	Total IgG consumed (g)	Apparent efficiency of absorption (%)
002	6.22	3.33	31.46	65.8
004	2.78	2.79	60.49	12.8
005	7.19	3.78	51.62	52.6
006	14.63	2.34	102.69	33.3
007	7.59	2.88	27.67	79.0
008	7.22	3.51	69.56	36.4
010	5.03	3.20	44.30	36.3
011	5.46	3.24	57.74	30.7
Mean	7.0	3.1	55.7	43.4
SD	3.5	0.5	23.7	21.2

DISCUSSION

This is the first study reporting the concentration of IgG in the serum of Icelandic dairy calves. Interestingly, there was already a minute concentration of IgG in serum samples taken at birth. This is not surprising, as traces of IgG can be found in newborn calves, up to a concentration of 1 mg ml^{-1} (Conneely et al. 2014). However, this level of IgG does not provide enough protection for the calf against microorganisms in its surroundings (Barrington and Parish 2001). The average concentration of IgG in serum samples taken at 24 hours postpartum was 8.02 mg IgG ml^{-1} ± 5.0, ranging between 2.78 and 20.20 mg IgG ml^{-1}. This is compatible with research done on Holstein calves both in Europe and the United States (Furman-Fratczak et al. 2011; Sakai et al. 2012), indicating that Icelandic calves take up sufficient IgG compared to other breeds although only two of the eleven calves reached the value of successful passive transfer of immunity. Numerous factors can increase the risk for failure of passive transfer of immunity (FTP), such as bacterial contamination of colostrum (Poulsen et al. 2010) and calving difficulties leading to poor vitality of the calf (Furman-Fratczak et al. 2011). Poor vitality of the calf not only negatively influences the amount of colostrum the calf can consume, but also the absorption of IgG through the intestinal mucosa, as reviewed by Weaver et al. (2000).

The average colostral IgG concentration observed in the Icelandic cows was considerably lower than in Holstein (79.48 mg ml^{-1}) and Jersey (72.9 mg ml^{-1}) cows (Furman-Fratczak et al. 2011; Morrill et al. 2015). Low median concentration (35 g L^{-1}) and variation (5-129 mg ml^{-1}) are known in Red Norwegian cows (Johnsen et al. 2019) and low concentrations (22 mg ml^{-1}) with wide range were described by Erhard et al. (1999), although without information on breed. The average colostrum IgG concentration in the present study was, therefore, only a little over half of the lowest previously reported concentration, ranging from 4.65 to 17.95 mg IgG ml^{-1}. Such variation is not unique, as considerable individual variation has been reported in Jersey cows (13-154 mg ml^{-1} in Morrill et al. 2015), even if the mean concentration was a very acceptable 72,9 mg ml^{-1}.

As there were only 11 calves and their dams in this study, conclusions must be drawn with care. However, the results show a significant influence of the absolute amount of IgG fed to each calf on the IgG serum concentration achieved. The results therefore give an indication of the ability of Icelandic calves to absorb a large part of the IgG ingested via colostrum. Birthweight was regrettably not registered for three of the calves, and, therefore, it was not possible to calculate the AEA for each calf. The AEA calculated with IgG intake measured by ELISA was widely distributed, ranging from 12.8% to 79.0%, but the average of 43.4% is high compared to the 21-28% recorded for Holstein and Jersey calves (Jones et al. 2004; Swan et al. 2007). Halleran et al. (2017) found a wide range of AEA values in Holstein calves and suggested that feeding larger volumes of colostrum and stricter genetic selection could increase the IgG concentration in serum. The study was conducted on 100 calves in 5 herds and the average AEA was 28%; however, the larger sample size and different analytical methods might affect the comparability of these studies.

As mentioned before, colostrum samples contained less than 50 mg IgG ml^{-1}, suggesting that the colostrum from these cows might not provide their calves with enough IgG to withstand disease. However, with low morbidity of neonatal calves in the herd, the relatively low concentration of IgG in their serum seems not to have a major effect on their health. The results rather suggest that the low risk of infections, due to the lack of harmful microorganisms in the environment, leads to lower IgG concentrations (Bush & Staley 2010). The relatively low IgG serum concentration in Icelandic calves seems to be sufficient for the infection pressure in their environment, although the concentration of other Ig types such as IgA, might also have a relatively bigger role than in other breeds (Ahmann et al. 2021). The cows in this study were kept in a freestall, as are most Icelandic dairy cows (73% of all cows in 2019), and milked automatically, as is the case for 40% of Icelandic dairy farms (Landssamband kúabænda 2020). According to Icelandic regulation, antibiotics are only used on an individual basis and based on a diagnosis by a veterinarian, and vaccines or immunomodulators are not used on the study farm (Reglugerð um heimildir dýralækna til að ávísa lyfjum nr. 539/2000).

Calves were given their first feeding of colostrum within one hour of birth. It is important to feed calves the appropriate amount of colostrum as soon after birth as possible. However, they should not be overfed in the first feeding and instead additional smaller feeds should be given during the first 24 hours (Chigerwe et al. 2008). In their study, Conneely et al. (2014) found that calves receiving colostrum corresponding to 10% of their bodyweight had a slightly lower serum concentration and AEA than calves receiving 8.5% of their bodyweight. The calves received the whole amount in one feed, which is less optimal with such quantities. In our study, calves had an average AEA of 43% and consumed colostrum corresponding to 13-21% of their birthweight during the first 24 hours, distributed over two to three feedings (3-8% at a time). Conneely et al. (2014) concluded that calves fed colostrum within two hours postpartum had a higher AEA and, after that, a higher colostral IgG concentration was needed to ensure similar serum concentrations. This indicates the importance of both the timing and amount of feeding provided to calves during their first hours of life.

Our results show a weak positive association between the IgG concentration in colostrum fed to calves and the concentration in their serum 24 hours after first feeding. A stronger correlation was seen when the total amount of IgG was calculated, verifying the high efficiency of absorption, and validating the method of smaller, more frequent feedings. The study design involved feeding the calves varying amounts of colostrum, thus following routine practice on the farm, instead of feeding a standard amount of pooled colostrum. Erhard et al. (1999) concluded that the absorption of IgG relies on other factors and not only colostrum quality. In a study involving colostrum supplement, serum IgG in calves fed a medium- or low-quality colostrum plus colostrum supplement was higher than for calves fed high-quality colostrum (McCoy & Hurley 1997). Other studies found that IgG in colostrum was absorbed more efficiently than IgG contained in a colostrum supplement (Morin et al. 1997). The absorption of IgG clearly relies on more factors than the amount of IgG available in the colostrum, importantly birthweight of the calf (Shivley et al. 2018), although birthweight was not shown to be a causal factor in this study.

In conclusion, our study showed that feeding calves on average 5.3 litres of colostrum in two to three feedings over 24 hours is highly effective. The average concentration in calf serum was considerably under the reference value for adequate transfer of immunity as defined in other breeds. This was also true of the mean colostrum IgG concentration, which was lower than the defined reference value for quality colostrum in other breeds. However, the average apparent efficiency of IgG absorption was high, enabling the calves to utilise a large proportion of the available colostral IgG. The absolute amount of colostral IgG fed to calves during the first 24 hours postpartum correlated well with their serum concentration of IgG. For future studies, it would be important to increase the sample size considerably, to include more herds, and to collect the birthweight and birth records for all calves studied.

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Short communications

Natural Regeneration of Lodgepole pine (*Pinus contorta*) in Steinadalur, SE-Iceland

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INTRODUCTION

Lodgepole pine (*Pinus contorta* Dougl.) is a coniferous tree species, native to the western parts of North America. It has been widely cultivated and is now found throughout the rest of America, in Europe and New Zealand (Simberloff et al. 2010). It has a wide ecological range and grows on various soil types, it is resistant to air pollution and spring frost and can adapt to different climatic conditions (Lotan & Critchfield 1990). Lodgepole pine produces viable seeds at an early age, commonly from 5 to 10 years in warmer climates and the percentage of germination is often high (Lotan & Critchfield 1990). The lodgepole pine is one of the most common tree species used in forestry in Iceland, with the first plantation taking place in 1940. The first seeds came from Smithers in British Colombia, north-western Canada (Bjarnason 1978) but the most common provenance of lodgepole pine used in Iceland is from Skagway in Alaska (Bragason 1995). The first self-regenerated plants in Iceland were recorded in Hallormsstaður before 1976, 20-25 years after the first planting took place there (Bjarnason 1978).

Lodgepole pine has been considered an invasive species in some parts of New Zealand, in Patagonia, Chile and in Argentina (e.g. Richardson et al. 1994, Nuñez et al. 2017). However, a recent study that took place in Sweden showed that the magnitude of the natural regeneration is limited at higher latitudes, and the species is not considered invasive in northern areas because its dispersal can be controlled (Jacobson & Hannerz 2020).

Steinadalur is located on the slopes of Mount Staðarfjall (928 m a.s.l.) in SE-Iceland (Figure 1). The average annual temperature in SE-Iceland is between 4,5 and 5 °C. In 1954, the planting of exotic tree species started at the foot of the mountain, 50 m a.s.l. and 4 km from the ocean. The plantation (0.4 ha) was originally fenced to exclude sheep grazing (Torfason 2007). In 1959, the first 300 3-year pine seedlings of Skagway provenance, coming from the Hallormsstaður nursery in East Iceland, were planted inside the fence. The planting continued until 1969 and the fence around the area had been extended at the end of planting, covering an area of 2.3 ha. In addition to lodgepole pine, also Sitka (*Picea sitchensis* (Bong) Carr.) and Norway (*Picea abies* (L.) Karst.) spruce were planted inside the fence (Torfason 2007). The first self-seeded pine plants outside the fenced area were found in 1985, 26 years after its first planting (Torfason 2007). The dominant tree outside the plantation is the native downy birch (*Betula pubescens* Ehrh.) which has recently

Figure 1. Photo taken from the slopes of Staðarfjall showing the old plantation and the dispersal of lodgepole pines towards southeast. Photo: Ólafur Eggertsson 27 July 2020.

spread throughout most of the area, most likely because of less sheep grazing (Figure 1). In this site, for some reason, the lodgepole pine has also had a more rapid natural regeneration and dispersal than has been observed around any other of the oldest plantations in Iceland. In 2010, Guðmundsdóttir (2012) estimated the size of the area with self-seeded lodgepole pine in Steinadalur and showed that the pines had spread from the original plantation of 2.3 ha to an area of 19.7 ha, with the most far-off seedling about 335 m from the original plantation.

The aims of this study were: 1) to quantify the distribution and density of the natural self-seeded regeneration of lodgepole pine in Steinadalur, and 2) to examine the changes that have taken place since last survey took place in 2010 (Guðmundsdóttir 2012). This paper is primarily based on the MSc thesis work of Delfina Andrea Castiglia (Castiglia 2020).

MATERIALS AND METHODS

The fieldwork took place during summer 2020. The area around the plantation was surveyed by systematically moving radially away from the plantation and the outermost limit of the natural lodgepole pine regeneration marked. Self-seeded lodgepole pines were also found further southeast, about 1.6 km away from the primary plantation, close to two cottages with lodgepole pines planted by them. The cottages were built in 1975 (www.skra.is). The same survey method was performed in that area. A high precision GPS (REACH RS2 Multi-band RTK GNSS) was used to record the location of the plants.

Moreover, a transect was placed southwest from the densest primary plantation, where the occurrence of self-seeded pines was most abundant in the area, towards an old growth downy birch forest. All trees were measured, collecting data on species, diameter and height, in a series of 200 m^2 circular plots separated by

20 m until no trees were found within the plots. A total of 11 permanent plots were placed, an iron bar was set in the middle of the plot and the position recorded with high precision GPS devices (REACH RS2 Multi-band RTK GNSS) for future surveying.

Microsoft Excel was used for processing the data collected in the field and Qgis software for producing explanatory maps e.g., using the "Distance matrix" tool of QGIS for calculating the average area increase of the regeneration.

RESULTS AND DISSCUSSION

Two distinct areas with self-seeded lodgepole pines were mapped in the study, a 66 ha area close to the primary plantation (site A) and a 33 ha area (site B) approx. 1.6 km south-east from it (Figure 2), with the total area being 99 ha where natural lodgepole pine regeneration was found. The average distance of plants from the primary plantation had increased from 270 m in 2010 to 500 m in 2020 (Figure 2). The annual rate of spread from the plantation during the period 1985-2010 was about 11 m and increased to 14 m considering the whole 1985-2020 period.

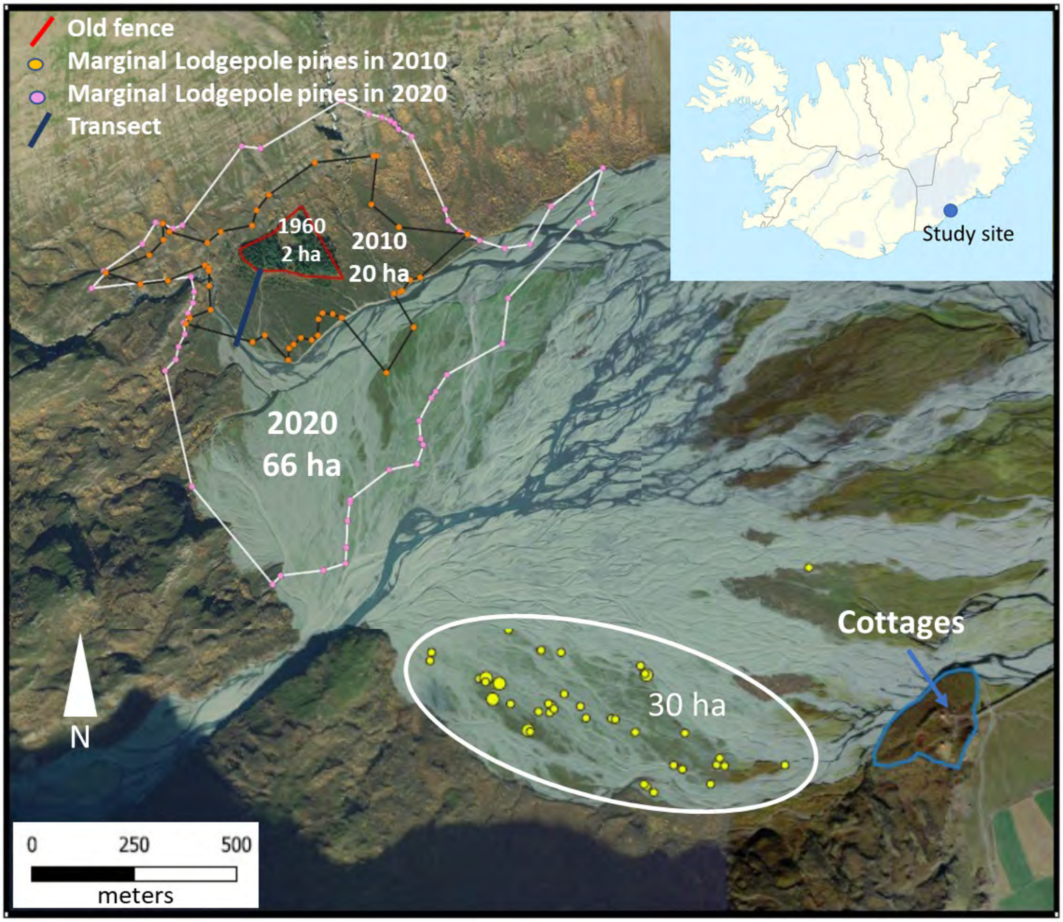

Figure 2. The location of the research site and an overview showing the distribution area of regenerated lodgepole pines in Steinadalur. The old plantation is defined by a red line (2 ha), the black line shows the boundaries of regeneration in 2010 (20 ha) and the white line the boundaries in 2020 (66 ha). The transect is shown as blue line. The red (site A) and blue (site B) lines specify the boundaries of areas where planted lodgepole pines can be found.

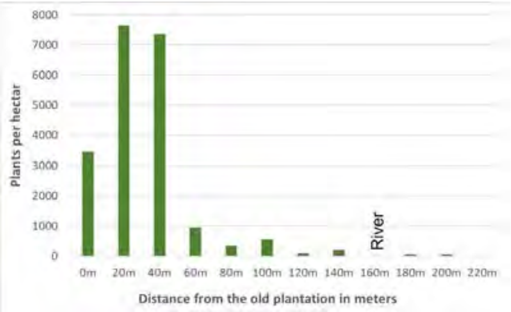

Figure 3. The number of natural lodgepole pine regeneration per hectare in relation to the distance from the plantation. The location of the transect can be seen in Figure 2.

Between 2010 and 2020, the rate was 23 m per year. The size of the distribution area was at least 66 ha in the summer 2020. If the regeneration in Steinadalur is split into two main portions, north and south respect to the old plantation, the average spread of the regeneration was 350 m and 610 m, respectively. The most far-off self-regenerated lodgepole pine in 2020 was about 760 m from the original plantation. In 2010 the most distant plant was about 335 m away (Guðmundsdóttir 2012).

The density of self-seeded pines was highest at 20-40 m from the plantation, with about 7500 plants per hectare, but decreased rapidly further away. At 200 meters the density was only 50 plants per hectare (Figure 3) and beyond this limit, the density was less than 50 plants per hectare. Consequently, we conclude that beyond 200 m from the primary plantation the density of self-seeded pines was less than 50 plants per ha. Within 60 m from the plantation, self-regenerated pines dominated, both in terms of density and size. However, as we moved further away the density of birch natural regeneration increased. The presence of self-seeded spruce in the plots was sporadic (Figure 4).

It cannot be excluded that the southernmost group of plants, located west of the cottages, were seedlings from the primary plantation, however they formed a discrete group well away from the main self-seeding area (Figure 2), therefore we consider it likely that they originated from the lodgepole pines planted next to the cottages. The average density of these pines was about 6 plants per ha.

As can be seen in Figures 1, 2 and 3, the pines distribution is rather anisotropic with most individuals growing on the poorly vegetated outwash plains, less on the hillside, and almost

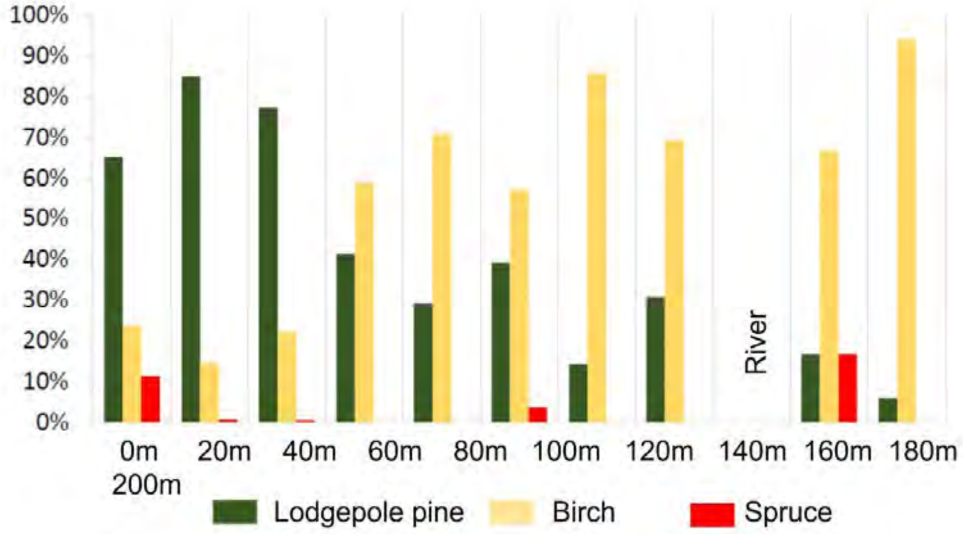

Figure 4. The frequency distribution of natural regeneration of the three most represented species in relation to the distance from the plantation. The location of the transect can be seen in Figure 2.

none where the vegetation cover is dense. No self-seeded pines were found inside the old growth birch stand.

In a study carried out in southern Chile on the dispersion of lodgepole pine, where the mean annual temperature was somewhat higher than in SE-Iceland (6-9 °C), the maximum density reached 5,319 trees per ha in the prairies where the farthest trees was recorded 310 m away from the seed source and 13,222 plants per ha in the comparatively less vegetated steppe where the furthest plant was 368 m away (Langdon et al. 2010). Comparing the results obtained in these two studies, three things stand out: i) the average regeneration success (seedling density) was much higher in the Chilean study, ii) the maximum distance of natural regeneration was higher in Steinadalur and iii) in both studies it is clear how the difference in the substrate and the presence of vegetation significantly affects seedling establishment. Regarding i), warmer climate resulting in more seed production can result in higher seedling density at the Chilean site. Regarding ii) higher wind speed a less vegetation cover might result in further spread of seeds at the Steinadalur site and regarding iii), the regeneration was much more successful in the gravel outwash plain south of the Steinadalur plantation than in the more dense herbaceous vegetation north of it. The dispersion capacity of lodgepole pine in Steinadalur was therefore in line with what has been found in other areas outside the natural range of the species (e.g. Nuñez et al. 2017). The maximum distance reached by dense regeneration (over 1000 plants/ha, Figure 3) was less than 60 m from the plantation, the absolute maximum distance of a single plant being 758 m, assuming that the southern-most group originated from the different sources, located at the cottages.

CONCLUSION

The dispersal of lodgepole pine regeneration in Steinadalur suggests that with time, in addition to an increase in the average density of plants, the expansion of pine will continue, especially on the poorly vegetated outwash plain. The purpose of this study was to describe the ongoing dynamics of natural pine regeneration, without going into the debate of its possible positive or negative consequences. It is important to study further the dispersal of self-seeded Lodgepole pines at other sites in Iceland to understand the dynamics and the impacts on native vegetation.

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Short communications

The pathogenicity of the blue stain fungus *Ophiostoma clavatum* in Scots pine seedlings

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INTRODUCTION

The aim of this research was to determine the pathogenicity of the bark beetle *Ips acuminatus* (Gyllenhal) associated fungus *Ophiostoma clavatum* Math.-Käärik (Kirisits 2004; Linnakoski et al., 2012, 2016) on Scots pine seedlings (*Pinus sylvestris* L.). This question arose as *I. acuminatus* has become more aggressive in Finland in the last two decades (Siitonen 2014), as well as in the alpine regions of Central Europe (Wermelinger et al. 2008), following the shift towards hotter and drier summers in these areas, which in turn has weakened the defence mechanisms of the pines (Chinellato et al. 2014, Wermelinger et al. 2008). The combination of increased bark beetle population size and availability of susceptible host trees (Allen et al. 2010, Siitonen 2014, Wermelinger et al. 2008), is believed to be the driving factor for increased tree mortality (Siitonen 2014, Wermelinger et al. 2008). It was, therefore, interesting to determine the pathogenicity of the fungus compared to mock-inoculated Scots pine seedlings, to see if it contributes to tree mortality.

MATERIALS AND METHOD

The research was carried out at the Forest Pathology Laboratory at the University of Helsinki, Finland. The fungal strain of *O. clavatum* originated from an adult beetle infesting Scots pine in Finland. The fungal cultures were grown in 70 mm petri dishes containing 2% malt extract agar (MEA) in an incubator in stable conditions at 25°C.

Frozen one-year old Scots pine seedlings were procured and placed in a fridge at 3-4°C with a diurnal light cycle. Two weeks later, the seedlings were planted individually in pots sized 10x10x10 cm and placed in an incubator room to adjust to potted life. In total, 90 seedlings were used. The potted seedlings were placed on a tray with 15 pots in each tray, and six trays in total. The trays were then placed on shelves within the temperature and light controlled incubator room. The incubator room had diurnal light cycles and the temperature was a steady 20°C. A fan kept the air moving, and the trays and the pots within the trays were rotated on weekly basis to ensure uniform conditions for every seedling.

After another two-week adjustment period, the inoculation of the seedlings was performed. The seedlings were randomly divided into three groups according to the treatment they would receive. One group was inoculated with *O. clavatum* and the other two groups were control groups, one mock-inoculated with 2% MEA and one left untouched with no inoculation or wounding on the bark. Before starting the inoculation, the height of the seedlings was measured and each seedling was examined to determine its quality, leaving 88 healthy seedlings, of which 66 were inoculated (*I*), ten mock-inoculated (*CM*), and 12 were control seedlings (*C*).

The inoculation was performed by stripping the woody part of the stems of needles on the first-year growth, on an area approximately 2 cm in length. A 3x4 mm lesion was cut into the bark to expose the sapwood on *I* and *CM* seedlings. To prevent contamination, the *CM* group was inoculated first with 2% MEA and then the test group was inoculated with the fungus by placing a piece of the malt agar or fungal cultures on the wound. Parafilm® was used to seal in the MEA and fungus.

Following the treatment, the seedlings were watered twice per week. Notes on the health of each seedling were taken once per week. Discolouration of needles, resin production around the lesions, and any other signs of stress were documented.

After eight weeks of observations, the seedlings' height was measured and each seedling was removed from their individual pots, the soil cleaned off, and the wounds examined. Both the *CM* and *I* seedlings' bark was first polished off with a fine-grained sandpaper (P400), and then pealed with a scalpel around the lesions to examine if any infection had manifested around the inoculation site. Any infection detected was measured using a digital Vernier calliper. Depending on the depth and size of the infected area, seedlings were assigned to one of four classes: 0 = no infection detected; 1 = minimal infection (barely visible < 0.5 mm); 2 = modest infection (infection surrounding the wound by 0.5-2 mm); and, 3 = considerable infection (infection growing > 2 mm along the stem from the wound). Additionally, random *I* seedlings were chosen, using the randomising command in Excel, and samples from the infected areas were taken to be regrown in agar plates and later reisolated to confirm infection of *O. clavatum*.

The seedlings were placed in a drying oven at 40°C for 48 hours after which they were weighed to determine their dry weight. Roots, stems, and needles were taken from the dry samples and weighed separately to establish if there was any difference in biomass allocation of infected and non-infected seedlings.

SAS Basic 9.4® software was used for statistical analysis. The variables analysed were: height at inoculation (H1), height at the end of the observation period (H2), height differences in cm (Hdiff), height differences percentage (Hdiff_rel), infection class (Infection), total dry weight (DW), dry needles (Needle), dry roots (Root), and dry stems and branches (Stem). Finally, the needle-mass ratio (NMR), root-mass ratio (RMR), and stem and branch mass ratio (SMR) were compared against each other and between treatments, as well as the root-shoot ratio (RSR). A Spearman´s ranking correlation was used and an ANCOVA test, for the statistical analysis.

RESULTS

During the eight-week observation period the seedlings showed no visual signs of stress, such as discolouration of the top needles. There was no visible discolouration in the mechanical wounding of *I* and *CM* seedlings, though dark and dried-up hyphae leftovers created a film at the top of many of the wounds; resin was visible in and around the wounds.

There was evidence of infection in 45%, or 30 of a total 66, of the *I* seedlings (data not shown). This left 55% with no infection (class 0). The distribution between the infection classes was 23%, 19%, and 3% (2 seedlings) for classes 1 to 3, respectively. Most of the infections were superficial with little or no depth within the sapwood of the seedlings. None of the mock-inoculated seedlings or the control group showed evidence of infection.

To determine if there was any relation to be found between the visually estimated infection class and other variables, the Spearman's rank correlation was used across all treatments (Table 1). The Spearman's test showed no significant correlation to be found between the infection class and other measured components.

Even though no significant relationship

was found between the infection class and the different variables, additional statistical analysis was applied, where initial size differences between the individual treatment plants were included in an ANCOVA model as a covariate. This test showed a significant difference in the total DW between the *I* and the *C* seedlings

Table 1. Spearman's rank correlation coefficients, N = 88, showing no relationship between the infection class of Scots pine seedlings infected by *Ophiostoma clavatum* and other categories. See text for definitions of variables.

	r	p
H1	-0,0508	0,6386
H2	-0,0753	0,4857
Hdiff	-0,0717	0,5069
Hdiff_rel	-0,0842	0,4353
DW	-0,1353	0,209
Needle	-0,0701	0,5163
Root	-0,117	0,2777
Stem	-0,0753	0,4854
NMR	0,04239	0,695
RMR	-0,0138	0,8983
SMR	-0,026	0,8101
RSR	-0,022	0,8386

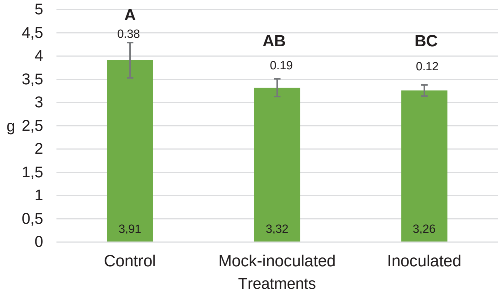

Figure 1: Results of ANCOVA test showing means and standard error of total dry weight (g) of Scots pine seedlings that had been inoculated by *Ophiostoma clavatum*, mock-inoculated and control with no treatment. Different letters indicate significant difference of P < 0.05.

Table 2. Results of Analysis of covariance (ANCOVA) where initial height (H1) is the covariate value on the difference Scots pine seedlings that had been inoculated by Ophiostoma clavatum, mock-inoculated and control group with no treatment. Dependent variables are: height at the end of the observation period (H2), the difference between the two height measurements in cm (Hdiff) and in percentage (Hdiff_rel), weight of the dry needles (Needle), dry roots (Root), and dry stem and branches (Stem), needle-mass ratio (NMR), root-mass ratio (RMR), stem and branches ratio (SMR), and root-shoot ratio (RSR). RMR, SMR, and RSR, were not determined because not all the treatment results had a normal distribution.

	Control		**Mock-inoculated**		**Inoculated**		**Significance**
Variable	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Pr > F**
H1	18,35	1,23	18,42	0,80	18,65	0,36	n/a
H2	20,33	1,37	20,26	0,87	20,71	0,41	0,7589
Hdiff	1,98	0,26	1,84	0,21	2,07	0,11	0,7589
Hdiff_rel	0,11	0,01	0,10	0,01	0,11	0,01	0,8227
Needle	2,20	0,21	2,01	0,08	2,04	0,06	0,3870
Root	0,81	0,05	0,79	0,04	0,78	0,02	0,7722
Stem	1,00	0,11	0,95	0,08	0,99	0,03	0,7561
NMR	0,55	0,02	0,54	0,02	0,53	0,00	0,6663
RMR	0,21	0,01	0,21	0,01	0,21	0,00	nd.
SMR	0,25	0,01	0,25	0,01	0,26	0,00	nd.
RSR	0,27	0,02	0,27	0,01	0,26	0,01	nd.

(Figure 1). There was, however, no significant difference between the *CM* seedlings and the other two groups. No other measured variables were significantly different between treatments in the ANCOVA analysis (Table 2).

DISCUSSION

Ophiostoma clavatum was shown to be of low virulence. Seedling mortality was not observed during the experiment. No measured growth components were significantly affected by the inoculation, apart from the difference between the total dry weight of the untreated *C* group and the *I* group. It was only the combined effect of the wounding (simulating bark-beetle effect) and the inoculation that led to significant growth reductions, while the *CM* was not significantly different from the *I* group or the *C* group. This suggests that the mechanical wounding and the fungus had only a small physiological effect on the growth of Scots pine seedlings, with no additional disturbances or stressors other than wounding and fungal infection.

Similar results have been found by Guérard et al. (2007) when inoculating 3-year old Sctos pine saplings with *O. bruenneo-ciliatum* (an *O. clavatum complex* species); the inoculation alone, without environmental stressors, did not seem to overly disturb the saplings. In another study, Krokene et al. (2000) showed that low intensity exposure to *O. canum* infection increased the resilience of Scots pine to more aggressive inoculation few weeks later. This result might suggest that the previous infection induced defence responses which then benefitted the tree in later infection. Largely, though, it has been found that the virulence of many *Ophiostoma* species has proven weak, contributing to tree mortality in only a limited way (Harrington 1993). Indeed the pathogenicity of most blue stain fungi is linked with the aggressiveness of the insect vector (Kirisits 2013), which is in turn then associated with population size and favourable environmental conditions (Siitonen 2014; Wermelinger et al. 2008). It can, therefore, be surmised that the single mechanical wounding of the seedlings in this experiment was not sufficient to severely affect the seedling.

In conclusion, the *O. clavatum* strain used in this study does not seem to seriously stress Scots pine seedlings, when kept in favourable conditions, in cases where a single infection is introduced through mechanical wounding under the bark. Further studies on the role environmental stressors play in the aggression of the fungus and how it manifests in its host are, however, warranted.

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Parasite composition of a raccoon transported to Iceland confirm its American origin

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ABSTRACT

In 2018, a live raccoon was detected by a mink hunting dog in a burrow on the coast close to Keflavík International Airport in Iceland and subsequently shot. Dissection confirmed this non-native vagrant to be a subadult, immature female. Presence of some subcutan and kidney fat reserves suggested transportation to Iceland as a stowaway hiding in goods in an aeroplane - rather than having been locked up starving for weeks in a container on a freight ship. Raccoons are native in the Nearctic but were released in Europe in the last century. Parasitological examinations of the raccoon in Iceland revealed the presence of at least 13 parasite species. Five of them are common in American raccoon populations (*Eimeria nuttally, Capillaria procyonis, C. putori, Molineus barbatus, Placoconus lotoris*) but do not occur in European conspecifics. This confirms the American origin of the animal.

Keywords: Iceland · Nearctic origin · parasite fauna · *Procyon lotor* · raccoon · translocation

YFIRLIT

Sníkjudýr sem fundust í þvottabirni á Íslandi staðfesta amerískan uppruna dýrsins

Árið 2018 fann minkahundur lifandi þvottabjörn *Procyon lotor* í holu við sjávarsíðuna skammt frá Keflavíkurflugvelli og var hann felldur. Krufning staðfesti að þarna var á ferðinni stálpuð, ókynþroska birna. Fitubirgðir undir húð og við nýru gáfu til kynna að birnan hafi tekið sér far til landsins falin í vörum flugvélar en ekki komið hingað sveltandi vikum saman í gámi flutningaskips. Náttúruleg heimkynni þvottabjarna eru Vestanhafs en á síðustu öld voru þeir fluttir til Evrópu þar sem þeir eru í dag víða algengir. Rannsóknir á sníkjudýrum birnunnar leiddu í ljós að minnsta kosti 13 tegundir. Fimm þeirra eru algengar í þvottabjörnum í Ameríku (*Eimeria nuttally, Capillaria procyonis, C. putori, Molineus barbatus, Placoconus lotoris*) en finnast ekki í þvottabjörnum í Evrópu. Því staðfestir sníkjudýrafána dýrsins það að birnan kom hingað frá Ameríku.

INTRODUCTION

The terrestrial mammalian fauna of Iceland is limited. The Arctic fox (*Vulpes lagopus*) was the only indigenous mammal when Norse settlers arrived with their livestock and pets in the late 9th century. Polar bears (*Ursus maritimus*), occasionally visiting Iceland through the centuries, have either been killed or swum or walked back to the pack ice (Skirnisson 2009). Humans unintentionally imported four rodent species and, in the late 18th century, reindeer (*Rangifer tarandus*) was introduced. In 1931, the American mink (*Neovison vison*) was imported for fur farming but soon escaped and, within few decades, a feral population had colonized all suitable habitat on the island (Skírnisson 1993) and, in recent decades, domestic rabbits (*Oryctolagus cuniculus*) have established stable populations locally in Iceland.

The importation of live animals is strictly controlled by the Icelandic Food and Veterinary Authority (MAST) to hinder the introduction of parasites and other disease agents to indigenous livestock and pets. Imported dogs and cats are quarantined, examined for contagious diseases and parasites, and systematically treated before being handed over to the owners free of any infections (Skirnisson et al. 2018).

Feral mink is usually considered a pest in Iceland and is frequently hunted with specially trained dogs that track and locate the animals in the wild. In 2018, a trained mink dog detected a raccoon (*Procyon lotor*) in a small hideaway close to the sea-shore in Hafnir, SW Iceland. This finding was unexpected as raccoons are neither kept as pets nor occur in the wild in Iceland. The raccoon was shot with a small shotgun used for mink hunting and afterwards handed over to MAST and taken to the Institute for Experimental Pathology (IEP) at Keldur. They examined the carcass for the presence of certain viral infections and non-indigenous parasites, for example *Trichinella* spp.

Raccoons are native and widespread wild mammals in North and Central America . have been introduced to other areas in the world, including Europe. Two pairs were brought to Germany in 1934 and deliberately released into the wilderness in northern Hesse (Fischer et. al 2015). A decade later, several raccoons escaped from a fur farm further to the east in Germany, in Brandenburg. In both areas feral populations became established and gradually spread to adjacent areas (Gey 1998). At present, they are widely distributed in several European countries, with the highest population density in Germany (Hohmann & Bartussek 2002).

In the 1990s, Gey (1998) studied the parasite fauna of two raccoon populations in Germany and detected five endoparasite species. However, reviewing the literature on the parasite fauna of native raccoons in America, he reported 110 species altogether, 60 endoparasites and 50 ectoparasites. Thus, the different parasite composition of raccoons living in Europe and America is well known; raccoons from the Nearctic frequently host several parasite species that are absent in Europe.

The objectives of the present study were to determine the sex, age and condition of the raccoon found in the coastal area in Iceland and to identify the parasite composition and food remains detected in the gastrointestinal tract. This paper will compare the findings to the continent-specific parasite fauna of raccoons in order to find out if the animal arrived in Iceland from America or Europe and consider the likely way of transportation to Iceland.

MATERIALS AND METHODS

The raccoon was shot on March 20, 2018 in a small burrow on the sea coast (63°56´54´´N, 22°38´32´´W) south of the village Hafnir, SW Iceland. The site is approximately 4 km southwest of Keflavík International Airport, and 8 km southwest of the harbour area in Helgavík. The carcass was brought to IEP Keldur the following day. To evaluate possible virological infections, samples were taken from the fresh carcass which was then put in a freezer at -18°C. Two weeks later, the carcass was thawed out overnight at room temperature. After photographing, weighing, sexing and taking standard body measurements the raccoon was examined for ectoparasites. Hairs were collected with a comb from different parts of the body and ears and examined directly under a stereo microscope. After digestion in 10% KOH (Foreyt 2001), hair samples were also examined under the microscope for the presence of ectoparasites.

During autopsy, inner organs were macroscopically examined for abnormalities. Tissue and muscle samples were systematically taken and fixed in 10% buffered formalin. Later, histological sections were prepared, stained with haematoxylin–eosin (H&E) stain (Suvarna et al. 2008) and examined microscopically for the presence of parasites. The gallbladder was cut up and examined for helminths under a stereoscope. The liver was cut into slices and visually checked for anomalies. Scrapings from the urinary bladder were examined for the presence of worms and eggs under the microscope.

The digestion method was applied to search for *Trichinella* sp. (Mayer-Scholl et. al 2017) with 39 grams of muscle used (tongue, diaphragm and masseter; 15, 16 and 8 grams, respectively).

The contents of the stomach, intestine and rectum were separately isolated and washed under tap water into a sieve with a 125 µm mesh size. All helminths were separated from indigestible prey remains under a stereoscope, identified to species or genus levels, and individuals of each prey species counted or their number roughly estimated (Gay 1998, Skirnisson 2016).

A faecal sample was taken from the rectum and examined for helminth eggs and protozoan cysts or oocysts by using the formalin-ethyl acetate sedimentation technique (FEAST) (Allen & Riedly 1970, Skírnisson et al. 2018).

Undigested food remains were compared to intact specimens in order to determine recently consumed prey.

RESULTS AND DISCUSSION

Sex, age and condition

The raccoon was a subadult female, weighing 3.0 kg. Head and body length was 44 cm, tail 24 cm, ear length 60 mm and hind foot 106 mm. Teeth were sharp. Undeveloped uterus and ovaries confirmed that the female was not sexually mature. According to Zeveloff (2002), approximately half of female raccoon yearlings do not mature and breed until their second year of life. The body mass and measurements were in accordance with data presented for subadult females in America (Chapman & Feldhamer 1982, Mech et al. 1968).

A few grams of subcutaneous fat were noted under the skin on the inside of the front legs and laterally on the belly. Approximately two grams of kidney fat were also present, while mesenteric fat was absent. The condition of the raccoon was estimated to be normal and the animal was not believed to have recently been starving.

Parasites and food composition

At least 13 distinct parasites were identified in the intestinal tract and in tissues of the raccoon, nine could be identified to species level, four to generic level and at least two distinct representatives of genus *Microsomacanthus* were found (Table 1). However, no ectoparasites were found, and no *Trichinella* sp. was detected. No parasites were found in the gallbladder and the liver.

No food remains were found in the stomach. However, remains of four distinct prey species were found in the small intestine and the rectum, remains that represent the prey selection of the animal earlier in the day or maybe on the day before its death. The most voluminous parts of these remains were formed by covert feathers, finely crushed leg bones and toes of a Purple sandpiper (*Calidris maritima*), a common shore bird on the shore where the raccoon was shot. Moreover, small-cut fragments of Great spider crab (*Hyas araneus*), shell fragments of a periwinkle (*Littorina* sp.), and few setae of an unidentified rainworm (Oligochaeta) were detected.

The parasite composition of the raccoon also suggested its earlier prey selection, as some of the parasites found were acquired by eating prey species on the shore that serve as intermediate hosts in the parasites' life cycle (Table 1). Thus, the presence of a fourth stage *Anisakis simplex* larva in the stomach of the raccoon confirmed consumption of a fish hosting the third stage parasite larvae some days earlier. The same fish, or maybe some other marine fish species on the shore, infected with metacercariae of *Cryptocotyle lingua* (Figure 1b) in its skin (black spot disease), had also been consumed. Thirdly, the presence of approximately 10,000 mature *Microphallus piriformis* (Figure 1a) in the small intestine confirmed that the raccoon had actively been eating periwinkles *Littorina* spp., infected with metacercaria of this small digenean. Fourthly, the presence of at least two distinct *Microsomacanthus* cestode species (one shown on Figure 1c) confirmed the consumption

Table 1. Parasites of a subadult female raccoon *Procyon lotor,* unintentionally transported to Iceland in late winter 2018 with information on the infection site of the parasite in the host, and if the parasite occurs in Iceland (Ic), America (Am) or Europe (Eu), according to Gey (1998) and Skirnisson (2016). Unknown intensity of infection is noted with "Present".

Parasite	Intensity	Infection site	Occurence
Protozoans			
Sarcocystis sp.	Present	Tongue and musculature*	Am, Eu,
Eimeria nuttally	Present	Faecal sample (rectum)	Am
Cestodes			
Microsomacanthus spp.	~100	Small intestine	Ic
Digeneans			
Microphallus piriformis	~10.000	Small intestine	Ic
Cryptocotyle lingua	~200	Small intestine	Ic
Nematodes			
Capillaria procyonis	Present	Tongue*	Am
Capillaria putori	51	Stomach	Am
Capillaria plica	Present	Urinary bladder	Am, Eu
Molineus barbatus	1	Small intestine	Am
Placoconus lotoris	63	Small intestine and colon	Am
Anisakis simplex	1	Stomach	Ic, Am, Eu
Acanthocephalans			
Polymorphus phippsi	2	Entangled in feathers in colon	Ic

*Confirmed with microscopical examination of histological samples

of some infected amphipods on the shore; amphipods are reported to host cysticercoids of several *Microsomacanthus* species in Iceland (Skírnisson 2016). Finally, two mature *Polymorphus phippsi* acanthocephalans were also identified. However, as they were detected within a bundle of feathers, rolled up in the lumen of the intestine, not free or attached to the intestinal wall of the raccoon, they are believed to have been parasites of the aforementioned Purple sandpiper.

In general, the above findings are consistent with reports on the general feeding habits and wide adaptations of raccoons and indicate that the animal did not have problems in finding appropriate food in the area. The raccoon had most likely survived for several days, even for some weeks, in Iceland when it was shot.

The seven remaining parasites found include two protozoans and five nematodes (Table 1). Tissue cysts of *Sarcocystis* sp. were detected in histological sections from the tongue and the pelvic musculature. In America, raccoons are reported to be an intermediate host for five, and final host for a further three, *Sarcocystis* species (Gey 1998). Which species occurred in the vagrant raccoon in Iceland remains unknown, however.

Figure 1. Photomicrographs of three indigenous parasite species detected in the intestine of a vagrant raccoon, *Procyon lotor*, that acquired helminth infections by consuming the respective intermediate hosts on the coast in Iceland (see text): **a** *Microphallus piriformis*, **b** *Cryptocotyle lingua*, **c** *Microsomacanthus* sp. Scale bars **a,b** 250 µm, **c** 100 µm.

A single eimeriid species was found. Given other evidence suggesting the origin of the raccoon (see below), it was assumed to be one of two eimerids, both with similar-sized oocysts, which infect raccoons in America. As no micropyle was detected on the oocysts examined in the present study, the species involved was identified as *Eimeria nuttally*. The other eimerid reported from raccoons, *Eimeria procyonis*, usually has an micropyle. *E. procyonis* was carried by raccoons over to Europe and is at present commonly identified from raccoons on both continents (Gey 1998).

Three *Capillaria* species are known to infect raccoons (Gey 1998). In America, *C. procyonis* occurs in the mouth epithelia, tongue and oesophagus and *C. putorii* parasitizes in the stomach and the intestine. Both species were found in the raccoon in Iceland (Table 1). However, these species are not reported in Europe. The third species, *C. plica*, was detected in scrapings from the urinary bladder of the raccoon in Iceland (Figure 2). This species occurs on both continents and is known to infect other species in Europe, e.g. red fox *Vulpes vulpes* (Gey 1998).

The two remaining nematode species found were *Molineus barbatus* and *Placoconus lotoris* (Figure 2, Table 1). Both are well known parasites of American raccoons and are not found in Europe (Gey 1998).

Based on the parasite composition of the vagrant raccoon transferred to Iceland and the geographical distribution of the distinct species (Table 1), it seems clear that the raccoon arrived from America.

Way of transportation

Raccoons can be transported to Iceland either in ships or by airplane. The harbour of Helgavík is located approximately 8 km away from the seashore where the raccoon was captured. Enquiries at the harbour office revealed that freight ships transporting containers from America have not been brought to the harbour for more than a decade. Therefore, importation by ship to Helgavík should be excluded. However, importation of a raccoon to Iceland in a container has already been documented (Anonymous 1998). In late October 1998, a raccoon, creeping on a wooden pallet close to the door of a container filled with hot tubs was observed when the container was opened. Almost four weeks earlier, the container had been loaded in Toronto, Canada, locked up, brought to New York harbour, taken aboard on a container ship and transported to Iceland.

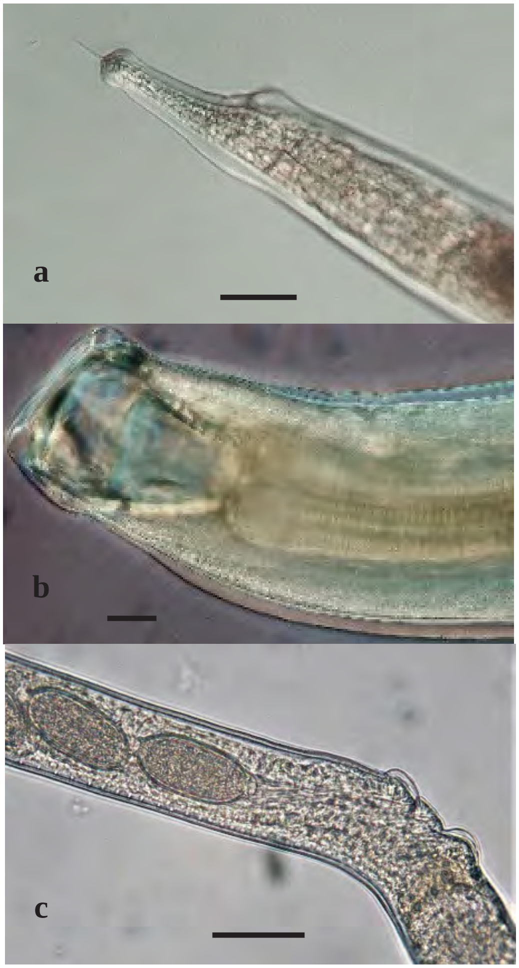

Figure 2. Photomicrographs of three intestinal nematodes detected in a vagrant raccoon, *Procyon lotor,* found on the coast close to Keflavík International airport in Iceland. Note that these parasites occur in raccoons in America but not in Europe (Gay 1998): **a** Posterior end of a female *Molineus barbatus*, **b** Buccal capsule of *Placoconus lotoris,* **c** Vulva region of *Capillaria putori*. Scale bar 50 µm.

When detected, the raccoon was still alive but very weak, unable to move and about to die after being locked up in the container for almost a month. In the present case, the good physical condition of the raccoon did not suggest recent starving in a closed container without access to food and water. On the contrary, the raccoon is considered to have come to Iceland by airplane by hiding in goods that were loaded onto the plane. Every day, several cargo and passenger planes leave from different airports in the USA, and are unloaded 6-8 hours later at the Keflavík International Airport, about 4 kilometres away from the site in Hafnir where the raccoon was detected.

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Interactions between nitrogen release from organic fertilisers and organic horticultural soils

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ABSTRACT

Mushroom compost has been one of the most commonly used fertilisers in organic greenhouse vegetable production in Iceland. However, mushroom compost contains conventional chicken manure. Therefore, the use of this fertiliser is prohibited in organic horticulture. In search of alternative nutrient resources, nine different substitutes for mushroom compost (plant compost, four composted animal manures, fishmeal, white clover residues, coarse meal of faba beans, 'Pioner complete 6-1-3®') were tested on two soils differing in organic matter in a pot experiment. Dry matter yield (DM) and the apparent N utilisation with the model plant perennial ryegrass was quantified in five cuts throughout 131 days. Both the DM yield and the apparent N utilisation was influenced by the fertiliser, the soil and their interaction. The N release of the tested alternative organic fertiliser resources was described by the N content and C:N ratio of the fertilisers, but was also affected by the soil. It was concluded that the composted animal manures provided an N release equal to mushroom compost and could therefore act as substitutes.

Keywords: C to organic N ratio; N content; nitrogen release; organic farming; organic fertiliser

YFIRLIT

Samspil köfnunarefnislosunar frá lífrænum áburði og lífrænum ylræktarjarðvegi
Sveppamassi hefur verið einn mest notaði áburður í lífrænni grænmetisframleiðslu á Íslandi. Hins vegar, þar sem hann inniheldur hænsnaskít sem ekki er lífrænt vottaður, er notkun þessa áburðar bönnuð í lífrænni garðyrkju. Þess vegna voru aðrar gerðir áburðar í stað sveppamassa (plöntumolta, fjórar mismunandi moltugerðir úr búfjáráburði, fiskimjöli, plöntuleifum af hvítsmára, möluðu fræi af hestabaunum, 'Pioner complete 6-1-3®') prófaðar í pottatilraun í tveimur gerðum af jarðvegi sem voru með mismikið magn af lífrænum efnum. Þurrefnisuppskera og niturnýting með rýgresi var mæld fimm sinnum á 131 dags vaxtartímabili. Bæði, þurrefnisuppskera og niturnýting var undir áhrifum af tegund áburðargjafa, jarðvegs og samspils þeirra. N nýting var lýst með N innihaldi og C:N hlutfalli áburðarins, en var einnig undir áhrifum af jarðvegi. Niðurstaðan var sú að moltan úr búfjáráburði gæfi jafnmikla niturupptöku eins og sveppamassi og gæti því verið notaður í staðinn.

INTRODUCTION

Organic fertilisers and crop residues are important nutrient sources for plants in sustainable crop systems (Whalen et al. 2013). Depending on the origin of the fertiliser, the proportion of N available to plants in organic fertilisers varies greatly (Gutser et al. 2005, Sradnick & Feller 2020). The inorganic N content in organic fertilisers ranges from 0% of total N in keratin-based fertilisers to 70% and more in liquid organic fertilisers. A considerable part of the N is organically bound. The organic bound N fraction of fertilisers is undergoing microbial decomposition and has to be first mineralised before available for plant uptake in the inorganic form. Different factors can affect N mineralisation, including soil properties (among other things rate of mineralisation of native soil organic matter) as well as the properties of the organic material (Magdoff 1978, Robertson & Groffman 2007). Consequently, the amount of plant-available N from organic fertilisers in the soil varies in the first months after application (Gutser et al. 2005).

In Iceland, mushroom compost was one of the most commonly used fertilisers in organic vegetable production until the end of June 2013. Since July 2013, the use of mushroom compost in organic production was forbidden according to the requirements for organic production set by Council Regulation *(EC) No 834/2007* of 28 June 2007 and implementing regulations (*No 889/2008* and *No 1235/2008*) as it contains conventional chicken manure. Although organic chicken husbandry has been practiced in Iceland since the beginning of 2016 (Hreiðarsson 2016), the conventional chicken manure in mushroom compost has not yet been replaced. Therefore, the exclusion of mushroom compost as a fertiliser in organic horticulture cannot be withdrawn. Thus, suitable substitutes for mushroom compost are required. To date, few studies on organic fertilisers focusing on yield of vegetable crops have been conducted in Iceland. In contrast, many studies focused on the effect of organic fertilisers in other European countries. However, the results of these studies cannot be transferred to Iceland as suitable organic fertilisers are not available in Iceland and the associated import costs are high. In addition, suitable organic fertilisers in European countries might give different results in Iceland. Experiments were mainly conducted with mushroom compost (Gunnlaugsson 1995, Gunnlaugsson 1997), mushroom compost in comparison to fishmeal (Gunnlaugsson & Guðfinnsson 2004) or a mixture of fishmeal and seaweed in comparison to NuGro (liquid fertiliser based on fishmeal) (Stadler et al. 2010). However, additional fertilisers might be suitable for substitution, e.g. horse and sheep manure from organic production or from conventional production after composting or crop residues.

Due to a high N requirement of vegetable crops, N fertilisers used for organic vegetable production should ensure high N turnover, fast N availability, and continuous N supply. Therefore, the prediction of N mineralisation in organic fertilisers is an important tool to optimise organic fertiliser application (Sradnick & Feller 2020). However, due to a lack of information on the amount of plant-available N that is mineralised within the growth period, knowledge to predict the N mineralisation of organic fertilisers is crucial (Sradnick & Feller 2020). Several studies agreed that the C to N or the C to N_{org} ratio of the organic fertiliser – plant residues, manure, mushroom compost – was the main factor influencing the net N release within the first months after application (Trinsoutrot et al. 2000, Kumar et al. 2003, Stadler et al. 2006, Chen et al. 2014). However, as N release depends on soil characteristics (Stadler 2006), it is therefore important to study the net N release from organic fertilisers in different soils.

The aim of this study was to investigate: (1) the effect of composted animal and crop residues, legumes and commercial organic fertilisers on DM yield and apparent N utilisation by ryegrass, (2) whether N release from these fertilisers can be predicted by their N content or C:N ratio, and (3) whether this relation is subject to modification by different soils.

MATERIAL AND METHODS

Fertilisers

Composted animal residues (cow, sheep, horse or chicken manure) from organic production, residues from the fish industry (fishmeal, Síldarvinnslan hf., Neskaupstaður, Iceland), crop residues (plant compost, white clover residues), coarse meal of faba beans (*Vicia faba* L.), the imported industrially processed plant residue ‘Pioner complete 6-1-3®’ (liquid fertiliser based on sugar beets and sugar cane, Azelis) were investigated in a pot experiment. Mushroom compost (Flúðasveppir ehf., Flúðir, Iceland) was commonly used as a fertiliser in organic vegetable production in Iceland and, therefore, is used as a reference. All kinds of composts were fully composted and mature. In contrast, white clover was cut into small cuttings and mixed while fresh (15.6% DS) into the soil.

Generally, residues with a small particle size showed a stronger and longer N immobilisation and subsequently lower N mineralisation than those with a large particle size (Corbeels et al. 2003). Therefore, to minimise particle size effects so as to better compare the investigated fertilisers, faba beans were coarsely milled and the composted plant and animal residues were sieved to pass through a 5.0 mm screen before chemical analysis.

The selected organic fertilisers show a wide range in N and C content and C:N ratio (Tab. 1). The N content was low in the plant compost (0.9% N), mushroom compost and composted animal manure (1.9-2.6% N). White clover residues, coarse meal of faba beans and Pioner complete 6-1-3® had intermediate values (3.6-5.4% N). The N content (10.9% N) of the commercial organic fertiliser of animal origin, fishmeal, was high. The C content of the investigated organic fertilisers varied considerably. The C:N ratio of composted animal manure, white clover residues and coarse legume meal was high (7.9-17.2). In contrast, the C:N ratio was much lower for Pioner complete 6-1-3® (2.8) and fishmeal (3.9). The P content in relation to the N content was very high for white clover residues (N:P ratio: 21), but relatively low for plant compost (N:P ratio: 2.2). The coarse meal of faba beans (N:P ratio: 7.4), cow compost (N:P ratio: 6) and remaining fertilisers (N:P ratio: 3-5) had medium values. The N:K ratio for all fertilisers was between 1-2, but higher for the coarse meal of faba beans and white clover residues (about 3.5) and for fishmeal (nearly 9).

Soils

Two loess soils (Vitric Andosols according to WRB), differing in the amount of organic matter and total nitrogen (N_t) as well as in the content of P and K, were selected from greenhouses (Tab. 2). The greenhouse soils were obtained

Table 1. N, C, P and K content and C:N, N:P and N:K ratios of the tested organic fertilisers.

Fertiliser	N content* (%)**	C content* (%)**	C:N ratio	P* (%)****	K* (%)****	N:P ratio	N:K ratio
Mushroom compost (reference)	1.91	22.2	11.6	0.44	1.04	4.3	1.8
Plant compost	0.89	10.5	11.8	0.40	0.46	2.2	1.9
Cow compost	1.92	21.7	11.3	0.58	0.89	3.3	2.2
Horse compost	1.98	34.0	17.2	0.54	1.91	3.7	1.0
Sheep compost	2.53	34.6	13.7	0.42	2.68	6.0	0.9
Chicken compost	2.57	20.3	7.9	0.80	1.26	3.2	2.0
White clover residues	3.56	38.5	10.8	0.17	1.05	20.9	3.4
Coarse meal of faba beans	4.29	42.7	10.0	0.58	1.24	7.4	3.5
Pioner complete 6-1-3®	5.40	15.2	2.8	1.25	3.75	4.3	1.4
Fishmeal	10.94	42.7	3.9	2.25	1.24	4.9	8.8

* Values are referring to DM (for ‘Pioner compete 6-1-3®’ was a fresh sample analysed)

** Chemical analysis according DIN ISO 13878

*** Chemical analysis according to DIN ISO 10694

**** Chemical analysis according to the German fertiliser ordinance (VDLUFA)

Table 2. Characteristics of the greenhouse soils used.

Soil name	C_{org}	N_t	C:N	pH ($CaCl_2$)	P (CAL)	K (CAL)
	(—— %	——)			(mg / 100 g dry soil)	
soil $_{\text{lower Corg}}$	6.8	0.6	10.7	6.4	27	18
soil $_{\text{higher Corg}}$	12.8	1.0	13.0	6.5	287	49

from organic vegetable growers from the 0-20 cm soil layer and sieved to pass through a 5.0 mm screen before chemical analysis. Both soils were under organic cultivation for more than 20 years. Soil $_{\text{higher Corg}}$ regularly received mushroom compost, while soil $_{\text{lower Corg}}$ received plant compost.

Pot experiment

A pot experiment with ten fertilisers (Tab. 1) and a treatment without fertiliser as a control (Tab. 2) was carried out with two different soils in winter 2012/2013 at the experimental greenhouse facilities of the Agricultural University of Iceland at Reykir, Hveragerði (21°12′W, 64°0′N), South Iceland. The experiment was set up in one greenhouse chamber (60 m^{-2}) which provided computer-controlled optimal microclimate conditions (18 °C / 15 °C (day / night), 240 W m^{-2}). 3.7 kg of moist (2.3 kg dry) soil $_{\text{higher Corg}}$, 4.0 kg of moist (2.8 kg dry) soil $_{\text{lower Corg}}$, respectively, was filled into five-litre pots with a height of 16 cm and a diameter of 20 cm. Eight hundred milligrams of fertiliser N (Tab. 1), equivalent to 255 kg N ha^{-1}, were mixed into the upper half of the soil in three replicates. In total, 66 pots were in the greenhouse chamber. Perennial ryegrass seeds (*Lolium perenne*, L. cv. ‘Birger’; 1.5 g) were sown at a depth of 0.5 cm two weeks after the addition of the fertiliser. The pots were covered with plastic until the germination of the ryegrass and were regularly watered to achieve a maximum 60% water holding capacity, which corresponded to 4.1 kg and 4.7 kg of soil (soil $_{\text{higher Corg}}$ / soil $_{\text{lower Corg}}$), respectively.

The ryegrass was cut to 1.5 cm stubble height 26, 48, 75, 106 and 131 days after sowing. Ryegrass was oven-dried for 24 h at 105 °C to determine the dry matter yield (DM). Samples were milled and their total N content was analysed according to the dry combustion DUMAS method using varioMax CN, Macro Elementar Analyser, Elementar Analysensysteme GmbH, Hanau, Germany to be able to determine N uptake in ryegrass.

The apparent N utilisation with the model plant perennial ryegrass was calculated over time as the additional N uptake of ryegrass compared to the control divided by the added fertiliser N. Residual soil mineral N after the final harvest was not included.

The apparent N utilisation was calculated as:

Apparent N utilisation (%) = (A – B) / C × 100,
A: Cumulative N uptake of fertilised ryegrass shoots at harvest in mg pot^{-1},
B: Cumulative N uptake of unfertilised ryegrass shoots (control) at harvest in mg pot^{-1},
C: N fertilised (= 800 mg pot^{-1}).

Statistical analyses

Statistical analysis was carried out using SAS (SAS 9.2; SAS Institute, Cary, NC). One factor analysis of variance with Tukey/Kramer HSD test at the significance level $\alpha = 0.05$ was applied for the comparison of the means between organic fertilisers. A two factor analysis of variance with Tukey/Kramer HSD was applied to determine how organic fertilisers and soils affect dry matter yield and apparent N utilisation and to determine whether or not there were interaction effects between organic fertilisers and different soils. Regression and correlation analyses were calculated using the SAS procedures “proc reg” and “prog corr”.

RESULTS

Dry matter yield

The pot experiment was conducted to determine the availability of organic-fertiliser N to ryegrass. DM yield was influenced both by the fertiliser and by the soil (Fig. 1). A fertiliser application to soil $_{\text{lower Corg}}$ increased DM yield compared to the unfertilised control in all five cuts, but the application of white clover residues resulted in a lower DM yield in the first cut than in the unfertilised treatment (Fig. 1a). In contrast, for white clover residues, the DM yield of the third and fourth cut was very high compared to the other fertiliser treatments. Fishmeal and Pioner complete 6-1-3® had an especially high DM yield during the first three cuts. Compared to these fertilisers, the application of mushroom, cow, sheep and horse compost resulted in lower DM yields during the first two cuts. In contrast, chicken compost and coarse meal of faba beans had only a slightly higher DM yield during the first two cuts than the unfertilised control, but after the second cut their DM yield was higher than those of the other composts. The DM yield of chicken compost at the fourth cut was still high, while all other composts gained just a small DM yield increase after the third cut. Plant compost was characterised by only a slightly higher DM yield during all five cuts than the unfertilised control. After the fourth cut, for all fertiliser treatments only a slight DM yield increase was achieved. After the fifth cut, DM yield was higher with fertilisers with a high N content. The highest DM yields were achieved with fishmeal (29 g pot^{-1}) and Pioner complete 6-1-3® (28 g pot^{-1}), followed by white clover residues (25 g pot^{-1}). Intermediate and not significantly different values of DM yields were obtained for composted animal residues (18-22 g pot^{-1}) and mushroom compost (19 g pot^{-1}). The lowest DM yields were achieved with plant compost (16 g pot^{-1}) and the control (14 g pot^{-1}); however, there were mainly no significant differences to the animal composts and to mushroom compost.

In contrast, soil $_{\text{higher Corg}}$ acted differently (Fig. 1b): a fertiliser application did not increase DM yield at any of the five cuts. Especially white clover residues, but also coarse meal of faba beans had a much lower DM yield than the unfertilised control during all cuts. In contrast to soil $_{\text{lower Corg}}$, the increase in DM yield was nearly comparable in all five cuts. After the last cut, the unfertilised control (36 g pot^{-1}) had a similar yield level to the composted animal fertilisers (31-40 g pot^{-1}), mushroom compost (34 g pot^{-1}) and plant compost (30 g pot^{-1}). However, when fertilisers with a high N content were applied, DM yield was significantly suppressed to 17-23 g pot^{-1} (white clover residues, coarse meal of faba beans, Pioner complete 6-1-3®), whereas DM yield obtained with fishmeal (29 g pot^{-1}) was higher.

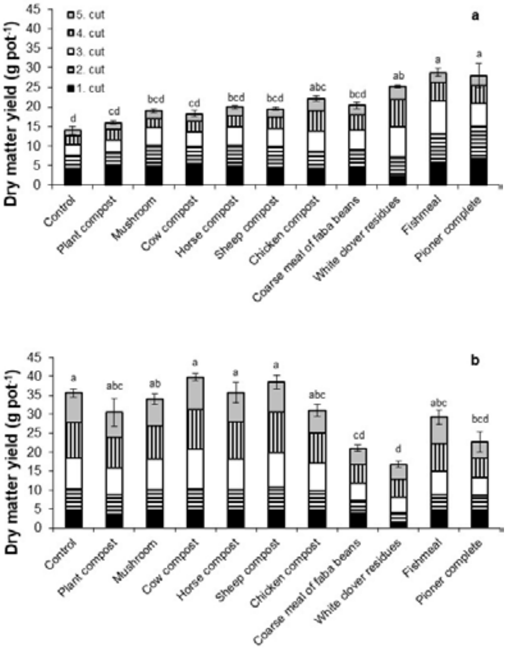

Figure 1. Dry matter yield of ryegrass after application of organic fertilisers to “soil $_{\text{lower Corg}}$” (a) and “soil $_{\text{higher Corg}}$” (b). Ryegrass was grown for 131 d at a maximum 60% water holding capacity and shoots were harvested five times. Differences between fertilisers are based on Tukey’s HSD test and different letters indicate statistically significant differences between means of total dry matter yield at the p = 0.05 level (n=3). Error bars indicate standard deviations of total dry matter yield.

The results of the two-way ANOVA revealed that there was a statistically significant difference in DM yield of ryegrass between both organic fertilisers (F(10)=3.08, p < 0.0048) and soils (F(1)=88.95, p < 0.0001) as well as their interaction (F(10)=9.94, p < 0.0001).

N utilisation

The time course of the apparent N utilisation in ryegrass differed strongly depending on the organic fertiliser used and showed a different pattern among soils (Fig. 2). For soil $_{\text{lower Corg}}$ (Fig. 2a), most of the N – in relation to the total mineralised N at the end of the experiment – was already released before the second cut (about 50% of the total N uptake) except for white clover residues, chicken compost and coarse meal of faba beans. Thereafter, the increase in the apparent N utilisation was markedly lower, but very high for white clover residues. During the whole experiment Pioner complete 6-1-3® had the highest N uptake of all the fertilisers (85%). The cumulative fertiliser N uptake of ryegrass was also high for fishmeal (75%) and white clover residues (80%). However, at the beginning of the experiment, fertiliser N was immobilised in the clover treatment, but after that apparent N utilisation increased rapidly. A medium apparent N utilisation in ryegrass (40-50%) was achieved for chicken compost and coarse meal of faba beans, but seems not to have been fully completed at the end of the experiment. The cumulative fertiliser N uptake was lowest (10-20%) for plant, sheep, cow and horse compost as well as for the reference fertiliser mushroom compost.

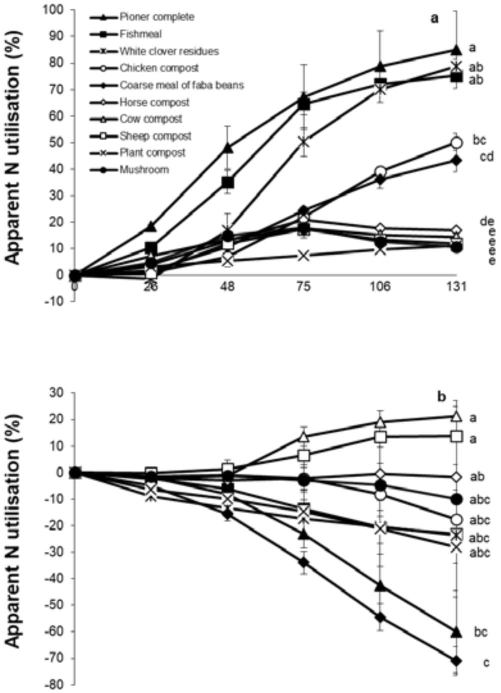

Figure 2. Apparent N utilisation of organic fertilisers by perennial ryegrass during the pot experiment with “soil $_{\text{lower Corg}}$” (a) and “soil $_{\text{higher Corg}}$” (b). Ryegrass was grown for 131 days at a maximum 60% water holding capacity, and shoots were harvested five times. Differences between fertilisers are based on Tukey’s HSD test and different letters indicate statistically significant differences between means at the p = 0.05 level (n=3). Error bars indicate standard deviations and are contained within the symbol if not indicated.

In contrast, soil $_{\text{higher Corg}}$ (Fig. 2b) was characterised by an immobilisation of N with nearly all fertilisers. The immobilisation even increased with longer ryegrass growing period and was highest for fertilisers with a high N content. At the end of the growth period, N immobilisation amounted to 60-70% for Pioner complete 6-1-3® and coarse meal of faba beans. Plant compost, white clover residues, fishmeal, chicken compost and mushroom compost had an N immobilisation of 10-30%, while horse compost immobilised only slightly. All of these fertilisers immobilised N during the whole growth period. In contrast, an application of sheep and cow compost to ryegrass was characterised at the beginning of the growth period by an N immobilisation, but after about 10 weeks of growth the immobilised N in these treatments mineralised and N utilisation amounted to 15-20% at the end of the growth period. Interestingly, this amount was comparable to the apparent N utilisation of the same fertilisers at soil $_{\text{lower Corg}}$.

The results of the two-way ANOVA revealed that there was a statistically significant difference in apparent N utilisation by ryegrass in all five cuts by both organic fertilisers (last cut: F(9)=3.65, p = 0.0021) and soils (last cut: F(1)=173.45, p < 0.0001) as well as their interaction (last cut: F(9)=13.78, p < 0.0001).

Relationship of N utilisation and fertiliser characteristics

As a general rule, it can be said that fertilisers with a higher N content showed a higher apparent N utilisation in ryegrass in soil $_{\text{lower Corg}}$ and a higher negative apparent N utilisation in ryegrass in soil $_{\text{higher Corg}}$. However, this relationship was very weak (Fig. 3a: r^2=0.50 *; Fig. 3b: r^2=0.12 $^{\text{n.s.}}$) and not fitting for all fertilisers (e.g. fishmeal).

The C:N ratio explained the relationship slightly better than N content, even though the relationship was still weak (Fig. 4a: r^2=0.61 **; Fig 4b: r^2=0.30 $^{\text{n.s.}}$). Generally it can be said that in soil $_{\text{lower Corg}}$, fertilisers with a high C:N ratio (e.g. composted animal manure) showed a low apparent N utilisation, whereas fertilisers with a low C:N ratio (e.g. fishmeal, Pioner complete 6-1-3®) are rapidly available for plants and resulted in a high apparent N utilisation. However, some exceptions can also be found here. For instance, white clover residues had a medium C:N ratio, but the apparent N utilisation was high. In contrast, in soil $_{\text{higher Corg}}$, fertilisers with a high C:N ratio (most of the composted animal manures) showed a higher N utilisation than fertilisers with a low C:N ratio (e.g. Pioner complete 6-1-3®).

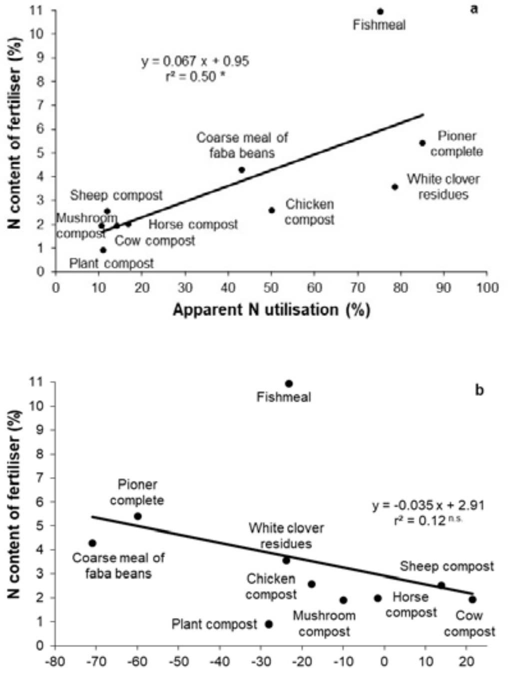

Figure 3. Relationship between apparent N utilisation of applied total N in perennial ryegrass and N content of organic fertilisers with "soil $_{\text{lower Corg}}$" (a) and "soil $_{\text{higher Corg}}$" (b). Apparent N utilisation was calculated based on the cumulative N uptake (five cuts) during 131 days of growth.

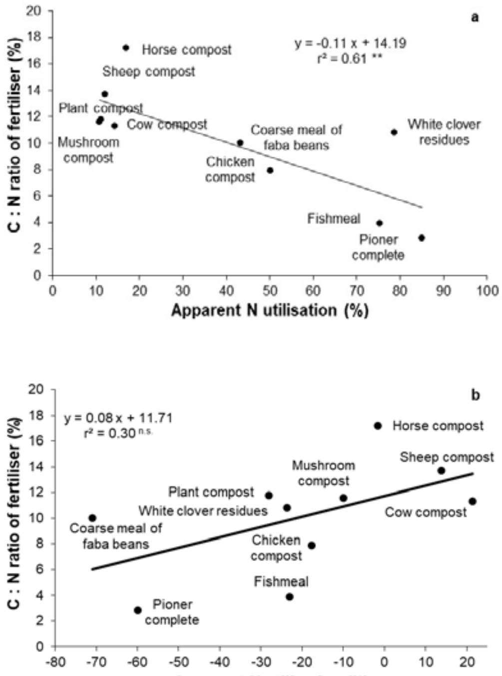

Figure 4. Relationship between apparent N utilisation of applied total N in perennial ryegrass and C:N ratio of organic fertilisers with "soil $_{\text{lower Corg}}$" (a) and "soil $_{\text{higher Corg}}$" (b). Apparent N utilisation was calculated based on the cumulative N uptake (five cuts) during 131 days of growth.

DISCUSSION

The tested organic fertilisers differed strongly regarding to DM yield and apparent N utilisation in ryegrass. N release was described by the N content and the C:N ratio of the tested fertilisers, though with a low correlation, suggesting that factors other than the N content and the C:N ratio must be responsible for a low, medium or high apparent N utilisation. Fertilisers that are characterised by a low N content, and thus a relatively high C:N ratio, resulted in a low and slow apparent N utilisation in soil $_{\text{lower Corg}}$, while this fertiliser group resulted in soil $_{\text{higher Corg}}$ in a slow but either low or slightly negative apparent N utilisation. In contrast, most of the fertilisers that featured a high N content, and thus revealed a low C:N ratio, provided a high amount of plant available N in soil $_{\text{lower Corg}}$. Consequently, the dynamics in DM yield during the different cuts reflect a fast N release. Therefore, the high apparent N utilisation rates can be explained by the high proportion of readily available N_{org} for microbial conversion in the soil (Stadler 2006). Furthermore, the results emphasised that the type and processing of organic N fertilisers was largely responsible for the N composition and mineralisation properties as was also observed by Stadler et al. (2006). For instance, the poorer performance of the N content with respect to C:N ratio in predicting the N release from fertilisers could be ascribed to the relatively high N content of fishmeal. However, a close relationship between the above mentioned fertiliser characteristics and their N mineralisation was found by Stadler et al. (2006) and Sradnick & Feller (2020). Therefore, fertiliser N content and C:N ratio are suitable indicators for predicting the N release from organic fertilisers, provided the fertiliser characteristics are sufficiently different (Stadler et al. 2006). Often a critical C:N ratio for net N immobilisation of 20-30 was reported (Trinsoutrot et al. 2000, Chen et al. 2014), while lower critical C:N ratios are also known. For example, Geisseler et al. (2021) mentioned a threshold of about 15 in their review. In contrast, in the present experiment, N immobilisation occurred in the more fertile soil already with a C:N ratio of much less than 10. Therefore, it can be assumed that, in addition to the C:N ratio of fertilisers, other factors might be attributed to N mineralisation / immobilisation processes of organic fertilisers. Potential interactions between organic fertilisers and soils can be expected. DM yield and apparent N utilisation in ryegrass was not only influenced by the fertiliser, but also by the soil and the interaction of fertiliser and soil. While application of organic fertilisers to soil $_{\text{lower Corg}}$ promoted plant growth and is characterised by an N mineralisation of all fertilisers, in soil $_{\text{higher Corg}}$ an immobilisation of N with nearly all fertilisers occurred. The immobilisation was even more pronounced for organic fertilisers with a high N content and consequently low C:N ratio and increased with a longer growing period possibly due to the inhibition of growth due to an excess supply of N. This is confirmed by the fact that cow and sheep compost were the only fertilisers which did not suppress growth (DM yield) and released N very slowly. Indeed, Ren et al. (2014) also stated that soils with higher C_{org} contents may immobilise more N. However, in the literature no obvious relation of soil organic matter to N mineralisation is reported (Bending et al. 2002, Stadler et al. 2006). Soil characteristics influenced N mineralisation more strongly for fertilisers with a lower N content (Stadler et. al. 2006). In contrast, the presented results showed that fertilisers with a high N content (e.g. fishmeal) caused a higher variation in N mineralisation depending on the C_{org} content of the soil (high N mineralisation in soil $_{\text{lower Corg}}$, N immobilisation in soil $_{\text{higher Corg}}$), whereas this variation was less pronounced for fertilisers with a lower N content (composted animal manures) because of less available N.

Due to the regular application of mushroom compost to soil $_{\text{higher Corg}}$, the total organic matter and mineralisable N of the soil increased. Therefore, it can be expected that the turnover of the organic substance might affect the N utilisation of organic fertilisers. Indeed, Thomsen et al. (2003) observed that the N turnover of organic matter added to soil in crop residues and manure affects the availability of N mineralised from both added and native soil organic matter. It is possible that too much N was applied to soil $_{\text{higher Corg}}$, e.g. in the form of fishmeal and Pioner complete 6-1-3®, due to the high availability of N from fertiliser applications in the form of mushroom compost from previous years and therefore, this soil was not able to deal with all the available N. Consequently, the newly applied fertiliser N was immobilised instead. Soil samples of mineral N could have given additional information. However, the N content in ryegrass, which was still high for all fertilisers in the fourth and fifth cut (around 4.5% N, data not shown) in soil $_{\text{higher Corg}}$ is emphasising a high N supply, while in soil $_{\text{lower Corg}}$, N content was dependent on the fertiliser treatment (4-4.5% for chicken compost, coarse meal of faba beans, Pioner complete 6-1-3® and plant compost; 1.5-2.5% for mushroom compost, sheep compost, unfertilised control and cow compost). Stadler (2006) observed that the lower apparent N utilisation of peas (3.7% N) was more pronounced in the higher C_{org} content soils. Moreover, Thönnissen et al. (2000) reported an often marginal effect of green manure on yield of tomatoes in fertile soils (high soil organic matter), but its high effect on poor soils (low soil organic matter). Magdoff (1991) obtained the highest amount of available N from soils with medium N_t and C_{org} content. He explained this result by the fact that at low organic matter contents, the mineralisation rate was high, but less N was available due to the low content of organic N. Soils high in N_t and C_{org} had low mineralisation rates which consequently resulted in low amounts of mineralised N as well. This might explain the high N mineralisation of the unfertilised control observed in soil $_{\text{higher Corg}}$. However, as soil mineral N content has not been measured before fertiliser application, no clear interpretation of this issue can be made. Magdoff (1978) concluded that soils with a high mineralisation rate of soil organic matter may also rapidly mineralise N from manure, whereas in a soil with a low soil N mineralisation rate, manure N release will be lower. However, this was not in line with the presented results.

Commercial organic fertilisers of animal origin (fishmeal) with a fast and high N release rate, as well as commercial industrially processed plant residues (Pioner complete 6-1-3®) should be highlighted as efficient organic fertilisers on the less fertile soil. Indeed, Sradnick & Feller (2020) also reported that due to their high release rate of inorganic N, the use of commercial organic fertilisers of animal origin meets the demand of plant species with high N requirements in vegetable production systems. Since their N to P ratio is relatively high, they qualify as a suitable fertilisation option in intensive organic production systems. The presented results further indicate that a higher efficiency of fertiliser could be reached by applying the aforementioned fertiliser group in more than one application for long-growing crops to better match the N release from fertilisers and the N demand of long growing crops.

Since only small amounts of organic N are available for N uptake from composted organic material (manure, plant residues) – except chicken compost – into the plant in the first four months of application, these organic fertilisers are only suitable for soil fertility improvement. Thus these soil amendments represent a fertiliser with long-term N mineralisation. As the total N utilisation of mushroom compost was comparable to the other composted animal manures, these fertilisers can act as a replacement for mushroom compost. However, despite the absence of livestock at most Icelandic vegetable producers, animal manure could be used from farms with organic animal production or from farms with conventional production after composting.

CONCLUSIONS

In the year of application, organic fertilisers were able to mineralise 10% (compost) to 80% (fishmeal, Pioner complete 6-1-3®) of the total N applied on the less fertile soil. Consequently, it can be assumed that the regular application of fertilisers with a low N mineralisation over several years will tend to increase the total soil organic matter and N that can potentially be mineralised. It should therefore be kept in mind whether long-term application of these fertilisers may require a modified application strategy. In addition, application strategies need to be adjusted depending on the selected greenhouse soil as, for the more fertile soil most fertilisers decreased the DM yield and consequently N was immobilised. Cow and sheep compost with a slow N release turned out to be the best suitable fertilisers on these soils. More research is needed to characterise soils that result after the application of organic fertiliser in N immobilisation processes. Fertiliser strategies need to be adapted to decrease and overcome high levels of N immobilisation and to maintain the recommended nutrient levels for optimal plant growth dependent on their soil fertility. However, for all greenhouse soils, the mineralisation / immobilisation properties of organic fertilisers can be predicted through the N content of the fertilisers and their C:N ratio, even though the precision of this relationship is influenced by the soil. Crucially, the prohibition of mushroom compost should not affect organic vegetable growers as there are at least equal (composted animal manures) fertilisers available.

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