Author(s): Jari Miina, Rainer Peltola, Pyry Veteli, Riikka Linnakoski, Marta Cortina Escribano,
Juho Haveri-Heikkilä, Pirjo Mattila, Pertti Marnila, Juha-Matti Pihlava, Jarkko Hellström,
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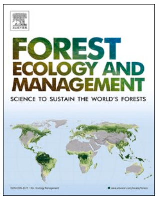

Inoculation success of *Inonotus obliquus* in living birch (*Betula* spp.)

Jari Miina [a], Rainer Peltola [b], Pyry Veteli [c], Riikka Linnakoski [c], Marta Cortina Escribano [a], Juho Haveri-Heikkilä [b], Pirjo Mattila [d], Pertti Marnila [e], Juha-Matti Pihlava [e], Jarkko Hellström [e], Tytti Sarjala [f], Niko Silvan [f], Mikko Kurttila [a], Henri Vanhanen [a,*]

[a] *Natural Resources Institute Finland (Luke), Joensuu, Finland*
[b] *Natural Resources Institute Finland (Luke), Rovaniemi, Finland*
[c] *Natural Resources Institute Finland (Luke), Helsinki, Finland*
[d] *Natural Resources Institute Finland (Luke), Turku, Finland*
[e] *Natural Resources Institute Finland (Luke), Jokioinen, Finland*
[f] *Natural Resources Institute Finland (Luke), Parkano, Finland*

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ABSTRACT

Inonotus obliquus is a pathogenic fungus—known as chaga in Asia and Russia and pakuri in Finland—that grows on broadleaves. Its sterile conks are used as an active ingredient in traditional healthcare products. Due to the higher value of pakuri than the tree it grows on, the cultivation of *I. obliquus* on living trees is expected to generate new and increased income opportunities for forest owners. So far, the success of large-scale inoculations of the fungus have not been studied. Therefore, the objectives of this study were to examine the probability of fungus infection and the appearance of sterile conks on birches, and to investigate the factors that affect the success of the inoculations. To this end, a total of 679 living birch trees (*Betula pendula* and *B. pubescens*) in 24 stands growing under different conditions in Finland were inoculated with two strains of *I. obliquus*, and re-examined after 4–5 years. The proportion of birches infected per stand was, on average, 79 ± 13%, whereas 6 ± 15% of birches per stand bore one or more conks. The size of the largest conk on a stem was, on average, 54 ± 64 cm^3. Part of the infected trees (13% of all trees studied) also showed signs of bulging and/or cracked bark, indicating conk formation under the bark. The main factors that improved the probability of successful infection and the incidence of conk on birches were southerly location, mineral soil texture, low mean tree diameter, high stand basal area, and inoculation at the beginning of the thermal growing season. Also, the fungal strain used had a significant effect on the probability of infection and bulging. Despite successful inoculation, the formation of conks was found to be a slow process. Therefore, the cultivation of *I. obliquus* may be difficult to integrate into the management of commercial forests, although set-aside, low-productive birch, especially *B. pubescens* stands, can be recommended for utilization in its cultivation.

1. Introduction

Consumer interest in the utilization of non-timber forest products (NTFPs) as food and nutritional supplements has been on the increase (Vidale et al. 2015), which has led to a rise in the application of multi-objective forest management; that is, managing forests for the production of both timber and NTFPs. For example, a new value chain, based on specialty mushroom production, has recently been introduced to Finnish forestry. The cultivation of *Inonotus obliquus* has been suggested for integration into the current management of birch (*Betula* spp.), with forest owners being able to utilize set-aside birch stands for its cultivation (Vanhanen et al. 2014, Verkasalo et al. 2017). *Inonotus obliquus* grows on broadleaved trees and forms sterile conks, known as chaga in Asia and Russia and Pakuri in Finland.

Inonotus obliquus has played an important part in ethnopharmacology, especially in Russia, for some years (Shikov et al. 2014). In Finland, the earliest industrial-scale production of sterile conks of *I. obliquus* for a tea product called Tikkatee (‘woodpecker tea’) began in the 1930s (Niemelä and Kotiranta 1983). There is a multitude of biologically-active compounds in the sterile conks of *I. obliquus* (Balandaykin and Zmitrovich 2015), with several studies having shown anti-inflammatory, anticancer and antidiabetic activities *in vitro* (Lishuai

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* Corresponding author.
E-mail address: henri.vanhanen@luke.fi (H. Vanhanen).

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et al. 2013, Kang et al. 2015) and in animal models (Mishra et al. 2012, Arata et al. 2016, Duru et al. 2019).

The fungus is found most commonly in birch stands in the circumboreal region of the Northern Hemisphere (Lee et al. 2008), but in some countries, it is has been red-listed as being rare (Piętka and Grzywacz 2006). The sterile conks on infected living trees consist of a charcoal-black mixture of woody material and fungal mass. Conk formation takes several years within the tree, with the conk eventually erupting through the bark. The fertile fruiting body of *I. obliquus* appears only after the host tree has died.

Conk formation requires an interaction between the fungus and the physiological defence mechanisms of the living host tree (Blanchette 1982). Formation of the appropriate bioactive compounds is thought to take place in the sclerotium on living trees, although the characteristics of *I. obliquus* mycelial culture, grown on an agar medium and in submerged culture (liquid fermentation in bioreactors), have also been studied (Kim et al. 2006, Chen et al. 2020). Research on the cultivation of wood-rotting culinary fungi on dead tree material has a long history (Badcock 1941), and fungiculture is currently a large-scale, global industry. Five genera—*Agaricus*, *Pleurotus*, *Lentinula*, *Auricularia* and *Flammulina*—constitute ca. 85% of the world́s mushroom supply, all cultivated on wood logs, sawdust or agricultural residues (Royse 2014). The inoculation of living trees with fungus is sometimes used to increase deadwood volume, aiming to create potential habitat trees that enable excavation by foraging and nesting by the primary cavity nester, the woodpecker. For example, Bednarz et al. (2013) found that fungal inoculation with *Fomitopsis pinicola* in western Washington state, USA resulted in visible conks or mycelia in about 27% of the treated trees inspected 8–9 years after inoculation. Also, Abrego et al. (2016) succeeded in reintroducing threatened deadwood-inhabiting fungi via inoculation in southern Finland. To the best of our knowledge, for culinary or medical fungi, the inoculation of *I. obliquus* is the only example of fungi cultivated on living trees.

To date, only naturally-growing *I. obliquus* have been collected from living trees for commercial use (Pilz 2004, Vanhanen et al. 2014), but for a viable value chain, a larger and more stable supply of raw material is needed. A growing demand for the raw material could be fulfilled by systematic, organized and sustainable cultivation, involving the inoculation of *I. obliquus*. So far, large-scale inoculations have not been conducted, although some inoculation experiments have been reported (Blanchette 1982, Piętka and Grzywacz 2006, Park et al. 2010, Silvan and Sarjala 2017).

In earlier cultivation attempts of *I. obliquus*, only in three of the studies, inoculation has been successful. In the study by Piętka and Grzywacz (2006), *I. obliquus* was inoculated as a conservation measure. The attempt was not successful which was, according to Piętka and Grzywacz (2006) due to other fungi outcompeting *I. obliquus*, or the defensive reaction of the trees generating false heartwood against fungal infection. The inoculations performed by Blanchette (1982) that were aimed to study the phytopathology of the species were successful. As with the study by Piętka and Grzywacz (2006), the defensive reaction of the host *Betula papyrifera* included occlusion of the xylem vessels and compartmentalization (Blanchette 1982). Despite these defensive reactions of the host tree against fungal infection, *I. obliquus* was able to evade and break down both the chemical and morphological barriers produced by the host (Blanchette 1982). In commercial cultivation experiments with *I. obliquus* executed by Park et al. (2010) and Silvan and Sarjala (2017), both were successful in generating infections, while the former also noted the formation of sterile conks three years post-inoculation.

The aim of this study was to examine the factors that affect the success rate of infection and the appearance of sterile conks on birches (*Betula pendula* and *B. pubescens*) inoculated with the fungal mycelium of *I. obliquus*. The success of the inoculation with two strains was measured 4–5 years after the inoculation of a total of 679 living birches in 24 stands in different locations that varied substantially in terms of their growing conditions. Using the resultant data, models were prepared to assess the probability of successful infection and the existence of sterile conks as a function of strain, the seasonal timing of inoculation, tree species, site and growing-stock characteristics. Based on the models, the characteristics most suitable for inoculation were determined. This study is the first of its kind to examine the large-scale commercial cultivation of *I. obliquus*.

2. Materials and methods

This study was executed according to the following steps: 1) the isolation of *I. obliquus* strains, and the preparation of inoculation plugs for use in the study (see Section 2.1.); 2) the establishment of inoculation trial sites in forests, acquired from private forest owners, and measurement of the site characteristics (see Sections 2.2. and 2.3.); 3) a follow-up on the trial sites 4 to 5 years after inoculation, and recording of the inoculation success at three different levels—visible infection, bulging of the bark and visible sterile conks (see Section 2.4.).

2.1. Isolation of I. obliquus strains

Samples of *I. obliquus* were collected from infected trees by drilling wood material from beneath growing conks, by kind permission of the landowner, the municipality of Liperi, North Karelia, Finland to MTT Agrifood Finland (since 1.1.2015, the Natural Resources Institute Finland, LUKE). The samples were plated on 2% malt-extract agar (Biokar Diagnostics), and fungal isolates were purified by sequential hyphal tipping, resulting in two isolates—J1 and J3 (leg. HV and RL). Mycelial DNA was extracted from the isolates using PrepMan Ultra Sample Preparation Reagent (ThermoFisher), and the extracted DNA was amplified by PCR with ITS1-F (CTTGGTCATTTAGAGGAAGTAA) and ITS4 (TCCTCCGCTTATTGATATGC) primers (White et al. 1990, Gardes and Bruns 1993). The sequencing was performed at Macrogen Europe Inc. The sequence data were compared to available data at the National Center for Biotechnology Information, which produced a 99% match to the *I. obliquus* sequences. The accession numbers are MW829454 and MW829455 respectively for J1 and J3 isolates. The isolates are maintained in the culture collection of the Natural Resources Institute Finland (Luke), Viikki, Helsinki.

After confirming the species identity by DNA analysis, inoculation plugs were produced by immersing birch plugs (10 mm in diameter, 40 mm long) into a sterile malt-extract solution (Biokar Diagnostics) for two days at the University of Eastern Finland. Following immersion, the plugs were canned and sterilized in an autoclave, and inoculated with malt-extract agar pieces overgrown by *I. obliquus* hyphae. The *I. obliquus* hyphae were allowed to overgrow the birch plugs, which were then kept at + 4 °C until used in the tree inoculations.

2.2. Study sites and measurement of site characteristics

The geographical coordinates (latitude, longitude), based on the national uniform coordinate system, were recorded for the locations of the 24 stands used in the study (Fig. 1). The climatic variables for the stands were based on measurements from meteorological stations interpolated onto a 10 × 10 km grid from the Finnish Meteorological Institute (Venäläinen et al. 2005). The climatic variables of the grid where the stand was located were used as the climatic data. The climatic variables used in the modeling were the mean annual effective temperature sum (lower threshold + 5 °C) and the mean annual rainfall of the years 2014–2018. The stands were located in the boreal coniferous zone, where the mean temperature sum of the years 2014–2018 varied between 880 and 1400 d.d., with the mean rainfall being 550–750 mm (Table 1).

The locations were privately-owned, often low-productive birch stands in eastern and northern Finland and Ostrobothnia (Fig. 1). The low productivity of the stands was reflected in the high proportion of

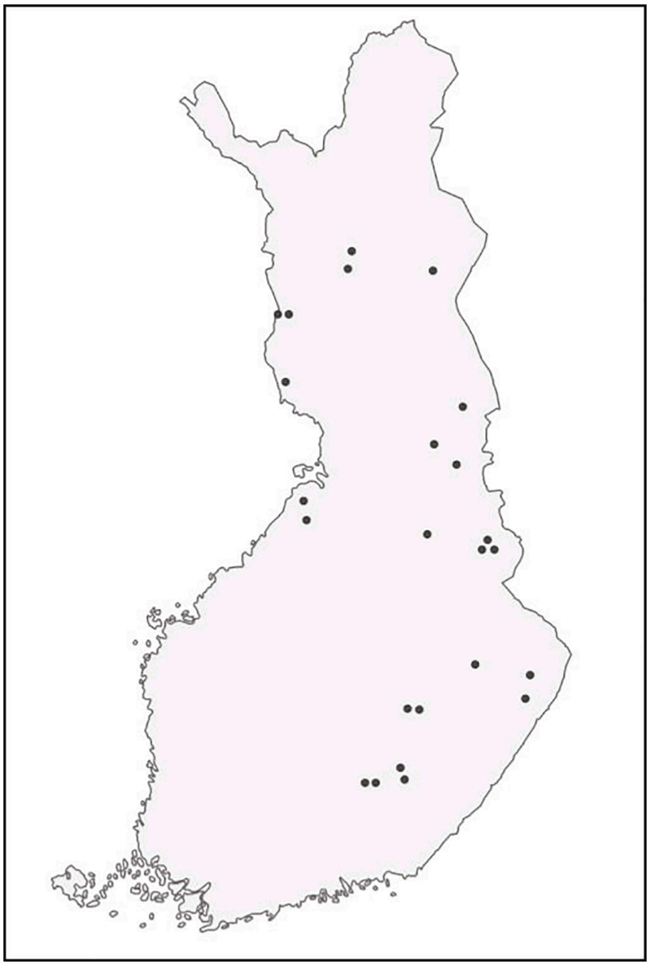

Fig. 1. Location of 24 stands where birch trees were inoculated with *I. obliquus* in Finland.

B. pubescens (73% of the inoculated birches), which has a lower commercial value than *B. pendula*.

The following site and soil characteristics were determined: site fertility (rich, medium or sub-dry); soil texture (mineral soil or peat); and wetness (i.e., whether the soil was wet or drained). The Finnish system of site fertility classification by Cajander (1926) was used, where OMT is herb-rich forest on mineral soil, mesotrophic mires and fens, MT is mesic forest on mineral soil and *meso*-oligotrophic peatlands, and VT is sub-xeric forest on mineral soil and oligotrophic peatlands. The wetness was affected by ditches, with only one stand being classified as wet due to paludification, made visible by its sphagnum vegetation.

The growing stock of the stand was measured on a relascope sample plot, by measuring the stand basal area (m^2/ha), the proportions of the basal areas by tree species, and the diameter of the median-basal-area tree (cm; below, referred as mean diameter). Also, the stand age (in years) and previous harvest activities (none, intermediate or regeneration harvest) over the last 25 years were determined.

2.3. Inoculation of trees

The birch trees were inoculated with *I. obliquus* by drilling five 11-mm-diameter holes in each trunk, with the heights of the inoculation points being at 1, 2, 3, 4 and 5 m. The inoculation plugs were installed in the holes and sealed with tree wound closures (Neudorf Malusan, EAN code: 4,005,240,008,348 or Cooper, EAN-code: 6414504287289). Inoculation was performed on 679 birches in a total of 24 stands in Finland (Fig. 1). The number of inoculated trees per stand varied from 20 to 40. The trees were inoculated in two successive years, starting in autumn 2013 and ending in October 2014. The seasonal timing of the inoculations were classed as spring, summer or autumn if done in May, June–August or October, respectively.

Table 1
Mean, standard deviation and range of main characteristics in the study material.

Variable	N	Mean	Std dev.	Minimum	Maximum
cr, %	679	40.8	19.6	0	90
No. of inoculated trees	24	28.3	10.1	11	40
T, years	24	56.7	22.6	25	90
D_{gm}, cm	24	18.1	5.6	9.0	29.5
G, m^2/ha	24	21.8	6.6	9	35
P_{birch} %	24	70.8	22.0	25.8	100.0
TS, d.d.	24	1177.1	163.0	878.5	1401.2
RF, mm	24	637.8	64.0	548.5	754.1
Latitude, 100 km	24	2.3	2.2	−0.8	5.9
Stand variable	N	%	Tree variable	N	%
Site fertility:			*Betula pendula*	181	27%
OMT	5	21%	Strain J1	220	32%
MT	16	67%	Inoculation:		
VT	3	13%	in May	79	12%
Soil texture:			in June–August	248	37%
Peat	3	12%	in October	352	52%
Mineral soil	21	88%	Status of tree:		
Cuttings:			Alive	670	99%
No cutting	11	46%	Infected	536	79%
Thinning	12	50%	Bulging	91	13%
Clearcutting	1	4%	Conk	30	4%

Notes: N—number of observations at tree or stand level; cr—live crown ratio; T—stand age; D_{gm}—diameter of median basal area tree (below, referred to as mean diameter); G—stand basal area; P_{birch}—proportion of birch trees of stand basal area; TS and RF—mean effective temperature sum and rainfall, respectively, during the last 5 years (2014–2018); latitude—distance in a northerly direction, centered on Jyväskylä in central Finland (6900 km from the Equator); OMT—herb-rich forests on mineral soil, mesotrophic mires and fens; MT—mesic forests on mineral soil and *meso*-oligotrophic peatlands; VT—sub-xeric forests on mineral soil and oligotrophic peatlands.

Two strains of *I. obliquus* (J1 and J3) were used in the inoculation, with J1 being applied to one-third of the trees. The inoculation was predominantly performed in October (52%).

2.4. Measurements of inoculated trees

The stands were inventoried in autumn 2018, four to five growing seasons after the establishment of the experiment. In each stand, two forest professionals, both instructed and trained so as to avoid between-investigator differences in the assessments, performed the measurements.

The inoculated birches were classified as alive or dead, and measured by tree species and live crown ratio. They were classed as infected if the tree showed signs of stem bleeding, bulging, cracking or visible conks (Fig. 2). Stem bleeding was indicated by dark-colored fluid oozing from the bark. If an infected tree showed signs of outward bulging and/or cracked bark, the tree was classed as bulging, and the length of the vertical portion of the bulging part of the stem was measured. Bleeding, bulging and cracking were determined to be the responses of the tree to fungal attack. The most evident sign of successful infection were conks, the numbers of which were counted on the stem. In addition, the volume of the largest conk on the stem was determined by assuming a cuboid shape and measuring its height, width and depth.

2.5. Statistical analyses

Tree-level analyses were conducted because, for example, in the same stand, two different tree species (*B. pendula* and *B. pubescens*) were

Fig. 2. Successfully inoculated (infected) birches, showing signs of stem bleeding (left), bulging and cracking (center) and visible conks (right). Photos: Pyry Veteli/Luke.

inoculated, with the trees being inoculated at different times of the year (spring, summer and autumn), and tree-level variables also being measured. The hierarchical structure of the data (i.e., trees within stands), and the consequently correlated observations were considered by including random stand effects in the intercept.

The probability that a birch tree was infected or bulging or had a conk was evaluated by the following logistic mixed model:

$$ln\left(\frac{\gamma_{ij}}{1-\gamma_{ij}}\right)=f(X,\beta)+u_i \tag{1}$$

where γ is the probability that the tree was infected, bulging or had a conk, $f(\cdot)$ is the fixed part of the model, X is a vector of fixed predictors, and β is a vector of fixed parameters. The subscripts i and j refer to the stand and tree, respectively. u_i is a random, normally distributed between-stand effect with a mean of zero and constant variance. A binomial assumption of the distribution of the error term, and a logit link function were used in the logistic modeling (McCulloch and Searle 2001).

Variables, including tree species, strain, the seasonal timing of inoculation, the number of post-inoculation growing seasons, site fertility, soil texture and cuttings, were used as categorical predictors, whereas stand basal area, mean diameter, location, climatic factors, etc. were considered as continuous ones. The logistic models were fitted using the GENLINMIXED command in IBM SPSS Statistics software, version 25. To be identified as important predictors, the fixed variables included in the models had to be significant at the 0.05 level.

To determine the quality of the predictions made using the logistic models, the probability of success (infected, bulging or conk) was predicted for each observation in the modeling data. The accuracy of the predicted classification as success or failure was analyzed using the observed success probability as the threshold. The overall accuracy was the ratio of the number of correctly classified observations relative to the total number of observations. The value kappa was a measure of the total accuracy that accounted for agreement occurring by chance. Cohen (1960) suggested the following interpretations: kappa values ≤ 0 indicate no agreement, 0.01–0.20 indicate none to slight, 0.21–0.40 fair, 0.41–0.60 moderate, 0.61–0.80 substantial, and 0.81–1.00 almost perfect agreement. The model predictions were used to illustrate the effects of different inoculating conditions on the probability of the successful inoculation of *I. obliquus* in living birches.

3. Results

3.1. Study sites and stand characteristics

The modeling data consisted of 679 trees growing on 24 stands, of which three stands (100 inoculated trees) were growing on peat and 21 stands (579 inoculated trees) on mineral soil (Table 1). In most stands, the site was of medium fertility (i.e., Myrtillus type sites, in the Finnish system of classification: Cajander 1926). Half the sites were drained, with only one stand being classed as wet.

The number of trees measured four and five growing seasons after inoculation was 575 (85%) and 104 (15%), respectively.

3.2. Probability of infection

From a total of 679 inoculated living birch trees studied in autumn 2018, 536 trees (79 ± 13%) showed at least one criterion of *I. obliquus* infection—stem bleeding, bulging or conk formation (Table 1). The success of inoculation varied considerably among the 24 stands, with the range being 53–100% inoculation success per stand.

A logistic mixed model with random stand effects (Eq. (1)) was prepared for the probability that a tree was infected (Table 2). The most powerful predictors of the logistic model were latitude, peaty soil and mean diameter. The following categorical predictors statistically significantly decreased the probability of successful infection: the tree species being *B. pendula*; the strain being J1; the soil texture being peat; and the site type being herb-rich or mesic. In addition, the less infected trees were found in stands where the mean diameter was high and the stand basal area was low. Also, a more northerly location decreased the probability of infection.

The odds of infection success when the tree species was *B. pendula* were about 59% lower than the odds with *B. pubescens* (Table 2, Fig. 3). Similarly, the use of strain J1 decreased the odds of success by about 45% compared to strain J3. A location 100 km towards the north

Table 2
Logistic mixed model (Eq. (1)) for the probability of a tree being infected. F-values were calculated to test the significance of the multi-categorical variables in the model. The modeling data consisted of 679 birch trees in 24 stands.

Variable	Estimate	Std err.	t-value	p	Exp (Est.)
Intercept	5.360	1.632	3.28	0.001	
B. pendula (ref. *B. pubescens*)	−0.895	0.287	−3.12	0.002	0.409
Strain J1 (ref. strain J3)	−0.603	0.266	−2.27	0.024	0.547
Peat (ref. mineral soil)	−1.492	0.404	−3.69	<0.001	0.225
Site type (ref. sub-xeric sites)			F = 3.59	0.028	
Herb-rich sites	−1.087	0.556	−1.96	0.051	0.337
Mesic sites	−1.193	0.452	−2.64	0.008	0.303
Stand basal area, m^2/ha	0.038	0.018	2.12	0.034	1.038
Mean diameter, cm	−0.086	0.024	−3.52	<0.001	0.918
Latitude, 100 km	−0.342	0.087	−3.92	<0.001	0.710
Random effect	Variance				
Stand (N = 24)	0.004				

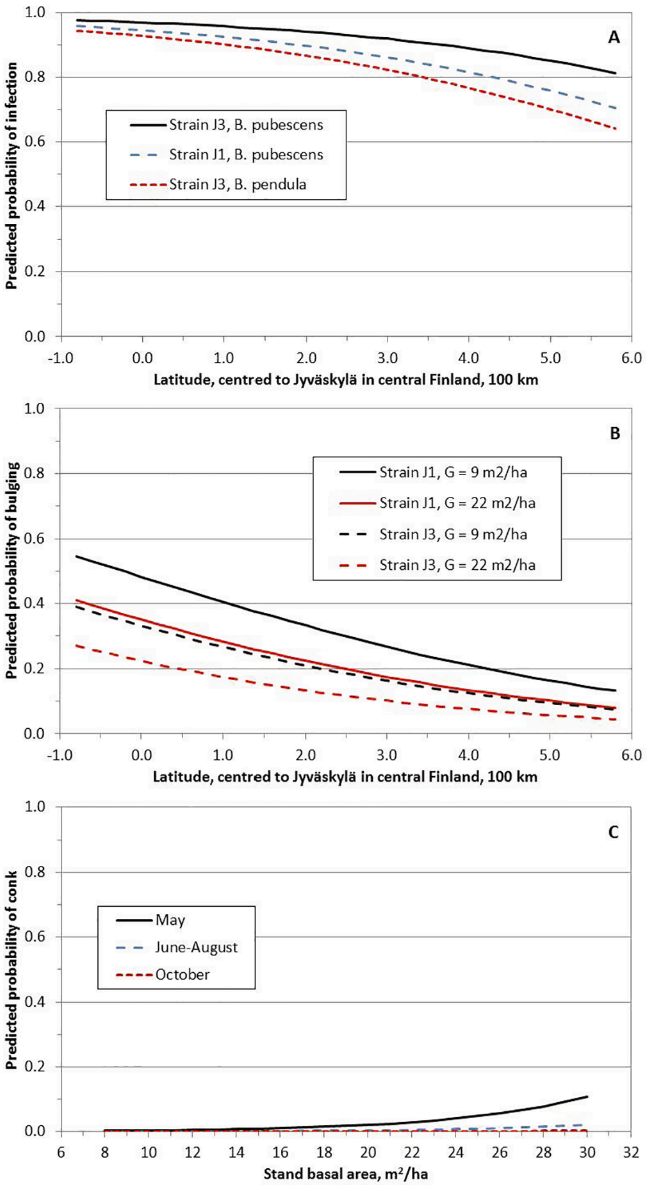

Fig. 3. Predicted probability of infection (A), bulging (B) and conk growth (C). Other predictors: (A) stand basal area (22 m^2/ha), mean diameter (18 cm), mineral soil (MT); (B, C) mineral soil.

decreased the odds of infection success by 29%. Stand basal area and mean diameter had minor effects on infection success, with an increase of 1 m^2/ha in stand basal area and 1 cm in mean diameter resulting, respectively, in a 4% increase and 8% decrease in the odds of infection success, when all other independent variables were held constant.

The accuracy of the predicted classification was calculated so that the observed success probability $536/679 = 0.79$ was used as a threshold. The agreement of the observed and predicted success of infection was only slight (Table 5), with the overall accuracy being 75% and the kappa value being 0.131.

3.3. *Probability of bulging*

Some of the infected trees showed signs of bulging bark, and this was used as an indicator of conk formation under the bark. The proportion of bulging birches per stand was, on average, 14 ± 15% (range 0–47%). From a total of 679 trees studied, 91 (13%) were classed as bulging (Table 1). The length of the vertical portion of the bulging part of the stem was measured to be, on average, 21 ± 26 cm (range 3–140 cm).

Strain, soil texture, stand basal area and the location of the stand were significant ($p < 0.05$) predictors in the logistic mixed model (Eq. (1)) for the probability that a tree stem was bulging (Table 3). Peaty soil and a more northerly location decreased the probability of bulging trees, similarly to the effect in infected trees, whereas strain J1 and the stand basal area had contradictory effects on bulging. Fewer bulging trees were found in stands with a high stand basal area and inoculated with strain J3. Again, the most powerful predictors from the logistic model were latitude and peaty soil, with the variance of the random stand effects being almost redundant.

The odds of bulging success when inoculated with strain J1 were about 88% higher than the odds with strain J3 (Table 3, Fig. 3). A location 100 km northwards decreased the odds of bulging success by 27%. Agreement between the observed and predicted (threshold $91/679 = 0.13$) success of bulging was only slight (Table 5), with the overall accuracy being 62% and the kappa value 0.198.

3.4. *Probability of conk formation*

Only 30 out of the 679 trees studied had one or more conks present on their stems (Table 1), with 18 trees bearing one conk, five trees bearing two conks, four bearing three conks, and three bearing four conks. The volume of the largest conk on the stem was, on average, 54 ± 64 cm^3 (range 1–294 cm^3). The production of conks varied considerably among the 24 stands, with the proportion of birches bearing conks in a stand being, on average, 6 ± 15% (range 0–72%).

Only soil texture, stand basal area and the seasonal timing of inoculation were significant ($p < 0.05$) predictors in the logistic mixed model (Eq. (1)) for the probability of a tree bearing one or more conks (Table 4). Peaty soil decreased, while stand density increased, the probability of conks, and similarly with infection. Conks were more likely to form on trees inoculated during the growing season (from May to August) than in October. The most powerful predictors were stand basal area and the seasonal timing of inoculation.

The odds of conks forming on birches inoculated in May were more than six and 30 times higher than the odds of conks forming on birches inoculated in June–August and October (Table 4, Fig. 3). Agreement between the observed and predicted (threshold $30/679 = 0.04$) incidence of conks was only fair (Table 5), with the overall accuracy being 94% and the kappa value 0.395.

4. Discussion

In this study, we were able to demonstrate the potential for large-scale commercial cultivations of *I. obliquus*. This finding opens the way for novel income sources based on non-timber products, especially from set-aside birch forests in Finland. Only three papers have addressed the inoculation of *I. obliquus* prior this study, to our knowledge.

The effects of different conditions (site and stand characteristics, seasonal timing of inoculation and strain) on the success of inoculation of *I. obliquus* in living birches (*B. pendula* and *B. pubescens*) were investigated. A total of 679 trees in 24 stands under different growing

Table 3
Logistic mixed model (Eq. (1)) for the probability of a tree bulging. F-values were calculated to test the significance of the multi-categorical variables in the model. The modeling data consisted of 679 birch trees in 24 stands.

Variable	Estimate	Std err.	t-value	p	Exp(Est.)
Intercept	−0.319	1.209	−0.26	0.792	
Strain J1 (ref. Strain J3)	0.629	0.290	2.17	0.030	1.876
Peat (ref. mineral soil)	−1.953	0.603	−3.24	0.001	0.142
Stand basal area, m^2/ha	−0.042	0.020	−2.14	0.033	0.959
Latitude, 100 km	−0.313	0.068	−4.61	<0.001	0.731
Random effect	Variance				
Stand (N = 24)	<0.001				

Table 4
Logistic mixed model (Eq. (1)) for the probability of a tree having a conk. F-values were calculated to test the significance of the multi-categorical variables in the model. The modeling data consisted of 679 birch trees in 24 stands.

Variable	Estimate	Std err.	t-value	p	Exp (Est.)
Intercept	−10.822	1.505	−7.19	<0.001	
Peat (ref. mineral soil)	−2.385	1.084	−2.20	0.028	0.092
Season (ref. October)			F = 12.34	<0.001	
May	3.543	0.724	4.90	<0.001	34.560
June–August	1.828	0.726	2.52	0.012	6.223
Stand basal area, m^2/ha	0.172	0.035	4.92	<0.001	1.188
Random effect	Variance				
Stand (N = 24)	0.019				

Table 5
Accuracy of the classification of the trees as being infected, bulging or having a conk. The predicted categories were calculated using the fixed part of the model (Eq. (1)).

	Predicted				
Observed	Infected	Not infected	Total	Accuracy	Kappa
Infected	480	56	536	90%	
Not infected	112	31	143	22%	
Total	592	87	679	75%	0.131
Observed	Bulging	Not bulging	Total	Accuracy	
Bulging	71	20	91	78%	
Not bulging	229	359	588	60%	
Total	300	379	679	62%	0.198
Observed	Conk	No conk	Total	Accuracy	
Conk	14	16	30	47%	
No conk	22	627	649	97%	
Total	36	643	679	94%	0.395

conditions in Finland were inventoried 4–5 years after inoculation with two strains of *I. obliquus*. From the field measurements, the success of inoculation was determined based on signs of infection (i.e., stem bleeding, bulging bark or conk formation) on the birch stem. The proportion of birches infected and bulging in the stand were, on average, 79 ± 13% and 14 ± 15%, respectively, whereas the stand-level proportion of birches bearing conks was 6 ± 15%. This level of success was not achieved by Piętka and Grzywacz (2006), who inoculated 30 living birches with a laboratory-grown strain of *I. obliquus*. In their study, no signs of conk formation were observed after three years, and *I. obliquus* could not be re-isolated from the inoculated trees. Similar findings to those from our study were reported by Ka et al. (2017). In their study, inoculated *Betula platyphylla* var. *japonica* formed their first conks 36 months post inoculation. Nine years after inoculation, the largest being 9 cm in diameter, demonstrating that the production of conks is successful by inoculating live trees, but is a slow process.

In the Ka et al. (2017) study, the forest variables could not be compared due to the low number of inoculated trees analyzed; however, in our study, we found that the success of inoculation varied considerably according to the stand conditions. Depending on the response variable, the main factors affecting tree-level inoculation success were latitude, soil texture, mean stand diameter and its basal area, and the seasonal timing of inoculation.

Here, the main assessment of inoculation success was based on the visual inspection of inoculated trees. Standing birch trees were inspected for the incidence of conks and other signs of infection, such as stem bleeding, bark bulging and cracks (Fig. 2). Most of the infected birches (420 trees) exhibited only stem bleeding. Only 30 trees showed the most evident sign of successful infection—conk formation. The most accurate diagnosis of *I. obliquus* infection would have required the extraction of wood samples and isolation of the fungus from all inoculated trunks (see, e.g., Luana et al. 2015). However, one year after inoculation, Laine et al. (2017) identified whether the trees in their study were infected or not using the same criteria we did, but also took a small set of drill samples from the trees classed as infected. After cultivation of the drill samples, and DNA identification of isolated *I. obliquus* strains, they confirmed that the isolated strains were identical to the inoculated strains. Based on their analysis, the share of infected trees was 75.5%, which is in concordance with the share of visually-confirmed tree infections (79%) in this study.

The study material was collected from cultivation areas of *I. obliquus* located in typical, privately-owned forests in different parts of Finland. Only balanced data from randomized controlled trials at different site types and locations would enable a truly reliable statistical comparison of the strains and tree species, for example. However, our survey data, with a reasonably high number of observations (679 trees in 24 stands), allowed examination of the main stand characteristics affecting the success of inoculation. In the future, an experimental design should be established to enable more thorough testing of different factors and their interactions.

In our study material, live crown ratio, as a tree-level variable, was not found to be a statistically significant predictor in the modeling. Stand-level characteristics (e.g., mean diameter, stand basal area, site type and location) were, however, found to be statistically significant predictors in terms of the inoculation success. In Finland, the temperature sum decreases with increasing latitude (i.e., northwards), and thus neither temperature nor rainfall improved the fit of the logistic models when latitude was used as a predictor.

Correlations among the proportions of infected, bulging and conk-bearing trees in the 24 stands were non-significant and low (-0.025–0.074). Obviously, the bark of trees bearing conks does not always bulge. In addition, it is unclear how long it takes for trees with stem bleeding or bulging bark to start forming conks. According to the personal field observations of the authors, trees naturally infected and killed by *I. obliquus* bear conks, but a longer follow-up period is needed to examine the succession of *I. obliquus* conk formation in inoculated trees.

Under natural conditions, *I. obliquus* conks usually start growing in dead branch stubs, cankers, frost cracks or other wounds in birches. In this study, the artificially-induced infection sites were much smaller (11-mm-diameter holes in the trunk) than naturally-occurring infection sites. Field observations have suggested that, if the stem wounds made during inoculation were completely closed, there were fewer signs of successful infection (e.g., stem bleeding or bulging), indicating that large, open infection sites may be more favorable for successful infection. On well-growing trees, stem wounds are expected to close rapidly. On the other hand, in our study material, a higher frequency of infected and bulging trees were found in the more southerly locations with better growing conditions than in the northerly ones (Tables 2 and 3). It should

Fig. 4. Scelerotium formation of *I. obliquus* under bulging bark. Photo not from study sites. Photo: Pyry Veteli/Luke.

be noted, however, that internal infection may exist behind a completely closed wound, without any visible signs of stem bleeding or bulging (Fig. 4).

The two strains used in the inoculation showed contradicting effects on the probability of infection and bulging. Strain J3 increased the probability of stem bleeding, whereas strain J1 resulted in a higher probability of bulging during the 4–5-year observation period. This short period, however, does not allow us to give recommendations regarding which of these strains will ultimately result in higher *I. obliquus* yields in birch trees. Silvan and Sarjala (2017) noted that rapid conk formation is a strain-specific attribute, a finding supported by our results. A follow-up period is needed to confirm whether the difference between the conk-forming features of different strains is permanent. If so, there is a need to compare more *I. obliquus* strains, and to study their properties in living birches in order to find optimal strains for conk production.

Tree species was only a significant predictor for the probability of infection, with *B. pubescens* being more vulnerable than *B. pendula*. Unfortunately, it was not possible to clarify the interaction between strain and tree species because only strain J3 was inoculated in both tree species.

As mentioned above, we were able to successfully induce the formation of conks of *I. obliquus*, thus generating the possibility for novel, non-timber forest-product-based income sources. *Inonotus obliquus* is an efficient pathogen, able to infect birch trees with bark injuries, so setting up commercial cultivations of *I. obliquus* could become a double-edged sword, either causing a threat to commercial birch forests or maintaining higher forest biodiversity by supporting the faunas that are dependent on dead or dying trees. Although *I. obliquus* is fairly common in Finland (Niemelä and Kotiranta 1983), there is a risk that commercial cultivations could pose a threat to commercial birch stands for timber, veneer and pulp. Thus, if such large-scale cultivations are set up, the harvesting of conk crops and inoculated trees prior to fruiting body formation should be considered, in order to prevent the unintentional spread of *I. obliquus* to commercial birch stands.

5. Conclusions

The main factors that improve the success rate of the infection and incidence of conk in birches were found to be southerly location, mineral soil texture, low mean diameter, high stand basal area, and inoculation at the beginning of the growing season. The effects of the two strains used in the inoculation were contradictory, with J3 significantly enhancing the probability of stem bleeding, and J1 significantly enhancing bulging. The joint effects of strain and tree species remain unclear, and will need to be examined in future studies.

This study took place 4–5 years after inoculation. Initially, the idea was that the bulging and conk formation would have been more rapid and common. However, the progress of conk formation was slower than expected, and the inoculated trees need to be re-inventoried in the future. Despite this, the results indicate that the cultivation of *I. obliquus* may be difficult to integrate into the intensive management of most productive (*B. pendula*) birch stands. On the other hand, the opportunity to increase the profitability of low-productivity (*B. pubescens*) stands on mineral soils still remains high. In addition, for certain set-aside stands, this also presents an opportunity, as it simultaneously accelerates the development of the deadwood component in these birch stands.

CRediT authorship contribution statement

Jari Miina: Conceptualization, Methodology, Validation, Formal analysis, Data curation, Writing - original draft, Visualization. **Rainer Peltola:** Conceptualization, Methodology, Validation, Writing - original draft, Funding acquisition. **Pyry Veteli:** Methodology, Investigation, Writing - original draft, Visualization. **Riikka Linnakoski:** Conceptualization, Validation, Formal analysis, Data curation, Resources, Writing - original draft. **Marta Cortina Escribano:** Writing - review & editing. **Juho Haveri-Heikkilä:** Methodology, Investigation, Writing - original draft. **Pirjo Mattila:** Writing - review & editing. **Pertti Marnila:** Writing - review & editing. **Juha-Matti Pihlava:** Writing - review & editing. **Jarkko Hellström:** Writing - review & editing. **Tytti Sarjala:** Writing - review & editing. **Niko Silvan:** Writing - review & editing. **Mikko Kurttila:** Writing - review & editing. **Henri Vanhanen:** Conceptualization, Methodology, Investigation, Resources, Data curation, Writing - original draft, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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