

The cultivation of oak seedlings inoculated with *Tuber aestivum* Vittad. in the boreal region of Finland

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Received: 19 December 2012 / Revised: 15 August 2013 / Accepted: 23 August 2013
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Abstract Despite recent findings, truffles are rarely found in Finland. In 2006, we began to explore the cultivation potential of *Tuber aestivum/uncinatum* in Finland. In 2006–2008, roughly 1,200 *Quercus robur* seedlings and 200 *Q. pubescens* seedlings were planted in 20 orchards. We aimed to challenge the southern European (France) tree provenances of oak seedlings in a boreal climate. Additional winter coverings made up of fabric or plastic and twigs prevented the seedlings' mortality even when the air temperature was below -30°C during the second winter. The results showed that the top soil temperature at 15 cm depth has to be above -5°C to guarantee the survival of seedlings. *Q. pubescens* was more sensitive to low soil temperatures than *Q. robur*. Morphological and PCR analysis of root samples collected over 2007–2010 confirmed the presence of *T. aestivum* in all orchards despite unfavorable temperatures during the winter time. The first *T. aestivum* sporocarps were found under *Q. robur* in October 2012 in the orchards established in 2006 on old agricultural land, showing truffle cultivation to be successful in the boreal climate.

Keywords Burgundy truffle · Truffle cultivation · Soil temperature · Winter protection · *Q. robur* · *Q. pubescens*

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Introduction

Truffles are the most expensive edible fungi in the world. They belong to the Tuberales family, grow in symbiosis with several trees, such as oaks (*Quercus* spp.), hazel (*Corylus avellana*), beech (*Fagus sylvatica*), and even birch (*Betula* spp.), and produce hypogeous sporocarps (Riouisset et al. 2001; Chevalier and Frochot 2002; Stobbe et al. 2012). Economically, the two most highly renowned truffle species are *Tuber melanosporum* Vitt., the Perigord black truffle, and *T. magnatum* Pico, the Italian white truffle. The summer truffle *T. aestivum* (syn. *T. uncinatum*; Wedén et al. 2005) also has significant commercial value (Mello et al. 2006; Hall et al. 2007; Streiblová et al. 2010).

The life cycle of a truffle involves an initial phase of growth as filamentous mycelium followed by a second phase of symbiotic association between fungal hyphae and host roots (ectomycorrhiza). If these first two stages are successful, the organization of hypogeous sporocarps may start (Peterson and Bonfante 1994). The last century has witnessed a continuously growing international market for truffles, while the world harvest of wild truffles has dropped dramatically from approximately 1,500 t to less than 100 t annually (Mello et al. 2006). This decline has led to the establishment of many truffle plantations worldwide (Mello et al. 2006; Hall et al. 2007) where truffle production is expected to begin in 5–7 years following orchard establishment (Chevalier and Frochot 2002; Sourzat 2000). Some findings have even suggested that the first production of these plants could begin in 3–4 years under optimal conditions (Lefevre et al. 2001; Streiblová et al. 2010). Currently, more than half the harvested truffles worldwide are produced in orchards (Hall et al. 2003). More than 80 % of French *T. melanosporum* production comes from truffle orchards (Mello et al. 2006).

The production of ectomycorrhizal plants in laboratory or greenhouse conditions was demystified and incorporated into

70 orchard preparation in the early 1970s in both France and
 71 Italy. The first successful harvest in France in the late 1970s
 72 led to a broad array of orchard set-ups (Lefevre et al. 2001).
 73 Following this period, much experience has accumulated on
 74 truffle cultivation and required growth conditions (Chevalier
 75 and Sourzat 2012). The most common, and the first species to
 76 be used in the commercial inoculation of plant seedlings, was
 77 *Tuber melanosporum* (Chevalier and Grente 1978). Other spe-
 78 cies, such as *T. magnatum* (Bencivenga and Granetti 1988) and
 79 *T. aestivum* (Chevalier and Frochot 2002), have gained culti-
 80 vation interest over the last few decades, either due to the drop
 81 in natural production or increased demands on the market that
 82 have necessitated overcoming problems of cultivation and
 83 inoculation techniques (Hall et al. 1998).

84 *T. aestivum* is naturally widespread in Europe and North
 85 Africa from 37° to 57° N, and can be found as far north as
 86 Gotland in Sweden (Jeandroz et al. 2008; Wedén et al. 2004b;
 87 Stobbe et al. 2012). *T. aestivum* is cultivated quite commonly
 88 in Europe (Streiblová et al. 2010). The most northern orchard
 89 before our trials was established in 1999 in Gotland, in a mild,
 90 sub-boreal climate (Wedén et al. 2004b). With regard to site
 91 characteristics, *T. aestivum* is less demanding in terms of
 92 environmental conditions compared to other commercial truff-
 93 fle species (Chevalier and Frochot 2002). The species also
 94 exhibits a broader range of symbiotic tree partners (Gardin
 95 2005), with many trees and shrubs successfully inoculated
 96 under laboratory conditions and transferred to orchards (Hall
 97 et al. 2007; Pruett et al. 2008). However, the presence of
 98 adequate exchangeable calcium in the soil is essential for *T.*
 99 *aestivum* growth (Chevalier 2012).

100 Finland is located between latitudes 60° and 70° N, and
 101 longitudes 20° and 32° E. As such, the country is not a
 102 traditional truffle-producing location, nor are truffles a part of
 103 the traditional Finnish kitchen. However, truffles have a long
 104 history in Finland, despite several truffle species (*T. borchii*
 105 Vittad, *T. maculatum*, *T. scruposum*, and *T. foetidum*, exclud-
 106 ing *T. aestivum*) having only been recently identified there
 107 (Shamekh et al. 2009; Orczán et al. 2010). Over the last
 108 decade, we have established the first truffle orchards in
 109 Finland in order to study seedling survival, growth, and truffle
 110 ectomycorrhiza development in a boreal environment with
 111 long winters and low winter soil temperatures. To overcome
 112 these environmental restrictions, a combination of different
 113 orchard adaptations and management methods were studied.

114 **Materials and methods**

115 Establishment of truffle orchards

116 Twenty truffle orchards were established over 2006–2008,
 117 mainly in Southern Savo. Six out of 11 orchards planted in
 118 2006 were included in the detailed analysis. Nine orchards

were planted from 2007–2008 that were also used for exam- 119
 ining the survival of seedlings. Altogether, approximately 120
 1,200 *Quercus robur* seedlings (provenance north-east 121
 France) inoculated with *T. aestivum/uncinatum* were planted. 122
 Roughly 200 *Q. pubescens* seedlings (originating from 123
 Southern France) were also planted. All seedlings were inoc- 124
 ulated under controlled conditions by Robin Pepinieres (Saint 125
 Laurent Du Cros, France) 1 year prior to planting. *Tuber* 126
aestivum sporocarps originating from mild climate areas in 127
 northern France were used for spore inoculation. 128

Seedlings were planted in rows with 3–5 m between plants 129
 and 4–6 m between lines to ensure good future shading with 130
 canopies and access to harrowing machines (Chevalier and 131
 Frochot 2002; Sourzat 2000). Lime was added prior to plant- 132
 ing to achieve a proper soil pH, and was continued during the 133
 following years. A total of 2.5 t ha⁻¹ of lime was added to 134
 increase the pH value of the soil by 0.1 at a depth of 10 cm. 135
 The soil between the rows was ploughed, harrowed, or 136
 weeded mechanically. The top soil was managed and 137
 protected as summarized in Table 1. Other orchards not shown 138
 in Table 1 had similar protection. 139

The seedlings were cultivated with or without grazing pro- 140
 tection tubes around the lower part of the trunk. When a 141
 protection tube was used, large side branches were removed. 142
 The orchards were irrigated by using a hose or bucket during 143
 the summer period, and especially during the first months after 144
 planting or when needed in years following. Fabric, plastic, 145
 twigs, or a sawdust layer (Table 1) was used for additional 146
 winter protection in a 1-m circular area around the seedlings 147
 from November until the snow melted in the spring. The 148
 selection of methods and soil protection was based on materials 149
 applied in plantation in Sweden (Wedén et al. 2004b, 2005) and 150
 locally available materials. Seedling survival was assessed after 151
 each winter. All orchards were maintained regularly by local 152
 land owners under the supervision of the Juva Truffle Center. 153

Environmental conditions 154

The soil properties were determined for all truffle orchards 155
 prior to the first liming and planting, as well as in the years 156
 following planting. The soil sample parameters were assessed 157
 according to Wedén et al. (2004b) by Savo Lab (Mikkeli, 158
 Finland). The soil temperature was measured by a datalogger 159
 (A-lab; Keuruu, Finland) for each orchard at two differently 160
 exposed points of 15 cm depth. A 15 cm depth was selected as 161
 the depth where truffle mycelium was most likely to develop 162
 (Suz et al. 2006). The temperature was measured every fourth 163
 hour throughout the year. 164

Analysis of ectomycorrhizae 165

To assess the survival of *T. aestivum* ectomycorrhiza on the 166
 roots of oak seedlings (*Q. robur*), root samples were taken in 167

Table 1 Summary of mulch, soil temperature, seedling survival and winter protection practices influencing soil temperature at 15 cm depth

Orchard number	Mulch (all year around)	Winter protection (October–Spring)	Lowest measured temperature at 15 cm depth: winter °C	Percentage of dead seedlings after winter 2007–2008 (<i>Q. robur</i> / <i>Q. pubescens</i>)
1	Fabric	Fabric and twigs	2006–2007, –1.5 2007–2008, –1.5	0 0
2	Sawdust	Plastic and twigs	2006–2007, –0.6 2007–2008, –0.25	0 0
3	No mulch	Twigs	2006–2007, –0.5 2007–2008, –4.4	0 0
4	No mulch	No protection	2006–2007, –1.5 2007–2008, –8.1	0 6/80
5	No mulch	Sawdust (thin layer)	2006–2007, –2.5 2007–2008, –5.2	0 2/15
6	Fabric	No	2006–2007, –2.0 2007–2008, –5.9	0 0/5

Each of these orchards had 50–100 *Q. robur* and 20 *Q. pubescens* seedlings

September of 2007 and 2010 from two orchards that had the highest mortality of seedlings in a given year (orchards 4 and 5; Table 1). Three seedlings per plantation were randomly selected for sampling among the vital plants. A part of the root system at about 10–15 cm depth (comprising approximately 1 L of soil volume around roots) was dug by a spatula. All parts of sampled roots were rinsed with water to remove any soil and soil particles attached to coarse and fine roots and kept in plastic tubes with 75 % ethanol until identification.

Prior to analysis, sampled parts of root systems were cut into 2-cm pieces and pooled for each year. Subsequently, pieces were randomly selected and analysed until the total count of fine roots reached 400 (Benucci et al. 2011). Three samples of vital ectomycorrhizae from each identified morphotype were removed at this point for DNA-based molecular identification.

For morphology-based identification, vital types of ectomycorrhiza, old mycorrhizal root tips and non-mycorrhizal root tips were differentiated, counted, and photographed under the Olympus SZX12 stereo-microscope (magnification ×3.5–45) and Olympus BX51 microscope (magnification ×100–2,000). Types of ectomycorrhiza were identified based on morphological and anatomical evaluation (Agerer 1987–2008; Agerer and Rambold 2004–2012).

For molecular identification, individual ectomycorrhizal root tips were used for DNA extraction with the Plant DNeasy Mini Kit (Promega). Extracted DNA was then resuspended in pre-warmed, sterile milli-Q water to the approximate final concentration of 100 ng/μl. General fungal primers ITS1 and ITS4 (White et al. 1990) were used for PCR amplification of the ITS region, including 5.8 S rDNA. Amplification reactions were performed according to Kraigher et al. (1995) in a PE 9700 DNA thermocycler with a lower annealing temperature. Negative controls lacking

fungal DNA were run for each experiment to check for any contamination of the reagents. Amplified DNA was separated and analysed as described by Grebenc et al. (2000). Amplified fragments were first separated and purified from the agarose gel using Wizard SV Gel and the PCR Clean-Up System (Promega), then sequenced at a commercial sequencing service (Macrogen). Sequencher 4.8 (GeneCodes) was used to identify the consensus sequence from the two strands of each isolate. The sequences were submitted to an EMBL database and compared to the GenBank (BLAST tools) to confirm their identity.

Results

All the orchard locations had been used prior as agricultural land for the production of various agricultural products (e.g., crops, grass, vegetables, and fruit trees), which made a decrease in the number of potential competing ectomycorrhiza propagules abundant in the forest soil quite likely (Hall and Yun 2001; Unterseher et al. 2012). The liming of ploughed local soils resulted in a pH increase of 0.8–1.3 in the year following the first application. In the next 2–3 years, additional liming raised the pH from a suboptimal to an optimal pH of 7.0–7.5 at all analysed sites (Table 2). The liming was continued after 2009 when the soil pH was not in the proper range. The pH values were also comparable in the other orchards (data not shown).

Mulching is known to promote *T. aestivum/uncinatum* colonization and reduction of contaminating ectomycorrhizal fungi (Zambonelli et al. 2005). Mulches affect soil temperature and moisture (Zambonelli et al. 2005). Commonly used (plastic in strawberry farming) and readily available (fabric, twigs, sawdust) materials were used in our orchards as

t2.1 **Table 2** Soil pH in four selected orchards with *Q. robur* planted in 2006. Lime was added yearly

t2.2 Orchard	2006 ^a	2007	2008	2009
t2.3 1	6.3	7.1	7.0	7.5
t2.4 2	5.1	6.0	6.4	7.0
t2.5 3	6.0	6.5	7.0	7.1
t2.6 4	5.8	6.5	7.0	7.4
t2.7 5	6.1	7.0	7.2	7.6
t2.8 6	5.5	6.6	7.0	7.3

^a 2006 soil pH value is before liming

233 protection (Table 1). Truffle cultivation has not historically
 234 been a part of Finnish agriculture. The first Finnish truffle
 235 orchard was the one established for this very study in the
 236 summer 2006. Different mulching and winter protection
 237 methods were applied to find out which one was suitable for
 238 the boreal climate. The effect of the protection methods was
 239 noted in examining the soil temperature and survival of the
 240 seedlings. The soil temperature at a depth of 15 cm dropped
 241 below 0 °C (−0.25 °C to −8.1 °C) at all measured sites during
 242 the winter of 2007–2008. The lowest temperatures (below
 243 −5 °C) were recorded on the sites where no additional protec-
 244 tion was applied, or only a thin layer of sawdust was used
 245 during the winter (Table 1). Fabric or plastic together with
 246 twigs was most efficient for additional winter protection to
 247 prevent low soil temperatures throughout January 2008
 248 (Table 1; Fig. 1).

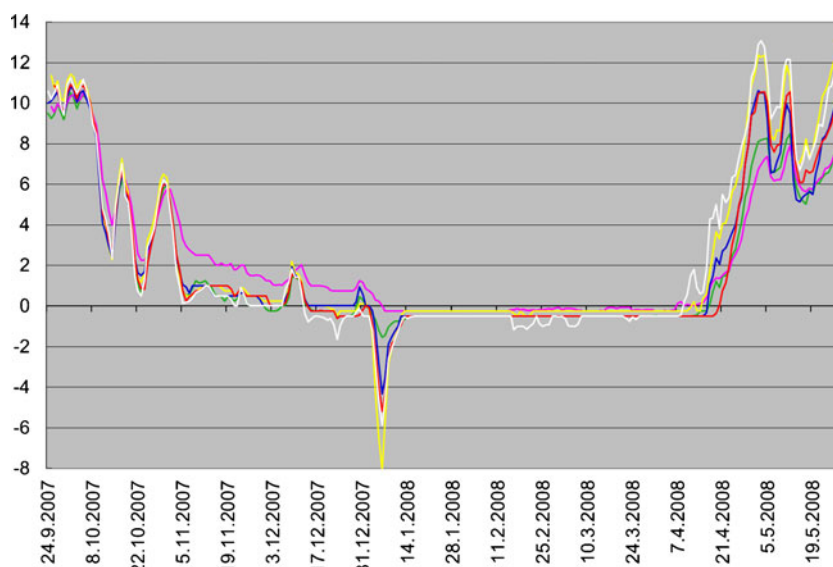
249 Low soil temperatures coincided with the mortality of seed-
 250 lings when the soil temperature at a depth of 15 cm dropped
 251 below −5 °C (Table 1). The mortality of Mediterranean prov-
 252 enances of *Q. pubescens* seedlings in two of the most affected
 253 plots was higher than with *Q. robur* (Table 1). While most *Q.*

254 *pubescens* seedlings died in orchard 4 during the winter of
 255 2007–2008, orchard 2, under 1 km distance from orchard 4,
 256 did not suffer any *Q. pubescens* deaths. Variation of the lowest
 257 soil temperature above −5 °C did not affect the survival of the
 258 seedlings. Protection was corrected for in orchard 4 (Table 1) in
 259 November 2007 by using sawdust and twigs. After this, no
 260 deaths of seedlings were detected in this orchard and other
 261 orchards save for 1–2 % of seedlings dying in some orchards
 262 over the following years due to attack by moles.

263 Microscopic investigation (Fig. 2) showed that *T. aestivum*
 264 ectomycorrhiza (sensu Agerer 1987–2008; Chevalier and
 265 Frochot 2002; Agerer and Rambold 2004–2012) was present
 266 at all studied sites during 2007–2011 with all diagnostic char-
 267 acters: the ramification of ectomycorrhizal roots absent or
 268 monopodial pinnate absent; shape of the unramified ends
 269 straight; surface irregularly hairy, cottony; mantle surface
 270 densely long-spiny under low magnification; colour of
 271 ectomycorrhizae ochre to yellow-brown and at the very tips
 272 the same colour or brighter, whereas older tips were darker
 273 brown; rhizomorphs were not observed; fungal cells of the
 274 outer and inner mantle layers formed pseudoparenchymatous
 275 mantle consisting of angular cells without septa. The mantle
 276 type of hypha was designated as “L” type after Agerer’s
 277 (1987–2008) categorization (Fig. 2b). Emanating hyphae were
 278 present and abundant, growing from different parts of
 279 ectomycorrhizae, and several emanating hyphae exhibited a
 280 curly shape. The emanating hyphae were not ramified, septae
 281 on emanating hyphae were present, and no special structures or
 282 clamp connections were observed. Anastomoses of emanating
 283 hyphae and rhizomorphs were not observed at higher
 284 magnifications.

285 Molecular characterization of the ITS region yielded com-
 286 plete ITS1, 5.8S rDNA and ITS2 sequences. The representa-
 287 tive sequence from three identical DNA sequences from the

Fig. 1 Soil temperature profile at six orchards planted in 2006. The temperature was measured at 15 cm depth in intervals of 4 h during the extreme low-temperature winter of 2007–2008. The colors for different orchards are as follows: 1 green; 2 pink; 3 blue; 4 yellow; 5 red; 6 white



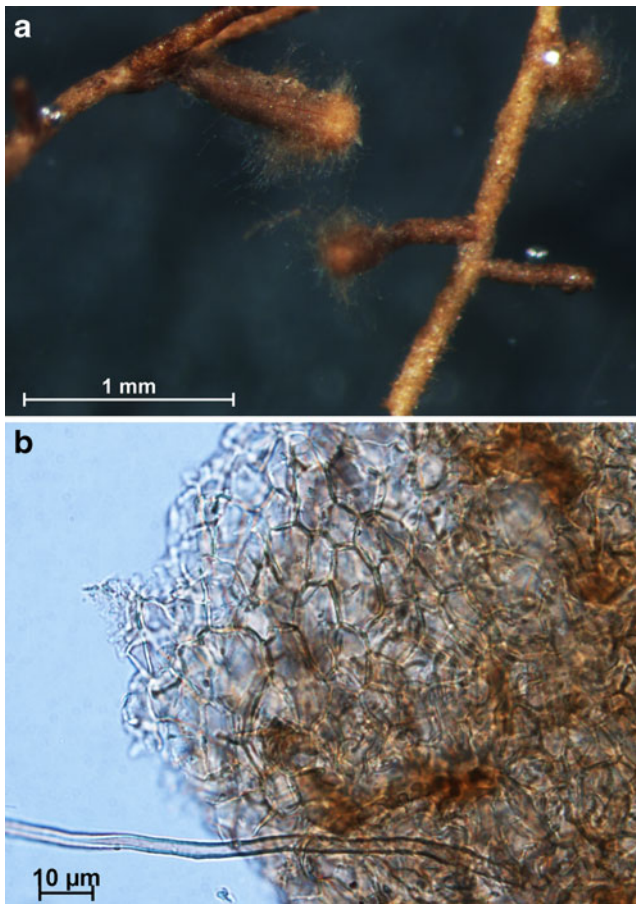


Fig. 2 a Morphology of anatomotype JUVA 01/2009 at ×45 magnification b Outer ectomycorrhiza mantle layers of anatomotype JUVA 01/2009, at ×1,000 magnification

288 analysed *T. aestivum* ectomycorrhiza was deposited at the
 289 EMBL database with accession number FN395017. The
 290 BLAST search gave multiple hits of query sequence with
 291 99 % similarity to several *T. aestivum* ascocarp sequences
 292 published by Paolocci et al. (2004).

293 The ectomycorrhizae of *T. aestivum* were present at all
 294 analysed sites 4 years after planting. The fine root analysis of
 295 growing seedlings at the two plantations with the highest
 296 mortality (namely, orchards 4 and 5; Table 1) indicated a
 297 decrease in the percentage of vital *T. aestivum* ectomycorrhizae
 298 among the total mycorrhizal population, the appearance of two
 299 additional types of mycorrhizae (including *Cenococcum*
 300 *geophilum*), and an increase of old ectomycorrhizal fine roots
 301 in the following years (Table 3).

302 In October 22, 2012, three truffle sporocarps from two
 303 orchards (established in 2006) in the city of Juva were found
 304 by the aid of a Lagotto truffle dog under *Q. robur*. Eleven
 305 orchards were screened. Two truffle sporocarps were
 306 harvested from orchard 2 and one from orchard 6. The pro-
 307 tection system varied in these two orchards. The sporocarps
 308 were growing under roughly 2-m-tall oaks at about 10–20 cm
 309 distance from the trunks, a similar distance to the first

Table 3 The ectomycorrhizal community, old ectomycorrhizal fine roots and non-mycorrhizal fine roots on vital oak seedlings (*Q. robur*)

Fine root/ectomycorrhiza	Sampling in year 2007	Sampling in year 2010	
<i>Tuber aestivum</i>	59.5 %	25.0 %	t3.3
Unknown type 1	0.0 %	5.5 %	t3.4
<i>Cenococcum geophilum</i>	0.0 %	1.0 %	t3.5
Old ectomycorrhizal fine roots	39.5 %	68.5 %	t3.6
Non-mycorrhizal fine roots	1.0 %	0.0 %	t3.7

The root samples were collected in years 2007 and 2010. Each value represents the percentage from 400 analysed fine roots in the pooled sample

cultivated truffles in Gotland (Wedén et al. 2009). The find-
 310 ings were at 5–10 cm depth. Each of the sporocarps weighed
 311 approximately 30 g, and showed morphological characteris-
 312 tics (Fig. 3a, b) and had the fresh aroma of *T. aestivum*.
 313 Previous use of the land in orchard 2 included the production
 314 of grains and grass, and in orchard 6, grains and cabbage.
 315

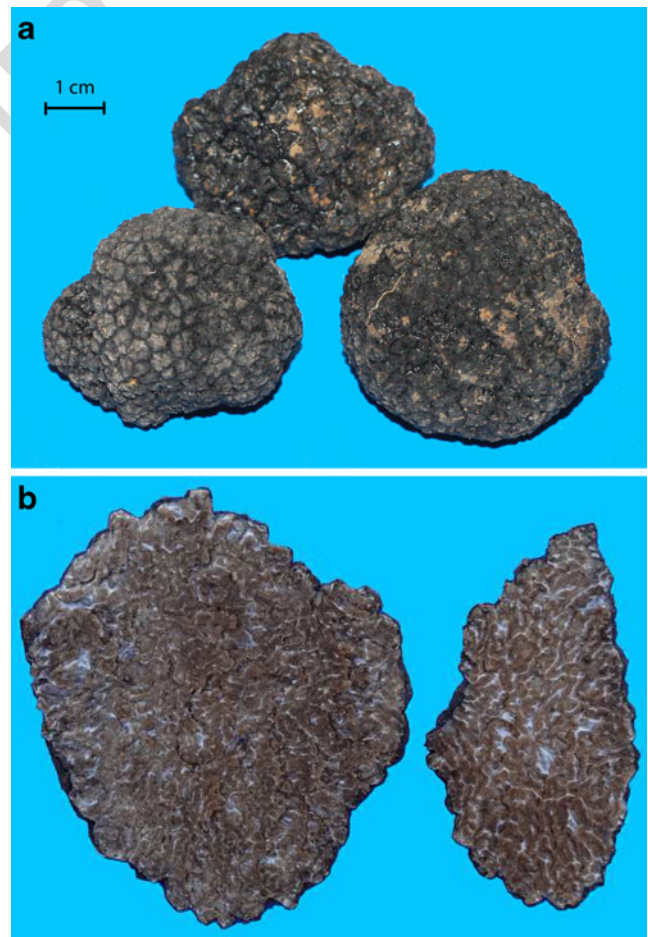


Fig. 3 The *T. aestivum* sporocarps found in October 2012 from the truffle orchards in Southern Savo. a Peridiums of all three sporocarps; the scale is in centimeters. b Cross-sections showing the spore tissue (gleba) of two sporocarps

316 **Discussion**

317 The conditions in Finland are challenging both for truffles and
 318 ectomycorrhiza host plants. *Quercus robur* grows in the
 319 hemiboreal zone in the southern coast of Finland (Koponen
 320 2004), whereas *Q. pubescens* is a southern European species.
 321 We have established truffle orchards in the southern boreal
 322 forest region of Finland using both tree species. Plantations
 323 were established in the regions that are outside the natural oak
 324 range roughly 100 km to the north of the coastal oak-growing
 325 zone. In our orchards, *Q. robur* was growing well, but not *Q.*
 326 *pubescens*. The average air temperature in the area (data for
 327 nearby city of Mikkeli) in January 2006 was -7.1 °C, while
 328 peak air temperatures can occasionally drop below -30 °C, as
 329 happened in 2007, over a year after plantation. This explains
 330 the sudden very low soil temperature shown in Fig. 1. In
 331 general, the thick snow layer protects the soil from the tem-
 332 perature decrease to the same levels as the air temperature.

333 Truffle species generally require a relatively high pH level
 334 (between 7 and 8) (Bencivenga and Granetti 1988; Rioussset
 335 et al. 2001; Mello et al. 2006). The optimal soil pH for the
 336 growth of *T. aestivum* is 6.8–8.0 (Chevalier and Frochot 2002;
 337 Wedén et al. 2004b). Thomas (2012) found the lowest optimal
 338 pH for commercial orchards to be 7.5. In our truffle orchards,
 339 pH elevated gradually during the first 3 years. Additional
 340 liming was used to obtain the optimal pH range as previously
 341 reported for many sites, including the unfavourable volcanic
 342 soils of New Zealand (Hall et al. 2007). The soil pH achieved
 343 the level of 7.0–7.5 in 1–3 years of liming depending on the
 344 site (Table 3). While the starting pH values in 2006 were in the
 345 range of 5.1–6.3, the pH values in the next year were in a
 346 range (6.5–7.1) adequate for *T. aestivum* growth (Chevalier
 347 and Frochot 2002; Thomas 2012).

348 The main challenge in truffle cultivation in Finland was the
 349 maintenance of soil temperature conditions that would ensure
 350 survival of *T. aestivum* ectomycorrhizae and the seedlings. Our
 351 results indicate that soil temperature at a depth where truffle
 352 mycelium is commonly present (Suz et al. 2006) should not
 353 drop below -5 °C, not even for a short time, as such temper-
 354 atures increased the mortality of both host tree species and
 355 likely reduced the number of vital *T. aestivum* ectomycorrhiza
 356 on surviving seedlings.

357 The temperature in the boreal region can drop severely. To
 358 overcome this problem, we applied several permanent or
 359 seasonal soil protection approaches. Soil protection was ap-
 360 plied similar to that of a previous truffle plantation in Gotland,
 361 Sweden (Wedén et al. 2004b, 2009), where it was primarily
 362 used not to protect the soil from low temperatures but to
 363 reduce competition from weeds and damage due to animal
 364 grazing. During the winter of 2006–2007 in the Finnish or-
 365 chards, no additional winter protection appeared to be neces-
 366 sary. The extremely low temperature in January 2008 required
 367 additional winter protection to keep the soil temperature high

enough. The combined use of plastic or fabric and twigs was 368
 most efficient in preventing the decrease of temperatures 369
 below -5 °C. Seedling survival was not generally affected 370
 by soil temperatures between -0.25 °C and -4.4 °C. Only 371
 when the soil temperature dropped below -5 °C was seedling 372
 death observed. 373

374 While *T. aestivum* ectomycorrhizae remained present 374
 4 years after planting, the amount of competing mycorrhizae 375
 increased. The frost might be one reason for the change in the 376
 ectomycorrhizal community in the Finnish orchards, since it 377
 has been reported that the low soil temperatures can reduce the 378
 percentage of *T. aestivum* ectomycorrhizae and support the 379
 development of a stress-tolerant type of ectomycorrhizae 380
 formed by *Cenococcum geophilum* (Hasselquist et al. 2005). 381
 However, the relatively low number of other species in the 382
 plantation (compared to the plantation in Benucci et al. 2011) 383
 was likely due to the history of the site and relatively un- 384
 favourable climate conditions. 385

386 The sporocarps collected in 2012 were identified based on 386
 the morphological characters of sporocarps (Fig. 3), microscop- 387
 y of spores (not shown), and the typical aroma of *T. aestivum*. 388
 The finding of the first sporocarps in October 2012 revealed 389
 that the Finnish truffle orchards started producing well-formed 390
 truffles 6 years after planting, much the same as the cultivated 391
T. aestivum sporocarps obtained in Gotland (Wedén et al. 392
 2009). The results of our work indicated that even a boreal 393
 area is suitable for the production of truffles, and it appears 394
 that *T. aestivum* is a promising truffle species for orchards in 395
 northern conditions. Geologically, Finland has Archaean bed- 396
 rock, whereas surficial deposits of the country have developed 397
 during the ice age (Nenonen and Portaankorva 2009). Gotland, 398
 which holds the nearest southern *T. aestivum* findings, is 399
 formed of Silurian shallow-water marine sediment (Kershaw 400
 1993). This comparison confirms the conclusion of Chevalier 401
 (2012) that the soil is not a limiting factor for the cultivation of 402
T. aestivum. The summer temperatures in southern Finland and 403
 Gotland are similar, whereas the autumn temperatures are 404
 higher in Gotland. For example, in October the average tem- 405
 peratures in Southern Finland are 4–6 °C, and in Gotland 406
 6–10 °C. Mean winter temperatures in Southern Finland are 407
 2–4 °C lower (Tveito et al. 2000). Precipitation is slightly 408
 higher in southern Finland than in Gotland (Tveito et al. 409
 2000). It appears that these climate differences did not affect 410
 the growth rate of the first cultivated *T. aestivum* sporocarps. In 411
 October 2012, when the fruit bodies were harvested, the 412
 average temperature in the Juva region was 4–5 °C. The 413
 production of *T. aestivum* fruit bodies occurs mainly when 414
 the temperature is between 3.5 and 15 °C (Wedén et al. 2004a; 415
 Bruhn et al. 2013). 416

417 In conclusion, the selection of proper tree species and 417
 provenances is needed to obtain positive results. These should 418
 be adapted to a shorter vegetation period and low winter air and 419
 soil temperatures. Our results show that southern European oak 420

421 seedlings and *T. aestivum* can adapt to the climate and ecolog-
 422 ical conditions of Finland. Our results also show that restric-
 423 tions caused by the northern climate and low soil temperatures
 424 can be overcome with proper soil and winter protection
 425 management.

426 **Acknowledgments** We would like to thank Rajupusu Leader ry and the
 427 Regional Council of South Savo for financial support, as well as the
 428 orchard owners for their kind cooperation. Thanks also to Mrs Heli
 429 Valtonen for her assistance. The partner from the Slovenian Forestry
 430 Institute was co-financed by the Ministry of Higher Education, Science
 431 and Technology through Research Programme P4-0107 and the Targeted
 432 Research Program Project CRP V4-0492.

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