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Review

Factors influencing fructification phenology of edible mushrooms in a boreal mixed forest of Eastern Canada

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ABSTRACT

Given the rise in commercialization of edible forest mushrooms in Eastern Canada, it is advantageous to understand the fruiting patterns of fungal species. The aim of this study is to understand the phenology of edible species within the mixed boreal forest. Weekly surveys were conducted in 481 quadrats during the mushroom growing season over three consecutive years (2005–2007). The initial fruiting dates, as well as the duration of fruiting, were examined relative to year and stand type. Species phenology was also considered in relation to soil temperature and moisture on temporal (interannual) and spatial scales (across sampling quadrats).

Our results show no significant influence of stand type on the phenology of edible ectomycorrhizal mushrooms. Neither the composition of dominant species nor the age or origin of the stand (natural regeneration or plantation) appears to have a direct influence on species phenology. On a temporal scale, the effect of year, as well as soil temperature and moisture, strongly explains the initial fructification date but is only weakly linked to the length of the fruiting. Soil conditions influence the phenology of all fungal species but each species has a specific response. For example, average soil moisture can either stimulate (e.g. *Boletus* aff. *edulis* and *Lactarius deterrimus*) or delay (i.e. *Cortinarius caperatus* and *Catathelasma ventricosum*) the initial fruiting date. On a spatial scale, soil conditions are correlated with the phenology of certain species; however, this source of variation seems less important to the overall phenology of all species.

The effect of year, coupled with soil temperature range (difference between maximum and minimum temperatures during the growing season), shows the strongest relations with the initial fruiting date in six of the seven species studied. That is, the wider the soil temperature range, the earlier the fruiting bodies appear. The stimulation of fructification by a marked decrease in temperature, a phenomenon known as cold-shock, is well known in the laboratory. According to our results, this phenomenon seems also important *in situ* to initiate fruiting of edible fungi in the mixed boreal forest and could help to optimize the harvest.

Contents

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1. Introduction

Understanding the ecology of edible forest mushrooms offers numerous opportunities to improve the management of their harvest (Pilz and Molina, 2002). Eastern Canada exemplifies a region with a strong potential to produce edible forest mushrooms, in which market development is under expansion and fruiting patterns of different species remain largely unknown. To overcome these limitations, it is important to describe and understand the fructification phenology pattern of fungi in relation to regional characteristics.

Several studies have shown that the productivity of epigeous species of fungi is linked to annual climate conditions, such as average monthly precipitation or accumulation, as well as average monthly temperature (e.g. Eveling et al., 1990; Ohenoja, 1993, 1995; Laganà et al., 2002; Salerni et al., 2002; Martínez de Aragón et al., 2007; Krebs et al., 2008). However, few studies have attempted to explain the dates or duration of fruiting in relation to climatic conditions (i.e. Straatsma et al., 2001; Mihail et al., 2007; Gange et al., 2007; Kauserud et al., 2008), and none have done so in the North American boreal forest. Furthermore, soil moisture retention has a greater influence on fungal productivity than does precipitation (Wilkins and Patrick, 1940; Laganà et al., 2002). Therefore, it is important to measure both moisture and temperature directly from the substrate on which the fruiting bodies appear in order to better explain mushroom phenology.

Productivity of fungal species is also determined by habitat characteristics. Generally, young forest stands display greater epigeous mushroom productivity than do more mature stands (Vogt et al., 1981; Senn-Irlet and Bieri, 1999; Bonet et al., 2004). Nevertheless, apart from casual observations, the effect of habitat type on the fruiting phenology of fungal species has not been specifically tested. It is vital to consider both habitat and geographic location of any species in order to determine the effects of climatic factors on the fruiting phenology of edible forest mushrooms (Ohenoja, 1993).

The overall goal of this study was to understand which factors influence the observed variation in the phenology of edible ectomycorrhizal mushrooms found in the Eastern Canadian boreal forest. More specifically, we tested the following hypotheses: (i) the phenology of each species (fruiting date and duration) varies from year to year and according to forest type and (ii) temperature and soil moisture (average and range) explain species phenology on an interannual scale (temporally) as well as regional scale regardless of the year under consideration (spatially).

2. Materials and methods

2.1. Study area

The study area (Fig. 1) is located in the Gaspé Peninsula, in the eastern part of the province of Québec, Canada (47°50′N–49°15′N, 64°08′W–67°37′W). The Gaspé Peninsula covers 22,000 km^2 and is a mountainous region expressing a maritime climate, with large local variation. The geological setting of the study sites is mostly located on sedimentary rocks covered by alteration till or marine-fluvial sandy deposits that developed primarily into podzolic soil type. Average annual temperature varies from 0 to 3.5° C and average annual precipitation fluctuates from 900 to 1300 mm. The coniferous boreal forest characterizes the Gaspé Peninsula interior (white birch – balsam fir bioclimatic domain) while the periphery of the peninsula contains a temperate mixed forest (yellow birch – balsam fir bioclimatic domain) (Robitaille and Saucier, 1998). Ninety-five percent of the entire territory is forested and slightly more than two-thirds of the stands are 50 years old or less (MRNF, 2005). The main coniferous tree species include balsam fir (*Abies balsamea* (L.) Mill.), black spruce (*Picea mariana* (Mill.) B.S.P.), white spruce (*P. glauca* (Moench) Voss.), eastern white-cedar (*Thuja occidentalis* L.) and white pine (*Pinus strobus* L.), while the dominant deciduous tree species include white birch (*Betula papyrifera* Marsh.), yellow birch (*B. alleghaniensis* Britton), trembling aspen (*Populus tremuloides* Michx.), sugar maple (*Acer saccharum* Marsh.) and red maple (*A. rubrum* L.).

2.2. Sampling design

Regionally, study sites were distributed using stratified random sampling, based on ecoforestry maps of the third decennial forestry inventory (MRN, 2000). This approach allowed us to sample forest stands that are regionally representative and have good mycological potential (as determined by the dominant tree species and their ectomycorrhizal associations). In particular, plantations of white spruce, Norway spruce (*Picea abies* (L.) Karst) and Jack pine (*Pinus banksiana* Lamb.), which represents <5% of the regional forest cover, were selected for sampling. Only the sites with most favorable moisture conditions, high stand density (60–100% of canopy cover) and low-grade slope (0–15°) were considered. Inaccessible stands, or those dominated by endomycorrhizal species (e.g. cedar stand), were excluded. Following validation in the field, the experimental design was based on a total of 24 study sites.

Within each study site, several quadrats were established according to a systematic sampling plan. As such, transects measuring 160–640 m per site allowed us to establish 481 quadrats of 5 m^2, spaced 20 m apart. Beginning at the center of each quadrat, the basal area of all woody species with a diameter at breast height (DBH) greater than 9 cm was measured using a factor 2 metric wedge prism. The age of each stand was estimated at 100 m intervals by selecting two trees (species and height representative of the stand) from which growth rings, obtained by a Pressler borer, were counted. This value was then also applied to the nearest quadrats. Stand origin (natural regeneration or plantation) was also noted. Taking these three factors into account (i.e. the proportions of tree basal area per species, estimated age and origin of the stand), 13 specific stand types were identified (Table 1). Two readings for temperature (T°C), and volumetric soil moisture (%) at a depth of 10 cm, were taken on a weekly basis in each quadrat throughout the growing season using a digital thermometer (DeltaTrak Waterproof Lollipop Min/Max Digital Thermometer, Model 11036) and a humidity probe (Field Scout Soil Moisture Meter, Model TDR 100).

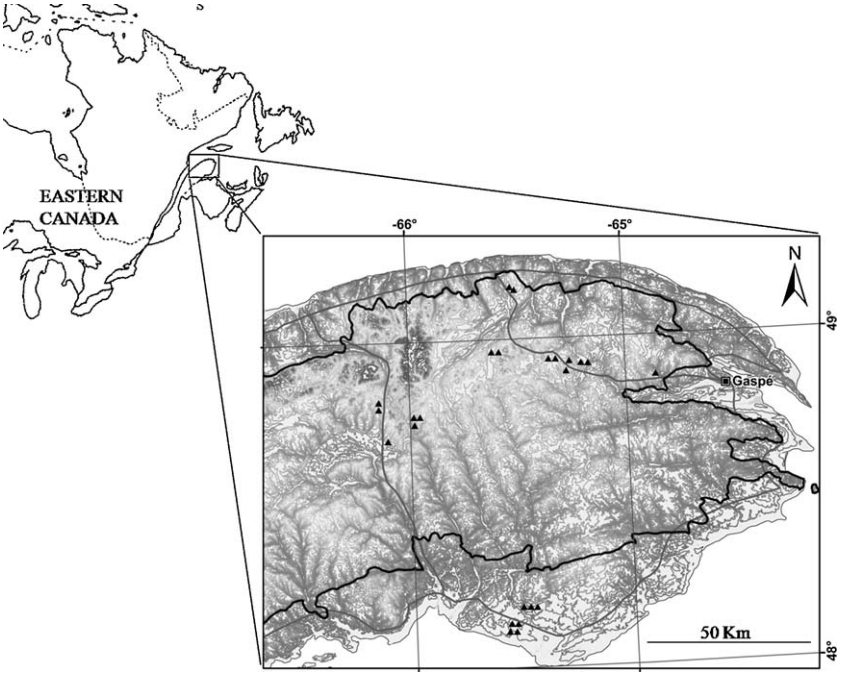

Fig. 1. Location of study sites (black triangles) in Gaspé Peninsula, Québec, Canada. Bold black line indicates bioclimatic domain edges (white birch – balsam fir bioclimatic domain is located in the center and while yellow birch – balsam fir bioclimatic domain is located in the periphery of the peninsula).

2.3. Fungi monitoring

Monitoring of quadrats took place weekly during three successive years (2005–2007), from mid July to the end of September, over 10–11 consecutive weeks. In total, 13,619 visits were conducted in the 481 quadrats to determine fungal species that were present. Fourteen edible species with a potential for commercial harvest were identified a posteriori and only the seven species that were encountered in more than eight quadrats were selected for analysis: *Boletus* aff. *edulis* Bulliard: Fries; *Catathelasma ventricosum* (Peck) Singer; *Hydnum repandum* Linnaeus: Fries; *Lactarius deterrimus* Gröger; *Lactarius thyinos* A.H. Smith; *Leccinum piceinum* Pilat and Dermek; *Cortinarius caperatus* (Persoon: Fries) Karsten.

We used the first date of mushroom appearance (day-of-year 1–365) to determine whether a species fructified early or late in the growing season. To determine persistence of a fungal species in any given quadrat, we calculated the duration of presence of all fruiting bodies of this species (number of days) by dividing weekly presence by total number of inventory weeks and we converted this frequency value into number of days. This was done by multiplying it by the total number of days inventoried per season in a given year, 66 days in 2005, 70 days in 2006 and 68 days in 2007.

Table 1
Forest stands sampled.

	Age[a]	Quadrat number	$IC_{95\%}$ basal area (%) of dominant species
Natural stands			
White spruce	Young	15	[80.1–96.6]
	Old	7	[75.6–89.6]
Black spruce	Young	8	[84.9–97.0]
	Old	98	[91.2–94.1]
Balsam fir	Young	25	[86.6–93.7]
	Old	65	[84.6–88.5]
Balsam fir with deciduous	Young	8	[45.2–57.3]
	Old	16	[51.0–61.2]
Mixed conifer	Young	25	[88.9–96.5]
	Old	65	[94.1–97.3]
Plantations			
White spruce	Young	58	[94.3–97.8]
Norway spruce	Young	51	[98.6–99.9]
Jack pine	Young	40	[96.4–99.6]

[a] Young: 30–45 years; old: 50–85 years.

2.4. Statistical analyses

We employed non-parametric tests for statistical analysis due to the fact that our data did not meet the required assumptions of parametric tests according to Shapiro–Wilk test, even following data transformation trials. Two-factor Scheirer–Ray–Hare tests, which are an extension of the Kruskal–Wallis test on ranks (Sokal and Rohlf, 1995), were applied in order to determine the effect of year ($n = 3$), stand type ($n = 13$) and their interaction on the fruiting date and duration of fruiting of target species. Post hoc comparisons of means using Behrens–Fisher tests allowed the comparison of means of different groups, which have different variance, where significant effects were noted. The same tests were applied to soil variables including average temperature and moisture, as well as temperature and moisture range (the difference between maximum and minimum values during the growing season).

The relationship between the phenological variables of a species and the variables of temperature and soil moisture were analyzed using multiple regressions by testing the coefficients of determination (r^2) with permutations (Legendre, 2005). Knowing that species fructification does not occur consistently within the same quadrat every year, we used the residuals of generalized linear models that took into account the effect of quadrat for all variables. Using these new weighted variables (residuals of generalized linear models), multiple regression models allowed us to test the effect of successive years combined with soil variables. This approach helped to highlight the importance of year, as well as the importance of specific soil variables, on the phenology of mushrooms, irrespective of the effect of quadrats.

Non-parametric Spearman correlations between soil variables (temperature and moisture) and species phenology variables were also calculated separately for each year (only in cases where the species was present in more than 10 quadrats during the same

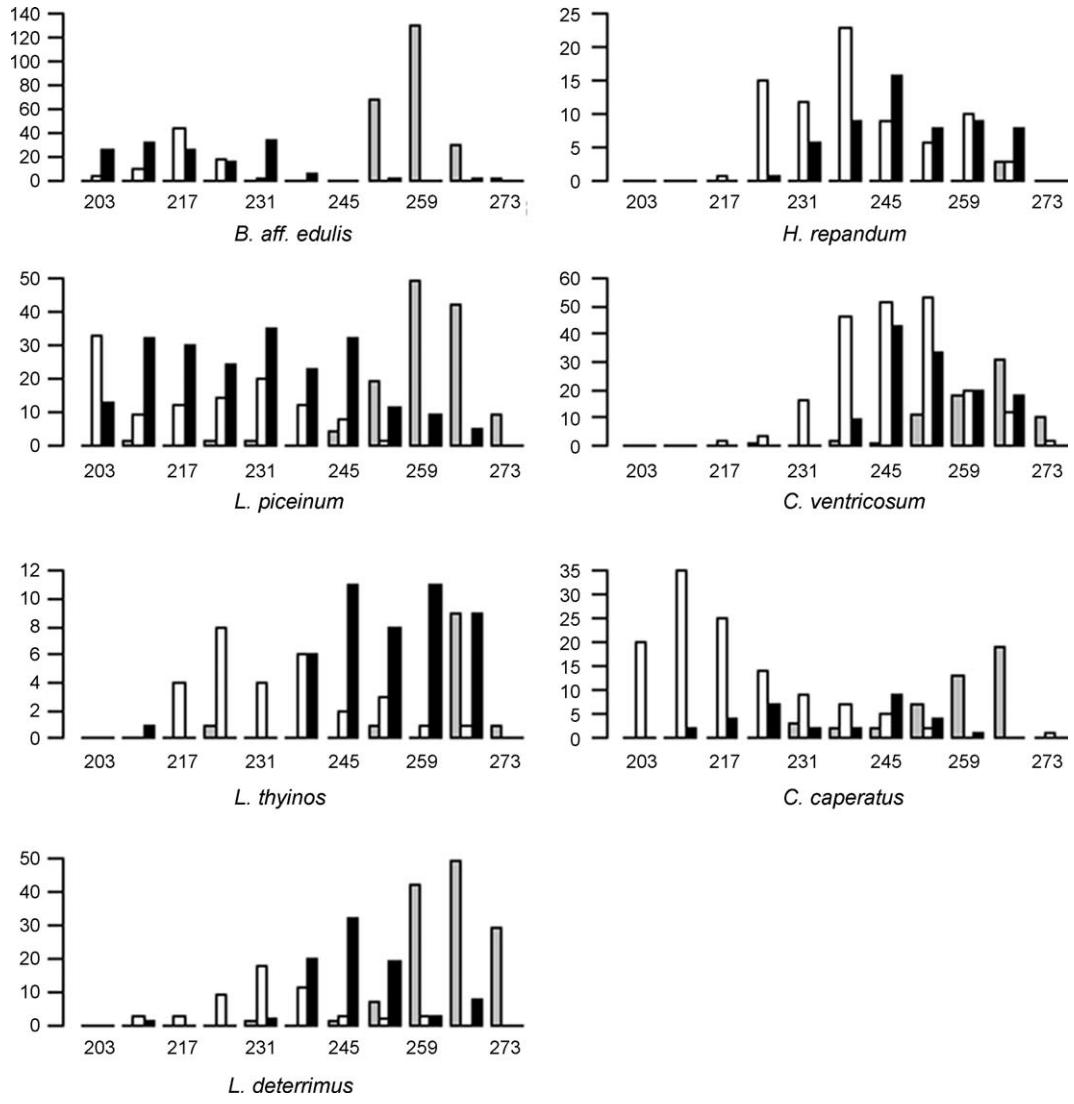

Fig. 2. Fructification patterns of seven edible forest mushroom species (weekly occurrence) from mid July to the end of September (day-of-year) for 2005 (in gray), 2006 (in white) in 2007 (in black) for 481 quadrats in the study area.

year). These analyses were used to assess if, in a given year, there was a spatial effect on variation in temperature and soil moisture on fruiting phenology.

For all multiple comparisons, we applied an alpha inflation correction procedure known as the False Discovery Rate (FDR) to correct for spurious correlations (Benjamini and Hochberg, 1995). All analyses were executed using the Pro-R software, version 2.10.0 (R Development Core Team, 2008).

3. Results

All seven species of edible ectomycorrhizal mushrooms studied displayed different fruiting patterns. Each species showed inter-annual variation in phenology in terms of fruiting date and the number of peaks of emergence throughout the growing season (Fig. 2).

3.1. Variation in soil temperature and moisture

Our *in situ* measurements (Table 2) show that soils were significantly warmer, with lower temperature differences in 2005, and significantly colder, with larger temperature differences in 2006. The soils were significantly wetter in 2007, with larger differences in moisture, and significantly drier in 2006, with smaller differences in moisture.

Stand type had a significant effect on temperature and soil moisture (mean and standard deviation) but multiple comparisons showed that not all stand types were different from one another. Our results suggest that year and forest stand type interact significantly with soil temperature or moisture variations (Table 2) and, thus, these interacting factors likely influenced the phenology of mushroom species that were studied. Although the interactions between year and stand type were significant, the extreme values (for the three years) were related to the same stand types.

3.2. Year and stand effect

Initial fruiting date was significantly different between years for all seven species of mushrooms, whereas stand type had no significant effect (Table 3a). Six of the seven species began fruiting significantly earlier in 2006 and only *B.* aff. *edulis* emerged significantly earlier in 2007.

Duration of fruiting was significantly different between years for five of the seven species studies, with the exception of *H. repandum* and *L. thyinos* (Table 3b). Fructification of *C. ventricosum* and *C. caperatus* was significantly longer in 2006, as it was for *B.* aff.

Table 2
Results from a two-factor Scheirer–Ray–Hare test on the effect of three different years (2005–2007) and 13 different forest types on soil temperature (°C) and soil moisture (%) during the inventory period (mid July to end of September). Means (±SE) for year and for forest type are presented. Post hoc comparison of means using a non-parametric multiple comparison method (Behrens–Fisher), with significant differences indicated by different letters (over the FDR).

$n = 1443$	Mean soil temperature (°C)	Soil temperature range (°C)	Mean soil moisture (%)	Soil moisture range (%)
Year	***H* = 75, df = 2**	***H* = 368, df = 2**	***H* = 651, df = 2**	***H* = 61, df = 2**
	***p* < 0.001**	***p* < 0.001**	***p* < 0.001**	***p* < 0.001**
2005	12.1 ± 0.05a	4.2 ± 0.06a	11.6 ± 0.3a	14.5 ± 0.3a
2006	11.5 ± 0.04b	6.1 ± 0.06b	10.0 ± 0.3b	15.5 ± 0.4a
2007	11.7 ± 0.05c	5.1 ± 0.06c	19.3 ± 0.4c	19.2 ± 0.5b
Interaction	***H* = 60, df = 24**	***H* = 152, df = 24**	***H* = 43, df = 24**	***H* = 241, df = 24**
	***p* < 0.001**	***p* < 0.001**	***p* = 0.018**	***p* < 0.001**
Stand type	***H* = 725, df = 12**	***H* = 122, df = 12**	***H* = 63, df = 12**	***H* = 127, df = 12**
	***p* < 0.001**	***p* < 0.001**	***p* < 0.001**	***p* < 0.001**
Y-nat – white spruce	12.9 ± 0.1a	5.7 ± 0.3a	15.1 ± 0.9abd	12.9 ± 0.6acdef
O-nat – white spruce	11.2 ± 0.3cde	4.7 ± 0.3cef	25.0 ± 4.4a	25.9 ± 3.9b
Y-nat – black spruce	10.9 ± 0.1e	5.7 ± 0.4f	10.7 ± 1.3bd	14.9 ± 1.5b
O-nat – black spruce	10.9 ± 0.04e	5.2 ± 0.1f	12.2 ± 0.4b	18.2 ± 0.6a
Y-nat – balsam fir	11.4 ± 0.2de	4.3 ± 0.2ef	17.7 ± 1.6abd	19.9 ± 1.4bd
O-nat – balsam fir	11.6 ± 0.06d	4.7 ± 0.1e	13.5 ± 0.5bd	17.8 ± 0.77b
Y-nat – balsam fir with deciduous	13.3 ± 0.1a	5.0 ± 0.4bc	17.4 ± 1.7abd	18.3 ± 1.7bc
O-nat – balsam fir with deciduous	12.3 ± 0.1bc	4.9 ± 0.2ad	15.0 ± 1.0acd	19.4 ± 2.1ab
Y-nat – mixed conifer	12.0 ± 0.1bd	4.8 ± 0.2bde	13.4 ± 0.8abd	15.4 ± 0.9ab
O-nat – mixed conifer	11.2 ± 0.06e	4.9 ± 0.1f	15.7 ± 1.0bc	18.5 ± 0.9be
Y-plant – white spruce	12.4 ± 0.05b	4.8 ± 0.1b	12.2 ± 0.3bd	12.5 ± 0.4a
Y-plant – Norway spruce	13.2 ± 0.03a	6.2 ± 0.1a	12.8 ± 0.3abd	11.8 ± 0.4a
Y-plant – Jack pine	11.5 ± 0.04d	5.7 ± 0.1e	11.2 ± 0.4bd	15.3 ± 0.5bf

Y: young; O: old; nat: natural stand; plant: plantation.

edulis, *L. deterrimus* and *L. piceinum* in 2007. The effect of stand type on duration of fruiting was only significant for *B.* aff. *edulis* and *L. piceinum*. However, multiple comparisons showed only differences among stand type for *B.* aff. *edulis*, between young plantations of Norway spruce and young natural stands of mixed conifers.

3.3. Effect of soil temperature and moisture

Initial fruiting date of all species was partially explained by the effect of year (r^2 of 0.16–0.75). Depending on the species, soil temperature and moisture (average and range) had also a significant effect on fruiting date (Table 4a). Year, coupled with temperature range of soil within quadrats, appeared closely related to the initial fruiting date for all species (r^2 of 0.49–0.82), except for *L. thyinos*, which was best accounted for by soil moisture range ($r^2 = 0.56$). On a spatial scale, the date of first fruiting was significantly correlated with: soil moisture range for *B.* aff. *edulis* in 2005 ($R_s = -0.48$; $p = 0.0001 <$ FDR; $n = 101$) and 2006 ($R_s = -0.56$; $p = 0.0001 <$ FDR; $n = 62$); average soil temperature for *B.* aff. *edulis* in 2005 ($R_s = -0.39$; $p = 0.0001 <$ FDR; $n = 101$) and 2006 ($R_s = -0.62$; $p = 0.0001 <$ FDR; $n = 62$), as well as for *L. piceinum* in 2006 ($R_s = 0.41$; $p = 0.001 <$ FDR; $n = 80$); average soil moisture for *L. deterrimus*

Table 3a
Results from a two-factor Scheirer–Ray–Hare test on the effect of three different years (2005; 2006; 2007) and 13 different stand types on initial fruiting date (day-of-year). Means (±SE) for year and stand type are presented. Post hoc comparison of means using a non-parametric multiple comparison method (Behrens–Fisher), with significant differences indicated by different letters (over the FDR).

	B. aff. *edulis*	*C. ventricosum*	*H. repandum*	*L. deterrimus*	*L. thyinos*	*L. piceinum*	*C. caperatus*
	$n = 228$	$n = 205$	$n = 59$	$n = 171$	$n = 28$	$n = 275$	$n = 112$
Year	**$H_{2,3} = 177$**	**$H_{2,6} = 83$**	**$H_{1,4} = 8.82$**	**$H_{2,9} = 114$**	**$H_{2,2} = 9.06$**	**$H_{2,8} = 156$**	**$H_{2,2} = 62$**
	$p < 0.001$	**$p < 0.001$**	**$p = 0.003$**	**$p < 0.001$**	**$p = 0.010$**	**$p < 0.001$**	**$p < 0.001$**
2005	249.5 ± 0.4a	257.1 ± 1.1a	–	258.1 ± 0.8a	251.8 ± 6.7a	253.8 ± 0.9a	250.3 ± 2.2a
2006	216.9 ± 0.4b	238.6 ± 0.1b	–	227.8 ± 1.8b	228 ± 3.7a	212.9 ± 1.5b	211.3 ± 1.5b
2007	207.2 ± 1.3c	243.9 ± 0.9c	–	239.4 ± 1.3c	238.2 ± 3.7a	218.1 ± 1.5b	227.8 ± 3.2c
Interaction	**$H_{2,3} = 2$**	**$H_{2,6} = 9$**	**–**	**$H_{2,9} = 1.0$**	**$H_{2,2} = 4.81$**	**$H_{2,8} = 1.9$**	**$H_{2,2} = 0.345$**
	$p > 0.999$	**$p > 0.999$**	**–**	**$p > 0.999$**	**$p = 0.310$**	**$p = 0.286$**	**$p > 0.999$**
Stand type	**$H_{2,3} = 7$**	**$H_{2,6} = 9$**	**$H_{1,4} = 1.13$**	**$H_{2,9} = 2$**	**$H_{2,2} = 2.44$**	**$H_{2,8} = 9$**	**$H_{2,2} = 2$**
	$p = 0.072$	**$p = 0.172$**	**$p = 0.89$**	**$p > 0.999$**	**$p = 0.300$**	**$p = 0.341$**	**$p = 0.420$**
Y-nat – white spruce	234.2 ± 4.7	246.4 ± 3.3	–	248.9 ± 4.5	–	238.5 ± 5.6	–
O-nat – white spruce	–	–	–	234.1 ± 5.8	–	–	–
Y-nat – black spruce	–	–	–	–	–	–	–
O-nat – black spruce	–	–	230 ± 3.4	–	–	218.8 ± 2.2	218.2 ± 1.8
Y-nat – balsam fir	–	244.4 ± 3.9	238.7 ± 4.6	237.7 ± 6.7	–	237.3 ± 8.6	–
O-nat – balsam fir	–	243.9 ± 2.2	232.4 ± 2.9	234.6 ± 4.8	–	239 ± 4.3	–
Y-nat – B-balsam fir with deciduous	–	–	–	243.3 ± 5.4	241.2 ± 5	235 ± 4.6	–
O-nat – balsam fir with deciduous	–	–	–	245.4 ± 4.3	229.3 ± 5.5	–	–
Y-nat – mixed conifer	234.1 ± 5	245 ± 3.1	239 ± 6	244.7 ± 4.7	237.4 ± 4.1	235.3 ± 5.1	–
O-nat – mixed conifer	–	236.6 ± 3.8	232.9 ± 2.2	241.5 ± 3.4	–	232 ± 5.2	–
Y-plant – white spruce	–	243.5 ± 1.2	–	248.3 ± 1.7	–	233.3 ± 3.2	–
Y-plant – Norway spruce	225.4 ± 1.7	247.4 ± 1.5	–	248.1 ± 3.6	–	–	–
Y-plant – Jack pine	–	–	–	–	–	224.3 ± 3	–

Y: young; O: old; nat: natural stand; plant: plantation.

Table 3b
Results from a two-factor Scheirer–Ray–Hare test on the effect of three different years (2005–2007) and 13 different stand types on fruiting period during the season (number of days). Means (±SE) for year and for stand type are presented. Post hoc comparison of means using a non-parametric multiple comparison method (Behrens–Fisher), with significant differences indicated by different letters (over the FDR).

	B. aff. *edulis*	*C. ventricosum*	*H. repandum*	*L. deterrimus*	*L. thyinos*	*L. piceinum*	C. caperatus
	$n = 228$	$n = 205$	$n = 59$	$n = 171$	$n = 28$	$n = 275$	$n = 112$
Years	$\mathbf{H_{2,3} = 16}$ **$p < 0.001$**	$\mathbf{H_{2,6} = 39}$ **$p < 0.001$**	$\mathbf{H_{1,4} = 2.85}$ **$p = 0.090$**	$\mathbf{H_{2,9} = 7}$ **$p = 0.033$**	$\mathbf{H_{2,2} = 0.037}$ **$p = 0.982$**	$\mathbf{H_{2,8} = 8}$ **$p = 0.020$**	$\mathbf{H_{2,2} = 15}$ **$p < 0.001$**
2005	14.1 ± 0.6a	10.0 ± 0.8a	–	10.8 ± 0.6a	12.7 ± 2.2	10.8 ± 0.5a	13.2 ± 1a
2006	8.4 ± 0.4b	15.1 ± 0.9b	13.8 ± 1.4	9.3 ± 0.9ab	11.2 ± 2.1	9.3 ± 0.5b	11.4 ± 0.7b
2007	15.5 ± 1.2ab	12.9 ± 0.9c	9.9 ± 1.6	11.4 ± 1b	17 ± 3.6	13.5 ± 0.8ab	8.9 ± 0.9c
Interaction	$\mathbf{H_{2,3} = 7}$ **$p = 0.292$**	$\mathbf{H_{2,6} = 15}$ **$p = 0.250$**	$\mathbf{H_{1,4} = 0.67}$ **$p = 0.950$**	$\mathbf{H_{2,9} = 18}$ **$p = 0.438$**	$\mathbf{H_{2,2} = 3.634}$ **$p = 0.458$**	$\mathbf{H_{2,8} = 21}$ **$p = 0.172$**	$\mathbf{H_{2,2} = 0.8}$ **$p = 0.900$**
Stand type	$\mathbf{H_{2,3} = 14}$ **$p = 0.002$**	$\mathbf{H_{2,6} = 7}$ **$p = 0.360$**	$\mathbf{H_{1,4} = 1.93}$ **$p = 0.750$**	$\mathbf{H_{2,9} = 11}$ **$p = 0.295$**	$\mathbf{H_{2,2} = 1.263}$ **$p = 0.532$**	$\mathbf{H_{2,8} = 17}$ **$p = 0.026$**	$\mathbf{H_{2,2} = 4.5}$ **$p = 0.100$**
Y-nat – white spruce	9.8 ± 1.2ab	13.5 ± 2.1	–	10.9 ± 1.6	–	9.9 ± 1.4a	–
O-nat – white spruce	–	–	–	13.5 ± 2.5	–	–	–
Y-nat – black spruce	–	–	–	–	–	–	–
O-nat – black spruce	–	–	9.6 ± 2.2	–	–	11.8 ± 0.7a	11.6 ± 0.6
Y-nat – balsam fir	–	11.3 ± 3.1	10.7 ± 2.2	12 ± 3.4	–	11.7 ± 2.8a	–
O-nat – balsam fir	–	8.9 ± 0.8	13.3 ± 2.2	10.8 ± 3	–	11.3 ± 1.5a	9.8 ± 0.6
Y-nat – balsam fir with deciduous	–	–	–	8.2 ± 1	14.6 ± 3	14.1 ± 2a	–
O-nat – balsam fir with deciduous	–	–	–	11 ± 2.2	18.6 ± 4.6	–	–
Y-nat – mixed conifer	9 ± 0.8b	12.1 ± 1.9	11.8 ± 3.5	11.7 ± 1.4	10.1 ± 1.6	10.4 ± 1.9a	10.7 ± 1.9
O-nat – mixed conifer	–	14.63 ± 3.4	13.6 ± 2.1	7.8 ± 0.7	–	8.2 ± 0.5a	–
Y-plant – white spruce	–	13.8 ± 0.8	–	11 ± 0.7	–	11.6 ± 1.1a	–
Y-plant – Norway spruce	14.2 ± 0.7a	13.3 ± 1	–	9.5 ± 1.1	–	–	–
Y-plant – Jack pine	–	–	–	–	–	11.8 ± 0.9a	–

Y: young; O: old; nat: natural stand; plant: plantation.

in 2005 ($R_S = -0.41$; $p = 0.0001 <$ FDR; $n = 80$), for *L. piceinum* in 2006 ($R_S = 0.52$; $p = 0.0001 <$ FDR; $n = 80$) and for *C. caperatus* in 2006 ($R_S = 0.44$; $p = 0.0002 <$ FDR; $n = 65$); soil moisture range for *C. caperatus* in 2006 ($R_S = 0.41$; $p = 0.0007 <$ FDR; $n = 65$) and in 2007 ($R_S = 0.70$; $p = 0.0003 <$ FDR; $n = 22$).

Duration of fruiting period was weakly correlated with the effect of year for all species (r^2 of 0.04–0.24), while the addition of soil variables contributed significantly to explain the presence of fungal species (Table 4b). The strongest coefficient of determination was found for the relationship between the duration of fruiting bodies of *L. thyinos* and the effect of year coupled with average moisture ($r^2 = 0.60$). Average soil moisture was significantly correlated, positively or negatively, with duration of fruiting for all species (except *C. ventricosum*). On a spatial scale, average moisture was the only soil variable that had an effect on duration of fruiting, and only in the case of *L. deterrimus* in 2005, ($R_S = 0.46$, $p = 0.0001 <$ FDR; $n = 80$).

4. Discussion

4.1. Interannual variation in species phenology

Patterns of fungal fructification vary greatly between species (interspecific variation) and between years within species (intraspecific variation). The phenological variability of a species is expressed by different number and time of emergence peaks, depending on the year (Fig. 2). For all species under study, the effect of year significantly explained part of the variation in initial fruiting date (r^2 of 0.16–0.75) as well as in length of the fruiting period (r^2 of 0.04–0.24).

The development of fungal fruiting bodies is dependent on the availability of surface water and soil temperature (Cooke, 1948; Slankis, 1974; Manachère, 1980) but not all species appear susceptible to the same limiting factors. In fact, soil temperature and moisture conditions, which vary significantly between years, appear to be specifically linked to the phenology of each species.

4.2. Soil water availability and stress

Precipitation is particularly important in initiating fungal fructification (Ohenoja and Metsänheimo, 1982; Mihail et al., 2007). Our results show that high average soil moisture promotes early fruiting of *B.* aff. *edulis* and *L. deterrimus*. According to Hall et al. (1998), fructification of the European variety of *B. edulis* may even be inhibited during very dry summers. The duration of fruiting is also slightly favored by high average soil moisture, particularly in the case of *L. thyinos*. However, excess water can stimulate the development of the mycelium at the expense of fungal fructification (Manachère,

Table 4a
Multiple regressions with permutations (r^2) between initial fruiting date and year, average soil temperature, and soil temperature range. Only the significant soil variables are presented ($p <$ FDR).

		Year only	Year + mean soil temperature	Year + soil temperature range	Year + mean soil moisture	Year + soil moisture range
B. aff. *edulis*	$n = 228$	0.75	–	(−)0.82	(−)0.78	0.77
C. ventricosum	$n = 205$	0.48	0.50	(−)0.60	0.51	0.51
H. repandum	$n = 59$	0.16	–	(−)0.61	–	–
L. deterrimus	$n = 171$	0.40	–	(−)0.54	(−)0.42	0.46
L. thyinos	$n = 28$	0.26	–	–	–	(−)0.56
L. piceinum	$n = 275$	0.44	0.45	(−)0.62	–	–
C. caperatus	$n = 112$	0.27	–	(−)0.49	0.36	0.30

Bracketed negative sign specifies the direction of the relationship.

Table 4b
Multiple regressions with permutations (r^2) between duration of fruiting and year, average soil temperature, and soil temperature range. Only the significant soil variables are presented (p < FDR).

		Year only	Year + mean soil temperature	Year + soil temperature range	Year + mean soil moisture	Year + soil moisture range
B. aff. *edulis*	n = 228	0.24	–	(−)0.29	0.25	–
C. ventricosum	n = 205	0.18	–	–	–	(−)0.20
H. repandum	n = 59	0.20	0.37	0.50	(−)0.36	(−)0.35
L. deterrimus	n = 171	0.04	0.08	(−)0.13	0.10	–
L. thyinos	n = 28	0.18	–	–	0.60	(−)0.32
L. piceinum	n = 275	0.13	–	–	0.17	–
C. caperatus	n = 112	0.17	–	–	(−)0.22	–

Bracketed negative sign specifies the direction of the relationship.

1980). As our results show, excess water may have a limiting effect on the fruiting phenology of certain species. High levels of average soil moisture within quadrats delay the initial fruiting of some species (i.e. *C. caperatus* and *C. ventricosum*) and reduces the duration of fruiting of other species (i.e. *C. caperatus* and *H. repandum*).

A wide soil moisture range, resulting in water stress, is also linked to species phenology. Water stress partly explains the shortened fruiting period and is related to delayed fruiting dates in some species (Tables 4a and 4b). In contrast, *L. thyinos* was positively related to water stress, and it seems that a wide soil moisture range stimulates initial fruiting date. Overall, interspecific fruiting phenology is affected differently by water availability and/or water stress in soil.

4.3. Soil temperature and the effect of cold-shock

The fruiting phenology of fungal species is sensitive to high temperatures. Elevated average soil temperature delays the initial fruiting date of *C. ventricosum* and *L. piceinum*. Furthermore, extreme variation in soil temperature during a growing season reduces the duration in which *B.* aff. *edulis* and *L. deterrimus* are present. High temperatures delay the fruiting of certain fungal species (Straatsma et al., 2001; Mihail et al., 2007; Kauserud et al., 2008) and according to Straatsma et al. (2001), an increase of 1 °C coincides with a one-week delay in fructification. However, temperature is not a limiting factor in the fructification of all species. For example, the duration of fruiting of *H. repandum* is positively correlated with high average soil temperature. The increased temperatures seen in the second half of the 20th century explain the expanded fruiting periods found in the United Kingdom (Gange et al., 2007) as well as the delayed fructification of species found in Norway (Kauserud et al., 2008). Thus, the effect of increased temperature rate on species fructification seems dependent on the autoecology of the species, in addition to the location under consideration.

The effect of year, coupled with soil temperature range, best explains the initial fructification of species. The increase in soil temperature range is the only factor apparently associated with the initial fruiting date of almost every studied species (except *L. thyinos*). A wide range of soil temperature during the whole growing season cannot be entirely considered as a causative of mushroom fruiting precocity based on our results. Nevertheless, this appears as a crucial factor in species phenology, acting as a hyphae stimulus and triggering fructification. It is well understood from laboratory experiments that a lowered incubation temperature of several degrees is required to initiate fruiting, a phenomenon known as cold-shock, for many saprophytic species including *Agaricus bisporus* (San Antonio, 1971), *Coprinus atramentarius* (Stott and Broderick, 1995), *Pleurotus ostreatus* (Penas et al., 1998), *Lentinula edodes* (Pire et al., 2001) and *Grifola frondosa* (Montoya Barreto et al., 2008). This phenomenon has also been suggested in natural settings for *B. edulis* (Hall et al., 1998), and Straatsma et al. (2001) postulated that the autumnal fructification peak is stimulated when average weekly temperatures fall below 14 °C. Our *in situ* data illustrating direct or indirect effects of temperature variation on the initiation of fructification of many ectomycorrhizal fungi species strongly supports the hypothesis of a cold-shock phenomenon in the mixed boreal forest.

4.4. Effect of stand type and spatial effect on the phenology of fructification

In addition to temporal variations (interannual scale), fruiting phenology can vary spatially within the same growing season. Certain factors related to soil temperature and moisture are correlated spatially with the initial fruiting date for some species, during some years (*B.* aff. *edulis*, *L. piceinum*, *L. deterrimus* and *C. caperatus*) and, in one instance, with the duration of fruiting (i.e. *L. deterrimus*; average soil moisture in 2005). However, these correlations are not found consistently each year and several soil factors that significantly influence species phenology on a temporal scale (interannual) are not correlated on a spatial scale (across quadrats). Therefore when considering the phenology of edible mushroom species, soil factors seem to play a less predominant role on the regional spatial scale than on the temporal scale.

Other features of forest habitats, such as composition, age and stand origin may also influence the phenology of fungal species. For example, *Amanita muscaria* reacts more quickly to precipitation in mature forests than it does in young stands (Last et al., 1981), and the date of fruiting can be stimulated or delayed for *Laccaria laccata*, depending on its host (Molina, 1982). The type of conifer stand did not have a significant effect on the phenology of any species studied, although *B.* aff. *edulis* demonstrated some variation in phenology in young plantations of Norway spruce; however this is likely attributed to significant differences in soil conditions. According to Wilkins and Harris (1946), forest type has no effect on the abundance peak of mushrooms within the same region. This is consistent with our results showing that the type of forest stand generally does not influence the phenology of edible mushroom species in the mixed boreal forest of Eastern Canada.

5. Conclusion

Variation in phenology of edible ectomycorrhizal mushrooms is principally due to soil temperature and moisture conditions on an interannual scale. Other factors, such as intraspecific genetic heterogeneity (Samson and Fortin, 1986; Selosse et al., 2001) or climatic conditions of the previous year (Kauserud et al., 2008), could also explain some of the observed variation in phenology. The phenological responses to soil conditions are specific to each species of fungi and any given factor can be either restrictive or favorable. Nonetheless, the date of initial fructification for six of the seven species studied responded uniformly to variation in soil temperature range. The possible effect of a large variation in soil

temperature, which would stimulate the initial fructification of edible mushrooms, supports the cold-shock hypothesis in the natural environment of the mixed boreal forest of Eastern Canada. Further field manipulative experiences would allow testing this hypothesis. Taking soil moisture and temperature conditions into account could contribute to the development of predictive models of fungal fruiting in order to optimize the management of harvesting edible forest mushrooms.

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