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Cultural studies of *Morchella elata*

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ABSTRACT

The *in vitro* growth of *Morchella elata* was characterized with respect to the effects of a variety of substrates, isolates, developmental status of the parental ascoma, temperature, and pH. Optimal substrates for growth included sucrose, mannose and lactose, but the growth of some isolates was substantially reduced in some composite media. Maltose and potato-dextrose media limited growth and caused changes in colony morphology; mycelial pigmentation was black in the case of maltose, and mycelial margins were plumose in potato-dextrose cultures. Rapid growth was most reliably achieved in a composite medium containing 1:1 sucrose:mannose. Isolates derived from single ascospores shortly after ejection from ascomata varied in ability to grow in the various substrates. This may be related to variable maturity or dormancy; increasing growth rates correlated with pileus length in the parental ascomata, and ascomata that initially produced slower-growing or abortive colonies produced faster-growing colonies after storage at 20 °C for 96 wk. The growth of *M. elata* derived from recently ejected ascospores was optimal at 16–24 °C or above for a faster-growing isolate, and 20–24 °C or above for a slow-growing isolate. Although neither isolate grew at 8 °C or below in an initial experiment, spawn cultured on puffed wheat at 28 °C produced mycelia that proliferated when transferred to soil media and incubated at 8 °C. Growth of *M. elata* in liquid cultures adjusted with potassium hydroxide was optimal at pH 7.0, and was relatively sensitive to more acidic or alkaline pH. When calcium carbonate was used to adjust pH, optimal growth shifted to pH 7.7 or above, suggesting that wood ash and other calcium compounds may not only stimulate growth in natural settings, but also alter the optimal pH for proliferation of *M. elata*. Further studies with other substrate combinations and incubation conditions will be necessary to fully understand the connections between *in vitro* growth and the ecological behaviour of the fungus.

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Morel (*Morchella* spp.) fruiting bodies are a well-known and important non-timber forest product in North America, where commercial production depends largely on harvest of wild mushrooms. Harvesting usually focuses on areas recently affected by forest fires (Amaranthus & Pilz 1994; Wurtz *et al.* 2005), where some species may fruit in prolific abundance (Duchesne & Weber 1993). In British Columbia (BC), morel harvests during 1992 were documented to produce at least 32,000 kg, despite a relatively low availability of burned areas and unsuitable weather that year (DeGeus 1993). Unfortunately, the actual cumulative economic benefit from this

resource is capricious and difficult to quantify or manage, because productive areas are usually ephemeral and difficult to predict. Although the habitat and behaviour of North American morel species have been extensively studied and reviewed (e.g. Obst & Brown 2000; Weber 1988), efforts to improve reliable harvesting of this non-timber forest resource will require a more substantial understanding of the ecological and physiological behaviour of these fungi (Kenney 1996). With such an understanding, it may be possible to better predict productive habitat or improve the productivity of prescribed burns used in forest management. For example, the

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intensity of prescribed burning is controlled in part by weather and moisture levels, and fire intensity in turn affects factors such as soil pH. Improved morel productivity could result if prescribed fires were timed to produce optimal soil conditions.

The objective of this research was to determine the cultural characteristics of several *M. elata* (*sensu* Weber 1988) isolates found near Victoria, BC, on Vancouver Island. *M. elata* is found in a variety of habitats there, including burned or disturbed areas, and forests and forest edges. Ascomata of *M. elata* collected by R. Winder and deposited in the DAVFP herbarium (Victoria, BC) include specimens repeatedly found in association with domesticated or wild members of the Rosaceae such as *Malus domestica* (apple), *Prunus laurocerasus* and *Holodiscus discolor* (ocean spray) (Natural Resources Canada 2005). *M. elata* is considered by some taxonomists to be synonymous with, or part of, a complex of black morel species or subspecies including *M. angusticeps* and *M. conica* (Bunyard *et al.* 1995; Wipf *et al.* 1999). The ascomata of *M. elata* are distinguished from other black morels by smooth, ivory-white stalks in younger fruiting bodies, by steel-grey tones in the ridges and pits of the pileus, and by the production of spores larger than those of *M. angusticeps* (Weber 1988). Previous investigations of various *Morchella* spp. have explored nutrient optima (Brock 1951; Robbins & Hervey 1959, 1965; Volk & Leonard 1989), the impact of nutrient gradients and flux (Ower *et al.* 1986, 1988, 1989; Amir *et al.* 1992, 1994, 1995; Amir 1993; Philippoussis & Balis 1995), pH and calcium concentration (Brock 1951; Robbins & Hervey 1965), and temperature or climatic impacts (Buscot 1993; Goldway *et al.* 2000; Obst & Brown 2000). More complex genotypic and developmental factors affecting cultural behaviour have also received attention. Gilbert (1960) observed 'recognizable differences' in three strains of *M. angusticeps* grown on agar or in liquid culture, but did not quantify them. Hervey *et al.* (1978) found that cultures of *M. esculenta* derived from single ascospores were variable regarding initial growth rates and the formation of aerial hyphae and sclerotia; again, the scope of variability was not quantified. Buscot (1993) quantified sclerotia formation in single ascospore isolates of *M. esculenta*, and observed developmental variation among isolates; it was proposed that the variability could reflect an ability to shift developmental patterns based on nutrient availability and different physiological requirements in varied habitats. Philippoussis and Balis (1995) also observed and quantified differences in sclerotia production by two isolates of *M. elata*, three isolates of *M. vulgaris*, and five isolates of *M. rotunda* (currently considered to be a synonym of *M. esculenta*).

Recent studies have suggested that there may be several cryptic species within the black morel group found in the Pacific Northwest (Pilz *et al.* 2004); hence the physiological and ecological similarities and differences of taxa in this group should be described. This study evaluated and compared the effects of physiological parameters, including substrate, temperature, pH and ascospore maturity, on the growth of *M. elata* isolates from Vancouver Island. To avoid problems caused by the uncertain taxonomic status of *M. elata*, a specific objective of the study was to characterize isolates corresponding to voucher specimens deposited in herbarium and culture collections.

Materials and methods

Isolate collection and stock cultures

An overview of experimental procedures is presented in Fig 1. Twelve ascomata of *Morchella elata* were collected in April 2003, at locations listed in Table 1, and stored at 5 °C for 1 d. Ascoma size and maturity were assessed by measuring the height of each pileus above the stipe. Portions of the pileus from each fresh ascoma were affixed under the cover of a plastic Petri dish with petroleum jelly (one portion per lid), and the lid was positioned over Petri dishes containing potato-dextrose agar (PDA). The PDA (BBL 4398196, Becton Dickinson, Cockeysville, MD) contained 4 g l⁻¹ potato infusion, 20 g l⁻¹ dextrose and 15 g l⁻¹ agar. After approximately 2 h of ascospore deposition (20 °C), the covers were replaced with clean lids and sealed with paraffin film. After being sampled for cultures, ascomata HA, HB, and HC were placed on separate covered plastic trays where they were allowed to deposit an additional collection of spores. The spore deposits were scraped into separate glass vials after 1 d. All ascomata were air-dried in separate paper bags (48 h) and stored in the bags for 22 m at 20 °C. The spores in vials were also stored at 20 °C. Meanwhile, the agar cultures were incubated (1 d) until colonies approximately 2 mm in diameter formed. With the aid of a microscope, eight colonies originating from single ascospores were individually excised from each plate with a dissecting needle and aseptically transferred to Petri dishes that contained malt extract agar (MEA) to form stock cultures. The MEA (Difco 0112-17-6, Difco Laboratories, Detroit, MI) contained 12.75 g l⁻¹ maltose, 2.75 g l⁻¹ dextrin, 2.35 g l⁻¹ glycerol, 0.78 g l⁻¹ peptone and 15 g l⁻¹ agar. The stock cultures were sealed with paraffin film and incubated at 20 °C until the colonies reached the edges of the plates. Pure cultures were stored at 15 °C. Agar and liquid cultures for subsequent experiments were made by aseptic transfer of 6-mm diam pieces cut from stock cultures with a sterile cork borer.

Data analysis

Unless otherwise stated, all statistical analysis was conducted with computer software (Statistica 6.1, Statsoft Inc., Tulsa, OK).

Isolate growth versus substrates

Stock cultures

To assess variation in growth rates observed during incubation of stock cultures, isolate growth was compared using a completely randomized experimental design. Treatments (ascoma origins) were imposed as a classification variable, with each stock culture (isolate) considered a replicate for its respective parental ascoma. There were 69 viable isolates from 12 ascomata (Table 2). Isolate cultures were returned from stock cultures to Petri dishes containing PDA. The resulting cultures were randomly arranged on a shelf and incubated at 15 °C. After 7 d, the longest and shortest radii were measured for each colony, and the growth of each culture was calculated from the mean radius. Data were subjected to analysis of variance. A nonlinear regression analysis was also used to compare the

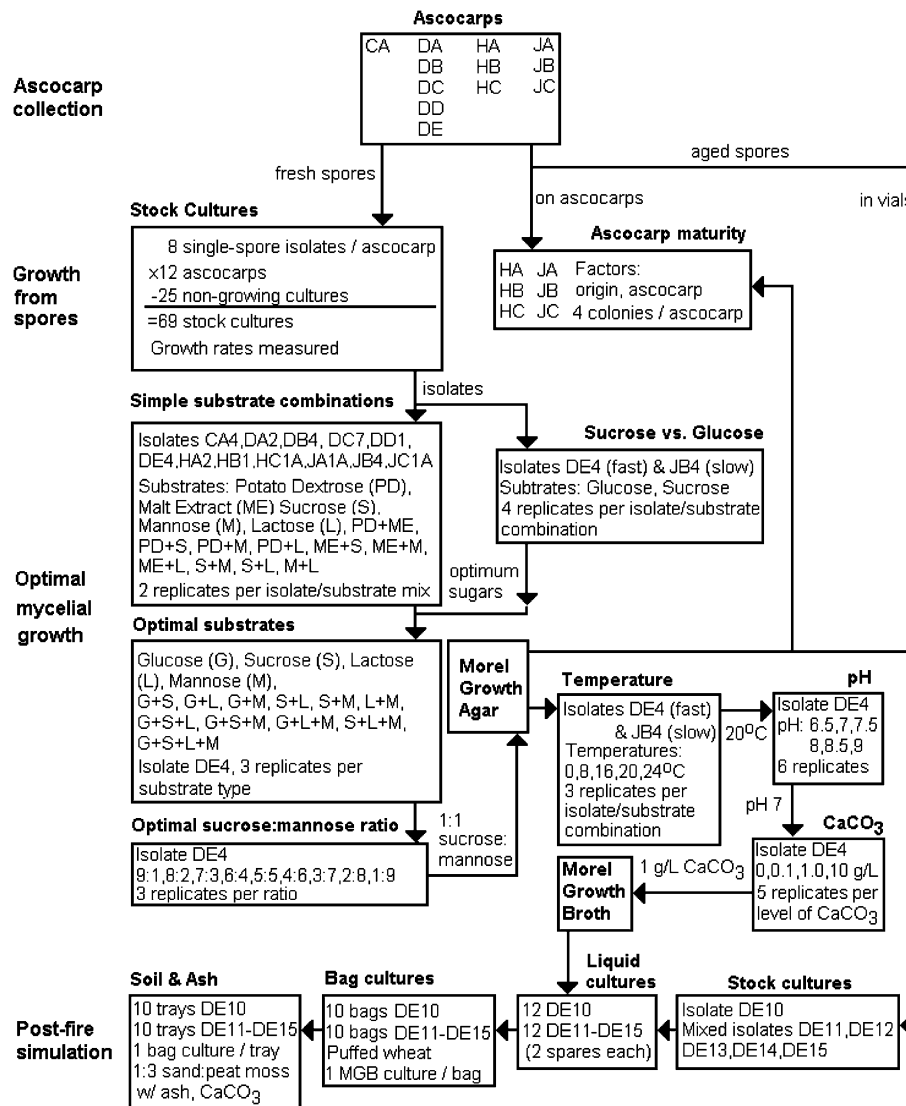


Fig 1 – Overview of experimental procedures.

mean growth rate of colonies from each ascoma with the height of the parental pileus. These analyses were performed with computer software (Sigmaplot 8.02, SPSS Inc., Chicago, IL). The three Langford ascomata were excluded from the regression analysis because they produced atypically dormant spores.

Simple substrate combinations

Variation in isolate growth on simple substrate combinations was determined using a 15×10 factorial experimental design. The first factor, substrate combination, consisted of one of 15 1:1 (v:v) combinations of PDA, MEA, or agar (15 g l^{-1}) that incorporated sucrose, lactose, or mannose (Fig 1). The latter three substrates were included because of their previously reported optimal effect on the growth of *Morchella esculenta* (Brock 1951). To supplement nitrogen, yeast extract was added to each type of medium, using an amount calculated as 1 % of the combined weight of substrate and agar. The mixtures were added to Petri dishes for a combined concentration of 0.037 M. For PDA, only

the amount of dextrose (glucose) present was used to calculate molarity. The second factor, isolate, consisted of the fastest-growing isolates from each of the ascomata (Table 2). Isolates from only nine parental ascomata were included in the test; colonies from ascomata JA and JC were dormant in the growth-rate test and therefore were excluded. After Petri dishes were inoculated with the isolates, the cultures were incubated for 7 d at 20°C , and colony growth rates were measured as before. Cultural characteristics including colour, abundance of aerial hyphae, abundance and size of sclerotia, and distribution of sclerotia throughout the colony were also noted. The experiment was repeated once and the combined data were subjected to a factorial analysis of variance.

Glucose versus sucrose

Because most isolates did not perform well on PDA, a 2×2 factorial experiment was employed to compare the growth of isolates without potato infusion, using glucose (dextrose) versus an optimal substrate (sucrose). The first factor, substrate,

Table 1 – Origin of *Morchella elata* isolates collected on Southern Vancouver Island in April, 2003, and their mean radial growth on potato-dextrose media

Location ^b	Habitat	Number of ascomata collected	Mean ^a radial growth (mm d ⁻¹)
1. Victoria, B.C. (48°27' N, 123°22'W)	Urban lawn	1	2.3 A
2. Sooke, B.C. (48°24' N, 123°41'W)	Rural garden near forest ^c	5	1.9 A
3. Sooke, B.C. (48°25' N, 123°41'W)	Rural lawn near forest ^c	3	0.9 B
4. Langford, B.C. (48°26' N, 123°31'W)	Urban roadside leaves ^d	3	0.1 B

a Means followed by the same letter are not significantly different according to the Newman-Keuls test ($P < 0.05$).

b Geographic longitude and latitude are rounded to the nearest minute.

c Ten metres from edge of forest wilderness.

d One hundred metres from edge of large forest.

was assayed in Petri dishes containing 15 g l⁻¹ agar with either 0.037 M sucrose or 0.037 M glucose, with both containing yeast extract as in the first combined nutrient experiment. The second factor (isolate) was tested by inoculating plates with either a relatively slow-growing (JB4) or relatively fast-growing (DE4) isolate. Each treatment combination was replicated four times. The cultures were incubated for 7 d at 20 °C, and colony growth rates were measured as in the PDA experiment. The data were subjected to a factorial analysis of variance.

Substrate selection for an optimal medium

A fractional 4 × 4 × 4 factorial experiment was employed to search for a combination of optimal substrates suitable for maximum growth of *Morchella elata*. The first factor,

primary substrate, included either glucose, sucrose, lactose or mannose. The second, third, and fourth factors, termed secondary, tertiary, and quaternary substrates, included the same substrates. Combinations of the second factor were omitted if the constituents duplicated those made with primary substrates. Similarly, combinations for the third factor were omitted if the constituents duplicated combinations with primary or secondary substrates, and combinations for fourth factor were omitted if the constituents duplicated combinations with primary, secondary, or tertiary substrates (Fig 1). Substrates were mixed in 15 g l⁻¹ agar in a 1:1:1:1 (w:w:w:w) ratio to achieve a combined 0.037 M concentration, and yeast extract equivalent to 1 % of the substrate weight was added to each medium combination before it was autoclaved and dispensed into Petri dishes. Each substrate combination was replicated three times. The dishes were inoculated with a fast-growing isolate (DE4), and incubated for 7 d at 20 °C. Colony growth was measured as in the PDA experiment, and the data were subjected to a fractional factorial analysis of variance. Because the experimental design was fractional, the analytical model used was limited to main effects and two- or three-way interactions.

For the optimal nutrient combination occurring in previous experiments (1:1 sucrose:mannose), a completely randomized experimental design was used to assay the optimal ratio of constituents. Sucrose and mannose were mixed in 15 g l⁻¹ agar at ratios of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9 to achieve a combined 0.037 M concentration. Yeast extract equivalent to 1 % of the substrate weight was added to each of the nine treatment combinations, each of which was replicated three times. The cultures were inoculated with a fast-growing isolate (DE4) and incubated for 7 d at 20 °C. The cultures were measured as in the PDA experiment, and the data were subjected to analysis of variance.

Ascoma maturity

The effect of ascoma maturity on growth rates was evaluated using a 2 × 3 factorial experimental design, where ascoma origin (three or four) was the first factor, and individual ascoma (three per treatment) was the second factor. Ascoma maturity was determined by measuring the length of the pileus from the stipe to the apex. An optimal growth medium was prepared using 15 g l⁻¹ agar, optimal substrates found in the previous substrate experiments (6.5 g l⁻¹ sucrose, 3.4 g l⁻¹

Table 2 – Designation of parental ascomata for *Morchella elata* isolates

Ascoma	Origin ^a	DAVFP no. ^b	Isolates	Best isolate ^c	UAMH no. ^d
CA	1	28858	CA1-CA5	CA4	10559
DA	2	28864	DA1-DA5	DA2	10560
DB	2	28865	DB1-DB6	DB4	10561
DC	2	28866	DC1-DC8	DC7	10562
DD	2	–	DD1-DD6	DD1	10563
DE	2	–	DE1-DE6	DE4	10564
HA	3	28860	HA1-HA6	HA2	10565
HB	3	28861	HB1-HB6	HB1	10566
HC	3	28862	HC1-HC5, HC1A	HC1A	10567
JA	4	28867	JA1-JA5, JA1A	JA1A	10568
JB	4	28868	JB1-JB5	JB4	10569
JC	4	28869	JC1-JC6, JC1A	JC1A	10570

a Numbers in this column correspond to the numbered origins in Table 1.

b Accession numbers for ascomata placed in the DAVFP herbarium (PFC, Victoria). Ascomata DD and DE were not deposited due to sample deterioration.

c Isolates with maximal growth. Rapidly growing isolates from ascomata JA, JC and HC are from stored ascospores; initial cultures were dormant or slow-growing.

d Accession numbers from the University of Alberta Microfungus Collection and Herbarium, Edmonton, Alberta, for the representative (best) isolate at left.

mannose), and 0.14 g l^{-1} yeast extract. This medium is referred to hereinafter as morel growth agar (MGA). Ascospores were scraped from each of the dried and stored fruiting bodies, which were in a semi-desiccated form 48 h after drying commenced. The spores were scraped into separate Petri dishes containing MGA. After 2 d incubation at 20°C , the radial growth of isolated, single-spore colonies was measured in each Petri dish (four replications per ascoma). The data were subjected to analysis of variance.

Isolate growth versus temperature

The growth of two isolates at various temperatures was assayed using a 2×5 factorial experiment. The first factor, inoculum type, involved inoculation of cultures with either a slow-growing (JB4) or fast-growing (DE4) isolate of *Morchella elata*. The second factor, temperature, was provided by incubators that maintained constant air temperature of 0, 8, 16, 20, or 24°C . Cultures were grown in Petri dishes with MGA. Each treatment combination was replicated three times. Cultures were incubated for 5 d, and measured as in the PDA experiment; data were subjected to a factorial analysis of variance.

Optimal pH and calcium

A completely randomized experimental design was used to evaluate impact of pH on growth of a fast-growing isolate of *Morchella elata* (DE4). Treatments consisted of six levels of pH: 6.5, 7.0, 7.5, 8.0, 8.5, or 9.0. For each treatment, three agar plugs of isolate DE4, each measuring 6 mm in diameter, were transferred to a 1 l Erlenmeyer flask containing 250 ml morel growth broth (MGB), which consisted of aqueous 0.037 M 1:1 (w:w) sucrose:mannose with 0.14 g l^{-1} yeast extract. The desired pH in each treatment was achieved after autoclaving by addition of aqueous potassium hydroxide or hydrochloric acid until the colour of paper indicator strips (ColorpHast®, E. M. Science, Gibbstown, NJ) matched the correct pH. Each treatment was replicated six times. The cultures were incubated at 20°C on a rotary shaker (100 rev min^{-1}) for 7 d, and the colonies removed by vacuum filtration through filter paper. After fresh weights were recorded, the colonies were dried in a food dehydrator (54°C) for 24 h, and dry weights were recorded. The data were subjected to analysis of variance.

To determine effect of calcium concentration on morel growth, a similar completely randomized experimental design was employed. Treatments consisted of four levels of calcium carbonate (0.0, 0.1, 1.0, and 10.0 g l^{-1}) in 1 l Erlenmeyer flasks containing 250 ml MGB. Each calcium carbonate level treatment was replicated five times. Isolate DE4 was transferred to the flasks and incubated as in the pH study. The filtration method used in the pH study was modified: accumulations of crystalline calcium carbonate were manually separated and rinsed from colonies after filtration to provide accurate biomass measurements. Fresh and dry weights were measured as in the pH study, and the data were subjected to analysis of variance.

Simulated post-fire conditions

A completely randomized experimental design was used to evaluate growth and potential fruiting of *Morchella elata* in simulated post-fire conditions. A bag culture method was employed to produce larger amounts of inoculum. Fresh isolates were used to minimize any effect from storage on artificial media. Spores from the deposit formed by dried ascoma DE (Table 2) were spread over the surface of MGA in Petri dishes. After incubation for 2 d (20°C), mycelia derived from a single spore, designated isolate DE10, and mycelia derived from five neighbouring spores, designated isolate group DE11-16, were transferred to MGA in Petri dishes. Resulting cultures were stored at 15°C and used as stock cultures for further inoculations. Twelve separate flasks with MGB were inoculated with isolate DE10 and 12 were inoculated with isolate group DE11-16, as in the pH growth study, with MGB modified by the addition of 10 g l^{-1} calcium carbonate. The MGB cultures were incubated on a rotary shaker (175 rev min^{-1}) for 14 d at 20°C . The cultures were used to inoculate culture bags according to the method of Winder (1999). Polypropylene bags ($20 \times 47 \text{ cm}$) with filter vents were filled with 200 g puffed wheat and 200 ml water, sealed with foam plugs held in place with plastic collars, and autoclaved at 121°C for 20 min. After the medium cooled, each of the 20 bags was inoculated with a separate MGB culture; four of the MGB cultures were not included due to contamination. The bags were resealed and incubated at 28°C for 50 d.

Bag cultures were transferred to a refrigerator ($2 \pm 2^\circ\text{C}$) and stored for 39 d. The cultures were removed from the bags and individually soaked for 1 min in approximately 3 l fresh tap water to remove frost and to mimic the effect of late winter rains. The cultures were placed in $48.5 \times 27 \times 7.5 \text{ cm}$ plastic trays with drain holes on the bottom, and covered with a 1:3 (v:v) mixture of dry coarse sand and dry sphagnum peat moss (Premier Pro-moss Emerald, Premier Horticulture, Rivière-du-Loup, Québec). To avoid sagging, each tray was placed inside a similar sized skeletal support tray with cross-bracing. In each tray set, a layer containing a mixture of 30 g calcium carbonate and 70 g ash and fine cinders from burnt stems of red alder (*Alnus rubra*) was sprinkled onto the surface, followed by 1.25 l distilled water. The tray sets were wrapped loosely in open plastic bags and placed in darkness in an incubator with controlled air temperature (8°C) for 20 d. They were each watered again (500 ml) after 7 d, and the trays were examined for the presence of mycelium, conidia, and primordia or fruiting bodies.

Results

Isolate growth versus substrates

Stock cultures

Isolate growth rates were variable and scattered (Fig 2). Origin of the ascomata had a significant ($P < 0.001$) effect on growth rates; most isolates from ascomata originating in Langford, BC, failed to grow after transfer from stock cultures (Table 1). Nonlinear regression analysis also showed a significant ($P < 0.031$) correlation between pileus height and mean growth

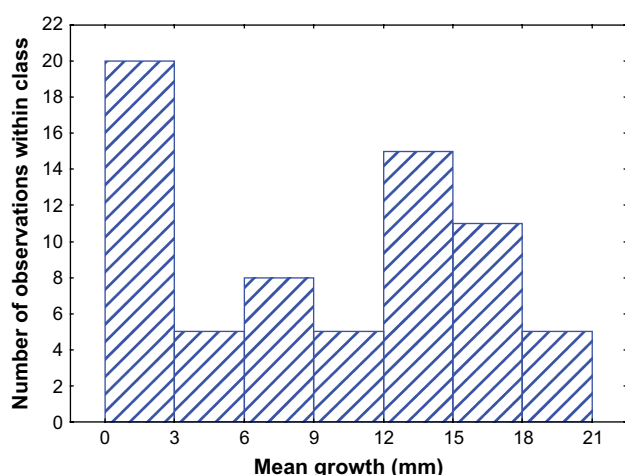


Fig 2 – Distribution of mean radial 7-d growth for 69 colonies of *Morchella elata* derived from isolates collected from Vancouver Island. Observations are grouped into incremental classes that span 3 mm.

rate (Fig 3); this regression accounted for about half of the variance in growth means ($R^2 = 0.51$).

Simple substrate combinations

Isolates displayed some individualistic responses to media combinations. The data were separated into a slower-growing cohort (Fig 4) and a faster growing cohort (Fig 5) for clearer presentation. There were significant main effects from both isolate ($P < 0.01$) and substrate combination ($P < 0.01$); but their interactive effect was also significant ($P < 0.01$); isolates from ascomata JB, HA, HB and HC were relatively insensitive to substrate changes and tended to grow slowly, while other isolates grew rapidly in the absence of maltose or PDA. Among slower isolates, isolate JB4 usually produced the fastest growth, but this was diminished in combinations of mannose and sucrose or lactose. All of the slower-growing isolates had reduced

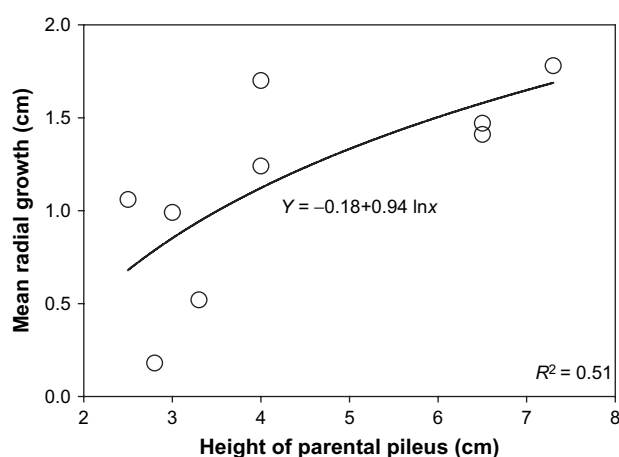


Fig 3 – Correlation ($P < 0.05$) between growth rates of *Morchella elata* isolates and height of parental pileus. Means refer to the group of isolates originating from one ascoma.

growth rates in media with maltose or PDA, except isolate JB4 in combined sucrose and maltose (Fig 4). In the faster-growing group, optimal growth rates generally occurred in media with mannose; sucrose and mannose; lactose, mannose and PDA; lactose and PDA, or; maltose and PDA. Growth rates were mixed on media containing maltose or sucrose and maltose, and they were generally slower on media with sucrose; sucrose and lactose; mannose and lactose; mannose and maltose; lactose and maltose; PDA, and; sucrose with PDA. Isolates CA1 and DC7 were particularly sensitive to substrate differences. For example, the growth rate of isolate CA1 was similar to other optimally growing isolates in media containing sucrose and mannose, but was much less than the optimally growing isolates when sucrose was combined with maltose.

There were consistent differences in cultural morphology in the various substrates. Cultures grown on PDA were typically light tan with some aerial hyphae and an accumulation of microsclerotia near the centre. After several weeks in PDA, larger sclerotia began to form some distance from the centre. Mycelial mats were very dense and mostly submerged in malt agar; the colonies developed a black pigmentation. Hyphae were also submerged and darkly pigmented (dark brown to black) in agar mixed with sucrose, mannose, or lactose, although mycelial growth was more dispersed, especially in lactose. Colonies growing in sucrose and mannose tended to form dense clumps of microsclerotia in short arcs some distance from the centre. In lactose, microsclerotia typically occurred all around the periphery of the colony or at the thickest parts of the mycelial mat. When sucrose or lactose was added to PDA, colony margins were plumose rather than even, and the agar surface became somewhat uneven with dense accumulations of microsclerotia at the centre. Cultures grown on a mixture of PDA and mannose produced diffuse microsclerotia at the agar surface, as well as a few submerged clusters of microsclerotia. Sucrose and mannose produced reddish-tan colonies when combined; these colonies had abundant aerial hyphae, but microsclerotia were infrequent, small and well-dispersed. When lactose and mannose were combined, colonies were much the same as those growing in only lactose, with slightly lighter (brown) pigmentation and somewhat denser growth that was more evenly dispersed. The combination of sucrose and lactose produced colonies with mostly submerged hyphae and no microsclerotia (Fig 6).

Glucose versus sucrose

In the absence of potato extract, cultures growing on glucose agar were slower than those growing on sucrose agar (Table 3). The main effects of substrate and isolate were significant ($P < 0.001$), as was the interactive effect ($P < 0.01$). As expected, isolate DE4 grew more rapidly than JB4. However, the growth of isolate DE4 was reduced only by 20 % on glucose, compared with 60 % for growth of isolate JB4.

Substrate selection for an optimal medium

In the first fractional factorial experiment, isolate DE4 grew most rapidly in cultures containing a combination of sucrose and mannose (Fig 7). Two significant main effects within the combinations were tested; growth in cultures containing

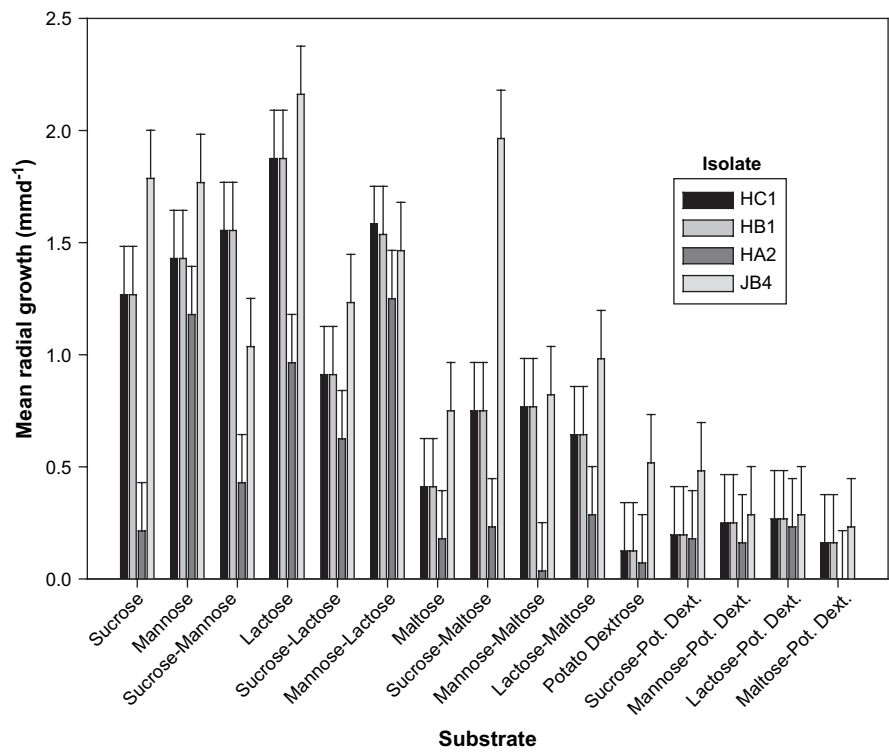


Fig 4 – Interactive effect of various substrates on slower-growing isolates of *Morchella elata*. Error bars show ±SE ($P < 0.05$).

either glucose ($P < 0.05$) or lactose ($P < 0.001$) was slightly reduced. There were significant two-way interactive effects ($P < 0.05$) between sucrose and the other sugars. While sucrose had no significant main effect, growth in the other substrates

was slightly enhanced in combinations with sucrose. There were also significant ($P < 0.05$) two-way interactions between glucose and either mannose or lactose. Growth reductions in glucose were eliminated in the presence of mannose, while

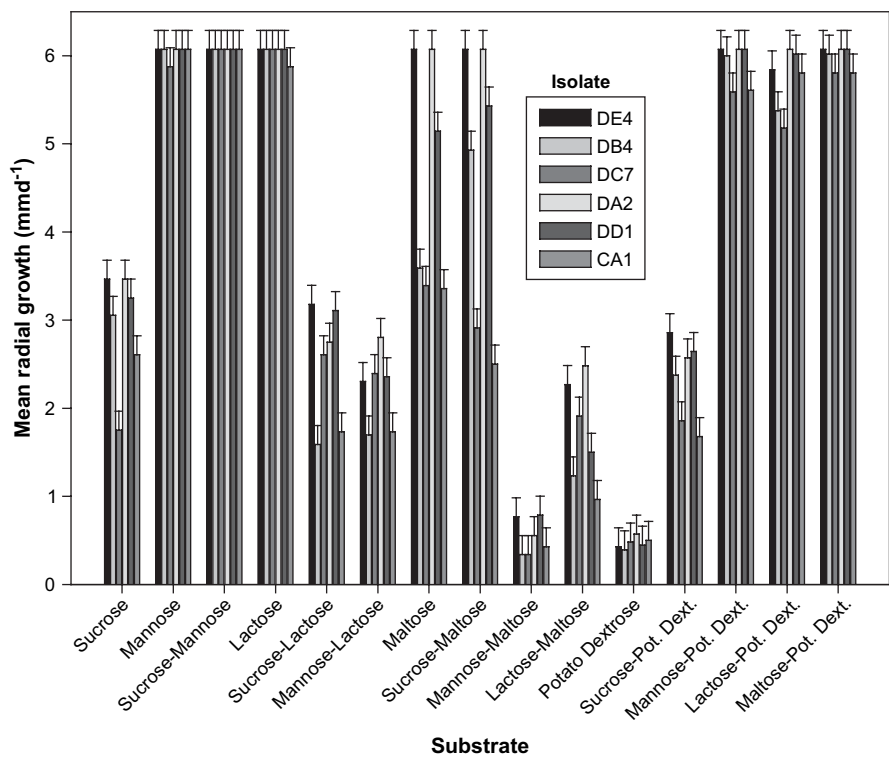


Fig 5 – Interactive effect of various substrates on faster-growing isolates of *Morchella elata*. Error bars show ±SE ($P < 0.05$).

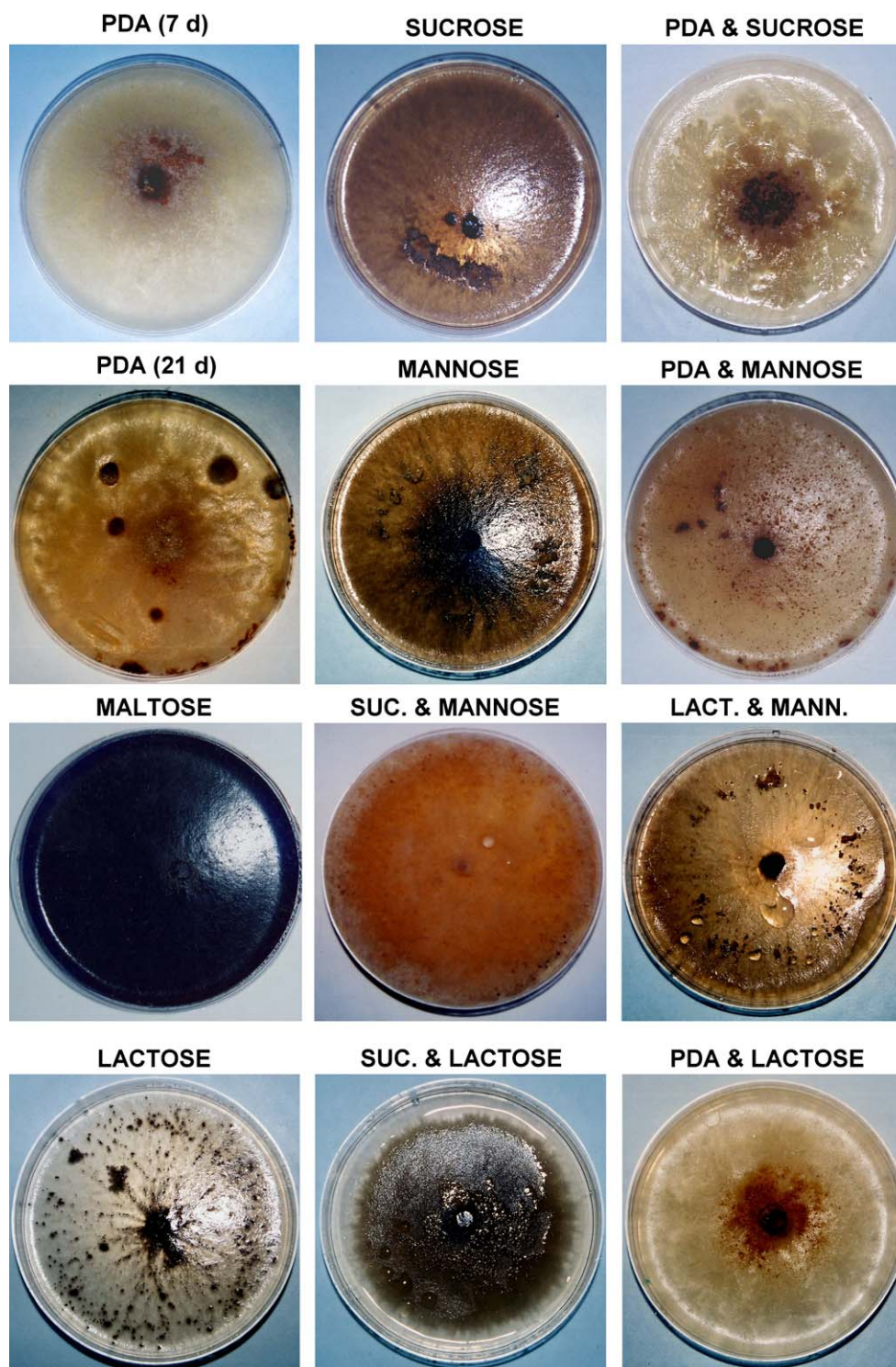


Fig 6 – Morphology of *Morchella elata* cultures grown in various media on 9-cm diam Petri dishes. Nutrients used in the media are indicated above each panel (Suc., sucrose; Lact., lactose; Mann., mannose). All cultures were photographed 7 d after inoculation, except the 21-d PDA culture (indicated above the panel).

growth reductions in combinations containing lactose occurred only when glucose was also present. In addition to these interactions, all three-way interactions were significant ($P < 0.005$). In these interactions, glucose and lactose negated

the stimulatory effect of sucrose in combinations with mannose; in the absence of sucrose, glucose removed the inhibitory effect of lactose, but this removal was not complete in the presence of mannose.

Table 3 – Effect of glucose versus sucrose on mean radial growth of two isolates of *Morchella elata*

Isolate	Substrate	Mean radial growth (mm d ⁻¹) ^a
DE4	Sucrose	5.6 A
	Glucose	4.7 B
JB4	Sucrose	0.7 C
	Glucose	0.3 D

^a Means followed by the same letter are not significantly different according to the Newman-Keuls test ($P < 0.05$).

When the growth of isolate DE4 was compared in media containing different ratios of sucrose and mannose, there was very little variation in growth. In the absence of mannose, growth was slightly, but significantly ($P < 0.01$), less than growth in media with 30 or 50 % mannose (Table 4). This result lead to the use of the 1:1 mannose:sucrose ratio in optimal media for subsequent experiments.

Ascoma maturity

After drying and storage, the fresh ascomata that initially produced slow-growing or abortive colonies (Table 1) produced colonial growth rates exceeding optimal rates from fresh spores. Mature ascospores from the Langford ascomata (JA–JC) produced colonies with a mean (\pm SE) growth of 2.8 ± 0.1 mm d⁻¹, while those from the lawn in Sooke (HA–HC) produced colonies with a mean growth rate of 2.7 ± 0.1 mm d⁻¹.

Isolate growth versus temperature

In the temperature experiment, the main and interactive effects of isolate and temperature were significant ($P < 0.01$).

Isolate JB4 grew more slowly throughout the range of temperatures tested, reaching a maximal growth rate at 16 °C while rapid-growing isolate DE4 achieved maximal growth rate at 20 °C (Table 5). A maximal optimum temperature for isolate DE4 was not detected within the range of temperatures assayed. Neither isolate grew at 8 °C in the time frame studied.

Optimal pH and calcium

When growth of isolate DE4 was compared at various potassium hydroxide-adjusted pH values, a distinct optimum occurred at pH 7–7.5. Growth in more acidic or alkaline pH conditions only produced about half of the mass that optimal cultures produced (Fig 8).

When the growth of isolate DE4 was compared in calcium carbonate, a significant increase in growth occurred at the highest (10 g l⁻¹) concentration (Table 6). Notably, growth at the next lowest concentration (1 g l⁻¹) was not significantly different than growth in controls, despite close correspondence to the optimal pH (7.0) found in the growth versus pH study. The growth rate in 10 g l⁻¹ calcium carbonate was likely maximal with respect to any further increases in concentration, because the calcium carbonate did not entirely dissolve at that level.

Simulated post-fire conditions

None of the trays simulating post-fire conditions produced ascomata or conidia. However, interestingly there was substantial mycelial growth in each of the inoculated trays used in the simulation of post-fire conditions, and this occurred during the incubation at 8 °C. This growth occurred despite earlier results from the previously mentioned temperature experiment, showing no growth of *Morchella elata* in agar

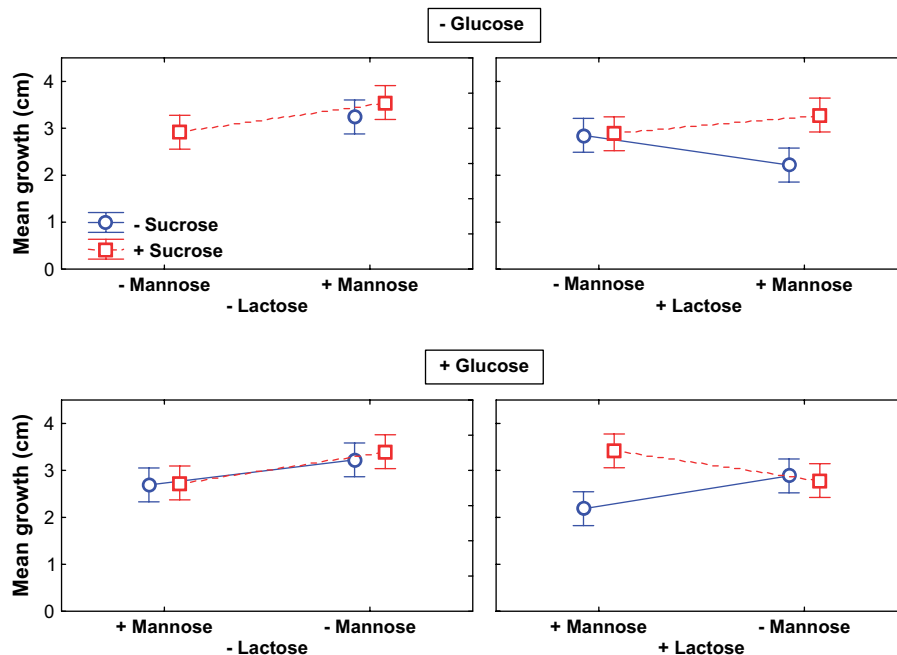


Fig 7 – Interactive effect of four sugars on mean radial growth (\pm SE, $P < 0.05$) in cultures of *Morchella elata* isolate DE4. Treatments are categorized by presence (+) or absence (–) of the sugar. Error bars show \pm SE ($P < 0.05$).

Table 4 – Effect of mannose and sucrose concentrations on mean radial growth of *Morchella elata* in agar media containing the two sugars

Sucrose (%)	Mannose (%)	Mean radial growth (mm d ⁻¹) ^a
0	100	3.4 AB
10	90	3.4 AB
20	80	3.6 AB
30	70	3.9 A
40	60	3.6 AB
50	50	3.9 A
60	40	3.4 AB
70	30	3.3 AB
80	20	3.7 AB
90	10	3.6 AB
100	0	3.0 B

a Means followed by the same letter are not significantly different according to the Newman–Keuls test ($P < 0.05$).

cultures at 8 °C. The growth in trays could be observed within a day of placing the inocula in the trays, and there were no observable differences related to quality or abundance of mycelia. The soil surface in each tray supported a proliferation of white and golden-coloured hyphae, with abundant tan-coloured microsclerotia typical of morel cultures. The microsclerotia were particularly concentrated where moisture condensed under the covering plastic bags. There were no larger sclerotia within the soils, and the puffed wheat in each tray was entirely consumed, except for a few hull remnants.

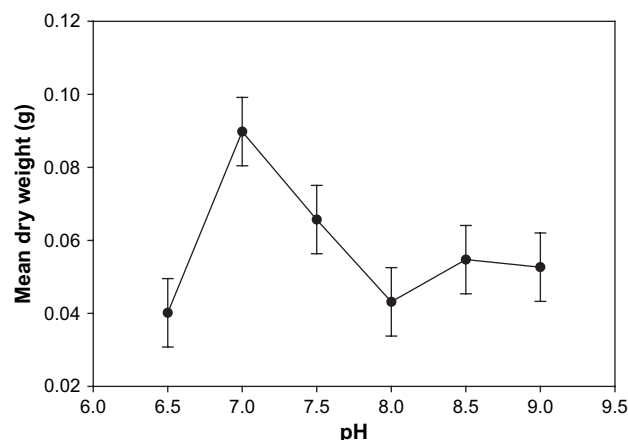
Discussion

Growth studies on single carbon sources essentially reflected earlier results obtained for *Morchella esculenta* (Brock 1951), but the response of *M. elata* to substrates was greatly influenced by isolate or presence of other substrates. The fungus flourished, for example, in combinations of optimal substrates such as mannose and sucrose, but performance was poor

Table 5 – Effect of temperature on mean radial growth of two isolates of *Morchella elata* in media containing sucrose and mannose

Isolate	Temperature (°C)	Mean radial growth (mm d ⁻¹) ^a
DE4	0	0.0 A
	8	0.0 A
	16	5.0 C
	20	9.0 D
	24	9.0 D
JB4	0	0.0 A
	8	0.0 A
	16	1.2 B
	20	0.8 B
	24	0.8 B

a Means followed by the same letter are not significantly different according to the Newman–Keuls test ($P < 0.01$).

**Fig 8 – Effect of pH on growth of *Morchella elata* isolate DE4 in liquid culture. Error bars show \pm SE ($P < 0.05$), and growth at pH 7 and 7.5 is significantly greater than growth at other pH values according to the Newman–Keuls multiple range test ($P < 0.01$).**

when these were mixed with another optimal substrate such as lactose. Maltose and potato dextrose appeared to stress the fungus, triggering slower growth and plumose culture margins or dark pigmentation. If morels form mycorrhizal-like root associations as suggested by other researchers (Dahlstrom et al. 2000), it may be that *M. elata* is responding to various root exudate compositions or root tissues with different strategies for growth and sclerotia formation. For example, mannose was an optimal substrate in this study, and it is reported that apple (*Malus domestica*), a plant often associated with morels (Weber 1988), produces root exudates that contain a major proportion of mannose and/or mannitol, depending on stage of growth (Wittenmayer & Szabó 2000). Further testing with other substrates and their composites would be needed to fully understand connections between *in vitro* growth and the behaviour of morels in the rhizosphere. Additional studies could test, for example, the effect of other carbohydrates, amino acids and inorganic nutrients. Clearly, future substrate assays should be performed with an assortment of isolates to ensure variable responses of particular isolates can be taken into account.

Researchers have previously noted arrested or variable growth in some morel isolates (Hervey et al. 1978). In this study, a shorter parental pileus was correlated with the production

Table 6 – Effect of increasing calcium carbonate concentration on growth of *Morchella elata* isolate DE4 in liquid culture

Calcium carbonate (g l ⁻¹)	pH	Mean culture dry weight (g) ^a
00.0	4.9	0.047 A
00.1	5.9	0.048 A
01.0	6.8	0.061 A
10.0	7.7	0.126 B

a Means followed by the same letter are not significantly different according to the Newman–Keuls test ($P < 0.05$).

of slower-growing isolates. Because morel ascomata elongate as they mature (Ower 1982), pileus length is related to ascoma maturity. However, the relatively low R^2 value of the correlation suggests that correlation with pit expansion or other improved measures of ascoma maturity would be useful in future assays. Spores from shorter ascomata eventually produced faster-growing colonies after the dried ascomata were stored for a period, possibly indicating a dormancy mechanism. Spore maturation could also be a factor in the improved germination. However, the ascomata were in a semi-desiccated form within 48 h after initiation of the original stock cultures, constraining the conditions for this to occur. It is known that some ascomycetes such as *Ascobolus* spp. and *Neurospora* spp. require a heat shock in order to break dormancy requirements (Dodge 1912; Shear & Dodge 1927). One alternative explanation for variable isolate performance in various media could be differences in maturity or dormancy mechanisms. Because more than one nutrient was involved, this would most likely involve a variable impact from spore maturation on different portions of morel metabolism. The variability could have derived from developmental and genetic differences. In one recent study, about 1–2 % of ascospores from *N. pannonica* were capable of spontaneous germination after ejection from asci; several exceptional genotypes or mutants also exhibited this lack of dormancy (Raju 2002). In this study, some isolates of *M. elata* grew optimally in maltose, whereas others did not. Their capacity for growth in different media was variable, yet all were derived from ascospores that were ejected from relatively large ascomata on the same date. It is possible that harvesting ascomata could cause premature ascospore release; further study of spores released *in situ* could elucidate this. Whatever the cause of the maturation effect, the results suggest that the general initial fitness of morel inoculum in natural areas may depend, in part, on ascoma maturity. The tendency of morel ascomata to 'blend in' with their environment by resembling pine cones, cinders, etc. (Weber 1988) may be linked to this requirement for full maturation.

The optimal temperature for growth of *M. elata* varied with isolate used, and there was no growth at 8 °C in the initial experiment. These effects may have been another consequence of isolate immaturity or dormancy; other studies have confirmed the ability of *Morchella* spp. to grow at cooler temperatures (Gilbert 1960; Schmidt 1983). In fact, isolate DE10 and isolate group DE11–16 grew at 8 °C in the subsequent soil experiment, after a long period of incubation in bulk media at higher temperatures. Beyond this incubation, the soil environment might also have stimulated growth at colder temperatures. Wood or its extracts are reported to stimulate morel growth (Robbins & Hervey 1959, 1965); silicon compounds present in the soil are another potential microbial stimulant (Wainwright *et al.* 1997).

The pH and calcium experiments demonstrated that *M. elata* grows at a pH optimum similar to that reported for *M. esculenta* (Brock 1951)—except the growth of *M. elata* at pH 8–9 produced a less pronounced alkaline 'shoulder'—rather than the bimodal optimum reported for *M. esculenta*. *M. elata* was also more sensitive to acidic conditions below the optimal pH of 7. Aside from effects due to species differences, the substitution of potassium hydroxide for sodium hydroxide during pH adjustment may have affected the response. Other

research has shown that calcium and manganese stimulate growth of *Morchella crassipes* (Robbins & Hervey 1965). Interestingly, the optimal pH for growth shifted to 7.7 or above when it was controlled by addition of calcium carbonate. This result suggests that the optimal pH for morels in natural environments may shift according to the availability of calcium and other inorganic ions, especially in burnt areas where these minerals are present in wood ash.

There could be several explanations for the absence of fruiting primordia or conidia in the simulated post-fire conditions used in this study. The puffed wheat substrate, incubation times, temperature regime, moisture regime, and absence of light may not have been conducive for the production of primordia or conidia. The isolates used in the study could also be unsuitable for fruiting.

If *M. elata* and other *Morchella* spp. share similar growth characteristics, they could also share similar habitats. However, Obst and Brown (2000) reported that black (or 'natural') morels, 'blonde' morels, and 'grey' (or 'firesite') morels in the Northwest Territories occurred in partially distinct habitats. Black morels were found in lower, moist areas, while blonde and grey morels were found in situations with better drainage. Keefer (2005) has also reported the heterogeneous distribution of morels after large fires in the Kootenay region of BC, and determined a potential association between morels and particular native herbs, shrubs and trees. Differential responses to the composite media used in this study might be linked to this type of plant association in the rhizosphere. Different moisture regimes were not addressed by this study, but they could also influence habitat, through direct effects on the fungus or by affecting the distribution of plant associates. Variable fire intensity must also be considered, because it creates differences in soil sterilization, ash deposition, and substrate abundance. McFarlane *et al.* (2005) and Keefer (2005) have reported that moderate levels of litter consumption are optimal for post-fire production of morels. This is possibly reflected in the results of the pH study, where optimal pH was neutral or near neutral, depending on the added base. With further study, interactions between ash deposition, pH, soil type, and morel growth or fruiting could provide a good predictive model for morel habitat. This could be further improved with a more complete characterization of optimal growth factors, potential plant associates, and how they relate to the natural habitat.

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