

Morchella conica exhibiting a long fruiting season

Martin GOLDWAY^{1,2*}†, Rachel AMIR^{1,2†}, Doron GOLDBERG^{1,2}, Yitzhak HADAR³ and Dan LEVANON^{1,4}

¹ Migal-Galilee Technology Center, Kiryat Shmona, 10200, Israel.

² Department of Biotechnology & Environmental Sciences, Academic College at Tel-Hai, Upper Galilee 10120, Israel.

³ Faculty of Agriculture, Otto Warburg Center for Biotechnology, Hebrew University, Rehovot, Israel.

⁴ Institute of Soil and Water, Volcani Center, Bet Dagan, Israel.

E-mail: Goldway@migal.co.il.

Received 1 December 1998; accepted 11 November 1999.

Morels are known to produce ascocarps during a few weeks in the spring. Here we present findings about a *Morchella conica* population which produces ascocarps during a period of 8–10 months, from early spring until mid-winter. To the best of our knowledge, this phenomenon has not been described previously. Field investigations correlated the ascocarp appearance with gradual drying of the soil. DNA-PCR fingerprinting indicated that these *Morchella* shared a similar genetic background.

INTRODUCTION

Morels (*Morchella* spp.) are among the most desirable edible mushrooms known. Ascocarps that reach the market are collected from the wild, although some success has been reported in cultivating morels (Ower 1982, Ower, Mills & Malachowski 1986, Ower, Mills & Malachowski 1989). Due to their strong aroma, much effort has been invested into growing them in liquid media (Kosaric & Miyata 1981) and on solid substrates (Ower 1982, Volk & Leonard 1989). This effort has been applied to various morphological structures, such as sclerotia, hyphae and mycelium (Kaul 1981, Volk & Leonard 1990, Buscot 1993, Amir *et al.* 1994). Their very short season and high price have encouraged researchers to study the biology and ecology of the species, especially the factors involved in its fruiting.

Morels are widely distributed in the temperate zones of the world. Ecological studies have been conducted in Europe, North America and the Himalayan foothills (Kaul *et al.* 1981, Batra 1983, Friedman 1986, Buscot 1989, Bartelli 1990). Morels appear from late April until the end of May, usually for about three weeks only. Schmidt (1983) and Buscot (1989) pointed out the role of temperature in their appearance; they demonstrated that the re-heating of the soil, after the snow season in early spring, encourages ascocarp formation. Temperature has also been shown to have an effect on spore germination, growth and development (Schmidt 1983). Ascospores of *Morchella esculenta* were not found to germinate until the soil temperature exceeded 10 °C. Mycelia and sclerotia, however, can colonize rye seeds placed in soil at

temperatures below 10 °C. Above this temperature only other saprotrophic fungi could be isolated from the rye seeds. This suggests that *Morchella* may be a less competitive colonizer of nutrient resources at higher temperatures. Schmidt (1983) proposed that *M. esculenta* is a psychrotolerant fungus, and that the fruiting of morels in spring may relate to their competitive abilities at low temperatures. Volk & Leonard (1990) suggested that the freezing and thawing associated with the winter and early spring lead to the formation of ascocarps. A correlation of fruiting with vernal post-thaw heat input has been reported for two species of morels in Europe (Buscot 1989).

In general, morels occur in a variety of habitats, including riverbanks, mountain slopes, pastures, burned-out forests and near plants that have been injured. They emerge in sand, moist soil with abundant organic matter, and in mud (Friedman 1986).

Two different types of environmental conditions have been discovered to encourage *Morchella* ascocarp formation. Morels can first fructify as pioneers on recently disturbed soils. For example, they become visible in the first spring following mechanical disturbance of the soil, after application of certain herbicides, after a deposition of vegetative wastes, after forest fires (Kaul *et al.* 1981), and even following volcanic devastation (Carpenter, Trappe & Ammirati 1987). Under these conditions the production of ascocarps declines rapidly in the years following the disturbance (Buscot & Roux 1987, Carpenter *et al.* 1987, Miller, Torres & McClean 1994). These observations reinforce the hypothesis that under these conditions morels are saprotrophic. The second case is the production of ectomycorrhiza with higher plants (Buscot & Roux 1987, Buscot & Kottke 1990, Buscot 1992). Morels are observed in

* Corresponding author.

† The first two authors contributed equally to this manuscript.

association with trees in undisturbed habitats, where only a few ascocarps are produced each spring over a period of several years (Buscot & Roux 1987, Buscot & Kottke 1990).

The present work describes a specific population of *Morchella conica* which produces ascocarps over more than eight months, from early spring to mid-winter. This population grows in the Dan Nature Reserve, northern Israel. In an attempt to shed light on this phenomenon, we examined the environmental parameters of the Dan niche, and also the genetic relatedness of these morels, as determined by their DNA fingerprints.

MATERIALS AND METHODS

General observation

The population of Dan morels was observed for 6 years, from 1991 to 1997, and the abiotic parameters and number of ascocarps were monitored over the first 3 years of this period. In an attempt to identify the environmental conditions that may confer the long season on the Dan morels, three parameters which influence the moisture of the banks of the small channels, i.e. soil temperature, rainfall, and the discharge of the main spring, were monitored at their location. Maximum–minimum thermometers were placed at the site of the *M. conica* population to measure soil temperature. No significant differences were noted between the temperatures, just beneath the litter and in the air at the height of 1 m. Furthermore, the latter temperatures were consistent with those registered by the Israeli national meteorological service at the Dan station. This enabled the temperature at the location of other morel populations, found outside the Dan Nature Reserve, to be estimated. The discharge of the main spring and the rainfall were recorded by professional hydrologists. The number of morels and abiotic parameters were measured every two weeks, since this is the period of time from the appearance of the ascocarp until its decay.

Conductivity measurements

Electrical conductivity (in terms of resistance) on the stream banks, from the water to the dry area, was measured by electrodes made of 10 cm nails. These were inserted 5 cm into the ground, with 5 cm between each nail. Each pair of nails was connected to an AC-operated LCR meter (Escort, model ELC-120).

Morchella isolates and DNA preparation

Isolates of *M. conica* were sampled from two sites in Israel, T1–8, from Dan, within the nature reservation, and K1 from a population located just outside the nature reserve, in a field recently sprayed with herbicide. The other *Morchella* species were obtained from the USA: *M. esculenta*, P3 C-280; *M. angusticeps*, P2; and *M. deliciosa*, WC190. P3 C-280 and P2 were purchased from Fungi Perfecti, USA; and WC190 was received from Pennsylvania State University, USA.

In order to examine fungal tissue, pieces from the internal part of the *Morchella conica* ascocarps were placed on potato dextrose agar (Difco) with chloramphenicol (300 mg l⁻¹). The

mycelia were transferred and placed on sterile cellophane, covering the solid media. The plates were incubated for four days at 28 °C. The hyphae were collected by scraping them from the cellophane.

To prepare specimens for DNA examination, about 2 mg of tissue was transferred to a microtube, and 30 µl lyzing buffer (Tris/HCl, pH 8, 250 mM; NaCl 250 mM; SDS 0.5%; EDTA 25 mM) and 3 mg acid-washed glass beads, 425–600 µ in size (Sigma G-8772), were added. The tube content was vortexed for 7 min. After centrifugation, the supernatant was extracted by phenol. DNA was precipitated with 0.2 M NaCl and 70% ethanol. A yield of about 3 µg DNA was obtained.

PCR-DNA fingerprint

To obtain PCR–DNA fingerprints, sets of partially degenerate, 17–18 base-long oligomers were used as primers in the PCR, which was: 5 min at 94 °, followed by 35 cycles of 1 min at 94 °, 2 min at 50 ° and 5 min at 74 °. Each sample was analyzed twice, with the same results in both experiments. The reaction samples were run on a 2% agarose gel with lambda/HindIII as a size marker. This is a variation on the RAPD technique (Williams *et al.* 1990, Welsh & McClelland 1990, Welsh, Peterson & McClelland 1991). Longer primers and a higher annealing temperature were used to increase the fidelity of the reaction. In order to compensate for the reduced probability of the longer primer finding a homologous site on the genome, a partially degenerate primer was utilized in each reaction. Primers were: 17602, 5'CCNGCG/ATTNCCNGTC/TTT 3'(256 oligomers); 17699, 5'GCNCAC/TTTC/TGAC/TC/AGNGA 3' (256 oligomers); and 17702, 5' TGT/CTCNACT/CTCNGCG/AAA 3' (128 oligomers). According to their PCR fingerprints, the similarity among strains was determined by the equation

$$BS = \frac{2 N_{ab}}{(N_a + N_b)}$$

where BS (band sharing) = level of band sharing between individuals a and b, N_{ab} = no. of bands shared by individuals a and b, and N_a and N_b = total no. of bands of individual a and b, respectively (Nei & Li, 1979).

RESULTS

The populations of Dan morels are located within a small area in the Dan Nature Reserve, which consists of a main spring that runs into small channels, which net the area. The abundant flora, especially trees [Syrian ash (*Fraxinus syriacus*), plane (*Platanus orientalis*), laurel (*Laurus nobilis*), and willow (*Salix acmophylla*)] create intensive shade on the bank. Morels appear only along the water channel banks in the wet soil. It was found that the Dan morels are associated with roots of *F. syriacus*, and the typical morphological structures, described by Buscot (1989), of mycelial muffs and connective mycelium, were identified (data not shown).

Eight sites along the channel banks were marked and observed carefully. These observations revealed that, as for other morels (Buscot 1989, Buscot 1992), the location did not change for 2 or 3 years, followed by their disappearance

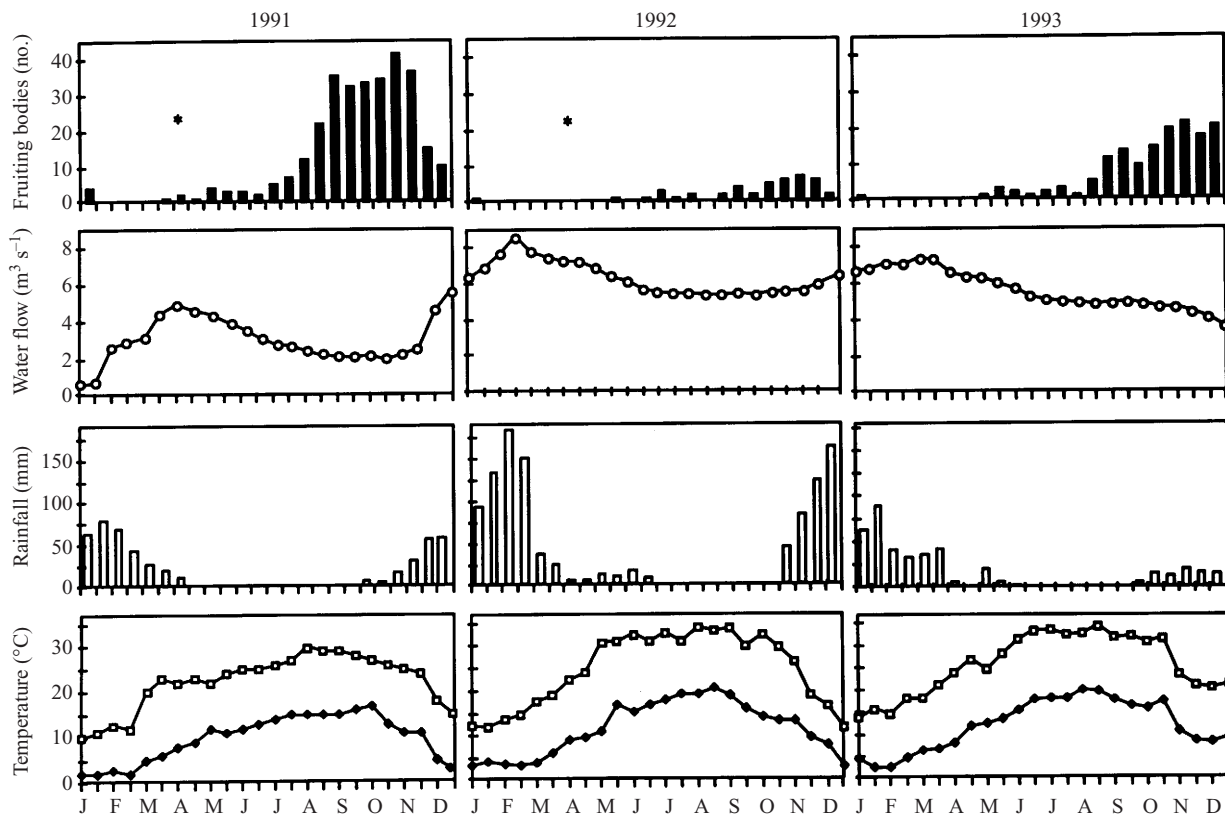


Fig. 1. The total number of Dan morel ascocarps, discharge of the main spring, precipitation, and soil temperature (averaged over 2 wk), max. (□) and min. (◆).

and the reappearance of new groups, at a distance not farther than 50 cm from the original location.

The number of morels varied over the three years of monitoring (Fig. 1). Fruiting season in all 3 years was found to be 8–10 months, from March, April or May to mid-January, peaking in the autumn, from September to December (Fig. 1). In addition, it was observed, that there were two typical short-season morel populations, in close proximity to the Dan Nature Reserve. One site was 200 m away from the Dan population, in an area which had been sprayed with the herbicide Atrazine. The other was 10 km from Dan, on soil that had been transported. At both sites, morels appeared for two consecutive years, for a period of about two weeks. At the first site, on an area of 2 m², 24 specimens emerged from mid-March of 1991, and 20 from mid-April of 1992 (asterisks in Fig. 1). At the second site, over an area of 3 m², ten specimens became visible from mid-March of 1994 and 12 from the beginning of March 1995. No morels were observed at these sites during the years that followed. Thus, morels found outside the Dan Nature Reserve exhibited a typically short fruiting season, as do morels elsewhere in Israel (N. Binyaminy, personal communications) and as has been reported for other *Morchella* worldwide (Buscot 1989).

During 1991–1993, abiotic parameters and the number of ascocarps were monitored, as indicated in Fig. 1. Soil temperatures were similar during each of the years, whereas the amounts of rainfall differed: 1991 and 1993 were drought years (467 and 413 mm, respectively) while 1992 had abundant precipitation (1056 mm) (Fig. 1). The discharge rate of the main spring is correlated to the rainfall, but the Dan Spring

also draws its water supply from thawing snow on Mt Hermon, so that variations in its flow rate are more moderate than are the variations in the precipitation.

A majority of ascocarps appeared when the moisture of the channel banks decreased. Drier weather and a decrease in the discharge of the main spring drastically affected the smaller channels, and this evidently induced the appearance of ascocarps in this area. When the discharge of the main spring was high (mid-January to April), morels were not seen. In April, although the discharge in the spring was still high, the channel banks began to dry slowly, in response to warmer and drier weather. During this period pioneer ascocarps began to emerge. As the discharge decreased, more ascocarps became visible (Fig. 1). It is clearly shown, that during 1992, when the discharge of the main spring was high, fewer ascocarps appeared than in 1991, a drought year. This trend, by which more morels emerge in drought years, than in wet years, was consistent during all 6 years of observations (data shown only for 3 years).

In order to strengthen the observation that there is a correlation between soil moisture and morel appearance, we calculated the inverse of the electrical conductivity (resistance) of the soil, as an indication of soil moisture. The resistance of the soil was measured on the banks, where morels were located, from the water to the dry area, at 30 sites. The value of the saturated soil was 0.93 ± 0.11 k Ω , while in the dry area the resistance was 5.22 ± 0.9 k Ω . Morels appeared only in narrow strips, where the soil moisture, in terms of resistance, was 2.45 ± 0.21 k Ω .

In an attempt to acquire some insight into the genotype of

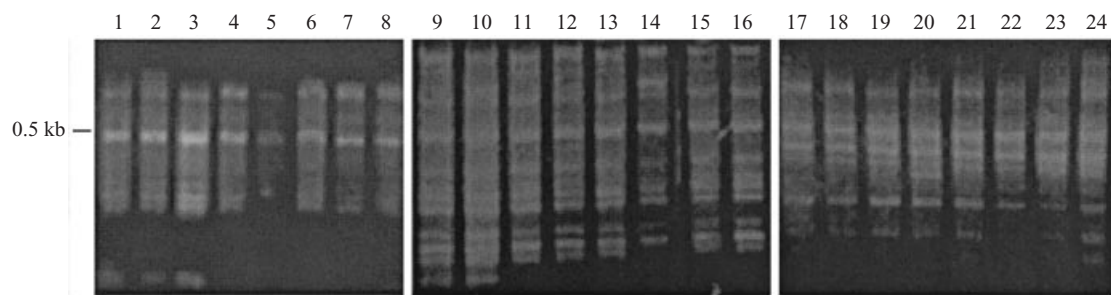


Fig. 2. DNA-PCR-fingerprint of Dan, long-fruited *M. conica* T1–8. Lanes 1–8, primer 17602; lanes 9–16, primer 17699; lanes 17–24, primer 17702.

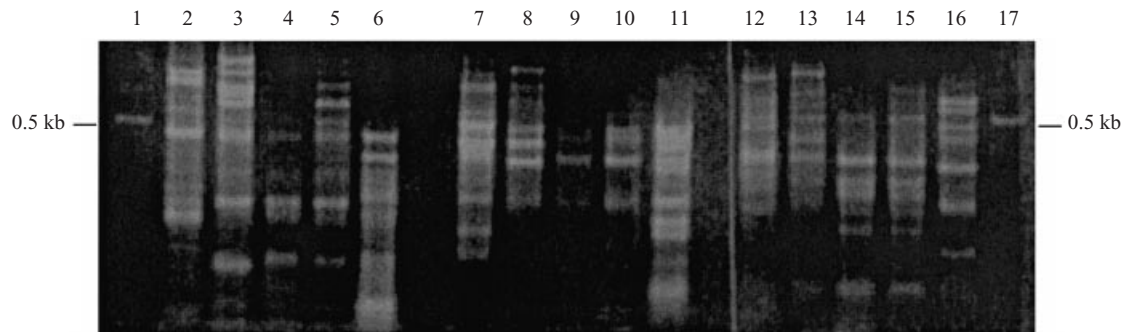


Fig. 3. DNA-PCR-fingerprints of Dan, long-fruited *Morchella conica* and other *Morchella* spp. Lanes 2–6, primer 17602; lanes 7–11, primer 17699; lanes 12–16, primer 17702. Lanes 1 and 17, size marker. Lanes 2, 7, 12: *M. conica* T3; lanes 3, 8, 13: *M. conica* K1; lanes 4, 9, 14: *M. esculenta* P3 C-280; lanes 5, 10, 15: *M. angusticeps* P2; lanes 6, 11, 16: *M. deliciosa* WC190.

Table 1. Coefficients of similarity for PCR fragment patterns of Dan long-fruited morels and other typically fruiting morels. Data based on Fig. 3.

Primer	Species	<i>M. conica</i> -T3	<i>M. conica</i> -K1	<i>M. esculenta</i>	<i>M. angusticeps</i>
17702	<i>M. conica</i> -K1	0.12	—	—	—
	<i>M. esculenta</i>	0.16	0.41	—	—
	<i>M. angusticeps</i>	0.12	0.94	0.61	—
	<i>M. deliciosa</i>	0.16	0.16	0.50	0.30
17602	<i>M. conica</i> -K1	0.31	—	—	—
	<i>M. esculenta</i>	0.4	0.22	—	—
	<i>M. angusticeps</i>	0.31	0.66	0.66	—
	<i>M. deliciosa</i>	0.22	0.4	0.33	0.53
17699	<i>M. conica</i> -K1	0.2	—	—	—
	<i>M. esculenta</i>	0.24	0.4	—	—
	<i>M. angusticeps</i>	0.37	0.4	0.93	—
	<i>M. deliciosa</i>	0.24	0.1	0.0	0
Average	<i>M. conica</i> -K1	0.21 ± 0.07	—	—	—
	<i>M. esculenta</i>	0.27 ± 0.09	0.34 ± 0.08	—	—
	<i>M. angusticeps</i>	0.27 ± 0.09	0.67 ± 0.19	0.73 ± 0.12	—
	<i>M. deliciosa</i>	0.21 ± 0.03	0.22 ± 0.11	0.28 ± 0.18	0.28 ± 0.19

the exceptionally long-season morels, we examined their genomic DNA-PCR fingerprints. Eight ascocarps were tested, using three different degenerate oligomers, and they had similar banding patterns (Fig. 2). The DNA-PCR fingerprint of the Dan morels suggested that these morels share a common genetic background. In order to determine whether this banding pattern was similar to or different from other *Morchella* spp. using the same primers, fingerprints of the Dan morels were compared to another Israeli *M. conica*, from near to the Dan morels in an open field, and three other *Morchella* species. Those of the Dan morels were distinct (Fig. 3). Calculation of the coefficients of similarity, based on PCR

fragment patterns showed a very high level of diversity (Table 1).

DISCUSSION

The population of morels in the Dan Nature Reserve is most unusual, both in its long fruiting season and in the variable environmental conditions extant at the time of ascocarps appearance. The morels in Dan produce ascocarps during an eight month period, throughout which the temperatures and the day lengths vary considerably. This is in contrast to other *Morchella* sites, in which the environmental conditions at the

time of ascocarp formation are more narrowly defined (Buscot 1989). Typically, morels appear in the early spring, when the soil temperature begins to rise, and the days become longer. These differences, between the Dan population and other sites, imply that ascocarp formation of morels is not restricted to the specific conditions governed by these two parameters. Alternatively, it may be argued, that these parameters do affect morels in general, but not the Dan morels.

Based on our findings in Dan morels and other morels in Israel, a possible mechanism responsible for the induction of their appearance may be suggested. We propose the hypothesis, that the dryness of the ground or the transition from saturated soil to relative aridity is a major factor in the induction of morel ascocarps. Observations in Dan indicated that (i) the highest number of morels appeared, when the moisture content of soil at the channel banks began to decrease, due to a low discharge of the springs or low rainfall; (ii) when the discharge of the main spring was high (from mid-January to April) morels were not detected; and (iii) morels emerged only in a narrow strip on the channel banks. Thus, the requirement for the induction of ascocarps was evidently fulfilled, when the moisture in the soil had decreased to a narrow range of values.

It seems that dryness of the soil also affects other morel populations in Israel. These populations became visible in the early spring, when the ground begins to dry, as a result of higher temperatures and the local desiccating east wind. A patent granted for morel cultivation (Ower *et al.* 1986, 1989) also points to the role of wetting and later drying of the substrate, in the induction of ascocarps. It is possible that morels, which appear in other parts of the world, are also induced by the drying of the ground or by reduced water availability. They emerge when the snow melts; during this period the daytime temperature is still cool and the night temperature below freezing. Freezing lessens the availability of water and may thus lead to the formation of ascocarps. Induction of ascocarps, after the substrate has changed from saturated to dry, is known to occur in other mushrooms, such as *Lentinus shiitake* (Yang 1994).

The DNA fingerprint of the Dan morels suggests that they are genetically related, since their PCR-DNA banding pattern is identical or very similar (Table 1). This assumption is supported by the finding that a neighbouring isolate of *M. conica* and other species of *Morchellaceae* had different banding patterns. To establish the possible interaction between the genetic background of the Dan morels and their long fruiting season, however, further investigations must be carried out.

The findings, that relatively flexible environmental conditions and a defined soil moisture can allow the production of ascocarps in *Morchella*, may simplify the cultivation of this valuable mushroom. In addition, this study offers new possibilities for trying to understand the mechanism of ascocarp formation.

ACKNOWLEDGEMENTS

We thank Lital Arazi, Guy Mayan, Tal Avraham, Liat Vaaknin and Hila Yehuda for their technical assistance. We are grateful to the Nature Reserves Authority for permission to examine the morels in Dan, especially to Dr Didi

Kaplan, the regional biologist. The research was supported by a grant of the Israel Ministry of Industry and Trade.

REFERENCES

- Amir, R., Levanon, D., Hadar, Y. & Chet, I. (1994) The role of source-sink relationship in translocation during sclerotial formation by *Morchella esculenta*. *Mycological Research* **98**: 1409–1414.
- Bartelli, I. (1990) May is morel month in Michigan. *Extension Bulletin E-614*. Michigan State University.
- Batra, L. R. (1983) Edible discomycetes and gasteromycetes of Afghanistan, Pakistan and north India. *Biologia* **29**: 293–304.
- Buscot, F. (1989) Field observations on growth and development of *Morchella rotunda* and *Mitrophora semilibera* in relation to forest soil temperature. *Canadian Journal of Botany* **67**: 589–593.
- Buscot, F. (1992) Stratégies écologiques et biologiques des morilles. *Cryptogamie Mycologia* **13**: 171–179.
- Buscot, F. (1993) Mycelial differentiation of *Morchella esculenta* in pure culture. *Mycological Research* **97**: 136–140.
- Buscot, F. & Kottke, I. (1990) The association of *Morchella rotunda* (Pers.) Boudier with roots of *Picea abies* (L.) Karst. *New Phytologist* **116**: 425–430.
- Buscot, F. & Roux, J. (1987) Association between living roots and ascocarps of *Morchella rotunda*. *Transactions of the British Mycological Society* **89**: 249–252.
- Carpenter, S. E., Trappe, J. M. & Ammirati, J. (1987) Observation of fungal succession in Mount St Helens devastation zone, 1980–1983. *Canadian Journal of Botany* **65**: 716–728.
- Friedman, S. A. (1986) Morels, may apples and the meaning of life. *Mushroom, the Journal of Wild Mushrooming* **4**: 5–9.
- Kaul, T. N. (1981) Cultural studies on morels. *Mushroom Science* **11**: 781–787.
- Kaul, T. N., Khurana, M. L., Kachroo, J. L., Krishna, A. & Atal, C. K. (1981) Myco-ecological studies on morel bearing in Kashmir. *Mushroom Science* **11**: 789–795.
- Kosaric, N. & Miyata, N. (1981) Growth of morel mushroom mycelium in cheese whey. *Journal of Dairy Research* **48**: 149–162.
- Miller, S. L., Torres, P. & McClean, T. M. (1994) Persistence of basidiospores and sclerotia of ectomycorrhizal fungi and *Morchella* in soil. *Mycologia* **86**: 89–95.
- Nei, M. & Li, W. H. (1979) Mathematical modeling for studying genetic variation in terms of restriction endonuclease. *Proceedings of the National Academy of Science, USA* **76**: 5269–5273.
- Ower, R. (1982) Notes on the development of the morel ascocarp: *Morchella esculenta*. *Mycologia* **74**: 142–143.
- Ower, R., Mills, G. & Malachowski, J. (1986) Cultivation of *Morchella*. *US Patent No. 4,549,809*.
- Ower, R., Mills, G. & Malachowski, J. (1980) Cultivation of *Morchella*. *US Patent No. 4,866,878*.
- Schmidt, E. (1983) Spore germination of and carbohydrate colonization by *Morchella esculenta* at different soil temperatures. *Mycologia* **75**: 870–875.
- Volk, T. & Leonard, T. (1989) Physiological and environmental studies of sclerotium formation and maturation in isolates of *Morchella crassipes*. *Applied and Environmental Microbiology* **55**: 3095–3100.
- Volk, T. & Leonard, T. (1990) Cytology of the life-cycle of *Morchella*. *Mycological Research* **94**: 399–406.
- Welsh, J. & McClelland, M. (1990) Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research* **18**: 7213–8.
- Welsh, J., Petersen, C. & McClelland, M. (1991) Polymorphisms generated by arbitrarily primed PCR in the mouse: application to strain identification and genetic mapping. *Nucleic Acids Research* **19**: 303–306.
- Williams, J., Kubelik, G. K., Livak, A. R., Rafalski, K. J. & Tingey, S. V. (1990) DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucleic Acid Research* **18**: 6531–6535.
- Yang, X. M. (1994) Integral cropping systems in mushroom production. In *The Biology and Technology of Lentinula Mushroom* (ed. X. C. Luo & M. Zang, eds): 197–220. China Agricultural Sciencetech Press, China.