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Spatial and temporal patterns of morel fruiting

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ABSTRACT

The biotic and abiotic factors conditioning morel fruit body production are incompletely known. We examined spatial and temporal patterns of *Morchella esculenta* fruiting over five years in a wooded site in Missouri, USA. Fruiting onset was inversely correlated with spring air and soil temperatures, whereas abundance was positively correlated with rain events (>10 mm) during the 30 d preceding fruiting. The two years with the greatest fruiting had the shortest fruiting seasons (6–7 d). Fruiting season length was positively correlated with soil warming, suggesting that a narrow range of optimum soil temperatures favour the explosive production of fruit bodies. All woody stems of at least 1 cm diam were mapped and stem diameter and crown condition were noted. Morel fruit bodies were significantly closer to stems of *Carya* spp., *Tilia americana* and *Ulmus americana* than predicted by the frequencies of these woody species or their contribution to the total basal area on the site. Although intra-annual clustering of fruit bodies was often observed, inter-annual clustering was not. The spatial pattern of *M. esculenta* fruiting appears to be associated with vegetation pattern, whereas the onset and abundance of fruiting are determined by the interaction of spring temperatures with availability of supporting precipitation.

Introduction

Fruit bodies of morel fungi (*Morchella* spp.) are highly prized for their culinary qualities. Despite the widespread appreciation of these prized edibles, many aspects of their biology and ecology are poorly understood. The identification of species has long been controversial (Weber 1988; Wipf *et al.* 1997; Kellner *et al.* 2005). For example, the distinction of North American *Morchella* species has been complicated by their phenotypic plasticity in response to environmental conditions. Species delineation has improved with the analysis of allozymes (Royse & May 1990), allelic variation (Gessner *et al.* 1987), and rDNA sequences (O'Donnell *et al.* 1997; Wipf *et al.* 1999). Using RFLP analysis of the 28S rDNA, Bunyard *et al.* (1994) distinguished the black morel group (*M. angusticeps*, *M. elata* and *M. conica*) from the yellow/tan group (*M. esculenta*, *M. crassipes* and *M. deliciosa*). RFLP analysis and sequencing of the rDNA ITS region distinguished *M. esculenta*, *M. crassipes* and *M. spongiola* within the European *M. esculenta* group (Kellner *et al.* 2005).

Many features of the morel life cycle remain poorly understood (Volk & Leonard 1990), including the importance of inbreeding *versus* out-crossing in determining the genetic structure of local populations. Unexpectedly high levels of polymorphism were found in a study of 34 RAPD loci from 57 *M. esculenta* fruit bodies (Dalgleish & Jacobson 2005) suggesting little inbreeding at the collection sites. Dalgleish & Jacobson (2005) suggested that although established populations may be characterized by high levels of out-crossing, single ascospores may establish new, inbreeding pioneer populations.

Although there are numerous anecdotal accounts of the specific environmental conditions under which *Morchella* spp. fruit bodies develop, detailed studies using site-specific

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environmental measurements are surprisingly rare. It is clear that the fruiting of some morel species is enhanced by periodic wildfires (Pilz et al. 2004). Buscot (1989) reported that the emergence of *M. rotunda* fruit bodies in France was preceded by the accumulation of 424–580 soil temperature degree-days (presumably measured on a 0 °C basis).

Folk wisdom regarding the vegetation associations of morel fruit bodies is voluminous in contrast with the very few analyses of fruit body proximity to specific plant taxa. Tiffany et al. (1998) analysed reports of woody species observed in close proximity to *Morchella* spp. fruiting bodies in Iowa, USA. They reported that fruiting bodies were most frequently associated with *Quercus* spp. and living and dead *Ulmus*. Wipf et al. (1997) summarized published reports of plant species observed in close proximity to morel fruiting bodies. Although the spatial proximity of morel fruit bodies to selected plant taxa is certainly suggestive of a preferential association, it is also possible that underlying biotic or abiotic factors independently favour both the development of morel fruit bodies and the presence of certain plant taxa.

We examined the spatial and temporal patterns of *Morchella esculenta* s.l. fruit body production over five years in a wooded site (*ca* 0.5 ha) with respect to local temperature and moisture fluctuations in order to characterize those conditions that may affect annual patterns of initiation, abundance, and intensity of morel fruiting. We also characterized spatial patterns of morel fruiting with respect to proximity to all woody stems of at least 1 cm diam. For each plant taxon present, we asked if morel fruit bodies were found in close proximity more (or less) frequently than one would predict based upon the proportional representation of that plant taxon in the woodland.

Materials and methods

Study site and field data collection

The study was conducted at the University of Missouri's Horticultural and Agroforestry Research Center (HARC), New Franklin, Missouri. The study site, *ca* 0.5 ha, was located on a north-facing hillside of unmanaged woodland fenced for deer exclusion. Each spring during 2001–2005 the site was repeatedly examined for morel fruit bodies. The locations of all morel fruit bodies were permanently marked and subsequently mapped. Representative fruit bodies were collected for pure culture isolation and identification, as noted below.

In 2003, all perennial woody vegetation with a stem diameter of at least 1 cm was mapped. For each stem, diameter at 140 cm (i.e. diameter at breast height, dbh), species identity and crown health were noted.

Air temperature at 1 m above the soil surface and soil temperature at 3 cm depth were recorded hourly from the site using a Campbell Scientific Micrologger (Campbell Scientific, Logan, UT). Daily precipitation was recorded by HARC staff within 850 m of the study site.

Statistical analysis

The association of morel fruit body production with precipitation and temperature during the five years of the study was assessed with Pearson's correlation coefficient (Sokal & Rohlf 1995) using the PROC CORR algorithm of SAS version 9.1 (SAS Institute, Cary, NC). In this analysis, annual morel fruit body production was represented by four metrics: total annual fruit body count (abundance), day of year (DOY) of first fruiting, fruiting season length (d), and fruiting intensity (fruit bodies d^{-1} from first to last observation). Precipitation was represented by 11 metrics summarizing both accumulated rainfall (mm) and number of precipitation events greater than selected thresholds: (1) total precipitation (mm) from DOY 1 until the onset of fruiting, (2–5) number of days from DOY 1 with precipitation exceeding four thresholds (5, 15, 25 or 50 mm) prior to the onset of fruiting, (6) total precipitation within 30 d preceding the onset of fruiting, (7–8) number of days with precipitation of at least 10 or 15 mm within 30 d preceding the onset of fruiting, (9) number of precipitation events of at least 10 mm within 30 d preceding the onset of fruiting, (10) total precipitation during fruiting season, and (11) total precipitation during the previous calendar year.

In the correlation analysis, temperature metrics were based on degree-days computed as the cumulative mean daily temperature above a threshold temperature. Air and soil temperatures were represented by 16 metrics: (1–8) air and soil temperature degree-days accumulated from DOY 1 until first fruiting above four thresholds (0, 2, 4 or 6 °C) and (9–16) air and soil temperature degree-days during the fruiting season divided by the length of the fruiting season, using the same four threshold temperatures.

The association of morel fruit bodies with woody vegetation was assessed using 131 mapped fruit bodies and 840 mapped woody stems. The contribution of each plant species to the woodland community was characterized as both stem frequency and as basal area (m^2 cross-sectional stem area at dbh) frequency. In the first analysis, the number of stems within 2 m of each mapped morel was determined for each plant species. Contingency table analysis and the chi-square statistic (Sokal & Rohlf 1995) were used to test the null hypothesis that the observed number of plant stems within 2 m of mapped morels was proportional to the frequency of occurrence of that woody species on the site. This analysis did not explicitly account for differences in tree size or crown condition. In all contingency table analyses, cells were combined to ensure that minimum expected values were at least 5 (Sokal & Rohlf 1995).

In a second analysis, a proximity index was computed as the distance between each stem and each morel divided by the stem diameter. This index weighted proximity of larger trees more heavily than that of smaller trees. For each mapped morel, the number of stems of each plant species within 0.5 m cm^{-1} dbh was noted. Contingency table analysis tested the null hypothesis that the number of plant stems of each species observed within 0.5 m cm^{-1} dbh of a morel was proportional to the basal area contribution of that woody plant species in the study area.

In order to assess the influence of crown condition on morel proximity, the stems of each woody species were divided into two groups: crown live or crown dying/dead. Contingency table analysis was used to test the null hypothesis that the observed number of dying/dead plant stems within 2 m of

a morel mapped in 2002–2004 was proportional to the proportion of dying stems for that plant species. A further contingency table analysis tested the null hypothesis that the number of dying/dead plant stems within 0.5 m cm^{-1} dbh of a morel mapped in 2002–2004 was proportional to the fraction of the basal area for that woody species contributed by dying/dead trees. We reasoned that trees classified as dead/dying in 2003 were likely dying in 2002 and had residual declining roots systems in 2004. Thus, morels mapped in 2001 and 2005 would have been much less likely to be influenced by these trees.

The degree to which morel fruit bodies appear in the same location each year was assessed. For each fruit body mapped during 2002–2005, the distance to the closest fruit body (i.e. minimum distance) of the previous year was calculated. The variation in the minimum distances was assessed graphically for evidence of inter-annual clustering.

Species identification

Fruit bodies collected for pure culture isolation were first split longitudinally with a sterile scalpel. The interior of the fruit body was briefly sprayed with 70 % ethanol before removal of small (*ca* 8 mm^3) tissue blocks for placement on Melin Norkrans Medium agar (Buscot 1992). Subcultures of four representative isolates (MO162-MO165) were grown on cellulose dialysis membrane overlaid on potato dextrose agar (PDA) at room temperature in the dark. Voucher cultures are all deposited with the Center for Forest Mycology Research, in the Forest Products Laboratory, Madison, WI. Accession numbers and isolate designations: FP-140146–FP-140149 for isolates MO162–MO165, respectively. The thallus was scraped off the membrane once it reached the plate margin, and genomic DNA was extracted using CTAB and the Nucleospin® Plant DNA extraction kit (Macherey-Nagel, Düren, Germany) following manufacturer's instructions. The ITS region was amplified using fungal specific primers ITS1F (5′CTTGGTCATTTA GAGGAAGTAA 3′) and ITS4 (5′TCCTCCGCTTATTGATATGC 3′) (Gardes *et al.* 1991; Gardes & Bruns 1993) in an MJ Research PTC-200 peltier thermal cycler (Bio-Rad Laboratories, Hercules, CA) with an initial 1 min 25 s denaturation step at 95 °C, followed by 35 cycles of 95 °C for 35 s, 55 °C for 55 s, and 72 °C for 45 s, and a final extension step at 72 °C for 10 min. Each 25 μl PCR reaction consisted of 2 mM dNTPs, 1.5 mM Mg^{2+}, 1 μM of each primer, 1 unit Taq (Invitrogen, Grand Island, NY), 1–5 ng DNA, PCR water to 25 μl. The 1–1.3 kb amplicons were visualized in 1 % agarose gel run in 1 × Tris, borate, ethylenediamine-tetraacetate (TBE) at 90 V.

The Nucleospin® Extract II kit (Macherey-Nagel Gmbh) was used to extract the ITS amplicon from the gel following manufacturer's instructions. The purified amplicon was then cloned into Promega pGEM® -T Easy Vector system I (Promega, Madison, WI) following manufacturer's instructions, and the insert was sequenced at The Ohio State University's Plant and Microbe Genomics Facility (http://www.biosci.ohio-state.edu/%7Epmgf/) using universal primers. The ITS sequences of the four isolates were then used as templates to search for homologous sequences in GenBank (http://www.ncbi.nlm.nih.gov) using the BLAST algorithm (Altschul *et al.* 1990).

Results

Temporal patterns of fruit body production

Over the five survey years, annual fruit body counts ranged from 8 to 58 (Table 1). There was notable inter-annual variation in fruit body count, fruiting season length, and the day of fruiting onset (Table 1). Of the four metrics used to characterize fruiting, total annual fruit body count and fruiting intensity (fruit bodies d^{-1}) were highly correlated ($r = 0.983$, $P = 0.003$), leaving three uniquely informative metrics to explore the influence of temperature and precipitation upon morel fruiting.

Of the 11 precipitation metrics assessed, total annual fruit body count was significantly correlated only with the number of precipitation events of at least 10 mm accumulation that occurred within 30 d of the onset of fruiting ($r = 0.907$, $P = 0.034$, Fig 1). The two years in which the most fruit bodies were observed had the shortest fruiting seasons (Table 1, Fig 2), although the overall correlation between total annual fruit body count and season length was not significant ($r = -0.753$, $P = 0.142$). Total annual fruit body count was not correlated with any of the air or soil temperature metrics examined.

The length of the fruiting season was significantly positively correlated with the extent of soil warming before onset of fruiting (Fig 3A, $r = 0.902$, $P = 0.036$), when soil temperature degree-days were calculated on a 0 °C basis. Fruiting season length was not significantly correlated with any precipitation metric.

The onset of morel fruiting was hastened by early spring warming. The onset of fruiting was significantly inversely correlated with air temperature before fruiting ($r = -0.905$, $P = 0.035$, Fig 3B) when air temperature degree-days were calculated on a 0 °C basis. Fruiting onset was also significantly inversely correlated with soil temperature before fruiting ($r = -0.936$, $P = 0.019$, Fig 3C) when soil temperature degree-days were calculated on a 2 °C basis. Fruiting onset was not significantly correlated with any precipitation metric examined.

Table 1 – Temporal features of morel fruiting during 2001–2005

Attribute	Year				
	2001	2002	2003	2004	2005
Number of fruit bodies	35	14	58	8	16
DOY of first fruiting	109	108	110	107	104
Length of fruiting season, days	6	10	7	13	15
Soil temperature degree-days at onset (0 °C)	365	435	441	477	583
Maximum air temperature during fruiting (°C)	28	29.9	22.1	31.8	28.3
Maximum soil temperature during fruiting (°C)	18.5	19	12.6	14.9	15.5
Minimum air temperature during fruiting (°C)	2.9	2.6	4	3.2	0.3
Minimum soil temperature during fruiting (°C)	9.7	9.9	10.7	10.2	10

DOY, day of year.

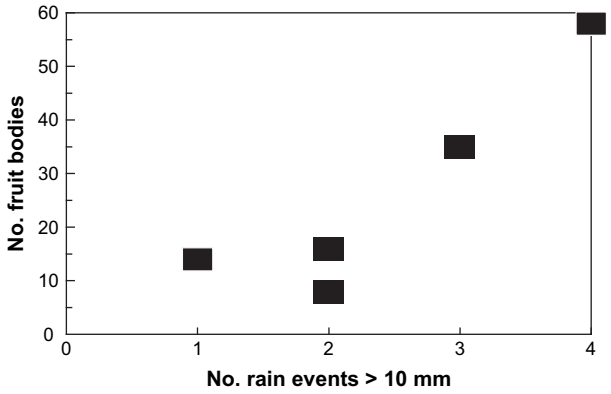

Fig 1 – Number of morel fruit bodies produced in 2001–2005 as a function of the number of rain events of at least 10 mm within 30 d preceding the onset of fruiting. Rain events are total precipitation (mm) from consecutive days with measurable precipitation.

During the fruiting season the minimum soil temperature was surprisingly constant across the five years of the study (Table 1). In contrast, the air temperature minima and maxima, as well as the soil temperature maxima during the fruiting season were quite variable (Table 1).

Spatial patterns of morel fruiting and associated vegetation

The woody vegetation of the study site comprised 27 species dominated by *Asimina triloba* and *Ulmus americana* based only on stem frequency. Based on basal area contribution, the vegetation was dominated by *U. americana* and *Tilia americana* (Table 2). Eleven species were represented only by healthy trees. *U. americana*, *A. triloba* and *Gleditisia triacanthos* had the greatest numbers of dying/dead trees. *U. americana*, *G. triacanthos*, *Prunus serotina*, and *Juglans nigra* accounted for the greatest basal areas of dying/dead trees (Table 2).

For several plant species in the woodland, the numbers of plant stems within 2 m of a mapped morel differed significantly from the values predicted under the null hypothesis of proportional representation based on their overall frequency of occurrence ($X^2 = 52.9$, D.F. $= 12$, $P < 0.001$).

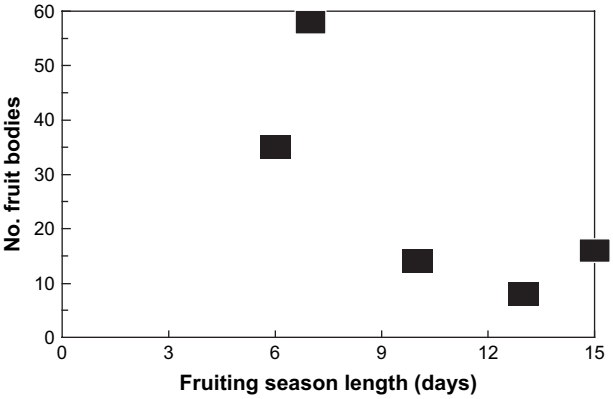

Fig 2 – Number of morel fruit bodies produced in 2001–2005 as a function of the length of the fruiting season (d).

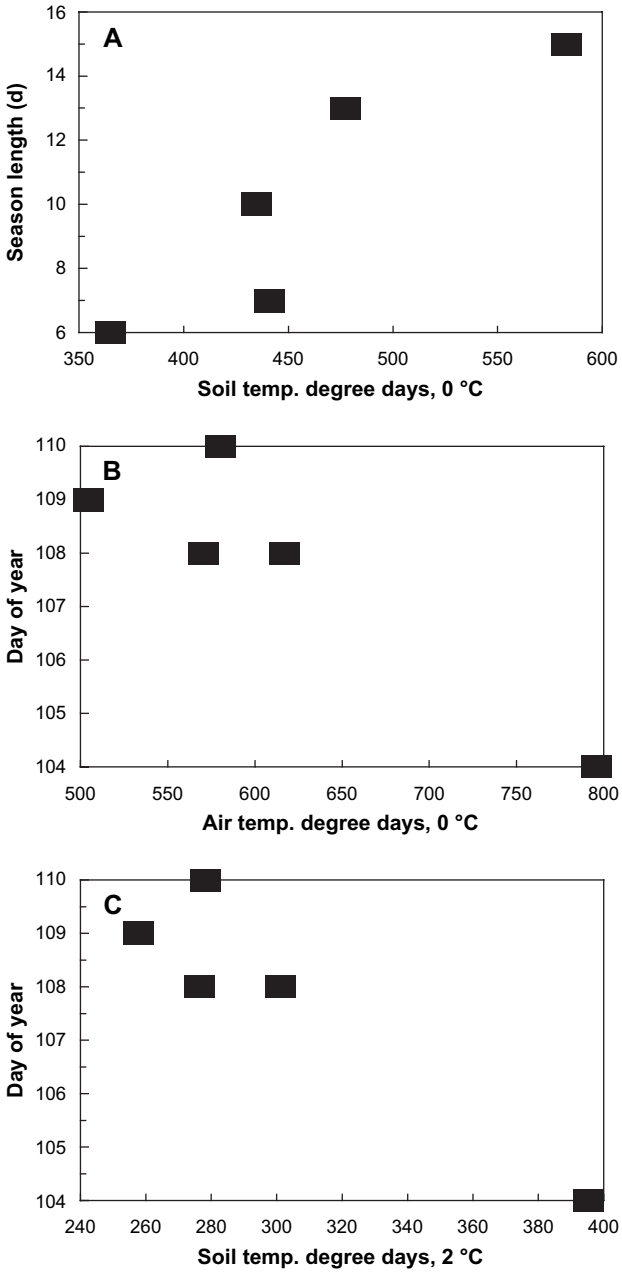

Fig 3 – Morel fruiting relative to temperature. (A) Length of fruiting season (d) as a function of soil warming represented as soil temperature degree-days above 0 °C accumulated by the onset of each fruiting season. (B–C) Day of year (DOY) of fruiting initiation as a function of cumulative air temperature degree-days above 0 °C from DOY 1 (B) or cumulative soil temperature degree-days above 2 °C from DOY 1 (C).

Significantly more stems than expected of *U. americana*, *Carya illinoensis*, and *T. americana* were found within 2 m of a mapped morel (Fig 4A), whereas significantly fewer stems than expected of *A. triloba*, *Quercus* spp., and *G. triacanthos* were recorded this close (Fig 4A).

The number of plant stems within 0.5 m cm^{-1} dbh of a mapped morel also differed significantly from values expected under the null hypothesis of proportional basal

Table 2 – Perennial woody vegetation associated with morel fruiting

Taxon	Acronym	Mapped stems		Diam (cm)	Basal area (cm^2)		Proportion morels near at least one stem	
		Total	Percent dead		Total	% dead	<2 m	<0.5 m cm^{-1} dbh
Asimina triloba	at	195	8.2	2.6 ± 0.07	1150.3	6.9	0.198	0.130
Carya cordiformis	cc	35	0	6.1 ± 0.97	1914.9	0	0.099	0.366
C. glabra	cg	39	2.6	5.9 ± 0.70	1648.4	0.6	0.107	0.374
C. illinoensis	ci	33	6.1	25.7 ± 1.91	20092.6	4.9	0.160	0.977
C. ovata	co	9	0	15.9 ± 3.60	2510.3	0	0.023	0.282
Celtis laevigata	cl	14	14.3	16.3 ± 4.89	6340.6	0.3	0.015	0.305
C. oxidentalis	ceo	3	0	11.9 ± 2.97	375.2	0	0	0.046
Cercis canadensis	red	3	0	3.5 ± 0.15	29	0	0	0
Cornus drummondii	cd	3	0	3.7 ± 1.16	39.2	0	0.015	0.008
Crataegus sp.	cra	3	33.3	6.9 ± 3.57	173.3	2	0	0.031
Diospyros virginiana	dv	2	50	3.1 ± 0.95	16	78.8	0	0
Fraxinus americana	fa	11	9.1	4.4 ± 0.66	208.7	10.2	0.015	0.015
F. pennsylvanica	fp	1	0	28.1	620.2	0	0	0.023
Gleditsia triacanthos	gt	42	26.2	18 ± 0.88	11759.9	14.8	0.038	0.740
Juglans nigra	jn	26	23.1	18.1 ± 1.64	8046.1	16.3	0.053	0.519
Juniperus virginiana	jv	9	0	1.3 ± 0.16	13.5	0	0.061	0.015
Ostrya virginiana	ov	13	0	9 ± 1.63	1145.5	0	0.008	0.153
Prunus serotina	ps	7	28.6	27.1 ± 5.69	5108.2	29.3	0.031	0.511
Quercus alba	qa	10	10	15.8 ± 2.27	2328.6	1.5	0.023	0.450
Q. imbricaria	qi	20	5	17.6 ± 2.03	6110.7	11.6	0.023	0.710
Q. palustris	qp	11	0	24.8 ± 3.79	6565.5	0	0.015	0.611
Q. rubra	qr	8	0	16.9 ± 5.03	2913.6	0	0.008	0.229
Q. velutina	qv	14	0	9.6 ± 1.06	1163	0	0.015	0.053
Tilia americana	ta	33	15.2	16.6 ± 2.15	11218.6	6.2	0.107	0.664
Ulmus americana	ua	275	27.6	9.6 ± 0.32	25776.3	39.2	0.618	0.901
U. rubra Muhl.	ur	5	40	21.5 ± 3.06	1958.8	58.7	0.053	0.176
Vitis sp.	vit	16	12.5	4 ± 0.21	207.3	11.8	0.076	0.031
Open – no woody stems	–	–	–	–	–	–	0.137	0

area representation for several plant species across the study site ($X^2 = 254$, D.F. = 21, $P < 0.001$). Significantly more stems than expected of *T. americana*, *U. americana*, *C. cordiformis* and *C. glabra* occurred within 0.5 m cm^{-1} dbh of a mapped morel (Fig 4B), whereas significantly fewer stems than expected of *Celtis laevigata*, *J. nigra*, *P. serotina*, *G. triacanthos*, *Q. rubra*, *Q. palustris*, and *Q. velutina* occurred this close to a mapped morel (Fig 4B).

The number of dying/dead plant stems within 0.5 m cm^{-1} dbh of a morel mapped in 2002–2004 differed significantly from values expected under the null hypothesis of proportional dying/dead basal area representation for several plant species ($X^2 = 127.44$, D.F. = 7, $P < 0.001$). Specifically, significantly more dying/dead stems of *C. illinoensis* were within 0.5 m cm^{-1} dbh of a mapped morel than expected, whereas significantly fewer dying/dead stems of *Q. imbricaria* were this close. For *G. triacanthos*, *J. nigra*, *P. serotina*, *T. americana*, and *Ulmus* spp., there was no statistically supported departure from the null hypothesis. For the remaining woody species, either too few stems were present on the study site or the proportion of dying/dead stems was too small to permit analysis.

Spatial clustering of morel fruit bodies became apparent during our study (Fig 5). We selected six clusters, designated A–F (Fig 5A), to be used for detailed analysis of the association of selected woody plant species with fruit body production (Fig 5B–D). Each of the morel clusters was associated with at least two *U. americana* stems (Fig 5B) and at least one stem of *Carya* spp. (Fig 5D). Cluster A was also associated with several *T. americana* stems (Fig 5C). We did not find a clear example of a cluster of morel fruit bodies associated with any single woody plant species.

Intra-annual clustering of morel fruit bodies was clearly evident (e.g. Clusters A, E and F, Fig 5A). To assess the occurrence of inter-annual fruit body clustering, we calculated the distance from each fruit body produced 2002–2005 to the nearest morel of the previous season, and used this 'minimum distance' as a measure of reappearance over time. We reasoned that if morels are repeatedly produced in the same location, the 'minimum distance' measures should be very short. The mean 'minimum distance' values ranged from 4.4 m (2003) to 11 m (2002). Although the smallest 'minimum distance' values were less than 1 m for each year (Fig 6), the largest 'minimum distance' values ranged from 12 m (2004) to 48.8 m (2002).

Species identification

The ITS sequences of three of the four isolates (MO162, MO164 & MO165) matched *Morchella esculenta*, with best matches characterized by minimum 97 % identities and E-values of 0. Isolate MO163 matched *M. spongiola* (best match with 97 % identity and E-value = 10^{-173}).

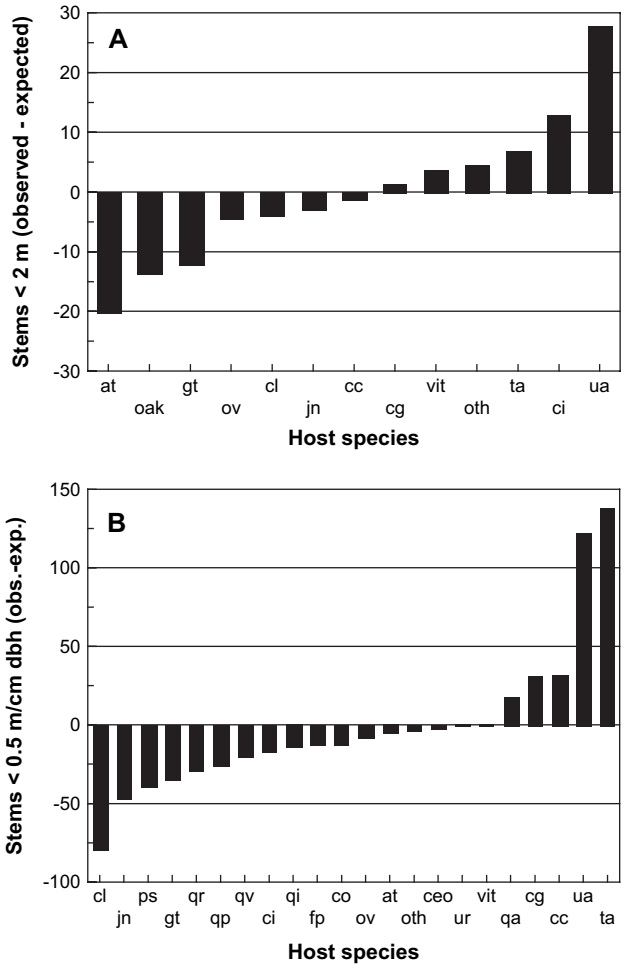

Fig 4 – The difference between the number of woody stems within 2 m (A) or within 0.5 m cm^{-1} dbh (B) of any of 131 mapped morels and the number of stems expected under the assumption of representation proportional to stem frequency on the site. Woody species designations are given in Table 2.

Discussion

The DOY of initiation of morel fruiting each spring was inversely correlated with both accumulated air and soil temperature degree-days. Further, the range of 365–580 soil temperature degree-days (0 °C basis) accumulated before annual onset of fruiting over the five years of our study was remarkably similar to the 425–580 soil temperature degree-days reported by Buscot (1989). Fruiting season length was shortest in the two years of highest annual fruit body production. Further, fruiting season length was significantly positively correlated with soil warming before the onset of fruiting. The minimum soil temperature during the five fruiting seasons was consistently in the range 9.7–10.7 °C suggesting a threshold temperature that must be maintained to sustain fruiting.

Although the onset of fruiting and the length of fruiting season were only related to warming spring temperatures, the total number of fruit bodies produced was only significantly correlated with precipitation in the 30 d preceding the onset of fruiting. Clearly morel fruit bodies require a timely moisture supply for development. The shorter duration of the two most productive fruiting seasons (2001 and 2003) may reflect the coincidence during these two years of appropriate temperatures and moisture levels. A major reason for the variability in annual morel abundance appears to be the independence of fruiting season timing (driven by temperature) and fruiting abundance (driven by precipitation). Thus, if a fruiting season is triggered in the absence of adequate supporting precipitation, little fruiting will take place.

The spatial patterns of morel fruit bodies were significantly related to those of the woody vegetation at the study site. Morel fruit bodies were significantly closer to stems of *Ulmus americana*, *Tilia americana*, and several *Carya* spp. than we would have expected based on the proportion of these species in the study area. Our observations on the proximity of morels to stems of *Ulmus* confirm a large body of documented observations (*e.g.*, Tiffany *et al.* 1998).

Contingency table analysis of the effect of tree mortality on morel fruiting suggests that mortality of different tree species affects fruiting differentially. In our study site, a greater number of dying/dead *C. illinoensis* stems than expected occurred within 0.5 m cm^{-1} dbh of a mapped morel. In contrast, fewer than expected dying/dead stems of *Quercus imbricaria* occurred this close to a morel.

When we examined the vegetation associations of six clusters of morel fruit bodies, in each case stems of at least two of the woody species noted above occurred within the cluster boundaries. Thus, we could not conclude that morel fruit bodies occur in exclusive proximity to any single woody species in our woodland. Our observations reveal that the spatial patterns of morel fruit bodies appear to be at least partially determined by more complex spatial patterns of the forest. It would be an oversimplification to conclude that morels occur in proximity to a single woody species, as the roots of the various species are intermingled in the soil throughout the forest floor.

Although intra-annual clustering of morel fruit bodies was quite apparent in years when fruiting was abundant, inter-annual clustering was not consistently observed. We hypothesize that observed spatial patterns of morel fruit bodies are not conditioned solely by proximity to any single woody species. If they were, we would expect to see significant inter-annual clustering of fruit bodies over ranges more completely representing the distributions of favourable woody species. We estimated the mean minimum distances among morel fruit bodies in successive years in the range of 4.4 to 11 m.

Three of four isolates examined were identified as *M. esculenta* based on ITS sequence similarity. The fourth isolate was most similar to the European *M. spongiola*. It is premature to conclude that we have found a European *Morchella* species in Missouri. Rather, we may have found a new or previously unsequenced North American species for which the closest sequenced relative is the European *M. spongiola*.

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The technical contributions of James J. Wetteroff Jr. in morel and vegetation mapping, and Duan Wang in molecular

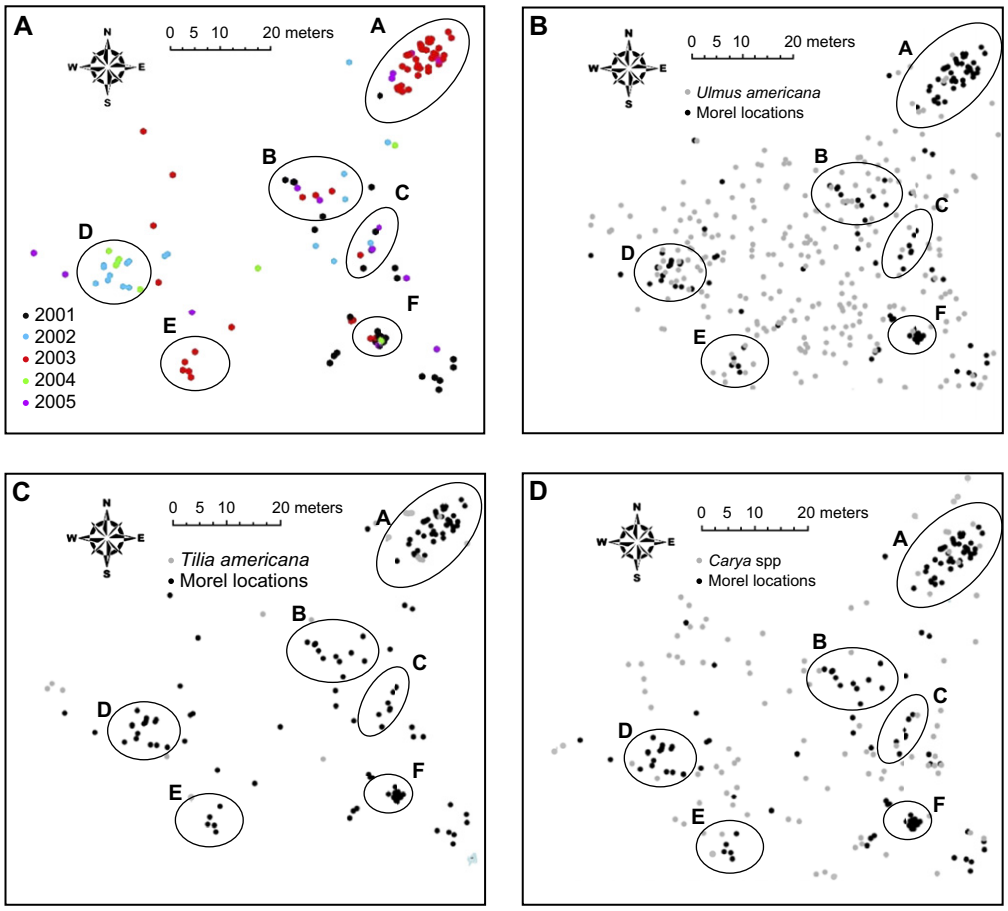

Fig 5 – Spatial pattern of morel fruit body production. Ellipses A–F indicate selected locations of clustered fruit body production. (A) Annual locations of morel fruit bodies. (B–D) Morel fruit body pattern, 2001–2005, relative to position of all *Ulmus americana* (B), *Tilia americana* (C), and *Carya* spp. (D) stems.

identification, are gratefully acknowledged. The assistance of M. Jamila Batchelder in preparing the maps of fruiting and vegetation patterns is much appreciated. We thank Karen Schwartz and Charles Shepley for field assistance. This research was partially supported by Missouri Agricultural Experiment Station Project PSSL0112. Contribution from the Missouri Agricultural Experiment Station. This work was partially funded through the University of Missouri Center for Agroforestry under cooperative agreements 58-6227-1-004 with the ARS and C R 826704-01-2 with the US EPA. The results presented are the sole responsibility of the authors and/or the University of Missouri and may not represent the policies or positions of the EPA. Any opinions, findings, conclusions or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the US Department of Agriculture.

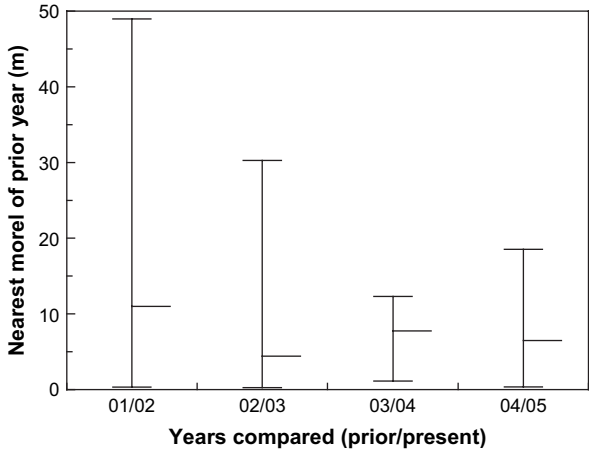

Fig 6 – Proximity of morels mapped in 2002–2005 to the nearest morel mapped the previous year. The top, intermediate and bottom cross bars represent the largest, mean and smallest 'minimum distance' values.

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