

## Occurrence of black truffles in Poland

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Ławrynowicz M., Krzyszczyk T., Fałdziński M.: *Occurrence of black truffles in Poland*. Acta Mycol. 43 (2): 143–151, 2008.

*Tuber aestivum* Vittad., *T. mesentericum* Vittad. and *T. bellonae* Quél. have been distinguished in recent collections of hypogeous fungi in Poland. Up till now only *T. mesentericum* has herbarial documentation. *T. aestivum* was confirmed to occur in Poland. Seven localities are documented with exiccates, but it is considered as a complex species. Some specimens have features of *T. uncinatum* Chat. not distinctively separated from these of *T. aestivum* Vittad., therefore are treated as its form. The studies based on rich material support the concept of *Tuber bellonae* as a separate species close to *T. mesentericum*.

**Key words:** *Tuber*, taxonomy, ecology, distribution

### INTRODUCTION

Hypogeous fungi and especially truffles have been of increasing interest in Poland in the recent years. The old data on the occurrence of truffles in Poland were set together by Lubelska (1953). She mentions 8 species of truffles, among them *Tuber aestivum* from 6 localities on the base of papers by: Caspary (1886), Błoński (1888), and Alexandrowicz & Błoński (1894). It was the only black truffle indicated; this taxon overlapped also *T. mesentericum*, but no herbarium material is now available to analyse this question.

After about hundred years *T. mesentericum* was discovered on Częstochowa Upland in two localities (Ławrynowicz 1988, 1990, 1999) in course of systematical searching of hypogeous fungi.

These fungi produce fruit bodies only in some places called hypogeous oasas or nests, where ecological conditions are suitable. Similarly, only in some years the climatic conditions unable formation of fruit bodies. The year 2007 was exceptional. The authors of this paper found independently black truffles in 13 sites. The analysis of the material permits to identify 4 taxons: *Tuber aestivum*, *T. aestivum* forma *uncinatum*, *T. mesentericum*, and *T. bellonae*; the latter as a new species for Poland.

## MATERIAL AND METHODS

The investigations were taken out at the calcareous hilly area, not exceeding 300 m a.s.l. On the base of type of ecosystem, density of vegetation cover, type of soil and features known as characteristic for truffle sites, the environment places for excavations were chosen. Numerous collections of different hypogeous species were made during several years of field searching, but in 2007, starting from the early summer, the fruit bodies of black truffles were to be found. Also in course of monitoring two above mentioned sites of *Tuber mesentericum* two new localities of *T. aestivum* were discovered in July 2007.

Altogether more than two hundred of fruit bodies were observed by two of us (TK and MF). In some cases a dog was helping to detect the ripe carpophores. Specimens were described immediately after taking out from the soil and in most cases documented at the sites concerned.

Fruit bodies were analysed taxonomically using the traditional procedure. The micromorphological and anatomical features of peridium cells, asci and ascospores were examined under Eclipse 600 E-microscope (Nikon) (200x, 400x, 1000x). Materials were identified according to Montecchi and Sarasini (2000), Rioussset et al. (2001) and some other authors indicated in the "References". The localities are indicated on three maps in the ATPOL system.

Abbreviations of collectors names: TK – Tomasz Krzyszczuk, MF – Marcin Fałdziński, MŁ – Maria Ławrynowicz. Other explanations for ATPOL system used for hypogeous fungi are according to Ławrynowicz (1989). Dried specimens were deposited in the Herbarium Universitatis Lodziensis (LOD).

## KEY TO SPECIES

- Fruit body black, purple- or black-brown, with warty surface; spores reticulate
1. Fruit body black becoming gray with cavity or depressions; warts small, obtuse ..... 2
  - 1\*. Fruit body black becoming brown; warts large, acute ..... 3
  2. Fruit body with distinct cavity; spores ellipsoid ..... *T. mesentericum*
  - 2\*. Fruit body with depressions; spores round ..... *T. bellonae*
  3. Gleba white becoming light yellow; spores with incomplete mesh reticulation up to 2  $\mu\text{m}$  high ..... *T. aestivum*
  - 3\*. Gleba yellowish becoming brown; spores with reticulum up to 4  $\mu\text{m}$  high ..... *T. aestivum* forma *uncinatum*

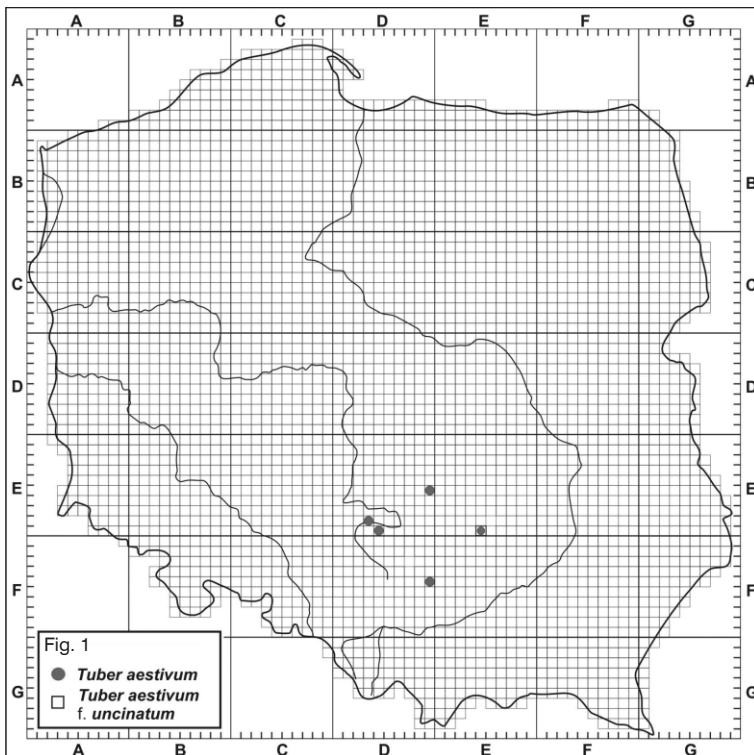
## DESCRIPTIONS OF THE SPECIES

*Tuber aestivum* Vittadini 1831

**Fruit bodies** globose or subglobose 2-5 cm in diam.; black, becoming brown when dried. **Peridium** strongly warty with pyramidal, irregularly polygonal warts 4-7 mm wide, truncate or depressed on top; with radial ridges and fine transversal striae. **Gleba** fleshy, compact, at first white becoming light brown to hazel brown, at maturity marbled with white, thin veins. Odour pleasant. **Asci** 60-100 × 55-75 μm globose or pyriform, with short stalks, 2-6 spored. **Spores** 20-45 × 15-35 μm at first hyaline becoming yellow to yellowish brown; ornamented by irregular reticulum with uncomplete alveolae, up to 2 μm high; 3-4 along the spore.

REMARKS. *T. aestivum* is a heterogenous species. Montecchi and Sarasini (2000) mention the results of studies indicating 18 various clones or breeds of this species but none of them reached the species level. The Polish collections of *T. aestivum* present great variability, as well. The study has just started. The collections from three localities contain fruit bodies of slightly different character, what allow considering them to be close to *Tuber uncinatum* Chat. according to Riousset et al. (2001).

ECOLOGY. The fruit bodies were found in association with *Fagus sylvatica*, *Quercus robur*, *Q. petraea* and variety of other deciduous trees on calcareous soil. The collections come from three uplands, mostly from the lower parts of the hills. The species was found in places of heavy human impact at the forest paths and tourist trails, in



the vicinity of houses or in the area facing a new arrangement of roads. It means that the occurrence of *T. aestivum* is seriously endangered (Pl. I 1, 3).

**DISTRIBUTION.** *T. aestivum* is considered to be the most widespread species among edible truffles in Europe, occurring from Mediterranean zone to South Scandinavia (Veden et al. 2001). Lubelska (1954) gives six localities of the species in Poland mainly on the basis of popular literature. Recent collections of *T. aestivum* are indicated in the Figure 1.

#### LOCALITIES

**DE 59** – Wyżyna Przedborska, Przedborski Landscape Park, Dobromierz by Przedbórz, *Fagus sylvatica*, Aug. 2007, leg. TK, LOD 22006.

**DE 84** – Wyżyna Krakowsko-Częstochowska, Jurajski Landscape Park, Mstów, Jul. 2007, *Quercus petraea*, *Fagus sylvatica*, leg. MŁ, LOD 22014. Res. Zielona Góra, *Fagus sylvatica*, *Carpinus betulus*, *Quercus robur*, Jul. 2007, leg. MŁ, LOD 22015.

**DE 95** – Wyżyna Krakowsko-Częstochowska, Jurajski Landscape Park, Ludwinów by Złoty Potok, *Fagus sylvatica*, Jul. 2008, leg. TK, LOD 22008.

**DF 49** – Wyżyna Krakowsko-Częstochowska, Złożeniec by Smoleń, *Fagus sylvatica*, *Quercus petraea*, *Carpinus betulus*, Jul. 2007, leg. TK, LOD 22010.

**EE 94** – Wyżyna Kielecko-Sandomierska, Dębina by Lisów, *Carpinus betulus*, *Quercus petraea*, *Pinus sylvestris*, Sept., Nov. 2007, leg. MF, LOD 22002.

*Tuber aestivum* Vittad. forma *uncinatum* (Chatin) Montecchi et Borelli 1990  
= *T. uncinatum* Chatin 1887.

**Fruit bodies** similar to those of *T. aestivum* Vittad. The differences are in smaller warts on the surface, darker colour of gleba, more intensive odour, spores ornamented with more distinct reticulum with meshes up to 4 µm high, curved in the upper part. The last feature was observed only in some spores. On the basis of three collections we concluded that *T. aestivum* forma *uncinatum* is a late autumn form of *T. aestivum* adapted to more humid sites (Pl. I 2, 4, 5, 6).

**REMARKS.** Recent collections of *T. aestivum* present a great taxonomical variability of this species. The most distinctly separated group is that showing the features of *T. aestivum* forma *uncinatum*. Continuation of collecting and taxonomical studies may bring additional arguments to verify our point of view in this question.

**ECOLOGY.** In humus-clay soil in calcareous area. Under *Carpinus betulus*, *Acer pseudoplatanus*, *Quercus petraea*, *Corylus avellana* and other trees. On hills up to 290 m a.s.l., but usually in the lower parts; autumn and winter mounts; in great quantities: more than a hundred of carpophores were collected in three localities.

**DISTRIBUTION.** The localities are indicated in Figure 1. The occurrence is endangered because of forest management practices and penetration by people from adjacent villages. The whole area should be protected.

#### LOCALITIES

**EE 94** – Wyżyna Kielecko-Sandomierska, Piotrkowice, *Acer pseudoplatanus*, *Quercus petraea*, *Carpinus betulus*, *Betula pendula*, Dec. 2007, leg. MF, LOD 22004.

Załatwie by Lisów, *Carpinus betulus*, Jan. 2008, leg. MF, LOD 22003. Małolipie by Skrzeczyce, *Carpinus betulus*, *Prunus spinosa*, *Rosa canina*. Jan. 2008, leg. MF, LOD 22005.

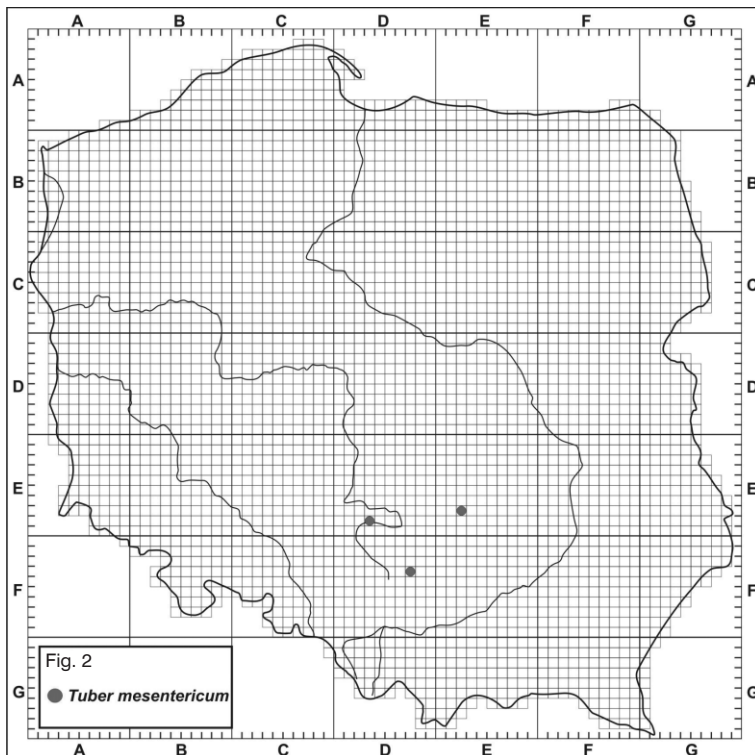
***Tuber mesentericum*** Vittadini 1831

= *Tuber bituminatum* Berkeley et Broom 1851.

**Fruit bodies** globose or subglobose with basal or side cavity 1-5 cm diam., firm, black or blackish blue when fresh, becoming blackish gray when dried. **Peridium** warty with rather small, obtuse warts 3-4 mm across. **Gleba** white when fresh becoming blue gray, yellowish to light brown when dried, marbled with whitish veins. Odour distinct, pleasant when not too much concentrated. **Asci** 60-100 × 55-80 μm, broadly ellipsoid, sometimes subglobose, with a short stalk, 1-5 spored. **Ascospores** 27-53 × 20-35 μm, ellipsoid or subglobose, hyaline when young, yellowish brown at maturity, ornamented with a coarse reticulum 3-5 μm high, usually 3-5 across the width of spore (Pl. II 3, 4).

REMARKS. *Tuber mesentericum* has been regarded as synonym of *T. aestivum* by several authors (e.g. Pegler et al. 1993), but the others confirmed it as separate species (Knapp 1951; Ceruti 1960; Montecchi, Sarasini 2000; Rioussset et al. 2001; Kers 2003).

*T. mesentericum* was distinguished from *T. aestivum* by having smaller fruit bodies, ornamented with smaller warts, obtuse or depressed at the apex, black colour of surface with black-blue reflex becoming black gray when dried, having cavity at the base or on side of carpophores. The spores of *T. mesentericum* are larger and ornamented with more complete reticulum than these of *T. aestivum*. Examination of rich materials collected by the authors supports the separation of these two species (Pl. II 2).



**ECOLOGY.** In humus-sandy soil, clay mixed with small calcareous stones. Under *Fagus sylvatica*, *Carpinus betulus*, *Corylus avellana*, *Quercus robur*, *Q. petraea*. *T. mesentericum* was found in different sites as *T. aestivum*. Even when the same locality has been stated for both species, they do not grow together but in different sites separated by distance of at least 50 m.

**DISTRIBUTION.** This is the only species of black truffles monitored in two localities since 1981 and 1997, respectively. Together with recent collections, it is known from five localities indicated in Figure 2. Exact distribution of the species in Europe is difficult to determine because *T. mesentericum* and *T. aestivum* had been treated as one taxon for a long time.

**LOCALITIES**

- DE 84** – Wyżyna Krakowsko-Częstochowska, Jurajski Landscape Park, Res. Zielona Góra (Ławrynowicz 1988, 1990), Jul. 2007, leg. MŁ, LOD 22017  
Wancierzów (Ławrynowicz 1999), Jul. 2007, leg. MŁ, LOD 22009.
- DF 37** – Wyżyna Krakowsko-Częstochowska, Jurajski Landscape Park, Hutki-Kanki by Olkusz, Jul. 2007, leg. TK, LOD 22017.
- EE 72** – Wyżyna Kielecko-Sandomierska, Res. Milechowy, *Carpinus betulus*, *Corylus avellana*, *Quercus petraea*, *Pinus sylvestris*, *Cornus* sp., Sept. 2007, leg. MF, LOD 22016.

***Tuber bellonae* Quélet 1887**

= *Tuber bituminatum* Berkeley et Broome var. *sphaerosporum* Ferry de la Bellone.

**Fruit bodies** irregularly globose, 3-5 cm in diam., with depressions or small cavity; black on surface. **Peridium** covered with pyramidal, obtuse warts. **Gleba** light, becoming yellowish brown. **Odour** intensive, not very pleasant when concentrated. **Asci** ovoid 77-115 × 77-95 μm with 1-6 spores. **Spores** globose or subglobose, 22.5-55 μm in diam.; ornamented with fine reticulum 5-7 μm high; alveoli 4-6 angled, 5-10 μm long (Pl. II 5, 6).

**REMARKS.** According to some authors (eg. Pacioni & Fantini 1997; Rioussset et al. 2001) *T. bellonae* possesses the features intermediate between *T. mesentericum* and *T. aestivum* forma *uncinatum*.

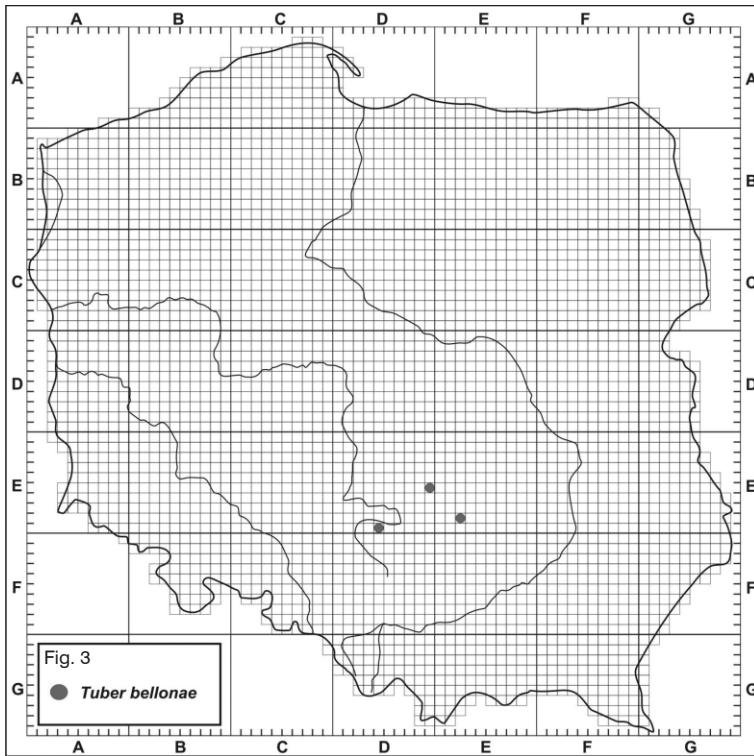
**ECOLOGY.** In humus-clay or clay soils mixed with small calcareous stones, usually covered with thin litter layer. *Tuber bellonae* has been found under *Quercus petraea*, *Corylus avellana* and *Fagus sylvatica* on calcareous soils (Pl. II 1).

**DISTRIBUTION.** As the species has not been distinguished as a separate taxon by many authors for a long time, its real distribution is impossible to determine. The collections comes from three localities in Poland indicated in the Figure 3.

**LOCALITIES**

- DE 59** – Wyżyna Przedborska, Przedborski Landscape Park, Dobromierz by Przedbórz, *Fagus sylvatica*, Aug. 2007; leg. TK, LOD 22011.
- DE 95** – Wyżyna Krakowsko-Częstochowska, Jurajski Landscape Park, Ludwinów on Złoty Potok, *Fagus sylvatica*, Jul. 2008, leg. TK, LOD 22007.
- EE 82** – Wyżyna Kielecko-Sandomierska, Grzywy Korzeczkowskie, *Quercus petraea*, *Corylus avellana*, Aug., Nov. 2007, leg. MF, LOD 22001.





### GENERAL REMARKS AND CONCLUSIONS

Increasing interest in hypogeous fungi in Poland results in discovering of several new localities and gathering big collections of carpophores, among them black truffles. This activity is of a great value for research and practice. The knowledge of occurrence of black truffles in natural habitats and of its distribution could help to better recognize the ecological requirements of particular truffle species.

The results of taxonomical, ecological and chorological analyses are concluded in the identification of four taxa: *Tuber bellonae* Quél., *T. mesentericum* Vittad., *T. aestivum* Vittad., and *T. aestivum* Vittad. forma *uncinatum* (Chat.) Montecchi et Borelli. The collected material, consisting of more than 200 fruit bodies, came from 13 localities in calcareous uplands in the South of Poland. Some collections were also signalled by Hilszczańska et al. (2008). In the present paper, *Tuber bellonae* is reported from Poland for the first time. *T. mesentericum* has been found and continuously observed since 1981; *T. aestivum* was mentioned in the literature in 19. century in 6 localities in different parts of Poland (Lubelska 1954). No exsiccates of the latter species coming from these sites were found neither in Polish nor in foreign herbaria, therefore it is impossible to know its exact taxonomical interpretation. The recent collections of fresh carpophores reveal intraspecific variation of *Tuber aestivum*, enabling us to distinguish a group of *uncinatum* in the rank of a form. The characteristic features of this taxon are attributed to the collections coming from the most north-eastern localities and autumn as well as winter period of occurrence.

Through examination of tens of carpophores revealed that part of them do not reach the maturity during the whole vegetation season. On the other hand it comes clear that the production of black truffle carpophores in Poland in the last years increases. Moreover, it seems that fruit bodies of *T. mesentericum* with cavity and *T. bellonae* with some depressions in carpophores are better adapted to humid and cold climatic conditions.

**Acknowledgments.** Many thanks to Dr. Izabela Kałucka for improving the English version of the paper, Barbara Grzesiak for technical assistance, and an anonymous reviewer for helpful remarks. The study was supported by the Polish State Committee for Scientific Research grant no. P04 G 074 25.

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## Występowanie czarnych truflí w Polsce

## Streszczenie

Grzyby podziemne, a wśród nich truflie są przedmiotem rosnącego zainteresowania z punktu widzenia naukowego i ekonomicznego, z uwagi na wysokie ceny jakie jadalne czarne truflie osiągają na rynkach światowych.

Centrum występowania czarnych truflí stanowi strefa śródziemnomorska. Do strefy umiarkowanej przenikają tylko niektóre gatunki, zaś najdalej, bo do południowej Skandynawii i na Wyspy Brytyjskie sięga trufla letnia *Tuber aestivum*. Gatunek ten wykazuje dużą zmienność cech taksonomicznych i bywa rozmaicie traktowany przez poszczególnych autorów zajmujących niekiedy skrajnie różne stanowiska. Jedni autorzy (np. Pegler i in. 1993) utrzymują *T. aestivum* jako jeden zbiorowy gatunek, inni zaś (np. Rioussset i in. 2001) wyróżniają kilka gatunków w grupie *T. aestivum*, czyli tych truflí, które mają czarne, grubo brodawkowane perydium i zarodniki z ornamentacją siateczkowatą.

Autorzy niniejszej pracy, dysponując bogatym materiałem w postaci świeżych owocników zebranych w roku 2007 na 13 stanowiskach, podjęli próbę taksonomicznej interpretacji występujących w Polsce czarnych truflí. W wyniku analizy makro- i mikromorfologicznej, a także ekologicznej i chorologicznej wyróżniono cztery taksony: *Tuber aestivum* Vittad. – trufla letnia, *T. aestivum* Vittad. forma *uncinatum* (Chat.) Mont. et Borelli – trufla letnia forma późna, *T. mesentericum* Vittad. – trufla wgłębiona i *T. bellonae* Quél. – trufla pośrednia. W pracy podano charakterystykę tych taksonów oraz ich rozmieszczenie w Polsce. *T. bellonae* został podany z Polski po raz pierwszy. Inne gatunki grzybów podziemnych, zebrane podczas badań terenowych będą przedmiotem kolejnych prac.



Plate I: 1. Site of *Tuber aestivum* in summer (photo MF). 2. Site of *T. aestivum* f. *uncinatum* in autumn (photo MF). 3. *T. aestivum* - surface and section of fruit body (photo TK). 4. *T. aestivum* f. *uncinatum* – section of fruit body (photo MF). 5. *T. aestivum* f. *uncinatum* - fruit body after taking out from the soil (photo MF). 6. Spores of *T. aestivum* f. *uncinatum* LOD 22003 (photo ME).

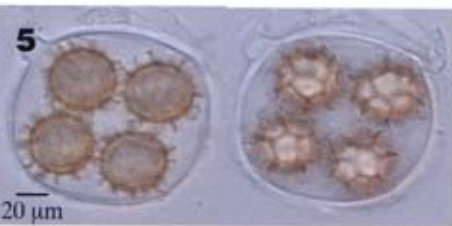
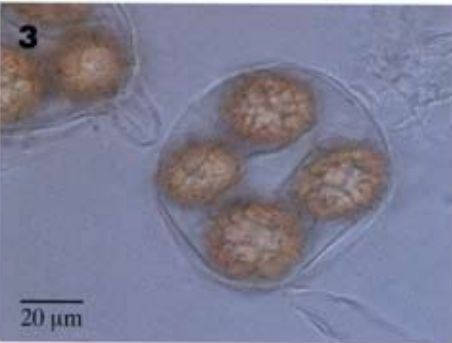


Plate II: 1. Site of *T. bellonae* (photo ME). 2. Comparison of surface sculpture of: (A) *T. aestivum* and (B) *T. mesentericum* (photo TK). 3. Spores in asci of *T. mesentericum* LOD 22016. 4. *T. mesentericum* – cross section of fruit body (photo TK). 5. Spores in asci of *T. bellonae* LOD 22001 (photo ME). 6. *T. bellonae* – fruit bodies with typical depressions (photo TK).