Morphological variations of *Tuber uncinatum* linked to climatic conditions

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Abstract: *Tuber uncinatum* ascocarps were harvested under different weather patterns between 2003-2009 in the East of England. Variations in ascocarp macromorphology and ontogeny were related to variations in climate, particularly precipitation patterns or extreme drought conditions based on observations on numerous samples. Unusual morphological patterns were linked to extreme climatic situations. Patterns of ascocarp growth dynamics and regeneration of the peridium after experimental dissection of ascocarp portions were studied in situ. Dry weather seems to promote the growth of the peridium, and wet weather the growth in volume of the gleba.

Résumé : *Tuber uncinatum* ascocarpes furent ramassés dans des périodes de temps différents entre 2003-2009 dans l'Est de L'Angleterre. Les variations de la macromorphologie et ontogénie des ascocarpes basées sur de nombreux exemplaires avaient un lien avec les différences climatiques, principalement le format et la durée des précipitations ou du temps extrêmement aride. Des modèles de morphologie furent reliés à des extrêmes de situations climatiques. Des modèles d'évolution de la croissance et de la régénération du peridium, après des expériences de dissections partielles de l'ascocarpe furent étudiées in situ. Un temps sec semble promouvoir la croissance du peridium, et un temps humide le volume de la gléba.

Zusammenfassung: Fruchtkörper von *Tuber uncinatum* wurden von 2003-2009 unter verschiedenen Witterungsbedingungen im Osten Englands gesammelt. Variationen der Makromorphologie und Ontogenie wurden auf Basis zahlreicher Einzelbeobachtungen den klimatischen Variationen, besonders den Niederschlagsverhältnissen, gegenübergestellt. Außergewöhnliche Fruchtkörperformen waren mit extremen Witterungsverhältnissen korreliert. Das Wachstum der Fruchtkörper sowie die Regeneration der Peridie nach experimenteller Entfernung von Fruchtkörperteilen wurden in-situ beobachtet. Trockene Witterung scheint das Wachstum der Peridie zu fördern, feuchtes Wetter die Zunahme des Volumens der Gleba.

According to established phylogenies, true truffles (genus *Tuber*) are evolutionarily derived from apothecioid ancestors. PARGUEY-LEDUC & al. (1989) confirmed MALENÇON's (1938) hypothesis by providing ontogenetic evidence, that an apothecioid stage is present in the early development of truffle ascocarps. The primordial apothecioid stage is transformed early in the ascocarp development by the closure of the peridium.

Studies of ascocarp growth have revealed the dependency on various environmental parameters (e.g., MONTANT & KULIFAJ 1990, GREGORI & al. 2008), but were usually limited to basic growth parameters only, disregarding the potential influence of climatic factors on ascocarp morphology. This study attempts to establish a link between developmental morphology (ontogenesis) at the macroscopic level and climatic conditions for *Tuber uncinatum* CHATIN. The objective of this initial investigation was (i) to fill a gap in the literature on the macromorphological structure of small immature specimens and (ii) to gain an insight on the effects of rainfall or drought on the ontogenesis.

It was hoped that the study of the natural precipitation pattern and its effect on truffle development might be useful for the water management of truffle plantations.

Material and methods

Origins, documentation and identification of studied specimens. About 300 truffle samples from a limited number of natural sites from the East of England were collected between 2003 and 2009 and photographed from various angles. Key specimens were dried whole or in slices, others kept frozen or in alcohol so as to keep their structure. Some specimens (98) have been kept whole or in part for review, 44 of which were viewed and confirmed by G. CHEVALIER and H. FROCHOT as *Tuber uncinatum*.

Based on observations on numerous specimens, hypotheses on the influence of weather on ascocarp form were developed. These hypotheses were tested by collecting truffles under particular meteorological conditions in 2009 (heavy rain and hail 16-18th June, followed by a long period of drought from early July to mid September).

For comparison, truffle specimens from France and Italy (2007-2010) were studied to check that the findings were not specific to the principal study area. A few specimens which were deformed by stones or thick roots were excluded from this study.

Collection sites, climate and geology. Site 1 is a garden near Cambridge, established on formerly arable land, covered with lawn and mixed trees (30 year old at most). Most trees are planted in a hedge near the edge of the property (130 m × 40 m): *Populus tremula*, *Fagus sylvatica* (pruned hedge), *Ulmus* spp., *Acer* spp., *Quercus cerris*, *Q. robur*, *Betula pendula*, *Corylus* spp., *Prunus laurocerasus*, *Chamaecyparis lawsonia*. A well revealed the water table at 300 cm depth. Site 2 comprises several isolated truffle areas scattered in the Brecklands (in Norfolk near Watton), a landscape dotted with collapsed periglacial pingos that had formed at the end of the last glaciation (SPARKS & al. 1972, WARMSLEY 2008), resulting in ponds of various sizes. The soil is typically very sandy in the uppermost horizons, with a gradually higher content of clay and chalk in lower horizons (40-60 cm). The chalk bedrock is found at various depths (30-150 cm, mostly 60-100 cm). *Tuber uncinatum* truffles were harvested mainly under *Quercus robur,* typically over 200 year-old and with an understorey of *Prunus spinosa*. The humus rich top soil was covered by leaf litter, moss and ivy. Many streams and ditches, ponds and wells reveal that the depth of the water table varies from 50-120 cm on average.

The East of England is the driest part of the United Kingdom in precipitation terms. Yearly average rainfall at site 1 averages 450-550 mm. Site 2 receives considerably more precipitation, averaging 720-960 mm (MET OFFICE 2010 a). For most of the period under study (2004-2006) exceptionally dry weather was recorded, alternating with short periods of excessive precipitation (MET OFFICE 2010 b, c).

The two truffle sites are on fractured cretaceous chalk bedrock. Quaternary bands of clay and windblown sand deposits contribute to the mineral soil matrix.

Truffle collecting. In the years 2003-2005 truffles growing at or near the surface were searched without dogs in site 1, independent of perfume. From 2006 dogs were trained and used for truffle search, resulting in many more finds, particularly in more natural areas (site 2), where it is difficult to detect truffles by eye.

Long-term observations on truffle development. Truffles found near the surface were covered with a bag of soil 20 cm square and 2-3 cm thick, and a weight (1 kg) resting on them. Truffle growth was observed daily to once a fortnight over up to four months, all observations were recorded on photographs. Ascocarp growth was measured by taking the diameter of the truffle as it emerged through the soil under the bag. The truffle, as it came though the soil made roughly half an oval. The furthest sides of the oval were marked by plastic pins and their distance measured. The pins were moved again as the truffle grew and they were found on the bare peridium. Samples of the gleba were taken fortnightly to assess the rate of maturity and the rate of healing of the peridium. The growth of the warts and the regeneration of the peridium were recorded. A minimum of three specimens were used for any measurement. Observations were successful during three periods of four months each (November-February, June-September, October-December). Many small truffles disappeared during the observation and no continuity was established for March-May.

Table 1. Estimated* number of specimens studied by ascocarp characteristic and depth. *These numbers are precise in case of rare types. In case of more common forms, they represent the minimal number of observations.

Ascocarp characteristic	Surface level	5-7 cm below ground	8-15 cm below ground	Early life	Immature	Near or at maturity
Double peridium	0	5*	0	5*	0	0
Double peridium with early lobes	0	4*	0	4*	0	0
Globose	32	5	5	20	5	17
Globose with constriction	1	55	59	5*	60	50
Globose with lobes	1	14	16	8*	18	5
Kidney shape	17	7*	0	2*	7	15
Total number of specimens	51	90	80	44	90	87

Some truffles were watered to keep the soil moist and others not, but covered as above, or not covered at all. All truffles were situated within 1.5 m of each other. Their dimensions were recorded on lifting them up.

Truffles growing deeply in the soil were most difficult to observe over time as the disturbance generally led to the disappearance of the truffle despite much care being taken.

Experimental excision of ascocarp portions. Some truffles had up to 1/3 of the ascocarp removed to take away clean parts or parts infested by larvae to see how they developed, if at all.

Results and discussion

Re-groupment by ascocarp characteristics and shape

Double peridium. Double peridium type is rare, and only a few observations could be made. The gleba is reduced to a thin line sandwiched between two layers of peridium (Fig. 1). This pattern was observed in very small truffles. When the layer of gleba is thicker, the form is intermediate between double peridium and ascocarp lobes (Fig. 2).

A small truffle with double peridium was observed to unfold itself absorbing water under the tap when being washed.

Globose. The globose form is the default unconstrained shape of truffles at maturity.

Surface truffles (half below ground at most). They were found under dense canopy, or in leaf litter are relatively small (3-4 cm), and totally globose (Fig. 3 a). They tend to be found in July and August as we search for the first truffles and retrain the dogs. Thirteen such truffles were found under a hazel at the same time. Four such globose ascocarps were found in leaf litter. In comparison to deeper truffles, they do not have necessarily a good perfume. The dogs failed to locate some in leaf litter.

Globose below-ground truffles. They are typically found at medium soil depth (5-7 cm below the ground). Under optimal conditions, with alternate damp and sunny weather and the soil remaining soft, the truffles seemed to grow fairly evenly, globose at maturity. This morphology is well documented (PEGLER & al. 1993, CHEVALIER & FROCHOT 1997, RIOUSSET & al. 2001). In-situ observations of four truffles of this kind over 4-5 months (from 3-5 cm soil depth, watered in dry weather) were successfully made (Fig. 3 b). Watered truffles grew to 8 cm on average whilst the others grew very little even when damp conditions returned and reached 3-4 cm at most.

Constricted forms (Fig. 4). Many ascocarps were found to have a constriction at some point in the otherwise globose form, resulting in an upside down pear shape structure. It seems as if at some time in the season the truffle grew more towards the surface, the least resistant path in the soil. The constriction can be narrow or very wide. If it is narrow, the truffle can be easily broken off at this point due to the direction of the sterile veins there. The narrower part of the pear shape truffle can be extremely small, reduced to a small ball or to be just a small elevation of the peridium, in the otherwise globose tuber.

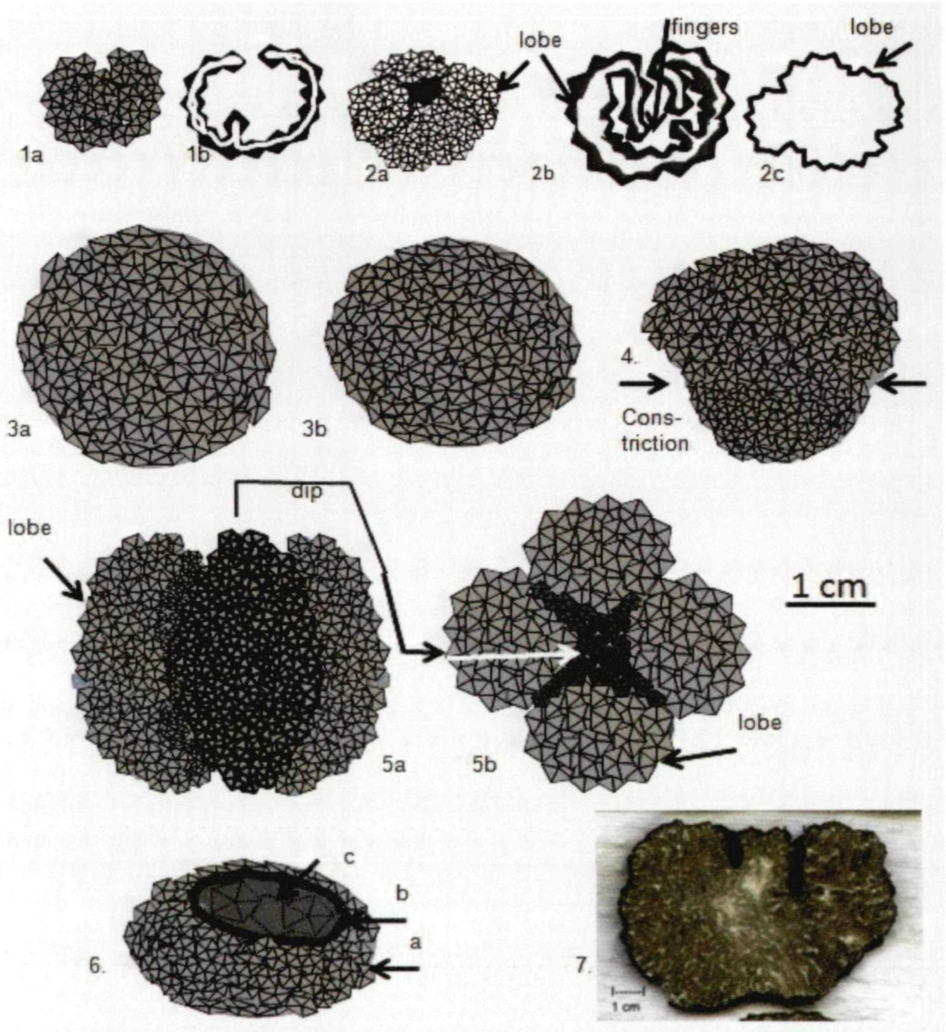
Lobed forms (Fig. 5). Irregularly formed ascocarps with many lobes are typically found in dry soil. The ascocarps appear shrunken. Very small warts can be seen where the lobes connect (Fig. 5 a). A top view of the truffle reveals a dip with smaller warts (Fig. 5 b), not a cavity, and within it sometimes a small or large dome showing a new spurt of growth. The opposite end is flatter, with lobes marked with just shallow dips converging. The slice of mature truffle shows the profile with a dip and dome. It was a fourlobed globose tuber (Fig. 7).

The herbarium specimens show that the number of lobes varies from 2-7. Possible remnants of these shapes can still be seen in large truffles when a later damp season allows continued growth, resulting in a more or less globose form. Specimens of this type were found in the Calestano truffle market in Italy (7. 11. 2010) where a very good harvest was praised after a damp autumn.

Kidney shape. The ascocarps are in the shape of a kidney with a shallow but oval crater oriented towards the surface (Fig. 6). This type was most often found at grass root level in parkland (site 1) in periods of drought when precipitation was limited to dew captured by grass strands.

A synthesis of observations on ascocarp development

The quest was to find out through which processes each series of ascocarp forms was attained. As a truffle, however lightly touched, stops growing, the samples represent a discontinuous time series. The numerous pictures helped to reconstruct continuous developmental series and to link morphological variation to weather patterns.



Figs 1-7. *Tuber uncinatum* ascocarps. – 1. With double peridium and cavity, *a* side view, *b* cross section. – 2. With double peridium and four lobes, *a* top view, *b* cross section, *c* top view contour. – 3. Globose without cavities, *a* round, *b* flattened. – 4. Globose with constriction. – 5. With four lobes and a dip, *a* side view, *b* top view. – 6. Kidney-shaped with hard ridge marking an oval crater with hard inflexible peridium lacking warts. – 7. Slice of mature ascocarp, globose with lobes and dome pushed out (site 2). – Bar: 1 cm.

Regeneration of the peridium. The formation of new peridium was observed. The quickly expanded truffle stated before, showed a band of gleba changing colour from tan to darker tan then black and recreated two rows of new warts (FRENCH 2008). In the experiment testing the healing process, the surface gleba in the cut started to change colour with red to tan from the 6^{th} day after the cut. The coloured surface increased from the centre of the cut. Hairline cracks then appeared in the shape of the base of a wart, marking out the forming of several warts. At the 12th day the whole cut area is of a much darker tan, and at the 14th day the colour was similar to mature peridium and the formation of warts could be seen. The regeneration of the peridium appeared to originate from the gleba surface and not from the neighbouring peridium.

Influence of humidity and drought. Truffles, like other fungi, absorb water from their environment and risk to dry out in periods of drought. The rapid absorption of temporally abundant water by a 3 cm unripe truffle was observed. The truffle gained an arc of exposed white gleba half its circumference a few days after a rainfall of 70 mm within 3 hours. It thus increased its visible volume by 10-15% within a week of the downpour. In the same period, one truffle, watered normally in the drought, partially uncovered every two weeks, increased its diameter by twice its normal fortnightly rate of 0.5 cm. The gleba did not appear on the exposed surface under the bag.

Truffles found amongst the humid grass roots in drought conditions had a different shape from the globose forms. The experiment of cutting 1/3 of ascocarps showed that the healed peridium does not have perfect warts, seems thicker and rigid in this case. As a result, the rest of the ascocarp grows over time leaving a ridge (Fig. 6 b) around a crater. The kidney shape of some truffles seems to be caused indirectly by scarification in response to superficial damage and is not a pattern of inherent, undisturbed truffle development.

Irregular shapes of ascocarps growing deeper in the soil seem to have been created by the alternate growth of the gleba and the peridium resulting from intermittent slight rain or dew in periods of drought. The most complex shapes were recorded under these conditions: short term access to limited water under drought conditions.

Ascocarps with mature double peridium entirely or in places were only found after excessive drought of 8-10 week period. If we dry an immature truffle, most of it collapses back into a cup. Whether this shrinking creates the double peridium truffle could not be tested under natural conditions. The question then arose as to whether the double peridium forms represent a normal developmental stage between the apothecioid stage and the mature globose forms, or rather a deformation due to unfavourable environmental conditions or ontogenetic defects. Observations on ascocarp development under more controlled conditions would be required to answer this question.

Conclusions

Lobed forms of ascocarps may be explained by exposure to drought. Double peridium specimens are characterised by a near complete reduction of gleba development, which was observed under conditions of extreme drought. Peridium development seems to be induced in dry weather by the increase of both the number and size of warts. Heavy rainfall has been seen to result in a sudden increase in volume of the ascocarp primarily affecting the gleba. The gleba has the potential to recreate peridium-like structures within 6-14 days.

The tendency to a globose form towards maturity is the default growth pattern in *Tuber uncinatum*. Deviations from the globose form can be interpreted as a result of interactions with the environment and mostly the weather pattern. The observations presented in this article aim to show the degree of plasticity of morphological features in response to extreme weather conditions and to improve the understanding of ascocarp development.

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References

CHEVALIER, G., FROCHOT, H., 1997: La truffe de Bourgogne, pp. 22-23 and fig. 5. – Levallois-Perret: Petrarque.

GREGORI, G., PONZIO, G., SBRISSA, F., VERLATO., G., GIOMARO, G., SISTI, D., 2008: Auxiometry of the *Tuber aestivum/uncinatum* carpophore. – In: Tuber 2008, 3° Congressio Internazionale di Spoleto Sul Tartufo, Abstracts, Spoleto.

FRENCH, M.-A., 2008: Sommaire d'observations personnelles sur les truffières, principalement sur pelouse en Grande Bretagne et leur système écologique (2003-2007). – In ROUSSET-ROUARD, Y., (Ed.) : L'avenir de la truffe face au réchauffement climatique, pp 117-124. – Paris : Albin Michel.

MALENÇON, M. G., 1938: Les truffes européennes. – Rev. Mycol. **3**, Mém. Hors série 1.

MET OFFICE, 2010 a: Regional climates. [http://www.metoffice.gov.uk/climate/uk/regional/]

— 2010 b: Historic station data [http://www.metoffice.gov.uk/climate/uk/stationdata/]

— 2010 c: Dry spell 2004/6. [http://www.metoffice.gov.uk/climate/uk/interesting/2004_2005dryspell.html]

MONTANT, C., KULIFAJ, M., 1990: L'ascocarpe du *Tuber melanosporum* VITT. Contrôle des facteurs externes agissant sur sa formation, sa croissance et sa maturation. – Compt. Rend. Acad. Sci. Paris **311**: 123-126.

PARGUEY-LEDUC, A., JANEX-FAVRE, M.-C., MONTANT, C., KULIFAJ, M., 1989: Ontogénie et structure de l'ascocarpe du *Tuber melanosporum* VITT. – Bull. Soc. Myc. France **105**: 227-246.

PEGLER, D. N., SPOONER, B. M., YOUNG, T. W. K., 1993: British Truffles. – Kew: Royal Botanic Gardens.

RIOUSSET, L. G, CHEVALIER, G., BARBET, M. C., 2001: Truffes d'Europe et de Chine. – Paris: INRA.

SPARKS, B. W., WILLIAMS, R. B. G., BELL, F. G., 1972: Presumed ground-ice depressions in East Anglia. – Proc. Roy. Soc. London, ser. A, **327**: 329-343.

WARMSLEY, A., 2008: The Norfolk 'Pingo' mapping project, 2007-2008. – Norwich: The Norfolk Wildlife Trust.

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