Five trillion basidiospores in a fruiting body of *Calvatia gigantea*

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De-Wei Li. 2011 – Five trillion basidiospores in a fruiting body of *Calvatia gigantea*. Mycosphere 2(4), 457–462.

Basidiospores in a fruiting body (38.5×37×22.5 cm in size) of *Calvatia gigantea* were quantified to be 5.1 trillion (5.1×10^{12}). The results suggested that Buller's study on a smaller fruiting body of 40×28×20 cm in size over-estimated the basidiospore number.

Key words – basidioma – puff ball – Lycoperdaceae – spores – sterigmal remnant

Article Information
Received 8 June 2011
Accepted 14 June 2011
Published online 21 September 2011
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Introduction

Calvatia Fr. is a cosmopolitan genus of medium- to large-sized puffballs with >35 species (Coetzee & van Wyk 2007). The genus is characterized by fruiting bodies that dehisce by irregular fragmentation of the peridium without a defined ostiolum (apical pore) (Gube 2007). *Calvatia gigantea* (Batsch) Lloyd, commonly called giant puffball, is widespread in the temperate zone (Kreisel 1994). It develops one of the world's largest basidiomata from a single primordium (Clémençon 1990). It is a saprobe on pastures, fertile meadows or in open woodland (Gube 2007). *C. gigantea* develops fruiting bodies singly, gregariously, or in fairy rings. The fairy rings were reported to associate with *Agrostis capillaris* L., *Festuca nigrescens* Lam. (*Festuca rubra* L. subsp. *commutata* Gaudin), or *Lolium perenne* L. on greens and fairways in golf courses (Arnold & Brien 1961) and also have been observed on pasture land and low maintenance turfgrasses. It is edible when its gleba is still white.

People are often fascinated by the unusually large size and edibility of its fruiting bodies. It is a trophy species to mushroom hunters. However, the number of basidiospores produced and released by the fruiting bodies of this giant puffball is the question people often ask. To hypersensitive individuals the spores of this species in the air may become a health concern, as *Calvatia* is one of the most prominent genera of the Basidiomycota responsible for the induction of fungal type I allergy (Simon-Nobbe et al 2008). *C. gigantea* does not have the bellows mechanism of basidiospore liberation (Ingold 1965), rather it is by irregular fragmentation of the peridium, exposing large spore masses to the air. Its basidiospores are freely blown away by wind (Ingold 1965) and, subsequently, this fungus releases a large quantity of basidiospores in to the atmosphere. Its basidiospores were frequently detected from air samples in the fall (personal observation).

A.H. Reginald Buller was the first person to quantify the number of basidiospores produced in a large fruiting body of *C. gigantea*. He determined the number to be seven trillion by counting the basidiospores from a single sample taken from the internal gleba (Buller 1909). In the past 100 years, Buller's study has not been repeated and his estimate remains to be verified due to the rarity of naturally mature and, more importantly, still intact fruiting bodies in the field. Usually fully ma-

ture fruiting bodies are either damaged by animals or have already dehisced by fragmentation.

A large fruiting body of *C. gigantea* developed on low maintenance turfgrass about 5 m from a mixed forest to the south and 3 m from a newly planted peach orchard to the north in a research farm of the Connecticut Agricultural Experiment Station, Valley Laboratory in Windsor, Connecticut, USA (Figs 1–2). When it was fully mature in the field and still unopened, the basidiospores in the fruiting body were quantified with a method to allow statistical analysis to be performed and to veryfy Buller's estimate.

Materials and Methods

The fruiting body had been observed daily in the field since it was spotted on 12 October 2011. The fruiting body was left in the field untouched to allow it to mature naturally so that the basidiospores produced in it would reach full potential. When the fruiting body was picked on 15 December 2010 it had become completely air-dried, dark brown in colour, but the peridium was still intact without any fragmentation or opening. Soil was gently brushed off from the base of the fruiting body. The fruiting body was measured as 38.5 × 37 × 22.5 cm in size, and 112 cm, 97 cm, and 90.5 cm in perimeter along X, Y, Z axes. The whole fruiting body weighed 298.4 g. The peridium was carefully peeled off from ¼ of the fruiting body and weighed 4.95 g. Thus, the whole peridium was 19.8 g. The gleba was olive in colour and weighed 278.6 g (fruiting body minus peridium = 298.4 g minus 19.8 g). The residual basidiospores were gently brushed off from the peridium pieces using a painting brush in a Hume hood prior to weighing the peridium pieces.

The fruiting body was cut in half. Nine samples were taken from the fruiting body, three from the outer area (0.069, 0.072, 0.074 g) (0–5 cm from peridium), three from an intermediate area (0.063, 0.1, 0.044 g), and three from the center (0.039, 0.055, 0.07 g) with a pair of tweezers. Each sample was soaked in 20 mL 70% isopropyl alcohol in a 150 mL flask. The flasks were sealed with a piece of 5×5 cm parafilm.

The spore suspension in a flask was vortexed five times, each lasted 10s, to suspend the spores into alcohol. A mini vortexer (Fisher Scientific) at a speed setting of 5 was used. One mL of the spore suspension was pipetted from the flask and was serially diluted 100× in two test tubes, each containing 9 mL distilled water. Prior to each pipetting, the spore suspension in a test tube was vortexed for 10s.

The spore suspension in the second test tube was pipetted onto an Improved Neubauer Hemacytometer (CA Hausser & Son, Philadelphia, USA). Basidiospores in one mm^2 area were counted. For each sample, spore counting was replicated eight times.

Formulas for calculating the total basidiospores in the fruiting body are listed as:

Spores/g = spore count $mm^{-2} \times 10^4 \times 20 \times 10^2$ (dilution factor)/sample weight = spore count $mm^{-2} \times 2 \times 10^7$/sample weight.

Total basidiospores = spore count $mm^{-2} \times 2 \times 10^7$/sample weight × 278.6g (gleba weight).

Data were analyzed using NCSS software (NCSS, 329 North 1000 East, Kaysville, Utah 84037) for ANOVA and Kukey-Kramer Multiple Comparison Test.

Results

Calvatia gigantea produced 5.1×10^{12} basidiospores in this fruiting body. Basidiospore number estimates from outer, intermediate, and center glebal areas were significantly different due to the differences in spore densities in the different areas of the fruiting body (Table 1). Basidiospore densities (spores/g) from outer, intermediate, and center glebal areas were also significantly different (Table 2). The basidiospore density in the intermediate area was significantly higher than in the outer and center areas. There were significant differences among samples collected from within a similar area also. The basidiospore density of the gleba in the fruiting body was not evenly distributed.

Discussion

Buller (1909) quantified the number of basidiospores produced in a large fruiting body

Figs 1–2 – *Calvatia gigantea*. **1** Immature fruiting body, **2** Mature fruiting body.

Table 1 Kukey-Kramer multiple comparison of basidiospore number estimates of *Calvatia gigantea* based on the samples collected from different glebal areas.

Gleba area	Basidiospore numbers ($\bar{x}$)	SD[1]
Outer	4.97×10^{12} a*	1.41×10^{12}
Intermediate	6.16×10^{12} b	1.59×10^{12}
Center	4.16×10^{12} a	1.82×10^{12}

*Treatments with different letters mean statistically significant difference. [1]SD: standard deviation.

Table 2 Kukey-Kramer multiple comparison of *Calvatia gigantea* spore density in different glebal areas.

Gleba area	Basidiospore numbers ($\bar{x}$)basidiospores/g	SD[1]
Outer	1.78×10^{10} a*	5.1×10^{9}
Intermediate	2.21×10^{10} b	5.7×10^{9}
Center	1.49×10^{10} a	6.6×10^{9}

*Treatments with different letters mean statistically significant difference. [1]SD: standard deviation.

of *C. gigantea* when he was teaching at the University of Birmingham, UK. He estimated that the fruit body of *C. gigantea* (40 × 28 × 20 cm, weighed 232 g) contained over seven trillion basidiospores by taking a single 0.1 g sample from the internal gleba and suspending it in 250 mL methylated spirit. Buller's fruiting body was smaller by 30% in size and lighter by 22% in weight than the specimen studied by the author. Buller's estimate of 7×10^{12} basidiospores (3×10^{10}/g) is higher than the 5.1×10^{12} basidiospores (1.7×10^{10}/g) calculated from the present study. It seems that Buller (1909) over-estimated the number within the fruiting body. Considering the time at which Buller's study was conducted, it is understandable that limitations existed in his study and this led to an over-estimation. Nobody should or can deny that Buller's study is instrumental. Several reasons might be responsible for the difference of quantifications of the basidiospores between the two fruiting bodies. The sample in Buller's study might be on the higher side of analytical variation or his sample came from a high spore density area, since basidiospore density in a gleba is not constant. The variation among the nine samples in the present study ranged from 2.98–7.66×10^{12} basidiospores. The two fruiting bodies may have grown on different substrates, which led to different basidiospore productivities and densities in the fruiting bodies. In Buller's study, the peridium was not weighed and excluded from the fruiting weight. When taking the weight of the peridium into consideration, the size of the fruiting body, and with a statistical analysis of multiple samples, Buller's estimate would be much lower. No doubt, *C. gigantea* is the most prolific fungal species for spore production in each fruiting body according to the reports available to date.

The exoperidium of *Calvatia* species is only one layer and it does not have the pseudoparenchymatous endostratum typical of all other Lycoperdaceae (Gube 2007). This is the reason that peridia of *C. gigantea* are thin and light in weight, only taking less than 10% of the whole fruiting body weight. Also, it does not have a sterile base. These factors make quantification of basidiospores in fruiting bodies relatively easy and Buller's study possible.

It is intriguing to find out that spore density was not evenly distributed in the fruiting body. When the gleba was cut open, it looked uniform in texture and colour. The unevenness of spore density is a crucial factor which can interfere with accuracy for the estimation of total spores, if sampling was not properly conducted. The unevenness might be due to several possible factors: uneven dehydration of the fruiting body, unstable spore development rate, irregular cellular/tissue structure etc. Gube (2007) studied the gleba development of several species of Lycoperdaceae and found that the gleba of *Calvatia gigantea* develops in a unique and previously unknown manner. The hymenium-forming palisade structures borne on fan-like branches of hyphae are different from those of species of Lycoperdaceae including other species of *Calvatia*. It is not clear whether the glebal development is playing any role in the unevenness of spore densities. What is the biological advantage of the unevenness to the species? Further study is necessary to determine the true reason for the unevenness.

Buller's fruiting body and the present one of *C. gigantea* are far from the biggest fruiting body ever observed. The largest one that the author observed in 1990's was the size of a small sheep. Apparently this size is not the record one. Bessey (1884) reported a specimen as 1.63 × 1.37 × 0.24 m, which was observed, photographed, and measured by Professor RE Call in New York State in 1877. This specimen was too big to collect and preserve in the 19th century. Bessey (1950) extrapolated that this fruiting body would produce approximately 160 trillion basidiospores. Bessey (1950) did not indicate how he did his calculation. It was quite possible that his extrapolation was based on Buller's study, since it was the only quantitative study of *C. gigantea* basidiospores. Bessey's extrapolation is too high according to the result of the present study. Murat et al (2008) stated that a Guinness World Record as the largest edible fungus is *Calvatia gigantea* and provided a web link (http://www.guinnessworldrecords.com/content_pages/record.asp?recordid=47431). However, the record of this fungus cannot be found by following this link. Searching the webpage of the Guinness World Records using different key words yielded nothing. In responding to my inquiry about the record to website_inquiry@guinnessworldrecords.com, the Guinness World Records confirmed their record "The world's largest edible fungi (us) was a giant puffball (*Calvatia gigantea*) measuring 2.64 m (8 ft 8 in) in circumference and weighing 22 kg (48 lb 8 oz). It was found by Jean-Guy Richard of Montreal, Quebec, Canada in 1987" (personal communication 2011). According to aforementioned information, this fruiting body is not the record holder. Carlyon (2011) cited a fruiting body of *C. gigantea* >150 cm in diameter, 45 cm in height and weighing more than 100 kg when fresh in her webpage of Giant Puffball. However, Carlyon's report cannot be verified by photo, specimens or collection information. If verifiable, this fruiting body could be the record. World Records Academy (2010) claimed that the world record of the largest puffball is the one found by Finley O'Neill in Slaithwaite, West Yorkshire, UK in October 2010 measuring 169 cm. However, according to the photo posted on the webpage, the measurement appears to be the circumference, rather than the diameter. Clearly this fruiting body is unusually large, but far from the world record of giant puffball. Thus, the verifiable record of this species is still the one found by Professor RE Call in New York State in 1877.

Airborne basidiospores of *Calvatia* are difficult to identify based on morphological characters under optical microscopy. Levetin et al (1992) conducted a morphological study on the basidiospores of *Calvatia craniiformis* (Schwein.) Fr., *C. cyathiformis* (Bosc) Morgan, *C. rubroflava* (Cragin) Lloyd, and *C. gigantea*. They found that each species showed unique ornamentation on spore surface under scanning electron microscopy (SEM), but only *C. cyathiformis* basidiospores were identifiable with sufficiently distinctive characters on spore trap slides under compound microscopy. Baseia

(2003) studied the ornamentations on basidiospores of four species of *Calvatia* in Brazil. Bates et al (2009) conducted an ultrastructural study on 28 species in *Lycoperdaceae* including eight species of *Calvatia* using SEM. These ultrastructural studies on basidiospores of a total of 13 species of *Calvatia* showed that ornamentation characters of basidiospores are taxonomically significant. Basidiospore pedicels and sterigmal remnants are taxonomically important in distinguishing certain *Lycoperdon* species (Bates 2004). However, the significance of these characters for differentiating *Calvatia* species is not fully studied. It is the author's opinion that sterigmal remnant characters, such as length, width, and detaching patterns are very useful for identification of airborne spores of *Calvatia* in combination with the characters of spore ornamentation, size, and colour.

Quantitative real time polymerase chain reaction might be an alternative method for detecting and quantifying *Calvatia* spp. However, up to date, primers and probes for quantitatively detecting *Calvatia* species have not been developed. Also, due to lack of study on the allergenic properties of *C. gigantea*, the health effects of its airborne spores are not fully understood. In the study by Levetin et al. (1992), allergen could not be extracted from basidiospores of *C. gigantea* in several attempts.

Calvatia is currently conserved against the earlier synonyms *Omalycus*, *Langermannia* and *Hippoperdon* (McNeill et al. 2006). However, it is not protected against *Lanopila*, another older synonym. Coetzee & van Wyk (2007) proposed to conserve *Calvatia* against *Lanopila*. Their proposal has been passed by the Nomenclature Committee for Fungi (Norvell 2010) and will obtain the final approval of XVIII International Botanical Congress in July, 2011 in Melbourne, Australia. Therefore, the valid scientific name for giant puffball should remain as *Calvatia gigantea* (Batsch) Lloyd, which has 12 synonyms.

Acknowledgements

I am very grateful to Dr James LaMondia and Mr James Preste for bringing my attention to the fruiting body.

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