Fruitbody Worlds, *Plastination of Mushrooms*

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Abstract: Plastination, a new method for the preservation of fungal fruitbodies, was investigated as a potential source for materials to aid in fungal biology education and mycological training. Cold-temperature and room-temperature protocols were followed to compare quality of preservation using different techniques. Preserved specimens from both protocols exhibited some slight changes in shape and darkening of colors, but many important morphological features of the fruitbodies were preserved. We conclude that plastination is a viable and useful method for the preservation of fungi.

Keywords: **fungal biology, K-12 education, mycology, preservation, undergraduate education**

An interesting, yet frustrating aspect of studying fungi is their apparent ephemeral nature. In summer, *Conocybe apala* dot a grassy landscape, but under the noonday sun they all seem to disappear as if by magic. It is known that one or more "individual" organisms producing these spore-bearing fruitbodies subsist in the soil, evidenced by the next day when more mushrooms appear as suddenly as the previous day's had disappeared.

On the far extreme of this is the woody polypore *Fomes fomentarius* where one can count the annual growth rings of a single fruitbody. They can grow for years or even decades and can persist long after the fungus is dead. In fact, a fruitbody of this fungus was found on the almost 5,000 year-old preserved remains of Ötzi, the Ice Man, and is thought to have been used as tinder in his time. Polypores like this, with their skeletal and binding hyphae, have been used in Biology classrooms to teach students about the sporocarp-producing members of Kingdom Fungi for generations, but how can students and interested parties see, touch, and learn about the common mushrooms, like *Conocybe apala*? One answer could be through the science and art of plastination.

In science, little is known about how long a fungus can live underground, and due to the difficulty of studying soil populations, the history of fungal taxonomy has mostly been predicated on the examination of the morphology of reproductive structures. Luckily, from an evolutionary point of view, classification based on morphology has been largely recapitulated by genetic analysis, meaning that we are able to accurately distinguish species for the most part based solely on the features of a mushroom or other kinds of reproductive structures. Being able to observe and characterize these fruiting bodies is therefore an essential skill for many kinds of mycologists. While pictures and diagrams are useful

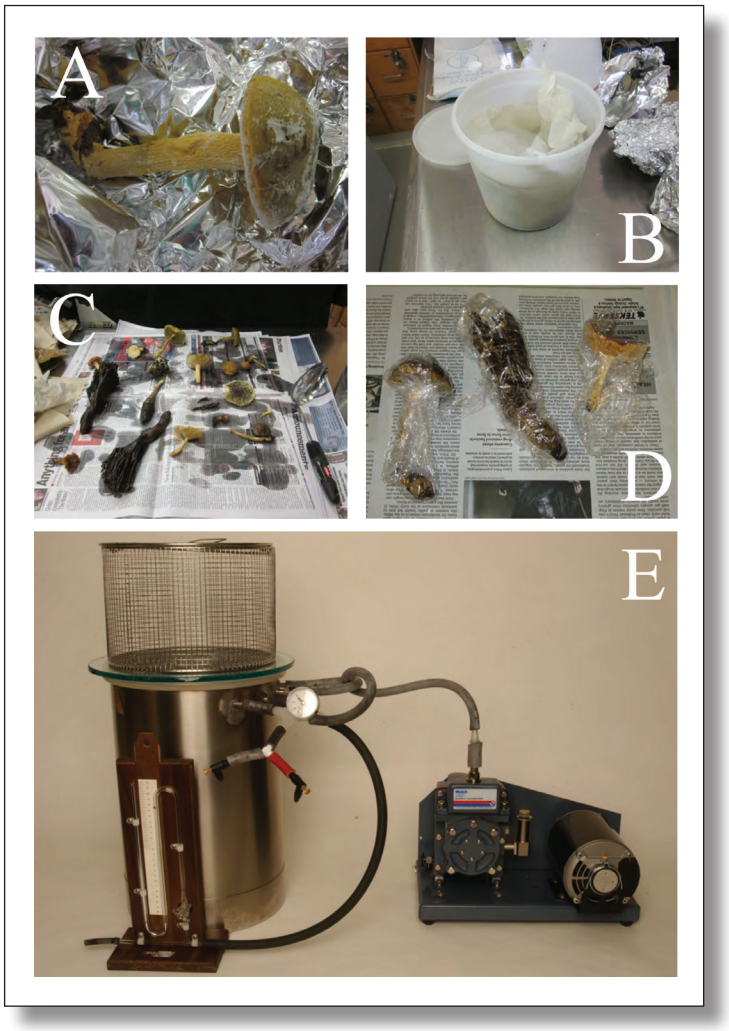

Figure 1. Equipment and stages of the plastination process. A) Frozen specimen ready for acetone treatment. B) Cold acetone bath. C) Specimens after removal from silicone bath. D) Specimens being cured with catalyst NC-S3 in plastic wrap. E) Impregnation setup: vacuum chamber, vacuum pump, basket for specimens, and Bennert manometer.

for developing a concept for these structures, there is no substitute for hands-on experience. These features can be preserved and observed through the process of plastination.

So what is plastination? Plastination is a method developed by Gunther von Hagens whereby cells of once living tissue are emptied of their contents and replaced with a curable polymer, like silicone, to preserve the tissue at a cellular level. The method was originally developed to produce anatomical teaching tools for medical students, and it is now an essential teaching tool for veterinary medicine and pathology as well. This technique has not transferred to the botanical world and only limited experimentation of plastinating plant and fungal tissue is reported. One notable exception is a presentation that was given by Diz and co-workers (2004) on the use of plastinated fungi in botanic classification in Spain. They report some problems with changes in shape and color during their process, but maintain that plastination is an effective tool for preserving fungi and can produce useful teaching materials. In 2011 a fungi module was being produced for a K-12 educational initiative at the University of Tennessee called Biology in a Box, and plastination was investigated as a method for producing teaching materials.

Figure 2. Plastinated specimens and representative photos of the species in living condition. A) *Ramaria grandis* (plastinated). B) *Ramaria grandis.* C) *Galiella rufa* (plastinated). D) *Galiella rufa* (photo by Dan Molter). E) *Pleurotus levis* (plastinated). F) *Pleurotus levis* (plastinated). G) *Pleurotus levis* (photo by Sylvia Hosler). H) *Lactarius volemus* (plastinated). I) *Lactarius volemus* (photo by Richard Kneal). J) *Amanita polypyramis* (plastinated). K) *Amanita polypyramis* gills with veil (photo by Patrick Harvey). L) *Amanita polypyramis* (photo by Patrick Harvey). M) *Russula sp.* (plastinated). N) *Russula sp.* O) *Scleroderma citrinum* (plastinated). P) *Scleroderma citrinum* (photo by Eric Smith).

Table 1. Plastinated species list.	
Calostoma cinnabarinum	*Amanita* sect. *Lepidella*
Amanita onusta	*Calvatia cyathiformis*
Galliela rufa	*Aureoboletus flaviporus*
Russula sp.	*Geastrum saccatum*
Austroboletus betula	*Hydnellum* sp.
Ramaria grandis	*Amanita polypyramis*
Infundibulicybe gibba	*Tylopilus plumbeoviolaceus*
Pleurotus levis	*Pleurotus ostreatus*
Lactarius volemus	*Laccaria* sp.
Scleroderma citrinum	*Retiboletus griseus*

Methods

The classic cold-temperature silicone protocol following DeJong and Henry (2007) and the newer room-temperature protocol following Henry (2007) were used to compare efficiency and quality following the four standard steps of plastination: 1) Specimen preparation; 2) Dehydration; 3) Impregnation; 4) Curing.

1) Specimen preparation: Both techniques followed the same protocol for this initial step as well as the Dehydration step. Fresh sporocarps of twenty species of Basidiomycota and Ascomycota were collected from the Great Smoky Mountains National Park and frozen in a –20° C freezer to preserve their form (Table 1) (Figure 1). Special care was taken to prop delicate specimens with aluminum foil around the base to prevent pileus and stipe deformation (Figure 1).
2) Dehydration: Frozen samples were immersed in cold (-20° C) 100% acetone and placed in a –20° C freezer. Acetone purity was measured with an acetonometer after 3 days and if acetone purity was ≤ 98%, the acetone was replaced with new cold acetone and the process continued until acetone purity was > 98% after 3 days time. This process took a week with two exchanges of acetone. Excess acetone was allowed to drain briefly before immersion into the new cold acetone bath.
3) Impregnation: Excess acetone was allowed to drain briefly from the dehydrated specimens, which were enclosed in a stainless steel mesh basket, before submersion in the silicone impregnation-mix.

4) **a.** For cold-temperature impregnation, specimens were submerged in a thoroughly-mixed 100:1 solution of cold (-15°C) NC-S10 silicone polymer and NC-S3 catalyst in a vacuum chamber. Specimens were allowed to equilibrate in the impregnation-mix overnight. The next day a vacuum pump was connected and pressure was decreased ~2/3 of an atmosphere (20 cm). Pressure/vacuum was monitored via gauge and Benneret manometer. After one day of equilibration at 20 cm Hg, pressure was slowly decreased to 0.3 cm Hg (~1 atm) over twenty days. Pressure was decreased incrementally by 1/2 to 1 cm every 4-5 hours. Impregnation rate was monitored by bubble formation. As pressure decreased, the acetone vaporized and bubbled out of the specimen through the polymer to be pumped off through the pump exhaust. When bubble formation decreased significantly or ceased, pressure was incrementally lowered 1/2 to 1 cm (as the acetone leaves the specimen cells, a void is created and the polymer-mix is drawn into the cells). This incremental process continued until bubbles in the silicone became infrequent ~ 2-3 weeks, then vacuum was released until pressure returned to atmosphere. Specimens were allowed to remain in the polymer-mix overnight and then were drained of excess silicone-mix for three to four days on paper towels with occasional wiping of excess silicone from specimens (Figure 1).

b. Room-temperature treatments followed a similar protocol to Cold-temperature impregnation. However, specimens were submerged in a thoroughly-mixed 10:1 solution of room-temperature NC-S10 silicone polymer and NC-S6 cross-linker in a vacuum chamber. Specimens were allowed to equilibrate in the impregnation-mix overnight. The next day a vacuum pump was connected and pressure was decreased ~1/2 of an atmosphere (35 cm). Pressure/vacuum was monitored via gauge and finally with a Benneret manometer. After two hours of equilibration at 35 cm Hg, pressure was slowly decreased to 1cm Hg over four days. Pressure was then decreased incrementally by 1/2 to 1 cm every 4-5 hours. Impregnation rate was monitored by bubble formation. As pressure decreased, the acetone vaporized and was pumped off. When bubble formation decreased significantly or ceased, pressure was incrementally lowered 1/2 to 1 cm. This incremental process continued until bubbles in the silicone became infrequent ~5-6 days. Vacuum was then released until pressure returned to atmosphere. Specimens were allowed to remain in the polymer-mix overnight and then were drained of excess silicone-mix for three to four days on paper towels with occasional wiping of excess silicone from specimens (Figure 1). The primary differences were the impregnation-mixture, shorter impregnation period (~8 days versus 2-3 weeks), and the process was carried out at room temperature. Also, pressure can be lowered faster in the room-temperature technique due to a lower viscosity of the impregnation-mix.

5) Curing:

a. For the cold-temperature process semi-dry specimens were cured using a gas curing procedure with NC-S6 cross-linker formula. Specimens were gently placed in a

Figure 3. Plastinated specimens exhibiting morphological features. A) *Aureoboletus flaviporus* exhibiting the preservation of a yellow, poroid hymenium. B) *Geastrum saccatum* exhibiting a peridium with an apical pore. C) *Calostoma cinnabarinum* exhibiting a reticulate, hygroscopic stalk. D) *Ramaria grandis* exhibiting coralloid branching. E) *Amanita* sect. *Lepidella* exhibiting skirt-like annulus and ornamented volva. F) *Hydnellum* sp. exhibiting a tooth-like hymenium.

closed container on paper towels (to absorb oozing polymer-mix). The specimens were manicured daily and one milliliter of NC-S6 was added daily to the container and the lid sealed. The NC-S6 cross-linker allows the silicone molecules to link in a 3-D meshwork, to maintain the structure of the tissue. After one week of curing, the specimens are ready to use and display.

b. For Room-temperature semi-dry specimens, the catalyst NC-S3 was used for curing. Specimens were lightly misted with NC-S3 using a spray bottle, making sure all surfaces were covered. Specimens were then wrapped in plastic wrap to seal the NC-S3 against the specimen. This process was repeated two or three times over one week. Between misting, the specimens were checked for excess polymer accumulation on the surface, which was removed using absorbent toweling. After specimens are cured, they are ready for use and display.

The resulting products of these two techniques are very similar in quality of structure, color, and appearance (Figure 2). The only major observed difference is that room-temperature specimens tend to exhibit a glossy lacquer over areas where too much curative agent was applied. This can be avoided by more frequent manicuring of the specimens, using less NC-S3, and more changes of the plastic wrap, but this also may present an opportunity for a different aesthetic with a glossy finish. Overall our results concur with the findings of Diz and others (2004). We notice a slight change in shape, with some shrinkage, and a significant change, usually darkening, of color. Additionally, some large specimens such as *Amanita polypyramis* and *Tylopilus plumbeoviolaceus* retained large amounts of silicone after Impregnation and became greatly deformed during curing. Otherwise, important features such as an annulus, volva, pileus ornamentation, and bruising all have been preserved in the process (Figure 3). Even the fishy odor of *Lactarius volemus* persists.

There are many potential applications for plastinated fungi in K-12 education, university biology labs, natural history museums, and amateur mycology clubs for display and hands-on education. Having authentic representations of rare or strange fungi that can usually only be seen in pristine wilderness or under specific environmental conditions is something students have not had access to. This is especially true for inner-city schools or places where fungal diversity is low or highly seasonal. Natural history museums might take advantage of plastinated mushrooms as a method for displaying local mycoflora, much the same way you see taxidermy animals and forest

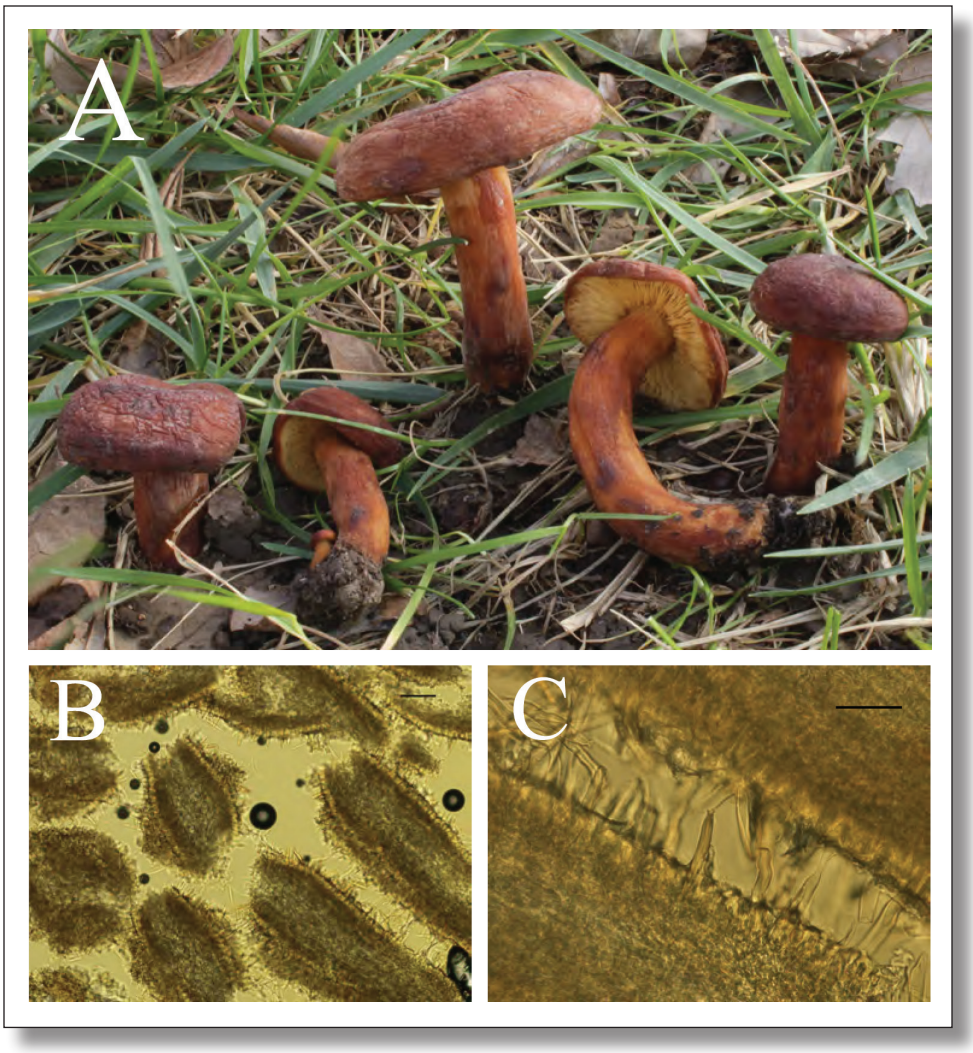

Figure 4. Micrographs and in situ style photograph of plastinated *Lactarius volemus*. A) "Field" photograph. B) Cross-section of gill at 40x magnification. C) Thick-walled cystidia at 1000x magnification. Scale bars are 100 µm.

dioramas being used. Plastination can allow students and the public to begin to explore the immense diversity of forms and functions of the kingdom Fungi through something much more interactive than a picture.

While there is no replacement for vouchered specimens in herbaria for scientific study, plastinated mushrooms might be useful to the scientific community as supplementary material for species description. Plastination can preserve general growth form and habit and be used to document variable growth forms. Additionally, because plastination acts at a cellular level, plastinated specimens can be sectioned and mounted for microscopy (Figure 4). Microfeatures are generally preserved through the plastination process, though much like macrofeatures, shape and color are affected. For example, basidia will lose their sterigmata. Also during the process spores are detached and removed from the specimen. Plastinated mushrooms provide another valuable form of documentation for species descriptions to supplement photos, drawings, genetic sequence, field notes, spore prints, and cultures.

The question has been raised whether a plastinated specimen from a holotype collection should be considered an epitype or holotype material itself. While the cell walls and structure are generally made from the original cellular material and indeed some cells seem to retain their cellular makeup, the contents of most cells have been emptied to be replaced with silicone. Can it still then be considered the original specimen? If not, one might view the plastic specimen as a very realistic sculpture, which would be good epitypic or supplementary material for species documentation. If we consider it to retain authenticity, a plastinated specimen meets the requirements of a holotype given by Article 8.4 of the ICN by being preserved permanently, arguably even better than a dried collection subject to fragility and insect infestation. This does not mean a collection of plastinated mushrooms should be chosen as a holotype collection, but wouldn't it be cool to have a plastinated fruitbody of the holotype of a newly described species?

If plastination is something that you may be interested in trying yourself, there is ample opportunity for experimentation and improvement of methodology. Given that we find little difference in quality between Cold-temperature and Room-temperature protocols, a room-temperature protocol might be ideal for ease and simplicity. If you choose to try these techniques, make proper arrangements to handle acetone safely. Equipment and material costs can be kept low depending on the size and amount of specimens you plan to plastinate in a given batch. Five-gallon vacuum chambers can be purchased for around $150 on online auction sites. Small quantities of acetone can be obtained from local hardware or farm supply stores or many chemical suppliers. Silicone and curative reagents can be purchased from Biodur in Germany or Silicones, Inc. (Dist. by Robert Henry) in North Carolina, with Silicones, Inc. being the least expensive buy. May the plastic mushrooms be with you!

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