Disinfection Treatment of Fungi Damaged Silver Gelatin Prints with Ethanol Vapours

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ABSTRACT

This study deals with fungi damaged photographic gelatin and disinfection methods. It is based on the conservation treatment of seven silver gelatin developing-out prints on baryta paper, belonging to the Historical Library of Paris (BHVP). These prints were water damaged. The emulsion has been weakened by flaking and hydrolysis of the constitutive gelatin. These damages are a consequence of fungal development on the emulsion.

When external conditions are not adapted to their growth, fungi will stay in dormancy waiting for more suitable external conditions to develop. Thus we considered the available disinfection treatment for photographs and decided to undertake further research and testing on the use of ethanol as an antifungal treatment before using it on the BHVP prints. This study was conducted at the Laboratoire Universitaire de Biologie et d'Ecologie Microbienne. The goal of the study is to determine a suitable method to apply a solution of deionised water and absolute ethanol (30:70 v/v) mixture in order to obtain a biocide effect on four fungal strains: *Alternaria alternata*, *Aspergillus niger*, *Chaetomium globosum*, and *Penicillium brevicompactum*. Three implementations were tested: solvent chamber, direct contact and direct contact followed by a mechanical removal. Different treatment times were tested for each method.

The deionised water and absolute ethanol mixture, applied for two hours in a solvent chamber, succeeded in inactivating the four tested fungal strains.

1. INTRODUCTION

This paper was based on the study and treatment of seven silver gelatin prints from the Bibliothèque Historique de la Ville de Paris (BHVP). The photographs were taken between 1930 and 1950 and printed in the early 1990s on a double weight baryta paper. They were then acquired by the BHVP in order to be exhibited in 1992-1993 for "Humanist photography 1930-1960 History of a movement in France". Following the exhibition, the prints were stored together in the "old kitchen" room in the BHVP's basement, in which they were successively water damaged in 1996 and 2001. As a result of the high humidity, the prints have been used as a substrate for fungal development. Thus we can now see filamentous fungi on the surface as well as characteristic damages resulting from their growth, hydrolysis of the gelatin and paper, resulting in brittleness and flaking of the materials.

For photographic collections, fungal development is a recurring problem. Most photographs are easily used as a substrate for fungal growth because they are made of proteinaceous materials such as gelatin or albumen, and polysaccharides such as cellulose; both material groups also being hygroscopic materials. Fungal development can result in the loss of parts or the totality of

the photographic image by hydrolysis of the substrate (paper and colloid). The prevention of such damages can be done with a strict control of the storage climate to avoid the fungal growth; however what are the options once the fungi have developed?

In this paper, we will present an overview of the available conservation treatments. Mechanical removal of the fungi is crucial but, as photographs are made of porous materials, disinfection treatments are equally as important to treat the fungi embedded in the materials. Ethylene oxide and gamma rays' effectiveness and long-term effects on the photographs have been researched fully and these methods are used in France for the treatment of large collections; however, they are not used for the treatment of smaller collections for budgetary reasons. For the treatment of smaller collections conservators use ethanol as an antifungal treatment event though its effectiveness has not yet been studied. Thus, we decided to study more in depth the use of ethanol as an antifungal treatment on silver gelatin photographs. Based on previous studies in the medical and food industries, this study focussed on an absolute ethanol-deionised water mix (70:30 v/v) where different implementation methods were tested in order to determine which mix was the most efficient.

2. ANTIFUNGAL TREATMENTS

Mechanical removal of fungi on a photograph is only a partial solution as the hyphaes, and sometimes spores, are embedded in the photograph's porous materials. Thus, the mechanical removal will not remove everything but it will reduce the amount of fungi on the object (Prevet 2016).

What do we do with the embedded fungi? When external conditions are unfavourable to fungi they go into dormancy, meaning their metabolic activity is at its lowest in order to avoid germinating in an unfavourable environment. The time fungi can stay in dormancy varies with each species and can last as long as 20 years (Florian 2002). Hence in a standard storage condition (18°C and 50% RH) the fungi are in dormancy. The embedded fungi are still viable and can activate, germinate and develop once the external conditions are more favourable. Consequently, it is important to disinfect the object to inactivate the fungi and avoid any development.

In France several antifungal treatments are used. For example, treatment using ethylene oxide or gamma ray have been the subject of various research projects and are used to treat fungi in archival collections.

Ethylene oxide fumigation is very efficient in killing fungi, insects, and bacteria (Flieder and Capderou 1999, 144–151); however it can cause damage to photographic prints. The gelatin peptic chains are broken, thus resulting in a high viscosity loss (Tomsŏvá, Ďurovič, Drábková 2016). As well, cellulosic materials are more hydrophilic after treatment, resulting in a higher sensitivity to further fungal attacks (Jacek 2004; Valentin 1986; Nittérus 2000a, 22–40). Ethylene oxide is also a very toxic compound: mutagen and carcinogenic for humans, that is forbidden to use in North America and several European countries (Trehorel 1988).
The dose of gamma rays needed to inactivate fungi is not consensual, and ranges from 4.5 kGy to 18 kGy (Flieder and Capderou 1999; Pavon Flores 1975). Gamma rays also cause damage to

photographic gelatin, such as viscosity loss after an exposure of 2.5 kGy of radiation (Tomsŏvá, Ďurovič, Drábková 2016) and increase of the print's density after an exposure of 90 kGy of radiation (Adamo et al. 2012). Even if this dose is much higher than the one needed to kill the fungi, it is important to keep in mind that exposure to gamma rays is cumulative. Thus if a photograph is treated several times in its life, the 90 kGy dose will be attained at some point. The radiation also degrades the photographic paper support which presents damage similar to the one caused by ageing (weakening and yellowing), resulting from cellulose depolymerisation and oxidization (Butterfield 1987; Nittérus 2000a, 25–40).

These two treatments are costly and more adapted to large collections. So, what are some available options for treating single items or a small collection of photographs?

Today, photograph conservators use pure ethanol or a deionised water – pure ethanol mixture of different ratios applied on the surface of the prints with a cotton swab. This method, while widely used, has not been tested and its fungicidal effects have not been studied. There is some research on the use of alcohols as a fungicide for paper objects. The use of a water-ethanol mixture with a 30:70 (v/v) ratio is the most recommended (Nittérus 2000, 101-105; Jacek 2004; Meier 2006; Sequeira et al. 2016), however authors disagree on its most effective implementation method between spraying, bathing, and vapour fumigating (Nittérus 2000b, 101–105; Bacílková 2006; Meier 2006).

3. ETHANOL AS AN ANTIFUNGAL TREATMENT: IMPLEMENTATION METHODS EVALUATION

In this study, we intended to clarify which implementation method of water-ethanol (30:70 v/v) mixture inactivates fungal growth on silver gelatin prints. Three implementation methods of this mixture were tested. In order to check if the cotton-swab application, corresponding to a short contact time with mechanical action, was efficient or not, we chose to test a short direct contact between the mixture and the photograph, with and without mechanical action, to determine the influence of this factor. The use of the mixture as vapour, previously tested, was chosen as our third implementation method because of the treatment possibilities it opens, such as treating photographs whose surfaces are too damaged for contact, and treating several photographs at the same time. Those three methods are not representative of today's practice in conservation labs but correspond to three levels of intervention on the prints: without contact, with contact, and with contact and mechanical action.

3.1. MATERIALS AND METHOD

3.1.1. Silver Gelatin Prints

Developing-out silver gelatin prints were selected as they are the most widely used black and white photographic printing technique throughout the 20th century. It was used by amateurs and professional photographers, thus it can be found in private and public, archival and museum collections. Glossy Ilford® Multigrad Classic baryta paper was selected for the tests. This paper is constituted of three layers: paper, baryta (barium sulphate in gelatin), and gelatin emulsion containing light sensitive silver halides. We decided to print a neutral light grey colour in order to work on a binder including metallic silver without impeding the observation of the fungal

development on the emulsion. The paper was exposed with an enlarger Omega® Super Chromega D Dichroic II for six seconds at f/32 without a contrast filter. It was developed with Kodak® Dektol (1:2 v/v) for two minutes, then plunged into a Tetenal® Indicet stop bath (1:19 v/v) for 30 seconds before fixing it for four minutes with Ilford® Rapid Fix (1:4 v/v). The print was then washed with cold flowing water for one hour and air dried overnight. The print was cut in square samples of 2.5 cm wide lengths. The samples were not sterilized because the autoclave temperature would have damaged the constituents.

3.1.2. Fungal Strains

Various fungi have been identified on silver gelatin prints; however, as the sampling is done with swabs, the identified species may not be the ones responsible for the prints′ deterioration (Schlocchi et al. 2013). For this study, four fungal species were selected within the most common encountered species on photographs (Lucas 2016, 289–294): *Alternaria alternata* FD412, *Aspergillus niger* FD255, *Chaetomium globosum* FD477, and *Penicillium brevicompactum* FD487, from the mycological collection of the LUBEM (Plouzané, France). These strains were identified on the basis of sequence analysis. Ribosomal DNA internal transcribed spacer (rDNA ITS) was used for identifying FD412 (accession number KY977416) and FD477 (accession number KY977415) whereas a portion of the beta-tubulin gene sequence was used for FD487 (accession number KY985235) and FD255 (accession number KY886458)

3.1.3. Inoculum Preparation

The cryopreserved strains were thawed, then plated on M2Lev medium (for 1 L of water: 20 g malt, 3 g yeast extract Biomerieux®, 15 g agar Biomerieux®) and incubated at 25°C for seven to 10 days. Spores were then harvested with a sterile sampling loop and suspended in 20% glycerol water, with two drops of Tween® 80 (Sigma-Algdrich®). The concentration of 5 x 107 spores/mL was determined with a haemocytometer. The suspension was diluted with sterile water in order to obtain a 5 x 105 spores/mL inoculum.

3.1.4. Sample Contamination

Each sample was inoculated with 10 µL of the inoculum. The samples were placed in Petri dishes, three samples per dish, with the gelatin emulsion facing up. The Petri dishes were placed over 300 mL of sterilized water in a closed Ikea® 365 + Food container (polypropylene and synthetic rubber) and incubated at 25°C for seven days. The Petri dishes were then removed from the box and incubated one more day to allow the gelatin emulsion to dry.

3.1.5. Ethanol Implementation Method

A mixture of sterilized water-absolute ethanol Carlo Reba Reagents® (30:70 v/v) was used as the treatment solution for the tests. The tests were conducted at a controlled temperature, between 15°C and 18°C.

3.1.5.1. Control samples

For each strain, three samples were contaminated but not treated with the treatment solution to serve as control samples.

3.1.5.2. Ethanol vapours

The Petri dishes containing the samples (three each) were placed open over 350 mL of the treatment solution in a closed Ikea® 365 + Food container. The container dimensions were 34 x 25 x 12 cm. Several exposure times were tested: 0.5, 1, 2, 4, 8, 16, and 24 hours. Those times were chosen based on previous research (Bacílková 2006; Dao et al. 2010).

3.1.5.3. Direct contact

The sample was transferred in a clean Petri dish with emulsion facing up, a 2.5 cm long square of sterilized Atlantis® microfiber cloth was placed on top. 300 mL of the treatment solution was added to the cloth with a pipette (the quantity has been determined empirically as the one required to soak the cloth). The Petri dish was closed to limit evaporation. Several contact times were tested: 15 seconds, 30 seconds, 1, 2, 4 and 8 minutes. These times are shorter than the vapour treatment times in order to be comparable to the conservation lab practice with cotton swabs. After the contact time, the cloth was removed by pulling it off gently.

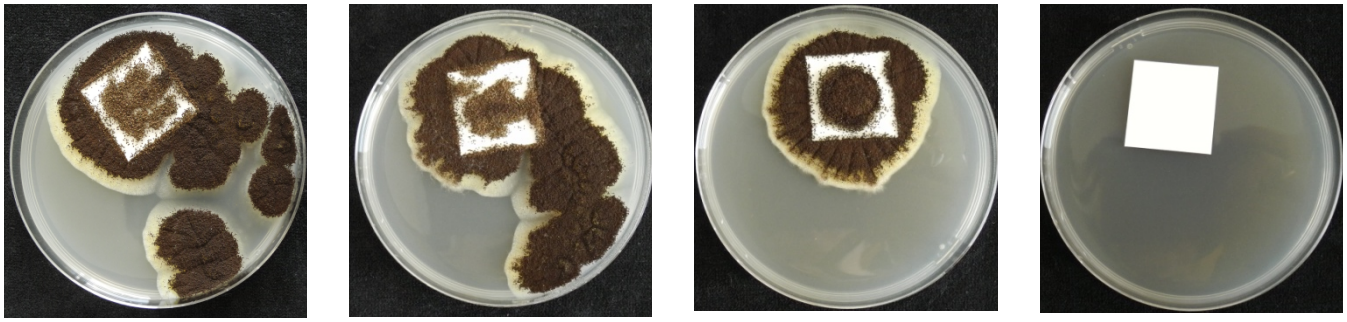
3.1.5.4. Direct contact followed by a mechanical removal

The treatment solution was applied in the same way and with the same contact times as the direct contact. The removal was done by swiping the cloth once over the emulsion.

3.1.6. Evaluation of Fungal Inactivation

Once treated, the sample was placed with the emulsion down on M2Lev medium (for 1 L of water: 20 g malt, 3 g yeast extract Biomerieux®, 15 g agar Biomerieux®), and then incubated at 25 °C. The colony diameter was observed visually after seven days. Its diameter was assessed qualitatively in comparison to the diameter of the untreated control samples. The samples were marked as "+ +" when the colony diameter was similar or larger than the control, " + " when inferior than the control, or "-" when no growth was visible (Fig. 1).

Fig. 1: Example of colony diameter for *Aspergillus niger*



Control | "++" similar or larger colony diameter than the control | "+" inferior colony diameter than the control | "-" no growth

When no growth was observed after seven days of incubation, the samples were incubated for an additional seven days and the colony diameter was observed again.

3.2. RESULTS

3.2.1. Ethanol Vapour Implementation

The results for the fungal growth at seven days after the water-ethanol vapour treatment are summarized in Table 1. This treatment prevented the fungal growth after seven days for all strains after two hours of treatment. *A. alternata* and *P. brevicompactum* are inactivated after one hour.

Table 1: Fungal growth at seven days (25°C) after water-ethanol vapour treatment (350 mL/0.1 m^3)

Strains	**Treatment times**						
	30 min	**1 h**	**2 h**	**4 h**	**8 h**	**16 h**	**24 h**
Alternaria alternata	+	-	-	-	-	-	-
Aspergillus niger	++	+	-	-	-	-	-
Chaetomium globosum	+	+	-	-	-	-	-
Penicillium brevicompactum	++	-	-	-	-	-	-

Evaluation: "++" similar/larger colony diameter than the control, "+"inferior colony diameter than the control, "-" no growth

3.2.2. Direct Contact Implementation

The results for the fungal growth at seven days after the water-ethanol direct contact treatment are summarized in Table 2. From 15 seconds to two minutes of treatment, all strains resumed growth, *A. alternata* was inactivated after eight minutes, and *P. brevicompactum* after four minutes.

Table 2: Fungal growth at seven days (25°C) after direct water-ethanol contact treatment (300 µL/6.5cm^2)

Strains	**Treatment times**					
	15 s	**30 s**	**1 min**	**2 min**	**4 min**	**8 min**
Alternaria alternata	++	++	++	+	+	-
Aspergillus niger	+	+	+	+	+	+
Chaetomium globosum	+	+	+	+	+	+
Penicillium brevicompactum	++	++	++	+	-	-

Evaluation: "++" similar/larger colony diameter than the control, "+"inferior colony diameter than the control, "-" no growth

3.2.3. Direct Contact Followed By A Mechanical Removal Implementation

The results for the fungal growth at seven days after the water-ethanol direct contact treatment and mechanical removal are summarized in Table 3. Up to four minutes of treatment, all strains resumed growth. At eight minutes, *A. alternata* and *P. brevicompactum* did not grow.

Table 3: Fungal growth at seven days (25°C) after direct water-ethanol contact and mechanical removal treatment (300 µL/6.5cm^2)

Strains	Treatment times					
	15 s	30 s	1 min	2 min	4 min	8 min
Alternaria alternata	++	++	++	++	+	-
Aspergillus niger	++	++	++	++	+	+
Chaetomium globosum	+	+	++	+	+	+
Penicillium brevicompactum	+	+	+	+	+	-

Evaluation: "++" similar/larger colony diameter than the control, "+"inferior colony diameter than the control, "-" no growth

3.2.4. Growth at 14 Days

The results obtained with the direct contact implementations are the same after 14 incubation days, i.e. the samples do not resume growth. Concerning the ethanol vapour implementation, *A. alternata* and *A. niger* samples do not resume growth; the *P. brevicompactum* samples treated for one hour resumed growth whereas no growth was visible at seven days; and the *C. globosum* samples treated for one hour shows a growth similar to the control sample. All samples treated for two hours or more are inactivated.

3.3. DISCUSSION

One of the major interests of the present study was the use of artificially contaminated silver gelatin prints. The development of all the tested species within a week at 25°C demonstrated that rapid decontamination (within 48 hours), or at least preservation, of photographs after flooding is required to avoid fungal growth.

The protocol allowed determination of fungal inactivation by ethanol vapours. Fungi are strictly aerobic organisms, however they can grow with oxygen levels as low as approximately 2% depending on the species (Nguyen Van Long and Dantigny 2016). Control samples demonstrated that the species grew at the surface of the agar medium, even if the sample was turned upside down, with the print over the mycelium. Therefore negative experiments that did not exhibit growth were not due to oxygen limitation but to fungal inactivation. Prior to ethanol treatments, the samples were entirely covered with mycelium and conidia. It can be expected that treatments that proved effective in the present study against heavy inoculum will be even more effective against lighter inoculum. The obtained results depend on the different tested strains. Indeed each strain has a different growth speed and sensitivity to external agents. In this experiment, *A. niger* and *C. globosum* showed more resilience to every tested treatment compared to the two other tested strains.

The vapour implementation showed the best results with the inactivation of all strains at 14 incubation days (25°C) after two hours of treatment. The samples treated by direct contact with the water-ethanol mixture showed consistent results within the two batches, with and without mechanical removal.

The mechanical removal of spores, by swiping the microfiber cloth on the sample surface, did not influence fungal growth in this experiment. Indeed, the results from this batch of samples are similar to the batch without cloth swiping. The spores' removal was partial, and the remaining spores resumed growth as the water-ethanol contact did not inactivate them; however, another study has shown that the mechanical removal of fungal spores, by reducing the number of spores on the surface, reduces the overall fungal activity on the heritage object (Prevet 2016).

For vapour implementation, the experiments that were negative after one week of incubation were re-incubated in the same conditions for another week. They remained negative thus suggesting that, in these conditions, all the mycelium and all the conidia were inactivated.

4. CONCLUSION

This study was based on the treatment of seven water damaged photographs from the Bibliothèque Historique de la Ville de Paris. As a result of the water damage, the gelatin emulsion had been used as a substrate by fungi. This led to one question: what do we do when we have a fungal growth on a photograph?

When external conditions are not favorable to its growth a fungi can go into dormancy, where it is still viable and can activate and grow if the conditions change. Thus, treatment of the contaminated photographs is crucial to avoid fungal growth and contamination of further objects. A thorough mechanical removal of the fungi will significantly decrease the amount of viable cells on the photographs (Prévet 2016); however, a disinfection treatment should be considered to address the fungi embedded in the materials.

Several disinfection methods are available to treat photographs. The present work was undertaken to clarify which implementation method of a water-ethanol (30:70 v/v) mixture inactivates fungal growth on silver gelatin prints. The efficiency of the fungal growth inactivation depends on the temperature and on the time of contact between the water-ethanol mixture and the fungus. In a heritage context, working at high temperature (over 20°C) is not possible because it would cause damage to the photograph's constituents. Consequently, contact time is the relevant parameter. Our results show that the longer times tested with vapour implementation successfully inactivated fungal growth. The four tested strains were inactivated after only two hours of exposure to water-ethanol vapours, however, the direct contact tests did not inactivate fungi as the contact times, of between 30 seconds and 8 minutes, were too short.
The vapour treatment shows promising use in heritage conservation as it could be used to treat storage spaces or larger volumes of photographic prints because ethanol vapour can reach any remote place. The lower flammability limit for ethanol is 3.3 kPa (Anonymous 2003) and it can be obtained at 25°C with an ethanol-water mixture close to 70:30 (v/v). For safety reasons, it is suggested to use an ethanol-water mixture close to 40:60 (v/v) but to extend the ethanol vapour application from 2 to 24 h (Dao et al. 2010).

In conclusion, before being used on photographs this treatment's long term effects on silver gelatin prints and other photographic techniques must be evaluated further. Some studies have already been performed regarding this matter. Firstly, the water-ethanol mixture was shown not to damage the paper (Sequeira et al. 2016; Weiß 2006); however, butanol vapours lead to a slight

modification in photographic gelatin structure resulting in a decrease in viscosity (Tomsŏvá et al. 2016). Complementary tests on the long-term effect of a water-ethanol (30:70 v/v) mixture on photographic materials should be performed.

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