THE EFFECT OF PHOSPHORESCENT AND FLUORESCENT MINERALS UPON THE GROWTH OF FUNGI IN CULTURES

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For a number of years there has been conducted a series of experiments at Purdue designed to determine the effect of various wave lengths of light upon the growth of fungi in culture. This report represents one phase of this rather extensive investigation.

Some minerals and chemical compounds possess the interesting property of emitting a phospherescent glow after they have been previously exposed to light. This glow produces sufficient illumination to permit one to distinguish between letters in an otherwise darkened room. The luminesence is most marked immediately after the exposure of the minerals to light, then slowly fades until after a few hours there is no visible light from this source.

Experiments with zinc sulphide.

Zinc sulphide when mixed with a slight amount of impurity such as bismuth sulphide is exceedingly phosphorescent after exposure to light. The luminescent quality is exhibited by this compound after an exposure of less than a minute to either daylight or to ordinary electric illumination. The phosphorescent property is retained with diminishing intensity throughout a period of several hours. The light given off includes the violet, indigo, blue, and some of the green of the spectrum. According to other investigators there is no indication of ultra violet being given off in the phosphorescent glow.

There seems to be no adequate explanation at the present time why impure zinc sulphide should possess this strange quality of luminesence. It has been suggested that the light stimulus to which the chemical is subjected acts with more or less explosive force on the atomic arrangement within the compound. The return of the various chemical units to their normal position within the molecule may account for the energy released in the form of light.

The following technique was followed in order to irradiate fungi with this type of light. The bottoms were cut from pyrex test tubes in such a manner that a small glass cup eight mm. in height was formed. These cups were sterilized in a Petri plate in the autoclave. Potato dextrose agar was poured into Petri plates and before the agar hardened the glass cups were pressed into the agar at the exact center of the plate. Agar plates so prepared were inoculated with the experimental fungus which was permitted to attain a colonial diameter of two cm. before it was irradiated. Approximately a gram of zinc sulphide was then placed in each cup, except those held as checks. The sulphide was then exposed to light in order to activate its phosphorescent qualities

and the preparations were set in a dark room and maintained at room temperatures. Re-exposure to light was made at approximately eight hour intervals. Checks were treated exactly as in the preparations just described except that zinc sulphide was not placed in the cups of these plates.

After several tests had been made in the manner described, the technique was altered in the following manner. Luminous paint, which derived its phosphorescent qualities from the presence of zinc sulphide, was obtained. This paint reacted toward light in exactly the same manner as that previously described for the chemical itself. All subsequent experiments with zinc sulphide were made using the paint rather than the mineral because of the greater ease in irradiating fungi when it was used. The paint was applied to either the upper or lower surface of the lid of the Petri plate in which the test fungus was growing. The effect of the luminescence on spore germination was likewise tested using luminous paint to smear either the lower or upper surface of the lids of culture chambers in which the germination of the spores was tested. The spores to be germinated were placed in water drops on clean glass slides in the moist chamber.

The following results were obtained when the phosphorescent light from zinc sulphide was permitted to irradiate either fungus cultures or fungus spores.

Cultures

Fusarium oxysporium.—Zinc sulphide in cups: Retarded growth and increased sporulation. Paint inside Petri dish cover: Growth entirely checked for 24 hrs.; after 24 hrs. growth was resumed at a slower rate but in the same manner as the check. Increased sporulation. Paint outside the Petri dish cover: No visible effects.

Helminthosporium inaequalis.—Zinc sulphide in cups: No marked retardation of growth. Increased sporulation. Paint inside Petri dish cover: Growth entirely checked over the first 24 hr. period and then resumed slowly. Greatly increased sporulation. Paint outside Petri dish cover: Growth not checked, but increased sporulation.

Colletotrichum lagenarium.—Zinc sulphide in cups: No effect. Paint inside Petri dish cover: Growth entirely checked for the first 24 hrs. and then slowly resumed. More aerial hyphae about the edge of the colony, which always seems to be directed outward toward the periphery of the plate. Greater activity of the acervuli in the center of the colony. Paint outside Petri dish cover: No visible effect.

Sclerotinia americana.—Paint outside Petri dish cover: Cultures so exposed averaged a colonial diameter of 70 mm. after 80 hrs. Check cultures entirely filled the plate in the same period of time and were therefore 90 mm. in diameter. Greatly increased sporulation. *Sclerotinia americana* was not exposed to luminous minerals in any other manner.

Penicillium digitatum.—Paint outside Petri dish cover: The only effect observed with this fungus was that the irradiated cultures were

distinctly more blue in color than those not irradiated. This was the only manner in which this fungus was irradiated.

Pythium deBaryanum.—This fungus was exposed to luminous paint applied to both inside and outside the Petri dish cover. No observable effect.

Spore Germination

Spores of *Alternaria solani* were chosen for this study because of their large size and because of the ease with which the spores are germinated. Checks gave a germination between 96 and 100% after 24 hrs. Paint on the inside of the lids of the culture chambers produced an effect whereby there was no germination after 24 hrs. and less than 10% after 60 hrs. Spores in chambers with luminous paint on the outside of the covers had the same percentage germination as did the checks.

Experiments with other minerals.

Samples of willemite, which is phosphorescent under certain circumstances, gave no visible phosphorescent glow even after exposure to sunlight. This mineral, it is said, can only be activated by a stream of electrons from an electron gun. With the assistance of the Department of Electrical Engineering, it is hoped that we may be able to test willemite in the same manner as we tested zinc sulphide. Willemite which gave off no visible luminesence had no effect upon fungi in culture.

Kunzite possesses fluorescent qualities. Since fluorescence is purely a physical phenomenon we did not anticipate that fungi in its presence would exhibit variation. Fungi were not affected by the near presence of kunzite.

It was thought that zinc sulphide, luminous paint, and willemite uninactivated and not giving off any visible light might be giving off some type of energy that, though not visible to the eye, might have some potency. To test this possibility photographic paper and very sensitive photographic film were subjected to the presence of zinc sulphide, zinc sulphide paint, and to willemite. The experiment was so devised that in some instances glass separated the minerals from the paper and the film. In other instances glass did not intervene. Paper and film were developed after exposure. The paper gave no indication of fogging. The film was fogged in every instance. It might be deduced from this experiment that energy in some invisible form is being released from the minerals under investigation, but proof of this deduction can only be ascertained after further tests.

In summary, it appears as if the radiations from phosphorescent minerals have but little effect upon the growth of fungi. When the exposure is made under extremely favorable circumstances the further development of most of the fungi tested was temporarily inhibited. It is possible that luminous paint possesses some fungicidal value.