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Ultraweak luminescence from germinating resting spores of Entomophthora virulenta Hall et Dunn

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Germinating resting spores of *Entomophthora virulenta* Hall et Dunn emit ultraweak luminescence with the intensity of the order 100 photons $\cdot s^{-1} \cdot cm^{-2}$ in the spectral region 200-750 nm. The emission kinetics and intensity depend on vitality and incubation temperature of the spores. The higher the ability of resting spores to germinate, the more intense the luminescence. Elevation of the incubation temperature to 50°C enhances ultraweak luminescence. The activation energy was found to be about 15 kJ $\cdot$ mol^{-1} and 5 kJ $\cdot$ mol^{-1} for nongerminating and germinating in 50% spores, respectively. The possibility of applying ultraweak luminescence as a simple assay for the spores vitality is discussed.

INTRODUCTION

There have been recent studies of the entomopathogenic fungi as biological control agents for insects (Soper et al. 1975; Majchrowicz and Soper 1979). *Entomophthora* species are probably the most pathogenic of all entomogenous fungi. One of these species, *E. virulenta*, is an aphid pathogen which easily produces resting spores in artificial media.

High germination and sporulation rates, feasibility of spore production

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using inexpensive media (Latgé et Bievré 1977; Majchrowicz and Soper 1979) and resistance of resting spores to temperature and chemicals (Krejzova 1977) favour practical use of *E. virulenta* as microbial insecticides for crop protection. The disadvantage is that we still do not know the metabolic rate of resting spores and actively groving vegetative mycelium and production of conidia.

It has been shown that germinating seeds of higher plants show an ultraweak luminescence (UWL) which correlates with their vitality and germination rate (Milczarek et al. 1973; Konecka et al. 1978; Grabikowski 1977). Germinating spores and growing mycelium of some fungi also appear to emit UWL (Sławiński et al. 1977).

To explore this situation further, the UWL of resting and germinating resting spores of *E. virulenta* with different vitality and at various temperatures was investigated.

MATERIALS AND METHODS

Entomophthora virulenta was isolated from Myzus persicae culture criginated from the state of in Maine the U. S. (Soper et al. 1974). Thick-walled, smooth hyaline resting spores were obtained in a form of powder from an egg yolk medium according to the methods described by Soper et al. (1975) and Majchrowicz and Soper (1979). The spores were kept four years at 5-8°C. Before these experiments the germination ability of the spores was carefully tested using the method of Majchrowicz and Soper (1979).

Apparatus for UWL measurements consisted of a sensitive photoelectric system, described previously (Sławiński et al. 1971). Samples of dry spores were placed on sterile Petri dishes inside of a light-tight camera. The number of counts (n) during 10 s time intervals was determined in 4-6 repetitions for each Petri dish. The air-dry weight of each sample of spores was 100 mg. Then, 3 ml of aqueous solution of streptomycin was added and the number of counts (n), proportional to the intensity of UWL, was repetetively determined versus time. During the measurements an emission level from the camera (a background) and from the control Petri dishes (water and antibiotics) was carefully and frequently checked. Materials and chemicals stored in dark compartments at a fixed temperature. All operations were performed in a weak dim red light. The temperature within the camera of the photon counter was constantly kept at 295°K.

Evaluation of the Petri dish-photocathode geometry used at maximum quantum efficiency of the photomultiplier, predicted the collection of approximately 2% of the total photons emitted. Calculations of the

absolute light intensity give values of the order from 80-600 photons per $cm^2 \cdot s$.

The results of UWL measurments were subjected to statistical analysis (W i l s o n, 1968). An average intensity of UWL was calculated from the formula:

$$I = I_t + I_b;$$

where I_t is an average total intensity, measured from the sample + interior of light-tight camera in cps (counts per second) and) I_b is an average intensity of apparatus background. All results are expressed as

$$I = \frac{k\sigma}{\sqrt{n}}$$

in which I is the mean and σ the standard deviation of n determinations; k is a factor dependent on n and the confidence level. In our studies n varied between 4 and 6, and the confidence level we chose was 90% (Fig. 1) and 95% (Fig. 2).

Germination of *E. virulenta* spores was performed in Petri dishes kept inside thermostats fixed at 0°, 20°, 50° and 80°C. After the appriopriate time of incubation (see Figs. 1 and 2) the Petri dishes were allowed to reach 20°C and then they were transferred into the light--tight camera of the measuring apparatus, where the temperature was always 25°C. After the measurements the dishes were transferred back to the thermostats (incubators). In the second series of experiments (temperature 25 and 50°C), 14 Petri dishes with 0% and 14 others with 50% germination ability were incubated at constant temperature for the duration of the experiment. Two Petri dishes were succesively tested after the appropriate time of incubation, and these were not transferred back for incubation. For control an additional 14 dishes with water and streptomycin were incubated and measured in the same way as the tested ones.

RESULTS AND DISCUSSION

In the first series of experiments, the intensity of UWL from *E. virulenta* spores with 0 and 50% vitality (germination ability) at four different temperatures was investigated. The intensity (I) — time (t) dependence curves are shown in Fig. 1.

It is evident that dry resting spores with 0% vitality exhibit intensity of UWL almost one half of those with 50% vitality. Addation of water results in a dramatic enhancement of UWL intensity by a factor of

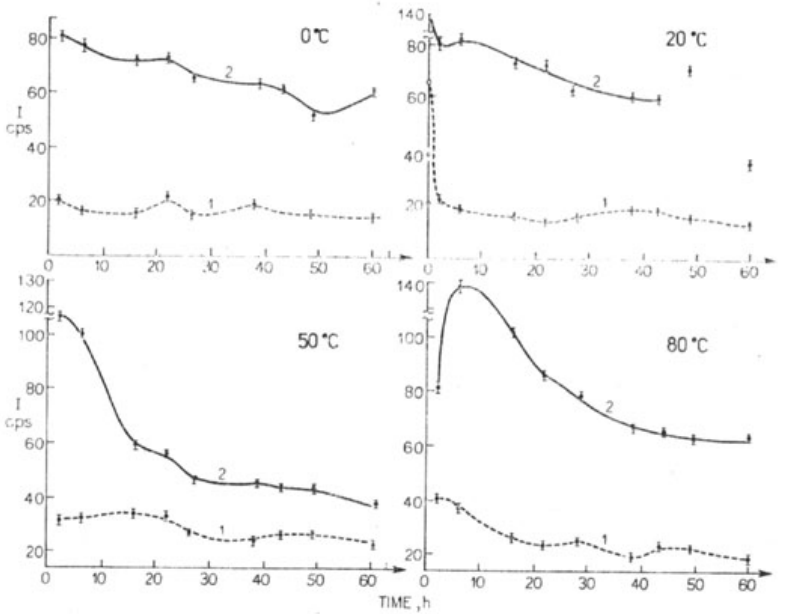

Fig. 1. Kinetics of ultraweak luminescence of germinating spores of *Entomophthora virulenta* at different temperatures

1 — spores with 0% germination ability, 2 — spores with 50% germination; *I* — average intensity of luminescence, h — time of incubation after addition of water + streptomycin and + intensity of luminescence for dry spores with 0% and 50% germination power, ○ and △ — intensity measured 1-3 min after addition of water + streptomycin

3.2 for both groups of spores. Non-germinating spores (0% vitality) always give UWL intensity lower by a factor of about 2-5 than those germinating in 50%. The differences are statistically significant. The change of incubation temperature from 0°C to 20°C does not affect *I* values of UWL. However, incubation of spores at 50 and 80°C distinctly enhances the light intensity. The ratio I_{50}/I_0 at 80°C is higher than that at 50°C. Averaged values of UWL intensity for 0% vitality are the same at both temperatures, while those for spores with 50% vitality are higher at 80°C. Therefore, one may conclude that incubation temperatures even as high as 80°C still enhance the rate of some metabolic processes, associated with generation of light quanta. The high chemical and temperature resistance of the resting spores from some species of the genus Entomophthora was also observed by Krejzova (1968). However, it is necessary to keep in mind that the light intensity was measured

at 25°C, while incubation temperatures were different. Thus, intensity of UWL reflects non-stationary (residual) processes induced by lower or higher incubation temperatures. Similar effects, manifested by "temperature hysteresis of UWL" have been observed for germinating seeds of rye, wheat, oat and pea (M i e l c z a r e k et al. 1974).

The data of Fig. 1 indicate that the maximum UWL occurs shortly after addition of water to the spores and then quickly decays. Measurements of UWL for longer times than 6 hours are not reasonable

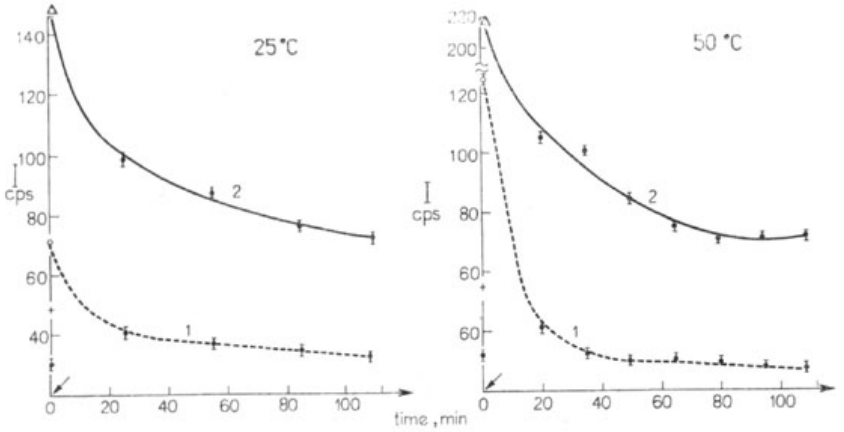

Fig. 2. Changes of ultraweak luminescence intensity during germination of spores of *E. virulenta* at 25 and 50°C. Arrows indicate addition of water + streptomycin

Description is the some as in Fig. 1

and therefore short-time experiments were performed.

Results presented in Fig. 2 clearly show that the fast increase of UWL intensity results from water penetration into spore membranes, where hydrophylic interactions occur. These physical-chemical proceses quickly stop which is evident in the case of non- living spores (0% germination). UWL decay is much slower for spores with 50% germination ability. This statement is valid for both incubation temperatures and is statistically significant.

The kinetics of UWL decay follows the first order reaction with two rate constants which depend on temperature and spore vitality (Table 1). k-values were roughly evaluated from the slope of $\Delta \ln I/\Delta t$. All the data clearly proves that differences between intensity and kinetics of UWL from non-vital and vital spores of *E. virulenta* are entirely distinct and statistically significant. Thus, UWL may serve as

Table 1

Rate constants of ultraweak luminescence decay of germinating resting spores of *Entomophthora virulenta*

Temperature °C / % of germinating spores	25		50	
	time, min			
	0-25	25-180	0-20	20-110
	k, min^{-1}			
0	$1.6 \cdot 10^{-2}$	$4.1 \cdot 10^{-3}$	$2.8 \cdot 10^{-2}$	$1.7 \cdot 10^{-3}$
50	$1.1 \cdot 10^{-2}$	$3.4 \cdot 10^{-3}$	$1.7 \cdot 10^{-2}$	$2 \cdot 10^{-3}$

a simple assay for spore vitality. A similar assay was reported for germination of higher plant seeds (Grabikowski 1977; Konecka et al. 1978).

The preliminary character of these experiments and the broad intervals of temperature do not permit one to conclude whether the relationship $I=f(T)$ obeys the *Arrhenius* law. Nevertheless, we roughly estimated activation energies E_a for the most proper intervals of T using the following formula:

$$E_a = \frac{(\ln I_2 - \ln I_1) T_1 T_2 R}{T_2 - T_1}$$

where I_1 and I_2 are average intensities of UWL at temperatures T_1 and T_2, and R is equal to 8.31 $J \cdot mol^{-1} \cdot {}^{\circ}K^{-1}$. Values of E_a computed

Table 2

Activation energy E_a of ultraweak luminescence of *E. virulenta*

% of germinating spores	Time of incubation	Temperature °C	E_a $kJ \cdot mol^{-1}$
0	1- 3 min	25-50	18.2 *
	35-50 min		5.8 *
	2 h	20-50	8.9
	6 h		14.2
	16.5 h		18.4
	22 h		16.7
50	1- 3 min	25-50	12.5 *
	35-50 min		4.1 *
	2 h	20-50	7.3
	6 h		5.1

* Values calculated from the second series of experiments (Fig. 2).

from kinetic data k instead of I are higher, however the relationship $(E_a)_{0\%} > (E_a)_{50\%}$ is also fulfilled. In average, E_a for non-germinating spores is 15.3 ± 4 kJ · mol^{-1} and for germinating in 50% is 4.6 ± 2 kJ · · mol^{-1}. These values are rather low, similar to those for radical or enzymatic processes (Vladimirov and Arczakov 1972). A low E_a indicates a low potential energy barrier for those processes which control the generation of UWL photons. It is seen that enzymatic processes associated with germination and mycelium growth have lower E_a than physical — chemical interactions in spore membranes.

Exergonic reactions which are the source of excitation energy are unknown. The first candidate may be the peroxidation of unsaturated fatty acids associated with peroxide breakdown and formation of ROO. radicals and excited molecular oxygen $^1O_2$. Latgé and Bievré, 1977) have found that the amount of lipids was 4 times larger at the prespore and spore stage than at the exponential mycelial stage of *E. virulenta*. The fine structure of the conidia of *E. apiculata* suggests that they are capable of a prolonged high metabolism rate of lipids. Microbody-like organelles in conidia might be glyoxysomes, containing enzymes necessary for converting lipid stores into energy (Lambiase and Yendol 1977). Model peroxidative reactions of this kind, accompanied by chemiluminescence, have been recently reported (Shoaf and Steele 1974; Sławińska and Sławiński 1975).

As the second possibility, the metabolism of carbohydrate might be mentioned. Tyrrel and Simpson (1975) have shown that the onset of germination in *E. pyriformis* correlates with the high increase of glycolytic enzyme activity, specially of aldolase, triosephosphate isomerase and dehydrogenase, enolase and pyruvate kinase.

As regards to the excitation energy in the first stage of spore germination, we hypothetize that there is a very small but definite probability of synchronized (coherent) H-bond formation (12-18 kJ · · mol^{-1} per one bond) during the penetration of membranes by water molecules. The emission of radiation with average $\lambda em = 500$ nm corresponds to the electronic excitation energy at least 240 kJ · mol^{-1}. Therefore, the generation of the excited state would require an energy amount equivalent to the formation of 10-14 H-bonds. Such a cooperative accumulation of energy high enough to populate electronic energy levels (2.5-4 eV) might account for UWL of biopolymers, seeds and spores treated with water. There are also some data indicating the possibility of photon storage in cell populations which might be pertinent to a growth regulation (Popp, Klima and Schmidt 1979).

Further research on UWL and the metabolism of conidia formation are in proggress.

Ultraluminescencja kiełkujących spor *Entomophthora virulenta* Hall et Duun

Streszczenie

Kiełkujące spory *Entomophthora virulenta* Hall et Dunn emitują ultrasłabą luminescencję o natężeniu rzędu 100 fotonów $\cdot s^{-1} \cdot cm^{-2}$ w zakresie widmowym 200-750 nm. Kinetyka i natężenie emisji zależą od żywotności spor i temperatury inkubacji. Im wyższa zdolność spor do kiełkowania, tym silniejsza luminescencja. Podwyższenie temperatury inkubacji do 50°C wzmaga ultrasłabą luminescencję. Obliczono, że energia aktywacji wynosi około 15 i 5 kJ $\cdot mol^{-1}$ odpowiednio dla martwych i kiełkujących w 50% spor. Dyskutuje się możliwość wykorzystania ultrasłabej luminescencji jako prostego testu określania żywotności spor.

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