Acta Phytopathologica et Entomologica Hungarica 37 (1–3), pp. 83–89 (2002)

Effect of UV and Visible Light Irradiation on Mycelial Growth and Sclerotium Formation of *Sclerotinia sclerotiorum*

P. NAGY and G. FISCHL

Georgikon Faculty of Agriculture, University of Veszprém, H-8360 Keszthely, Deák F. u. 57., Hungary

We examined effects of UV and visible light irradiation on mycelial growth and sclerotium formation of *Sclerotinia sclerotiorum.* The irradiation with UV had no effect on the production of sclerotia. However it had strong inhibitory action on the growth of mycelia. In cultures of the fungus irradiated with yellow and green light more but small sclerotia developed mainly in the perimeter of culture at the time of irradiation and between this circle and the wall of Petri dishes. In cultures irradiated with red and blue light, a few, but large sclerotia developed.

Keywords: *Sclerotinia sclerotiorum,* formation of sclerotium, UV irradiation.

Effect of light on plant and animal organisms is well known. The effect of light on phytopathogenic microscopic fungi is not so well known, although the morphogenetic effect of visible or near UV irradiation on specific microscopic species of *Deuteromycetes* has been widely examined. The general morphogenetic effects are related to the reproduction and formation of conidiophores and conidia, and various rough morphological changes including the morphological change of spores and sporangiophores as well as the zone of culture (Leach, 1971). The most frequently examined morphogenetic effect is the induction of sporulation. Numerous fungi of *Deuteromycetes* are known in which sporulation was induced by the near UV irradiation and inhibited by blue light (Lukens, 1963; Leach and Trione, 1965; Kumagai and Oda, 1969; Tan and Epton, 1974; Vakalounakis and Christias, 1981).

The morphological effect of light on *Ascomycetes* is much less known and examinations may not be exact in all cases. As regards *Ascomycetes* Leach (1971) pointed out that (i) only the UV radiation stimulates the formation of ascocarpium, (ii) there are fungi which respond to the UV radiation as well as to blue light. It was pointed out for some species that the UV radiation stimulates the formation of apothecium (Leach, 1971) or perithecium (Leach and Trione, 1966; Leach, 1972).

From the point of view of plant protection the stimulatory effect of UV radiation on sporulation could be important because with decreasing ozone shield the intensity of near UV radiation is increasing and this process may promote the reproduction of fungi which may cause yield loss.

Sclerotinia sclerotiorum, which was choosed to the subject of experiments belongs to the *Ascomycetes.* It is able to cause great losses on more 400 dicotyledonous plants. This species may produce apothecium by sexual reproduction but this process can rarely

0238–1249/2002/$ 5.00

observed. However, persistent sclerotia are often formed which induces the formation of mycelium under suitable circumstances. This is the reason that the effect of UV and visible radiation on growth of mycelia and development of sclerotia was examined.

Materials and Methods

To observe effect of natural light, cultures was inoculated in three repetition. The cultures were grown in Czapek nutrient medium in Petri dishes 90 mm of diameter. Three cultures were grown in continuous darkness (0/24), three cultures were grown under natural illumination and in darkness only in night (12/12). Three cultures were grown under continuous artificial illumination (24/0). In this last case the exposure to illumination was 1000 lux at the level of the cultures.

For the UV irradiation Hg vapour lamp was used and for illumination by yellow light Na lamp was used. In this latter case the exposure of illumination was 5 time greaters then the exposure of 40 W incandescent lamp which was used earlier. In the case of illumination by yellow and green lights the 0.8 and 4 h^s exposition time was used instead of 1 h, because it was shown in former experiments that the number of the produced sclerotia at this dose was significantly greater than in other cases.

For the illumination by other wavelength ranges, 40 W incandescent lamps were used, which emitted only the known wavelength range. The used colours, exposures and doses are summarised in *Table 1.*

Table 1

The physical parameters of illumination

Wawelength (nm)	Exposure (lux)	Intensity (mW/cm^2)	Exposition time (h)		Dose (mJ/cm^2)	
760–640 (red)	20	0.008	0.5	1	14.4	28.8
589.6 (yellow)	1000	0.095	0.1	0.8	34.2	273.6
540–490 (green)	35	0.004	0.5	4	7.2	57.2
490–420 (blue)	30	0.007	0.5	1	12.6	25.2
254 (UV)	8000	–	0.5	1	–	–

Exposure was measured by PU 150 photometer, and the intensity was measured by a LI-COR 185B radiometer.

At the level of the cultures there was no observable temperature increase arising as a result of radiation. The dependence of growth of colonies was determined. For this reason the diameters of the cultures were measured in two directions perpendicular each to other and the average of this values was calculated. For describing of growth of colonies the so-called logistic function was used, which describes the growth of living organisms in limited living-room (Wilson and Bosset, 1981).

$$D = \frac{K}{\left(1 + \frac{K}{D_0} - 1\right) \cdot e^{-r \cdot t}} \quad (1)$$

Where D is the diameter of the culture (in general the appointed number of population), K is the diameter of Petri dish (in general the carrying capacity of environment), D_0 is the diameter of culture at time zero (in general the appointed number of population at the zero time), r is the growing rate and t is the time.

Results

1. Sclerotinia cultures growing under 0/24, 12/12 and 24/0 light regime

The number of sclerotia was counted at the 7th day after the inoculation. The number of sclerotia was compared with the Mann-Whitney U-test (this test essentially agrees on Wilcoxon rank sum test). The control grown under continuous darkness. The number of sclerotia increased significantly with the increase of the exposition time. The sclerotia was dried for 24 h on air at room temperature after 19 days of irradiation, and their mass was compared with the Student's *t*-test. Control culture were grown in continuous darkness. The number of sclerotia formed in various treatments, the average masses and the results of statistical tests is seen in *Table 2.*

Table 2

The number and average mass of developed sclerotia

Repetition	0/24 h	12/12 h	24/0 h
1	7	19	39
2	18	19	12
3	5	10	34
Average number	10	16	28,3
U-test		*	*
Average mass [mg]	22,70	14,97	5,79
Variancy [mg]	17,70	12,59	4,81
t-test		*	***
Total mass [mg]	681,1	718,6	491,8

Abbreviations: *: $p < 0.05$, ***: $p < 0.001$

According to the Student's *t*-test significant differences existed between the mean masses of various treatments and control.

As a result of growth analysis the logistic function described here seems suitable for describing the growth of fungi. In *Fig. 1* growth of a culture of *Sclerotinia* grown in continuous darkness can be seen. For comparison the straight line fitted to the data can also be seen.

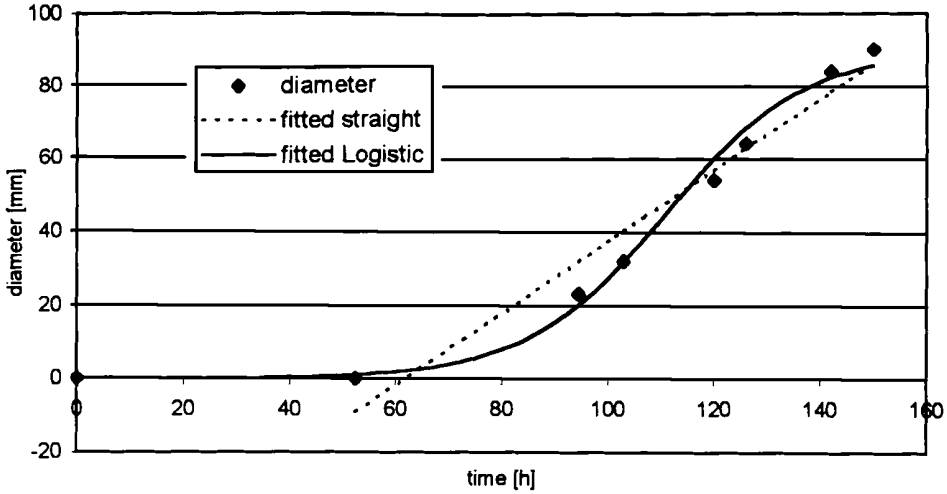

Fig. 1. Growth of cultures of *Sclerotinia* in function of time

2. Effect of visible light and UV radiation on the formation of sclerotia

The number of sclerotia was counted on the seventh day after inoculation. In cultures irradiated with UV the number of sclerotia was counted three days later, because at that time sclerotia developed for full size. The sclerotia developed at the wall of Petri dishes in cultures grown in darkness. In cases of various irradiations sclerotia developed at the perimeter of the cultures as well as near the wall of Petri dishes and in the area between this two circle. To compare the number of sclerotia of treated and the control samples the Mann-Whitney U-test was used.

From each culture 10 average characteristic sclerotia were chosen on the 17th day after irradiation, and the weight of mass was measured after 24 h drying on free air at room temperature. The average mass of control and treated cultures were compared with the help of the Student's *t*-test. In all cases when the F-test resulted in significant differences, the Welch-test were used instead of the *t*-test. The number of sclerotia developed after one hour illumination, the average mass of sclerotia and the results of statistical tests can be seen in *Table 3.*

The total sclerotium mass can be seen in *Fig. 2.* in all wavelength region.

Table 3

The number and average mass of developed sclerotia in one hour illumination of cultures of *Sclerotinia*

Repetition	1 h illumination with Red light	0,8 h illumination with Yellow light	4 h illumination with Green light	1 h illumination with Blue light	1 h irradiation with UV	Control
1	0	18	1	0	7	17
2	7	0	18	7	0	12
3	7	17	17	2	9	8
4	2	12	15	7	15	17
Average	4	11.75	12.75	4	7.75	13.5
U-test	*	NS	NS	*	*	
Average	28.26	12.37	16.44	16.37	19.67	11.99
Variancy	21.17	5.50	8.34	8.81	10.35	5.97
t-test	*	NS	NS	NS	NS	

Abbreviations: NS: There is no significant difference, *: $p < 0.05$

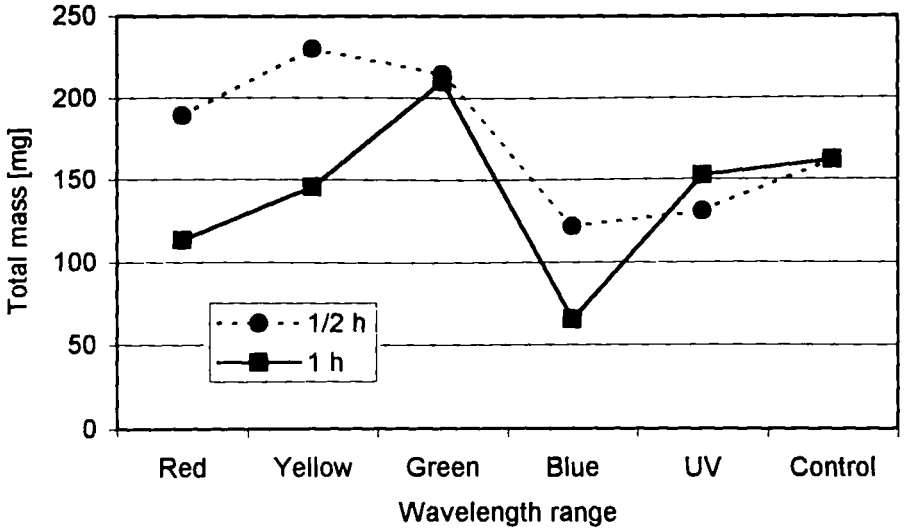

Fig. 2. The total mass of developed sclerotia in function of irradiation wavelength

Discussion

From the sclerotium production of cultures grown in 0/24, 12/12 and 24/0 light regime on the ground of data listed in *Table 2* can be ascertained, that the average sclerotium mass significantly decreases with increasing of exposition time. The significance level at the 12/12 sample is 95% and at the 24/0 sample 99.9%.

The total sclerotium mass can be seen in the last row of *Table 2.* The total sclerotium mass of cultures grown in 24/0 light regime is significantly lower than in the other cases. The average mass of sclerotium gives an opposite tendency than the average number of sclerotium. In this case when relatively many sclerotia developed, the average mass is small, and inverse.

It is generally accepted, that the growth of mycelia is directly proportional to the time of growth (Zándoki et al., 2001), that is when the diameters of cultures is measured, than growth belonging to the same time interval is averaged. The logistic function describing the growth of living organisms in limited living-room is applicable for the growth of mycelia as can be ascertained from *Fig. 1.* The fit of logistic function is better, than the fit of straight line. The square of correlation coefficient of linear function is only 0.95, while the square of correlation coefficient of logistic function is 0.97. The simple exponential function is yet fit better than the linear function, because the square of correlation coefficient is 0.96.

From data listed in *Table 3* for the cultures illuminated by light belonging to the various wavelength range the following qualitative observations can be taken.

(i) The average mass of sclerotium gives an opposite tendency than the average number of sclerotium.

(ii) In the cultures illuminated by yellow and green light developed more, but smaller sclerotia, in great rate in the perimeter of mycelia at the illumination, as well as in the area between this perimeter and the wall of Petri dish.

(iii) In the cultures illuminated by red and blue light relatively few but great sclerotia developed.

From the results of statistical tests listed in *Table 3* significant differences can be observed in average masses of sclerotia in cultures illuminated only by red light. The average mass is greater than it is in the control cultures. There is no significant difference between the average number of sclerotia of cultures illuminated by yellow and green light. In all other cases the average number of sclerotia is less than in the control cultures.

The total mass of sclerotia can be seen for all wavelength region in *Fig. 2.* In the cultures illuminated by blue light for an hour the total sclerotium mass is less, in the cultures illuminated by green light is more than in the control culture.

The number and average mass of sclerotia illuminated by yellow and green light are similar to the culture grown in the 0/24, 12/12 and 24/0 light regime according to the results. (The number of sclerotia is increasing, the average mass is decreasing compared to the cultures illuminated by red and blue light.) It seems, that in white light the effect of yellow and green components are more dominant than the effect of red or blue components.

It is interesting that from *Fig. 2* the UV radiation on the production of sclerotia has no effect. At this time is known (Nagy et al., 2000; Nagy and Fischl, 2001), that the growth of mycelia is strongly inhibited by UV radiation.

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