INDUCING SPORULATION IN ARTIFICIAL CULTURE OF *ALTERNARIA SOLANI* (ELL. & MARTIN) JONES & GROUT

by

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(with 4 figs)

INTRODUCTION

It has been reported that *Alternaria solani* the inciting organism of Early-blight of potato does not sporulate on undisturbed artificial culture media. The same difficulty was encountered by the present authors in the taxonomic studies of the organism, screening out the potato varieties for their resistance and evaluating the efficacy of different fungicides against Early-blight. In order to obtain a heavy crop of spores of *Alternaria solani* various methods were tried and in the present paper an account is given of a new method which proved effective in producing satisfactory amount of spores in P.D.A. (Potato Dextrose Agar) cultures.

Previous workers (RANDS 1917; KUNKEL 1918) resorted to special treatments of the culture like shredding of the mycelium, exposing the cultures to sun-rays, etc., for inducing sporulation in the fungus. RAMSEY & BAILEY (1930) obtained sporulation in *Macrosporium tomato* by exposing its cultures to ultra-violet irradiation. According to them there is a definite stimulation of spore production in the cultures of the organism on exposure to these rays and the spore production is greatest when filters with lower limits of transmission ranging between 2535—288 Å are used. MCCALLAN & CHAN (1944) while confirming the findings of RAMSEY & BAILEY concluded that sporulation in the cultures of *Alternaria solani* increases as the wave length of transmitted rays decreases. They obtained 10,000,000 spores per petridish on exposing the cultures to irradiations from an open mercury arc lamp.

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Material and Method

The fungus was isolated from the infected potato leaves at Simla and for the sake of record had been designated as isolate No. 43. It was grown on Potato Dextrose Agar medium in petridishes of 9 cm diameter and was subjected to various treatments after the culture was two weeks old. The fungus was incubated throughout at 27°C. The petridish cultures were exposed to direct sun-rays for varying periods between 11—14 hours of the day at Simla. The cultures were also exposed to infra-red rays by keeping them at the distance of 24" from the lamp, for a period varying from 15—80 minutes. Philips infra-red lamp of the type 13373 F 1479 was used in these experiments which were replicated thrice along with the controls. The cultures were exposed to direct sun-rays and infra-red rays for fixed periods at 24 hours interval. To avoid the effect of any external source of light the petridishes were thoroughly wrapped in black paper during the incubation period. The paper wrappers were removed only during the exposure of the cultures to sun-rays and infra-red rays. To study the effect of dextrose on the degree of sporulation seven lots of P.D.A. were prepared in which the proportion of dextrose varied from 0.0 to 3.0 per cent.

EXPERIMENTAL RESULTS

1. Effect of total darkness and sun-rays

Inoculated P.D.A. petridishes were incubated at 27°C in total darkness by wrapping them in dark papers. The fungus grew up to the margin of the dish quickly and within 10 days the surfaces of all the petridishes were covered with the mycelium. Thereafter the cultures were exposed to sun-rays for 15, 30 and 45 minutes respectively. The treatment lasted for 10 days but no spores were produced.

Table I.

Effect of total darkness and sun-rays on sporulation

Sl. No.	No. of replication	Treatments	Observation
1.	4	Total darkness + 15 minutes of sun-rays	No sporulation
2.	4	Total darkness + 30 minutes of sun-rays	No sporulation
3.	4	Total darkness + 45 minutes of sun-rays	No sporulation

As evident from Table I, sun-rays alone are not effective in the production of spores.

2. Effect of total darkness and infra-red rays

In this treatment inoculated P.D.A. dishes were incubated in total darkness at 27°C for 10 days. Later, they were exposed to infra-red

TABLE II.

Effect of total darkness and infra-red rays

Sl. No.	No. of replication	Treatments	Observation
1.	4	Total darkness + 15 minutes of infra-red rays	No sporulation
2.	4	Total darkness + 30 minutes of infra-red rays	No sporulation
3.	4	Total darkness + 45 minutes of infra-red rays	No sporulation

Table II indicates that alone infra-red rays are not sufficient for producing spores.

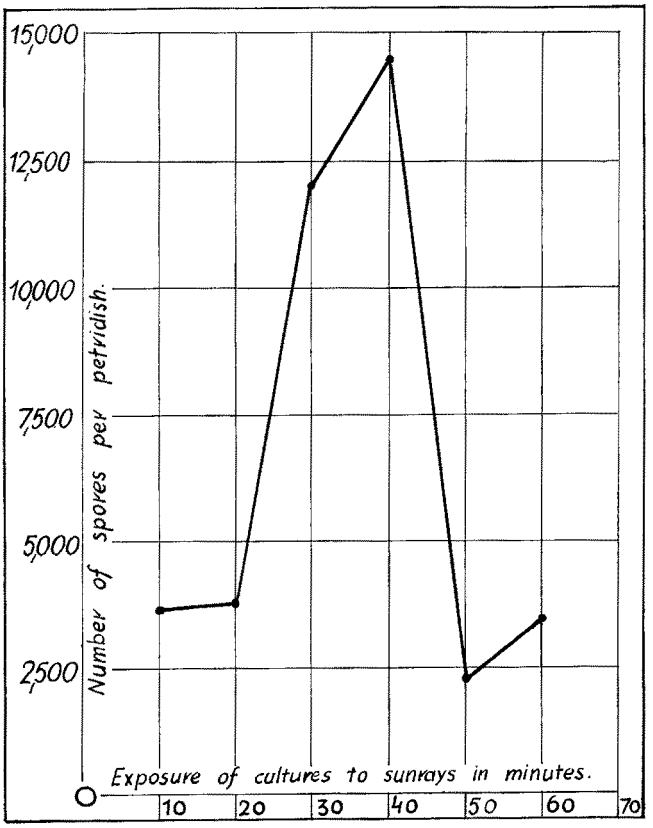

Fig. 1: Effect of time of exposure to sun-rays on the degree of sporulation in the cultures which were later on exposed to infra-red rays for 60 minutes.

rays for 15, 30 and 45 minutes respectively. The results are summerized in Table II.

3. Effect of sun-rays and infra-red rays

Since the treatments mentioned in Table I and II failed to induce sporulation, a combination of sunrays and infra-red rays was tried. Different batches of cultures were exposed to sun-rays for 60 minutes and to infra-red rays for 15, 30 and 45 minutes respectively. The degree of sporulation was maximum in those cultures which were exposed to infra-red rays for 45 minutes and mininum in those which were exposed for 15 minutes.

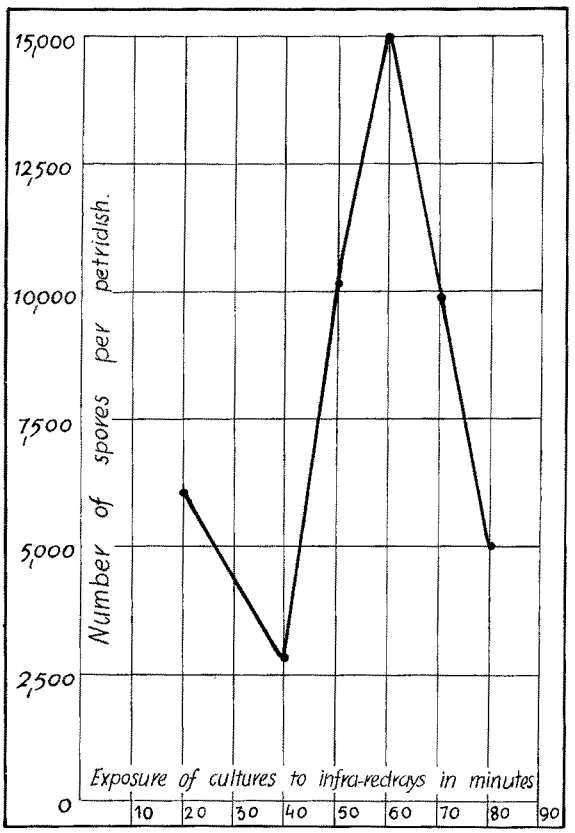

Fig. 2: Effect of time of exposure to infra-red rays on the degree of sporulation in the cultures already exposed to sun-rays for 40 minutes.

4. Optimum period for exposing to sun-rays

Since treatment with sun-rays and infra-red rays was found to have beneficial effect on spore formation, it was worth-while to

attempt to find out the optimum time for exposing the cultures to sun-rays for maximum spore formation. Batches of fully grown cultures were exposed to sun-rays for 10, 20, 30, 40, 50 and 60 minutes respectively and each was then exposed to infra-red rays for 60 minutes. After a few treatments all the cultures showed sporulation, but the degree of sporulation varied with different batches. Maximum spore formation was observed in the batches which were exposed to sun-rays for 40 minutes. The results have been depicted in Figure 1.

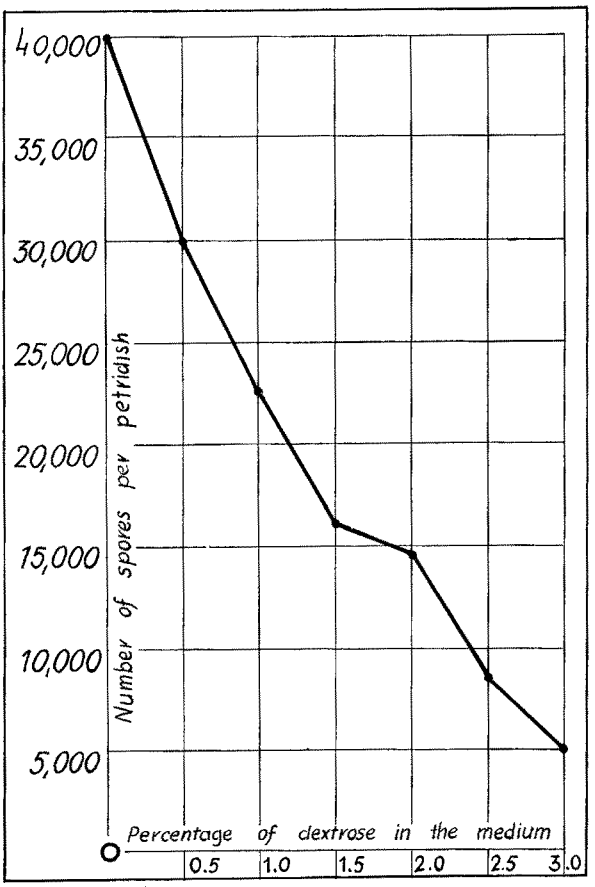

Fig. 3: Effect of concentration of dextrose in P.D.A. on the degree of sporulation.

5. Optimum time for exposure to infra-red rays

From the previous experiment it was found that a 40 minute exposure to sun-rays is most suitable for getting a heavy spore crop. A similar experiment was laid out to determine the optimum time of exposure to infra-red rays. Four batches of the cultures were

subjected to sun-rays for 40 minutes and thereafter to infra-red rays for 20, 40, 60 and 80 minutes respectively. The results have been shown in Figure 2.

The maximum sporulation was induced in the batch which was exposed to infra-red rays for 60 minutes.

6. Effect of dextrose on sporulation

Concentration of dextrose in the medium is one of the most important factors which affects the degree of sporulation. P.D.A. media were prepared having different concentrations of dextrose ranging from 0.0 to 3.0 %. The regular treatment of sun-rays and infra-red rays was started after the fungus had grown fully. The 40 minutes of exposure of sun-rays was followed by an exposure to infra-red rays for 60 minutes. The results are presented in Figure 3. The degree of sporulation was inversally proportional to the concentration of dextrose.

7. Multi inoculation of potato agar (No dextrose) media

During the studies it had been observed that spore production was more on the growing points of the mycelium and, therefore, it was thought that the inoculation of the media plates at several places might produce large amount of spores. The medium was inoculated at 5 different places and incubated as usual. Within 5 days the surface of the petridish was covered with mycelial growth. It was then exposed to sunrays and infra-red rays for 40 and 60 minutes respectively. The treatment was given for three times.

A marked increase in the sporulation resulted in multi-inoculated petridishes. An average of about 1,50,000 spores were produced per petridish.

Spore yield

For counting the spores equal blocks of the medium along with spores were removed from the petridishes, with the help of a cork borer of 4 mm diameter. From each dish 5 blocks were removed at different places. On each block the number of the spores was counted. Average number of spores in a unit area was calculated. On the basis of unit area the number of spores in a petridish was calculated.

Spore Morphology

The spores are club shaped with 2 to 9 cross septa and a few longitudinal septa, measured from 12.5—28.3 × 101.4—340.6 μ, beaked, dark brown coloured.

Summary

Alternaria solani, the casual organism of Early blight of potato, does not sporulate on undisturbed artificial culture media. Sporula-

tion in P.D.A. cultures of the organism can be induced by exposing them to sun-rays for 40 minutes followed by an exposure of 60 minutes to infra-red rays. The degree of sporulation is inversely proportional to the concentration of dextrose in the medium.

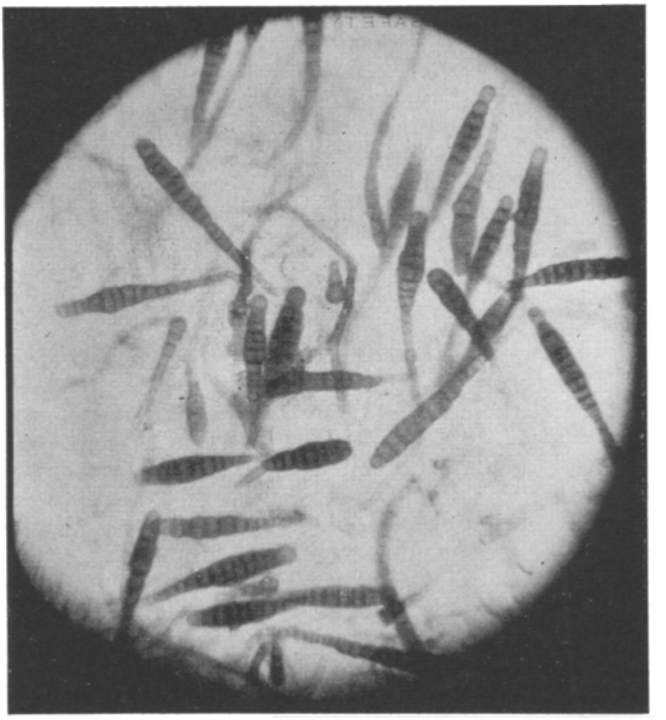

Fig. 4: Spores of *Alternaria solani* as produced by treating the cultures of the organism to sun-rays followed by infra-red rays.

ACKNOWLEDGEMENT

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