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STUDIES ON NECTRIA GALLIGENA BRES.

Part 1. The Effect of Temperature, Light and Media on Growth and Sporulation

A.J. AL-ZARARI

Department of Plant Protection, College of Agriculture and Forestry,
University of Mosul, Hammam Al-Alil, Iraq

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Abstract. The response of *Nectria galligena* Bres. to different temperatures, light and media on growth and sporulation was determined. Maximum growth occurred at 15 and 20 while at 4, 10 and 30° very little growth was observed. Growth at 25° was of intermediate order. Sporulation did not occur under continuous darkness on any media. Spores were produced on PDA and MA under continuous light conditions and higher number of spores were produced on PDA . Sporulation also occurred when the fungus was exposed to 15 hr of light and 9 hr of dark conditions. The amount of spore production and septation of spores varied on different media. Maximum number of spores were obtained on PDA but mostly with one septum while on SEA minimum number of spores were produced but mostly with five septa. Spore production seems to be stimulated by light. Significant difference was observed on the growth of the fungus in different media. Growth was maximum in WOA and minimum in SEA while on PDA and MA it was of intermediate order.

Apple canker, *Nectria galligena* Bres. was reported by Plowright [1] from England, Lortie and Kuntz [2] from Canada, Gyorfi [3] from Hungary, Agarwala and Gupta [4] from India, Ghillini and Mazzini [5] from Italy and Najjar [6] from Syria. Hoffman [7] reported that strong light inhibits spore germination, Hall [8] showed that *Sclerotinia fructicola* sporulated well in darkness, much less or not at all during light periods. On the contrary a number of reports suggest that spore germination is increased by light or even some require light for sporulation: Gassner and Niemann [9], Hutchinson and Ashton [10], Yarwood [11] and Ziegler [12]. A large number of investigators have reported the effect of temperature and different media on the growth of fungi which will not be mentioned here due to lack of space. Effect of temperature and different media on growth of *Nectria galligena* and of light on sporulation is reported in this investigation.

MATERIALS AND METHODS

Malt extract agar medium was prepared according to the DIFCO manual and used to observe the effect of temperature as it produces good growth of the fungus giving distinct colonies. Fifteen ml of the medium was poured in each plate. The plates were inoculated with 5 mm cores taken from an actively growing colony of the fungus. The plates were kept under temperatures ranging from 4-30°. Ten plates were kept for each temperature.

To observe the effect of media, malt agar, potato dextrose agar, wheat oat meal agar and shoot extract agar were used. MA and PDA were prepared according to DIFCO manual while WOA was prepared by cooking 35 g oat meal and wheat meal separately in 500 ml distilled water. The two solutions were filtered through cheese cloth, mixed and 1.5% agar was added. The volume was made upto 1 l and sterilized at 15 lb pressure for 20 min. SEA was prepared by chopping the apple tree shoots and extracted by boiling and filtering through filter paper. 1.5% agar was added and sterilized. Hundred g chopped pieces were used for 1 l dilute media. All plates were inoculated with 5 mm core taken from an actively growing colony of the fungus and kept at 20°. Diameter of colonies was recorded at 48 hr in intervals.

To observe the effect of light on sporulation the media MA, PDA, WOA and SEA were prepared and used as described. Three light regimes were used i.e. continuous dark, continuous light and 15 hr light followed by 9 hr darkness. Fluorescent light (380-750 nm) was used. The plates were inoculated with the fungus as in previous experiment and were given a pretreatment of 12 days of dark incubation period to establish growth before placing in three regimes. Each treatment was replicated five times.

RESULTS AND DISCUSSION

The fungus grew well at temperatures of 15°, 20°, and 25°. There was very little growth at 4° and 30° while at 25° it was moderate (Fig. 1). At 15° and 20° there seems

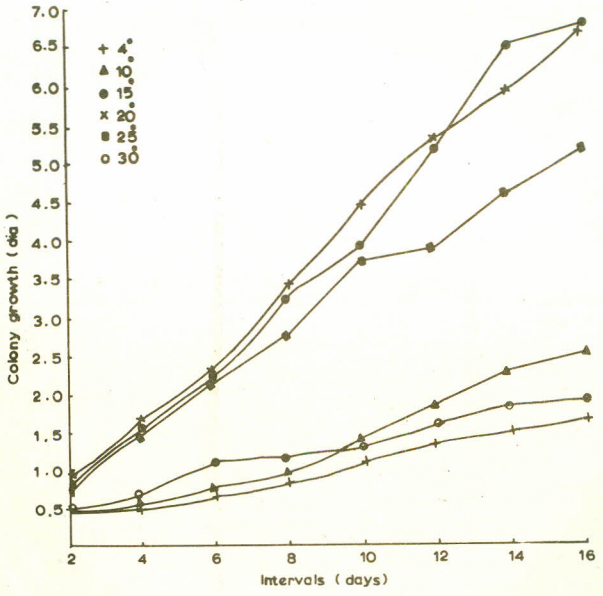

Fig. 1. Effect of different temperatures on growth of *N. galligena*.

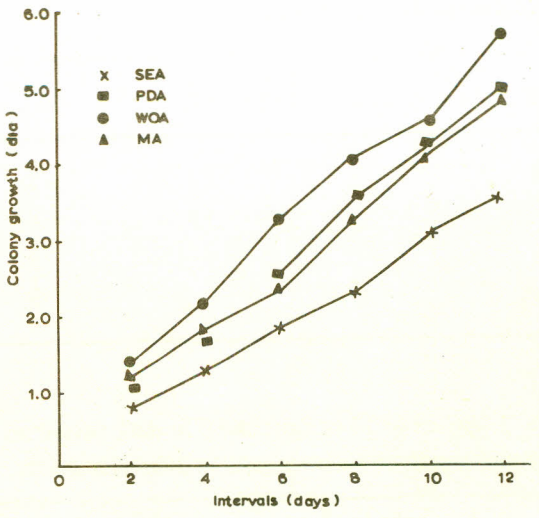

Fig. 2. Influence of various media on growth of *N. Galligena*.

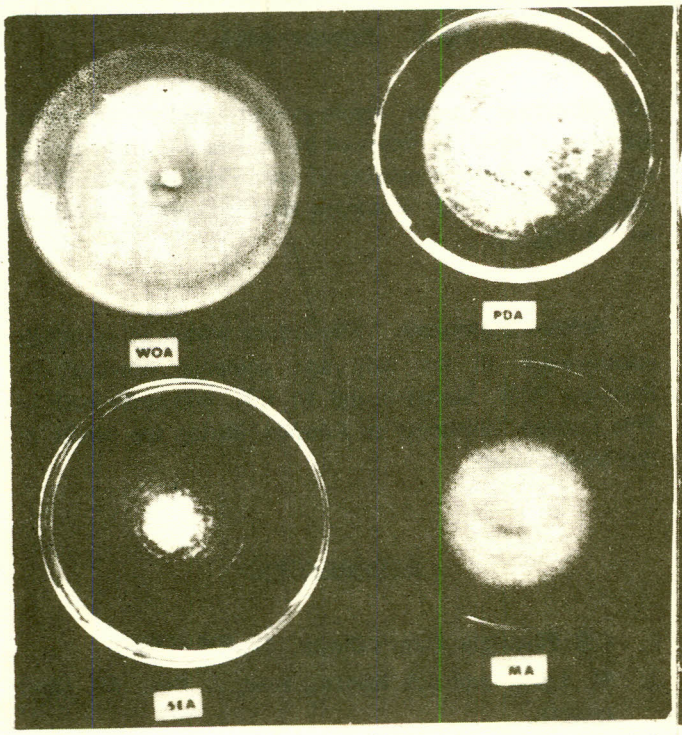

Fig. 3. Effect of continuous darkness on growth of *N. galligena* in different media (Observe no sporulation).

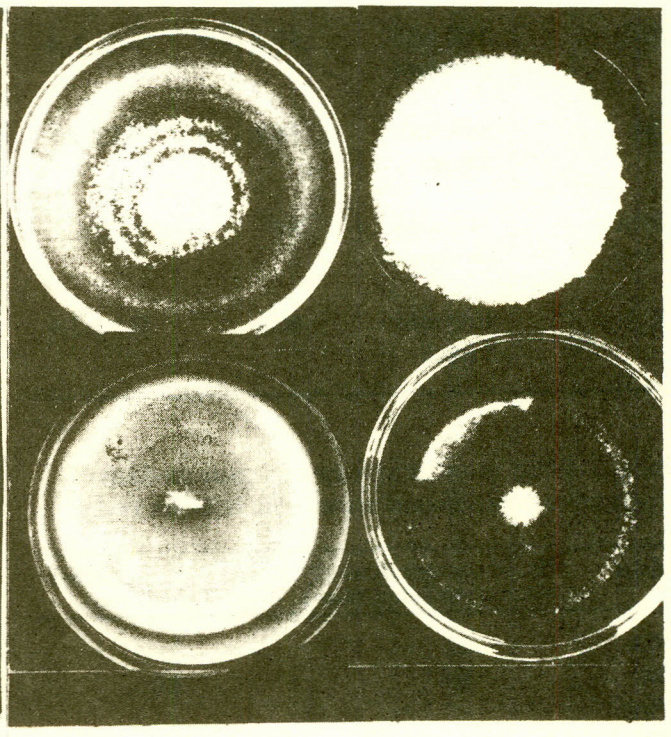

Fig. 4. Effect of continuous light on growth of *N. galligena* in different media. (Note the formation of spores on PDA and MA.)

to be a sharp rise of growth once the growth is established. However, growth at 4^{o}, 10^{o} and 30^{o} does not show any significant increase even after 16 days of incubation.

Maximum growth was observed on WOA but there was no aerial myclium. Minimum growth occurred on SEA while growth on MA and PDA was of intermediate order (Fig. 2).

No spores were produced under continuous dark conditions on any of the media (Fig. 3) and MA (Fig. 4). Spores were produced on all media kept under 15 hr light and 9 hr dark regimes. The shortest period of sporulation in this regime was observed on MA medium followed by PDA (Table 1). After 3 weeks of incubation spore production was estimated by macerating each fungal colony in 100 ml

Table 1. Effect of continuous darkness, continuous light and 15 hr light-9 hr dark period on minimum period for sporulation in different media.

Media	Continuous dark (days)	Continuous light (days)	15 hr light 9 hr dark(days)
PDA	-	15	5
MA	-	17	4
SEA	-	-	9
WOA	-	-	8

Table 2. Effect of 15 hr light and 9 hr dark period on septation and length of macroconidia in different media.

Media	Mode of septation	Length of macroconidia (microns)	Other septation
PDA	84% with 1 septum	12.2-16.5	0, 2, 3, 4
MA	86% with 5 septa	46.2	0,1,2,3,4,6
WOA	74% with no septa	9.9	1, 2, 3
SEA	90% with 5 septa	46.2	0,1,2,3,4

Table 3. Effect of continuous light on spore production, length of macroconidia and septation in different media.

Media	No. of spores/square of haemocytometer(mean)	Average microconidial length in microns
PDA	0.7	9.75
MA	0.4	6.6
WOA	-	-
SEA	-	-

water for 5 min. Using haemocytometer slide, ten counts were made per sample and the mean for haemocyto meter squares calculated for each plate. The results are shown in Tables 2 and 3. It may be observed that on PDA 84% macroconidia have 1 septum, on MA 86% have 5 septa, on WOA 74% have no septum and on SEA 90% have 5 septa. Spore production is stimulated by light but it must be followed by darkness to form conidia on all media but the number of spores and septation differ with the media used. Zonation was also noticed especially on MA (Fig. 5).

Fig. 5. Zonation on MA.

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