Effect of Culture Medium and Light on Sporulation of Two Peanut Leaf Spotting Fungi, **Cercospora Arachidicola** Hori and **Cercosporidium Personatum** (Beck & Curtis) Deighton[1]

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ABSTRACT

Cercospora arachidicola and **Cercosporidium personatum** sporulated abundantly on certain culture media, while on others they produced none to few conidia. On some media which failed to support sporulation, the addition of an aqueous extract of peanut leaves markedly increased sporulation.

Light was not required for sporulation by **C. arachidicola** but was essential for sporulation by **C. personatum.** No conidia were produced by **C. personatum** in suitable media in continuous darkness or orange light, while greatly reduced sporulation occurred in yellow, blue, green, or red light. The pathogenicity of **C. personatum** grown under various wavelengths of light was unaffected.

A characteristic of many *Cercospora* species is that they sporulate sparingly if at all on standard laboratory media (Anzalone and Plakidas, 1957). Nagel (1934) reported that sporulation in certain species of *Cercospora* varied quantitatively according to the medium used. Woodroof (1933) and Jenkins (1938) noted that *Cercospora arachidicola* and *Cercosporidium personatum (Cercospora personata)* grew very slowly and produced few conidia on potato-dextrose agar. Roldan and Querijero (1939) failed to induce sporulation by *C. personatum* on several types of media. Hebert (1944) reported that the peanut-leafspot fungi grown on bean-agar medium produced variants of fluffy, non-sporulating mycelia which overgrew the original sporulating mycelia. Unless special precautions were taken in transferring the sporulating cultures, the non-sporulating variants predominated, and as a result, the sporulating cultures were lost.

Miller (1953) found spore formation by *C. arachidicola* closely related to rate of growth, and that 2% glucose was optimum for sporulation on his basal agar medium. More conidia were produced on semi-solid media than in liquid ones. Miller's procedure was not adaptable to developing a routine laboratory medium for preparing large quantities of inoculum. Of the different media tested, he found that a mixture of germinating barley, yeast extract, sweet potatoes, and basal agar nutrients in a peanut-hull base was an effective medium for growth and sporulation of *C. arachidicola* and *C. personatum. Shanta* (1956) cultured *C. personatum* on a synthetic medium with added yeast extract. He reported that sporulation occurred in cultures initiated from a spore but not in cultures initiated from mycelia.

Landers (1963, 1964a) found that *C. arachidicola* grew and sporulated well in a medium composed of 5% wheat starch, 0.5% yeast extract, and 0.5% KH_2PO_4 adjusted to pH 4.5 with HCl. Landers (1964b) also developed a chemically defined medium suitable for nutritional studies in which he added the vitamins biotin, niacin, and thiamin. He found that thiamin was the growth-limiting factor for *C. arachidicola. Smith* (1971) combined the ingredients of two media successfully used by Abdou (unpublished PhD thesis) for the culture of sporulating *C. arachidicola.*

It is the purpose of this paper to present the original substantiating data hitherto available only as a thesis cited in Smith's paper and to record effects of other factors influencing sporulation of *C. arachidicola* and *C. personatum in vitro.*

Materials and Methods

The **Cercosporidium personatum** and **Cercospora arachidicola** cultures employed in this study were collected from infected peanut leaflets in North Carolina. Infected leaflets were washed in running tap water for about 5 min, immersed for 1 min in 10% Clorox (0.5% NaOCl), and rinsed in sterile water several times. The leaflets with **C. personatum** lesions were inverted and those with **C. arachidicola** lesions were placed upright on a wire screen or filter paper in a moist chamber and incubated under continuous fluorescent light at room temperature for 48-72 hr to induce sporulation. Conidia from the leaflet lesions were streaked across the surface of agar plates from which, after 5-6 days, monosporous cultures from single colonies were established on slants of peanut leaf-agar (WA + PLX) prepared as

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follows: Peanut leaf extract (PLX) 100 ml, agar 12.0 g, and distilled water 900 ml.

To prepare the peanut leaf extract, 300 g green, fresh peanut leaves with no prior fungicidal treatment were washed in tap water for 10-15 min. They were then boiled in 1000 ml distilled water for 30 min with intermittent agitation. The extract was filtered through several layers of cheesecloth. Both **C. arachidicola** and **C. personatum** were found to sporulate freely at room temperature under continuous illumination on this medium. Since all isolates were found to be highly pathogenic to the cultivated peanut, single monosporous cultures of **C. arachidicola** and **C. personatum** were utilized throughout the study.

The ability of the fungi to sporulate was tested on the following media alone and with the addition of 100 ml of the peanut leaf-extract per 900 ml basal medium: Water agar (WA), oatmeal agar (OMA), Difco cornmeal agar (CMA), Difco lima bean agar (LBA), mycophil-agar (My. A), made up of phytone (10.0 g), dextrose (10.0 g), agar (20.0 g) and distilled water (1 L), and a synthetic medium (SA), composed of dextrose (10.0 g), NH_4NO_3 (3.0 g), KH_2PO_4 (1.32 g), $MgSO_4.7H_20$ (0.52 g), 1% $FeCl_3$ (0.6 ml), 1% $ZnSO_4$ (0.6 ml), agar (16.0 g) in 1 L distilled water. The cultures were incubated at room temperature under continuous fluorescent light. The relative sporulation was measured by crushing two single-spore colonies from each culture medium into a drop of water on a slide. The mycelium was then removed, a cover slip added, and the number of conidia per microscopic field (X100) determined for 10 fields. This procedure was replicated three times.

In order to study the effect of light on sporulation by **C. personatum**, WA + PLX plates were seeded with **C. personatum** conidia and incubated at room temperature for 1 week either under continuous fluorescent light or covered with aluminum foil to provide complete darkness for the desired interval of exposure.

The effect of visible light on sporulation **in vitro** was determined in unfiltered light and under the following color filters: blue, 480 nm; green, 520 nm; yellow, 580 nm; orange, 600 nm; and red, 640 nm.

To determine whether light affected the culture medium rather than the fungus directly, petri dishes containing unseeded WA + PLX medium were placed beneath the different filters for 1 week using the same light source and temperature as previously described. After this treatment, each plate was seeded with conidia and incubated at room temperature under continuous light or darkness for another week.

To determine if light of any of the wavelengths tested affected sporulation of the progeny of the irradiated colonies, WA + PLX plates were seeded with conidia taken from those colonies grown under each light filter and incubated under continuous light for 1 week.

Since illumination with different wavelengths of light caused striking changes in the sporulation of **C. personatum**, the pathogenicity of conidia produced under these conditions was tested. Conidia from exposed colonies were harvested to prepare a standardized conidial suspension (15,000 conidia/ml), and plants of a highly susceptible cultivar of **A. hypogaea** (P.I. 262074) were inoculated.

The isolate of **C. arachidicola** used in these studies was highly stable in culture. No change in sporulation or pathogenicity of this isolate was noted even when cultures were kept in the refrigerator for 4-5 months on WA + PLX medium. The isolate of **C. personatum** was highly unstable if transfers to fresh medium were not made at 10-day intervals. Old cultures became covered with the white, non-sporulating mycelium. To maintain this isolate for longer periods without losing its ability to sporulate, a liquid medium composed of 100 ml peanut leaf-extract, 30 ml potato broth, 1 g agar, and 870 ml distilled water was developed. This medium was seeded with conidia and incubated at room temperature for 2-3 weeks: meanwhile the flasks were shaken vigorously at least once daily. After this incubation period, the fungus developed into separate non-sporulating colonies suspended in the medium. If the culture remained still, some colonies floated to the surface and began to sporulate. If stored in the refrigerator, the cultures remained viable and gave pathogenic and sporulating colonies after 7 months. To start fresh cultures, a bacterial transfer loop was used to lift out some colonies with a minimum amount of the medium and transfer them to a fresh WA + PLX slant.

Results

EFFECT OF MEDIA ON SPORULATION

The results given in Table 1 show that (1) *C. arachidicola* sporulated on all media used except on WA and SA; (2) sporulation of *C. arachidicola* was abundant enough for mass inoculation experiments on the following media: WA + PLX, OMA + PLX, OMA, and LBA + PLX; (3) *C. arachidicola* sporulation was also quite satisfactory on SA

Table 1. Effect of media on sporulation of Cercospora arachidicola and Cercosporidium personatum.

Medium	*Cercospora arachidicola*				*Cercosporidium personatum*			
	Number conidia/ field*	Conidial length† (μ) Average	Range	Growth‡	Number conidia/ field*	Conidial length† (μ) Average	Range	Growth‡
WA	0	--	--	++	0	--	--	++
WA + PLX	44.5	102	52-133	++	39.4	64	44-73	++
OMA	41.3	94	40-120	++++	37.4	78	60-93	+++
OMA + PLX	43.5	97	60-120	++++	39.6	81	60-80	+++
My. A	16.4	92	52-119	++++	0.7	21	16-20	+++
My. A + PLX	20.8	100	52-133	++++	1.2	25	20-27	+++
CMA	6.4	97	86-106	++	15.8	66	53-80	+++
CMA + PLX	20.2	93	66-160	++++	34.7	70	53-80	++
LBA	18.8	106	52-159	+++	2.5	41	24-80	++
LBA + PLX	39.9	98	60-146	+++	7.2	41	33-80	++
SA	0	--	--	+	0	--	--	+
SA + PLX	21.2	96	53-106	++++	0	--	--	++

*Averages of 10 fields (X100) in each of three replications.

†Average of 10 spores in each of three replications.

‡+ = diameter of colonies 0.5-1 mm, ++ = 1-2 mm, +++ = 2-3 mm, and ++++ = 3-4 mm.

+ PLX, My. A + PLX, CMA + PLX, LBA, and My. A media; however, on CMA medium sporulation was comparatively very low; (4) sporulation of *C. personatum* was affected differently by the various media. Excellent sporulation was found on OMA + PLX, WA + PLX, OMA, CMA, + PLX, and CMA media. It is of interest that some media; *i.e.*, My. A, My. A + PLX, LBA, and LBA + PLX, which produced good to abundant sporulation of *C. arachidicola*, gave very poor sporulation of *C. personatum*. Also, the addition of PLX to SA medium stimulated sporulation of *C. arachidicola* but not of *C. personatum*.

Jenkins (1938) reported that conidia of *C. arachidicola* measure 37-108 μ in length and those of *C. personatum* measure only 18-60 μ. From Table 1 it appears that conidial length of these fungi, especially *C. personatum*, was affected by the medium on which the fungus grew.

EFFECT OF LIGHT ON SPORULATION

Preliminary tests indicated that *C. arachidicola* sporulated normally in both continuous light and continuous darkness. On the other hand, *C. personatum* produced no conidia if grown in complete darkness, even when the medium was favorable for sporulation.

The results presented in Table 2 indicate that *C. personatum* did not sporulate at all if cultures were grown in complete darkness or if supplied with only 1 day of light at the beginning of incubation. There was a progressive but abrupt reduction in the number of conidia produced when the period of darkness increased at any time during incubation. Growing the fungus in continuous darkness for 2-3 days caused drastic reduction in conidial production and made the cultures useless as inoculum. When cultures were subjected to continuous darkness for 7 days followed by 4-5 days of continuous light, the colonies sporulated poorly and white, non-sporulating fluffy variants began to grow in the cultures. To obtain a good yield of conidia, the fungus must be grown in continuous daylight, at least during the first 6 days of incubation. Within a 7-day period, light is apparently more essential after the growth of the colonies is established than at the beginning of incubation; that is, 2 days light at the end of incubation gave 10 times more conidia than 2 days light at the beginning of incubation, and 3 days light at the end of incubation produced 14 times as many conidia as 3 days light at the beginning of incubation.

Table 2. Effect of light and darkness on sporulation of Cercosporidium personatum during 7 days incubation.

Sequence of light and dark (24-hr periods)	Avg number of conidia/field*
7 continuous light	31.27
7 continuous dark	0.00
1 light + 6 dark	0.00
6 dark + 1 light	0.80
2 light + 5 dark	0.36
5 dark + 2 light	3.76
3 light + 4 dark	0.50
4 dark + 3 light	6.98
4 light + 3 dark	9.33
3 dark + 4 light	7.77
5 light + 2 dark	10.63
2 dark + 5 light	9.97
6 light + 1 dark	28.41
1 dark + 6 light	29.14

*Average of 10 microscopic fields for each of three replications (total 30 fields).

Although darkness inhibited conidial formation of *C. personatum* in culture media, it did not inhibit sporulation of this fungus on naturally infected peanut leaves. Peanut leaflets infected with this fungus placed in a moist chamber under continuous darkness for 48-72 hr showed sporulation. However, tufts of white sterile conidiophores appeared intermixed with the dark fertile conidiophores. These sterile conidiophores did not appear under light conditions.

EFFECT OF CONTINUOUS LIGHT OF DIFFERENT WAVELENGTHS ON SPORULATION OF *C. personatum*

Exposure of *C. personatum* to continuous orange light during incubation completely inhibited conidial formation (Fig. 1). Sporulation was also much reduced under yellow, green, blue and especially red light, under which the fungus produced only about one-third as many conidia as in unfiltered light.

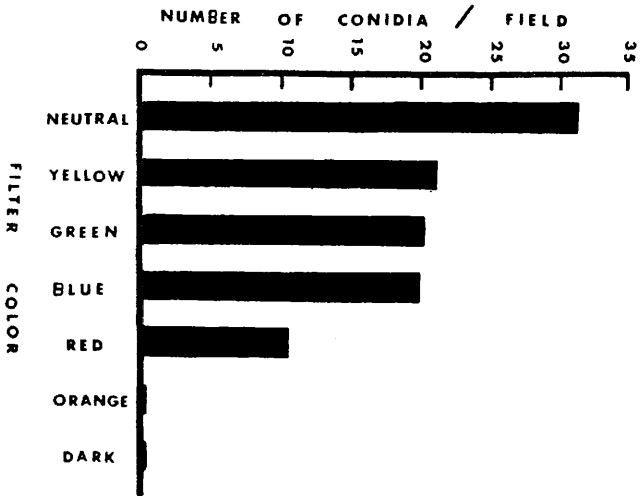

Fig. 1 Effect of culture medium and light on sporulation of two peanut leaf spotting fungi, Cercospora arachidicola Hori and Cercosporidium personatum (Beck and Curtis) Deighton.

Plates of uninoculated medium left under the various filters for 1 week, when inoculated showed no effect of the medium on germination, growth, or sporulation of the fungus.

After incubation the conidia formed under various filters germinated, grew and sporulated normally, averaging 31-35 conidia/field.

After 2-3 weeks incubation under greenhouse conditions favorable for successful infection, all plants inoculated with conidia formed under the various wavelengths of light were severely infected.

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