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INDUCING SPORULATION IN ALTERNARIA SOLANI I. EFFECT OF WATER TREATMENT

by

BHAWANI PRASAD, B. L. DUTT & B. B. NAGAICH

ABSTRACT

The sporulation was induced when fully grown cultures were given dip or spray treatment with distilled water (cold or hot) and thereafter, kept partially covered at different temperatures. Cultures dipped in cold water (4° C) for 4 minutes or sprayed with cold water (4° C) or hot water (58° C) and thereafter incubated at room temperature (13—26° C) in diffused sunlight, produced maximum number of spores within 60 hours. Incubating water treated cultures in diffused sunlight or complete darkness and age and scraping of the cultures had a considerable effect upon intensity of sporulation. The cultures yield a number of subsequent crops of spores when scraped and given dip treatment with cold or hot water, after obtaining each crop of spores.

INTRODUCTION

Laboratory screening of the potato varieties and fungicides against *Alternaria solani* (ELL. & MART.) JONES & GROUT, the early blight inciting organism, can be facilitated by obtaining satisfactory amount of spores in the cultures of the pathogen. Previously, various methods which included mutilation of the mycelium, exposing cultures to different light sources, dehydration of the medium and chemical treatment of the culture etc., have been tried for inducing and increasing sporulation of *A. solani* in culture (RANDS, 1917; KUNKEL, 1918; DILLON WESTON, 1936; MCCALLAN & CHAN, 1944; BEECHER, 1945; CHARLTON, 1953; LUKENS, 1960; GUPTA & PUSHKARNATH, 1962; LUDWIG et al, 1962; SINGH. 1967; BARKSDALE, 1968). Recently, DOUGLAS & PAVEK (1971) have reported method which consists of mixing culture strips with water, pouring the liquid on to a fresh plate of P.D.A. and incubation of the plates at 20° C in an incubator with fluorescent light. In the present paper an account of two new methods for obtaining abundant sporulation in *A. solani* is given.

MATERIAL AND METHODS

A mono-conidial isolate which produced orange to pink coloura-

Division of Plant Pathology Central Potato Research Institute, Simla, India.
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tion in P.D.A. (Patoto Dextrose Agar) culture was used in the experiments. Cultures were grown on 0.5.cm deep P.D.A. medium in glass petridishes of 9 cm diameter. P.D.A. plates were inoculated in the centre and incubated at 26° C for about 9 days. The fully grown cultures, thus, obtained were placed on the laboratory benches and were treated with cold or hot distilled water in two different ways. In the first method, hot or cold water was poured in the cultures and maintained for different durations. In the second method, the cultures were only sprayed with cold or hot water (2 ml per petridish), after opening the lids. Water treated plate cultures were stacked inside the incubators or on the laboratory benches in such a manner that they remained partially covered.

The spores were removed from the cultures by adding water to the cultures and by rubbing them with a smooth rubber fitted at the end of a glass rod. Average number of spores per plate was estimated after making spore counts with the help of a Sedgwick Cell.

Experimental

Effect of incubating water treated cultures in diffused sunlight or in darkness

The cultures were divided into four batches and were treated with cold water (8° C) or hot water (64° C). The first and the second batches were given dip treatment with water for 7 minutes while the other two batches were only sprayed. Thereafter the treated cultures were incubated at room temperature (13-26°C) in diffused sunlight. A similar set of water treated cultures were placed in complete darkness at 13-26° C (room temperature), 14° C, 20° C and 26° C. Untreated cultures placed either in light or in darkness served as control. Spore formation started 18 hours after the teatment with water and maximum spore formation occurred in cultures within 60 hours. Only the cultures given dip treatment with water at 8° C or spray treatment with water at 8° C or 64° C produced spores. Maximum spore formation occurred in the cultures placed in diffused sunlight. The cultures placed in darkness although produced spores required higher temperature for sporulation (Table I). Since the cultures placed in diffused sunlight produced maximum spores, in further experiments the cultures were incubated in diffused sunlight after they were subjected to the various treatments.

Effect of treatment with water at different temperatures

The cultures were dipped in water at 4, 14, 24, 48, 58, 68 or 78° C for 7 minutes or were sprayed. It was observed that the dip treatment was effective at 4° C to 24° C. The sporulation was completely inhibited when the temperature of water was raised to 48° C. On the

TABLE – I

Effect of incubating water-treated cultures in diffused sunlight and darkness.

Treatment of culture	Culture placed in light at 13—26° C	Spore yield in thousands Culture placed in darkness at 13—26° C	14° C	20° C	26° C
1. Dipped in cold water	660.0	12.4	—	74.0	458.0
2. Dipped in hot water	—	—	—	—	—
3. Sprayed with cold water	895.0	45.2	—	112.2	462.2
4. Sprayed with hot water	912.0	105.0	—	295.0	410.0
5. Untreated (control)	—	—	—	—	—

— indicates no sporulation

other hand in the case of spray method, the sporulation was observed when water at any temperature was applied. Highest amount of spores was, however, obtained when water was either at 4° C or at 58° C (Table II).

TABLE – II

Effect of application of water at different temperatures

Temperature of water	Spore yield in thousands Dip-method	Spray-method
4° C	948.0	1219.6
14° C	373.2	798.0
24° C	245.2	533.0
48° C	No Sporulation	994.3
58° C	No Sporulation	1297.8
68° C	No Sporulation	901.2
78° C	No Sporulation	750.0

Effect of duration of dip-treatment

The cultures were dipped in cold water (4°C) for one to sixteen minutes durations. It was observed that spores were formed even in cultures dipped for one minute only. However, maximum spore formation occurred in cultures given dip treatment for 4 minutes. If the cultures are dipped for longer durations the sporulation is decreased (Table III).

TABLE – III

Effect of dipping cultures in cold water (4° C) for different durations.

Duration (in minutes)	Spore yield in thousands
1	996.0
4	1109.6
8	932.0
12	752.0
16	516.0

Effect of age of the culture

Eight and eleven days old cultures were obtained by incubating the dishes with 2-point inoculations, for 8 and 11 days, respectively. Five days old cultures were obtained by incubating the dishes with 5-point inoculations, for 5 days. All the cultures were treated with cold water (4° C) or hot water (58° C). A similar set of untreated cultures served as control. Five to eight days old cultures produced more spores than 11 days old cultures (Table IV). Further, it is also evident that 11 days old cultures produce a heavy crop of spores, if spray treatment is given.

TABLE – IV

Effect of cold and hot water treatments on sporulation in cultures of different age.

Treatment of culture	Spore yield in thousands in water treated cultures		
	5 days old	8 days old	11 days old
1. Dipped in cold water.	1116.0	1240.4	378.0
2. Dipped in hot water.	—	—	—
3. Sprayed with cold water	1290.8	1278.2	630.0
4. Sprayed with hot water	1148.0	1349.6	915.0
5. Untreated (control)	—	—	—

— indicates no sporulation.

Subsequent crops of spores

After obtaining the first crop of spores from the cultures used in the previous experiments, a number of subsequent crops of spores were obtained from the same culture by scraping the mycelium

and repeating the dip treatment after every 60 hours duration. The subsequent crops of spores were heavier than the first crop (Table V).

TABLE V

Effect of water treatment on sporulation in different spore crops.

Treatment of culture	Spore yield in thousands			
	1st crop	2nd crop	3rd crop	4th crop
1. Dipped in cold water	1240.0	2151.6	2160.0	2193.6
2. Dipped in hot water	No Sporulation	2815.2	2733.6	2623.2
3. Untreated (control)	No Sporulation	—	—	—

DISCUSSION

Various factors viz. sunlight, temperature, composition of the media and mutilation of the mycelium etc., have been reported to induce sporulation in cultures of *A. solani* (RANDS, 1917; KUNKEL, 1918; DILLON WESTON, 1936; MCCALLAN & CHAN, 1944; BEECHER, 1945; CHARLTON, 1953; GUPTA & PUSHKARNATH, 1962; LUDWIG *et al*, 1962; LUKENS, 1966; SINGH, 1967; BARKSDALE, 1968). According to LUDWIG *et al.* (1962) some 'antisporulating agent present in *A. solani* can be removed by scraping and washing the cultures in running tap water for 24 hours. Cold and hot water treatments used in the present studies probably served as shock or injury treatments of varying degrees which stimulated the formation of spores to different extents. Further, it is also possible that the water treatments may have provided the necessary moisture for sporulation or they might have counteracted or even checked the formation of some antisporulant. The washing off of the antisporulant may be possible to some extent in the dip method but may not be so in the spray method. However, the spray treatment has been found to be better than the dip treatment in the present studies. Therefore, it is presumed that induction of sporulation is mainly because of providing additional moisture and slight injury to the mycelium.

Post treatment incubation in diffused sunlight and scraping the cultures were additional factors which increased the intensity of sporulation. Although, the beneficial effect of scraping can be explained on the basis of injury or mutilation of the mycelium, it is difficult to explain the stimulatory effect of diffused sunlight from the present information and requires further investigation.

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