INFECTIVITY AND *IN VITRO* PRODUCTION OF SWEET POTATO SCAB FUNGUS (*Sphaceloma batatas* Saw.) INOCULUM

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ABSTRACT

Sweet potato stem agar and exposure to continuous darkness supported the most abundant conidial production of *Sphaceloma batatas*. Saw. Sweet potato stem agar produced 19 times more conidia than sweet potato tuber agar and almost 3 times more conidia than potato dextrose agar. Sporulation in carrot agar peaked at 12-15 days after seeding and harvestable conidia generally declined after 18 days of incubation. Multipoint seeding of agar slants hastened sporulation and increased conidial production by almost 6 times compared to single-point seeding. Infectivity of conidia decreased with age of agar culture probably due to the drastic decline in conidial viability with age. Conidia from 1-week old agar cultures were most infective on sweet potato internodes and leaves.

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KEY WORDS: Sweet potato scab. *Sphaceloma batatas* Saw. Conidial production *in vitro*. Infectivity.

INTRODUCTION

Sphaceloma batatas Saw., the causal organism of sweet potato stem and foliage scab, is a very slow-growing fungal pathogen in artificial culture (Lao, 1978; Lao and Divinagracia, 1979). Its growth in common agar media is poor such that it could easily be overgrown by other fungi. Thus, it is difficult to isolate the fungus in pure culture. As in other fastidious fungal pathogens, investigations on the conditions that

enhance growth and reproduction of *S. batatas in vitro* are practically wanting. Information derived from these is important particularly for studies on varietal screening, genetics of host-parasite interaction, yield loss assessment, and fungicide bioassays where conidial inoculum must be supplied in sufficient quantities from time to time.

Lao and Divinagracia (1979) identified suitable techniques for isolation of *S. batatas* and cultural factors that would favor growth in agar cultures. Based on dry weights, sweet potato tuber decoction agar, carrot agar, malt agar, yeast extract agar and oatmeal agar supported significantly greater mycelial yields than six other agar media tested. Moreover, temperature ranging from 25-30°C, pH of agar media from 6.0-8.5, and exposure to either continuous light or 24-hr alternate light and dark regimes relatively enhanced mycelial growth. Although few conidia were produced on 4-week old agar cultures, Lao and Divinagracia (1979) failed to make actual conidial counts over time. This information is basically important in mass production of conidial inoculum. The study was therefore conducted to determine the influence of the kind of culture media, age of culture, period of incubation between seeding of agar slants and counting of conidia, photoperiod, and seeding method on the quantity and quality of conidia used as inoculum.

MATERIALS AND METHODS

Effect of Culture Media Used on Sporulation

The agar media tested to determine the superior medium for *S. batatas* spore production were potato dextrose agar, malt agar, glycerine agar, onion agar, carrot agar, prune juice agar, sweet potato leaf agar, sweet potato stem agar and sweet potato tuber agar.

The experiment was conducted using the completely randomized design. The culture media represented the treatments and each treatment was replicated 6 times. Treatment means were compared using the Duncan's Multiple Range Test.

Potato dextrose agar, malt agar and prune juice agar were prepared based on the procedure described by Tuite (1969); glycerine agar according to the method of Winston (1923); and carrot agar and onion agar following the process used by Paningbatan and Ilag (1981). Sweet potato leaf agar (SpLA), sweet potato stem agar (SpSA) and sweet potato tuber agar (SpTA) were prepared using 100 g of leaves, young stems, and tuberous roots of sweet potato cultivar BNAS-51, respectively. Chopped leaves, stems and tubers were separately boiled for 15-20 minutes in 500 mL distilled water and the decoctions were used in culture media preparation. Twenty grams of agar (Bacto-Agar) was used for each culture medium

and distilled water was added to make one liter.

Each culture slant was filled with 20 mL water with 500 ppm Tween 80. Fungal colonies were then gently scraped to suspend conidia. Conidia were counted weekly for 4 weeks using a hemacytometer and a microscope.

Effect of Photoperiod on Sporulation

Five light regimes (21-hr light period, continuous darkness, 9-hr light period and 15-hr darkness, 12-hr light period and 12-hr darkness, and natural daylight) were used to determine the effects of photoperiod on conidial production using carrot agar as the standard culture medium. Due to regular electric interruption of about 3 hours during the course of the experiment, exposure of cultures to 21-hr light period was made instead of a continuous light regime. Except for natural photoperiod, a 140-foot-candle fluorescent lamp served as the source of light. Ambient temperature varied from 23-29°C. Conidial yield was taken at 3-day interval until 27 days. Replication and sampling procedure were the same as those used in the experiment on the effect of culture media on sporulation.

Effect of Seeding Method on Sporulation

Two seeding methods, single point and multipoint (Tuite, 1969), were used and their effect on conidial production of the fungus grown in carrot agar slants was compared. Single-point seeding is the usual means of transferring an agar block of fungal culture either for maintenance or for inoculum increase. Multipoint seeding involves the aseptic transfer of a certain amount of water-suspended conidia or hyphal fragments of the fungal pathogen for the same purpose (Paningbatan and Ilag, 1981). Two mL of sterile distilled water was added to 1-week old test tube slant cultures of the scab fungus and the colonies were gently scraped with a wire loop. The suspension of conidia and fragmented mycelia served as the inoculum for seeding fresh agar slants. Each seeding method was replicated 6 times in a completely randomized design.

Sampling procedures and statistical analysis were the same as those used in the first two experiments.

Effect of Age of Culture on Infectivity of Conidia

Conidia harvested from 1-week, 2-week, 1-month, 2-month and 3-

month old carrot agar cultures of *S. batatas* were used as inoculum in this experiment. The conidial concentration of the different treatments was uniformly adjusted to 5×10^6 per mL sterile tap water. A drop of Tween 80 was added per 100 mL of inoculum suspension to enhance attachment of conidia to infection courts (Paningbatan, 1980). Two-month old BNAS-51 (a susceptible sweet potato variety) plants grown in 30-cm clay pots inside the screenhouse were inoculated using an atomizer until inoculum run-off. A randomly chosen vine tip per plant constituted one of 10 replicates per treatment. Only the vine tips of newly-opened leaves were tagged. Fourteen days after inoculation, lesion counts per 5 cm^2 leaf area and per 5 cm of the youngest internode next to the tagged leaf were taken. Incubation period (defined here as the time from inoculation to initial appearance of symptoms) was also observed.

Effect of Age of Culture on Conidial Germination

Conidia from 3-week, 6-week, 2-month and 4-month old carrot agar cultures of *S. batatas* were tested for germination. The conidia were suspended in sterile distilled water. Two drops of the suspension were placed on sterile glass slides and incubated for 18 hrs in petri dishes lined with moist filter paper. Each treatment was replicated 10 times. The incubation temperature ranged from 23-30°C. The percent germination (number of germinated conidia whose germ tubes were measurable and with sizes at least equal to the conidial diameter) was observed under a microscope. The actual values were subjected to arc sin transformation for analysis of variance and comparison of means.

RESULTS AND DISCUSSION

Effect of Culture Media Used on Sporulation

Among the culture media tested, sweet potato stem agar (SpSA) supported the most abundant conidial production of *S. batatas* followed by sweet potato leaf agar (SpLA) based on average conidial counts in 4 weeks (Table 1). Conidial yield in SpSA (788×10^4 conidia per mL) was almost 3 times more than that in potato dextrose agar (271×10^4 conidia per mL), almost 2 times more than that in carrot agar (491×10^4 conidia per mL) and about 5 times more than that in malt agar (160×10^4 conidia per mL). Potato dextrose agar is the general culture medium for fungi (Tuite, 1969). Carrot agar was earlier found to support the greatest mycelial growth of the scab pathogen among the 12 agar media tested by Lao and Divinagracia (1979). Although it enhanced dry mycelial yield of the fungus, carrot agar reduced conidial

Table 1. Sporulation of *Sphaceloma batatas* in various culture media at different periods of incubation between seeding of agar slants and counting of conidia.[1]

Culture Medium	Number of Conidia (x 10,000) per mL				
	Incubation Period (week)				Mean[2]
	1	2	3	4	
Potato Dextrose Agar	244.0	231.0	301.0	308.5	271.1d
Malt Agar	521.0	58.5	30.0	31.0	160.1e
Glycerine Agar	4.0	2.5	0	0	1.6i
Onion Agar	1.0	3.5	39.0	327.5	92.7f
Carrot Agar	147.5	656.0	611.0	551.0	491.4c
Prune Juice Agar	66.0	5.0	0	14.0	21.2h
Sweet Potato Leaf Agar	561.0	525.0	702.5	614.0	600.6b
Sweet Potato Stem Agar	784.0	816.5	787.5	762.5	787.6a
Sweet Potato Tuber Agar	71.5	31.5	31.5	31.5	41.5gh
Mean[2]	262.7b	259.0b	278.0a	293.5a	

[1]Each slant was filled with 20 mL water with 500 ppm Tween 80. Weekly values are means of six replicates.

[2]Means followed by the same letter are not significantly different at 5% level, DMRT.

production by 38% relative to SpSA (Table 1). The data therefore indicate that carrot agar induces vegetative growth better than asexual reproduction of *S. batatas* in artificial culture. Glycerine agar exhibited distinctively poor colony growth and least conidial yield. The pathogen's sporulation in sweet potato tuber agar was remarkably lower than that in SpSA by a factor of 19.

Averaged across the nine culture media, conidial production reached maximum values at 3-4 weeks after seeding. However, maximum sporulation over a time period was characteristically different for each medium. For instance, SpSA produced the highest number of conidia at 2 weeks after seeding whereas malt agar and carrot agar had peak conidial yields at 1 and 2 weeks after seeding, respectively.

Many culture media derived in part from natural host plants of the pathogenic fungi have been reported to induce asexual sporulation *in vitro*. Examples are peanut leaf oatmeal agar for *Cercospora arachidicola* (Paningbatan, 1980), winged bean leaf extract agar for *Pseudocercospora psophocarpi* (Pua

and Ilag, 1980), and mungbean leaf juice-oatmeal agar for *Cercospora canescens* (Mew et al., 1975). Tuite (1969) also enumerated many more culture media partly derived from host plant sources.

Effect of Photoperiod on Sporulation

S. batatas produced the most abundant conidial yield when kept continuously in the dark (Table 2). Culture slants exposed to 21-hr light period gave the least number of conidia. Exposure to continuous darkness significantly improved conidial yield by 204% relative to that of the 21-hr light regime. This indicates that light suppresses sporulation of the scab fungus contrary to its effect on sporulation of *Cercospora* spp. (Paningbatan, 1980). The result supports the observation of Lao and Divinagracia (1979) that exposure to continuous light enhanced the pathogen's ability to produce mycelia better than exposure to darkness. Thus, the light requirements of *S. batatas* for vegetative growth and sporulation *in vitro* may be different.

Based on the overall means per incubation period, the peak of sporulation was noted at 12 days after seeding. Sporulation started in 6-day old cultures but harvestable conidia started to decline at 15 days after seeding (Table 2).

Effect of Seeding Method on Sporulation

Multipoint seeding of culture slants increased conidial production of the pathogen by over 6 times relative to that of single-point method (Table 3). The peak of sporulation was observed after 2 weeks in multipoint seeding. This was one week earlier than that in single-point seeding. This result may be due to the relatively wider area occupied by numerous *S. batatas* colonies in the multipoint seeding method which could have resulted in more efficient utilization and in early depletion of nutrients in the medium. As Tuite (1969) observed, enhanced conidial production in some fungi is accompanied by nutrient depletion.

Effect of Age of Culture on Infectivity of Conidia

Infectivity of conidia declined with age of agar culture (Table 4). Conidia from 1-week old *S. batatas* cultures were the most infective inoculum based on frequency of lesions formed either on the leaf or internode. The mean number of lesions formed on leaves (19.4 lesions/5 cm^2) and internodes next to the leaves (18.9 lesions/5 cm) inoculated with conidia taken from 1-week old cultures were respectively 97 and 63 times more than those inoculated with conidia from 3-

Table 2. Conidial production of *Sphaceloma batatas* on carrot agar slants under different light regimes and incubation periods.

Treatment[1]	Number of Conidia (x 10,000) per mL[2]									Mean[3]
	Days of Incubation									
	3	6	9	12	15	18	21	24	27	
21-L	0	33.5	319.0	333.5	381.0	245.0	184.0	211.5	150.0	206.4c
CD	0	128.5	889.0	1221.0	1055.0	1005.0	420.0	467.5	461.0	627.4a
9L 15D	0	79.5	587.5	890.5	946.0	508.5	344.0	407.5	288.5	450.2b
12L 12D	0	63.5	641.0	873.5	593.5	578.5	326.5	377.5	327.5	420.2b
Natural daylight	0	27.5	334.0	616.5	523.5	708.5	205.0	410.0	271.5	344.1b
Mean[3]	0g	66.5f	554.1c	786.9a	699.8ab	609.1bc	295.9de	374.8d	281.7de	

[1]21-L = 21-hr continuous light (140 footcandles); CD = continuous darkness; 9L 15D = 9-hr light period and 15-hr darkness; 12L 12D = 12-hr light period and 12-hr darkness.

[2]Each culture slant was filled with 30 mL water to suspend conidia. Average of six replications.

[3] Means with the same letter are not significantly different at 5% level, DMRT.

Table 3. Effect of seeding method on conidial production of *Sphaceloma batatas* grown on carrot agar slants.[1]

Seeding Method	Number of Conidia (x 10,000) per mL[2]				
	Incubation Period (week)				Mean
	1	2	3	4	
Single point	28.35c	164.15b	372.50a	336.65a	225.40
Multipoint	895.00c	1641.65a	1502.50a	1815.00b	1463.54

[1]Each culture slant was filled with 20 mL of water with 500 ppm Tween 80 to evenly suspend conidia.

[2]Means of six replications. In a row, means with the same letter are not significantly different at 5% level, DMRT.

Table 4. Infectivity and incubation period of *Sphaceloma batatas* on BNAS-51 sweet potato plants after inoculation with conidia taken from carrot agar cultures of different ages.

Age of Culture	Infection Frequency [1]		Incubation Period (days)	
	Leaf (lesions/5 cm^2)	Internode (lesions/5 cm)	Leaf	Internode
1 week	19.4a	18.9a	9.0a	9.2a
2 weeks	3.4b	5.0b	9.0a	9.0a
1 month	0.7c	1.1c	9.0a	8.7a
2 months	0.3d	0.6d	8.0a	7.7a
3 months	0.2e	0.3d	8.1a	8.4a

[1] Means of 10 replications. In a column, means with the same letter are not significantly different at 5% level, DMRT.

month old cultures. Incubation period of the scab fungus was not affected by the age of agar cultures. Infectivity of conidia from 3-month old cultures was reduced by about 99 and 98% on sweet potato leaves and internodes, respectively, relative to infectivity of conidia from 1-week old cultures.

Effect of Age of Culture on Conidial Germination

Based on germination test of conidia incubated for 18 hrs, the significant reduction in conidial infectivity with age of culture was directly related to the drastic decline in conidial viability with age (Fig. 1). This could explain why conidia from 3-month old cultures hardly initiated infection on susceptible tissues (Table 4). Based on the results, conidia from 1- to 2-week old agar cultures should be used as inoculum in studies about fungicidal evaluation, pathogenic variation, yield loss assessment, and screening for disease resistance.

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