EVALUATION OF MEDIA FOR SPORANGIAL PRODUCTION AND RADIAL GROWTH OF PHYTOPHTHORA CACTORUM[1]

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ABSTRACT - Six media were compared for sporangial production and radial growth of *Phytophthora cactorum* (Lebert & Cohn) Schroeter. The media included: frozen lima bean agar (FLB), clarified V-8 juice agar (V-8 juice), fresh potato dextrose agar (PDA), Difco corn meal agar (DCM), Difco lima bean agar (DLB), and a semi-synthetic medium (GN) containing salts, thiamine, glucose and nitrate. Sporangial production was greatest on FLB, followed by V-8 juice; all isolates consistently produced more sporangia on these two media than on the other media tested. Sporangial production was substantially less on DCM, while DLB, PDA, and GN gave very poor sporangial production. The best radial growth occurred on DLB, followed by V-8 juice, FLB, DCM, PDA, and GN, respectively. There was no significant difference in radial growth on V-8 juice and DLB, nor on V-8 juice and FLB; however, growth was significantly less (P = 0.05) on all other media.

Index terms: fungus, asexual reproduction, natural media.

AVALIAÇÃO DE MEIO PARA PRODUÇÃO DE ESPOROS E CRESCIMENTO RADIAL DE PHYTOPHTHORA CACTORUM

RESUMO - Seis meios de cultura foram avaliados em sua eficiência para produzir esporângios de *Phytophthora cactorum* (Lebert & Cohn) Schroeter. Os meios incluíram: Feijão-lima-ágar (FLB) gelado; suco V-8-ágar clarificado (suco V-8); batata-dextrose-ágar (PDA) fresco; Farinha de milho-ágar, da Difco (DCM); Feijão-lima-ágar, da Difco (DLB); e um meio semi-sintético (GN) contendo sais, tiamina, glucose e nitrato. A produção de esporângios foi maior em FLB, seguido por suco V-8; todos os isolados de *Phytophthora cactorum* produziram consistentemente mais esporângios nestes dois meios. A produção de esporângios foi substancialmente menor em DCM, enquanto que em DLB, PDA e GN foi mínima. O maior crescimento radial do fungo ocorreu em DLB, seguido por suco V-8, FLB, DCM, PDA e GN, respectivamente. Não houve diferença significativa (P = 0,05) no crescimento radial em suco V-8 e DLB, nem em suco V-8, e FLB; entretanto, o crescimento foi significativamente menor (P = 0,05) em todos os outros meios de cultura.

Termos para indexação: fungo, reprodução assexuada, meios naturais.

INTRODUCTION

To promote sporangial production, *Phytophthora cactorum* and other *Phytophthora* species were grown on a variety of defined (Hendrix 1964) and undefined media (Chee & Newhook 1965, Harnish 1965, Hyre & Cox 1931, McIntosh 1969, Miller 1955, Schiffmann-Nadel & Cohen 1966, Tucker 1931 e Waterhouse 1931). Wilson (1914) found media made with grain or leguminous seeds. (e.g. peas, beans, and oat) gave the most satisfactory growth for *Phytophthora* spp. Waterhouse (1931) found that many of these same media provided good sporangial production and concluded that the moisture content of the media probably regulated the production of sporangia. Tucker (1931), in his studies of the genus *Phytophthora*, found oatmeal agar to be satisfactory for early and abundant production of sporangia; however, he concluded that no single medium was most favorable for sporangium formation, and that different isolates of the same species showed decided variation in sporangial production on the same medium. Harnish (1965), studying the effect of light on production of oospores and sporangia by four isolates of *P. cactorum*, also found considerable variability between isolates when grown on clarified lima bean agar.

Difco lima bean and V-8 juice agars are two of the most commonly used media for production of sporangia by *P. cactorum;* however, an assortment of other media are routinely employed to maximize sporangial production. While sporangial production on various media has been documented by several

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authors (Chee & Newhook 1965, Harnish 1965, Hyre & Cox 1931, Miller 1955, Schiffmann-Nadel & Cohen 1966, Tucker 1931, Waterhouse 1931), there are no studies in which sporangial production in *P. cactorum* has been quantitatively evaluated on various media. Indeed, what quantitative studies have been done to evaluate media for sporangial production by *Phytophthora* spp have usually entailed such a variety of methods for growing the organism and counting sporangia that quantitative comparisons between species and media are impossible to make. Also, many studies have only included one isolate of the respective *Phytophthora* species, thus not allowing for differences between isolates in their production of sporangia.

Considering the importance of *P. cactorum* as a plant pathogen and the frequent need to consistently produce large numbers of sporangia for taxonomic, physiological, etiological, and epidemiological purposes, a detailed, quantitative study of those media commonly used to promote sporangial production appeared warranted.

MATERIAL AND METHODS

Isolates of *P. cactorum* were furnished by the following personnel from their respective geographical locations (isolate designations follow each source): Dr. C.N. Clayton, Department of Plant Pathology, North Carolina State University at Raleigh (North Carolina); Dr. D.L. McIntosh, Research Station, Summerland, B.C., Canada (Canada); Dr. Herb Aldwinckle, Geneva Experiment Station, New York (Geneva); Dr. J. Sewell, East Malling Research Station, Maidstone, Kent, England (England); Dr. S.M. Mircetich, Plant Pathology Department, University of California, Davis, California (California); Dr. Zofia Borecki, Institute of Pomology 96-100 Skierniewice, V.I., Pomologiczna 18, Poland (Poland). Stock cultures were maintained on PDA slants at room temperature (20° - 25° C).

Pathogenicity tests were conducted both on six-day-old safflower seedlings (cv. Nebraska-10) and on four to five-month-old Grimes Golden apple seedlings in the cotyledon stage. Seedlings were placed in paper cups containing 225 ml of sterile distilled water with ca 1,200 zoospores/ml. After four days, the seedlings were removed, surface sterilized with 5% sodium hypochloride for 1 minute, rinsed twice in sterile distilled water, blotted dry, and plated to PDA medium containing 10 ppm of pimaricin, 200 ppm of vancomycin, and 180,000 units of polymyxin B sulfate.

The semi-synthetic medium (GN) was that used by Hendrix (1964) to study sterol induction of reproduction and stimulation of growth of species of *Pythium* and *Phytophthora*. Frozen lima bean agar (FLB) was prepared by steaming 142 g of frozen Fordhook lima beans in 400 ml of distilled water for 30 minutes, filtering through cheese cloth, then bringing the volume up to 1 liter. After adding 10 g of agar, the solution was autoclaved for 30 minutes. Clarified V-8 juice agar (V-8 juice) was prepared by mixing 4.5 g of $CaCO_3$ with 300 ml of V-8 juice (Campbell Co.), centrifuging at 3,000 RPM for 5 minutes and diluting 200 ml of the supernatant with 800 ml of deionized water. Agar (15 g) was added and the medium then autoclaved for 30 minutes. Fresh potato dextrose agar (PDA) was prepared by steaming 200 g of peeled, sliced potatoes in 400 ml of distilled water for 30 minutes, filtering through cheese cloth, and bringing the volume up to 1 liter. Dextrose (10 g) and agar (12 g) were added before autoclaving for 30 minutes. Difco corn meal agar (DCM) and Difco lima bean agar (DLB) were prepared according to manufacturers instruction.

Inoculum disks (6 mm diameter), taken from the margin of seven-day-old cultures of *P. cactorum* grown on DCM, were placed in the center of petri dishes with the different media. Cultures were grown at 25° + 0.5 C under continuous illumination. Light was provided by two Champion f90 t 17/w, white fluorescent tubes suspended at a height of 45 cm. Each experiment was arranged in a completely randomized design with three replicates per isolate per media.

Media were evaluated for sporangial production and radial growth after seven days of incubation. Evaluation of radial growth was determined by measuring the colony diameter. Sporangial production was evaluated as follows: three plugs (6 mm diameter) were taken in a triangular fashion 5 mm in from the margin of seven-day-old cultures. Plugs were placed in vials containing 1% lactophenol to kill mycelia and to prevent contamination. From the middle of each plug, a 1 mm wide strip was taken and cut into four 1 sq mm blocks. Each block was then placed on a slide, covered with a cover slip, and gently squashed to avoid disruption of sporangia. The total number of sporangia per block were then counted using X400 magnification. Thus a total of 12 sq mm per petri plate were counted. The average number of sporangia per sq mm of each culture was then calculated.

RESULTS

Results of sporangial production on different media by *Phytophthora cactorum* are shown in Fig. 1. There was significant difference ($P = 0.05$) among the media tested in supporting sporangial production, and also in sporangial production by different isolates (Table 1). Of the six media tested,

FLB supported maximum sporangial production, followed by V-8 juice then DCM, with significant difference at P = 0.05. DLB, GN, and PDA gave the poorest production of sporangia; however, the difference was not significant at P = 0.05 among the media in this last group (Table 1).

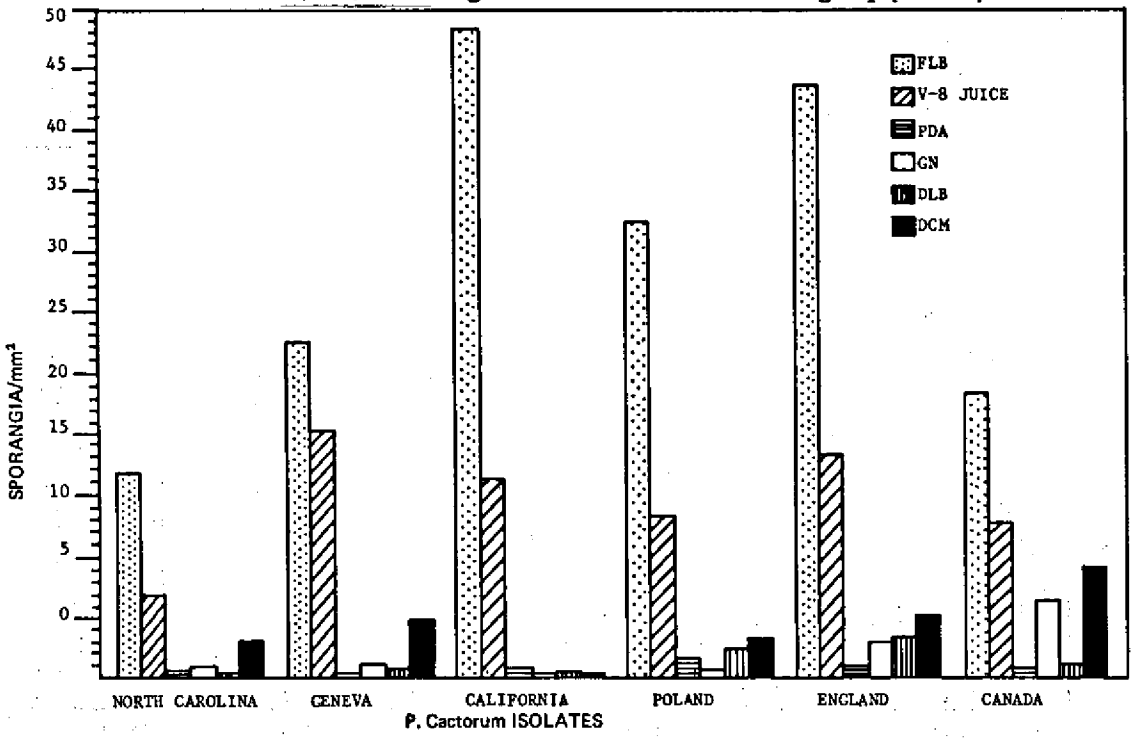

FIG. 1. Sporangial production of *Phytophthora cactorum* isolates after one week growth on frozen lima bean agar (FLB), clarified V-8 juice agar (V-8 juice), potato dextrose agar (PDA), Difco lima bean agar (DLB), Difco corn meal agar (DCM), and semi-synthetic medium (GN).

TABLE 1. Sporangial production by *Phytophthora cactorum* isolates after 7 days growth on various media.

Culture Media[a]	*Phytophthora cactorum* isolates						Media Means[b]
	England	California	Poland	Geneva	Canada	North Carolina	
	Sporangia per square millimeter						
FLB	48.2	55.4	36.7	29.8	23.3	16.7	35.0A
V-8 Juice	17.9	16.3	13.1	20.1	9.5	6.9	14.5B
DCM	5.3	0.7	2.8	4.8	9.0	3.1	4.3C
GN	3.0	0.3	0.3	0.8	6.7	0.6	2.0CD
DLB	3.3	0.2	2.2	0.7	1.0	0.5	1.3D
PDA	0.7	0.3	1.3	0.3	0.8	0.3	0.6D
Isolate Means[b]	13.1A	12.2AB	9.4BC	9.4BC	9.0C	4.7C	

[a] FLB = frozen lima bean agar; V-8 juice = clarified V-8 juice agar; DCM = Difco corn meal agar; GN = semi-synthetic medium; DLB = Difco lima bean agar; and PDA = fresh potato dextrose agar

[b] Values followed by the same letter do not differ significantly at P = 0.05.

Radial growth of *P. cactorum* on the different media are shown in Fig. 2. Radial growth was greatest on DLB, followed by V-8 juice, FLB, DCM, PDA, and GN agar, respectively (Table 2). There was no significant difference between V-8 juice and DLB, nor between V-8 juice and FLB; however, DCM, PDA, and GN were significantly (P = 0.05) less supportive of radial growth than DLB, V-8 juice, and FLB (Table 2).

DISCUSSION AND CONCLUSIONS

Studies with *P. cactorum* frequently require large quantities of sporangia to be readily availa-

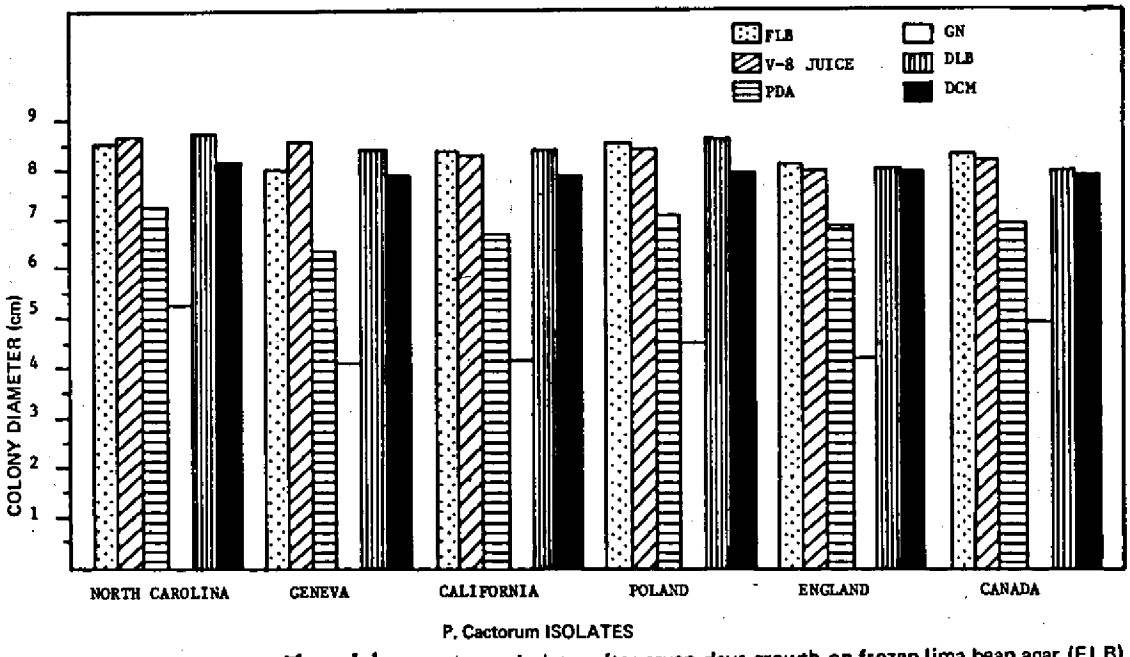

FIG. 2. Colony diameter of *Phytophthora cactorum* isolates after seven days growth on frozen lima bean agar (FLB), clarified V-8 juice agar (V-8 juice), potato dextrose agar (PDA), and semi-synthetic medium (GN).

TABLE 2. A comparison of colony diameter of *Phytophthora cactorum* isolates after 7 days growth on various media.

Culture Media[a]	*Phytophthora cactorum* isolates						Media Means[b]
	North Carolina	Poland	Canada	California	Geneva	England	
	Colony diameter (mm)						
DLB	87.6	87.5	81.5	86.3	81.6	85.1	85.1A
V-8	87.0	85.5	83.0	83.8	86.6	81.0	84.5AB
FLB	85.5	86.1	84.6	84.3	84.3	81.0	83.9B
DCM	81.6	80.4	80.4	80.0	80.1	81.0	80.7C
PDA	73.0	71.3	70.0	67.8	64.6	69.5	69.4D
GN	53.6	45.6	50.0	43.0	41.6	42.5	46.1E
Isolate Means[b]	78.1E	76.2D	74.9C	74.1B	73.5AB	73.0A	

[a] FLB = frozen lima bean agar; V-8 juice = clarified V-8 juice agar; DCM = Difco corn meal agar; GN = semi-synthetic medium; DLB = Difco lima bean agar; and PDA = fresh potato dextrose agar.
[b] Values followed by the same letter do not differ significantly at P = 0.05.

ble. While several media are routinely used for production of sporangia, consistently, large quantities of sporangia are not obtained. This is in part due to the varied effects that light, moisture, oxygen, temperature, and nutrients have on sporangial production and, in part, due to the capricious nature of the organism.

Hyre & Cox (1931), working with ten different media, found oat agar and lima bean agar to be best for maximum sporulation of *P. phaseoli*. Of the six media evaluated in our study, the most consistent and highest yields of sporangia were obtained on FLB and V-8 juice, with FLB the highest yields (Fig. 2 and 3). These findings are in agreement with Miller (1955), who reported sporulation of *P. infestans* on V-8 juice agar to be almost equal to that on fresh lima bean agar. Likewise, the poor sporangial production on PDA is in agreement with results by Chee & Newhook (1965) for *P. cinnamomi*, in which they observed that sporangia were produced sparingly and inconsistently on both fresh and Difco PDA. Of interest was the difference in sporangial production between FLB and DLB, with FLB giving almost a 30-fold increase in sporangia as compared to DLB. This is especially noteworthy since DLB is frequently used for sporangial production with many *Phytophthora* spp. A possible reason for this large difference is that in the processing of commercially prepared lima bean agar, sporangial promoting substances are removed and/or destroyed. Schiffmann-Nadel & Cohen (1966) also found poor sporangial production on eleven comercially prepared (Difco) agar culture media. Only five of the eleven media supported sporangial production of *P. citrophthora*, and of these five, only Bacto-agar and lima bean agar gave appreciable numbers of sporangia.

Tucker (1931) concluded that no single media was best for sporangial production among species of *Phytophthora*. However, from previous studies (Hyre & Cox 1931, Miller 1955, Schiffmann-Nadel & Cohen 1966) and our results, it does appear that certain media, e.g. FLB and V-8 juice, are superior for sporangial production, with possibly a universal similarity among species of *Phytophthora* as to which media best support sporangial production.

Also noteworthy is the apparent lack of correlation between sporangial production and radial growth (Fig. 3). However, this same lack of correlation was reported by Schiffmann-Nadel & Cohen (1966), with *P. citrophthora*, in which eleven media were tested and no correlation between sporangial production and radial growth was found. Apparently, separate and unique substances are required for vegetative vs. reproductive growth of *Phytophthora* spp.

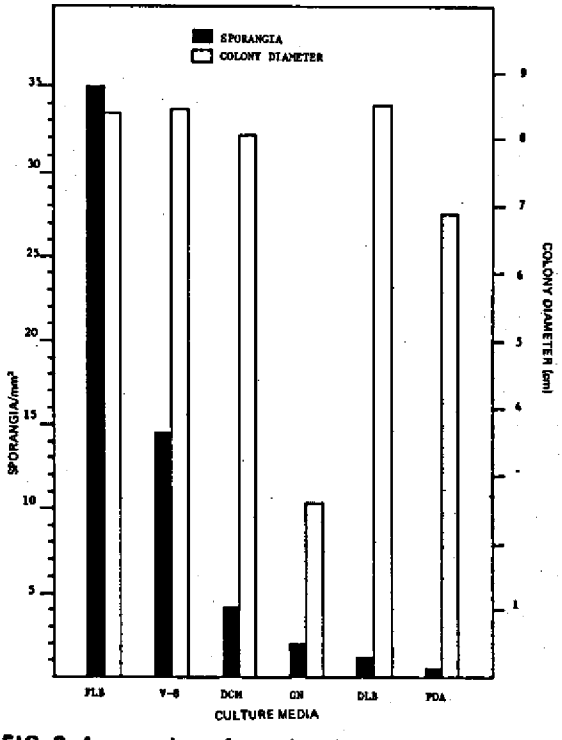

FIG. 3. A comparison of growth and sporangial production with six isolates of *Phytophthora cactorum* on frozen lima bean agar (FLB), clarified V-8 juice agar (V-8 juice), potato dextrose agar (PDA), Difco lima bean agar (DLB), Difco corn meal agar (DCM), and semi-synthetic medium (GN).

A difference in sporangial production among the isolares of *P. cactorum* used was also apparent. For example, the isolate from England showed an almost three-fold increase in sporangia when compared to the North Carolina isolate. A similar difference in sporangial production among *Phytophthora* isolates also was noted by Tucker (1931), who found that different isolates of the same species showed decided variation in that a particular isolate would sometimes produce sporangia

sparsely on a medium which was favorable for sporulation with other conspecific isolates.

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