CULTURE OF THE SWEET POTATO SCAB FUNGUS *(Sphaceloma batatas* Saw.)

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Portion of MS thesis in Plant Pathology conducted by the senior author in UP Los Baños.
Funded by Philippine Council for Agriculture and Resources Research.

ABSTRACT

Various methods were tried to isolate the causal fungus of sweet potato stem and foliage scab. The following isolation techniques were successful: (a) small pieces of diseased tissues from stems and leaves were planted in petri dishes containing either Leonian's, onion or potato dextrose agars and incubated at various temperatures; and, (b) free hand sections of plant tissues through the lesion were transferred on malt, carrot, sweet potato leaf decoction and potato dextrose agar drops on glass slides and kept in a humid atmosphere in petri dishes. The fungus grew well on sweet potato decoction agar, carrot agar, malt agar, yeast extract agar, and oatmeal agar. Its growth was better at 25 and 30 C than at 15 and 20 C on both malt and carrot agars; no growth occurred at 10, 35 and 40 C. Its growth was optimum at pH 6.0 to 8.5 and grew better when exposed to alternate light and dark and continuous light than under natural light and continuous dark. The fungus was induced to produce conidia.

INTRODUCTION

The culture of a facultative pathogen in artificial media is necessary in the investigation of various factors involved in its growth. Cultural and physiological studies have become routine for some pathogens but such is not the case with the scab fungus of sweet potato. Its inherent characteristics, such as difficulty of isolation, slow growth, polymorphic conidia and uncertain existence of a perfect stage made such studies quite rigorous and extremely difficult.

Studies that have been conducted on the scab of sweet potato did not deal thoroughly on the culture of the causal fungus. Few related studies dealt with the isolation of the fungus. Previous investigators (Goto, 1937; Anonymous, 1955; and Divinagracia, 1976) succeeded in isolating the organism. Experiments on the bud-atrophy disease of sweet potato (synonym: sweet potato stem and foliage scab) in Taiwan (Anonymous, 1955) included investigations about the effect of temperature on spore formation and pH on growth of the pathogen. This study was conducted to determine ways of isolating the fungus and determine its cultural and physiological characteristics.

MATERIALS AND METHODS

Isolation. — Sweet potato cuttings from different sweet potato varieties bearing scab lesions were collected. Different methods were employed in isolating the fungus and the most promising ones were tried several times. The diseased tissues used in all cases were rinsed with sterile distilled water (SDW) then dipped in 1:1000 $HgCl_2$ for about one minute and washed in SDW again. The methods used were as follows:

(1) Small pieces of diseased tissues from stem and leaves were planted in Petri dishes containing different kinds of artificial media and incubated at various temperatures until fungal growth could be observed (Baines, 1937).

(2) A suspension of scrapings obtained from the lesions was mixed thoroughly in varying dilutions of melted agar and placed in sterile Petri dishes (Baines, 1937).

(3) Small slices of tissues were placed on hanging drops of agar on cover slips suspended on glass rings inside a moist chamber (Plakidas, 1948).

(4) Free-hand sections of plant tissues through the lesion were transferred to malt, carrot and sweet potato leaf decoction agar drops on glass slides kept in humid atmosphere in Petri dishes (Bitancourt and Jenkins, 1939).

(5) Thin lesion sections were transplanted to the edge of

agar slants (Bitancourt and Jenkins, 1937).

(6) Scrapings of lesions were placed in Petri dish and poured with potato-dextrose or glycerin agar (Bitancourt & Jenkins, 1937).

Pure cultures obtained were transferred to agar slants of selected media and kept in suitable temperatures. All cultures were properly identified and grouped by varietal source of isolates. These served as sources of inocula for subsequent experiments.

Cultural and Physiological Characteristics. — Malt agar, unless otherwise specified, placed in flat rum bottles was used in most of these tests because in it the fungus produces a distinct reddish color which often becomes visible before other growth characters are advanced enough to be distinctive. Each bottle was seeded by planting an inoculum of 1 mm in diameter as near as possible to the center of the medium. Unless otherwise specified, the cultures were replicated eight times and incubated in continuous darkness at 30 C.

Colony diameter was measured on the 3rd, 4th and 5th week after inoculation. Dry weights of the cultures were obtained on the 5th week of incubation by removing the colony from the bottle with the use of a flattened needle or forcep. The agar adhering to the culture was removed by scraping with a scalpel before the colonies were placed on filter papers previously dried and weighed. These were then dried in an oven at 80 C for 24 hr before weighing again.

Effect of culture medium. Twelve agar media were used as follows: (1) Leonian's agar (LA), (2) glycerine agar (GA), (3) onion agar (OA), (4) Molisch medium (MM), (5) potato dextrose agar (PDA), (6) malt agar (MA), (7) sweet potato decoction agar (SPDA), (8) yeast extract agar (YEA), (9) carrot agar (CA), (10) Czapek's agar (CzA), (11) oatmeal agar (OMA) and (12) prune agar (PrA). Fifty ml of each medium were placed in flat bottles.

Glycerine agar and Molisch medium were prepared following the method described by Winston (1923). Leonian's agar, onion agar and malt agar were prepared according to the methods described by Riker and Riker (1936). Carrot agar was made based on the method described by Plakidas (1948). Potato dextrose agar, yeast extract agar, Czapek's agar, oatmeal agar, and prune agar were prepared according to the methods described by Tuite (1969).

Sweet potato decoction agar was prepared as follows: 200 g of young leaves of sweet potato were boiled in 500 ml of water, filtered through a cheesecloth and the filtrate added to agar melted in 500 ml of water. The volume of the filtrate was restored to its original

volume before it was added to the melted agar. An isolate from variety Bentong, herein designated as isolate Bentong #2, was used in this experiment.

Effect of temperature. The cultures were incubated from 10 to 40 C at intervals of 5 degrees.

Effect of pH. The pH range tried were from 5.5 to 8.5 at 0.5 unit interval. The media were adjusted to the desired pH level using 1/10N NaOH or 1/10N HCl.

Effect of light. The following light treatments were used: (1) continuous light, (2) continuous dark, (3) alternate light and dark, and (4) natural light. Cultures exposed to light were placed in an incubator with a built-in fluorescent light (3120 lux). Cultures kept in the dark were kept in the same incubator but were wrapped with aluminum tin foil then covered with black plastic bags. The covers were removed and returned every 24 hr for alternate light and dark treatment. Natural light treatment consisted of exposing cultures to natural light in a laboratory.

Inducement of conidial production. — Several trials were performed to induce sporulation (Whiteside, 1975) using 3 isolates, namely: Bentong #1, Bentong #2, and UPCA #47.

RESULTS AND DISCUSSION

The sweet potato scab fungus was isolated with extreme difficulty. The fungus also grew very slowly. Of the isolation techniques tried, only the following succeeded:

(a) Small pieces of diseased tissues from stems and leaves planted in Petri dishes containing either Leonian's, onion or potato dextrose agars and incubated at various temperatures, and,
(b) free hand sections of plant tissues through the lesion transferred to malt, carrot and sweet potato leaf decoction and potato dextrose agar drops on glass slides and kept in a humid atmosphere in Petri dishes.

The fungus was first isolated in onion agar and Leonian's agar. It was done using the first method above in which four days after incubation at room temperature (about 30 C), reddish growth developed at the margins of the lesions. The fungus was transferred to slants of the same medium after another four days. The fungus which grew very slowly gave rise to convolute and somewhat reddish brown colonies about ten days after. In another series of isolation trials, the fungus was again isolated from UPCA #47 using the second method also described above. The fungus grew on PDA drops 16 days after incubation.

The three isolates at any age possessed the same cultural characteristics as described by other workers (Bitancourt & Jenkins, 1937; Baines, 1937; Bolton & Racicot, 1951; Kemp, 1953; Kurata, 1960; Whiteside, 1975). The fungal colony at about a month old did not differ from a three-month old colony in form, color and other characteristics, except in size (Figure 1). The characteristic features of the fungus in each medium are shown in Table 1 and Figure 2. The colonies were

Table 1. Cultural characteristics of *Sphaceloma batatas* colonies grown on various agar media for 5 weeks.

Media	Color of colony [1]	Morphology of colony and other characters
Leonian's Agar	vinaceous red	convolute, compact, raised; felty on the surface
Glycerine Agar	Hay's maroon	smooth, compact, margins appressed, imparted color to medium; not felty
Onion Agar	cinnamon brown center; chalcedony yellow margins	convolute, compact, raised; some colonies felty
Molisch Medium	cinnamon brown center; chalcedony yellow margins	smooth, compact, raised; felty on the surface
Potato Dextrose Agar	cinnamon brown center; chalcedony yellow margins	convolute, compact, raised; surface felty
Malt Agar	ox-blood red center fading towards margin	convolute, compact, raised; surface covered with felt
Sweet Potato Decoction Agar	olivaceous black center; mineral gray margins	smooth, compact, margins appressed; felty on the surface
Yeast Extract Agar	Dresden brown	slightly convolute, compact, raised; surface felty
Carrot Agar	orange-vinaceous at center; and over green sides	cònvolute, compact, raised; surface with white felt; sectored
Czapek's Agar	vinaceous-cinnamon	convolute, compact, raised; surface with white felt; surface with white felt; sectored
Oatmeal Agar	chocolate brown center, the rest white	convolute, compact, raised; felty surface
Prune Agar	chestnut brown center, mineral gray margins	convolute, compact, raised; felty surface

[1]Color readings based on Ridgway's color guide.

significantly wide on sweet potato decoction agar and carrot agar, followed by those on malt agar, Leonian's agar, onion agar, prune agar, Czapek's agar, yeast extract agar, Molisch medium, glycerin agar, potato dextrose agar, and oatmeal agar, in descending order (Figure 3). The dry weights of the mycelia also varied among the media used (Table 2). The trend of dry weights did not correspond with the colony diameters because of the 'piled-up' character of the colonies. Carrot agar produced significantly heavier colonies than the rest while the lightest colony was produced on glycerine agar.

Table 2. Mycelial dry weights (mg) of *Sphaceloma batatas* on various agar media.

Medium	Mean [1]
Carrot Agar	63 a
Yeast Extract Agar	47 b
Oatmeal Agar	43 b
Leonian's Agar	26 c
Czapek's Agar	25 cd
Molisch Medium	24 cd
Onion Agar	23 cd
Potato Dextrose Agar	23 cd
Sweet Potato Dextrose Agar	23 cd
Malt Agar	22 cd
Prune Agar	21 cd
Glycerine Agar	4 d

[1] Data taken 5 weeks after seeding. Average of 4 replications. Means with common letter are not significantly different at 5% level using Duncan's Multiple Range Test.

The widest colony diameter was at 25 C followed by those at 30, 20 and 15 C for both media (Figure 4). No growth occurred at 10, 35 and 40 C. The highest dry weights on malt agar were at 25 and 30 C and very low at 20 and 15 C (Table 3). On carrot agar, however, the dry mycelial weights at 25 C were significantly heavier than those at 30, 20 and 15 C. As in malt agar, no growth took place at 10, 35 and 40 C (Table 3).

Table 3. Mycelial dry weights (mg) of *Sphaceloma batatas* on malt and carrot agars at varying temperatures.

Temp °C	Mean [1]	
	Malt agar	Carrot agar
10	0 c	0 d
15	9 b	15 c
20	25 b	18 c
25	69 a	87 a
30	89 a	33 b
35	0 c	0 d
40	0 c	0 d

[1] Data taken 5 weeks after seeding. Average of 4 replications. Means with common letter are not significantly different at 5% level using Duncan's Multiple Range Test.

The fungus was less sensitive to changes in hydrogen-ion concentrations as shown by the data on colony diameters and dry weights. Growth was optimum at pH 6.5 to 7.5 (Figure 5). The highest weight

of mycelia was obtained at pH 6.5 (Table 4). The least growth was at pH 5.5. The findings correspond with those reported in Taiwan (Anonymous, 1955) for a similar disease of sweet potato.

Table 4. Mycelial dry weights (mg) of *Sphaceloma batatas* at varying hydrogen-ion concentrations.

pH of medium	Mean [1]
5.5	51 c
6.0	86 ab
6.5	103 a
7.0	90 ab
7.5	86 ab
8.0	75 b
8.5	78 b

[1] Data taken 5 weeks after seeding. Average of 4 replications. Means with common letter are not significantly different at 5% level using Duncan's Multiple Range Test.

There were significant variations in the colony diameters of the fungus exposed to different light conditions (Figure 6). Cultures exposed to alternate light and dark and continuous light produced the optimum growth. Those exposed under natural light and continuous dark had significantly less growth than the above treatments. The mycelia from cultures exposed to alternate light and dark and continuous light were also significantly heavier than those at the latter two treatments (Table 5). This is in contrast to many other fungi which tend to have profused vegetative growth under constant dark.

Table 5. Mycelial dry weights (mg) of *Sphaceloma batatas* at different light treatments.

Light treatment	Mean [1]
Continuous Light	115 a
Continuous Darkness	73 b
Alternate Light and Dark	122 a
Natural Light	86 b

[1] Data taken 5 weeks after seeding. Average of 4 replications. Means with common letter are not significantly different at 5% level using Duncan's Multiple Range Test.

In the above tests, spore counts were omitted for failure to get a homogenous suspension of the typical ovoid-elliptical and hyaline conidia on the fourth week when the colony was large enough to be plugged. It was observed that, at this age, there were very few spores produced.

In the microcolonies produced by the Bentong #1 isolate, the typical ovoid and hyaline conidia were absent even after 24 hr while in Bentong #2 and the UPCA #47, the typical ovoid hyaline conidia was released in about 18 hr.

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Fig. 1. Malt agar cultures of *Sphaceloma batatas* (2 1/2X).

Fig. 2. Characteristic of *Sphaceloma batatas* on 12 agar media grown in continuous darkness at 30 C for 5 weeks (1/10X).

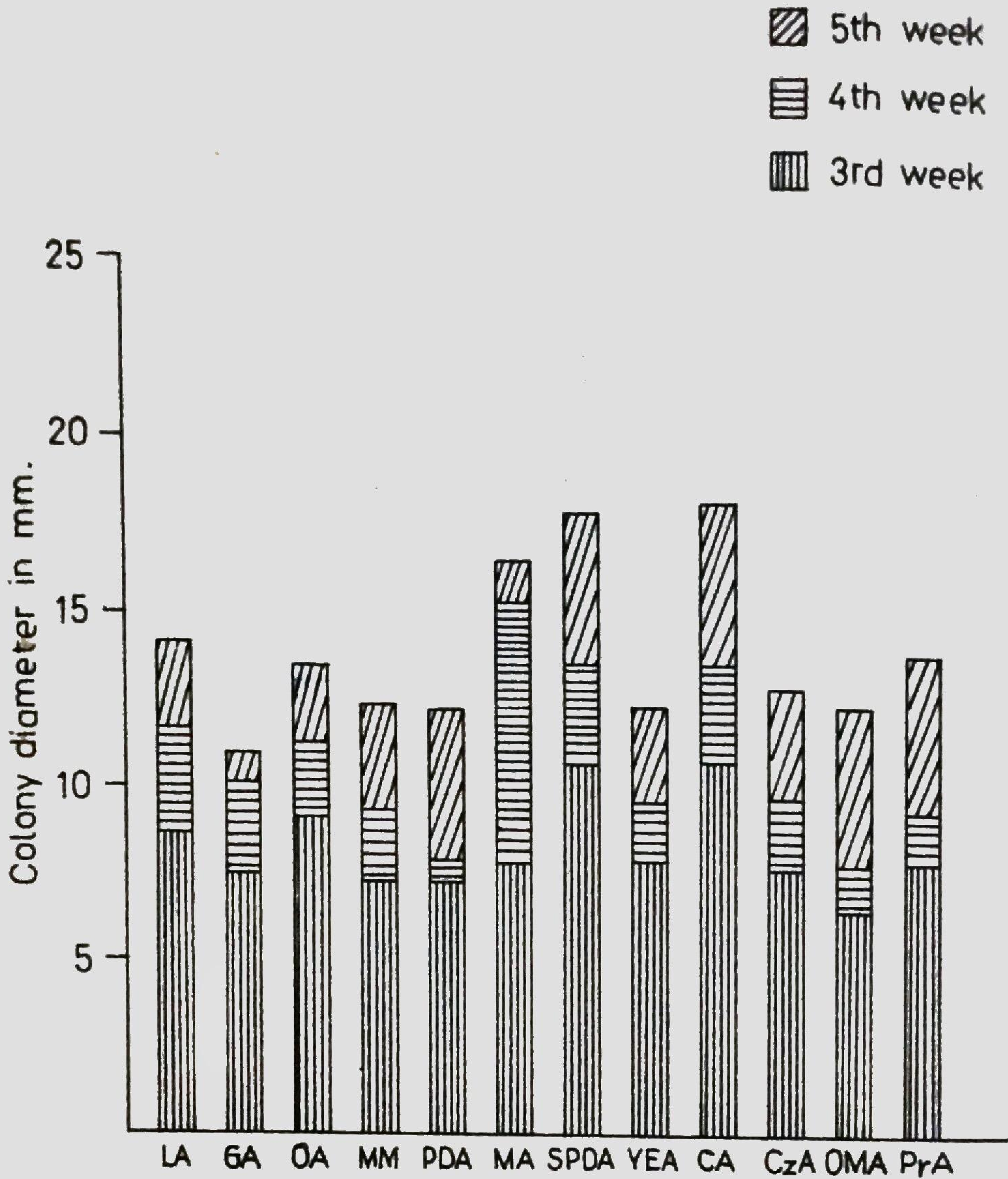

Fig. 3. Colony diameter of *Sphaceloma batatas* on various agar media.

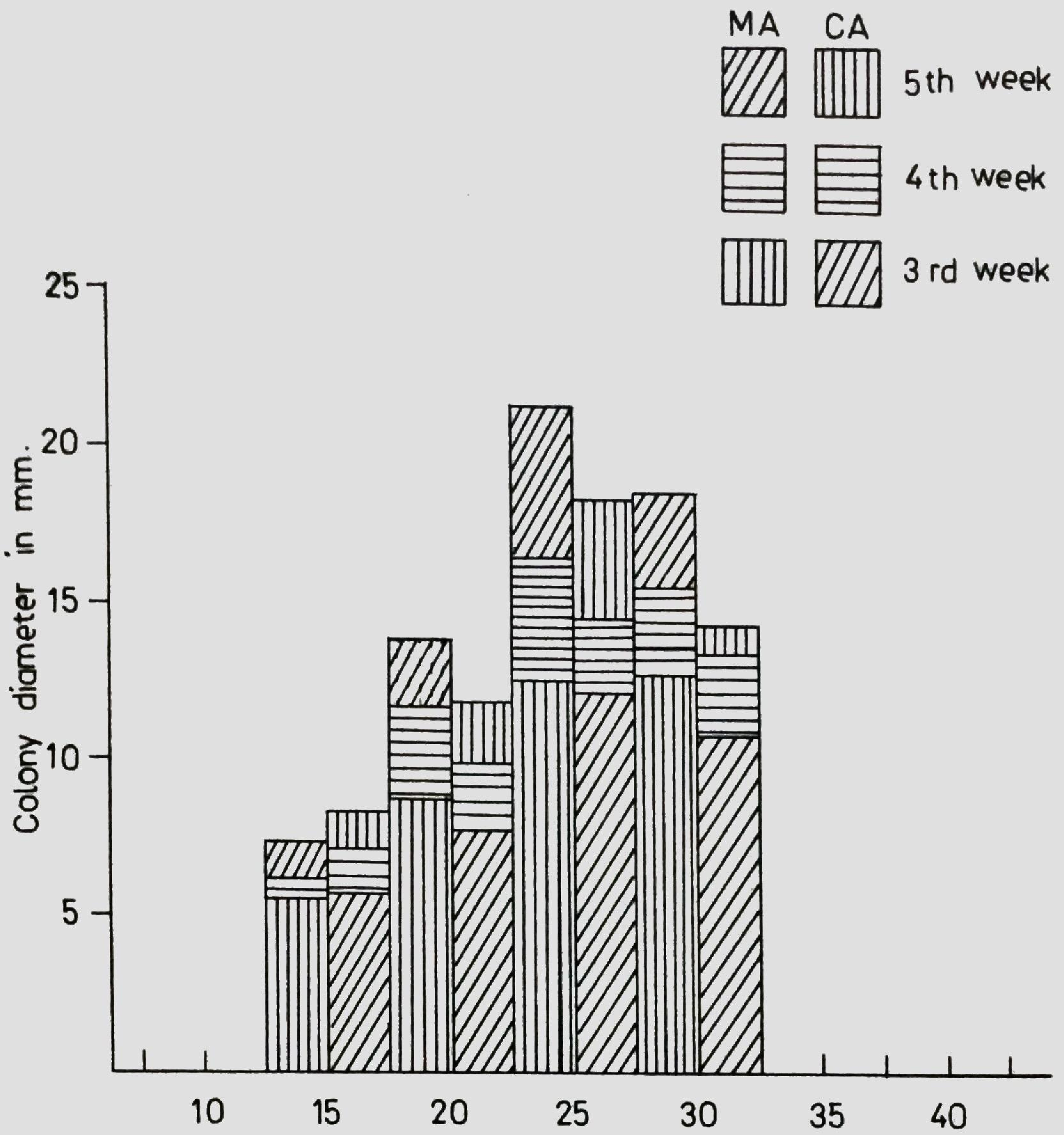

Fig. 4. Colony diameter of *Sphaceloma batatas* at varying temperatures in malt agar and carrot agar.

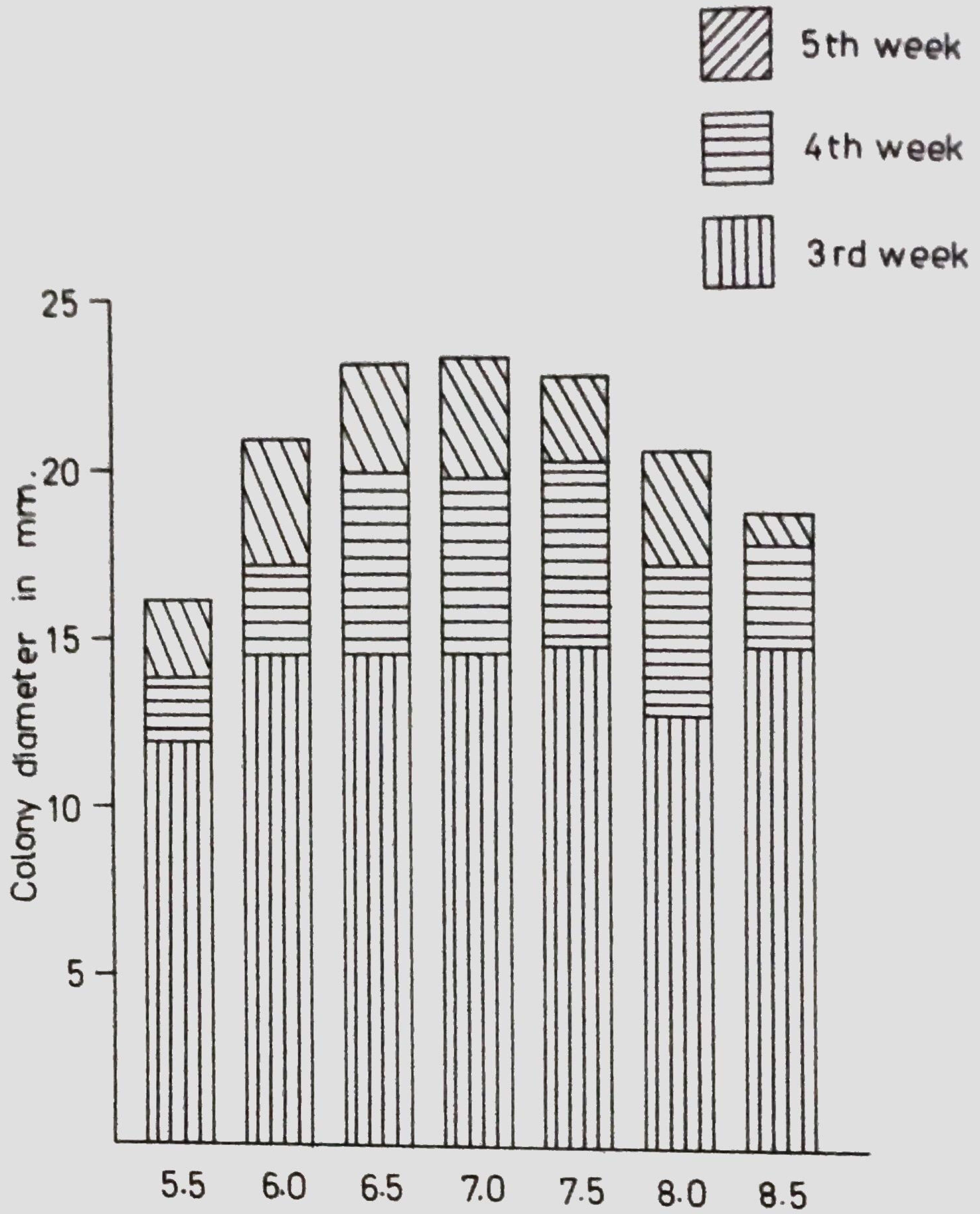

Fig. 5. Colony diameter of *Sphaceloma batatas* at varying hydrogen-ion concentrations.

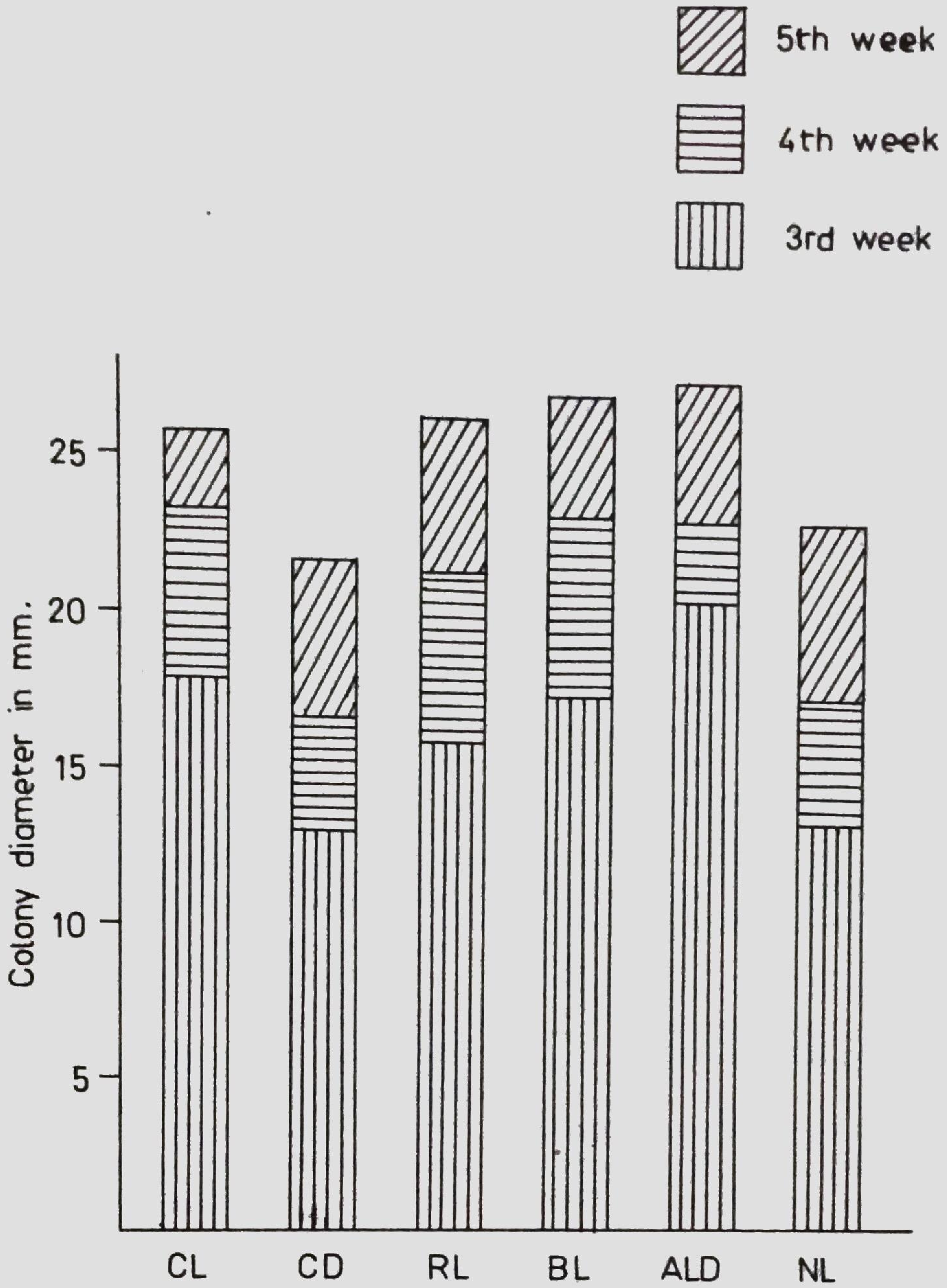

Fig. 6. Colony diameter of *Sphaceloma batatas* at different light conditions.