BIOLOGICAL STUDIES OF DIAPORTHE VEXANS (SACC. AND SYD.) GRATZ.

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THESIS

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BIOLOGICAL STUDIES OF *DIAPORTHE VEXANS* (SACC. AND SYD.) GRATZ.

by

EDWARD EUGENE BUTLER

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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E. E. B.

I. INTRODUCTION

Present information reveals that Diaporthe vexans (Sacc. and Syd.) Gratz is pathogenic only to the eggplant, Solanum melongena L. The organism is present wherever the eggplant is grown, sometimes causing infections that result in serious crop losses. All parts of the plant above ground are susceptible to infection by Diaporthe vexans. The fruit is especially susceptible in the field, in transit, and in storage. The names most commonly used to designate the disease are damping-off, stem blight, leaf spot, and fruit rot, depending upon the plant part affected.

Several articles, Harter (1914), Edgerton and Mooreland (1921), Nolla (1929), and Palo (1936) have been written regarding the symptoms and control of the disease and the taxonomy of the fungus. Existing literature evidences, however a dearth of information relative to the physiology of Diaporthe vexans.

This thesis will deal mainly with the effect of pH and temperature upon growth, and light upon the growth and sporulation of the organism. Studies have been included to ascertain the ability of the organism to assimilate various sugars and various forms of nitrogen. Little attention has been given to the symptoms, epiphytology, control and other aspects of the disease except where there is need in solving the problems which are purely mycological.

II. REVIEW OF LITERATURE

A. History and Nomenclature of the Fungus.

Diaporthe vexans (Sacc. and Syd.) Gratz has appeared in the literature since the late 19th century under the following names:

1891 Phoma solani Hals. N. J. Agr. Exp. Sta. 12th Ann. Rpt. 1891, p. 277. nom. nud. Sacc. 1895, Syll. Fungorum, 11:490. Not Cooke and Hark. 1884, in Grevillea, 13:16. (fide Harter).

1891[1] Phyllosticta hortorum (not Speg.).

1899 Phoma vexans (Sacc. and Syd.) Syll. Fungorum, 14:889.

1905 Ascochyta hortorum (Speg.) C. O. Smith. Delaware Agr. Exp. Sta. Bul. 70.

1914 Phomopsis vexans (Sacc. and Syd.) Harter. Jour. Agr. Res., 2:331-338.

1942 Diaporthe vexans (Sacc. and Syd.) Gratz. Phytopathology, 32:540-542.

Halsted (1891) reported a fungous disease of Solanum melongena which caused a damping-off of eggplant seedlings in the hot-bed. He assigned Phoma solani Hals. as the casual organism. He did not make a diagnosis but made reference to the fungus as follows: "The fungus that causes the death of the subject is so small that without the aid of a microscope nothing more is seen of it than a number of black specks embedded in the substance of the diseased portion." "The dark spots are spaces where the fungus threads have formed multitudes of spores inclosed in a dense mass of surrounding substance."

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[1] Explanation given in text, p. 3, 5.

Saccardo (1895), who evidently secured specimens from Halsted, gave the following brief morphological description of Phoma solani Hals: "... perithecia innata, depressa. Oblonga, late sperta; sporulae oblongae, stipitellatae." Later Saccardo (1899) changed the epithet, Phoma solani Hals., to Phoma vexans (Sacc. and Syd.) upon discovering that the name Phoma solani (Cooke and Hark.) had been previously employed for another organism.

Halsted (1891), in addition to describing an organism causing damping-off of eggplant seedlings, also, attributed Phyllosticta hortorum Speg. as the causal agent of a fruit rot of eggplant. Referring to Phyllosticta hortorum he states, "The fungus which causes large brown and lifeless patches in the leaves is one that has been known for considerable time...." Spegazzini (1881) described Phyllosticta hortorum as the fungus causing a leaf spot of Solanum melongena in Italy. The pycnidia measured 80-90 microns in diameter and the conidia 4-6 x 2-2.5 microns. Soon after Spegazzini's diagnosis, Phyllosticta hortorum was assigned as the causal agent of a leaf disease of eggplant in America, with symptoms as indicated by Halsted.

Smith (1904) described Ascochyta lycopersici as causing a leaf spot of Solanum melongena. He stated that the fungus differed from Phyllosticta hortorum in character of leaf spot, septation of spore, and size of spore. Smith noted that in material collected by Halsted the spores of Phyllosticta hortorum agreed in size with those given by Saccardo, i. e., 4-6 x 2-2.5 microns, while those of the Ascochyta he found were

6-12 x 3.5-4 microns.

The following year (1905), Smith described a leaf spot disease of eggplant assigning Ascochyta lycopersici Brun. as the causal organism. However, he noted changes in the character of the spot, the number of pycnidia produced, and alteration of spore size from the previous year. The following statements made by Smith (1905) indicate that the fungus which he had under observation in 1903 and 1904 was possibly Phyllosticta hortorum Speg. "A disease of eggplants has been described by Halsted of New Jersey which seems to be quite similar to this trouble. In fact, were it not for the fact that the fungus described in this paper has uniseptate spores I should regard them as identical. The septate spore characteristic does not always show plainly and can be easily overlooked, and in fact, in some cases could not be found at all in specimens that were examined." "The pycnidia containing the septate spores are only found in old cultures and then not very abundant." "The writer is quite certain that the fungus described in America as Phyllosticta hortorum Speg. is the same as Ascochyta lycopersici Brun. He has never seen an authentically determined specimen of Ascochyta lycopersici for comparison."

Despite the apparently weak evidence, Smith proposed that the name be changed to Ascochyta hortorum (Speg.) Smith. However, since the new name was based upon somewhat uncertain taxonomic characters, pathologists in general did not accept the suggested change from Phyllosticta hortorum Speg. to Ascochyta hortorum (Speg.) C. O. Smith.

Harter (1914) believed that Phyllosticta hortorum, Phoma solani, and Ascochyta hortorum were the same fungus. To ascertain the truth of his theory he conducted cross-inoculation experiments with Phyllosticta hortorum and Phoma solani. His results showed that the two organisms were identical morphologically and that both organisms were capable of producing a fruit rot, stem blight, and a rapid damping-off of eggplant seedlings. The symptoms produced on all parts of the eggplant by the two fungi were indistinguishable and the incubation period was about the same. He also made thorough morphological studies of fungi identified as Phyllosticta hortorum and Phoma solani and in each case found the organisms to possess characteristics typical of the genus Phomopsis.

Harter, doubting somewhat, that Phyllosticta hortorum Speg. occurs in the United States, sent typical specimens of the fungus to Spegazzini for examination. Spegazzini pointed out the main differences between his fungus and the fungus forwarded by Harter, stating that Harter's specimens were not Phyllosticta hortorum. Hence, Harter concluded that Phyllosticta hortorum had not been recorded in this country. Thus, the diseases of Solanum melongena attributed to Phyllosticta hortorum, Phoma solani, and Ascochyta hortorum from 1881 until approximately 1914 were in reality probably due to Phomopsis vexans (Sacc. and Syd.) Harter. Harter believed that the results obtained by Smith (1904) (1905) were due to the presence of an Ascochyta and, at the same time, the so-called Phyllosticta hortorum.

Edgerton and Mooreland (1921) found a species of Diaporthe appearing frequently on agar cultures of Phomopsis vexans, but they were unable to infect eggplant seedlings with the ascospores. Gratz (1942) found perithecia in six week old cultures of Phomopsis vexans growing on 2% potato dextrose agar. He was able to induce typical leaf and stem lesions by inoculating seedlings with suspensions prepared from crushed perithecia, or with ascospore suspensions made from the exudate taken from the tip of the perithecial beaks. He did not, however, observe perithecia growing upon the host. Based upon the results of these successful inoculations of eggplant seedlings with ascospores, Gratz proposed the binomial Diaporthe vexans (Sacc. and Syd.) Gratz as the perfect stage of Phomopsis vexans (Sacc. and Syd.) Harter.

B. Description of the Fungus.

1. Vegetative structures. Palo (1936) described the mycelium as consisting of "...fine hyaline, septate hyphae which ramify within the diseased parts of the host tissues. The hyphae vary from 1.5 to 3.3 microns in diameter. In artificial culture some of the hyphal threads may grow as large as 4.2 microns in diameter." In general, hyphal measurements are not considered of taxonomic importance and are not given detailed consideration by other mycologists or plant pathologists.

2. Reproductive structures. Asexual:-Since the asexual reproductive structures are, perhaps, solely responsible for the propagation of Diaporthe vexans they have been given much attention by those who have studied the organism.

The pycnidia were first described by Harter (1914) as "irregularly shaped or flattened with a well developed beak." Edgerton and Mooreland (1921) state that the pycnidia are variable in shape depending upon the part of the plant upon which they grow and upon the amount of moisture present. They describe them, however, as flattened but sometimes more or less sub-globose, the top often prolonged into a beak. Palo (1936) describes the pycnidia as sub-globose but states that some may be lenticular-like or flask-shaped, while others are irregular and beaked. Nolla (1929) states that the pycnidia are typically beaked and quotes the original description given by Harter. In his monograph of the genus Phomopsis, Diedicke (1911) states that the pycnidia of most species are flat when covered with the host tissue, becoming conical and beaked when exposed. They may, however, remain flat.

Typically, pycnidia contain a single cavity, (Stevens, 1925), but Edgerton and Mooreland (1921) have, upon occasion, found compound ones which often have more than one beak at the top. They, also, found inclusions projecting into certain pycnidia from the base.

In respect to the host tissue, the pycnidia may be innate or erumpent. Harter (1914) gave the following description: "On the foliage and stems pycnidia loosely gregarious in more or less definite spots, on fruit compact, at first buried, later erumpent...." According to Edgerton and Mooreland (1921) the pycnidia are generally embedded or partly so in the host tissue but if very moist conditions are present during development they may all be on the surface. Palo (1936) states that

the pycnidia are first formed within the host tissues but as they grow in size they break through the epidermis. According to Nolla (1929) they are formed just beneath the epidermis of the host, their beaks, when developed, extending beyond the surface.

Edgerton and Mooreland (1921) noted that beaks are usually better developed on the pycnidia on old stems. They also give the pycnidial wall measurements as varying in thickness from 10 to 30 microns with the thicker portion of the wall at the base of the pycnidium. Harter (1914), on the other hand, found a "thick, black wall at the top of the pycnidia becoming less noticeable at the base." The latter is given some confirmation by Diedicke (1911) who noted that a thickened sclerotia-like wall occurs typically at the apex of many species of Phomopsis.

Considerable variation exists in the size of the pycnidia as reported by various workers. These differences are recorded in Table I.

Evidence points to the fact that a true ostiole does not exist in the pycnidia of Diaporthe vexans. Harter (1914) makes no reference to a pycnidial opening. Edgerton and Mooreland (1921) seem to consider the beak and pycnidial opening as synonymous terms. They state "...the larger pycnidia may have more than one opening or beak at the top." According to Palo (1936) the pycnidia do bear ostioles; however, he is not supported in this contention by other workers. In regard to the genus Phomopsis, Diedicke (1911) states that

various types of mouths are present among the species, and Clements and Shear (1931) use "Pycnidia without ostiole" as a distinguishing feature. Also, Saccardo (1906) states that the pycnidia of the genus Phomopsis are not regularly ostiolate.

Two types of spores are formed within the pycnidia; short elliptical spores, Phoma or Phyllosticta type (so-called because of the resemblance to spores of the genera Phyllosticta and Phoma), and long narrow spores which often appear boomerang-like (Fig. 1). The latter type are referred to by various authors as Septoria-type, Phlyctaena-type, and Stylospores. Harter (1914) described both types as follows: "...pycnospores subcylindrical, somewhat acute...continuous, hyaline, 2-guttulate, rarely 3...stylospores filiform, curved, rarely straight...." Edgerton and Mooreland (1921) refer to the elliptical spores as Phyllosticta type, describing them as small, elliptical in shape, single-celled, hyaline, and usually containing two prominent guttulae. Because of their apparent similarity to the genus Phlyctaena they applied this name to the filiform spores which they described as "...hyaline, continuous and usually sickle-shaped, though they may be nearly straight at times." The two spore types may be borne in separate pycnidia or together in the same pycnidium

Although various workers have reported variation in spore size (Table I), they concur in regard to the morphology of the two types.

Evidently Harter (1914) and Edgerton and Mooreland (1921)

Table I. SPORE AND PYCNIDIAL MEASUREMENTS[1] RECORDED BY VARIOUS WORKERS

Source	Phoma-type spores	Stylospores	Pycnidia		
			Location	Diameter	Height
Carter (1914)[1]	5-8 x 2-2.8	Length: 13-28	Leaves & stems Fruit	60-200 100-250	
Edgerton (1921)	6-8 x 3-4	18-32 x 1-1.8	Leaves & fruit Old stems (dead) Pure culture	125-300 700 -	110-250 800-950 up to 1200
Nolla (1929)	5-8 x 1.3-3				
Palo (1936)	5-8 x 1.7-3.6	13.3-18.3 x 1.0-1.7 Avg. 15.6 x 1.3	Leaves Stems Fruit Pure culture	72-162 114-228 120-245	76-144 92-304 94-298 up to 1500

did not experience difficulty in securing stylospores, but Nolla (1929) and Palo (1936) report that stylospores are not frequently found. There is general agreement, however, that stylospores are more abundant on old stems and mummied fruit.

Harter (1914) referred to the conidiophores as "awl-shaped" and described them as simple, short, straight or slightly curved, hyaline and continuous. This stout, awl-shaped conidiophore, according to Diedicke (1911), is characteristic of the genus Phomopsis but, he states that only the Phomatype spores are borne upon these structures, the stylospores being borne upon shorter, conical shaped conidiophores.

Sexual:-Gratz (1942), who proposed the binomial Diaporthe vexans for the perfect stage of Phomopsis vexans (Sacc. and Syd.) Harter, described the perithecia, asci, and ascospores as follows: "...perithecia, occurring usually in clusters, were from 130 to 350 microns in diameter. The beak-like structures, ostioles, were carbonaceous, sinuate, irregular and from 80 to 500 microns long. The asci produced in these perithecia were 8-spore, clavate, sessile, 28-44 x 5-12 (av. 36 x 8.9) microns, hyaline, with thin walls, and apex slightly thickened and pierced by a narrow pore. The spores were biseriate, hyaline, narrowly ellipsoid to bluntly fusoid, quite uniform in size, 9-12 x 3.0-4.4 (av. 10.8 x 3.7) microns, bicellular, constricted at the septum with each cell usually containing two guttulae. In all respects, this fungus, isolated on two occasions from eggplant stems exhibiting characteristic symptoms of "tip over," from Marion County, Florida, is, in appearance, a typical Diaporthe."

3. The Organism in Pure Culture. Diaporthe vexans is not nutritionally fastidious, growing well on most ordinary culture media. Palo (1936) grew the organism upon a number of culture media. These are listed in Table II with the relative ability of each to produce pycnidia and to support mycelial growth. His investigation also showed that growth on all agar media was white, distinctly lobed at edges of colonies, and in some cases distinctly zonate. He also reports that growth was flat in general, but aerial tufts of hyphae were usually produced on papaya and potato dextrose agar.

TABLE II. RELATIVE VALUES OF VARIOUS MEDIA (Palo 1936) FOR PRODUCTION OF PYCNIDIA AND MYCELIUM.

Medium	Mycelium	Pycnidia
Steamed corn meal	3[a]	4
Steamed rice	3	4
Steamed eggplant stems		4
Steamed string beans		4
Papaya agar	4	2
Potato dextrose agar	4	2
Oatmeal agar	3	3
Prune agar	3	3
Eggplant agar	3	3
String bean agar	2	1
Corn meal agar	1	1
Cucumber seed agar	1	1
Leonian's m. extract agar[b]	3	3

[a] (1) Poor; (2) Fair; (3) Good; (4) Excellent.

[b] KH_2PO_4 -1.2 gr., $MgSO_4$-.6 gr., Peptone-.6 gr., Maltose-6.0 gr., Malt extract-6.0 gr., Distilled H_2O-1000 cc, Agar 1.5-2.0%.

Howard and Desrosiers (1941) inoculated sterile vegetative structures of a large number of field and garden crops and found that the fungus would grow on all substrata tried. They also found that pycnidial production was greater on cauliflower petioles, carrot roots, and beet roots than on eggplant tissues. Nolla (1929) grew the organism upon oatmeal agar, corn flour agar and 1% dextrose nutrient agar. He reported that luxuriant growth was obtained with the latter medium, filling a dish, 90 mm. in seven days; and that he obtained poor growth upon oatmeal agar and corn flour agar. Nolla states that "Stromata began to develop in these media on the fifth day and large numbers had appeared on the twentieth day. The stromata are black. One to several pycnidia arise in each stroma in these media. The pycnidia are typically beaked." Harter (1914) agrees that the fungus forms a stroma in culture and produces beaks 1 mm. or more in length.

Edgerton and Mooreland (1921) found that the fungus produces both kinds of spores in culture and Harter reported stylospore formation on sterile corn meal. The investigations of Nolla and Palo (1936), however, showed that stylospores were almost lacking on agar cultures although the Phoma-type were produced abundantly on many media. There is no record of stylospore germination.

Edgerton and Mooreland give the optimum temperature for growth as "around 29° C." but state that good growth occurs between 21 and 32° C. Chupp (1925) gives "approximately 85° F." (29.4° C.) as the optimum temperature, remarking that it will

grow at slightly higher and considerably lower temperatures. Palo (1936), who did his experimental work at Manila, Philippine Islands, incubated his cultures at room temperatures. The average temperature at Manila for the months of January, April, July, and October over a period of 47 years was recorded as 26.8° C. (Yearbook of Agr. U.S.D.A., 1941).

Edgerton and Mooreland (1921) found that the fungus grows well on slightly acid or slightly alkaline media but not on strongly acid media.

Nolla (1929) stated, "We have not found much variation in our fungus." He discovered two kinds of spots on the host; from each type of lesion not less than fifteen isolations were made and compared on three media. Nolla did not observe differences that would indicate the presence of two strains. Edgerton and Mooreland, however, after studying a hundred or more different cultures, noted variation in manner of infection, rate and manner of growth and in ratio of the two kinds of spores.

4. Susceptible Hosts. As pointed out earlier, the eggplant (Solanum melongena L.) appears to be the only known host of Diaporthe vexans. In cross inoculation experiments the following plants were inoculated with spore suspensions of the organism giving negative results: Lycopersicon esculentum, Capsicum annuum, Datura tatula, Ipomoea batatas (Harter, 1914), Solanum indicum, Solanum pyroconthum, Solanum mammosum (Howard and Desrosiers, 1941), and Solanum tuberosum

(Edgerton and Mooreland, 1921). The latter authors inoculated unknown species of Solanum but they were unable to induce infection.

III. EXPERIMENTAL

A. Materials and General Methods.

1. Source of Isolates. Cultures of Diaporthe vexans used for experimental work were isolated from fruit and stems of diseased specimens of Solanum melongena.

Stems of Solanum melongena bearing many pycnidia of Diaporthe vexans were obtained from fields near Gainesville, Florida. The fungus was isolated from these stems and grown in pure culture. From this culture a single spore was isolated which produced the strain used throughout the course of this work. A second strain similarly isolated from fruit purchased on the local market was carried in vitro but not used in each experiment. The strain isolated from stems will be referred to in this paper as A and that from the fruit as B.

A section of stem, 50 mm. in length, heavily infested with pycnidia, was immersed in a 1-1000 solution of mercuric chloride. At the end of 30 seconds the section was removed and rinsed twice with sterile distilled water. Individual pycnidia were removed with a dissecting needle under low magnification of a binocular dissecting microscope. Five pycnidia were placed in a 4 c.c. sterile water blank and crushed with a sterile glass rod. A 4 mm. wire loop of the

resulting spore suspension was added to each of 15 tubes containing 10 c.c. of potato dextrose agar[1] which had been cooled in a water bath to approximately 42° C. The tubes were rotated rapidly to disperse the spores and the agar suspension was poured into Petri dishes, and incubated at 26° C.

Eggplant fruits showing typical rotting but not fruiting bodies of the organism were also used for isolation purposes. An attempt was made to free the surface of the fruit from contaminating organisms by the following method: the fruit was washed thoroughly with a solution of sodium lauryl sulfonate and rinsed with sterile distilled water. The surface was then flooded with a 1-50 solution of 10% Roccal (Alkyl-dimethyl-benzyl-ammonium chlorides) by using saturated cheese cloth. The disinfectant was removed after three minutes with sterile distilled water. The fruit was then cut into two parts with a sterile scalpel and small pieces of diseased tissue removed and placed directly on potato dextrose agar in Petri dishes. Ten plates were prepared in this manner, two tissue sections per plate, and incubated at 26° C.

Pure cultures of the organism were obtained by the two methods just described.

2. Single Spore Technique. A method similar to that outlined by Thom and Raper (1945) was used to isolate single spores for preparation of stock cultures.

A single pycnidium, from a culture on potato dextrose

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[1] 120 gr. potatoes, 20 gr. dextrose, 18 gr. agar, 1000 c.c. H_2O.

agar, was crushed aseptically with a glass rod in a test tube containing 2 c.c. of sterile distilled water. Subsequent dilutions were made in 4 c.c. sterile water blanks until a 2 mm. transfer loop contained an average of one to three Phoma-type spores. The Van Tieghem cell provided an excellent aid for observing each loop of suspension under high-power of the microscope. The drop of spore suspension was placed near the center of a clean cover glass and inverted over the glass ring. Addition of petroleum jelly to the upper and lower edges of the ring served to prevent evaporation of the liquid.

A 2% agar solution was cleared by the method given by Riker and Riker (1936) and a plate was poured containing 10 c.c. of agar. On the bottom of the Petri dish several circles (approx. 7 mm. in diameter) were drawn with a wax pencil. A drop of spore suspension was then placed on the agar above each circle and the plate incubated at 26° C. At four hour intervals the area within each circle was searched under low-power of a microscope for germinating spores. When a circle was found containing only one germinating spore, the location was marked with ink on the surface of the plate. The section of agar above the ink was removed with a small razor-blade-type scalpel and transferred to a freshly poured plate of cornmeal agar. The presence of a single germinating spore was confirmed under low-power of a microscope.

3. Infection Experiments. Seedlings of Solanum melongena used for inoculation purposes were grown in a sandy soil in four-inch clay pots. The pots and soil were sterilized in an

autoclave for two hours at 15 pounds pressure. The seed, before planting, was treated with tetramethyl thiuram disulphide (arasan) as a protectant against seed-borne infection.

Three separate inoculation experiments were conducted. In the first, a five millimeter cube of agar from a rapidly growing culture of the organism on potato dextrose agar was placed near the middle of each seedling leaf. Each leaf was previously punctured with a sterile dissecting needle at the point where the agar was to be placed. The control plants were treated in the same manner, a five millimeter cube of sterile potato dextrose agar being placed over the puncture. Eighteen 24-day-old seedlings were used; nine were inoculated, and nine were used as control plants. The pots bearing seedlings (three per pot) were placed in a 12-inch clay dish containing one-half inch of water. The pots were covered with a bell jar and incubated in the greenhouse. The bell jar was removed after 48 hours. Temperature was recorded with a Taylor thermograph.

Thirty 21-day-old seedlings, grown under the same conditions as those for the first experiments, were used in the second tests. Each pot contained three seedlings; 21 were inoculated and nine were used for control plants. A spore suspension in sterile distilled water was prepared by crushing pycnidia grown on sterilized carrot roots. Five c.c. of the supernatant fluid containing the spores was aseptically pipetted into a sterile atomizer jar. After establishing the fact that spores were being released from the atomizer, the

seedling stems and leaf surfaces were sprayed with the suspension. The control plants were sprayed with sterile distilled water. The pots were then placed in 12-inch clay dishes, one per dish, containing one-half inch of tap water and each covered with a large lantern-chimney type glass, moist chamber. The sides of the chambers were lined with water-saturated paper towels; petroleum jelly was applied to the upper edges and the top covered with a glass plate. The seedlings were incubated in the greenhouse without artificial light at 21° C. At the end of 48 hours the glass plates were removed.

In the third experiment 15 pots were used, each containing three 30-day-old seedlings. Thirty seedlings were inoculated and 15 were used as control plants. Essentially the method was that employed in the second experiment with the following modifications. The spore suspension was applied to both upper and lower leaf surfaces (not injected) by using a hypodermic needle and syringe. The stems, however, were injected about one inch from the soil line with a small quantity of the suspension. The glass plates covering the chambers were removed after 48 hours but replaced 24 hours later so that there was a one-inch opening at the top of the chamber. At 12 hour intervals the seedlings were sprayed with sterile distilled water to increase the humidity. The saturated paper toweling was removed at the end of 96 hours. The pots were incubated at an average temperature of 27° C. and subjected to continuous artificial light from two 500 watt bulbs

placed three feet above the pots. The pots were arranged in three rows of five pots each.

B. Identification of the Organism.

Following are the characteristics of the organism observed in pure culture and on the host which were used to identify the fungus as Diaporthe vexans (Sacc. and Syd.) Gratz. When grown in the dark on potato dextrose agar, cornmeal agar, prune agar, and rice agar the organism produces a white, somewhat lobed, zonate colony. On most natural media the mycelium is flat; however, numerous aerial tufts occur on potato dextrose agar. This compares favorably with the description and plates given by Palo (1936). Stromata are produced in culture, each bearing from one to several pycnidia (Nolla, 1929). The pycnidia are subglobose to beaked (Harter, 1914) with the latter type appearing more frequently in older cultures. The morphology of pycnidia occurring on the host is similar to that observed in pure culture.

Phoma-type spores (Fig. 1,A) and conidiophores similar to those described by Harter (1914) were found in all material examined on the host and in culture. However, stylospores (Fig. 1,B) which are said to be characteristic of the genus Phomopsis (Harter, 1914), were found only in cultures of the organism growing on sterilized, four-months old eggplant stems. The spore measurements compared favorably with those given by Harter (1914) and others (Table I). These measurements are given in Table III and are based upon 50 Phoma-type spores and 25 stylospores. All measurements were made

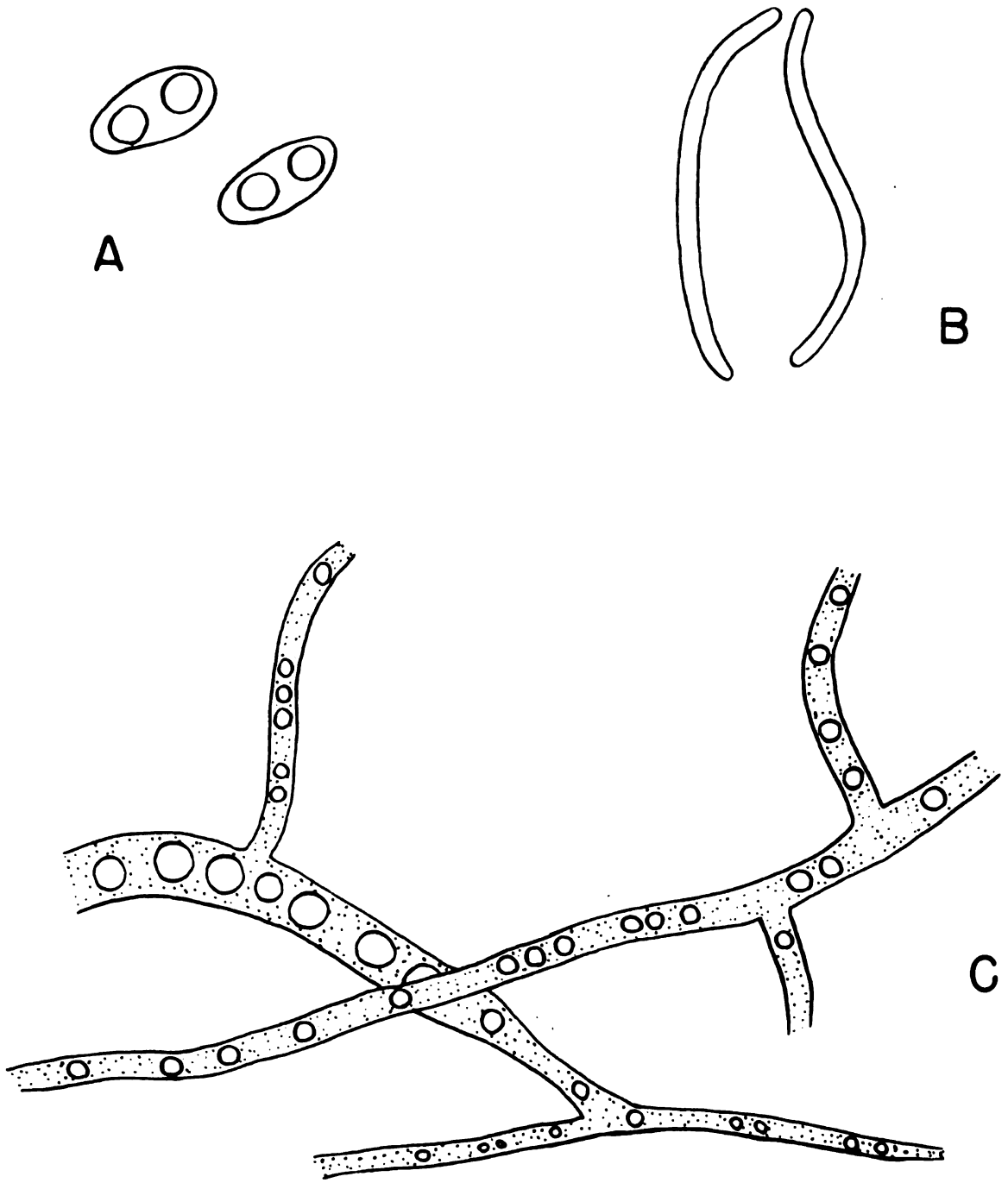

FIGURE 1. - (A) PYCNOSPORES (B) STYLOSPORES (C) HYPHAE

at x960 using spores from pycnidia formed on sterilized egg-plant stems. Several hundred spores from several pycnidia were observed in order to establish the range of size. Stylospores were not observed in strain B.

TABLE III. SUMMARY OF SPORE MEASUREMENTS (IN MICRONS) OF THE STRAINS USED DURING THE COURSE OF THIS STUDY.

Strain	Stylospores	Phoma-type spores	
		Average	Range
A	1-1.8 x 16-25	2.4 x 5.8	4.8-6.9 x 1.9-3.2
B	Not observed	2.2 x 6.1	4.8-7.8 x 1.8-3.0

In order to establish definitely that the organism in question was Diaporthe vexans, a series of three inoculation experiments were conducted with three-week-old seedlings of Solanum melongena. The first two experiments conducted at an average temperature of 21° C. were unsuccessful, the typical leaf spot or stem blight disease failing to develop at the end of 30 days. In the third experiment, conducted at an average temperature of 27° C. disease symptoms appeared at the end of five days and all inoculated seedlings showed visible signs of infection at the end of 10 days. Strain A and strain B were used, both producing typical symptoms.

On the leaves the disease first appeared at the end of five days as small circular, grayish-brown spots (Fig. 2), in many cases surrounded by a circular water-soaked area. These spots enlarged rapidly causing necrotic areas, curling, yellowing, and dropping of leaves. Necrotic areas appeared on

Figure 2.-Seedlings of Solanum melongena seven days after inoculation with a spore suspension of Diaporthe vexans. (A) and (T) show yellowing of leaves and typical grayish-brown spots. Lesions present on (S) were not revealed in photographing. Plant (G) was not inoculated.

the stems at points where spores were injected. These lesions were brown and elongate. At the end of seven days the stems were not girdled.

The organism was isolated from the typical lesions and grown in pure culture. Under the conditions of the experiment, pycnidia were not observed on the host. However, when surface-sterilized, diseased leaves were placed on potato dextrose agar and incubated at 26° C., numerous characteristic fruiting bodies developed after 48 hours on the leaves.

C. Studies on Temperature and Hydrogen-ion Concentration.

The effect of temperature and hydrogen-ion concentration upon growth was determined by using as a criterion the mean diameter of colonies on a synthetic medium at varying pH and temperature levels. A modification of Coon's (1916) synthetic solution was used, with 15 grams of agar added per 1000 grams of water. The formula is as follows:[1]

Sucrose	7.20 gr.
Dextrose	5.60 gr.
$MgSO_4$	1.23 gr.
KH_2PO_4	2.72 gr.
KNO_3	2.02 gr.
Bacto agar	15.00 gr.
Distilled water	1000 c.c.

The medium was prepared on the basis of 2500 c.c., all materials being carefully weighed on an analytical balance. The $MgSO_4$, KH_2PO_4, KNO_3, agar, and 2500 c.c. of water were placed in a 3000 c.c. pyrex flask and autoclaved at 15 pounds pressure for twenty minutes. This preheating facilitated

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[1] Reaction of medium after autoclaving at 15 pounds for 15 minutes was pH 6.4.

solution of the agar and removal of the precipitate that would occur upon subsequent heatings. The sugars were added after the medium had cooled to 60° C. at room temperature. No attempt was made to filter the medium since a clear solution could be obtained by removing the supernatant fluid with a pipette without disturbing the precipitate.

It was desired that the initial reaction for each series of plates at the six temperature levels studied range from pH 4.0 to pH 9.0 at intervals shown in Table IV. The hydrogen-ion concentration was adjusted as follows.

In to each of six 500 c.c. flasks was pipetted 300 c.c. of the stock medium. These flasks, together with the remaining stock medium, were placed in a water bath maintained at 50° C. until ready for use. Ten c.c. portions of the stock

TABLE IV. INDICATORS USED TO ADJUST THE REACTION OF MEDIUM.

pH Desired	Indicator	Useful pH Range
4.0	Bromphenol Blue	3.0-4.6
5.0	Methyl Red	4.4-6.0
6.0	Chlorphenol Red	5.2-6.8
7.0	Bromthynol Blue	6.0-7.6
8.0	Cresol Red	7.2-8.8
9.0	Oleo Red B	8.6-10.2

solution were then placed in each of six test tubes and incubated in a beaker of water at approximately 60° C. Five-tenths c.c. of the appropriate sulfonephthalein or other pH indicator (Table IV) was added to each tube. The tubes were then placed in a Lamotte comparator and N/5 HCl or N/5 NaOH

added from a one c.c. pipette until the end point for each pH level was reached.

The amount of normal acid or base necessary to adjust the pH of the 300 c.c. of medium in each of the six flasks was computed from these preliminary tests. These quantities were recorded.

Sufficient amounts of NaOH were added to the flasks with medium in the neutral or alkaline range to compensate for the anticipated drop in pH during sterilization. These quantities were estimated based upon previous experience. The pH of the medium from each flask was then ascertained colorimetrically[1] from 10 c.c. samples. The data was recorded. Sterile distilled water was added to each flask where necessary, to equalize the volumes where they had been altered by adding acid or base. The content of each flask was pipetted into 20 c.c. test tubes, exactly 12 c.c. per tube. The tubes were plugged and autoclaved at 10 pounds pressure for 12 minutes. The pH was again determined and recorded. Since the pH values were approximately the ones desired to experimentally establish the relation of hydrogen-ion concentration to the growth of Diaporthe vexans, additional adjustment was not made.

After 48 hours (tubes kept in a moist airtight container) the medium was liquefied in an Arnold sterilizer (100° C.) and 18 plates poured from the tubes at each of the six pH levels. Hydrogen-ion concentrations were determined immediately from the remaining portions of the stock medium. The plates were

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[1] The Lamotte comparator was used.

inoculated with a two millimeter mycelial disc cut with a sterile glass tube from the periphery of a five day old culture of the organism (incubated at 26° C.) growing on the stock medium in Petri dishes. Three plates from each pH level were incubated in DeKhotinsky Constant Temperature Tanks at the following temperatures: 2.5° C., 10° C., 26° C., 28° C., 30° C., and 34° C. The plates at 2.5° C. were placed in an ordinary laboratory refrigerator. Temperature variation in the temperature tanks was ±.25° C.

At the end of five days, growth was determined by measuring the diameter of the colonies. If the colony was not circular, the largest diameter was measured and recorded.

The final reaction for the medium in each series was as follows: pH 4.2, 5.3, 6.2, 7.0, 7.8, and 8.8. As anticipated, a reduction occurred during autoclaving in the hydrogen-ion concentration of the alkaline media. The pH of each series before autoclaving was as follows: pH 4.2, 5.3, 6.2, 7.4, 8.4, 9.6. Slight caramelization of the medium occurred at pH 8.8 as indicated by the brown coloration. No perceptible change in reaction occurred during the period of 48 hours following preparation and before pouring the plates, nor after heating the medium to the melting point.

The maximum growth of Diaporthe vexans, as determined by colony measurements, occurred when the initial reaction of the medium was adjusted to pH 6.2 and the temperature maintained at 26-28° C. (Table V). This is illustrated graphically in Figures (3) and (4).

TABLE V. GROWTH OF DIAPORTHE VEXANS AFTER FIVE DAYS AT VARIOUS TEMPERATURES AND HYDROGEN-ION CONCENTRATIONS.

Reaction of Medium (pH)	Temperature (degrees C.) and Diameter (mm.) of Colonies					
	2.5	10	26	28	30	34
4.2	-	-	39	38	34	5
	-	-	38(39)[1]	39 (39)	28 (30.6)	4 (4.3)
	-	-	40	40	30	4
5.3	-[2]	-	41	44	36	6
	-	-	43(40.6)	40 (41)	38 (37.8)	6 (5.6)
	-	-	38	39	39	5
6.2	-	4.5	45	46	39	17
	-	5.0 (4.5)	43(43.6)	46 (45.3)	40 (39.6)	18 (17)
	-	4.0	43	44	40	16
7.0	-	+	39	35	32	11
	-	+	39(39.3)	37 (36)	30 (30.6)	12 (12)
	-	+	40	36	30	13
7.8	-	+	33	30	22	7
	-	+	32(31.3)	31 (32)	25 (22)	8 (8)
	-	+	29	34	19	9
8.8	-	+	36	29	21	-
	-	+	35(34.3)	31 (30.3)	21 (22.3)	-
	-	+	32	31	25	-

[1] Numbers in parentheses refer to mean diameter of colonies.

[2] (-) No growth; (+) perceptible growth but not measurable.

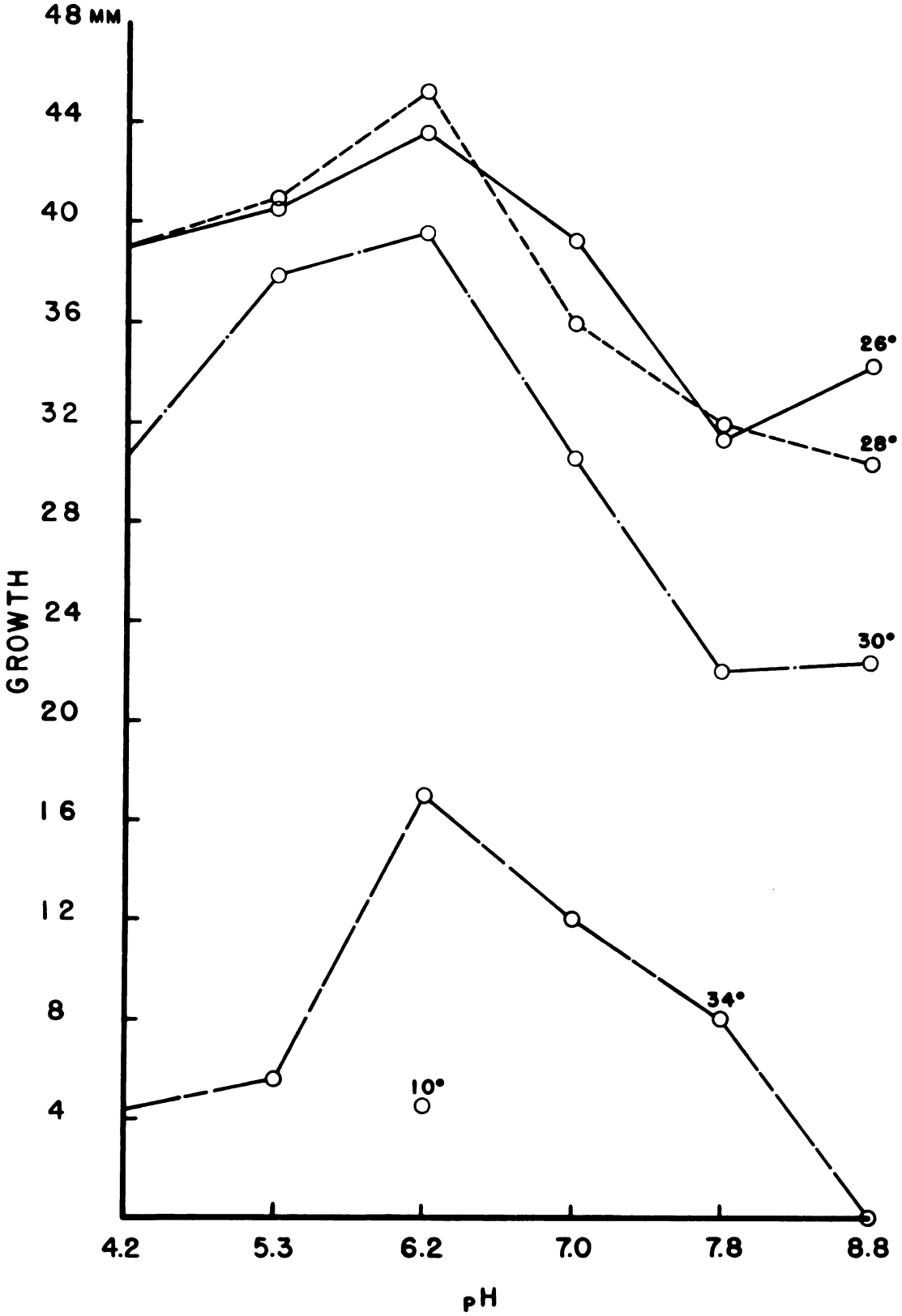

FIGURE 3.-THE RELATION OF HYDROGEN-ION CONCENTRATION TO THE GROWTH OF DIAPORTHE VEXANS AT VARIOUS TEMPERATURES ON A SYNTHETIC MEDIUM. MEAN DIAMETER OF COLONIES AFTER FIVE DAYS. GROWTH AT 10°C. OCCURRED ONLY AT pH 6.2.

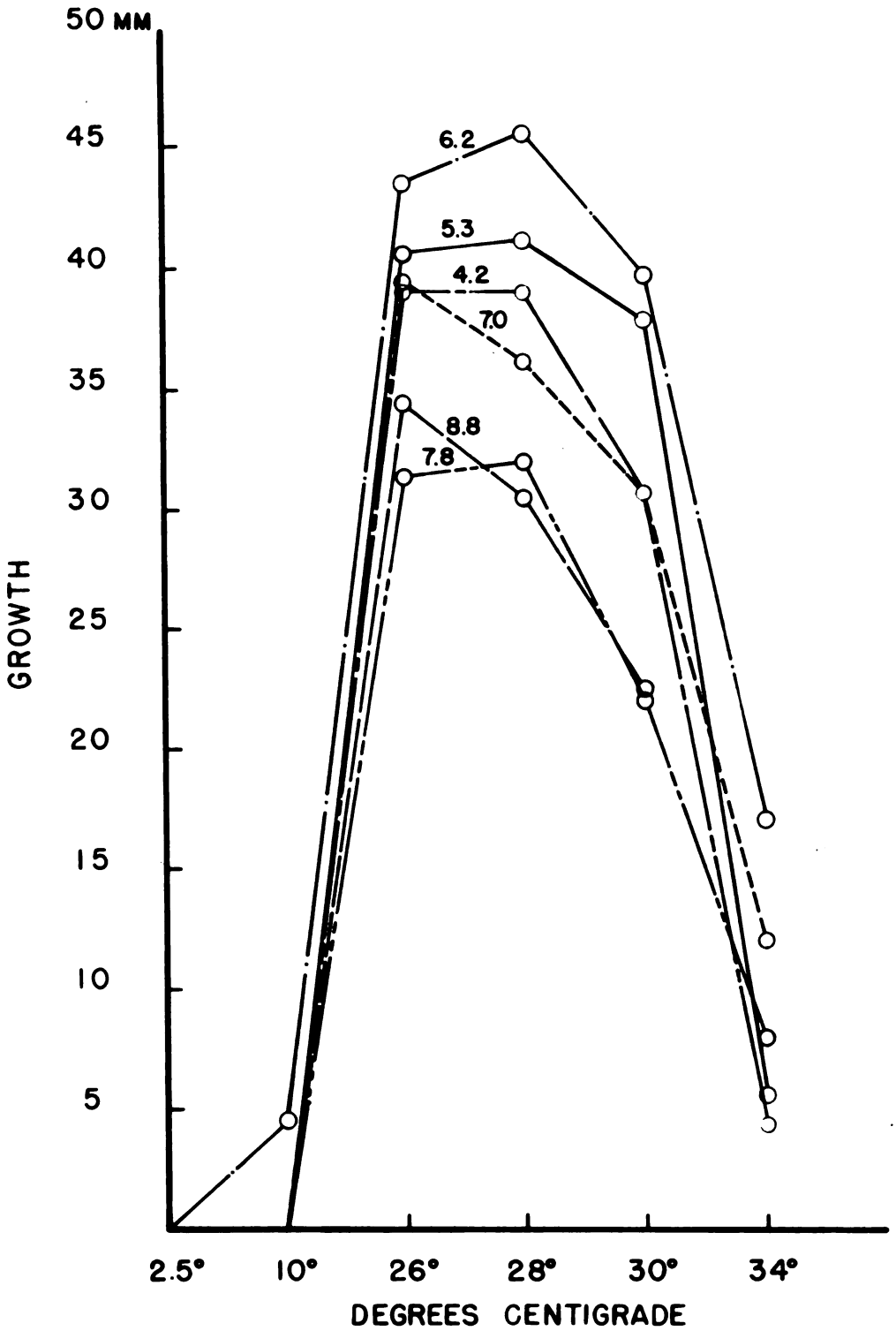

FIGURE 4.- THE RELATION OF TEMPERATURE TO THE GROWTH OF DIAPORTHE VEXANS ON A SYNTHETIC MEDIUM AT VARIOUS HYDROGEN-ION CONCENTRATIONS. MEAN DIAMETER OF COLONIES AFTER FIVE DAYS.

The influence of hydrogen-ion concentration upon growth was most marked at temperatures adverse to growth. At 10° C. measurable growth occurred only at pH 6.2 and at 34° C. a marked increase was noted (Fig. 3).

In general, growth at pH 4.2 appeared superficial and the mycelium was very fine. The colonies were measurable, but the mycelium was barely visible unless the readings were made in bright light. A similar condition existed where the initial reaction of the medium was pH 7.8 or 8.8. At these levels the hyphae were course, sparse, and the colonies very irregular. This was especially true at 30° and 34° C. At 30° C., pH 5.3, the colony characteristics were identical to those at pH 4.2 previously discussed. However, at 26° C. the plates with an initial reaction of pH 5.3 showed a very white, luxuriant growth. This situation also occurred at 28° C., pH 7.0.

D. Effect of Light upon Growth and Pycnidial Production.

An experiment to determine the effect of light, within certain limits of intensity, upon mycelial growth was conducted as follows: potato dextrose agar[1], synthetic agar (p. 35), and cornmeal agar[2] were used as growth media. Two hundred c.c. of each medium was prepared in 500 c.c. flasks and the reaction adjusted to pH 6.0 with approximately N/5 HCl and N/5 NaOH. The colorimetric method (p. 25) was used to determine the reaction. The media were then autoclaved for 10 minutes at 12 pounds pressure and the reaction determined a second time.

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[1] 120 gr. sliced potatoes, 15 gr. dextrose agar, 1000 c.c. water.

[2] Difco cornmeal agar, 20 gr. per 1000 c.c. water.

Eight plates were poured from each flask, each plate containing approximately 20 c.c. of agar, and inoculated with Diaporthe vexans, using inoculum and method cited in pH studies (p. 26).

Twelve plates, four from each medium, were placed in a closed metal box, and 12, those to receive light, were spread upon a standard drawing board (26 x 20 inches). The plates were incubated near the center of a greenhouse (approx. 20 x 15 x 12 ft.), the drawing board resting upon the rectangular steel box. Artificial light was not supplied. However, after 48 hours, due to low temperature and insufficient light the plates were moved to a warmer room and subjected to artificial light. The metal container was placed contiguous to the plates on the end of the drawing board which in turn was seated on a greenhouse bench. The plates were subjected to light from a 250 watt bulb suspended 40 inches above the plates. This artificial light was supplied from midnight to six a.m. each day, the plates received daylight at other times. The temperature was recorded with a Taylor thermograph placed on the board. The temperature in the metal container and at the level of the plates was recorded at intervals with a mercury thermometer.

The results of this experiment are summarized in Table VI. Artificial light may either stimulate or retard the growth of Diaporthe vexans in vitro, depending upon the nature of the substrate. Under the conditions of this experiment growth was stimulated on potato dextrose agar and a synthetic agar

medium, but retarded on cornmeal agar. Colonies produced on cornmeal agar in light were exceptionally regular. This was also true for colonies produced on potato dextrose agar in the dark.

TABLE VI. THE GROWTH OF DIAPORTHE VEXANS ON VARIOUS MEDIA IN LIGHT AND DARK. DIAMETER OF COLONIES AFTER SEVEN DAYS. AVERAGE TEMPERATURE : 24° C., pH 6.0

Medium	Light	Dark
Potato dextrose agar	65 65 63 (64.3) C[2]	45 45 (43.7)[1] 43 42
Cornmeal agar	21 21 (21.0) 21 21	31 26 (29) 31 C
Synthetic agar[3]	40 43 (43) 47 42	24 25 (25.3) 27 C

[1] Numbers in parentheses refer to mean diameter of colonies.

[2] Indicates contamination.

[3] Formula given on page 24.

Experiments conducted to note the effect of light upon the production of reproductive structure *in vitro* were conducted as follows: In the first experiment, 12 tubes of cornmeal agar (reaction adjusted to pH 6.0) were inoculated with the organism (p. 35 par. 1). Six of these tubes were placed in a metal box (12 x 4 x 3.5 inches) containing absorbent

cotton, in such a way that they were in contact with the metal lid. The other six were seated on the lid directly above the inner tubes. Continuous artificial light was supplied from a shaded 100 watt bulb placed 17 inches above the tubes. A sterile thermometer was immersed in the agar of a separate tube (inoculated with the organism) and placed with the tubes exposed to light. Equipment was not available for accurately measuring the light received by each tube; however, a range of incident light values was established from tube one to six by using a Weston light meter. A mirror was laid on the tubes to facilitate reading the light meter scale and the meter moved from one limit of the tubes to the other. The values were recorded.

After eight days the number of fruiting bodies in each of the 12 tubes were counted and recorded.

It is demonstrated by this experiment that the formation of pycnidia is stimulated by exposure to artificial light. At the end of eight days the tubes exposed to the light contained numerous pycnidia (Fig. 9), while those incubated in darkness did not produce fruiting bodies. The light received by the tubes ranged from 110 foot candles for tube one to 140 for tubes four and five, to 130 for tube six. The number of pycnidia formed in each tube was as follows:

Tube No.	1	2	pycnidia
" "	2	25	"
" "	3	39	"
" "	4	41	"
" "	5	41	"
" "	6	32	"

In a second experiment, 500 c.c. of a synthetic agar[1] were prepared, and 15 c.c. poured into each of 20 Petri dishes. The plates were inoculated with a 2 mm. loop of spore suspension and incubated in a manner similar to that given on p. 32 par. 2. Ten plates were kept in total darkness and 10 were exposed to light. Artificial light was supplied on a 24 hour basis from a shaded 500 watt bulb placed 40 inches above the plates. At the end of 10 days the number of pycnidia on each plate was observed. Hundreds of typically beaked pycnidia were produced on the 10 plates exposed to light, while no pycnidia were produced in any of the plates incubated in darkness. Perithecia did not develop under the conditions of these experiments.

E. Carbon Utilization.

To ascertain the ability of the fungus to utilize carbon from various sources when supplied separately in vitro, the following methods were employed. Varying quantities of sugars (Table VII) were incorporated in a "sugar-free" synthetic medium.[2] Two liters of the medium containing the basic compounds were prepared in a 3000 c.c. flask and autoclaved at 15 pounds for 20 minutes to allow for precipitation. Fifty c.c. aliquots of this basic medium were pipetted into each of twenty-five 200 c.c. flasks. Each series of five flasks contained a carbohydrate at five different concentrations

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[1] Sucrose 30.80 gr., Asparagine .33 gr., K_2HPO_4 2.610 gr., $MgSO_4$ 1.23 gr., H_2O 1000 ml., Reaction adj. to pH 6.2.

[2] Formula given on p. 24.

TABLE VII. SOURCES AND QUANTITIES OF CARBOHYDRATES EMPLOYED IN PREPARATION OF MEDIA.

Source	Mol. Conc.	Flask No.	Gr. per flask (50 ml. aliquot)	Gr. per Liter	Gr. Carbon per Liter
Glucose M.W.[1] 198.17	.03	1	.297	5.95	2.15
	.06	2	.594	11.89	4.33
	.09	3	.892	17.84	6.48
	.12	4	1.189	23.79	8.63
	.15	5	1.486	29.74	10.79
Galactose M.W. 180.15	.03	6	.270	5.40	2.15
	.06	7	.540	10.80	4.33
	.09	8	.810	16.21	6.48
	.12	9	1.080	21.61	8.63
	.15	10	1.350	27.01	10.79
Sucrose M.W. 342.2	.03	11	.510	10.27	4.32
	.06	12	1.030	20.53	8.64
	.09	13	1.540	30.80	12.96
	.12	14	2.050	41.06	17.27
	.15	15	2.560	51.32	21.59
Maltose M.W. 342.2	.03	16	.510	10.27	4.32
	.06	17	1.030	20.53	8.64
	.09	18	1.540	30.80	12.96
	.12	19	2.050	41.06	17.27
	.15	20	2.560	51.32	21.59
Lactose M.W. 342.2	.03	21	.510	10.27	4.32
	.06	22	1.030	20.53	8.64
	.09	23	1.540	30.80	12.96
	.12	24	2.050	41.06	17.27
	.15	25	2.560	51.32	21.59

[1] Molecular Weight

(Table VII). The medium was agitated vigorously to insure that the sugar was completely in solution. From each flask 36 c.c. was pipetted into three tubes, 12 c.c. per tube. The pipettes used to transfer the medium to test tubes were calibrated to yield 12 c.c. of medium. They were washed with boiling distilled water after the transfer from each flask. The tubes and remaining stock media were autoclaved at 12 pounds for 10 minutes. Ten c.c. of the remaining stock medium from each flask was used for pH determination; the colorimetric method was employed.

The contents of the tubes were poured into Petri dishes and inoculated with 2 mm. discs containing the mycelium, cut from the periphery of a seven day old culture on the stock medium. All the plates were incubated at 26° C. A sufficient amount of water was placed in the incubator to prevent excessive loss of moisture from the medium.

At the end of five days the diameter of each colony was measured and recorded.[1]

Experimental results indicate that Diaporthe vexans is able to utilize sucrose, glucose, maltose, lactose and galactose as a source of carbon when these carbohydrates are supplied singly in a synthetic agar medium (Table XIII). The greatest growth, as determined by colony measurements, occurred when sucrose was supplied in a .09 molar concentration (30.80 gr. per liter). Using units of growth obtained per gram of elemental

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[1] Many colonies were circular, others somewhat elliptical. In the latter cases the greatest spread was recorded as the diameter.

carbon supplied as a basis, glucose was nearly twice as efficient as other sugars. Also on this basis, the six-carbon compounds were more efficient, after five days of growth, than the 12-carbon compounds. The most efficient utilization of carbon occurred when galactose, glucose, lactose, maltose, and sucrose were supplied in .03 molar concentrations (Tables VIII-XII). This efficiency decreased progressively as the molarity of the solution increased.

Poor growth was obtained when galactose and lactose were employed singly as the source of carbon (Fig. 5). The growth values obtained for these two sugars were nearly identical (Table XIII). At the end of 12 days the colonies were approximately one-half as large as those obtained from sucrose, maltose or glucose (Fig. 6).

Colony appearance, particularly zonation, varied with the sugar supplied. This is clearly illustrated in Figure 6.

F. Nitrogen Utilization.

An experiment was conducted to determine the ability of the organism to use nitrogen from various compounds when incorporated separately in a "nitrogen-free" synthetic agar medium.[1] Two liters of this basic medium, without sugar, were prepared and autoclaved at 15 pounds for 20 minutes to allow for precipitation. Maltose was added after the solution had cooled to approximately 60° C. The medium was not filtered.

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[1] $MgSO_4$ 1.23 gr., KH_2PO_4 2.72 gr., Maltose 40.00 gr., agar 15 gr., H_2O 1000 c.c.

TABLE VIII. ASSIMILATION OF GALACTOSE BY DIAPORTHE VEXANS DURING FIVE DAYS OF GROWTH AT 26° C.

Gr. of Galactose per liter of medium	Molar concentration of Galactose	Gr. of carbon supplied per liter of medium	Growth: mean diameter (mm) of colonies	Growth (mm) per gram of carbon supplied
5.40	.03	2.15	18.3	8.5
10.80	.06	4.33	19.3	4.5
16.21	.09	6.48	18.3	2.8
21.61	.12	8.63	18.0	2.1
27.01	.15	10.79	17.3	1.6

TABLE IX. ASSIMILATION OF LACTOSE BY DIAPORTHE VEXANS DURING FIVE DAYS OF GROWTH AT 26° C.

Gr. of lactose per liter of medium	Molar concentration of lactose	Gr. of carbon supplied per liter of medium	Growth: mean diameter (mm) of colonies	Growth (mm) per gram of carbon supplied
10.27	.03	4.32	18.3	4.3
20.53	.06	8.64	16.6	1.9
30.80	.09	12.96	16.3	1.3
41.06	.12	17.27	17.0	1.0
51.32	.15	21.59	17.0	0.8

TABLE X. ASSIMILATION OF SUCROSE BY DIAPORTHE VEXANS DURING FIVE DAYS OF GROWTH AT 26° C.

Gr. of Sucrose per liter of medium	Molar concentration of sucrose	Gr. of carbon supplied per liter of medium	Growth: mean diameter (mm) of colonies	Growth (mm) per gram of carbon supplied
10.27	.03	4.32	28.6	6.6
20.53	.06	8.64	32.3	3.7
30.80	.09	12.96	40.3	3.1
41.06	.12	17.27	34.3	2.0
51.32	.15	21.59	29.3	1.4

TABLE XI. ASSIMILATION OF GLUCOSE BY DIAPORTHE VEXANS DURING FIVE DAYS OF GROWTH AT 26° C.

Gr. of glucose per liter of medium	Molar concentration of glucose	Gr. of carbon supplied per liter of medium	Growth: mean diameter (mm) of colonies	Growth (mm) per gram of carbon supplied
5.95	.03	2.15	30.0	13.9
11.89	.06	4.33	38.3	8.8
17.84	.09	6.48	30.0	4.6
23.79	.12	8.63	37.3	4.3
29.74	.15	10.79	35.0	3.2

TABLE X. ASSIMILATION OF SUCROSE BY DIAPORTHE VEXANS DURING FIVE DAYS OF GROWTH AT 26° C.

Gr. of Sucrose per liter of medium	Molar concentration of sucrose	Gr. of carbon supplied per liter of medium	Growth: mean diameter (mm) of colonies	Growth (mm) per gram of carbon supplied
10.27	.03	4.32	28.6	6.6
20.53	.06	8.64	32.3	3.7
30.80	.09	12.96	40.3	3.1
41.06	.12	17.27	34.3	2.0
51.32	.15	21.59	29.3	1.4

TABLE XI. ASSIMILATION OF GLUCOSE BY DIAPORTHE VEXANS DURING FIVE DAYS OF GROWTH AT 26° C.

Gr. of glucose per liter of medium	Molar concentration of glucose	Gr. of carbon supplied per liter of medium	Growth: mean diameter (mm) of colonies	Growth (mm) per gram of carbon supplied
5.95	.03	2.15	30.0	13.9
11.89	.06	4.33	38.3	8.8
17.84	.09	6.48	30.0	4.6
23.79	.12	8.63	37.3	4.3
29.74	.15	10.79	35.0	3.2

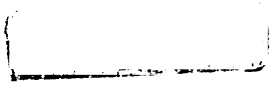
TABLE XII. ASSIMILATION OF MALTOSE BY DIAPORTHE VEXANS DURING FIVE DAYS GROWTH AT 26° C.

Gr. of Maltose per liter of medium	Molar concentrations of Maltose	Gr. of Carbon supplied per liter of medium	Growth: mean diameter (mm) of colonies	Growth (mm) per gram of carbon supplied
10.27	.03	4.32	21.3	5.1
20.53	.06	8.64	28.3	3.3
30.80	.09	12.96	24.5	1.9
41.06	.12	17.27	30.6	1.8
51.32	.15	21.59	28.6	1.3

TABLE XIII. GROWTH OF DIAPORTHE VEXANS AFTER FIVE DAYS AT 26° C. USING VARIOUS SUGARS AS A SOURCE OF CARBON

Molar concentration of sugar	Carbohydrate and diameter (in millimeters) of colonies				
	Maltose	Glucose	Sucrose	Galactose	Lactose
.03	23,21,21 (21.3)[1]	30,29,31 (30)	29,29,28 (28.6)	16,18,18 (18.3)	18,17,18 (18.3)
.06	29,28,28 (28.3)	37,40,38 (38.3)	32,32,33 (32.3	20,19,19 (19.3)	17,18,15 (16.6)
.09	24,22,26 (24.5)	29,30,31 (30)	40,41,40 (40.3)	18,21,16 (18.3)	17,17,15 (16.3)
.12	31,31,30 (30.6)	37,38,37 (37.3)	35,35,33 (34.3)	20,17,17 (18.0)	18,17,16 (17.0)
.15	30,28,28 (28.6)	34,36,35 (35)	31,28,29 (29.3)	16,20,16 (17.3)	17,18,16 (17.0)

[1]Mean Diameter of Colonies



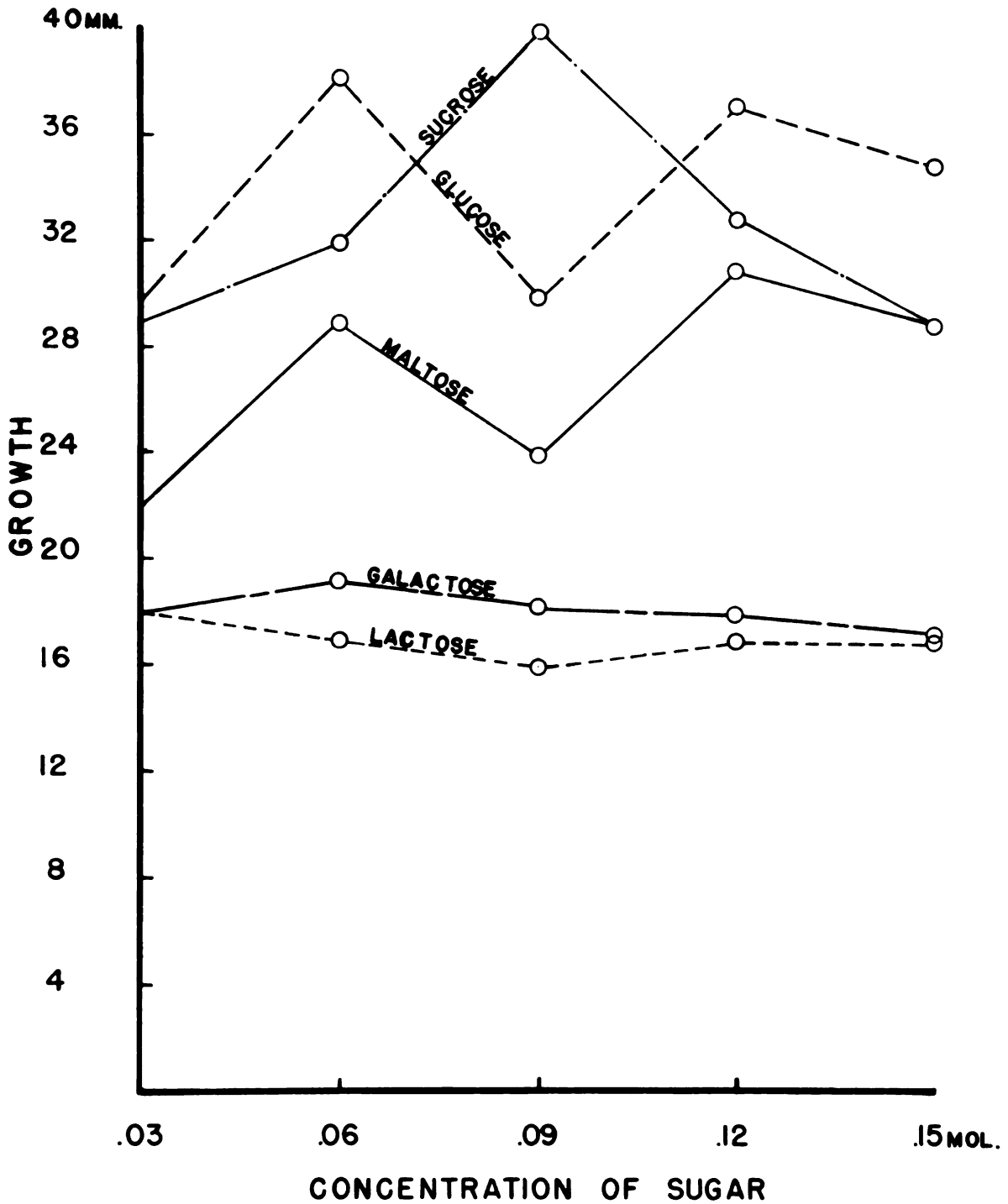


FIGURE 5.—THE EFFECT OF VARYING QUANTITIES OF SUGARS UPON GROWTH OF DIAPORTHE VEXANS. MEAN DIAMETER OF COLONIES AFTER FIVE DAYS AT 26°C.

Figure 6.- Photograph to illustrate colony characteristics of Diaporthe vexans on a synthetic medium containing various sugars in .12 mol. concentrations, after 12 days at 26° C. Initial reaction: pH 6.4. (A) Sucrose. (B) Maltose. (C) Glucose. (D) Lactose. (E) Galactose. Note colony zonation typical of each sugar, i.e., very fine in (D) to very broad in (A). Growth on plate (B) is typical of that on Potato dextrose agar.

Fifty c.c. of the clear medium was pipetted into each of twenty-four 200 c.c. flasks containing a quantity of a nitrogenous compound (Table XIV).

The methods employed from this point to inoculation, incubation, and recording growth, were identical to the procedures outlined for the carbohydrate work (pp. 35-37) except for the following: The pH of the tubed media was altered during autoclaving. The method employed to adjust the reaction to the desired level was as follows. Methyl Red, in .6 c.c. quantities, was added to each tube which required readjustment. All the tubes were maintained at approximately 60° C. in a water bath. A tube containing 12 c.c. of stock medium was adjusted to pH 5.0 using Methyl Red and the Lamotte comparator. Quantities of N/5 HCl and N/5 NaOH were added aseptically to each tube until the color change was equal to that of the tube previously adjusted to pH 5.0. To each tube not receiving Methyl Red .6 c.c. of sterile distilled water was added.

Although growth was recorded after five days, the plates remained in the incubator for a period of 16 days.

The results are summarized as follows. When ammonium sulphate, potassium nitrate, asparagine, and peptone were employed singly as the source of nitrogen, asparagine gave the greatest growth (Table XV). Asparagine proved also to be the most efficient source of nitrogen when millimeters of colony growth per gram of nitrogen supplied, were used as a basis for comparison (Table XV). Peptone was the least efficient source of nitrogen, although very good growth occurred

TABLE XIV. SOURCES AND QUANTITIES OF NITROGEN EMPLOYED IN PREPARATION OF MEDIA.

Compound	Mol. wt.	% Nitrogen	Flask no.	Grams of compound per flask (50 ml. aliquot)	Grams of compound per liter	Grams of nitrogen supplied per liter
a-Asparagine	132.1	21.20	1	.0165	.330	.070
			2	.0330	.660	.140
			3	.0660	1.320	.280
			4	.0990	1.980	.420
			5	.1320	2.640	.560
			6	.1980	3.960	.840
Potassium Nitrate	101.1	13.80	7	.0254	.507	.070
			8	.0507	1.014	.140
			9	.1015	2.029	.280
			10	.1522	3.043	.420
			11	.2029	4.058	.560
			12	.3043	6.087	.840
Ammonium Sulfate	132.1	21.20	13	.0165	.330	.070
			14	.0330	.660	.140
			15	.0660	1.320	.280
			16	.0990	1.980	.420
			17	.1320	2.640	.560
			18	.1980	3.960	.840
Peptone		16.16[1]	19	.10	2.0	.323
			20	.3	6.0	.969
			21	.5	10.0	1.616
			22	.7	14.0	2.262
			23	.9	18.0	2.908
			24	1.20	24.0	3.878

[1] See Table XV.

TABLE XV. THE GROWTH OF DIAPORTHE VEXANS USING VARIOUS COMPOUNDS AS A SOURCE OF NITROGEN.

Source of nitrogen	% Nitrogen	Gr. of compound per liter of medium	Gr. of nitrogen per liter of medium	Diameter (mm.) of colonies after 5 days at 26° C.	Growth (diameter of colony in millimeters) per gr. of Nitrogen
a-Asparagine	21.20	.330	.070	40,41, 37 (39.3)[1]	561
		.660	.140	38,38 36 (37.3)	266
		1.320	.280	35,39,40 (38.0)	136
		1.980	.420	42,35,35 (37.3)	89
		2.640	.560	45,45,43 (44.3)	79
		3.960	.840	40,38,38 (38.6)	46
Potassium Nitrate	13.80	.507	.070	29,29,25 (27.6)	394
		1.014	.140	24,25,28 (25.6)	183
		2.029	.280	28,27,28 (27.6)	102
		3.043	.420	30,34,33 (32.3)	77
		4.058	.560	35,35,35 (35.0)	62
		6.087	.840	36,34,33 (34.0)	40
Ammonium Sulphate	21.20	.330	.070	13,13,15 (13.5)	193
		.660	.140	19,16,14 (16.0)	114
		1.320	.280	15,15,13 (14.3)	51
		1.980	.420	18,15,13 (15.3)	36
		2.640	.560	20,20,19 (19.6)	23
		3.960	.840	18,17,18 (17.6)	21
Peptone	Total nitrogen 16.16[2]	2.0	.323	15,15,14 (14.6)	45
		6.0	.969	42,42,39 (41.0)	42
		10.0	1.616	34,27,33 (31.3)	19
		14.0	2.262	35,37,34 (35.3)	16
		18.0	2.908	35,35,33 (34.3)	12
		24.0	3.878	35,38,35 (36.0)	9

[1] Mean diameter of colonies.

[2] "Typical analysis of peptone," Manual of Dehydrated Culture Media and Reagents, p.176, Difco Laboratories: Detroit, 1944.

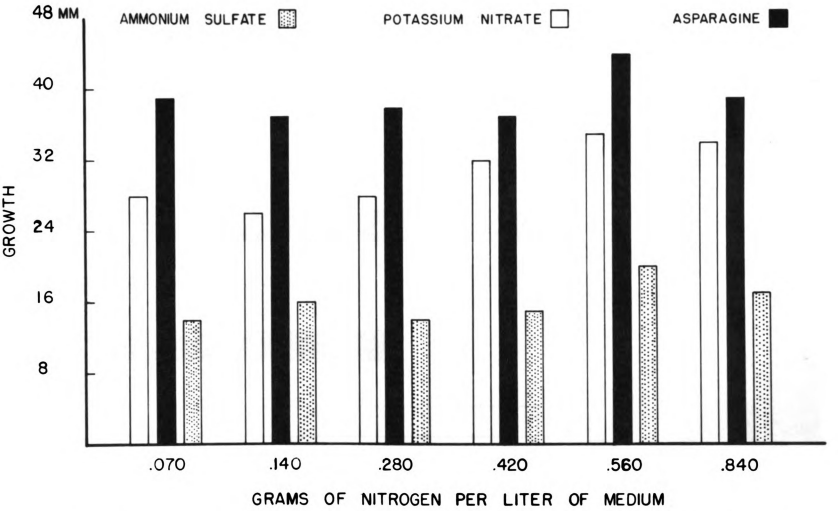


FIGURE 7. - THE EFFECT OF VARIOUS SOURCES OF NITROGEN ON THE GROWTH OF DIAPORTHE VEXANS. MEAN DIAMETER OF COLONIES AFTER FIVE DAYS AT 26° C.

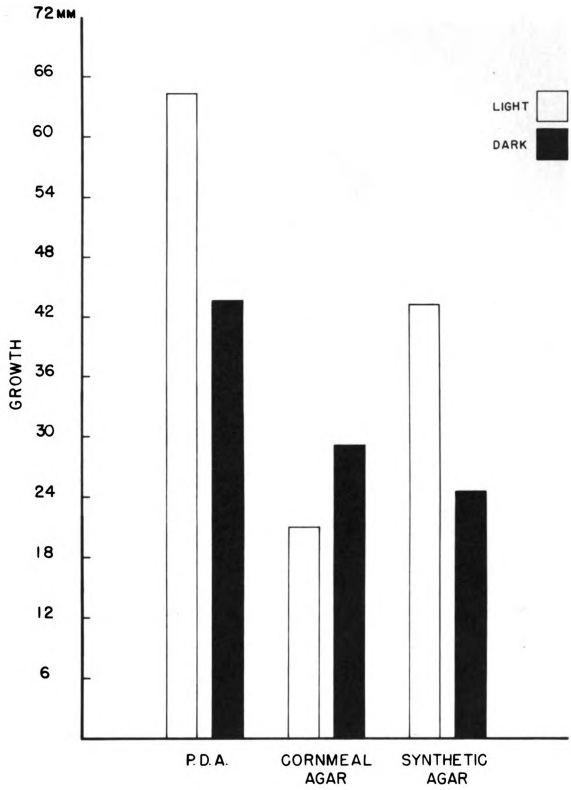


FIGURE 8.- EFFECT OF LIGHT UPON MYCELIAL GROWTH ON POTATO DEXTROSE AGAR, CORNMEAL AGAR, AND A SYNTHETIC MEDIUM. MEAN DIAMETER OF COLONIES AFTER SEVEN DAYS. AV. TEMP. 24°C.

Figure 9.- The relation of light to the formation of pycnidia on cornmeal agar at (approx.) 27° C. Initial reaction: pH 6.0. Reading from left to right, tubes (1) and (2) received continuous light for eight days (Pycnidia numerous). Tubes (3) and (4) were incubated in total darkness (Pycnidia absent). Note luxuriant mycelial growth in tubes (3) and (4) as compared to the weak growth in tubes exposed to the light. Streaks on the agar were made in an attempt to remove moisture for photographing.

when six grams were supplied per liter of medium.

Within limits, growth increased with an increase in the amount of nitrogen but a marked decrease occurred in the efficiency of nitrogen utilization.

Poorest growth was obtained with ammonium sulphate. When this compound was employed, colonies were irregular, without zonation, and appeared like glistening yeast or bacterial colonies. Colonies on plates containing potassium nitrate were extensive but did not give rise to the luxuriant mycelial growth which occurred when asparagine or peptone were employed as the source of nitrogen. In the plates containing asparagine, there was a tendency for production of black stromatic mycelium which was not prevalent with other nitrogen sources.

In the case of asparagine, potassium nitrate, and ammonium sulphate, greatest growth occurred when the compounds were supplied in quantities yielding .560 grams of nitrogen per liter (Table XV).

IV. DISCUSSION

A. Experimental Methods.

Existing literature indicates that the majority of experimental work dealing with the metabolism of fungi has involved the use of growth as a means of measuring the effect of unknown factors. In general two methods for determining growth response are used. The fungus is grown in nutrient solution, the mycelial mats extracted, dried and weighed, or the nutrient solution is solidified with agar and growth

recorded as a function of colony area or diameter.

The former method is outlined by Klotz (1923), Webb and Fellows (1926), Talley and Blank (1941) and others. Although this method (dry-weight of mycelium) is preferred and more widely used than measuring growth response as a function of colony diameter on a solid medium, it presents sources of experimental error which must be considered. Robbins and Schmidt (1939) washed the mycelial mats of Ashbya gossypii in Gooch crucibles. They experienced difficulty in removing sugar from the mycelium during the washing process and report this responsible for erratic data. Horr (1936) also filtered and dried mycelial mats of Aspergillus niger in Gooch crucibles. He stated, "This method does not give an accurate measurement because of the loss of spores through the filter."

Although dry-weight of mycelium is considered a more efficient method of measuring growth response to various stimuli, the use of colony-diameter on solid substrate is widely used, especially among plant pathologists (Fulton, 1948 and Tyler and Parker, 1945). However, this method introduces a greater number of unknowns into the system; e.g., agar generally contains a variety of minerals available to the organism (Schopfer, 1943) and Robbins (1941) states that biotin was present in the agar he used to grow Fusarium avenaceum. In the presence of dilute acids, agar (a mixture of polysaccharides) may break down to assimilable carbohydrates if heated to high temperatures.

If it were possible to accurately measure the growth of a given organism on a solid medium the results might not be comparable to those obtained from nutrient solutions containing the same components. Webb and Fellows (1926) grew Ophiobolus graminis in liquid media and on various agars adjusted to different levels of hydrogen-ion concentration. They concluded that the growth of O. graminis depends to a large extent upon the physical nature of the medium.

Worley (1939) pointed out that the actual fungus growth of a colony takes place in three dimensions; the order of these according to their importance being radial, tangential, and vertical. She states that after colonies have obtained relatively large diameters, growth approaches a linear function.

Although the writer was aware of the many limitations and merits of each of the general experimental methods outlined in the previous paragraphs, it was not deemed feasible to grow the organism in nutrient solution, using dry-weight of the mycelial mat as a basis for comparison, since suitable equipment was not available.

B. Temperature and Hydrogen-ion Concentration.

It has been shown by various workers that temperature and the nutritional and physical nature of the media may modify the influence of the hydrogen and hydroxyl ions on the growth of fungi in pure culture. In view of this fact it can be seen that any information relative to the tolerance of Diaporthe vexans to hydrogen and hydroxyl ions would be

largely confined to the scope of the experiments outlined in this paper. Failure of various workers to agree on the pH range that will support growth in a given fungus may be attributed to differences in culture media, temperature, length of incubation period, possible strain variation of the fungus, and other reasons. Although the limits of experiments involving hydrogen-ion and temperature studies are acknowledged by many workers, the work is continued because many practical applications have been found in the field of plant pathology.

The increase in growth from pH 7.8 to pH 8.8 at 26° C. and 30° C. (Fig. 3) may be attributed to the slight caramelization of the medium which occurred in the media at pH 8.8 upon autoclaving. Webb and Fellows (1926) found this to be true when Ophiobolus graminis was grown on slightly caramelized Czapek's solution and alkaline potato dextrose solution. However, Wolpert (1924) obtained a similar curve when Polystictus versicolor was grown in Richard's solution, apparently without caramelization.

The accuracy of the colorimetric method of pH determination used to adjust the reaction of media is approximately ±.1 pH unit. The use of an electrical potentiometer was desired but was not available. However, from the standpoint of time and convenience, the colorimetric method is superior.

C. Carbon Utilization.

Figure 5 illustrates graphically that sucrose and

glucose are excellent sources of carbon for the growth of Diaporthe vexans, while maltose is intermediate and galactose and lactose are poor sources.

For most fungi galactose and lactose are poor sources of carbon (Steinberg, 1939). However, there are a few organisms which can use galactose and lactose. Ledeborer (Steinberg, 1939) reported that Ceratostomella ulmi can readily use galactose and lactose as sources of carbon, and Moscher, Saunders, Kingerly and Williams (1936) state that galactose is a good source of carbon for Trichophyton interdigitale (animal parasite).

As previously pointed out (p. 37) Diaporthe vexans can assimilate galactose; however, the effects upon growth were somewhat drastic. The organism developed abnormal mycelium and growth was comparatively static when galactose was supplied as the source of carbon (Fig. 6, E). The mycelium was highly irregular and the hyphae were much thickened. Also, the colonies were incised and did not exhibit zonation.

As a hydrolytic product of lactose, it is possible that galactose also exerted an effect upon the growth obtained when the former was supplied as the source of carbon. If during the process of heating (118° C., 10 min.) in acid solution, lactose was hydrolized to any extent, the presence of the three molecules (lactose, glucose, galactose) did not yield a synergic effect in relation to their influence on growth. In some instances, however, it has been shown that if galactose is supplemented with one or more other sugars,

growth is greater than if either sugar is used alone. Horr (1936) stated, "In my work it is found that when dextrose, levulose, or mannose is added to galactose, there is a decided acceleration in the growth of Aspergillus niger and Penicillium glaucum, as indicated by the weight of dry matter formed." Working with Aspergillus niger, Steinberg (1935) noted that greater yields of growth were obtained if mannitol and lactose were used together as a source of carbon than if they were supplied alone. He attributes the poor growth obtained when mannitol or lactose were used singly, to the inadequate molecular configuration of the single source. A comparison of the data in Tables V and XIII shows that if glucose and sucrose are used together as a source of carbon for the growth of Diaporthe vexans, greater growth is obtained than if either source is supplied alone.

Although the growth curves in Figure 5 are informative, it should be pointed out that the disaccharides supplied exactly twice the carbon as the hexoses. The relative efficiency of each sugar in respect to carbon utilization can be visualized from the graph. Full interpretation of the curves presented in Figure 5 would prove very difficult. The reasons for bimodal parallel patterns in the case of glucose and maltose is unknown to the author. Why is there a sharp rise in the sucrose curve at .09 molar concentration, and a depression in the curves of maltose and glucose at the same concentration? Before attempting to answer this question, the author would like to point out that Horne and Williamson

(1923) obtained a similar curve when they grew Eidamia viridescens on a nutrient agar medium containing .01, .02, .1, .20 and 1.0 molar concentration of sucrose. After three days the average diameter of colonies was 49, 53, 49, 57 and 26 mm. respectively. After four days, however, though the same curve existed, there was a tendency to reach a peak at .20 molar concentration of sucrose.

There is a possibility that the previous questions could be at least partially answered if data relative to the final osmotic values and hydrogen-ion concentration of the media were available. The author is of the opinion, however, that if the drop is not due to experimental error, the explanation is one of an intricate biochemical nature.

If the average osmotic values of the hyphal protoplasm were very high, e.g., 25 atmospheres, which is not uncommon among higher plants (Meyer and Anderson, 1939), then it is conceivable that a theoretical total change of 2.688 atmospheres in solutions whose actual osmotic values were approximately 10 atmospheres, would not be responsible for the differences in growth rates as indicated in Figure 5. Weimer and Harter (1923) grew Fusarium acuminatum, Diplodia tubericola, Rhizopus tritici in solutions with a maximum osmotic pressure of 81.33 to 101.46 atmosphere and obtained good growth.

If, on the other hand, the osmotic values of the hyphal protoplasm were as low as 12 atmospheres, a maximum shift of

2.688 atmospheres might give considerable differences in growth responses. The final pH of the medium at the .09 molar level might have been one corresponding to the isoelectric point of the mycelium. In this case absorption of water and consequently growth might have been retarded.

When maltose and glucose were supplied in concentrations equal to .09 molar concentration the colonies, especially on maltose, were irregular, i.e., much more lobed and asymmetric as compared to the very regular colonies on the same sugars at other levels.

In general the author has observed that when good growth was obtained on any medium, there was a tendency toward symmetrical, nearly circular colonies and when poor growth occurred, there was a tendency toward irregular, abnormal colonies. If the hydrogen-ion concentration was either very low, pH 4.2, or very high, pH 7.8-8.8, the colonies were irregularly incised (not lobed) and the mycelium was much thickened. If the temperature was very high, 34° C., regardless of pH level, the mycelium was highly irregular. If ammonium sulphate was the source of nitrogen employed, the colonies were small, appeared solid and pearl-like. When galactose was the carbohydrate source, the colonies were highly irregular (Fig. 6).

The condition of these colonies which developed on media receiving glucose and maltose in .09 molar concentrations leads the author to believe that the unusual curve is not due to experimental error.

The ability of Diaporthe vexans to utilize various sugars

when supplied singly, *in vitro*, may be an important factor related to virulence of the organism or serve to differentiate between strains, but certainly, the fungus will not likely find a pure source of available carbon in nature. The absorption, and assimilation of carbohydrates by fungi involve many intricately, interrelated factors. Some of the known factors which definitely play a role in the ability of fungi to utilize carbohydrates *in vitro* are as follows: temperature, hydrogen-ion concentration of medium, balance of mineral elements in medium, nitrogen source, osmotic pressure of medium, growth substances, e.g., thiamin, biotin, etc., and radiant energy.

D. *Nitrogen Utilization*.

Robbins (1937) classified fungi into groups on the basis of their ability to utilize various forms of nitrogen, and Steinberg (1939), after reviewing 99 articles relative to metabolism of fungi, was able to place all the organisms encountered in one of the categories outlined by Robbins. The results shown in Figure 7 indicate that *Diaporthe vexans* belongs to a group capable of utilizing organic, nitrate and ammonium nitrogen, although the organism grew poorly when the nitrogen was supplied in the ammonium form.

The initial reaction of the medium under the conditions of this experiment was pH 5.0. It is possible that ammonium sulphate would have given good growth at lower hydrogen-ion concentrations. Mehlich (1939) noted that *Cunninghamella*

blakesleeana was able to utilize ammonium nitrogen only, at pH 5.0 or above, but utilized nitrate nitrogen and asparagine over a wide range of hydrogen-ion concentrations ranging from pH 2.9 to pH 8.6. This increase in nitrogen availability in response to alteration of the media reaction, seems to depend upon the organism. Fellows (1936) grew Ophiobolus graminis in a number of nutrient solutions containing ammonium nitrate, ammonium sulphate, ammonium carbonate and ammonium chloride. He adjusted the reactions to levels ranging from pH 4.8 to pH 8.4 and obtained no significant growth differences when the cultures were compared to the control which contained no nitrogen.

It is also possible that ammonium nitrogen could be utilized if maltose was replaced with a more suitable carbohydrate or combination of sugars. Hagem (Steinberg, 1939) found that several species of Mucor were able to utilize ammonium or nitrate nitrogen with glucose, but required nitrate nitrogen when glycerol was employed as a source of carbon.

The synthesis of amino acids depends largely upon the availability of carbohydrates or their derivatives, and a suitable source of nitrogen. Talley and Blank (1941) and others have shown that if available carbohydrates are not the limiting factor in a system where other requirements are met, the rate of growth, within limits, may be regulated by increasing the nitrogen supply. This is clearly illustrated for Diaporthe vexans in the case of peptone (Fig. 7). A sharp rise in growth occurred when the amount of nitrogen was

increased from .323 to .969 grams per liter. When quantities greater than .969 grams per liter were supplied, no significant increase occurred. In the case of ammonium sulphate and potassium nitrate an increase in growth is noted when the nitrogen supply is increased from .070 to .560 grams per liter (Table XIV). However, evidence indicates that good growth may be obtained when asparagine is supplied in less than .070 grams per liter, since there is no significant growth differences at higher values. The author has no explanation for the slightly higher growth values obtained when nitrogen was supplied at .560 grams per liter (Fig. 7).

The role of asparagine in the metabolism of higher plants has been extensively studied (Robinson, 1929) and a great deal more is known concerning its role here than in the metabolism of fungi. In green plants, asparagine appears to be a precursor of proteins and an important translocation form of nitrogen. Hydrolysis of proteins in germinating seeds produces quantities of asparagine.

It was believed by Czepak and others (Klotz, 1923) that asparagine plays much the same role in the metabolism of Aspergillus niger as it does in higher plants. Loew (Klotz, 1923) stated that a fungus fed asparagine and sugar forms an aldehyde of aspartic acid which is then condensed to make the protein molecule. After reviewing much of the available experimental data regarding nitrogen metabolism, Steinberg (1939) did not support the viewpoint of Czepak.

Experimental evidence indicates that asparagine is a

superior source of nitrogen for the growth of many fungi. The reason is attributed mainly to its close relation to aspartic acid and the readily available amino nitrogen.

Figure 7 reveals that asparagine is an excellent source of nitrogen for the growth of Diaporthe vexans. Although the growth (Table XV) as measured by colony diameter was significantly greater than that produced by ammonium sulphate and potassium nitrate, the author is of the opinion that if dry weight of mycelium had been used as a criterion, asparagine would prove far superior to the other sources of nitrogen used. In each case the mycelium was very dense and tufted as compared with the "weak" colonies of potassium nitrate and ammonium sulphate.

Relatively recent work has shown that the stimulation of growth imparted to many fungi when asparagine is supplied as the source of nitrogen may be due to complex relationships with certain growth substances. Herrick and Alexopoulos (1942) grew Stereum gausapatum in vitro using varying quantities of nitrogen with and without thiamin. Their results are summarized as follows: asparagine without thiamin yielded practically no growth; however, excellent growth was obtained in the presence of thiamin. The growth in solutions containing only the nitrate ion was negligible even when thiamin was present. Schopfer (1943), using results of experimental work with Phycomyces blakesleeanus as a basis, states that "apparently a relationship exists between the asparagine content of the medium and the optimal dosage of active thiamin."

Schopfer and Blumer (1942) have shown that under certain conditions, purified biotin acts as a growth factor for Trichophyton album. They state that when cultures are supplied with asparagine, biotin is required only during the early stages of development. The growth of controls soon equaled those supplied with biotin. However, when colonies are supplied with ammonium sulphate and ammonium citrate, biotin is required until a later stage of growth. Perlman (1948) grew Memnoniella echhinata and Stachybotrys atra in nutrient solution, with and without biotin. He stated that "When 100 mg. of (synthetic) dl-aspartic acid was added to a liter of medium, the biotin requirements were reduced to approximately 5 per cent of normal." Perlman also cites other references to the "stimulating" effect of aspartic acid.

In acid solution thiamin and biotin will both withstand autoclaving for the period carried out in these experiments. It is possible, as pointed out by Robbins (1941), that agar may contain biotin and it is also known that thiamin and biotin are present in agar or may be in asparagine.

Convincing evidence is presented in the previous paragraphs that both aspartic acid and asparagine are capable of acting as a substitute for biotin. If Diaporthe vexans is unable to synthesize biotin then it is possible that the exceedingly large amount of growth obtained with asparagine as a nitrogen source can be attributed, to some extent at least, to the ability of asparagine to partially replace biotin. Evidence presented by Steinberg (1942) strongly supports the

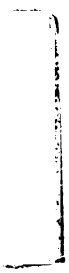
author's belief that the additional carbon supplied by asparagine is not the growth stimulating factor.

E. Light.

During the early phases of experimental work much difficulty was encountered in securing pycnidia for single-spore isolations and subsequent infection experiments. The author, believing that the absence of pycnidia was perhaps a matter of nutrition, grew the fungus on several natural culture media. No media proved to be superior for pycnidial production. In fact, a given medium would yield pycnidia at one time and not another, apparently under the same conditions. It was soon discovered that the plates exposed to light produced pycnidia, while pycnidia were absent in the plates kept in total darkness. To verify this observation it was decided to secure experimental proof that light would stimulate the production of pycnidia.

It is acknowledged by the author that slight temperature differences existed between the tubes or plates incubated in the light and those incubated in the dark. However, since no accurate means were available for regulating this factor, an exact comparison was not obtainable. Other experiments outlined in this thesis in which the cultures were incubated in darkness at various temperatures and hydrogen-ion concentrations, did not give rise to the formation of pycnidia.

For other fungi it has been shown that hydrogen-ion concentration and temperature (Henry and Andersen, 1948) and



nutrition (Coons, 1916) play an important role in the formation of fruiting structures. During the course of this experimental work, the hydrogen-ion concentration, temperature, supply of nitrogen, and carbohydrate supply were varied through a range of values. The cultures were incubated in the dark and no pycnidia developed regardless of the incubation period. Coons (1916), working with Plenodomus fuscomaculans, pointed out that the nutritional and environmental limits within which pycnidia were formed were much narrower than those required for vegetative growth. It is conceivable, then, that the optimum nutritional and environmental requirements have not yet been met for Diaporthe vexans.

Smith (1936) made an extensive survey of the literature regarding the effects of radiation upon fungi. She states that, "Throughout most of the work there has been an inadequate control of environmental conditions" and that "these uncontrolled factors have often led to unfair interpretation of results." The work has been mainly qualitative in nature, involving the use of X-rays, ultra-violet radiation and the visible spectrum.

The mechanism by which light stimulates or retards fungous growth is unknown. Coons and Levin (1920) speculate that light serves to unlock the reserves of cellular food, thus furnishing the energy for the fruiting process. However, it is obvious that many factors must be considered. The complexity of the problem is clearly seen by taking note of the factors involved in experimental work. Smith (1936) lists some of these as follows: "the wave lengths emitted by the source,

the intensity of the source, the nature of any absorbing media enterposed, temperature, composition of the medium, hydrogen-ion concentration of the medium, and age of the culture."

According to Stevens (1930), who exposed plate cultures of Diaporthe vexans to ultra-violet light, "pycnidia with oblong spores were very rare on the non-irradiated side and very abundant on the irradiated region." This article by Stevens is the only one the author has been able to find where Diaporthe vexans was the subject of irradiation experiments. However, Levin (1917) and Coons and Levin (1920) show that light (from 100 watt bulb) will induce the formation of pycnidia in many of the Sphaeropsidales. Levin (1917) grew Ascochyta, Phoma, Sphaeropsis and Fusicoccum on cornmeal agar in light and dark and found that pycnidia developed in the presence of light while none were formed in the dark. A greater number of light-positive Sphaeropsidales were discovered by Coons and Levin (1920).

Coons (1916) showed that Plenodomus fuscomaculans when grown in corn broth gave more vegetative growth in the dark than in the presence of light. The author obtained the same results when Diaporthe vexans was grown in light and dark on cornmeal agar (Table VI).

Nearly all of the experimental work with Diaporthe vexans has been conducted in tropic or semi-tropic climates where there was little need for incubation in the dark. The author attributes this "light-incubation" or possible strain variations

as the basic reason that other workers have not reported difficulty in obtaining pycnidia on natural or artificial culture media.

Evidence that light may play an important role in pycnidial formation, even under natural conditions, is given by Palo (1936) who states that pycnidia "... may be found on both surfaces of the leaf, but they are usually produced more abundantly on the upper than on the lower surface."

In older cultures of the organism the author has observed that pycnidial necks exhibit a positive phototropic response to both artificial light and diffuse daylight. The statement of Backus (1937), that "there have been numerous accounts of phototropic responses shown by fungi" indicates that the phototropic response in the pycnidial necks of Diaporthe vexans is not an unusual occurrence.

F. Practical Applications.

It seems advisable that since the organism will not grow well, in vitro, over a wide range of conditions, that a specific synthetic medium be recommended for the growth of Diaporthe vexans.

A possible medium which may serve as an initial point for further physiological studies or as an economical medium for laboratory use is as follows:

Glucose	6.00 gr.
Asparagine	.33 gr.
KH_2PO_4	2.72 gr.
$MgSO_4$	1.23 gr.
Agar	15.00 gr.
H_2O	1000 c.c.

The final reaction after autoclaving would be near pH 5.0, being largely controlled by the presence of the dihydrogen phosphate. Although good growth would probably occur at this reaction (pH 5.0), the author is of the opinion that a reaction approaching pH 6.2 would be more suitable. For best growth cultures should be incubated at temperatures ranging from 26° to 28° C.

It is possible that since the organism did not sporulate in darkness on the various media employed during the course of experimentation, that light is also required for the formation of pycnidia on the host. If this is true, storage of eggplant fruit in such a way as to exclude light might prevent formation of fruiting bodies and spread of the organism. However, even in the presence of light, temperature would play an important role. Under the conditions of these experiments the organism did not grow when incubated at 2.5° C. and growth occurred only at 10° C. when the reaction of the medium was adjusted to pH 6.2. The author believes that these temperature relationships can be applied to the storage of the eggplant fruit so as to prevent much of the fruit rot now existent.

V. SUMMARY AND CONCLUSIONS

A review of the literature on Diaporthe vexans is given.

A single strain of Diaporthe vexans was grown in vitro on various synthetic and natural media and experiments were conducted to determine (1) the effect of hydrogen-ion concentration and temperature upon growth, (2) the ability of

the organism to obtain carbon from various sources, (3) the ability of the organism to obtain nitrogen from various sources, and (4) the effect of light upon vegetative growth and formation of pycnidia.

Fungous growth was obtained when the initial reaction of the medium ranged from pH 4.2 to pH 8.8. The best growth, however, occurred only at pH values from 5.3 to 6.2, with the optimum at pH 6.2. At pH values above the neutral point, growth decreased with an increase in the hydroxyl-ion content of the medium.

Temperature exerted a marked effect upon the pH range at which growth took place. At 10° C. significant growth occurred only at pH 6.2. Temperature did not affect the optimum hydrogen-ion concentration of the medium, nor was the optimum temperature significantly altered by variations in the hydrogen-ion concentration. Growth was retarded at 34° C. No growth was observed at 2.5° C. At initial pH values suitable for growth, the optimum temperature ranged from 26° C. to 28° C.

Greatest growth occurred when sucrose (30.80 gr. per liter) was supplied as the source of carbon. Galactose and lactose retarded growth of the organism when compared to other carbon sources. Glucose was the most efficient form of carbon. Greater growth was obtained when sucrose and glucose were supplied together than if an equal quantity of either was supplied.

The relative efficiency with which the organism utilizes carbohydrates decreased significantly with an increase in the molarity of the sugar solution.

When ammonium sulphate, potassium nitrate, asparagine, and peptone were employed singly as the source of nitrogen, asparagine gave the greatest growth. Moderate growth was obtained with potassium nitrate and peptone, and poor growth with ammonium sulphate. Within limits, growth increased with an increase in the amount of nitrogen but a marked decrease occurred in the efficiency of nitrogen utilization. Asparagine was the most efficient form of nitrogen. If other growth requirements were met, an increase in the available nitrogen gave a greater increase in growth than an increase in available carbohydrates.

Under the conditions of these experiments, pycnidia did not develop in darkness, but were produced profusely on cornmeal agar and synthetic agar exposed to artificial light. The influence of light upon the vegetative growth of Diaporthe vexans is variable, depending upon the nature of the substrate.

A positive phototropic response to artificial and diffuse daylight was observed for the perithecial necks of Diaporthe vexans.

A synthetic medium is recommended for growth of the organism.

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