Growth Responses of the Pecan Scab Fungus, *Fusicladium effusum*, On Various Common and Exotic Agar Media

by

George L. Barnes

Department of Botany and Plant Pathology

Technical Bulletin T-110, September 1964

CONTENTS

ACKNOWLEDGEMENT

The author acknowledges assistance during part of this investigation by I. N. Adams, former National Science Foundation Research Participant from Taft College, Taft, California.

Growth Responses of the Pecan Scab Fungus, *Fusicladium effusum*, on Various Common and Exotic Agar Media

by

George L. Barnes

Department of Botany and Plant Pathology

The pecan scab fungus, *Fusicladium effusum* Wint., has been considered to be a difficult fungus to culture. As reported by Nolen (10), it was first cultured by Rand prior to 1913 on potato agar and on cornmeal agar. Nolen reported also that the fungus grew well and sporulated freely at 68 and 77 degrees F. on cornmeal agar, nutrient cornmeal agar, and potato-dextrose agar (PDA) containing two percent dextrose. The fungus grew poorly on prune agar, potato agar, rice tubes, cornmeal tubes, and PDA containing four percent dextrose. Later, Demaree (9) reported that *F. effusum* grew slowly in culture, but the medium was not described. However, a photograph of the fungus growing on lima bean agar accompanied the text.

No additional literature appeared until 1951 when Taylor (12) reported that the fungus grew on nutrient-cornmeal agar, potato-dextrose agar, and malt agar but grew slightly better on cornmeal agar. Spores were produced, but only in trace amounts, on these media. Taylor also noted that there was some variation in growth rates between isolates of the fungus obtained from different pecan varieties. Later, Converse (8) reported good growth on agar media made from oatmeal, Chinese chestnuts, potatoes, lima beans, cornmeal, prunes, carrots, V-8 juice, pecan leaf pieces, and variations of a synthetic nutrient solution containing different carbon sources. Only oatmeal agar and Chinese chestnut agar allowed sporulation in his study.

No mention of pH or comparative growth measurements, outside of sporulation, was made by any of the authors except Taylor. Taylor reported that one isolate of the fungus developed one centimeter colonies

Research reported herein was done under Station Project 806.

from single spore plantings on cornmeal agar after 28 days at 75 degrees C. All of these reports indicate that the fungus grows very slowly and that the fungus does not sporulate well, or consistently, on the media tested.

A good method for mass producing spores is needed to meet spore needs for epidemiological studies. The present work was undertaken to determine whether the fungus would grow well on additional agar media and to determine whether the fungus would sporulate well and consistently on these media. A wide variety of media have been tested. Details are discussed herein. A preliminary report was published earlier (2).

MATERIALS AND METHODS

Cultures of *F. effusum,* derived originally from a single-spore isolate (Converse W-11), were used throughout this work. The original isolate was obtained from a lesion on a Western variety pecan tree, *Carya illinoensis* (Wang) H. Koch.

A wide variety of commercially-prepared and laboratory-prepared agar media were tested during this investigation. They are listed in Tables 1-5. Distilled water was used in all cases. All pH adjustments were made with 1M or 2M NaOH and HCl. Measurement of pH was made with a Beckman Zeromatic temperature-compensated pH meter.

Because wide variations in depth of agar media influence radial growth (4, 5, 6), approximately 30 milliliters of sterile media were added to each sterile Petri dish (15 x 90 millimeters). Growth of *F. effusum* apparently was uniform for any particular medium when volumes in excess of 20 milliliters were used. A growth period of 21 days was arbitrarily selected for the tests to allow for the slow growth habit of the fungus. The approximate 30 milliliters volume of media in each dish helped to prevent excessive drying and shrinking of media during the long growth period. Each dish of medium was "inoculated" with 7-millimeter discs cut from the peripheries of 4-6 week old colonies on peptone-dextrose agar. Three discs were placed upside down on the medium in each dish and spaced in a triangular arrangement. All dishes were held at room temperature (24-30 degrees C.)—optimum growth temperature is 25 degrees C. (10). Colony diameter was used as a reliable criterion of growth (4). Because of the usual uniform roundness of the colonies which developed on the media, a single diameter measure-

ment for each colony was recorded at the end of each test. For any slightly off-round colonies, an average of two measurements was recorded. Irregularly-shaped colonies were not measured.

RESULTS

Growth varied considerably between the various agar media during preliminary testing (Table 1). The commonly-used, laboratory-prepared PDA supported more growth than most of the commercially-prepared media. Chinese chestnut agar and oatmeal agar, reported by Converse (8) to be the best media in his work, supported less growth than agar media made from Difco nutrient agar and dextrose, peptone and dextrose, V-8 juice, casein hydrolysate and dextrin, V-8 juice and dextrose, and tomato paste and dextrose. Spores were produced inconsistently, and only in trace amounts, on Chinese chestnut agar, PDA, and peptone-dextrose agar. No spores were observed on the remaining media. Differential growth responses were observed on media containing different carbohydrates. Limited observations indicate that autoclaved dextrose and dextrin are readily utilized, several other carbohydrates are utilized to a lesser degree, and galactose is either not utilized or a toxic reaction product is formed during autoclaving.

Variations of the two better media from the preliminary work, Difco nutrient agar plus dextrose and peptone-dextrose agar, were tested at three pre-autoclaving (initial) pH values (ipH) 4.5, 6.8 and 8.0. Dextrose concentration was varied in the Difco nutrient agar plus dextrose series. More growth occurred at ipH 4.5 and 6.8 than at ipH 8.0 regardless of dextrose concentration (Table 2). The colonies in this series did not sporulate and were flat and dark gray over black. In the peptone-dextrose agar series, the concentrations of peptone and dextrose were varied. *F. effusum* grew well on each medium (Table 3). Varying the proportions of dextrose and peptone had little effect on radial growth. *F. effusum* colonies in this series were mounded and had a superficial, frosty-gray, non-sporulating mycelium over a black base.

Later, combinations of yeast extract, dextrose, and peptone were tested. Combinations of all three materials supported excellent growth at ipH 6.8 and 8.0 and good growth at ipH 4.5 (Table 4). No sporulation was detected. Colonies up to 30 millimeters in diameter were obtained on one of the media. The colonies in this series resembled those in the peptone-dextrose series.

During the work with media containing peptone, a peptone manufactured by Conray Products Company was used initially. When a Difco Laboratories peptone was substituted, slightly less growth resulted. Because of the differential response, many additional peptone sources were tested. A wide range of growth responses was obtained (Table 5).

DISCUSSION

Differential growth responses of *F. effusum* on media containing different carbohydrates demonstrated that the source of carbon is important in nutritional studies with the fungus. Though it appeared that dextrose and dextrin were preferentially utilized over a number of other carbohydrates, little weight can be given to the order of apparent preference. First, other carbohydrates were provided by the peptone sources, and secondly, autoclaving probably hydrolyzed some of the more complex carbohydrates. Also, heating carbohydrates in the presence of nitrogenous substances may cause reactions producing inhibiting or growth-promoting materials (7). A preliminary study on nutrition of filter-sterilized carbohydrates to determine intrinsic utilization by *F. effusum* has been completed (3).

In the Difco nutrient agar plus dextrose series, more growth occurred with two percent dextrose than with one and four percent dextrose at ipH of 4.5; but the growth was equivalent, regardless of dextrose concentrations, at ipH 6.8 and 8.0. The results at ipH 4.5 are in agreement with those obtained by Nolen (10) when culturing *F. effusum* on PDA containing two and four percent dextrose. Dextrose concentration, therefore, may be less important than pH.

In the peptone-dextrose agar series, variations of the peptone and dextrose concentrations had little effect on growth. Williams (13) obtained similar results with 36 fungi on similar media. When yeast extract was added to the peptone-dextrose combinations in the current study, the fungus grew very well at ipH 6.8 and 8.0. Yeast extract is an excellent source of the B-complex vitamins. It contains more of these vitamins than purified peptone (personal communication—C. W. Christensen, Difco Laboratories). This fact may partially account for the increased growth when yeast extract is a component of a medium.

The differential growth on media containing different peptones demonstrated the importance of choice of peptone when culturing *F. effusum*. If other fungi respond likewise, it would indicate that the

source and type of peptone should be clearly specified when reporting results obtained with peptone-containing media. The presence and quantity of various sugars, vitamins, trace elements, various nitrogen sources, etc. in the peptones apparently account for the differential responses. Typical chemical analyses of some Difco peptones have been published (1). Christensen (Difco Laboratories) has provided the author additional analyses. Stokes et al. (11) reported that peptones contain varying amounts of B-complex vitamins. Though the components vary in amount from one peptone to another, it is impossible to correlate growth of *F. effusum* with one or more of the components because of a lack of sufficient, detailed analytical data and the complexity of factors involved.

Though a wide variety of media at various pH levels which will allow poor-to-excellent growth of the fungus have been found, none have been observed to allow consistent and good sporulation. A number of factors could be responsible. The fungus may have been maintained on artificial media too long, the media may be too rich in nutrients, an unknown sporulation factor may be missing, etc. Additional research on nutrition of *F. effusum* to obtain more detailed data on growth and reproduction of this pathogenic fungus is needed.

SUMMARY AND CONCLUSIONS

The pecan scab fungus, *Fusicladium effusum* Wint., has been considered to be a difficult fungus to culture. To determine whether the fungus could be induced to sporulate in culture and be grown at a more rapid rate than reported previously, numerous agar media were assayed at 24-30 degrees C. Colony diameters were measured 21 days after planting mycelial discs on the media in Petri dishes. Many of the media tested were found to be favorable for good-to-excellent growth of the pecan scab fungus. Growth varied with pH. Some media favored growth in the acid range, while others favored growth in the neutral to alkaline range.

Maximum diameters of 27-30 millimeters were obtained on certain media containing peptone, dextrose and yeast extract. Less satisfactory media included agar media made from peptone and dextrin, peptone and sucrose, Difco nutrient agar and dextrose, Chinese chestnuts, oatmeal, V-8 juice, and Difco malt agar. Among the poorest media were Difco cornmeal agar, malt extract agar, pecan kernel agar, Difco lima bean agar, Difco nutrient agar, peptone-lactose agar and peptone-galactose agar. No sporulation was detected on most of the media. Trace

amounts of spores were produced inconsistently on Chinese chestnut agar, potato dextrose agar, and peptone-dextrose agar.

Colony morphology varied with the types of media. Some media produced flat, dark gray or black colonies; and others produced mounded, dark gray or frosty-gray colonies. Differential growth responses were obtained on different peptone sources.

No medium has been developed which will allow consistent and good sporulation. However, the preliminary information on utilization of carbohydrates and peptone sources reported here will be useful in future studies on host-parasite relationships. The development of suitable media for producing large colonies of *F. effusum* will be useful in future work on mass production of spores for studies on epidemiology of pecan scab.

LITERATURE CITED

1. Anonymous. 1953. Difco Manual, 9th ed., p. 265. Difco Laboratories, Inc., Detroit.
2. Barnes, G. L. 1963. Growth of the pecan scab fungus, *Fusicladium effusum,* on common and exotic agar media. (Abstr.) Phytopathology 53:621.
3. _____ and I. N. Adams. 1964. A preliminary investigation on carbohydrate nutrition of the pecan scab fungus, *Fusicladium effusum* Wint. Proc. Okla. Acad. Sci. 44 (In press).
4. Brancato, F. P. and N. S. Golding. 1953. The diameter of the mold colony as a reliable measure of growth. Mycologia 45:848-884.
5. Brown, W. 1923. Experiments on the growth of fungi on culture media. Ann. Botan. 37:105-129.
6. Chaudhuri, H. 1923. A study of the growth in culture of *Verticillium albo-atrum,* B. et Br. Ann. Botan. 37:519-539.
7. Cochrane, V. W. 1958. Carbon nutrition, pp. 55-98. *In* V. W. Cochrane, Physiology of Fungi, Wiley, New York.
8. Converse, R. H. 1956. The production and germination of conidia of *Cladosporium effusum* (Wint.) Demaree in the laboratory. (Abstr.) Phytopathology 46:9.
9. Demaree, J. B. 1928. Morphology and taxonomy of the pecan-scab fungus, *Cladosporium effusum* (Wint.) comb. nov. J. Agr. Research. 37:181-187.
10. Nolen, R. E. 1926. Pecan Scab. Fla. Agr. Expt. Sta. Bull. 181.
11. Stokes, J. L., Marion Gunness and J. W. Foster. 1944. Vitamin content of ingredients of microbiological culture media. J. Bacteriol. 47:293-299.
12. Taylor, O. C. 1951. Studies of the pathogen, *Cladosporium effusum* (Wint.) Demaree, and host-parasite relations in pecan scab. Master's thesis, Okla. State University.
13. Williams, J. W. 1935. Effect of variation of ratios of dextrose to peptone on colonies of certain pathogenic fungi. Arch. Dermat. Syph. 32:893-914.

Table 1. Colony diameters of *Fusicladium effusum* after 3 weeks' growth on various agar media during preliminary screening of media.

Medium	Number colonies measured	Average colony diameter
		mm.
Nutrient Agar (Difco) + Dextrose (2%), pH 6.3	54	27.3
Peptone-Dextrose Agar (1%:2%:1.5%)	182	25.3
V-8 Juice-$CaCO_3$ Agar	39	25.2
Casein Hydrolysate-Dextrin Agar	51	24.8
V-8 Juice-Dextrose Agar, pH 6.0	48	24.8
Tomato Paste-Dextrose Agar, pH 4.4	65	24.5
Chinese Chestnut Agar (White)[1]	104	23.7
Chinese Chestnut Agar (Red)[2] + Coconut "milk" (10%)	12	23.7
Chinese Chestnut Agar (Red)[2]	29	23.3
V-8 Juice-Dextrose Agar, pH 4.4	77	22.9
V-8 Juice Agar, pH 4.0	36	22.9
Chinese Chestnut Agar (White)[1] + Coconut "milk" (10%)	16	22.7
Oatmeal Agar	48	22.6
Potato-Dextrose Agar + Coconut "milk" (10%)	16	22.3
Mycological Agar (Difco), pH 7.0	70	22.3
Tomato Paste-Oatmeal Agar, pH 6.8	71	22.3
Peptone-Sucrose Agar (1%:2%:1.5%)	37	22.2
V-8 Juice Agar, pH 4.5	49	22.2
Casein Hydrolysate-Dextrose Agar	50	21.7
V-8 Juice Agar, pH 6.0	43	21.7
Neopeptone-Dextrose Agar (1%:4%:1.5%)	29	21.6
Tomato Paste-Oatmeal Agar, pH 4.5	84	21.6
Potato-Dextrose Agar	34	21.3
Cornmeal-Dextrose Agar	44	21.3
Malt Agar (Difco), pH 5.5	26	20.9
Casein Hydrolysate-Gum Tragacanth Agar	38	20.6
Peptone Agar (2%:1.5%)	48	20.0
Potato-Gum Tragacanth Agar + Coconut "milk" (10%)	20	19.9
Cornmeal Agar (Difco), pH 6.0	41	19.3
Malt Extract Agar	41	19.3
Pecan Kernel Agar	55	19.0
Casein Hydrolysate-Glycogen Agar	27	18.6
Peptone-Lactose Agar	56	18.4
Lima Bean Agar (Difco), pH 5.0	35	17.3
Casein Hydrolysate-Amylo Pectin Agar	53	17.0
Yeast Extract Agar	40	16.3
Peptone Agar (1%:1.5%)	39	15.5
Potato-Gum Tragacanth Agar	10	15.2
Casein Hydrolysate-Pectin Agar	50	15.1
Casein Hydrolysate-Soluble Starch Agar	54	14.5
Coconut "milk" (10%) Agar	10	13.8
Nutrient Agar (Difco), pH 6.8	27	13.2
Casein Hydrolysate Agar	35	9.9
Water Agar	27	9.7
Peptone-Galactose Agar (2%:2%:1.5%), pH 7.2	47	8.2
Peptone-Galactose Agar (1%:0.4%:1.5%)	58	7.5

[1]Red pericarp not included.
[2]Red pericarp included.

Table 2. Growth of *Fusicladium effusum* on a nutrient agar and dextrose medium, with varying amounts of dextrose, at three pH levels, after three weeks.

Medium	pH (before autoclaving)	Number colonies measured	Average colony diameter
			mm.
Nutrient Agar (Difco) + dextrose[1] (2.3%: 1%)	4.5	84	26.7
	6.8	36	26.6
	8.0	69	24.8
Nutrient Agar (Difco) + dextrose[1] (2.3%: 2%)	4.5	95	28.1
	6.8	61	26.4
	8.0	47	24.1
Nutrient Agar (Difco) + dextrose[1] (2.3%: 4%)	4.5	44	26.9
	6.8	55	26.8
	8.0	59	24.6

[1]Dextrose, C. P. (Pfanstiehl Laboratories, Inc.)

Table 3. Growth of *Fusicladium effusum* on peptone-dextrose agar combinations at three pH values after three weeks.

Medium	pH (before sterilization)	Number colonies measured	Average colony diameter
			mm.
Peptone - Dextrose Agar II (1%:1%:1.5%)	4.5	33	28.1
	6.8	49	27.9
	8.0	55	28.2
Peptone - Dextrose Agar I (1%:2%:1.5%)	4.5	57	28.4
	6.8	34	25.3
	8.0	52	27.4
Peptone - Dextrose Agar VII (1%:3%:1.5%)	4.5	48	28.4
	6.8	60	27.3
	8.0	56	27.9
Peptone - Dextrose Agar III (1%:4%:1.5%)	4.5	64	25.3
	6.8	86	26.8
	8.0	76	26.2
Peptone - Dextrose Agar IV (2%:1%:1.5%)	4.5	67	23.4
	6.8	45	26.1
	8.0	50	28.3

Table 3. (Continued)

Medium	pH (before sterilization)	Number colonies measured	Average colony diameter
			mm.
Peptone - Dextrose Agar V (2%:2%:1.5%)	4.5	49	23.9
	6.8	44	28.6
	8.0	75	27.3
Peptone - Dextrose Agar IX (3%:1%:1.5%)	4.5	40	26.7
	6.8	57	27.4
	8.0	37	26.2
Peptone - Dextrose Agar VI (3%:2%:1.5%)	4.5	67	24.9
	6.8	27	28.1
	8.0	41	23.4
Peptone - Dextrose Agar VIII (3%:3%:1.5%)	4.5	69	25.7
	6.8	18	27.1
	8.0	56	26.1

Table 4. Growth of *Fusicladium effusum* on agar media made from various combinations of yeast extract, peptone and dextrose at three pH levels, after three weeks.

Medium	pH (before sterilization)	Number colonies measured	Average colony diameter
			mm.
Yeast Extract-Dextrose Agar I (1%:2%:1.5%)	4.5	47	27.4
	6.8	60	27.4
	8.0	57	28.1
Yeast Extract-Dextrose Agar II (2%:2%:1.5%)	4.5	61	25.4
	6.8	46	29.1
	8.0	76	28.1
Yeast Extract-Peptone - Dextrose Agar I (0.5%:0.5%:2%:1.5%)	4.5	12	24.7
	6.8	27	28.6
	8.0	43	29.2
Yeast Extract-Peptone - Dextrose Agar III (1%:1%:2%:1.5%)	4.5	84	25.7
	6.8	50	29.3
	8.0	67	26.5

Table 5. Colony diameters of *Fusicladium effusum* after three weeks' growth on pH adjusted peptone-dextrose agar media[1] containing different peptone sources.

Peptone Source	pH (after sterilization)	Number colonies measured	Average colony diameter
			mm.
Peptone, U.S.P.[2]	6.8	64	27.7
Proteose Peptone No. 2[3]	6.4	62	27.4
Proteose Peptone No. 3[3]	7.2	52	26.8
Bacto-Peptone[3]	6.7	43	26.0
Proteose Peptone No. 4[3]	7.2	66	25.8
Peptone, U.S.P.[4]	6.5	48	25.7
Bacto-Casitone[3]	7.2	61	25.7
Bacto-Tryptose[3]	7.2	53	25.1
Bacto-Gelatone[3]	6.5	55	25.1
Yeast Extract[3]	7.1	75	25.1
Bacto-Pantone[3]	6.7	79	24.9
Proteose-Peptone[3]	7.0	58	24.6
Neopeptone[3]	6.6	58	24.6
Bacto-Protone[3]	6.4	80	23.4
Bacto-Tryptone[3]	7.1	42	22.7
Bacto-Lactaltone[3]	6.8	51	21.8
Casein Hydrolysate (Enzymatic)[5]	6.3	79	21.7
Bacto-Soytone[3]	7.2	64	20.6
Bacto-Casamino Acids[3]	6.6	62	16.2

[1]Peptone-dextrose Agar (2%:2%:1.5%)
[2]Conray Products Co.
[3]Difco Laboratories, Inc.
[4]Matheson, Coleman and Bell
[5]Nutritional Biochemicals Corp.

9-64, 1½M