GROWTH OF *DIPLODIA ZEAE* IN SHAKER CULTURE

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ABSTRACT. — Using Czapek-Dox solution, *Diplodia zeae* grew best when initial pH levels were about 4.5. Mycelial dry weight increased with the addition of yeast extract to this solution. The growth-stimulating effect of yeast extract could not be replaced with ash of yeast extract. Fresh weights of mycelium grown in continuous light were greater than that grown in the dark. Growth of mycelium in light or dark did not appear to affect dry matter production.

Diplodia zeae (Schw.) Lev. causes seedling blight and ear, root and stalk rot of corn. There are relatively few reports regarding the factors affecting growth of this fungus in pure culture. Durrell (1913) reported that *D. zeae* grew well on a number of standard media such as cornmeal, oatmeal, and bean stems. Using synthetic media, he found that the fungus grew well on those with sucrose, dextrose, maltose, and lactose, but made slowest growth on the latter. Pure cellulose agar induced profuse growth and sporulation. Kinsel (1937) and Stevens and Larsh (1939) were able to grow the fungus in media with different carbon sources. Wilson (1942) also grew the fungus in media containing different carbohydrates and in different samples of the same carbohydrate. He obtained evidence that biotin might be a growth factor of *D. zeae*. Semeniuk (1940) reported that *D. zeae* grew poorly in Czapek-Dox liquid medium, plain agar, and glucose agar, but that the fungus grew better with additions of small amounts of water extracts of potato, carrots, and other organic materials. Biotin was not found to be a required growth substance.

This paper presents data on the effect of pH, yeast extract, ash from yeast extract, and light on the growth of *D. zeae* in shaker culture.

MATERIALS AND METHODS

A small piece of *D. zeae* and underlying potato dextrose agar was taken from near the edge of a rapidly-growing, four-day-old colony and was transferred to a 125 ml Erlenmeyer flask containing 50 ml of Czapek-Dox Broth (Fisher) liquid medium of pH 4.5 after autoclaving. All flasks were placed on a rotary shaker at 110 rpm with the temperature between 27-29°C.

To determine the effects of pH and light on growth, fragmented mycelium was grown for seven days in 50 ml of Czapek-Dox solution adjusted to give pH values of 3.5, 4.5, 5.5, 6.5, and 7.5 after autoclaving. Three flasks from each pH level were wrapped in aluminum foil and covered with a loose aluminum foil cap. These flasks were used for the dark treatment. Three other flasks from each pH level were used for a light treatment. In that group, aluminum foil caps were used to avoid any aeration differences in the two treatments. All flasks were agitated on a rotary shaker at 110 rpm under 300-foot-candles of illumination from Gro-Lux fluorescent tubes (Pappelis et al., 1964). The pH of the medium of all flasks was estimated daily using short range pH paper and drops of medium obtained with a sterile loop. The fresh and dry weights of the mycelium were determined after seven days of growth. Fresh weights were obtained after carefully washing and decanting three times with 50 ml of distilled water to remove all medium, filtering on a Buchner funnel, washing several times with water, and aspirating for three minutes after the final washing. Aspiration was with a Buchler Water Booster which provided a constant 40 psi for uniform aspiration. Dry weights were determined after 24 hours drying at 70°C.

The effect of yeast extract (Baltimore Biological Laboratories) on growth of

D. zeae was determined using 0.0, 0.1, 0.5, and 1.0 g of yeast extract per liter of Czapek-Dox solution. The pH of the media was adjusted to 4.5 since this pH gave good growth in previous experiments. In a separate experiment the fungus was grown nine days in Czapek-Dox media with ash from 0.5 g of yeast extract per liter and compared to growth in media with 0.5 mg yeast extract per liter of Czapek-Dox solution. Dry weight and pH were determined after 3, 4, 6, and 8 days of incubation. The mycelium was separated from the growth medium as described above.

Results and Discussion

The effect of light and pH on the fresh and dry weights of *D. zeae* mycelium are presented in Table 1. During the first 6 days of growth, the change in pH of the growth medium in the light and dark treatments were similar and, thus, only the pH values of medium in the light treatment are reported. Regardless of initial pH, growth medium in all treatments had changed to pH 6.8 by the fourth day and remained relatively unchanged thereafter. An increase to a higher pH was noted in the medium of the dark treatment by the seventh day.

Growth, as measured by fresh weight of the mycelium, was affected by both initial pH of the growth medium and by light. Estimates of the mycelial growth observed in the flasks during the 7 days indicated most rapid growth occurred in the low pH treatments in both light and dark. After 6 days, mycelial growth in the high pH treatments was similar in amount to that observed in the low pH treatments after one or two days of growth. However, the mycelium in the high pH treatments differed in appearance, being "milky" rather than forming round, loose colonies. Fresh weight of light-grown mycelium after 7 days of growth was greatest in the pH 4.5 medium; whereas, in the dark, the greatest growth

Table 1.—Daily Averages for pH of *Diplodia zeae* Growth Medium[1], and Final Fresh and Dry Weights of *D. zeae* Mycelium Grown in Czapek-Dox Solution in Light and Dark Treatments[2]. Each Average Represents 3 Flasks.

pH after autoclaving	Days after start of experiment: 1 pH	2 pH	3 pH	4 pH	5 pH	6 pH	7 pH L	7 pH D	7 g F wt L	7 g F wt D	7 g Dry wt L	7 g Dry wt D
4.5[3]	4.3	5.5	6.0	6.8	6.9	6.8	6.8	6.7	6.43	2.13	0.69	0.56
4.5	5.2	5.5	6.0	6.8	6.8	6.9	6.9	7.1	7.27	1.97	0.68	0.63
5.5	5.6	6.2	6.3	6.6	6.9	6.8	6.9	7.1	4.96	1.95	0.41	0.58
6.5	6.6	6.8	6.8	6.8	6.9	6.9	7.0	7.0	4.50	1.90	0.55	0.61
6.5[4]	6.9	7.0	6.9	6.8	6.9	6.9	6.9	7.3	3.70	1.80	0.62	0.63

[1] The averages of *D. zeae* growth medium pH for light and dark treatments generally varied ± 0.1 pH units during the first 6 days of growth. Thus, only the light treatment averages are reported.

[2] Dark treatment (D) was aluminum foil wrapped flasks with foil caps over cotton plugs; light treatment (L) was 300 foot candles of Grow-lux light with aluminum foil caps over cotton plugs.

[3] The pH 3.5 medium changed to pH 4.5 with autoclaving.

[4] The pH 7.5 medium changed to pH 6.5 with autoclaving.

was at 3.5. The amounts of fresh weight decreased when initial pH was increased in both treatments. Dry weight of mycelium grown in the light and dark did not differ greatly in the pH treatments except at pH 5.5.

When data concerning light and dark grown mycelium are compared little difference in dry weight production occurred but from 2 to 4 fold differences occurred in fresh weights, the greater gains occurring in the light. The greatest differences were observed in the lower pH treatments.

Similar differences in fresh weights of mycelium grown in light and dark were observed in earlier experiments with *D. zeae*. In those preliminary studies, the growth medium of the light treatment was observed to be more viscous than that of the medium in the dark and created problems during filtration and washing of the mycelium. The chemical nature of the viscous matter was not determined. Growth medium of dark treatments was easily filtered and presented no problem when mycelium was washed. In the reported experiment, the growth medium was diluted with 50 ml of distilled water and decanted three times before being transferred to the Buchner funnel for final washing under reduced pressure. In these conditions, mycelium was obtained relatively free from the viscous material. No other problems were encountered in this step to suggest that differences in fresh weights were due to external rather than internal moisture. The earlier work also showed mycelium dry weights to be greatest in the low pH treatments. From the data presented in this paper, it is possible that this was due to incomplete removal of the viscous material adhering to the mycelium.

Addition of yeast extract to the Czapek-Dox media resulted in greater mycelial dry weights (Table 2). Throughout the sampling period, dry weight as well as pH increased with increased amounts of yeast extract in the growth medium. The addition of 0.5 g of yeast extract per liter was considered sufficient to stimulate growth in future studies. In a separate study, ash of 0.5 g of yeast extract per liter of growth medium resulted in 160 mg of dry mycelium after 9 days of growth while 660 mg dry mycelium was obtained in medium containing 0.5 g of unashed yeast extract. This suggests that the growth factor for *D. zeae* in yeast extract is not a mineral nutrient. The nature of the stimulus from the organic matter contained is not known.

Robins et al. (1963) recently reported that the response of *Polyporus schweinitzii* Fr. to yeast extract or to the growth factors prepared from it was definitely affected by light. At high yeast extract levels, light-exposed cultures yielded higher dry weights than dark-grown cultures while the reverse was true at low levels of yeast extract. Growth of cultures in media containing ferulic acid instead of yeast extract was less in the light than in the dark except at high levels of this compound. When

TABLE 2.—Average pH of Medium and Dry Weight of *Diplodia zeae* mycelium Grown in Czapek-Dox Solution with Different Concentrations of Yeast Extract. Medium pH after Autoclaving was 4.5. Flasks were Wrapped in Aluminum Foil.

Yeast Extract g/l	Days after inoculation							
	2		4		6		8	
	pH	D Wt mg	pH	D Wt mg	pH	D Wt mg	pH	D Wt mg
0.0	5.2	10	5.9	78	6.1	149	6.1	146
0.1	5.9	77	6.4	213	6.6	273	6.6	280
0.5	6.2	104	6.5	265	6.7	328	6.9	450
1.0	5.9	137	6.7	345	6.8	422	7.1	505

sodium oleate, ferulic acid, and isoeugenol were added instead of yeast extract, the growth curves for the cultures grown in the light and dark were similar to those for yeast extract media under light and dark treatment. This was significant since oleic and ferulic acids were isolated from yeast extract. The dry matter yields obtained for *Poria ambigua* Bres. grown in light and dark was similar to that reported for *Polyporus schweinitzii* (Robbins and Hervey, 1960).

The observation of increased fresh weights for *D. zeae* grown in the light are unique to the work presented in this study. What role light-induced changes in *D. zeae* may play in the physiology of parasitism in root or stem rot of corn (Craig and Hooker, 1961; Koehler, 1960) remains for future studies.

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