Nitrogen Source Corrects a Potato-Dextrose Agar Medium Deficient in Supporting Mycelial Growth of *Monilinia* spp.

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ABSTRACT

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Mycelial growth of *Monilinia* spp. was compared on one freshly prepared and two commercial potato-dextrose agars. Growth on the agar made by BioQuest was found to be atypical. Addition of various ingredients to this agar indicated a deficiency in the amount of nitrogen needed for typical mycelial growth of the fungi. Subsequent analysis of the three potato-dextrose agars showed that the one made by BioQuest contained the least nitrogen. When an organic source of nitrogen was added to it, typical mycelial growth of *M. laxa* and *M. fructicola* was restored.

Additional key words: brown rot

Potato-dextrose agar (PDA) is routinely used as a medium for the isolation and identification of *Monilinia* spp. *M. laxa* (Aderh. & Ruhl.) Honey can easily be differentiated from *M. fructicola* (Wint.) Honey by cultural characteristics on this medium (5). However, cultural characteristics varied when these fungi were grown on PDA from different sources, and growth was drastically reduced on one of the media (6).

The variation is important because PDA is used in monitoring fungicide resistance (7). Use of the medium that limited growth could provide results suggesting extreme sensitivity to a fungicide. We attempted to find the basis for this variation so that it could be prevented in the future. One laboratory and two commercial preparations of PDA were studied, although most tests were done with one commercial formulation (9).

MATERIALS AND METHODS

Commercial PDA formulations were obtained from Difco Laboratories, Detroit, MI 48232 (PDA-Difco), and BioQuest, Division of Becton, Dickinson and Co., Cockeysville, MD 21030 (PDA-BBL). Our laboratory medium (PDA-P) contained 15 g of agar, 10 g of glucose, and infusion materials from 200 g of fresh potatoes per liter of distilled water. The infusion was prepared by autoclaving 200 g of peeled, diced, fresh potatoes in 1 L of distilled water and holding them in cheesecloth for 10 min. The solid potatoes were removed and the liquid infusion added to 15 g of melted agar and 10 g of glucose; volume was adjusted with distilled water to 1 L, and the mixture was autoclaved for 15 min at 121 C under 15 psi.

PDA-Difco and PDA-BBL were prepared by adding 39 g of the dehydrated formulation to 1 L of distilled water and autoclaving for 15 min. According to the labels, both commercial formulations contained the same amount of dextrose (20 g), agar (15 g), and potato infusion (4 g). PDA-Difco contained infusion from 200 g of potatoes in 1 prepared liter of medium. The BBL-PDA label did not state how many potatoes were used to prepare the infusion.

In experiment 1, three Difco defined media and Difco yeast extract (1) were added to PDA-BBL at the rate of 10 g/L of PDA-BBL. The three defined media were Bacto yeast nitrogen base without amino acids and ammonium sulfate, which contains salts and vitamins; Bacto yeast nitrogen base without amino acids, which contains enough ammonium sulfate to act as a nitrogen source; and Bacto vitamin-free yeast base, a vitamin-free source of amino acids, nitrogen, and carbon. In experiment 2, ammonium nitrate (NH_4NO_3), ammonium sulfate [$(NH_4)_2SO_4$], potassium nitrate (KNO_3), and asparagine were added to PDA-BBL at the rate of 10 g of total nitrogen per liter.

The amended PDA-BBL was inoculated with mycelial disks (4-mm diameter) of *M. fructicola* and *M. laxa* taken from PDA-BBL containing no additives. The plates were incubated in the dark at 24 C for 2–7 days and mycelial growth was measured daily.

Table 1. Mycelial growth of *Monilinia fructicola* and *M. laxa* on PDA-BBL amended with Difco defined media and yeast extract

	Average growth (mm)[w] on PDA-BBL amended with				
Fungus	Nothing	YNB w/o A.A. & $(NH_4)_2SO_4$[x]	YNB w/o A.A.[y]	Vitamin-free YB[z]	Yeast extract
M. fructicola	14.6 c	29.5 c	35.2 b	44.8 a	39.2 b
M. laxa	25.8 bc	20.2 c	47.7 a	44.3 a	33.0 b

[w] Data are averages from five plates in one experiment. Values within each row not followed by the same letter are significantly different ($P = 0.05$) according to Duncan's multiple range test.
[x] Yeast nitrogen base without amino acids and ammonium sulfate.
[y] Yeast nitrogen base without amino acids.
[z] Vitamin-free yeast base.

Table 2. Mycelial growth of *Monilinia fructicola* and *M. laxa* on PDA-BBL amended with various sources of nitrogen

	Average growth (mm)[y] on PDA-BBL amended with					
Fungus	Nothing	KNO_3	$(NH_4)_2SO_4$	NH_4NO_3	Asparagine	Yeast extract
M. fructicola	14.6 d	11.6 de	8.4 e	30.0 c	46.2 b	54.2 a
M. laxa	25.8 c	32.2 b	38.2 a	38.0 a	31.3 b	23.4 c[z]

[y] Data are averages from five plates in one experiment. Values within each row not followed by the same letter are significantly different ($P = 0.05$) according to Duncan's multiple range test.
[z] Mycelium forms a very thick mat.

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PDA-P, PDA-Difco (control code 637836), and PDA-BBL (lot BODFXZ, expiration 2/81) were analyzed for total nitrogen by the Kjeldahl method (2).

RESULTS

The *Monilinia* spp. grew very slowly on PDA-BBL without additives (Table 1). With the addition of yeast nitrogen base without amino acids and ammonium sulfate, *M. laxa* grew at about the same rate as on PDA-BBL alone, while *M. fructicola* grew slightly faster. Both fungi grew well on PDA-BBL with vitamin-free yeast base. With the addition of yeast nitrogen base without amino acids, *M. laxa* grew at an excellent rate, similar to that of *M. fructicola* on yeast extract.

When various forms of nitrogen were added to PDA-BBL (Table 2), Difco yeast extract supported extensive growth of *M. fructicola* but not of *M. laxa*. The addition of asparagine resulted in the most typical growth of both *Monilinia* spp., and ammonium nitrate provided typical growth of *M. laxa* (Fig. 1). *M. laxa* typically grows on PDA as colonies with lobed margins, whereas *M. fructicola* produces colonies with entire margins (5).

The average total nitrogen contents (five determinations) of the three PDA were: PDA-P, 0.75 ± 0.02%; PDA-Difco, 0.56 ± 0.02%; and PDA-BBL, 0.12 ± 0.01%.

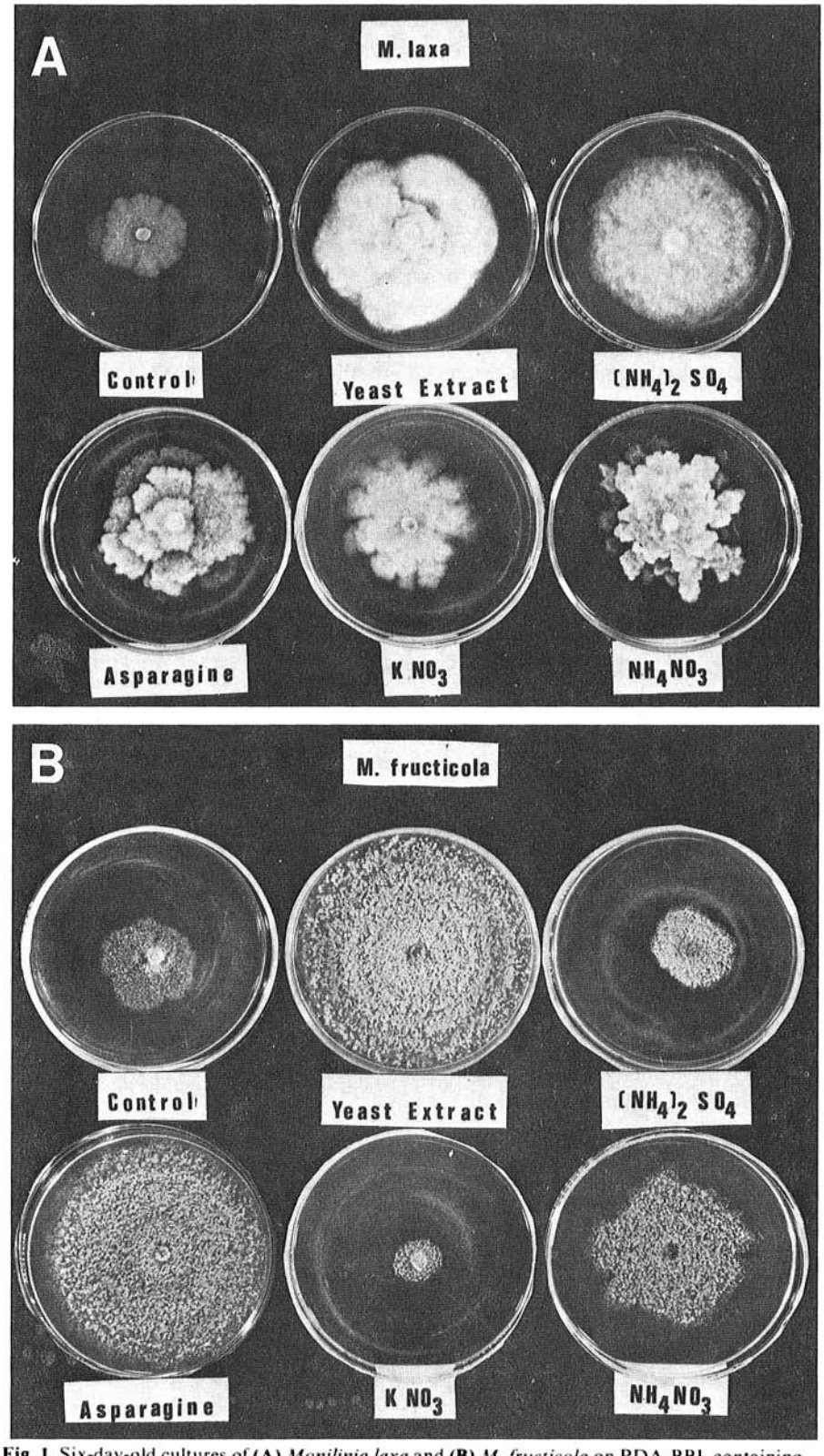

Fig. 1. Six-day-old cultures of **(A)** *Monilinia laxa* and **(B)** *M. fructicola* on PDA-BBL containing various sources of nitrogen.

DISCUSSION

PDA-BBL lacked sufficient nitrogen for typical growth of the *Monilinia* spp. As noted previously (6), PDA-P was the best growth medium of those tested, probably because it contained the highest amount of total nitrogen.

Hall (3) found that poor growth of *M. fructicola* on media containing potassium nitrate is not a pH effect. He concluded that *M. fructicola* uses organic in preference to inorganic nitrogen, which may explain why asparagine was the best source of nitrogen of the additives tested. The nitrogen nutrition of *M. laxa* (Fig. 1) was more complex than that of *M. fructicola*, producing different colony characteristics as the source of nitrogen changed. These fungi also appear to differ in their nitrogen requirements: *M. laxa* grew well on the medium amended with KNO_3, but *M. fructicola* did not.

Rosenberger and Meyer (8) suspected that commercial PDA preparations contain substances that are inhibitory to *M. fructicola*. We believe that the amount of total nitrogen in commercial PDA could cause variable growth in *Monilinia* spp., as well as in other fungi. Harding (4) found that freshly prepared PDA is superior for the growth of *Bipolaris sorokiniana* to two commercially available preparations containing potato infusions. We suggest that the labels on commercial formulations of PDA state the total nitrogen content of the product.

LITERATURE CITED

1. Anonymous. 1953. Difco Manual of Dehydrated Culture Media and Reagents for Microbiological and Clinical Laboratory Procedures. Difco Laboratories, Detroit, MI. 350 pp.
2. Anonymous. 1960. Official Methods of Analysis of the Association of Official Agricultural Chemists. 9th ed. p. 94.
3. Hall, R. 1967. Carbon and nitrogen nutrition of *Monilinia fructicola*. Aust. J. Biol. Sci. 471-474.
4. Harding, H. 1979. Effects of culture media containing potato extracts on colony morphology of *Bipolaris sorokiniana*. (Abstr.) Proc. Can. Phytopathol. Soc. 46:57.
5. Hewitt, W. B., and Leach, L. D. 1939. Brown rot Sclerotinias occurring in California and their distribution on stone fruit. Phytopathology 29:337-351.
6. Ogawa, J. M., Gilpatrick, J. D., Uyemoto, J. K.,

and Abawi, G. S. 1978. Variations in fungal growth on various preparations of potato-dextrose agar media. Plant Dis. Rep. 62:437-441.
7. Ogawa, J. M., Manji, B. T., Bose, E. A., Szkolnik, M., and Frate, C. A. 1978. Methods for detection of benomyl-tolerant *Monilinia fructicola*. (Abstr.) Phytopathol. News 12:180.
8. Rosenberger, D. A., and Meyer, F. W. 1979. Inhibitory effect of some potato-dextrose agar preparations on germination of pycnidiospores of *Leucostoma* species. Plant Dis. Rep. 63:793-795.
9. Sholberg, P. L., and Ogawa, J. M. 1979. Nitrogen requirement in a certain potato dextrose agar for mycelial growth of *Monilinia* species. (Abstr.) Phytopathology 69:920.