CARBON AND NITROGEN NUTRITION OF *MONILINIA FRUCTICOLA**

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Little work on the response of *Monilinia fructicola* (Wint.) Honey to defined carbon and nitrogen sources has been reported. In the present study three Australian isolates of the fungus were examined to determine satisfactory carbon and nitrogen sources on which to culture the species and possible interactions between carbon and nitrogen sources. The isolates, designated 30A, 36A, and 61A, were all pathogenic to the fruit of *Prunus persica* (L.) Batsch. but were culturally distinct.

Four sources of carbon, namely D-glucose (10·0 g/l), L-arabinose (6·6 g/l), D-xylose (6·6 g/l), and sucrose (9·5 g/l), and four sources of nitrogen, namely L-asparagine (2·0 g/l), glycine (2·6 g/l), ammonium chloride (1·6 g/l), and potassium nitrate (3·1 g/l), were used. In each medium there was one carbohydrate at a concentration equivalent in carbon to 10·0 g/l of glucose and one nitrogen source at a concentration equivalent in nitrogen to 2·0 g/l of asparagine. The basal medium consisted of 1·0 g KH_2PO_4, 0·5 g $MgSO_4.7H_2O$, 1 mg ferric citrate, 5 μg biotin, 100 μg thiamine hydrochloride, and 1 litre glass-distilled water. All glassware was acid-washed and rinsed with glass-distilled water and all reagents, apart from Oxoid Czapek Dox medium, were B.D.H. laboratory reagent grade.

The media were adjusted to pH 5·5 with 6N NaOH or 6N HCl, as required, sterilized by filtration to avoid possible degradation of sugars to toxic products during autoclaving (Cochrane 1958), and dispensed in lots of 30 ml into Erlenmeyer flasks (250 ml capacity). To prepare inoculum, single-spore colonies on Oxoid Czapek Dox agar slopes were subcultured to liquid Oxoid Czapek Dox medium and incubated at 25°C with continuous rotary shaking in continuous light for 10 days. A portion of this mycelium was subcultured to further lots of Czapek Dox liquid medium and incubated as before. The resultant mycelial mats were washed with 200 ml of sterile glass-distilled water and macerated in sterile glass-distilled water for 5 min. To minimize variation between replicates of liquid cultures of fungi Ward and Colotelo (1960) suggest that the dry weight of the inoculum should not be less than 2 mg. Accordingly, each flask was inoculated with 1 ml of a mycelial suspension adjusted to contain 2 mg dry weight of mycelium per millilitre and incubated with continuous rotary shaking at 25°C in continuous light. Dry weights of total growth per flask were determined 5 days after inoculation.

The mean results of one experiment with six replicates per treatment are presented. The mean final pH of each medium after supporting growth of isolate 30A, being representative of data for all isolates, is presented in Table 1. Changes

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in pH depended largely on the nitrogen source and were virtually independent of the isolate of the fungus and the carbon source. In the presence of asparagine, glycine, and potassium nitrate the pH rose from 5·5 to about 7 during the 5 days' incubation whereas the pH fell to about 5 in the presence of ammonium chloride.

The effect of each combination of carbohydrate and nitrogen source on dry weight production of each isolate is seen in Figure 1. The data were subjected to an analysis of variance (Steel and Torrie 1960, p. 205). A very low mean square value (6·5) was obtained for replicates, indicating the small variation that occurred between replicates. Differences in dry weight production between isolates, between nitrogen sources, and between carbon sources were significant at the 5% level of probability.

The relative amounts of growth supported by the four sugars depended to some extent on the nitrogen source. However, where abundant growth was made, i.e. where asparagine was present, the sugars generally fell into the following order: glucose > sucrose > xylose > arabinose. This order agrees with most published

TABLE 1

pH OF MEDIA AFTER SUPPORTING GROWTH OF ISOLATE 30A FOR 5 DAYS

Carbon Source	Nitrogen Source			
	Asparagine	Glycine	NH_4Cl	KNO_3
Glucose	7·0	6·9	5·0	6·0
Sucrose	7·2	7·2	5·1	6·7
Xylose	7·3	7·5	4·8	6·8
Arabinose	7·5	7·2	5·3	7·0

studies on other fungi (Cochrane 1958). In contrast to the present results Lilly and Barnett (1953) observed that, in the presence of asparagine, mineral salts, thiamine, and biotin, arabinose supported more growth of *M. fructicola* than did xylose.

Asparagine supported far more growth than the other sources of nitrogen tested. This concurs with the observation that *M. fructigena* makes very little growth with glycine, ammonium chloride, or potassium nitrate as the sole nitrogen source (Cole 1956). The limited amounts of growth made by *M. fructicola* in media containing one of the latter three nitrogen sources cannot be attributed to detrimental changes in pH of the media (Table 1). Moreover, this fungus grows quite vigorously in a medium containing asparagine and pectin at pH 3·5 (Hall, unpublished results). It appears that *M. fructicola* prefers organic to inorganic sources of nitrogen. Asparagine probably acted as a source of carbon also. This was not its major effect, however, because the type of sugar present with asparagine greatly influenced the dry weight of mycelium produced.

Firm conclusions cannot be drawn from the data as they refer only to one experiment. However, a statistically significant interaction was found, at the 5%

level, between carbon source and nitrogen source. Several studies have shown interactions between different concentrations of carbon and nitrogen when reproduction of fungi is considered (Hawker 1957) but there is little information on the relation of fungal growth to different combinations of several carbon and nitrogen sources.

A mixture of sugars often supports a level of growth greater than the total growth supported by individual components of the mixture (Steinberg 1950). That is, the ability of sugars to support growth of fungi may be greatly influenced by the presence of other carbohydrates in the medium. Similarly, the significant interaction detected here between carbon source and nitrogen source implies that the relative amounts of growth made in the presence of a particular sugar depended on the

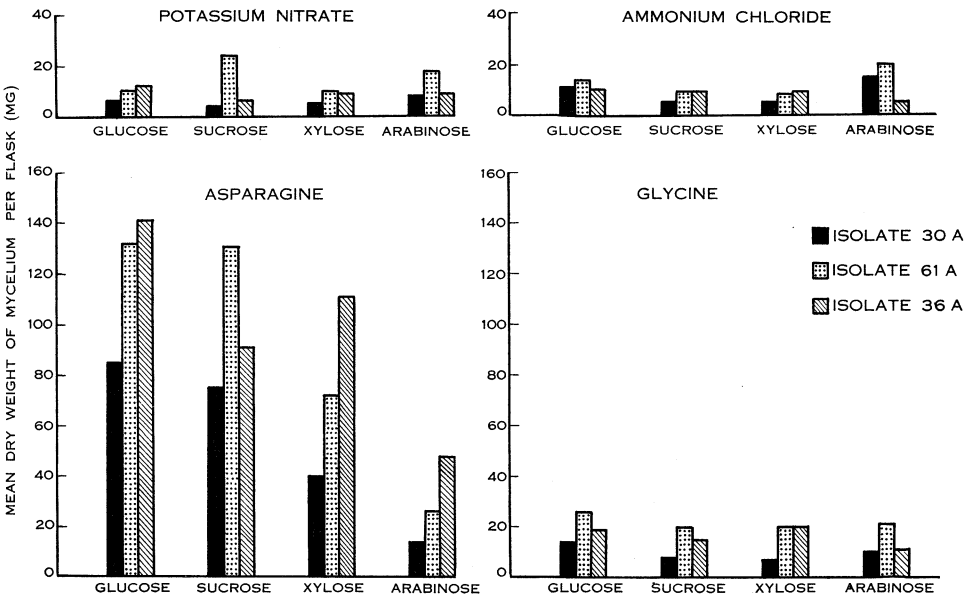

Fig. 1.—Growth of three isolates of *M. fructicola* in response to all combinations of four sugars with four nitrogen sources. The sugar is shown below each group of three columns and the nitrogen source is shown above each group of 12 columns.

nitrogen source. For example, in the presence of potassium nitrate there was little difference between the sugars in the amount of growth they supported whereas with asparagine as the nitrogen source there was a four-fold difference between glucose and arabinose. Thus, as in the nutrition of higher plants (Brown 1963), interactions between chemical factors may occur in the nutrition of fungi and other microorganisms.

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