THE EFFECT OF RESPIRATORY INHIBITORS ON THE OXYGEN UTILIZATION OF THREE ISOLATES OF *APHELENCHUS AVENA* UNDER AEROBIC AND ANAEROBIC CONDITIONS

BY

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The effect of culture age, temperature and anaerobiosis on inhibition of respiration by cyanide, the nematicide EDB, and other respiratory inhibitors was studied in three isolates of *Aphelenchus avenae*. Two isolates differed in their responses to cyanide, the nematicide, temperature and to hyperbaric oxygen. Respiration in isolate F was strongly inhibited by cyanide but the depression of respiration in isolate M following prolonged incubation at 30°C was partly relieved by cyanide. Isolate F respiration was inhibited by EDB whereas "young" cultures of isolate M showed respiratory stimulation. Salicyl hydroxamic acid (SHAM), carbon monoxide, and p-chloromercuribenzoate had similar effects on both isolates. These results provide preliminary evidence for the existence of several respiratory pathways which differ in significance in isolates of *A. avenae*.

**Keywords:** physiology, respiration, metabolic inhibitors, nematicide, free-living nematode.

Understanding of the respiratory metabolism of plant parasitic nematodes could be improved by studying related free-living species such as *Aphelenchus avenae*, which are easily cultured. Metabolic inhibitors, whose use is essential to elucidate respiratory pathways, have, however, affected closely related species differently. For example, the rate of oxygen utilization (QO2) of *Caenorhabditis briggsae* was 70% inhibited by cyanide, but the inhibition was not permanent since only 14% inhibition remained following the removal of the inhibitor (Bryant *et al.*, 1967). It was further observed that *C. briggsae* "survived for 7-10 days in cyanide with reduced activity before dying". Similarly, the state-3 mitochondrial respiration of *C. elegans* was 85% inhibited by 20 μM KCN (Murfitt *et al.*, 1976). By contrast, the state-3 succinate oxidation by a mitochondrial preparation of *Turbatrix aceti* required 111 μM NaCN for complete inhibition and 10 μM sodium azide and up to 74 μM cyanide had little effect (Rothstein, *et al.*, 1970).

Metabolic inhibition by haem protein blockage (e.g. CN− inhibition of cyt a-a3) was proposed by Castro (1964) as a mechanism for the mode of action of alkylhalide nematicides, for which Evans & Thomason (1971) produced sup-
porting circumstantial evidence. Marks (1971) showed that the alkylhalide nematicide EDB stimulated the QO₂ of the third stage *Caenorhabditis* sp., con-
forming with observations on EDB-treated cockroaches (Morikawa, 1964), but
the QO₂ of *A. avenae* was “not significantly influenced” by EDB (Marks,
1971). Awan (1975), however, found the QO₂ of *A. avenae* was depressed
21.9% by EDB and 17% by halothane.

In view of the need for further information on the respiratory physiology of
stylet-bearing nematodes we examined and characterised several isolates of *A.
avenae* (Mendis & Evans, 1984a). This paper describes the differential effects of
metabolic inhibitors and the nematicide ethylene dibromide (EDB) on the
respiration of three different isolates of *A. avenae*.

MATERIALS AND METHODS

The three isolates of *A. avenae* used were isolates IC and F (parthenogenetic),
and isolate M (amphimictic). Details of their origin and culture, together with
methods of harvesting and respiratory assay, are given in Mendis & Evans
(1984a), except where otherwise stated.

For assessing the rate of O₂ consumption (QO₂) a Clark-type oxygen ele-
ctrode (Rank Bros., Bottisham, Cambridge, U.K.) was employed, with stir-
ring. After initial zeroing with Na₂S₂O₄, the electrode was calibrated before
each measurement using 3 mls of sterile distilled water (SDW) at the test
temperature (usually 30°C) to a constant baseline, following which the
calibrating water was removed and immediately replaced with 3 ml of the
nematode suspension. The polarographic recording was made once the test
sample reached the test temperature. Following assays, the dry weight of the
nematode samples were determined to constant weight over P₂O₅. Unless
otherwise stated, this was the general procedure for all QO₂ measurements.

Chemicals used were sodium cyanide, ethylene dibromide, carbon monox-
ide (B.D.H.Ltd), salicylhydroxamic acid, and p-chloromercuribenzoic acid
(Sigma London Chemical Co.).

RESULTS

1. *The effect of culture age on the cyanide sensitivity of the QO₂ of three isolates of A.
avenae*

The three isolates were grown synchronously and harvested after 40, 50, 60,
70, 90, 120 and 140 days, then prepared as standard suspensions for QO₂
measurement at 30°C. Sodium cyanide (NaCN, 1 M solution) was then added
to the suspension to give a final concentration of 3 mM and the QO₂ measured
again. Three batches from the same harvest-times were assessed and 6
replicate assays conducted with each batch.

Isolates were similar in their QO₂ with age of culture but differed in the
degree of respiratory inhibition seen immediately following the addition of