Nematologica 22 (1976): 343-351. E. J. Brill, Leiden

IN VITRO STUDY OF TOXICITY OF SOLUBLE SULPHIDES TO THREE NEMATODES PARASITIC ON RICE IN SENEGAL

BY

R. FORTUNER and V. A. JACQ

Laboratoire de Nématologie et Laboratoire de Microbiologie des Sols
O.R.S.T.O.M. B.P. 1386. Dakar, Sénégal

Toxicity of sulphides to three species of nematodes parasitic on rice from Senegal (*Hirschmanniella oryzae, H. spinicaudata* and *Tylenchorhynchus mashhoodi*) was studied *in vitro* using three different methods.

A Sulphide Time Unit (STU) was defined related to the average concentrations of sulphides and their variations during the tests.

With hydrogen sulphide dissolved in water, the very low pH increased nematode mortality. When hydrogen sulphide was dissolved in a buffered medium used for bacterial culture, mortality was lower but a shock effect appeared due to sudden contact with high concentrations of sulphides and toxicity of some of the components of the medium. When hydrogen sulphide was produced by sulphate reducing bacteria a similar shock effect was still observed, but only when high concentrations were reached within 1 or 2 days: in such experiments nematodes were killed by low STU, and differences were observed in resistance of the three species. However, when sulphide accumulated more slowly, i.e., when lethal concentrations were reached in 3 or more days, the sensitivity of the three species was similar: 50% mortality was obtained at about 120-130 STU.

The possibility of using sulphate reducing bacteria for biological control of parasitic nematodes of rice is discussed.

A survey of root parasitic nematodes of rice in Senegal has shown that in some fields, particularly in the Casamance region, few or no nematodes were found (Fortuner & Merny, 1973). In four of these fields surveyed for sulphate reducing bacteria (Jacq, 1972) their activity was found to be very high. This suggested that the very low nematode populations were possibly due to the nematicidal properties of hydrogen sulphide. This phenomenon was first reported from Louisiana rice fields by Rodriguez-Kabana *et al.* (1965) who also established *in vitro* the relation between H_2S concentration and the time required to kill *Tylenchorhynchus martini.*

The present study was undertaken to determine *in vitro* the lethal dose of sulphides against the three most common species of rice parasitic nematodes in Senegal: *Hirschmanniella spinicaudata* (Schuurmans Stekhoven), *Hirschmanniella oryzae* (van Breda de Haan) and *Tylenchorhynchus mashhoodi* Siddiqi & Basir. They were subjected for 1 to 6 days to various concentrations of sulphides produced in three different ways:

— hydrogen sulphide dissolved in deaerated water;
— hydrogen sulphide dissolved in a sterile buffered medium used for sulphate reducing bacteria culture;
— hydrogen sulphide produced by sulphate reducing bacteria in the same buffered medium.

MATERIALS AND METHODS

Nematodes were obtained from stock cultures maintained at the O.R.S.T.O.M. laboratory at Dakar, originating from specimens from the Casamance and the Senegal River regions in Senegal. Two to four lots extracted at different times were used for each trial.

Nematodes were extracted from soil by elutriation (Seinhorst, 1962) or from rice roots by a mistifier apparatus (Seinhorst, 1950) and put in deaerated water. About one hundred nematodes in 1 ml of water were injected with a syringe into each test flask. A total of 650 flasks (excluding controls) were subjected to various concentrations and for various times to sulphide produced by the following methods.

Hydrogen sulphide dissolved in deaerated water

4N-hydrochloric acid (HCl) was reacted with iron monosulphide (FeS) at 60° C; the gaseous hydrogen sulphide produced was conducted by a nitrogen stream into vessels containing 100 ml of deaerated water through which it bubbled and dissolved. By varying the durations of bubbling and reagent contents hydrogen sulphide concentrations between 0.1 and 60 ppm $S^=$ were produced.

Soluble concentrations of sulphides were measured at 22° (Chaudhry & Cornfield, 1966). Aliquots of less than 2.2 ml were pipetted into 2.7 ml plastic test tubes containing 0.5 to 1 ml of a nematode suspension. The tubes were then filled with deaerated water, avoiding air bubbles under the caps to prevent oxidation of sulphide. The tubes were incubated at 32°. Sulphide and pH were measured again at the end of the test.

Hydrogen sulphide dissolved in a sterile buffered medium used for bacterial culture

0.5 to 5 ml of hydrogen sulphide solution obtained as described above were syringed through the rubber membrane of the screw cap into 11 ml flasks containing about 0.5 ml of nematode suspension. Sterilised Starkey's medium modified by Pichinoty (1966), used for cultivation of sulphate reducing bacteria, was added to fill the flasks in a manner to avoid air bubbles. The flasks were incubated at 32°. Sulphide concentrations were measured every day withdrawing 1 ml samples with a syringe without opening the flasks. Each flask was then refilled with 1 ml of newly prepared hydrogen sulphide solution. The pH was measured at the end of the tests.

Hydrogen sulphide produced by sulphate reducing bacteria in a buffered medium

Flasks containing the nematode suspension and Starkey medium were prepared as described above, then inoculated with 0.5 to 2 ml of 2-3 days old pure culture of a *Desulfovibrio* strain (S6) isolated from a rice field at Savoigne, Senegal. The flasks were filled completely to ensure anaerobiosis and maintained at 32°. The bacteria produced hydrogen sulphide which dissolved in the medium. Concen-