Nematodes: Biological Control in Rice Fields: Role of Hydrogen Sulfide

Abstract. *In flooded rice fields a decline in total nematode populations began shortly after the onset of soil anaerobiosis and was correlated with a sustained increase in concentration of molecular hydrogen sulfide in the soil-water phase. Laboratory tests showed that hydrogen sulfide at concentrations found in flooded fields killed 100 percent of nematodes in 5 to 10 days. The effect of hydrogen sulfide and its pattern of occurrence in the soils of water-saturated rice fields suggest that this compound can be a significant factor in the etiology and control of certain plant diseases.*

The natural control of nematode populations in flooded Louisiana rice fields, first observed in 1955 (*1*), was linked in laboratory studies with microbiological factors (*2*). Johnston (*3*) demonstrated that the metabolic fatty acids produced by *Clostridium butyricum* Prazmowski isolated from a rice soil were toxic to nematodes in vitro and to populations of nematodes incubated under the artificial conditions of anaerobiosis in soil samples supplemented with organic matter and submerged.

Measurements of microbiological and chemical variables in soil samples drawn at intervals from flooded rice fields over a 2-year period have demonstrated that the key factor in the reduction of nematode populations, postulated for mixtures of fatty acids (*3*), is hydrogen sulfide (H_2S).

Field and laboratory data show a straightforward effect of H_2S on nematodes. However, soil under conditions of anaerobiosis presents patterns of great complexity, and, in order to test thoroughly the evidence for the role of H_2S, we examined many other variables for their possible effects on nematodes.

Variables measured on field samples were *p*H, oxidation-reduction potential (*Eh*), organic carbon (*4*), total nitrogen (Kjeldahl), percentage of moisture, fatty acids (gas-liquid chromatography, *5*), H_2S, populations and species of the genus *Clostridium*, and total free-living and plant-parasitic nematodes. Variables tested against nematodes in vitro were H_2S, fatty acids, *p*H, oxygen, carbon dioxide, nitrogen, hydrogen, and natural gas containing methane.

We present evidence first on the measurement, production, and role of H_2S in reducing nematode population in rice fields, and then on other variables involved in soil anaerobiosis.

Determinations of H_2S in the range of 0.1 to 40 parts per million (ppm) were made with a polarograph constructed for the purpose. Theoretical details and practical limitations were in agreement with a technique outlined for measurement of H_2S in water associated with petroleum deposits (*6*). A series of standard H_2S concentrations established by iodometric titration (Fig. 1) show the limits of sensitivity of the method which, by extrapolation, can extend to 0.01 ppm of H_2S. Duplicate 500-ml samples of water-saturated soil from each sampling were diluted with distilled water, connected to a trap containing alkali, and heated to boiling for 15 minutes. The molecular H_2S was trapped in flasks containing 100 ml of 1*N* NaOH; the flasks were stoppered until the H_2S determination was made.

Soil was sampled from duplicate plots (3 by 3 m) in nine rice fields selected at random in 1963 to cover a range of soil types, and on four sites from single plots during the 1964 rice-growing season. Soil samples were composited from cylindrical cores (7.6 by 10.9 cm long) taken at random from the plots and then packed and sealed in quart jars. Single cores were removed to pint jars for *p*H and *Eh* determinations.

Nematodes were extracted from the soil in six replicate sets of 200-ml samples according to Seinhorst (*7*) and counted by routine methods (*1*). Swarming populations of the common stunt nematode of rice fields, *Tylenchorhynchus martini* Fielding 1956 (*8*), were used in laboratory assays of the listed variables, because of their uniformity, high vigor, and equivalence to nonswarming populations in their reactions to toxicants (*9*).

Data from three of the 1964 field plots (Fig. 2) demonstrate a relation between increase in H_2S concentrations and decrease in total nematode populations. The first traces of H_2S (0.1 to 1 ppm) appeared in the plots 5 to 7 days after flooding. This was followed by the appearance of deposits of metal sulfides on rice roots and by the lowering of *Eh* values to about 200 mv (2 to 4 weeks). An irridescent, rust-colored pellicle appeared at 4 to 6 weeks on the soil surface in the two plots which were free of weeds; it was stabilized by rice roots ramifying on the surface.

Current theory holds that H_2S formation in submerged soils results from the action of sulfate-reducing bacteria on organic substrates (*10*). We have isolated an organism, tentatively identified as *Desulfovibrio desulfuricans* (Beijerinck, 1895) Kluyver and van Niel, 1936, from "reduced" zones of 1963 Wild plot soil (Midland-Crowley silt loam) submerged under laboratory conditions; the initial culture was made on Van Delden's medium. Strong hydrogen- and metal-sulfide production was accompanied by formation of a typical pellicle on the surface of the medium. Analysis of the pellicle indicates the presence of iron oxides and hydroxides in addition to other constituents. An oily, irridescent substance, which exerts a cementing effect on these constituents, may be composed primarily of hydrocarbons (*10*). We have obtained experimental support for a subsidiary theory of H_2S production, which links the phenomenon of anaerobiosis with occurrence of fatty acids in rice soils (*11*).

Nematodes present on the sites were those that occur commonly in rice fields (*12*). Spiral, lesion, and ring nematodes, which occurred infrequently and in small numbers, generally could not be detected at higher H_2S concentrations. Fluctuations in populations of total plant parasites and of *Tylenchorhynchus martini* and *Radopholus oryzae* (V. Breda de Haan 1902) Thorne 1949 paralleled fluctuations of total nematodes, a phenomenon common to all populations extracted from rice fields (*1*). Characteristic variability of total nematode populations from the three plots was examined by analyses of variance on six replicate counts at each sampling, and subsequently by Duncan range tests (*13*) on sampling means ($X^{1/2}$ transformation). Sampling means, in general, differed significantly at the 1-percent level from the mean of the highest population in each plot. The population peak in the Richard plot (sampling No. 4) resulted from a stimulus

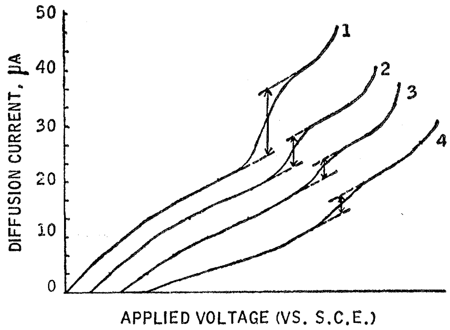

Fig. 1. Standard curves for H_2S in normal NaOH; pH 12, 25°C. Diffusion current at 0.84 volt (versus standard calomel electrode) is proportional to HS^- concentration. Steps in curves, denoted by arrows, represent H_2S concentrations of 13.43, 6.71, 3.35, and 1.67 ppm.

of plant growth caused by the application of mineral fertilizer high in nitrogen.

The percentage reduction of plant-parasite populations on 12 field sites in 1963 and 1964 was significantly greater than that of total nematode populations. This may be attributed to the greater resistance of saprozoic nematodes to H_2S, since they comprise a large proportion of the population in rice soils and show greater resistance than other nematodes to nematocides (*1*).

The rice varieties grown were principally Nato and Bluebonnet, medium- and long-grain varieties; both mature in 120 to 135 days. Rice fields are drained approximately 2 weeks before harvest, and, in the absence of flooding for a second crop, there is usually a marked increase in the nematode populations (both total and plant parasitic) from August to November in the stubble fields, the increase being stimulated by renewed plant growth. One of the four 1964 sites (Davis) was drained in midseason, with concomitant increase in nematode populations and the disappearance of H_2S.

When nematodes die in H_2S solutions there are characteristic morphological changes, the most prominent being globule formation in the intestinal region and lateral shrinkage of the esophagus. The typical time-concentration curve of 100 percent kill of *T. martini* in large swarms (2000 to 4000 specimens) in sealed vials (Fig. 3) provides a basis for interpretation of field results. Incomplete killing at H_2S concentrations above 10 ppm in the field plots is attributable at present to greater resistance of the nematode egg stage and to the effect of oxygen in lowering H_2S concentrations in the vicinity of rice roots.

Attempts to disprove a nematocidal role for H_2S in soils low in organic matter and in sand have consistently yielded negative results. The progressive decline in populations beginning 20 to 30 days after flooding (Fig. 2) may be taken to reflect the action of variables other than H_2S on nematodes under conditions of anaerobiosis. Repeated tests with a suspension of *T. martini* incubated in vitro in an atmosphere of hydrogen, carbon dioxide, or natural gas containing methane have shown that hydrogen and natural gas are harmless. Mortality of 20 to 30 percent under carbon dioxide seems irrelevant, since the highest concentrations of this gas in submerged rice fields occur soon after flooding (*14*), when nematode populations are high.

The fatty acid composition of rice fields consisted primarily of acetic acid, with lesser amounts of propionic acid and traces of butyric acid (*15*). Mixtures of acids simulating the pH range existing in the rice fields had no effect on *T. martini*, which tolerates soil acidities in vitro ranging from pH 3.5 to 11.5 (*16*), a range much greater than that in rice fields. The soil pH should not markedly influence dissociation of H_2S, since this compound has very small ionization constants (8.4×10^{-8}, 1.2×10^{-13}) and exists almost entirely in the molecular state.

The low concentrations and high volatility of mercaptans (methyl mercaptan, 0 to 0.12 ppm) studied in paddy soils by Takai and Asami (*17*), probably precludes any nematocidal importance in rice fields. We have confirmed the presence of oxygen around rice roots (*18*) by chronoamperometric methods (*19*), using an oscilloscope. Concentrations in soil around rice plants grown in plastic cylinders were nearly equivalent to values for oxygen-saturated water in air (8.38 ppm at 25°C) at all depths of root penetration from 0 to 15 cm. Oxygen is a disturbance factor in anaerobiosis, sufficient to sustain nematode activities (*20*).

Evidence that lack of oxygen is not repressive to the short-term survival of nematode populations in rice fields comes from studies in vitro. Swarming and nonswarming populations of *T. martini* in aqueous suspensions

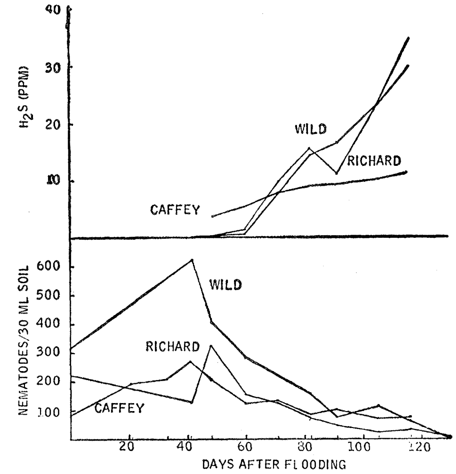

Fig. 2. Total nematode populations in three Louisiana rice fields in 1964 and H_2S concentrations in the soil-water phase (days—initial sampling to flooding: Caffey, 38; Wild, 41; Richard, 51).

were incubated under nitrogen for periods of 2 weeks. Absence of oxygen was assured by prolonged sweeping of the nematode suspensions with nitrogen before the chambers were sealed and confirmed periodically by chronoamperometric measurements with platinum electrodes immersed in the suspensions and compared with a sodium sulfide standard.

Hydrogen sulfide has been credited with a minor role in plant disease control, primarily because laboratory investigations of its mechanism of action and fungicidal efficiency have demonstrated that other constituents of

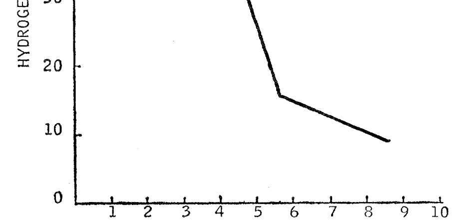

Fig. 3. Relation between H_2S concentration and time required for 100 percent kill of swarming populations of *T. martini* in sealed vials.

sulfur-bearing fungicides possessed higher inherent toxicity (*21*). Japanese workers (*22*) detected low concentrations of H_2S in rice soil incubated in the laboratory, and subsequent investigations have shown that Akiochi disease, a physiological disorder of rice in Japan, is caused by H_2S (*23*).

Our results provide the first instance of real biological control of nematode populations on a broad-scale field basis. They support a rationale for investigation of the role of H_2S in the control of soil-borne diseases and as an etiological agent in physiological diseases of rice and other crop plants.

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24. Supported in part by PHS grant EF-00252 to J.P.H. We thank M. Yates, MicroTek Instruments, Inc., for assistance in gas chromatography of fatty acid samples; J. E. Sedberry, Jr., and R. H. Brupbacher, L.S.U., for facilities and advice; M. de los Reyes, A. Contino, R. Espino, R. Valdes-Fonte, A. Rovira, and A. Lichea; and H. R. Caffey, S. P. Firmin, G. Martin, G. C. Meaux, and M. Veillon of L.S.U. for assistance in planning the field experiments.

18 February 1965

Signal Detection in Fixed-Ratio Schedules

Abstract. *A psychophysical choice technique can be used to measure discrimination of the stimuli produced by two fixed-ratio schedules. As the difference between the two ratios is reduced, the number of errors in discrimination increases. The analysis differentiates between discrimination and response bias, which are frequently confused in animal psychophysics.*

The theory of signal detectability was originally developed to specify the electronic detection of radar signals in noise. Psychophysical methods based on this theory have been applied in sensory psychology to measure detection of auditory signals in noise by human observers (*1*). Psychophysical methods are usually applied to exteroceptive stimuli whose properties can be physically specified. We demonstrate that an analysis adapted from the theory of signal detectability can also be applied to detection of stimuli resulting from different reinforcement schedules.

In a fixed-ratio schedule of reinforcement, a hungry animal is reinforced with food at the completion of a fixed number of responses counted from the preceding reinforcement. Ferster and Skinner (*2*) point out that either the number of responses in the fixed ratio or the time required to emit them could serve as a discriminative stimulus. The present study makes no attempt to specify the nature of such discriminative stimuli, but does attempt to demonstrate that the discrimination of these stimuli may be studied by means of psychophysical methods.

Two adult White Carneaux pigeons were maintained at 80 percent of their "free feeding" weights. The pigeons were tested in a chamber containing three plexiglass keys mounted on one wall. Each key was illuminated from behind by a light bulb, each of the three bulbs being of a different color. A food magazine located below the center key was raised to permit access to grain as reinforcement.

There were two steps in the procedure. In the first step the bird pecked the center key the number of times specified by a fixed-ratio schedule. In the second step the bird pecked one of the two side keys. During the first step, the center key was illuminated with white light and one of two fixed-ratio requirements was in effect. The schedule for each trial was selected by a pseudorandom series (*3*). We will refer to the shorter fixed ratio as the "signal" and the longer fixed ratio as the "noise." The fixed ratio required under the signal schedule was varied during the experiment, while the noise schedule remained constant at fixed ratio 50.

When the bird had pecked the center key the required number of times, the light for the center key went off and the two side keys were illuminated. Reinforcement of a peck on the side key was contingent upon discriminating which schedule had been in effect on the center key. If the bird had just pecked the center key the number of times specified by the signal schedule, a peck on the left key was reinforced while a peck on the right key darkened the box and delayed reinforcement for 60 seconds. If the bird had just pecked the center key the number of times specified by the noise schedule, the reinforcement contingencies were reversed. Each animal was given 100 trials a day, 50 with a signal schedule on the center key and 50 with a noise schedule.

Training began with fixed ratio 5 as the signal and fixed ratio 50 as the noise. The signal-noise difference was gradually reduced as the discrimination improved. Both birds met a criterion of 90 percent responses for two consecutive days with fixed ratio 35 as the signal, and a signal-noise difference of 15. The discrimination was established in about 120 sessions. After the criterion was met, the psychophysical function for the discrimination of ratios was obtained by daily increasing the signal ratio in increments of 2 until the percentage of correct responses fell below 60 percent. Four determinations were made at each of the signal ratios in the following series: ascending, descending, ascending, descending.

Figure 1 presents the results for bird 5488. Each datum point is the percentage of the correct responses for each of the four determinations at each signal condition. The percentage decreases as the signal ratio approaches the noise ratio. The results for bird 4800 are similar. The percentage of correct responses for both birds falls