NEMATODE DAMAGE ASSESSMENT PROBLEMS AND SOLUTION FOR A RICE NEMATODE: *HIRSCHMANNIELLA ORYZAE*

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Evaluation of pathogenicity of plant nematodes poses difficult methodology problems. Most plant nematodes are obligate parasites and cannot be cultivated on artificial media. Nematodes are invisible to the naked eye and in their majority they make no macroscopic damage. Most nematode attacks on plants result in heavy yield losses unaccompanied by any visible symptom. There are no leaf spots, no discoloration or change of color, no malformations, but only a general reduction of the plant vigor that may be too slight to be readily noticed. As a result, plant nematodes are often considered as parasites of little economic importance according to the common wisdom that what you cannot see cannot hurt you. Not so with nematodes that can reduce by a third, a half, or more the yield of an apparently healthy field. It is one of the tasks of plant nematologists to prove to sceptical farmers and agronomists the reality of heavy nematode related yield losses.

Traditionally, yield losses have been demonstrated by i) histopathological studies; ii) chemical field tests; and iii) pot inoculations.

1. Histopathological Studies

The first step in demonstrating the pathogenicity of a nematode is to make sure that it is able to inflict damage to plant tissues. Inoculations, extractions, and staining experiments show where and how the parasite attack the roots or the aerial parts of the plant. The potential noxiousness of a nematode depends, up to a point, on its mode of attack. A parasite that feeds in the central cylinder is more likely to do heavy damage than one like *H. oryzae* that remains in the cortex. However, a cortical attack, if sustained by a sufficient number of nematodes, can result in total destruction of the cortex and death of the root. Histopathology may indicate a potential threat to a plant, it does not evaluate the extent of this threat.

2. Chemical Field Tests

Many studies pretend to obtain a direct estimate of yield losses by comparing, in a statistically significant field experiment, control plots to plots treated with a suitable nematicide. Such experiments often show a high yield increase correlated to the control of the nematodes. For example, chemical control of *H. oryzae* in Japan resulted in a 38% yield increase (Kawashima & Hori, cited in Siddiqi, 1973). However, it is not possible to conclude that nematodes were the sole responsible of the observed yield loss. In addition to the target pest, nematicides may also control other pests. Some chemicals have a direct fertilizer-like action on the plant. This results in a yield increase in the absence of any pathogen. On the other hand, some pesticides may be phytotoxic at higher concentrations. They also may destroy natural enemies of the nematode. Finally, pesticides may have a positive and/or a negative effect on the plant when they interfere with harmful or with beneficial soil bacteria.

3. Pot Experiments

To avoid the side effects of chemicals, plant growth in pots inoculated with a nematode can be compared to the growth in non-inoculated control pots. Such experiments often show a high correlation between number of inoculated nematodes and plant growth reduction. For example, Babatola & Bridge (1979) demonstrated a yield reduction of 30 to 38% in pots inoculated with 1,000 or 5,000 _H. oryzae_ specimens. These results are inconclusive because pots provide a very artificial medium for the culture of the plant and its parasite. Pots are small and roots cannot develop properly. The hypothesis that a healthy root growth can "outrun" a nematode attack is largely unproven, but it remains that roots developping in a crowded space may be more susceptible to nematode attacks. Other environmental factors, and particularly temperature and the watering of the pot may also affect the plant and lower its natural resistance to parasites. The parasites themselves suffer from the same environmental factors and from the extraction/inoculation trauma. More than half the inoculum disappears after a few days, and populations remain low until the next generation is produced (Fortuner, 1977b). Pot experiments may also be biased by "overkill" when an enormous inoculum (up to 10,000 nematodes per pot) is used. However, a higher inoculum does not always result in a higher established population in the pot. An inoculum of 300 _H. oryzae_ produced a final pot population of 9,000 after three months. Final populations were not higher when a two and a half time bigger inoculum was used (Fortuner, 1977b). Because we ignore what happens in a pot after it has been inoculated, results of pot experiments also are suspect.

Histopathological studies are inconclusive, field pesticide tests are tainted by side effects, pot experiments are too artificial. Are we doomed to never know for sure how much damage a nematode attack actually do?

4. Microplots Experiments

When I started working in Africa on the rice root nematode, _H. oryzae_, I quickly discovered that numerous observations hinted at the possibility of a high damage to the rice by this nematode, but also that, for the reasons explained above, no single study was perfectly satisfactory. What was needed was an experiment medium small enough to be under complete control, and at the same time large enough to imitate natural field conditions. Helped by my colleagues at ORSTOM (particularly Michel Luc and Georges Merny), I decided to use the microplot design elaborated for population dynamics studies by Seinhorst and by Oostenbrink, in

Holland. I conducted some experiments with *H. oryzae* that were published in two articles (Fortuner, 1974;1977a). Probably because these publications are in French, they did not receive much attention from the scientific community. The method used and the results obtained in these tests are briefly summarized below.

4.1. Methods

Fig. 1 shows the microplots at Dakar (Senegal) ORSTOM nematology laboratory. The plots are cement cubes one cubic meter in capacity, where rice can be cultivated under flooded conditions. The plots were initially filled with soil disinfected with metham-sodium. Because soil of all plots were similarly treated, any possible side-effect from the chemical would occur in all plots. Specimens of *H. oryzae*, obtained from a local rice field, were cultivated in pots in the greenhouse. Nematodes were extracted from the culture pots several times at a few months interval, and reinoculated to more pots to increase the available populations. Each successive extraction was made by elutriation where nematodes are washed from soil, soil debris, and soil microorganisms by water. Finally, large numbers of *H. oryzae* were inoculated to five microplots. Five other plots were used as non-inoculated plots. No fertilizer was added to the soil.

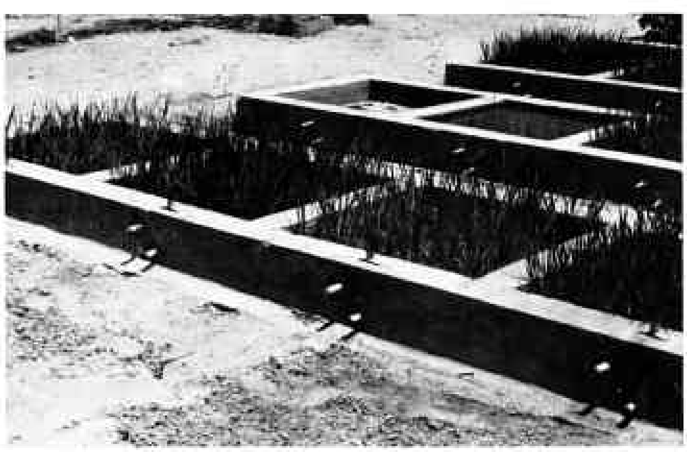

Fig. 1 - General view of the microplots at Dakar (Senegal).

Fig 2 - Difference in growth in plots inoculated and non-inoculated with *H. oryzae*.

Two successive rice cultures (from July to November 1972 and from November 1972 to May 1973) were necessary to establish nematode populations in inoculated plots at a level similar to local field population levels (2,750 nematodes per liter of soil, and 100 per gram of roots in Senegal Delta, Fortuner, 1976). Observations were made during a third cultivation, from May to September 1973, which is the normal rice growing season in many parts of Senegal. Rice was sown directly into the plots at a density of 25 plants per square meter, identical to local field density. Observations were made only on the nine plants in the middle of each plot.

4.2. Results

4.2.1. First Experiment Without Fertilization

Plants in all plots were seemingly healthy, without any disease symptoms from nematode or other pests. Rice plants in inoculated plots were markedly smaller and less vigorous than plants in control plots (Fig. 2). Highly significant differences between inoculated and control plants occurred in number of tillers per plant (10.2 vs. 13.6), number of panicles (10.1 vs. 12.3), plant height (78.5 cm vs.87.0), plant weight (43.9 g vs. 74.1), and panicle weight (2.71 g vs. 3.11).

Grains from each plant (nine central plants per plot) were harvested separately, dried, and weighed. Dry weight per plant was highly significantly different between inoculated plots (22.3 g) and non-inoculated ones (31.6 g). This represents a yield loss of 41.7%. The germinative power of the grains was not reduced by the nematodes.

Nematode populations at harvest were 3,200 to 6,000 nematodes per liter of soil, and 5 to 30 per gram of root.

4.2.2. Effect of Fertilization.

A second test was made with twenty microplots where rice was cultivated under similar conditions. Half the plots were inoculated with _H. oryzae_. In each series, five plots received a fertilization formula (135, 40, and 50 kg/ha of the elements N, P, and K) commonly used in Senegal rice fields.

Observations were made after two preliminary rice cultures, from April to September 1975.

As during the first test, presence of nematodes reduced rice growth, whether fertilization was applied or not. Yields in dry weight of grain per plant were as follows:

Treatment	Grain Dry Weight (g)	Significativity
1. No fertilization, nematodes	24.9	a
2. -d°- , no nematodes	36.1	bc
3. Fertilization , nematodes	33.7	b
4. -d°- , no nematodes	41.5	cd

Nematode populations at harvest were 1,500 to 2,500 nematodes per liter of soil, and 90 to 410 per gram of root.

Yield losses in non-fertilized plots (treatments 1 and 2) is 45%. this compares well with the 42% observed in the first test.

In fertilized plots, nematodes reduce yield by 23% (treatment 3 and 4). It can be noted that, when nematodes are present, fertilization significantly increases yield (treatment 1 and 3, +35%) but, without nematodes, the effect of fertilization is not significant (treatment 2 and 4). It can also be noted that treatment 2 and 3 are not significantly different. From plots with the lowest yield (treatment 1, no fertilizer, nematodes present), the increase in yield obtained by fertilization only (treatment 1 and 3, +35%) is not significantly different from the increase in yield that result from the absence of the nematodes (treatment 1 and 2, +45%). Naturally, the best yield is obtained when both nematodes are absent and fertilization is used (treatment 4).

In conclusion, H. oryzae significantly reduces yield, even when the rice is grown in the best conditions with adequate fertilization. Nematode damage caused by H. oryzae on rice is so high that removal of the nematode produces an increase in yield comparable to the increase produced by fertilization, a common agricultural practice.

5. Discussion

Microplot experiments are proposed as a reliable method to assess nematode damage to plants because i) plants are grown almost under field conditions. Soil temperature, watering, and volume of soil available to the roots are similar to natural conditions; ii) nematode populations are able to get over the trauma of initial inoculation and get established in the plots at normal field population levels.

It is unlikely that other pathogens (bacteria or virus for example)were introduced in the plots with the nematode inoculum because i) nematodes from the field are cultivated in the greenhouse during several cultures prior to inoculation. Between each successive culture, nematodes are extracted by an elutriation method that washes off most surface contaminants; and ii) bacterial, fungal, or virus diseases are usually visible macroscopically by the characteristics spots, changes in color, or malformations they cause. No such symptom was ever observed in the experiment microplots. Nevertheless, it would be better to raise the last doubts by a proper control of the absence of contamination of the inoculum.

It has been argued that the successive rice over rice over rice cultures are damaging to the plants. In fact, because the control plots bear a similar continuous rice culture, any problem related to rice monoculture would exist in inoculated and control plots alike. Also, adverse effects of monoculture is probably for the most part linked to the establishment of high populations of pathogenic nematodes. This is precisely the point we want to investigate.

Microplot experiments have demonstrated conclusively the damage caused by H. oryzae to rice culture in Senegal. It is hoped that similar tests will be conducted to assess rice damage associated with the related nematode H. belli in California.

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