Proceedings of the research planning conference on Root-knot nematodes, Meloidogyne spp. June 7-11, 1976.- Ibadan (Nigeria), International Institute of Tropical Agriculture.
Phase III of the International Meloidogyne project. Contract no AID/ta-c-1234

MELOIDOGYNE RESEARCH AT O.R.S.T.O.M., DAKAR

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The Nematology Laboratory of O.R.S.T.O.M. (Office de la Recherche Scientifique at Technique Outre - Mer) was created by M. Luc in Abidjan, Ivory Coast, in 1955. As a result of a survey of Senegal made by C. Netscher in 1964, ORSTOM decided to create another nematology laboratory in Dakar, Senegal, primarily to study nematodes of semi-arid areas. This laboratory was inaugurated in June 1970, and at the present time there is a staff of seven nematologists of whom six are engaged in research concerning the genus Meloidogyne.

A major activity of the Nematology Section of ORSTOM has been to conduct surveys in different African countries to define the most urgent problems in agriculture caused by plant-parasitic nematodes. These surveys established that Meloidogyne spp. were abundant in Madagascar, Ivory Coast, Canary Islands, Congo, Central African Republic, Senegal, Mauritania and the Gambia. A recent survey has demonstrated that Meloidogyne is also frequently encountered in the Cape Verde Islands.

Concerning the distribution of species of Meloidogyne in West Africa, the following picture is provided (identification based on perineal patterns): M. incognita and M. javanica are common in the Congo, Central African Republic, Ivory Coast, Senegal, Mauritania, and the Gambia; whereas M. arenaria is less frequently found. In many cases populations were composed of two species, and often such mixed populations contained individuals showing characters intermediate between the species present.

Since Chitwood reestablished the genus Meloidogyne in 1949 the possibility was created to determine the host ranges of the different species, on the basis of which a crop rotations could be envisaged in soils where the identity of the root-knot nematode was known. However, the occurrence in the field of mixed populations and the presence of individuals possessing intermediate characters necessitated a more profound study of the subject. Therefore, more than sixty single-egg mass populations were established to study their morphology and karyology in order to obtain a better comprehension of intraspecific variation and specific differentiation within the genus.

In addition to the difficulties caused by the great variability of

perineal patterns, information obtained from Senegalese isolates suggested great physiological variability. Therefore, host range studies, including plant species used by Sasser (2) to differentiate between species of Meloidogyne as well as resistant tomato varieties, were made with the single egg-mass cultures maintained in the laboratory. To avoid contamination between different isolates, they were aseptically reared on tomato root cultures. Unfortunately for this study, all these monoxenic cultures were accidentally destroyed before all measurements and tests were completed. At the present time a less ambitious program has been started in which 14 single-egg-mass cultures will be compared. Nevertheless, a number of measurements of juveniles from different isolates was made. These showed that contrary to the findings of Whitehead (4) it was not possible to distinguish between M. javanica and M. incognita on the basis of juvenile length because a clear overlapping of this character existed. When comparing lengths of juveniles produced by sister females (derived from the same eggmass) of a M. javanica culture, using Whitehead's criterion, it was shown that certain females belonged to M. incognita whereas others belonged to M. javanica. This result was rather astonishing, because in the related genus Heterodera it had been shown that, using the same techniques, stabilization of the variability of juvenile length was observed after four generations of inbreeding. Inbred lines of this amphimictic species were obtained by having females fertilized by their brothers during four successive generations.

Great differences exist between the reaction of different Meloidogyne populations towards the same crop because of interspecific variation and intraspecific variation. For example, only three of six isolates from Florida of M. arenaria, the "peanut root-knot nematode," were parasitic on peanut; and resistance-breaking biotypes ("B-races") of M. incognita and M. javanica have been described on resistant tomato by Riggs & Winstead (1) and by several other workers since.

Because of the great practical importance of this subject experiments were carried out in Senegal involving plants having potential use in crop rotations in vegetable production. Although results in general agreed with those of Sasser, a number of exceptions must be mentioned: M. arenaria from Senegal has consistantly failed to reproduce on peanut; strawberry originally not attacked in a field infested with M. javanica became heavily infected after this crop had been grown continuously for 12 months. Furthermore, resistance-breaking biotypes of both M. incognita and M. javanica were developed on resistant tomato.

Detailed studies were undertaken to better understand the mechanism of resistance or susceptibility to species of Meloidogyne in several plants including peanut, millet, Hibiscus sabdariffa and resistant tomato.

Peanuts are readily penetrated by juveniles of all isolates of Meloidogyne tested, including those containing M. arenaria. The juveniles provoke a hypersensative reaction within the root which results in the death of the nematodes. Plants that are heavily invaded are severely damaged by the extensive root necrosis. Population studies have shown that Meloidogyne populations decrease more rapidly in the presence of peanut than when no plants are present. Heavily infested fields are virtually cleaned of root-knot nematodes by a crop of peanuts, and a susceptible crop grown after peanut performs much better than when grown after a susceptible crop.

Although millet reduced populations of Meloidogyne in the field, it was found to be a host even though development rate and rate of reproduction were low.

Hibiscus sabdariffa, reported to be resistant to Meloidogyne, was classified as a poor host supporting limited reproduction of both M. incognita and M. javanica. Histological examination of the basal portion of the stem of H. sabdariffa grown in Meloidogyne - infested soil revealed stem galling, synctial development, and the presence of mature females and egg masses of both species.

The reactions of more than 70 isolates of Meloidogyne on resistant tomato were studied. B-races were selected from 10 of these populations by repeated reinoculation of juveniles recovered from the resistant varieties. One isolate each of M. incognita and M. javanica were capable of attacking resistant tomato immediately although no selection pressure had been exerted by resistant tomatoes. The remaining populations either did not attack the resistant varieties at all or occasionally produced one or two galls each containing a female with an egg mass. Subsequent inoculation of resistant tomato with juveniles derived from these egg masses produced no increase in pathogenicity. In two of the ten isolates from which B-races could be developed, indications were obtained that pathogenicity increased with each generation. B-races could be obtained from certain single egg mass cultures.

Two tomato varieties containing different genes for resistance

(Small Fry with gene L Mi R_1 and Nematex with gene L Mi R_2 (3) were tested against several Meloidogyne isolates. A B-race capable of attacking both varieties was observed in one isolate each of M. incognita and M. javanica. In addition, a wild population of M. incognita was found that was capable of attacking Small Fry but not Nematex indicating that even within B-races physiologic variation exists. A systematic search for biological races capable of attacking the different known resistant genes in tomato is in progress.

The variability in behavior towards resistant tomato shown by certain single egg mass cultures as well as in variability in juvenile length previously mentioned, suggests that in parthenogenetic species of Meloidogyne mechanisms, yet unknown, exist that assure sufficient variability to enable the organisms to adapt to changing environmental conditions.

In order to study the relationships between species and the physiological variations (biotypes) within species, an enzymatological and serological research product was initiated using the Meloidogyne material available at Dakar. Research was initiated, techniques developed and tested, but the untimely death of the researcher forced the abandonment of this work.

Survival of Meloidogyne under the semi-arid conditions of Senegal has been studied since 1972. Climatically there is a four-month long wet period followed by a dry season of eight months. Before dependable results could be obtained, an accurate method of recovering the maximum numbers of Meloidogyne had to be developed. All extraction methods had been tested and were unsatisfactory because in Senegal egg masses with adhering sand particles were associated with the large sand particle fraction of the soil. A method combining elutriation and mist extraction techniques was developed to recover active juveniles, individuals present as eggs in egg masses and loose eggs. Using this technique Meloidogyne population dynamics were followed during the dry season in fields containing, or not containing, roots from a previous crop infected with Meloidogyne. Population counts taken at regular intervals during the dry season indicate that nematodes disappear rapidly in the 0-20 cm horizon; however, in the 20-40 cm horizon a few Meloidogyne survive a dry season. Evidence indicates that eggs within egg masses are responsible for survival of Meloidogyne in the dry season; juveniles do not survive dry conditions for a long time. Egg masses associated with roots did not possess greater resistance to drying than those found free in the soil.

Field observations suggested that movement of root-knot nematodes was greater than generally believed. Therefore, a program was started in early 1973 in which attraction of Meloidogyne juveniles to plants was studied. Initial results have shown that soil in which plants of resistant or susceptible tomato or peanut had been grown actively attracted Meloidogyne juveniles. In vitro studies showed that Meloidogyne juveniles were attracted to nutrient agar on which tomato roots had been grown aseptically.

Horizontal and vertical movement (migrations?) of M. javanica juveniles was studied in the presence or absence of a susceptible variety of tomato (Roma). Nematodes were placed at different distances from the roots which were isolated by a screen with openings of 35 um that permitted passage of nematodes. Results showed that M. javanica juveniles placed 75 cm vertically and 50 cm horizontally from the roots were capable of penetration in large numbers and that migration was greater in the presence of plants. Juveniles of M. javanica were shown to have a great capacity of movement, with 20% capable of moving vertically 50 cm in three days. In addition, movement towards roots did not appear to be greater in rhizosphere soil than in sterile soil. A susceptible variety of tomato (Roma) exerted a greater attraction than a resistant variety (Rossol).

More recently a program has been initiated to investigate physiological aspects of penetration of root-knot nematodes. At the present time techniquesare being developed to measure food reserves (proteins, glucosides and lipids) of Meloidogyne juveniles.

To test the validity of some of these findings some field experiments have been made in Senegal. One example, it was shown that under field conditions resistant tomato varieties yielded 30% more than comparative susceptible varieties when grown in moderately infested soil and that yields of resistant varieties equal those of susceptible varieties grown in fumigated soil.

Comparing development of Meloidogyne in a resistant and susceptible tomato variety growing in parallel rows in an infested field it was shown that under natural conditions all infection sites of susceptible plants were galled and contained giant cells, no necrosis was observed. In the resistant varieties galling and giant cell formation were rare; necrosis occurred at a high percentage of infection sites. Fewer juveniles penetrated in the resistant variety and very few exhibited development. Several B-races were isolated from the resistant varieties.

In Savaigne (northern Senegal) soils of fields of a tomato cannery

were found to be infested with Meloidogyne down to a depth of 1.5 m. Treatments with DD at a depth of 30 cm and with a double dosage (one at 30 and one at 60 cm) did not show any difference in infestation of tomatoes subsequently grown. Both treatments protected one crop fairly well. An equally good result was obtained either by maintaining a clean fallow for three months during the rainy season or by growing a crop of peanut.

A crop rotation trial at Camberene showed that a crop of peanut or strawberry grown in heavily infested land prior to tomato greatly increased the yield potential, as compared with a previous crop of tomato. Incidence of Fusarium was delayed for 20 days in plots where Meloidogyne populations had decreased due to the effect of previous crops.

Results obtained in the field so far are too fragmentary to draw general conclusions, but it may be anticipated that when a continued effort is made to test results obtained in the laboratory in field trials, a pattern of integrated control of root-knot nematode in annual crops may be developed. However, it should be emphasized that the variability of Meloidogyne isolates necessitates that rotation should be developed and tested locally. From our observations so far it has become clear that much emphasis should be placed on prevention rather than curative measures.

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