NATURE OF COFFEE RESISTANCE TO TWO COSTA - RICAN *Meloidogyne s p* POPULATIONS

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Introduction

Creation of coffee varieties resistant to root-knot nematodes started little time ago in Central America, through a project financed by European Union. Usually, resistance phenomenons are more likely studied through the point of view of their influence on nematode populations. The histological study here presented allows to link macroscopic symptoms (number and size of galls) observed during the screening of several C. *arabica* and C. *canephora* introductions, with microscopic characteristics that tell about the nature of the encountered coffee resistance towards *Meloidogyne arabicida* and *Meloidogyne exigua*.

Materials and methods

Nematodes under study

Population # 1 comes from Costa Rica. Possibly, it would be a new species described by Lopez and Salazar (1991) under the name of *Meloidogyne arabicida sp* n. The population we used comes from "Hacienda Juan Viñas" in Jiménez county (altitude: 1000 m).

Population # 2 comes from CICAFE farm in Heredia (altitude 1100 m) and is identified as *Meloidogyne exigua*

Although those two populations belong to apparently different species, they induce a same genotipic reaction (see presentation of Bertrand *et al*, at this conference).

Plant material

During different resistance trials, we tested several types of plant material towards populations 1 and 2. C. *arabica is* represented by control varieties (Caturra, Catuai), by Ethiopian cultivars from ORSTOM and FAO field collections and by several Catimor lines. C. *canephora is* represented by five accessions coming from Congo.

Control varieties and Catimors were analyzed in the field (6 years old adult plants) and at the nursery (8 months old plants). The other ones were studied at a young stage (between 8 and 18 months) and came from the field or the nursery.

Macroscopic observations

We adopted a classical qualitative scale called Gall Index (GI) and adapted from Arango *et al* (1982) as follow:

GI 0: no gall

GI 1: 1 or 2 very small galls

GI 2: 3 to very small to small galls

GI 3: 11 to 30 small and medium galls

GI 4: 31 to 100 small, medium and large galls

GI 5: more than 100 small, medium and large galls .

Very small galls are barely visible bumps on thin roots. The difference of diameter between a healthy and an affected cylinder vary between 0.2 and 0.5 mm (measured with a caliper).

Small galls are galls which relative diameter can reach 0.5 to 3 mm

Medium galls are galls which relative diameter vary between 3 and 5 mm Large galls are galls which relative diameter is superior to 5 mm

This scale has been verified by Rafinon (1994) and is highly correlated with the number of nematodes (eggs and larvae) for those types of nematodes.

Microscopic observations

For each GI used in the macroscopic observations we sampled representative roots. In every case, they are young white roots without any apparent necrosis. The sections were made at the spot of the gall. In case of no visible gall (it is the case for resistant varieties), the sections were made randomly. For each section we studied: the presence of nematodes, the presence of well developped or mishaped giant cells, the presence of egg masses.

Histological techniques

The samples were fixed in FAA (Formaldehyde, alcohol, acetic acid and distilled water) for 48 hours. They were then dehydrated in a series of ethanol (70%, 80%, 90%, 95%, 100%, 100%), one hour in each bath, embedded in HistoresinTM at 4°C overnight, and then molded. Sections 3 ,um thick were cut and stained with a quadruple stain (CIRAD, 1989).

Fresh samples were also stained in acidic fushin-lactophenol in order to evaluate internal of external presence of egg masses.

Results

Observed symptoms in susceptible plants

We did not find any susceptible plant in C. *canephora* All the susceptible plants described all come from C. *arabica* (Catimors, Ethiopians and control varieties). Staining of fresh and sectionned material shows that we are in presence of two species which sare the characteristic of burying most of their egg masses inside the roots. This is different from the symptoms observed by Anzueto (1993) or Peña (1994) with Guatemalan populations *(M. sp)* and Salvadorian populations *(M. incognita)* in which egg masses are mostly external.

Within susceptible plants (GI 3 to 5), the histological patterns are identical, being with *M. arabicida* or with *M. exigua.* The sectionned galls reveal several females deeply buried in the root tissues at the vascular bundle level. These females are connected to large to very large egg masses. The giant syncitial cells are well formed and very aboundant. It is possible to observe a dense cellular content and the presence of several nuclei. The cortex is devoid of nematodes and is constituted of living cells (Figs. 1, 2. 3).

Observed symptoms in very resistant plants (GI 0).

The very resistant plants are found within C. *canephora* and principally within Catimor varieties. It is most likely that the Catimors resistance genes come from C. *canephora.* However, we observed one Ethiopian plant classified as resistant (T4900 - E531).

As there is no gall, we decided to observe certain points where the root is naturally (or not) thicker. In some cases, nematodes fragments are observed only at the periphery of the root (Fig. 4), but in most of the cases, no nematode is observed (Fig. 5). No difference is noted between resistant Canephoras, Catimors or the E531 accession.

Observed symptoms in resistant plants (GI 1 or 2). As above, those plants are found within C. *canephora* and Catimor varieties. On the chosen scale, the GI is 1 or 2. Small galls were sampled and sectionned.

Case 1: In some plants it is possible to observe a normal development of the female. The difference with the susceptible controls is that the average number of female per gall is down to 1 or two. The egg masses are often reduced. For those trees, there would be a simple reduction of the female proliferation. This phenomenon is observed in C. *canephora* as well as in C. *arabica* (Catimor varieties) (Fig. 6).

Case 2: In a few plants, one can observe a small number of syncitial cells (sometimes, just one is left). However, the female seems to develop. Nevertheless, we did not see any egg mass (Figs. 7, 8).

Case 3: Contrarily to case 2, the symptoms are localised in the periderm cells. One can see that the cellular enlargement process was stopped since those cells are empty, without any cellular content. Remains of nematodes can be observed (Fig. 9).

Conclusion

The qualitative scale used to classify the plants according to the gall number index (GI) matches with characteristical microscopic symptoms.

For a GI from 3 to 5, we confirm that the plants are equally susceptible. The differences in the gall numbers are to be connected with the variations unavoidable when an artificial inoculation method is used and with the differences of growth of the root systems.

For a GI =0, two resistance phenomenons are possible:

A post-infection resistance mechanism; in this case, a penetration is observed since fragments of nematodes were detected, but they don't penetrate deeply. Since the nematode cannot reach its nutritional site, it cannot complete its normal development cycle.

An hypersensibility mechanism; in some cases, a necrose is observed in the periderm, that could be explained by a tentative penetration of the nematode. More accurate studies must he lmdertaken in order to define thi~ mechaniim

For a GI of 1 and 2, several phenomenons coexist:

A normal development of the nematode, but with a reduction in the number of the females formed. It would be a case of partial resistance.

An abnormal nematode development due to the presence of mishaped giant cells.

An interrupted development of the nematode due to the failure in the formation of giant cells.

Litterature

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