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Resistance of cultivated coffee (*Coffea arabica* and *C. canephora*) trees to corky-root caused by *Meloidogyne arabicida* and *Fusarium oxysporum*, under controlled and field conditions.

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Abstract

The coffee disease complex known as corky-root, composed of the root-knot nematode *Meloidogyne arabicida* and *Fusarium oxysporum*, causes serious damage to *Coffea arabica* in Costa Rica. Resistance to *Meloidogyne arabicida* alone and to the field population (*M. arabicida*, *M. exigua*, *F. oxysporum*) was evaluated under controlled and field conditions. The studied material consisted of subspontaneous-derived *C. arabica* accessions, *C. canephora* accessions, introgression lines derived from the interspecific Timor Hybrid (*C. arabica* × *C. canephora*) and F1 hybrids from crosses between subspontaneous-derived accessions and cultivars. Accessions resistant to *M. arabicida* and to the field population were identified at varying frequencies in the different groups of study materials. The results showed that resistance to corky-root is heritable and that genetic resistance to *M. arabicida* is an effective strategy against corky-root disease. By using *C. canephora* rootstocks, it was possible to substantially reduce mortality in the field and reduce by half the number of plants with corky-root symptoms. The results were used to define appropriate strategies for the sustainable management of coffee corky-root disease resistance.

Keywords: *Coffea arabica*; *Coffea canephora*; Corky-root disease; Genetic resistance; *Meloidogyne arabicida*

1. Introduction

Coffee is the world's most valuable agricultural export commodity. Coffee cultivation is an important factor in social stability because of the numerous jobs generated. Commercial coffee production relies on two species: *Coffea arabica* (75%) and *C. canephora* (25%). *C. arabica* is mainly grown over 1000 m a.s.l., while *C. canephora* is grown in lowlands. *C. arabica* is characterised by low genetic diversity that is attributable to its allotetraploid ($2n = 4x = 44$) origin, self-fertility and evolution (Lashermes et al, 1999). In contrast, *C. canephora* is a diploid ($2n = 2x = 22$) and a self-incompatible species characterised by high genetic diversity (Dussert et al., 1999).

Root-knot nematodes of the genus *Meloidogyne* are frequently and abundantly found in Arabica coffee plantations in Central America (Campos et al., 1990; Anzueto et al., 1993). In certain areas, the prevailing nematodes are highly destructive, leading to coffee tree death. In other areas, nematode attacks only affect coffee tree yields. In Costa Rica, *C. arabica* cultivation is threatened by a root disease known as corchosis (Bertrand et al., 2000a, b). Diseased plants showed a progressive decline starting with leaf chlorosis, followed by flower and fruit fall, eventually leading to plant death in 2–4 yr. The root systems of the diseased plants showed reduced growth and many galls leading to an extensive development of corky tissue on the main and secondary roots. Root galls are caused by two root-knot nématodes: *Meloidogyne exigua* and *M. arabicida,* but only *M. arabicida* is associated with corky tissue galls (López and Salazar, 1989). Hernández et al. (1996) recently confirmed that *M. arabicida* isolated from

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coffee roots with corky-root symptoms had a specific esterase phenotype (M1F1) and an original perineal pattern. In Costa Rica, *M. exigua* is the root-knot nematode most commonly found on coffee roots, but it is unable to cause severe damage to coffee trees under field conditions (Bertrand et al., 1999). Combined inoculations of *M. arabicida* or *M. exigua* and *Fusarium oxysporum* under controlled conditions showed that *F. oxysporum* alone was non-pathogenic (Bertrand et al., 2000a, b). *M. exigua* or *M. arabicida* alone induced galls and a reduction in shoot height, but did not induce any corky-root symptoms. Only the combination *M. arabicida* with *F. oxysporum* could induce corky-root symptoms on roots. These observations led to the conclusion that corky-root disease has a complex etiology and emphasised the dominant role of *M. arabicida* as a predisposing agent to subsequent *F. oxysporum* invasions.

Genetic resistance is an essential component in the integrated control of root nematodes. It avoids the need for nematicides, which are usually toxic, pollutants and not particularly effective on the endoparasitic nematodes of perennial plants. Our study was conducted in such a context. The resistance of *C. arabica* and *C. canephora* accessions was tested against the root-knot nematode *M. arabicida* alone and against the field population under controlled and field conditions. Introgression lines derived from a natural interspecific hybrid (*C. arabica* × *C. canephora*) and F1 hybrids between cultivars and wild coffee were included in the study for a characterisation of their resistance to corky-root disease. The results were used to define appropriate strategies for sustainable management of coffee corky-root disease resistance.

2. Material and methods

2.1. Material evaluated under controlled conditions

Three groups of genetic materials were evaluated under controlled conditions: subspontaneous-derived accessions of *C. arabica*, accessions of *C. canephora* var. Robusta and introgression lines of *C. arabica* derived from a natural interspecific hybrid (*C. arabica* × *C. canephora*), known as the Timor Hybrid (Bettencourt, 1973). The subspontaneous-derived accessions were collected on farms in the *C. arabica* centre of diversity, in Ethiopia, by the FAO (1968) and ORSTOM (Guillaumet and Hallé, 1978), and in Sudan by Thomas (1942). These accessions were selected from the CATIE field genebank (Turrialba, Costa Rica). The other accessions studied (i.e. *C. canephora* and introgression lines) were kept at the ICAFE experiment centre (Barva de Heredia, Costa Rica). The accessions studied were represented by the open-pollinated progeny of a single tree.

Resistance to *M. arabicida* alone was evaluated in six subspontaneous-derived accessions (seven individuals), two *C. canephora* accessions, and the introgression line cv. Costa Rica 95, grown in Costa Rica. Resistance to the field population was evaluated in 21 *C. arabica* subspontaneous-derived accessions (25 individuals), three *C. canephora* accessions, including the root-stock cultivar cv. Nemaya (Bertrand et al., 1999), and 10 introgression lines derived from the Timor Hybrid.

2.2. Material evaluated under field conditions

Two trials were conducted at the Hacienda Juan Viñas (Costa Rica) in a field showing severely diseased plants naturally infected with root-knot nematodes. A mixed population of *M. arabicida* and *M. exigua* was identified by isozyme markers (Hernández et al., 1996). In both trials, cv. Caturra and cv. Catuai were used as the control for susceptibility to *M. arabicida* and *M. exigua*. Trial 1 was composed of four subspontaneous-derived accessions and five F1 hybrids between cv. Caturra or cv. Catuai and subspontaneous-derived accessions. Sixteen 8-month-old seedlings per accession were planted out in the field. The study was laid out in a randomised complete block design with four replicates of four trees per accession, without border rows. A total of 176 trees was studied. In trial 2, cv. Caturra grafted on *C. canephora* (T3757) was compared to non-grafted cv. Caturra. Each material was represented by eight rows of ten trees each. The rows were alternated without border rows. In both experiments, the distances between and along the rows were 2 and 1 m, respectively. Plants received 18N–3P–10K–8Mg–0.5B nutrient, with a 500 kg/ha dose in the first year and 1000 kg/ha in the following years, 250 kg/ha/yr of nitrogen and two applications per year of copper hydroxide at a rate of 1.5 kg/ha/yr to prevent leaf and fruit diseases.

2.3. Resistance evaluation under controlled conditions

The evaluation of resistance to *M. arabicida* alone and to the field population was carried out in a greenhouse at a mean temperature of 24°C, at CATIE. The isolate was obtained from a single egg mass extracted from pre-washed coffee roots displaying corky-root symptoms. The isolate presented the esterase phenotype and the perineal pattern of *M. arabicida*. The nematodes were multiplied on tomato roots (*Lycopersicon esculentum* cv. Nainemor). To provide nematode inoculum, 10-week-old tomato roots were cut with a scalpel into 1–2 cm segments and blended in a Waring blender for 40 s (Barker, 1985). The nematode suspension was passed through a 75 μm pore sieve, nested over a 38 μm pore sieve to collect free eggs that were then rinsed carefully

with tap water. The inoculum was applied to the collar of each coffee seedling, using a micropipette, when the plants were four months old (two pairs of leaves). The nematode inoculum concentration was determined from a 1 ml aliquot and adjusted to give a dose of 2000 ± 200 second-stage juveniles (J2) per pot. For all the treatments, there were 28–40 replicates. Six months after inoculation, the plants were removed from the pot and the roots were washed free of soil. Numbers of root galls were assessed using a six point gall index scale (GI): GI = 0, 0 galls; GI = 1, 1–2 very small galls; GI = 2, 3–10 very small galls; GI = 3, 11–30 small and large galls; GI = 4, 31–100 small and large galls; GI = 5, over 100 small and large galls. It was considered that plants scoring 0, 1 and 2 in this index were resistant and those scoring 3, 4 and 5 were susceptible.

The field population came from a severely infested plot in which all the coffee trees were affected by corky-root symptoms. The plot was located at the Juan Viñas Hacienda at 900 m a.s.l. in a humid tropical climate without a dry season and a mean temperature of 20–22°C. Nematodes were extracted from roots by the mist technique (Hooper, 1970). Once the coffee seedlings reached the cotyledonary leaf stage, they were transferred to 250 ml pots containing a 2:1 mixture of disinfected soil and sand (pH 5.2 to 5.6) in a greenhouse at a mean temperature of 24°C, at CATIE. The inoculation conditions were the same as those defined for *M. arabicida* alone. There was a large quantity of *F. oxysporum* microconidia ($93{,}000 \pm 22{,}000$ spores ml^{-1}) in the aqueous solution used for inoculation. Observations were carried out 9 months after inoculation. Corky-root development was recorded as a percentage of the entire root covered by corky-root. Plants with corky-root development were considered susceptible and plants without corky-root were considered resistant.

2.4. Resistance evaluation under field conditions

Dead plants were noted 5 and 4 yr after planting, in trial 1 and trial 2, respectively. The living plants were carefully removed to observe their root system. Root knot severity was rated by the gall index previously described. GI was scored for each secondary root and the mean of GI observed in secondary roots was calculated per root system. Corky-root development was recorded as the percentage of the root system covered by corky-root symptoms.

2.5. Analysis of data

Data were analysed by a one-way analysis of variance. Means were compared using the least significant difference test (LSD) at $P = 0.05$. Data for the percentage of mortality, the percentage of plants with corky-root and the percentage of corky-root development were transformed to Arcsin($\sqrt{x}\%$) for analysis.

3. Results

3.1. Resistance to M. arabicida under controlled conditions

Among the six subspontaneous-derived accessions tested, three accessions (T16737, T17204 and T17205) proved to be totally resistant to *M. arabicida*, with 88% of immune plants (Table 1). The two *C. canephora* accessions also proved to be resistant, with 81% of gall-free plants. One of them (T3757) was the rootstock used in trial 2. However, the introgression line cv. Costa Rica 95 was as susceptible to the nematode as the cv. Caturra control.

3.2. Resistance to the field population under controlled conditions

Four of the 21 subspontaneous-derived accessions (T2744, T16690, T16725, T17177) revealed susceptibility to corky-root similar to that of the cv. Caturra control, with 100% of affected plants (Table 2). Five accessions (T4662, T4900, T16712, T16737, T17205) had 100% of plants without corky-root symptoms. Accession T17204 revealed resistance polymorphism, with one individual without corky-root symptoms and the other in 1R:2S segregation. The other subspontaneous-derived accessions had varying proportions of resistant (46%) and susceptible (54%) plants. From 10% to 35% of the root system on susceptible plants was affected by corky-root, i.e. similar proportions to those found on the cv. Caturra control (10–25%).

One hundred and fifty-seven of the 196 individuals of the three *C. canephora* accessions evaluated (80%) did not reveal any corky-root symptoms (Table 2). On the susceptible plants, the symptoms were only slight and only affected small areas. Only 1–10% of the root system was affected, on average, on the susceptible plants, as opposed to 10–30% for the cv. Caturra control.

Two of the ten introgression lines evaluated (T17934, T18141) had 75% of resistant plants (Table 2). The other eight introgression lines proved to be as susceptible as the cv. Caturra control. The percentage of the root system affected by corky-root in the susceptible plants was the same as the cv. Caturra control (10–30%).

3.3. Field resistance

In trial 1, it was possible to distinguish between two groups of materials, based on mortality after 5 yr of

Table 1
Gall index for a pure population of *M. arabicida* under controlled conditions, among six subspontaneous-derived accessions (seven genotypes), two *C. canephora* accessions and one introgression line

Identification	Accession	Gall index						
		0	1	2	3	4	5	Total
C. arabica from Sudan	T2744 a1					34	4	38
C. arabica from Sudan	T2744 a8				12	24	4	40
C. arabica from Ethiopia	T4579 a1				15	14	8	37
C. arabica from Ethiopia	T4759 a6				4	16	15	35
C. arabica from Ethiopia	T16737 a2	38	2					40
C. arabica from Ethiopia	T17204 a2	38	2					40
C. arabica from Ethiopia	T17205 a4	19	6	2	1			28
C. canephora	T3580 (1–3)	34	3	1				38
C. canephora	T3757 (2–1)	29	10	1				40
Introgression line	cv. Costa Rica 95				4	16	18	38
Susceptible control	cv. Caturra				1	2	33	36

exposure to parasite pressure in the field (Table 3): accessions for which all the plants had survived, and those with over 12.5% mortality. The cv. Caturra and cv. Catuai controls were in the second group. Two subspontaneous-derived accessions (T16693, T16704) and two F1 hybrids, (cv. Caturra × T16693) and (cv. Catuai × T4816), recorded no mortality. However, galls were found on the roots of all the subspontaneous-derived accessions and the F1 hybrids, though there was no significant difference between accessions for the gall index. However, major differences were found for corky-root symptoms, with 0–100% of plants affected depending on the accessions. The cv. Caturra and cv. Catuai controls had 100% affected plants and around half of their root system was affected. Two F1 hybrids, (cv. Catuai × T2744) and (cv. Catuai × T4579), proved to be as susceptible as the controls. This result was expected, as the two subspontaneous-derived parents were susceptible to the pure *M. arabicida* population (Table 1), as was the cv. Catuai cultivar (Bertrand et al., 2000a, b). Two subspontaneous-derived accessions (T16704, T16712) and two F1 hybrids, (cv. Caturra × T16693) and (cv. Catuai × T4816), did not have any corky-root symptoms.

3.4. Effect of grafting in the field

Cv. Caturra grafted on a *C. canephora* rootstock revealed lower mortality than non-grafted cv. Caturra after 4 yr in the field, 5% and 30%, respectively (Table 4). The gall index for the rootstocks (0.3) was statistically lower than that for ungrafted cv. Caturra (2.3). The proportion of plants affected by corky-root symptoms among the rootstocks (21%) was half of that recorded on ungrafted cv. Caturra (42.8%). However, the symptoms on plants susceptible to corky-root were similar for both rootstocks and cv. Caturra.

4. Discussion

Three of the six subspontaneous-derived accessions evaluated in this study proved to be resistant to a pure *M. arabicida* population. A high frequency of resistant accessions had already been found for another nematode in Guatemala (*Meloidogyne sp.*) in wild coffee trees collected from the centre of diversity of the *C. arabica* species (Anzueto et al, 2001). Location of these genes in the genome will show whether they are the same resistance genes. The three subspontaneous-derived accessions resistant to *M. arabicida* proved to be resistant to corky-root under controlled conditions. Resistance to the nematode *M. arabicida* consequently affords a high level of resistance to corky-root. The F1 hybrid derived from the cross between cv. Caturra and one of the resistant accessions (T16693) proved to be resistant under parasitic conditions in the field. On the other hand, two hybrids derived from parents susceptible to *M. arabicida* proved to be susceptible in the field. These results show that resistance to corky-root is heritable and that genetic resistance to *M. arabicida* is an effective strategy against corky-root disease. Such resistance can be exploited in creating vigorous F1 hybrids resistant to corky-root.

The three *C. canephora* accessions studied proved to be resistant to *M. arabicida*. Resistance to other root-knot nematodes has been detected in *C. canephora* coffee trees, such as for *M. incognita* and *M. exigua* (Gonçalvez and Pereira, 1998; Anzueto, 1993; Bertrand et al., 2000a, b). Nevertheless, around 19% of plants developed small galls after inoculation with *M. arabicida*. A similar proportion of plants with corky-root symptoms was found to be susceptible to the disease under controlled conditions (20%). The existence of small galls caused by *M. arabicida* could be enough to enable corky-root development. Even so, the disease

Table 2
Number of plants without and with corky-root symptoms under controlled conditions, among 21 subspontaneous-derived accessions of *C. arabica* (25 individuals), three *C. canephora* accessions and ten introgression lines

Identification	Accession	Without symptoms	With symptoms	Total
C. arabica from Ethiopia	T2724 a1	4	33	37
C. arabica from Ethiopia	T2724 a2	4	28	32
C. arabica from Sudan	T2744 a1	0	27	27
C. arabica from Sudan	T2744 a8	0	23	23
C. arabica from Ethiopia	T4662 a1	29	0	29
C. arabica from Ethiopia	T4759 a6	4	26	30
C. arabica from Ethiopia	T4900 a2	31	0	31
C. arabica from Ethiopia	T16689 a1	6	16	22
C. arabica from Ethiopia	T16690 a1	0	30	30
C. arabica from Ethiopia	T16693 a5	22	7	29
C. arabica from Ethiopia	T16694 a8	21	7	28
C. arabica from Ethiopia	T16695 a2	26	12	38
C. arabica from Ethiopia	T16704 a7	13	18	31
C. arabica from Ethiopia	T16705 a2	21	3	24
C. arabica from Ethiopia	T16712 a1	35	0	35
C. arabica from Ethiopia	T16712 a4	26	0	26
C. arabica from Ethiopia	T16714 a6	14	17	31
C. arabica from Ethiopia	T16725 a1	0	30	30
C. arabica from Ethiopia	T16729 a4	22	8	30
C. arabica from Ethiopia	T16737 a2	28	0	28
C. arabica from Ethiopia	T16739 a2	14	19	33
C. arabica from Ethiopia	T17204 a2	29	0	29
C. arabica from Ethiopia	T17204 a3	10	19	29
C. arabica from Ethiopia	T17205 a4	25	0	25
C. arabica from Ethiopia	T17177 a2	0	27	27
Susceptible control	cv. Caturra	0	30	30
C. canephora	cv. Nemaya	102	19	121
C. canephora	T3580 (1–3)	25	11	36
C. canephora	T3757 (2–1)	30	9	39
Susceptible control	cv. Caturra	0	35	35
Introgression line	T5175	0	27	27
Introgression line	T5296	0	28	28
Introgression line	T17924	0	23	23
Introgression line	T17931	0	24	24
Introgression line	T17934	21	7	28
Introgression line	T17935	0	25	25
Introgression line	T18141	22	6	28
Introgression line	T18135	0	27	27
Introgression line	cv. Costa Rica 95	0	28	28
Introgression line	cv. IAPAR59	0	35	35
Susceptible control	cv. Caturra	0	25	25

covered a smaller root area in *C. canephora* than in the susceptible cv. Caturra cultivar.

Resistance to corky-root proved to be variable in the introgression lines studied. Cv. Costa Rica 95, which was found to be susceptible to *M. arabicida* confirmed its susceptibility with respect to the field population. Two of the ten introgression lines revealed a good level of resistance to corky-root, with a similar number of affected plants (23%) to that found in *C. canephora*. The frequency of corky-root resistance in the introgression lines was lower than that found for *M. exigua* by Bertrand et al. (2001a, b). Eight of the ten introgression lines susceptible to corky-root in this study had been found to be resistant to *M. exigua*. This comparison suggests that the genes of resistance to *M. arabicida* and *M. exigua* are different in *C. canephora*. Pedigree selection by selfing, which was undertaken using the Timor Hybrid, has made it possible to preserve a large proportion of lines with resistance to *M. exigua,* but may have led to counter selection of resistance to *M. arabicida*. This could be linked to an unfavourable agronomic trait, inherited from the *C. canephora* parent of the Timor Hybrid. Identification of molecular markers associated with resistance to *M. arabicida* will

Table 3
Mortality, gall index and corky-root symptom development under field conditions, 5 yr after planting, among four subspontaneous-derived accessions and five F1 hybrids (Trial 1)

Identification	Accession	Mortality(%)	Gall index	Plants with corky-root symptoms (%)	% of root system with corky-root
C. arabica from Ethiopia	T16693	0.0b	3.2	25.0b	40.0a
C. arabica from Ethiopia	T16704	0.0b	3.5	0.0c	0.0b
C. arabica from Ethiopia	T16712	12.5a	3.1	0.0c	0.0b
C. arabica from Ethiopia	T16725	18.7a	3.0	30.8b	28.0a
F1 hybrid	Caturra × T16693	0.0b	3.3	0.0a	0.0b
F1 hybrid	Caturra × T16725	12.5a	3.4	50.0b	35.0a
F1 hybrid	Catuai × T2744	12.5a	3.2	100.0a	43.0a
F1 hybrid	Catuai × T4579	18.7a	3.1	100.0a	32.0a
F1 hybrid	Catuai × T4816	0.0b	3.4	0.0b	0.0b
Susceptible control	cv. Caturra	25.0a	3.2	100.0a	62.6a
Susceptible control	cv. Catuai	25.0a	3.1	100.0a	45.6a
P		0.05	NS	0.000	0.000

P, probability level of ANOVA; NS, not significant ($P > 0.05$).

Table 4
Mortality, gall index and corky-root symptom development under field conditions, 4 yr after planting, observed in cv. Caturra grafted on *C. canephora* root-stock (T3757) and non-grafted cv. Caturra (Trial 2

Material	Mortality (%)	Gall index	Plants with corky-root symptoms (%)	% of root system with corky-root
Grafted	5.0b	0.3b	21.0	22.0 ± 18
Non-grafted	30.0a	2.3a	42.8	29.0 ± 12
P	0.004	0.001	0.04	NS

P, probability level of ANOVA; NS, not significant ($P > 0.05$).

make it possible to control the transfer of desired DNA fragments from *C. canephora*. Such a marker-assisted selection method is being developed for resistance to *M. exigua* (Fernandez et al., 2001).

When faced with the complex population of field parasites (*M. exigua*, *M. arabicida*, *F. oxysporum*), the subspontaneous-derived accessions and their F1 hybrids revealed a high gall index ($\geqslant 3$), even in accessions for which no plant had shown corky-root symptoms. The galls observed in accessions resistant to *M. arabicida* and to corky-root where therefore caused by the other nematode present in the field, *M. exigua*. Indeed, these origins are known to be 100% susceptible to that nematode (Bertrand et al., 2001a, b). Nevertheless, the existence of a complex population of field pathogens does not prevent identification of accessions resistant to corky-root several years after planting. A comparison of cv. Caturra grafted on a rootstock resistant to *M. arabicida* with non-grafted material revealed the advantage offered by grafted plants in terms of mortality and the number of galls 4 yr after planting out. Around 20% of the rootstocks were affected by corky-root. That proportion of *C. canephora* plants affected in the field was similar to that of plants developing small galls after inoculation with *M. arabicida* only, and affected by corky-root after inoculation with the field population. Use of rootstocks therefore seems to be an alternative to the dissemination of resistant cultivars. This solution will have to be accompanied by phytosanitary measures in the nursery, to prevent gall formation during the early stages of development.

In view of these results, it is possible to propose breeding strategies adapted to growing conditions. Adaptation of *C. canephora* to hot zones means that rootstocks of this species can be used below 800 m, as demonstrated by the Nemaya variety, selected for its resistance to nematodes in El Salvador and Guatemala (Bertrand et al., 2000a, b). For cooler zones, above 800 m, the simplest strategy seems to be the selection of F1 hybrids from subspontaneous-derived accessions resistant to *M. arabicida*. In the longer term, the selection of introgression lines combining resistance to *M. exigua* and *M. arabicida* may also be envisaged. The first generation plants, whose coffee quality is likely to be altered by the existence of numerous introgression fragments, could be selected for use as rootstocks.

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