CULTIVAR RELEASE

Crop Breeding and Applied Biotechnology
23(3): e46322339, 2023
Brazilian Society of Plant Breeding.
Printed in Brazil
http://dx.doi.org/10.1590/1984-70332023v23n3c32

IAC Herculândia – a *Coffea canephora* rootstock multiresistant to *Meloidogyne* species

Oliveiro Guerreiro Filho[1*], Luiz Carlos Fazuoli[1], Masako Toma Braghini[1], Paulo Boller Gallo[1], Claúdio Marcelo Gonçalves Oliveira[2], Lilian Padilha[3], Bárbhara Joana dos Reis Fatobene[1], Vinicius Teixeira Andrade[1], Larissa de Brito Caixeta Vasconcelos[1] and Juliana Magrinelli Osório Rosa[2]

Abstract: *IAC Herculândia is a Coffea canephora cultivar multiresistant to Meloidogyne exigua, M. incognita, and M. paranaensis to be used as a rootstock for Coffea arabica cultivars. It is a synthetic cultivar resulting from recombination among the clonal cultivars IAC WG, IAC FEBS, IAC PM, IAC LCCBF, and IAC ARM.*

Keywords: *Coffee breeding, nematode resistance, resistant cultivars, root-knot nematodes*

INTRODUCTION

The high occurrence of plant-parasitic nematodes in coffee, previously limited to the western regions of the state of São Paulo and the north of the state of Paraná, has been rapidly spreading to the other Brazilian Arabica coffee production regions (Santos et al. 2018, Terra et al. 2019).

Preventive control of *Meloidogyne* spp. through use of healthy certified seedlings is more efficient and economical than curative treatments. Although the use of chemical and biological control agents is recommended for the management of crops diagnosed with these parasites, their effectiveness is limited in perennial species such as *C. arabica*. This can be explained by the fact that reduction in pathogen populations allows recomposition of the plant root system, which results in a renewed increase in the nematode population density (Arita et al. 2020, Fatobene et al. 2022).

Conversely, planting susceptible cultivars of Arabica coffee grafted onto resistant rootstock has a preponderant role in reducing and maintaining low levels of nematode populations, especially in crop renewal areas that have already been infested. This practice has been adopted since the late 1980s through use of the rootstock Apoatã IAC 2258 (Fazuoli et al. 1987) in association with the hypocotyledonary grafting technique developed by Reina (1966) and adapted by Moraes and Franco (1987). However, plantations established with seedlings grafted onto the Apoatã IAC 2258 cultivar require about 20% replanting, in part due to the segregation rate for susceptibility.

The cultivar IAC Herculândia was developed to increase the frequency of resistance alleles against the nematodes *Meloidogyne exigua*, *M. incognita*, and *M. paranaensis* in a new rootstock. In addition to reducing the need for

***Corresponding author:**
E-mail: oliveiro.guerreiro@sp.gov.br
ORCID: 0000-0002-8228-2886

Received: 04 August 2023
Accepted: 18 August 2023
Published: 20 August 2023

[1] Instituto Agronômico de Campinas, Centro de Café Alcides Carvalho, Avenida Barão de Itapura, 1481, Botafogo, 13020-902, Campinas, SP, Brazil
[2] Instituto Biológico, Centro Avançado de Pesquisa e Desenvolvimento em Sanidade Agropecuária, Alameda dos Vidoeiros, 1097, Sítios de Recreio Gramado, 13101-680, Campinas, SP, Brazil
[3] Empresa Brasileira de Pesquisa Agropecuária, Embrapa Café, Avenida W3 Norte, Parque Estação Biológica, 70770-901, Brasília, DF, Brazil

replanting in infested areas, this strategy will allow the planting and management of Arabica coffee without the adoption of any extra technological level.

BREEDING PROCESS

The project to obtain the IAC Herculândia cultivar began with identification of *Coffea canephora* plants resistant to different species of the genus *Meloidogyne*. As an initial strategy, open-pollinated clones and progenies were evaluated in infested areas, mostly located in the state of São Paulo (SP), Brazil.

The resistance of clones and half-sib progenies that survived in experimental fields infested by species of the parasite was confirmed in a greenhouse. This was achieved through controlled infestations with the nematode population collected in these fields and identified according to species and physiological race. Genotypes validated as resistant were propagated sexually or by cuttings and were evaluated in subsequent experiments. They were planted in fields also infested by species and populations of root-knot nematodes (RKN) characterized by esterase isoenzyme polymorphism (Carneiro and Almeida 2001) and SCAR DNA markers (Randig et al. 2004).

Over approximately 30 years, around 400 parent plants were evaluated that are currently kept in clonal gardens established by Dr. Wallace Gonçalves in Campinas, Mococa, and Herculândia in the state of São Paulo. During this time, in addition to coffee plants being evaluated for genetic resistance to RKN, they were evaluated in the field in terms of vigor, longevity, plant reaction to pruning, yield potential, flowering time, early maturation, and fruit and seed size.

In the final phase, 10 mother plants were selected according to the set of variables considered in the selection process. In order to confirm the multiple resistance reported in previous experiments, these mother plants were vegetatively propagated and their clones subjected to infestation by the nematodes *M. exigua* Est E2 race 1 (from Campinas, SP, lat 22° 52’ 03’’ S, long 47° 04’ 58’’ W, alt 674 m asl), *M. incognita* Est I2 race 1 (from Marília, SP, lat 22° 16’ 31’’ S, long 49° 54’ 18’’ W, alt 624 m asl), and *M paranaensis* Est P2 (from Pompéia, SP, lat 22° 7’ 55’’ S, long 50° 8’ 18’’ W, alt 592 m asl). The identity of the nematode populations was confirmed by the Est isoenzyme profile and by the SCAR markers.

The same 10 mother plants were recombined in a diallel experiment carried out in Campinas, SP, in order to determine compatibility and to generate interclonal hybrids for analysis of multiple resistance to the three species of *Meloidogyne*. More than 100,000 flowers were artificially pollinated over two years of studies. The results published by Gonçalves et al. (2021) confirmed the multiple resistance of the parents and 80% of the interclonal hybrid progenies. They responded differently regarding compatibility in crosses between them, providing support for the choice of five clones to compose a synthetic variety.

The clonal cultivars IAC WG, IAC FEBS, IAC PM, IAC LCCBF, and IAC ARM were selected due to the lack of genetic barriers among them, with all possible combinations being compatible with each other. Furthermore, they showed high frequency of individuals with multiple resistant to the three nematode species in each of the full-sib hybrid progenies generated in these crosses (Gonçalves et al. 2021).

Of the total number of F_1 hybrids generated in combinations between the five parents, 93% had a reproduction factor lower than 1 when inoculated with *M. paranaensis* and 100% when inoculated with *M. exigua* and *M. incognita* (Table 1), revealing that resistant to the three nematodes, according to Oostenbrink (1966).

During the experimental and pre-commercial phases, the clonal cultivars received different names: IAC 5 and CcK1 (IAC WG), GCR1C9A and CcR2 (IAC FEBS), HE 164 and CcR7 (IAC PM), HE 143 and CcR9 (IAC LCCBF), and HE 177 and CcR10 (IAC ARM). The final name of each clonal cultivar is made up of the acronym IAC, which begins the name of cultivars developed by the Agronomic Institute of Campinas, together with acronyms using the first letter of names of five distinguished people who contributed to the development of coffee growing in Brazil: Wallace Gonçalves (WG), Francisco Eduardo Bernal Simões (FEBS), Paulo Makimoto (PM), Luiz Carlos Camargo Barbosa Ferraz (LCCBF), and Ailton Rocha Monteiro (ARM), respectively.

IAC Herculândia is a synthetic cultivar resistant to the nematodes *M. exigua*, *M. incognita*, and *M. paranaensis*, resulting from intercrosses between genetically compatible clonal cultivars of *C. canephora* called IAC WG, IAC FEBS,

Table 1. Resistance of F_1 hybrids obtained from the clonal cultivars IAC WG, IAC FEBS, IAC PM, IAC LCCBF, and IAC ARM of *Coffea canephora* to the nematodes *Meloidogyne exigua, M. incognita*, and *M. paranaensis*, evaluated by the Reproduction Factor (RF) and Reduction in the Reproduction Factor (RRF) variables. Adapted from Gonçalves et al. (2021)

Intercross	*M. paranaensis*[1]			*M. incognita*			*M. exigua*		
	RF[2]		RRF[3]	RF		RRF	RF		RRF
	Mean	RF<1	Mean	Mean	RF<1	Mean	Mean	RF<1	Mean
		%	%		%	%		%	%
IAC WG × IAC FEBS / IAC FEBS × IAC WG	0.63	92	96	0.03	100	100	0.00	100	100
IAC WG × IAC PM / IAC PM × IAC WG	0.83	85	94	0.03	100	100	0.00	100	100
IAC WG × IAC LCCBF / IAC LCCBF × IAC WG	0.46	92	97	0.01	100	100	0.00	100	100
IAC WG × IAC ARM / IAC ARM × IAC WG	0.58	88	96	0.00	100	100	0.00	100	100
IAC FEBS × IAC PM / IAC PM × IAC FEBS	0.47	92	97	0.09	100	100	0.00	100	100
IAC FEBS × IAC LCCBF / IAC LCCBF × IAC FEBS	0.82	92	94	0.02	100	100	0.00	100	100
IAC FEBS × IAC ARM / IAC ARM × IAC FEBS	0.02	100	100	0.01	100	100	0.01	100	100
IAC PM × IAC LCCBF / IAC LCCBF × IAC PM	0.33	94	98	0.01	100	100	0.00	100	100
IAC PM × IAC ARM / IAC ARM × IAC PM	1.37	96	90	0.02	100	100	0.00	100	100
IAC LCCBF × IAC ARM / IAC ARM × IAC LCCBF	0.11	96	99	0.01	100	100	0.02	100	100
Mean	0.56	93	96	0.02	100	100	0.02	100	100
Mundo Novo IAC 515-20[4]	14.74	0		17.40	0		15.92	0	

[1] Identity of nematodes *M. exigua* Est E2 race 1 (Campinas, SP; lat 22° 52' 03'' S, long 47° 04' 58'' W, alt 674 m asl), *M. incognita* Est I2 race 1 (Marília, SP; lat 22° 16' 31'' S, long 49° 54' 18'' W, alt 624 m asl), and *M. paranaensis* Est P2 (Pompéia, SP; lat 22° 7' 55'' S, long 50° 8' 18'' W, alt 592 m asl) confirmed by the profile of the esterase enzyme (Est) (Carneiro and Almeida 2001) and SCAR markers (Randig et al. 2004). [2] RF = Reproduction factor: RF < 1 = Resistant; RF > 1 = Susceptible, according to Oostenbrink (1966). [3] RRF = Reduction in the Reproduction Factor (RRF), calculated by the equation $RRF = \frac{RFc - RFh}{RFc} \times 100$, where RFc is the average reproduction factor of the control Mundo Novo IAC 515 -20, and RFh is the average reproduction factor of the F_1 hybrid (Moura and Regis 1987). Plants with values between 0 and 75 % are considered susceptible (S); between 75.1 and 90 %, moderately resistant (MR); between 90.1 and 95 %, resistant (R); between 95.1 and 99.9 %, highly resistant (AR); and 100%, immune (I), according to the classification adapted from Moura (1997). [4] Experimental control.

IAC PM, IAC LCCBF, and IAC ARM. It is recommended for use as a rootstock with Arabica coffee cultivars grown in soils infested by these RKN.

The parental clonal cultivars of the IAC Herculândia cultivar were obtained by associating individual selection methods with progeny tests and clonal selection. Evaluations were carried out in a greenhouse and, above all, in fields infested by different populations of *M. exigua*, *M. incognita*, and *M. paranaensis*, mostly located in the state of São Paulo, as detailed below and shown schematically in Figure 1.

About the clonal cultivars

IAC WG: The *C. canephora* accession IAC 68 was introduced in 1932 in the IAC coffee germplasm bank, and it is still maintained in the field today. In the early 1980s, seeds of the coffee plant IAC 68-5 were sent to IAPAR, in Londrina, PR, Brazil (currently IDR), and evaluated for about 10 years in relation to resistance to races 1, 2, 3, and 4 of *M. incognita*. In 1990, IAC 68-5 clonal seedlings characterized as resistant to the four races of *M. incognita* were sent to the IAC, in Campinas, SP, and have been identified since then as IAC 5. Clones and open-pollinated progenies of this coffee plant were evaluated in infested fields between 1990 and 2010 in relation to resistance, yield potential, vigor, size, maturation cycle, and fruit size. The experiments were carried out in different locations in coffee-producing regions of the state of São Paulo, such as Ribeirão Corrente, Itirapuã (*M. exigua*), Gália, Garça, Lucélia, Marília (*M. incognita*), Cássia dos Coqueiros, Adamantina, Parapuã, Tupã, Pompéia, Vera Cruz and Herculândia (*M. paranaensis*). In subsequent experiments related to interclonal compatibility and validation of resistance in the greenhouse, the clone IAC 5 was identified as CcK1 (Gonçalves et al. 2021). The clonal cultivar IAC WG was obtained from vegetative propagation of clone IAC 5 (CcK1) (Figure 1).

IAC FEBS: In 1974, seeds from the coffee plant T-3755 of *C. canephora* var. Robusta, from the collection of the Centro Agronómico Tropical de Investigación y Enseñanza - CATIE, Turrialba, Costa Rica, were brought to the coffee germplasm bank of the IAC and registered as accession IAC 2291. The material considered resistant to root-knot nematodes was selected by Lourival Carmo Monaco and has the cultivar BP 46, selected in Indonesia, as its ancestor. In 1975, a progeny trial was carried out in soil free of nematode infestation in Limeira, SP, and one of the trial treatments was represented by a half-sib progeny of accession IAC 2291. In 1976, seeds from the accession IAC 2291, which stood out for its initial vigor and rapid vegetative growth, were used in the formation of seedlings and subsequent planting in an area infested by *M. incognita* race 2 at Fazenda Suissa, Guaimbê, SP. In 1978, a progeny of a coffee plant of accession IAC 2291 was selected from the Guaimbê experiment and planted at Fazenda Santo Antônio, Marília, SP, in an area infested by *M. incognita* race 1. The surviving coffee clones in this experiment that stood out for their vegetative vigor and yield potential were multiplied and planted in Garça, SP. In subsequent experiments related to interclonal compatibility and validation of resistance in the greenhouse, the GCR1C9A clone was identified as CcR2 (Gonçalves et al. 2021). The vegetative propagation of this clone generated the clonal cultivar called IAC FEBS (Figure 1).

IAC PM: In 1980, a coffee progeny of accession IAC 2291 selected in the experiment carried out in Guaimbê, SP, described above, was planted at Fazenda Santa Marina in Vera Cruz, SP, in an area infested with *M. incognita* race 1. In 1990, the clones of IAC 2291 from this experimental area that survived and that stood out for their vegetative vigor, as well as for their potential for fruit production, were multiplied and planted at Fazenda Santo Antônio, Herculândia, SP, in an area infested with *M. paranaensis*. One of these clones established at Herculândia and identified as HE 164 was included in subsequent experiments. The objective was to determine the genetic compatibility with other clones and validate the resistance observed in the field in a greenhouse. In these experiments, the HE 164 clone was identified as CcR7 (Gonçalves et al. 2021). The denomination IAC PM was assigned to the clonal cultivar obtained from the vegetative propagation of clone HE 164 (CcR7) (Figure 1).

IAC LCCBF: The clonal cultivar IAC LCCBF was obtained from the vegetative propagation of clone HE 143, which was also selected in the experiment conducted at Fazenda Santo Antônio, Herculândia, SP (Figure 1). The ancestor of this

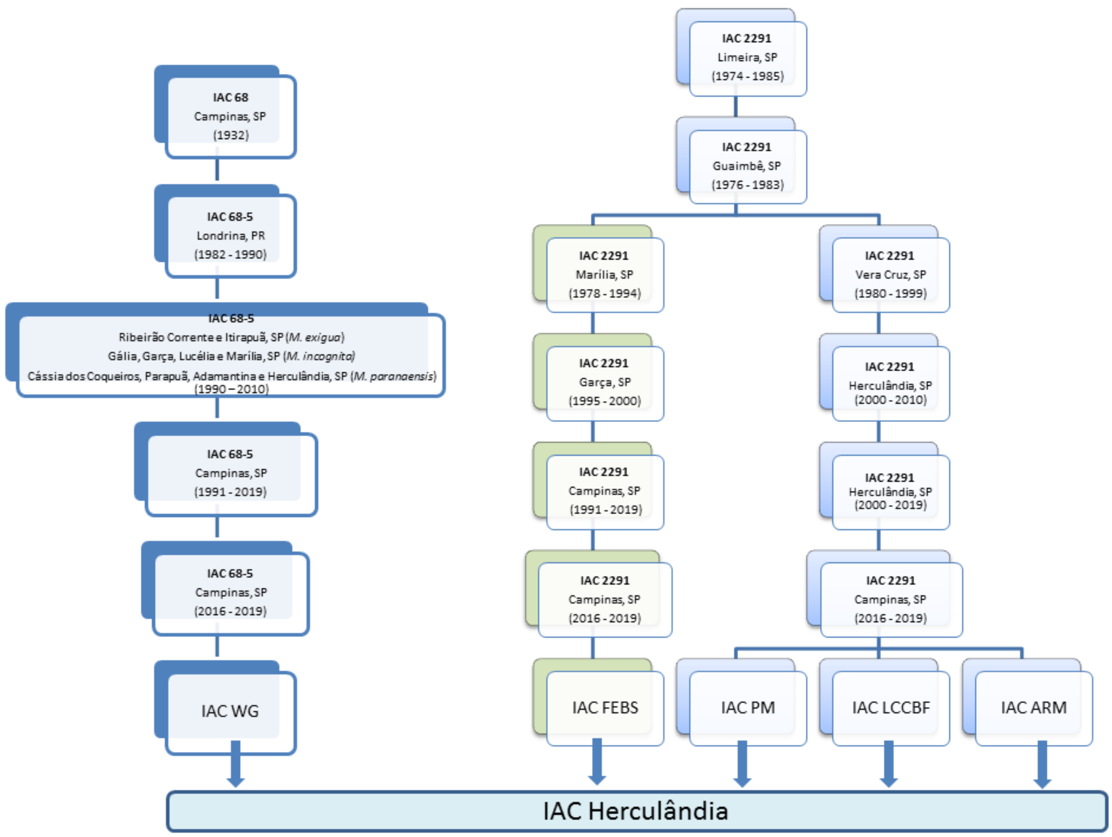

Figure 1. Genealogy of clonal cultivars IAC WG, IAC FEBS, IAC PM, IAC LCCBF, and IAC ARM. They are parents of the rootstock cultivar IAC Herculândia of *Coffea canephora*.

clonal cultivar is the same as that of the IAC PM cultivar.

IAC ARM: This clonal cultivar has the same origin as the IAC PM and IAC LCCBF cultivars and is also a clone, named HE 177, from one of the IAC 2291 coffee plants that survived in the experiment conducted in Vera Cruz, SP. Clone HE 177 was multiplied and planted in 1990 in an area infested with *M. paranaensis* in Herculândia, SP. IAC ARM is the name of the clonal cultivar obtained from the vegetative propagation of the HE 177 coffee plant (Figure 1).

Resistance analysis in a greenhouse

Experiments with artificial inoculations of nematodes collected in different regions of the state of São Paulo were carried out in a greenhouse between 1991 and 2014 at the IAC in Campinas. The clonal cultivars IAC WG and IAC FEBS were resistant to two populations of *M. paranaensis*, coming from Herculândia and Cássia dos Coqueiros. They were also resistant to four populations of *M. incognita*, three populations of race 1 (from Lucélia, Ribeirão Corrente, and Marília), and a population of race 3 from Garça (Fatobene et al. 2019).

Subsequently, greenhouse experiments conducted between 2016 and 2019 in Campinas, using artificial inoculations, confirmed the resistance of the clonal cultivars IAC WG, IAC FEBS, IAC PM, IAC LCCBF, and IAC ARM to the nematodes *M. exigua* Est E2 race 1 (Campinas, SP, lat 22° 52’ 03’’ S, long 47° 04’ 58’’ W, alt 674 m asl), *M. incognita* Est I2 race 1 (Marília, SP, lat 22° 16’ 31’’ S, long 49° 54’ 18’’ W, alt 624 m asl), and *M. paranaensis* Est P2 (Pompéia, SP, lat 22° 7’ 55’’ S, long 50° 8’ 18’’ W, alt 592 m asl) (Gonçalves et al. 2021).

Complete diallel crosses

Between 2016 and 2018, studies conducted by Gonçalves et al. (2021) in combinatorial analysis in a complete diallel evidenced gametophytic genetic compatibility among the clonal cultivars IAC WG, IAC FEBS, IAC PM, IAC LCCBF, and IAC ARM. All twenty possible combinations among the five cultivars used as a male or female parent resulted in the production of fruit and seeds.

Progenies of interclonal F_1 hybrids generated in these studies were evaluated for simultaneous resistance to the three nematode species (Table 1). In the crosses, regardless of the combination performed among the five parental clones, the set of hybrids generated showed, on average, 100% of individual plants resistant to *M. exigua* and *M. incognita*. Progenies of F_1 hybrids that have the clonal cultivars IAC WG, IAC FEBS, IAC PM, IAC LCCBF, and IAC ARM as one of the parents showed 89%, 94%, 92%, 93%, and 97% of individual plants resistant to *M. paranaensis,* respectively (Table 1).

PERFORMANCE

IAC Herculândia is a synthetic cultivar of *C. canephora* with multiple resistance to nematodes of the genus *Meloidogyne*, and it is recommended as a rootstock for susceptible cultivars of Arabica coffee. The performance of each of the possible combinations of intercrosses among the five recombinant parents AC WG, IAC FEBS, IAC PM, IAC LCCBF, and IAC ARM is shown in Table 1.

All individuals were resistant to the species *M. incognita* and *M. exigua*, with a reproduction factor (RF) ranging from 0 to 0.09 and reduction in the reproduction factor (RRF) equal to 100% in experiments carried out under controlled conditions with inoculation of nematodes on coffee plants grown in pots. The average percentage of coffee plants resistant to *M. paranaensis*, with RF < 1, was equal to 93%, with values ranging from 85% to 100%. The average value of RRF was equal to 96%, with values ranging from 90% to 100%. The data, adapted from Gonçalves et al. (2021), were obtained from artificial inoculations of the nematodes *M. exigua*, *M. incognita*, and *M. paranaensis*, with *C. arabica* Mundo Novo IAC 515-20 used as a susceptible control.

OTHER TRAITS

In hypocotyledonary grafting, the rootstock IAC Herculândia is compatible with the *C. arabica* cultivars IAC Ouro Verde, Catuaí Vermelho IAC 99, Mundo Novo IAC 379-19, IAC Catuaí SH3, IAC 125 RN, IAC Obatã 4397, and Obatã IAC 1669-20. Compatibility with other Arabica coffee cultivars registered in the Brazilian Ministry of Agriculture (Ministério da Agricultura, Pecuária e Abastecimento - MAPA) has not been evaluated.

Like the parental clones, coffee plants of the IAC Herculândia cultivar have a dense and voluminous root system.

The rootstock cultivar IAC Herculândia was tested against the nematode species described here and was not evaluated for other soil pests.

SEED MAINTENANCE AND DISTRIBUTION

The cultivar IAC Herculândia was registered by the Brazilian Ministry of Agriculture in the National Cultivar Registry (MAPA/RNC) under no. 52409. The IAC is responsible for the production and distribution of seeds.

REFERENCES

Arita LY, Silva AS and Machado ACZ (2020) Efficacy of chemical and biological nematicides in the management of *Meloidogyne paranaensis* in *Coffea arabica*. **Crop Protection 131**: 105099.

Carneiro RMDG and Almeida MRA (2001) Técnica de eletroforese usada no estudo de enzimas dos nematoides de galhas para identificação de espécies. **Nematologia Brasileira 25**: 35-44.

Fatobene BJR, Andrade VT, Gonçalves W and Guerreiro Filho O (2019) *Coffea canephora* clones with multiple resistance to *Meloidogyne incognita* and *M. paranaenses*. **Experimental Agriculture 55**: 443-451.

Fatobene BJR, Oliveira CMG, Rosa JMO, Tomazini MD, Vasconcelos LBC and Guerreiro Filho O (2022) Manejo de nematoides parasitos do cafeeiro. In Santinato F (ed) **A moderna cafeicultura brasileira: tecnologias que afetam a produtividade**. Funep, Jaboticabal, p. 525-544.

Fazuoli LC, Lima MMA, Gonçalves W and Costa WM (1987) Melhoramento do cafeeiro visando resistência a nematoides: utilização de porta-enxertos resistentes. In **Anais do Congresso Paulista de Agronomia.** AEASP, Piracicaba, p. 171-180.

Gonçalves W, Andrade VT, Fatobene BJR, Caixeta LB, Padilha L, Oliveira CMG, Rosa JMO, Rodrigues LMR and Guerreiro Filho O (2021) Selection strategy of a *Coffea canephora* rootstock with simultaneous nematode resistance to *Meloidogyne exigua*, *M. incognita* and *M. paranaensis*. **European Journal of Plant Pathology 160**: 81-95.

Moraes MV and Franco CM (1987) **Método expedito para enxertia em café**. IBC, Campinas, 16p.

Moura RM (1997) Gênero *Meloidogyne* e a meloidoginose. Parte II. **Revisão Anual de Patologia de Plantas 5**: 281-315.

Moura R and Regis EMO (1987) Reações de cultivares de feijoeiro comum (*Phaseolus vulgaris*) em relação ao parasitismo de *Meloidogyne javanica* e *M. incognita.* **Nematologia Brasileira 11**: 215-225.

Oostenbrink M (1966) Major characteristics of the relation between nematodes and plants. **Mededelingen van de Landbouwhogeschool te Wageningen 66**: 1-46.

Randig O, Carneiro RMDG and Castagone-Sereno P (2004) Identificação das principais espécies de *Meloidogyne* parasitas do cafeeiro no Brasil com marcadores SCAR café em multiplex-PCR. **Nematologia Brasileira 28**: 1-10.

Reina EH (1966) La técnica del injerte hipocotiledonar del cafeto para el control de nematodos. **Turrialba 7**: 5-11.

Santos MFA, Salgado SML, Silva JGP, Correa VR, Mendonça JSF and Carneiro RMDG (2018) *Meloidogyne incognita* parasitizing coffee plants in southern Minas Gerais, Brazil. **Tropical Plant Pathology 43**: 95-98.

Terra WC, Salgado SML, Fatobene BJR and Campos VP (2019) Expanded geographic distribution of *Meloidogyne paranaensis* confirmed on coffee in Brazil. **Plant Disease 103**: 589.