

## RESEARCH NOTE/NOTA INVESTIGATIVA

### RESISTANCE OF SEMI-WILD *COFFEA ARABICA* L. FROM ETHIOPIA TO A ROOT-KNOT NEMATODE, *MELOIDOGYNE KONAENSIS*

Sayaka Aoki<sup>1\*</sup>, Brent Sipes<sup>1</sup>, Carlos Astorga<sup>2</sup>, Chifumi Nagai<sup>3</sup>

<sup>1</sup>Department of Plant and Environmental Protection Sciences College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, Honolulu, HI 96822. 808-621-1353; <sup>2</sup>Laboratorio de Biotecnología, Tropical Agricultural Research and Higher Education Center (CATIE), Turrialba, Costa Rica; <sup>3</sup>Hawaii Agriculture Research Center, Kunia, HI 96759; Corresponding author: sipes@hawaii.edu

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#### ABSTRACT

Aoki, S., B. Sipes, C. Astorga, and C. Nagai. 2012. Resistance of semi-wild *Coffea arabica* L. from Ethiopia to a root-knot nematode, *Meloidogyne konaensis*. *Nematropica* 42:131-136.

The Kona coffee root-knot nematode (*Meloidogyne konaensis*) infects coffee, reduces yield and shortens tree life. Ten accessions of semi-wild Ethiopian *Coffea arabica* imported from CATIE, Costa Rica were compared to the well characterized susceptible *C. arabica* ‘Yellow Catuai’ and ‘Typica’ and tolerant *C. liberica* var. *dwevrei* ‘Fukunga’ for resistance to *M. konaensis*. Three thousand eggs were inoculated on the coffee seedlings. Eggs and second-stage juveniles were collected 8 months later and Reproductive factor (Rf) values were calculated. The final nematode levels observed in the semi-wild Ethiopian accessions were 13- to 15-fold lower than in the two susceptible controls (P=0.05). All Ethiopian accessions tested, except ET 8, had Rf values <1, indicating that the accessions are resistant.

Key words: coffee, coffee diseases, Ethiopian, host-plant resistance, screening

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#### RESUMEN

Aoki, S., B. Sipes, C. Astorga, and C. Nagai. 2012. Resistencia de *Coffea arabica* L. semi-salvaje de Etiopia to a root-knot nematode, *Meloidogyne konaensis*. *Nematropica* 42:131-136.

El nematodo agallador del café Kona (*Meloidogyne konaensis*) infecta al cafeto, reduce la producción y acorta la vida del árbol. Se comparó la resistencia a *M. konaensis* de diez accesiones de *Coffea arabica* semi-salvajes de Etiopía, importadas de CATIE, Costa Rica, con el comportamiento de las variedades susceptibles *C. arabica* ‘Yellow Catuai’ y ‘Típica’ y de la variedad tolerante *C. liberica* var. *dwevrei* ‘Fukunga’. Las plántulas se inocularon con 3,000 huevos. Ocho meses después de la inoculación, se colectaron los huevos y juveniles de segundo estadio para calcular los valores de factor reproductivo (Rf). Los niveles de población de nematodos observados en las accesiones semi-salvajes de Etiopía fueron 13 a 15 veces menores que los observadas en los dos controles susceptibles (P=0.05). Todas las accesiones de Etiopía evaluadas, except ET 8, tuvieron valores de Rf <1, indicando que las accesiones son resistentes.

Palabras clave: café, enfermedades del café, Etiopía, resistencia vegetal, evaluación de resistencia

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Coffee is an important agricultural commodity, ranking second in international trade (Vieira *et al.*, 2006). The genus *Coffea* contains approximately 103 species (Jaramillo *et al.*, 2009), yet current commercial production is based on only *Coffea arabica* L. (Arabica coffee) and *C. canephora* Pierre ex Forehner (Robusta coffee) (Vieira *et al.*, 2006). *Coffea arabica* accounts for 70% of worldwide coffee production (Gichuru

*et al.*, 2008). Hawaii is the only state in the United States of America where coffee is grown commercially. Even though Hawaii's coffee production is small, constituting less than 1% of total world commercial coffee production (Fleming and Mauri, 2001), Hawaii, especially in the Kona and Ka'u districts on the island of Hawaii, produces coffee with high beverage quality ranking among one of the best in the world

(Bittenbender and Smith, 2004).

Hawaii has been free from most of the serious coffee diseases, but the Kona district, like many coffee producing areas, has devastating root-knot nematode problems (Schmitt *et al.*, 2001). Kona coffee decline is caused by the root-knot nematode *Meloidogyne konaensis* (Eisenback *et al.*, 1994). A rapid increase in coffee hectares in the 1990s planted with seedlings from infested farms probably spread the nematode further. By 2001, 85% of the coffee in Kona was infected with *M. konaensis* and the nematode caused an estimated 20-25% reduction in coffee yields (Nelson *et al.*, 2002). Kona 'Typica' was the most susceptible *C. arabica* accession among those grown in Hawaii (Bittenbender *et al.*, 2001).

The eradication of root-knot nematodes from a field is extremely difficult (Seracin *et al.*, 1999) and chemical treatments to reduce nematode damage are hazardous to humans and expensive (Anzueto *et al.*, 2001). So far, resistance and tolerance are the main management tactics used commercially to reduce root-knot nematode damage in Hawaii and other coffee growing areas of the world.

Nematode-tolerant coffee cultivars used around the world are hybrids resulting from crosses between relatively disease resistant *C. canephora* (Berthaud *et al.*, 1988; Jaramillo *et al.*, 2009) and susceptible *C. arabica* (Gichuru *et al.*, 2008; Vieira *et al.*, 2006). 'Timor hybrid,' a natural hybrid between *C. arabica* and *C. canephora*, and 'Nemaya,' a hybrid within *C. canephora*, are used as resistance rootstock against several root-knot species in Central America (Nelson *et al.*, 2002). *Coffea canephora* hybrid 'Apoata' is used as a rootstock in Brazil for control of *M. incognita* and *M. paranaensis* (Campos *et al.*, 2005; Bittenbender *et al.*, 2001). 'Fukunaga', a cultivar of *C. liberica* var. *dewevrei*, is used as root stock in Hawaii for its partial resistance and tolerance to *M. konaensis*. Currently, Hawaii's coffee production depends solely on root stocks of 'Fukunaga' *C. liberica* var. *dewevrei* (Zhang and Shmitt, 1995; Bittenbender *et al.*, 2001). A serious problem would occur if a new root-knot species were introduced into Hawaii or the current nematode overcomes the resistance in 'Fukunaga'.

Today, only 'Typica' and 'Bourbon' constitute the genetic basis of almost all *C. arabica* cultivars that are planted in the world, resulting in a very narrow genetic base of the commercial *C. arabica* cultivars. To conserve the genetic variation of *C. arabica* and enhance the genetic variability in commercial plantings (Chaparro *et al.*, 2004; Fleming and Mauri, 2001), native wild accessions of *C. arabica* were collected from Ethiopia by the Food and Agriculture Organization of the United Nations (FAO) in the 1960s. These accessions have been maintained in several institutes including the Tropical Agricultural Research and Higher Education Centre (CATIE) in Costa Rica. These Ethiopian coffee seedlings had been screened against a few root-knot nematode species including *M.*

*paranaensis* and *M. incognita* and nine accessions have confirmed resistance against these two species (Anzueto *et al.*, 2001; Boisseau *et al.*, 2009). No information on behavior of the accessions against *M. konaensis* is available. The objective of this experiment was to identify new sources of resistance to *M. konaensis* in 10 semi-wild *C. arabica* accessions.

Seed of 10 semi-wild accessions of Ethiopian *C. arabica* were imported into Hawaii in 2006 from CATIE by Hawaii Agriculture Research Center (Table 1).

Fourteen seedlings of each accession were selected for uniformity. Seedlings of *C. arabica* 'Yellow Catuai', obtained from Kauai, and seedlings of *C. arabica* 'Typica', obtained from Waialua, Oahu were included as susceptible controls. Coffee seedlings were transplanted into 15-cm dia clay pots filled with a steam-sterilized soil and silica sand mixture (1:1). The plants were maintained in the greenhouse at 13°C to 27°C under 20% shade.

Nematode cultures for inoculum were maintained on 'Typica' coffee plants. Infected plants were removed from pots and the soil gently removed. Nematode eggs were extracted by blending the roots in 0.5% NaOCl for two pulses of 30 seconds each (Hussey and Barker, 1973). Nematode eggs were collected on nested 150-µm and 25-µm aperture sieves. The eggs were subjected to sucrose density centrifugation (Jenkins, 1964), counted and adjusted to 3000 eggs/ml. Eleven 30-cm coffee seedlings of each accession at the 10-leaf stage were inoculated with 3000 eggs/plant. Three seedlings from each accession received an equal volume of water to serve as uninoculated controls.

The seedlings were arranged in a randomized strip plot on benches and maintained in the greenhouse for eight months. Plants were fertilized with Miracle-Gro Acid Loving Plant Food 30-10-10 (SCOTTS, Marysville, OH) every two weeks. Scales and aphids were controlled by sprays of soapy water and manual wiping of stems and leaves with sponges.

Plant height was recorded at harvest 8 months after inoculation. Plant shoots were excised at the soil line and the fresh and dry weight were recorded. The roots were rinsed free of soil, weighed and cut into 2-cm long pieces. The roots were then blended in a 0.5% NaOCl solution for 30 seconds to extract eggs and second-stage juveniles (J2). The eggs and (J2) were collected on nested 20-µm and 150-µm aperture sieves, rinsed, centrifuged, and counted (Hussey and Barker, 1973; Jenkins, 1964). The reproductive factor (Rf) was calculated (final nematode population (Pf) divided by 3,000 for each plant). The genotypes were considered resistant when  $Rf < 1$ , and susceptible when  $Rf > 1$  (Seinhorst, 1965).

In a second experiment, nematode reproduction on *C. liberica* var. *dewevrei*, 'Fukunaga' from Kona, Hawaii Island was compared to that on *C. arabica* 'Typica'. 'Fukunaga' was included to serve as a comparative tolerant control. Ten 10- to 15-cm tall

seedlings with 3-5 leaves of 'Fukunaga' and 'Typica' were transplanted into 15-cm dia clay pots filled with a steam-sterilized soil and silica sand mixture (1:1). Inoculum was prepared from 'Typica' cultures of *M. konaensis* as described previously. Because seedlings were smaller than in the first trial, a lower inoculum level was used. The eggs were adjusted to 1,000 eggs/ml, and 1 ml inoculated to the roots of each plant; three control plants from each of the cultivars received water. Plants were arranged in a strip-plot design and maintained in the greenhouse for eight months.

Eight months after inoculation, coffee shoot height, shoot and root wet and dry weights were recorded. The roots were rinsed free of soil, weighed and blended in a 0.5% NaOCl solution for 30 seconds to extract eggs. The eggs were collected on nested 150- $\mu$ m and 20- $\mu$ m aperture sieves, rinsed and centrifuged (Jenkins, 1964). Eggs were counted, and Rf calculated (final nematode population (Pf) divided by 1,000 for each plant).

The Rf values of Typica from the two experiments were compared and found to be similar. The two data sets were then tested for homogeneity of error variance and found to not differ. Pf data were log-transformed ( $\log_{10}(x+1)$ ) to improve normality of the data. Data were analyzed for variance and where appropriate, mean separation procedures conducted.

All of the semi-wild Ethiopian accessions provided higher levels of resistance to *M. konaensis* than the commonly used 'Fukunaga' root-stock (Fig. 1 and Fig. 2). Nine of the semi-wild Ethiopian accessions were resistant to *M. konaensis* (Rf < 1.0) and only one accession was susceptible (Rf > 1.0). Dry shoot weights of inoculated ET25, ET28, ET52, ET57 and ET32B decreased 21% compared to the uninoculated plants (Fig. 3). This decrease indicated that these accessions, while resistant to *M. konaensis*, may be intolerant to infection by the nematode. Inoculated plant dry shoot weights of ET8, ET11C, ET15, ET17 and ET25-B were 22% greater compared to the uninoculated shoot weight of each plant. The inoculated fresh root weight of ET15 and ET 17 was higher than other accessions ( $P=0.05$ ) (Fig. 4). This increased weight was probably due to the root-knot galls induced by *M. konaensis*. The same result was seen in Typica which had a high Rf and high root weight, indicating the greater fresh root weight might be attributable to root compared to intolerant 'Yellow Catuai', where the roots simply did not grow.

These semi-wild Ethiopian accessions hold much utility for nematode management in coffee. The accessions could be used as production cultivars, rootstocks, and more importantly, as sources of resistance in coffee breeding programs.

The root-knot resistant semi-wild accessions of *C. arabica* identified in this and other research (Anzueto *et al.*, 2001; Boisseau *et al.*, 2009) will provide resistance that can be incorporated into current commercial cultivars such as 'Typica' and 'Bourdon'. Introducing the resistance from semi-wild *C. arabica* will address many of the concerns and challenges arising from

using resistance from other wild *Coffea* spp. The resistance available in the semi-wild *C. arabica* offers a sustainable and environmentally-sound method for controlling root-knot nematodes in coffee plantations.

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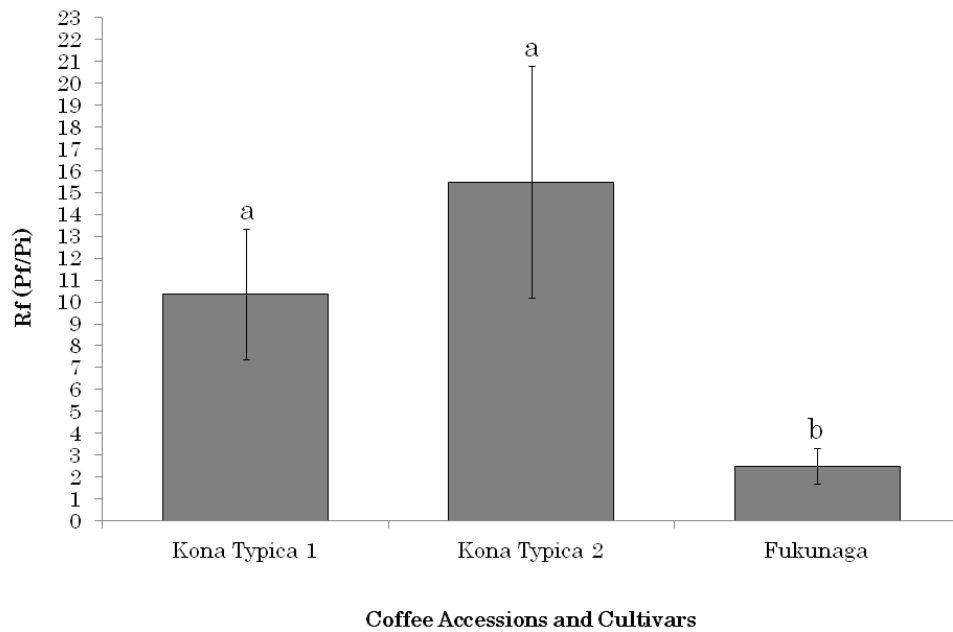


Fig. 1. *Meloidogyne konaensis* reproductive factors (Rf) on two susceptible (Rf >1.0) *Coffea arabica* 'Typica' from Experiment 1 and 2 and *C. liberica* var. *dewevrei* 'Fukunaga'. Bars indicate standard error.

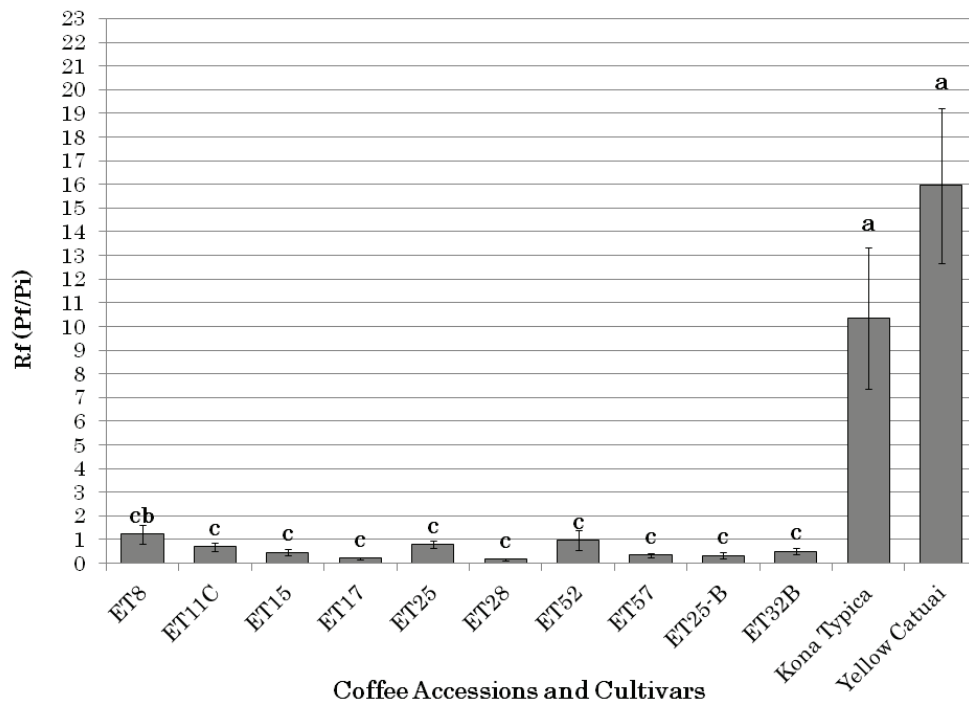


Fig. 2. *Meloidogyne konaensis* reproductive factors (Rf) on ten semi-wild Ethiopian (ET) accessions of *Coffea arabica*, and two susceptible (Rf >1.0) *C. arabica* 'Typica' and 'Yellow Catuai'. Bars indicate standard error.

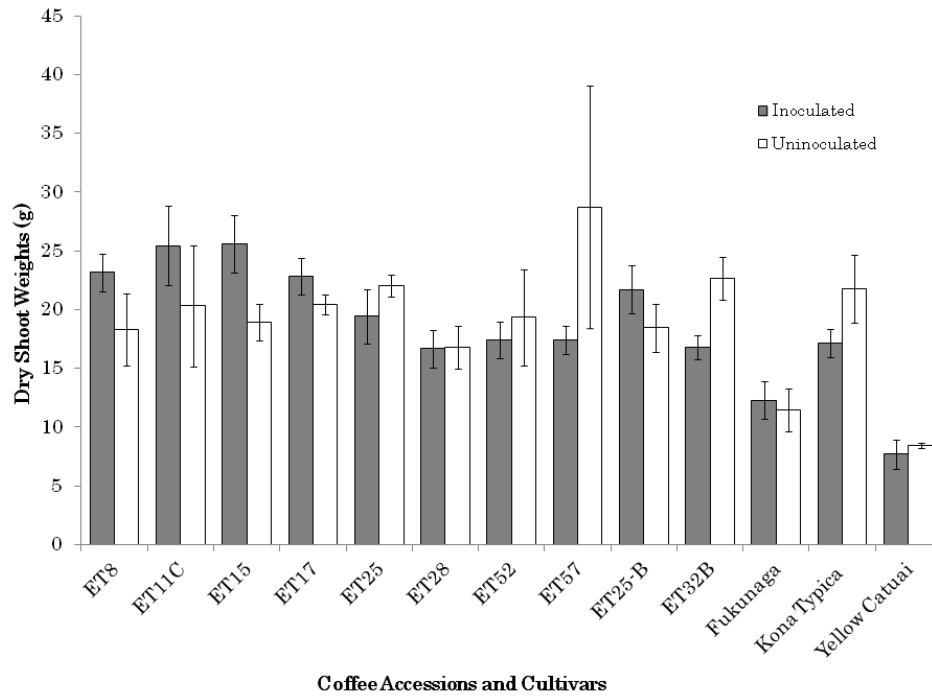


Fig. 3. Dry shoot weights of ten semi-wild Ethiopian (ET) accessions of *Coffea arabica*, two susceptible ( $R_f > 1.0$ ) *C. arabica* 'Typica' and 'Yellow Catuai' and *C. liberica* var. *dewevrei* 'Fukunaga' inoculated and uninoculated with *Meloidogyne konaensis*. Bars indicate standard error.

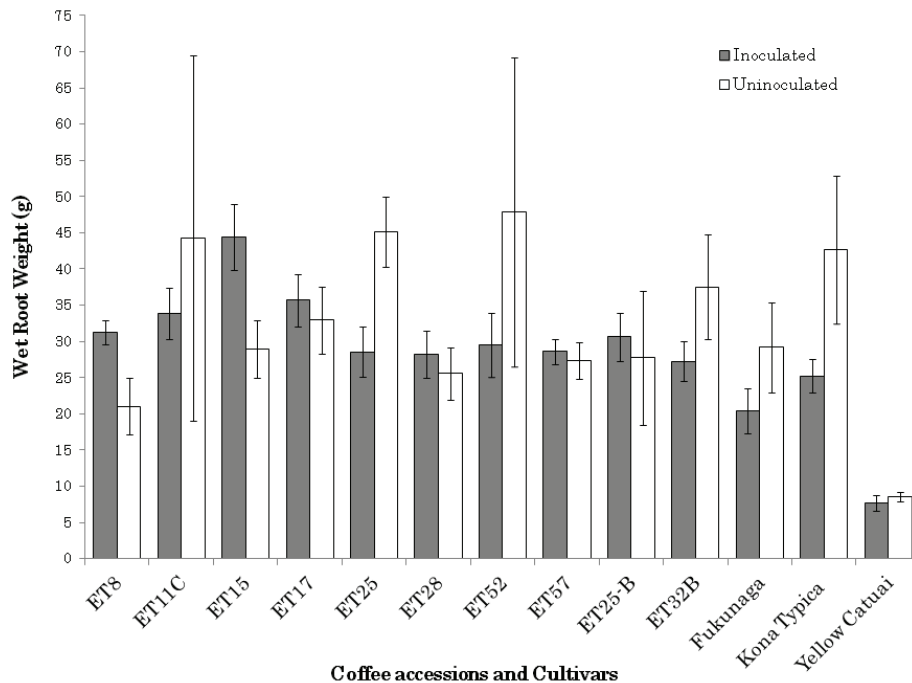


Fig. 4. Fresh root weights of ten semi-wild Ethiopian (ET) accessions of *Coffea arabica*, two susceptible ( $R_f > 1.0$ ) *C. arabica* 'Typica' and 'Yellow Catuai' and *C. liberica* var. *dewevrei* 'Fukunaga' inoculated and uninoculated with *Meloidogyne konaensis*. Bars indicate standard error.



Table 1. Two well characterized cultivars, a rootstock cultivar and ten CATIE accessions of *Coffea arabica* evaluated for resistance to *Meloidogyne konaensis*.

	Accession #	Type or Cultivar
<i>C. arabica</i> (Ethiopian)	ET8	T.16694-8
	ET11C	T.16700-7
	ET15	T.16704-7
	ET17	T.16706-6
	ET25	T.16712-1
	ET28	T.167-15
	ET52	T.16733-2
	ET57	T.16737-2
	ET25-B	T.17204-2
	ET32B	T.17205-4
<i>C. liberica</i> var. <i>dewevrei</i>	-	'Fukunaga'
<i>C. arabica</i>	-	'Yellow Catuai'
<i>C. arabica</i>	-	'Typica'

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