PLANT-PARASITIC NEMATODES ASSOCIATED WITH RICE IN ECUADOR

Carmen Triviño Gilces[1]*, Daniel Navia Santillán[1], and Luis Velasco Velasco[1]

[1]Instituto Nacional de Investigaciones Agropecuarias (INIAP), Estación Experimental Litoral Sur, Crops Protectión Department, Box. 09 -017069, Guayaquil, Ecuador. *Corresponding author: carmen.trivino@iniap.gob.ec; trivino.carmen@gmail.com

ABSTRACT

Triviño, C., D. Navia-Santillán, and L. Velasco. Plant-parasitic nematodes associated with rice in Ecuador. Nematropica 46:45-53.

The aim of this work was to analyze the frequency of occurrence, distribution, and population densities of plant-parasitic nematodes associated with rice in Guayas, Los Ríos, Manabí, El Oro, and Loja provinces, Ecuador. A total of 331 samples of roots and soil and 210 samples of paddy panicles were collected in 46 rice-growing areas. Nematodes were extracted from 10 g roots, 100 cm^3 soil, and 100 seeds. The root-knot nematode, *Meloidogyne graminicola*, occurred at greatest frequency and had the highest population densities both in roots and soil. In irrigated rice plantations, *Hirschmanniella oryzae* was found most often in rainfed lowland rice, and *Pratylenchus* spp. were present at greatest frequency. Other nematodes identified in soil samples were species of *Helicotylenchus*, *Criconemoides*, and *Tylenchorhynchus*. *Aphelenchoides besseyi* was detected in dry seeds collected in the five provinces at varying population densities.

Key words: irrigated and rainfed lowland plantation, nematode survey, *Oryza sativa*, root-knot nematode, white-tip nematode.

RESUMEN

Triviño, C., D. Navia-Santillán, y L. Velasco. Nematodos fitoparásitos asociados al cultivo de arroz en Ecuador. Nematropica 46:45-53.

El objetivo de este trabajo fue analizar la frecuencia de la ocurrencia, distribución y densidad poblacional de nematodos fitoparásitos asociados al cultivo de arroz en las provincias de Guayas, Los Ríos, Manabí, El Oro y Loja, en Ecuador. En 46 áreas de arroz seleccionadas, se recolectaron 331 muestras de raíces y suelo, y 210 muestras de panículas. Los nematodos fueron extraídos de 10 g raíces, 100 cm^3 suelo y 100 semillas secas (cinco repeticiones cada una). El nematodo encontrado con mayor frecuencia, distribución y altas densidades poblacionales en raíces y suelo fue el agallador de raíces, *Meloidogyne graminicola*. En plantaciones bajo riego estuvo presente *Hirschmanniella oryzae* y en plantaciones sembradas en secano estuvo *Pratylenchus* spp. Otros nematodos identificados en el suelo fueron especies de *Helicotylenchus*, *Criconemoides* y *Tylenchorhynchus*. *Aphelenchoides besseyi* fue detectado en semilla seca colectada en las cinco provincias muestreadas, con distribución heterogénea y densidad poblacional variada.

Palabras claves: plantaciones bajo riego y secano, muestreo de nematodos, *Oryza sativa*, nematodo agallador de raíces, nematodo de la punta blanca.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important crops in Ecuador with 411,459 ha cultivated. Growers produce about 1,565,535 tons annually with an average yield of 4.22 tons per hectare (INEC, 2012). Ninety-two percent of Ecuador's rice area is in the provinces of Guayas and Los Ríos where 60% is planted in irrigated areas and 40% in rainfed lowlands (INEC, 2012).

Plant-parasitic nematodes have been implicated as a major constraint to rice production all over the world. In a survey done in rice plantation in Liberia, *Helicotylenchus dihystera*, *Hemicycliophora typica*, *Meloidogyne incognita*, *Mesocriconema curvatum*, *M. onoense*, and *Xiphinema ifacolum* were identified

(Lamberti *et al*., 1991). The root-knot nematode, *M. graminicola* Golden and Birchfield, 1965, is widespread in much of the world and is capable of causing growth retardation of rice seedlings in nursery seedbeds (Padgham *et al*., 2004). The impact of this nematode on rice yield has been well established with reported yield losses of 20 to 90% (Arayarungsarit, 1987). It has been reported to cause yield losses in upland, rainfed lowland, and irrigated rice, but yield losses are limited under continuous flooding (Prot *et al*., 1994; Prot and Matias, 1995; Padgman *et al*., 2004). *Meloidogyne graminicola* is widespread and causes yield reduction to rice grown in Japan, Bangladesh, Nepal, Pakistan, and other countries in South and Southeast Asia (Bridge and Page, 1982; Munir *et al*., 2000; Pokharel *et al*., 2005), Florida (Handoo *et al*., 2003), as well as in Costa Rica (Montero and Salazar, 2006).

Yield loss due to *M. graminicola* is greatest under non-irrigated conditions (Plowright and Bridge, 1990; Soriano *et al*., 2000). Early flooding immediately after sowing to limit invasion of roots by the nematode and to promote good establishment of the rice crop appears necessary to prevent or minimize yield losses due to *M. graminicola* in irrigated rice. In Ecuador the population density of *M. graminicola* in soil was reduced 89% by rotation with three continuous cycles of soybean and cotton (soybean-cotton-soybean), but maize and sorghum were good hosts (Triviño, 1994).

The rice root nematode, *Hirschmanniella oryzae* (Van Breda de Haan, 1902), Luc and Goodey, 1964, is another important nematode pest of the rice crop in many countries. It has been reported to cause yield losses of 10 to 30% in China (Liao *et al*., 2000). This nematode has been associated with rice, wheat, maize, and legumes in Bangladesh, Nepal, and Pakistan (Munir *et al*., 2000). In Venezuela, the most important nematodes are *Hirschmanniella* spp. (*H. oryzae*, *H. spinicaudata*, *H.caudacrena*), *Tylenchorhynchus annulatus*, *Meloidogyne salasi*, *Mesocriconema onoense*, *Helicotylenchus concavus*, and *Pratylenchus zeae* (Medina *et al*., 2009a). In the Philippines, the most prevalent nematodes found in lowland and upland rice ecosystems were *M. graminicola*, *H. oryzae*, and *H. mucronata*; other nematodes identified were *Aphelenchoides*, *Pratylenchus*, *Tylenchorhynchus*, *Helicotylenchus*, *Cricinemoides*, and *Rotylenchus* (Pascual *et al*., 2014).

The white tip nematode, *Aphelenchoides besseyi* Christie 1942, is the causal agent of white tip disease of rice. This nematode is widely distributed in many rice growing areas of Africa, North, Central and South America, Asia, Europe, and the Pacific. Yield losses vary with cultivar, year, temperature, cultural practices, and other factors (Tulek and Cobanoglu, 2010). This nematode feeds on the host leaves and young tissues causing whitening of the tips of the leaves (Yoshida *et al*., 2009) during the growing season. It then infects the rice panicle and remains coiled up in a dormant condition on the rice paddy seeds. In addition to potential yield and quality reductions in the rice crop, detection of white-tip nematodes in rice seed are of phytosanitary importance for seeds certification and quarantine programs (Rajan and Lal, 2006). *Aphelenchoides besseyi* lowers the number of seeds produced, and grains are smaller than normal (Kepenekci, 2013). In Costa Rica, *A. besseyi* was found in rice seeds at variable population densities (Montero and Salazar, 2006), and in Japan, a field survey revealed that infestation levels of this nematode varied among paddy fields even in a restricted area (Hoshino and Togashi, 1999). In infested fields, the average yield losses range from 10 to 30%, however, maximum losses of up to 70% for the most susceptible cultivars have been reported (Prot, 1992). This nematode also may cause variability in rice grain physical properties including decreasing moisture content, grain length, thickness, and diameter (Asadi *et al*., 2015). Maize and sorghum are other reported hosts of *A. besseyi* in Brazil (Tenente *et al.*, 2004).

The objective of this work was to analyze the frequency of the occurrence, distribution, and plant-parasitic nematode population densities in rice plantations from Guayas, Los Ríos, Manabí, El Oro, and Loja provinces of Ecuador.

MATERIALS AND METHODS

Sites and sampling methods

The survey was conducted in five provinces of Ecuador where rice is cultivated. A total of 331 composite rice root and soil samples and 210 composite rice grain samples were collected from 24 and 22 arbitrarily selected rice areas, in Guayas, Los Ríos, Manabí, El Oro, and Loja provinces (Table 1). In Guayas, Manabí, El Oro, and Loja provinces, 96% of the samples came from irrigated rice fields grown in monoculture and 4% from rainfed lowland plantations. In Los Ríos province 25% of the samples were collected from irrigated rice fields and 75% were rainfed fields grown in intercrop cycles with soybean and maize (Table 2). Both root and soil samples were collected from 10 arbitrarily-selected individual plants per field ranging in size from 1 to 17 ha. A kilogram of soil was collected in the rizosphere, placed individually in a labeled plastic bag, and transported to the laboratory within 24- to 48-hr after collection. A composite of 50 rice

Table 1. Frequency of occurrence (percentage) of plant-parasitic nematodes detected in root and (or) soil samples from rice in Ecuador.

Province and Region	Number of fields sampled	Altitude of fields sampled (m.a.s.l)	*Meloidogyne graminicola*	*Hirschmanniella oryzae*	*Pratylenchus* spp.	*Helicotylenchus* spp.	*Criconemoides* spp.	*Tylenchorhynchus* spp.
			----------%----------					
Guayas:								
Simón Bolivar	10	27-36	100	40	90	100	90	10
El Triunfo	10	29-67	100	90	0	0	30	0
Nobol	10	9-21	100	100	0	0	0	10
Taura	10	10-14	100	90	0	80	30	70
Palestina	10	5-23	100	100	0	20	0	0
General Vernaza	10	8-15	100	0	0	85	80	100
Naranjal	10	11-18	100	75	0	60	0	60
Samborondón	10	4-18	100	95	0	30	0	100
Santa Lucía	10	10-22	100	100	0	0	0	0
Yaguachi	10	9-10	100	85	25	0	0	0
Jujan	10	11-18	100	20	0	40	40	90
Los Ríos:								
Babahoyo	11	6-26	100	0	0	0	0	0
Montalvo	10	17-20	10	0	100	0	0	0
Mocache	11	20-44	82	0	100	0	0	0
Palenque	9	12-40	100	0	100	0	0	0
Quevedo	5	15-42	100	0	100	0	0	0
Valencia	8	20-45	100	0	100	0	0	0
Vinces	3	17-38	100	0	0	0	0	0
Manabí:								
Rocafuerte	57	2-35	11	62	0	20	0	0
Sucre	56	2- 9	27	80	0	13	0	0
El Oro:								
Santa Rosa	3	11-17	100	100	0	0	0	0
Arenillas	26	2-24	100	100	0	0	0	0
Loja:								
Macará	11	403-475	100	64	0	0	0	0
Zapotillo	11	225-242	100	64	0	0	0	0

Table 2. Frequency of rice cultivars (percentage) and cropping history in selected area sampled in Ecuador.

	Percentage per province				
Cultivar	Guayas	Manabí	Los Rios	El Oro	Loja
INIAP 11	12.7	60.5		17.2	4.6
INIAP 12	2.7				23.0
INIAP 14	43.6	5.3	55.2	10.4	
INIAP 15	3.6	29.0	12.0		
INIAP 16	1.0				22.5
INIAP 415	4.5	2.6	3.5		
F 21		2.6	1.7	10.0	
FD 50	10.9		25.9	12.0	
FL 09				50.4	
Millares					36.4
Tinajones					13.5
Conejo	11.8				
Donato	1.0		1.7		
1001	6.4				
IR 43	1.8				
Crop cycles/year	Rice-rice (90%) Rice-rice-rice (6%) Rice-corn (4%)	Rice-rice (90%) Rice-bean (3%) Rice-corn (4%) Rice-vegetables (3%)	Rice-rice (25%) Rice-corn (40%) Rice-soybean (35%)	Rice-rice (96%) Rice-corn (4%)	Rice-rice (97%) Rice-vegetables (2%) Rice-peanut (1%)

panicles was collected from ten different points in the same field during the harvesting period. In the laboratory, all the seeds within a composite sample were mixed and, five subsamples of 100 seed were placed in labeled individual Petri dishes until processed. The most common cultivars that were surveyed (70% of fields) were INIAP 11, INIAP 14, INIAP 15, FL 09, and F 21 (Table 2).

Nematode extraction

Ten grams of roots from each sample were washed, cut to about 1-cm length, and thoroughly mixed. The roots were triturated with 100-ml water in a kitchen blender for 20 seconds at low speed, and the resulting mixture was washed from the blender through nested sieves of 250-, 150-, and 25-µm opening (60-, 100-, 500-mesh, respectively). The residue on the 250- and 150-µm sieves was washed for 1 min, and then placed into a 250-ml beaker in 100-ml water. Nematodes from two 2-ml aliquots were identified through a Wilovert inverted microscope. Population densities of all plant-parasitic nematodes were determined and adjusted to number of nematodes per 10-g fresh roots. The absolute frequency of occurrence was calculated as a percentage (number of samples containing a species or genus/number of samples collected).

Nematodes from soil were extracted by a modified Baermann funnel method (Hooper, 1986). Each sample was thoroughly mixed and two subsamples of 100 cm^3 each were used for the extraction, identification and quantification of the nematodes. The soil was placed in two aluminum dishes covered with a plastic mesh of 1-mm opening which supported a tissue paper, water was added until the soil was covered, and samples were incubated for 72 hr. The volume of water was reduced to 100 ml using a 25-µm sieve, and nematodes were identified by morphological characteristics and counted with an inverted microscope.

Aphelenchoides besseyi *extraction from seed*

Aphelenchoides besseyi were extracted from 100 seeds (5 replicates per plantation). The seeds were crushed with mortar and pestle, placed in a Petri dish containing 25-ml water, and incubated. At 2-hr intervals, nematodes were collected and counted and fresh water was placed onto the crushed seeds. Counts continued every 2 hr until there were no nematodes detected.

Table 3. Population density range and (mean) of plant-parasitic nematodes detected in rice in Ecuador.

Province and region	Nematodes per 10 g fresh root			
	Meloidogyne graminicola	*Hirschmanniella oryzae*	*Pratylenchus* spp.	*Helicotylenchus* spp.
Guayas:				
Simón Bolivar	6500-174730 (21592)	0-775 (107)	100-3460 (490)	0
El Triunfo	2400-47370 (15334)	0-175 (107)	0	0
Nobol	4500-83980 (12248)	50-2815 (884)	0	0
Taura	2600-43200 (10098)	0-775 (115)	0	0
Palestina	1500-26530 (9150)	0-725 (198)	0	0
General Vernaza	1300-48317 (8241)	0	0	50-4535 (977)
Naranjal	1800-38680 (7025)	0-320 (94)	0	0
Samborondón	850-11997 (4071)	0-145 (41)	0	0
Santa Lucía	1600-5882 (3682)	0-2412 (288)	0	0
Yaguachi	1200-16525 (3501)	0-250 (146)	0-140 (23)	0
Jujan	1250-8465 (1948)	0-50 (5)	0	0-50 (5)
Los Ríos:				
Babahoyo	3500-17500 (8927)	0	0	0
Montalvo	1600-19525 (3273)	0	1000-17500 (4418)	0
Mocache	2200-26570 (4878)	0	750-10277 (2731)	0
Palenque	3500-104750 (12392)	0	600-8287 (3552)	0
Quevedo	10000-125787 (44509)	0	1000-7595 (3417)	0
Valencia	3000-76552 (27129)	0	400-4470 (1561)	0
Vinces	10000-50850 (33507)	0	0	0
Manabí:				
Rocafuerte	50-1100 (59)	50-332 (76)	0	50-215 (12)
Sucre	400-16000 (2395)	50-1050 (187)	0	50-100 (27)
El Oro:				
Santa Rosa	500-2000 (1167)	50-200 (133)	0	0
Arenillas	1800-21050 (3681)	50-400 (121)	0	0
Loja:				
Macará	3599-71100 (27377)	50-150 (64)	0	0
Zapotillo	2000-21700 (5023)	50-300 (82)	0	0

RESULTS

Frequency of occurrence of plant-parasitic nematodes

In the 331 root samples analyzed, *M. graminicola* was the most abundant nematode in all provinces surveyed except Manabí, and was found in 89% of the samples that were collected (Table 1). In Manabi, *H. oryzae* was the most frequently detected nematode (71%). This nematode also was found in 72% of the samples in Guayas, 100% of the samples

collected in El Oro, and 64% of Loja samples. In the root samples from Los Ríos province, *Pratylenchus* spp. were found in 71% of the sites sampled. The most frequently detected plant-parasitic nematodes in soil samples were *Helicotylenchus*, *Criconemoides*, and *Tylenchorhynchus*.

Nematode population densities in root samples

The highest population densities of *M. graminicola* in roots were found in Simon Bolivar at the Guayas province, and Quevedo and Palenque at Los Ríos province with 21,592, 44,509, and 12,392 second-stage juveniles (J2) per 10 g roots (Table 3). *Hirschmanniella oryzae* was found in Nobol, Santa Lucía at Guayas province and Sucre Canton in Manabí province, with 884, 288, and 187 individuals per 10 g roots. *Pratylenchus* spp. were common in Los Ríos with population densities ranging from 1,561 to 4,418 per 10 g roots. Lesion nematodes were not detected in roots of samples from Manabí, El Oro, and Loja provinces.

Nematode population densities in soil samples

In the soil samples, the highest averages of *M. graminicola* population densities were found in Valencia, Babahoyo, and Quevedo in Los Ríos province (Table 4). The highest population density of *H. oryzae* was found in Simon Bolivar (1,357/100 cm^3 soil) followed by El Triunfo and Palestina in Guayas province, with 955 and 937 nematodes per 100 cm^3 soil, respectively. *Pratylenchus* spp. were found in Los Ríos province, and the highest populations were observed in Montalvo, Mocache, Palenque, Valencia, and Quevedo. The ectoparasitic nematodes *Helicotylenchus*, *Criconemoides*, and *Tylenchorhynchus* spp. also were identified in the Guayas province and the highest populations of *Helicotylenchus* were observed in General Vernaza, Jujan, and Palestina with 3,437, 1,885, and 1,655 nematodes per 100 cm^3 soil. In Manabí province this nematode was found in the Sucre canton with a population of 2,007/100 cm^3 soil. The highest average population density of *Criconemoides* spp. was found in General Vernaza at Guayas province, and the highest average *Tylenchorhynchus* spp. was observed in Samborondon in the same province.

Aphelenchoides besseyi *densities in seed samples*

In the 210 paddy panicle samples analyzed, the white tip nematode, *A. besseyi*, was found in 17% (Table 5). The nematode was detected in seed in all regions surveyed, with a relatively high incidence (87%). The highest population density was also found in El Oro with 8,800 *A. besseyi* per 100 seeds.

DISCUSSION

Results of this survey demonstrate that a diversity of nematode genera are present in rice plantations of Ecuador. The root-knot nematode, *M. graminicola*, was the most widespread and abundant nematode, occurring in both rainfed lowland and irrigated areas. This nematode has been reported to cause damage in rice plantations from the Philippines (Prot *et al*., 1994), Bangladesh, Nepal, Pakistan (Munir *et al*., 2002; Padgham *et al*., 2004; Pokharel *et al*., 2005), and Costa Rica (Soriano *et al*., 2000). In this study all the commercial rice sampled was infested by *M. graminicola*. Rice growers in Guayas province where *M. graminicola* was found in all the collected samples may have cause for concern because the population densities were generally very high. Most of the rice plantations in the region sow seed directly into the field and the farmers grow two to three continuous cycles, practices that could explain the high incidence and densities. Manabí province had a relatively low level of infestation by *M. graminicola*. In this area most of the farmers grow rice during the rainy season and vegetables or beans in dry season. *Hirschmanniella oryzae* was found only in irrigated plantations where its presence was common, possibly because it is favored by higher humidity and the practice of intercropping with poor hos ts.

The lesion nematode, *Pratylenchus* spp., in this survey was frequently found in rainfed lowland rice, especially in Los Ríos province where rice is usually intercropped with corn or soybean, both excellent hosts of the nematode. Some species of *Pratylenchus* are known to be pathogenic on rice, and in general, all species of *Pratylenchus* extracted from the roots, should be considered as potential constrains to crop yield (Lamberti *et al*., 1991; Medina *et al*., 2009a, 2009b). *Tylenchorhynchus*, *Helicotylenchus*, and *Criconemoides*, as well as *Pratylenchus* spp., are known to attack vegetables and other crops, which could explain their presence in many of these plantations. Identification of the species will be necessary before their pathogenicity and potential threat to rice can be estimated. *Aphelenchoides besseyi*, causal agent of the leaf white tip disease (Hoshino and Togashi, 1999; Montero and Salazar, 2006; Rajan and Lal, 2006; Yoshida *et al*., 2009; Kepenekci, 2013) was detected in all five provinces surveyed. This could have implications both in terms of yield and quality of the rice grain, and as a potential regulatory issue in marketing the crop.

Table 4. Population density range and (mean) of plant-parasitic nematodes detected per 100 cm^3 soil.

Province/Places	*Meloidogyne graminicola*	*Hirschmanniella oryzae*	*Pratylenchus* spp.	*Helicotylenchus* spp.	*Criconemoides* spp.	*Tylenchorhynchus* spp.
Guayas:						
Simón Bolivar	8500-13845 (1905)	50-1357 (159)	50-600 (209)	50-345 (35)	50-327 (92)	0-102 (10)
El Triunfo	250-2087 (1075)	100-955 (620)	0	0	0-42 (12)	0
Nobol	750-8520 (2158)	50-255 (132)	0	0	0	0-40 (5)
Taura	450-4000 (1154)	50-425 (97)	0	50-195 (54)	0-62 (14)	100-610 (138)
Palestina	1250-5945 (2803)	100-937 (177)	0	100-1655 (174)	0	0
General Vernaza	400-6492 (1100)	0	0	150-3437 (584)	250-3062 (407)	50-257 (107)
Naranjal	800-8705 (1910)	50-145 (52)	0	50-120 (45)	0	50-935 (138)
Samborondón	1000-4947 (1682)	245 (57)	0	257 (36)	0	1060 (283)
Santa Lucía	400-3212 (1183)	0- 82 (40)	0	0	0	0
Yaguachi	50-367 (85)	0-47 (25)	50-400 (65)	0	0	0
Jujan	50-655 (147)	0	0	50-1885 (337)	0-27 (8)	0-175 (48)
Los Ríos:						
Babahoyo	200-2625 (1113)	0	0	0	0	0
Montalvo	150-850 (295)	0	400-3250 (1239)	0	0	0
Mocache	4400-012 (796)	0	100-2290 (800)	0	0	0
Palenque	3450-677 (745)	0	300-1967 (895)	0	0	0
Quevedo	1000-1012 (757)	0	100-975 (389)	0	0	0
Valencia	7500-182120 (23812)	0	100-1250 (512)	0	0	0
Vinces	150-940 (477)	0	0	0	0	0
Manabí:						
Rocafuerte	0-100 (6)	128 (115)	0	0-112 (37)	0	0
Sucre	0-1100 (120)	353 (90)	0	0-2007 (107)	0	0
El Oro:						
Santa Rosa	0-300 (100)	0-100 (83)	0	0	0	0
Arenillas	50-1250 (360)	0-85 (25)	0	0	0	0
Loja:						
Macará	750-6300 (2895)	0-350 (45)	0	0	0	0
Zapotillo	100-3100 (873)	0-250 (73)	0	0	0	0

Table 5. Frequency of occurrence (percentage) and population density range and (mean) of *Aphelenchoides besseyi* detected in rice seed in plantation of Ecuador.

Provinces/Places	Number of fields sampled	Altitude of fields sampled (m.a.s.l)	% Frequency of occurrence	*Aphelenchoides besseyi* per 100 seeds
Guayas:				
Daule	10	11-20	0	0
Nobol	10	9 -21	30	2-113 (15)
Taura	10	1- 10	10	0-50 (5)
El Triunfo	10	29 - 67	10	1-63 (8)
Santa Lucía	10	7 - 18	30	5-91 (14)
Yaguachi	10	9-10	0	0
Colimes	10	13-26	0	0
Palestina	10	5-23	0	0
Los Ríos:				
Babahoyo	11	5-26	10	0-13 (3)
Ventanas	10	17-25	10	3-265 (17)
Puebloviejo	9	10-24	10	2-120 (7)
Urdaneta	9	13-20	10	0-27 (3)
Montalvo	10	17-22	20	8-276 (17)
Valencia	7	20-45	14	2-48 (5)
Quevedo	7	15-42	14	0-13 (3)
Manabí:				
Rocafuerte	14	2-35	17	11-324 (34)
Sucre	17	2-9	18	20-1469 (120)
El Oro:				
Santa Rosa	4	11-17	100	2420-8800 (5060)
Jumón	4	9-20	100	1000-3000 (2400)
Arenillas	13	2-24	62	1100-8400 (3340)
Loja:				
Macará	8	403-475	0	0
Zapotillo	7	225-242	29	20-650 (250)

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