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Distribution and Prevalence of Parasitic Nematodes of Cowpea (*Vigna unguiculata*) in Burkina Faso

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Abstract: A comprehensive survey of the plant parasitic nematodes associated with cowpea (*Vigna unguiculata*) production fields was carried out in the three primary agro-climatic zones of Burkina Faso in West Africa. Across the three zones, a total of 109 samples were collected from the farms of 32 villages to provide a representative coverage of the cowpea production areas. Samples of rhizosphere soil and samples of roots from actively growing cowpea plants were collected during mid- to late-season. Twelve plant-parasitic nematode genera were identified, of which six appeared to have significant parasitic potential on cowpea based on their frequency and abundance. These included *Helicotylenchus*, *Meloidogyne*, *Pratylenchus*, *Scutellonema*, *Telotylenchus*, and *Tylenchorhynchus*. *Criconemella* and *Rotylenchulus* also had significant levels of abundance and frequency, respectively. Of the primary genera, *Meloidogyne*, *Pratylenchus*, and *Scutellonema* contained species which are known or suspected to cause losses of cowpea yield in other parts of the world. According to the prevalence and distribution of these genera in Burkina Faso, their potential for damage to cowpea increased from the dry Sahelian semi-desert zone in the north (annual rainfall < 600 mm/year), through the north-central Soudanian zone (annual rainfall of 600-800 mm/year), to the wet Soudanian zone (annual rainfall ≥ 1000 mm) in the more humid south-western region of the country. This distribution trend was particularly apparent for the endoparasitic nematode *Meloidogyne* and the migratory endoparasite *Pratylenchus*.

Key words: Agro-climatic zones, Burkina Faso, cowpea, *Criconemella*, detection, *Helicotylenchus*, *Meloidogyne*, nematode survey, *Pratylenchus*, *Rotylenchulus*, *Scutellonema*, *Telotylenchus*, *Tylenchorhynchus*, *Vigna unguiculata*, West Africa.

Cowpea (*Vigna unguiculata* L. Walp.) is the primary source of plant proteins in the diet of rural populations of the Sahelian countries of sub-Saharan Africa. It is generally cultivated for the quality of its seeds which have a protein content of 22 to 33%, including a high content of lysine, an essential amino acid for human metabolism that is deficient in cereals (Quin, 1997; Steele, 1976). Cowpea also represents a monetary source of income for the farmers of West and Central Africa, where it is the most economically important indigenous African legume crop (Langyintuo et al., 2003). However, the yields are highly variable under the rain-fed, limited-resource production conditions employed. In Africa, the average cowpea grain yields vary from 50 to 550 kg per ha, typically being only about 10-20% of cowpea yield potential attainable under optimal growing conditions (Hall et al., 2003). West and Central Africa annually produce about 2.6 million tonnes of cowpea grain on 7.8 million hectares, accounting for about 69% of world production and 80% of the world's cowpea production area (Langyintuo et al., 2003). The principal cowpea producing countries in West Africa are Nigeria, Niger, Burkina Faso, Mali, Senegal, and Ghana. In addition to unpredictable drought conditions, which constitute a major constraint in these Sahelian zone rain-fed cropping areas, the production of cowpea is significantly limited by attacks from insects, diseases, and parasitic plants like *Striga* and *Alectra* (Singh, 2002).

Several species of nematodes are known to cause losses to cowpea throughout the world. Caveness and Ogunfowora (1985) listed 55 species of plant parasitic nematodes associated with cowpea production. The root-knot nematodes *Meloidogyne incognita* and *M. javanica* are documented to cause major losses, with *M. incognita* indicated to be the most detrimental species to cowpea (Sarmah and Sinha, 1995). An estimated 59% loss of cowpea grain yield caused by *M. incognita* was reported for Nigeria (Ogunfowora, 1976), while Olowe (1978) found a seedling mortality rate of up to 80% for cowpea grown in soil with a *M. incognita* population density of 1300 second-stage juveniles/L. A comprehensive survey of cowpea growing areas in Nigeria revealed root-knot nematodes present in 100% of the 248 farms included, with *M. incognita*, *M. javanica*, and *M. arenaria* present in 52%, 44%, and 4% of the samples, respectively (Olowe, 2004). Good sources of host resistance to root-knot nematodes are available in cowpea and have been bred into some cultivars (Ehlers et al., 2002). Field and greenhouse studies in Senegal by Sarr et al. (1989a,b) indicated that several nematodes may be implicated in suppression of cowpea grain and root weights, including a mixed population of *Pratylenchus sefaensis*, *Helicotylenchus dihystera*, *Hoplolaimus pararobustus*, *Scutellonema cavenessi*, and *Tylenchorhynchus* sp., representing nematodes found associated with fields used for cowpea production in rotation or mixed cropping with peanut, sorghum, and (or) millet (Sarr et al, 1989b). Field experiments with DBCP and metam-sodium indicated growth stimulation of cowpea in treated plots in soils containing *S. cavenessi*, *H. dihystera* and *Tylenchorhyncus* sp. in Senegal and also population increases on cowpea of *H. dihystera*, *H. pararobustus*,

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Sadly, Dr. Abdoussalam Sawadogo died unexpectedly while the manuscript was under review, and this work is dedicated to the memory of Burkina Faso's senior nematologist, a good colleague and friend.
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S. cavenessi, *T. gladiolatus*, and *Peltamigratus macbethi* (Sarr et al., 1989a). Large multiplication rates of *S. cavenessi* were found in non-treated plots in two cowpea field locations in Senegal, and a significant increase in yield was found in nematicide-treated plots at the same sites (Sarr et al., 1989a).

The extent to which cowpea growth and yield are impacted by nematodes in Burkina Faso is not known. Other studies have revealed the presence in Burkina Faso farmer fields of nematodes known or suspected to parasitize cowpea on crops such as peanut, sorghum, millet, and vegetables grown in cowpea rotations (A. Sawadogo, pers. comm.). However, their direct association with cowpea has not been assessed. This study reports on the findings of a comprehensive survey of nematodes in samples of rhizosphere soil and cowpea roots from cowpea production fields representing the main growing regions in Burkina Faso.

Materials and Methods

Collection of field samples: The survey was carried out through the three main agro-climatic zones of Burkina Faso described by Sivakumar and Gnoumou (1987) (Fig. 1). These zones include the Sahelian zone characterized by an average annual rainfall lower than 600 mm/year, the northern Soudanian center zone with an average rainfall of 600 to 800 mm/year, and the southern Soudanian humid zone with average annual rainfall of at least 1000 mm. The composite sample collected from each field consisted of 5 individual subsamples of soil and roots each taken with a small shovel from the cowpea root zone area of a separate plant in the top 25 cm of soil and placed in a plastic bag. A total of 109 composite samples were collected from 109 cowpea fields from 32 villages, to provide a representative survey across the country (Fig. 1).

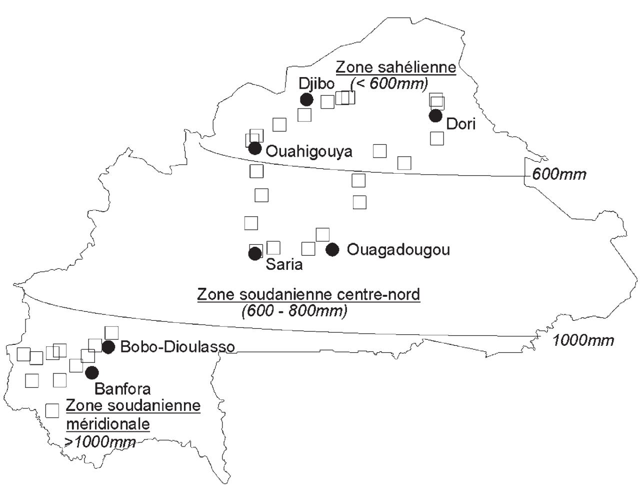

Fig. 1. Distribution of the survey sites for soil and root sample collection in cowpea fields within each of the three agro-climatic zones in Burkina Faso. Sample sites of the 32 villages are indicated by open squares, and townships by closed circles. Average annual rainfall totals for each zone are indicated in mm.

Nematode extraction: Nematodes were extracted from samples using two methods. The root and soil fractions of each sample were separated, and the soil fraction was mixed thoroughly before extraction. Nematodes in the soil fraction of the samples were extracted following the elutriation method of Seinhorst (1962) from a 250-cm^3 aliquant of each sample. Numbers of nematodes in the soil samples were converted to numbers of nematodes per liter of soil (N/L) of a given species or genus. Nematodes were extracted from the roots following the method of Seinhorst (1950) by cutting roots into 2-3-cm long pieces and holding them for 2 weeks in a mist chamber. The fresh roots were weighed, and the numbers of nematodes of a given species or genus in each sample were converted to numbers per gram of fresh roots (N/g).

Data analysis: The data were analyzed according to the prevalence of nematode populations based on two factors: frequency and abundance. The frequency (F) of the nematode genus or species was determined from the relationship between the number of samples (e) in which the nematode was observed divided by the total number of samples taken (E), multiplied by 100 to express as a percentage. The abundance (A) of the nematode genus or species was expressed as a decimal logarithm (loga x + 1) based on the average number of nematodes per liter of soil (N/L) or per gram of fresh roots (N/g). Abundance was calculated as the sum of N/L or N/g for all samples containing that nematode, divided by the number of positive samples for that nematode. Thresholds above which a nematode was considered as abundant or frequent were determined according to the limits established by Fortuner and Merny (1973), who applied these thresholds following criteria applied to survey data for nematodes associated with cultivated rice in Senegal and Gambia, West Africa. A nematode was regarded as abundant in roots if abundance $\geq$ 1.3 (20 individuals/g of roots). A nematode was regarded as abundant in soil if abundance $\geq$ 2.3 (200 individuals/L of soil). A nematode was regarded as frequent in the soil or the roots when it was observed in at least 30% of the samples.

Results

Nematode inventory: Plant-parasitic nematodes from 12 genera were found in soil and (or) cowpea root samples collected from cowpea production fields representing the main growing regions of Burkina Faso. These genera, represented by at least one species each, were *Meloidogyne, Pratylenchus, Scutellonema, Telotylenchus*, *Hoplolaimus*, *Helicotylenchus*, *Criconemella, Xiphinema, Rotylenchulus*, *Paratrichodorus, Paratylenchus*, and *Tylenchorhynchus*. At the species level, a limited number of specimens from several random samples revealed the presence of *Helicotylenchus dihystera*, *Scutellonema clathricaudatum*, *Pratylenchus sefaensis*, and *Rotylenchulus reniformis*. The species representing the other genera found in the survey samples were not identified.

Nematodes parasitizing cowpea: The potential for damage to cowpea caused by plant parasitic nematodes was evaluated according to the method of Fortuner and Merny (1973) based on nematode frequency and abundance. This classification made it possible to distinguish 4 groups of nematodes by sectioning plots of abundance by frequency into quadrants based on the threshold values (Fig. 2 - 5). The first group, within the upper right quadrant of each plot is comprised of nematodes with high prevalence based on abundance and frequency values both above the assigned thresholds. Six nematode genera met these criteria when analyzed with the complete national survey data from soil and root samples (Fig. 2 A, B), and included *Helicotylenchus* sp., *Meloidogyne* sp., *Pratylenchus* sp., *Scutellonema* sp., *Telotylenchus* sp., and *Tylenchorhynchus* sp. The first four genera were found in root samples as well as soil samples, and represent endoparasitic and migratory endoparasitic nematodes attacking cowpea. In soil samples, *Pratylenchus*, *Tylenchorhynchus*, *Scutellonema*, and *Helicotylenchus* were found at frequencies of 77%, 79%, 90% and 99%, respectively. In root samples, *Meloidogyne* and *Pratylenchus* were highly abundant whereas *Helicotylenchus* and *Scutellonema* were less abundant (Fig. 2B). The nematodes *Meloidogyne* sp. and *Rotylenchulus reniformis* observed in the upper left quadrant were abundant but not very frequent in soil samples (Fig. 2A). The 18% and 34% frequency of *Meloidogyne* sp. in soil and root samples, respectively (Fig. 2 A, B), reflected a strong zonal distribution of root-knot nematodes identified in the nationwide survey. By taking into account the high populations in the roots, *Meloidogyne* sp. was classified in the first group. *Rotylenchulus reniformis* is a semi-endoparasite but was not found in

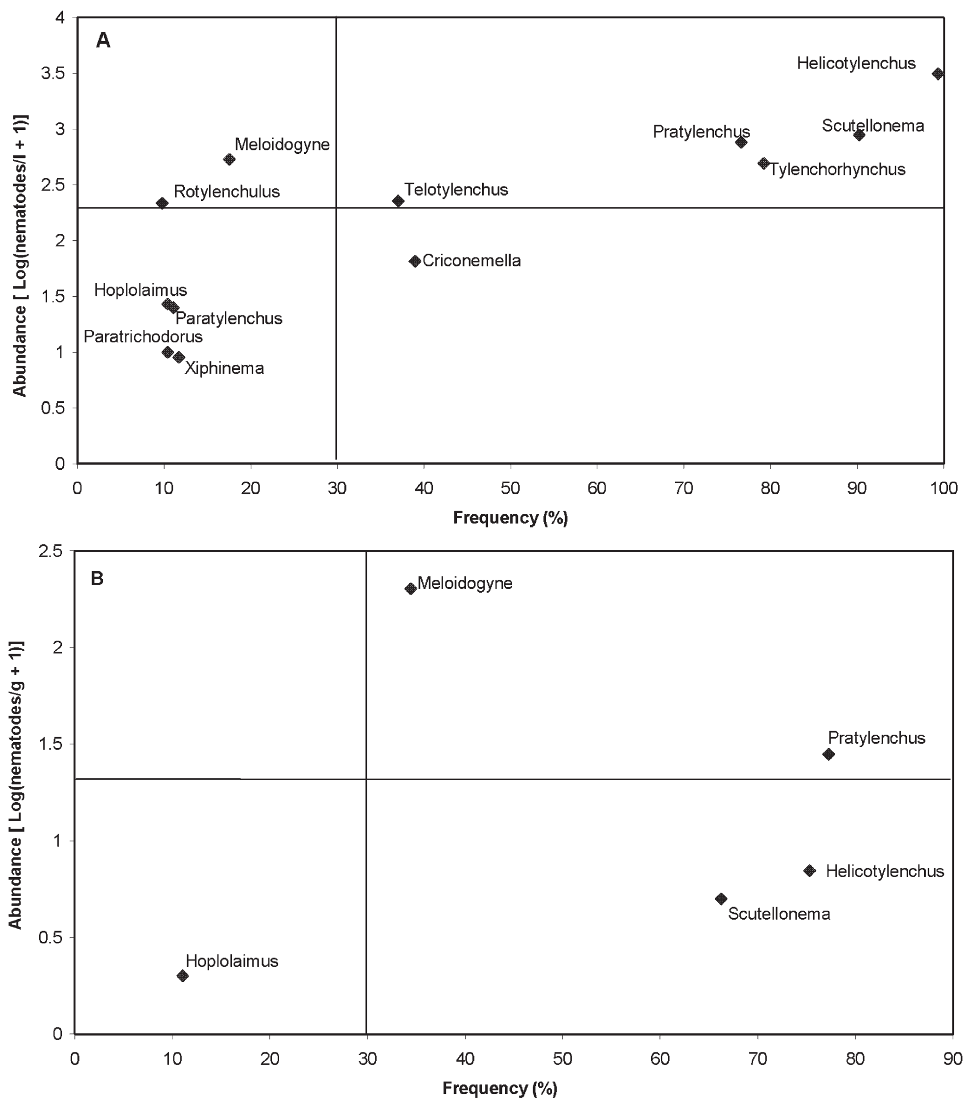

FIG. 2. Frequency (percentage of positive samples) and abundance (mean numbers per sample) of the plant-parasitic nematode genera associated with cowpea in soil (A) and root (B) samples in a national survey of Burkina Faso (overall mean of the three agro-climatic zones).

cowpea root samples. The relatively high abundance of *R. reniformis* in soil samples coupled with low frequency (10%) placed this species in the second group (upper left quadrant, Fig. 2A), and it can be regarded as an active parasite of cowpea but its development seems especially related to specific conditions of cowpea production and location. The ectoparasitic nematode *Criconemella* sp. was the only nematode in the lower right quadrant indicating a high frequency among cowpea fields but a relatively low abundance in soil samples (Fig. 2A). The nematode genera grouped in the lower left quadrant (Fig. 2 A, B) included species of *Hoplolaimus*, *Paratylenchus*, *Paratrichodorus*, and *Xiphinema*. These nematodes were not prevalent, being characterized by both low frequency and abundance in soil samples; among these, *Hoplolaimus* sp. was present in low numbers in a few root samples (Fig. 2B).

Nematode distribution according to agro-climatic zone: In the dry semi-desert Sahelian zone, all 12 genera found in the national survey were also found in cowpea fields in this region (Fig. 3 A, B). Four of the six most prevalent genera observed at the national level were found at high frequency and abundance, including *Helicotylenchus*, *Pratylenchus*, *Scutellonema* and *Tylenchorhynchus* (Fig. 3 A, B). Root-knot nematodes (*Meloidogyne* sp.) were found in very low frequency and abundance in soil and root samples in this zone. *Telotylenchus* also had a low prevalence in soil in this arid zone (Fig. 3A).

The prevalence of nematode genera in the Soudanian north-center agro-climatic zone was overall similar to that of the semi-desert Sahelian zone for both soil and root sample data (Fig. 4 A, B). However, there was an increase in both the frequency and especially the

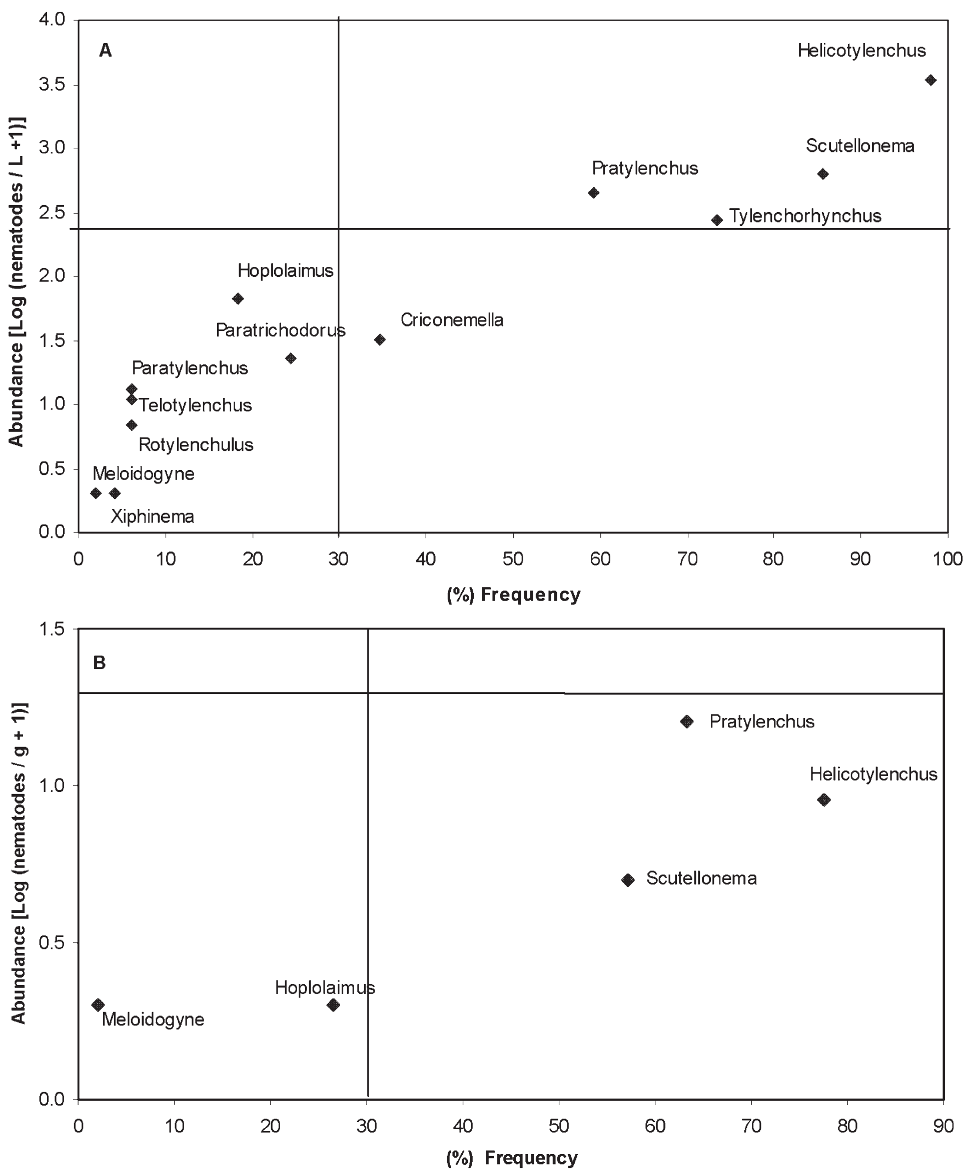

Fig. 3. Frequency (percentage of positive samples) and abundance (mean numbers per sample) of the plant-parasitic nematode genera associated with cowpea in soil (A) and roots (B) of the northern semi-desert Sahelian zone of Burkina Faso.

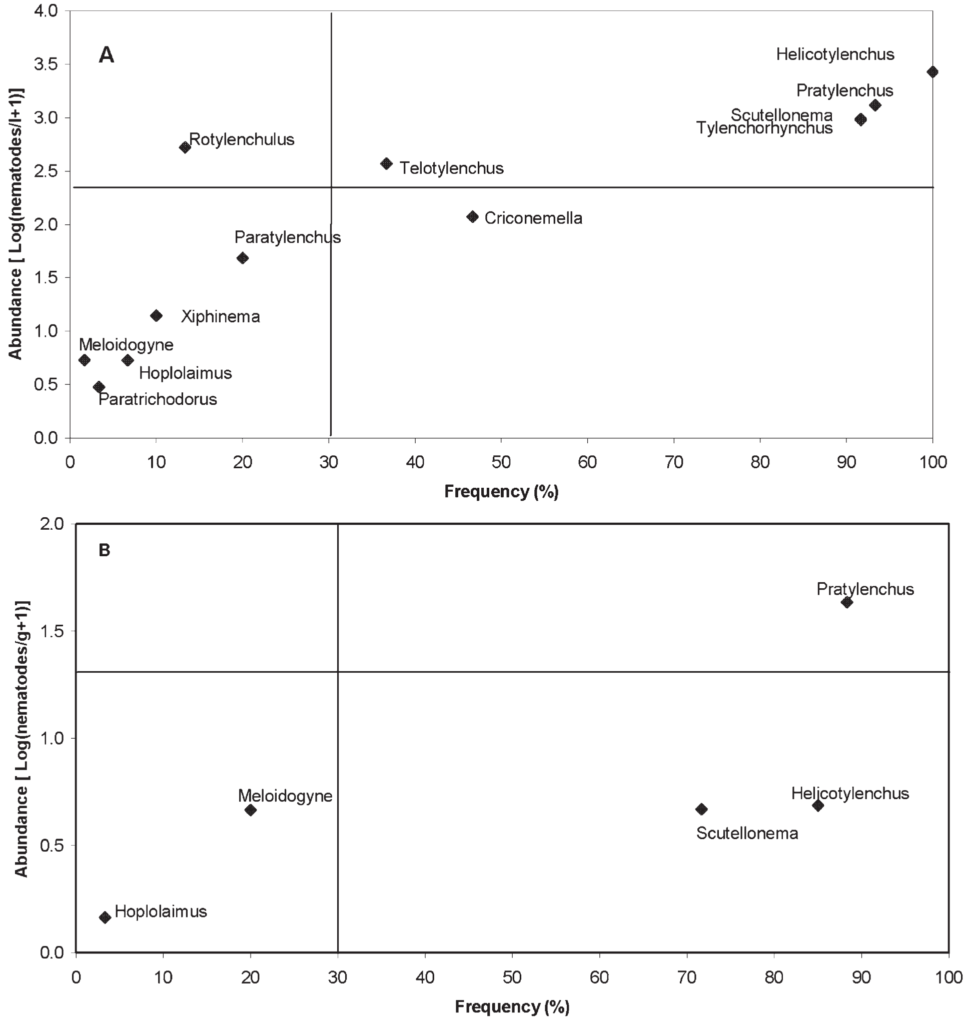

FIG. 4. Frequency (percentage of positive samples) and abundance (mean numbers per sample) of the plant-parasitic nematode genera associated with cowpea in soil (A) and roots (B) in the north-central Soudanian zone of Burkina Faso.

abundance of *Telotylenchus* in this zone and also an increased abundance of *R. reniformis* in soil samples compared to the northern Sahel region (Fig. 4A). *Pratylenchus* sp. appeared as the major migratory endoparasite in this zone based on root sample data (Fig. 4B). The frequency and abundance of *Meloidogyne* sp. in root and soil samples remained relatively low (lower left quadrant, Fig. 4A and 4B), however, the values in both soil and root samples reflected an increase in prevalence compared to that in the northern Sahel (Fig. 4 A, B).

In the southern-most humid south-Soudanian zone of the country, all 12 genera found in the national survey were represented (Fig. 5 A, B). A significant increase in the frequency (56% of soil and 89% of root samples) and the abundance of root-knot nematodes (*Meloidogyne* sp.) in both soil and root samples compared to the other agro-climatic zones established it as the most prevalent nematode in cowpea fields in this region. *Meloidogyne* sp. replaced *Pratylenchus* sp. as the major endoparasite of cowpea in this zone, although *Pratylenchus*, *Helicotylenchus*, and *Scutellonema* spcies were still frequent and abundant in soil samples (Fig. 5A) and frequent but less abundant in root samples (Fig. 5B) in this humid zone.

DISCUSSION

The survey results identified twelve plant parasitic nematode genera associated with cowpea production fields across Burkina Faso. The twelve genera were widely distributed, being found in each of the three major agro-climatic zones in which cowpea is produced, and as such they can be expected to be encountered in any cowpea field across the country. Beyond the broad distribution of these nematode genera, their relative prevalence indicated distinct rankings of potential importance as cowpea parasites, and also distinct effects of agro-climatic conditions which influenced their relative prevalence and potential pathogen status between growing regions.

Considering the frequency and abundance estimates of these nematodes in soil and cowpea root samples at

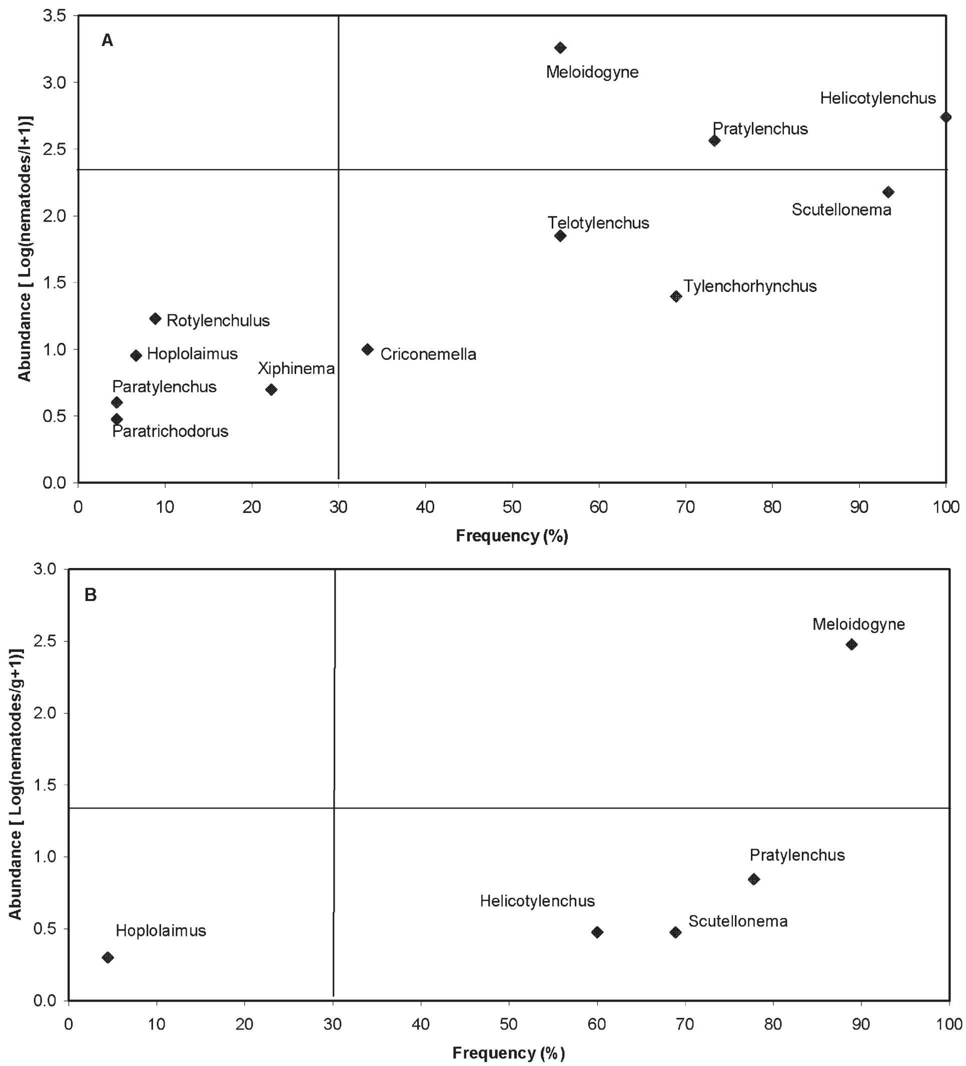

FIG. 5. Frequency (percentage of positive samples) and abundance (mean numbers per sample) of the plant-parasitic nematode genera associated with cowpea in soil (A) and roots (B) of the humid south-Soudanian zone of Burkina Faso.

a nationwide level, we conclude that six genera of nematodes appear to have a high parasitic potential on cowpea. Of these, *Meloidogyne, Pratylenchus*, *Scutellonema*, and *Helicotylenchus* are endoparasites that were found in cowpea roots, and *Tylenchorhynchus* and *Telotylenchus* are ectoparasites that were found to be highly prevalent in cowpea rhizosphere soil. These nematodes were distinguished from the other genera by their active development on cowpea and are the most likely to cause or contribute to yield losses on cultivated cowpea. With the exception of *Telotylenchus*, species of these genera have been demonstrated to cause damage to cowpea or have been implicated in causing damage to cowpea through field or greenhouse studies (Baujard & Martiny, 1995a,b,c,d; Sarr et al, 1989a,b; Ehlers et al., 2002; Hall et al., 2003). We found *Helicotylenchus dihystera* in the survey samples, and this species is common in West Africa, particularly in the cowpea cropping systems that include peanut, sorghum, and millet. Baujard & Martiny (1995e) working with Senegal populations of *H. dihystera* found them to have high populations and multiplication rates on cowpea and millet and injury to millet and peanut, but no assessment of effects on cowpea growth were made.

The lesion nematode *Pratylenchus sefaensis* found in the survey has been reported previously in West Africa from cowpea rotations in Nigeria (Egunjobi et al., 1986) and Senegal (Sarr et al., 1989b). This nematode was shown to interact with root-knot and reniform nematodes in mixed infections on cowpea and maize in Nigeria, where its effects on cowpea were considered to be minor (Egunjobi et al., 1986). In mixed inoculations with *P. sefaensis* as part of a typical Senegal field nematode community including *Hoplolaimus*, *Helicotylenchus*, *Scutellonema*, and *Tylenchorhynchus*, decrease in cowpea growth was reported, although tests with three common varieties of cowpea from Senegal indicated that cowpea was a poor host for *P. sefaensis* (Sarr & Baujard, 1988). While these other studies suggested that *P. sefaensis* is likely to be a minor nematode parasite of cowpea, our survey data indicated this lesion nematode species was highly prevalent in cowpea fields in Burkina Faso, occurring at both high frequency and abundance in soil and root samples.

The high prevalence of *Scutellonema clathricaudatum* in soil samples in each of the cowpea production regions and also its high frequency and moderate abundance in cowpea roots indicated that this nematode is a prominent cowpea parasite. Previous work on this nematode in West Africa by Baujard & Martiny (1995c) revealed that it replaces *S. cavenessi* as the primary species of this genus to the east of Senegal, predominating in Mali, Niger, and Burkina Faso, where it has adapted to both semi-arid and rainy tropical regions. *Scutellonema clathricaudatum* was shown to have a high multiplication rate on cowpea, and also on millet and sorghum grown in cowpea rotations (Baujard & Martiny, 1995c). Effects of this species on cowpea growth have not been reported, but it should be considered to be parasitic on cowpea based on its prevalence and root infection identified in the current survey, and more research is warranted to assess its damage potential on cowpea.

The high prevalence of *Meloidogyne* in samples from the humid south-Soudanian region of Burkina Faso indicated that root-knot nematodes are a significant problem in cowpea fields. In this region we observed significant symptoms of root galling in many fields. Among five species of root-knot nematode identified on vegetable fields in Burkina Faso (A. Sawadogo, unpublished results), two species, *M. incognita* (63%) and *M. javanica* (27%), were the most prevalent in the country. These common root-knot species are likely to have been the primary species found in the survey samples, although species identification for *Meloidogyne* was not conducted in the current study. Both species are known to be serious pathogens of cowpea in many cowpea growing areas (Ehlers et al. 2002; Gallaher & MacSorley, 1993; Hall et al., 2003; Olowe, 2004). Effective resistance to root-knot nematodes is available in cowpea (Ehlers et al. 2002; Hall et al., 2003), and trials to breed resistance into varieties adapted for Burkina Faso and other areas of West Africa are underway. The survey data showed a clear trend of increasing prevalence of *Meloidogyne* with increases in the rainfall, soil moisture and humidity profiles of the agro-climatic zones. In the dry zone in the northern region of the country root-knot nematode occurred in less than 5% of samples, whereas in the humid southwestern region at least 90% of cowpea fields were infested. The very hot and long (8 months) dry season in the semi-desert northern part of the country could explain the low frequency of root-knot nematode in this area. A more urgent need to develop adequate root-knot nematode management strategies for protection of cowpea, including the breeding of adapted resistant varieties, is apparent for the wetter regions. The regional distribution pattern for root-knot nematode in the Burkina Faso survey data contrasted with the situation in Nigeria in which all 248 cowpea fields sampled in a national survey were positive for *Meloidogyne*, with *M. incognita* and *M. javanica* being the primary species present (Olowe, 2004). The northern part of Nigeria is not as dry as the northern region sampled in Burkina Faso, which may in part explain the ubiquitous nature of the distribution across Nigeria.

The reniform nematode *R. reniformis* is known to attack and cause growth reduction of cowpea (Yassin & Ismail, 1994), the host from which it was first described (Linford and Oliveira, 1940), and was shown to have high multiplication rates on cowpea in Senegal, where it was found restricted to fields under irrigation even though it enters anhydrobiosis during the dry season (Baujard & Martiny, 1995f). However, its status as a pathogen of cowpea in Burkina Faso is difficult to gauge, because our survey data revealed a significant abundance of the nematode in soil samples, but no detection in cowpea root samples and a relatively low frequency among samples. It is likely that more extensive sampling of fields with a high abundance of reniform nematode would reveal parasitism of cowpea varieties.

Criconemella sp. was the only nematode with a high frequency among cowpea fields but a relatively low abundance in soil samples, suggesting that it is a parasite of cowpea but not that significant under the conditions of cowpea production of Burkina Faso. Of the remaining nematodes found in the survey, species of *Hoplolaimus*, *Paratylenchus*, *Paratrichodorus*, and *Xiphinema* were not prevalent. Their low abundance suggests that they are not significant parasites of cowpea and probably cause little or no direct damage to cowpea crops in this region of Africa, as also suggested by results with *H. pararobustus* in cowpea field tests in Senegal (Baujard & Martiny, 1995d).

In conclusion, the results of this survey revealed the significant prevalence of a group of seven genera of nematodes in cowpea production areas of Burkina Faso. Species of *Meloidogyne, Pratylenchus, Scutellonema, Helicotylenchus, Tylenchorhynchus, Telotylenchus* and *Criconemella* were found in soil and root samples. The first three of these genera are known to contain species causing yield losses to cowpea throughout the world. Their distribution according to the agro-climatic zone indicated that the pressure that these parasites exert increases passing from the northern Sahelian zone to the southern humid zone of the country. Although in the case of *Meloidogyne* the severe pathogenicity to cowpea is well documented and management options including host resistance are available, the pathogenicity of the other nematode species found in this survey parasitizing cowpea needs to be determined in order to prioritize and guide development of management strategies.

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