RESEARCH/INVESTIGACIÓN

NEMATODES ASSOCIATED WITH INVASIVE SPOTTED KNAPWEED

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ABSTRACT

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Freedom from plant-parasitic nematodes associated with the native ranges of plants might contribute to plant invasions in new areas. However, nematode damage surveys and diversity inventories are largely limited to crop plants. No invasive plant has ever been compared in its native and invaded ranges for damage due to plant-parasitic nematodes. The objective of this study was to determine nematode taxa and trophic groups associated with spotted knapweed (*Centaurea stoebe*) in its invaded range in western North America. Three sites invaded by spotted knapweed were selected near Moscow, Idaho, USA. Soil samples, roots, and shoot systems of *C. stoebe* were collected in June 2012 at the young-rosette stage, in July 2012 at flowering, and in August 2012 at bolting and nematodes extracted. Sixteen nematode taxa were identified from soil samples. Bacterial-feeding and omnivorous predator nematodes were the most dominant groups, whereas plant-parasitic nematodes were found to have the lowest population levels. *Aphelenchoides*, *Aphelenchus*, *Ditylenchus,* and *Tylenchus* were characterized as fungal-feeding nematodes. *Aphelenchus* (400 individuals/100 g of soil) was the most prevalent fungivorous genus found in the bulk soil, followed by *Aphelenchoides* (120 nematodes/100 g of soil). Even though almost all nematode trophic groups coexisted with spotted knapweed in both the rhizosphere and in the bulk soil, no nematodes were obtained from root or leaf samples. *Centaurea stoebe* could be in a state of nematode release in western North America relative to its native range in Eurasia. The next step in the native range is to see if the plant-parasitic nematodes known to occur in Eurasia with *C. stoebe* actually damage or in some way limit the plant.

*Key word*s: *Centaurea stoebe,* nematodes, rhizosphere

RESUMEN

García-De la Cruz, R., G. R. Knudsen, L.-M. C. Dandurand, L. K. Carta, and G. Newcombe. 2019. Los nematodos asociados con knapweed manchado invasor. Nematropica 49:200-207

Liberación de nematodos fitopatogenos en su rango nativo podría contribuir a la invasión de plantas. Sin embargo, en la literatura los daños y la diversidad de nematodos están limitadas hacia cultivos. Hasta ahora en nuestro conocimiento no se han comparado ninguna especie de planta en su hábitat natural y su rango invasivo en cuanto a daños provocados por nematodos fitoparásitos. El objetivo del estudio fue tomar el primer paso para probar la hipótesis de liberación de nematodos mediante el aislamiento, identificación y determinación de los grupos tróficos de nematodos asociados a *Centaurea stoebe*, en el oeste de Norte América. Se seleccionaron tres sitios invadidos por *C. stoebe*, cerca de Moscow Idaho, USA. Se tomaron muestras de suelo, raíces y hojas de *C. stoebe*, en el verano de 2012, en las siguientes épocas: Junio (plantas jovenes-rosetas), Julio (floracion) y Agosto (llenado de semilla). Se determinaron 16 taxas de nematodos. Los nematodos bacterivoros y onmivoros fueron los grupos más dominantes y los fitoparasitos fueron los menos abundantes. Los generos de nematodos fungivoros mas predominantes fueron, *Aphelenchus, Aphelenchoides, Ditylenchus* y *Tylenchus*. El genero mas dominante de los nematodos fungivoros fue *Aphelenchus* (con hasta 400 individuos/100 g de suelo), seguido por *Aphelenchoides* (120 individuos/100 g de suelo). Aun cuando se detectó la coexistencia de todos los grupos tróficos de nematodos asociados a *C. stoebe,* no se detectó ningún nematodo de raíz y hojas. Estos resultados sugieren que *C. stoebe* podría estar en un estado de liberación de nematodos fitoparasitos, relativo a su habitad de origen en Eurasia. El siguiente paso, en su hábitat nativo de *C. stoebe*, seria ver si existen nematodos fitoparasitos que en alguna forma ocasionen un daño y que puedan limitar su crecimiento.

Palabras clave: *Centaurea stoebe*, nematodos, rizosfera

INTRODUCTION

Centaurea stoebe L. ssp. *micranthos* (Gugler) Hayek (spotted knapweed), native to southeastern Europe, is now found in various locations around the world, from North America to New Zealand, and in Europe from western France to northwestern Kazakhstan and southwestern Siberia (Oschman, 2001). The invasion of spotted knapweed in North America began in the late 1890s, and it has since infested millions of hectares, especially in the northwestern part of the continent (Sheley *et al*., 1998). The economic effects of this invasion are approximately $150 million annually (Pimentel *et al*., 2000). Currently, exotic spotted knapweed threatens managed and natural ecosystems in the Pacific Northwest (Sheley *et al*., 1998).

In natural systems, invasions of this noxious weed since 1980 have altered ecosystem function through changes in native biodiversity and trophic structure, ecosystem processes, and both biotic and abiotic soil disturbance regimens (Tyser and Key, 1988). Some theories explain the robust success of this invasive plant in terms of plant-soil biota positive feedback (Callaway *et al*., 2004), including release from enemies, enhanced mutualism by mycorrhiza or endophytes (Klironomos, 2002; Newcombe *et al*., 2009), or novel weapons such as allelochemical toxins (Callaway and Ridenour, 2004). Enemy release is integrated into many of the other hypotheses of invasiveness.

Enemy release is usually thought of as the absence in the invaded range of arthropods and pathogens that limit the plant in its native range. Plant-parasitic nematodes, thus far, have not been included in studies of enemy release. Plant-parasitic nematodes are associated with *C. stoebe* in its native range in Europe (Geisen *et al*., 2018) but damage due to those nematodes was not assessed. No nematode damage surveys or diversity inventories have been conducted in the invaded range of *C. stoebe* in the Pacific Northwest of North America.

For inventories, nematologists have classified the roles of nematodes in the soil food web according to the mode of nematode feeding in different trophic groups, such as fungivorous, bacterivorous, omnivorous, predatory, or plant-parasitic (Bongers and Bongers, 1998; Bongers, 1999; Bongers and Ferris, 1999; Yeates *et al*., 1993; Yeates, 1999). However, some nematodes are active in different ways within each trophic food network. Nematodes can be grouped functionally if they respond similarly to different ecosystem function (e.g. food web enrichment, environmental perturbation, succession, etc.) (Neher, 2001; Neher, 2010; Yeates *et al*., 1993; Yeates *et al*., 1999). Many species of nematodes are plant-parasitic, causing plant diseases in important crops. However, some nematodes are targets of biocontrol, whereas others are predators of plant-parasitic nematodes or beneficial nematodes.

Generally, nematode density in soil is about 50 million/m^2 in the upper soil surface (10 cm). About 15, 000 nematode species have conservatively been described so far. Typically, 3,000 nematodes can be isolated from 100 g of soil (Yeates *et al.*, 1993; Yeates *et al*., 1999). To test the hypothesis of nematode enemy release, a nematode inventory was performed with samples from plant tissue, both above and below ground, and of both rhizosphere and bulk soil.

MATERIALS AND METHODS

Three sites were selected to assess various environmental conditions under which spotted knapweed thrives. Site 1 was located in Bovill, ID (46º 51' 36'' N, 116º 23' 39' W) elevation 2,874 m. Site 2 was in Deary, ID (46º 79' 77'' N, 116º 55' 90'' W) at the same elevation and Site 3 was at Moscow Mountain, ID (46º 47' 45.90'' N, 116º 54' 39'' W) at an elevation of 4,983 m. A common observable feature in all these sites was the long-term presence of spotted knapweed. The approximate size of each site was 1 km^2.

Soil and spotted knapweed plant samples were collected in Summer 2012 three times from June through August. Five soil and knapweed samples were taken from each location. Soil samples were collected using a trowel to a depth of 30 cm. Roots with adhering rhizosphere soil were separated from bulk soil by sifting (mesh size 20 mm) and removing the roots with tweezers. Soil samples were bulked at each site and mixed by hand with 1 kg per soil sample. Composite soil and rhizosphere soil were stored on ice in coolers until they arrived at the laboratory. Next, the samples were placed into a cool room until processing. Then, three subsamples of soil were used for both nematode extraction and soil serial dilution. Ten-g soil samples collected at each site were dried in an oven at 100°C for 24 hr to determine soil moisture.

Nematodes were extracted from 100 g soil subsamples using the Baermann extraction method for 48 hr, and nematodes were then counted at 40× magnification using a dissecting microscope. Nematodes were morphologically characterized and then separated to allow identification. Samples of nematodes were sent to the USDA-ARS Nematology Laboratory for identification to genus level. Nematodes were classified into trophic groups following Yeates *et al.* (1993) as bacterial- or fungal-feeding (BF, FF respectively), plant parasites (PP), and omnivores/predators (OP, nematodes feeding at several trophic levels). A *Ditylenchus* (Tylenchida) with unconfirmed feeding habits (plant-parasitic or fungal-feeding) was considered a fungal-feeding. Leaves and roots of spotted knapweed also were used for nematode extraction using a technique described by Jagdale and Grewal (2006). The characteristics of the nematode communities were described as: i) total number of nematodes (per 100 g dry soil), ii) trophic group structure: bacterial-feeding (BF), fungal-feeding (FF), plant-parasitic (PP) and omnivores-predators (OP), and iii) ecological indices: F/B=FF/BF and trophic diversity. Nematode diversity was measured with the Shannon diversity index ($H' = -\sum Pi$ (ln Pi), where Pi is the proportion of the genus ni in the total nematode community (Shannon, 1948).

RESULTS AND DISCUSSION

No nematodes were recovered from the spotted knapweed roots and leaves. Nematodes were only recovered from the soil samples. A total of 16 nematode taxa were identified to genus. The mean numbers of nematodes found in both bulk and rhizosphere soil samples combined ranged from 1 to 44 individuals/100 g dry soil. Bacterial-feeding nematodes were the dominant trophic groups at all sites. In general, OP and BF followed an increasing trend as described for the total number of nematodes. The OP population increased from June to July fivefold and then decreased by August (Fig. 1). *Microdorylaimus* was the only genus found in all sites in this category. The number was similar at Site 1 and Site 2. However, FF and BF were generally slightly more abundant at Site 3 (Fig.1). *Aphelenchus*, *Aphelenchoides*, *Ektaphelenchoides*, *Tylenchus,* and *Ditylenchus* were grouped as FF feeders. The mean numbers of FF found in the bulk soil samples ranged from 40 to 151 individuals/100 g dry soil (Fig. 1). The most dominant was *Aphelenchus* (50%), followed by *Aphelenchoides* (30%) in all sample times. The population of FF increased by about 80% from June to July, and then decreased to 40% by August (Fig. 2). The same pattern was observed for BF nematodes. The most predominant genus of BF was *Acrobeloides* (about

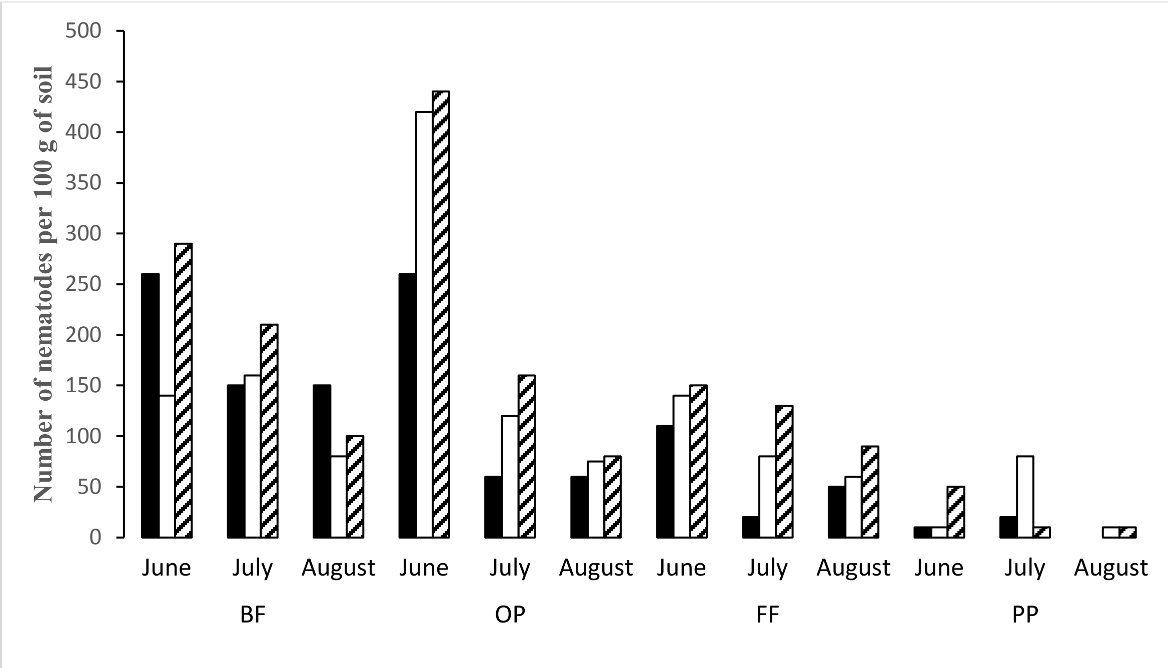

Figure 1. Nematode trophic groups isolated from bulk and rhizosphere soil in three sites infested with spotted knapweed. Sampling were performed on June (rosette stage), July (flowering stage), and August 15, 2012 (bolting stage). Bovill, ID (■), Deary, ID (□), Moscow Mountain, ID (▨).

35% of total samples), followed by *Panagrolaimus* (25%), *Eucephalobus* (15%), *Wilsonema* (15%), and *Protorhabditis* (10%) in all locations. We also found *Plectus, Prismatolaimus,* and *Ethmolaimus* classified as BF (Yeates, 1993). While PP were present in very small numbers, they were observed in all samples (10 individuals/100 g soil). The plant-parasitic nematodes were identified as *Paratylenchus* and *Tylenchorhynchus*. Means of the Shannon diversity index of these nematode genera were 2.21, 2.73, and 2.93 for Sites 1, 2, and 3, respectively. All five nematode trophic groups were present in Site 3. Besides greater diversity, Site 3 had greater numbers of nematodes than the two lower elevation sites.

This is the first report of plant-parasitic nematode *Paratylenchus* consistently present in association with *C. stoebe*. *Paratylenchus* was also common in Swiss alpine grasslands at similar elevations to the first two sites in this study (Kergunteuil *et al.,* 2016). Absent from these sites was the northern root-knot nematode, *Meloidogyne hapla,* which has been reported to affect *C. repens* but not *C. stoebe* (Faulkner and McElroy, 1964).

Dorylaimid nematodes, including *Microdorylaimus,* were the most abundant nematode trophic group across all the sites and times sampled. Opportunistic nematodes, such as *Acrobeloides* (BF), were also highly abundant. As soil dehydrated with each successive sampling some species may have declined due to drier conditions. Populations of fungal-feeding nematodes and predators coincidentally decreased from June to August when moisture was more limited. However, other parameters besides soil moisture could also account for this decrease. The FF trophic group was present in all sites across the three sample times. Fungal-feeding nematodes increased in July, just as spotted knapweed was at the flowering stage.

Aphelenchus, *Aphelenchoides,* and *Ditylenchus* have been reported to be cultivated on diverse fungi (Bae and Knudsen, 2001; Hasna*, et al*., 2007; Hasna *et al*., 2008; Jun and Kim, 2004; Ruess *et al*., 2005). However, some FF species have also evolved to be important plant pathogens such as *Ditylenchus dipsaci*, *Aphelenchoides*

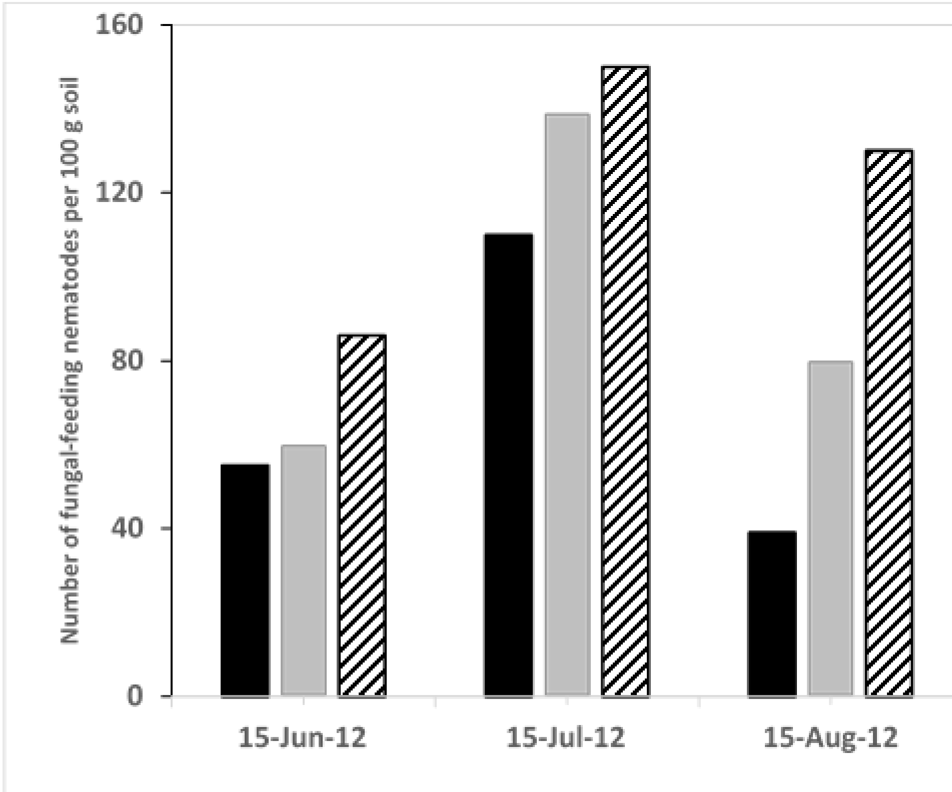

Figure 2. Populations of fungal-feeding nematodes isolated from sites infested with spotted knapweed in Bovill, ID (■), Deary, ID (□), and Moscow Mountain, ID (▨).

ritzemabosi, and *A. fragariae* (Jagdale and Grewal, 2006). Bacterial-feeding nematode populations can also decrease, and FF nematode populations can increase under acidification or heavy metal-induced stress conditions such as the disruption from invasive species (Bongers and Bongers 1998). Previous studies about the relationship of soil biota and invasive plant species have shown that non-indigenous plant species receive a positive benefit from soil biota feedback by increasing their composition and structure (Kliromonos, 2002; van der Stoel *et al.,* 2002; Reinhart and Callaway, 2006).

This study suggests that spotted knapweed has not yet influenced the development of free-living nematode communities in these three geographical locations, in terms of abundance and diversity, and across nearly all nematode trophic levels. However, there is a relatively low abundance of plant-parasitic nematodes (PP) across all sites. If *C. stoebe* has truly been released in its invaded range from the damaging nematodes of its native range, then we should find no nematodes likely to damage the plant in our sampling of the invaded range. This study shows that expected result, but it should now be followed up with a study of the native range in which we would expect to find damaging nematodes.

The diversity of nematode genera changed with time, but one particular type of fungal-feeding nematode, *Aphelenchus avenae*, was always most numerous. *Aphelenchus avenae* was capable of reducing disease caused by *Pythium* and *Pyrenochaeta* (Barnes *et al*., 1981) All of these fungal-feeding nematodes have the potential to decrease the effectiveness of applied biocontrol fungi, and represent targets for testing with experimental formulations of biocontrol organisms (Garcia *et al*., 2016).

Further research is needed to investigate the role of the trophic groups of nematodes, especially FF nematodes, and fungi interaction *in planta*. We also reported previously that this would enhance our understanding of how spotted knapweed communities are assembled, and how these relationships may affect the potential efficacy of some potential plant pathogens such as *Sclerotinia sclerotiorum* (Garcia *et al*., 2016) in the biological control of invasive knapweeds. More effective strategies for integrated weed management can be developed by applying ecological concepts related to nematode coexistence with other soil fauna such as fungi, bacteria, other microorganisms, as well as plants.

"The enemy release" hypothesis is a major hypothesis in invasion ecology and posits that the absence of enemies in the exotic range of an alien species is a cause of its invasion success" (Heger and Jeschke, 2014). The results of this study from three separate sites are consistent with this hypothesis because of the absence of nematodes in plant tissue and the greater abundance of nematodes in bulk soil than rhizosphere soil. Our results show low abundance of plant-parasitic nematodes compared to free-living nematodes. This may contribute to spotted knapweed proliferation. Conversely, Beckstead and Parker (2003) found negative effects of soil-borne pathogens, particularly nematodes and fungi, on germination and early growth of invasive marram grass (*Ammophila arenaria)*. That *C. stoebe* might be limited by nematodes in its native range is suggested by a recent study (Geisen *et al*., 2018).

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