DIEGO ARRUDA HUGGINS DE SÁ LEITÃO

MIGRAÇÃO ASCENDENTE DE *Meloidogyne* spp. EM FUNÇÃO DA TEMPERATURA E ESTÍMULO VEGETAL

Recife – PE

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Tese apresentada ao Programa de Pós-Graduação em Engenharia Agrícola na Área de Concentração em Engenharia de Água e Solo, da Universidade Federal Rural de Pernambuco (UFRPE), como requisito para obtenção do título de Doutor em Engenharia Agrícola.

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Migração ascendente de *Meloidogyne* spp. em função da temperatura e estímulo vegetal

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Recife - PE
Fevereiro, 2019

“A man who fears battle wins no victories”

- **A Storm of Swords**

George R. R. Martin

Aos meus pais, **Christiane Arruda Vasconcelos e Bruno Huggins de Sá Leitão**, aos meus irmãos **Danielle, Giovanni e Júlia,** ao meu sobrinho **Vinícius**, pelo suporte, amor e conselhos durante a minha vida e trajetória na pós-graduação.

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Resumo Geral

Leitão, D. A. H. S., 2019. Migração ascendente de *Meloidogyne* spp. em função da temperatura e estímulo vegetal. Tese de doutorado, Universidade Federal Rural de Pernambuco, Recife, PE, Brasil.

Os nematoides das galhas, *Meloidogyne* spp., são a principal ameaça à agricultura mundial, deste modo compreender o comportamento dos nematoides durante o ciclo de vida é crucial para um manejo apropriado. A migração de juvenis de segundo estádio (J2s) entre os poros do solo é mediada pela condições edáficas e exsudatos radiculares, influenciando a taxa de sucesso de infecção. A presente tese de doutorado abordou o efeito da temperatura e do estímulo vegetal na migração ascendente de J2s de *M. incognita* e *M. floridensis* ao longo do tempo. Colunas de solo foram contruídas utilizando-se três aneis de PVC (4 x 4,4 d cm) em cima de um anel de 2 x 4,4 d cm, o qual foi utilizado como anel de inoculação. As colunas foram preenchidas com solo arenoso e mantidas a 10% de umidade e 1,2 Kg dm^{-3} de densidade do solo. Copos de isopor sem o fundo contendo aproximadamente 300 g do mesmo solo e uma planta de tomate ou de *Tagetes patula* foi colocado no topo da coluna; copos sem plantas foram usados como controle. O Capítulo 1 aborda a revisão de literatura acerca *Meloidogyne* spp. e os fatores que contribuem ou dificultam a migração de J2s na matriz do solo. O Capítulo 2 aborda a migração de J2s das duas espécies sob tomate (*Solanum lycopersicum*) e *Tagetes patula* a 20 ºC. O Capítulo 3 foca na migração de *M. floridensis* sob diferentes estímulos vegetais e a duas temperaturas, 20 e 26 ºC. Os resultados mostram que ambas as espécies são capazes de migrar longas distâncias para parasitar raízes, ainda que *M. floridensis* seja mais móvel que *M. incognita* a 20ºC. A temperatura é o principal fator governante na migração de *M. floridensis*, visto que os padrões de migração sob estímulo vegetal foram similares ao controle. Plantas de *Tagetes patula* podem ser utilizadas no manejo de *Meloidogyne* spp. já que poucos juvenis foram capazes de penetrar as raízes. Os achados do presente estudo podem contribuir para entender como nematoides migram sob diferentes condições.

Key words: Colunas de solo, *Meloidogyne floridensis*, *Meloidogyne incognita*, mobilidade, nematoide das galhas, *Tagetes patula*.

General Abstract

Leitão, D. A. H. S., 2019. Upward migration of *Meloidogyne* spp. as a function of temperature and plant stimulus. Doctoral Thesis, Federal Rural University of Pernambuco, Recife, PE, Brazil.

Root-knot nematodes, *Meloidogyne* spp., are the main threat for global agriculture, thus understanding the behavior of nematodes throughout their life cycle is crucial for a proper management. The migration of infective second-stage juveniles (J2s) within soil pores is mediated by soil conditions and root exudates, which influences infection success rate. This doctoral thesis addressed the effect of temperature and plant stimulus on the vertical migration of J2s of *M. incognita* and *M. floridensis* over time. Soil columns were assembled by taping together three PVC rings (4 x 4.4 d cm) on top of one 2 x 4.4 d-cm ring, which served as an inoculation ring. The columns were filled with very sandy Candler soil and maintained at 10% water content and 1.2 Kg dm^{-3} bulk density. Bottomless Styrofoam cups containing ca. 300 g of the same soil and either a plant of tomato and *Tagetes patula* were placed on top of the columns; cups without plants were used as control. Chapter 1 focuses on literature review about *Meloidogyne* spp. and the factors that contribute or hinder the migration of J2s along the soil matrix. Chapter 2 addresses the migration of J2s of both species under tomato (*Solanum lycopersicum*) and *Tagetes patula* at constant temperature of 20 ºC. Chapter 3 focuses on the migration of *M. floridensis* under different plant stimuli and at two temeperatures, 20 and 26 ºC. The results show that J2s of both species are able to migrate over long distances in order to parasitize host roots, even though *M. floridensis* was more motile than *M. incognita* at 20ºC. Temperature is the main factor ruling *M. floridensis* migration, since migration patterns under plant stimuli were similar to host-free conditions. *Tagetes patula* plants might be used for *Meloidogyne* spp. management because fewer juveniles were able to penetrate their root systems. The findings of this study may help future research to understand how nematodes migrate under different conditions.

Key words: Mobility, *Meloidogyne floridensis*, *Meloidogyne incognita*, root-knot nematodes, soil columns, *Tagetes patula*.

SUMÁRIO

Introdução Geral

A produção agrícola do Brasil vem ganhando força nos últimos anos, mesmo em época de crise econômica. Uma das razões para tal comportamento é a expansão de áreas de cultivo no território nacional. Contudo, reduções na produção nordestina podem estar relacionadas a diversos fatores, como irregularidade de precipitação e ataque de pragas agrícolas. Um desafio ainda recorrente em todas as áreas agricultáveis no mundo é o ataque de nematoides parasitos de planta, especialmente os nematoides das galhas (*Meloidogyne* spp.). Atualmente, eles são considerados uma das principais limitações para a segurança alimentar mundial.

A capacidade de se movimentar e migrar para regiões com condições ideais é uma característica fundamental para a sobrevivência de qualquer organismo vivo. Ecologicamente, o movimento resulta de interações entre fatores externos e intrínsecos aos animais. A maioria dos nematoides se movimenta através da propulsão ondulatória, com contração e relaxamento dos músculos dorsoventrais, seguindo uma trajetória sinusoidal. Após eclodirem dos ovos, os juvenis de segundo estádio (J2s) movem-se livre e ativamente no filme de água aderido às partículas do solo em direção às raízes das plantas hospedeiras.

Quando em movimento no solo, os J2s estão sujeitos às variações ambientais. A temperatura é um dos principais fatores que exercem grande influência no ciclo de vida dos nematoides das galhas, os quais possuem uma faixa ótima para os diferentes estádios do ciclo. Por serem organismos poiquilotérmicos, o metabolismo aumenta com o aumento da temperatura do solo e, consequentemente, pode favorecer a migração dos J2s. Contudo, os J2s dependem de reservas lipídicas para sobreviver no solo até encontrar raízes de uma dada planta hospederia; portanto, quanto mais tempo permanecerem no solo, maior é o consumo de lipídios, tornando-os inviáveis para infecção.

A quimiotaxia, orientação em resposta a algum estímulo químico, é a principal via com a qual os nematoides são capazes de localizar uma possível planta hospedeira. Após a percepção dos exsudatos radiculares, através dos órgãos sensoriais, os J2s apresentam movimento coordenado em direção às raízes, caracterizando assim a migração no solo. Os exsudatos radiculares desencadeiam respostas distintas para as diferentes espécies de nematoides parasitos de planta, podendo ser atrativos, repelentes ou neutros. Há registros de extratos de uma mesma planta hospedeira possuir

características atrativas em baixa concentração, porém repelentes quando em alta concentração.

Portanto, o conhecimento sobre a migração de nematoides sob diferentes condições de temperatura e estímulo vegetal podem auxiliar na melhoria da eficiência das técnicas de manejo integrado atualmente utilizadas pelos pesquisadores e agricultores. Ensaios em ambientes controlados são de fundamental importância para se responder algumas questões iniciais, com posterior utilização das metodologias a nível de campo.

Hipóteses:

- A migração de *Meloidogyne floridensis* é diferente de *M. incognita*;
- Altas temperaturas favorecem a migração dos juvenis de segundo estádio das duas espécies;
- A presença de estímulo vegetal favorecerá a migração vertical de ambas as espécies;
- Plantas de *Tagetes patula* serão capazes de repelir os juvenis de segundo estádio de ambas as espécies.

Objetivo geral:

Estudar a migração ascendente de *Meloidogyne* spp. em colunas preenchidas com solo arenoso em função da temperatura e presença de estímulo vegetal ao longo do tempo.

Objetivos específicos:

- Comparar o efeito da presença e ausência de plantas hospedeiras sobre a taxa de migração de juvenis de segundo estádio de *M. floridensis* e *M. incognita* a 20 ºC;
- Avaliar a migração de *M. floridensis* sob temperaturas diferentes e estímulos vegetais;
- Identificar padrões de distribuição de ambas as espécies ao longo da coluna e ao longo do tempo, com e sem a presença de estímulo vegetal.

CAPÍTULO I

1. Nematoides do solo

1.1. Aspectos gerais

Os nematoides do solo pertencem taxonomicamente ao filo Nematoda, podendo ser encontrados em ambientes marinhos, cavernas profundas e ao longo do perfil de solos naturais e agrícolas (AHMED et al., 2015; van MEGEN et al., 2009). Esses microrganismos apresentam grande diversidade de tamanho (300 µm a 4 mm), ecologia, morfologia e ciclo de vida (BLAXTER, 2011). Estima-se que 80% dos metazoários sejam nematoides (BONGERS; BONGERS, 1998); sendo, portanto, os organismos mais abundantes do reino Metazoa (ABEBE; MEKETE; THOMAS, 2011; AHMED et al., 2015; MIR; TANVEER, 2016; REYNOLDS et al., 2011; ZHAO et al., 2014).

Contudo, os nematoides estão entre os organismos menos estudados (ABEBE; MEKETE; THOMAS, 2011), contendo em torno de 27.000 espécies catalogadas (AHMED et al., 2015). Esse número representa apenas uma pequena fração ($\leq$ 3%) do total de espécies, o qual é estimado em 1 milhão (HUGOT; BAUJARD; MORAND, 2001; LAMBSHEAD, 2004). Em termos de densidade populacional, pode-se contabilizar de 6 x 10^4 a 10^8 indivíduos por metro quadrado de solo (BOAG; YEATES, 1998; BONGERS, 1990; LAMBSHEAD, 2004; VERVOOT et al., 2012; WASILEWSKA, 1979). Adicionalmente à grande abundância, a diversidade trófica dos nematoides é outra característica muito importante desses organismos. Esses invertebrados participam de todos os fluxos de energia da cadeia alimentar do solo, sendo eles: herbívoro, bacteriófago e fúngico, além de apresentarem mais dois hábitos alimentares, onivoria e predação (AHMED et al., 2015; FREITAS et al., 2009; LEVI et al., 2012; MIR; TANVEER, 2016; VERVOOT et al., 2012; YEATES et al., 1993).

Os nematoides herbívoros (ou, comumente chamados, parasitos de planta) representam uma grande parcela de todas as espécies descritas (MITREVA et al., 2005), representados por mais de 50 gêneros (COSTA; LILLEY; URWIN, 2007). Do ponto de vista agrícola, os nematoides parasitos de planta são importantes economicamente, pois causam perdas significativas na produção de diferentes culturas de interesse nacional (LEITÃO, 2015; SGRIGNOLI et al., 2014). Atualmente, eles são considerados uma das principais limitações para a segurança alimentar mundial (BUCKINGHAM; PARTRIDGE; SATELLE, 2014; JONES et al., 2013; KHAN et al., 2012; OLIVEIRA; MONTEIRO; BLOK, 2011). Perdas anuais causadas por nematoides parasitos de planta

equivalem a cerca de 9-15% da produção mundial, acarretando prejuízos em torno de US$ 173 bilhões no mundo (ELLING, 2013; NICOL et al., 2011).

1.2. *Meloidogyne* spp.

Taxonomicamente, o gênero *Meloidogyne* faz parte da classe Chromadorea, ordem Tylenchida, subordem Tylenchina, infraordem Tylenchomorpha, superfamília Tylenchoidea, família Meloidogynidae e subfamília Meloidogyninae (MANTELIN; BELLAFIORE; KYNDT, 2017) e são mundialmente conhecidos como nematoides das galhas devido ao engrossamento das raízes da planta hospedeira após o parasitismo (MARIN et al., 2017). A primeira descrição de uma espécie de *Meloidogyne* no Brasil foi realizada em 1887 por Göldi, o qual observou perdas de produção em cafeeiro e nomeou o nematoide como *M. exigua* (MOENS; PERRY; STARR, 2009). Inicialmente, *Heterodera marioni* era mundialmente usado para designar os nematoides das galhas, porém Chitwood (1949) redescreveu quatro espécies de nematoides das galhas e concluiu que tais nematoides diferiam do gênero *Heterodera*, portanto a taxonomia publicada por Göldi (*Meloidogyne*) foi utilizada para designar os nematoides das galhas (MOENS; PERRY; STARR, 2009).

Meloidogyne spp. é a principal ameaça dentre os gêneros de nematoides para a agricultura mundial (JONES et al., 2013), devido a sua ampla distribuição mundial e polifagia (BRUINSMA; ANTONIOLLI, 2015). Atualmente, mais de 100 espécies de nematoides das galhas estão descritas na literatura (SEID et al., 2015) parasitando mais de 2.000 culturas (MOREIRA et al., 2018), porém a maior parte dos estudos concentram-se em *M. arenaria*, *M. hapla*, *M. incognita* e *M. javanica* (ELLING, 2013), as quais causam a maior parte dos danos às culturas (MOENS; PERRY; STARR, 2009). Recentemente, algumas espécies emergentes estão ganhando notoriedade, devido às grandes perdas culturais localizadas, como por exemplo *M. enterolobii* em goiabeiras no Brasil (CARNEIRO et al., 2011), *M. minor* em relva na Europa (MORRIS et al., 2011) e *M. floridensis* em pessegueiros nos Estados Unidos da América (BRITO et al., 2015).

Uma característica comum a todas as espécies do gênero é o clico de vida, o qual apresenta seis estádios fenológicos: ovo, quatro juvenis (J1, J2. J3. J4) e adultos (macho ou fêmea), com duração média de 21 a 28 dias (FERRAZ; BROWN, 2016). Durante cada passagem de um estádio para outro seguinte, ocorre ecdise (NIBLACK; LAMBERT; TYLKA, 2006; TURNER; ROWE, 2006); isto é, troca de cutícula (CARES; BALDWIN, 1995). O ciclo tem início com a fêmea depositando ovos,

envoltos em uma matriz gelatinosa (WARMERDAM et al., 2018), a qual protege os ovos de condições ambientais adversas e predação (EVANS; PERRY, 2009). O número médio de ovos produzidos pode chegar a 500 por fêmea sob condições favoráveis (CALDERÓN-URREA et al., 2016). Dentro do ovo, ocorre o processo de embriogênese e a formação do juvenil de primeiro estádio (J1), onde o mesmo sofre a primeira ecdise originando o juvenil de segundo estádio (J2) (MOENS; PERRY; STARR, 2009), o qual eclode do ovo sob condições ótimas de umidade e temperatura (ELLING, 2013). A partir da eclosão dos J2s, inicia-se a fase ativa do ciclo de vida de *Meloidogyne* spp. (DAS; WESEMAEL; PERRY, 2011).

Os J2s são os únicos estádios infectivos (BLEVE-ZACHEO et al., 2007), também chamados de pré-parasitas (FERRAZ; BROWN, 2016), os quais movem-se na solução do solo em busca de fontes de alimentação (CASTAGNONE-SERENO; DANCHIN, 2014; WALLACE, 1966). A infecção começa quando os J2s, guiados pelos exsudatos radiculares, encontram e penetram a zona de elongação da raiz (ELLING, 2013; MOREIRA et al., 2018), através de compostos secretados pelos estiletes que degradam a parede celular das células vegetais (ABAD et al., 2009). Após a penetração, os J2s movem-se ao longo do córtex, em direção ao meristema radicular para poder entrar no cilindro vascular (WARMERDAM et al., 2018). Por serem endoparasitas sedentários obrigatórios, *Meloidogyne* spp. é capaz de interagir com a planta hospedeira para sua própria vantagem (ABAD et al., 2009; SILVA et al., 2016).

Posteriormente, inicia-se o processo de alimentação e a diferenciação das células do parênquima, originando as chamadas células nutridoras ou gigantes (CASTAGNONE-SERENO; DANCHIN, 2014; MARIN et al.., 2017; MOENS; PERRY; STARR, 2009), através da secreção pelo estilete de substâncias provenientes da glândula esofagiana dentro das células da raiz, as quais fornecem os nutrientes necessários para o desenvolvimento dos J2s (MARIN et al., 2017; SHI et al., 2018). Durante essa fase, ocorre hiperplasia e hipertrofia dos tecidos circundantes aos J2s, o que origina as famosas galhas, sintoma típico de infecção de *Meloidogyne* spp. (ABAD et al., 2009; MOREIRA et al., 2018). Posteriormente, os J2s perdem a mobilidade e se tornam sedentários pelo resto do ciclo (ELLING, 2013; FERRAZ; BROWN, 2016), ocorrendo mais duas ecdises, passando pelo juvenis de terceiro (J3s) e quarto estádio (J4s) até atingirem a fase adulta reprodutiva depois da última ecdise (ABAD et al., 2009). Em condições adversas, por exemplo, superpopulação, escassez de alimento e temperaturas extremas, ocorre reversão sexual (CHITWOOD; PERRY, 2009) e parte dos juvenis fêmeas irão se transformar em machos e sair das raízes (ELLING, 2013).

1.3. Meloidogyne incognita

M. incognita pode ser encontrado em quase todo o mundo, especialmente em regiões tropicais, porém com menor dispersão em regiões temperadas (KARSSEN; WESEMEAL; MOENS, 2013). Possuem reprodução por partenogênese mitótica obrigatória (CASTAGNONE-SERENO; DANCHIN, 2014), sendo capazes de parasitar mono e dicotiledôneas (HUNT; HANDOO, 2009; KARSSEN; WESEMAEL; MOENS, 2013). Morfologicamente, os J2s são vermiformes com comprimento e diâmetro do corpo entre 350-450 e 10.9-13,6 µm, respectivamente (CHITWOOD, 1949; HUNT; HANDOO, 2009). Dentre as espécies do gênero, *M. incognita* é considerada uma espécie modelo devido ao grande avanço e descobertas em diversos estudos (ELLING, 2013).

1.4. Meloidogyne floridensis

M. floridensis é uma espécie recente, tendo sido descrita no início dos anos 2000 a partir de material vegetal de pessegueiros resistentes à *M. incognita* e *M. javanica* (HANDOO et al., 2004), por isso ficou conhecida mundialmente como o nematoide das galhas do pessegueiro. Atualmente, registros de parasitismo de *M. floridensis* estão restritos a condados da Flórida (BRITO et al., 2015; SMITH et al., 2015; NYCZEPIR; THOMAS, 2009) e da Califórnia, Estados Unidos da América (WESTPHAL et al., 2019). A reprodução se dá por partenogênese meiótica (HANDOO et al., 2004), com supressão da segunda divisão de maturação (CHITWOOD; PERRY, 2009). Apesar disso, *M. floridensis* é considerado um táxon irmão de *M. incognita* (ADAMS; DILLMAN; FINLINSON, 2009). Os J2s de *M. floridensis* são morfologicamente similares aos de *M. incognita*, com comprimento e diâmetro do corpo entre 310-482 e 12-16 µm, respectivamente (HUNT; HANDOO, 2004; STANLEY et al., 2009). Possui grande amplitude de hospedeiros, incluindo culturas economicamente importantes, como tomate (CHURCH, 2005) e melância (STANLEY et al., 2009), até diversas espécies de ervas daninhas (BRITO et al., 2015; RICH et al., 2009).

2. Movimentação de nematoides no solo

A capacidade de se movimentar e migrar para regiões com condições ideais para pleno desenvolvimento, fugir de predadores, localizar sítios de alimentação e indivíduos para reprodução é uma característica fundamental para a sobrevivência de qualquer organismo vivo (COHEN; BOYLE, 2010; GOLDMAN, 2014; GOLDMAN; HU, 2010; WALLACE, 1968). Ecologicamente, o movimento resulta de interações entre fatores externos (ambiente) e intrínsecos aos animais (BARTUMEUS; LEVIN, 2008). Tais eventos de locomoção ocorrem em diferentes escalas espaciais e temporais (GOLDMAN, 2014; MIRBAGHERI et al., 2015). Cada espécie animal se movimenta diferentemente (WALLACE, 1968) segundo diversos propósitos (BEJAN; MARDEN 2006), tais como nado dos espermatozoides até o óvulo, voo de uma águia (GOLDMAN, 2014) e o caminhar/correr dos animais bípedes. É importante salientar que, durante a evolução dos tipos de movimentos citados, houve uma otimização da relação entre distância percorrida e custo de energia relacionado ao movimento (BEJAN; MARDEN, 2006; COHEN; BOYLE, 2010; WILSON et al., 2013).

De acordo com a própria anatomia e habitat em que vivem, os animais podem se locomover através de nado, rastejo, voo, caminhada e corrida (WALLACE, 1968). De todas as classes de movimento, a propulsão ondulatória é marcadamente a mais difundida entre as espécies animais (COHEN; BOYLE, 2010; DEMIN; VITYAEV, 2014; GRAY, 1953). Aspectos envolvidos no movimento ondulatório (físico-mecânicos e fisiológicos) foram avaliados em maiores detalhes por outros autores em diversas espécies animais, incluindo minhocas (DORGAN; LAW; ROUSE, 2013), lagartos (BAUMGARTNER et al., 2008; HOSOI; GOLDMAN, 2015; MALADEN et al., 2009), enguias (LAUDER; TYTELL, 2006) e nematoides (BERMAN et al., 2013; BILBAO et al., 2013; BURR; ROBINSON, 2004; COHEN; BOYLE, 2010; COHEN et al., 2012; CROLL, 1977; CRONIN et al., 2005; ERB; LU, 2013; FEENY et al., 2013; FELTHAM; CHAPLAIN, 2000; FELTHAM et al., 2002; FERNANDES; DESPLAN, 2015; GART; VELLA; JUNG, 2011; GRAY, 1953; GRAY; LISSMANN, 1964; PROT, 1980; ROBINSON; PERRY, 2006; WALLACE, 1968; YUAN; RAIZEN; BAU, 2015).

Salvo algumas exceções (GRAY, 1939), a maioria dos nematoides se movimenta através da propulsão ondulatória (BURR; ROBINSON, 2004; HELMS et al., 2015; ROBINSON; PERRY, 2006; YUAN; RAIZEN; BAU, 2015). Movimentos ondulatórios são formas adaptativas de organismos terrestres para se locomoverem sobre ou através de meios granulares (GRAY, 1953), como solo, sangue e tecidos vegetais (BURR; ROBINSON, 2004). Cohen e Boyle (2012) citam que para se realizar

o movimento ondulatório, o organismo não necessita de nenhum membro, apenas o próprio corpo e, mesmo organismos que possuem membros (i.e. lagartos), podem escolher por não os utilizar quando "nadam" em meios granulares.

Os corpos dos nematoides quando em movimento originam formas curvas criando ondas que se propagaram retrogradamente às cabeças dos mesmos, quando movem-se para frente, ou progressivamente, quando movem-se para trás (BURR; ROBINSON, 2004; KARBOWSKI et al., 2006). As ondas são o produto de eventos de contração e relaxamento dos músculos dorsoventrais (BILBAO et al., 2013; GART; VELLA; JUNG, 2011; ROBINSON; PERRY, 2006), controlados pelo sistema neuromuscular (GART; VELLA; JUNG, 2011; KARBOWSKI et al., 2006). Vale salientar que as propriedades das ondas são específicas a cada nematoide (BOCKGARD, 2011) e também dependem do meio em que se locomovem.

Como resultado desse tipo de movimento e interações de fatores intrínsecos (sistema neuromuscular, esqueleto hidrostático, cutícula) e fatores extrínsecos (condição do habitat), os nematoides seguem uma trajetória sinusoidal (GOLDMAN, 2014; KARBOWSKI et al., 2006; NIEBUR; ERDÖS, 1991), utilizando a cabeça para abrir caminho entre os grãos do solo (WALLACE, 1958a) (Fig.1). Os princípios envolvidos na propulsão ondulatória foram revisados por Cohen e Boyle (2012), Gray e Lissmann (1964) e Wallace (1968).

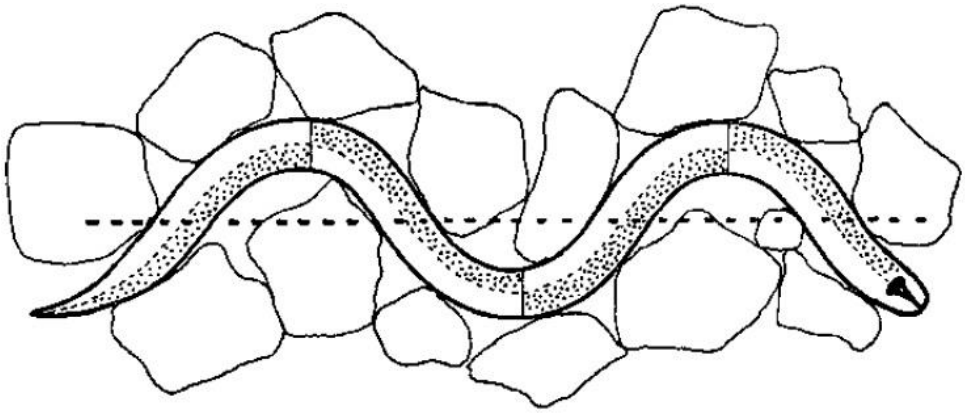

Figura 1. Movimento sinusoidal de um nematoide entre partículas de solo. Notam-se três curvas ao longo do comprimento do corpo do nematoides, cada uma funcionando como uma unidade propulsora. As áreas hachuradas representam músculos contraídos e as áreas brancas representam músculos relaxados durante a propulsão ondulatória. (Fonte: WALLACE, 1960).

3. Fatores condicionantes ao movimento de nematoides

Como citado anteriormente, parte do ciclo de vida de nematoides parasitos de planta se desenvolve no perfil do solo (FAJARDO; ABALLAY; CASANOVA, 2011). Durante a fase de J2s, os nematoides não se alimentam (DUTTA et al., 2012), porém movem-se livre e ativamente no filme de água aderido às partículas do solo (WALLACE, 1966). Assim, os J2s se locomovem por propulsão ondulatória através dos espaços porosos do solo (BONGERS; FERRIS, 1999) com o objetivo de encontrar raízes de plantas hospedeiras, parceiros sexuais e fugir de predadores, podendo assim continuar o ciclo de vida.

Estudos pioneiros acerca da movimentação de nematoides e localização de raízes de plantas hospedeiras foi realizada por Steiner (1925). O autor supracitado elaborou as primeiras hipóteses sobre o assunto, sendo: i) os nematoides podem localizar raízes de plantas hospedeiras a grandes distâncias; ii) as raízes produzem substâncias, as quais são carreadas pela água do solo, que servem como estímulo para os nematoides; iii) os nematoides respondem a esses estímulos através dos anfídios, os quais são órgãos sensoriais. Prot (1980) avaliou as hipóteses de Steiner através de uma revisão de literatura e concluiu que Steiner estava certo em vários aspectos.

Além dos anfídios, os nematoides possuem outros órgãos sensoriais que respondem a estímulos, tais como os fasmídios (BEHN, 2012; MBEGA; NZOGELA, 2012; PROT, 1980). Esses órgãos sensoriais entram em contato com substâncias exógenas produzidas pelas raízes e/ou organismos do solo, levando ao reconhecimento e localização da planta hospedeira (HAEGEMAN et al., 2012; RASMANN et al., 2012). Embora a quimiotaxia seja a mais importante via de orientação a estímulos para os nematoides (ANDALÓ; MOREIRA; MOINO JUNIOR, 2014), os mecanismos de atração dos exsudatos radiculares ainda não estão totalmente elucidados na literatura.

Adicionalmente aos exsudatos radiculares, os nematoides podem chegar até as raízes simplesmente por movimentos aleatórios no solo, através de substâncias liberadas por microrganismos se alimentando das raízes ou por gradientes de atributos edáficos (e. g. concentração de gases, pH, temperatura, umidade, dentre outros) (BEHN, 2012; DAVIS; EARL; TIMPER, 2014; DIEZ; DUSENBERY, 1989; MORRIS et al., 2011; SALAME; GLAZER, 2015; SPENCE; LEWIS; PERRY, 2008).

3.1. Atributos edáficos

Algumas hipóteses não consideradas por Steiner (1925) estão relacionadas com o ambiente no qual os nematoides se movem, ou seja, como os atributos edáficos

influenciam a locomoção desses microrganismos. Atualmente, sabe-se que o ambiente edáfico exerce enorme influência na capacidade de sobrevivência dos nematoides (CARRILLO; HALLEM; 2015; GALLARDO et al., 2015; HUNT et al., 2001; MBEGA; NZOGELA; 2012; MOORE et al., 2010; WINTER; RAJCAN; SHELP, 2006; ZAKI; KHAN; ABID, 2012), incluindo as formas infectivas de nematoides parasitos de planta e entomopatogênicos.

Devido à grande complexidade de interações entre as propriedades do solo condicionantes ao movimento de nematoides, Hunt et al. (2001) mencionaram que não há um modelo geral que descreva a locomoção de nematoides no solo. Vários pesquisadores estudaram o movimento, migração e dispersão dos nematoides em diversas condições, segundo as características físico-químicas e hidráulicas do meio (Tabela 2). Os parâmetros representados corroboram Hunt et al. (2001), os quais citaram diferenças entre os aparatos experimentais (dimensão e orientação da coluna e número e dimensão das seções nas colunas); esse fato também dificulta a criação de um modelo genérico para locomoção de nematoides em solo.

3.1.1. Atributos físicos

A estrutura do solo (porosidade + textura) exerce grande influência na capacidade de locomoção dos nematoides (WALLACE, 1968; YOUNG et al., 1998). O solo é considerado um substrato particulado, mas se os poros forem muito menores que o diâmetro do corpo dos nematoides, ele se torna efetivamente “sólido”, em relação ao movimento desses microrganismos (ROBINSON; PERRY, 2006). Por isso, o tamanho dos agregados do solo é determinante para a locomoção dos nematoides (BURR; ROBINSON, 2004). Portanto, solos bem estruturados possuem poros que permitem melhor transporte, aumentando o movimento dos nematoides parasitos de planta (PINO, 1994).

Tabela 2. Estudos sobre locomoção de nematoides parasitos de planta em relação aos atributos do meio e dimensões das colunas de solo (comprimento x diâmetro), quando utilizadas

Atributo	Meio	Dimensão das colunas (cm)	Táxon de Nematoide	Referências
Porosidade				
	Solo e bolas de vidro	11,0 x 2,0 11,0 x 5,0	*Meloidogyne incognita*	Fujimoto et al. (2009, 2010)
	Solo	-	*Heterodera schachtii* e *Ditylenchus dipsaci*	Wallace (1958a, b)
	Areia	-	*M. javanica*	Wallace (1966)
	Ágar e água	-	11 taxa, incluindo *H. schachtii*	Wallace (1958c)
Umidade				
	Solo	50,0 x 8,5	*M. chitwoodi* e *M. hapla*	Pinkerton et al. (1987)
	Solo	-	*H. schachtii* e *H. rostochiensis*	Wallace (1958a, 1960)
	Areia	9,5 x 1,6	*M. javanica*	Prot (1979)
Textura				
	Solo	21,0 x 2,0	*M. incognita*	Prot e van Gundy (1981)
	Solo	-	*H. rostochiensis*	Wallace (1960)
Percolação de água				
	Solo e bolas de vidro	11,0 x 2,0 11,0 x 5,0	*M. incognita*	Fujimoto et al. (2009, 2010)
	Solo	30,0 x 26,0*	*Radopholus similis*	Chabrier et al. (2008)
	Areia	9,0 x 0,5	5 taxa, incluindo *H. rostochiensis*	Wallace (1959a)
Temperatura				
	Solo	50,0 x 8,5	*M. chitwoodi* e *M. hapla*	Pinkerton et al. (1987)
	Solo	20,0 x 2,8	*Pratylenchus penetrans*	Pudasaini, Viaene e Moens (2007)
	Areia	15 x 15, 20 x 3,8	5 taxa, incluindo *M. incognita*	Robinson (1994)
	Areia	-	*M. javanica*	Wallace (1966)
	Água	-	*M. minor*	Morris et al. (2011)
Gases				
	Areia	4,0/7,2 x 3,8	5 taxa, incluindo *M. incognita*	Robinson (1995)
	Ágar	-	*P. penetrans*	El-Sherif e Mai (1969)
	Ágar	-	*M. incognita*	Pline e Dusenbery (1987)
Sais				
	Areia	9,5 x 1,6	*M. javanica*	Prot (1979)
	Ágar e gel	-	*M. incognita*	Castro et al. (1989a), Hida et al. (2015), Qi et al. (2015)
	Ágar	-	*H. glycines*	Huettel e Jaffe (1987)

* - O experimento foi realizado em campo utilizando cilindros metálicos com as dimensões citadas.

A mobilidade de animais parasitos de planta é fortemente influenciada pelo tipo de solo (ERB; LU, 2013). Nematoides parasitos de planta se adaptam melhor a solos porosos (textura arenosa/franco-arenosa) (GALLARDO et al., 2015; PROT; van GUNDY, 1981); os autores observaram que *Pratylenchus* spp. e *Meloidogyne* spp. se desenvolvem melhor nos solos citados. Esse fato é decorrência da maior macroporosidade de solos arenosos (FAJARDO; ABALLAY; CASANOVA, 2011). Porém *Heterodera rostochiensis* não apresentou diferença de locomoção em solos arenosos ou argilosos (WALLACE, 1960).

Diante disso, pode-se perceber que a movimentação de nematoides está diretamente ligada ao tamanho das partículas do solo. *H. schachtii* (nematoide dos cistos da beterraba) move-se mais rápido em solo com partículas de diâmetro entre 150-250 µm (Wallace, 1958a-c). Esses estudos demonstraram que a textura age indiretamente no movimento de nematoides, enquanto que a variação no tamanho das partículas possui maior influência. Posteriormente, Wallace (1960) acrescenta o fato de que, nos poros produzidos por partículas com diâmetro 200 µm, os nematoides estão sujeitos às forças da tensão superficial do filme d'água, sem quebrar a continuidade do mesmo; facilitando a locomoção. De fato, Neher et al. (1999) verificaram que os espaços entre os agregados proporcionam melhores condições para os nematoides, quando comparados com os espaços intra-agregados.

Recentemente, Fujimoto et al. (2009, 2010) avaliaram a migração de J2s de *M. incognita* em colunas preenchidas com solo (areia ou Andisol) ou bolas de vidro, apresentando diferentes porosidades e distribuição de poros ($\leq$ 20 µm, 20-130 µm e $\geq$ 130 µm). As colunas preenchidas com as bolas de vidro (Ø = 800 µm) apresentaram porosidade total de aproximadamente 40%, com maior proporção de poros $\geq$ 130 µm; nas colunas com Andisol, a porosidade total foi de aproximadamente 65%, com maior proporção de poros $\leq$ 20 µm; a coluna preenchida com areia apresentou características intermediárias. Os resultados obtidos por Fujimoto et al. (2009) revelaram que o movimento de nematoides foi diferente apenas nas colunas preenchidas por Andisol. Os autores indicaram que esse comportamento é devido a estrutura do Andisol e complexa distribuição dos poros. Poros com diâmetros menores exercerão maior resistência, limitando o movimento efetivo dos nematoides (WALLACE, 1968).

Adicionalmente, os nematoides só serão capazes de atravessar poros com diâmetro mínimo igual ao diâmetro do corpo dos mesmos. No experimento, Fujimoto et al. (2009) utilizaram colunas com Andisol contendo mais de 40% de poros $\leq$ 20 µm; considerando um diâmetro médio de 12,3 µm para *Meloidogyne* spp. (CHITWOOD,

1949), pode-se afirmar que a massa de solo não permitiu a migração dos J2s de *M. incognita* (FUJIMOTO et al., 2009). O mesmo tamanho de poros é prejudicial ao movimento de espécies do gênero *Heterodera* (WALLACE, 1958a).

Fujimoto et al. (2010) utilizaram um aparato experimental diferente para descrever a migração de J2s de *M. incognita* em colunas preenchidas com areia e Andisol. O aparato consiste em uma coluna de solo segmentada (11 seções iguais), no centro da qual os J2s eram inoculados. Os autores consideraram o movimento negativo quando os nematoides se movimentavam para esquerda, e positivo quando migravam para direita, da coluna. Os autores concluíram que o movimento foi maior nas colunas com areia, ocorreu distribuição praticamente uniforme em ambos os meios e os nematoides migraram em direção a regiões não saturadas.

Os nematoides parasitos de planta possuem uma faixa ótima de temperatura para os diferentes estádios do ciclo de vida (FERRAZ; BROWN, 2016). Em geral, o ciclo de vida pode se prolongar em temperaturas pouco elevadas (NOEL, 1993). No geral, para nematoides, os intervalos de temperaturas entre 5-10 ºC, 15-22 ºC, 24-30 ºC e 33-39 ºC são, respectivamente, a faixa mínima para sobrevivência, a faixa para o exercício normal das atividades metabólicas, a faixa ideal ou ótima para a sobrevivência e a faixa onde há limitação nas atividades; acima disso, a temperatura é limitante para sobrevivência (FERRAZ; BROWN, 2016).

O movimento de espécies de *Meloidogyne* é o mais estudado, dentre os parasitos de planta, visto a quantidade de estudos com esse gênero (Tabela 2). Segundo Robinson e Perry (2006), algumas espécies de nematoides parasitos de planta possuem preferência por determinada temperatura. As espécies *M. hapla* e *M. chitwoodi* foram estudadas por Pinkerton et al. (1987) em relação a migração em três temperaturas (12, 18 e 24 ºC). Os autores observaram comportamento diferente entre as espécies; *M. hapla* migrou menos (acima de 20 cm apenas em 18 ºC) em relação a *M. chitwoodi* (acima de 35 cm).

Robinson (1994) observou que *M. incognita* migrou em direção a fonte de calor independentemente da orientação de colunas preenchidas com areia; adicionalmente, os autores concluíram que as resposta de espécies de nematoides parasitos de planta a gradientes de temperatura é diferente. Com relação a *M. javanica*, a migração foi maior na temperatura de 25 ºC, onde 80% dos J2s migraram para camadas mais profundas de colunas preenchidas com areia (WALLACE, 1966). *M. minor*, uma espécie emergente na Europa, foi estudada por Morris et al. (2011) em temperaturas entre 4-30 ºC; os autores observaram que os J2s se moviam em todas as

temperaturas analisadas, porém as maiores taxas (movimentos da cabeça a cada 5 min) foram observadas entre 15-25 ºC.

Avaliando a migração de *Pratylenchus penetrans* nas temperaturas de 11, 16 e 21 ºC, Pudasaini, Viaene e Moens (2007) observaram que os J2s migraram no máximo 11 cm quando submetidos a menor temperatura e 16 cm nas temperaturas de 16 e 21 ºC; é importante salientar que maior proporção, mesmo que pequena, de J2s foi encontrada na temperatura de 21 ºC.

3.1.2. Atributos hidráulicos

A dispersão hídrica é uma das principais vias de transporte da microbiota do solo (DIGHTON et al., 1997; LEHMAN, 1994), incluindo nematoides (BAXTER et al., 2013). No decorrer ou após um evento de precipitação, a água é percolada através do solo e provoca migração de nematoides para camadas mais profundas (WALLACE, 1959a). Contudo, organismos podem apresentar reotaxia positiva, ou seja, nadar contra o fluxo (YUAN; RAIZEN; BAU, 2015).

O experimento de Wallace (1959a) foi um dos pioneiros a avaliar a influência de percolação de água na movimentação de nematoides. As espécies utilizadas nesse experimento foram *Heterodera rostochiensis*, *Ditylenchus dipsaci*, *Panagrolaimus rigidus*, *Panagrellus redivivus* e *Diplogaster lheritieri*. O autor verificou que o movimento descendente aumenta à medida que a densidade do fluxo aumenta e que essa relação é linear a partir de 5000 mm h^{-1}. Adicionalmente, foi observado que *H. rostochiensis* pode atravessar poros menores quando a densidade do fluxo é baixa.

Na última década, estudos sobre a percolação de água e o movimento de nematoides parasitos de planta estão ganhando notoriedade. Chabrier et al. (2008) avaliou a disseminação de *Radopholus similis* (nematoide cavernícola) após simulação de chuva de diferentes intensidades e durações ao longo de um perfil de solo extraído do campo. Maiores proporções de *R. similis* foram encontradas na segunda camada avaliada (5-10 cm) após chuva de 60 mm h^{-1} durante 72 min; contudo, as camadas mais profundas não apresentaram diferenças significativas. Os autores citam que os nematoides podem ter escapado da percolação por migrarem para capilares finos, impossibilitando a recuperação deles mesmo que a chuva dure 12h ou mais.

Os experimentos de Fujimoto et al. (2009, 2010) – descritos anteriormente – também avaliaram a capacidade do nematoide de resistir a um fluxo contínuo. No

primeiro experimento, os autores observaram que os J2s de *M. incognita* resistiram a velocidade de fluxo, variando de 328,8 a 531,6 mm h^{-1}, sugerindo que os nematoides são capazes de resistir à maioria dos fluxos de água em condições naturais, os quais são da ordem de mm h^{-1} (Fujimoto et al., 2009). No segundo experimento, os autores observaram que os nematoides atingiram a extremidade de saída da coluna no máximo 3 dias após a inoculação e acrescentaram que os nematoides não são capazes de se movimentar ativamente contra o fluxo, apenas resistir a ele. Contudo, espécimes de *Caenorhabditis elegans* foram capazes de se movimentar contra um fluxo 276 mm h^{-1} (10 vezes maior que a velocidade de nado desses microrganismos) (YUAN; RAIZEN; BAU, 2015). Estes autores concluíram que a reotaxia resulta de interações puramente mecânicas, e não neurobiológicas, como quimiotaxia, termotaxia e eletrotaxia.

Nos trabalhos com percolação de água com fluxo contínuo, o solo encontra-se completamente saturado. Porém, sabe-se que mudanças na espessura do filme d'água aderido às partículas primárias (ou seja, variação na umidade do solo) está relacionada com a mobilidade (MBEGA; NZOGELA, 2012; PROT, 1980) e esta relação é específica ao grupo trófico dos nematoides. Indivíduos de C. *elegans* não foram capazes de migrar para regiões distantes em média de 30 mm do ponto de inoculação em ágar com potencial de -2 kPa, possivelmente devido a descontinuidade dos filmes d'água (YOUNG et al., 1998). Neher et al. (1999) observaram que o número de nematoides bacteriófagos diminuía à medida que se aumentava (valor absoluto) o potencial mátrico do solo; enquanto que nematoides predadores foram beneficiados por potenciais menores que -20 kPa. Wallace (1960) observou que *H. rostochiensis* apresentou comportamento similar aos predadores do estudo anterior.

Prot (1979) observou que J2s de *M. javanica* se distribuíam igualmente em colunas sem gradiente de umidade, mas se concentravam na camada mais úmida quando aplicado um gradiente (e.g. 6% $\longrightarrow$ 14%). Os autores atribuíram esse resultado ao comportamento dos nematoides de se movimentar para regiões com filmes d'água mais espessos. Indivíduos de *H. schachtii* se locomoveram mais rapidamente em solos parcialmente saturados, em comparação com solos completamente saturados e secos (WALLACE, 1958a), reforçando que a espessura do filme é fundamental para o movimento dos nematoides. Wallace (1959b) descreve as forças atuantes em *H. schachtii* imerso em diferentes espessuras de filme d'água.

Pinkerton et al. (1987) avaliaram o movimento de J2s de *M. chitwoodi* em diferentes condições de umidade: abaixo (4, 6 e 8%) e acima (10, 12 e 14%) da capacidade de campo e observaram que os nematoides foram capazes de se movimentar

melhor em solo com umidade entre 10 a 14%. Os autores também avaliaram três regimes de irrigação (não irrigado, 25 ml de água 24 h após inoculação e 25 ml de água 24 e 48 h após inoculação). Os resultados desse experimento indicaram que houve movimento ascendente de *M. chitwoodi* devido ao movimento descendente de percolação da água, com 1 e 34% dos nematoides recuperados na seção distante 10 cm do ponto de entrada nos tratamentos com irrigação 24 h e 24 e 48h após inoculação, respectivamente (PINKERTON et al., 1987). Wallace (1960) cita que os nematoides se orientam para camadas mais úmidas, e não em relação a gravidade.

3.1.3. Atributos químicos

A atração ou repelência de organismos a estímulos químicos é chamada de quimiotaxia. Os nematoides possuem vários órgãos sensoriais, através dos quais eles percebem estímulos químicos do ambiente (MBEGA; NZOGELA, 2012; PERRY, 1996; PROT, 1980). A orientação química é fundamental para que os nematoides se movimentem em direção às raízes e a parceiros sexuais e se afastem de predadores ou regiões com condições desfavoráveis à sobrevivência desses microrganismos (RIGA, 2004). Dentre os fatores químicos percebidos pelos nematoides estão gradientes de gases (principalmente gás carbônico) e sais solúveis.

Gás carbônico (CO_2, também conhecido como dióxido de carbono) é produzido por todas as plantas, sendo considerado o principal produto metabólico em termos quantitativos (PROT, 1980). Esse gás contribui em diversos aspectos do parasitismo de nematoides, incluindo localização do hospedeiro e movimento no solo (ROBINSON, 1995); diversas espécies são atraídas por dióxido de carbono (ROBINSON; PERRY, 2006). Maiores informações podem ser encontradas em Carrillo e Hallem (2015) e Riga (2004).

Um dos primeiros estudos da influência de um gradiente de CO_2 em uma espécie de nematoide parasito de planta mantido em ágar foi realizado por Pline e Dusenbery (1987). Os autores observaram que a taxa de migração de *M. incognita* foi de 0,7 cm h^{-1} sob condições favoráveis e citaram que o CO_2 liberado pelas raízes permite que os nematoides migrem em uma linha reta em direção à região com maiores concentrações. Ao contrário, El-Sherif e Mai (1969), ao contrário, não observaram atração de *P. penetrans* por fontes de CO_2, a não ser quando um gradiente de temperatura ocorria simultaneamente em ágar. Gradientes efetivos para atrair nematoides parasitos de planta (*M. incognita* e *R. reniformis*) encontrados por Robinson

(1995) foram de 0,12-0,65 % cm^{-1} em areia. Esses estudos evidenciam a necessidade de se estudar a atração de CO_2 utilizando diferentes tipos e porosidades de solo. Huettel e Jaffe (1987) foram uns dos primeiros pesquisadores a avaliar o comportamento quimiotático de machos de *H. glycines*. Os resultados encontrados mostraram que os indivíduos foram altamente atraídos por glicerol, em diferentes concentrações; moderadamente atraídos por KOH, e repelidos por HCl.

A sensibilidade a estímulos provocados por gradientes de sais em solução proporciona orientação dos nematoides em direção às raízes de plantas hospedeiras (PROT, 1980). Em recente revisão sobre nematoides em ambientes salinos, Zaki, Khan e Abid (2012) citam que o excesso de sais solúveis é prejudicial a todos os estádios do ciclo de vida de nematoides parasitos de planta. Concentrações de sais excessivas provocam estresse hídrico no nematoide através da diminuição do potencial osmótico da água (WALLACE, 1968), diminuindo a capacidade de locomoção. Utilizando glicerol para diminuir o potencial osmótico de ágar, Wallace (1966) observou considerável diminuição na movimentação de J2s de *M. javanica* nas concentrações de 0,3-1 M. Este autor ainda cita que glicerol em concentrações maiores que 0,1 M (potencial osmótico de -202 kPa) inibe o movimento e migração de J2s. Wallace (1968) afirma que os nematoides raramente encontram condições osmóticas dessa magnitude.

Diferentes tipos de sais ou solução de sais ($\approx 10^{-2}$ M) foram utilizados para avaliar a movimentação horizontal de J2s de *M. javanica* em colunas preenchidas com areia (PROT, 1979). Observou-se migração dos juvenis pra camadas de menor concentração da solução nutritiva de Hoagland, e dos sais KH_2PO_4, KNO_3, $Ca(NO_3)_2$ e $MgCl_2$; essas camadas coincidem com menores teores de umidade. No entanto, quando em presença de $MgSO_4$, $FeSO_4$ e $FeCl_2$, os nematoides moveram-se para regiões mais salinas e de maior umidade; esse comportamento pode ser explicado por uma hidrotaxia superior a uma repelência aos sais. Castro et al. (1989a) classificou diferentes cátions e ânions em relação a maior ou menor capacidade de repelência em J2s de *M. incognita*.

Recentemente, estudos sobre quimiotaxia de nematoides parasitos de planta utilizaram aparatos experimentais em forma de microcanais ou seções para avaliar a movimentação em direção favorável ou contrária a maior concentração do agente químico. Hida et al. (2015) observaram que KNO_3 possui propriedades puramente atrativas (9,9 mM), puramente repelentes (990 mM) ou uma combinação das duas (50 e 99 mM) em relação ao movimento de J2s de *M. incognita*. Qi et al. (2015) avaliaram a quimiotaxia de *M. incognita* em resposta a 48 tipos de sais através do índice quimiotático criado por eles. Os resultados indicaram linearidade na relação entre a

concentração da maioria dos sais e o índice quimiotático, ou seja, valores negativos do índice indicam repelência, enquanto que valores positivos indicam atração. Esses resultados podem auxiliar na tomada de decisão para o controle de nematoides parasitos de planta em condições reais, mantendo uma concentração repelente a nível de campo (HIDA et al., 2015).

3.2. Atributos da planta hospedeira

A rizosfera pode ser definida como a camada próxima (escala de mm) à superfície das raízes de determinada planta, onde diversos processos biológicos são realizados (BAETZ; MARTINOIA, 2014; CURTIS, 2008). As plantas produzem diversos tipos de compostos que são liberados para a rizosfera, tais como moléculas lipofílicas, açúcares e aminoácidos (baixo peso molecular) e polissacarídeos e proteínas (alto peso molecular) (HAICHAR et al., 2014; RASMANN et al., 2012). Os nematoides coevoluíram juntamente com as plantas para reconhecer e responder a estímulos oriundos do hospedeiro (CURTIS, 2008; DUTTA et al., 2012), sendo a quimiotaxia a via primária pela qual os nematoides localizam as plantas hospedeiras (REYNOLDS et al., 2011).

Assim como os nematoides são atraídos por gradientes relacionados a presença de gases e sais, os produtos metabólicos das raízes também geram respostas quimiotáticas nos fitonematoides. Tais produtos (exsudatos) provocam diferentes respostas em nematoides parasitos de planta. Alguns desses compostos podem desencadear a eclosão de ovos e atrair ou repelir certas espécies de nematoides (HILTPOLD; JAFFUEL; TURLINGS, 2014). Os exsudatos atrativos auxiliam na locomoção de nematoides parasitos de planta em direção à superfície da raiz a procura de regiões adequadas para a penetração (DUTTA et al., 2012; MBEGA; NZOGELA, 2012).

Em relação ao movimento de nematoides, os exsudatos radiculares são classificados em atrativos a longas distâncias, atrativos a curtas distâncias e atrativos locais (PERRY, 2005). Os primeiros atraem os nematoides em direção a regiões com presença de hospedeiros; os atrativos a curtas distâncias guiam os nematoides para as raízes em si, enquanto que os atrativos locais permitem que os nematoides endoparasitos migrem para sítios de invasão favoráveis nas raízes (CURTIS, 2008; MBEGA; NZOGELA, 2012; SPENCE; LEWIS; PERRY, 2008; REYNOLDS et al., 2011).

Castro et al. (1989b) verificaram que diferentes frações de exsudatos de uma mesma planta hospedeira (pepino) apresentaram comportamentos de atração e repelência em *M. incognita*. O comportamento quimiotático de *M. incognita* também foi estudado com relação a frações de exsudatos de tomateiros (DIEZ; DUSENBERY, 1989). Os J2s foram fortemente repelidos pelo exsudato concentrado; à medida que o exsudato era diluído, o efeito de repelência se tornou mais fraco.

Atualmente, bioensaios sobre atração de nematoides parasitos de planta a exsudatos de diferentes plantas vêm ganhando atenção mundial. Spence, Lewis e Perry (2008) avaliaram a eficácia desses ensaios laboratoriais em predizer resultados a nível de campo, visto que as condições utilizadas em ágar ou outro tipo de substrato pode não ser semelhante às condições encontradas no perfil do solo (DUTTA et al., 2011; PERRY, 2005; REYNOLDS et al., 2011).

Reynolds et al. (2011), através de simulações e bioensaio, comprovaram que o comportamento quimiotático de nematoides parasitos de planta pode levá-los à fonte da substância atrativa pelo caminho mais curto. Quando os autores simularam uma atração a um composto químico, o nematoide se movimentaria pelo labirinto de canais pela via mais curta e não fariam curvas "erradas". No bioensaio, Reynolds et al. (2011) utilizaram três tipos de hospedeiras: tomate (boa hospedeira para *M. incognita*), arroz (boa hospedeira para *M. graminicola*) e mostarda (não hospedeira para ambas as espécies) e controle (sem planta) para avaliar a atração por vias curtas e longa em olfatômetros em forma de Y utilizando gel plurônico (para imitar as condições edáficas). Maior quantidade J2s de *M. incognita* migraram em direção às raízes de tomateiro, quando comparados com arroz; não foi observado nenhum movimento significativo em direção às raízes de mostarda (semelhante ao controle). Em relação a *M. graminicola*, observou-se maior quantidade de J2s próximos a raízes de arroz; o mesmo comportamento foi observado em relação a mostarda e controle.

O estudo de Dutta et al. (2011) corrobora os resultados descritos anteriormente. Em bioensaio sobre atração de *M. incognita* e *M. graminicola* a raízes de tomate e arroz, os autores observaram que J2s da primeira espécie foram atraídas para raízes de tomate (boa hospedeira) e J2s da segunda espécie foram atraídas para raízes de arroz (boa hospedeira). Ambas as espécies apresentaram o mesmo comportamento em relação a raízes de mostarda. Esses resultados indicam que os nematoides são capazes de perceber sinais de compostos metabólicos produzidos pelas raízes e se movimentam em direção às boas hospedeiras. Maiores informações podem ser encontradas em outros

estudos (HAICHAR et al., 2014; HILTPOLD; JAFFUEL; TURLINGS, 2014; RASMANN et al., 2012).

Plantas antagônicas ou armadilha são uma alternativa viável para o controle de nematoides parasitos de planta por apresentarem efeitos nematicidas e/ou nematostáticos (MUNHOZ et al., 2017). *Tagetes* spp. possui efeito supressivo em diversos nematoides parasitos de planta, especialmente *Meloidogyne* e *Pratylenchus* (KALAISELVAM; DEVARAJ, 2011), através do uso de extratos e óleos de diversas partes da planta ou incorporado ao solo (ADEKUNLE; ACHARYA; SINGH, 2007; MARAHATTA et al., 2012; MUNHOZ et al., 2017; NATARAJAN et al., 2006). Há registros de melhor efeito supressivo de *Tagetes patula* quando comparado com outras espécies (KALAISELVAM; DEVARAJ, 2011; MARAHATTA et al., 2012; MUNHOZ et al., 2017). Contudo, poucos estudos avaliaram a influência de raízes de *Tagetes* spp. na migração de nematoides parasitos de planta. Não foi observada diferença na migração de J2s de *M. chiwoodi* e *P. penetrans* injetados em colunas de solo (2 cm de comprimento x 0,8 cm de diâmetro interno) contendo extratos de tomate e *T. patula* (NJEŽIĆ; SUTTER; MOENS, 2014). Mais recentemente, Francilino et al. (2017) observaram significativa repelência de raízes trituradas de *T. patula* em indivíduos de *P. coffeae* em colunas de resina acrílica (11 cm x 2,2 cm de diâmetro interno) preenchidas com areia lavada, em comparação com um efeito atrativo de iscas de inhame (boa hospedeira). Marahatta et al. (2012) cita que o efeito supressivo é mais evidente na presença de raízes em desenvolvimento ativo.

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Migration of second-stage juveniles of *Meloidogyne floridensis* and *M. incognita* under different plant stimuli

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CAPÍTULO II

Leitão, Diego Arruda Huggins de Sá. Dr. Universidade Federal Rural de Pernambuco. Fevereiro de 2019. Migração ascendente de *Meloidogyne* spp. em função da temperatura e estímulo vegetal. Drª. Elvira Maria Régis Pedrosa.

Migration of second-stage juveniles of *Meloidogyne floridensis* and *M. incognita* under different plant stimuli

Abstract: Several researches have been performed on pest management but only a few considered their migration through soil. The vertical migration of J2s of *Meloidogyne floridensis* and *M. incognita* was investigated in sandy soil columns under different plant stimuli. Segmented 14-cm long PVC pipes were assembled by taping together three 4.4-cm-d x 4-cm long rings on top of one 2-cm long ring (inoculation ring). The columns were filled with sterilized soil at 1.2 kg dm^{-3} and 10% soil water content. On top of each column a bottomless Styrofoam cup was attached containing one 4-week-old tomato (cv. Cobra) or *Tagetes patula* (variety 'Petite') plant, and no plant as the control. Freshly hatched J2s (1,000±100 per column) were injected into the middle of the inoculation ring and the columns were placed on growth chambers at 20 °C under a completely randomized block design with four replications. The columns were disassembled 3, 6 and 9 days after inoculation. J2s were extracted from the soil of each ring and cup separately and roots were stained to observe penetration. Data was subjected to a repeated measure MANOVA with posterior chi-square test to compare J2s distribution along the columns. No preferential migration was observed for both active species, but *M. floridensis* was more motile than *M. incognita*, with 4.4% and 1.6% of active J2s, respectively, migrating over 13 cm. Root weight was significantly higher at the end of the experiment and *M. floridensis* migration was correlated to this variable. Penetration was greater under tomato regardless of nematode species. Although *Tagetes patula* can lure juveniles of *Meloidogyne floridensis* and *M. incognita* to their rhizosphere, a few were able to parasitize their roots.

Key words: Root-knot nematodes, mobility, soil columns, *Tagetes patula*.

Introduction

Plant-parasitic nematodes (PPN) are major biotic stress factors of economically important agricultural crops (Sobkowiak et al, 2018). Their parasitism can cause yield suppressions of hundreds of billions of dollars (Kohli et al., 2018), almost half of which may be attributed to *Meloidogyne* spp. (Fujimoto et al., 2010). *Meloidogyne incognita* is

the main soil-borne pest of Solanaceae (Barbary et al., 2015) and it was recently ranked the most invasive pathogen worldwide (Bebber et al., 2014). On the other hand, *M. floridensis* has primarily been reported parasitizing *M. incognita*-resistant peach rootstocks in Florida counties (Sharpe et al., 1969), but it has been further found in tomato (Church, 2005) and several other plant species (Brito et al., 2015; Smith et al., 2015).

Managing these agricultural pests remains a global challenge. The primary control strategy is the application of chemical nematicides, especially low molecular weight soil fumigants and contact carbamates (Hussain et al., 2017). However, in recent years there has been an intensive banishment or restriction on their use (Jiang et al., 2018) due to the hazardous effects on non-target microorganisms, fauna and flora, human beings, and ecosystems, such as ozone depletion and groundwater contamination (Collange et al., 2011; Kearn et al., 2014; Pudasaini et al., 2007). Thus, the adoption of multiple strategies may be a solution to manage PPN (Pudasaini et al., 2007).

Been et al. (2005) have pointed out that a knowledge-based integrated pest management system (IPMS) is needed and they suggested one for *Globodera* spp., using accurate population dynamics estimates, cultivars resistance degree and control strategies effectiveness. *Meloidogyne* spp. management may take advantage of such system. Although IPMS requires host-pathogen interaction input data (Pudasaini et al., 2007), it does not cover the role of nematode migration on the efficiency of control measures as *Meloidogyne* spp. may migrate or reside in areas far from the site of nematicide application (Ntalli and Caboni, 2012).

Second-stage juveniles (J2s), which are the sole infective stage of *Meloidogyne* spp. (Bleve-Zacheo et al., 2007), need to move through soil pores in order to penetrate host roots, and their behavioral responses are altered by soil conditions (Fujimoto et al., 2009), including the gradient of root exudates dissolved in the soil solution. Root exudates play a crucial role on J2s migration, as they are the main source of chemical cues to guide nematodes to the root tips to start the infection process. Such exudates range from polysaccharides and proteins to small lipophilic molecules (Haichar et al., 2014; Rasmann et al., 2012), which may be repellent, attractant or neutral to nematode migration (Hiltpold et al., 2014). Reynolds et al. (2011) have observed that *M. incognita* and *M. graminicola* migrated towards preferred hosts (tomato and rice, respectively) and no significant attraction behavior was observed towards mustard (non-host for both species). Migratory PPN, *Pratylenchus coffeae* and *P. penetrans*, have shown preferred

migration towards susceptible host as well (Francilino et al., 2017; Pudasaini et al., 2007, respectively).

Tagetes spp. are commonly known as natural nematicides (Murga-Gutiérrez et al., 2012). They can suppress a wide range of PPN, especially root-knot nematodes and root lesion nematodes (Kalaiselvam and Devaraj, 2011), by using different parts of the plant amended into the soil or their extracts, oils and leachates (Adekunle et al., 2007; Marahatta et al., 2012; Munhoz et al., 2017; Natarajan et al., 2006). Regarding *Meloidogyne* species, it is reported that *Tagetes* spp. are non-host for *M. floridensis* (Brito et al., 2015) and resistant to *M. incognita* (Buena et al., 2008). Best suppression results were observed when using *Tagetes patula* in contrast with other species (Kalaiselvam and Devaraj, 2011; Marahatta et al., 2012; Munhoz et al., 2017). Nježić et al. (2014) migration study showed that *Tagetes patula* diffusates applied to sandy soil columns did not influence the rate of vertical migration of *M. chitwoodi* and *P. penetrans*. On the other hand, Francilino et al. (2017) have observed a repellent effect of chopped *Tagetes patula* roots on the horizontal movement of *P. coffeae* in sand-filled columns. Nonetheless, there is no literature on the effect of *Tagetes patula* plants on *M. floridensis* and *M. incognita* vertical migration within the soil.

Nematodes have co-evolved together with plant species to recognize and respond to host stimuli (Dutta et al., 2012), thus chemotaxis is reported to be the leading factor of nematodes' host location (Reynolds et al., 2011). Steiner's hypothesis (Steiner, 1925) on the ability of nematodes to locate and move through soil to infect hosts have led to several studies about chemotactic responses. However, chemotaxis assays have evaluated two-dimensional migration of J2s on agar-filled plates (Castro et al., 1989; Dusenbery, 1983; Hewlett et al., 1997; Huettel and Jaffe, 1987), which does not have a similar realism to the three-dimensional soil condition (Spence et al., 2008). Column bioassays, on the other hand, have focused on implementing such 3D conditions by using columns filled with sand or soil as the migration medium. In order to evaluate the migratory ability of PPN, host plants are placed in one end of the columns and J2s are inoculated on the opposite end and allowed to migrate over different distances and periods of time.

Several species of nematodes have been used on column bioassays. Wallace's research was the pioneer to evaluate how the soil medium and water flow influence the migration of infective juveniles of *Heterodera schachtii* and *Ditylenchus dipsaci* without a host plant (Wallace, 1958a-c, 1959a). Later, Prot (1976, 1978) and Prot and van Gundy (1981) have introduced an attractive host plant to the system and evaluated

the host-finding ability of *M. javanica* and *M. incognita* under different soil conditions (water content, temperature) and distances to the tomato roots (up to 50cm). These studies highlight that J2s of *Meloidogyne* are able to migrate over long distances to infect good host roots but their migration patterns may be species-specific. Pinkerton et al. (1987) observed no preferential migration of *M. chitwoodi* on columns with and without tomato plants, while *M. incognita* migrated towards a good host in sand-filled pipette-bulbs (Dalzell et al., 2011). However, up to this date there is no information regarding *M. floridensis* migration.

Therefore, the aim of this research was to evaluate the vertical migratory ability of *M. incognita* and *M. floridensis* J2s under tomato and *Tagetes patula* stimuli. We have tested the hypothesis that: i) both species are able to migrate long distances; ii) migration patterns are different under tomato and *Tagetes patula* stimuli; iii) *Tagetes patula* is less attractive than tomato to both species.

Material and Methods

Nematode inoculum

Meloidogyne incognita and *M. floridensis* populations were maintained and multiplied on tomato (*Solanum lycopersicum* cv. Cobra) in 25-cm-diameter clay pots containing sandy soil in the greenhouses at the University of Florida, Gainesville, FL. When plants were 60 days old, their root system were washed free of debris with tap water and chopped into 2-cm long pieces, then grinded in 0.52% NaOCl solution for 20 s in a metal blender. The solution was poured into a 200-mesh sieve nested in a 500-mesh sieve and washed thoroughly with tap water for 3 min to remove NaOCl excess (Hussey and Barker, 1973). The egg solution was poured into a modified Baermann funnel at 27 °C in 2-ply "Kleenex" tissue paper (Rodríguez-Kábana and Pope, 1981). The J2s that hatched during the first 24 h were discarded. For the migration assays, freshly hatched J2s were collected for 3 days and maintained under refrigeration until the beginning of the experiment.

Tomato and *Tagetes patula* plants

Seeds of tomato and *T. patula* (variety "Petite") were sown, one seed per cell, on seedling trays containing vermiculite as substrate, and kept under greenhouse conditions. For the migration assays, 4-week-old germinated tomato and *Tagetes patula* plants were used.

Experimental device

The juveniles' migration was studied using polyvinylchloride (PVC) columns (Fig. 1) based on the specifications of Pinkerton et al. (1987). The columns were assembled by taping together three 4-cm long PVC ring on top of a 2-cm long ring; the latter was drilled 1 cm above its base to produce a hole used for inoculating J2s into the columns. Each column was 14-cm long with 4.4-cm internal diameter and 213-cm^3 internal volume. The columns were filled with heat-sterilized Candler sand (soil characterization is shown in Table 1) collected from a peanut field in Levy County, FL, and kept at 1.2 Kg dm^{-3} soil bulk density and 10% water content by weight, which were similar to field conditions. The base of the inoculation ring was covered with 15-µm mesh to keep the juveniles in the system. When the columns were completely filled, a parafilm was placed on top of them to prevent evaporative water loss and maintain the water content until the beginning of the experiment.

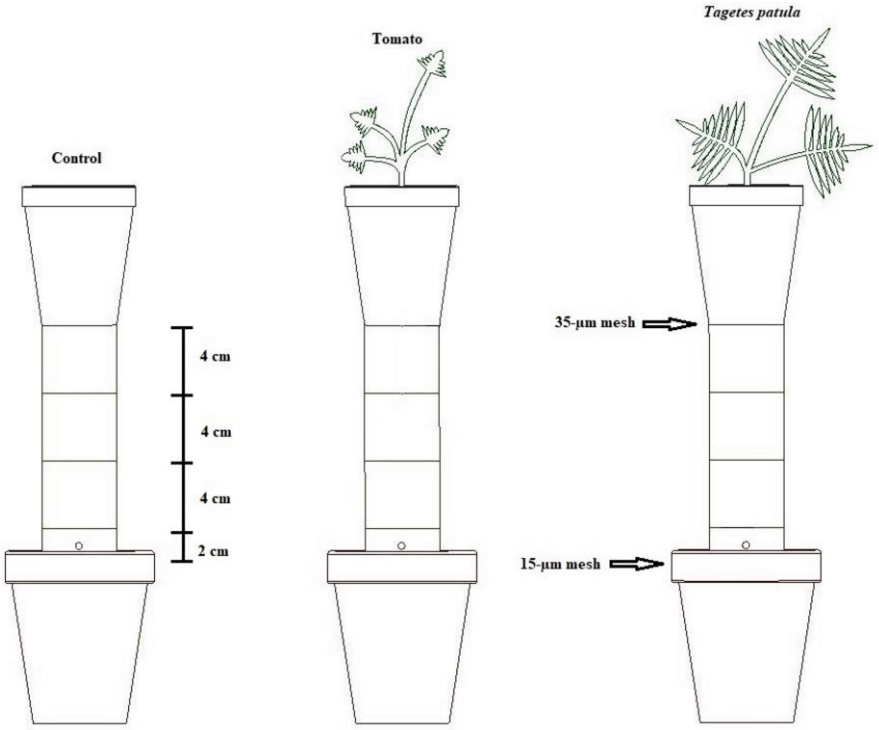

Figure 1. Experimental device used in the experiment. Column is composed of three 4.4-cm-d x 4-cm long rings taped together to one 2-cm long ring (inoculation ring) filled with sandy soil. Four-week-old tomato or *Tagetes patula* plant, or no plant, were on the top of the columns as migration stimuli. Freshly hatched juveniles were injected 1 cm above the base of the columns through an inoculation port.

Table 1. Physical and chemical attributes of Candler soil used on nematode migration assays.

Physical attributes	Unit	Depth (m)	Chemical attributes	Unit	Depth (m)
		0.00-0.40			0.00-0.40
Sand	%	96	pH	(1:2)	5.6
Silt	%	2	Extractable P	mg Kg^{-1}	>135
Clay	%	2	Extractable K	mg Kg^{-1}	13
Texture		Sandy	Extractable Mg	mg Kg^{-1}	0
Bulk density	Kg dm^{-3}	1.2	Extractable Ca	mg Kg^{-1}	164
Water content	%	10	Organic Matter	%	0.27

P: Phosphorus; K: Potassium; Mg: Magnesium; Ca: Calcium

A bottomless Styrofoam cup containing approximately 300 g of the same Candler soil at 10% water content with either one tomato (attractive stimulus) or one *Tagetes patula* (repellent stimulus) plant was taped to the top of the columns; cups without plants served as control. A 35-µm nylon mesh was placed between the cups and the top ring of each column (Prot, 1976), thus preventing root growth into the columns and allowing juveniles to migrate through. The fully assembled columns were transferred to growth chambers kept at 20 °C with 16h light/8h dark photoperiod regime for 24 h prior to J2s inoculation.

The J2s collected from modified Baermann funnels were counted under a stereomicroscope at 100× magnification and the suspension was adjusted to at least 500 ± 50 J2 ml^{-1}. Approximately 1,000 ± 100 mobile J2s were inoculated into each column through the hole on the inoculation ring (1 cm above the column base); the holes were sealed right after the inoculation. No water was added to the columns at that moment to avoid unnecessary nematode percolation. In order to keep the soil at 10% water content, the columns were weighted daily, and irrigation was performed to replace the amount of water lost by evapotranspiration (Pudasaini et al., 2007). The irrigation water was maintained in wash bottle kept inside the growth chambers at 20 °C, thus there was no temperature fluctuation during and after irrigation. The experimental design was a completely randomized block design with four replicates. No application of fertilizers was used during the experimental period in order to observe the sole effect of root exudates.

The columns were dismantled 3, 6 and 9 days after inoculation (DAI) following each ring and the Styrofoam cup, from each of which the nematodes were extracted by centrifugal-flotation technique (Jenkins, 1964). Nematodes were counted under stereomicroscope to determine the number of recovered and active juveniles in each

ring; active juveniles showed some movement regardless of its intensity. Concomitantly, tomato and *Tagetes patula* root systems were washed free of debris and stained with acid fuchsin (Byrd et al., 1983) to observe nematode penetration, and fresh shoot and root weights were determined.

Statistical Analysis

Nematode data were $\sqrt{x+0.5}$-transformed prior to statistical analysis to meet MANOVA assumptions. The effect of plant stimuli, distance migrated (section), and time on *M. floridensis* and *M. incognita* upward migration were analyzed through a repeated measure MANOVA. When significant effects were observed, a chi-square (X^2) test was performed to compare the distribution of J2s along the columns; while Tukey's multiple comparison test was used for J2s inside the roots. Pearson's correlation coefficients between plant variables and nematodes inside roots were also determined. All statistical analyses were performed in RStudio environment (RStudio Team, 2015).

Results

Upon termination of each extraction, no nematodes were found trapped in the 15-µm or 35-µm mesh nor on the walls of the PVC rings. The distribution of recovered J2s along the columns was influenced by the plant stimuli ($P < 0.05$, Table 2), with ferwer juveniles being recovered from the control columns; however the presence of tomato or *Tagetes patula* roots did not influence the rate of migration of active J2s as compared to the control treatment ($P \geq 0.05$). There was a significant interaction between nematode species and distance migrated (section) as well as time and section for juveniles of *M. incognita* and *M. floridensis* ($P < 0.0001$, Table 2).

Table 2. Repeated measure MANOVA summary of the effects of plant stimuli, distance migrated (section) and time on recovered and active second-stage juveniles of *Meloidogyne incognita* and *M. floridensis* vertical migration along PVC columns filled with sandy soil at 20 ºC.

		Recovered J2s				Active J2s			
Source	df	SS	MS	F	p-value	SS	MS	F	p-value
Block	3	56.70	18.90	5.86	0.0007	64.90	21.60	7.61	<0.0001
Nema	1	116.90	116.90	36.23	<0.0001	43.20	43.20	15.18	0.0001
Stimulus	2	22.60	11.30	3.50	0.0317	15.60	7.80	2.75	0.0660
Section	4	2899.60	724.90	224.76	<0.0001	1632.70	408.20	143.49	<0.0001
Nema:Stim	2	3.80	1.90	0.60	0.5490	3.70	1.90	0.66	0.5185
Nema:Sec	4	895.60	223.90	69.43	<0.0001	524.30	131.10	46.08	<0.0001

Stim:Sec	8	11.20	1.40	0.45	0.8927	10.20	1.30	0.45	0.8903
Nema:Stim:Sec	8	18.40	2.30	0.71	0.6826	12.10	1.50	0.53	0.8308
Time	2	16.40	8.20	2.53	0.0818	20.70	10.40	3.64	0.0276
Time:Nema	2	0.80	0.40	0.14	0.8739	1.10	0.50	0.19	0.8283
Time:Stim	4	10.00	2.50	0.78	0.5400	12.90	3.20	1.13	0.3421
Time:Sec	8	330.40	41.30	12.80	<0.0001	177.40	22.20	7.80	<0.0001
Time:Nema:Stim	4	11.20	2.80	0.87	0.4855	6.80	1.70	0.60	0.6636
Time:Nema:Sec	8	42.20	5.27	1.64	0.1147	37.90	4.70	1.67	0.1064
Time:Stim:Sec	16	60.80	3.80	1.17	0.2897	49.20	3.10	1.08	0.3736
Time:Nema:Stim:Sec	16	43.20	2.70	0.83	0.6458	39.40	2.50	0.87	0.6091

df: Degree of freedom; SS: Sum of squares; MS: Mean square; *P*>F: Significance level of F test

A steady migration pattern was observed for *M. floridensis*, while most of *M. incognita* J2s remained in the inoculation ring. More than 40% of *M. floridensis* J2s were recovered at the inoculation ring, while twice as much was observed for *M. incognita* (Fig. 2A). As the distance from the inoculation ring increased, there was a decrease on the number of recovered J2s of both species; however, 5% of J2s of M. *floridensis* were found at distances over 13 cm (Fig. 2A). Although many J2s did not move away from the inoculation ring, 1.6% and 4.4% of active J2s of *M. incognita* and *M. floridensis*, respectively, were able to migrate over 13 cm towards the upper end of the columns (Fig. 2B). Regarding *M. incognita*, 14% of active J2s migrated 1-5 cm, 8% migrated 5-9 cm, 2% migrated 9-13 cm; while 33%, 21% and 10% of active J2s of *M. floridensis* (Fig. 2B).

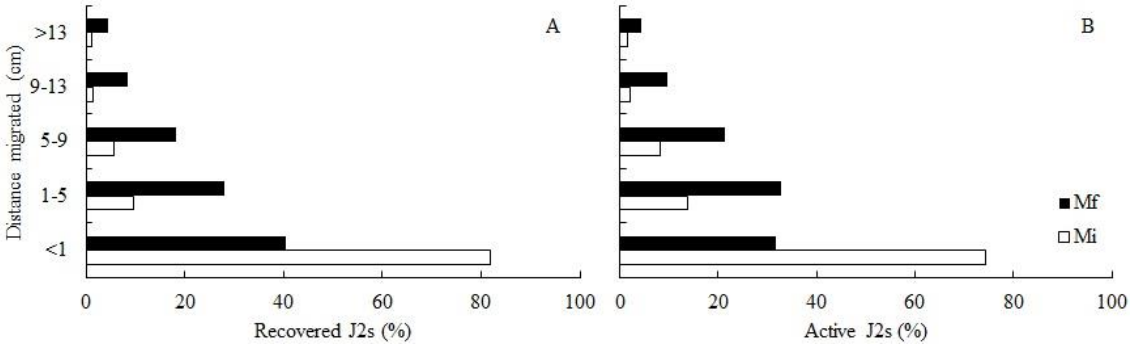

Figure 2. Distribution of recovered (A) and active (B) second-stage juveniles (J2s) of *Meloidogyne floridensis* (Mf) or *M. incognita* (Mi) along sandy soil columns at 20 °C, composed of 4-cm long x 4.4-cm internal diameter rings. (n=9)

The highest percentages of juveniles were found at the inoculation ring at all extraction times; nonetheless, there was a gradual decrease on the number of J2s recovered over time at that section (Fig. 3). Although very few J2s (<0.5%) were able to reach the top of the column 3 DAI, the vast majority remained at the inoculation ring

throughout the experiment, with an average of 84%, 64% and 54% at 3, 6 and 9 DAI, respectively (Fig. 3A). An increase on the number of recovered J2s was observed on the upper sections over time, with almost 6% at the top of the columns at 9 DAI. At 3 DAI almost 80% of active J2s remained at the inoculation ring and almost none were observed at distances greater than 9 cm. At 6 DAI, 34% of active J2s migrated 1-9 cm and 2.7% reached the Styrofoam cup. At 9 DAI, 56% of active J2s were distributed throughout the length of the column, with nearly 4% at the Styrofoam cup section (Fig. 3B).

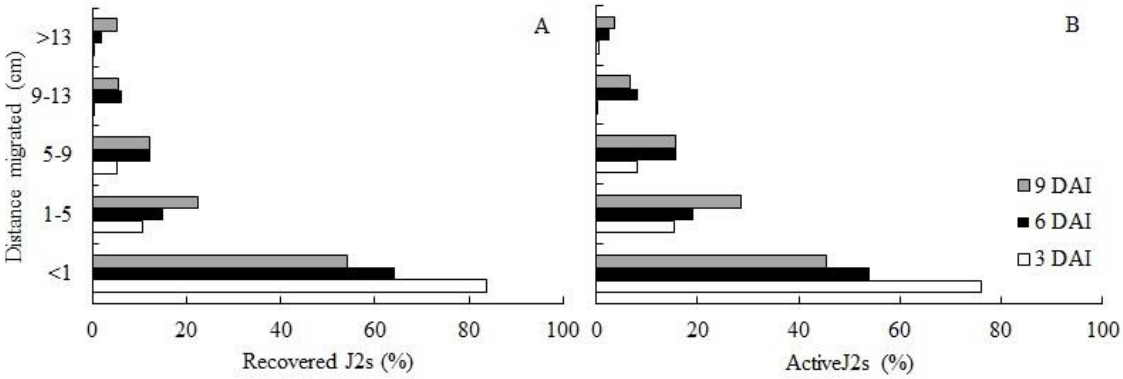

Figure 3. Distribution of recovered (A) and active (B) second-stage juveniles (J2s) of *Meloidogyne floridensis* and *M. incognita* along sandy soil columns at 20 °C, composed of 4-cm long x 4.4-cm internal diameter rings. Bars represent the average percentage of juveniles recovered at 3, 6 and 9 days after inoculation (DAI). (n=6)

Juveniles of *M. incognita* and *M. floridensis* showed different penetration rates ($P < 0.01$) and the penetration was significantly different for tomato and *Tagetes patula* plants ($P < 0.01$). Time alone had a significant effect on nematode penetration ($P < 0.0001$), and its interaction with nematode species ($P < 0.05$) and stimulus ($P < 0.01$) were also significant (Table 3).

Table 3. Repeated measure MANOVA summary of penetration of second-stage juveniles (J2s) of *Meloidogyne floridensis* and *M. incognita* into roots placed at 13 cm distance from the inoculation point at 20 ºC over time.

		J2s inside roots			
Source	df	SS	MS	F	p-value
Block	3	0.70	0.23	0.79	0.5092
Nema	1	3.53	3.53	11.89	0.0016
Stimulus	1	4.89	4.89	16.48	0.0003
Nema*Stim	1	0.00	0.00	0.00	0.9833
Time	2	18.01	9.01	30.34	<0.0001
Time*Nema	2	3.08	1.54	5.19	0.0110
Time*Stim	2	3.32	1.66	5.60	0.0081

Time*Nema*Stim	2	0.19	0.10	0.32	0.7289

df: Degree of freedom; SS: Sum of squares; MS: Mean square; *P*>F: Significance level of F test

Both nematode species were not able to parasitize the roots of either plant at 3 DAI (Fig. 4), in spite of 0.4% of total active J2s being able to migrate >13 cm (Fig. 3A). At 6 DAI, the population of J2s of *M. incognita* inside the roots was the lowest (average of 0.5 J2s/root system) but did not differ from the population of *M. floridensis* (2.25 J2s/root system). The highest number of J2s parasitizing roots of both plant species was observed at 9 DAI for *M. floridensis*, with an average of 8 J2s per root system (Fig. 4).

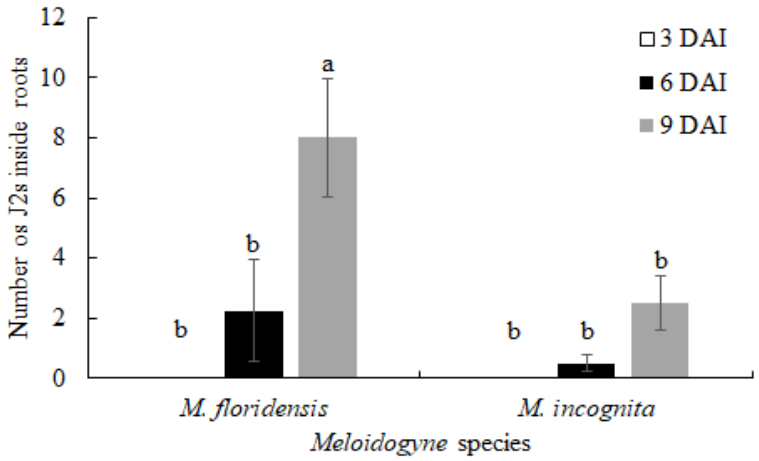

Figure 4. Number of second-stage juveniles (J2s) of *Meloidogyne floridensis* and *M. incognita* parasitizing tomato and *Tagetes patula* roots at 3, 6 and 9 days after inoculation (DAI). Figures followed by the same letter do not differ significantly according to Tukey's multiple comparison test (P ≥ 0.05).

Overall, the greatest average number of J2s inside roots was observed at 9 DAI under tomato (Fig. 5). The average number of J2s inside tomato roots at 6 DAI was exactly the same (2.5 J2s per root system) as those parasitizing *Tagetes patula* roots at 9 DAI. This behavior highlights that tomato is more attractive than *Tagetes patula* to *Meloidogyne* spp, possibly due to the former good host status for both species.

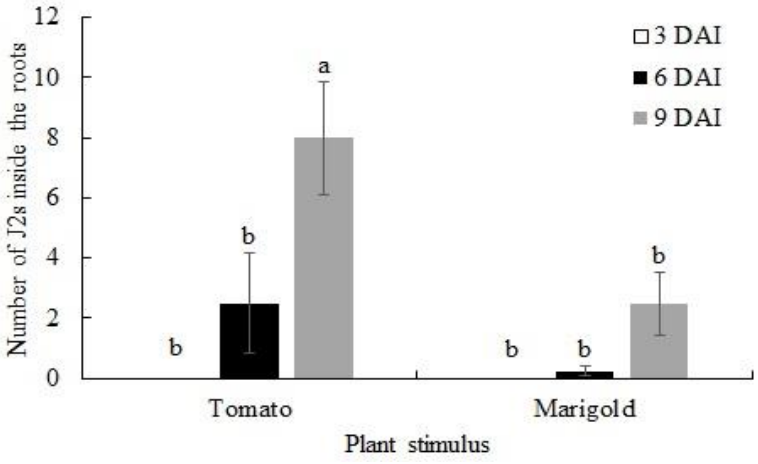

Figure 5. Number of second-stage juveniles (J2s) of *Meloidogyne floridensis* and *M. incognita* parasitizing tomato or *Tagetes patula* roots at 3, 6 and 9 days after inoculation (DAI). Bars followed by the same letter do not differ significantly according to Tukey's multiple comparison test ($P \geq 0.05$).

The lowest value of fresh root weight and *Tagetes patula* was recorded at 3 DAI. Even though there was no application of fertilizers, the plants gained weight over time. The number of J2s of *M. floridensis* inside roots was positively correlated to both root and shoot weights, with r = 0.53 ($P < 0.01$) and r = 0.48 ($P < 0.05$), respectively (Fig. 6A); however, J2s of *M. incognita* did not show any correlation to plant growth variables, $P \geq 0.05$ (Fig. 6B).

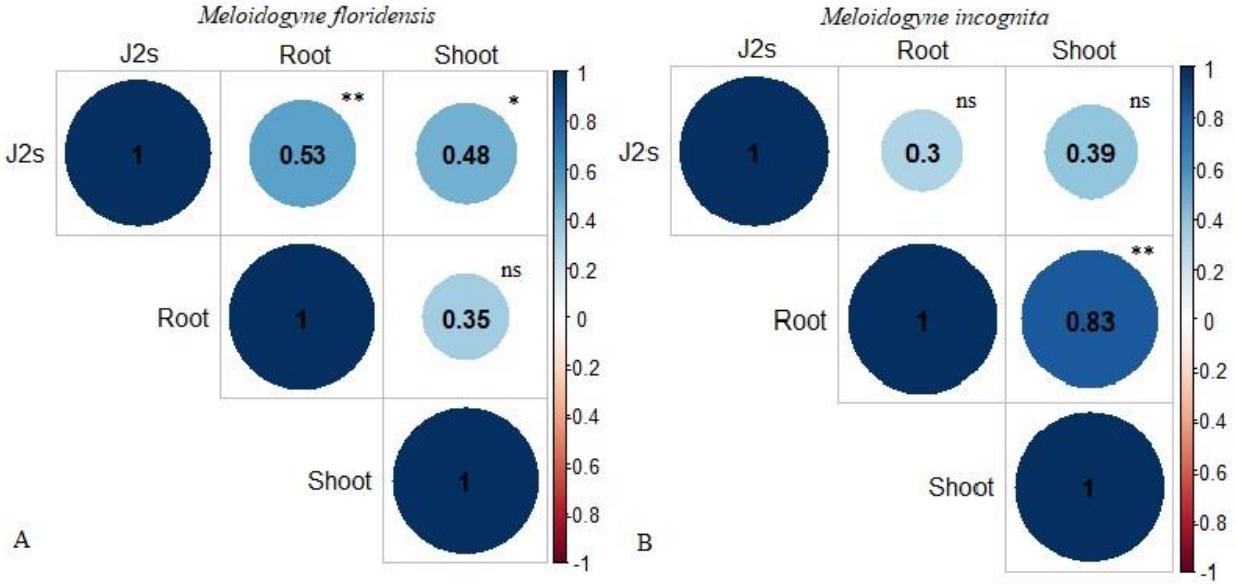

Figure 6. Correlation coefficients between second-stage juveniles (J2s) of *Meloidogyne floridensis* (A) and *Meloidogyne incognita* (B) and root and shoot weights of tomato and *Tagetes patula* plants. * – $P < 0.05$; ** – $P < 0.01$; ^{ns} – $P \geq 0.05$.

Discussion

The ability of organisms to move and migrate towards food srouces and mating individuals or away from harmful conditions is crucial for their survival (Cohen and Boyle, 2010; Goldman, 2014; Goldman and Hu, 2010; Wallace, 1968). Migration is a result of interactions among environmental factors and the animal's intrinsic characteristics (Bartumeus and Levin, 2008), thus each species move differently (Wallace, 1968) according to several purposes (Bejan and Marden, 2006) and those migration events occur in several spatial and temporal scales (Goldman, 2014; Mirbagheri et al., 2015).

Active juveniles of plant-parasitic nematodes can migrate randomly or following a stimulus gradient (Davis et al., 2014; Rasmann et al., 2012; Spence et al., 2008). In our experiment, less than 1% of inoculated J2s of *M. floridensis* and *M. incognita* were able to migrate over 13 cm vertically regardless of stimuli. Pinkerton et al. (1987) have found similar recovery rates for *M. chitwoodi* in 45-cm long columns and Pudasaini et al. (2007) have found similar migration patterns for *P. penetrans* in columns with different lengths. Contrarily, Prot (1976) have recovered 50% of *M. javanica* parasitizing tomatoes placed 50 cm from the inoculation ring and Prot and van Gundy (1981) observed that more than 30% of *M. incognita* J2s were able to migrate 20 cm and parasitize tomato plants. Those differences may be due to the soil texture and the column dimensions. Since nematode migration is influenced by soil physical attributes (soil pore size and connectedness, water content and percentage of silt and clay), coarser soils might hinder J2s movement towards plant roots. Wallace (1968) stated that nematode migration is dependent on pore size and J2s body diameter relation, as well as the thickness of water films adhered to soil particles. In our experiment, we have used a very sandy soil (96% of sand), which does not hold as much water as the soil from the studies mentioned. Additionally, clay particles can hold root exudates and guide nematode towards root penetration sites (Prot and van Gundy, 1981).

The distance between the inoculation port and the host root is also important because nematodes might get disoriented, consume all their energy reserves before reaching the roots and become inactive or die (Pudasaini et al., 2007; Rocha et al., 2016). Another important factor governing nematode migration studies is the columns' internal diameter. Prot (1976) used columns with 1.2 cm internal diameter; in our experiment, the columns internal diameter was 4.4 cm. Smaller diameters will probably restrict nematode horizontal dispersal and impose vertical migration (Spence et al.,

2008); therefore, it would be expected a greater number of nematodes at the top of the experimental device if we have used smaller columns.

Although the migration rate of *M. floridensis* and *M. incognita* along the columns with plant stimuli did not significantly differ from the control treatments, the downward percolation of irrigation water might have enhanced the juveniles' upward migration. Some studies highlight that entomopathogenic and PPN migrate towards and aggregate in wetter soil layers (Prot, 1978, Salame and Glazer, 2015, Wallace, 1960). Pinkerton et al. (1987) did not observe a preferential migration of *M. chitwoodi* in columns with and without tomato plants; however, a greater number of J2s reached the top of the control columns at 9 DAI, probably due to a moisture gradient created by top irrigation water.

Although *Tagetes* spp. has been reported as a non-host for *M. floridensis* (Brito et al., 2015), showing very low reproductive factor (Kokalis-Burelle and Nyczepir, 2004), and highly suppressive for *M. incognita* (Buena et al., 2008; Murga-Gutiérrez et al., 2012), especially in vermiform stages (Marahatta et al., 2012), this does not mean J2s will not migrate towards its roots, as we observed in our experiment. In fact, only a few J2s were able to migrate > 13 cm and penetrate *Tagetes patula* roots; however, they did not show signs of further development – no late J2s were observed during the experiment for both *Meloidogyne* species. Nježić et al. (2014) have observed no differences on the migration of *M. chitwoodi* in soil columns with tomato or *Tagetes patula* extracts and suggested that *Tagetes* spp. can be used as a trap crop to decrease root-knot nematode population under field conditions. It is important to highlight that *Tagetes* spp. plant extracts may not be as efficient *in vivo* as it is *in vitro* (Tsay et al., 2004) because the compounds present in such extracts need to be photoactivated (Munhoz et al., 2017). Moreover, Marahatta et al. (2012) points out that *Tagetes* spp. are more suppressive when actively growing roots are present. The use of trap crops may be a useful control method by luring nematodes to their root system (Rasmann et al., 2012).

In our experiment, the number of J2s recovered decreased as a function of distance migrated; at distances over 13 cm (Styrofoam cups) approximately an average of 9 and 14 active J2s of *M. incognita* and *M. floridensis*, respectively, were counted in the soil samples regardless of plant stimuli, while 420 and 98 active J2s were recovered in the inoculation ring. Horizontal and vertical migration studies with *Meloidogyne* and *Pratylenchus* spp. point out that most of the inoculated nematodes remain close to the inoculation ring in the short term (Fujimoto et al., 2010; Francilino et al., 2017; Nježić

et al., 2014; Pudasaini et al., 2007; Prot and van Gundy, 1981). By the end of the studies, however, J2s populations were almost evenly distributed within the columns; our data show that an even distribution of J2s of both species would be expected if we have allowed the nematodes to migrate longer than 9 days.

The development of tomato and *Tagetes patula* plants was evaluated through fresh shoot and root weights at 3, 6 and 9 DAI. Although tomato fresh shoot weight was significantly greater than *Tagetes patula* plants, they did not differ over time. Root weights, on the contrary, have increased significantly over time. Root growth is crucial because their exudates were the only chemical cues J2s were exposed to during the experiment, and as roots grow, they release the allelochemicals necessary for nematode migration. When roots stops growing, the attractiveness to nematodes ceases, as it is proportional to the growth rate of the host root system (Prot, 1980). In fact, J2s of *M. floridensis* found inside the roots were positively correlated to the fresh root weight, which means that their migration rate might have been enhanced by continuous root growth during the experiment. On the other hand, the upward migration of *M. incognita* was not correlated to the root weight, suggesting that this species does not need any stimulus to migrate, as observed for *M. chitwoodi* (Pinkerton et al., 1987) and *M. arenaria* (Santos, 1973). These results reinforce the need for further studies about the migration of *Meloidogyne* spp. under different conditions and stimuli to understand their behavior within the soil.

Conclusions

Meloidogyne floridensis and *M. incognita* are able to migrate over 13 cm and parasitize roots of tomato and *Tagetes patula* plant;

M. floridensis and *M. incognita* upward migration occurs regardless of plant stimuli, but *M. floridensis* is more affected by plant stimuli than *M. incognita*;

Although *Tagetes patula* can lure juveniles of *M. floridensis* and *M. incognita* to their rhizosphere, few ones are able to parasitize their roots.

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Temperature: a driving factor for *Meloidogyne floridensis* migration

CAPÍTULO III

Leitão, Diego Arruda Huggins de Sá. Dr. Universidade Federal Rural de Pernambuco. Fevereiro de 2019. Migração ascendente de *Meloidogyne* spp. em função da temperatura e estímulo vegetal. Drª. Elvira Maria Régis Pedrosa.

Temperature: a driving factor for *Meloidogyne floridensis* migration

Abstract: The peach root-knot nematode, *Meloidogyne floridensis*, is an emerging species restrained to Florida state in the US and may become a huge threat to peach growers if measures to avoid contamination and spread are not taken. The influence of temperature and plant stimuli on the vertical migration of second-stage juveniles (J2s) of *M. floridensis* was studied using soil column migration assays. A series of three 4.4-cm-d x 4-cm long PVC rings were taped together on top of one 2-cm long ring (inoculation ring) to assemble the columns and then filled with heat-sterilized sandy soil and kept at 1.2 kg dm^{-3} and 10% water content by weight. On top of the columns a 300 cm^3 Styrofoam cup containing either a 4-week-old tomato or a *Tagetes patula* plant was attached, and host-free cups were used as control. Approximately 1,000±100 freshly hatched J2s were injected into the columns and then transferred to growth chambers at 20 and 26 ºC under a completely randomized block design with four replicates. Nematodes were allowed to migrate for 3, 6, 9 and 12 days after inoculation (DAI) and then the columns were disassembled. Juveniles were extracted from the soil of each ring and cups separately and counted under stereomicroscope. Soil nematode data were subjected to a repeated measure MANOVA, and a chi-square test was performed to compare J2s distribution along the columns according to significant interactions. Roots were stained, and Tukey's multiple comparison test was performed on J2s inside roots. The presence of host stimuli did not improve J2s migration rate as compared to host-free conditions. Temperature influenced the distribution of J2s along the columns, but no effect was observed for juveniles inside host roots. Greater percentages of J2s at distances greater than 13 cm and inside host roots occurred over time, which followed an increase in both shoot and root weights.

Key words: Peach root-knot nematodes, mobility, plant stimuli, *Tagetes patula*

Introduction

Plant-parasitic nematodes are ubiquitous soil-dwelling microorganisms associated to economically important agricultural crops (Bernard et al., 2017) in tropical, subtropical and temperate regions (Brito et al., 2008). Their parasitism may cause considerable yield suppressions, 9-15% of worldwide production (Nicol et al., 2011), thus representing a major constraint for global food security (Coyne et al., 2009). *Meloidogyne* spp. has been recently ranked first among genera of plant-parasitic nematodes that threaten world agriculture (Jones et al., 2013). Also known as root-knot nematodes, *Meloidogyne* spp. contain over 100 species described up to this date (Moreira et al., 2018, Seid et al., 2015).

A new species of root-knot nematode, *Meloidogyne floridensis*, has been recently described parasitizing *M. incognita*- and *M. javanica*-resistant peach rootstocks in Florida (Handoo et al., 2004), thereafter known as peach root-knot nematode. In 2005, the first report of *M. floridensis* naturally parasitizing tomatoes in Florida was published (Church, 2005). Further studies have shown that several other horticultural and weed species are host, like corn and watermelon (Brito et al., 2008, 2010, 2015; Stanley et al., 2009), and non-host, such as *Tagetes* spp. (Kokalis-Burelle and Nyczepir, 2004) to the peach root-knot nematode, indicating that its host range might be different from other *Meloidogyne* spp. (Esfahani, 2009). Currently, the peach root-knot nematode geographical distribution is restrained to some Florida counties, but due to its ability to overcome peach resistance (Smith et al., 2015; Nyczepir and Thomas, 2009), *M. floridensis* is a major emerging species in the US.

In order to parasitize roots, second-stage juveniles (J2s) probe their stylet on the cell walls of a host plant, secreting different proteins and enzymes (Abad et al., 2009). However, before they get inside the roots, freshly-hatched J2s migrate through the porous space of the soil matrix and are subjected to its environmental conditions, some of that reported to influence J2s migration and survival (Prot 1980, Wallace, 1968).

Temperature is one of the main factors that plant-parasitic nematodes face throughout their life cycles (Karssen and Moens, 2013). Each developmental stage has an optimum temperature range (Ferraz and Brown, 2016). Mobility under temperature gradient showed that *M. incognita* moved towards warmer areas (Robinson, 1994). Prot and van Gundy (1981b) reported that *M. incognita* migration reached a maximum at 20 ^{o}C, while *M. hapla* migrated well under lower temperatures, and *M. javanica* migrated

further at 25 ºC (Wallace, 1966). These results imply that there is an optimal temperature range for migration and it is species-specific (Robinson and Perry, 2006).

Researchers have primarily addressed plant-parasitic nematode migration by assessing their chemotactic responses to organic and inorganic stimuli in agar plate experiments (El-Sherif and Mai, 1969; Wallace, 1958c). Later, three-dimensional approaches (i.e. soil columns) were introduced to migration assays, which enhanced the realism of their results to those under field conditions (Spence et al., 2008). Wallace was one of the first researchers to evaluate nematodes' migratory behavior in relation to soil physical attributes (i.e. pore diameter) under various water suctions, and his results highlighted that nematode movement is not directly affected by soil texture but subjected to pore size distribution (Wallace, 1958a-b, 1959a).

Studies that focus on plant-parasitic nematodes migration, specially *Meloidogyne* spp., in column assays under plant stimuli are increasingly gaining notoriety. The rationale behind such soil column assays is to allow J2s to migrate towards host roots for several days under controlled conditions. In general, the vertical migration is determined by counting the number of J2s at different distances from the inoculation point and time intervals observing their distribution along the column length or counting the J2s that were able to penetrate host roots. Prot (1976) have reported that J2s of *M. javanica* were able to migrate 50 cm, both horizontal and vertically, and parasitize tomato plants. Dalzell et al. (2011) reported that *M. incognita* shows a preferential migratory pattern towards tomato when compared to treatments with no stimulus. However, no preferential migration was observed for *M. chitwoodi* in columns with and without tomato as an attractant (Pinkerton et al, 1987).

Although experimental assays have broadened our knowledge on nematode migratory abilities, little is known about *M. floridensis* behavior within the soil. Therefore, the aim of this research was to study how temperature and plant stimuli (tomato and *Tagetes patula*) influence the peach root-knot nematode migration over time. We hypothesized that: i) high temperature will increase *M. floridensis* migration, ii) migration patterns will be different under each stimulus, and iii) *M. floridensis* will be able to migrate and parasitize tomato (attractive host) but will be repelled by *Tagetes patula* root exudates.

Material and Methods

Nematode inoculum

Meloidogyne floridensis populations were maintained on tomato (*Solanum lycopersicum* cv. Cobra) grown in sandy soil-filled clay pots in the greenhouses at the University of Florida, Gainesville, FL. Sixty-day old plants were selected, and their roots were taken out of the pots, washed free of debris with tap water and chopped into 2-cm long pieces. A 0.52% NaOCl solution was added together with the root pieces to a metal blender for grinding for 20 s. The blended solution was then poured over a combination of 200-mesh and 500-mesh sieves and washed thoroughly with tap water for 3 min to remove NaOCl excess (Hussey and Barker, 1973). The egg solution was poured into a modified Baermann funnel at 27 °C in 2-ply "Kleenex" tissue paper (Rodríguez-Kábana and Pope, 1981). The second-stage juveniles of *M. floridensis* (J2s) which hatched during the first 24 h were discarded. For the migration assays, freshly hatched J2s were collected for 72 h and kept under refrigeration until the beginning of the experiment.

Tomato and *Tagetes patula* plants

Tomato and *Tagetes patula* (variety "Petite") seeds were sown into vermiculite on seedling trays and kept under greenhouse conditions. One seed per cell of each plant species was sown on separate seedling trays. Four-week-old tomato and *Tagetes patula* plants were used in the migration assay.

Experimental apparatus

The migration of *M. floridensis* was evaluated using polyvinylchloride (PVC) rings taped together to assemble segmented columns (Fig. 1). Each column consisted of one 2-cm long ring (inoculation ring), three 4-cm long rings, and a Styrofoam cup on the upper end. A hole was drilled 1 cm above the base of the inoculation ring in order to inject J2s into the experimental apparatus. Each column was 14-cm long with 4.4-cm internal diameter and 213-cm^3 internal volume. Candler sand (96% sand, 2% silt, 2% clay, 0.27% organic matter) collected from a peanut field in Levy County, FL, was heat-

sterilized and then used to fill the columns at 1.2 Kg dm^{-3} soil bulk density and 10% water content by weight, which were observed at field conditions. A 15-µm nylon mesh was used to cover the inoculation ring to avoid juvenile's dispersion out of the apparatus. Right after assembling the columns, a parafilm sheet was used to prevent evaporative water loss until the beginning of the experiment.

A bottomless Styrofoam cup containing approximately 300 g of the same Candler soil at 10% water content with either one tomato (good host) or one *Tagetes patula* (non-host) plant was taped to the top of the columns; host-free cups were used as control. A 35-µm mesh covered the base of the cups (Prot, 1976) in order to inhibit root growth into the columns whilst allowing juveniles migration. The fully assembled columns were placed inside growth chambers and kept at 20 and 26 °C with a photoperiod regime of 16h light/8h dark for 24 h prior to J2s inoculation. The growth chamber conditions were maintained throughout the experiment.

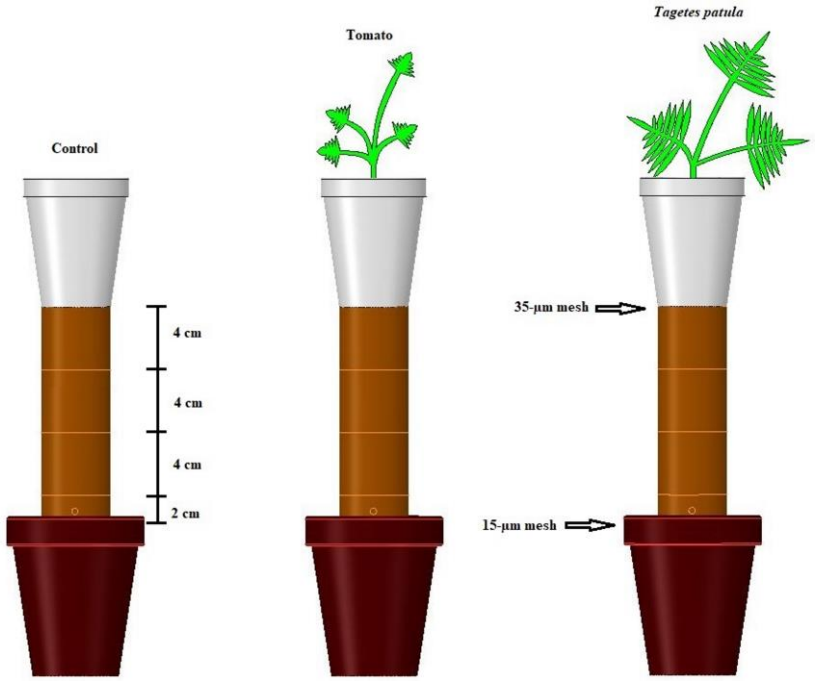

Figure 1. Experimental apparatus used in vertical migration assay of second-stage juveniles of *Meloidogyne floridensis*. Column consists of three 4.4-cm-d x 4-cm long rings taped together to one 2-cm long ring filled with sandy soil. Four-week-old tomato or *Tagetes patula* plants were transplanted to Styrofoam cups and taped to the upper end of the columns – cups with no plants were used as control. Freshly hatched juveniles were injected through an inoculation port, 1 cm above the base of the column.

Experiment conduction

The experiment was carried out in a completely randomized block design with four replicates. Juveniles were collected from modified Baermann funnels and counted under a stereomicroscope (100× magnification). The suspension was adjusted to 500 ± 50 J2 ml^{-1}. Freshly hatched mobile J2s (*ca.* 1,000 ± 100) were injected into the columns through the inoculation port; the holes were sealed with water-proof tape right after. No water was added during the first 24 h to prevent nematode percolation. Columns were watered daily to maintain the soil water content at 10% by weight; the amount of water added was equal to that lost by evapotranspiration (Pudasaini et al., 2007). To avoid temperature fluctuation inside the columns during and after daily irrigation, a water bottle was kept inside both growth chambers.

The columns were disassembled 3, 6, 9 and 12 days after inoculation (DAI) and soil from each ring and the Styrofoam cup was used to extract nematodes by centrifugal-flotation technique (Jenkins, 1964). After each extraction the nylon meshes (35 and 15 µm) were checked under a stereomicroscope for trapped J2s. Percentages of recovered and active J2s for each section of the column was determined. The root systems of tomato and *Tagetes patula* plants were washed free of debris with tap water and stained with acid fuchsin (Byrd et al., 1983) in order to evaluate the juveniles' penetration, additionally fresh shoot and root weights were determined.

Statistical Analysis

Nematode data were $\sqrt{x+0.5}$-transformed prior to statistical analysis to meet MANOVA assumptions. The effects of plant stimuli, distance migrated (section), temperature and time on *M. floridensis* vertical migration were analyzed through a repeated measures MANOVA. A chi-square (X^2) test was performed to compare the distribution of J2s along the columns for significant effects and interactions; while Tukey's multiple comparison test was used for J2s inside the roots. Additionally, Pearson's correlation coefficients between plant variables and nematodes inside roots were also determined. All statistical analyses were performed in RStudio environment (RStudio Team, 2015).

Results

The presence of tomato or *Tagetes patula* roots did not improve the migration rate of J2s when compared to control treatments ($P \geq 0.05$). The main effects of temperature, distance migrated (section) and time were significant on the vertical migration of recovered J2s ($P < 0.0001$), as well as the interaction among time, temperature and section ($P < 0.05$). The distribution of active J2s along soil columns was significantly influenced by the interactions of time and temperature ($P < 0.05$) and time and section ($P < 0.0001$, Table 1). No J2s were found trapped in either nylon meshes.

Table 1. Repeated measure MANOVA summary of the effects of plant stimulus, distance migrated (section), temperature and time on vertical migration of recovered and active second-stage juveniles (J2s) of *Meloidogyne floridensis* along PVC columns filled with sandy soil.

		Recovered J2s				Active J2s			
Source	df	SS	MS	F	p-value	SS	MS	F	p-value
Block	3	166.05	55.35	20.22	<0.0001	167.94	55.98	22.24	<0.0001
Temperature (Temp)	1	60.22	60.22	22.00	<0.0001	79.11	79.11	31.43	<0.0001
Stimulus (Stim)	2	6.72	3.36	1.23	0.2941	2.38	1.19	0.47	0.6248
Section (Sec)	4	966.36	241.59	88.26	<0.0001	696.60	174.15	69.18	<0.0001
Temp:Stim	2	1.42	0.71	0.26	0.7713	0.44	0.22	0.09	0.9160
Temp:Sec	4	13.32	3.33	1.22	0.3038	10.88	2.72	1.08	0.3650
Stim:Sec	8	15.04	1.88	0.69	0.7030	11.52	1.44	0.57	0.8012
Temp:Stim:Sec	8	33.20	4.15	1.52	0.1497	32.56	4.07	1.62	0.1181
Time	3	41.46	13.82	5.05	0.0019	64.92	21.64	8.60	<0.0001
Time:Temp	3	11.82	3.94	1.44	0.2308	28.26	9.42	3.74	0.0114
Time:Stim	6	18.66	3.11	1.14	0.3413	31.62	5.27	2.10	0.0532
Time:Sec	12	271.08	22.59	8.25	<0.0001	199.32	16.61	6.60	<0.0001
Time:Temp:Stim	6	17.70	2.95	1.08	0.3753	9.18	1.53	0.61	0.7235
Time:Temp:Sec	12	64.56	5.38	1.96	0.0266	39.36	3.28	1.31	0.2134
Time:Stim:Sec	24	72.00	3.00	1.10	0.3444	50.40	2.10	0.83	0.6935
Time:Temp:Stim:Sec	24	56.40	2.35	0.86	0.6606	62.40	2.60	1.03	0.4217

df: Degree of freedom; SS: Sum of squares; MS: Mean square, *P*>F: Significance level of F test

At 3 DAI, more than 50% of recovered J2s were found at the inoculation ring regardless of the temperature; however none were observed above 9 cm at 26 ºC, while less than 1% reached the top of the columns at 20 ºC (Fig. 2A). At 6 DAI, J2s still concentrated at the inoculation ring, but *ca.* 8% were found at 9-13 cm at 26 ºC; the percentage of recovered J2s at 20 ºC decreased at the inoculation ring and became more

or less evenly distributed from 1-13 cm (Fig. 2B). There was a significant increase (10%) on the percentage of J2s migrating more than 13 cm at 20 °C at 9 DAI, whereas J2s were still concentrated within the first 9 cm of the columns at 26 °C (Fig. 2C). By the end of the experiment, the distributions of recovered J2s were uniformly distributed along the columns, with similar recovery rates at distances of 9-13 and <13 cm at both temperatures (Fig. 2D).

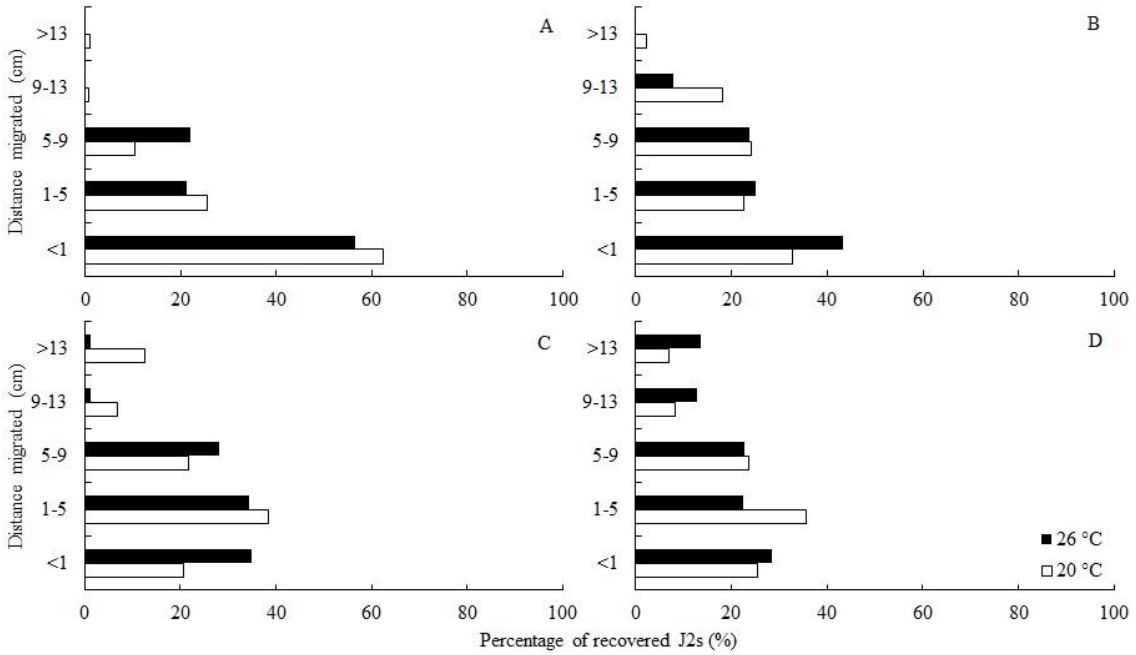

Figure 2. Distribution of recovered second-stage juveniles (J2s) of *Meloidogyne floridensis* as a function of distance migrated and temperature at 3 (A), 6 (B), 9 (C) and 12 (D) days after inoculation. Nematode distribution along the column (4-cm long rings taped together) over time and at both temperatures was statistically different according to X^2 test ($P < 0.01$).

At 20 ºC, the percentage of J2s recovered from the soil decreased from 27% to 21% at 3 and 12 DAI, respectively; whereas, there was a 30% reduction on the percentage of recovered J2s at 26 ºC. At 3 and 6 DAI a greater percentage of J2s were recovered from columns at 26 ºC, however this behavior changed from 9 DAI onward, with 21% and 8% of active J2s recovered at 20 and 26 ºC, respectively, at 12 DAI. (Fig. 3).

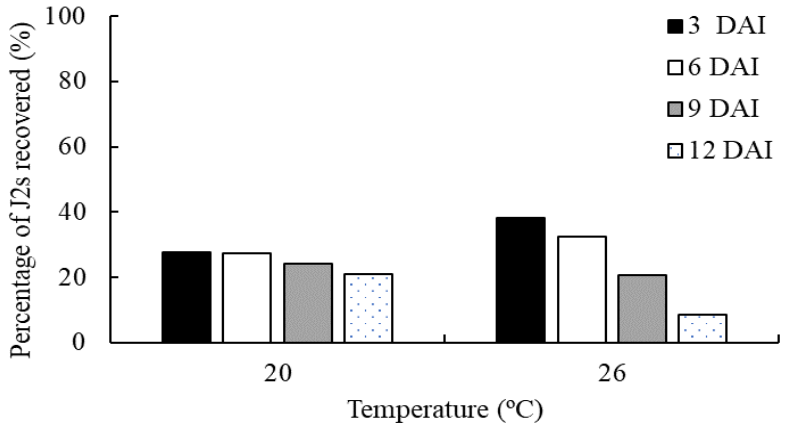

Figure 3. Percentage of active second-stage juveniles (J2s) of *Meloidogyne floridensis* recovered at 3, 6, 9 and 12 days after inoculation (DAI) at 20 and 26 ºC from sandy soil PVC columns, composed of 4-cm long x 4.4-cm internal diameter rings. Nematode distribution over time and at both temperatures was statistically different according to X^2 test ($P < 0.01$).

The distribution pattern of active J2s along the soil columns changed over time. At 3 DAI, more than 50% was recovered from the inoculation ring, while the remaining were concentrated at distance of 1-9 cm; only 1% of active J2s were able to migrate more than 9 cm. At 6 DAI, 26 to 28% of J2s were recovered from the first three rings, indicating a progressive and even vertical migration along the first 9 cm; even so, only 1.4% were able to reach the upper end of the columns. A turning point occurred at 9 DAI, where *ca.* 40% of active J2s migrated 1-5 cm and 7% were recovered from the soil inside the Styrofoam cup, which means a migration of more than 13 cm. A similar behavior was observed at 12 DAI, with almost 9% of MfJ2s found at the cups (Fig. 4).

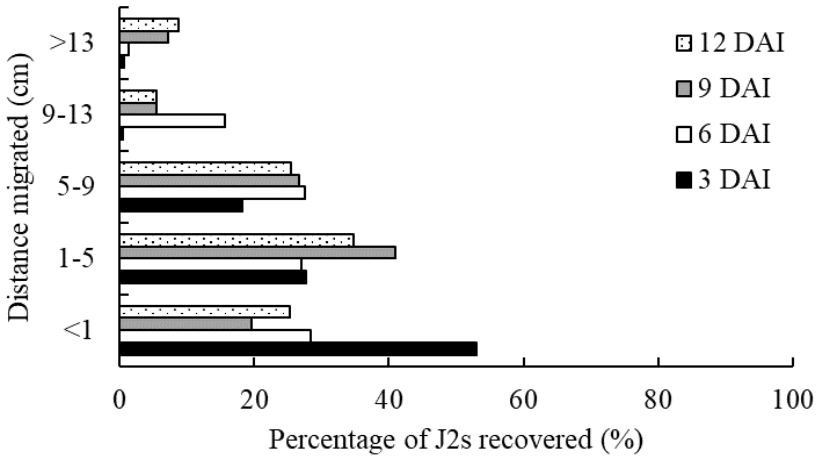

Figure 4. Distribution of active second-stage juveniles (J2s) of *Meloidogyne floridensis* as a function of distance migrated at 3, 6, 9 and 12 days after inoculation (DAI). Nematode distribution along the columns (4-cm long rings taped together) over time was statistically different according to X^2 test ($P < 0.01$).

There was no effect of temperature on plant variables or penetration of J2s ($P > 0.05$), nor significant interactions between any of the factors ($P > 0.05$, Table 2). The plants of tomato and *Tagetes patula* showed significantly different shoot weight ($P < 0.0001$) and it was affected by time ($P < 0.01$). The effect of time was also observed for root weight ($P < 0.0001$). The penetration of J2s was significantly different under tomato and *Tagetes patula* plants ($P < 0.01$) and it also differed over time ($P < 0.0001$, Table 2).

Table 2. Repeated measures MANOVA summary of the effects of temperature and plant stimuli on penetration of second-stage juveniles (J2s) of *Meloidogyne floridensis* over time.

		J2s inside roots			
Source	df	SS	MS	F	*P*>F
Block	3	14.60	4.87	5.18	0.0037
Temperature (Temp)	1	0.19	0.19	0.20	0.6556
Stimulus (Stim)	1	14.62	14.62	15.57	0.0003
Temp*Stim	1	2.26	2.26	2.41	0.1279
Time	3	27.89	9.30	9.90	<0.0001
Time*Temp	3	3.05	1.02	1.08	0.3663
Time*Stim	3	5.97	1.99	2.12	0.1111
Time*Temp*Stim	3	0.63	0.21	0.22	0.8800

df: Degree of freedom; SS: Sum of squares; MS: Mean square; *P*>F: Significance level of F test

Although there was no addition of fertilizers throughout the experiment, root weight increased over time, reaching a maximum of 6.69 g at 12 DAI, which significantly differed from the values at 3 and 6 DAI. J2s were able to parasitize both plant species, however a greater number of J2s were observed inside tomato roots (average of 7 J2s/root system), whereas less than 2 J2s, on average, were counted inside *Tagetes patula* roots (Fig. 5A); no further development was observed inside *Tagetes patula* roots (data not shown). Regarding their penetration over time, less than 0.1% of J2s were able to migrate >13 cm through the column and parasitize roots regardless of the stimulus and temperature at 3 DAI, while a peak of approximately 9 J2s inside roots were observed at 9 DAI (Fig. 5B).

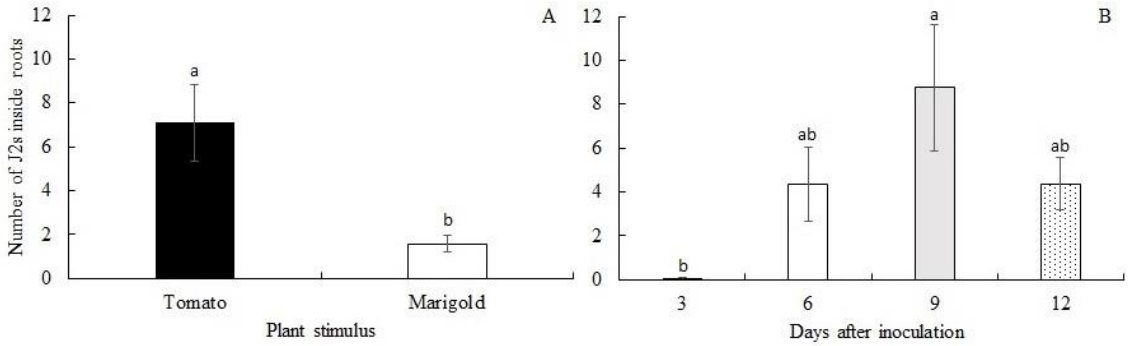

Figure 5. Penetration of second-stage juveniles (J2s) of *Meloidogyne floridensis* on tomato and *Tagetes patula* plants (A) and over time (B) after vertically migrating over 13 cm in sandy soil-filled PVC columns. Figures followed by the same letter do not differ significantly according to Tukey's multiple comparison test ($P \geq 0.05$).

Discussion

Chemotaxis is reported to be the main component of the host-finding ability of J2s of plant-parasitic nematodes (Reynolds et al., 2011), thus it is a key factor to completing their life cycle. Right after hatching, J2s display random movements until a chemical attractant is perceived by their sensory organs (Curtis, 2008; Haegeman et al., 2012; Perry, 1996; Rasmann et al., 2012). From then on, they will show coordinated movement towards the attractant source (i.e. host plant roots) (Bilgrami and Gaugler, 2004) or away from unfavorable conditions (Wang et al., 2018), which is defined as migration (Robinson and Perry, 2006).

Preferential migration of *Meloidogyne incognita* and *M. javanica* in soil columns towards suitable hosts was previously reported (Dalzell et al., 2011; Prot, 1976); however, the migration rate of *M. chitwoodi* under host-free conditions was not different from those under tomato plants (Pinkerton et al., 1987). Similarly, the presence of tomato or *Tagetes patula* plants on the top of the columns did not contributed significantly to the vertical migration of *M. floridensis* J2s compared to control treatments in our experiment. Feltham and Chaplain (2000) described a migration model for nematodes in restricted domains and pointed out that nematodes may present a biased-random motion strategy, that is, juveniles would migrate with a bias towards a host root while simultaneously searching for better food sources. This assumption might explain the results obtained in our experiment.

Although the great majority of J2s were recovered near or at the inoculation ring, the percentage of J2s at distances greater than 13 cm significantly increased over time, with less than 1% and 9% of total active J2s found at 3 and 12 DAI, respectively. Some studies have reported greater recovery rates of juveniles near the source of plant

stimuli. Prot (1976) has observed that 20% of inoculated juveniles of *M. javanica* were able to migrate 50 cm in soil columns with 1.2-cm d rings. Dalzell et al. (2011) reported a recovery rate of nearly 80% of *M. incognita* near tomato rhizosphere in a pipette-bulb assay. On the other hand, Pinkerton et al. (1987) mentioned that less than 0.1% of inoculated juveniles of *M. chitwoodi* and *M. hapla* were able to move 45 cm in soil columns with 8.5-cm d rings. Such different findings might be related to the apparatus dimensions and/or soil physical attributes.

Under natural conditions, the movement of nematodes takes place within an infinite domain (Feltham et al., 2002). *Rotylenchulus reniformis* individuals were able to rapidly colonize a cotton field by migrating to adjacent rows because of lateral root expansion (Moore et al., 2010). However, the evaluation of nematode migratory ability under laboratory conditions occurs in bounded domains and without root growth into the system. Although soil columns have introduced a 3D aspect to migration assays, the dimensional restraint imposed by such columns favors vertical rather than horizontal migration (Spence et a., 2008); therefore, the smaller the internal diameter of the apparatus, the greater the upward migration of juveniles. According to Hunt et al. (2001), the differences on experimental apparatus and conditions for nematode migration assays make it difficult to create a general model to describe nematode migration within the soil.

The classic experiments of Wallace (1958a-c, 1959a-b) have demonstrated that the movement of different plant-parasitic nematodes is dependent on soil attributes, such as pore space and water content. Since nematodes are unable to significantly displace soil particles with their heads, pores smaller than the nematode's body width will hinder their migration (Wallace, 1968). Sandy soils are thought to improve nematode migration (Gallardo et al., 2015) because they have wider pores (Rinaldi et al., 2014). Juveniles of *M. incognita* were unable to migrate in soils with high clay and silt contents (Prot and van Gundy, 1981a), even with water flow (Fujimoto et al., 2009, 2010). These results imply that there is an optimal clay(+silt) content for nematode migration. As a matter of fact, Prot and van Gundy (1981a) have evaluated the migration of *M. incognita* under increasing clay percentages and observed a greater migration rate after adding 5% of clay to pure sand. A model created by Hunt et al. (2001) predicted a faster movement of nematodes in soils with intermediate textures (less than 80% and 30% of sand and clay, respectively).

While moving within the soil, plant-parasitic nematodes are subjected to environmental changes, including temperature oscillations. Several studies have reported that temperature highly influences the migration distance of plant-parasitic juveniles, and when a gradient is present, they show thermotaxis (Dusenbery, 1988) and tend to migrate towards the warmer end (Robinson, 1994). Prot and van Gundy (1981b) have observed maximum values of *M. incognita* vertical migration at 20 ºC, while *M. chitwoodi* and *M. hapla* migrated furthest at 18 ºC (Pinkerton et al., 1987) and *M. javanica* at 25 ºC (Wallace, 1966). Migratory endoparasitic nematodes have shown similar behavior, i.e. *Pratylenchus penetrans* migration was better at 21 ºC (Pudasaini et al., 2007).

In our experiment, temperature did not influence the penetration of *M. floridensis* J2s since they are protected from environmental stress when inside healthy host roots (Adam et al., 2014; McSorley, 2003). However, the recovery rate of active J2s from the soil decreased faster at 26 ºC, suggesting penetration of juveniles into tomato or *Tagetes patula* roots or a greater mortality/inactivity rate of *M. floridensis* J2s. In fact, high temperature may be lethal to nematodes even if exposed for a short period of time (Wallace, 1966). As nematodes are poikilothermic animals (Pudasaini et al., 2007), juveniles metabolism increases concomitantly with temperature and their consumption of lipids reserves also increases (Das et al., 2011; Rocha et al., 2016).

Concomitantly to nematode migration, the development of tomato and *Tagetes patula* plants was evaluated by measuring fresh shoot and root weights over time, and both plants were able to develop regardless of temperature with gain in weight by the end of the experiment. Because there was no addition of fertilizers, the only signaling cues were derived from root exudation, which is correlated to root growth and in turn to the attractiveness to plant-parasitic nematodes (Prot, 1980).

Tomato is considered a universal host for several species of *Meloidogyne* (Seid et al., 2015), including *M. floridensis* (Brito et al., 2015). However, Cetintas et al. (2007) reported that *M. floridensis* J2s showed significant infectivity levels on tomato cv. Florida 47, whereas they were not pathogenic on cv. Solar Set. The authors have ruled out low inoculum viability because J2s produced high galling index on tomato cv. Solar Set grown in clay pots. An average of 7 J2s of *M. floridensis* were found inside tomato (cv. Cobra) roots in our experiment, which is comparable to the results observed in microplots experiments using tomato cv. Solar Set (Cetintas et al., 2007). Other tomato cultivars have been reported as susceptible (cv. Rutgers and Talladega) or

resistant (cv. Crista) to *M. floridensis* (Stanley et al., 2009). The rationale behind the discrepancies on host status of different tomato cultivars to *M. floridensis* is still unknown and should be studied in order to improve peach root-knot nematode management at field scale.

Although we have hypothesized that J2s of *M. floridensis* would be repelled by *Tagetes patula* exudates, a few J2s were able to penetrate their roots but did not show further development, reinforcing *T. patula* non-host status (Kokalis-Burelle and Nyczepir, 2004). The chemotactic responses of active J2s of plant-parasitic nematodes lead them to the vicinity of host root tips or away from non-host plants (Wang et al., 2018). Nematode's host-finding ability is a complex mechanism that rely on a blend of attractant and repellent compounds exuded by the same plant species (Chaisson and Hallem, 2012; Dutta et al., 2011). Different concentrations of extracts from tomato (Diez and Dusenbery, 1989) and cucumber (Castro et al., 1989b) have shown both attractive and repellent properties to *Meloidogyne* spp.

The behavior towards host roots may be species-specific, for example narrow host range *Meloidogyne* spp. (i.e. *M. graminicola*) might respond differently to chemical cues from root exudates when compared to broader host range species, like *M. incognita* (Reynolds et al., 2011). This behavior was also reported for migratory endoparasitic nematodes. The vertical migration of individuals of *P. penetrans* was evaluated under different hosts and column lengths, where a preferential movement under good hosts (maize and bean) was observed regardless of the distance between host and inoculation point (Pudasaini et al., 2007), while *P. coffeae* was highly attracted to yam baits and repelled by *Tagetes patula* chopped roots incorporated into one end of the soil column (Francilino et al., 2017). Further studies need to focus on understanding how plant-parasitic nematodes perceive exudate compounds as repellent or attractive from a given host (Dutta et al., 2012).

Conclusions

Temperature directly affects the migration of *M. floridensis* J2s within the soil, but has no influence on J2s after penetration on host tissue;

M. floridensis J2s can migrate great distances (>13 cm) regardless of plant stimuli due to their random movement during their host-finding phase;

Tagetes patula plants did not repel J2s of *M. floridensis* nor hinder their migration.

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Conclusões gerais

CAPÍTULO IV

Juvenis de segundo estádio de *Meloidogyne floridensis* e *M. incognita* são capazes de migrar longas distâncias sem a presença de plantas hospedeiras, ainda que em baixas percentagens;

Sob as mesmas condições, *M. floridensis* mostrou-se mais ágil que *M. incognita*;

A temperatura de 26ºC apresentou-se como fator determinante para a migração de *M. floridensis* sob três estímulos vegetais diferentes ao longo do perfil das colunas de solo;

A presença de *Tagetes patula* não repeliu os juvenis de *M. floridensis* e *M. incognita*, porém poucos penetraram as raízes.