DANIELE DE BRUM

EARLY DETECTION OF *Meloidogyne exigua* AND BACTERIA DIVERSITIES ASSOCIATED TO EGG MASSES OF THE PATHOGEN IN COFFEE

LAVRAS – MG

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DANIELE DE BRUM

DETECÇÃO PRECOCE DE *Meloidogyne exigua* E DIVERSIDADE DE BACTÉRIAS NA MASSA DE OVOS DO PATÓGENO EM CAFÉ

EARLY DETECTION OF *Meloidogyne exigua* AND BACTERIA DIVERSITIES ASSOCIATED TO EGG MASSES OF THE PATHOGEN IN COFFEE

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LAVRAS –MG
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À minha mãe Beatriz, à meu irmão Dionatan, à meus sobrinhos Isadora e Matheus e à meu marido Marco

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"preparem-se sofredores do mundo o tempo não é linear..."

(Valter Hugo Mãe)

"Se as coisas são inatingíveis...ora!
Não é motivo para não querê-las
Que tristes os caminhos, se não fora
A presença distante das estrelas"

(Mario Quintana)

RESUMO GERAL

Nematoides do genero *Meloidogyne* causam severos danos a cultura do cafeeiro. Dentre as espécies, *Meloidogyne exigua* esta amplamente disseminada nas lavouras brasileiras. Dentro de um planejamento de manejo integrado da doença, a detecção precoce do nematoide é um dos maiores desafios para os produtores. Além disto, o controle biológico é um dos pilares no manejo integrado e pesquisas envolvendo interações entre o patógeno e microrganismos que habitam a massa de ovos do nematoide são essenciais. Assim, o objetivo deste trabalho foi: desvendar as bactérias que habitam a massa de ovos de *M. exigua,* em plantas de café sintomático e assintomático quanto ao ataque do nematoide, atraves de sequenciamento do gene 16S; e, avaliar o potencial do sensoriamento remoto como ferramenta para o diagnóstico precoce de *M. exigua,* por meio de imagens multiespectrais coletadas com drone em dois períodos do ano (maio – estação seca, outubro –estação chuvosa)**.** Foi constatado diferenças significativas na composição bacteriana e diversidade nas massas de ovos de plantas sintomáticas em comparação com as assintomáticas. As famílias *Pseudomonadaceae, Burkholderiaceae, Flavobacteriaceae, Rhizobiaceae, Micrococcaceae* e *Bacteroidaceae* foram mais abundantes nas amostras assintomáticas enquanto *Chitinopagaceae, Glycomycetaceae, Micropepsaceae, Beijerinckiaceae* e *Enterococcaceae* foram mais abundantes nas sintomáticas. Os gêneros *Pseudomonas, Sphingobacterium, Flavobacterium, Corynebacterium* e *Virgibacillus* foram encontrados em maior abundância nas amostras assintomáticas, enquanto apenas *Tumebacillus* e *Bacillus* foram significativos nas amostras sintomáticas. A qualidade do inóculo das massas de ovos utilizadas nos ensaios foi testada em tomateiros quanto à infectividade e reprodução. Ovos por grama de raízes foram significativamente reduzidos pelo inóculo de cafeeiros assintomáticos em comparação com os sintomáticos, enquanto o peso da raiz foi significativamente maior em tomates infectados com inóculo de cafeeiros assintomáticos em comparação com os sintomáticos. Apartir dos resultados obtidos no trabalho com sensoriamento remoto, foi verificado que os parâmetros de imagem investigados demonstraram eficácia na detecção da infestação por *M. exigua* em lavoura de café durante os dois períodos distintos do ano. Em ambos os períodos foi observada correlação positiva e estatisticamente significativa entre o número de galhas do nematóide e a faixa vermelha. O índice de vegetação GNDVI apresentou correlação moderada e negativa com o número de galhas de nematóides, e o índice NGRDI apresentou forte correlação negativa com o número de galhas de nematóides em ambos os períodos do ano avaliados. O GNDVI foi fortemente correlacionado negativamente com o número de galhas do nematoide durante o período seco. Sob a perspectiva de inovação, esta pesquisa destaca-se por trazer novas informações relevantes a cerca de bactérias que habitam a massa de ovos de *M. exigua* e o potencial do sensoriamento remoto para detectar o patógeno no campo.

Palavras-chave: nematoide das galhas. *Coffea arabica*. Microbioma. Sequenciamento. Drone. Sensoriamento remoto.

ABSTRACT

Nematodes of the *Meloidogyne* genus cause severe damage to coffee crops. Among the species, *Meloidogyne exigua* is widely disseminated in Brazilian crops. Within integrated disease management planning, early detection of the nematode is one of the biggest challenges for producers. Furthermore, biological control is one of the pillars in integrated management and research involving interactions between the pathogen and microorganisms that inhabit the nematode egg mass are essential. Thus, the objective of this work was: to reveal the bacteria that inhabit the egg mass of *M. exigua*, in symptomatic and asymptomatic coffee plants regarding nematode attack, through sequencing of the 16S gene; and, evaluate the potential of remote sensing as a tool for the early diagnosis of *M. exigua*, through multispectral images collected with a remotely piloted aircraft (RPA) in two periods of the year (May – dry season, October – rainy season). Significant differences were found in the bacterial composition and diversity in the egg masses of symptomatic plants compared to asymptomatic ones. The families *Pseudomonadaceae*, *Burkholderiaceae, Flavobacteriaceae, Rhizobiaceae*, *Micrococcaceae* and *Bacteroidaceae* were more abundant in asymptomatic samples while *Chitinopagaceae, Glycomycetaceae, Micropepsaceae, Beijerinckiaceae* and *Enterococcaceae* were more abundant in symptomatic ones. The genera *Pseudomonas*, *Sphingobacterium, Flavobacterium, Corynebacterium* and *Virgibacillus* were found in greater abundance in asymptomatic samples, while only *Tumebacillus* and *Bacillus* were significant in symptomatic samples. The quality of the inoculum of the egg masses used in the trials was tested on tomato plants for infectivity and reproduction. Eggs per gram of roots were significantly reduced by inoculum from asymptomatic compared to symptomatic coffee plants, while root weight was significantly greater in tomatoes infected with inoculum from asymptomatic compared to symptomatic coffee plants. Based on the results obtained in the work with remote sensing, it was verified that the image parameters investigated demonstrated effectiveness in detecting infestation by *M. exigua* in coffee crops during the two different periods of the year. In both periods, a positive and statistically significant correlation was observed between the number of nematode galls and the red band. The GNDVI vegetation index showed a moderate and negative correlation with the number of nematode galls, and the NGRDI index showed a strong negative correlation with the number of nematode galls in both periods of the year evaluated. GNDVI was strongly negatively correlated with the number of nematode galls during the dry period. From the perspective of innovation, this research stands out for bringing new relevant information about bacteria that inhabit the *M. exigua* egg mass and the potential of remote sensing to detect the pathogen in the field.

Keywords: Plant parasitic nematodes. *Coffea arabica*. Microbiome. Sequencing. Drone. Remote sensing.

INDICADORES DE IMPACTO

No trabalho de tese desenvolvido, os autores estudaram bactérias associadas ao nematoide parasita de plantas de café, conhecido popularmente por nematoide das galhas, nome científico *Meloidogyne exigua*. Este nematoide causa prejuízos econômicos por reduzir a produtividade das lavouras cafeeiras e, consequentemente, impactar o desenvolvimento socioeconômico da comunidade onde as áreas estão localizadas. Neste sentido, o objetivo do trabalho foi conhecer quais bactérias estavam associadas a massa de ovos produzidas pelo nematoide, *M exigua* e, compreender se as bactérias poderiam interferir na reprodução e infecção do nematoide. Além disto, estudou-se a possibilidade de identififar, precocemente, a presença de *M. exigua* em área infestada com o nematoide através de imagens aéreas coletadas com drone. No trabalho foram identificadas bactérias relacionadas ao controle biológico de doenças de plantas e a promoção de crescimento vegetal. Os resultados demonstram o potencial impacto tecnológico visando o manejo sustentável de nematoides no cafeeiro com a possibilidade, por exemplo, de desenvolver produtos para o controle biológico. Além disto, foi possível correlacionar o número de galhas de *M.exigua* com índices de vegetação criados a partir das imagens de cafeeiro infectado com nematoide. Por contribuir com a detecção precoce do nematoide, o trabalho de tese desenvolvido mostra as possibilidades para que o manejo do patógeno seja feito antes que ocorra infestação na área total, reduzindo tanto o número de entrada de máquinas na lavoura quanto a aplicação de produtos, gerando impactos econômicos, tecnológicos e ambientais. Além do envolvimento da discente de doutorado, docentes e técnicos do programa de pós-graduação em fitopatologia/UFLA, o trabalho também contribuiu com a formação de uma aluna de iniciação científica do curso de Agronomia da UFLA, e permitiu o intercâmbio internacional da doutoranda, por 8 meses, na Inglaterra. Os impactos da tese estão alinhados aos objetivos de desenvolvimento sustentável (ODS) da ONU, número 2 e número 12 pelo potencial ao desenvolvimento da agricultura, produção e consumo mais sustentável.

IMPACT INDICATORS

During the PhD thesis, the authors studied bacteria associated with the coffee plant parasitic nematode, popularly known as root-knot nematode, scientific name *Meloidogyne exigua*. This nematode causes economic losses by reducing the productivity of coffee plants and, consequently, impacting the socioeconomic development of the community where the areas are located. In this sense, the objective of the work was to identify which bacteria were associated with the egg mass produced by the nematode, *M. exigua*, and to understand whether the bacteria could interfere in the reproduction and infection of the nematode. In addition, the possibility of early identification of the presence of *M. exigua* in an area infested with the nematode through aerial images collected with a drone was studied. In the work, bacteria related to the biological control of plant diseases and the promotion of plant growth were identified. The results demonstrate the potential technological impact aimed at the sustainable management of nematodes in coffee plants with the possibility, for example, of developing products for biological control. Furthermore, it was possible to correlate the number of *M. exigua* galls with vegetation indexes created from images of coffee trees infected with nematodes. By contributing to the early detection of nematodes, the thesis work developed shows the possibilities for managing the pathogen before infestation occurs in the entire area, reducing both the number of machines entering the crop and the application of products, generating economic, technological and environmental impacts. In addition to the involvement of the doctoral student, professors and technicians from the plant pathology postgraduate program at UFLA, the work also contributed to the training of a scientific initiation student in the Agronomy course at UFLA, and allowed the doctoral student to spend 8 months abroad in England. The impacts of the thesis are aligned with the ONU sustainable development goals, numbers 2 and 12, due to the potential for the development of sustainable agriculture, production and consumption.

SUMÁRIO

1. INTRODUÇÃO GERAL 14
REFERÊNCIAS 17
ARTIGO 1 19
1. Introduction 21
2. Metodology 23
2.1 Study area 23
2.2 Soil and root sampling 24
2.3 Extraction of Meloidogyne exigua egg masses from Coffea arabica roots 24
2.4 DNA Extraction and Polymerase Chain Reaction 25
2.5 Bioinformatics Analysis 26
2.6 Greenhouse experiments for infectivity and reproduction of M. exigua in tomato plants 26
2.7 Statistical analysis 27
3. Results 28
3.1 Physical and chemical soil properties 28
3.2 Bacterial diversity and abundance in egg masses and rhizosphere soil from symptomatic and asymptomatic coffee roots infected with M. exigua 28
3.3 Infectivity and reproduction of Meloidogyne exigua 33
4. Discussion 33
References 37
ARTIGO 2 42
5. INTRODUCTION 43
6. MATERIAL AND METHODS 45
6.1 Study site and soil sampling 45
6.2 Meteorological data 46
6.3 Field data collection 46
6.4 Aerial data collection 48
6.5 Image processing 49
6.6 Statistics and data analysis 50
7. RESULTS 50
7.1 Number of nematode galls and the temperature of coffee plants in two seasons of the year 50

7.2 Correlations between the number of nematode galls and the spectral and textural characteristics of images of coffee plants captured during two seasons 51
8. DISCUSSION 55
9. CONCLUSIONS 59
REFERENCES 61
CONSIDERAÇÕES FINAIS 63

1. INTRODUÇÃO GERAL

Nematoides são os animais mais abundantes da Terra, sendo encontrados em diversos ambientes, onde desempenham inúmeras funções (TOPALOVIĆ; HUSSAIN; HEUER, 2020; VAN DEN HOOGEN et al., 2019). Ao interagirem com as plantas, muitos nematoides tornaram-se patogênicos a elas (EVES-VAN DEN AKKER, 2021). Esse grupo de fitoparasitas que compreende 15% dos nematoides descritos são chamados de fitonematoides ou nematoides fitoparasitas.

Os nematoides pertencentes ao gênero *Meloidogyne*, popularmente conhecidos como nematoides de galhas, constituem o grupo de fitonematoides de maior importância econômica, pois, anualmente, são responsáveis por causar perdas estimadas em US$ 173 bilhões (ELLING, 2013; MOENS; PERRY; STARR, 2009).

Meloidogyne exigua Goeldi, 1887, é a espécie tipo do gênero *Meloidogyne* e foi descrita primeiramente no Brasil, em lavouras de café, no Rio de Janeiro, na segunda metade do século XIX (CAMPOS; VILLAIN, 2005; GOELDI, 1887). No Brasil, *M. exigua* tem grande importância econômica, pois encontra-se amplamente disseminado em todas as regiões produtoras de *Coffea arabica* (VILLAIN; SALGADO; TRINH, 2018). Na região Sul de Minas, responsável por 35% da produção de café arabica no Brasil, *M. exigua* foi encontrado em 25% das lavouras (CASTRO et al., 2008; CONAB, 2024). Em outras regiões importantes para a produção de café, como Zona da Mata e Triângulo Mineiro a disseminação é ainda mais alta, alcançado 50% das lavouras (TERRA et al., 2019). As perdas de produtividade em lavouras de café infestadas com *M. exigua* geralmente não excedem os 10%. Entretanto, há dados de reduções de 45% na produtividade (BARBOSA et al., 2004). O patógeno está distribuido pela América Central e América do Sul (ELLING, 2013), possui vários hospedeiros como plantas daninhas, seringueira, pimentão, tomate, cana-de-açúcar entre outros (FILHO et al., 2019).

Considerando a importância socioeconômica do cafeeiro para o Brasil, é essencial entender a relação *C. arabica*-*M. exigua*, compreendendo a ecologia dos processos que envolvem a interação planta-patógeno. O ciclo de vida de *M. exigua* inicia-se com o desenvolvimento embrionário, dentro do ovo, que leva a formação do juvenil do primeiro estádio que passa por ecdise (troca de cutícula), resultando no juvenil de segundo estádio (J2). Então, o J2 eclode do ovo invade a raiz na zona de elongação, migrando intercelularmente até a região da coifa de onde migra para a região do cilindro vascular. No cilindro central o J2 seleciona de 5 a 7 células para a sua alimentação. Essas células passam por um processo de hipertrofia e seu tamanho aumenta em até 100x. Tal estrutura permanente de alimentação do J2 é chamada de sítio de alimentação, sendo a sua manutenção fundamental para a sobrevivência do nematoide. O J2 após a indução do sítio de alimentação mostra-se gradualmente mais robusto com corpo salsichóide e perde a mobilidade, tornando-se sedentário. Assim, o J2 com o corpo bem desenvolvido sofrerá a 2ª ecdise passando a juvenil de terceiro estádio e logo em seguida sofrerá a 3ª ecdise passando a juvenil de quarto estádio. Após a 4ª ecdise forma-se o adulto, as fêmeas permanecem sedentárias, enquanto os machos se tornam vermiformes e deixam a raiz da planta. Uma característica peculiar do nematoide é que durante a fase adulta, as fêmeas do nematoide depositam os ovos em uma matriz gelatinosa composta, principalmente, por glicoproteína, polissacarídeos, enzimas e galactose (SHARON; SPIEGEL, 1993), denominada massa de ovos. Esta substância é produzida pelas glândulas retais da fêmea e liberada pela abertura anal do nematoide (MAGGENTI; ALLEN, 1960). Neste local forma-se um ambiente abundante em nutrientes e apropriado para o desenvolvimento de microrganismos.

A diversidade de microrganismos que habitam a massa de ovos e o papel que eles desempenham na biologia do nematoide e na interação nematoide-planta ainda são pouco conhecidos (BENT et al., 2008). Por exemplo, até o momento, apenas um trabalho, que utilizou métodos dependentes de cultivo de microrganismos, avaliou a diversidade de microrganismos habitantes na massa de ovos de *M. exigua* em raízes de cafeeiro (COSTA et al., 2015). Entretanto, nos últimos anos com avanços de novas técnicas de sequenciamento do gene 16S rRNA é possível realizar estudos que visam identificar bactérias associados às diferentes fases de vida de fitonematoides, assim como a relação do nematoide com organismos de diferentes nichos ambientais como solo, rizosfera e plantas (CASTILLO; VIVANCO; MANTER, 2017; YERGALIYEV et al., 2020). Lamelas et al., 2020 sequenciaram o gene 16S rRNA para analisar a comunidade bacteriana associada a *Meloidogyne paranaensis* e os diferentes estágios da doença em plantas de café. Este foi o primeiro trabalho, empregando sequenciamento para identificar bactérias associadas a um nematoide parasita do cafeeiro. Os autores conseguiram, ainda, prever o perfil metabólico de bactérias em raízes de cafeeiro saúdaveis e infectadas com o nematoide. Estudos recentes provaram que microrganismos associados a cutícula do J2 de *M. hapla* induzem resistência em plantas de tomate após a penetração do J2 (TOPALOVIĆ et al., 2020). Topalović et al., 2019 demonstraram que as bactérias com maior afinidade para se aderir a cutícula do J2 de *M. hapla* pertencem ao gênero *Microbacterium, Sphingopyxis, Brevundimonas, Acinetobacter e Micrococcus*, revelado pelo sequenciamento do gene 16S rRNA . Brown et al., 2015, por meio de técnicas de hibridização, sequenciamento e genômica funcional comparativa, encontraram bactérias endosimbiontes no intestino do nematoide

Xiphinema americanum, desempenhando papéis na absorção nutricional do nematoide. Para entender a interação entre nematoides do gênero *Meloidogyne* spp. e plantas hospedeiras, (Wolfgang et al., 2019) estudaram o microbioma de plantas de tomate infestadas e saudáveis, assim como o solo da rizosfera dessas plantas. Como resultado, os autores destacam diferenças de diversidade bacteriana entre raízes infestadas e não infestadas, com destaque para *Pseudomonas, Variovorax e Comamonas* encontradas em raízes saudáveis além da informação de que 16,5% das bactérias isoladas por meio convencional produziram compostos orgânicos voláteis tóxicos a *Meloidogyne*.

Os estudos com microbioma de massas de ovos de *Meloidogyne* spp. ainda são escassos e há muito o que descobrir sobre a diversidade e a função de microorganismos neste ambiente na regulação da população do patógeno. Em lavouras de café infestadas com *Meloidogyne* spp. é comum existirem, dentro de um mesmo talhão, plantas que apresentam sintomas mais drásticos de depauperamento e outras que mesmo infestadas apresentam sintomas leves da doença na parte aérea (COSTA et al., 2015). Entretanto, a explicação para este cenário carece de um explicação cientifica, podendo este fenômeno estar atrelada a fatores químicos e/ou biológicos. No presente trabalho lançamos a hipótese de que a variação na intensidade da doença observada em plantas de café infestadas com *M. exigua* está associada a variação na abundância/diversidade de bactérias presente nas massas de ovos de *M. exigua.*

Ainda, dentro do escopo desta pesquisa, também realizado em lavoura de café infestada com *M.exigua,* buscou-se avaliar o potencial do sensoriamento remoto como ferramenta para o diagnóstico precoce da presença do nematoide através de imagens multiespectrais coletadas com drone. Segundo (Shao et al., 2023), o sensoriamento remoto é uma tecnologia que permite a detecção de fitonematoides pela mudança na sintomatologia da parte aérea das plantas, sendo um método rápido e que não danifica as plantas para realizar o diagnóstico. Groover & Lawrence, 2020 usaram imagens de drones para rastrear a presença de *M. incognita* e *Belonolaimus longicaudatus* em grama bermuda. Os autores destacaram a importância e o potencial do sensoriamento remoto como ferramenta para rastrear o nematoide das galhas em condições de campo. Žibrat et al., 2021, através de imagens hiperespectrais determinaram a presença de *M. luci* em tuberculos de batata severamente afetados pelo nematoide e, também, naqueles que ainda não apresentavam sintomas visuais quanto ao ataque do patógeno. Neste sentido, considerando a magnitude da cultura do cafeeiro para o Brasil e a ameaça que *M. exigua* representa para as plantas, especialmente quando detectado tardiamente, o trabalho conduzido caracteriza-se como um avanço na área nematológica visto a necessidade de métodos eficazes e que conduzam a sustentabilidade de manejo, com aplicações de produtos de forma localizada, evitando que os patógenos se espalhem por toda a lavoura.

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ARTIGO 1

(Versão preliminar, conforme as normas da revista Phytobiomes Journal)

Bacteria associated with *Meloidogyne exigua* egg masses using next generation sequencing

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Abstract

Bacteria associated with nematode egg masses and rhizosphere soil may alter plant pathosystems. Using a metataxonomic approach based on sequencing the *16S rRNA* gene of bacteria from *Meloidogyne exigua* egg masses, we found significant differences in bacterial composition and diversity in the egg masses of symptomatic coffee plants compared to asymptomatic ones, for the first time in field conditions. The families *Pseudomonadaceae, Burkholderiaceae, Flavobacteriaceae, Rhizobiaceae, Micrococcaceae*, and *Bacteroidaceae* were more abundant in egg masses sampled from asymptomatic plants, while *Chitinopagaceae, Glycomycetaceae, Micropepsaceae, Beijerinckiaceae,* and *Enterococcaceae* were more abundant in samples from symptomatic plants. The genera *Pseudomonas, Sphingobacterium, Flavobacterium, Corynebacterium,* and *Virgibacillus* were found in greater abundance in egg masses from asymptomatic plants while only *Tumebacillus* and *Bacillus* were significantly more abundant in samples from symptomatic plants. The inoculum quality of egg masses used in the assays was tested in tomato plants for infectivity and reproduction. The reproduction index of *Meloidogyne exigua*, represented by nematodes eggs per gram of roots, was significantly lower when using nematode inoculum from asymptomatic coffee plants compared to using inoculum from symptomatic plants. On the other hand, the root weight of tomato plants infected with inoculum from asymptomatic coffee plants was significantly higher than that of plants infected with inoculum from symptomatic plants. However, there was no significant difference in the infectivity index (number of galls per root system) of tomato plants when inoculated with inoculum from either source ($p \leq 0.05$). If found bacteria in egg masses and rhizosphere soil of symptomatic coffee plant roots are involved in increasing disease severity of *M. exigua* in coffee should be clarified in future researches.

Key words: *Coffee arabica*; soil microbial ecology; biological control; microbiome; bionematicide

1. Introduction

The cultivation of coffee (*Coffea arabica*) in Brazil is important from economic and social perspectives, as the country is the largest producer and exporter of the grain in the world. In 2024, the estimated production reached 42.10 million 60-kg bags, with a cultivated area of 1.8 million hectares, and gross revenue of $21.26 billions (Conab, 2024). Despite the historical relevance of coffee production for the country, the development of this crop has always been threatened by plant-parasitic nematodes, particularly by species belonging to the root-knot nematodes (*Meloidogyne* spp.) (CAMPOS; VILLAIN, 2005).

Among the *Meloidogyne* spp., *M. exigua* is the most widely disseminated root-knot nematode in coffee production areas in Brazil (CASTRO et al., 2008; SALGADO; TERRA, 2021). This soil-borne pathogen generally causes small to large, rounded galls, mostly on new, whitish roots, which turn dark brown as the root becomes older (VILLAIN; SALGADO; TRINH, 2018). Coffee plants infected with *M. exigua* show reduced absorption of water and nutrients (PEREIRA et al., 2021). Damage levels vary but can reach a 45% reduction in productivity, as observed by Barbosa et al. (2004) in a plantation in the state of Rio de Janeiro.

The *M. exigua* life cycle occurs in two distinct environments: in the soil, during the migratory juvenile stage, the second stage juvenile (J2), and inside the host tissue, during the sedentary life stages. In these environments, the nematode interacts with several microorganisms, and the resulting ecological relationships can affect both the nematode and its host plant (COSTA et al., 2015; TOPALOVIĆ et al., 2020). During the adult stage of *Meloidogyne* spp., nematode females lay eggs in a gelatinous matrix composed mainly of glycoprotein (SHARON; SPIEGEL, 1993) and lectin (SPIEGEL; COHN, 1982), which can hold more than 500 eggs. The gelatinous matrix is produced in the female's rectal glands and released through the nematode's anal opening (MAGGENTI; ALLEN, 1960). Orion et al.

(2001), demonstrated the role of the gelatinous mass surrounding *M. incognita* egg mass in resistance to egg-predatory microorganisms.

Over the past two decades, researchers have made important progress in understanding the microbial composition associated with *Meloidogyne* egg masses using culture-dependent method (PAPERT; KOK; VAN ELSAS, 2004). For example, Kok; Papert (2001) isolated and identified 70 bacteria species associated with the *M. fallax* egg mass. Similarly, Costa et al. (2015) and Estupinan-Lopez et al. (2018) isolated fungi and bacteria from egg masses of *M. exigua* and *M. paranaensis* from coffee plants. However, simply isolating microorganisms in culture media is not enough to unravel all the diversity that occurs in egg masses. In recent years, cultivation-independent methods based on profiling of marker genes or high throughput metagenome sequencing have made possible to understand a broader composition of microbial communities associated with different development stages of plant-parasitic nematodes (TOPALOVIĆ et al., 2022).

Using pyrosequencing Cao et al. (2015) assessed the microbial composition of *M. incognita* at different life stages, including egg masses. The authors demonstrated that the female, second-stage juvenile (J2) and egg masses harbored a similar microbiome. Lamelas et al., (2020), through sequencing of the *16S rRNA* gene, explored the microbiome of females and eggs of *M. paranaensis* as well as coffee roots infested by the nematode. The authors argue that the bacterial community found in the study was an important component of the disease caused by the nematode and may have favoured its progress. Knowing the microorganisms associated with the *M. exigua* egg masses is a process to advance studies on the biology and ecology of this pathogen, leading researchers to better understand this pathossistem. Therefore, in this study we hypothesized that there is variation in the composition of the bacterial communities that inhabiting *M. exigua* egg masses which is host-depended.

The objective of this study was to identify, through *16S rRNA* amplicon sequencing, the bacterial community associated with the *M. exigua* egg masses collected from roots of symptomatic and asymptomatic *Coffee arabica* plants.

2. Metodology

The work was carried out at the Federal University of Lavras, in the Laboratory of Nematology, located in the Department of Phytopathology, and in the Molecular Microbiology laboratory, located in the Department of Biology.

2.1 Study area

Sampling of coffee roots and soil was done at the experimental area of the Federal University of Lavras (-21.23112, -44.99482), Lavras, Minas Gerais State, in the Southeast region of Brazil. The study area was chosen due to its nematological interest, as the coffee plants were infested with *Meloidogyne exigua* at varying levels of severity. The purity of the root-knot nematode population was confirmed by isoenzyme electrophoresis(CARNEIRO; ALMEIDA, 2001). The area is cultivated with 12 years old Arabica coffee plants from Topazio variety. The plantation undergoes conventional management, receiving regular applications of fertilizers, insecticides, and fungicides. The crop did not exhibited any symptoms of fungal, bacterial, or viral diseases, or attacks by insect pests. However, in the study area, some plants parasitized by *M. exigua* showed symptoms such as defoliation and chlorosis, whereas some parasitized plants showed minimal symptoms as evaluated using the Boldini scale for leaf coverage (BOLDINI, 2001). The scale ranges from 1 to 5 (Figure 1), where 1 represents 0-20% leaf coverage, 2 represents 21-40%, 3 represents 41-60%, 4 represents 61-80%, and 5 represents 81-100% leaf coverage. (Supplementary Table 1). The height of the plants in the study area was also measured (Supplementary Table 1).

2.2 Soil and root sampling

Roots were obtained in February 2020, during the rainy season, in a year of positive bienniality, meaning that the coffee plants had higher productivity. Six root samples from asymptomatic as symptomatic coffee were collected in the borderline of the trees, at a depth of 0-20 cm (n=12). Each sample contained roots of two selected plants with approximately 300 g of roots from each one. Additionally, soil under the influence of the roots (SUIOR) was sampled from each plant to compare the bacterial composition in the two environments. For this, the same procedure described for roots was carried out for the SUIOR collections, with approximately 100g of soil being sampled in the root zone of each plant. After collection, the samples were stored in a refrigerator (4 ºC) until subsequent analysis. To determine the physical and chemical properties of the soil, additional collections were carried out at each sampling point, at a depth of 0-20cm. The collected samples were sent to the Soil Analysis Laboratory in the Soil Department of the Federal University of Lavras, where they were processed and evaluated for pH, cation exchange capacity (CTC), organic matter (OM), organic carbon (CO), base saturation (V%), aluminium saturation (m%), macronutrient composition (P, K, Mg, S, Ca), micronutrients (Mn, Fe, Cu, B, Zn, Na) and the percentage of silt, clay, and sand.

2.3 Extraction of Meloidogyne exigua egg masses from Coffea arabica roots

To remove egg masses from inside the roots, each root sample was carefully washed with tap water. Next, root fragments were immersed in 70% alcohol for 30 seconds, followed by immersion in 1% sodium hypochlorite for 1 minute, and washed 4 times in sterilized water. Under a stereoscopic microscope, egg masses were collected from infected root tissues using a sterilized needle. Forty egg masses per sample were obtained from the roots and transferred to microtubes containing sterilized milli-Q water. The egg masses were subsequently used for DNA extraction. In addition, fifteen egg masses were extracted for the infectivity experiment, conducted in a greenhouse (topic 2.5).

2.4 DNA Extraction and Polymerase Chain Reaction

Total DNA from egg masses and soil samples (0.25g per sample) was obtained using the Power Soil1 DNA Isolation Kit (MoBio) according to the manufacturer's recommended protocol. DNA quality was verified by spectrophotometry in Nanodrop (Thermo Fisher Scientific, Waltham, MA, USA). After the extraction step, the DNA samples were stored at -80 °C until further processing. The DNA was amplified in a 25 µL reaction using the Invitrogen™ Platinum™ Hot Start PCR Master Mix (Thermo Scientific™) according to the manufacturer's protocol. The V4 region of the 16S rRNA gene was amplified using the primers CCTACGGGAGGCAGCAG (forward) and ATTACCGCGGCTGCTGG (reverse). The polymerase chain reaction (PCR) products were evaluated using electrophoresis on a 1.2% (w/v) agarose gel and stained with red gel in 1 × TAE buffer. Finally, the bands were visualized under ultraviolet light and amplification confirmed (bands ± 300bp). The PCR products were purified to remove primers and short fragments using magnetic beads (MagSi-DNA NGSPREP Plus, Magnitivo). The final DNA concentration was quantified using a fluorometer (Qubit 2.0) and dsDNA BR Assay Kit (Invitrogen, Carlsbad, CA, USA).

Multiple samples were PCR-amplified using barcoded primers. The second PCR was carried out as previously described (Dobbler et al. 2017). The PCR products were purified with the Agencourt® AMPure® XP Reagent (Beckman Coulter, Brea, CA, USA), quantified using the Qubit Fluorometer 4.0, with a DNA High Sensitivity Assay Kit (Invitrogen, Carlsbad, CA, USA), and combined in equimolar ratios. This composite sample was used for library preparation with the Ion OneTouch™ 2 System fitted with the Ion PGM™ Hi-Q™ View OT2 400 Kit using the Ion 316™ Chip v2 (Thermo Fisher Scientific, Waltham, MA, USA), following the manufacturer's recommendations.

2.5 Bioinformatics Analysis

The bioinformatics analysis of sequences was carried out using the BMP Operating System (BMPOS) (Pylro et al. 2016). Pre-processing of 16S rRNA gene data and diversity estimation were performed with VSEARCH ver. 2.3.4 (Rognes et al. 2016) and QIIME ver. 1.9.1 (Caporaso et al. 2010), respectively. Sequence clustering followed the UPARSE method and was classified into operational taxonomic units (OTUs) at an identity threshold of 97% similarity (Edgar 2013). Representative sequences of OTU groups were used to assign the taxonomic category using the SILVA database (Quast et al. 2013). The 16S rDNA datasets were rarefied to the same number of sequences per database (Lemos et al. 2011) and used to construct dissimilarity matrices generated by Binary and Bray–Curtis distances using the 'phyloseq' package in R. The statistical significance among treatments was calculated using permutational multivariate analysis of variance (PERMANOVA) with 10,000 permutations using the 'Adonis' function. The dataset was summarized at the family level, and changes in microbial diversity were measured using the alpha diversity metric, Chao1, Simpson, Shannon and Observed index; beta diversity (principal coordinate analysis-PCoA analyses) of the bacterial in egg masses and SUIOR was compared using a non-parametric test of analysis of similarity (ANOSIN). Community composition was analysed using the `phyloseq` (McMurdie and Holmes 2013) and `metacoder` (Foster et al., 2016) R packages. For the metacoder R package, OTUs of the bacterial community between symptomatic and asymptomatic samples were compared.

All data were analysed using R software (R Core Team 2020) and the Microbiome Analyst platform (https://www.microbiomeanalyst.ca) (Chong et al. 2020; Botina et al., 2023).

2.6 Greenhouse experiments for infectivity and reproduction of M. exigua in tomato plants

For this experiment, 5 egg masses were used to quantify the average number of eggs in each mass. It was found that each egg mass had approximately 200 eggs. Therefore, it was necessary to collect 15 egg masses to have a total of 3,000 *M. exigua* eggs per plant for the experiment. The egg masses were stored in sterilized test tubes containing sterilized water. A tomato seedling of the Santa Clara variety, approximately 15 days old, with three pairs of true leaves, was transplanted into a 300 mL plastic cup filled with Multiplant® substrate. Five days after transplanting, the 15 egg masses were inoculated into the substrate containing the tomato plants. Six replications were used for symptomatic plants and 6 replications for asymptomatic plants.

To compose the control treatment, which represented the quality of the nematode inoculum, coffee roots containing galls were collected from symptomatic and asymptomatic plants. Following, using the Hussey; Barker (1973) method, eggs were released from the gelatinous matrix and 3000 eggs were inoculated in tomato plants with 6 replications. After 45 days of plant inoculation, the tomato plants were evaluated for the number of nematode eggs per gram of root, number of galls and weight of roots. The experiment was repeated in July 2020, during the winter season and dry period, using inoculum from the same area and collected from the same plants, as previously described. Greenhouse conditions were 25±C and plants were fertilised as needed.

2.7 Statistical analysis

In the greenhouse assay, the experiments were conducted in a completely randomized design, and the results of the repetitions of each experiment were subjected to combined analysis. The data were previously subjected to the normality test (Shapiro-Wilk) and homogeneity test (Bartlett). Next, the F test was applied, using analysis of variance. When the F test was significant ($P < 0.05$), the means for the different treatments were compared using the Tukey test ($P < 0.05$). The R software (R Core Team, 2020) was used to run the statistical tests.

3. Results

3.1 Physical and chemical soil properties

There was low variability among soil properties collected in symptomatic and asymptomatic coffee samples (Table 1). Slightly lower pH was observed in soil with asymptomatic plants compared to soil with symptomatic plants. A similar trend was observed for macronutrients, micronutrients, and textural variables.

Table 1. Soil properties from experimental area of the Federal University of Lavras. Lavras, Minas Gerais, Brazil. Soil sampled in coffee plants infected with Meloidogyne exigua. Organic matter (OM), organic carbon (CO), base saturation (V%), aluminium saturation (m%), macronutrient composition (P, K, Mg, S, Ca), micronutrients (Mn, Fe, Cu, B, Zn, Na). As – Asymptomatic sample. S- Symptomatic sample. No significance (P <0,05) was obtained by t test between means of asymptomatic and symptomatic treatments.

Samples	Soil properties																			
	Ph_H2O	Pmehlich	S	K	Na	Ca	Mg	Al	MO	CO	B	Cu	Fe	Mn	Zn	V	m	Clay	Silt	Sand
		mg/dm3		mg/dm3		cmol/dm3			g/dm3		mg/dm3					%				
AS	4.9	5	45.6	0.12	0.01	1.1	0.5	0.2	3.7	2.1	0.26	3.3	34.9	4.5	2.4	23.93	10.36	75.3	5.6	19.1
AS	4.5	3.4	17.6	0.33	0.01	0.5	0.2	1	3.3	1.9	0.19	2.6	42.1	2.3	1.3	10.68	9.02	78.6	4.3	17.1
AS	4.9	4.9	30.4	0.2	0.01	1.9	0.5	0.2	3.6	2.1	0.17	2.8	30.1	8.1	3.7	31.41	7.12	73.6	6	20.4
AS	5.4	8.5	16.2	0.4	0.01	2.9	0.8	0.1	4.3	2.5	0.57	3.4	24.8	10.9	5	51.96	2.38	67	7.6	25.4
AS	4.9	5.1	38.9	0.16	0.01	1.8	0.5	0.1	3.6	2.1	0.35	3.2	31.1	8.1	3.6	30.99	3.89	73.5	6.1	20.4
AS	5	5.3	21.7	0.17	0.01	1.8	0.6	0.2	3.6	2.1	0.38	3	29.9	5.5	4.2	33.16	7.19	76.8	4.4	18.7
S	5.8	4.1	55.6	0.39	0.01	2.7	0.6	0	3.2	1.8	0.15	3	25.4	6.1	3.2	59.68	0	72.9	7.4	19.7
S	5.1	7.1	42.7	0.22	0.01	2	0.5	0.1	4.3	2.5	0.17	2.4	29.9	2.8	1.6	37.24	3.53	72.9	7.4	19.7
S	5.4	4.6	17.7	0.41	0.01	2.3	0.5	0	3.8	2.2	0.15	3.4	32.2	4.7	2.5	45.87	0	74.5	5.8	19.7
S	5.1	4.1	64.2	0.15	0.02	0.7	0.3	0.7	3.9	2.3	0.23	2.3	34	1.6	1.1	13.34	7.43	75.9	7.5	16.6
S	4.8	4.4	54	0.13	0.01	0.7	0.2	0.4	3.1	1.8	0.29	2.2	36.8	2.8	8	16.67	7.78	74.1	5	20.9
S	4.9	2.6	47.3	0.18	0	0.8	0.2	0.4	3.3	1.9	0.33	2.1	43.7	2.3	4.9	20.42	5.32	76.8	5.5	17.7

3.2 Bacterial diversity and abundance in egg masses and rhizosphere soil from symptomatic and asymptomatic coffee roots infected with M. exigua

From the sequencing of the 16S gene egg masses, a total of 603,328 sequences were obtained after quality filtering, with an average of 50,277 sequences per sample representing 241 bacterial operational taxonomic units (OTUs). For rhizosphere soil 97,718 sequences were obtained representing 537 OTUs. The phylum *Firmicutes* and *Actinobacteriota* were among the most abundant in *M. exigua* egg mass samples, on the other hand *Proteobacteria,* *Actinobacteriota, Chloroflexi* and *Acidobacteria* were most abundant in samples from rhizosphere soil (Figure 3).

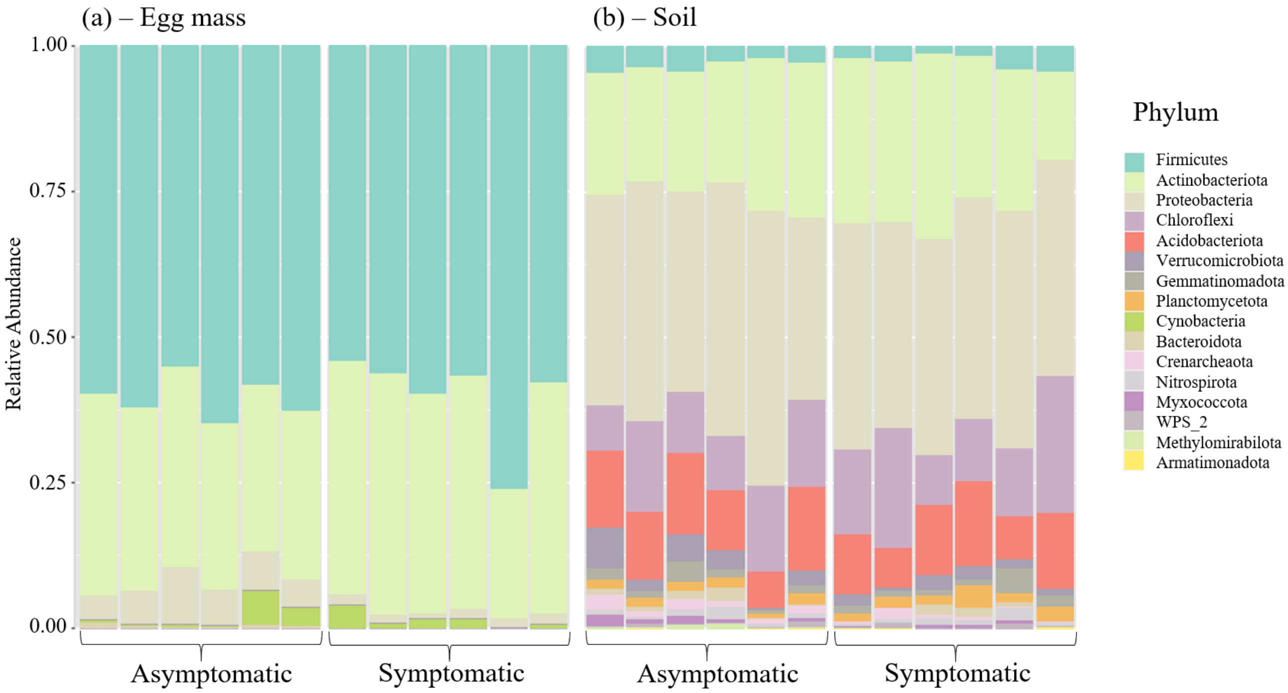

Figure 3. Relative abundance of Operational Taxonomic Unit (OTUs) at the phylum level from egg mass (a) and soil under the influence of the roots (SUIOR) (b) from symptomatic and asymptomatic *Coffea arabica* plants.

There was a significant difference in the composition of the bacterial community ($p < 0.003$) in samples from *M. exigua* egg masses collected from symptomatic and asymptomatic plants (Figure 4a). The same trend was not observed for samples from SUIOR ($p < 0.11$) (Figure

4b). As stated previously, differences in bacterial composition were observed depending on the health status of coffee plants only in the egg mass environment ($p < 0.003$).

(a) – Egg mass (b) – Soil

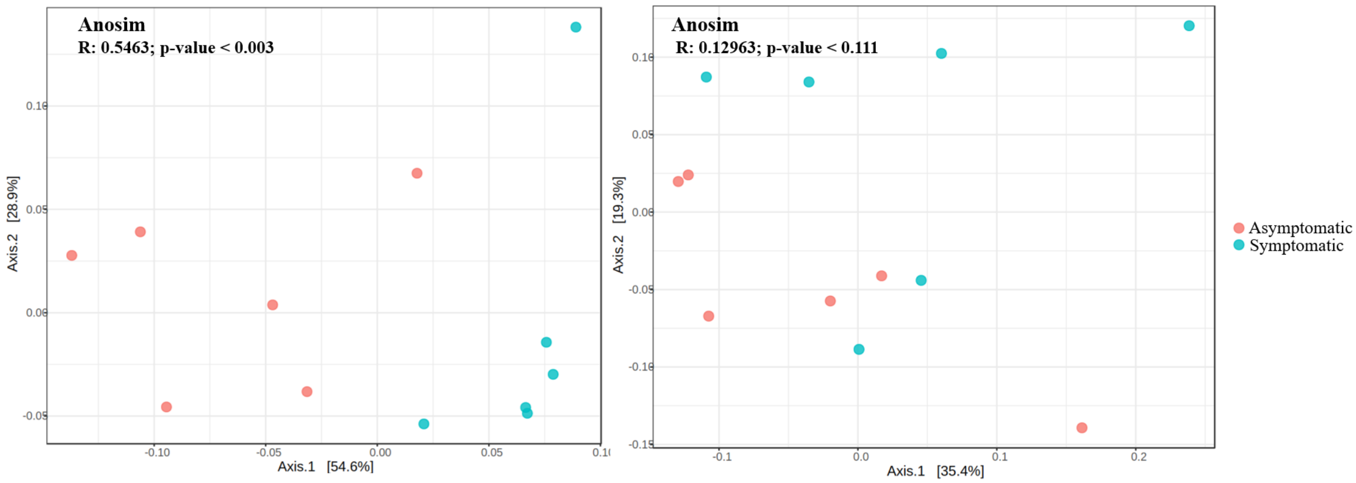

Figure 4. Principal-component analysis (PCA) analysis of the bacterial community in egg mass (a) and soil under the influence of the roots (SUIOR) (b) from symptomatic (blue dots) and asymptomatic (orange dots) *Coffea arabica* plants. Global R= 0.75: well-separated groups; global R= 0.5: groups with overlap but clearly differentiated; global R= 0.25: not well-separated groups.

Several families were more abundant in samples from asymptomatic plants (Figure 5, branches and nodes coloured in red), including *Pseudomonadaceae, Burkholderiaceae, Flavobacteriaceae, Rhizobiaceae, Micrococcaceae,* and *Bacteroidaceae*. In contrast, only a few families, including *Chitinopagaceae, Glycomycetaceae, Micropepsaceae, Beijerinckiaceae,* and *Enterococcaceae* were more abundant in symptomatic plants (Figure 5, branches and nodes coloured in blue). When comparing the taxonomic differences between bacterial communities in egg masses from symptomatic and asymptomatic plants, many taxa were equally abundant (Figure 6, branches and nodes coloured in gray). However, there was a greater abundance of genera (statistically significant) in asymptomatic samples, including *Pseudomonas, Sphingobacterium, Flavobacterium, Corynebacterium,* and *Virgibacillus* (Figure 6, branches and nodes coloured in red) as compared to symptomatic ones which included *Tumebacillus* and *Bacillus* (Figure 6, branches and nodes coloured in blue).

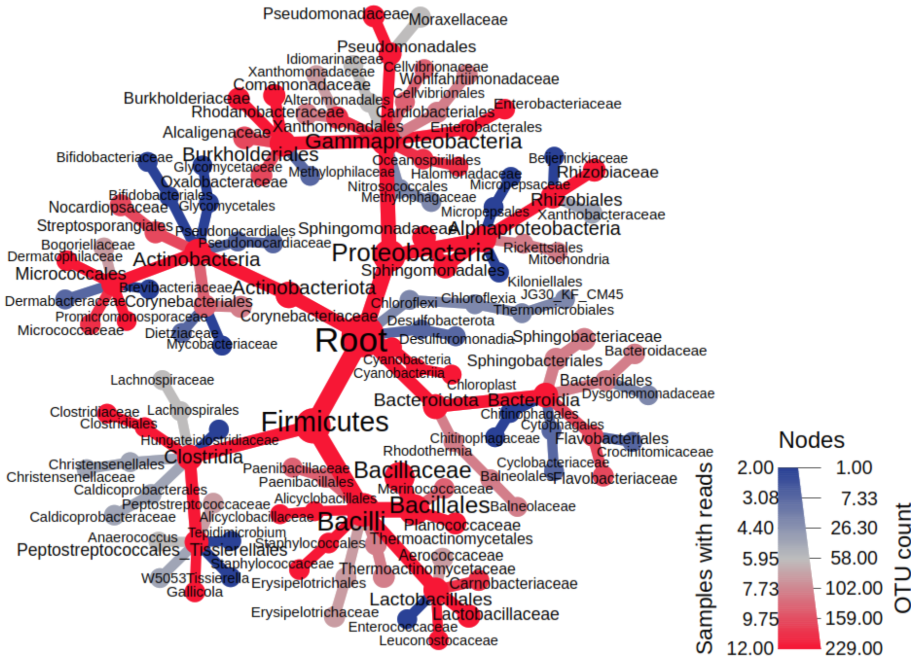

Figure 5. Differential heat tree showing relative abundance of bacteria among all the taxa, up to the family level for egg masses between asymptomatic (coloured in red) and symptomatic (coloured in blue) coffee plants. For each taxon, a Wilcoxon Rank Sum test was used to assess differences between the median abundances of samples in each treatment (plant health status). The branches indicate the association between taxa and the node sizes indicate the OTU counts per taxon. Taxa coloured in blue are more abundant in symptomatic plants, while those coloured in red are more abundant in asymptomatic plants. Taxa coloured in gray were equally detected in both symptomatic and asymptomatic plants.

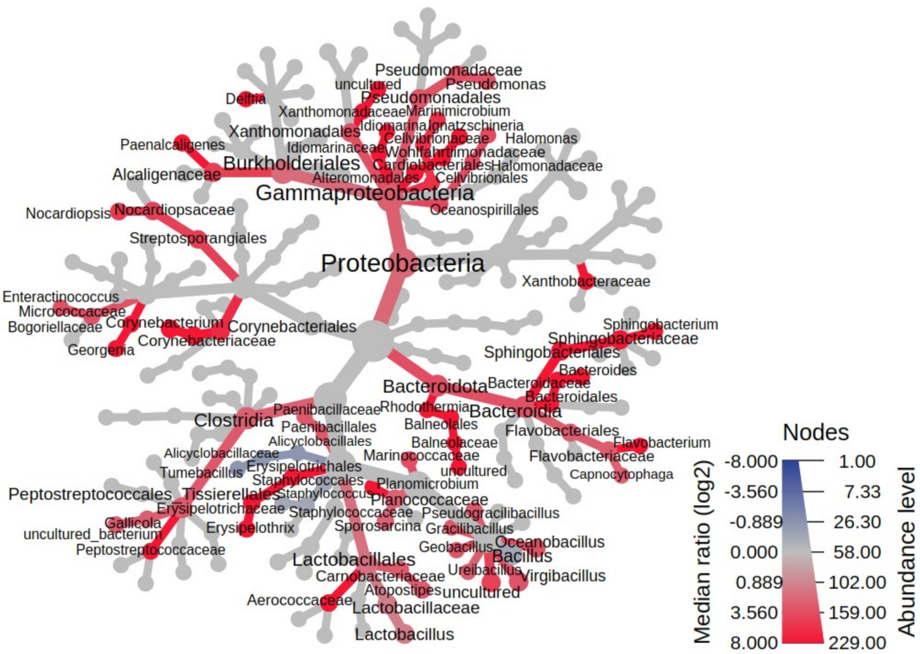

Figure 6. Differential heat tree showing differences in bacterial composition to the genus level for egg masses. The comparisons were made among the health status of plants symptomatic and asymptomatic. For each taxon, a Wilcoxon Rank Sum test was used to test for differences between the median abundances of samples in each treatment. Taxa coloured in red are statistically more abundant in the asymptomatic, while those coloured blue are statistically more abundant in the symptomatic. The branches indicate the association between taxa and the node sizes indicate the OTU counts per taxon. Taxa coloured in in gray were equally detected in both symptomatic and asymptomatic plants.

The alpha diversity of the bacterial community in each sample was revealed by indices of Shannon, Simpson, Chao and Observed. Samples from the egg mass of asymptomatic plants presented the highest diversities of Shannon (P<0.001) (Figure 7c), Observed and Chao (P<0.001) (Figure 7ab) indexes followed by Simpson ((P<0.001) (Figure 7d) index. When we compared diversity indexes based on soil samples collected from symptomatic and asymptomatic plants, no significant differences were observed in the Observed ((P<0.001)), Chao (P<0.001) Shannon (p= 0.29795) and Simpson ((P<0.001)

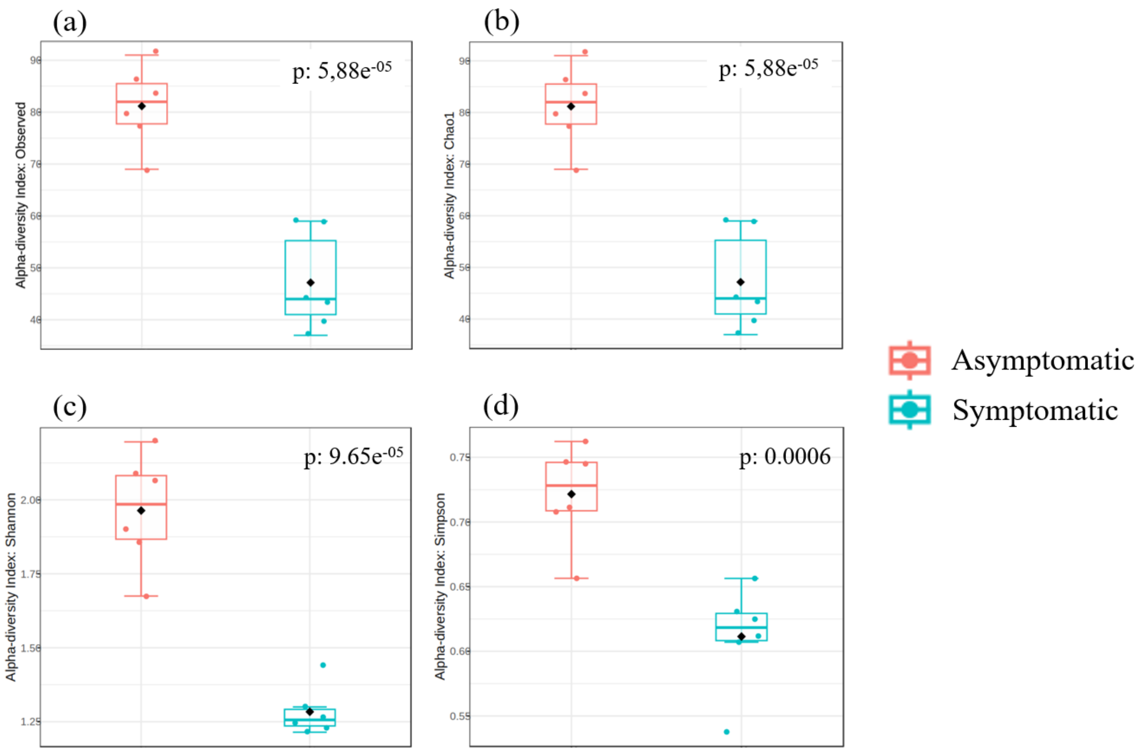

Figure 7. Alpha diversity indices for the class of bacteria inhabiting the *Meloidogyne exigua* egg mass, from symptomatic and asymptomatic *Coffea arabica* plants. a: Observed, p: 5,88e^{-05}; b: Chao, p: 5.88e^{-05}; c: Shannon, p: 9.65e^{-05}; d: Simpson, p: 0.0006.

3.3 Infectivity and reproduction of Meloidogyne exigua

Inoculum quality was evaluated by infectivity and reproduction of *M. exigua* in tomato plants. In both assays, conducted at different times, the number of galls per gram of tomato root was similar in both treatments (symptomatic and asymptomatic plants) (Table 2). For the variables number of eggs per gram of root and root weight, a statistical difference was verified between treatments (P <0.05) in the two assays. In the first one, there was a 61.3% reduction in the number of eggs and a 37% increase in the weight of tomato roots when tomato plants were inoculated with egg mass from asymptomatic coffee plants compared to plants that received egg mass from symptomatic plants in the first experiment. In the second assay, there was a 67.86% reduction in the number of eggs and a 24.79% increase in the weight of tomato roots inoculated with egg mass from asymptomatic coffee plant roots. For control, it was verified that there were high infection and reproduction when compared to plants that received egg masses (asymptomatic or symptomatic) and also lower root weight (Table 2).

Table 2. Infectivity and reproduction of *Meloidogyne exigua* in tomato plants, Santa Clara variety, inoculated with 3000 *M. exigu*a eggs from egg masses of symptomatic and asymptomatic *Coffea arabica* plant roots.

Treatments	**Galls/g root**		**Eggs/g root**		**Root Weight**	
	Experiment 1	**Experiment 2**	**Experiment 1**	**Experiment 2**	**Experiment 1**	**Experiment 2**
Control	20.66a	39.69a	1149.33a	1015.33a	12.31ab	12.31ab
Asymptomatic	7.66b	17.33a	411.14b	207.31c	14.59a	13.79a
Symptomatic	13.33b	29.55a	1061.43a	645.03b	10.65b	11.05b

Different letters indicate significance (P <0,05) of Tukey test between means of asymptomatic and symptomatic treatments.

4. Discussion

The main objective of this work was to identify the bacteria that inhabit the *Meloidogyne exigua* egg masses and compare the abundance and bacterial diversity from samples collected in coffee plants showing different health status. So, for the first time, the complexity of bacterial diversity in the *M. exigua* egg masses was evaluated using next generation sequencing. For this purpose, the 16S RNA gene was sequenced from the egg masses collected from Arabica coffee roots. At the same time, the 16S RNA gene was also sequenced from samples of the infested coffee soil.

In *Coffea arabica* plantations infested by *M. exigua*, it is common to observe among infested plants both depleted and healthy plants within the same field plot (COSTA et al., 2015). With this in mind, we selected a *M.exigua*-infested coffee field for our study, with similar soil physicochemical conditions but varying levels of suppressiveness against the nematode. Previous research using cultivation-dependent methods has suggested that bacteria present in the *M. exigua* egg mass play a fundamental role in the disease's development in the field (COSTA et al., 2015; ESTUPIÑAN-LÓPEZ et al., 2018). Recent technological advances have allowed for study of egg mass microbiomes using culture-independent methods (PENT et al., 2018; XIA et al., 2019). Until now, only Lamelas et al. (2020) have investigated the bacterial microbiome associated with phytonematodes in coffee. Their study was the first to sequence the bacterial microbiome at different stages of the *M. paranaensis* life cycle, including egg masses in healthy and infected coffee tissues. The authors did not find significant differences in alpha diversity at any stage of the nematode's life cycle, unlike our work, where we found differences in the diversity and bacterial composition of egg masses from symptomatic and asymptomatic coffee plants. The dominant phyla found in the *M. exigua* egg masses were *Firmicutes, Actinobacteria,* and *Proteobacteria*. The *Firmicutes* phylum includes several genera that are important for the biological control of phytonematodes, such as *Bacillus* (CHINHEYA; YOBO; LAING, 2017). Within the phylum *Proteobacteria*, classes such as *Alphaproteobacteria* and *Gammaproteobacteria* encompass bacterial genera known to be plant pathogens, nitrogen-fixing bacteria, and bacteria with potential for biological disease control. Particularly noteworthy within Proteobacteria is the bacterial family *Burkholderiaceae*, which comprises a diverse group of bacteria, including *Paraburkholderia* and *Pseudomonas*, with characteristics that promote plant growth and improve stress tolerance, such as the production of indole acetic acid, the solubilization of phosphates, the solubilization of potassium and the production of 1-aminocyclopropane1-carboxylate (ACC) deaminase (RASCOVAN et al., 2016). Nitrogen-fixing and cellulose-degrading bacteria are rhizobacteria or endophytic bacteria that have been previously associated with plant parasitic nematodes (LI et al., 2023). In our study, this group of bacteria was found to be more abundant in egg mass samples from asymptomatic plants, including the cellulose-degrading bacterium *Sphingobacterium* within the *Sphingobacteriaceae* family. Also, *Corynebacterium*, a nematode pathogenic bacterium within the phylum *Actinobacteria* was found in greater abundance in egg mass samples from asymptomatic plants. Actinomycetes have been identified as associated with eggs and females of *Meloidogyne* spp. from various host plants using crop-dependent methods (SUN et al., 2006).

The egg mass and soil environments had different bacterial compositions, as expected. The rhizosphere soil samples had a greater predominance of the phylum Proteobacteria, a group commonly found in different types of soil. This was also found by (PAPERT; KOK; VAN ELSAS, 2004), who used the PCR-DGGE technique of the *16S rRNA* gene to describe differences in the bacterial community between the egg mass of *M. fallax* and the rhizosphere soil of tomato and potato plants. According to the authors, this difference is likely due to differences in the nutritional composition of the nematode egg mass and root exudates found in the rhizosphere environment. Although the data presented in this study demonstrated interesting differences in the bacterial composition of the *M. exigua* egg masses from symptomatic or asymptomatic coffee plants, the same trend did not occur in soil samples collected from symptomatic and asymptomatic plants. This may be because the soil environment is more complex, with more factors and microorganisms involved in the interaction with bacteria, resulting in a more buffered bacterial community (BONITO et al., 2019).

As previous studies have highlighted, interactions between nematodes and bacteria can be mutualistic, symbiotic, pathogenic, or parasitic (PROENÇA et al., 2010). Studies on bacteria associated with phytonematodes have mainly focused on antagonists due to their importance in regulating pathogen populations. Consistent with the molecular analysis of this study, our greenhouse results showed that several bacterial families with known biological control potential against *M. exigua* were more abundant in the egg mass collected from asymptomatic coffee roots (Fig 4). Some isolates of these bacteria can act synergistically, enhancing the effect against nematodes through direct suppression, promoting plant growth, and facilitating colonization of the rhizosphere. In previous research, Costa et al. (2015) demonstrated that bacteria isolated from the egg mass of *M. exigua* produce volatile organic compounds toxic to its juveniles, causing greater J2 mortality compared to fungi evaluated in the same experiment. Although further studies are needed to understand the mutualistic, symbiotic and others relationships between *M. exigua* and its associated bacteria, our results, particularly those regarding the infectivity and reproduction of tomato plants with *M. exigua* egg masses, provide new insights for nematode management.

Based on current results and recent studies with other plant parasitic nematodes (LARTEY et al., 2023; TOPALOVIĆ et al., 2022), we can suggest that bacteria associated with *M. exigua* egg masses could protect the host by producing nematicide compounds or other mechanisms such as volatile organic compounds (WOLFGANG et al., 2019). Knowledge of the bacteria associated with *M. exigua* egg masses can be considered an important step in exploring microbial associations for potential use in nematode control or even in enhancing disease severity, especially in those found in symptomatic coffee plant egg masses.

Further studies are still needed to explore the functional characterization of these bacterial consortia and reveal their biological roles in nematode hosts and their potential as new targets for controlling plant parasitic nematodes or enhancing of the disease caused by them, known as disease complex. Maybe a balance among benefits and plant pathogenic bacteria which live in the egg masses of *M. exigua* define the host healthy of declining.

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Annex

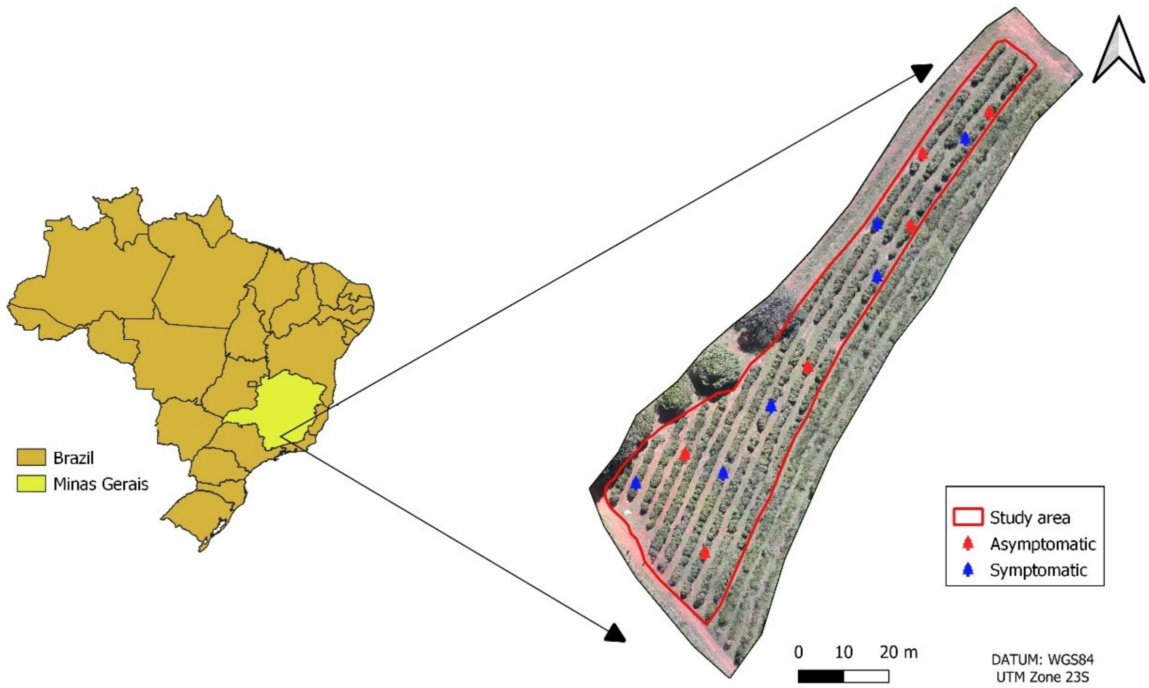

Figure 2: Map of the study area in Minas Gerais State/ Brazil on the left and, on the right, representation of coffee root sites circled by red lines and red and blue dots pointing out for symptomatic and asymptomatic sampling plants. DATUM WGS84: global reference system. UTU zone (Universal Transverse Mercator): coordinate system.

Supplementary table 1: Scale of coffee tree foliage proposed by Boldini (2001) and measurement of the height of coffee plants, in meters.

COFFEE PLANTS	BOLDINI SCALE	HEIGHT (m)
Asymptomatic	5	2,50
Asymptomatic	5	2,53
Asymptomatic	5	2,43
Asymptomatic	5	2,46
Asymptomatic	5	2,50
Asymptomatic	5	2,41
Symptomatic	2	2,25
Symptomatic	3	2,27
Symptomatic	3	2,39
Symptomatic	2	1,76
Symptomatic	3	2,14
Symptomatic	3	2,35

ARTIGO 2

(Versão preliminar. Submetido conforme as normas da revista Precision Agriculture)

Early detection of *Meloidogyne exigua* in coffee plants using multispectral images obtained by remotely piloted aircraft and thermal images

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Abstract

One of the challenges on the disease caused by plant parasitic nematodes is to the early diagnoses of them to have more benefits of the control practices available today. In this paper, the technologies to do was improved using multispectral images collected by remotely piloted aircraft (RPA) and thermal images during two periods of the year in coffee plantation infested by *Meloidogyne exigua.* Hight correlation in the red bands was obtained among values of the spectra bands between vegetation index and number of galls on coffee roots (*C. arabica*, Topazio variety) during both periods studied (May – dry season, October- initiated of rainy season) when the whole data collected was processed with Pix 4D software. The Green Normalized Difference Vegetation Index (GNDVI) showed a strong negative correlation with the number of galls in the rainy season. The information generated herein may support the development of methodologies for monitoring coffee plantations infested by plant parasitic nematodes. This study is a pioneer in correlating *M. exigua* infectivity data with vegetation indices generated by RPA, as research carried out to date did not include the variable number of galls or eggs in their studies.

Keywords: Unmanned aircraft systems; Vegetation indices; Coffea arabica, Coffee farming.

5. INTRODUCTION

Coffee is one of the most economically important crops in Brazil (Santos et al., 2023). In fact, Brazil ranks first in the production of Arabica coffee worldwide, contributing 46.4% of the global yield. This value is based on estimated total worldwide production of coffee, in 2023/2024, of 96.33 million 60-kg bags (USDA, 2023). The southern region of Minas Gerais state is the largest coffee producer in Brazil. This crop is essential for the socioeconomic condition of this region holding numerous families in the countryside involved in food production and promoting the development of the communities avoiding movement to big cities.

Among the challenges that coffee producers face, plant diseases caused by phytonematodes cause severe losses of coffee crops. The main species that cause disease in this crop are those of the genus *Meloidogyne*, which are known as root-knot nematodes. *Meloidogyne exigua*, *M. paranaensis* and *M. incognita* are the main species found in Brazilian coffee plantations (Terra et al., 2018). Early diagnosis of infection with these pathogens is an important part of management practices, but it is challenging because the symptoms of infection manifest only when the plant is already severely affected (Pan et al., 2014). Given this limitation, it is necessary to develop tools for the early diagnosis of nematode infection.

Remote sensing is a tool that can be used for the diagnosis of nematode infection because it can detect changes in the symptoms of aerial plant parts without damaging the plants, and it is faster and less expensive than other diagnostic tools (Shao et al., 2023). In this context, remotely piloted aircraft (RPAs) are platforms that are equipped with multispectral sensors that can remotely obtain information about coffee plantations, allowing the acquisition of high-resolution images at various wavelengths. This approach for collecting information has been investigated and studied by Bento et al. (2023), Santana et al. (2023) and Santos et al. (2022), who used such platforms for various applications related to coffee farming.

For detecting nematodes in crops, Norman and Fritz (1965) were among the first to try to detect *Radopholus similis* with infrared sensors. Nutter et al. (2002) identified and quantified *Heterodera glycines* using remote sensing and geographic information systems (GIS). Moreover, Pan et al. (2014) used hyperspectral remote sensing technology to detect disease caused by nematode infection in pine trees. Furthermore, Joalland et al. (2017) investigate the use of visible light imaging, thermometry and spectroscopy to detect the presence of *Heterodera schachtii* in sugar beet. Additionally, Skurdal et al. (2023) studied the Normalized Difference Vegetation Index (NDVI) of multispectral images of soybean plants, the number of *Heterodera glycines* eggs and soil nutritional parameters. These authors found that the NDVI was satisfactory for the determination of nematode population density during the planting, crop development and harvesting phases.

However, studies are scarce on coffee plants on remote sensing and RPA in areas infested with root-knot nematodes. Martins et al. (2017) showed that classification of multispectral images by the RapidEye sensor could determine the spatial distribution of healthy coffee plants that were moderately infested or severely infested by *Meloidogyne* sp. Moreover, Pereira et al. (2022) used multispectral images to evaluate the growth parameters of coffee plants infested with nematodes. These authors highlighted the need to improve data analysis approaches in order to fully realize the potential of remote sensing in monitoring coffee plants that are infested with nematodes. In addition, the aforementioned studies focused only on spectral information from orbital images or RPAs; however, the use of texture as a way of representing spatial information has not yet been explored (Martins et al., 2023).

Furthermore, the use of thermal images, despite being a powerful and promising tool for early detection of diseases in plants, has not yet been tested for diseases in coffee plants. Some recent work was carried out for fruit trees and rice and proved to be efficient in detecting diseases in the plants that were tested (Martinez, et al., 2023; Singh et., 2023; Bhakta, et al., 2023; Camino et al., 2021).

Given the damage caused by plant parasitic nematodes to crops of relevant socioeconomic importance, early detection of the pathogen is valuable, especially in a global scenario of climate change and environmental overheating. This measure, combined with other sustainable management practices, mitigates the damage caused by recurring applications of chemical products in situations where the disease is already present in crops.

In this context, the objective of this study was to associate the infection of coffee plants with nematodes and parameters of images collected by a RPA system, considering spectral and textural information, and thermal images of infested plants that were collected at two seasons of the year.

6. MATERIAL AND METHODS

6.1 Study site and soil sampling

The study was conducted in an experimental area (Figure 1) of the coffee sector of the Department of Agriculture-DAG, Federal University of Lavras-UFLA, in Lavras-MG. The area was grown with coffee (*Coffee arabica* L.), Topázio cultivar, in a space of 1.80 m between rows and 0.70 m between plants. The crop showed healthy, with no symptoms of fungal, bacterial, viral or insect pest infestation. The topography includes a low slope, and the chemical properties of the soil were determined (Table 1).

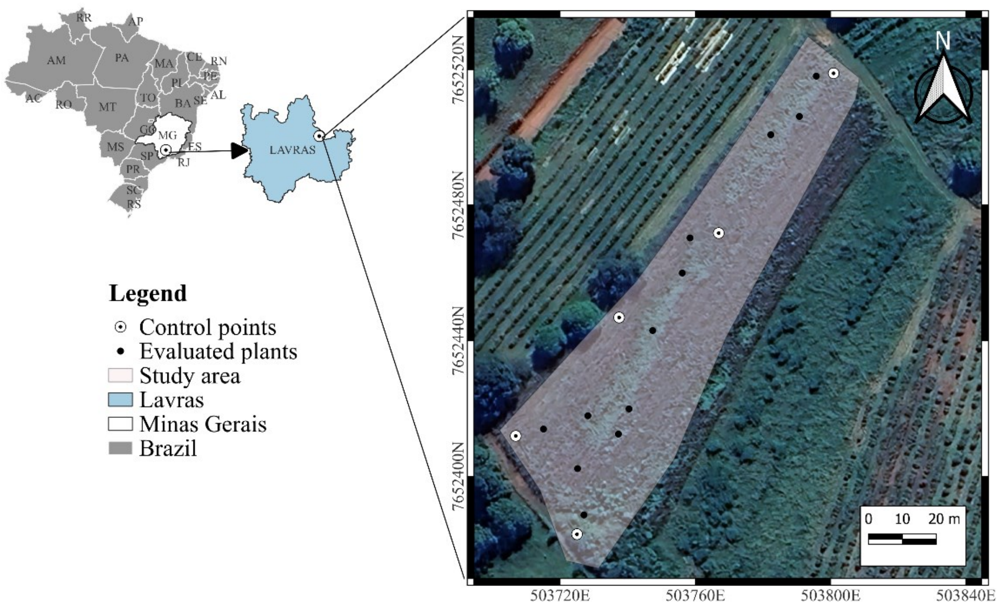

Figure 1. Geographical location of the study area in Lavras (Brazil).

6.2 Meteorological data

The climate of the region, according to the Koppen classification, is Cwa, and the region is characterized by a dry season in the winter and a rainy season in the summer, with an average temperature of 20°C and average annual rainfall of 1153 mm (ALVARES et al., 2013).

The monthly meteorological data of total precipitation (mm), minimum temperature (T min, in °C), maximum temperature (Tmax, in °C) and relative humidity (RH, in %) were obtained at the meteorological station of the National Institute of Meteorology (INMET), located in the UFLA, from 01/01/2020 to 12/31/2020 (Figure 2).

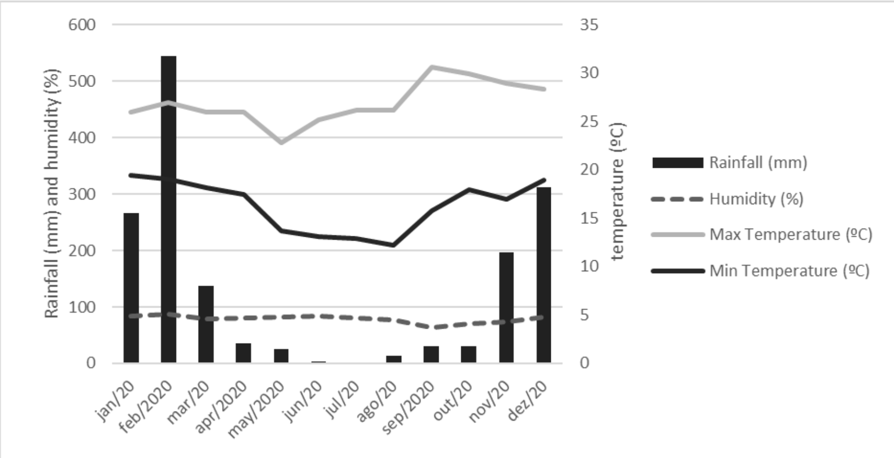

Figure 2. Graphic representation of the meteorological variables that were recorded monthly in Lavras, Minas Gerais, from January 2020 to December 2020.

6.3 Field data collection

The study area was chosen due to nematode incidence since coffee plants were infested with root-knot nematodes (*Meloidogyne* spp.). Initially, a nematological survey of the study area was conducted to identify the nematode species established in the field. Based on the results of isoenzyme electrophoresis, *M. exigua* was detected throughout the crop.

In this area, twelve coffee plants and 5 control points were sampled and georeferenced (Figure 1). To obtain this information, a DGPS differential global positioning system (Trimble Navigation Limited, Sunnyvale, California, USA) was used.

To estimate the number of nematode galls, approximately, 300 grams of roots were collected from each plant at the coffee plant canopy projection at a depth of 0-20 cm. The roots were subsequently sent to the nematology laboratory of the Federal University of Lavras, where they were washed with tap water; then, the number of galls per gram of roots was estimated.

For the analysis of soil fertility, 200-gram soil samples were collected at a depth of 0-20 cm. The samples were collected from the projection of the coffee plant canopy, and 10 samples were collected randomly distributed sites throughout the area; the samples were placed into a container for homogenization, and a composite sample was taken. It was subsequently sent to the agricultural analysis laboratory of the company 3rlab in Lavras-MG.

Leaf analysis was performed to determine if there were nutritional symptoms in the plants (Table 2). Then ten leaves were collected per plant from the third or fourth pair of leaves in the middle one third of the plant height at four cardinal directions to generate a sample. The analyses were done for nitrogen, phosphorus, calcium, potassium, magnesium, sulfur, boron, copper, iron, manganese and zinc and the results were also used for correlation analysis. To characterize the symptoms on the aerial parts of the plants, a foliage scale proposed by Boldini (2001) was used (Table 2).

A FLIR E75 thermographic camera with a spectral range of 7.5 to 14.0 µm (Figure 3c) was used to obtain the temperature of the twelve plants that were included in the study. The thermal images were collected on May 1, 2020 (dry season), and October 28, 2020 (rainy season), between 9:30 am and 11:30 am. The data were manually collected at an average height of 1.50 m above the ground, and images of the central area of the plants were obtained. The thermographic images were stored on an SD card and analyzed using FLIR Tools software.

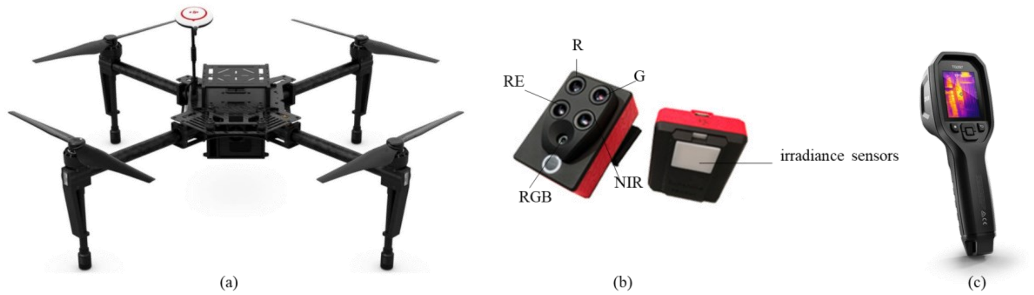

Figure 3. Equipment: (a) RPA (Remotely Piloted Aircraft) Matrice 100 used for aerial survey; (b) Parrot Sequoia TM camera (image and irradiance sensors); (c) FLIR E75 thermal imager.

Table 1. Chemical properties of the soil.

Variable	Value
pH	5.0
P available (mg.dm^{-3})	12.2
K (mg.dm $^{-3}$)	91.6
S (mg.dm $^{-3}$)	39.2
Na (mg.dm $^{-3}$)	0.01
Ca (cmolc.dm $^{-3}$)	1.7

Mg (cmolc.dm $^{-3}$)	0.3
Al (cmolc.dm $^{-3}$)	0.4
B (mg.dm $^{-3}$)	0.16
Cu (mg.dm $^{-3}$)	3.0
Fe (mg.dm $^{-3}$)	37.1
Mn (mg.dm $^{-3}$)	5.5
Zn (mg.dm $^{-3}$)	4.9

Table 2. Leaf chemical properties and foliage scale (proposed by Boldini).

Sample	Foliage scale	Galls	N	P	Ca	K	Mg	S	B	Cu	Fe	Mn	Zn
			g/Kg						mg/Kg				
1	3	76	33.2	1.9	16.4	38.3	3.1	3	72.2	53.8	244.7	208.8	27.8
5	3	376	29.5	1.9	20.5	39.8	4.3	3.6	64.5	74	280.2	119.8	24.4
6	3	17	32.7	1.9	15.5	35.5	3.2	3.1	84.3	46.4	260.2	227.6	26
8	5	332	27.8	1.8	19.2	30.8	2.6	2.8	82.7	73.8	364.9	178.5	32.7
9	5	265	26.2	1.6	20.6	17	4.1	3	65.4	54.9	283.4	169.1	27.7
10	4	64	26.8	2	11.7	26.7	2.2	2.2	46.8	24.2	343.5	77.8	28.8
13	3	466	30.1	1.5	22	31.3	3.9	3	97.3	38.5	239.1	245.4	21.5
14	4	76	27.8	1.3	22.1	27.9	4.1	3.2	72.3	69.3	230.3	174.3	21.2
15	4	2	31.2	1.8	12.3	42.7	2.4	2.7	60.9	24.4	226.5	203.3	25
18	3	508	30.2	2.3	11.8	42	2.6	3.1	122	22.4	228.1	110.3	21.2
19	4	29	29.9	2	13.6	29.9	2.5	2.7	62.7	33.9	237.4	136.4	24.2
20	5	3	22.8	1.8	9.6	28.5	2.4	2.4	72.9	39.6	220.7	88.2	28.1

6.4 Aerial data collection

Aerial images were collected on the same day as the field collection. So, images and field samples were obtained on May 1, 2020 (dry season), and October 28, 2020 (rainy season), between 11:30 am and 1:30 pm. The RPA used had a rotary wing with four propellers (quadricopter) and four engines powered by a battery that were remotely controlled by the DJI brand Matrice 100 (Figure 3a), and this RPA was controlled by means of a remote control that was integrated with a global positioning satellite system (GNSS). The aircraft had a fully customizable flight deck, with an expandable compartment for two batteries; it had flight autonomy of 40 minutes when carrying the lowest payload; and it performed autonomous and programmable flights. The RPA had a structure for camera stabilization and damping that was oriented perpendicular to the ground, called gimbal, to which a Parrot SequoiaTM multispectral camera (MicaSense, Seattle, WA, USA) with five image sensors was attached; these 5 sensors included one 16-MP (RGB) visible sensor and four 1.2 MP sensors, namely Green (G) (530–570 nm), Red (R) (640–680 nm), Red Edge (RE) (730–740 nm), Near-Infrared (NIR) (770-810 nm) (Figure 3b). The flight plan was set to an altitude of 40 m above ground level, with 80% frontal and 50% lateral overlap and a speed of 3 m/s.

6.5 Image processing

The images were processed using the Pix4Dmapper Pro software, educational version, according to the methodology described by Santos et al. (2022). The orthomosaics were georeferenced in QGIS software version 3.10 (Quantum GIS) in the UTM cartographic projection on the SIRGAS 2000 datum, zone 23S, performing a first order polynomial transformation with the nearest neighbor resampling method, using 5 control points (GCPs) distributed in the area. For image processing, arithmetic operations were performed with the R, NIR, RE and G spectral bands using a raster calculator, resulting in vegetation indices (VIs), namely, Green Normalized Difference Vegetation Index (GNDVI), Normalized Difference Red Edge (NDRE), Normalized Difference Vegetation Index (NDVI), Optimized Soil Adjusted Vegetation Index (OSAVI) and Normalized Green Red Difference Index (NGRDI), as described in Table 3. The indices were selected based on scientific literature and studies on coffee plantations. In addition to the VIs, the bands were also individually analyzed (for R, NIR, RE and G).

In addition to the spectral band and VI data, the texture parameter values of the images were obtained. For the eight textural parameters proposed by Haralick et al. (1973), the following were calculated: mean, variance, homogeneity, contrast, dissimilarity, entropy, angular second moment and correlation. The GLCM package (Zvoleff, 2020) was used for this purpose. Variations in window sizes (3 × 3, 5 × 5 and 7 × 7 pixels) and angular directions starting from the North (0°, 45°, 90°, 135° and "invariance") were tested. These parameters were obtained in bands that correlated (above 0.5) with the number of galls.

The zonal statistics were calculated in a 0.5-meter buffer around the points of the twelve plants sampled to obtain descriptive statistics of the VI and spectral information.

Table 3. Vegetation indices applied to the images obtained using the bands of the multispectral camera.

Vegetation Indices	*Equation*	*Source*
Green Normalized Difference Vegetation Index (GNDVI)	$\text{GNDVI}=\frac{\text{NIR-G}}{\text{NIR+G}}$	Gitelson et al. (1996)
Normalized Difference Red Edge (NDRE)	$\text{NDRE}=\frac{NIR - RE}{\text{NIR + RE}}$	Gitelson and Merzlyak (1994)
Normalized Difference Vegetation Index (NDVI)	$\text{NDVI}=\frac{\text{NIR} - \text{R}}{\text{NIR} + \text{R}}$	Rouse et al. (1974)
Optimized Soil Adjusted Vegetation Index (OSAVI)	$\text{OSAVI}=\frac{\text{NIR} - \text{R}}{\text{NIR} + \text{R} + 0.1}$	Steven (1998)
Normalized Green Red Difference Index (NGRDI)	$\text{NGRDI}=\frac{G - R}{\text{G + R}}$	Tucker (1979)

6.6 Statistics and data analysis

For the statistical analysis of the number of galls in the dry and rainy seasons, the means were assessed using paired t tests at a 5% significance level. For the statistical analysis of the plant temperatures in the dry and rainy seasons, we verified whether the means differed by performing a t test for two independent samples at a 5% significance level. Descriptive statistics (mean, median, maximum, minimum, first and third quartile) were calculated to support the exploratory analysis of the data.

The gall number data were correlated with VIs, plant temperature values, plant nutritional information and image spectral and textural information using Pearson's correlation (R). To assess whether the estimates were significant (p value < 0.2), Student's t test was used. All the statistical analyses were performed using R statistical software (R Core Team, Vienna, Austria).

7. RESULTS

7.1 Number of nematode galls and the temperature of coffee plants in two seasons of the year

The average number of galls on coffee plant roots in the dry season was significantly lower than the average number of galls on coffee plant roots in the rainy season (Figure 4) ($t(11) = -4.9924$; $p < 0.001$).

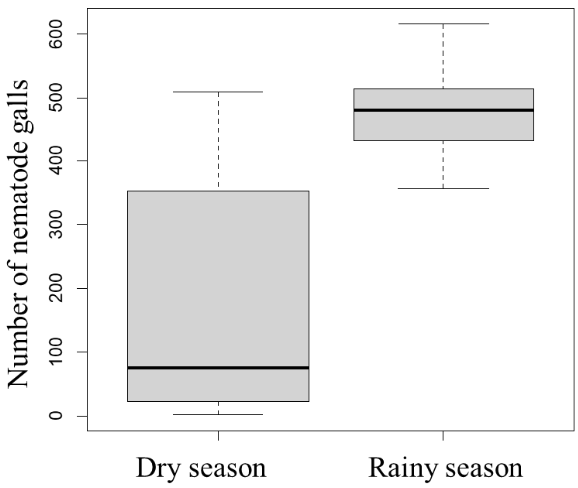

Figure 4. Box plot of the number of nematode galls in the dry season and that in the rainy season, showing a statistically significant difference according to paired t tests ($p < 0.001$).

The coffee plant temperature data exhibited a normal distribution during the two periods that were analyzed, and the variances were homogeneous, meeting the assumptions of the t test. There was a significant difference ($t(22)= -18.98$; $p < 0.001$) in coffee plant temperature between the two periods that were studied. On average, higher temperatures were observed in the rainy season than in the dry season (Figure 5).

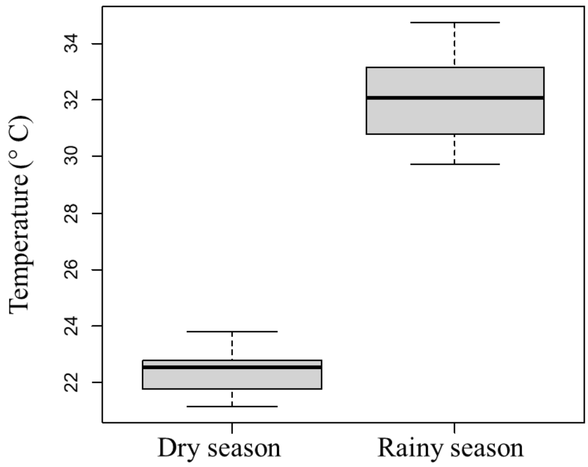

Figure 5. Box plot of the coffee plant temperatures in the dry season and in the rainy season; the results were significantly different according to paired t tests ($p \leq 0.05$).

7.2 Correlations between the number of nematode galls and the spectral and textural characteristics of images of coffee plants captured during two seasons

For Moore (2007), correlation measures the direction and degree of a linear relationship between two quantitative variables. To quantify this relationship, the following classification was adopted: R ranging from 0.10 to 0.30 (weak), R ranging from 0.31 to 0.60 (moderate) and R ranging from 0.61 to 1 (strong). Figure 6 shows that the correlations between the number of nematode galls and the G and R bands, plant temperature in the dry period, textural characteristics (variance, mean entropy, dissimilarity, correlation and contrast), and leaf nutrients (Ca, S and B) were positive and moderate, as indicated by the blue colour. The correlations between the number of galls and the textural characteristics at the second moment and homogeneity were negative and moderate, respectively, as indicated by the light red colour. The correlation between the number of galls and NDVI was negative and moderate, and the correlation between the number of galls and GNDVI was negative and strong.

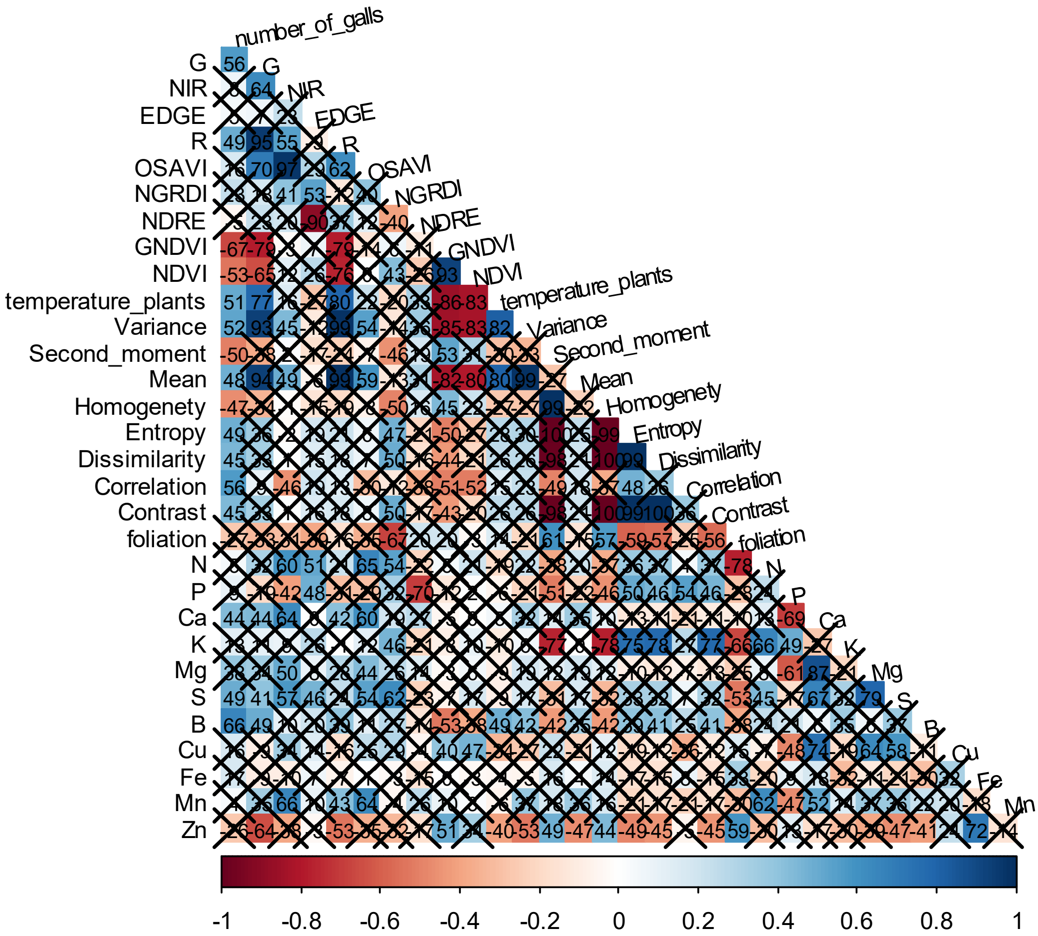

Figure 6. Pearson correlation matrix between the number of nematode galls and the spectral, textural, temperature and nutritional information of the plants for the day of the dry period. Positive correlations are displayed in blue, and negative correlations are displayed in red. The intensities of the two colors are proportional to the correlation coefficients. Nonsignificant ($p>0.20$) differences are represented by x.

According to Figure 7, in the rainy season, the correlations between the number of nematode galls and the GNDVI and NDVI were negative and moderate, respectively. The correlation between the number of galls of nematodes and the contrast agent was positive and moderate. The correlations between the number of nematode galls and the R band, variance and mean were positive and strong. The correlation between the number of nematode galls and the NGRDI was negative and strong.

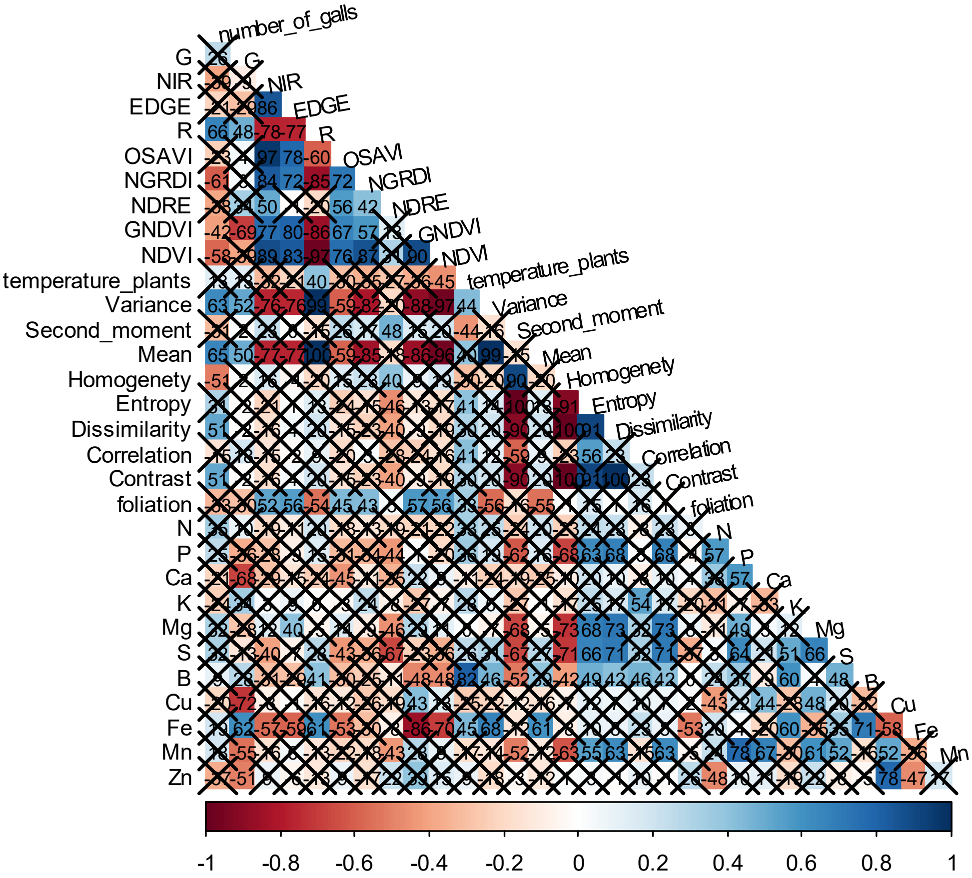

Figure 7. Matrix of Pearson's correlation between the number of nematode galls and the spectral, textural, temperature and nutritional information of the plants during the rainy season. Positive correlations are displayed in blue, and negative correlations are displayed in red. The intensities of the two colors are proportional to the correlation coefficients. Nonsignificant ($p>0.20$) differences are represented by x.

Based on these results, to analyze the texture characteristics of the images, this study considered only those analyses with significant variations ($p \leq 0.05$) and with positive and/or negative correlation coefficients greater than 0.50. Therefore, there was a relationship between the number of galls and the texture parameter of the green band in the dry period (Table 3), between the number of nematode galls and the texture parameters of the red band in the dry period (Table 4) and between the number of nematode galls and the texture parameters of the red band in the rainy season (Table 5). All these parameters were adequate for the early detection of the pathogen in the area.

Table 3. Pearson correlation between the number of nematode galls and texture parameters of the green band in the dry period.

Green Band – Dry season									
Window	Shift	Variance	Second moment	Mean	Homogeneity	Entropy	Dissimilarity	Correlation	Contrast
3x3	0	-0.0716	-0.3385	0.5668	-0.4414	0.3478	0.4414	-0.3711	0.4414
	45	0.5927	-0.3084	0.5632	-0.3219	0.3204	0.3214	-0.1712	0.3184

	90	0.5928	-0.2560	0.5602	-0.2026	0.2786	0.2026	0.0150	0.2026
	135	0.5928	-0.3212	0.5575	-0.3879	0.3422	0.3899	-0.6267	0.3988
	Inv.	0.5927	-0.3084	0.5620	-0.3652	0.3242	0.3656	-0.4970	0.3676
5x5	0	0.5926	-0.2963	0.5666	-0.4456	0.3365	0.4456	-0.4030	0.4456
	45	0.5927	-0.3084	0.5632	-0.3219	0.3204	0.3214	-0.1712	0.3184
	90	0.5928	-0.2283	0.5608	-0.2179	0.2759	0.2179	0.0617	0.2179
	135	0.5928	-0.2846	0.5583	-0.3904	0.3326	0.3922	-0.4667	0.4007
	Inv.	0.5927	-0.2725	0.5624	-0.3715	0.3153	0.3719	-0.3758	0.3737
7x7	0	0.5934	-0.2934	0.5676	-0.4404	0.3606	0.4404	-0.2502	0.4404
	45	0.5935	-0.2784	0.5651	-0.3313	0.3365	0.3309	-0.0222	0.3289
	90	0.5936	-0.2401	0.5623	-0.2286	0.3057	0.2286	0.2278	0.2286
	135	0.5936	-0.2905	0.5598	-0.3876	0.3568	0.3894	-0.2391	0.3976
	Inv.	0.5935	-0.2773	0.5637	-0.3700	0.3418	0.3704	-0.1177	0.3724

Table 4. Pearson correlation between the number of nematode galls and texture parameters of the red band in the dry period.

Red Band – Dry season									
Window	Shift	Variance	Second moment	Mean	Homogeneity	Entropy	Dissimilarity	Correlation	Contrast
3x3	0	0.5205	-0.4207	0.4792	-0.3342	0.4073	0.3300	0.2561	0.3093
	45	0.5205	-0.4207	0.4792	-0.3342	0.4073	0.3300	0.2561	0.3093
	90	0.5206	-0.4658	0.4780	-0.4527	0.4559	0.4527	-0.0687	0.4527
	135	0.5206	-0.4672	0.4773	-0.4646	0.4568	0.4634	0.3514	0.4575
	Inv.	0.5206	-0.4471	0.4783	-0.4219	0.4355	0.4199	0.3004	0.4098
5x5	0	0.5214	-0.4462	0.4796	-0.3427	0.4291	0.3387	0.2591	0.3189
	45	0.5214	-0.4462	0.4796	-0.3427	0.4291	0.3387	0.2591	0.3189
	90	0.5215	-0.4847	0.4788	-0.4540	0.4662	0.4540	0.1580	0.4540
	135	0.5215	-0.4835	0.4779	-0.4560	0.4724	0.4548	0.4927	0.4490
	Inv.	0.5215	-0.4673	0.4787	-0.4212	0.4509	0.4192	0.3258	0.4096
7x7	0	0.5225	-0.4701	0.4806	-0.3602	0.4497	0.3569	0.3680	0.3406
	45	0.5225	-0.4701	0.4806	-0.3602	0.4497	0.3569	0.3680	0.3406
	90	0.5225	-0.5031	0.4799	-0.4660	0.4814	0.4660	0.3011	0.4660
	135	0.5226	-0.4995	0.4793	-0.4568	0.4850	0.4557	0.5596	0.4499
	Inv.	0.5226	-0.4874	0.4796	-0.4298	0.4675	0.4282	0.4195	0.4200

Table 5. Pearson correlation between the number of nematode galls and texture parameters of the red band in the rainy season.

Red Band – Rainy season									
Window	Shift	Variance	Second moment	Mean	Homogeneity	Entropy	Dissimilarity	Correlation	Contrast
3x3	0	0.6357	-0.2409	0.6439	-0.1055	0.2464	0.1055	0.1491	0.1055
	45	0.6356	-0.2694	0.6455	-0.1495	0.2797	0.1495	0.2159	0.1495
	90	0.6355	-0.3289	0.6499	-0.4059	0.3132	0.4059	0.0647	0.4059
	135	0.6354	-0.3515	0.6543	-0.5260	0.3437	0.5260	-0.3177	0.5260
	Inv.	0.6355	-0.2991	0.6485	-0.3015	0.2971	0.3015	0.0150	0.3015
5x5	0	0.6352	-0.2283	0.6442	-0.1075	0.2299	0.1075	0.1397	0.1075
	45	0.6352	-0.2503	0.6458	-0.1446	0.2597	0.1446	0.3120	0.1446
	90	0.6351	-0.3083	0.6498	-0.3955	0.2963	0.3955	0.0929	0.3955
	135	0.6350	-0.3317	0.6543	-0.5234	0.3224	0.5234	-0.2120	0.5234

	Inv.	0.6351	-0.2805	0.6486	-0.2957	0.2782	0.2957	0.0743	0.2957
7x7	0	0.6352	-0.2206	0.6453	-0.1056	0.2213	0.1056	0.1052	0.1056
	45	0.6351	-0.2401	0.6467	-0.1336	0.2475	0.1336	0.3286	0.1336
	90	0.6350	-0.2888	0.6506	-0.3770	0.2851	0.4955	0.0650	0.4955
	135	0.6349	-0.3116	0.6546	-0.5130	0.3083	0.5130	-0.1472	0.5130
	Inv.	0.6350	-0.2660	0.6494	-0.2833	0.2665	0.2833	0.0572	0.2833

8. DISCUSSION

The infestation of crops with plant parasitic nematodes negatively impacts agricultural production in Brazil, which is one of the main suppliers of food worldwide. Among them, coffee has a significant socioeconomic importance in the country. Since the pathogen is often detected at late stages of infection, when the plants already exhibit severe disease and evident symptoms, growers predominantly resort to chemical management practices. Such approaches are associated with high costs and unfavorable environmental implications. These studies give some support for producers in monitoring crops and plan farms costs.

During the development of this study, the plants did not show symptoms of nutritional deficiency, confirmed by soil and leaf analysis. (Table 1 and 2); additionally, the plants were not affected by pest in-sects, and no severe symptoms of nematode infestation were observed in aerial plant parts. The presence of this pathogen becomes evident due to the formation of root galls. In May, during the dry season (Figure 2), weather conditions included low humidity and mild temperatures, which were not favorable for the pathogen. In the rainy season (Figure 2), which is characterized by increased temperatures and higher soil moisture, the weather conditions were conducive to the development of the nematode. These results corroborate the results of Marcuzzo et al. (2000), who showed that nematode number markedly increased during the rainy season, starting in October, and that the population (eggs in the survival phase) stabilized during the dry season (May, June and July).

In the dry season, environmental conditions are unfavorable to nematodes, and although the pathogen is present in the area, we did not observe the symptoms of plant yellowing that were observed in the rainy season. During this drought period, the positive correlation between plant temperature and the number of galls was moderate (Figure 6). Thus, given the difficulty in detecting symptoms in aerial plant parts at times of low nematode infestation, the use of a thermographic camera has become a promising approach for the direct and early diagnosis of plant parasitic nematodes infection in the field. These findings may be crucial for coffee management, especially during different climatic periods and for different coffee leaf temperatures, and may provide important information for decision making regarding the control of nematodes and other factors that affect coffee production. Thus, these results represent advances in the use of thermal infrared remote sensing in precision coffee farming.

Notably, for thermographic studies, one should consider the use of different thermal cameras to collect images since uncooled thermal cameras with small and lightweight designs are used in RPAs; however, these cameras may have measurement deviations, whereas the cameras used in this study are robust cameras that contain built in sensors, ensuring cooling and accurate measurements. Research by Santos et al. (2023) confirmed this discussion, and these authors observed significant differences between the temperatures of coffee plants that were observed by two different cameras, FLIR DUO (noncooled camera) and FLIR E75 (cooled camera), in May (winter). In addition, climatic conditions must be considered, as noncooled chambers may exhibit temperature variations according to environmental conditions, as indicated by the study by Santos et al. (2023).

In both periods, there was a positive and significant correlation between the number of nematode galls and the red band (Figure 6 and 7). A moderate correlation was observed in the dry season, and a strong correlation was observed in the rainy season. The rainy season is considered a period of high infestation of plants by nematodes; in addition, the onset of yellowing symptoms in the aerial parts of the plants was observed.

According to Kirkpatrick et al. (1991), root-knot nematodes induce anatomical changes in roots, as shown by an increase in root diameter or by the development of root galls. This process results in hypertrophy of the cells of the central cylinder, which, in turn, exerts pressure on the xylem vessels, compromising and reducing the efficiency of water and nutrient transport (Kirkpatrick et al. (1991). Thus, the aerial parts of plants manifest symptoms of yellowing and defoliation, resulting in greater reflectance in the red band when the plant is infested. This behavior is associated with nutritional imbalances caused by the presence of chlorophylls a and b, which affect plant vigor (Jensen, 2009).

The results of the present study corroborate those of Martins et al. (2017), who mapped the root-knot nematode with multispectral images in an area that was planted with coffee and infested with *Meloidogyne.* The authors concluded that the red band was helpful for discriminating healthy coffee plants from infested coffee plants. These results may be useful for identifying plants that are infested with nematodes, even in the early stages of infestation, as in the present study, since certain changes in the color of the leaves and in the general health of the plant may be indicators of stress caused by the infestation. Plants under stress often reflect or absorb light differently than healthy plants. In addition, the strong correlation of the red band in both study periods may reduce equipment costs since a conventional camera that captures visible images (RGB), which is less expensive and easier to operate than multispectral or hyperspectral cameras, is sufficient. In the literature, several plant diseases caused by fungi and bacteria have been detected via remote sensing in the visible spectrum (Carvalho et al., 2021; Miranda et al., 2020; Oliveira, et al., 2020).

In the dry period, the green band was moderately positively correlated with the number of galls on the nematode. The positive correlation may suggest that areas with a lower number of nematode galls also have a lower reflectance in the green band. This could be interpreted as a response of the vegetation to the stress caused by the nematode, leading to changes in reflectance. This result supports the choice of VIs considering only those that have red and green bands in their equation.

Regarding the VIs, the GNDVI was strongly correlated with the number of galls in the dry period (Figure 6) and negatively correlated with the number of galls in the rainy period (Figure 7). This result is significant because the strong relationship between the variables suggested that the GNDVI may be a promising metric for the early identification and diagnosis of coffee plant infection by nematodes. A negative correlation indicates that as the GNDVI decreases, the number of galls also tends to increase. The strong correlation suggested a consistent relationship between the two variables, which may be valuable for producers in the early detection of problems related to nematodes during the dry season. Abreu Júnior et al. (2022) reported similar correlations between the GNDVI and coffee yield in an area that was infested by nematodes.

For the rainy season, the NGRDI was strongly negatively correlated with the number of nematode galls and VI (Figure 7). The negative correlation suggested that as the number of nematode galls increased, the NGRDI tended to decrease. This inverse relationship is an important indicator because it suggests that areas with lower NGRDI values may be associated with greater nematode infestation. However, for the correlation results between galls and VIs, it is important to consider other factors that may influence these relationships and validate the results in future studies under different conditions and in different locations. In addition, it may be useful to compare the performance of the GNDVI and NGRDI with that of other metrics to ensure a more effective approach for the early diagnosis of nematodes.

The NDVI also showed a moderate and negative correlation with the number of galls in both periods (Figure 6 and 7). This result suggested that as the NDVI decreases, the number of galls tends to moderately increase. This pattern may indicate that areas with lower vegetative vigour, as reflected by a lower NDVI, are more likely to have a greater infestation of nematodes. In the literature, the NDVI is often traditionally used to perform various analyses related to the conditions of vegetation of various types (Rousse et al., 1973). However, few studies have be done on using IV to identify nematodes in coffee plants. Furthermore, this work considered the variable number of galls of *M. exigua*, as it adds greater reliability to the results as it is the main symptom of the nematode associated with damage to the root system of plants. Concomitantly, the studies by Xavier et al. (2019) are particularly relevant because they indicate the satisfactory potential of the NDVI derived from Sentinel 2 satellite images to be used for detecting nematodes. This literature provides support and context to this research, highlighting the relevance of the obtained results. It is important to continue validating and refining these results, considering different conditions and contexts, and to observe the behavior of the NDVI in both periods because, in that study, the authors presented similar results and it was not possible to differentiate the two periods.

According to the analyses of the plant leaf information, during the dry period, there was a positive and moderate correlation between the number of nematode galls and the leaf nutrients (Ca, S and B) (Figure 6). In the dry season, although the pathogen was present in the area, the environmental conditions were unfavorable to the nematode, and consequently, the plant did not experience nutritional maladjustment caused by the nematodes. However, during the rainy season, neither the foliage nor leaf information of the plants was significantly different. These results complement the results obtained by Martins et al. (2017), who concluded that biophysical characteristics do not allow the differentiation of healthy coffee plants from those infested with nematodes. This occurs because root damage becomes visible in the aerial parts of plants at different stages of development, especially in the rainy season when the crop shows higher levels of infestation and chlorosis.

In this study, the information generated from the texture variables that were obtained during RPA image processing showed that the highest correlations were observed between the red and green bands and between the number of galls and the 3x3 pixel window and an angle of 135°. Martins et al. (2023), who studied coffee plants, also used the smallest moving window size (3 × 3 pixels) but with an angle of 45°. However, in the present study, the highest correlation values were observed at an angle of 135°. These results suggest that these specific settings were the most effective for capturing and correlating relevant information on the presence of galls caused by the nematode *M. exigua*. These observations are crucial for optimizing future studies that involve spectral and textural variables that are derived from aerial images to construct prediction models, as suggested in the study by Martins et al. (2023).

Considering the importance of coffee to Brazil and the relevance of the damage caused by *Meloidogyne* spp. to the plants, and mainly reflect the costs of conventional diagnosis and its impacts on plants, the results of this study are satisfactory for the early detection of this pathogen in the crop. Additionally, this investigation is highly relevant for coffee management, especially on different climatic contexts with variations of the leaf temperatures of coffee plants. These results are a crucial source of information to support decisions regarding nematode control.

9. CONCLUSIONS

The imaging parameters that were investigated in this study demonstrated the efficacy of detecting nematode infestation in coffee plantations during two distinct periods of the year (the dry and rainy reasons). In both periods, a positive and statistically significant correlation was observed between the number of nematode galls and the red band.

There was a positive and moderate correlation between leaf temperature and the number of galls on the plants during the dry period. This result indicates that the use of thermographic cameras may be a promising approach for the early diagnosis of nematode infection directly in the field, as it is difficult to detect symptoms in aerial plant parts at times of low infestation (dry season).

In the context of VIs, the GNDVI showed a moderate and negative correlation with the number of nematode galls, and the NGRDI showed a strong negative correlation with the number of nematode galls in both seasons. The GNDVI was strongly negatively correlated with the number of nematode galls during the dry period, indicating its potential for use as a promising metric for the early identification and diagnosis of this nematode in coffee plants.

In addition, the information derived from the texture variables, which were processed from the images obtained by RPA, revealed a marked correlation between the red and green bands and the number of galls. This correlation was especially evident when considering the 3x3 pixel window and the 135° angle. These findings strengthen the potential usefulness of spectral and textural variables and specific image analysis settings in the accurate identification of nematode infestations in coffee plants.

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Author Contributions

Conceptualization, D.d.B., L.M.d.S. and M.A.Z.; methodology, L.M.d.S., M.A.Z. and D.d.B.; software, L.M.d.S., M.A.Z. and F.A.F.; validation, D.d.B., L.M.d.S. and M.A.Z.; formal analysis L.M.d.S., F.A.F., M.A.Z., and D.d.B.; investigation, L.M.d.S., D.d.B., P.F.P.F. and W.C.T.; resources, G.A.e.S.F., P.F.P.F., and V.P.C.; data curation, L.M.d.S., P.F.P.F. and D.d.B.; writing-original draft preparation, L.M.d.S. and D.d.B.; writing-review and editing, L.M.d.S., D.B.M., M.A.Z., G.A.e.S.F. and W.C.T.; visualization, L.M.d.S., F.A.F. and M.A.Z.; supervision, G.A.e.S.F., W.C.T. and V.C.P.; project administration, G.A.e.S.F.; funding acquisition, G.A.e.S.F.. All authors have read and agreed to the published version of the manuscript.

Declarations

Conflict of Interest: The authors declare no conflict of interest.

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CONSIDERAÇÕES FINAIS

Pela primeira vez, sequenciamento de nova geração foi utilizado para estudar bactérias associadas a massa de ovos de *Meloidogyne exigua*. A composição bacteriana na massa de ovos de *M. exigua* foi diferente conforme a sintomatologia do cafeeiro (sintomático e assintomático) assim como a infectividade e reprodução do nematoide em plantas de tomate.

Pesquisas futuras devem ser conduzidas para afirmar se, bactérias encontradas nas massas de ovos e no solo da rizosfera das raízes sintomáticas do cafeeiro estão envolvidas no aumento da severidade de *M. exigua* no café e, se bactérias encontradas nas raízes assintomáticas do cafeeiro, estão envolvidas no controle do nematóide.

Índices de vegetação desenvolvidos a partir de imagens coletadas por drone foram efetivos em detectar *M. exigua* em cultivo de café durante dois períodos do ano.