

Characterising the interactions between major nematode pathogens and the host coffee plant

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Abstract

Coffee is a hugely significant agricultural crop, produced by millions of growers worldwide. Production is threatened by numerous pests, pathogens, and increasingly unpredictable climactic conditions such as prolonged periods of drought. Nematode pests, distributed on a global scale, damage production by causing reduced coffee bean yield, and can cause plant death. The work described here investigates the interaction between the two major nematode species *Meloidogyne incognita* and *Pratylenchus coffeae* and commercially grown coffee cultivars. Various aspects of plant health under infection were measured in order to characterise the tolerance status of each cultivar to the two nematode species. The effect of drought on these cultivars was also investigated. Variable tolerances to infection and drought were observed between cultivars through photosynthetic rate, fresh weight and leaf water content measurements. Robusta cultivars exhibited strong resistance to nematode infection and reproduction in roots. Drought stress was observed to be a greater limiting factor to plant growth than nematode infection. The Robusta cultivar FRT49 and Arabica both showed stable photosynthetic rate measurements under infection and drought treatments, implying good performance in the field under these stresses. Stronger photosynthetic performance at lower soil moisture was seen in FRT79, suggesting that this cultivar may be useful in selective breeding for a drought tolerant rootstock. Reduced *P. coffeae* populations in FRT65 roots under drought conditions also suggest that this cultivar may have application in limiting the proliferation of this species in the field, although at the cost of coffee bean yield. The observations made here into the early stages of nematode infection and coffee plant development can be used to inform the application of specific cultivars in breeding programs aimed at producing new nematode and drought tolerant rootstock material.

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1. Introduction

1.1. The coffee industry

1.1.1. The importance of coffee

Coffee is one of the world's most important agricultural crops, with an estimated 1.5 billion cups of coffee being consumed worldwide every day (Luttinger and Dicum, 2011). The value of the coffee bean market was estimated at \$10,471 million in 2017, and is predicted to reach a value of £15,635 million by 2024 (Allied Market Research, 2018). Globally, coffee is produced by more than 50 equatorial countries, with 125 million people working in the industry (Fairtrade, 2018). Global production of coffee has steadily increased in previous decades as a result of innovations in agricultural practices. Between 1990 and 2018, total global production increased from 93 to 159 million bags (ICO, 2018^a). The cultivation of coffee forms a large basis of the economy for many developing countries; for example, coffee accounted for around 17.5% of total exports in Ethiopia and Uganda, and 41% in Burundi in 2018. Conversely, the European Union and North America (USA and Canada) constitute 65% of coffee imports, although this figure represents a lower share of the import market than in 1996 (ICO, 2018^a). The trade is also important in providing social benefits, where community cohesion is aided by the economic stability and rural employment opportunities that cultivation provides (ICO, 2014). Like many other agricultural crops, innovations in plant breeding and the use of pesticides have largely been responsible for increased production in coffee plantations, whilst also improving the quality of the harvested bean that is essential to the processing and taste of the finished product. Despite the developments in agricultural practice over previous decades, new challenges to coffee cultivation are now arising.

1.1.2. Cultivated coffee varieties

Cultivated coffee is comprised of two agriculturally produced species - *Coffea arabica* and *Coffea canephora*. *Coffea liberica* is also grown in places such as Java, as a more robust crop replacing *C. arabica* plants that were devastated by disease, but this species makes up only around 2% of overall production due to its low quality, is generally not traded and consumed only by local populations (Rodrigues Jr. *et al.*, 1975, Mordor Intelligence, 2018). Arabica coffee is favoured for the high quality bean that is produced, and as a result can be sold for a higher price (ICO, 2018^b). However, Arabica cultivation is more difficult due to the crops greater susceptibility to pests and diseases, variability in climatic conditions, and the narrower range of altitudes at which satisfactory growth is achieved (Zullo *et al.*, 2011). In 1927, a natural hybrid cross of *C. arabica* and *C. canephora* was discovered on the Southeast Asian island of Timor, which exhibited resistance to leaf rust, a major fungal disease of coffee (Fragoso *et al.*, 1972). This hybrid was then used as a rootstock that could be grafted onto other coffee cultivars that showed weaker resistance to pests and diseases, in order to produce modern cultivars of Robusta coffee that show enhanced

resistance traits (Rutherford, 2006). The beans produced by the Robusta progeny contained a much higher caffeine content, providing the plant with greater resistance to common pests and diseases through its toxic effects on predators (Filho and Mazzafera, 2003). However, the taste and quality of the coffee bean produced is regarded as inferior to that of Arabica coffee, due to lower sucrose content and higher levels of caffeoylquinic and feruloylquinic acids (Farah *et al.*, 2006). Robusta coffee therefore commands a lower price on the world's coffee markets, which is usually negated by higher yields. Robusta production is increasing as a result of greater yields, with the International Coffee Organization projecting an increase of 12.1% of total Robusta output in the 2017-2018 year (ICO, 2018^c). Robusta coffee production is also projected to increase to around 38% of total production in 2018, a marked increase from the historic output of 20-25% of the market (Coffee Research, 2006). This market trend likely represents the preference of growers for a hardier crop.

1.1.3. Coffee production

Coffee is a perennial crop that has traditionally been grown on hillside terrain in polyculture with other species that provide shaded cover to the crop. Many growers are now using more intensive, full-sun monoculture systems in an effort to maximise yields (Fain *et al.*, 2018, Jordan, 2017). In general, shade-cover systems require much lower chemical inputs and less crop management, but produce reduced yields as a result of lower sunlight levels and nutrient acquisition by other species of plants growing in the plantation (Perfecto, 2005). Intercropping coffee with other crops such as banana and black pepper is common, allowing the grower more control over their product when the price of coffee fluctuates, as well as promoting biodiversity and providing ecosystem services (Jha *et al.*, 2014). Most coffee berries are typically harvested by hand; for those with the financial capital, mechanised harvesting systems are available, although there are some concerns regarding yield losses as some berries are left unharvested (Santos *et al.*, 2010). Coffee berries are then laid out to dry in the sun before processing.

Cultivated coffee is highly sensitive to climatic conditions, with humidity and the altitude of the crop particularly affecting yield and bean quality (Bosselman *et al.*, 2009, Avelino *et al.*, 2005). The variety of coffee grown depends largely on geographical location, with Robusta generally being more tolerant of drought, but more sensitive to low temperatures (DaMatta and Ramalho, 2006). The vast majority of Arabica coffee is produced in Brazil, historically the largest producer of coffee globally. In Vietnam, the second largest producer, Robusta accounted for 97% of coffee production in 2012, although some Arabica is also grown in cooler regions of the country (Reuters, 2012). Whilst countries in Central and South America have historically produced the majority of the world's coffee, Southeast Asian countries such as Indonesia and Vietnam have significantly increased their production in recent years (ICO, 2018^a). Vietnam in particular has been able to expand its output so significantly through intensive use of monocultures and high chemical inputs, resulting in Vietnamese growers typically producing at levels multiple times higher than neighbouring countries

Thailand and Laos (Gro-Intelligence, 2016). Current data on total coffee production indicate that exports by South American countries are remaining relatively stable, whilst outputs in India, Vietnam, Costa Rica and Honduras have all increased by over 10% since 2014 (ICO, 2018^d). This suggests a general trend towards increasing production in Asia and Central America, whilst production in South American countries is stable or increasing to a much lesser degree. This may reflect growers' preference to grow other crops in response to depressed coffee market prices, caused in part by the massive expansion in output by Vietnam in the past two decades (The Economist, 2013).

1.1.4. Common pests and diseases of coffee

C. arabica and *C. canephora* are both susceptible to a variety of pests and diseases at differing severities. The most significant pests, the coffee berry borer (*Hypothenemus hampei*) and coffee leaf rust (*Hemileia vastatrix*) are now widespread in all coffee producing regions (Talhinhas *et al.*, 2017). The coffee berry borer, the most common insect pest, is found in Central and South America, Africa and Asia, and causes significant problems to growers by destroying the coffee berry whilst it is developing. Coffee berry borer incidence in over 90% of fruits has been reported in Hawaii, Tanzania and Malaysia, showing that this pest is a major problem in separate distant regions (Follett *et al.*, 2016, Vega, 2004). It has previously been estimated that this pest causes around \$500 million of crop losses annually (Baker, 1999). Berry borer infestation may be more difficult to diagnose and control than other pests, as the species exhibits a cryptic life-habit where infection of the coffee berry may not be easily identified, and whilst insecticide applications are commonly used, the insect can avoid contact with the chemical when remaining sedentary inside the fruit (Williams *et al.*, 2013). Coffee berry borer populations exhibit low genetic diversity, raising fears that the acquisition of genetic resistance to insecticides would spread rapidly throughout populations (Benavides *et al.*, 2014). In 2015 the draft of the berry borer genome was published, which will allow a much more detailed understanding of the genetic basis of host plant resistance, and allow for precise targeting in coffee breeding programmes and new biocontrol methods (Vega *et al.*, 2015).

Coffee leaf rust is a fungal disease, distributed throughout the Americas, Africa and Asia (Ameson, 2000). Coffee leaf rust can be devastating to coffee plantations; in Sri Lanka, *C. arabica* cultivation was once widespread, before the whole industry was devastated by an outbreak of leaf rust (Cressey, 2013). Crop losses of 30% can result from severe infections, such as during the outbreak of leaf rust in Colombia and Central America between 2008 and 2013 (Cristancho *et al.*, 2012). Poor hygiene and quarantine practices, as well as the inevitable transport of disease-causing fungal spores during the movement of coffee products between different locations has allowed leaf rust to become endemic to all coffee producing regions (Brown and Hovmøller, 2002). The reproductive potential of coffee leaf rust is dependent on variations in temperature, with a more temperature range allowing for faster growth and spore production (Avelino *et al.*, 2015). This problem is likely to

be exacerbated by increasing global temperatures, as well as expanding the suitable host range for the disease. Whilst there are recent reports of sufficient control of leaf rust through fungicide application, evolution of the fungus to acquire resistance to fungicides is also presenting new problems for growers (Capucho *et al.*, 2013, Cressey, 2013). Unlike coffee berry borer, leaf rust shows higher genetic diversity across populations, which may reduce the spread of resistance, but will also present challenges in generating resistant cultivars, as there is greater potential for resistance to be overcome by the pathogen (Zambolim, 2016).

Alongside these major pests, coffee plantations can also experience bacterial blights and attack by coffee leaf miner (*Leucoptera coffeella*). Controlling pests and diseases in a perennial crop system presents great challenges, especially as many smallholders do not have sufficiently large budgets to pay for agronomical expertise or chemical products to control pests (Dorsey, 1999). Whilst there have been many advances in chemical usage, plant breeding and land management practices, the limited budgets of many smallholders growing coffee limits the effectiveness with which the plantations can be properly managed. As coffee plantations are expected to have a productive lifespan of around 20 years, options in controlling soil-borne pathogens are limited as growers are unable to disrupt the soil system to any great extent, and are reluctant to take any pest control measures that may damage the crop through phytotoxicity (Souza, 2008). Despite coffee's history as a relatively pathogen-free crop, the increasing preference for intensive monoculture systems in an effort to produce higher yields is increasing the potential for resistance acquisition to current control methods, and repeated evolution to overcome previously resistant cultivars and fungicide controls has been observed (Ligabo *et al.*, 2015, Talhinas *et al.*, 2017). The continued intensive application of pesticides has negative impacts on the environment and human health, is also an unsustainable option for most low budget growers (Staver *et al.*, 2001). Research into protecting coffee from pests and diseases is key to securing sustainable production in the future. This research focuses on characterising the impact of a globally distributed group of pests - plant parasitic nematodes.

1.2. Plant parasitic nematodes

1.2.1. Feeding strategies and symptoms of infection

Plant parasitic nematode species are obligate parasites, relying on the host plant as the nutrient source essential to their survival and reproduction (Williamson and Gleason, 2003). Plant parasitic nematodes feed on plant tissues by acquiring nutrients from the root system through a variety of strategies, which are detailed in following chapters. Diversity in feeding strategies has allowed plant parasitic nematodes to interact with a huge range of host plants, which is a major contributing factor to their success and widespread global distribution. Many factors affect the success of nematode species in specific environments, particularly macronutrient concentrations, humidity and soil

composition (Andaló *et al.*, 2017). Plant parasitic nematodes feeding on plant roots may not necessarily produce visible above-ground effects on the host plant, making the diagnosis of infection difficult, particularly under low to moderate levels of infection. Young coffee plants are also more likely to become stunted and die as a result of nematode infection, as the underdeveloped root system cannot support the plant when infected (Vovlas and Di Vito, 1991). Symptoms such as leaf yellowing, wilting and loss of vigour may present at sufficiently high levels of infection (Kawabata *et al.*, 2018). Nematode populations can also persist in the soil for long periods of time in the absence of a host plant, provided humidity is sufficient (Kung *et al.*, 1991). As a result, controlling these pests can be very difficult once they have been introduced.

1.2.2. Root lesion nematodes - *Pratylenchus* spp.

Root lesion nematodes comprise the *Pratylenchus* genus, containing around 50 species (Ryss, 2002). Root lesion nematodes species are migratory endo-ectoparasites; these nematodes are able to feed on the plant's cells from outside of the plant whilst also being able to move through the root cortex to new feeding sites (Smiley, 2015). Root lesion nematodes reproduction can occur either within the host plant root tissues, or in the soil surrounding the root system (Han *et al.*, 2017). Root lesion nematodes lay eggs individually in the soil, as opposed to some nematode species that form egg masses containing hundreds of eggs (Karakas, 2009). As a result, root lesion nematodes may reproduce at a slower rate than other genera of nematode. The life-cycle and infective pathway of root lesion nematodes is shown in Figure 1.1.

1.2.3. Infection

Plants infected with root lesion nematodes species show a characteristic dark-stained necrotic region on the root's exterior, a result of the host plants' response to the action of cell wall degrading enzymes secreted through the nematode's stylet (Popeijus *et al.*, 2000). Root lesion nematodes infection can cause the plant to experience a variety of negative effects, including water availability stress and nutrient loss, as well as increasing the susceptibility of the plant to attack by other pathogens, such as root rot caused by fungal *Rosellinia* species (Jackson-Ziems, 2016, Singh and Phulera., 2015). Root lesion nematodes exhibit a wide host range, and are readily able to move to the root systems of new plants; this is in part due to their small size of 0.35-0.6mm, which allows them to move easily in different soil types (Jones and Fosu-Nyarko, 2014, Sher and Allen, 1953). This feature of root lesion nematode biology presents a particular challenge to controlling populations in agricultural systems. Crop losses resulting from infection with *Pratylenchus* spp have been estimated at 5-10% in some states in the USA in maize, 5.9% in tobacco crops in Canada, and up to 27% in wheat in Australia (Olthof *et al.*, 1973, Koenning *et al.*, 1999, Nicol *et al.*, 1999). In Robusta coffee plantations in East Java, yield loss estimates of up to 78% have been attributed to *Pratylenchus* spp. (Indarti and Putra, 2017).

1.2.4. *Pratylenchus coffeae*

Pratylenchus coffeae is commonly known as the banana root nematode, despite also being one of the most damaging nematode pests to coffee cultivation. This species is the most commonly reported nematode species on cultivated coffee worldwide, with the global distribution shown in Figure 1.2 (Luc *et al.*, 2005). *P. coffeae* is commonly found in Vietnam, being present in 11 of 15 samples taken by Trinh *et al.*, with authors also reporting a higher incidence of the species in sandy soils (Trinh *et al.*, 2009). The prevalence of *P. coffeae* also correlates positively with soil zinc and manganese content, as reported in Costa Rica (Avelino *et al.*, 2009). Other authors have reported the presence of *P. coffeae* on 5.1% of roots sampled in Brazil (Kubo *et al.*, 2003).

An optimum temperature range for reproduction of 25-30°C has been observed for *P. coffeae*, explaining the distribution in tropical and sub-tropical areas (Radewald *et al.*, 1971). *P. coffeae* is unlikely to survive in soils where temperatures fall below 10°C or exceed 32°C, and where humidity is lower than 2% (Souza, 2008). However, the presence of this species in countries such as Austria illustrates the ability of *P. coffeae* to survive in a wide range of environments, which is a major factor in its persistence as a pest. Reproductive potential in *P. coffeae* is markedly lower than in many sedentary endoparasitic nematodes, as eggs are laid individually in the soil and not in large numbers in egg masses (Jones and Fosu-Nyarko, 2014). The life cycle of *P. coffeae* usually takes between 4 and 8 weeks depending on environmental suitability, which is slower than many other nematode species (Davis and MacGuidwin 2000). *P. coffeae* infection on coffee plants causes extensive root damage through penetration of root cells and subsequent migratory endoparasitic behaviour within the roots, where cells within the root cortex are explored by the nematode in search of nutrients (Vaast *et al.*, 1998). Alongside dark purple and black root lesion formation, other symptoms of infection include leaf discoloration, plant wilting and internal rotting (CABI, 2018^a). The lack of gall-formation on plant roots can make the identification of *P. coffeae* on coffee roots more difficult than other species of nematode, particularly for untrained growers. However, a clear sign of the presence of *P. coffeae* in a plantation is the degenerative characteristics manifesting in young trees that have been recently transplanted from the nursery; this also suggests that poor sanitation and pest control practices in nurseries aid the dissemination of this species (Waller *et al.*, 2007).

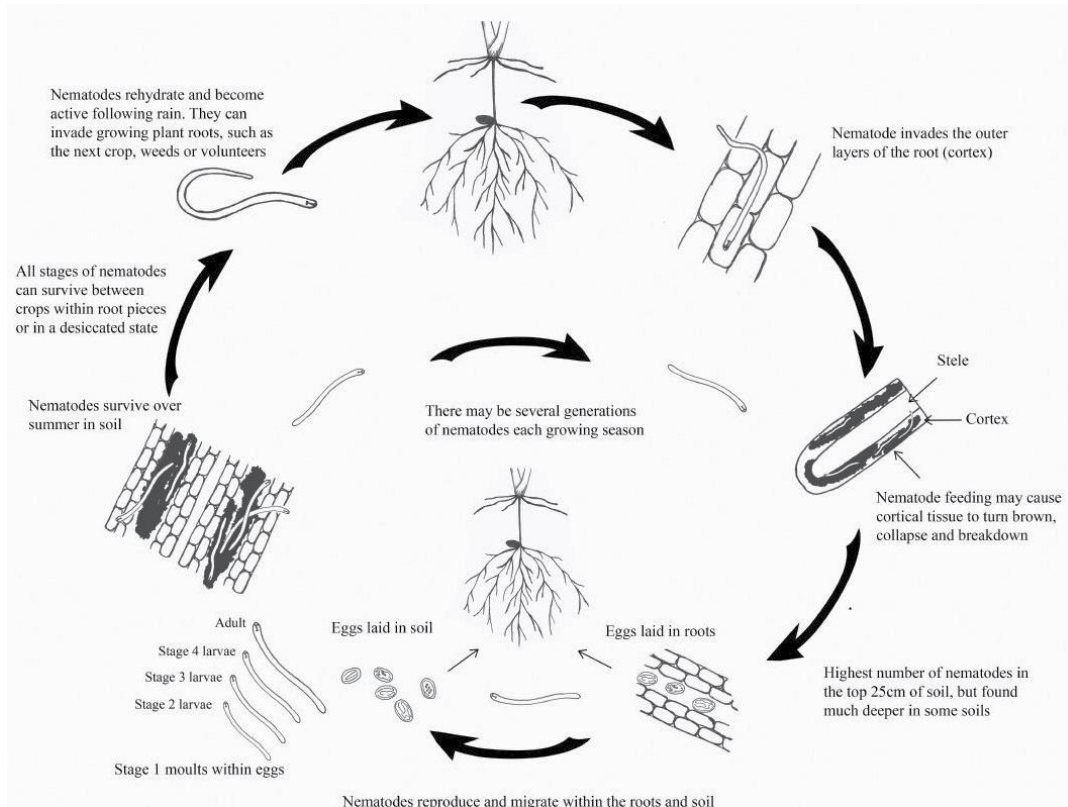


Figure 1.1. – The life cycle of root lesion nematodes. Root lesion nematodes hatch from eggs laid in the soil or within the plant roots, and transition through three larval stages before becoming adults. All stages of the life cycle (except the egg and stage 1 larvae) are able to infect plant roots. Infective stage nematodes invade the outer root cells of the host plant and migrate through them, feeding on the cortical tissues through the stylet, which produces the characteristic dark purple/black root lesion response. Root lesion nematodes may also remain dormant in the soil until conditions are amenable to their activity and reproduction (Agrios, 2005).

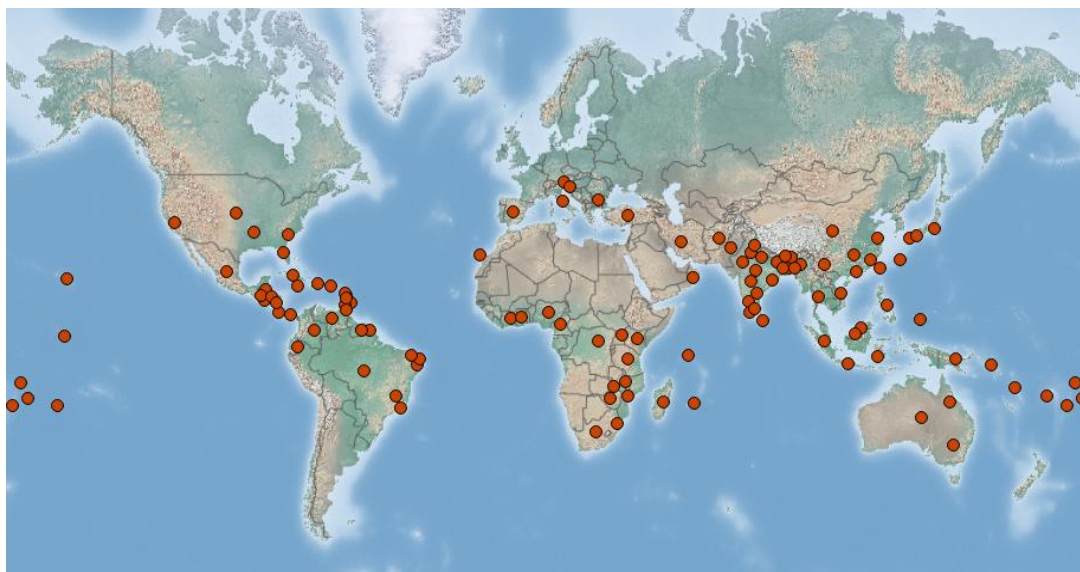


Figure 1.2. – The global distribution of *P. coffeae*, which has been reported in most coffee producing countries (CABI, 2018^a).

1.2.5. Root-knot nematodes - *Meloidogyne* spp.

Root-knot nematodes, *Meloidogyne* spp., are found on nearly all subtropical agricultural crops, and present huge problems in crop damage and yield losses in all equatorial regions. Root-knot nematodes are sedentary endoparasites, forming feeding sites inside the roots and feeding from the plants' vasculature (Absmanner *et al.*, 2013). Most root-knot nematodes produce a root-galling response in the host plant, where a feeding site is created in the root cortex after being induced by the secretion of salivary proteins from the nematode's stylet, resulting in the manipulation of the host plant's cellular processes (Favery *et al.*, 2016). Once the feeding site has formed around the nematode inside the root, the animal may remain there for extended periods of time, feeding from the vasculature of the root (Bartlem *et al.*, 2013). Root-knot nematode reproduction occurs within the host plant, forming an egg mass, which can produce greater



Figure 1.3. - Comparison of uninfected lettuce roots (right) to roots infected with *Meloidogyne hapla* (left), where extensive root galls have formed (Mitkowski *et al.*, 2003).

numbers of progeny than root lesion species, creating the opportunity for exponential population growth in a relatively short amount of time if the pest is uncontrolled (Fourie *et al.*, 2010). The distinct appearance of root galling is shown in Figure 1.3., and the reproductive strategy of root-knot nematodes is shown in Figure 1.4. The galling response exhibited by the host plant allows for easier identification of infection by *Meloidogyne* spp. compared to *Pratylenchus* spp.

Meloidogyne spp. have been reported in nearly all coffee producing regions. In Minas Gerais, a major producing region in Brazil, root-knot nematodes were detected in 37% of field samples taken in coffee plantations, with the same survey also giving first reports of *M. exigua* and *M. paranaensis* in two other regions (Santos *et al.*, 2018). Similarly, in Espírito Santo, another major producing region in Brazil, root-knot nematodes were detected in 66% and 100% of sites sampled respectively in certain areas of the state (Barros *et al.*, 2014). Recent first reports of *Meloidogyne* spp. in Africa, and the discovery of new species *Meloidogyne daklakensis* in Vietnam also illustrate the ongoing problem in characterising root-knot nematodes distribution in coffee producing regions (Jorge Junior *et al.*, 2016, Trinh *et al.*, 2018). Coffee yield losses attributed to *Meloidogyne* spp. of 20% in Uganda and 45% in Brazil have been previously reported (Okech *et al.*, 2004, Barbosa *et al.*, 2004).

1.2.6. *Meloidogyne incognita*

Meloidogyne incognita is thought to be the most widely distributed PPN species of tropical and subtropical agricultural crops, and for this reason is also the species that has been most studied (CABI, 2018^b). Locations where *M. incognita* has been reported are shown in Figure 1.5. *M. incognita* exhibits a shorter life-cycle than *P. coffeae*, with egg laying females being observed at a minimum of 25 days from hatching (Ibrahim and El-Saedy, 1987). As a result, proliferation of *M. incognita* in agricultural systems can occur extremely quickly. Whilst juveniles and female *M. incognita* show similar body sizes to other nematode genera, males typically exhibit a larger body length of 1.2mm – 1.7mm, allowing for identification in samples where other species may be present (Whitehead, 1968). Infection with *M. incognita* can lead to plant death if uncontrolled.

The genome of *M. incognita* was sequenced in 2008, which has allowed for great advances in understanding the ability of this species to manipulate the host plants cellular processes, using a diverse suite of cell wall degrading enzymes. The authors reporting the first draft genome in 2008 also hypothesized that many of the cell wall modifying enzymes found in *M. incognita* were derived by horizontal gene transfer from infective bacteria (Abad *et al.*, 2008). The sequencing of the *M. incognita* genome has allowed for the characterization of genes such as MiISE5, which suppresses cell death responses in the host plant cells that the nematode infects, thus allowing cellular processes to continue and provide a continuous nutrient supply to the feeding site (Shi *et al.*, 2018). Study into the genetics of *M. incognita* can be used to inform experimental aims for the control of other nematode species.

1.2.7. *Meloidogyne paranaensis*

Due to similarities in morphologies, feeding behaviour and reproductive strategy, *M. paranaensis* was not distinguished from *M. incognita* until 1974, and is often referred to as a ‘minor’ *Meloidogyne* species in the scientific literature, despite its damaging effects (Lordello *et al.*, 1974). The morphology and life-cycle of *M. paranaensis* are very similar to other *Meloidogyne* spp., however field observations suggest that *M. paranaensis* infection does not produce the typical root-galling response in host plants caused by other members of the genus, but instead produces more generic symptoms such as chlorotic spot formation on leaves and plant wilting (Carneiro *et al.*, 1996). The results of these non-specific symptoms have likely lead to many cases of misdiagnosis where *M. paranaensis* is present. *M. paranaensis* shows a less extensive global distribution than *M. incognita*, and is currently only reported in Central and South America (Elling, 2013). This species has not yet been reported in Asia, and good quarantine practice will hopefully prevent its introduction; however, other root-knot nematode species such as *M. enterolobii* and *M. graminicola* are present in Vietnam, suggesting that *M. paranaensis* will likely become established if introduced (Iwahori *et al.*, 2009, Bellafiore *et al.*, 2015). Where it does occur, *M. paranaensis* is one of the most damaging

Meloidogyne spp. to coffee production, with estimated crop losses of 50% in coffee in Paraná state, Brazil resulting from infection (Carneiro *et al.*, 1996). Whilst coffee is the major host for this species, it also exhibits a wide host range with other agricultural crops and weeds (Souza, 2008).

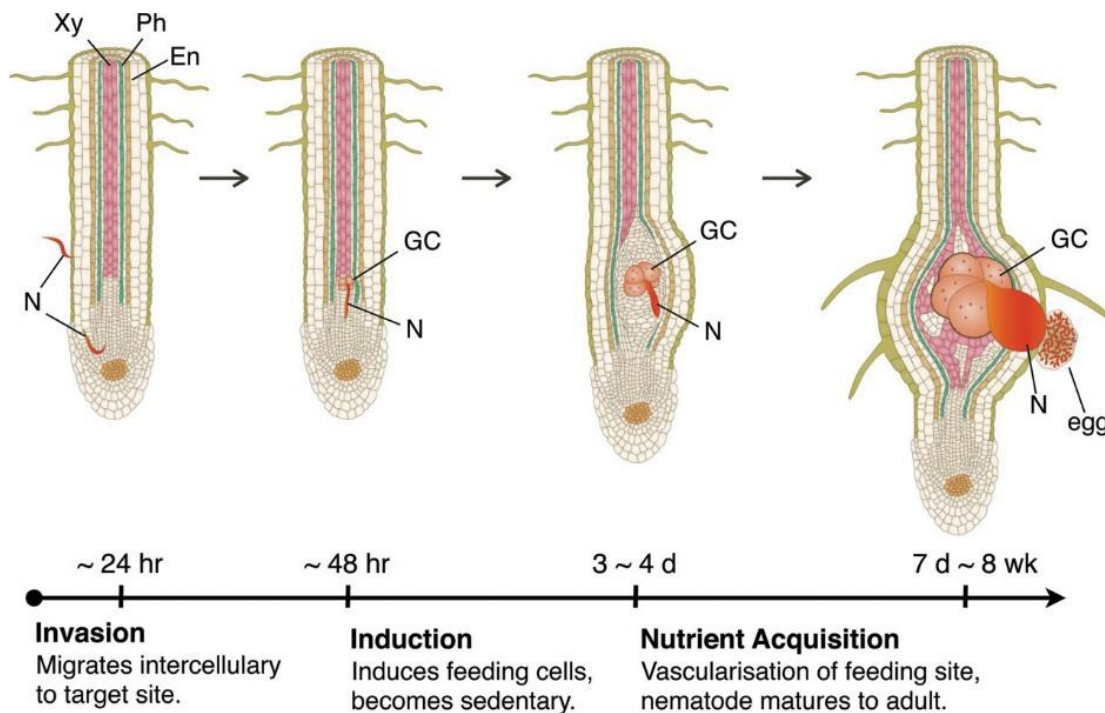


Figure 1.4. – Sedentary endoparasitic nematode life cycle. Infective stage juveniles invade the outer root cortex cells and migrate into the host plant root. The nematode then forms a feeding site at target cells in the endodermis, where they induce the formation of giant cells through targeted changes to the plants cellular activity. The nematode then remains sedentary in the feeding site, where it develops further and produces an egg mass on the exterior of the plant root. This produces the typical root-galling response. N – nematode second stage juveniles, Xy – xylem, Ph – phloem, En – endodermis, GC – giant cells (Bartlem *et al.*, 2013).

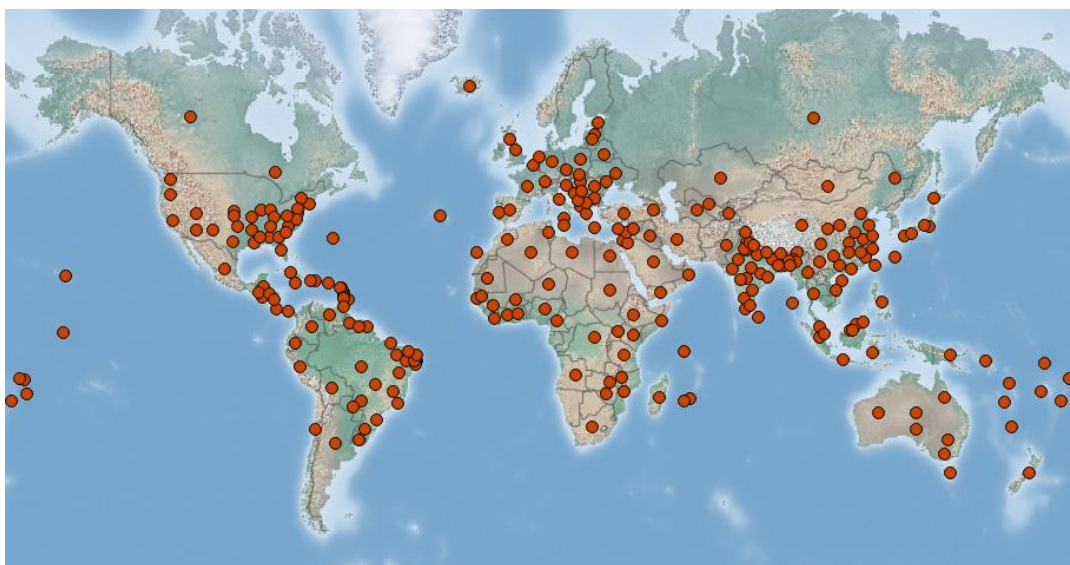


Figure 1.5. – Global distribution of *Meloidogyne incognita*. This species is thought to be the most widely distributed plant parasitic nematode globally (CABI, 2018^b).

1.3. Nematodes in agriculture

1.3.1. Nematodes and coffee production

It is estimated that plant parasitic nematodes are responsible for annual crop losses of around \$157 billion, and pose a particular problem to coffee, cotton, tomato and potato growers (Abad *et al.*, 2008, Youssef, 2013). The need to effectively control these pests, therefore, is clear. Synthetic nematicides have traditionally been used as a control, but repeated application has detrimental effects on both soil and human health, and may produce phytotoxic effects in the coffee crop (Mian and Kabana, 1982, Cepeda-Siller *et al.*, 2018). Many commonly used nematicides supplied as granular products lose efficacy in controlling nematode populations, as much of the product is dissolved into water and lost to non-target areas (Souza, 2008). As coffee is a perennial species, crop rotation strategies are extremely difficult, and may be ineffectual due to the large host range of many nematode species that infect coffee.

1.3.2. Nematode resistant cultivars

Plant breeding to produce nematode resistant coffee cultivars has been a major area of interest to provide long term, reliable control. Due to more widespread distribution, most research into resistant cultivars has targeted *Meloidogyne* spp., and relatively successful results highlighting key clones exhibiting improved resistance have been produced (Lima *et al.*, 2015, Rezende *et al.*, 2017, Santos *et al.*, 2017). The identification of the *Mex-1* gene, which confers resistance to *Meloidogyne* species by producing a hypersensitive response in *C. arabica* and *C. canephora*, was an important development in the understanding of nematode resistance in coffee (Anthony *et al.*, 2005). The hypersensitive response involves the programmed cell death of root cortex cells at and around the nematode invasion site, which prevents the further penetration of the parasite into the host plant by removing the nutrient supply, and hence preventing further development of the nematode (Lam *et al.*, 2001). It is thought that the *Mex-1* gene is responsible for preventing invading nematodes from reaching the inner tissues of the roots, and hence reducing the potential for feeding and reproduction.

Pathogenicity assays screen for particular genotypes that show heightened resistance to nematode infection. For example, the *C. arabica* clone 'UVF 408-28' caused an 87% reduction in the population of *M. incognita* when compared to a susceptible genotype, with a hypersensitive response in the host plant being shown to provide this resistance (Albuquerque *et al.*, 2010). Other infection assays have highlighted semi-wild Ethiopian *C. arabica* varieties as showing heightened resistance to *M. incognita* (Anzueto *et al.*, 2001). Robusta genotypes showing resistance to *P. coffeae* have also been characterised (Wiryadiputra, 1996).

The characterisation of resistance related genes in coffee allows for specific targeting in the breeding of resistant cultivars. However, these cultivars will need to be continuously tested and

improved through field trials, as nematodes have been observed to break resistance in response to prolonged interaction with resistant cultivars (Saucet *et al.*, 2016). As genetic variability in commercially grown coffee is low (particularly in *C. arabica*), there are concerns that plantations growing monocultures of genetically identical material will provide the conditions for resistance acquisition in pests and diseases (Lashermes *et al.*, 2000). As a result, there is growing interest in the use of wild coffee cultivars that show greater genetic variation as sources of material for new resistance breeding programs (dos Reis Fatobene *et al.*, 2017, Aerts *et al.*, 2017). The authors also highlight the importance of preserving vulnerable wild coffee populations for this purpose.

1.3.3. The impact of climate change on coffee production

Climate change is forecast to cause major instability in coffee producing regions. Anticipated global temperature increases are forecast to drastically reduce the area of land that is suitable for coffee production; for example, areas at less than 1300m altitude in Uganda are expected to become unsuitable for coffee production using current agricultural practices (Jassogne *et al.*, 2013). Temperature increases may also create more suitable conditions for pest reproduction, as reproductive potential correlates with increasing temperatures in some pests (Jaramillo *et al.*, 2009). Less predictable weather patterns and changes in the length of the wet and dry seasons in coffee producing regions are also expected, which will make the management of plantations much more difficult (Baker and Hagggar, 2007). Increasingly severe drought periods are likely to reduce yields in coffee producing regions (Yara, 2018). Prolonged periods of drought can also reduce the ability of the soil to take up water, increasing the potential for flooding and concurrently providing favourable conditions for the spread of fungal diseases (Rosenzweig *et al.*, 2001). Crop management problems are exacerbated by the fact that around 80% of coffee producers are smallholders, with limited resources available to spend on chemicals, machinery and labour (Fairtrade, 2018). There is therefore no guarantee that these increasingly trying conditions will be overcome with any reliability. As a result, it is essential to properly characterise the major biotic and abiotic challenges facing coffee production, and work to produce effective solutions to these problems.

1.3.4. Drought-resistant cultivars and practices

Coffee plants require relatively high amounts of water to produce satisfactory yields, placing high demands on water availability in growing regions (Carr, 2001, D. Lovarelli *et al.*, 2016). Irrigation systems are too expensive for many growers, and huge amounts of water may be lost as run-off on hillside terrain (Wang *et al.*, 2015). As a result, plant breeding is the main focus of producing drought tolerance in coffee production systems. As rainfall is forecast to decrease in many areas, there is a need to produce cultivars that are more tolerant of drought.

Investigation into the tolerance of drought stress in different cultivar clones has highlighted certain clones that show an increased ability to tolerate insufficient water availability. *C. canephora* clone

120 exhibited greater water use efficiency compared to other cultivars through improved control of stomatal closure; similarly, clones 14 and 73 were shown to increase ABA-signalling under drought stress, causing the expression of a suite of stress response genes (DaMatta *et al.*, 2003, Vieira *et al.*, 2013, Tuteja, 2007). Recent grafting studies, where clone 120 was used as a rootstock onto which clone 109 (a drought-sensitive *C. canephora* cultivar) was grafted. When clone 120 was used as a rootstock, the amount of time taken to reach a severe water deficit level was 7 days longer than when clone 109 was self-grafted, indicating that drought tolerant clones can be useful in enhancing the ability of coffee plants to withstand prolonged periods of drought (Silva *et al.*, 2018). Elevated levels of ABA in the plants grafted with clone 120 have been confirmed by other authors, supporting further a role in drought tolerance (Silva *et al.*, 2018). Under extended periods of drought stress, ABA has been shown to influence the closing of guard cells in leaves, limiting water loss through transpiration, and also restricts the growth of new tissues throughout the plant when water is limited (Sreenivasuluab *et al.*, 2012).

Acclimation to drought stress has also been demonstrated in *C. canephora*, where clone 120 (drought tolerant) and clone 109 (drought sensitive) were put under drought conditions recurrently. The tolerant clone 120 exhibited greater water potentials in leaves with each successive drought period, showing that coffee plants can acclimate to drought conditions and have a 'memory' of osmotic stress, with the authors suggesting controlled changes occurring to the plants metabolism, particularly increased RuBisCo activity, to be the mechanism by which drought tolerance is enhanced (Menezes-Silva *et al.*, 2017). This finding is important in understanding the biological basis of drought tolerance and breed cultivars that are primed to endure drought stress more successfully.

Drought resistance in coffee can also be modulated through the application of chemicals, with authors reporting increases in water use efficiency when nitrogen fertilizers are applied to the crop (Salamanca-Jimenez *et al.*, 2016). Tolerance of osmotic stress is also affected by the rhizosphere surrounding the plant, with root association with AMF known to increase root water uptake in the host plant (Augé *et al.*, 2001).

1.4 Aims of the project

The work outlined in this thesis aims to investigate the impact of infection with two major nematode species on Nestlé coffee cultivars. The hypothesis that coffee cultivars will respond differentially to infection with *M. incognita* and *P. coffeae*, and that these differences will be observable through the various aspects of plant physiological performance measured, will be tested. The objectives of this project can be summarised as follows:

- Investigate the impact of nematode infection on coffee cultivars through glasshouse trials and monitoring of photosynthetic activity
- Compare the ability of these cultivars to tolerate prolonged drought conditions
- Evaluate the impact of simultaneous nematode stress and water restriction on coffee cultivars
- Assess inter-specific competition between *P. coffeae* and *M. incognita* for plant root resources by co-infecting coffee plants with both species
- Measure nematode attraction to root exudates of different coffee cultivars through agar plate assays

2. Materials and Methods

2.1.1. Origin of plant material and maintenance in glasshouse

Robusta coffee plantlets (FRT11, FRT23, FRT49, FRT65, FRT79) were produced in Tours, France by Nestlé Research Centre staff. Nemaya coffee plantlets were grown from seed provided by CIRAD (France) at The University of Leeds. Arabica coffee plantlets were sourced from the Eden Project. Robusta plants were propagated from cuttings in tissue culture before being transferred to soil trays and grown in a glasshouse under 28°C, 100%RH and 12 light regime conditions. All plants were then transported to the University of Leeds via courier and transferred to pots containing a 2:1 mixture of potting compost: vermiculite to promote root growth. Plants were maintained in glasshouse conditions of 28°C, 80%RH and a 12 hour light regime and watered daily.

2.1.2. Maintenance culture and propagation of nematodes on host plants

Meloidogyne incognita and *Meloidogyne paranaensis* populations obtained in Brazil were used to infect the root systems of *Solanum lycopersicum* (tomato, cv. Ailsa Craig). *Solanum lycopersicum* were grown in potting compost and maintained in a glasshouse at 25°C, 50-60% RH and a 16 hour light regime. Plants were grown from seed for at least four weeks before being infected. Plants were infected by mixing infected root material from previously infected plants into the potting compost mixture before potting. New plants were infected every two weeks to maintain the supply of nematodes.

Pratylenchus coffeae obtained in Ghana and cultured at Leeds University were used to infect the root system of *Musa acuminata* (banana, Dwarf Cavendish cv.). *Musa acuminata* were grown in 50/50 potting compost:sand mix and maintained in a glasshouse at 25°C, 75-80% RH and a 12 hour light regime. Populations of *P. coffeae* were maintained on the plants for a minimum of 6 months before extraction.

2.1.3. Maintenance of *Pratylenchus coffeae* on carrot discs

To increase the numbers of nematodes available, *P. coffeae* populations were also maintained on sterile carrot discs on 2% water agar plates. Prior to application to the carrot disc, nematodes were sterilised in 1.5ml microcentrifuge tubes through the following antibiotic treatments. Tubes were micro centrifuged between each step, and solution removed before the next antibiotic was applied:

1. 0.1% Kanamycin (30 minutes)
2. 0.1% Penicillin G + Streptomycin sulphate (30 minutes)
3. 50 µg/ml Amphotericin (30 minutes)
4. 0.1% CTAB antiseptic agent (5 minutes)
5. Nematodes were then rinsed five times in sterile tap water

Carrot discs were cut from store-bought carrots and sterilised in 10% sodium hypochlorite solution for 30 minutes. Sterilised carrot discs were placed onto agar plates in a laminar flow hood, and around 1000 nematodes were pipetted underneath the carrot discs. Plates were then sealed with biofilm and incubated at 25°C for a minimum of 6 weeks.

2.1.4. Extraction of nematodes from plant roots and carrot discs

To extract 2nd-stage juveniles (J2s) of *M. incognita* and *M. paranaensis*, tomato plants were removed from soil at 8 weeks post infection and the root systems were washed and cut into pieces of 3-4 cm in length. Roots were then soaked under a constant mist of water at 25°C for 3 days, where the water was filtered through a nylon mesh and tissue paper before being funnelled into a 50ml tube. Tubes were replaced daily to maximise nematode yield.

Mixed infective stages of *Pratylenchus coffeae* were extracted from carrot discs after 6 weeks incubation by rinsing the agar plate with tap water and collecting the water in a 50ml tube.

Following extraction, all nematodes were used immediately or incubated at 10°C.

2.1.5. Randomisation and arrangement of coffee plants

Coffee plants in glasshouse were randomised using a Latin square prior to the start of the trial to avoid bias in selection. Plants were labelled and placed in saucers within plastic trays.

2.1.6. Infection of coffee plants with infective stage nematodes

To infect coffee plants, four 1ml pipette tips were placed into the soil immediately surrounding the root system. A water suspension containing nematodes was pipetted into the tips, and the solution was allowed to diffuse into the soil. Tap-water was repeatedly washed through the pipette tip to ensure that all nematodes had left the tip and entered the soil. Pipette tips were also placed into the soil of uninfected control plants and washed through with water to replicate the same mechanical procedure in experimental and control groups.

2.2.1. F_v/F_m measurements of PSII efficiency

Measurements of photosynthetic efficiency in PSII (photosystem II) were taken using the F_v/F_m measure on the OS30p chlorophyll fluorometer (Opti-Sciences, Hoddesden, UK). Plastic clips with light-excluding foam were clipped onto leaves with the shutter closed for 30 minutes to prevent light entry and allow the leaf portion to enter the dark-adapted state (Figure 2.1.). The F_v/F_m reading was then taken by calibrating the sensor in ambient light, inserting the sensor into the clip and taking a fluorescence reading at 700-750nm wavelength.



Figure 2.1. – Plastic clips were attached to apical leaves for 30 minutes to exclude light. F_v/F_m measurements were

2.2.2. Soil moisture measurements

Readings were taken using the SM200 soil moisture sensor probe and HH2 meter (Concord Scientific Devices, India). The probe was fully inserted into the soil (Figure 2.2.), and four measurements were recorded from each pot to give an average. An analogue DC voltage reading is taken by the probe, which is converted to a soil moisture reading based on known calibration values. The probe was cleaned with white roll after each measurement to remove any soil and moisture. The setting used on the HH2 meter was 'Organic Soil'.



Figure 2.2. – The SM200 probe was used to measure soil moisture content. Four measurements were taken per pot, and average values used.

2.2.3. Maintenance of soil moisture content

Plants maintained under drought conditions were provided with water only when soil moisture readings showed an average value of <15%. If the average soil moisture reading was <15%, then 50ml water was supplied to the saucer containing the plant to maintain the soil moisture at the 15% stress level. Plants that were not maintained under drought stress were watered daily by filling the saucer with water.

2.2.4. Root analysis for nematode content

To analyse nematode presence in the root system, plants were cleaned of soil, the root system was chopped up into pieces of 2-3cm in the same procedure as nematode extraction (2.1.4.). Roots were soaked in the misting chamber for 3 days, after which tubes containing nematodes in suspension were taken and stored at 4°C (Figure 2.3.). To analyse the number of nematodes extracted from each root system, tubes were agitated to homogenise nematode concentration in the suspension, and 20µl samples were pipetted onto a watch glass. Numbers of each species were identified visually. Five 20µl aliquots were used to analyse numbers of nematodes, counting the total number in each aliquot and using average values to determine the total number of nematodes in suspension.



Figure 2.3. – Plant root systems were continuously soaked in the misting chamber for 3 days to allow for maximal nematode recovery.

2.2.5. Calculation of relative water content

For each plant, a leaf was removed and fresh weight was measured immediately. Leaves were then placed in a petri dish of tap water for three hours to allow maximal absorption of water and for the leaf to become turgid. Leaves were then dried of any surface moisture and turgid weight was recorded. Leaves were then desiccated overnight in a 65°C incubator to remove all water content, and dry weight was recorded the following day. These values were used to calculate the relative water content through the equation:

$$\text{Relative water content (\%)} = \left[\frac{\text{Fresh weight} - \text{Dry Weight}}{\text{Turgid Weight} - \text{Dry Weight}} \right] \times 100$$

This value indicates the water content of the leaf as a percentage of the maximum potential water content, and hence provides insight into the status of water availability in the plant.

2.2.6. Plant fresh weight

Plants were weighed at the end of the trial period. Plants were removed the pot, and root systems were cleaned of all soil. Weights were then recorded using the Sartorius 1413MP8-1 balance (Sartorius, Germany).

2.3.1. Root exudate production

Root exudate solutions were produced by soaking cleaned coffee roots in tap water at 4 °C for 12 hours (80 g root tissue/litre).

2.3.2. Agar plate preparation

Agar plates were prepared using 2% agar, 0.25% Tween 20 and 0.168mM HEPES. Tween 20 was used as a detergent to allow the nematode aliquot to associate with the agar medium more readily. HEPES 99% was used as a pH buffer to maintain a pH of 6 in the agar medium. Root exudate plates were prepared using the same medium, substituting tap water in the agar plate for the root exudate solution. Agar and root exudate mixes were sterilised via autoclaving before being poured into 5cm plates in a laminar flow hood.

Plugs were then cut from the agar plates using 1 ml pipette tips to produce two wells at opposing ends of the agar plate, 3 cm apart. On each plate, tap water was pipetted into one well, and a plug of the root exudate agar inserted into the other well. Plates were incubated at room temperature for 30 minutes before nematode application to allow root exudate to diffuse into the agar medium.

2.3.3. Nematode application

Nematode aliquots were pipetted into the centre of the agar plate in a 20µl droplet containing ~100 infective stage nematodes. Plates were incubated at room temperature throughout the experiment. Six repetitions per species were conducted in each experiment.

2.3.4. Nematode visualisation and counting

A guide plate was made containing 1.5cm zones around each exudate plug. Experimental plates were placed on top of the guide plate. The total number of nematodes on the plate was counted initially, and nematode movement towards the tap water and root exudate plugs was recorded after 24 hours incubation at room temperature. Agar plates were visualised using a Leica M165 microscope.

2.4.1. Data Analysis

All data was recorded contemporaneously in Microsoft Excel. Statistical analysis was conducted using SPSS Statistics developed by IBM. Statistical tests used were 'Means comparison', 'One way ANOVA' and 'Univariate analysis'. LSD post-hoc tests were conducted when evaluating statistical significance between experimental groups. Statistically significant results showed $p > 0.05$.

Analysis for photosynthetic rate measurements was split into three groups: 0-7, 7-28 and 28-56 days post infection. These time periods were used as analysis revealed statistically significant differences between experimental treatments during these periods, which may not have been seen

over the whole trial period. Outlier analysis was performed, and extreme values were not included in analysis.

3. Results

3.1. Preliminary infection trial (63 days)

To establish a reliable method of nematode infection of coffee plants in pots, a preliminary infection assay was conducted. Three cultivars (FRT11, FRT23 and Nemaya) were used to test the effects of infecting coffee plants with 2000 *M. incognita* or *P. coffeae* on F_v/F_m , and also to show that the method of nematode inoculation resulted in the establishment of populations in host plant roots.

The recovery of differential numbers of nematodes depending on coffee cultivar and species suggested that susceptibility to nematode species differed between cultivars. The total number of *M. incognita* recovered from FRT23 roots was significantly greater than Nemaya roots. *P. coffeae* recovery did not differ between cultivars. No statistically significant differences were observed between the total number of *P. coffeae* recovered from Nemaya and FRT23. Statistical tests could not be performed for FRT11 as the number of surviving plants at the end of the trial was less than 3 (Figure 3.1.). Nematode recovery from plant roots supported the infection protocol and collection method, demonstrating that nematodes were able to successfully colonise and become established in coffee plant roots when inoculation with 2000 nematodes was performed using the filter tip method. This finding provided the basis for further study into the effects of nematode infection using this protocol.

To quantify the impact of nematode infection on PSII efficiency, F_v/F_m , an indicator of photosynthetic rate and plant health status, was recorded weekly for a period of 9 weeks. This period was chosen to allow nematode populations to complete at least two life cycles, and confirm that populations could reproduce under the growth conditions. Uninfected control groups for FRT11 and FRT23 could not be included due to limited plant material at the time of the trial. Infection with *M. incognita* and *P. coffeae* appeared to affect F_v/F_m differentially between cultivars, with *M. incognita* infection being associated with reduced F_v/F_m in Nemaya and FRT11. Infection with *P. coffeae* was related to changes in F_v/F_m in FRT23. In Nemaya, F_v/F_m fell significantly between 0-7 DPI under infection with *M. incognita*, but recovered to pre-infection levels at 14 DPI. This effect was not seen in the uninfected control plants or plants infected with *P. coffeae*. Infection with *M. incognita* was associated with a significantly lower F_v/F_m in the period 7-60 DPI in Nemaya. F_v/F_m over the 7-60 DPI period in plants infected with *M. incognita* was significantly lower than in uninfected plants. F_v/F_m in Nemaya plants also appeared to fall in the 0-7 DPI period following infection with *M. incognita*, but was not statistically different from *P. coffeae* and uninfected groups. Nemaya plants infected with *P. coffeae* did not show significantly reduced F_v/F_m compared to the uninfected control group (Figure 3.2.). In FRT23, *M. incognita* infection was associated with a greater reduction in F_v/F_m than *P. coffeae* infection in the period 0-14 DPI. The significant difference in F_v/F_m that occurred between the two infected groups was not seen in the 14-60 DPI period or across the whole

trial period (Figure 3.3.). In FRT23, F_v/F_m fell significantly from pre-infection levels when plants were infected with *P. coffeae* between 0-7 DPI. F_v/F_m in FRT23 did not differ significantly from pre-infection levels under *M. incognita* infection (Figure 3.4.).

Changes in F_v/F_m as a result of infection with *M. incognita* and *P. coffeae* species in the preliminary infection trial were used as the basis for further investigation into the effects of nematode infection.

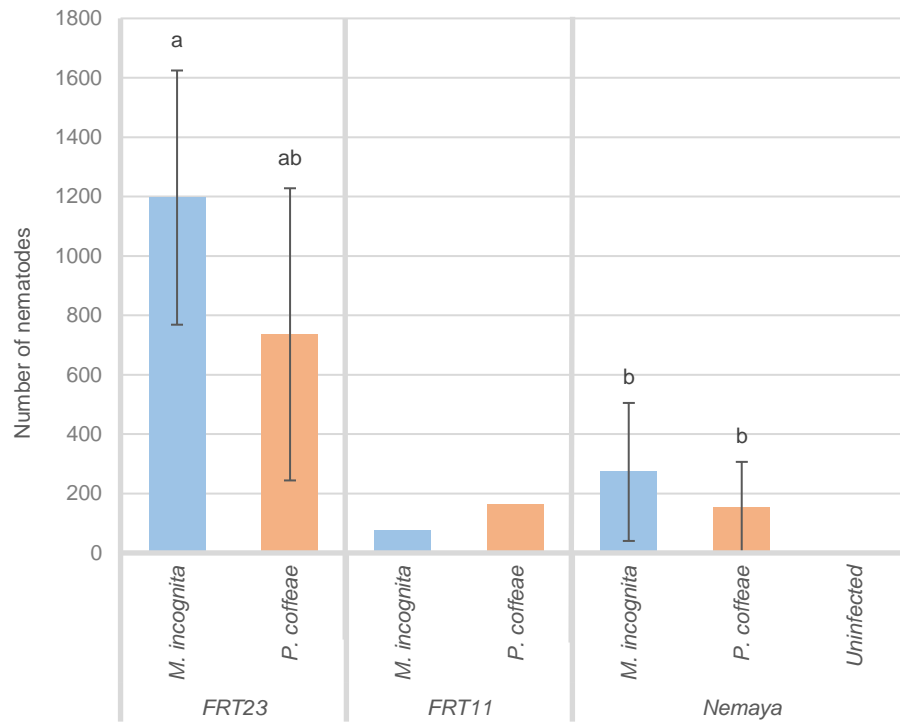


Figure 3.1. – Total number of nematodes recovered from plant root systems at the end of the 9 week period. Infective species and cultivar name are shown on the x-axis. SEM bars are shown. For FRT23 and Nemaya, n=6. Error bars could be not included for FRT11 as n=2.

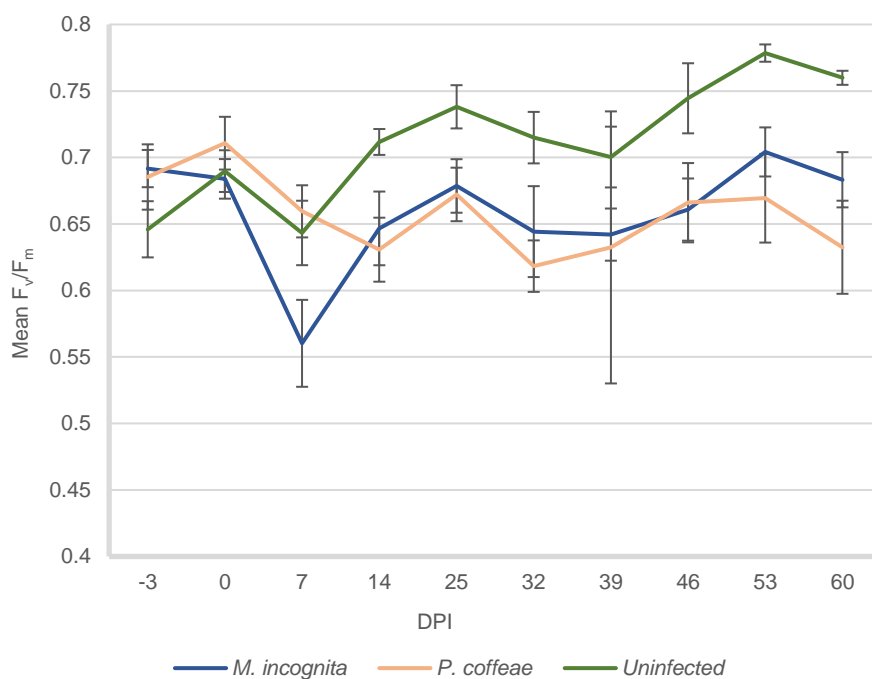


Figure 3.2. – Mean F_v/F_m in Nemaya plants under infection with *M. incognita*, *P. coffeae* or uninfected treatment over the 9 week preliminary trial period. SEM bars are shown (n=6). One way ANOVA test was performed and $p < 0.05$ was used as the confidence interval for significant difference.

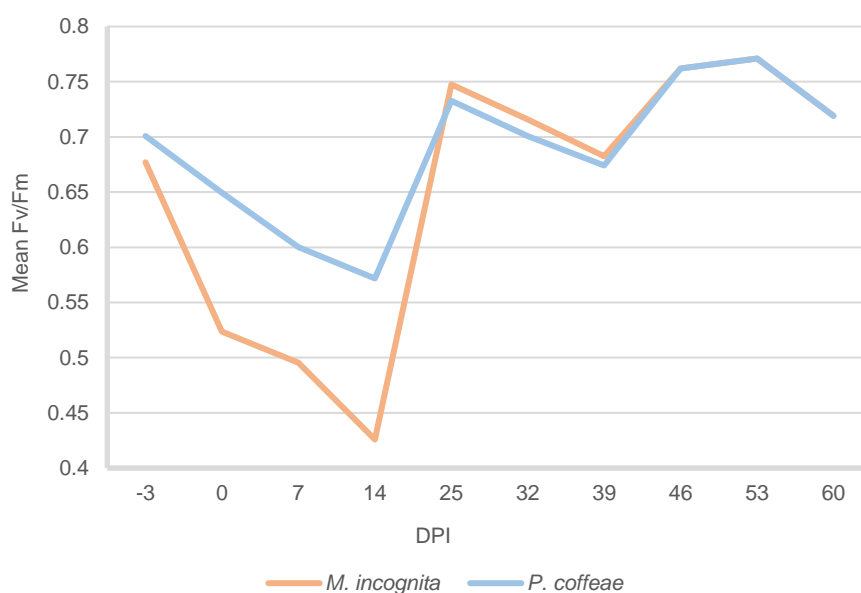


Figure 3.3. – Mean F_v/F_m in FRT11 under infection with *M. incognita* and *P. coffeae* species over the 9 week preliminary trial period. SEM could not be calculated as $n=2$ for each group in this trial. One way ANOVA test was performed and $p < 0.05$ was used as the confidence interval for significant difference.

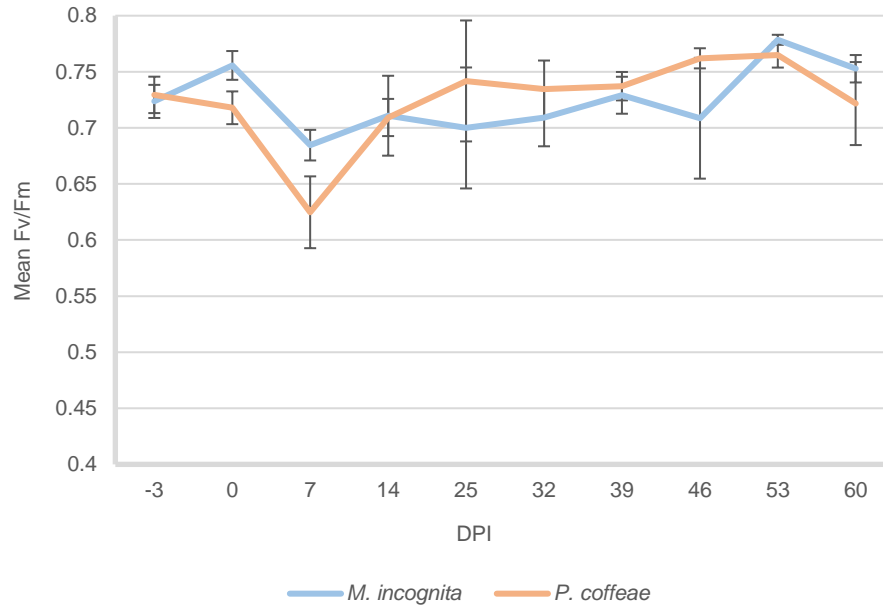


Figure 3.4. – Mean F_v/F_m in FRT23 under infection with *M. incognita* and *P. coffeae* over the 9 week preliminary trial period. SEM bars are shown (n=6). One way ANOVA test was performed and p<0.05 was used as the confidence interval for significant difference.

3.2. – Nematode intra- and inter-specific competition trial (28 days)

After observing differences in F_v/F_m and root invasion susceptibility between cultivars, an infection trial was conducted to investigate the effects of nematode infection on additional coffee cultivars. Plants were infected with either 2000 or 4000 *M. incognita* or *P. coffeae* to compare the impact of higher inoculation numbers on F_v/F_m , nematode recovery from roots and total plant fresh weight. To assess inter-specific competition between the two nematode species for host plant root feeding sites, plants were also infected with *M. incognita* and *P. coffeae* simultaneously, using 2000 of each species. FRT65, Nemaya and Arabica cultivars were tested in this trial.

Nematode infection was associated with reduced F_v/F_m in FRT65 (Table 3.1., Figure 3.5.). Between 7-28 DPI, all groups infected with nematodes showed a lower F_v/F_m than the uninfected control group: *M. incognita* (2000), *M. incognita* (4000), *P. coffeae* (2000), *P. coffeae* (4000) and *M. incognita* / *P. coffeae* (2000/2000). Between 0-7 DPI, infection with *M. incognita* (2000) was associated with significantly lower mean F_v/F_m compared to the uninfected control group and plants infected with *M. incognita* (4000). There were no other statistically significant differences in F_v/F_m between treatments during this period. Over the whole trial period, there were no significant differences in mean F_v/F_m between experimental groups. In Nemaya (Table 3.2., Figure 3.6.), plants infected simultaneously with *M. incognita* / *P. coffeae* showed lower F_v/F_m than groups infected with single nematode species during the 0-7 DPI period (*M. incognita* (4000), *P. coffeae* (2000), *P. coffeae* (4000)). F_v/F_m in plants co-infected with both species did not differ significantly from plants infected singly with *M. incognita* during the 0-7 DPI period. The reduction in F_v/F_m associated with simultaneous infection was not observed over the 7-28 DPI period. Nemaya plants infected with *M. incognita* (2000) showed lower F_v/F_m in the 7-28 DPI period compared to the uninfected control group and group infected with *M. incognita* (4000). Simultaneous infection with both nematode species was associated with significantly lower F_v/F_m than infection with *M. incognita* (4000) over the whole trial period. This effect was also observed in the 0-7 DPI period. There were no other statistically significant differences between treatments over the whole trial period. In Arabica (Table 3.3., Figure 3.7.), F_v/F_m did not differ significantly between groups under different infection treatments. No differences in F_v/F_m were observed between infected plants and the uninfected control group over 0-7, 7-28 DPI or whole trial period.

The total number of nematodes recovered from plant roots was greater in Arabica plants; when infected with 2000 and 4000 *P. coffeae*, significantly higher numbers of nematodes were recovered from Arabica plants than both Nemaya and FRT65. When infected with 4000 *M. incognita*, significantly higher numbers of nematodes were recovered from Arabica roots than Nemaya, but not FRT65. There was no significant difference between *M. incognita* recovery from FRT65 and Arabica

roots under this treatment. Similarly, there were no significant differences in *M. incognita* recovery between cultivars when 2000 *M. incognita* were applied. When 4000 *M. incognita* were applied to Arabica plants, significantly more nematodes were recovered at the end of the 28 day period than when 2000 *M. incognita* were applied. Higher levels of nematode inoculation did not significantly affect nematode recovery in Nemaya and FRT65 (Figure 3.8.).

Analysis of nematode prevalence in roots of plants simultaneously infected with both nematode species did not produce an observable competitive effect in any cultivar tested. LSD post hoc analysis did not report significant differences in the numbers of *M. incognita* and *P. coffeae* recovered from plant roots in any cultivar.

To assess the impact of nematode infection on plant growth, the total fresh weight of each plant was recorded at the end of the 28 day trial period. Due to the age of each cultivar differing slightly, comparisons in plant weight were not made between cultivars. Initial plant weight was not taken so as not to disrupt the coffee plantlets. Plant size within each cultivar was very similar, and as the trial was randomised there was no bias in plant selection. Therefore, comparing the final total plant fresh weight at the end of the trial was a valid means of testing the effect of nematode treatment on growth. Dry weights were not taken as the root system was used for nematode analysis, and leaves were used for RWC calculation.

(Figure 3.9. A) Nematode infection was associated with reduced plant fresh weight in Nemaya. Mean fresh weight of Nemaya plants was significantly lower than the uninfected control group when plants were infected with *M. incognita* (4000), *P. coffeae* (2000), *P. coffeae* (4000) and simultaneously infected with *M. incognita* / *P. coffeae* (2000/2000). Infection with *M. incognita* (2000) was not associated with significantly different plant fresh weights to the uninfected control group. Plants infected with *M. incognita* (4000) showed significantly lower fresh weights than plants infected with *M. incognita* (2000). (Figure 3.9. B) *M. incognita* infection was associated with significantly higher fresh weight than *P. coffeae* infection in FRT65. Infection with *M. incognita* (4000) caused significantly higher plant fresh weight than infection with *P. coffeae* (4000). LSD post hoc analysis did not report any other significant differences in fresh weight between treatments in FRT65. (Figure 3.9. C) Higher numbers of *M. incognita* were associated with higher fresh weight in Arabica. Infection with *M. incognita* (4000) caused higher plant fresh weight than both uninfected plants and plants infected with *M. incognita* (2000) in Arabica. Simultaneous infection with both species and infection with *P. coffeae* at both severities did not cause a significantly different plant fresh weight from uninfected plants.

Table 3.1. – Comparison of mean F_v/F_m values in FRT65 over 0-7 DPI, 7-28 DPI and whole trial periods. Superscript letters indicate significant differences between treatments. Comparisons between treatments have been made within each chosen time period, and not between time periods. One way ANOVA was performed to test for statistical significance ($n=7$). $p<0.05$ was used as the confidence interval for significant difference.

Cultivar	Treatment	Mean F_v/F_m		
		0 – 7 DPI	7 – 28 DPI	Whole trial
FRT 65	<i>M. incognita</i> (2000)	0.738 ^b	0.695 ^a	0.714 ^a
	<i>M. incognita</i> (4000)	0.762 ^a	0.705 ^a	0.728 ^a
	<i>P. coffeae</i> (2000)	0.750 ^{ab}	0.683 ^a	0.710 ^a
	<i>P. coffeae</i> (4000)	0.761 ^{ab}	0.703 ^a	0.727 ^a
	<i>M. incognita</i> / <i>P. coffeae</i> (2000/2000)	0.753 ^{ab}	0.689 ^a	0.717 ^a
	Uninfected	0.763 ^a	0.736 ^b	0.749 ^a

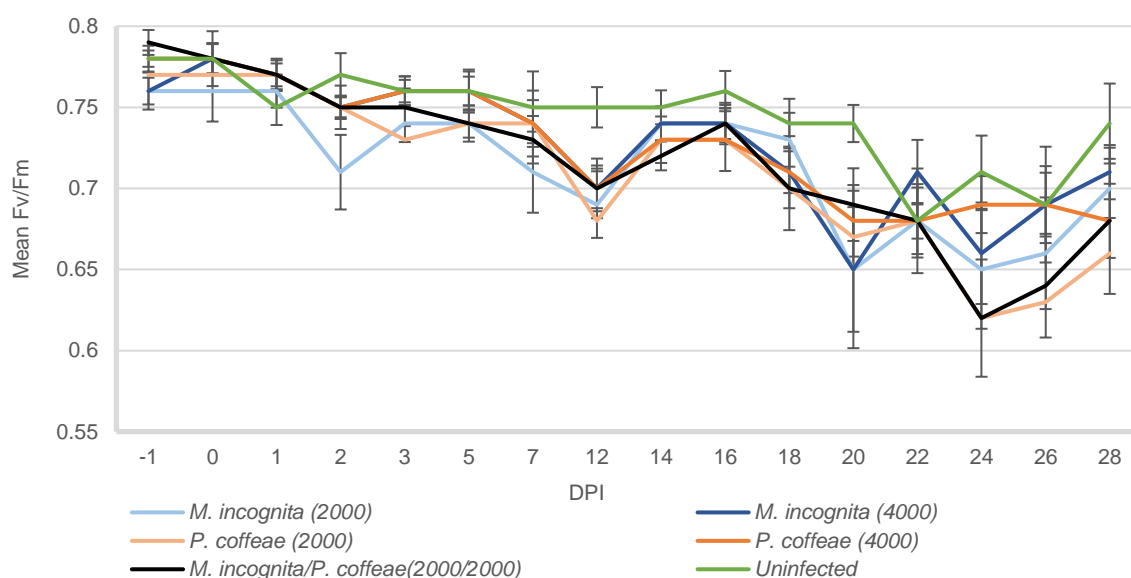


Figure 3.5. – Comparison of mean F_v/F_m readings for FRT65 for the whole trial period. SEM error bars are shown ($n=7$). One way ANOVA was performed to test for statistical significance. $p<0.05$ was used as the confidence interval for significant difference.

Table 3.2. – Comparison of mean F_v/F_m values in Nemaya over 0-7 DPI, 7-28 DPI and whole trial periods. Superscript letters indicate significant differences between treatments. Comparisons between treatments have been made within each chosen time period, and not between time periods. One way ANOVA was performed to test for statistical significance ($n=6$). $p<0.05$ was used as the confidence interval for significant difference.

Cultivar	Treatment	Mean F_v/F_m		
		0 – 7 DPI	7- 28 DPI	Whole Trial
Nemaya	<i>M. incognita</i> (2000)	0.7358 ^{ab}	0.6690 ^a	0.6928 ^{abc}
	<i>M. incognita</i> (4000)	0.7390 ^a	0.6971 ^b	0.7085 ^{ab}
	<i>P. coffeae</i> (2000)	0.7472 ^a	0.6739 ^{ab}	0.7007 ^{abc}
	<i>P. coffeae</i> (4000)	0.7372 ^a	0.6643 ^{ab}	0.6922 ^{abc}
	<i>M. incognita</i> / <i>P. coffeae</i> (2000/2000)	0.7023 ^b	0.6530 ^{ab}	0.6746 ^c
	Uninfected	0.7233 ^{ab}	0.6961 ^b	0.7037 ^{abc}

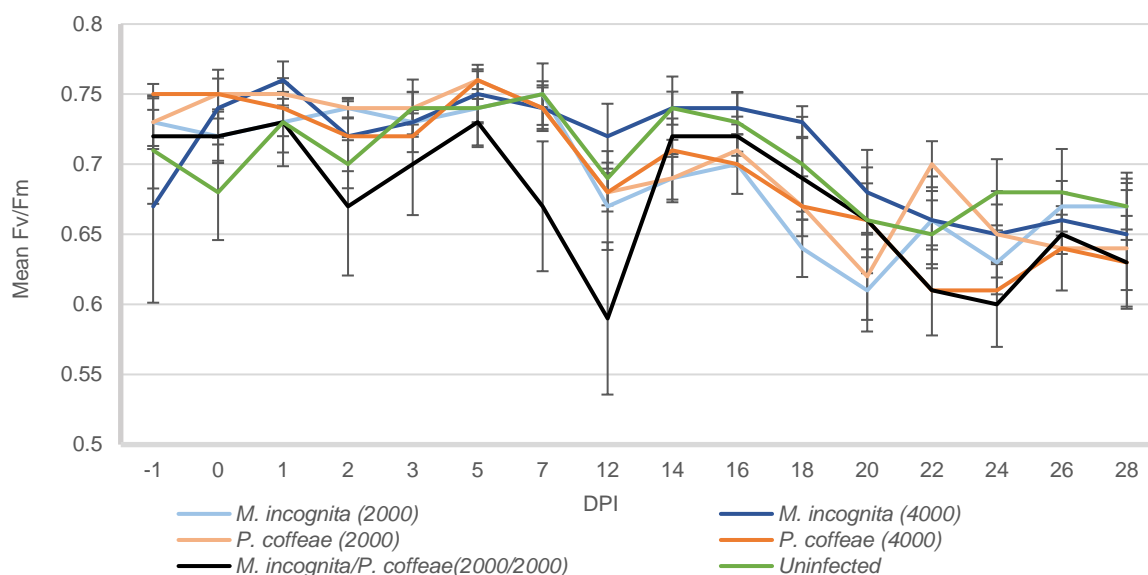


Figure 3.6. – Comparison of mean F_v/F_m readings for Nemaya for the whole trial period. SEM error bars are shown ($n=6$). One way ANOVA was performed to test for statistical significance. $p<0.05$ was used as the confidence interval for significant difference.

Table 3.3. – Comparison of mean F_v/F_m values in Arabica over 0-7 DPI, 7-28 DPI and whole trial periods. Superscript letters indicate significant differences between treatments. Comparisons between treatments have been made within each chosen time period, and not between time periods. One way ANOVA was performed to test for statistical significance ($n=7$). $p<0.05$ used as the confidence interval for significant difference.

Cultivar	Treatment	Mean F_v/F_m		
		0 – 7 DPI	7 – 28 DPI	Whole Trial
Arabica	<i>M. incognita</i> (2000)	0.7440 ^a	0.6848 ^b	0.7082 ^a
	<i>M. incognita</i> (4000)	0.7509 ^a	0.6930 ^b	0.7152 ^a
	<i>P. coffeae</i> (2000)	0.7479 ^a	0.6876 ^b	0.7102 ^a
	<i>P. coffeae</i> (4000)	0.7532 ^a	0.6996 ^b	0.7199 ^a
	<i>M. incognita</i> / <i>P. coffeae</i> (2000/2000)	0.7558 ^a	0.6858 ^b	0.7110 ^a
	Uninfected	0.7405 ^a	0.6987 ^b	0.7139 ^a

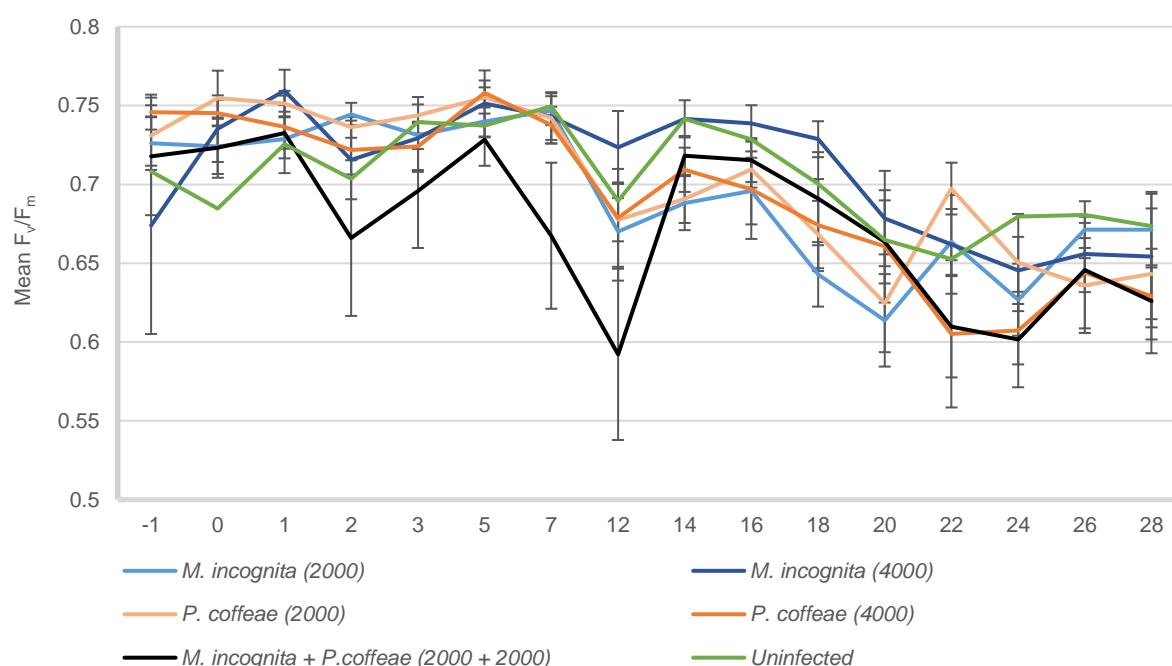


Figure 3.7. – Comparison of mean F_v/F_m readings for Arabica for the whole trial period. SEM error bars are shown ($n=7$). One way ANOVA was performed to test for statistical significance. $p<0.05$ was used as the confidence interval for significant difference.

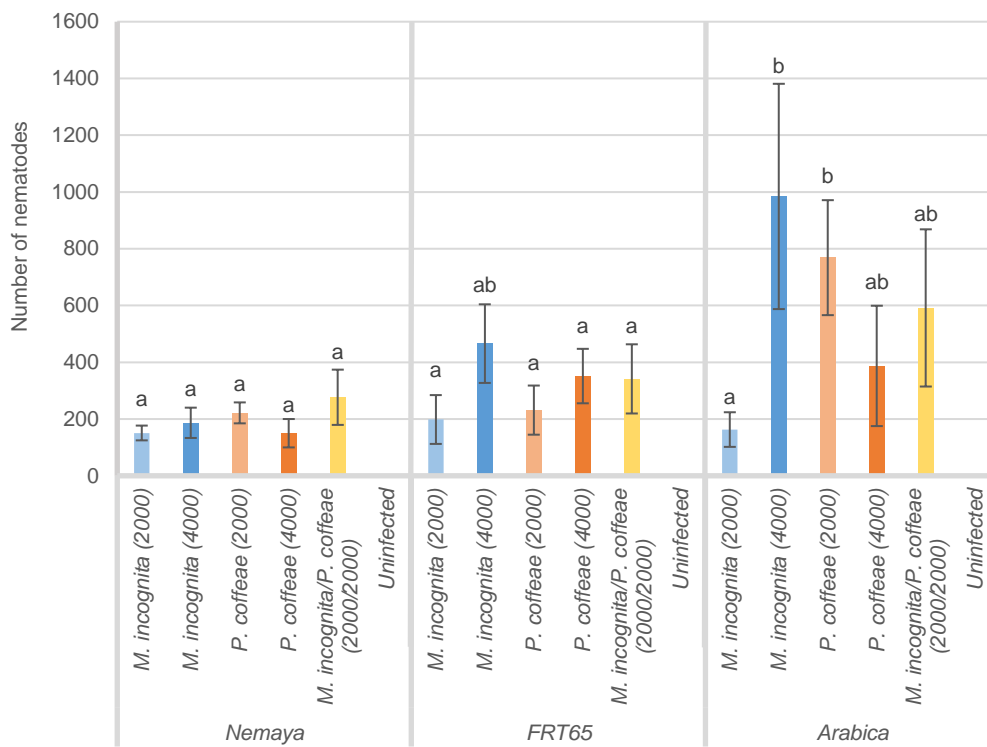


Figure 3.8. – Comparison of total numbers of nematodes recovered from root systems. Cultivars and infection treatment are shown on the x-axis. SEM bars are shown. For FRT65 and Arabica, n=7, for Nemaya n=6. Data labels indicate significant differences in nematode recovery between cultivars for each treatment. One way ANOVA was performed to test for statistical significance. $p < 0.05$ was used as the confidence interval for significant difference.

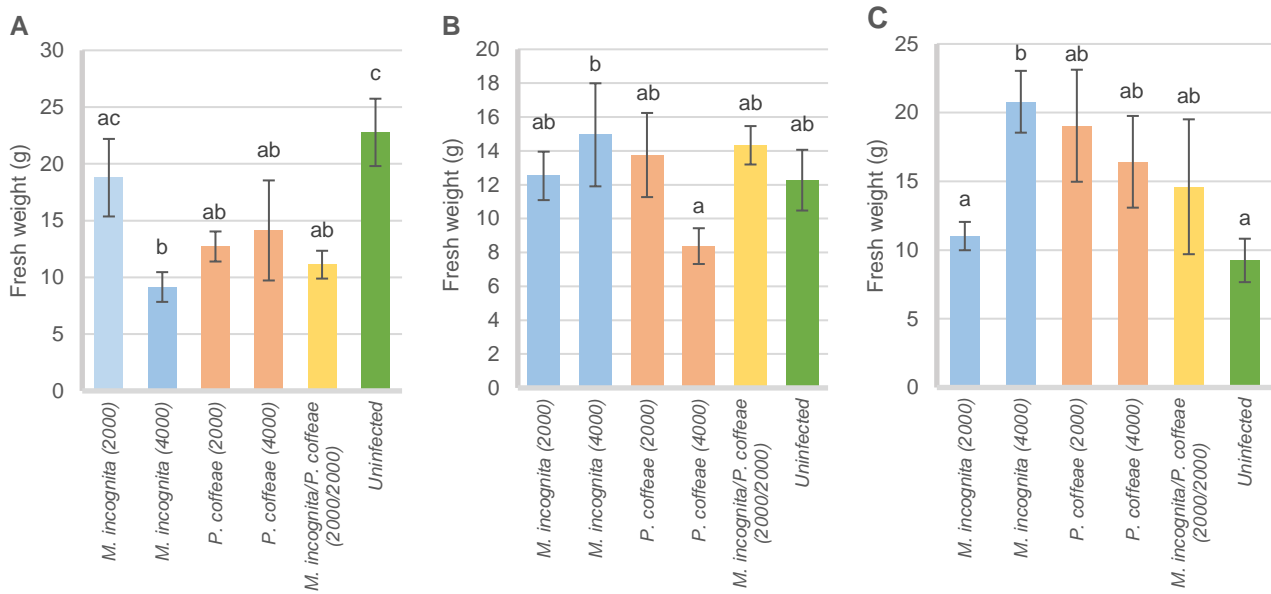


Figure 3.9. – Mean plant fresh weights at the end of the trial period for Nemaya (A), FRT65 (B) and Arabica (C) cultivars. SEM bars are shown. For FRT65 and Arabica, $n=7$, for Nemaya $n=6$. Data labels indicate statistically significant differences between treatments. One way ANOVA was performed to test for statistical significance. $p<0.05$ was used as the confidence interval for significant difference.

3.3. Preliminary drought trial to establish protocol (20 days)

To establish an effective protocol for a drought stress trial, plants were placed under four different severities of drought stress. F_v/F_m was measured by recording F_v/F_m , allowing for the relationship between drought stress and F_v/F_m in coffee to be observed, and for the identification of a suitable level of drought stress to be chosen for the subsequent trial. The cultivar FRT23 was used in the preliminary drought trial. Soil moisture was used as the measure of water availability to the plant. The four soil moistures that were tested were 40%, 30%, 20% and 10% of soil saturation.

Plants maintained under different soil moisture contents showed differences in F_v/F_m in FRT23. Plants maintained at 10% soil moisture showed significantly lower F_v/F_m compared to all other groups. Plants kept at 20% soil moisture showed significantly lower F_v/F_m than plants kept at 40%, but did not differ significantly from plants kept at 30%. As a result of this preliminary investigation, a soil moisture content of 15% was selected for use in the following trial, in order to provide adequate drought stress without being so severe as to cause plant death (Figure 3.10.).

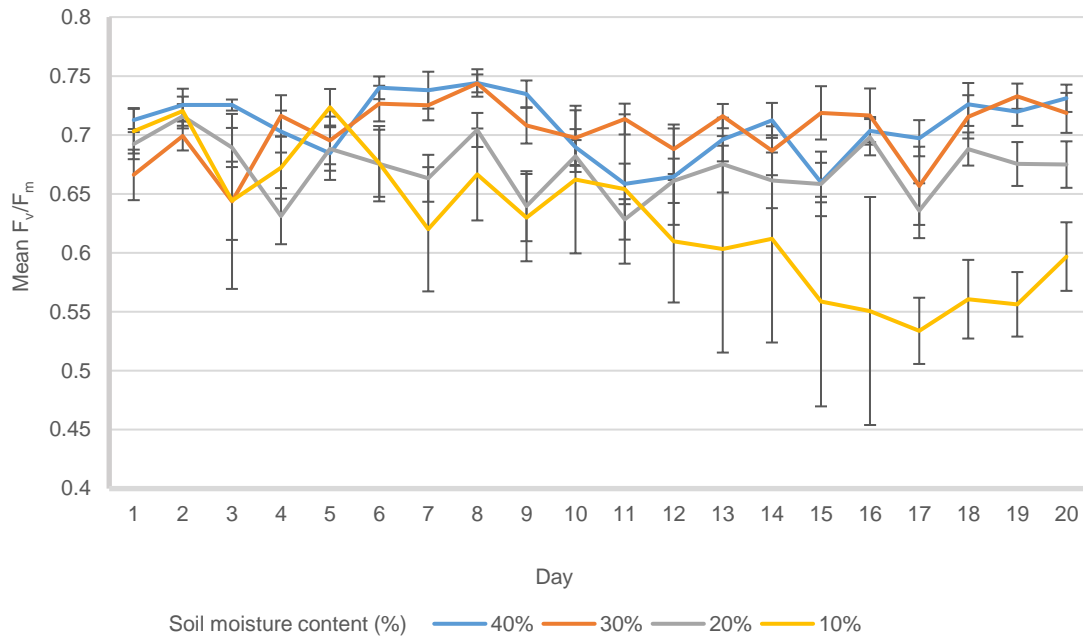


Figure 3.10. – Mean F_v/F_m over time for FRT23 plants maintained at four different soil moisture contents, shown below the x-axis ($n=8$). One way ANOVA test was performed to test for statistical significance. $p < 0.05$ was used as the confidence interval for significant difference.

3.4. Investigation into the interaction between drought and nematode infection (56 days)

To assess the interaction between drought, nematode infection and the physiological effects on coffee cultivars, an infection trial was conducted. Plants were infected with 2000 nematodes of either *M. incognita* or *P. coffeae* and maintained under watered or drought regimes. An uninfected control group was included for each regime. Plants maintained under the watered regime were watered daily by supplying water to the saucer containing the plant pot. Four soil moisture readings were taken daily per plant pot to record water availability. Plants kept under drought were only supplied with water when soil moisture readings were <15%. The trial was conducted for 56 days. F_v/F_m was analysed over 0-7, 7-28 and whole trial (0-56) DPI periods as differences were observed between experimental groups during these periods. Cultivars tested in this trial were FRT65, FRT79, FRT49 and Arabica.

F_v/F_m was measured by recording F_v/F_m . In FRT65, infection with *M. incognita* was associated with reduced photosynthetic rate under both watered and drought conditions. In the 0-7 DPI period, under the watered regime, plants infected with *M. incognita* showed reduced F_v/F_m compared to the uninfected control group. Under the drought regime, plants infected with *M. incognita* also showed reduced F_v/F_m compared to the uninfected control group. In the 7-28 DPI period, *M. incognita* infection under drought was associated with lower F_v/F_m than all other treatments. The same reduction in F_v/F_m was also seen when analysed across the whole trial period. Compared to the uninfected control groups, *M. incognita* infection was associated with lower F_v/F_m under both watered and drought regimes. No statistically significant differences were observed between F_v/F_m in uninfected plants and plants infected with *P. coffeae* under watered or drought regimes. (Table 3.4.). In FRT79, infection with *M. incognita* under drought was associated with higher F_v/F_m than plants that were supplied with water. Over all periods, plants infected with *M. incognita* under drought showed significantly higher F_v/F_m than plants infected with *M. incognita* under the watered regime. Infection with *M. incognita* did not cause a significant reduction in F_v/F_m compared to the uninfected control groups under watered or drought treatments. *P. coffeae* infection only caused significant reductions in F_v/F_m under drought conditions in FRT79. Under drought, *P. coffeae* infection caused significantly reduced F_v/F_m in the 0-7 DPI and 7-28 DPI periods compared to the uninfected control group. There were, however, no significant differences in F_v/F_m in plants infected with *P. coffeae* under each water regime. Infection with *M. incognita* or *P. coffeae* did not significantly reduce F_v/F_m under watered conditions during any time period compared to the uninfected control group. During all time periods, uninfected plants under the drought regime showed higher F_v/F_m than both infected and uninfected groups that were supplied with sufficient water (Table 3.5.).

Nematode infection was not associated with any change in F_v/F_m in FRT49. No statistically significant differences were observed between nematode treatments and uninfected control groups for FRT49 over any time period. F_v/F_m did not differ between plants under water and drought regimes in FRT 49 (Table 3.6.).

In Arabica plants, there were no significant differences in F_v/F_m between group treatments in the 0-7DPI period. During the 7-28 DPI period, Arabica plants under drought infected with *P. coffeae* showed a significantly higher F_v/F_m compared to the uninfected control group. *P. coffeae* infection did not cause a significant difference in F_v/F_m from uninfected plants under the water regime. In the 7-28 DPI period, *M. incognita* infection was associated with significantly lower mean F_v/F_m than *P. coffeae* infection under drought. This effect was not reproduced in plants under the watered regime. In infected groups, no changes in F_v/F_m were observed in comparison to uninfected groups when plants were supplied with water. Nematode infection did not affect F_v/F_m significantly over the 0-7 DPI or whole trial periods, and was not associated with significant differences in F_v/F_m compared to control groups under both water and drought regimes (Table 3.7.).

The total numbers of nematodes recovered from plant roots was higher in Arabica roots under two treatments. Under the watered regime, more *P. coffeae* were recovered from Arabica roots than all other cultivars. Under the drought regime, more *M. incognita* were recovered from Arabica roots than all other cultivars. Under the drought regime, more *P. coffeae* were recovered from Arabica roots than FRT65 roots. Under drought, Arabica roots showed greater numbers of *P. coffeae* than FRT65 roots, but there were no significant differences in *P. coffeae* numbers between Arabica and FRT79 and FRT49 cultivars. Under the water regime, there were no significant differences in *M. incognita* recovery between any cultivars (Figure 3.11.).

The total number of nematodes recovered from plant roots only differed between treatments in FRT79 (Figure 3.12. A) More *P. coffeae* were recovered from FRT79 roots when plants were kept under drought conditions compared to the watered regime. *P. coffeae* recovery was greater than *M. incognita* under drought conditions. Under the water regime, there was no significant difference between numbers of *M. incognita* and *P. coffeae* recovered. (Figure 3.12. B) No significant differences were observed between nematode recovery from FRT49 root systems. (Figure 3.12. C) No significant differences were observed between nematode recovery from FRT65 root systems (Figure 3.12. D) No significant differences were observed between nematode recovery in Arabica root systems.

The final total fresh weight of cultivars was affected differentially depending on the water regime and nematode species infecting the plant. Again, dry weights were not taken as root systems were used

for nematode analysis, and leaves were used for RWC calculation, and final fresh weight values given here represent the total fresh weight at the end of the trial period. (Figure 3.13. A) Drought did not significantly impact fresh weight in FRT79. In uninfected, *M. incognita* and *P. coffeae* infected groups, there were no significant differences in plant weight under watered and drought regimes. Plants infected with *P. coffeae* under the water regime showed significantly higher fresh weight than uninfected plants and plants infected with *M. incognita* under drought conditions. Nematode infection was not associated with changes in fresh weight compared to the uninfected control under both watered and drought regimes (Figure 3.13. B) FRT49 infected with *P. coffeae* under the water regime showed significantly higher fresh weights than plants under all other nematode treatments and water regimes. Infection with either species did not significantly affect plant weight under drought conditions. Infection with *M. incognita* did not significantly affect plant weight under the water regime in FRT49. (Figure 3.13. C) 'FRT 65' plants infected with *M. incognita* showed greater fresh weight when watered in comparison to plants under drought conditions. Similarly, plants infected with *P. coffeae* showed greater weight when watered daily as opposed to under drought. In 'FRT 65', infection with both *M. incognita* and *P. coffeae* was not associated with significantly different total fresh weight when compared to the uninfected control group under both water and drought regimes. (Figure 3.13. D) Uninfected Arabica plants under drought regime showed lower fresh weight than uninfected plants that were watered daily. The uninfected control group under the water regime showed significantly higher total fresh weight than all groups under drought. Infection with *M. incognita* was associated with lower fresh weight than uninfected plants under the water regime, but not under drought. Under both water and drought, *P. coffeae* infection did not cause a significant difference in total fresh weight compared to the uninfected control group.

Cultivars also responded differentially to drought and nematode treatment in the relative water content of leaves. (Figure 3.14. A) In FRT79, nematode infection was associated with higher relative water content under both water and drought regimes compared to uninfected plants. Plants infected with *P. coffeae* showed significantly higher relative water content values than the uninfected control group under both water and drought regimes. This result was reproduced under infection with *M. incognita*. Drought did not impact relative water content in 'FRT 79'. (Figure 3.14 B) In FRT49, nematode infection and drought did not influence relative water content. No significant differences in relative water content were observed as a result of infection with nematodes or drought treatment in this cultivar. (Figure 3.14. C) In FRT65, infection with both *M. incognita* and *P. coffeae* was associated with higher relative water content compared to uninfected plants under both water and drought regimes. There were no significant differences in relative water content between plants infected with *M. incognita* or *P. coffeae* under both water and drought regimes. Uninfected plants under drought regime showed lower relative water content than uninfected plants that were watered daily. (Figure 3.14 D) *M. incognita* infection had differential effects on relative water content in

Arabica. Under the water regime, infection with *M. incognita* did not cause a significant change in relative water content compared to the uninfected group. However, under drought conditions, *M. incognita* infection caused significantly lower relative water content compared to uninfected plants. Infection with *P. coffeae* was not associated with differences in relative water content to uninfected plants under water or drought regime. Relative water content in plants under *P. coffeae* infection were similar under water and drought regimes.

Comparison of relative water content between cultivars revealed differential effects depending on treatment and water regime. Arabica showed higher relative water content in leaves than FRT79 and FRT65 cultivars. Under drought, uninfected Arabica plants showed higher relative water content than all other cultivars. Under both water and drought regimes, Arabica plants infected with *P. coffeae* showed lower relative water content compared to FRT65. *M. incognita* infection affected relative water content differentially in Arabica under water and drought regimes. When watered, *M. incognita* infected Arabica plants showed higher relative water content than FRT79 and FRT49 cultivars. However, when plants were kept under drought, Arabica leaves showed lower relative water content than FRT65 (Table 3.8.).

Table 3.4. – Comparison of mean F_v/F_m values in FRT65 over 0-7 DPI, 7-28 DPI and whole trial periods. Superscript letters indicate significant differences between treatments (n=10). Comparisons between treatments have been made within each chosen time period, and not between time periods. LSD post hoc test was performed to test for statistical significance. $p < 0.05$ used as the confidence interval for significant difference.

Cultivar – FRT65		Mean F_v/F_m		
Regime	Treatment	0 – 7 DPI	7 – 28 DPI	Whole trial (0-56 DPI)
Watered	Uninfected	0.732 ^a	0.731 ^a	0.719 ^a
	<i>M. incognita</i>	0.708 ^b	0.714 ^a	0.698 ^b
	<i>P. coffeae</i>	0.730 ^a	0.733 ^a	0.725 ^a
Drought	Uninfected	0.741 ^a	0.726 ^a	0.722 ^a
	<i>M. incognita</i>	0.707 ^b	0.691 ^b	0.692 ^b
	<i>P. coffeae</i>	0.735 ^a	0.734 ^a	0.717 ^a

Table 3.5. – Comparison of mean F_v/F_m values in FRT79 over 0-7 DPI, 7-28 DPI and whole trial periods. Superscript letters indicate significant differences between treatments (n=5). Comparisons between treatments have been made within each chosen time period, and not between time periods. LSD post hoc test was performed to test for statistical significance. $p < 0.05$ used as the confidence interval for significant difference.

Cultivar – FRT79		Mean F_v/F_m		
Regime	Treatment	0 – 7 DPI	7 – 28 DPI	Whole trial (0-56 DPI)
Watered	Uninfected	0.698 ^a	0.699 ^a	0.692 ^a
	<i>M. incognita</i>	0.687 ^a	0.680 ^{ac}	0.681 ^a
	<i>P. coffeae</i>	0.704 ^a	0.691 ^{ac}	0.691 ^a
Drought	Uninfected	0.711 ^b	0.743 ^b	0.710 ^b
	<i>M. incognita</i>	0.718 ^b	0.724 ^b	0.712 ^b
	<i>P. coffeae</i>	0.699 ^a	0.700 ^a	0.695 ^{ab}

Table 3.6. – Comparison of mean F_v/F_m values in FRT49 over 0-7 DPI, 7-28 DPI and whole trial periods. Superscript letters indicate significant differences between treatments (n=5). Comparisons between treatments have been made within each chosen time period, and not between time periods. LSD post hoc test was performed to test for statistical significance. $p < 0.05$ used as the confidence interval for significant difference.

Cultivar – FRT49		Mean F_v/F_m		
Regime	Treatment	0 – 7 DPI	7 – 28 DPI	Whole trial (0-56 DPI)
Watered	Uninfected	0.714 ^a	0.697 ^a	0.665 ^a
	<i>M. incognita</i>	0.723 ^a	0.707 ^a	0.704 ^a
	<i>P. coffeae</i>	0.701 ^a	0.693 ^a	0.697 ^a
Drought	Uninfected	0.701 ^a	0.686 ^a	0.667 ^a
	<i>M. incognita</i>	0.715 ^a	0.704 ^a	0.704 ^a
	<i>P. coffeae</i>	0.729 ^a	0.704 ^a	0.668 ^a

Table 3.7. – Comparison of mean F_v/F_m values in Arabica over 0-7 DPI, 7-28 DPI and whole trial periods. Superscript letters indicate significant differences between treatments (n=10). Comparisons between treatments have been made within each chosen time period, and not between time periods. LSD post hoc test was performed to test for statistical significance. $p < 0.05$ used as the confidence interval for significant difference.

Cultivar – Arabica		Mean F_v/F_m		
Regime	Treatment	0 – 7 DPI	7 – 28 DPI	Whole trial (0-56 DPI)
Watered	Uninfected	0.676 ^a	0.704 ^{abc}	0.675 ^a
	<i>M. incognita</i>	0.702 ^a	0.696 ^{ac}	0.686 ^a
	<i>P. coffeae</i>	0.713 ^a	0.706 ^{abc}	0.705 ^a
Drought	Uninfected	0.704 ^a	0.699 ^{ab}	0.686 ^a
	<i>M. incognita</i>	0.689 ^a	0.691 ^{bc}	0.686 ^a
	<i>P. coffeae</i>	0.711 ^a	0.720 ^b	0.698 ^a

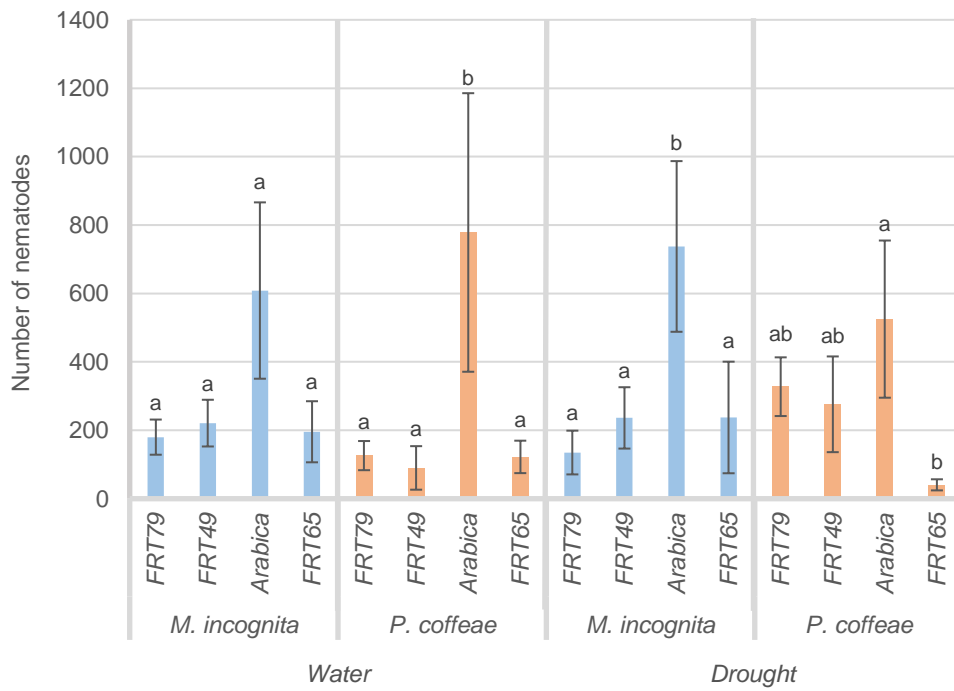


Figure 3.11. – Total number of nematodes recovered from plant root systems at the end of the 56 day trial period. Cultivar, nematode treatment and water regime are shown on the x-axis. SEM bars are shown. For FRT79 and FRT49, n=5. For Arabica and FRT65, n=10. Data labels indicate statistically significant differences within cultivars. LSD post hoc test was performed to test for statistical significance. $p < 0.05$ used as the confidence interval for significant difference.

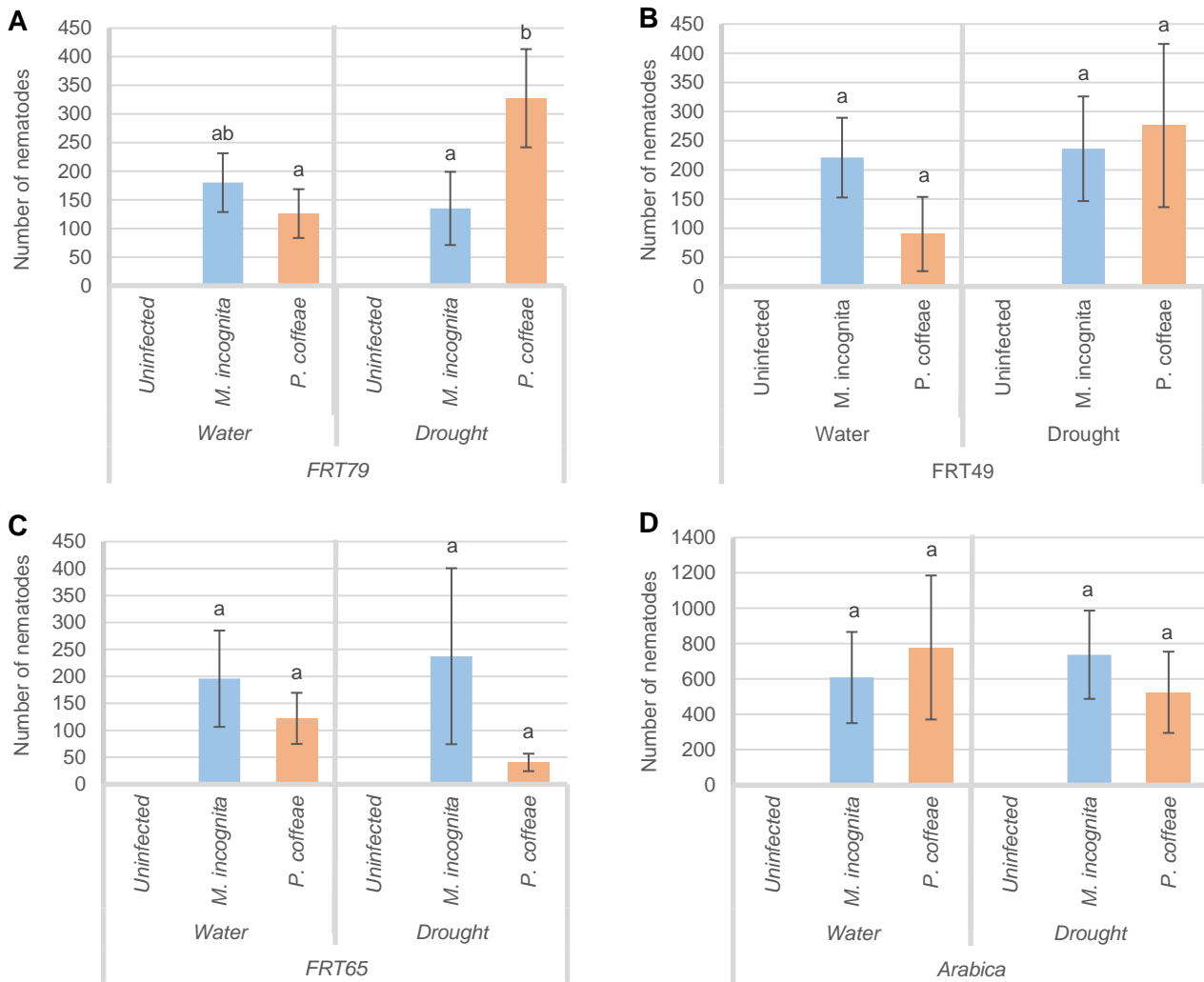


Figure 3.12. – Total number of nematodes recovered from (A) FRT79, (B) FRT49, (C) FRT65 and (D) Arabica root systems at the end of the 56 day trial period. Nematode treatment and water regime are shown on the x-axis. SEM bars are shown. For FRT79 and FRT49, n=5. For Arabica and FRT65, n=10. Data labels indicate statistically significant differences between groups. LSD post hoc test was performed to test for statistical significance. $p < 0.05$ used as the confidence interval for significant difference.

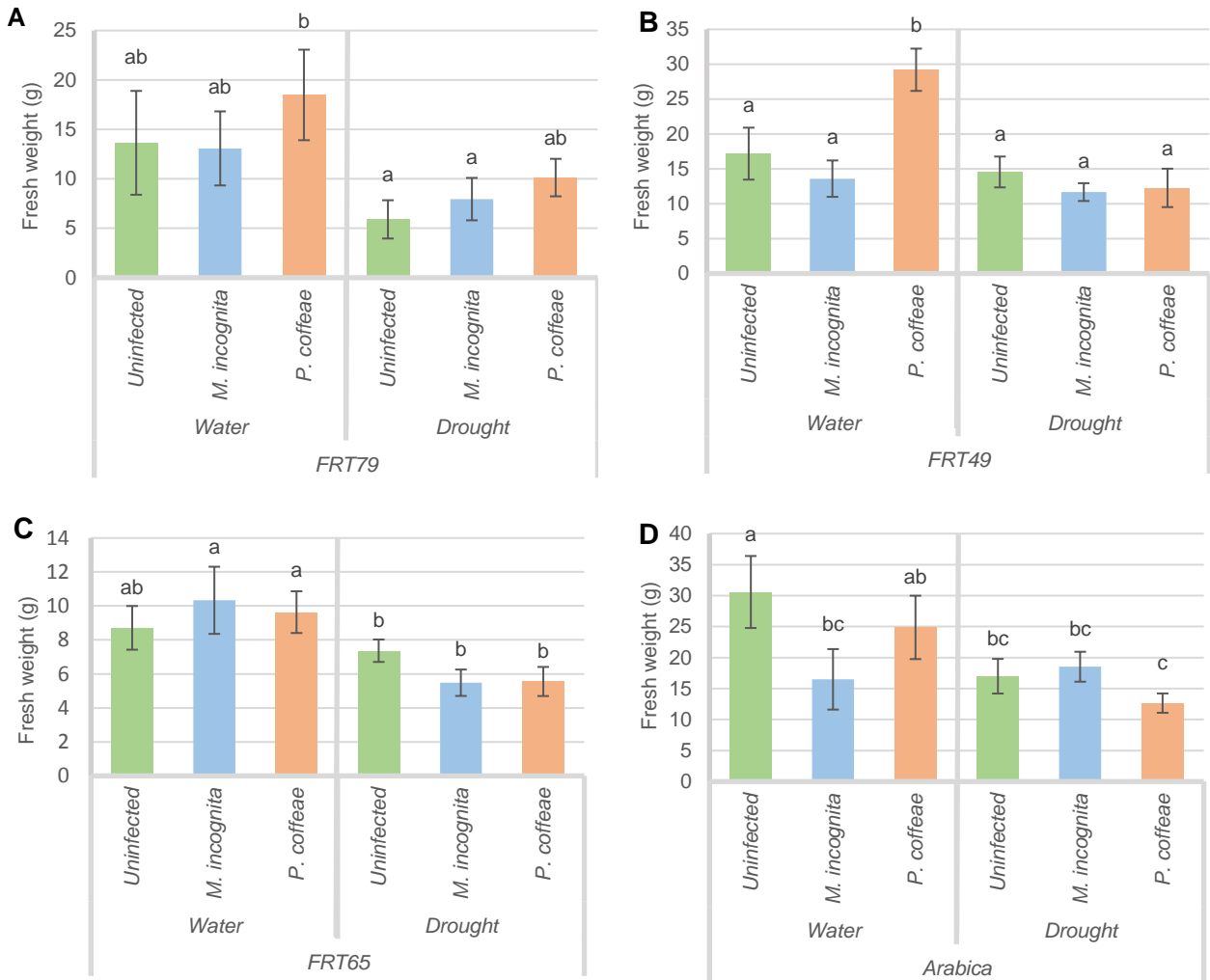


Figure 3.13. - Mean fresh weight of (A) FRT79, (B) FRT49, (C) FRT65 and (D) Arabica plants at the end of the 56 day trial period. Nematode treatment and water regime are shown on the x-axis. SEM bars are shown. For FRT79 and FRT49, n=5. For Arabica and FRT65, n=10. Data labels indicate statistically significant differences between groups. LSD post hoc test was performed to test for statistical significance. $p < 0.05$ used as the confidence interval for significant difference.

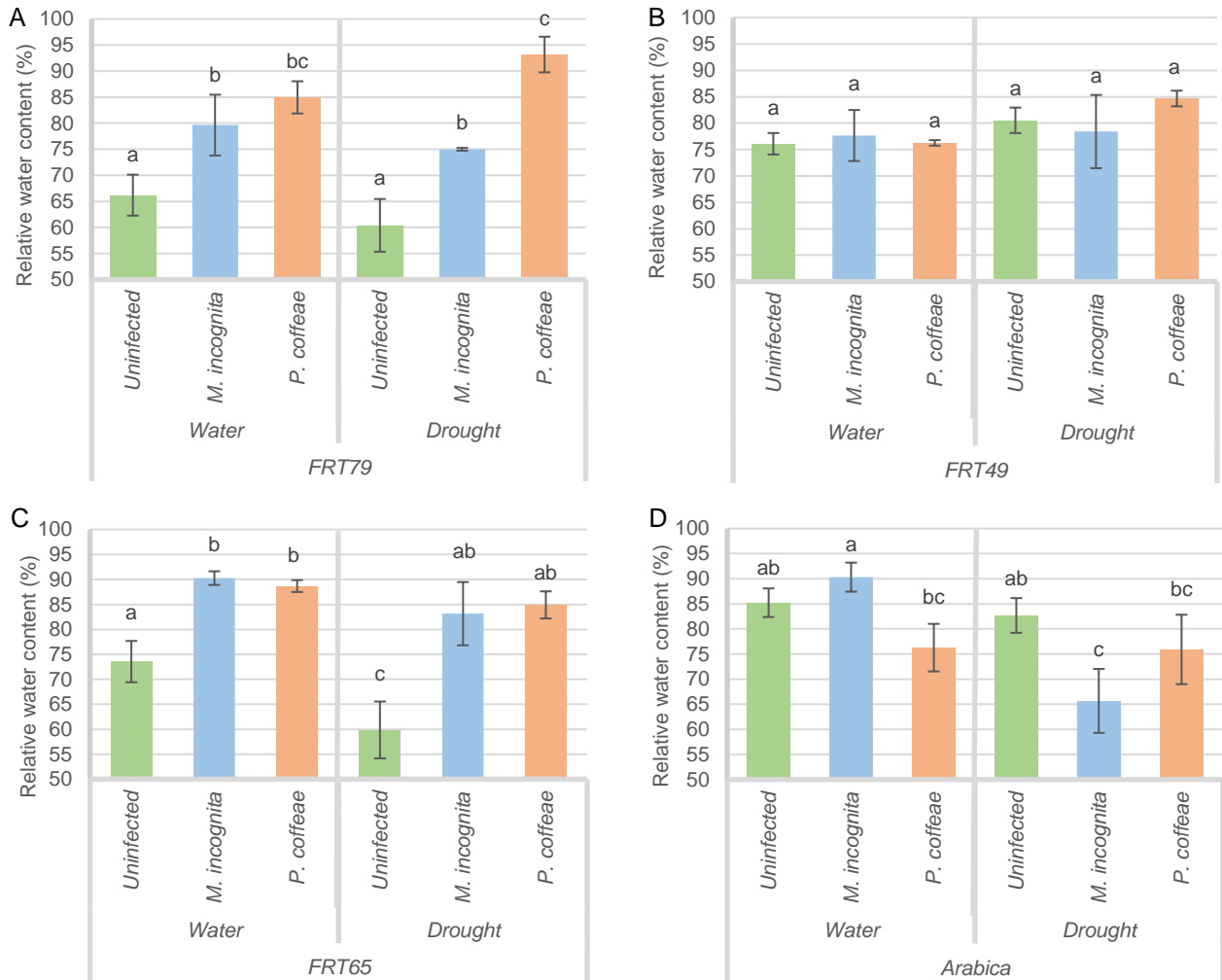


Figure 3.14. – Relative water content of leaves in (A) FRT79, (B) FRT49, (C) FRT65 and (D) Arabica cultivars. Nematode treatment and water regime are shown on the x-axis. SEM bars are shown. For FRT79 and FRT49, n=5. For Arabica and FRT65, n=10. Data labels indicate statistically significant differences between groups. LSD post hoc test was performed to test for statistical significance. $p < 0.05$ used as the confidence interval for significant difference.

Table 3.8. – Comparison of relative water content values between cultivars. Water regime, nematode treatment and cultivar are shown. Relative water content (%) values are shown. Superscript letters indicate statistically different values between cultivars under the same infection and water regime. Statistical comparisons have been made between each cultivar under the same nematode treatment and water regime only. LSD post hoc test was performed to test for statistical significance. $p < 0.05$ used as the confidence interval for significant difference.

		Relative water content (%)			
Regime	Treatment	FRT79	FRT49	FRT65	Arabica
Water	Uninfected	66.19 ^a	76.10 ^{ab}	73.55 ^a	85.22 ^b
	<i>M. incognita</i>	79.64 ^a	77.67 ^a	90.27 ^{ab}	90.31 ^b
	<i>P. coffeae</i>	84.96 ^{ab}	76.27 ^{ab}	88.68 ^a	76.27 ^b
Drought	Uninfected	60.39 ^a	80.54 ^a	59.86 ^a	82.68 ^b
	<i>M. incognita</i>	75.00 ^{ab}	78.41 ^{ab}	83.15 ^a	65.66 ^b
	<i>P. coffeae</i>	93.18 ^{ab}	84.67 ^{ab}	84.90 ^a	75.91 ^b

3.5. Nematode root exudate attraction assays

To gain insight into the attraction of nematodes to the roots of different coffee cultivars, agar plate assays were performed. Nematode movement towards either the water plug or root exudate plug was recorded after 24 hours incubation at 25°C. Nematodes were counted as having moved towards each plug if they entered the 1.5cm zone around the plug after 24 hours. Nematodes that did not enter either zone were not counted as being significantly attracted to either plug, as movement outside of these zones could be considered not to be targeted towards either the exudate or water control plug.

(Figure 3.15. A) *P. coffeae* attraction did not differ between coffee root exudates. No significant differences in attraction were found between root exudates. *P. coffeae* did not show greater attraction to any root exudates compared to the water control. (Figure 3.15. B) Attraction of *M. incognita* was highest in FRT23 and FRT65 root exudates. FRT23 was the only root exudate that attracted *M. incognita* more than the water control plug. (Figure 3.15. C) *M. paranaensis* showed less attraction to Nemaya root exudate compared to the water control. This significant difference in attraction between the root exudate plug and the water control was seen only in Nemaya root exudate. There were no significant differences in *M. paranaensis* attraction between other cultivars and water control plugs (Figure 3.15.).

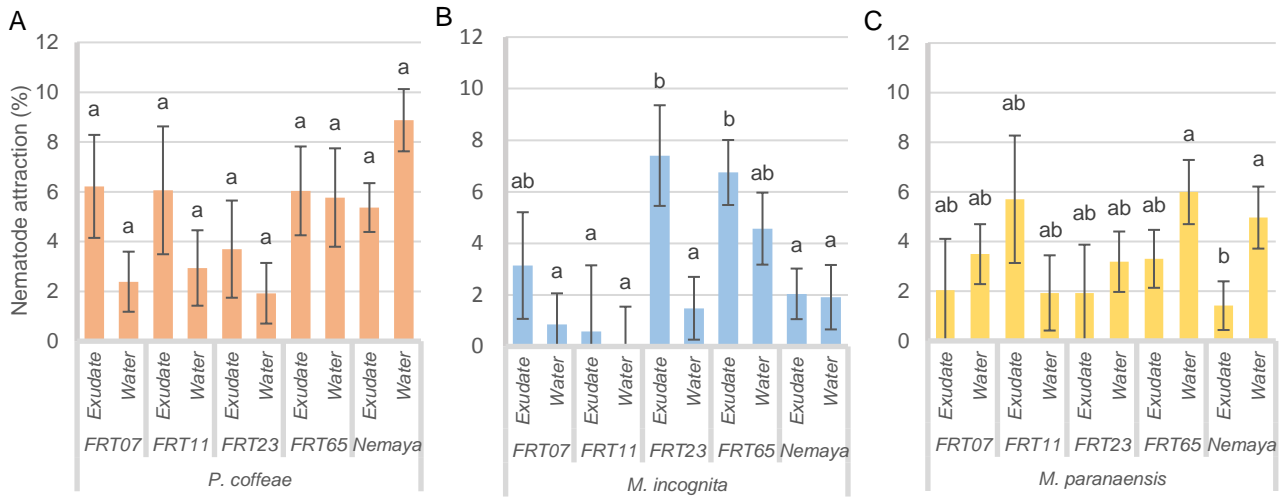


Figure 3.15. – Mean percentage attraction of *P. coffeae* (A), *M. incognita* (B) and *M. paranaensis* (C) to coffee root exudates and water control plugs at 24 hours after application to the agar plate. Cultivar is shown on the x-axis. SEM bars are shown (n=6). LSD post hoc test was performed to test for statistical significance. $p < 0.05$ used as the confidence interval for significant difference.

3.6. Summary of Results

Table 3.9. – Summary of results for nematode competition trial (3.2).

	FRT65	Arabica	Nemaya
F_v/F_m	<i>M. incognita</i> infection reduced F _v /F _m in the 0-7 DPI period. F _v /F _m was reduced under all nematode infections in 7-28 DPI period.	F _v /F _m did not differ between infected and uninfected plants.	F _v /F _m was reduced under infection with <i>M. incognita</i> (2000) and co-infection with <i>M. incognita</i> / <i>P. coffeae</i> .
Nematode recovery	Nematode recovery did not differ between experimental groups.	Nematode recovery was greater in Arabica for <i>M. incognita</i> (2000) and <i>P. coffeae</i> (2000).	Nematode recovery did not differ between experimental groups.
Total fresh weight	Plant fresh weight was higher in plants infected with <i>M. incognita</i> (2000). Infection with <i>P. coffeae</i> (4000) was associated with lower fresh weight than <i>M. incognita</i> (4000).	<i>M. incognita</i> (4000) infection was associated with higher fresh weight than infection with <i>M. incognita</i> (2000) and uninfected plants.	Plant fresh weight was lower under infection with <i>M. incognita</i> (4000), <i>P. coffeae</i> (2000), <i>P. coffeae</i> (4000) and <i>M. incognita</i> / <i>P. coffeae</i> co-infection.

Table 3.10. – Summary of results for nematode infection and drought trial (3.4).

	FRT65	FRT79	FRT49	Arabica
F_v/F_m	<i>M. incognita</i> was associated with lower F _v /F _m than uninfected plants under water and drought conditions.	<i>M. incognita</i> was associated with higher F _v /F _m under drought compared to watered plants.	Nematode infection did not affect F _v /F _m . Drought also had no effect on F _v /F _m	F _v /F _m did not differ in infected and uninfected plants under water or drought regimes. F _v /F _m was higher in plants under drought infected with <i>P. coffeae</i> than watered plants infected with <i>M. incognita</i>
Nematode recovery	Infective nematode species and water regime did not significantly affect numbers of nematode recovered from roots.	More <i>P. coffeae</i> were recovered from roots under drought conditions than under the water regime. <i>P. coffeae</i> recovery was also greater than <i>M. incognita</i> recovery under drought conditions.	Infective nematode species and water regime did not significantly affect numbers of nematode recovered from roots.	Nematode recovery from Arabica was greater under <i>P. coffeae</i> /water and <i>M. incognita</i> /drought than all other cultivars. More <i>P. coffeae</i> were recovered from Arabica roots than FRT65 roots under drought.
Total fresh weight	Plants infected with <i>M. incognita</i> under water regime showed greater weight than under drought. This result was replicated under <i>P. coffeae</i> infection.	Drought did not impact fresh weight. <i>P. coffeae</i> infected plants showed higher weight under watered conditions than under drought.	<i>P. coffeae</i> infection was associated with higher weight than all other treatments, including uninfected plants.	All groups under drought showed lower weight than uninfected plants under water regime. Compared to uninfected plants, <i>M. incognita</i> infection was associated with lower weight under water regime, but higher weight under drought. <i>P. coffeae</i> infection did not cause a significant difference in weight compared to uninfected plants under either water regime.
Relative water content	Infection with <i>M. incognita</i> and <i>P. coffeae</i> were both associated with higher weight under water and drought conditions compared to uninfected plants.	Infection with nematode species was associated with higher relative water content under water and drought regimes. Drought did not impact relative water content.	Nematode infection or drought did not significantly impact relative water content. There were no significant differences between groups.	<i>M. incognita</i> infection was associated with lower relative water content under drought conditions, but not under the water regime. <i>P. coffeae</i> infection was not associated with changes in relative water content compared to uninfected plants under either water regime.

4. Discussion

4.1. Robusta cultivars showed strong nematode resistance

Resistance to nematodes can be defined as the ability of the host plant to prevent infection and reproduction in the root tissues, whilst tolerance is exhibited when the host plant is able to maintain satisfactory yield and agronomic traits despite the presence of an infective population (Cook and Evans, 1987, Trudgill, 1991). Nemaya is a resistant rootstock used for its high yield under infection the field (Souza, 2008). The Robusta cultivars tested showed similar nematode populations in roots to Nemaya, suggesting good resistance (Figures 3.8. and 3.11.). In many *C. canephora* cultivars, a hypersensitive-like response in root cells resulting from the expression of resistance genes such as *Mex-1* is the major mechanism by which nematode establishment and reproduction in roots is limited (Anthony *et al.*, 2005). A lack of *Mex-1* and other resistance-related genes in Arabica may have allowed for easier infection by infective stage nematodes, resulting in the greater root populations observed under several treatments in this cultivar.

4.2. Nematode infection affected photosynthesis differentially

Changes to photosynthetic rate occur as a result of nematode infection, with both *Meloidogyne* and *Pratylenchus* spp. having previously been reported to impair photosynthesis in coffee through nutrient and water loss to the infective population (Kubo *et al.*, 2003, Mazzafera *et al.*, 2004, Hurchanik *et al.*, 2004). Measuring F_v/F_m in response to nematode infection provides insight into the nematode tolerance status of the host plant. F_v/F_m measurements show that FRT49 and Arabica maintained stable PSII activity under infection, while FRT65 and FRT79 showed reductions that suggest poorer nematode tolerance (Tables 3.3., 3.4., 3.5., 3.6., and 3.7.). Nutrient and water loss to the nematode population were sufficient to reduce F_v/F_m in FRT65 and FRT79, whilst infected FRT49 and Arabica maintained F_v/F_m at similar levels to uninfected plants.

Long term infection is associated with reduced leaf chlorophyll content, water content and nutrient availability, all of which inhibit photosynthesis (Lu *et al.*, 2014). Decreasing carbon assimilation as a result of *Meloidogyne* and *Pratylenchus* spp. infection has previously been reported in crops such as green bean, barley and soybean, and prolonged infection would be expected to reduce plant growth and coffee bean yield (Mazzafera *et al.*, 2004, Melakeberhan *et al.*, 1985, Forti *et al.*, 2015, Umesh *et al.*, 1994). Therefore, the maintenance of stable F_v/F_m measurements under nematode infection implies better agronomic performance. This is important for the characterisation of these cultivars, as photosynthetic activity acts as a predictor of growth and yield (Araus *et al.*, 1998, Flagella *et al.*, 1995). The observation of stable F_v/F_m ratios in FRT49 and Arabica suggests that yield of the crop would be affected less by nematode infection. In Arabica, the significantly greater

populations of nematodes than Robusta cultivars in roots did not impair PSII efficiency, indicating good tolerance of both nematode species.

4.3. Drought stress alone did not reduce F_v/F_m

Drought is considered to be the most important abiotic stress factor, causing growth limitation and reduced yields (Trenberth *et al.*, 2014). Stomatal closure, carbohydrate accumulation in leaf tissues and enzyme inhibition are proposed mechanisms by which drought inhibits photosynthesis (Cornic, 2000, Chaves and Oliveira, 2004, Lawlor, 2002). Drought stress was not sufficient to cause reduced F_v/F_m in this study, supporting previous findings that F_v/F_m is not significantly affected by moderate drought stress alone (Lima *et al.*, 2002, DaMatta *et al.*, 2002). Reduced F_v/F_m at 10% soil moisture in the preliminary drought trial (3.3.) was likely a result of the severe stress that this water limitation placed on the plant. F_v/F_m readings in FRT79 was higher under drought in two treatments, suggesting strong performance at lower soil moistures and hence highlighting the potential usefulness of this cultivar in areas where drought conditions are common (Table 3.5.).

4.4. Drought stress increased the impact of nematode infection on F_v/F_m in two cultivars

The damage threshold of the host plant is reduced under drought, allowing for successful infection to occur at lower nematode population densities (Smiley, 2015). Drought and nematode infection would be expected to cause greater plant stress, and therefore reduce growth and yield further than either factor in isolation, as each stress is thought to be independent and additive, with the presence of one stress increasing the severity of the other (Davis *et al.*, 2014). This effect was observed, with the severity of *M. incognita* and *P. coffeae* infection increasing under drought in FRT65 and FRT79 respectively (Tables 3.4. and 3.5.). This finding supports observations of the additive effects of stress also seen in rice (Audebert *et al.*, 2000). Additive stress effects were not seen in FRT49 and Arabica, with both cultivars showing strong tolerance to drought and infection with regard to photosynthesis (Tables 3.6. and 3.7.). As Arabica evolved in a region with an extended dry season, greater tolerance to prolonged periods of drought should be expected (Willson, 1999).

Stable F_v/F_m readings under infection in both watered and drought conditions in FRT49 and Arabica indicated tolerance of both abiotic and biotic stress. These results imply the maintenance of good yields in these cultivars under nematode infection and water limitation, therefore providing the basis for their use in areas that are both infested with nematodes and at risk of extended periods of drought.

4.5. Drought stress had a greater impact on final plant fresh weight than nematode infection

In three cultivars, lower weight in uninfected plants under drought, compared to *P. coffeae* infected plants that were watered, suggests that water availability was a more important determinant factor for growth than infective status (Figure 3.13. A, B, C). The stimulation of growth at low levels of nematode infection and with less pathogenic species has been observed in grape and tomato (McKenry and Anwar, 2006, Corbett *et al.*, 2011). Increased plant weight under *P. coffeae* infection and water availability suggests that *P. coffeae* may be less pathogenic to *Coffea* spp. than *M. incognita*, causing some stimulation of growth in response to root tissue damage when water was sufficient in the soil. FRT49 exhibited the least variation in plant weight under infection and drought treatments, further demonstrating a robust response to stress and supporting its use in locations where both stresses exist (Figure 3.13. B). As initial plant fresh weights could not be taken, the percentage change in fresh weight could not be calculated. However, these values provide comparisons between treatments that may be useful in indicating the future growth of each cultivar under each nematode treatment and water regime.

4.6. Leaf water content was higher under infection in two cultivars

Relative water content in coffee leaf tissues has been shown to remain high under all but the most severe drought conditions, as *Coffea* spp. are not considered to be drought-tolerant, but water-conserving, retaining water in the tissues whilst drought stress negatively impacts the plants metabolic processes (DaMatta, 1993). This finding was supported by relative water content measurements, with values largely being similar between infective treatments under drought or water regimes, suggesting drought conditions alone had a much smaller effect on leaf water content (Figure 3.14.). Drought tolerance traits are associated with stomatal control of water use in coffee, with stomatal conductance reducing as xylem pressure (an indicator of water availability) decreases (Pinheiro *et al.*, 2005). Reduced CO₂ flow into the plant as a consequence of stomatal closure reduces respiration potential, limiting carbon fixation and plant growth (Bird, 1974).

As nematode infection reduces water availability to the host, stomatal closure, and therefore a higher leaf water content as a result of infection would be expected in leaves. FRT65 and FRT79 exhibited elevated leaf water content under nematode infection, which may be indicative of the plant's strategy to conserve water and prevent loss to the infective nematode (Figure 3.14. A, C). Reduced CO₂ flow into the plant as a result of this may also explain the lower F_v/F_m readings observed in these cultivars (Tables 3.4. and 3.5.). A lesser degree of leaf water content change under infection in FRT49 suggests that this cultivar did not experience water stress to the same extent as the other cultivars, and therefore stomatal conductance remained high under both nematode and drought stresses (Figure 3.14. B). Higher leaf water content did not correlate with

increased F_v/F_m , suggesting that water retention through stomatal closure in FRT79 and FRT65 limited CO_2 availability and therefore photosynthetic efficiency (Figure 3.14. A, C). This further supports the use of FRT49 or Arabica in nematode infested fields, as the lesser extent of relative water content change in leaves suggests a more robust response to infection.

4.7. Nematode reproductive potential was limited at higher inoculation densities

Higher inoculation densities did not produce more severe effects on photosynthetic rate in this study (Tables 3.1., 3.2. and 3.3.). Declines in nematode populations at high initial densities have been reported, which may explain why larger inoculant populations did not lead to greater effects on photosynthetic rate (Oostenbrink, 1966). Intraspecific competition within populations for root invasion sites is likely to have limited infective potential, so that the number of infective nematodes exceeded the number of available root infection sites, supporting observations made by Umesh *et al.* (1994). Reproductive potential of the population may have been more limited at the greater inoculation density. As plants used in this trial were relatively young, the size of the root system may not have provided sufficient invasion sites for the inoculant population.

4.8. *M. incognita* infection caused opposing effects on plant weight in Nemaya and Arabica

Plants inoculated with the higher density of *M. incognita* exhibited reduced final plant fresh weight in Nemaya compared to those inoculated with the lower population density, supporting evidence of correlation between larger inoculant populations and reduced root weights reported by Vovlas and Di Vito (1991) (Figure 3.9.). In Arabica, final plant fresh weight was greater under higher *M. incognita* inoculation density in comparison to the lower density, most likely due to greater population establishment in Arabica roots, leading to great giant cell formation and root galling (Figure 3.8.). As *M. incognita* populations under the higher inoculation density were significantly lower in Nemaya than Arabica, this evidence supports the use of Nemaya as a resistant rootstock in nematode infested plantations.

4.9. FRT65 limited reproduction of *P. coffeae* under drought conditions

Due to differences in infective strategy, *P. zae* reproduction has been reported to be up to 50 times more successful than *M. incognita* in wheat (Kagoda *et al.*, 2015). Under drought, FRT65 limited *P. coffeae* establishment more successfully than other cultivars, although plant growth was limited under these conditions (Figures 3.11. and 3.13.). This observation supports the use of FRT65 as a rootstock to limit *P. coffeae* population development in the field, although yield losses would be predicted based on the plant weight and relative water content values recorded (Figures 3.9., 3.13. and 3.14.).

4.10. Coffee root exudates affected nematode chemotaxis differentially

Chemicals exuded by plant roots have been observed to have attractive, repellent, or neutral effects on nematode attraction (Prot, 1980, Diez and Dusenbery 1989, Wang *et al.*, 2018). Plant species and the composition of soil biota such as microbial pathogens and mycorrhizal fungi are important factors influencing nematode chemotaxis (Bais *et al.*, 2006). These chemotaxis assays were performed in agar to prevent interactions with soil biota that would interfere with nematode attraction. Greater attraction of *M. incognita* to FRT23 and FRT65 exudates than other cultivars suggest the chemical profile of root exudate from these cultivars cause attraction, although as attraction to FRT65 was not greater than water, this could not be confirmed for this cultivar as the result may have arisen from random movement (Figure 3.15. B). Nemaya, used in the field for good performance under nematode infection, showed lower attraction of *M. paranaensis* to its root exudate than water (Figure 3.15. C). Nematode repellence from the roots of this cultivar may be a factor in the reported strong performance in the field under infection, although this was not observed for *M. incognita* or *P. coffeae*.

The lack of difference in attraction between root exudates and water in most cases suggest that the chemical composition of root exudate did not result in a net attraction or repulsion of nematodes. Previous work has suggested that the CO₂ environment in the rhizosphere influences nematode movement, and as exudate agar was used instead of live plant material, this gradient would not have been established in the agar and may have limited attraction (Curtis, 2008). Exudated compounds such as amino acids and sugars have been shown to attract nematodes, whilst other exudates such as enzymes, antibiotics and mucilage cause repellance. The microniche concept suggests that different cells within the root have different chemical exudation profiles that specify where nematode root invasion occurs. Using the whole root exudate may have provided the nematode with a mixture of attractant and repellent chemicals, resulting in the lack of difference in attraction between root exudate and water seen in most cases (Pierson, 2000). Future investigation into nematode chemotaxis in response to coffee root exudate should involve the characterisation of the compounds contained in the exudates from each coffee cultivar, followed by chemotaxis assays investigating the strength of attraction or repellence to each compound. This data could then be used in conjunction with the quantity of each compound in the root exudate, therefore allowing the overall attraction or repellence to each exudate to be quantified.

4.11. Summary and Future Work

These nematode pests are distributed on a global scale and affect thousands of plant species. The methods of investigation into plant health used in this work can be applied to many different crop systems, and can be used to predict crop yields under different pathogen and climactic conditions.

This information can then be used to inform the planning and implementation of crop breeding programmes. Developing cultivars that show strong performance under pathogen attack and adverse environmental conditions is essential in all agricultural crops. In coffee, the work of organizations such as World Coffee Research and Global Coffee Platform aims to provide international coordination in research, and sharing of *Coffea* spp. genetic rootstock material, which has previously been lacking in breeding superior cultivars. Using physiological approaches, such as the techniques outlined in this work, in combination with the genetic profiling of new cultivars to screen for specific resistance to the pests that are prevalent in coffee producing areas will provide a powerful approach to producing new cultivars that effectively reduce the impact of specific pests and diseases. These approaches will also allow for quicker screening of potentially useful cultivars, and allow the time taken for the development of new cultivars through crop breeding to be significantly reduced. Developing and characterising cultivars with desirable traits targeted to the biotic and abiotic conditions of specific regions and crops is likely to be the most significant route through which the agricultural challenges of the future are tackled.

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