Research article

Survival of *Pratylenchus brachyurus* under dry soil conditions

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A B S T R A C T

Pratylenchus brachyurus, a root-lesion nematode, depends on host plants for growth and survival. Weeds, volunteer plants, and crop root residues may act as reservoirs for the parasite in the field, but little is known about the ability of *P. brachyurus* to survive in the absence of a host. This study aimed to evaluate *P. brachyurus* survival and infectivity in artificially and naturally infested soil under dry conditions. Two experiments were conducted, the first using artificially infested soil and the second using naturally infested soil. Soil samples were inoculated with a nematode suspension or infected root fragments. At 0, 30, 60, and 90 days post-inoculation, pots were planted with nematode-susceptible maize and soybean. Fallow pots were also analyzed. Nematode survival, infectivity, and morphology were determined 30 days after planting. *P. brachyurus* showed enhanced survival in soil in the presence of root fragments. However, inoculation method had no effect on the ability of surviving nematodes to infect host roots. Parasites showed signs of anhydrobiosis (C-shaped or tightly coiled body) after 90 and 120 days under dry conditions.

1. Introduction

Pratylenchus brachyurus (Godfrey) Filipjev and Schuurmans Stekhoven is a parasite of important food crops, such as maize and soybean. The distribution of nematodes in the field depends on interactions between biotic, abiotic, and management factors. No-till farming, a practice adopted in many countries, has contributed to the persistence of plant-parasitic nematodes in the soil, especially in Brazil, where maize and soybean are grown in succession (Dias-Arieira et al., 2012).

P. brachyurus population densities reduce during the off-season when no crops are cultivated. However, survival is ensured by the presence of hosts in the field, such as maize and soybean roots, weeds, and volunteer plants. Even after the roots are degraded, nematodes remain in the soil, and their longevity depends on soil moisture and accumulated energy reserves (McSorley, 2003; Neves et al., 2012).

The increase of organic matter in the soil and low moisture may reduce pathogen activity and population levels (Perry and Moens, 2011). Plant-parasitic nematodes, however, have developed mechanisms to respond to changes in environmental conditions, especially when adverse conditions interfere with their life cycle (Wang et al., 2009). Such a phenomenon is known as cryptobiosis and can occur in response to moisture stress (anhydrobiosis), cold stress (cryobiosis), and oxygen shortage (anoxybiosis) (Erkut and Kurzchalia, 2015).

Anhydrobiosis is a physiological state of desiccation-induced dormancy, characterized by a drastic reduction in metabolism and cessation of movement and feeding. The mechanisms involved in anhydrobiosis include decreased cuticle permeability and packing of tissues and organelles. Some nematode species may increase the production of non-reducing sugars, such as trehalose (Watanabe, 2006), to maintain membrane lipids and proteins within cells, preserving structural integrity despite water loss (Crowe, 2002; Erkut and Kurzchalia, 2015). When exposed to dry conditions, nematodes can coil their bodies via muscle and cuticle contraction (Treonis and Wall, 2005; Otsubo et al., 2006; Tsai, 2008; Neves et al., 2012), thereby reducing their surface area (Perry and Moens, 2011).

Several species of the genus *Pratylenchus* have developed the ability to remain in an anhydrobiotic state in the soil or in host roots for long periods, including *Pratylenchus penetrans* (Cobb) Filipjev and Schuurmans Stekhoven, *Pratylenchus thornei* Sher and Allen, *Pratylenchus mediterraneus* Corbett, *Pratylenchus coffeae* (Zimmermann) Filipjev and Schuurmans Stekhoven, *Pratylenchus jordanensis* Hashim, and *P. brachyurus* (Glazer and Orion, 1983; Townshend, 1984; Mani, 1999; Tsai, 2008; Neves et al., 2012). However, little is known about the ability of *P. brachyurus* to survive in the soil under dry conditions.

The objectives of this study were to determine the ability of *P. brachyurus* to survive in artificially and naturally infested soils and

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investigate the effects of dry soil conditions on nematode survival, infectivity, and morphology.

2. Material and methods

2.1. Survival and infectivity of P. brachyurus in artificially infested soil

Two experiments were conducted in a greenhouse and temperature-controlled room, with temperatures of 17–31 °C and relative humidity of 58–76%.

In Experiment 1, treatments were arranged in a $2 \times 3 \times 4$ factorial in a completely randomized design with six replications. The first factor was the inoculation method (nematode suspension or infected root fragments), the second factor was the cover crop (fallow, maize, or soybean), and the third factor was the period of incubation of parasites in non-irrigated soil prior to planting (0, 30, 60, or 90 days).

The population of *Pratylenchus brachyurus* used in this study was obtained from naturally infected soybean plants cultivar NA 7337 grown in a commercial farm in Brazil (17°47′53″S 50°55′41″W). *Pratylenchus brachyurus* individuals were identified based molecular characters (Machado et al., 2007).

Soybean roots were washed, cut into 2 cm pieces, and crushed in a blender (Coolen and D'Herde, 1972). The density of the inoculum was adjusted to 800 nematodes mL^{-1} using a Peters' counting chamber. Half of the experimental units were inoculated with 5 mL of the nematode suspension, totaling 4000 nematodes per pot.

The same plant material was used to obtain infected root fragments. Roots were cut into 3 cm pieces, and an aliquot was collected for determination of nematode quantity, performed as described above. Roots were found to contain 1143 nematodes g^{-1}. The other half of the experimental units received the addition of 3.5 g of root, totaling 4000 nematodes per pot.

Each experimental unit consisted of a pot containing 200 cm^3 of a 2:1 (v:v) mixture of soil and sand, previously treated by solarization (Ghini, 1997). The substrate was composed of 21% organic matter, 70% sand, 5% silt, 25% clay and pH 4.9. Inoculation was performed by adding the nematode suspension or root fragments into a hole in the soil and covering the hole with soil. Pots were incubated in a temperature-controlled room at 25 °C under a photoperiod of 12 h light and 12 h darkness, without any irrigation.

At 0, 30, 60, and 90 days post-inoculation, pots were planted with nematode-susceptible plants to assess the infective capacity of surviving individuals. Seeds of soybean (*Glycine max*) cultivar NA 7337 and maize (*Zea mays*) cultivar VT PRO2 were disinfected with sodium hypochlorite (1%) for 1 min and germinated in seed trays of moist, coarse sand in a temperature-controlled room at 25 °C. Three days after sowing, when the primary root of seedlings was about 3 cm in length, seedlings were transplanted, one per pot, into nematode-infested soil. Pots planted with seedlings were kept in a greenhouse and were irrigated as necessary, while those without plants remained in the same place, but without irrigation.

At 30 days post-transplantation (i.e., at 30, 60, 90, and 120 days post-inoculation), root and soil samples were collected for analysis of nematode population density. Plant roots were washed carefully and weighed, determining fresh weight. Nematode extraction was performed as described above, according to Coolen and D'Herde (1972). Nematodes were extracted from soil samples (200 cm^3) by centrifugation in a solution of sucrose (Jenkins, 1964). The number of nematodes was counted using a Peters' chamber and an inverted microscope 40x objective.

2.2. Survival and infectivity of P. brachyurus in naturally infested soil

A second experiment was performed to evaluate the survival ability of *P. brachyurus* in naturally infested soil. Experiment 2 was arranged in 3 × 4 factorial design with six replications. Cover crop (fallow, maize, or

soybean) was the first factor, and the period of nematode incubation in dry soil (0, 30, 60, or 90 days) was the second.

Naturally infested soil and soybean roots were obtained from the same commercial farm indicated in Experiment 1. Soil was collected 90 days after soybean was planted. The organic matter, sand, silt, clay and pH were 15%, 92%, 2%, 6% and 4.7, respectively. For inoculum quantification, 20 samples of about 3 kg of soil and roots, divided into 4 subsamples, were collected from the rhizosphere of symptomatic plants. In the laboratory, samples were mixed, and roots separated by sieving through a 20-mesh sieve. Soil samples (100 cm^3) were subjected to nematode extraction following the method of Jenkins (1964). Nematode population density averaged 100 nematodes 100 cm^{-3} soil. Roots were cut into 3 cm pieces and subjected to nematode extraction according to Coolen and D'Herde (1972). Roots contained 1143 nematodes g^{-1}.

Soil and root fragments (1.3 L in total) were added to plastic pots to represent an initial nematode population of 5300 nematodes per pot. Incubation periods and experimental procedures were the same as described for Experiment 1, except that the volume of soil analyzed at 30 days post-transplantation was 1.3 L.

2.3. Effect of dry soil conditions on P. brachyurus

Changes in nematode morphology in response to dehydration were analyzed in Experiments 1 and 2. At 30 days post-transplantation, the found individuals in anhydrobiosis were prepared in temporary slides and analyzed under an inverted microscope 100x objective.

2.4. Statistical analysis

Data were subjected to analysis of variance at the 5% probability level. Differences in nematode population density in roots between inoculation methods (Experiment 1) and cover crops (Experiments 1 and 2) were compared by the Bonferroni *t*-test. Mean nematode population densities in soil (fallowed, under maize, or under soybean) in both experiments were compared by Tukey's test at the 5% probability level. Linear and quadratic regressions were used to describe the relationship between nematode population density and incubation period. Statistical analyses were performed using Sisvar (Ferreira, 2011).

3. Results

3.1. Survival and infectivity of P. brachyurus in artificially infested soil

The interaction effects of inoculation method, incubation period, and cover crop on nematode survival (nematode population density in soil) were significant ($P \leq 0.05$). However, the results are presented separately for clarity. Soils inoculated with infected roots at the time of planting maize (Figure 1a) and soybean (Figure 1b) had significantly higher nematode populations than soils inoculated with nematode suspensions. Nematode survival did not differ significantly between inoculation methods for the other incubation periods. Fallow soil inoculated with infected roots had significantly higher nematode populations than fallow soil inoculated with nematode suspension, regardless of incubation period (Figure 1c).

Nematode survival in soils under maize showed a quadratic relationship with incubation period; inoculation with root fragments was estimated to result in the lowest population density after 101 days of incubation, and inoculation with suspension was estimated to produce the lowest population density after 154 days (Figure 1a). In soils under soybean, it was estimated that nematode population would be lowest with 111 days of incubation for samples inoculated with infected roots and highest with 49 days of incubation for samples inoculated with nematode suspension, reducing thereafter (Figure 1b). The relationship between nematode population and incubation period in fallow soil could not be described by linear or quadratic equations (Figure 1c).
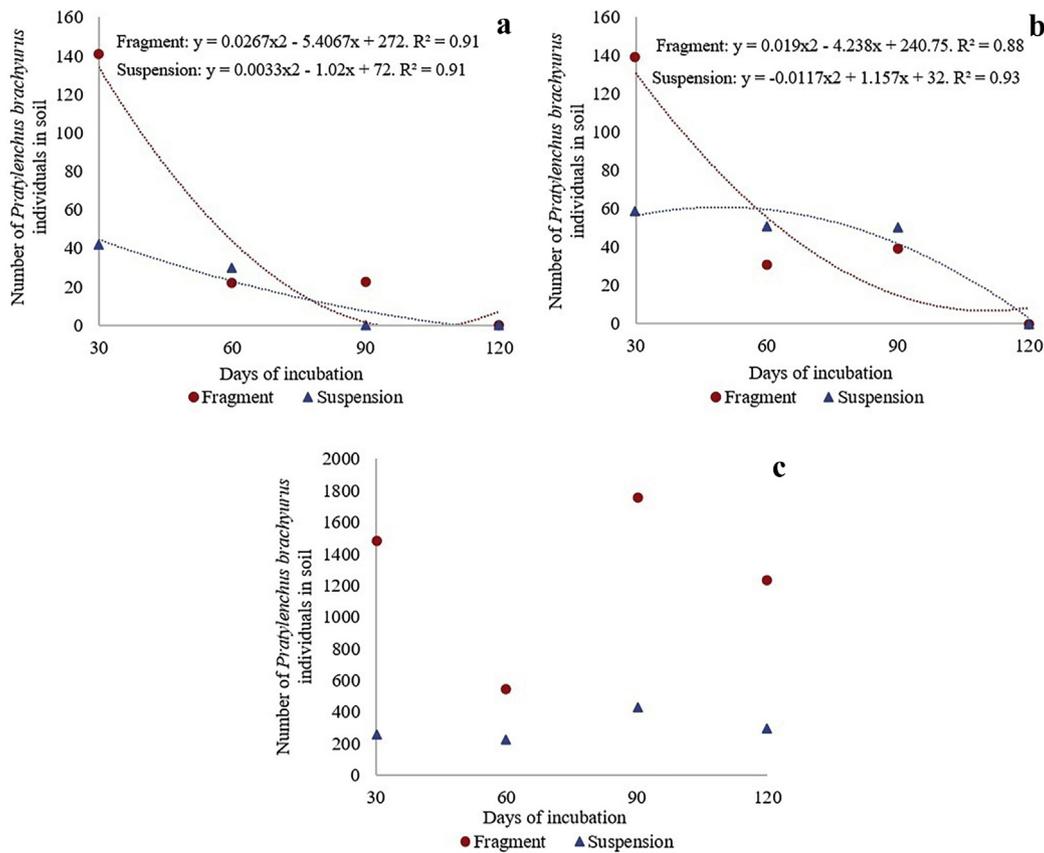
Figure 1. Effect of inoculation method on *Pratylenchus brachyurus* survival in fallow soil (a), soil in which maize (b) and soybean were cultivated (c) after incubation under dry conditions.

Nematode population density was significantly higher in fallow soil than in soil under maize or soybean (not differing significantly between the two), regardless of incubation period and inoculation method (Figure 2). Regression analysis showed that maize and soybean results could be described by quadratic equations, whereas fallow soil results could not be described by either linear or quadratic terms.

The interaction effects of variables on nematode infectivity (nematode population in roots) were also significant (Figures 3 and 4). In maize (Figure 3a) and soybean (Figure 3b) planted immediately after inoculation (day 0), nematode populations were significantly higher by inoculation with infected roots than with nematode suspension (P ≤ 0.05). Nematode population density did not differ significantly between

inoculation methods for the other incubation periods for maize (Figure 3a), whereas population densities significantly when inoculating with infected roots than with suspension in soybean planted on day 60 of incubation. For other incubation periods, nematode densities in soybean roots did not vary between inoculation methods (Figure 3b).

The relationship between nematode infectivity in maize roots and incubation period was best described by a quadratic equation. Nematode population density was estimated to be lowest with 62 days of incubation for soil inoculated with infected roots and with 72 days of incubation for samples inoculated via nematode suspension (Figure 3a). A quadratic equation was also used to describe nematode infectivity in soybean roots for inoculation with infected roots. Nematode populations were
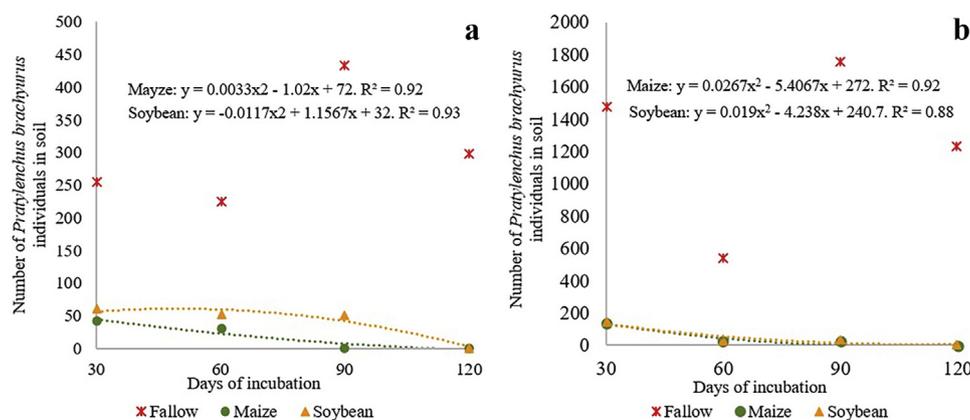

Figure 2. Effect of cover crop on *Pratylenchus brachyurus* survival in soils inoculated with nematode suspension (a) and infected root fragments (b) after incubation under dry conditions.
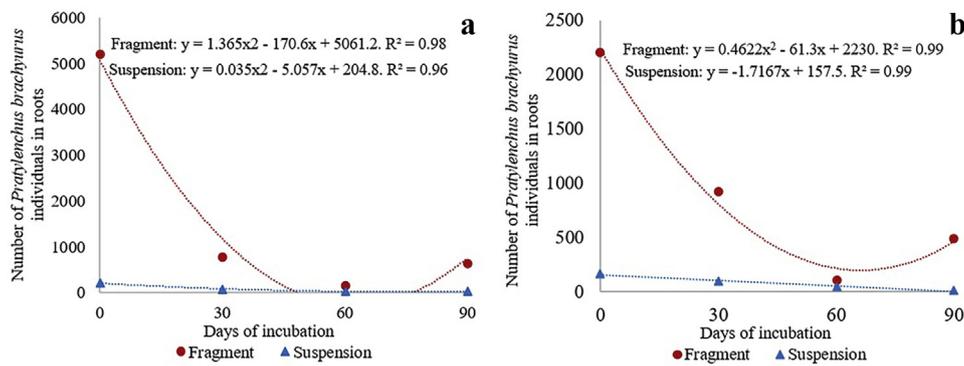
Figure 3. Effect of inoculation method on *Pratylenchus brachyurus* infectivity in maize (a) and soybean (b) roots after incubation under dry conditions.
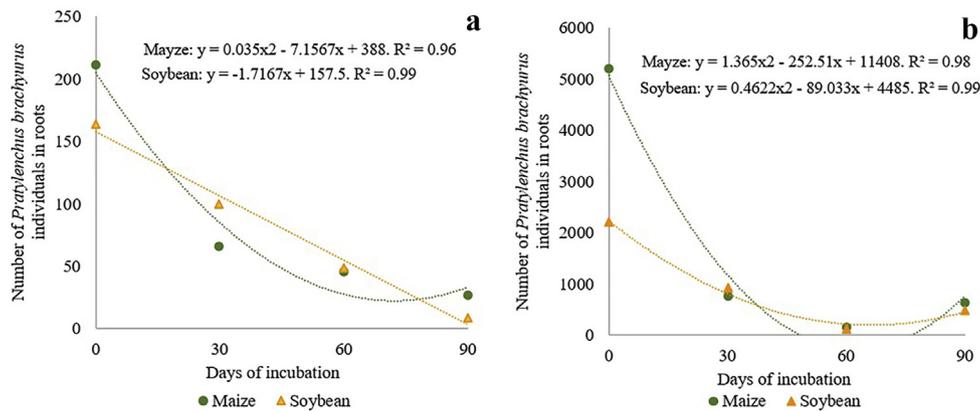

Figure 4. Effect of cover crop on *Pratylenchus brachyurus* infectivity in the roots of crops planted in pots inoculated with nematode suspension (a) and infected root fragments (b) following incubation under dry conditions.

estimated to reach the lowest densities with 66 days of incubation. Population density decreased linearly with incubation time in soybean inoculated with the nematode suspension (Figure 3b).

The nematode population densities in maize and soybean roots inoculated with the nematode suspensions did not differ significantly (Figure 4a). For pots inoculated with infected roots, population density was significantly higher in maize than in soybean planted on day 0 of incubation but did not differ significantly between crops for other incubation periods (Figure 4b). The relationship between nematode population density in maize roots and incubation period was best described by quadratic equations. A linear equation was used to describe nematode population density in soybean planted in soil inoculated with nematode suspension (Figure 4b), and a quadratic equation best described nematode population densities in soybean planted in soil inoculated with infected roots (Figure 4a).

3.2. Survival and infectivity of P. brachyurus in naturally infested soil

Interaction effects between incubation period and cover crops were observed (Figure 5) in the second experiment (P ≤ 0.05). Overall, data corroborate those of the first experiment. Nematode population was significantly higher in fallow soil than in soil in which maize or soybean were grown for 30 days after a 60- or 120-day incubation period (Figure 5). Relationships between nematode incubation period and nematode population were best described by quadratic equations. Population densities in fallow soil, soil under maize, and soil under soybean were estimated to be lowest with 72, 105, and 96 days of incubation, respectively.

Nematode populations were significantly higher in maize roots than in soybean roots in non-incubated pots but did not differ significantly

between crops for other incubation periods (Figure 6). Incubation time had a quadratic effect on nematode population densities in maize and soybean roots.

3.3. Effects of dry soil conditions

P. brachyurus juveniles were found in a quiescent state in soils incubated for 90 and 120 days without irrigation. The cuticle was intact and shrunken, suggestive of anhydrobiosis (Figure 7). Nematodes in open-C (Figure 7a and b) and closed C-shapes (Figure 7e) and tightly coiled
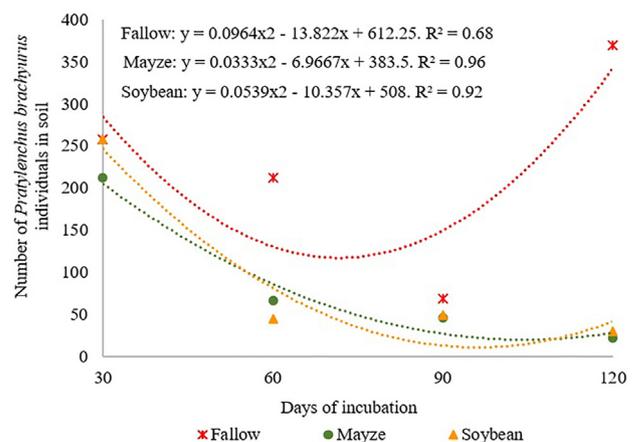

Figure 5. *Pratylenchus brachyurus* population densities in naturally infested soil after incubation under dry conditions.
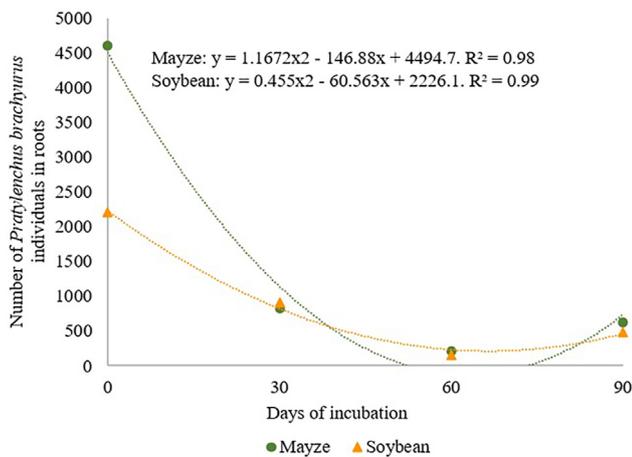
Figure 6. *Pratylenchus brachyurus* population densities in maize and soybean roots grown in naturally infested soil after incubation under dry conditions.

(Figure 7c and 7d), or coiled in the caudal end (Figure 7f) were observed. Vacuoles were observed inside the body of dead nematodes, and the esophagus and intestines were not visible (Figure 7). No differences in nematode shape were found between incubation periods or inoculation methods.

4. Discussion

The results showed that nematodes were able to survive better under dry soil conditions in the presence of root fragments, corroborating the findings of Neves et al. (2012). This result supports that no-till planting may favor the persistence of *P. brachyurus* in the soil throughout the off-season, as root residues, especially those of maize, can act as reservoirs (Inomoto, 2008).

Nematodes living within plant roots are protected against desiccation until senescence. However, nematodes present in the soil are affected by environmental conditions, such as increased temperature and reduced soil moisture, and can thus suffer rapid dehydration (McSorley, 2003). It is possible that, within the root, nematodes were able to control water loss better and retain infectivity (Perry and Moens, 2011). Plant-parasitic nematodes depend on the moisture level of the environment (whether soil or roots) to control their water loss. In the absence of root residues, low soil moisture greatly affects nematode population densities (Asmus and Ishimi, 2009).

Nematodes can be found at different stages of development within plant roots. Eggs are well adapted to survive and, in the case of *Pratylenchus*, have a longer life span than mobile forms (Perry and Moens, 2011). Juveniles hatch with a finite lipid reserve, sufficient to reach and infect the host plant (Christophers et al., 1997). *P. thornei* juveniles were shown to lose 89% of their lipid reserves in six weeks at 10 °C. The loss is greater in females than males because of their higher metabolic activity (Storey et al., 1982).

Management practices that stimulate nematode activity in the absence of host plants might increase parasite mortality. Nematodes use lipid reserves more quickly under high moisture than low moisture conditions (Storey et al., 1982). For instance, irrigation during the dry season in an area without the presence of hosts can stimulate juvenile hatching and increase metabolic activity, causing nematodes to consume the stored energy.

Nematode population density in the soil was highest in the present study in fallow pots than in pots planted with maize or soybean. Because they are obligate parasites, nematodes will migrate to and penetrate the roots of susceptible plants and remain inside while conditions are favorable for growth.

After incubation under dry conditions, the remaining nematode populations were able to infect host roots, even though their density had decreased substantially, suggesting that *P. brachyurus* specimens remained active and motile. The results agree with those observed for *P. penetrans* (Townshend, 1984), *P. thornei* (Thompson et al., 2017), and *Rotylenchulus reniformis* Linford and Oliveira (Torres et al., 2006).

P. brachyurus with anhydrobiotic characteristics were found in fallow soil inoculated with infected roots and nematode suspensions. The specimens were morphologically similar to other plant parasites in

Figure 7. *Pratylenchus brachyurus* individuals extracted from fallow pots incubated under dry conditions for 90 and 120 days. a and b, open-C shaped; c and d, coiled; e, closed-C shaped; f, coiled in the caudal end.

anhydrobiotic states (Glazer and Orion, 1983; Towson and Apt, 1983; Townshend, 1984; Otsubo et al., 2006; Tsai, 2008), suggesting that coiling is a strategy against desiccation. Most nematodes can undergo a quiescent state at some point in the life cycle, but relatively few species are able to enter anhydrobiosis (McSorley, 2003). The low number of nematodes found in an anhydrobiotic state was probably due to the fact that the soil was very dry at the beginning of the experiment, leading to rapid dehydration and death. Similar results were reported for *P. penetrans* (Townshend, 1984).

The presence of vacuoles in nematodes suggested that all lipid reserves had been used. This condition may have triggered a reduction in metabolic activity during anhydrobiosis, similar to that observed in starving nematodes (Glazer and Orion, 1983). Lipid reserves are crucial for maintaining infectivity. In particular, neutral lipids are an important source of energy for nematode infection, movement, and feeding (Campos et al., 2006). Nematodes under stress conditions tend to decrease their movements and preserve energy, postponing host infection (Freire et al., 2007).

Coiling was partially but not solely responsible for nematode survival under dry conditions. *Aphelenchus avenae* Bastian individuals exposed to osmotic stress showed decreased motility, independent of being coiled or not. Decreased motility induces the physiological changes that accompany stress. Therefore, coiling is not critical to survival but rather the biochemical changes that induce the anhydrobiotic state (Otsubo et al., 2006).

We highlight that the experimental procedure adopted in this study simulated the environmental conditions of the dry season in a tropical country, characterized by lack of rainfall, high temperatures, and low soil moisture in the surface layer. Our results indicated that the presence of root fragments favors nematode persistence in the soil. Furthermore, the results showed that nematodes can survive, albeit in small numbers, for relatively long periods in the absence of hosts. To the best of our knowledge, this is the first report on the ability of *P. brachyurus* to infect maize and soybean after a period of dry conditions.

5. Conclusion

P. brachyurus has greater survival ability in the presence of root fragments than in the absence of host tissues. Nematode infectivity was maintained up to 90 days under dry soil conditions. A possible survival strategy of root-lesion nematodes may be the induction of an anhydrobiotic state.

Declarations

Author contribution statement

Lilianne M. Ribeiro: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Hercules D. Campos: Conceived and designed the experiments; Analyzed and interpreted the data.

Danilo L. Neves: Performed the experiments.

Claudia R. Dias-Arieira: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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