Meloidogyne enterolobii – Survival and distribution under temperate climate conditions within Europe.

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Summary- *Meloidogyne enterolobii* is one of the most destructive plant-parasitic nematodes (PPN). It was recently detected or intercepted in several European countries, raising concerns about its spread, mainly due to climate change which favors the proliferation of parasites. In this study, the survival of second-stage juveniles (J2) of three (3) root-knot nematode (RKN) species (namely: tropical PPN *M. enterolobii* as preferred species, *M. incognita* as tropical control RKN and *M. chitwoodi* as a control temperate RKN) was examined *in vitro* at four (4) different temperatures (5, 12, 20 and 25 °C) and five (5) periods (2, 4, 6, 8 and 10 weeks) in the absence of their host. The infectivity of the surviving nematodes was evaluated by inoculating them into tomato seedlings following two scenarios (inoculum obtained after extraction of the nematodes using the automatic zonal centrifuge and the inoculum obtained without extraction of the nematodes after incubation). One week post-inoculation, nematodes inside the roots were counted under a microscope after staining to facilitate observation. Data collected after nematode extraction showed significant variations in survival depending on species, temperatures, and incubation periods. In the final weeks of incubation, *M. enterolobii* showed greater resilience to high temperatures, while *M. chitwoodi* was more sensitive. In general, higher temperatures reduced nematode survival over time. For *M. enterolobii*, a significant difference was noted between 25 °C and 20 °C after 4 weeks of incubation. Survival generally declined over several weeks, influenced by nematode lifespan and the effectiveness of incubation conditions, with complex and non-linear interactions. All three Meloidogyne species had higher survival rates at temperatures below 20 °C, but the number of surviving nematodes declined sharply over time. Observation of the stained roots revealed great variability in the percentage of nematodes present inside the roots. Both studies confirm that temperature was a critical factor for root-knot nematode infectivity. In the infectivity test carried out after extraction of the nematodes, *M. enterolobii* had reached its maximum infectious power at 20 °C after two and six weeks incubation, while *M. incognita* and *M. chitwoodi* present peaks at different temperatures and periods, highlighting the optimal conditions of infectivity specific to each species. In the nematode infectivity test without extraction, *M. enterolobii* showed maximum and significant infectivity at 5 °C after seven (7) weeks of incubation, with more constant infectivity across temperatures after eight (8) weeks of incubation. *M. incognita* presented a relatively constant infectivity regardless of temperatures, with a slight drop at 25 °C after 7 weeks incubation and a significantly higher infectivity at 5 °C after 8 weeks observation. In contrast, *M. chitwoodi* reached a peak at 20 °C after 7 weeks and remained constant regardless of temperature after 8 weeks incubation. After ten weeks, no nematodes were observed in the roots, regardless of species or temperature. The climate projections for the cities of Gent and Bonn were produced using data from local weather stations and climate models based on different socio-economic scenarios (Shared Socio-economic Pathway: SSP). Projections of the future scenarios indicate a significant increase in Heat accumulation by 2085, stability in the Chill accumulation, and a reduction in the Frost duration, highlighting the importance of adapting nematode management strategies to these futures weather conditions. Given the survival and remaining infectivity of *M. enterolobii* at lower temperatures as shown in this research, its establishment in temperate regions is an eminent threat.

Keywords- *M. enterolobii*, *M. incognita, M. chitwoodi*, incubation, temperature, Climate change.

Introduction

Problem

Agriculture plays a crucial role in the world, and it is of great importance as it is the primary source of food and plays an essential multifunctional role. It contributes to food, the economy, the preservation of the environment and the well-being of local populations. Therefore, it is important to promote sustainable agricultural practices to guarantee food security and long-term prosperity. However, while robust agriculture guarantees food security by ensuring a constant supply of high-quality food products, it should be noted that many biotic and abiotic factors can significantly reduce crop yields (Bolaji Umar et al., 2022; Francini & Sebastiani, 2019). Among the biotic factors, we have the plant-parasitic nematodes (PPN), specifically the root-knot nematodes (RKN: *Meloidogyne spp.)*, which represent a major challenge for global agriculture, both in terms of yield and food security and among the most devastating we find *M. enterolobii* (Jones et al., 2013). This nematode was first described in the early 80's by Yang & Eisenback (1983) and was previously restricted to tropical and subtropical regions (Castagnone-Sereno, 2012). It has just aroused concern due to its destructive potential and by the fact that this nematode has already been detected in several European countries (Santos et al., 2019), which attracts the attention of scientists who fear a possible spread on European territory. The emergence of climate change exacerbates the issue, as it promotes the proliferation of parasites that affect cultivated plants, such as nematodes (Gendron St-Marseille et al., 2015). Several species of PPN, including Meloidogyne (*M. incognita*), Pratylenchus (*P. brachyurus, P. vulnus*), Helicotylenchus (*H. pseudorobustus, H. multicinctus*) and Ditylenchus (*D. dipsaci*), have been spotted migrating towards more northern areas of the hemisphere due to global warming (Bebber et al., 2013; Dutta & Phani, 2023).

The problem linked to *M. enterolobii* mainly concerns the negative impacts of this nematode on crops because it can infect the roots of many plant species, causing deformations of the roots, which can lead to considerable yield losses. Recent studies have reported the presence of *M. enterolobii* in Switzerland (Kiewnick et al., 2008) and Portugal (Santos et al., 2019). Genotypes of tomato (*Solanum lycopersicum*) and pepper (*Capsicum annuum*) carrying the Mi-1, N and Tabasco genes are susceptible to parasitic attacks by *M. enterolobii* (Brito et al., 2007), although they are genes that confer resistance against the most economically important species of RKN such as *M. incognita*, *M. javanica* and *M. arenaria* (Thies et al., 2008). The situation becomes even more alarming with the phenomenon of climate change which can potentially have consequences on the behavior, distribution, and effects of this nematode on crops. Tropical Meloidogyne species can move northwards, and temperate and tropical root-knot nematodes can have more generations per year (Wesemael et al., 2011).

Discussions about the devastating effects of global warming on the planet, supported by solid scientific evidence and increasingly frequent extreme weather events, have intensified (Intergovernmental Panel on Climate Change (IPCC), 2023). Recent reports highlight a concerning rise in temperatures across Europe (Copernicus, 2024). In this context, climate change poses significant threats to agriculture and food security through extreme weather events and disruptions in ecosystems (Carty, 2013; Duchenne et al., 2020). Notably, PPN are impacted by these changing environmental conditions, potentially leading to increased populations and crop damage (Gendron St-Marseille et al., 2015).

To address these challenges, scientists utilize weather station data to develop climate models projecting future scenarios (Luedeling, 2024). These models, incorporating Representative Concentration Pathways (RCPs) (van Vuuren et al., 2011)and Shared Socioeconomic Pathways (SSPs), aid in anticipating the impacts of climate change on agriculture and other sectors (Meinshausen et al., 2020). Consequently, it is crucial for researchers and farmers to closely monitor nematode presence and activity in their respective regions and adjust management strategies accordingly to minimize potential crop damage.

The importance of such research extends beyond immediate agricultural concerns. By understanding nematode responses to changing environmental conditions and integrating this knowledge into climate models, researchers contribute to broader efforts in developing adaptation and mitigation strategies. These strategies not only safeguard agricultural productivity but also enhance resilience in ecosystems and contribute to global food security. In this research, we investigated the responses of *M. enterolobii* to climate change, serving as a vital component in addressing the multifaceted challenges posed by climate change, benefiting both current and future generations.

Hypotheses

i. Temperature can influence the survival and infectivity of *M. enterolobii* differently.
ii. The survival and infectivity of *M. enterolobii* differ depending on the incubation period.

Objectives

Learn more about the behavior of *M. enterolobii* in temperate conditions and determine the influence of temperatures below 20 °C on the infectivity and survival of this nematode.

Specific objectives

i. Evaluate the effect of temperature on the survival and infectivity of *M. enterolobii*.
ii. Evaluate the effect of time on the survival and infectivity of *M. enterolobii*.
iii. Simulate climate data to make projections about the future climate of 2 cities (Gent and Bonn) in Europe.

MATERIALS AND METHODS

The study was conducted at ILVO (Flanders Research Institute for Agriculture, Fisheries and Food) during the period from February 2024 to May 2024 (4 months). To ensure optimal conditions (temperature, humidity), this study was conducted under controlled laboratory conditions to guarantee the reproducibility of the experiments and the reliability of the results.

Nematode species

For this experimental trial, three (3) species of RKN were chosen, namely: the tropical PPN *M. enterolobii* (as the preferred species), *M. incognita* (as control tropical RKN) and *M. chitwoodi* (as control temperate RKN). Initially, these nematodes were collected from plant material such as tomatoes and butter cup

(*Ranunculus hispidus*) infected with the RKN previously mentioned and cultivated at ILVO. These roots were cut into small pieces and then placed on a modified Baermann funnel (Baermann, 1917). The freshly (<24h) harvested J2s were then used to set up our test.

Survival test

The test was carried out with 4 different temperatures (5 °C, 12 °C, 20 °C and 25 °C) and 3 species of RKN: a species under investigation (namely *M. enterolobii)* and two (2) species as controls (a tropical RKN namely *M. incognita* and a temperate RKN namely *M. chitwoodi*). After extraction of the nematodes in the Bearman funnel, an estimation of the volume of solution containing 100 nematodes was made after counting 4 sub-samples of 5 ml of the solution collected. Subsequently, this volume was injected into the soil sample contained in the cylindrical tubes (3.21 cm of diameter, 5.27 cm of height and 40 ml of volume), noted that each tube contained an average of 32 grams of soil at a humidity of 20%. The cylindrical tubes were covered with parafilm (to limit evaporation) in which small holes were created to maintain an aerobic condition so that the nematodes had enough oxygen during the incubation period. Then the tubes were placed in incubators fixed at the different temperatures mentioned above. For this experiment, a total of 4 replicates were chosen, each week the humidity of the tubes was brought to 20% by taking their weights separately and adding water to return to the initial weight. Every 2 weeks (more precisely: 2, 4, 6, 8 and 10 weeks post-incubation), the extraction of nematodes was done using the automated zonal centrifuge (Hendrickx, 1995).The solutions obtained after each extraction were placed in an observation room at ambient temperature to allow the nematodes to acclimatize to the environment and left to rest for 4 hours so that the nematodes could sink to the bottom of the beaker before reducing the volume of the liquid supernatant to 40 ml. Subsequently, the nematodes (alive and dead) were counted with the aid of a stereomicroscope to study the survival of the nematodes. This was done based on mobility. The immobile nematodes were touched using a “fishing needle” to observe their reactions before confirming whether the nematodes were dead. The survival of the nematodes was determined after counting all the nematodes present in each bottle (divided into two categories: alive and dead).

Infectivity test

Seedlings of tomato (variety: Marmande) which is considered as favorable host for our target RKN (Kiewnick et al., 2008) were used to assess the infectivity. Root-knot nematodes generally prefer light, well-drained soils (Kim et al., 2017). Therefore, the tomato seedlings were grown in Ray Leach Cone-Tainers to which 63 grams of loamy-sandy soil were added (respecting the 2:1 ratio). The loamy soil was collected from the ILVO farm and was previously sterilized (at 100 °C for 24 hours) to destroy all pathogens present in this soil. After mixing the soil with the sand, the humidity was conducted to 25% and then the tubes were filled and sowing of tomato seeds was done. The seedlings were subsequently placed in the growth chamber where the temperature and light were adjusted to promote germination and plant growth. Noted that during the germination test the tomato seed had a Faculty of Germination (FG) of 100% and a Mean Time of Germination (MTG) of 5 days.

After each microscopic observation to determine nematode survival at different temperatures, the 4 replicates per treatment were bulked to ensure that approximately the same number of nematodes were inoculated into each plant. At the time of inoculation, the tomato plants were at the true leaf stage (aged between 25 and 30 days after germination). The aim of this test is to determine whether after the incubation period the nematodes remain infectious and to determine whether the different temperatures and the

duration of incubation influence the infectivity of the 3 species of RKN differently. Inoculation was carried out by direct injection of the inoculum near the root system of the seedlings by creating small holes very close to the crown of the seedlings. Subsequently, the seedlings were left in the growth chamber for a week and then the nematodes inside the roots were stained (Byrd et al., 1983) to facilitate the observation of nematodes in the roots.

A similar experiment was conducted to assess the infectivity using a higher number of nematodes. In this experiment, 500 nematodes were incubated per tube instead of the previous 100, maintaining the same temperature conditions for an incubation period of 7 and 8 weeks (4 replicates per treatment). Following the incubation period, soil samples from the tubes were mixed with the soil in which tomato seedlings had been planted. The plants were then left for one (1) week before staining.

The root staining process was performed as follows. First, the roots were gently removed from the tubes, washed, cut into 1 to 2 cm pieces, and then placed in a glass beaker with a capacity of 150 ml. To clear the roots, a solution consisting of 25 ml of water and 10 ml of 5.25% NaOCl (Sodium hypochlorite) was poured into the beaker, where the roots were immersed and held for 4 minutes, stirring regularly. Afterwards, the NaOCl solution was carefully removed from the beaker, followed by thorough rinsing of the roots under running water to remove any remaining residue. After rinsing, excess water was removed by drainage to avoid excess moisture. Subsequently, 1 ml of a staining solution (consisting of 3.5 g of fuchsin, 250 ml of acetic acid and 750 ml of DH_20) was added to the roots immersed in 30 ml of water. The mixture was brought to boil and held at this temperature for 30 seconds. After boiling, the solution was allowed to cool until it reached room temperature, followed by rinsing the roots thoroughly again to remove any residual coloring. Next, 20 to 30 ml of glycerol was added to the roots and then the mixture was heated to boiling. Then the roots were transferred to petri dishes for observation under a microscope and counting of the nematodes. It should be noted that the petri dishes which were not observed on the same day were hermetically closed with parafilm and then placed in the refrigerator, thus ensuring their conservation for future use.

Simulation of future climate data

The data already collected by the weather stations constitutes a valuable resource for understanding past and present climate trends. Using this data as a basis, researchers develop sophisticated climate models to simulate various scenarios and project potential climate developments (De Ridder et al., 2020). In this research, climate projections were made for two (2) different cities in Europe namely Gent and Bonn (Figure 1) where climate data were already collected from two (2) weather stations (namely: SEMMERZAKE for Gent and Koln Bonn for Bonn) which were chosen based on their proximity to the city under study and the number of years covered by the data collected (Tables 1 and 2).

Table 1: Weather stations closest to the city of Gent.

Name of the weather station	Countries	Latitudes	Longitudes	Start period	End period	Distance (km)
Melle	Belgium	51	3.82	26/10/2009	26/05/2024	9.48
Semmerzake	Belgium	50.9	3.67	01/01/1973	26/05/2024	13.8
Semmerzake (Bafb)	Belgium	50.9	3.65	10/05/2004	26/05/2024	14.3
Gent/Industrie-Zone	Belgium	51.2	3.8	30/10/1979	31/12/1997	15.5
Westdorpe	Netherlands	51.2	3.87	01/09/1994	26/05/2024	22.4
Hoofdplaat	Netherlands	51.4	3.67	01/07/2001	05/07/2016	35.6
Cadzand Wp	Netherlands	51.4	3.38	01/09/1996	26/05/2024	44.4
Vlissingen	Netherlands	51.4	3.6	10/05/2004	26/05/2024	45.5
Vlissingen	Netherlands	51.4	3.6	02/01/1928	26/05/2024	45.5
Wevelgem	Belgium	50.8	3.2	30/10/1977	14/05/2003	45.5

Table 2: Weather stations closest to the city of Bonn.

Name of the weather station	Countries	Latitudes	Longitudes	Start period	End period	Distance (km)
Bonn/Friesdorf(Aut)	Germany	50.7	7.15	01/02/1936	31/12/1992	4.86
Bonn-Hardthoehe	Germany	50.7	7.03	23/05/1975	23/12/1997	5.78
Bonn-Roleber	Germany	50.7	7.2	05/07/2001	31/12/2008	7.05
Koln Bonn	Germany	50.9	7.14	01/01/1931	30/04/2024	15.4
Butzweilerhof(Bafb)	Germany	51	6.9	09/01/1978	23/08/1995	31.5
Norvenich	Germany	50.8	6.66	01/01/1973	30/04/2024	33.1
Mendig	Germany	50.4	7.32	02/01/1973	1/12/1997	43.3
Nuerburg-Barweiler	Germany	50.4	6.87	01/04/1995	31/12/1997	43.6
Blankenheim	Germany	50.4	6.65	02/10/1978	04/05/1984	44.5
Nuerburg	Germany	50.3	6.95	09/01/1930	31/12/1992	45.4

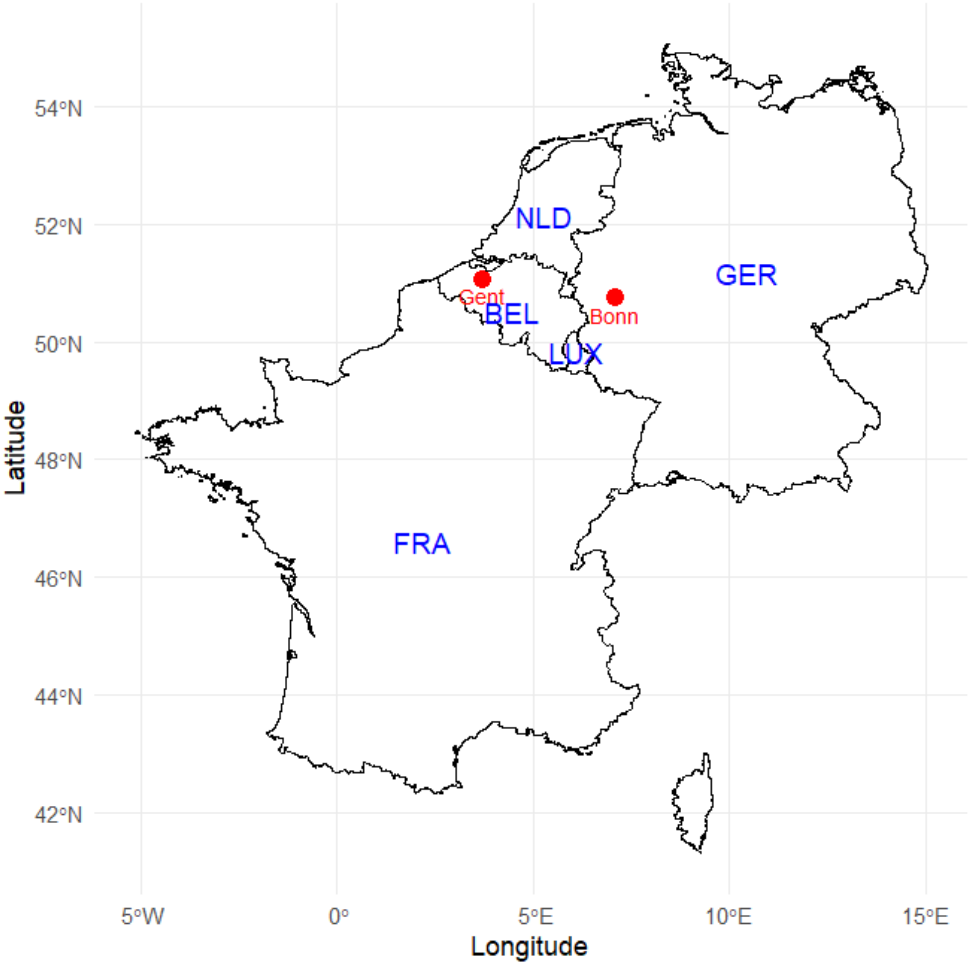

Figure 1: Location of the cities (Gent and Bonn) for which a climate simulation was done.

Both Gent and Bonn have temperate climates. Gent experiences mild conditions and significant year-round rainfall, with an average temperature of approximately 11 °C and about 786 mm of precipitation annually (Climate-Data.org, n.d.-b). Bonn experiences a hot summer and a cold winter with significant precipitation throughout the year, an average temperature of 10.6 °C, and around 847 mm of annual precipitation (Climate-Data.org, n.d.-a). A future climate projection for Gent and Bonn would help nematology researchers anticipate changes such as warmer temperatures and increased precipitation. This would enable them to develop nematode management strategies in agricultural and environmental systems.

To make future projections of climate change in the two (2) locations cited above, the use of SSP (SSP1 which concerns the Sustainable development trajectory, SSP2 which concerns the Intermediate trajectory and SSP5 which concerns Environmental degradation trajectory) was made which represents the most relevant projected global socioeconomic changes until 2100 within climate change scenarios (Intergovernmental Panel on Climate Change (IPCC), 2021). These scenarios were used as a basis for the assessment of possible futures based on different socio-economic contexts and greenhouse gas emissions (GHG) and aerosols. Meteorologists and climatologists generally recommend averaging 30 years of

weather data to characterize a location's climate (climatedata.ca, n.d.). When we add 30 years to the current situation it takes us to the 2050s, being the halfway point of the interval 2001 to 2100. Considering that the data downloaded from the future climate database are raw runs of GCMs (General Climate Models), which means that they include the usual ups and downs of weather records. If we just used the year 2100, we might get an unusually cold or warm year, depending on what the model did during that run. Therefore, it is crucial to use an average of data spanning the last 30 years of our interval from 2001 to 2100, which corresponds to 2085 (considering the interval 2070 to 2100 covering an estimation period stable 31 years). Therefore, since creating a scenario for 2100 is not feasible, the scenarios were created for the years 2050 and 2085. Therefore, the comparison (for the past and present) was made for a period from 1970 to 2024 and futuristic projections were made for the year 2050 (midway to the interval 2001 to 2100) and the year 2085 (year closer to 2100 for which we were able to stimulate the most data from the GCM) (Intergovernmental Panel on Climate Change (IPCC), 2021), which could be used as reference years to make projections on the Heat accumulation (expressed in Growing Degree Hours “GDH”), Chill (expressed in Chill Portion “CP”) and Frost duration (expressed in Hours).

Data analysis

The data was collected on Microsoft Excel and the analysis was carried out on R software version 4.3.2 to perform statistical tests (calculation of averages, standard deviation, p-value, etc.) (R Core Team, 2023). Packages like chillR (Luedeling, 2012), ggplot (Wickham, 2009; Wilkinson, 2011) were used respectively in the simulation of climate projections and the preparation of graphs.

Results

RKN Survival Study

Significant differences in survival, depending on the species, temperatures, and incubation periods were observed (Table 3). Each nematode species responds differently to temperature and over time. At the last 2 weeks of incubation, *M. enterolobii* showed greater resilience to high temperatures (20 and 25 °C) than the other species, while *M. chitwoodi* was more resilient to lower temperatures (5 and 12 °C) (Table 4 and Figure 2). Although higher temperatures are generally associated with a decrease in the number of surviving nematodes, which has intensified over time, exceptions to this trend lead to complexity in their response. For example: at 2 and 4 weeks incubation, more *M. incognita* survivors were observed in samples exposed to 20 °C than in those exposed to 12 °C, although these data were not significantly different according to Tukey HSD with a probability of 5% (p-value > 0.05). This was not the case for *M. enterolobii*, where at 4 weeks incubation, a strong significant difference was observed between nematodes exposed to 25 °C and those exposed to 20 °C (Table 4). From the second (2^{nd}) to the 10^{th} week incubation the greatest numbers of survivors, for *M. enterolobii* and *M. chitwoodi* were obtained for the nematodes exposed to temperature below 20 °C. At 4 weeks of incubation, the number of surviving *M. enterolobii* in samples incubated at 20 °C were significantly lower than the other temperatures. At 6 weeks of incubation, the number of surviving *M. enterolobii* in samples incubated at 5 °C were significantly higher than the other temperatures. At 2 weeks of incubation, a significant reduction in the number of surviving *M. chitwoodi* was observed with an increase of the temperature. At 6 weeks of incubation the number of surviving *M. chitwoodi* in samples incubated at 5 °C were significantly higher than other temperatures and at 8 weeks of incubation, the number of surviving *M. chitwoodi* in samples incubated at temperatures below 20 °C were significantly higher than other temperatures.

For *M. incognita*, the greatest numbers of survivors were initially obtained at 5 °C, then from the 6th to 10th week more survivors were obtained at 12 °C. At 2 weeks of incubation, the number of surviving *M. incognita* in samples incubated at 25 °C was significantly lower than the other temperatures. At 8 weeks of incubation, the number of surviving *M. incognita* in samples incubated at 12 °C were significantly higher than the other temperatures.

A gradual decrease in the number of surviving nematodes was observed over several weeks (Figure 2), with variations depending on species and temperature. This trend is likely influenced by factors such as the inherent lifespan of nematodes and the effectiveness of incubation conditions. Interactions between nematode species, temperatures and incubation periods were found to be complex and often exhibited non-linear patterns, indicating potential advantages of certain species under specific conditions. Notably, during the 8th and 10th week incubation, a higher number of surviving *M. chitwoodi* was systematically observed compared to the other two species at temperatures below 20 °C, while the opposite trend was observed for *M. enterolobii* and *M. incognita* (Figure 2). However, in most cases these differences in the data were not statistically significant (Table 4). It should be noted that the temperature had a great influence on the percentage of humidity retained in the tubes and therefore had an influence on the survival of the nematodes, given that evaporation was higher in the samples exposed to temperatures above 12 °C (Table 5). For this reason, each week 0.5 ml of water was added to each tube incubated at temperatures below 20 °C and 1 ml to those incubated at temperatures above or equal to 20 °C.

Table 3: Significance of main and interaction effects (ANOVA) on the survival of three *Meloidogyne* spp.

Source of variation	Df	Sum Sq	Mean Sq	F value	P value
Species	2	427	214	4.101	0.0179 *
Temperatures	3	7010	2337	44.851	< 2e-16 ***
Times	5	308923	61785	1185.974	< 2e-16 ***
Species:Temperatures	6	946	158	3.028	0.0073 **
Species:Times	10	1078	108	2.07	0.0281 *
Temperatures:Times	15	5283	352	6.761	6.67e-12 ***
Species:Temperatures:Times	30	3155	105	2.019	0.00225 **
Residuals	216	11253	52		

Table 4: Nematode survival in soil after inoculation with 100 second-stage juveniles according to species, temperatures, and incubation periods

	Temperatures			
Species	5 °C	12 ° C	20 °C	25 ° C
	2 weeks incubation			
M. chitwoodi	A 42 ± 1.63 a	A 39.5 ± 3.42 ab	A 17 ± 4.16 bc	A 16.5 ± 2.52 c
M. enterolobii	A 33.5 ± 13.80 a	AB 24 ± 20.33 a	A 16 ± 5.16 a	A 10.5 ± 6.19 a
M. incognita	A 36 ± 10.58 a	B 13.5 ± 8.54 a	A 14 ± 10.46 a	A 11.5 ± 5.26 b
	4 weeks incubation			
M. chitwoodi	A 38 ± 5.42 a	A 31 ± 14.65 a	A 12 ± 14.88 a	A 20 ± 23.04 a
M. enterolobii	A 35 ± 14.65 a	A 18 ± 7.83 a	B 5.5 ± 5.51 b	A 14 ± 8.16 a
M. incognita	A 33 ± 9.02 a	A 17.5 ± 6.40 a	A 22 ± 16.57 a	A 11.5 ± 10.38 a
	6 weeks incubation			
M. chitwoodi	A 28.5 ±12.58 a	A 11 ± 3.46 b	A 6.5 ± 6.61 b	A 3 ± 2.58 b
M. enterolobii	A 36.5 ±5.00 a	A 11.75 ± 5.19 b	A 12.5 ± 5.00 b	A 3.5 ± 1.91 b
M. incognita	B 10 ± 1.63 a	A 22 ± 2.83 a	A 12.5 ± 9.29 a	A 7.5 ± 12.48 a
	8 weeks incubation			
M. chitwoodi	A 17 ± 8.87 a	A 24.5 ± 2.52 a	A 3.5 ± 1.91 b	A 1.5 ± 1.91 b
M. enterolobii	A 7.5 ± 1.00 a	B 12 ± 3.65 a	A 9.5 ± 3.00 a	A 8.5 ± 2.52 a
M. incognita	A 8 ± 4.32 b	AB 20 ± 7.12 a	A 2.5 ± 3.00 b	A 4 ± 3.27 b
	10 weeks incubation			
M. chitwoodi	A 3.5 ± 3.00 ab	A 7 ± 4.76 a	A 0.5 ± 1.00 ab	A 0 ± 0.00 b
M. enterolobii	A 2.5 ± 3.00 a	A 2 ± 1.63 a	A 1.5 ± 1.91 a	A 2 ± 2.83 a
M. incognita	A 2 ± 1.63 a	A 4.5 ± 2.52 a	A 2 ± 1.63 a	A 2 ± 4.00 a

The Values in the table are the mean ± Standard Deviation of 4 replicates. For the same week at the same temperature, the means accompanied by the same capital letter in a column are not significantly different at 5% probability according to Tukey HSD (p-value > 0.05) and, the means accompanied by the same small letter in a line are not significantly different at 5% probability according to Tukey HSD (p-value > 0.05). It is important to note that the capital letters allow the comparison of the Means between the 3 species (*M. chitwoodi, M. enterolobii* and *M. incognita*) for the same temperature and the small letters allow the comparison of the response of the same species to the different temperatures for the same week.

Table 5: Effect of temperature on evaporation from tubes and volume of water added after each week of incubation.

Temperatures	Weight Initial (grams)	Weight (grams) After 1 Week Tubes with *M. enterolobii*	Weight (grams) After 1 Week Tubes with *M. incognita*	Weight (grams) After 1 Week Tubes with *M. chitwoodi,*	Volume Water Added (ml)
5 °C	32	31.5	31.7	31.6	0.5
12 °C	32	31.2	31.4	31.5	0.5
20 °C	32	30.6	30.4	30.5	1
25 °C	32	30.1	30.1	30.3	1

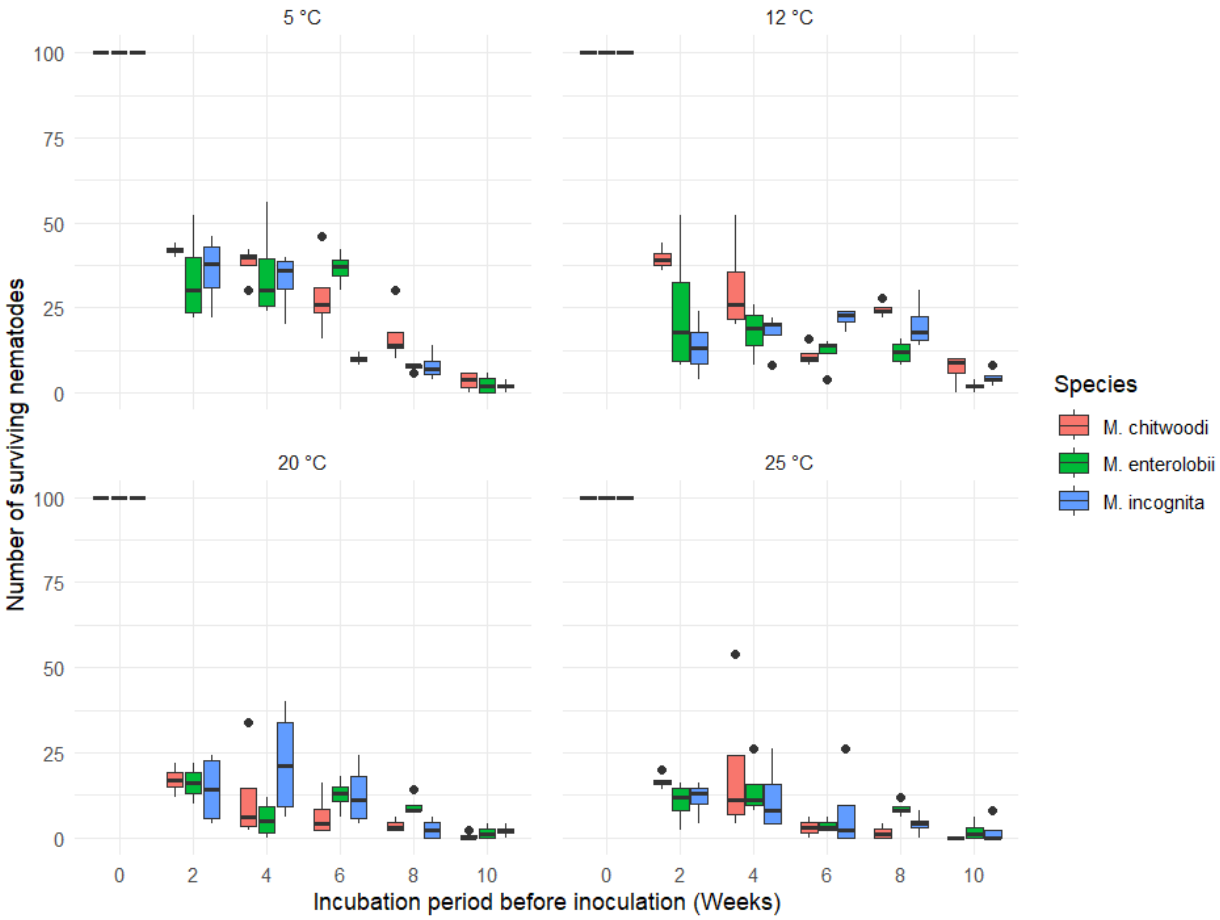

Figure 2: Boxplot of the number of surviving nematodes over time for different temperatures.

Infectivity study on tomatoes

Inoculation with nematodes extracted using the automatic zonal centrifuge.

The observation of the stained roots revealed a high variation in the percentage of nematodes found inside the roots. Temperatures and incubation time had significant effects on the infectivity of the three nematode species (Table 6). Species showed different responses to temperature and time, with significant interactions observed between the 3 factors (species, temperatures and time). From the 2nd week to the 8th week incubation, some of the nematodes inoculated were able to penetrate the roots. However, it is important to note that the highest proportions of infectivity were observed for *M. incognita* (Table 7 and Figure 3). At 2 weeks of incubation, a non-significant infectivity peak was observed at 20 °C for *M. enterolobii*, at 12 °C for *M. incognita*, and at 25 °C for *M. chitwoodi*. This trend varies at 4 weeks incubation where a non-significant infectivity peak was observed at 12 °C, at 25 °C and at 20 °C for *M. enterolobii, M. incognita* and *M. chitwoodi*, respectively. At 6 weeks of incubation and at 20 °C, the infectivity of *M. chitwoodi* was significantly lower than the other 2 species. Peak infectivity of *M. enterolobii* and *M. incognita* at 20 °C was significantly higher that nematodes exposed to temperatures below 20 °C, while *M. chitwoodi* reached a non-significant peak at 5 °C compared to the other temperatures. At 8 weeks incubation, a non-significant infectivity peak was observed for *M. enterolobii* at 12 and 20 °C, for *M. incognita* at 25 °C, and for *M. chitwoodi* at 20 °C. Infectivity generally decreased with time, and after 10 weeks of incubation, no infectivity was detected for any species and temperature.

Table 6: Significance of main and interaction effects (ANOVA) on the infectivity of three *Meloidogyne* spp. after incubation at different temperatures.

Source of variation	Df	Sum Sq	Mean Sq	F value	P value
Species	2	579	289.6	1.538	0.21753
Temperatures	3	4637	1545.7	8.212	3.75e-05 ***
Times	4	8476	2119.1	11.259	3.56e-08 ***
Species:Temperatures	6	1365	227.6	1.209	0.30358
Species:Times	8	4507	563.4	2.993	0.00356 **
Temperatures:Times	12	6409	534.1	2.837	0.00137 **
Species:Temperatures:Times	24	8360	348.4	1.851	0.01273 *
Residuals	180	33880	188.2		

Table 7: Infectivity (%) in tomato seedlings one week after inoculation of the surviving second-stage juveniles of *Meloidogyne* spp. incubated at 4 different temperatures during 5 different periods.

	Temperatures			
Species	5 °C	12 °C	20 °C	25 °C
2 weeks incubation				
M. chitwoodi	A 1.14 ± 2.27 a	A 4.51±5.93 a	A 10.33±9.12 a	A 40.77±26.26 a
M. enterolobii	A 3.74±5.24 a	A 10.00±20.00 a	A 38.64±7.80 a	A 9.38±18.75 a
M. incognita	A 13.94±5.63 a	A 31.67±28.09 a	A 25.00±21.52 a	A 20.83±25.00 a
4 weeks incubation				
M. chitwoodi	A 11.19±7.57 a	A 14.17±24.09 a	A 24.63±20.43 a	A 17.92±22.17 a
M. enterolobii	A 3.85±7.69 a	A 8.52±11.79 a	A 0.00±0.00 a	A 3.85±7.69 a
M. incognita	A 0.00±0.00 a	A 0.00±0.00 a	A 2.50±5.00 a	A 18.27±23.79 a
6 weeks incubation				
M. chitwoodi	A 3.85±4.44 a	A 0.00±0.00 a	B 0.00±0.00 a	A 0.00±0.00 a
M. enterolobii	A 0.00±0.00 b	A 3.57±7.14 b	A 41.87±12.95 a	A 16.67±33.33 ab
M. incognita	A 5.00±10.00 b	A 0.00±0.00 b	A 50.00±35.36 a	A 20.19±25.00 ab
8 weeks incubation				
M. chitwoodi	A 1.67±3.33 a	A 0.00±0.00 a	A 8.33±16.67 a	A 0.00±0.00 a
M. enterolobii	A 0.00±0.00 a	A 6.25±12.50 a	A 6.25±12.50 a	A 0.00±0.00 a
M. incognita	A 7.69±15.38 a	A 6.25±12.50 a	A 0.00±0.00 a	A 16.67±33.33 a
10 weeks incubation				
M. chitwoodi	A 0.00±0.00 a	A 0.00±0.00 a	A 0.00±0.00 a	A 0.00±0.00 a
M. enterolobii	A 0.00±0.00 a	A 0.00±0.00 a	A 0.00±0.00 a	A 0.00±0.00 a
M. incognita	A 0.00±0.00 a	A 0.00±0.00 a	A 0.00±0.00 a	A 0.00±0.00 a

The Values in the table are the mean ± Standard Deviation of 4 replicates. For the same week at the same temperature, the means accompanied by the same capital letter in a column are not significantly different at 5% probability according to Tukey HSD (p-value > 0.05) and, the means accompanied by the same small

letter in a line are not significantly different at 5% probability according to Tukey HSD (p-value > 0.05). It is important to note that the capital letters allow the comparison of the Means between the 3 species (*M. chitwoodi, M. enterolobii* and *M. incognita*) for the same temperature and the small letters allow the comparison of the response of the same species to the different temperatures for the same week.

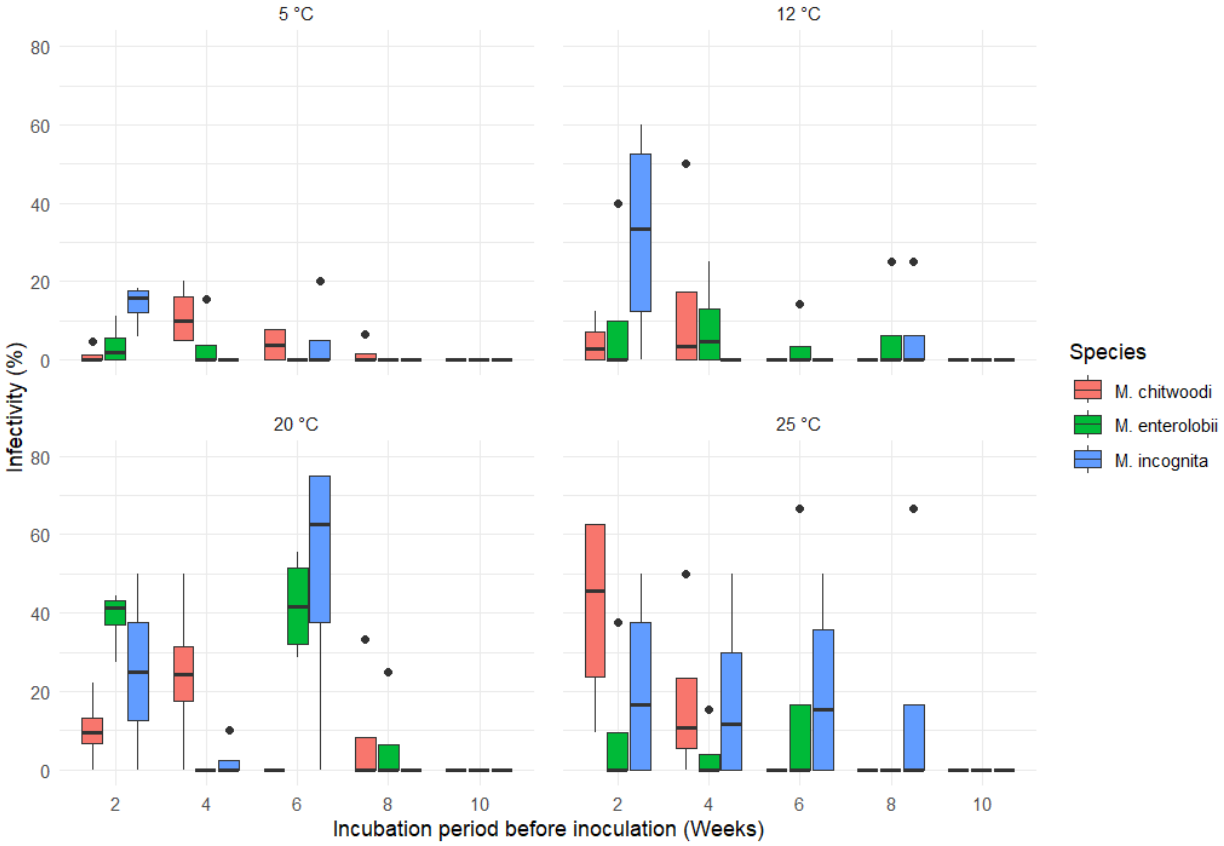

Figure 3: Nematode Infectivity in tomato seedlings after incubation at different temperatures during different weeks.

Inoculation without extraction of the nematodes

For this infectivity test, 500 nematodes were put in each tube then placed in incubators regulated at temperatures of 5, 12, 20 and 25 °C for 7 weeks and 8 weeks. The results, obtained after observation of the stained roots, revealed a strong variation in the percentage of nematodes found inside the root tissue and confirm that temperature can significantly affect the infectivity of the 3 selected species of RKN on tomato plants (Table 8). *M. enterolobii* initially exhibited significantly higher infectivity at lower temperatures, while *M. incognita* maintains more constant infectivity over a range of temperatures and presented a significantly higher infectivity at 5 °C compared to 20 °C at 8 weeks of incubation. *M. chitwoodi* exhibits non-significant variabilities, peaking at certain temperatures and periods (Table 9 and Figure 4). After 7 weeks incubation, the infectivity of nematodes (*M. enterolobii*) incubated at 5 °C was significantly higher than those incubated at 12 °C, 20 °C, and 25 °C. For *M. incognita*, the infectivity was relatively constant across temperatures, with a slight decrease observed for nematodes incubated at 25 °C. For *M. chitwoodi*, infectivity varied, with a non-significant peak obtained for nematodes incubated at 20 °C after 7 weeks. After 8 weeks incubation, the infectivity for *M. enterolobii* appeared much more constant across temperatures compared to the data from the 7 weeks. *M. incognita* showed higher infectivity at 5 °C and a significant reduction at 20 °C. *M. chitwoodi* infectivity remained relatively constant across temperatures. Note that after 7 weeks incubation, the infectious power of *M. enterolobii* was significantly higher than the 2 other root-knot species. During this infectivity test, the highest proportion of nematodes was found in

seedlings that initially had a much more developed root system, which probably had a strong influence on the statistical analysis and responsible for the significant differences observed between the samples.

Table 8: Significance of main and interaction effects (ANOVA) on the infectivity of three *Meloidogyne* spp. after incubation at different temperatures during 7 or 8 weeks.

Source of variation	Df	Sum Sq	Mean Sq	F value	P value
Species	2	0.57	0.283	0.091	0.9128
Temperature	3	54.61	18.205	5.878	0.00120 **
Times	1	1.35	1.354	0.437	0.51063
Species:Temperature	6	60.44	10.074	3.253	0.00693 **
Species:Times	2	35.15	17.576	5.675	0.00514 **
Temperature:Times	3	27.36	9.120	2.945	0.03860 *
Species:Temperature:Times	6	105.00	17.500	5.650	7.49e-05 ***
Residuals	72	222.99	3.097		

Table 9: Infectivity (%) in tomato seedlings one week after inoculation with 500 second-stage juveniles of *Meloidogyne* spp. incubated at 4 different temperatures during 7 and 8 weeks.

	Temperatures			
Species	5 °C	12 °C	20 °C	25 °C
7 weeks				
M. chitwoodi	B 1.75 ±1.34 a	A 1.15 ±0.34 a	A 5.2 ±2.46 a	A 2.15 ±3.91 a
M. enterolobii	A 7.15 ±1.35 a	A 0.65 ±0.5 b	A 0.9 ±0.88 b	A 0.25 ±0.3 b
M. incognita	B 1.1 ±1.44 a	A 0.95 ±0.68 a	A 1.3 ±0.68 a	A 0.8 ±-0.33 a
8 weeks				
M. chitwoodi	A 1.75 ±2.06 a	A 1.3 ±0.75 a	A 1.2 ±1.90 a	A 1.95 ±2.37 a
M. enterolobii	A 2 ±1.76 a	A 2.1 ±0.84 a	A 1.5 ±1.29 a	A 2.75 ±2.22 a
M. incognita	A 6.35 ±4.06 a	A 2.35 ±1.53 ab	A 0.45 ±0.52 b	A 2.5 AB ±1.19 ab

The Values in the table are the mean ± Standard Deviation of 4 replicates. For the same week at the same temperature, the means accompanied by the same capital letter in a column are not significantly different at 5% probability according to Tukey HSD (p-value > 0.05) and, the means accompanied by the same small letter in a line are not significantly different at 5% probability according to Tukey HSD (p-value > 0.05). It is important to note that the capital letters allow the comparison of the Means between the 3 species (*M. chitwoodi, M. enterolobii* and *M. incognita*) for the same temperature and the small letters allow the comparison of the response of the same species to the different temperatures for the same week.

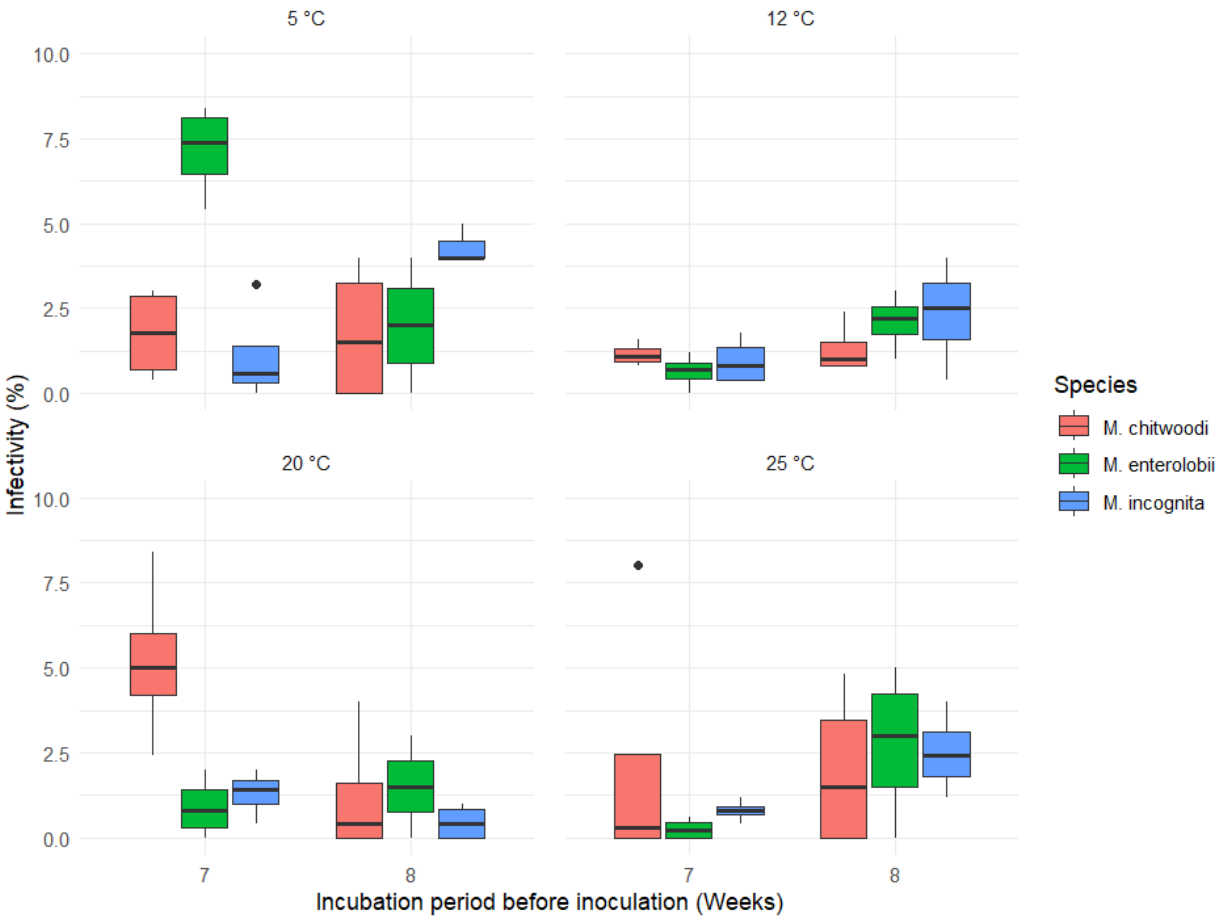

Figure 4: Nematode Infectivity in tomato seedlings after incubation at different temperatures during different weeks.

Simulation of future climate data

To conduct future projections, the chillR package in the R software was employed (Luedeling, 2012). This facilitated simulating future scenarios for the 2 selected temperate locations (Gent and Bonn) up to 2100. The projections were based on data collected from selected meteorological stations, chosen based on their proximity to the study sites and the time interval covered by these data, hence the blue points in Figures 5 to 10 representing observed data (current and past data) and the blue boxplots representing the past simulated in the same Figures. The current and past scenarios reveal that for both sites, the average Heat accumulation approaches 5000 GDH (Growing Degree Hours) annually, with an average Chill accumulation not exceeding 100 CP (Chill Portion), and an average Frost duration of up to 1000 hours. However, future projections show a significant increase in Heat accumulation for the year 2085 compared to 2050, considering SSP5 (Shared Socioeconomic Pathways 5) for both cities. It is noteworthy that an increase in future Heat accumulation (in 2050 and 2085) has been observed compared to current and past scenarios, across all SSPs. Chill accumulations in future scenarios are very similar to those in current and past scenarios (for both observed and simulated data). However, the future Frost duration for both cities is lower (around 500 hours) compared to current Frost duration or that of past years, which could be directly linked to the future increase in Heat accumulation.

In Figures 5 to 10, the acronyms GCM (Global Climate Models) represent various climate models developed by different institutions and research organizations around the world. Each model aims to simulate the Earth's climate system and its interactions between the atmosphere, oceans, land surfaces and ice (Flato, 2011; Qin et al., n.d.;). A brief explanation of each acronym:

- ACCES-CM2: Australian Community Climate and Earth System Simulator - Coupled Model Version 2.
- CMCC-ESM2: CMCC (Centro Euro-Mediterraneo sui Cambiamenti Climatici) Earth System Model Version 2.
- CNRM-CM6-1-HR: Centre National de Recherches Météorologiques (CNRM) Coupled Model Version 6.1 - High Resolution.
- AWI-CM-1-1-MR: Alfred Wegener Institute Climate Model Version 1.1 - Medium Resolution.
- CanESM5: Canadian Earth System Model Version 5.
- CIESM: Coupled Institute Earth System Model.
- CNRM-ESM2-1: CNRM Earth System Model Version 2.1.
- EC-Earth3-Veg-LR: EC-Earth Consortium Earth System Model Version 3 with dynamic vegetation - Low Resolution.
- INM-CM4-8: Institute for Numerical Mathematics Climate Model Version 4.8.
- MIROC6: Model for Interdisciplinary Research on Climate Version 6.
- FGOALS-g3: Flexible Global Ocean-Atmosphere-Land System Model Version 3.
- FIO-ESM-2-0: First Institute of Oceanography Earth System Model Version 2.0.
- INM-CM5-0: Institute for Numerical Mathematics Climate Model Version 5.0.
- MPI-ESM1-2-LF: Max Planck Institute Earth System Model Version 1.2 - Low Frequency.
- IPSL-CM6A-LR: Institute Pierre Simon Laplace Climate Model Version 6A - Low Resolution.
- IRM-ESM2-0: Institute of Resource Ecology and Marine Sciences Earth System Model Version 2.0.
- EC-Terre3-CC: EC-Earth Consortium Earth System Model Version 3 - Carbon Cycle.
- GFDL-ESM4: Geophysical Fluid Dynamics Laboratory Earth System Model Version 4.
- MIROC-ES2L: Model for Interdisciplinary Research on Climate Earth System Model Version 2 - Low Resolution.
- NESM3: Norwegian Earth System Model Version 3.

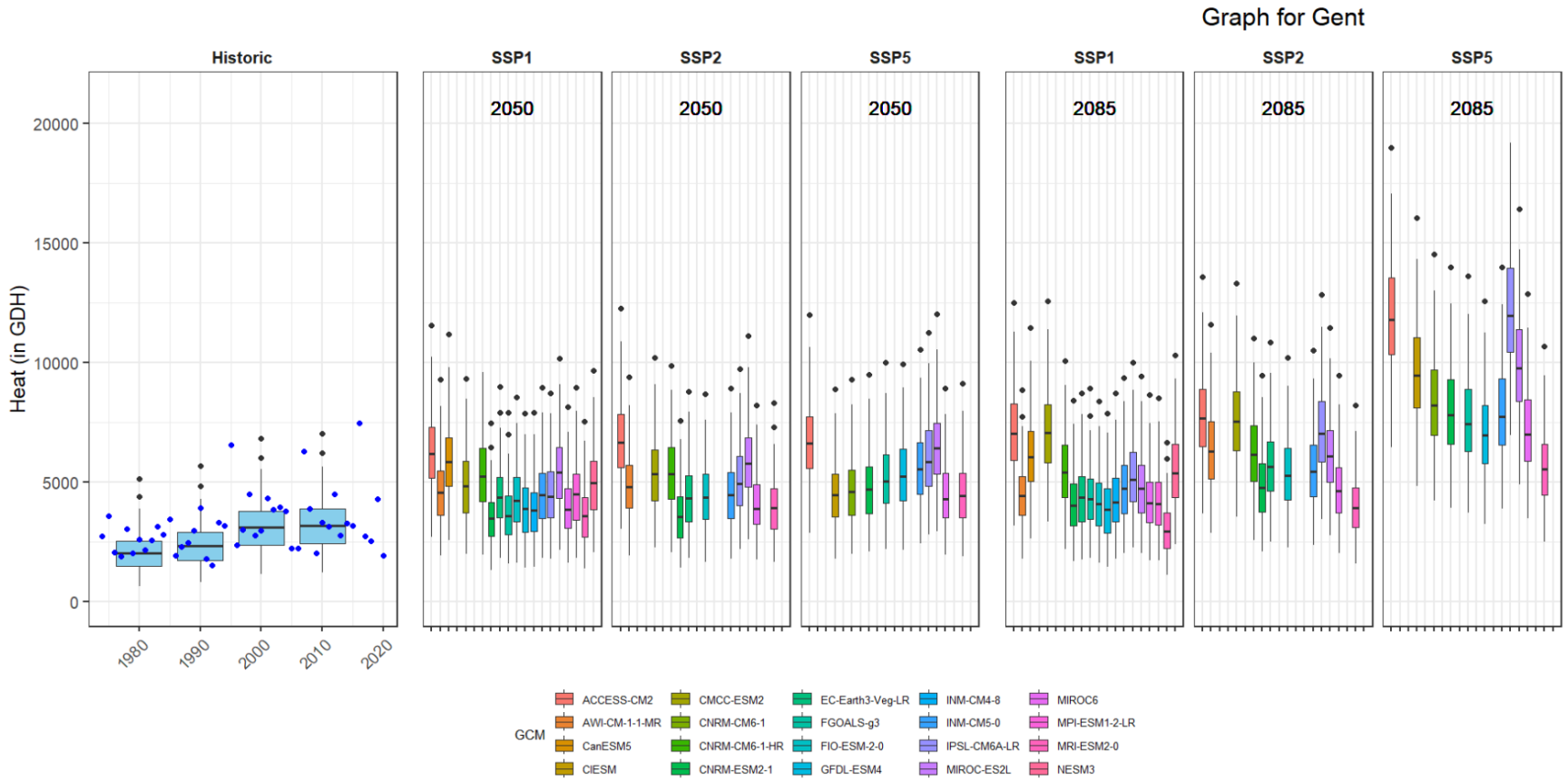

Figure 5: Projected Heat Scenarios for Gent's Future

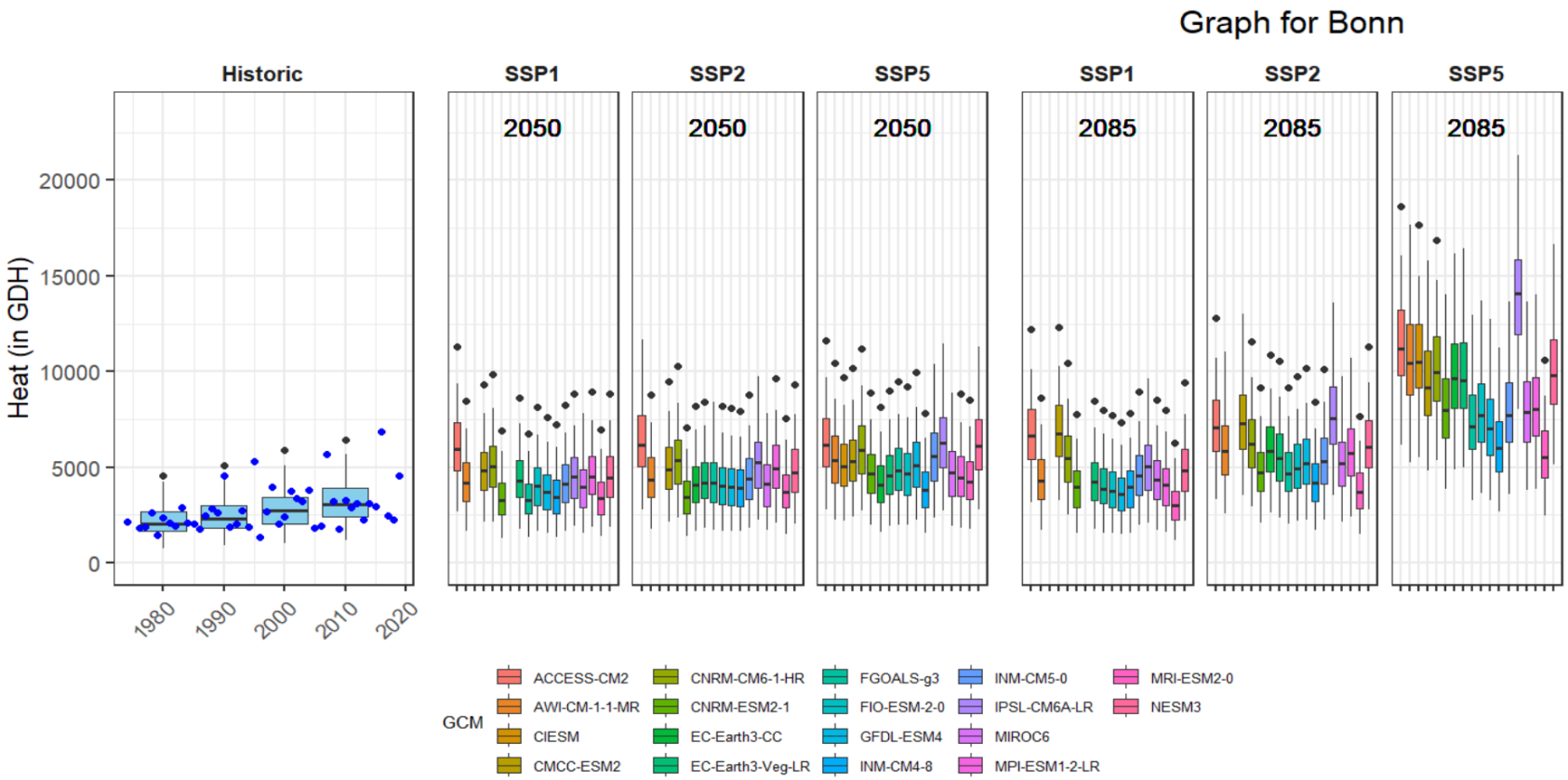

Figure 6: Projected Heat Scenarios for Bonn's Future

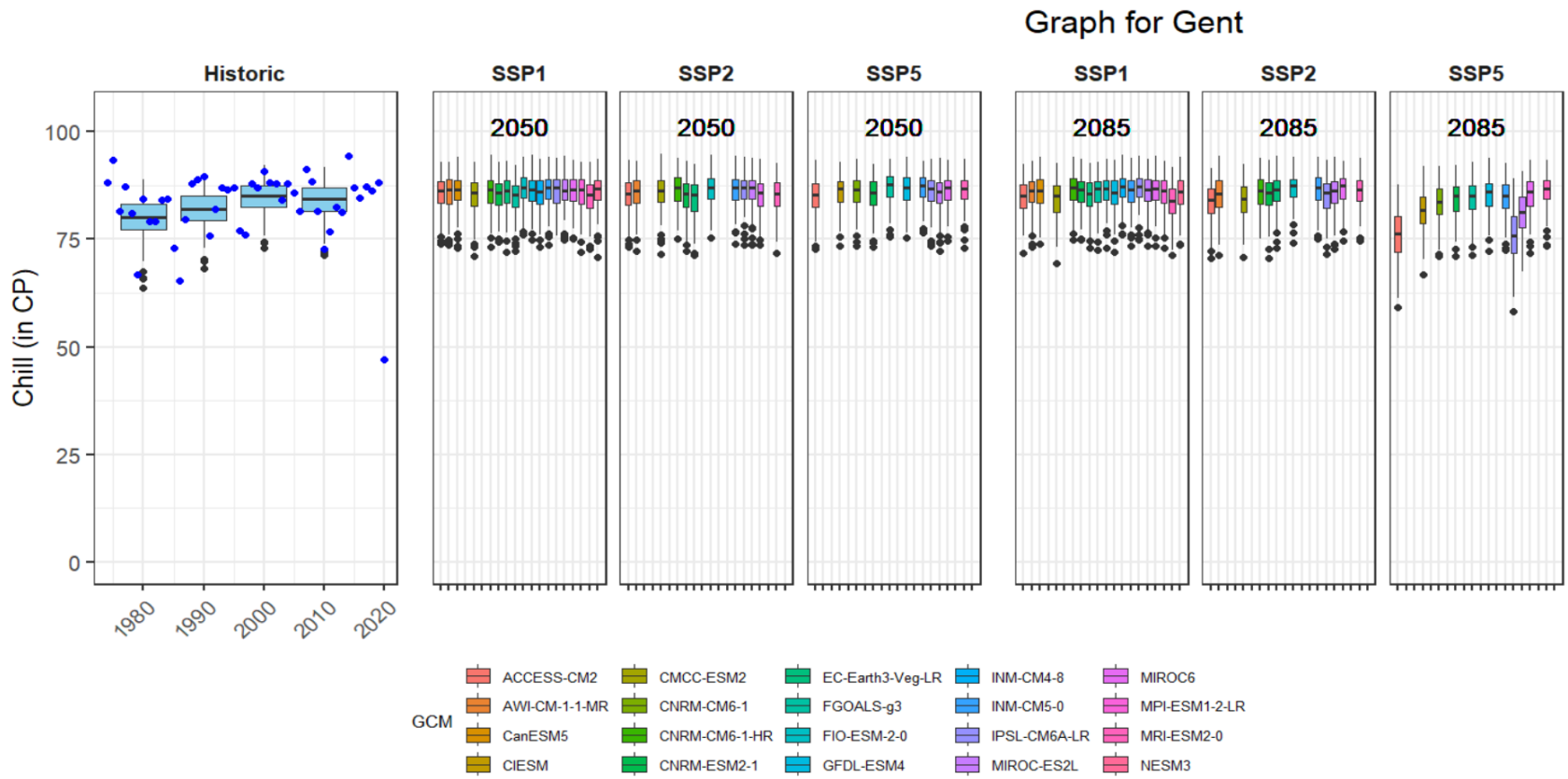

Figure 7: Projected Chill Scenarios for Gent's Future

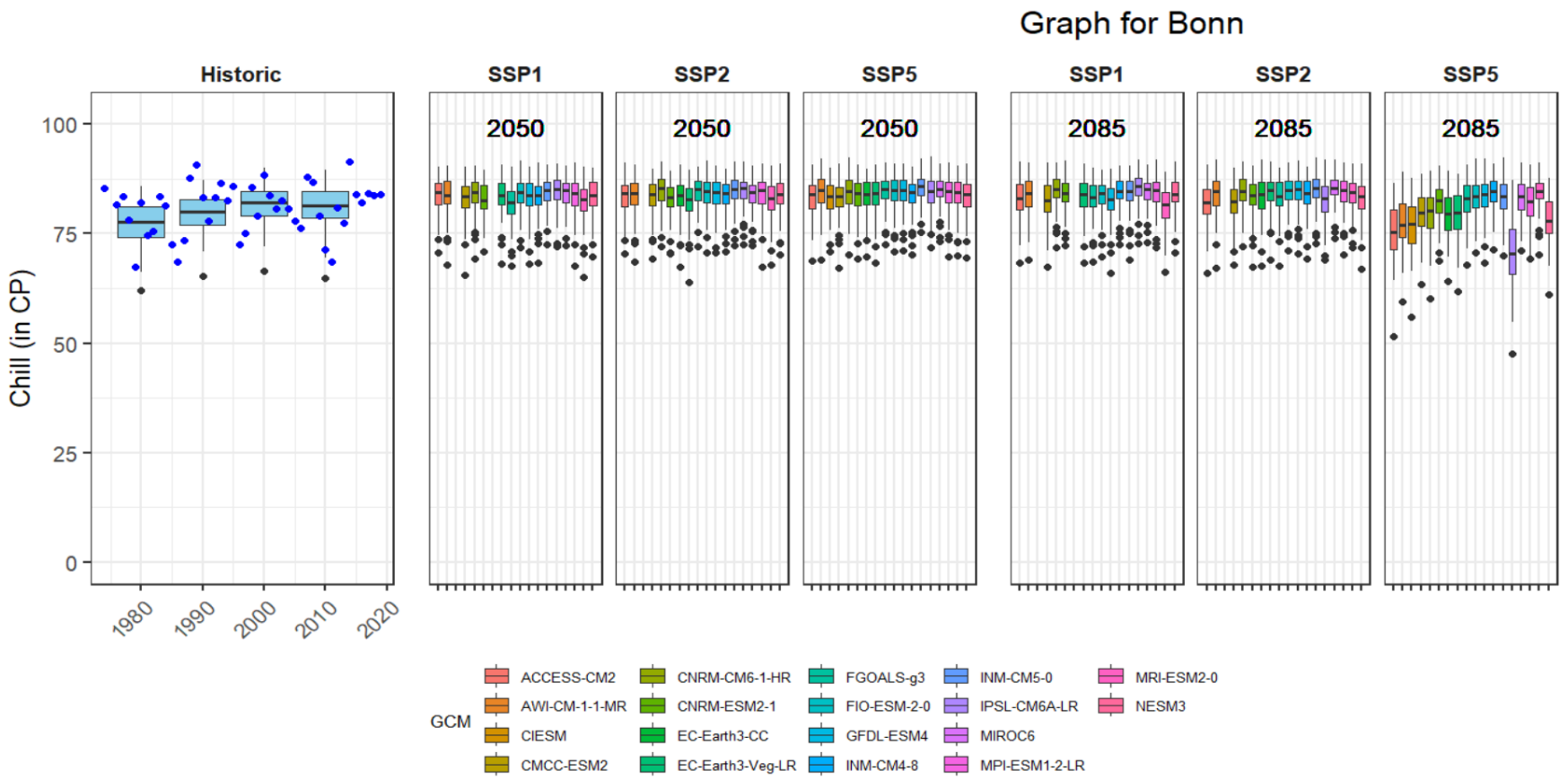

Figure 8: Projected Chill Scenarios for Bonn's Future

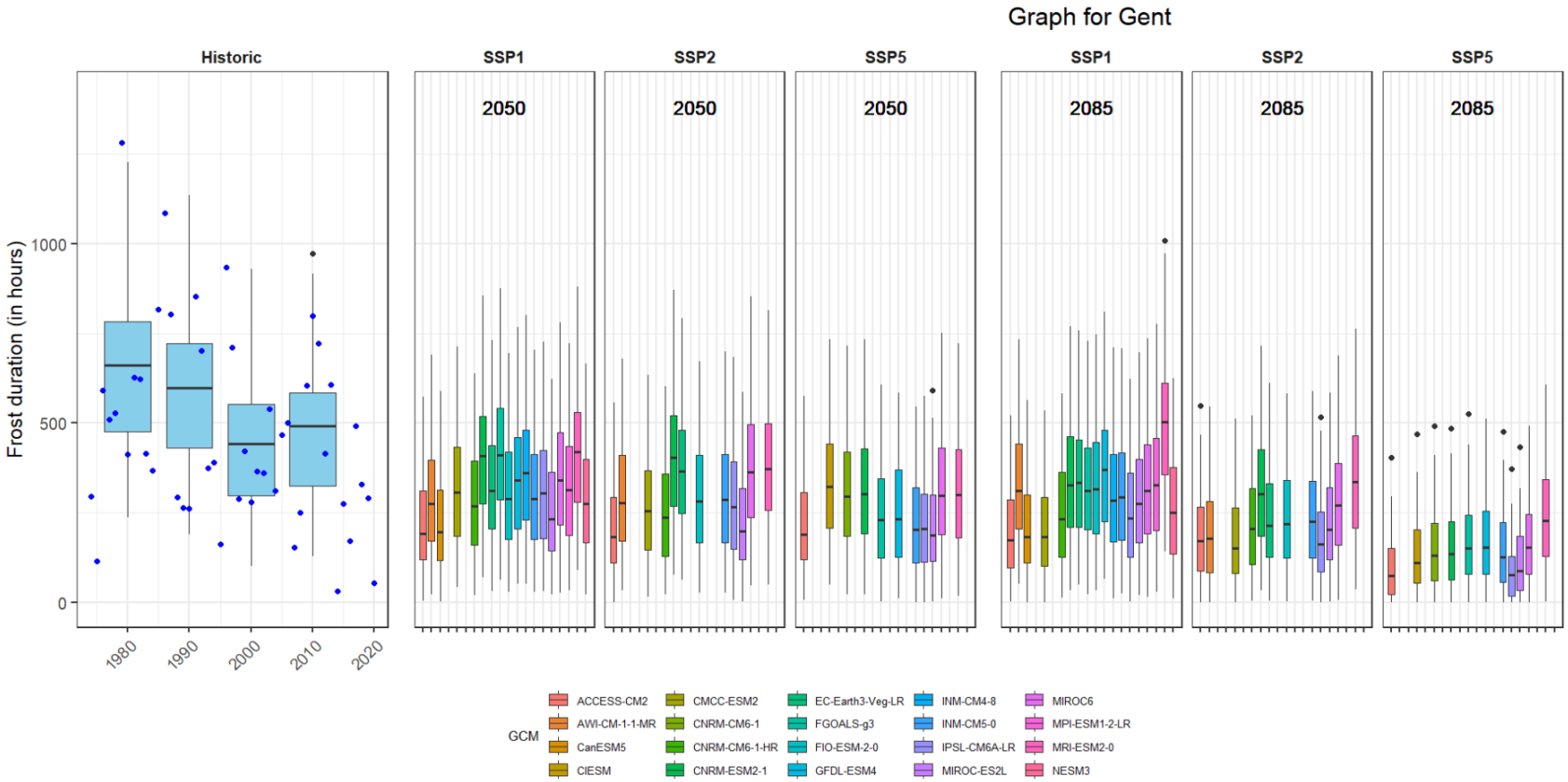

Figure 9: Projected Frost Scenarios for Gent's Future

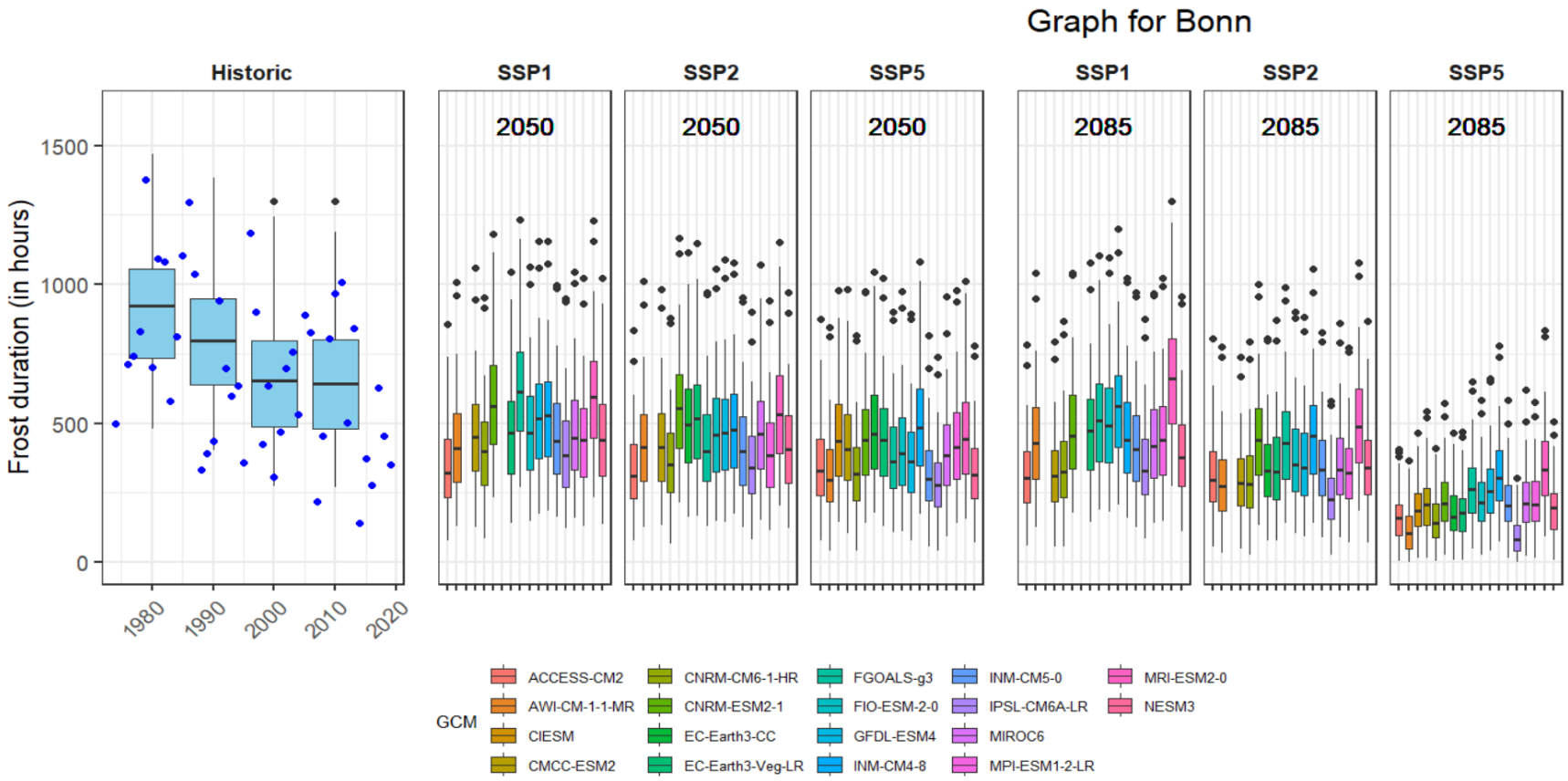

Figure 10: Projected Frost Scenarios for Bonn's Future

Discussion

The ability of nematodes to survive temperature fluctuations is crucial for their maintenance and adaptation in diverse environments (Pradhan et al., 2023; Velloso et al., 2022). This study on the behavior of *M. enterolobii* to different temperatures and incubation periods provided us essential information on its survival and infectivity, especially at temperatures below 20 °C. The data collected confirm that the temperature and incubation periods may affect Meloidogyne species differently, as suggested by previous work by Teklu, Schomaker and Been (2018), which highlighted the impact of storage duration or incubation period on the survival and infectivity of root-knot nematodes, more specifically *M. chitwoodi*.

In the final weeks of incubation, *M. enterolobii* showed resilience to higher temperatures (20 and 25 °C) compared to the other 2 species, consistent with its renowned tropical and subtropical habitat (Castagnone-Sereno, 2012). On the other hand, for the same period *M. chitwoodi* showed better survival rates at lower temperatures, which agrees with the results of the study carried out on survival and infectivity of 3 species of Meloidogyne at different temperature regimes, including *M. chitwoodi* (Wesemael et al., 2012), but opposed with those found by Eslah et al. (2023). These discrepancies highlight the differentiated impact of temperature on nematode survival, particularly between *M. enterolobii* and other species.

During the ten-weeks incubation period, all nematode species showed a gradual decline with significant variations in their survival. All 3 Meloidogyne species had higher survival rates at temperatures below 20 ºC and might be linked to the different levels of evaporation in incubators. However, the quantity of surviving nematodes declined sharply over time, highlighting the importance of temperature and duration of incubation to determine nematode viability. This tendency to obtain the greatest number of nematodes surviving at cooler temperatures (below 20 °C) could be linked to the fact that evaporation was more intense at the highest temperature (20 and 25 °C). Even if the humidity level was adjusted to 20% each week, this did not prevent the desiccation and a previous study proved that nematodes require a minimum level of humidity in their environment to survive (Dávila-Negrón & Dickson, 2013; Guiran & Demeure, 1978).

The two infectivity tests revealed that the infectivity of nematodes can be significantly influenced by temperature, with marked variations between species, the number of nematodes used and the duration of incubation (Velloso et al., 2022). Both studies confirm that temperature was a critical factor for root-knot nematodes infectivity. Inoculation made with nematodes extracted from the automatic zonal centrifuge showed greater fluctuations in optimal infectivity temperatures over time, while inoculation with 500 nematodes showed more constant infectivity for some species, with variations marked for the different temperatures. However, it should be mentioned that the development of the root system of tomato seedlings could exert an influence which is at the origin of that variation observed in the number of nematodes found in the root tissues of the plant, given that seedlings with a much more developed root system tended to contain more nematodes, for all species or temperatures combined.

For the infectivity test carried out after extraction of the nematodes, *M. enterolobii* had reached its maximum infectious power at 20 °C after 2 and 6 weeks of incubation, while *M. incognita* and *M. chitwoodi* present peaks at different temperatures and periods, highlighting optimal conditions of infectivity specific to each species. For the nematode infectivity test without extraction, *M. enterolobii* showed maximum and significant infectivity after 7 weeks of incubation at 5 °C, with more constant and non-significant infectivity across temperatures after 8 weeks of incubation. *M. incognita* exhibits relatively constant and non-significant infectivity across temperatures, with a slight decline at 25 °C after 7 weeks incubation and a significantly higher infectivity after 8 weeks at 5 °C. On the other hand, *M. chitwoodi* reached a non-significant peak at 20 °C after 7 weeks and remained constant across temperatures at 8 weeks incubation. These results suggest that even if temperature impacts the survival of nematodes, they still preserve their

infectious potential (Teklu et al., 2018), which is crucial for understanding their spread and their impact in the context of climate change.

Climate projections for Gent and Bonn indicate significant changes in temperature patterns through 2100. According to these projections, an increase in Heat accumulation and a decrease in the duration of frosts are expected by 2085. These changes could have major implications on the survival and spread of nematodes, particularly *M. enterolobii*, in these regions (Wesemael et al., 2011). Higher temperatures could favor *M. enterolobii*, potentially increasing its infectivity, survival rates or increasing its resilience, which would further complicate its management in agricultural systems. In contrast, species like *M. chitwoodi*, which thrive at lower temperatures, may become less problematic as climates warm, keeping in mind that this nematode remains infectious even at the highest temperatures (Teklu et al., 2018). However, given that the root-knot nematode's life cycle occurs entirely within the soil, it follows that soil conditions could profoundly influence the spread of *M. enterolobii* (Pan et al., 2023).

These results highlight the need for adaptive management strategies in agricultural systems, considering the differential responses of nematode species to temperature changes. The resilience of *M. enterolobii* to higher temperatures suggests that it may become more problematic under future climate conditions, particularly in regions experiencing significant warming. Conversely, the greater sensitivity of *M. chitwoodi* to high temperatures could limit its distribution and impact in warmer climates. Therefore, monitoring and managing nematode populations will require a nuanced understanding of how different species respond to different climatic conditions. An increase in the Heat accumulation can lead to an increase in precipitation and temperature, which provides a better survival condition for PPNs as well as their host plants. Recent studies have highlighted the presence of *M. enterolobii* in European countries such as Switzerland and Portugal (Kiewnick et al., 2008; Santos et al., 2019). With the projections of future climate scenarios made in this study, by 2085 a strong Heat accumulation is predicted for cities like Gent and Bonn, which can lead to better conditions for adaptation of *M. enterolobii* in these regions. This prediction agrees with the study carried out on the potential distribution of *M. enterolobii* in future climate scenarios, reported by the 2090s this nematode showed a tendency to expand and move towards higher latitudes (Pan et al., 2023). As has been the case for other species of tropical nematodes in past years which have been able to migrate (Bebber et al., 2013; Dutta & Phani, 2023). Moreover, recent study indicates that numerous tropical species, both on land and in the ocean, are likely to cope with global warming more effectively than previously anticipated (Chevalier et al., 2024).

To improve the robustness and relevance of future research, several recommendations can be made in response to the weaknesses and criticisms of the current study. It is crucial to expand sampling and experimental conditions by including a variety of geographic sites and complementing *in vitro* studies with experiments in real field conditions. This would provide a better understanding of local variations in nematode survival and infectivity and ensure the practical applicability of the results.

Longer term studies should be conducted to observe the effects of seasonal and annual climatic variations on nematode populations, including other environmental variables such as soil moisture, pH and the presence of beneficial microorganisms. Increasing the number of replicates in the experiments and the number of nematodes, providing the humidity of the samples in the incubators to 20% at least two (2) times a week and the use of more advanced statistical methods would allow to reinforce the robustness of the conclusions and to identify the complex interactions between the factors studied.

It is also recommended to test nematode infectivity on a wider diversity of host plants to assess the specificity and variability of plant-nematode interactions, performing cross-studies with resistant and susceptible host plants to better understand the mechanisms of resistance. In addition, it is extremely

important to inoculate the plants with nematodes without extraction and to ensure that the seedlings are at approximately the same stage of development (same size, same development of the root system, and if possible same age). By addressing these recommendations, future studies will be able to overcome the identified weaknesses, produce more robust and applicable results, and thus better contribute to the effective management of nematodes in the context of climate change.

Conclusion

This study highlights the significant impact of temperature and incubation periods on the survival and infectivity of 3 species of root-knot nematodes: *M. enterolobii*, *M. incognita* and *M. chitwoodi*. *M. enterolobii*, one of the extremely destructive plant-parasitic nematodes (Jones et al., 2013), showed greater resilience to high temperatures over time, notably at 20 and 25 °C, compared to *M. incognita* and *M. chitwoodi*, which is consistent with its tropical and subtropical origins (Castagnone-Sereno, 2012). In contrast, *M. chitwoodi* showed better survival rates at lower temperatures, consistent with its preference for temperate habitats.

Research highlights the crucial role of temperature in root-knot nematode infectivity (Teklu et al., 2018), with distinct optimal conditions for each species. This variability in response to temperature and incubation duration is essential to understanding the spread and potential impact of these nematodes in the context of climate change. These divergent responses highlight the importance of developing appropriate management strategies. Understanding these dynamics is crucial to anticipate the impact of climate change on nematode infestations and to develop effective control measures.

Climate projections for Gent and Bonn suggest a significant increase in heat accumulation and a decrease in the duration of frosts by 2085, which could favor the proliferation of *M. enterolobii* in these regions (PAN et al., 2023; Wesemael et al., 2011). Higher temperatures could favor the proliferation of this nematode (Gendron St-Marseille et al., 2015), thus complicating management efforts in agricultural systems. In contrast, in the long term *M. chitwoodi* may become less of a problem in warmer climates due to its sensitivity to high temperatures. However, in the short term *M. chitwoodi* may have many more generations per year due to the possibility of extended growing seasons and therefore more time for the nematodes to reproduce. These projections provide a framework for anticipating potential changes in the differentiated behavior of these three nematode species in response to climate change. The predicted climatic changes highlight the urgency of developing robust control measures adapted to the specific behaviors of these nematodes under future environmental conditions. Given the survival and remaining infectivity of *M. enterolobii* at lower temperatures as shown in this research, its establishment in temperate regions is an eminent threat.

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