Guy Medina

Seagrasses response to climate change

University of Algarve
Faculty of Science and Technology
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Master in Marine Biology

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Abstract:

Context: Increasing sea surface temperatures endangers coastal and marine communities around the globe. One of the communities expected to be greatly influenced by rising sea water surface temperature is seagrasses community. Seagrasses are marine macrophytes inhabiting's subtidal and intertidal coastlines in temperate to tropical bioregions around the world. Seagrasses are angiosperms (flowering plants) consisting of approximately fifty species belonging to ten genera. There are several threats to seagrasses populations and ecosystems worldwide today such as pathogens and parasites, climate change and rise in sea water temperatures. As sea surface temperatures are expected to increase and the tendency of heat waves expected to become more frequent with greater magnitude, seagrasses populations and particularly edge populations are predicted to be negatively influenced. In addition seagrasses population's ability to resist pathogens and parasites might be reduced due to the negative effects of heat waves, effecting their survival.

Objective: The study aims to evaluate ability of seagrasses to endure future heat waves reflected as increase in sea water temperature, and examine the effects of such sudden temperature increase on growth, quantum yield and survivability of seagrass species *Cymodocea nodosa* from: Mauritania and southern coast of Portugal, which represents the Atlantic southern and northern distribution edges respectively. It is expected that Mauritania population will be better adapted to higher constant temperature than Ria Formosa population due to their natural environment (Mediterranean vs temperate). In addition, to examine the diversity of oomycete infections, their proliferation and effects on the seagrass populations being subjected to heat wave in terms of percentage of infection and the variety of infections with the expectation that as the temperatures increase the resistance to pathogens will decrease in both populations.

Materials and Methods: In each experiment 45 specimens were divided randomly and equally into 3 treatment groups in three independent tanks: control, 28°C, 31°C. 28°C and 31°C groups were experiencing a gradual ascent in temperatures from 17°C to 28°C and 31°C respectively followed by a gradual decrease in temperature to 17°C. Each heatwave treatment consisted of five independent experimental units containing three shoots each. One shoot was sampled from each treatment at three different periods:

Acclimation (T6), End of heatwave (T14) and Recovery (T25) when each number represents days from the beginning of the experiments. PAM (pulse-amplitude modulated) fluorometry method was used to calculate photosynthetic quantum yield. The leaf puncturing technique was used to estimate leaf growth and software Image J was used to evaluate percentage of infection and growth from photographs. Isolation techniques were used to estimate the oomycete diversity of both seagrass populations.

Results: Our study results suggest that Mauritania population has a higher thermal tolerance than Ria Formosa population based on the results in Fig.2 at day 26 in recovery period. In addition results regarding quantum yield suggest that at day 6 in acclimation phase Mauritania population had a higher quantum yield values compared with Ria Formosa population in all treatment groups. In terms of diversity of infections the results imply that Mauritania population has a greater variety of infections compared with Ria Formosa population based on control and 28°C groups at the end of heatwave phase (T14) in Fig.6.

Conclusion: Our study results suggest that as anticipated Mauritania population may have a better response to future heatwaves than Ria Formosa population in terms of survivability and quantum yield. In addition the results imply that Mauritania population have greater variety of infections compared with Ria Formosa population and that Mauritania pathogens may have a better thermal tolerance compared with Ria Formosa pathogens. However no significant difference were observed regarding growth, percentage of infection and weight.

Keywords: *Seagrasses*, *Cymodocea nodosa*, *Temperature*, *Pathogen, Heatwave, Eco physiology.*

Introduction:

Seagrasses are marine macrophytes and aquatic angiosperms with terrestrial ancestors who found their path into the oceans approximately 90-100 million years ago {Beer et al 2014}. Despite their terrestrial origin, seagrasses adapted with great success to marine environments and can be found across temperate to tropical coastlines of the world {Short et al 2007}, {Beer et al 2014}. Seagrasses inhabits six global bioregions: four temperate and two tropical, which includes: the temperate North Atlantic, temperate North Pacific, Mediterranean, temperate southern Oceans, Tropical Atlantic and Tropical indo-Pacific {Short et al 2007}. There are four native species of seagrasses in Europe with depth ranges spannings from the intertidal zone and to depths of 50-60 meters {Borum et al 2004}.

The taxa referred to as seagrass relates to a very exclusive plant families (such as *Zosteraceae*, *Cymodoceaceae*, *Posidiniaceae*, and *Hydrocharitaceae*) classified within the superorder Alismatiflorae commonly known as Helobiae {Larkum et al 2006}. There are approximately 50 species of seagrasses worldwide that belong to 10 genera which belongs to the monocotyledonous higher plants {Beer et al 2014}.

Seagrasses dominated systems possess small biomass proportion in comparison with terrestrial ecosystems, however they are highly productive and plays a major role in marine ecosystems {Short et al 2007}. Seagrasses provides various vital environmental functions and utilities: nutrient cycling, coastlines protection and habitat for a wide range of species {York et al 2013}. It is estimated that around 50% of the world fisheries benefit from their service as a habitat and nursery for a wide range of species and seagrasses are considered to be one of the largest carbon sinks in the world {York et al 2013}. Seagrasses grow in a form of meadow and canopy as a result of their vegetative growth, in the juncture between the water column and the sediment in subtidal or tidal areas {Beer et al 2014} {Larkum et al 2006}. These meadows and canopy networks solidify the sediment offering sub layer for attachments, limiting irradiance, generating shelters from predation and variety of habitats that were not present in the previous barren sediment {Borum et al 2004}. Seagrasses meadows therefore contributes to an increase in biodiversity in the region as well as providing protection from coastal erosion by consolidating the sediment compared with scarce terrain{Borum et al 2004}{Beer et al 2014}. The height of the seagrass canopy can range from several centimeters to more than a meter (depend on the species) and so does the scope of their rhizomes {Larkum et al 2006}. Seagrasses are commonly known as ecosystem engineers for their ability to alter their surrounding physical and chemical environmental conditions {Bos et al 2007}. Seagrass leaf canopies assist diminishing flow velocity of waves and currents and therefore slowing water movement, which in turn increase sedimentation of suspended particles in the water column{Bos at el 2007}{Borum et al 2004}{Larkum et al 2006}. The seagrass network of rhizomes and roots helps to maintain and deposit the particles within the sediment, which encourage the activity of microbial benthic communities involved in mineralization and recycling of organic matter{Larkum et al 2006}. The removal of the particles from the water column enhance water transparency and increase the availability of light in the water which improve photosynthesis for the seagrasses themselves in the area{Borum et al 2004} and reducing problems of eutrophication{Short et al 2007}. Seagrasses are photosynthetic organisms that fix carbon dioxide and turn it into organic carbon for their own use such as growth while releasing oxygen in the process {Borum et al 2004}. Seagrasses have large rate of primary production which means high rates of oxygen production emitted into the surrounding water as a byproduct of the photosynthesis process{Borum et al 2004}. Seagrasses primary production constitutes only 1% of the total primary production in the oceans yet seagrasses are accounted for 12% of the total amount of carbon deposited in ocean sediments, which makes them important regulators in the global carbon cycle{Borum et al 2004}.

During the last century in many parts of the world different seagrasses species have experienced mass mortality events referred to as wasting disease, which has been attributed to a pathogenic stramenopile protist of the *Labyrinthula* genus {Bishop2013} {Sullivan et al 2013} {Trevathan-Tackett et al 2018}. In recent history, one of the largest abrupt and destructive mass mortality events occurred during the 1930s, with the severe loss of *Zostera marina* seagrass beds across the North Atlantic coasts which was related to *Labyrinthula zosterae* {Garcias-Bonet et al 2011}{Sullivan et al 2013}. The seagrass destruction was followed by sharp decrease in populations of limpets, waterfowl and scallops {Bishop2013}. It took close to forty years for the seagrass population to gain the initial population size {Bishop2013}. Throughout the years there were several cases of mass mortality of seagrasses all over the world when in many of them there was involvement of *Labyrinthula* species {Garcias-Bonet et al 2011}. Seagrass losses due to pathogens and other factors are still being reported around the world {Sullivan et al 2013}. Between 1879 and 2009 approximately 29% of the global coverage of seagrass was lost {Sullivan et al 2013} and according to estimations, the current disappearance rate of seagrass coverage worldwide stands somewhere between 2-5% annually {Martin et al 2016} and expecting to increase, although there are studies {De los Santos et al 2019} suggesting that overall decline is not the current state of european seagrasses and that deterioration of seagrass populations is reversible feasible process. Although *Labyrinthula* species were isolated from lesions of a wide variety of seagrasses all over the world, massive outbreaks of wasting diseases are quite rare{Sullivan et al 2013}{Garcias-Bonet et al 2011}. It is speculated that exposure of seagrasses to biological and anthropogenic environmental stressors over long periods of time compromise their health, making them more vulnerable to pathogenic infection {Trevathan-Tackett et al 2018} {Sullivan et al 2013} {Garcias-Bonet et al 2011}. Beside *Labyrinthula* species, *Phytophthora* species and *Halophytophthora* species are recently suggested as potential pathogens of seagrass species {Govers et al 2016}.

One of the rising threats to aquatic ecosystems worldwide and to seagrasses in particular is global climate change and increase in sea surface temperatures {Olsen et al 2012} {Reusch et al 2008}. A study {Massa et al 2009} aimed to explore the thermal tolerance of *Zostera noltii* in the southern coast of Portugal discovered that above 37°C there was a sharp decline in photosynthetic capacity which was followed by the death of the shoot. Another research {Moore et al 2014} designed to examine the effects of temperature on *Zostera marina* in the York river at Chesapeake bay, implies that short time exposure to quick elevation in temperatures in a range of 4-5 degrees beyond the average recorded during the summer period, may cause a massive die off events and to disappearance of this species. A research {George et al 2018} conducted on four different species of seagrasses exhibits the effect of temperature stress on biomass and photosynthesis. The study examined the effect of exposure to five different maximal temperature peaks for three midday hours for seven consecutive days. At 45°C severe decline in photosynthetic efficiency was measured in all species. Biomass reduction was noticed in all species at 40°C and 45°C. The study findings suggested that even tropical seagrass species can experience severe stress by an increase of a few degrees beyond the daily maximum temperature. These studies demonstrate the detrimental effects of heatwaves and potential future hazard on seagrass communities around the world.

Our study focuses on the seagrass species *Cymodocea nodosa*. *Cymodocea nodosa* commonly known as "seahorse grass" is a dioecious species and can be found throughout the Mediterranean Sea, the Atlantic coast of North Africa, southern coast of Portugal and the Canary Islands {Gkafas et al 2016} {Cabaco et al 2010}{Cancemi et al 2002}. This species is considered a pioneer species for its ability to settle in a wide range of different habitats such as coastal waters, lagoons and estuaries and can be found in various depths: from shallow areas and up to 50-60 meters deep{Cancemi et al 2002}{Borum et al 2004}. In terms of morphology *Cymodocea nodosa* have 2-5 leaves with 2-4 mm width and with length that can vary between 10-45 cm {Borum et al 2004}. The shoots are connected to a vertical rhizome that attached to a horizontal rhizome (in a pink or white color), and the roots are scattered over both kind of rhizomes when each rhizome section include one root {Borum et al 2004}.

Our study aimed to examine the thermal tolerance response of *Cymodocea nodosa* from two locations: Mauritania and the southern coast of Portugal which are known to be the Atlantic southern and northern limits respectively {Alberto et al 2008}. We wanted to examine differences in response to thermal stress in terms such as: photosynthetic quantum yield, weight, growth and survival. We focus on edge populations since these populations are more susceptible to environmental changes and pressures that shapes and define their natural borders{Mota et al 2018}. Previous conservation and evolutionary studies suggested that separate edge populations may have unique genetic and phenotypic attributes which derive from low genetic diversity {Mota et al 2018}. Rapid environmental stressors may trigger distinct reactions between edge populations which may be used as an early alarming indicators of disruption in the ecological system and may assist in predicting future response to climate change{Mota et al 2018}. Our hypothesis is that Mauritania species will endure thermal stress better compared with Ria

Formosa species due to their natural environment (mediterranean compared with temperate).In addition, we also want to investigate this species host-pathogen relationship in response to an increase in temperature. We assume that as the temperature increase, the resistance to pathogens will decrease. Due to the aforementioned importance of seagrasses to the marine environment it is crucial to assess and predict the future damage that seagrasses meadows might sustain and its consequences.

Materials and Methods:

Our first experiment was conducted on seagrass species *Cymodocea nodosa* that were gathered from Banc d'Arguin Mauritania, transferred to Portugal during March 2020 and were kept in acclimation for 13 consecutive days prior the experiment. Our second experiment was conducted on seagrass species of *Cymodocea nodosa* that were gathered from Ria Formosa lagoon during May 2020 and were kept in acclimation for 14 consecutive days prior the experiment. Banc d'Arguin Mauritania is known as the southern limit for this species which resides in mediterranean climate while Ria Formosa lagoon known as the northern limit for this species and reside in temperate climate.

Acclimating: Once the specimens were transferred into the acclimation chamber they were planted into sand within a plastic container already containing sea water that was replaced every three days. Forty five shoots were allocated for the study and both leaves and roots were clipped to an even length of 12 and 3-4 cm length respectively. The acclimation chamber temperature was kept constant at 14°C throughout the period prior the experiment (15 days for Mauritania experiment and 9 days for Ria Formosa experiment) as well as the light intensity (between 20-30 ppm) and air circulation within the chamber. The temperature in the acclimation chamber was raised to 17°C several days preceding the specimen's relocation to the lab. These conditions were maintained the same for Mauritania and Ria Formosa experiments.

Heatwave experiment: Three separate and independent thermal baths (xL) (Huber Pilot one Variostat) were placed in the lab seven days prior the study, each tank had an independent temperature control system in order to assure constant water temperature throughout the experiment. The forty five shoots were distributed randomly and equally among the three tanks. Five beakers (1L glass beaker) each filled with 5 cm of sand were placed in each tank. In each beaker three random shoots were planted within even distance from each other and were completely submerged with sea water. A small aeration tube was inserted into each beaker providing air at a constant rate in order to maintain a constant and even water flow in each beaker. An Aquaray Led panel (Marine white) was placed over each water tank in order to maintain a constant and even light intensity (30umol m2 s-1) to all the beakers in all the tanks during the experiment. Half of the sea water volume was exchanged every three days in all the beakers during the experiment. The seawater was extracted from a container holding seawater (salinity between 35 and 38 ppm) from Ria Formosa which was refilled once a week. The extraction was performed using a filter to exclude external contaminants.

The temperature in all the water tanks began at 17°C at the same time from T0 (the first day of the experiment) to T6 (day six from the beginning of the experiment). The factor temperature was divided into three levels: 17, 28 and 31°C, which represents the maximal temperature that the plants were experiencing in the three independent water tanks. One water tank remained the entire study at 17°C and was used as control group while the temperature in the other tanks were raised gradually to either 28 or 31°C and decreased gradually back to 17°C. In tank reaching 31°C temperature was increased daily from: 17°C, 20°C, 23°C, 26°C,28°C, to- 31°C as can be seen in Fig.1. The specimens experienced four consecutive days at 31°C followed by a daily decrease of temperature from: 31°C, 28°C, 26°C, 23°C, 20°C, to- 17°C. In the 28°C heatwave treatment, temperature was increased daily from: 17°C, 20°C, 23°C, 26°C,to-28°C and decreased daily as follow: 28°C, 26°C, 23°C, 20°C, to-17°C as can be seen in Fig.1. The specimens in this tank experienced 28°C for six consecutive days before the temperature decrease. The temperature increase and decrease in 28°C and 31°C tanks was parallel and simultaneously except for the maximal temperature. Shoots were sampled at three phases during the study: the end of the acclimation period (referred to as T6), end of heatwave (referred to as T14) and the recovery phase (referred to as T25/T26). Overall the acclimation period lasted 6 days, the heatwave period 8 days and the recovery period 11(Mauritania) or 12(Ria Formosa) days. The two experiments were executed in identical ways.

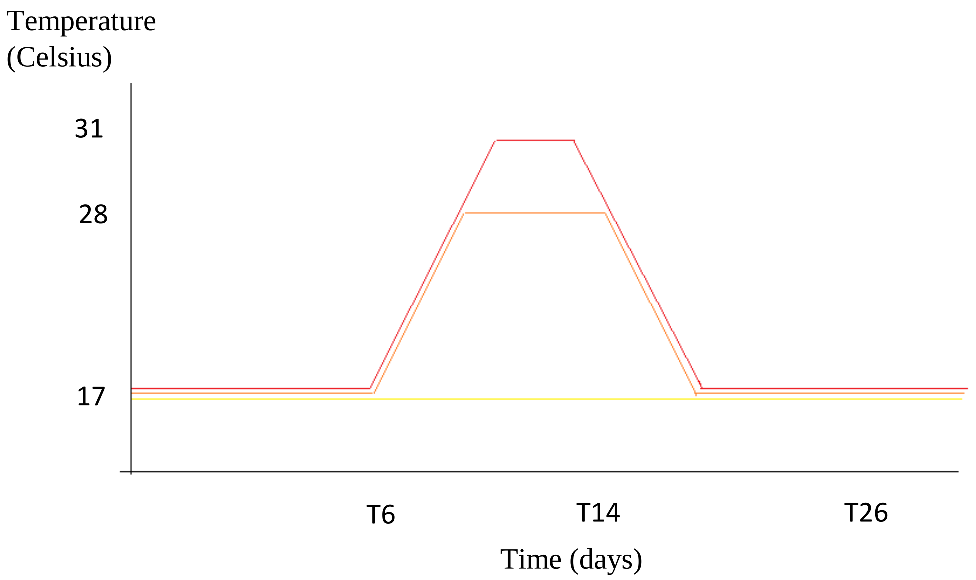

Fig.1. Illustrates the heatwave experiment conduct in both Mauritania and Ria Formosa experiments. T6, T14, T26 represents the three sampling phases acclimation, end of heatwave and recovery respectively. The colors red, orange and yellow relates to 31°C, 28°C and control tanks respectively. Mauritania experiment lasted for 25 days compared with 26 days in Ria Formosa, for demonstration purposes recovery phase is labeled T26.

<u>Methods:</u> In all the sampling phases specimens were weighted, photosynthetic quantum yield was measured and photos of the plants were taken in order to calculate growth and the degree of the infection. A scale (Acculab ALC-150.3) was used to weight the plants in grams up to the third decimal point (mg). A camera was used to take the pictures with the use of the software digicam. The quantum yield was calculated using fluropen software and PAM (pulse-amplitude modulated) fluorometer. In each shoot sampling, the specimen was removed from the beaker and was dark acclimated for three minutes. After three minutes a reading took place using PAM fluorometer and fluropen software to measure Fv/Fm values (quantum yield). Afterwards the shoot was weighted using a scale, was dissected and the leaves were separated and placed next to each other on a glass plate with a ruler next to the plate. A camera combined with digicam software were used to take pictures of the glass plate and the ruler which later was used to calculate growth and estimate the percentage of infection. Growth per leaf was calculated using the formula: (length of the shoot tip from the punctured hole)/ (length of the shoot base from the punctured hole) {Short et al 2001}, average growth of all the leaves was considered the average growth of the shoot and average growth of all the shoots in treatment group was

considered growth per treatment. Percentage of infection per leaf was visually estimated, when the average percentage of infection of all the leaves was considered average infection of the shoot and average infection of all the shoots in treatment group was considered average percentage of infection per treatment.

The infected sample tissues (roots and leaves) from the plants were dried using paper tissue and then dissected into smaller pieces and divided between two selective media: PARPNH selective media for Halophytophthora and SSA selective media (with the same antibiotics as PARPNH) for Labyrinthula {Garcias-Bonet et al 2011}. PARPNH selective media consist of unclarified V8-agar with 10 µg ml^{-1} pimaricin, 200 µg ml^{-1} ampicillin, 10 µg ml^{-1} rifampicin, 25 µg ml^{-1} pentachloronitrobenzene (PCNB), 50 µg ml^{-1} nystatin and 50 µg ml^{-1} hymexazol {Man int Veld et al 2019}{Laboratories of Molecular Biotechnology and Phytopathology, IBB-CGB plant and animal genomic group(University of Algarve) 2011}. The antibiotics aforementioned were dissolved in 5-10 ml of 80% ethanol and stirred on magnetic stirrer until dissolution. The nystatin and hymexazol were dissolved in 100 ml of sterile distilled water with temperature above 45°C using magnetic stirrer until they dissolved {Laboratories of Molecular Biotechnology and Phytopathology, IBB-CGB plant and animal genomic group (University of Algarve) 2011}. All antibiotics were added to the V8-agar when the temperature was at 45°C and the vial containing the agar was shaken to evenly distribute the antibiotics {Laboratories of Molecular Biotechnology and Phytopathology, IBB-CGB plant and animal genomic group (University of Algarve) 2011}. SSA without antibiotics was used as culture media for Labyrinthula growth {Garcias-Bonet et al 2011}.

Statistics:

All the results were statistically analyzed using primer 6 software using the three factors: population, treatment and time. Each factor was divided into levels as followed: population- (Mauritania, Ria Formosa), treatment-(Control, 28°C, 31°C), time-(acclimation (T6), end of heatwave (T14), recovery (T26)). Using primer 6 software a three way ANOVA was performed on 6 variables(Number of live replicates, Fv/Fm, weight, growth, type of infection and percentage of infection) using the aforementioned factors to test similarity. The full detailed analysis can be seen in the appendix.

Results:

Shoot survival was similar for both populations except at day 26 after recovery (T26) when number of live shoots was found to be five time higher in 28°C group in Mauritania population compared with Ria population (p=0.05, Fig.2). Marginal significant distinction was observed between Mauritania and Ria populations in factor treatment groups (p=0.0509, Fig.2).

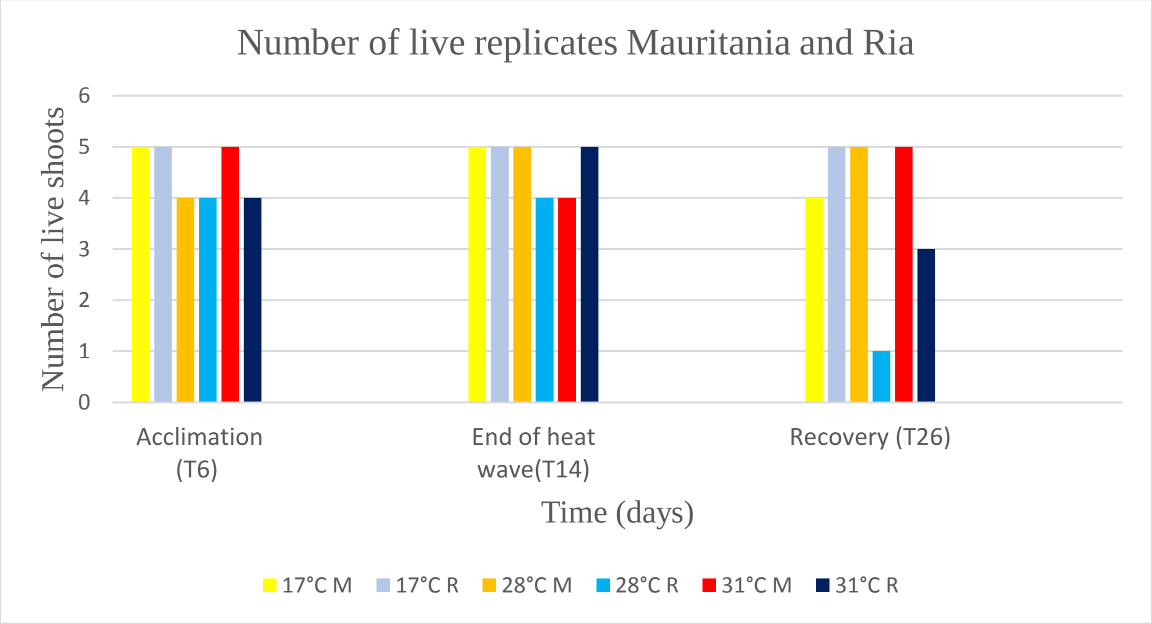

Figure 2. Represents number of live shoots of *Cymodocea nodosa* per treatment groups per sampling period, when each treatment group contains 5 shoots. 17°C, 28°C, 31°C relates to control, 28°C group and 31°C group respectively, when each number represents the maximal temperature for treatment group. The labels M and R refers to Mauritania and Ria Formosa respectively. Mauritania experiment lasted for 25 days compared with 26 days in Ria Formosa, for demonstration purposes recovery phase is labeled T26.

All Fv/Fm values were similar in both populations except at day 6 after acclimation (T6) when Fv/Fm values were found to be higher at Mauritania population compared with Ria Formosa population (p=0.05, Fig.3). Examination of Fig.3 reveals that Fv/Fm values in all treatment groups are higher in Mauritania population compared with Ria Formosa population.

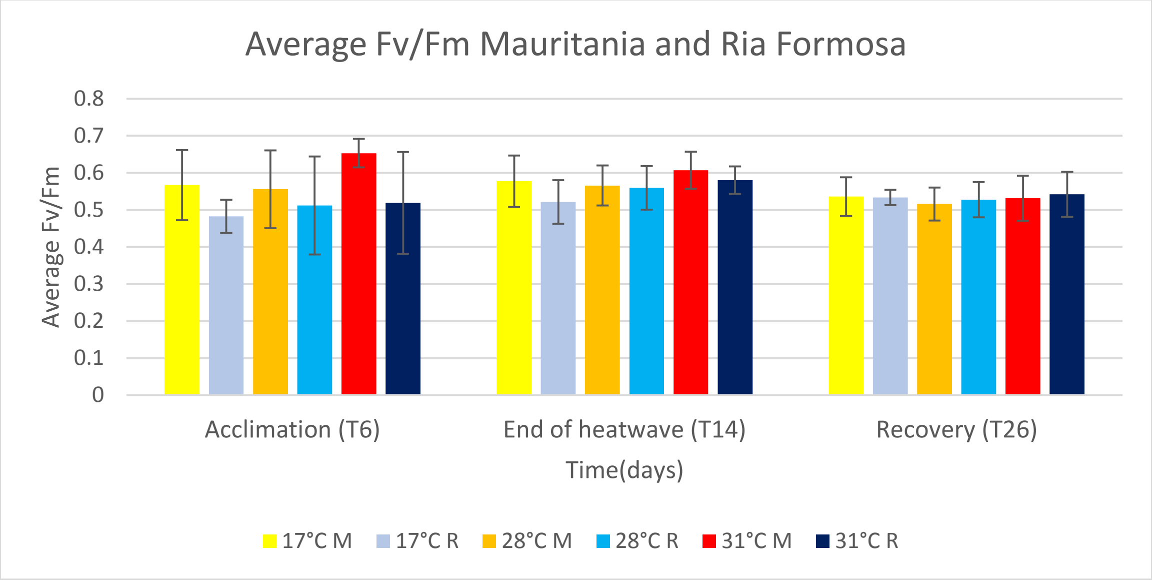

Figure 3. Represents the average Fv/Fm of *Cymodocea nodosa* per treatment group per sampling period, when each treatment group contains 5 shoots. 17°C, 28°C, 31°C relates to control, 28°C group and 31°C group respectively, when each number represents the maximal temperature for treatment group. The labels M and R refers to Mauritania and Ria Formosa respectively. The error bars represents the standard deviation (STD). Mauritania experiment lasted for 25 days compared with 26 days in Ria Formosa, for demonstration purposes recovery phase is labeled T26.

All weight values were found without significant difference between the two populations (p=0.05, Fig.4), however marginal meaningful distinctions were found between the two populations and factor time (p=0.0596, Fig.4), and between factors: populations, time and treatment groups (p=0.079, Fig.4).

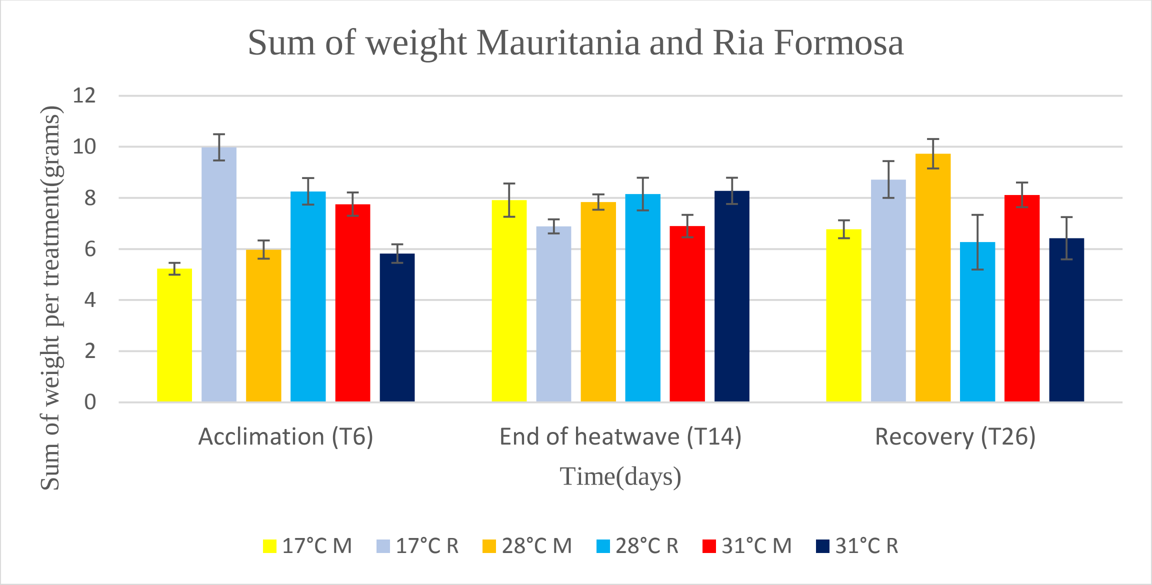

Figure 4. Represents the sum of weight of *Cymodocea nodosa* per treatment group per sampling period, when each treatment group contains 5 shoots. 17°C, 28°C, 31°C relates to control, 28°C group and 31°C group respectively, when each number represents the maximal temperature for treatment group. The labels M and R refers to Mauritania and Ria Formosa respectively. The error bars represents the standard deviation (STD). Mauritania experiment lasted for 25 days compared with 26 days in Ria Formosa, for demonstration purposes recovery phase is labeled T26.

Examination of growth data did not find meaningful statistical difference between the two populations (p=0.05, Fig.5). However, observation at the end of heatwave (T14) between two populations suggest that the growth rate in Mauritania population may be greater compared with Ria Formosa population in all treatment groups. The results at the recovery period imply that Ria Formosa population may have a greater rate of growth compared with Mauritania population. It is important to mention that the leaf puncture technique was performed successfully on 14/30 individuals in Mauritania study and on 17/30 individuals in Ria Formosa study. It is also important to mention that the numbers of individuals between the treatments groups was uneven. These reservation need to be taken into account when examining the growth per treatment.

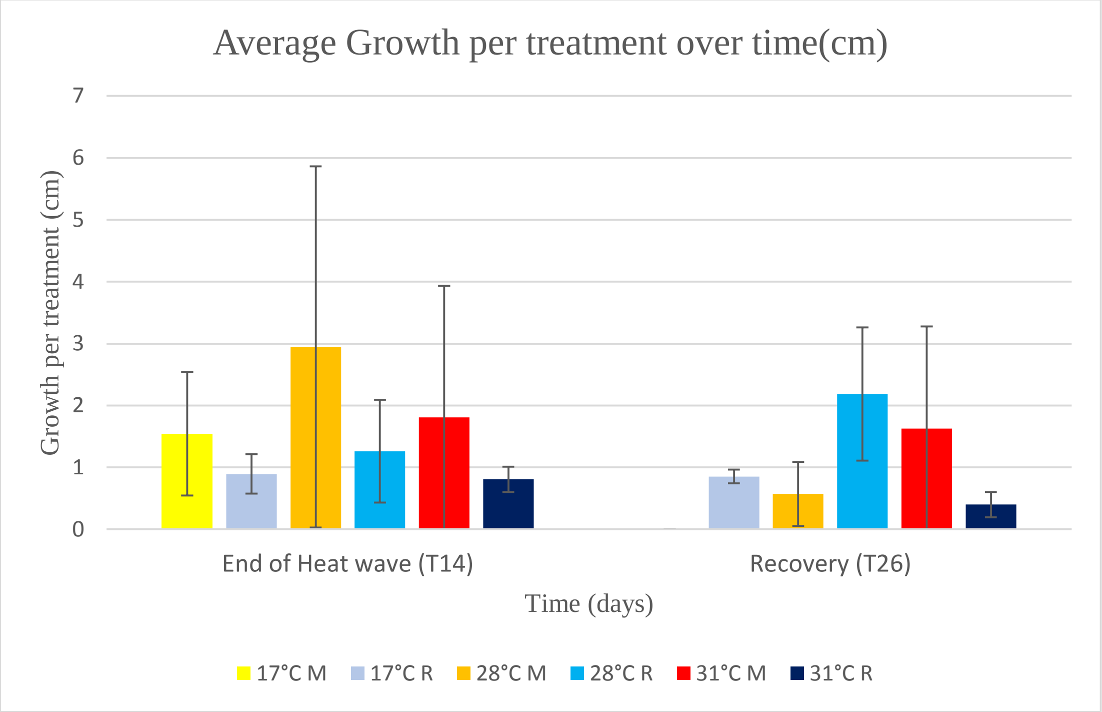

Figure 5. Represents the average growth of *Cymodocea nodosa* (cm) per treatment group per sampling period from Mauritania and Ria Formosa. 17°C, 28°C, 31°C relates to control, 28°C group and 31°C group respectively, when each number represents the maximal temperature for treatment group. The labels M and R refers to Mauritania and Ria Formosa respectively. The error bars represents the standard deviation (STD). Mauritania experiment lasted for 25 days compared with 26 days in Ria Formosa, for demonstration purposes recovery phase is labeled T26.

Types of pathogenic infection between the two populations was found without significance with the exception at 14 days from the beginning of the experiment after the end of heatwave (T14), in the control group and 28°C group. Ria Formosa population had 3 replicates infected with Halophytophthora species in the control group at T14, compared with Mauritania population which had 3 replicates infected with Halophytophthora species and Labyrinthula species, 1 replicate infected with Halophytophthora species and another replicate infected with Labyrinthula species (p=0.05, Fig.6). In 28°C group Mauritania population had five replicates infected with Halophytophthora species compared to zero replicates infected in Ria Formosa population (p=0.05, Fig.6).

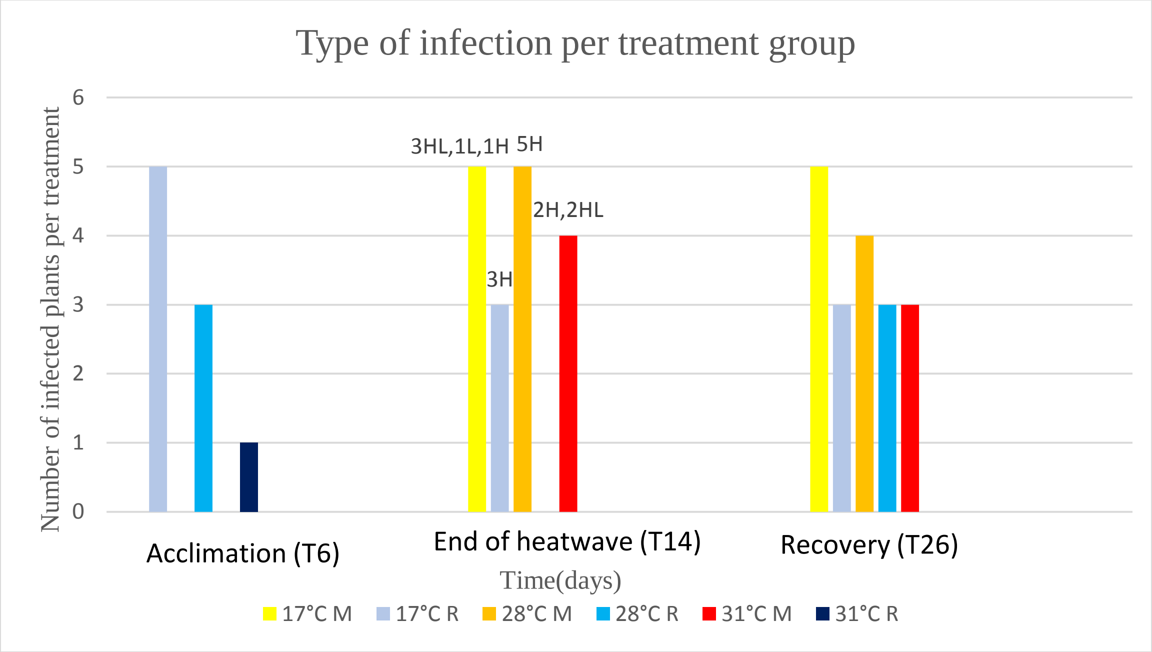

Figure 6. Represents the type of pathogenic infection of *Cymodocea nodosa* per treatment group, per sampling period, when each treatment group contains 5 shoots. 17°C, 28°C, 31°C relates to control, 28°C group and 31°C group respectively, when each number represents the maximal temperature for treatment group. The labels M and R refers to Mauritania and Ria Formosa respectively. The labels H, L and HL represents the pathogen species: Halophytophthora, Labyrinthula and Halophytophthora + Labyrinthula respectively. Mauritania experiment lasted for 25 days compared with 26 days in Ria Formosa, for demonstration purposes recovery phase is labeled T26. Note: at T6 there is no data regarding Mauritania population. At T14 and T26 the missing bars meaning no infection.

Examination of the percentage of infection values did not discovered any meaningful difference between the two populations (p=0.05, Fig.7). However, close examination of Fig.7 imply that in the control group of Mauritania population the percentage of infection increases over time while in Ria Formosa control group the percentage of infection does not seem to change drastically over time.

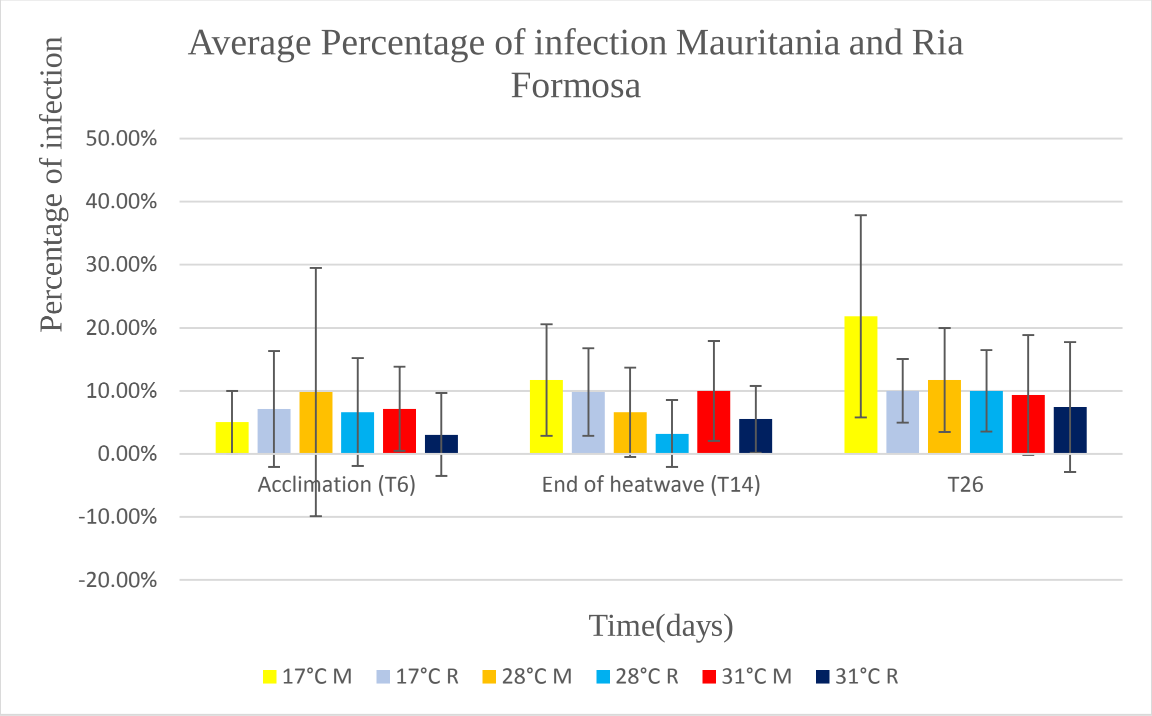

Figure 7. Represents the average percentage of infection of *Cymodocea nodosa* per treatment group per sampling period, when each treatment group contains 5 shoots. 17°C, 28°C, 31°C relates to control, 28°C group and 31°C group respectively, when each number represents the maximal temperature for treatment group. The labels M and R refers to Mauritania and Ria Formosa respectively. The error bars represents the standard deviation (STD). Mauritania experiment lasted for 25 days compared with 26 days in Ria Formosa, for demonstration purposes recovery phase is labeled T26.

Discussion:

This study results suggest that there are some meaningful distinctions between Mauritania and Ria Formosa populations regarding: Fv/Fm values, Survivability and types of pathogenic infections.

Our study results imply that at acclimation phase (T6) Fv/Fm (quantum yield) values of Mauritania population were significantly higher than those of Ria Formosa population in all tested groups. At the acclimation phase the specimens from both populations were not subjected yet to any sort of heat stress, therefore it is a possibility that these differences derive from the populations unique traits and attributes. As previous study {Lee at el 2007} suggested, minimum light requirements for seagrass development can change within the same species as a result of acclimation to the environment local light regime. It is possible that Mauritania population adjusted faster and better to the artificial light regime in the study compared with Ria Formosa population due to the difference between their natural environments (mediterranean vs temperate). Regarding survivability our study results suggest that significant distinction between the two populations was found at recovery phase (T26) in 28°C group. In this test group from Mauritania the number of live shoots was five time higher compared with Ria Formosa population. This result imply that Mauritania population may have better response to heatwave scenario compared with Ria Formosa population. A study {Chefaoui et al 2016} aimed to predict the potential distribution of *Cymodocea nodosa* in the Mediterranean and Atlantic coastlines showed that the most relevant environmental factors affecting the distribution of this species were sea surface temperature and salinity. The study suggested that suitable environments for these species are in temperature range between 5.8-26.4°C. Additional study {Olsen et al 2012} conducted on two species of Mediterranean seagrass (*Cymodocea nodosa and Posidonia oceanica*) aimed to explore growth and demography as response to experimental warming suggested that increasing the temperature up to 29-30°C improved some of the plant performance indicators but beyond 30°C all the indicators were decreased. The results of these two studies ({Chefaoui et al 2016},{Olsen et al 2012}) and our study results imply that Ria Formosa edge population may be susceptible to future heatwaves with temperatures of 28°C and above. According to our expectations Mauritania population results imply that this population has a better thermal tolerance to heatwave compared with Ria Formosa population.

Comparison between the types of infections between the two populations suggest that the variation of infection is greater in Mauritania population than in Ria Formosa population between the control and the 28°C groups at the end of the heatwave phase (T14). Comparison between 28°C group reveals that while in Mauritania population all the replicates were infected with Halophytophthora species, none of the replicates from Ria Formosa species were infected. This may suggest that pathogens species from Ria Formosa are more susceptible to a rapid temperature elevation compared with Mauritania pathogens. Another possible explanation could be that Ria Formosa population has a better resistance to its pathogens compared with Mauritania population. The results between the two populations regarding the control group at the end of the heatwave (T14) indicate that Mauritania population may have a greater variety of pathogens compared with Ria Formosa population. In the control group from Ria Formosa only 3/5 replicates were infected with Halophytophthora species compared with Mauritania population where all the replicates were infected as follow: 1/5 with Halophytophthora species, 1/5 with Labyrinthula species, 3/5 with Labyrinthula + Halophytophthora species. Our initial expectations were that the resistance to pathogens will decrease as the temperature will increase regardless of the population tested. However, our results from Ria Formosa suggest that increased temperature may assist reducing the risk of pathogens while Mauritania population results are not conclusive. A study {Sullivan et al 2018} that investigated host pathogen dynamics of seagrass diseases under future global change suggested that climate related effects of diseases are likely to vary between different species and locations around the world as was observed in our study results. Additional study {Olsen et al 2012} also suggest that contrary to what has been predicted, warming may assist diminishing the threat of wasting disease in some seagrass species.

Statistical analysis of the sum of weight between the two populations (Fig. 4) found the results to be without statistical significance. As previously mentioned in both trials all the plants were clipped prior to the experiment in both roots and leaves to an even length of 3-4 and 12 cm respectively. However, the plants were not weighted before the beginning of the experiments and therefore we cannot assume that their initial weight is calibrated. In addition, in each sampling the plant that was retrieved and weighted was destroyed and did not return into the experiment, which means that in each sampling the results that we received derived from a different individual with distinct unknown initial weight.

Analysis of the growth values did not find any meaningful distinction between the two populations. However, the results in Fig.5 suggest that the growth rate in Mauritania population is greater than Ria Formosa population at end of heatwave phase (T14) in all the tested groups.

There was no significant difference between the two populations regarding the percentage of infection.

Conclusion:

Our study results suggest that there may be a difference between the two populations in several points in terms of Fv/Fm, survivability and the variety of pathogens. Our study results confirm to some degree our initial expectation that Mauritania population will be better adapted than Ria Formosa temperature to higher constant temperatures as was observed in the survivability results. In addition Mauritania population may have better adapted to our artificial light regime based on the Fv/Fm values. However our initial assumption that the resistance to pathogen will decline as the temperature rise have been refuted based on our results. Ria Formosa results regarding type of infection suggest that increase of temperature may reduce pathogen risk while Mauritania results were not conclusive. In addition there was no significant difference between the two populations throughout the experiment regarding the percentage of infection. This important issue require more research as it have a major impact on marine environments and human society.

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Appendix:

Statistical analysis:

Number of live replicates:

PERMANOVA
Permutational MANOVA

Resemblance worksheet
Name: Resem5
Data type: Similarity
Selection: All
Resemblance: S17 Bray Curtis similarity

Sums of squares type: Type III (partial)
Fixed effects sum to zero for mixed terms
Permutation method: Permutation of residuals under a reduced model
Number of permutations: 9999

Factors

Name	Abbrev.	Type	Levels
Population	Po	Fixed	2
Treatment	Tr	Fixed	3
Time	Ti	Fixed	3

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Po	1	444,44	444,44	4,2353	0,0436	8208
Tr	2	666,67	333,33	3,1765	0,0424	9043
Ti	2	518,52	259,26	2,4706	0,088	9943
PoxTr	2	666,67	333,33	3,1765	0,0509	9616
PoxTi	2	518,52	259,26	2,4706	0,096	9942
TrxTi	4	148,15	37,037	0,35294	0,8482	9950
PoxTrxTi	4	1037	259,26	2,4706	0,0488	9958
Res	72	7555,6	104,94			
Total	89	11556				

Details of the expected mean squares (EMS) for the model

Source	EMS
Po	1*V(Res) + 45*S(Po)
Tr	1*V(Res) + 30*S(Tr)
Ti	1*V(Res) + 30*S(Ti)
PoxTr	1*V(Res) + 15*S(PoxTr)
PoxTi	1*V(Res) + 15*S(PoxTi)
TrxTi	1*V(Res) + 10*S(TrxTi)
PoxTrxTi	1*V(Res) + 5*S(PoxTrxTi)
Res	1*V(Res)

Construction of Pseudo-F ratio(s) from mean squares

Source	Numerator	Denominator	Num.df	Den.df
Po	1*Po	1*Res	1	72
Tr	1*Tr	1*Res	2	72
Ti	1*Ti	1*Res	2	72
PoxTr	1*PoxTr	1*Res	2	72
PoxTi	1*PoxTi	1*Res	2	72
TrxTi	1*TrxTi	1*Res	4	72
PoxTrxTi	1*PoxTrxTi	1*Res	4	72

Estimates of components of variation

Source	Estimate	Sq.root
S(Po)	7,5446	2,7467
S(Tr)	7,6132	2,7592
S(Ti)	5,144	2,268

```
S(PoxTr)        15,226    3,9021
S(PoxTi)        10,288    3,2075
S(TrxTi)       -6,7901   -2,6058
S(PoxTrxTi)     30,864    5,5556
V(Res)          104,94    10,244
```

Number of replicates (Pop x Ti) pairwise comparison:

```
PERMANOVA
Permutational MANOVA

Resemblance worksheet
Name: Resem5
Data type: Similarity
Selection: All
Resemblance: S17 Bray Curtis similarity

Sums of squares type: Type III (partial)
Fixed effects sum to zero for mixed terms
Permutation method: Permutation of residuals under a reduced model
Number of permutations: 9999

Factors
Name         Abbrev.   Type    Levels
Population   Po        Fixed        2
Treatment    Tr        Fixed        3
Time         Ti        Fixed        3

PAIR-WISE TESTS

Term 'PoxTi' for pairs of levels of factor 'Population'

Within level 'T6' of factor 'Time'
                                                     Unique
Groups                            t       P(perm)    perms   P(MC)
Mauritania, Ria Formosa      0,57735     0,6055      5062   0,566

Denominators
Groups                       Denominator    Den.df
Mauritania, Ria Formosa      1*Res              24

Average Similarity between/within groups
                 Mauritania    Ria Formosa
Mauritania          95,556
Ria Formosa         93,926          91,746
```

```
Within level 'T14' of factor 'Time'
                                                        Unique
Groups                               t        P(perm)    perms   P(MC)
Mauritania, Ria Formosa      6,2563E-8          1          204       1

Denominators
Groups                       Denominator   Den.df
Mauritania, Ria Formosa      1*Res             24

Average Similarity between/within groups
                 Mauritania    Ria Formosa
Mauritania          95,556
Ria Formosa         95,852          95,556

Within level 'T26' of factor 'Time'
                                                    Unique
Groups                            t      P(perm)    perms     P(MC)
Mauritania, Ria Formosa      2,6726      0,0143      7072    0,0107

Denominators
Groups                       Denominator   Den.df
Mauritania, Ria Formosa      1*Res             24

Average Similarity between/within groups
                 Mauritania    Ria Formosa
Mauritania          95,556
Ria Formosa         86,222          82,857
```

Number of replicates (Pop x Tr) pairwise comparison:

```
PERMANOVA
Permutational MANOVA

Resemblance worksheet
Name: Resem5
Data type: Similarity
Selection: All
Resemblance: S17 Bray Curtis similarity

Sums of squares type: Type III (partial)
Fixed effects sum to zero for mixed terms
Permutation method: Permutation of residuals under a reduced model
Number of permutations: 9999

Factors
Name           Abbrev.   Type    Levels
```

```
Population     Po        Fixed     2
Treatment      Tr        Fixed     3
Time           Ti        Fixed     3

PAIR-WISE TESTS

Term 'PoxTr' for pairs of levels of factor 'Population'

Within level 'Treatment 2' of factor 'Treatment'
                                                  Unique
Groups                         t     P(perm)     perms    P(MC)
Mauritania, Ria Formosa     1,069    0,3045      6181    0,2919

Denominators
Groups                      Denominator   Den.df
Mauritania, Ria Formosa     1*Res             24

Average Similarity between/within groups
              Mauritania    Ria Formosa
Mauritania       95,556
Ria Formosa          92          88,571

Within level 'Treatment 1' of factor 'Treatment'
                                               Unique
Groups                        t    P(perm)     perms    P(MC)
Mauritania, Ria Formosa     2,5    0,0174      7286    0,0217

Denominators
Groups                      Denominator   Den.df
Mauritania, Ria Formosa     1*Res             24

Average Similarity between/within groups
              Mauritania    Ria Formosa
Mauritania       95,556
Ria Formosa      86,222          82,857

Within level 'Control' of factor 'Treatment'
                                            Unique
Groups                      t  P(perm)     perms    P(MC)
Mauritania, Ria Formosa     1   0,4134      1122    0,3292

Denominators
Groups                      Denominator   Den.df
Mauritania, Ria Formosa     1*Res             24

Average Similarity between/within groups
```

```
              Mauritania   Ria Formosa
Mauritania      95,556
Ria Formosa     97,778          100
```

Number of replicates (Pop x Tr x Ti) pairwise comparison:

```
PERMANOVA
Permutational MANOVA

Resemblance worksheet
Name: Resem5
Data type: Similarity
Selection: All
Resemblance: S17 Bray Curtis similarity

Sums of squares type: Type III (partial)
Fixed effects sum to zero for mixed terms
Permutation method: Permutation of residuals under a reduced model
Number of permutations: 9999

Factors
Name         Abbrev.   Type    Levels
Population   Po        Fixed        2
Treatment    Tr        Fixed        3
Time         Ti        Fixed        3

PAIR-WISE TESTS

Term 'PoxTrxTi' for pairs of levels of factor 'Population'

Within level 'Treatment 2' of factor 'Treatment'
Within level 'T6' of factor 'Time'
                                              Unique
Groups                        t   P(perm)     perms     P(MC)
Mauritania, Ria Formosa       1      1          1      0,3487

Denominators
Groups                        Denominator   Den.df
Mauritania, Ria Formosa       1*Res              8

Average Similarity between/within groups
              Mauritania   Ria Formosa
Mauritania       100
Ria Formosa     93,333        86,667

Within level 'Treatment 2' of factor 'Treatment'
```

```
Within level 'T14' of factor 'Time'
                                               Unique
Groups                          t   P(perm)    perms   P(MC)
Mauritania, Ria Formosa         1        1         1   0,347

Denominators
Groups                          Denominator   Den.df
Mauritania, Ria Formosa         1*Res              8

Average Similarity between/within groups
                Mauritania    Ria Formosa
Mauritania          86,667
Ria Formosa         93,333            100

Within level 'Treatment 2' of factor 'Treatment'
Within level 'T26' of factor 'Time'
                                                    Unique
Groups                              t    P(perm)    perms     P(MC)
Mauritania, Ria Formosa         1,633     0,4429        2    0,1423

Denominators
Groups                          Denominator   Den.df
Mauritania, Ria Formosa         1*Res              8

Average Similarity between/within groups
                Mauritania    Ria Formosa
Mauritania             100
Ria Formosa         86,667             80

Within level 'Treatment 1' of factor 'Treatment'
Within level 'T6' of factor 'Time'
                                                         Unique
Groups                                  t     P(perm)    perms   P(MC)
Mauritania, Ria Formosa         1,9992E-9           1        2       1

Denominators
Groups                          Denominator   Den.df
Mauritania, Ria Formosa         1*Res              8

Average Similarity between/within groups
                Mauritania    Ria Formosa
Mauritania          86,667
Ria Formosa         89,333         86,667

Within level 'Treatment 1' of factor 'Treatment'
Within level 'T14' of factor 'Time'
```

```
                                                Unique
Groups                         t   P(perm)     perms    P(MC)
Mauritania, Ria Formosa        1       1          1     0,3518

Denominators
Groups                         Denominator    Den.df
Mauritania, Ria Formosa        1*Res              8

Average Similarity between/within groups
                Mauritania    Ria Formosa
Mauritania          100
Ria Formosa      93,333          86,667

Within level 'Treatment 1' of factor 'Treatment'
Within level 'T26' of factor 'Time'
                                                Unique
Groups                         t   P(perm)     perms    P(MC)
Mauritania, Ria Formosa        4   0,0474         3     0,0049

Denominators
Groups                         Denominator    Den.df
Mauritania, Ria Formosa        1*Res              8

Average Similarity between/within groups
                Mauritania    Ria Formosa
Mauritania          100
Ria Formosa      73,333          86,667

Within level 'Control' of factor 'Treatment'
Within level 'T6' of factor 'Time'
                                                                Unique
Groups                                  t             P(perm)    perms    P(MC)
Mauritania, Ria Formosa        Denominator is 0

Denominators
Groups                         Denominator    Den.df
Mauritania, Ria Formosa        1*Res              0

Average Similarity between/within groups
                Mauritania    Ria Formosa
Mauritania          100
Ria Formosa         100            100

Within level 'Control' of factor 'Treatment'
Within level 'T14' of factor 'Time'
                                                                Unique
```

```
Groups                                      t            P(perm)    perms    P(MC)
Mauritania, Ria Formosa        Denominator is 0

Denominators
Groups                         Denominator    Den.df
Mauritania, Ria Formosa        1*Res               0

Average Similarity between/within groups
                    Mauritania     Ria Formosa
Mauritania             100
Ria Formosa            100             100

Within level 'Control' of factor 'Treatment'
Within level 'T26' of factor 'Time'
                                              Unique
Groups                         t   P(perm)     perms    P(MC)
Mauritania, Ria Formosa        1       1           1     0,3517

Denominators
Groups                         Denominator    Den.df
Mauritania, Ria Formosa        1*Res               8

Average Similarity between/within groups
                    Mauritania     Ria Formosa
Mauritania           86,667
Ria Formosa          93,333            100
```

Fv/Fm:

```
PERMANOVA
Permutational MANOVA

Resemblance worksheet
Name: Resem1
Data type: Similarity
Selection: All
Resemblance: S17 Bray Curtis similarity

Sums of squares type: Type III (partial)
Fixed effects sum to zero for mixed terms
Permutation method: Permutation of residuals under a reduced model
Number of permutations: 9999

Factors
Name          Abbrev.   Type    Levels
Population    Po        Fixed      2
Treatment     Tr        Fixed      3
```

Time	Ti	Fixed	3

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Po	1	249,09	249,09	4,9649	0,026	9922
Tr	2	172,72	86,358	1,7213	0,1864	9948
Ti	2	193,39	96,693	1,9273	0,1472	9948
PoxTr	2	48,735	24,368	0,4857	0,6256	9950
PoxTi	2	302,15	151,08	3,0113	0,0489	9959
TrxTi	4	80,442	20,11	0,40084	0,8215	9951
PoxTrxTi	4	64,759	16,19	0,32269	0,8756	9961
Res	70	3511,9	50,17			
Total	87	4618,1				

Details of the expected mean squares (EMS) for the model

Source	EMS
Po	1*V(Res) + 43,784*S(Po)
Tr	1*V(Res) + 29,195*S(Tr)
Ti	1*V(Res) + 29,211*S(Ti)
PoxTr	1*V(Res) + 14,597*S(PoxTr)
PoxTi	1*V(Res) + 14,605*S(PoxTi)
TrxTi	1*V(Res) + 9,7401*S(TrxTi)
PoxTrxTi	1*V(Res) + 4,8701*S(PoxTrxTi)
Res	1*V(Res)

Construction of Pseudo-F ratio(s) from mean squares

Source	Numerator	Denominator	Num.df	Den.df
Po	1*Po	1*Res	1	70
Tr	1*Tr	1*Res	2	70
Ti	1*Ti	1*Res	2	70
PoxTr	1*PoxTr	1*Res	2	70
PoxTi	1*PoxTi	1*Res	2	70
TrxTi	1*TrxTi	1*Res	4	70
PoxTrxTi	1*PoxTrxTi	1*Res	4	70

Estimates of components of variation

Source	Estimate	Sq.root
S(Po)	4,5432	2,1315
S(Tr)	1,2395	1,1133
S(Ti)	1,5927	1,262
S(PoxTr)	-1,7676	-1,3295
S(PoxTi)	6,9089	2,6285
S(TrxTi)	-3,0862	-1,7568
S(PoxTrxTi)	-6,9775	-2,6415
V(Res)	50,17	7,0831

Fv/Fm (pop x Ti) pairwise comparison:

```
PERMANOVA
Permutational MANOVA

Resemblance worksheet
Name: Resem1
Data type: Similarity
Selection: All
Resemblance: S17 Bray Curtis similarity

Sums of squares type: Type III (partial)
Fixed effects sum to zero for mixed terms
Permutation method: Permutation of residuals under a reduced model
Number of permutations: 9999

Factors
Name        Abbrev.  Type   Levels
Population  Po       Fixed       2
Treatment   Tr       Fixed       3
Time        Ti       Fixed       3

PAIR-WISE TESTS

Term 'PoxTi' for pairs of levels of factor 'Population'

Within level 'T6' of factor 'Time'
                                              Unique
Groups                          t   P(perm)    perms   P(MC)
Mauritania, Ria Formosa    2,2439    0,0307     9907  0,0331

Denominators
Groups                     Denominator  Den.df
Mauritania, Ria Formosa    1*Res            24

Average Similarity between/within groups
              Mauritania  Ria Formosa
Mauritania        90,839
Ria Formosa        87,65       87,157

Within level 'T14' of factor 'Time'
                                              Unique
Groups                          t   P(perm)    perms   P(MC)
Mauritania, Ria Formosa    1,3333    0,1967     9894  0,1924
```

```
Denominators
Groups                        Denominator   Den.df
Mauritania, Ria Formosa       1*Res             22

Average Similarity between/within groups
               Mauritania   Ria Formosa
Mauritania       94,249
Ria Formosa      94,042          93,71

Within level 'T26' of factor 'Time'
                                                      Unique
Groups                               t      P(perm)    perms   P(MC)
Mauritania, Ria Formosa        0,39611       0,708      9918   0,705

Denominators
Groups                        Denominator   Den.df
Mauritania, Ria Formosa       1*Res             24

Average Similarity between/within groups
               Mauritania   Ria Formosa
Mauritania       94,476
Ria Formosa      95,137         95,392

Weight:

PERMANOVA
Permutational MANOVA

Resemblance worksheet
Name: Resem2
Data type: Similarity
Selection: All
Resemblance: S17 Bray Curtis similarity

Sums of squares type: Type III (partial)
Fixed effects sum to zero for mixed terms
Permutation method: Permutation of residuals under a reduced model
Number of permutations: 9999

Factors
Name         Abbrev.   Type    Levels
Population   Po        Fixed        2
Treatment    Tr        Fixed        3
Time         Ti        Fixed        3

PERMANOVA table of results
```

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Po	1	328,84	328,84	0,89359	0,3752	9942
Tr	2	318,25	159,12	0,4324	0,7585	9945
Ti	2	710,05	355,03	0,96475	0,3982	9945
PoxTr	2	1560	780	2,1196	0,0955	9943
PoxTi	2	1872,7	936,34	2,5444	0,0596	9961
TrxTi	4	629,4	157,35	0,42759	0,8751	9935
PoxTrxTi	4	2874,5	718,64	1,9528	0,079	9951
Res	72	26496	368			
Total	89	34790				

Details of the expected mean squares (EMS) for the model

Source	EMS
Po	1*V(Res) + 45*S(Po)
Tr	1*V(Res) + 30*S(Tr)
Ti	1*V(Res) + 30*S(Ti)
PoxTr	1*V(Res) + 15*S(PoxTr)
PoxTi	1*V(Res) + 15*S(PoxTi)
TrxTi	1*V(Res) + 10*S(TrxTi)
PoxTrxTi	1*V(Res) + 5*S(PoxTrxTi)
Res	1*V(Res)

Construction of Pseudo-F ratio(s) from mean squares

Source	Numerator	Denominator	Num.df	Den.df
Po	1*Po	1*Res	1	72
Tr	1*Tr	1*Res	2	72
Ti	1*Ti	1*Res	2	72
PoxTr	1*PoxTr	1*Res	2	72
PoxTi	1*PoxTi	1*Res	2	72
TrxTi	1*TrxTi	1*Res	4	72
PoxTrxTi	1*PoxTrxTi	1*Res	4	72

Estimates of components of variation

Source	Estimate	Sq.root
S(Po)	-0,87019	-0,93284
S(Tr)	-6,9625	-2,6387
S(Ti)	-0,43241	-0,65758
S(PoxTr)	27,466	5,2408
S(PoxTi)	37,889	6,1554
S(TrxTi)	-21,065	-4,5896
S(PoxTrxTi)	70,127	8,3742
V(Res)	368	19,183

Growth:

PERMANOVA

```
Permutational MANOVA

Resemblance worksheet
Name: Resem4
Data type: Similarity
Selection: All
Resemblance: S17 Bray Curtis similarity

Sums of squares type: Type III (partial)
Fixed effects sum to zero for mixed terms
Permutation method: Permutation of residuals under a reduced model
Number of permutations: 9999

Factors
Name        Abbrev.  Type   Levels
Population  Po       Fixed       2
Treatment   Tr       Fixed       3
Time        Ti       Fixed       2

PERMANOVA table of results
                                                          Unique
Source      df      SS       MS   Pseudo-F  P(perm)   perms
Po           1  609,79   609,79    0,42133   0,6695    9957
Tr           2  2325,5   1162,8    0,80339   0,5157    9954
Ti           1  815,76   815,76    0,56363   0,5612    9942
PoxTr        2  3025,1   1512,5     1,0451   0,3702    9949
PoxTi        1  986,39   986,39    0,68153    0,506    9947
TrxTi        2  390,96   195,48    0,13506   0,9845    9938
PoxTrxTi**   1  2084,9   2084,9     1,4405   0,2395    9956
Res         20   28946   1447,3
Total       30   40514

** Term has one or more empty cells

Details of the expected mean squares (EMS) for the model
Source     EMS
Po         1*V(Res) + 9,1429*S(Po)
Tr         1*V(Res) + 7,0737*S(Tr)
Ti         1*V(Res) + 10,225*S(Ti)
PoxTr      1*V(Res) + 4,0091*S(PoxTr)
PoxTi      1*V(Res) + 4,5714*S(PoxTi)
TrxTi      1*V(Res) + 3,6245*S(TrxTi)
PoxTrxTi   1*V(Res) + 2,2857*S(PoxTrxTi)
Res        1*V(Res)

Construction of Pseudo-F ratio(s) from mean squares
```

```
Source      Numerator     Denominator   Num.df  Den.df
Po          1*Po          1*Res              1      20
Tr          1*Tr          1*Res              2      20
Ti          1*Ti          1*Res              1      20
PoxTr       1*PoxTr       1*Res              2      20
PoxTi       1*PoxTi       1*Res              1      20
TrxTi       1*TrxTi       1*Res              2      20
PoxTrxTi    1*PoxTrxTi    1*Res              1      20
```

Estimates of components of variation

```
Source         Estimate   Sq.root
S(Po)           -91,604    -9,571
S(Tr)           -40,227   -6,3424
S(Ti)           -61,767   -7,8592
S(PoxTr)         16,266    4,0331
S(PoxTi)        -100,83   -10,041
S(TrxTi)        -345,38   -18,584
S(PoxTrxTi)      278,93    16,701
V(Res)           1447,3    38,044
```

<u>Type of infection:</u>

PERMANOVA
Permutational MANOVA

Resemblance worksheet
Name: Resem3
Data type: Similarity
Selection: All
Resemblance: S17 Bray Curtis similarity

Sums of squares type: Type III (partial)
Fixed effects sum to zero for mixed terms
Permutation method: Permutation of residuals under a reduced model
Number of permutations: 9999

Factors

```
Name         Abbrev.   Type    Levels
Population   Po        Fixed        2
Treatment    Tr        Fixed        3
Time         Ti        Fixed        3
```

PERMANOVA table of results

```
                                                       Unique
Source        df     SS        MS     Pseudo-F  P(perm)   perms
Po             0      0                No test
```

```
Tr              1   427,52   427,52    1,4486     0,2374     9927
Ti              1   1220,7   1220,7    4,1363     0,0483     9914
PoxTr           1   36,943   36,943    0,12518    0,7467     9924
PoxTi**         1     375      375     1,2706     0,2706     9933
TrxTi           3   3530,2   1176,7    3,9872     0,0169     9956
PoxTrxTi**      0      0               No test
Res            32   9444,1   295,13
Total          43    19714
```

** Term has one or more empty cells

Details of the expected mean squares (EMS) for the model

```
Source      EMS
Po
Tr          1*V(Res) + 14,845*S(Tr)
Ti          1*V(Res) + 6,8354*S(Ti)
PoxTr       1*V(Res) + 3,5821*S(PoxTr)
PoxTi       1*V(Res) + 3,75*S(PoxTi)
TrxTi       1*V(Res) + 3,9389*S(TrxTi)
PoxTrxTi
Res         1*V(Res)
```

Construction of Pseudo-F ratio(s) from mean squares

```
Source      Numerator   Denominator   Num.df   Den.df
Po                                       0        0
Tr          1*Tr        1*Res            1       32
Ti          1*Ti        1*Res            1       32
PoxTr       1*PoxTr     1*Res            1       32
PoxTi       1*PoxTi     1*Res            1       32
TrxTi       1*TrxTi     1*Res            3       32
PoxTrxTi                                 0        0
```

Estimates of components of variation

```
Source           Estimate   Sq.root
S(Po)             No test
S(Tr)              8,9182    2,9863
S(Ti)              135,41    11,637
S(PoxTr)          -72,076   -8,4898
S(PoxTi)           21,299    4,6151
S(TrxTi)           223,82    14,961
S(PoxTrxTi)       No test
V(Res)             295,13    17,179
```

Type of infection (Tri x Ti) pairwise comparison:

PERMANOVA

```
Permutational MANOVA

Resemblance worksheet
Name: Resem3
Data type: Similarity
Selection: All
Resemblance: S17 Bray Curtis similarity

Sums of squares type: Type III (partial)
Fixed effects sum to zero for mixed terms
Permutation method: Permutation of residuals under a reduced model
Number of permutations: 9999

Factors
Name          Abbrev.   Type    Levels
Population    Po        Fixed        2
Treatment     Tr        Fixed        3
Time          Ti        Fixed        3

PAIR-WISE TESTS

Term 'TrxTi' for pairs of levels of factor 'Treatment'

Within level 'T14' of factor 'Time'
                                                       Unique
Groups                              t      P(perm)    perms    P(MC)
Treatment 2, Treatment 1        1,972      0,1699         3   0,0907
Treatment 2, Control          0,77402      0,4538       201   0,4608
Treatment 1, Control           4,1161      0,0055       110   0,0018

Denominators
Groups                      Denominator   Den.df
Treatment 2, Treatment 1    1*Res              7
Treatment 2, Control        1*Res              9
Treatment 1, Control        1*Res             10

Average Similarity between/within groups
               Treatment 2   Treatment 1   Control
Treatment 2         66,667
Treatment 1             75           100
Control             74,792        77,083    71,667

Within level 'T26' of factor 'Time'
                                                       Unique
Groups                              t      P(perm)    perms    P(MC)
Treatment 2, Treatment 1      0,41551      0,7565       248   0,7652
```

Treatment 2, Control	1,7035	0,1264	215	0,1183
Treatment 1, Control	1,258	0,2322	9127	0,2337

Denominators

Groups	Denominator	Den.df
Treatment 2, Treatment 1	1*Res	7
Treatment 2, Control	1*Res	8
Treatment 1, Control	1*Res	11

Average Similarity between/within groups

	Treatment 2	Treatment 1	Control
Treatment 2	86,667		
Treatment 1	77,619	72,381	
Control	68,333	76,25	81,429

Within level 'T6' of factor 'Time'

Groups	t	P(perm)	Unique perms	P(MC)
Treatment 2, Treatment 1	Denominator is 0			
Treatment 2, Control	Denominator is 0			
Treatment 1, Control	Denominator is 0			

Denominators

Groups	Denominator	Den.df
Treatment 2, Treatment 1	1*Res	0
Treatment 2, Control	1*Res	0
Treatment 1, Control	1*Res	0

Average Similarity between/within groups

	Treatment 2	Treatment 1	Control
Treatment 2	0		
Treatment 1	100	100	
Control	100	100	100

Percentage of infection (log transformation):

PERMANOVA

Permutational MANOVA

Resemblance worksheet

Name: Resem4

Data type: Distance

```
Selection: All
Transform: Log(X+1)
Resemblance: D1 Euclidean distance

Sums of squares type: Type III (partial)
Fixed effects sum to zero for mixed terms
Permutation method: Permutation of residuals under a reduced model
Number of permutations: 999

Factors
Name   Abbrev.        Type    Levels
Population      Po      Fixed       2
Treatment       Tr      Fixed       3
Time   Ti       Fixed       3

PERMANOVA table of results
                                                 Unique
Source df           SS         MS          Pseudo-F       P(perm)         perms
Po         1         2,2302             2,2302            1,5423           0,204    998
Tr         2         5,9571             2,9786            2,0598           0,138    999
Ti         2         10,697             5,3486            3,6987           0,023    999
PoxTr   2         1,2388            0,61939          0,42832          0,652    999
PoxTi   2        0,18697         9,3487E-2      6,4649E-2        0,934    999
TrxTi    4         4,6941             1,1735           0,81152          0,503    999
PoxTrxTi          4          2,4283            0,60707          0,41981          0,816    998
Res       72        104,12             1,4461
Total    89        131,55

Details of the expected mean squares (EMS) for the model
Source EMS
```

```
Po      1*V(Res) + 45*S(Po)
Tr      1*V(Res) + 30*S(Tr)
Ti      1*V(Res) + 30*S(Ti)
PoxTr   1*V(Res) + 15*S(PoxTr)
PoxTi   1*V(Res) + 15*S(PoxTi)
TrxTi   1*V(Res) + 10*S(TrxTi)
PoxTrxTi        1*V(Res) + 5*S(PoxTrxTi)
Res     1*V(Res)

Construction of Pseudo-F ratio(s) from mean squares
Source Numerator      Denominator   Num.df          Den.df
Po      1*Po    1*Res      1        72
Tr      1*Tr    1*Res      2        72
Ti      1*Ti    1*Res      2        72
PoxTr   1*PoxTr         1*Res      2        72
PoxTi   1*PoxTi         1*Res      2        72
TrxTi   1*TrxTi         1*Res      4        72
PoxTrxTi        1*PoxTrxTi    1*Res      4        72

Estimates of components of variation
Source   Estimate        Sq.root
S(Po)    1,7426E-2       0,13201
```

Tables:

Table 1. Represents the status of each shoot at a given time from Mauritania and Ria Formosa.

Replicate	Population	Time			Treatment
		T6	T14	T26	
15	Mauritania	1	1	1	31°C
14	Mauritania	1	1	1	31°C
13	Mauritania	1	1	1	31°C
12	Mauritania	1	1	1	31°C

11	Mauritania	1	0	1	31°C
10	Mauritania	1	1	1	28°
9	Mauritania	1	1	1	28°C
8	Mauritania	1	1	1	28°C
7	Mauritania	1	1	1	28°C
6	Mauritania	0	1	1	28°C
5	Mauritania	1	1	1	17°C
4	Mauritania	1	1	1	17°C
3	Mauritania	1	1	0	17°C
2	Mauritania	1	1	1	17°C
1	Mauritania	1	1	1	17°C
15	Ria Formosa	0	1	1	31°C
14	Ria Formosa	1	1	1	31°C
13	Ria Formosa	1	1	1	31°C
12	Ria Formosa	1	1	0	31°C
11	Ria Formosa	1	1	0	31°C
10	Ria Formosa	1	1	1	28°C
9	Ria Formosa	1	1	0	28°C
8	Ria Formosa	1	1	0	28°C
7	Ria Formosa	0	1	0	28°C
6	Ria Formosa	1	0	0	28°C
5	Ria Formosa	1	1	1	17°C
4	Ria Formosa	1	1	1	17°C

3	Ria Formosa	1	1	1	17°C
2	Ria Formosa	1	1	1	17°C
1	Ria Formosa	1	1	1	17°C

Table 2. Represents the average Fv/Fm from Mauritania and Ria Formosa per time and per treatment.

Population	Treatment	Temperature	Time	Fv/Fm
Mauritania	Treatment 2	17	T6	0,653
Mauritania	Treatment 1	17	T6	0,5556
Mauritania	Control	17	T6	0,5668
Mauritania	Treatment 2	31	T14	0,607
Mauritania	Treatment 1	28	T14	0,5656
Mauritania	Control	17	T14	0,5772
Mauritania	Treatment 2	17	T25	0,5316
Mauritania	Treatment 1	17	T25	0,5158
Mauritania	Control	17	T25	0,5356
Ria Formosa	Treatment 2	17	T6	0,5184
Ria Formosa	Treatment 1	17	T6	0,5122
Ria Formosa	Control	17	T6	0,4826
Ria Formosa	Treatment 2	31	T14	0,5802
Ria Formosa	Treatment 1	28	T14	0,5595
Ria Formosa	Control	17	T14	0,5214
Ria Formosa	Treatment 2	17	T26	0,542
Ria Formosa	Treatment 1	17	T26	0,5276

Ria Formosa	Control	17	T26	0,5332

Table 3. Represents the sum of weight per time and per treatment from Mauritania and Ria Formosa.

Replicate	Population	Treatment	Temperature(Celsius)	Time	weight	SUM weight
15	Mauritania	Treatment 2	17	T6	1,548	7,756
14	Mauritania	Treatment 2	17	T6	1,850	
13	Mauritania	Treatment 2	17	T6	1,361	
12	Mauritania	Treatment 2	17	T6	0,900	
11	Mauritania	Treatment 2	17	T6	2,097	
10	Mauritania	Treatment 1	17	T6	1,277	5,973
9	Mauritania	Treatment 1	17	T6	1,033	
8	Mauritania	Treatment 1	17	T6	0,687	
7	Mauritania	Treatment 1	17	T6	1,648	
6	Mauritania	Treatment 1	17	T6	1,328	
5	Mauritania	Control	17	T6	0,998	5,227
4	Mauritania	Control	17	T6	1,341	
3	Mauritania	Control	17	T6	0,747	
2	Mauritania	Control	17	T6	1,191	
1	Mauritania	Control	17	T6	0,95	
15	Mauritania	Treatment 2	31	T14	1,539	6,895
14	Mauritania	Treatment 2	31	T14	1,533	
13	Mauritania	Treatment 2	31	T14	1,949	
12	Mauritania	Treatment 2	31	T14	0,924	
11	Mauritania	Treatment 2	31	T14	0,95	
10	Mauritania	Treatment 1	28	T14	1,641	7,839
9	Mauritania	Treatment 1	28	T14	1,576	
8	Mauritania	Treatment 1	28	T14	1,964	
7	Mauritania	Treatment 1	28	T14	1,548	
6	Mauritania	Treatment 1	28	T14	1,11	
5	Mauritania	Control	17	T14	1,614	7,916
4	Mauritania	Control	17	T14	0,822	
3	Mauritania	Control	17	T14	2,58	
2	Mauritania	Control	17	T14	1,259	

1	Mauritania	Control	17	T14	1,641	
15	Mauritania	Treatment 2	17	T25	1,198	8,118
14	Mauritania	Treatment 2	17	T25	2,113	
13	Mauritania	Treatment 2	17	T25	1,09	
12	Mauritania	Treatment 2	17	T25	2,087	
11	Mauritania	Treatment 2	17	T25	1,63	
10	Mauritania	Treatment 1	17	T25	1,418	9,731
9	Mauritania	Treatment 1	17	T25	2,702	
8	Mauritania	Treatment 1	17	T25	2,227	
7	Mauritania	Treatment 1	17	T25	2,061	
6	Mauritania	Treatment 1	17	T25	1,323	
5	Mauritania	Control	17	T25	1,632	6,77
4	Mauritania	Control	17	T25	1,193	
3	Mauritania	Control	17	T25	0,838	
2	Mauritania	Control	17	T25	1,393	
1	Mauritania	Control	17	T25	1,714	
15	Ria Formosa	Treatment 2	17	T6	1,379	5,819
14	Ria Formosa	Treatment 2	17	T6	1,067	
13	Ria Formosa	Treatment 2	17	T6	1,397	
12	Ria Formosa	Treatment 2	17	T6	0,568	
11	Ria Formosa	Treatment 2	17	T6	1,408	
10	Ria Formosa	Treatment 1	17	T6	1,478	8,257
9	Ria Formosa	Treatment 1	17	T6	2,469	
8	Ria Formosa	Treatment 1	17	T6	1,088	
7	Ria Formosa	Treatment 1	17	T6	1,801	
6	Ria Formosa	Treatment 1	17	T6	1,421	

5	Ria Formosa	Control	17	T6	2,161	9,98
4	Ria Formosa	Control	17	T6	1,119	
3	Ria Formosa	Control	17	T6	2,284	
2	Ria Formosa	Control	17	T6	2,025	
1	Ria Formosa	Control	17	T6	2,391	
15	Ria Formosa	Treatment 2	31	T14	2,164	8,278
14	Ria Formosa	Treatment 2	31	T14	0,86	
13	Ria Formosa	Treatment 2	31	T14	1,516	
12	Ria Formosa	Treatment 2	31	T14	2,033	
11	Ria Formosa	Treatment 2	31	T14	1,705	
10	Ria Formosa	Treatment 1	28	T14	0,988	8,151
9	Ria Formosa	Treatment 1	28	T14	2,187	
8	Ria Formosa	Treatment 1	28	T14	2,378	
7	Ria Formosa	Treatment 1	28	T14	1,531	
6	Ria Formosa	Treatment 1	28	T14	1,067	
5	Ria Formosa	Control	17	T14	1,122	6,886
4	Ria Formosa	Control	17	T14	1,265	
3	Ria Formosa	Control	17	T14	1,843	

2	Ria Formosa	Control	17	T14	1,271	
1	Ria Formosa	Control	17	T14	1,385	
15	Ria Formosa	Treatment 2	17	T26	2,464	6,418
14	Ria Formosa	Treatment 2	17	T26	1,755	
13	Ria Formosa	Treatment 2	17	T26	1,043	
12	Ria Formosa	Treatment 2	17	T26	0,401	
11	Ria Formosa	Treatment 2	17	T26	0,755	
10	Ria Formosa	Treatment 1	17	T26	2,008	6,269
9	Ria Formosa	Treatment 1	17	T26	0,461	
8	Ria Formosa	Treatment 1	17	T26	0,925	
7	Ria Formosa	Treatment 1	17	T26	0,168	
6	Ria Formosa	Treatment 1	17	T26	2,707	
5	Ria Formosa	Control	17	T26	1,811	8,719
4	Ria Formosa	Control	17	T26	2,958	
3	Ria Formosa	Control	17	T26	1,375	
2	Ria Formosa	Control	17	T26	1,146	
1	Ria Formosa	Control	17	T26	1,429	

Table 4. Represents the average growth (cm) per treatment over time.

Population	Plant ID	Plant growth	Time	Treatment	Average Growth
Mauritania	DSC_0065	0,095026455	T14	31°C	1,806981
Mauritania	DSC_0066	1,135343182	T14	31°C	
Mauritania	DSC_0068	4,190574832	T14	31°C	
Mauritania	DSC_0070	0,192963657	T14	28°C	2,946201
Mauritania	DSC_0072	2,510204912	T14	28°C	
Mauritania	DSC_0073	7,059654655	T14	28°C	
Mauritania	DSC_0074	2,021982561	T14	28°C	
Mauritania	DSC_0075	2,005958709	T14	17°C	1,544517
Mauritania	DSC_0077	2,230552219	T14	17°C	
Mauritania	DSC_0079	0,397038865	T14	17°C	
Mauritania	DSC_0126	2,793936425	T25	31°C	1,62804
Mauritania	DSC_0128	0,462143273	T25	31°C	
Mauritania	DSC_0130	0,935630977	T25	28°C	0,569742
Mauritania	DSC_0135	0,203852963	T25	28°C	
Ria Formosa	DSC_0085	0,249351442	T14	31°C	0,809831
Ria Formosa	DSC_0086	1,537940397	T14	31°C	
Ria Formosa	DSC_0089	0,642201835	T14	31°C	
Ria Formosa	DSC_0090	0,661429486	T14	28°C	1,261867
Ria Formosa	DSC_0091	2,456632673	T14	28°C	
Ria Formosa	DSC_0092	0,735074627	T14	28°C	
Ria Formosa	DSC_0093	1,194329573	T14	28°C	
Ria Formosa	DSC_0096	0,422961389	T14	17°C	0,894036
Ria Formosa	DSC_0097	1,104510777	T14	17°C	
Ria Formosa	DSC_0098	1,066617165	T14	17°C	

Ria Formosa	DSC_0099	0,982053495	T14	17°C	
Ria Formosa	DSC_0170	0,400386259	T26	31°C	0,400386
Ria Formosa	DSC_0174	1,697432729	T26	28°C	2,185029
Ria Formosa	DSC_0177	3,418528252	T26	28°C	
Ria Formosa	DSC_0178	1,439126564	T26	28°C	
Ria Formosa	DSC_0179	0,773468518	T26	17°C	0,852867
Ria Formosa	DSC_0180	0,932266447	T26	17°C	

Table 5. Represents the variety and type of infections from Mauritania and Ria Formosa.

Replicate	Population	Treatment	Time	parasite species
15	Mauritania	31°C	T14	
14	Mauritania	31°C	T14	H
13	Mauritania	31°C	T14	HL
12	Mauritania	31°C	T14	H
11	Mauritania	31°C	T14	HL
10	Mauritania	28°C	T14	H
9	Mauritania	28°C	T14	H
8	Mauritania	28°	T14	H
7	Mauritania	28°C	T14	H
6	Mauritania	28°C	T14	H
5	Mauritania	17°C	T14	HL
4	Mauritania	17°C	T14	HL
3	Mauritania	17°C	T14	HL
2	Mauritania	17°C	T14	L
1	Mauritania	17°C	T14	H
15	Mauritania	31°C	T25	
14	Mauritania	31°C	T25	L

13	Mauritania	31°C	T25	
12	Mauritania	31°C	T25	L
11	Mauritania	31°C	T25	HL
10	Mauritania	28°C	T25	
9	Mauritania	28°C	T25	H
8	Mauritania	28°C	T25	L
7	Mauritania	28°C	T25	HL
6	Mauritania	28°C	T25	HL
5	Mauritania	17°C	T25	H
4	Mauritania	17°C	T25	H
3	Mauritania	17°C	T25	L
2	Mauritania	17°C	T25	HL
1	Mauritania	17°C	T25	H
15	Ria Formosa	31°C	T6	
14	Ria Formosa	31°C	T6	H
13	Ria Formosa	31°C	T6	
12	Ria Formosa	31°C	T6	
11	Ria Formosa	31°C	T6	
10	Ria Formosa	28°C	T6	
9	Ria Formosa	28°C	T6	H
8	Ria Formosa	28°C	T6	H
7	Ria Formosa	28°C	T6	H
6	Ria Formosa	28°C	T6	
5	Ria Formosa	17°C	T6	H

4	Ria Formosa	17°C	T6	H
3	Ria Formosa	17°C	T6	H
2	Ria Formosa	17°C	T6	H
1	Ria Formosa	17°C	T6	H
15	Ria Formosa	31°C	T14	
14	Ria Formosa	31°C	T14	
13	Ria Formosa	31°C	T14	
12	Ria Formosa	31°C	T14	
11	Ria Formosa	31°C	T14	
10	Ria Formosa	28°C	T14	
9	Ria Formosa	28°C	T14	
8	Ria Formosa	28°C	T14	
7	Ria Formosa	28°C	T14	
6	Ria Formosa	28°C	T14	
5	Ria Formosa	17°C	T14	
4	Ria Formosa	17°C	T14	
3	Ria Formosa	17°C	T14	H
2	Ria Formosa	17°C	T14	H

1	Ria Formosa	17°C	T14	H
15	Ria Formosa	31°C	T26	
14	Ria Formosa	31°C	T26	
13	Ria Formosa	31°C	T26	
12	Ria Formosa	31°C	T26	
11	Ria Formosa	31°C	T26	
10	Ria Formosa	28°C	T26	H
9	Ria Formosa	28°C	T26	H
8	Ria Formosa	28°C	T26	
7	Ria Formosa	28°C	T26	
6	Ria Formosa	28°C	T26	L
5	Ria Formosa	17°C	T26	H
4	Ria Formosa	17°C	T26	H
3	Ria Formosa	17°C	T26	
2	Ria Formosa	17°C	T26	
1	Ria Formosa	17°C	T26	H

Table 6. Represents the average percentage of infection per time and per treatment from Mauritania and Ria Formosa.

	T6	T14	T26

Control M	5,00%	11,70%	21,80%
Control R	7,10%	9,80%	10,00%
Treatment 1 M	9,80%	6,60%	11,70%
Treatment 1 R	6,60%	3,20%	10,00%
Treatment 2 M	7,16%	10,00%	9,32%
Treatment 2 R	3,06%	5,50%	7,40%