

## Effect of *Bursaphelenchus* spp. inoculation on carbohydrate concentrations of different pine species in forest stands of Düzce Forest Directorate

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**Abstract:** The pinewood nematode (*Bursaphelenchus xylophilus*) is one of the most important pathogens of conifer forests worldwide that causes the pine wilt disease. This problem has increased the scientific interest in *Bursaphelenchus* species both in the world and in Turkey. Previously, six *Bursaphelenchus* species were isolated from wilted pine trees in Turkey. The main goal of this study was to investigate the effects of nematode inoculation on the total carbohydrate concentrations of tree species. Native six *Bursaphelenchus* species were inoculated on three pine species (*Pinus pinaster*, *P. nigra*, and *P. sylvestris*) under natural stand conditions. Two different doses of nematodes (2000 and 20,000) were used for inoculation. For each treatment, three trees of each species were inoculated. The total carbohydrate concentrations were differed significantly among tree species, nematode species and between nematode doses. The total carbohydrate concentration values were higher in *P. pinaster* than both *P. nigra* and *P. sylvestris*. These results may suggest that pine species responded to nematode inoculation by changing the amount of TCC.

**Key words:** *Bursaphelenchus*, pine, carbohydrate concentration, inoculation

### 1. Introduction

Stress is defined as any biotic and abiotic environmental factors potentially unfavorable to plants (Rhodes and Nadolska-Orczyk 2001). These factors play very important roles in the changes and development of forest ecosystems (Shibata et al., 2010). The growth and development of trees in forests are governed by the interactions between these components. Stress in a plant can be divided into two basic categories as abiotic and biotic (Verma et al., 2013; Gull et al., 2019). Biotic stress is caused by biological agents like insects, viruses, bacteria, fungi, nematodes, arachnids, and weeds or intraspecific competition for available resources (Hill et al., 1998; Verma et al., 2013; Singla and Krattinger, 2016). However, abiotic stress is produced by drought, extremes in temperature, submergence, waterlogging, salinity/alkalinity, heavy metals, radiation, etc. (Gull et al., 2019; Hasanuzzaman et al., 2020). When a plant is exposed to stress, several changes can be observed in plant physiology such as water and nutrient absorption, photosynthesis, respiration, growth, and reproduction (Salisbury and Ross, 1994; Çepel, 1995; Lambers et al., 1998; Verma et al., 2013).

Plant-parasitic nematodes are one of the major biotic factors causing serious damage to a wide range of crop and

forest species worldwide (Lambert and Bekal, 2002; Ruehle, 2003; Cram and Fraedrich, 2012; Espada et al., 2018). The estimation of crop losses in the agricultural sector due to nematodes represents 8.8%–14.6% of total crop production (Singh et al., 2013). Moreover, the cost of crop damage by plant-parasitic nematodes has been estimated at about 80–118 billion dollars per year (Sasser, 1987; Nicol et al., 2011; Bernard et al., 2017).

*Bursaphelenchus xylophilus* (Steiner and Buhner 1934) Nickle 1970 (Nematoda: Aphelenchoididae) (the pinewood nematode) is one of the top 10 nematodes of scientific and economic importance according to a survey conducted by researchers working with plant parasitic nematodes (Jones et al., 2013). This nematode is the causal pathogen of pine wilt disease (Ekino et al., 2020) which is one of the most significant and devastating diseases affecting the genus *Pinus* L. worldwide (Webster and Mota, 2008). At the beginning of the 20th century, the nematode has been accidentally introduced to Japan via timber exports (EPPO, 2013) and also spread to other East Asian countries (Wu et al., 2013). In Europe, the first discovery of the pinewood nematode was reported on *P. pinaster* (Aiton 1789) in Portugal (Mota et al., 1999). The introduction of this nematode

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into nonnative regions has caused huge wood product losses and irreversible changes in forest ecosystems (Mota et al., 2009). Due to this serious threat, the scientific interest to this group of nematodes has increased. In Turkey, several survey studies have been conducted and six *Bursaphelenchus* species were previously reported (Akbulut et al., 2006, 2007a, 2008, 2013; Dayı et al., 2014). In addition, pathogenicity studies were also carried out in both greenhouse and under field conditions (Akbulut et al., 2007b, 2015; Dayı and Akbulut, 2012; Öztürk et al., 2019).

A close relationship has been observed between the development of pine wilt disease and environmental factors (Mamiya, 1983). Both high-temperature and water stress accelerate the development of disease in pine trees (Ikeda et al., 1990; Sikora and Malek, 1991). It has also been reported that the number of susceptible pine trees to nematodes increases when the temperature is above 20 °C (Braasch and Enzian, 2004).

It is well known that soluble sugars (sucrose, glucose, and fructose) play a role in plant metabolism and supports plant growth and development (Halford et al., 2011; Lastdrager et al., 2014; Sami et al., 2016; Yasseen et al., 2018; Ciereszko, 2018). Due to its regulatory functions, it affects all life cycle stages of plants and controls the growth and development processes (Wind et al., 2010; Stokes et al., 2013). The soluble sugar content plays a very important role in carbohydrate metabolism and is closely related to photosynthesis and production (Wilcox, 2001). When plants are exposed to stress, a significant decrease occurs in photosynthetic source tissues and reduces the supply of soluble sugars to sink tissues (Rosa et al., 2009). In addition, plants adapt themselves to stress by accumulating various organic solutions such as sugar and proline (Gill et al., 2001). There is a strong relationship between soluble

sugar concentration and stress tolerance (Morkunas and Ratajczak, 2014).

The present study was conducted to evaluate changes in total carbohydrate concentration (TCC) amounts due to the biostress induced by two inoculum doses of six different *Bursaphelenchus* species inoculated on three pine species under field conditions.

## 2. Materials and methods

### 2.1. Site description

The study was conducted in forested areas of the Düzce Forest District Directorate (40°48'N, 31°15'E for *P. pinaster*; 40°48'N, 31°16'E for *P. nigra*; 40°40'N, 31° 9'E for *P. sylvestris* stand) (Figure 1). In selected experimental plots, *P. pinaster* plots were established by planting *P. nigra* and *P. sylvestris* plots naturally. Some descriptive values of trees located in experimental plots are given in Table 1.

To evaluate the physiological responses of three pine species to six native *Bursaphelenchus* species (*B. mucronatus* (Mamiya & Enda, 1979), *B. sexdentati* (Rühm, 1960), *B. anamurius* (Akbulut, Braasch, Baysal, Brandstetter, & Burgermeister, 2007), *B. vallesianus* (Braasch, Schönfeld, Polomski, & Burgermeister, 2004), *B. andrassyi* (Dayı, Calin, Akbulut, Gu, Schröder, Vieira, & Braasch, 2014), and *B. hellenicus* (Skarmoutsos, Braasch, & Michalopoulou, 1998)) a total of 36 trees (6 nematode species x 3 tree species x 2 doses) were selected for two inoculum doses (2000 nematodes for low and 20 000 for high) from each tree species. In addition, three trees were served as a control group for each tree species. Studies were carried out between June, 2015 and May, 2016. During the study period, temperature and relative humidity data were obtained by the data logger (Cem DT-172) located at each tree species stand.

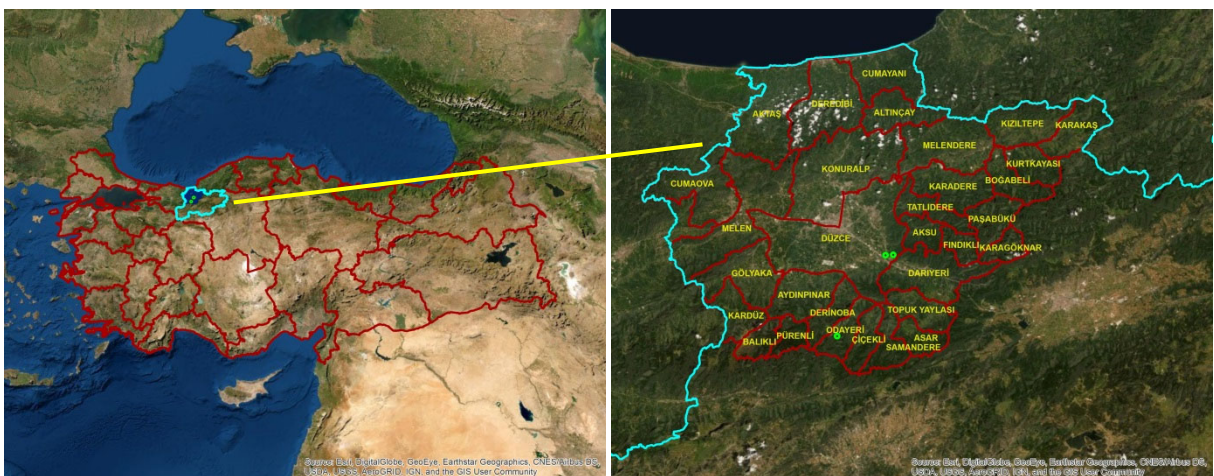


Figure 1. Study areas.

**Table 1.** Mean ( $\pm$ SD) diameter, height, age, and bark thickness of tree stands.

Tree species	Diameter ( $d_{1.30}$ )	Height (m)	Age (Year)	Bark thickness (mm)
<i>P. pinaster</i>	35.2 $\pm$ 6.5	23.2 $\pm$ 1.2	25.8 $\pm$ 0.9	3.2 $\pm$ 0.7
<i>P. nigra</i>	25.7 $\pm$ 3.0	20.6 $\pm$ 1.4	40.1 $\pm$ 1.3	2.4 $\pm$ 0.4
<i>P. sylvestris</i>	31.2 $\pm$ 4.1	23.6 $\pm$ 0.9	42.0 $\pm$ 1.4	2.0 $\pm$ 1.5

## 2.2. Nematode culturing and inoculation

Before the inoculation study, nematode species were reared on grey mold, *Botrytis cinerea* Pers., cultures on potato dextrose agar in Petri dishes at 25 °C for 7–8 days, and were extracted using the tray method. Each *Bursaphelenchus* species were placed into a 500-mL beaker and a homogeneous nematode suspension was obtained with the help of a magnetic stirrer. Five 0.5-mL samples were taken from the beaker. The nematode samples were placed in a scaled (1 cm  $\times$  1 cm) petri dish. Then the nematode density in the solution was calculated under a microscope. These processes were performed separately for both inoculum densities.

A method used in previous studies was taken into account for the inoculation of nematodes into trees. For this purpose, a 2.5-mL nematode suspension (mixed-stage nematodes) was inoculated into the hole (4–5 cm deep) on the trunk at 1.5 m above the ground of level, then holes were covered with styrofoam and grafting wax. Trees used as control groups were inoculated with distilled water (2.5 mL).

## 2.3. Carbohydrate concentration

After inoculation, trees were monitored once a month for 6 months. The needle samples were taken from tree branches with long pruning shears by climbing the trees. Three branch samples from each tree were taken from the southern sides and the middle or top parts of the trees. Needles were removed from the branches and wrapped in aluminum foil, then brought to the laboratory in a sample transport container and analyzed to determine the TCC amount. Collected samples were stored in refrigeration condition (–86 °C) until processing.

The total carbohydrate concentration was determined according to Dubois et al. (1956). For this purpose, glucose stock (20  $\mu$ g/mL) was prepared. First 0.02 g of glucose was weighed and 1 L of distilled water was added. Then, the mixture was homogenized in a magnetic stirrer. For the preparation of the 5% phenol solution, 5 g of phenol was weighed and completed with 100 mL of distilled water. To reset the photometer, 1 mL of H<sub>2</sub>O, 1 mL of 5% phenol (Surechem Products LTD., P 1922), and 5 mL of H<sub>2</sub>SO<sub>4</sub> were added into a tube and cooled for 15 min. One milliliter of glucose stock, 1 mL of 5% phenol, and 5 mL of H<sub>2</sub>SO<sub>4</sub> (Sigma, 33, 974-1) were added into a tube and cooled for 15 min for measurement.

Needle samples taken from the trees were cut into pieces and placed into small paper bags and then dried at 60 °C for 48 h. Then, 0.025-g samples were weighed from the extracted samples and placed in Eppendorf tubes. The processes were repeated 3 times for each sample. Samples were stored at –30 °C until shredding. When all samples were ready, 1 mL of 80% ethanol was added to deep freeze Eppendorf tubes at –86 °C to disintegrate in the tissue disintegrant. Thereafter, it was centrifuged at 8000 rpm for 10 min and 1 mL of supernatant was taken from the filtrate and then transferred to Eppendorf tubes, and stored at –30 °C until the processing time.

Into 10 mL capped glass tubes, 950  $\mu$ L of purified water was added, later 50  $\mu$ L (contained in –30 °C in-house extract) was taken and added to the glass vials and vortexed. After adding 1 mL of 5% phenol into the tube, it was vortexed again. Subsequently, 5 mL of sulfuric acid was added to the mixture and vortexed again. The reaction mixture was then allowed to cool for 15 min. Carbohydrate concentration was measured by reading the absorbances at 490 nm in 3-mL cuvettes against a blank.

## 2.4. Statistical analysis

The factorial ANOVA analysis was used to evaluate the effects of nematode species on TCC amounts of pine species. All statistical analyses were performed using the SPSS Statistical Package. The results were considered significant at  $p \leq 0.05$ .

## 3. Results

All data were tested for normality with the Kolmogorov–Smirnov test (Achim and Zöfel 2000). The distributions of the total carbohydrate concentration (TCC) significantly deviated from normality (Kolmogorov–Smirnov tests,  $p < 0.05$ ). Therefore, the nonparametric Kruskal–Wallis test was used to investigate significant differences in the TCC among tree species. It was found that the TCC was significantly different among tree species ( $\chi^2 = 435.911$  SD = 2,  $p = 0.000$ ) (Table 2). The Mann–Whitney U test was used for nonparametric comparisons. Bonferroni correction for multiple comparisons were applied; corrected  $\alpha = 0.0167$  (0.05/3). The TCC was significantly different between *Pinus pinaster* and *P. nigra*; *P. pinaster* and *P. sylvestris*; *P. nigra* and *P. sylvestris* (respectively;  $U = 101,080$ ,  $p = 0.000$ ;  $U = 178,834.500$ ,  $p = 0.000$ ;  $U = 134,992.000$ ,  $p = 0.000$ ). The TCC was higher in *P. pinaster* than in both *P. nigra* and *P. sylvestris* (Table 2).

According to the results of the Kruskal–Wallis H test, the effect of nematode species on TCC was significantly different ( $\chi^2 = 44.573$  SD = 6,  $p = 0.000$ ). Pairwise comparisons with significant differences between nematode species are given in Table 3. The highest amount of TCC was measured in *P. sylvestris* trees inoculated with *B. hellenicus* in May and the control group of *P. nigra* trees in August. In *P. pinaster* stand, the highest TCC was measured in trees inoculated with *B. sexdentati* and the control group in November. However, the lowest amount of TCC in *P. pinaster* stand was measured in trees inoculated with *B. mucronatus* in September. In addition, the lowest amount of TCC in *P. nigra* and *P. sylvestris* stands was measured in trees inoculated with *B. vallesianus* in May and October, respectively (Figure 2).

The use of two different nematode doses for inoculation significantly affected the amount of TCC among pine species ( $\chi^2 = 26.593$  SD=2,  $p = 0.000$ ). In general, the highest TCC was measured in control groups in all three species. However, the lowest TCC was measured in trees inoculated with a high dose of nematodes (Figure 3).

Average temperatures in *P. pinaster* and *P. nigra* stands were above 20 °C between July and September except for *P. sylvestris* stand (Figure 4). There was a significant and negative correlation between the TCC and monthly temperatures for *P. pinaster* ( $r = -0.085$ ,  $p = 0.024$ ). No relationship was found between the TCC and the relative humidity ( $r = 0.040$ ,  $p = 0.287$ ) for *P. pinaster*. The TCC and the relative humidity were correlated negatively and positively for *P. nigra* ( $r = -0.195$ ,  $p = 0.000$ ) and *P. sylvestris* ( $r = 0.077$ ,  $p = 0.041$ ), respectively. A positive correlation was found between the TCC and monthly temperatures for *P. nigra* ( $r = 0.155$ ,  $p = 0.000$ ). The total carbohydrates and monthly temperatures were negatively correlated for *P. sylvestris* ( $r = -0.220$ ,  $p = 0.000$ ).

#### 4. Discussion

Stress induced by abiotic or biotic factors such as temperature, drought, nutritional substrate availability, pathogen activities can cause changes in plant physiology including metabolic activities of plant tissues (Bolla et al., 1987; Salisbury and Ross, 1994; Çepel, 1995; Lambers et al.,

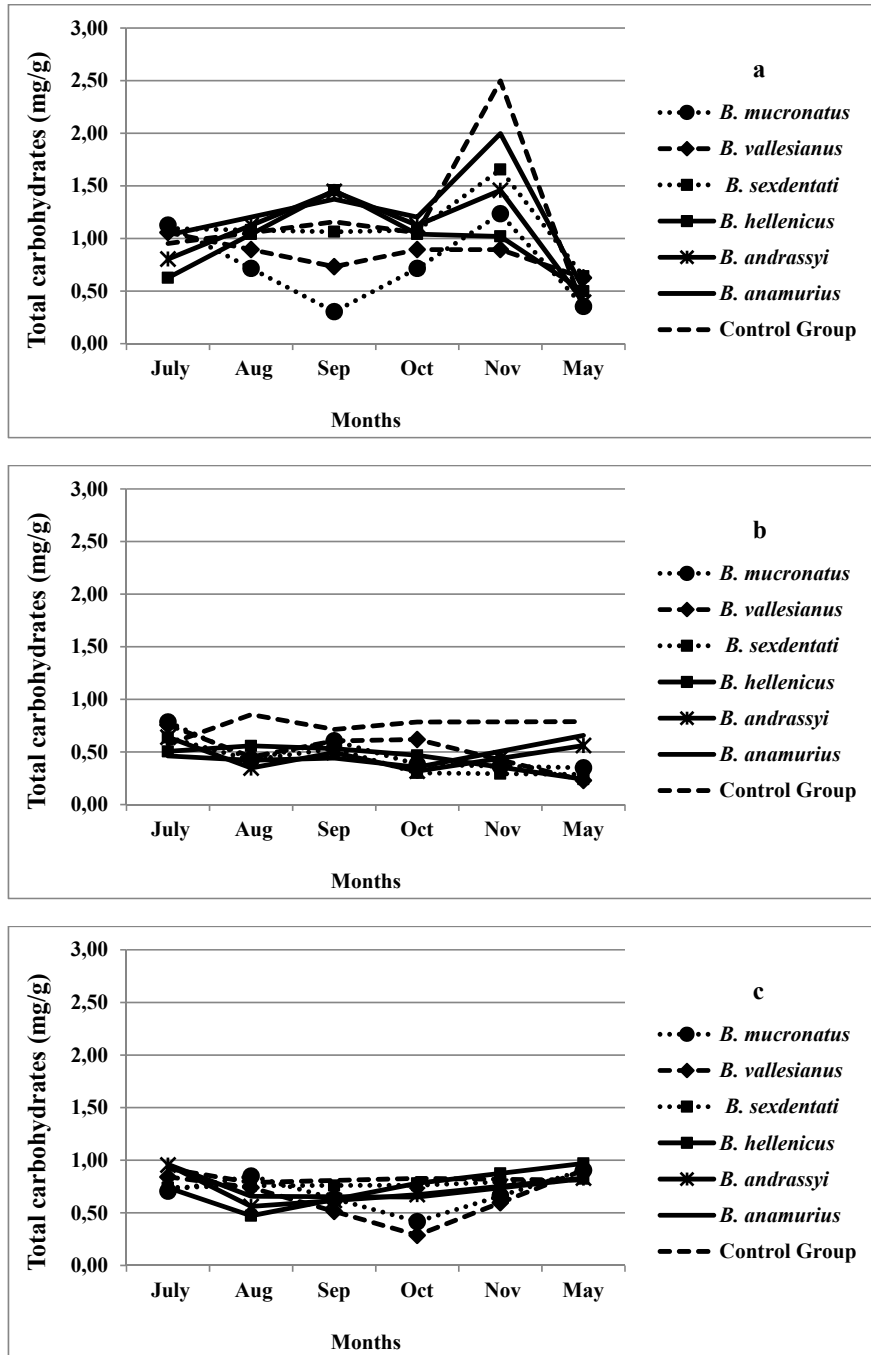
**Table 2.** Kruskal–Wallis nonparametric test results.

Tree species	N	Mean rank	SD	$\chi^2$	p
<i>P. pinaster</i>	702	1356.76	2	435.911	0.000
<i>P. nigra</i>	702	687.78			
<i>P. sylvestris</i>	702	1115.95			

**Table 3.** Pairwise comparisons of nematode species (independent-samples Mann–Whitney U test).

Nematode comparison*	Z	U	Adjusted p value
1 with 3	-3.15	44981.0	0.002
1 with 5	-2.65	46170.0	0.008
1 with 6	-3.65	43797.0	0.000
1 with 7	-6.00	17488.5	0.000
2 with 3	-2.07	47568.0	0.039
2 with 6	-2.67	46120.0	0.008
2 with 7	-5.13	18757.5	0.000
3 with 7	-3.00	21865.5	0.003
4 with 6	-2.16	47350.0	0.031
4 with 7	-4.70	19389.5	0.000
5 with 7	-3.92	20523.0	0.000
6 with 7	-2.49	22603.5	0.013

\*1 = *B. mucronatus*; 2 = *B. vallesianus*; 3 = *B. sexdentati*; 4 = *B. hellenicus*; 5 = *B. andrassyi*; 6 = *B. anamurius*; 7 = Control Group

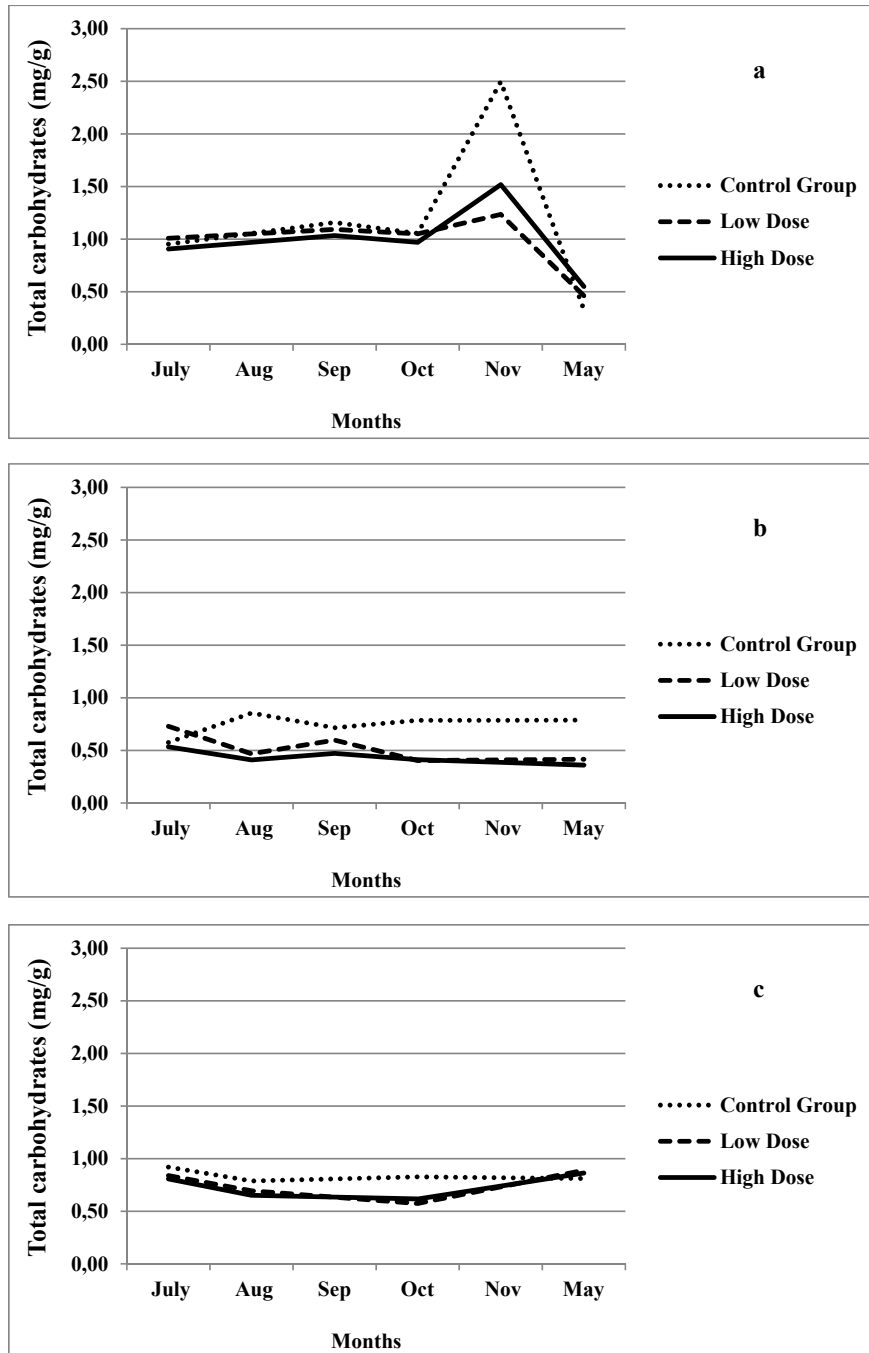


**Figure 2.** Effects of nematode species on TCC in *P. pinaster* (a) *P. nigra* (b), and *P. sylvestris* (c) stands.

1998; Verma et al., 2013). Plant-parasitic nematodes lead to stress in host plant species. *Bursaphelenchus xylophilus* is one of the most significant and devastating plant parasitic nematodes affecting the genus *Pinus* L. worldwide. Pine seedlings respond to the stress after inoculation of *B. xylophilus* by initial decreasing in both reducing and nonreducing carbohydrates (Bolla et al., 1987). They

suggested that the nematode parasitism has caused several changes in pine seedlings (disruption of nutrient and water movement, and declining photosynthetic activity due to leaf chlorosis resulting from *B. xylophilus* infection) (Bolla et al., 1987).

In the current study, the TCC differed significantly among tree species and *P. pinaster* had the highest TCC.



**Figure 3.** Effects of nematode doses on TCC in *P. pinaster* (a) *P. nigra* (b), and *P. sylvestris* (c) stands.

The TCC was significantly different among nematode species and inoculation doses. These differences were mainly found between *B. mucronatus* and 3 other *Bursaphelenchus* species and *B. vallesianus* and 2 other species (Table 3). These results suggested that pine species responded to nematode inoculation by changing the amount of TCC. No mortality was observed in nematode

inoculated trees. In a previous study, it was reported that six native *Bursaphelenchus* species inoculated to pine trees under natural stand conditions were not pathogenic to pine trees (Öztürk et al., 2019). They suggested that inoculated nematode species were not successful in colonizing and killing trees under natural stand conditions or successful colonization occurred but the population was not able

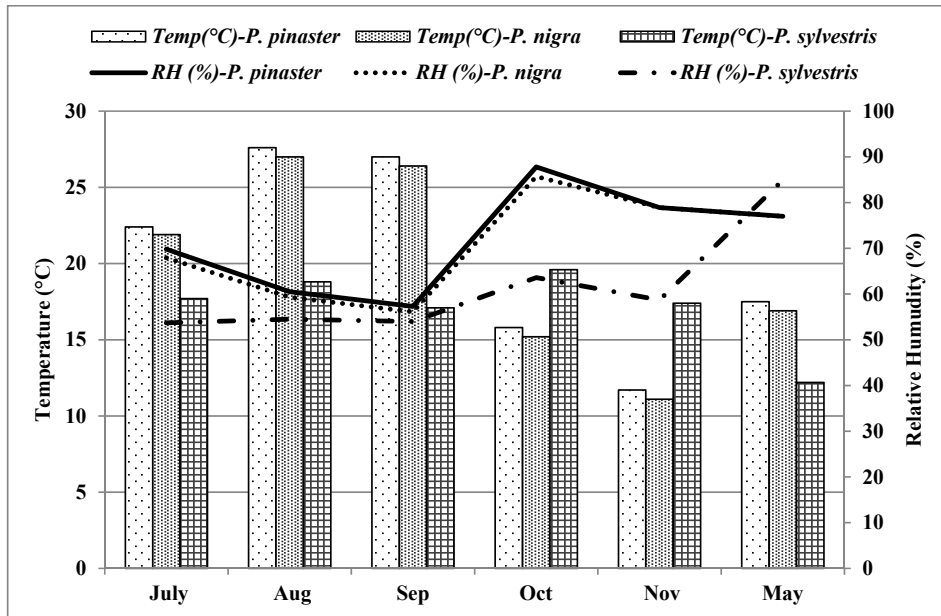


Figure 4. Monthly mean temperature and relative humidity of each tree stand during study period.

to survive for a long time in healthy host trees. In the current study, similar results were found. Due to nematode inoculation, the TCC initially decreased and fluctuated during the study period. Nematodes may cause temporary stress but not enough to kill trees at the end of the study.

Carbohydrates in plants are a physiological measure of stress tolerance and are used as energy reserves under stress conditions (Watschke et al., 1972, 1973; Beard, 1973; Howard and Watschke, 1985, 1991; Hull, 1992; Maness, 2010). Studies have shown that changes in the carbohydrate contents of plants are closely related to the weather temperature (Liu and Huang, 2000; Huang and Gao, 2000; Liu and Huang, 2001). The reduction in total carbohydrate content may result from an imbalance between carbon production in photosynthesis and its consumption in respiration (Liu et al., 2011). Energy and nutrient stresses caused by biotic and abiotic stress factors in plants lead to changes in growth functions (Lastdrager et al., 2014). Sugars, which have an effect on the growth and development of plants, are active components that reflect the energy status of plants and play a protective role against stress factors (Lastdrager et al. 2014; Ciereszko, 2018).

In previous studies, sugar accumulation has been observed under biotic and abiotic stress conditions (Ciereszko, 2009; Morkunas and Ratajczak, 2014; Żebrowska et al., 2017; Łukaszuk et al., 2017). Lower sugar levels were observed in plant tissues under abiotic stress conditions (Ciereszko, 2009; Sami et al., 2016). A study was conducted to determine the level of soluble sugars (sucrose, glucose, and fructose) on leaves of 10-day-old *Pisum sativum* L. seedlings infected with 10, 20, and 30 apterous adult females of the *Acyrtosiphon pisum* Harris

(Morkunas et al., 2015). They reported that the lower total sugar content was observed at 72 and 96 h after infestation by *A. pisum* compared to the control groups of *P. sativum*.

The total carbohydrates in plants decrease seasonally from the beginning of the vegetation period to the middle of summer, then increase gradually and reach their highest level in the winter period (Kulaç et al., 2012; Wang and Zwiazek, 1999). In the current study, the TCC fluctuated from July to May (Figure 3). Different patterns were observed among tree species. Unfortunately, no data is available between November and May due to severe weather conditions in the location of the *P. sylvestris* stand.

It was reported that carbohydrate concentrations initially decreased due to the decrease or loss of transpiration as a result of *B. xylophilus* infection (Bolla et al., 1987). It was found that high-dose inoculated trees had a lower sugar concentration than that of low-dose inoculated trees. In the current study, the TCC increase in control groups of pine species as a result of high transpiration due to high temperatures in November.

The present study was carried out due to limited information available about the responses of the trees to biotic stress infected with *Bursaphelenchus* spp. under natural stand conditions. Forests are complex ecosystems that comprise abiotic and biotic components. These components are connected with each other in an ecosystem. In this context, it is important to investigate the effects of biotic and abiotic factors and their results in the protection and utilization of forested areas. Forest pests and diseases will be more effective to the plants directly or indirectly due to the changing balances of the world's ecosystems.

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## References

- Achim B, Zöfel P (2000). SPSS Version 10 Einführung in die moderne Datenanalyse unter Windows. Munich: Addison Wesley (in German).
- Akbulut S, Vieira P, Ryss A, Yüksel B (2006). Preliminary survey of the pinewood nematode in Turkey. Bulletin OEPP/EPPO Bulletin 36 (3). doi: 10.1111/j.1365-2338.2006.01056.x
- Akbulut S, Braasch H, Baysal İ, Brandstetter M, Burgermeister W (2007a). Description of *Bursaphelenchus anamurius* sp n Nematoda Parasitaphelenchidae from *Pinus brutia* in Turkey. Nematology 9 (6):859-867. doi: 10.1163/156854107782331207
- Akbulut S, Yüksel B, Serin M, Baysal İ, Erdem M (2007b). Pathogenicity of *Bursaphelenchus mucronatus* in pine seedlings under greenhouse conditions. Turkish Journal of Agriculture and Forestry 31: 169-173.
- Akbulut S, Elekçioğlu H, Keten A (2008). First record of *Bursaphelenchus vallesianus* Braasch Schönfeld Polomski and Burgermeister in Turkey. Turkish Journal of Agriculture and Forestry 32 (4): 273-279.
- Akbulut S, Braasch H, Cebeci H (2013). First report of *Bursaphelenchus hellenicus* Skarmoutsos Braasch Michalopoulou 1998 Nematoda Aphelenchoididae from Turkey. Forest Pathology 43 (5): 402-406.
- Akbulut S, Yüksel B, Serin M, Erdem M (2015). Comparison of pathogenic potential of *Bursaphelenchus* species on conifer seedlings between greenhouse and outdoor conditions. Phytoparasitica 43 (2): 209-214.
- Beard JB (1973). Turfgrass science and culture. Prentice Hall, Englewood Cliffs, N.J.
- Bernard GC, Egnin M, Bonsi C (2017). The impact of plant-parasitic nematodes on agriculture and methods of control, In book: Nematology - Concepts, Diagnosis and Control, doi:10.5772/intechopen.68958.
- Braasch H, Enzian, S (2004). The pinewood nematode problem in Europe present situation and outlook, Proceedings of International Workshop Portugal, 77-91.
- Bolla RI, Fitzsimmons K, Winter REK (1987). Carbohydrate concentration in pine as affected by inoculation with *Bursaphelenchus xylophilus* 1. Journal of Nematology, 19 (1): 51-57.
- Ciereszko I (2009). Sucrose metabolism in plant tissues under stress conditions: key enzymes, localization and function. In: Maksymiec W, editor. Compartmentation of responses to stresses in higher plants, true or false. Kerala: Transworld Research Network; p. 193-218.
- Ciereszko I (2018). Regulatory roles of sugars in plant growth and development. Acta Societatis Botanicorum Poloniae 87 (2). doi: 10.5586/asbp.3583, 87 (2)
- Cram MM, Fraedrich SW (2012). Nematode damage and management in North American forest nurseries. Tree Planters' Notes 55 (1): 27-35.
- Çepel N (1995). Orman Ekolojisi 4. Baskı. İstanbul Üniversitesi Orman Fakültesi Yayını, No: 433, İstanbul (in Turkish).
- Dayı M, Akbulut S (2012). Pathogenicity testing of four *Bursaphelenchus* species on conifer seedlings under greenhouse conditions. Forest Pathology 42 (3): 213-219.
- Dayı M, Calin M, Akbulut S, Gu J, Schröder T et al. (2014). Morphological and molecular characterisation of *Bursaphelenchus andrassyi* sp n Nematoda Aphelenchoididae from Romania and Turkey. Nematology 16: 207-218.
- Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956). Colorimetric method for the determination of sugars and related substances. Analytical Chemistry 28: 350-356.
- Ekino T, Kirino H, Kanzaki N, Shinya R (2020). Ultrastructural plasticity in the plant-parasitic nematode, *Bursaphelenchus xylophilus*. Scientific Reports 10 (1): 11576.
- EPPO (2013). European and mediterranean plant protection organization, PM 7/4 (3) *Bursaphelenchus xylophilus*. Bulletin OEPP/EPPO Bulletin 43 (1): 105-118.
- Espada M, van den Akker SE, Maier T, Vijayapalani P, Baum T et al. (2018). STATAWAARS: a promoter motif associated with spatial expression in the major effector-producing tissues of the plant-parasitic nematode *Bursaphelenchus xylophilus*. BMC Genomics 19 (1): 553. doi: 10.1186/s12864-018-4908-2
- Gill PK, Sharma AD, Singh P, Bhullar SS (2001). Effect of various abiotic stresses on the growth, soluble sugars and water relations of Sorghum seedlings grown in light and darkness. Bulgarian Journal of Plant Physiology 27 (1-2): 72-84.
- Gull A, Lone AA, Islam Wani NU (2019). Biotic and abiotic stresses in plants. In: Abiotic and Biotic Stress in Plants. doi:10.5772/intechopen.85832.
- Halford NG, Curtis TY, Muttucumaru N, Postles J, Mottram DS (2011). Sugars in crop plants, Annals of Applied Biology - AAB 158 (1): 1-25. doi: 10.1111/j.1744-7348.2010.00443.x
- Hasanuzzaman M, Borhannuddin Bhuyan MHM, Zulfiqar F, Raza A, Mohammad Mohsin S et al. (2020). Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the Crucial Role of a Universal Defense Regulator. Antioxidants (Basel) 9 (8): 681. doi: 10.3390/antiox9080681

- Hill J, Becker HC, Tigerstedt PMA (1998). Quantitative and ecological aspects of plant breeding. Chapman and Hall, London, UK.
- Howard HF, Watschke TL (1985). Mechanisms of relative high temperature tolerance among Kentucky bluegrass cultivars. p.126. In 1985 Agronomy Abstracts, ASA, Madison, WI
- Howard HF, Watschke TL (1991). Variable high-temperature tolerance among Kentucky bluegrass cultivars. *Agronomy Journal* 83: 689-693.
- Huang B, Gao H (2000). Growth and carbohydrate metabolism of creeping bentgrass cultivars in response to increasing temperatures. *Crop Science* 40: 1115-1120
- Hull R (1992). Energy relations and carbohydrate partitioning in turfgrass, p. 175-205. In: D.V Waddington, R.N. Carrow, and R.C. Sherman.(eds.). *Turfgrass*. American Society of Agronomy, Madison, Wis.
- Ikeda T, Kiyohara T, Kusunoki M (1990). Change in water status of *Pinus thunbergii* Parl. inoculated with species of *Bursaphelenchus*. *Journal of Nematology* 22: 132-135.
- Jones J, Haegeman A, Danchin E, Gaur H, Helder J et al. (2013). Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular Plant Pathology* 14: 946-961. doi: 10.1111/mpp.12057
- Kulaç S, Nzokou P, Guney D, Cregg BM, Turna I (2012). Growth and physiological response of Fraser fir (*Abies fraseri* (Pursh) Poir.) seedlings to drought stress: seasonal and diurnal variations in photosynthetic pigments and carbohydrate concentration. *Hortscience* 47: 1512-1519.
- Lambers H, Chapin FS, Pons TL (1998). *Plant physiological Ecology*. Springer-Verlag, New York. Larcher, W. (1995) *Physiological Plant Ecology*. III Ed. pringer-Verlag, Berlin.
- Lambert K, Bekal S (2002). Introduction to plant-parasitic nematodes. *The Plant Health Instructor*. doi: 10.1094/PHI-I-2002-1218-01
- Lastdrager J, Hanson J, Smeekens S (2014). Sugar signals and the control of plant growth and development. *Journal of Experimental Botany* 65 (3): 799-807.
- Liu X, Huang B (2000). Carbohydrate accumulation in relation to heat stress tolerance in two creeping bentgrass cultivars. *Journal of the American Society for Horticultural Science* 125: 442-447.
- Liu B, Li YS, Liu XB, Wang C, Jin J et al. (2011). Lower total soluble sugars in vegetative parts of soybean plants are responsible for reduced pod number under shading conditions. *Australian Journal of Crop Science* 5 (13): 1852-1857.
- Liu X, Huang B (2001). Seasonal changes and cultivar difference in turf quality, photosynthesis, and respiration of creeping bentgrass. *HortScience* 36: 1131-1135.
- Łukaszuk E, Rys M, Możdżeń K, Stawoska I, Skoczowski A et al. (2017). Photosynthesis and sucrose metabolism in leaves of *Arabidopsis thaliana* aos, ein4 and rcd1 mutants as affected by wounding. *Acta Physiologiae Plantarum* 39: 17.
- Mamiya Y (1983). Pathology of the pine wilt disease caused by *Bursaphelenchus xylophilus*. *Annual review of Phytopathology* 21: 201-220.
- Maness NO (2010). Extraction and analysis of soluble carbohydrates. *Methods in molecular biology* (Clifton, N.J.) 639: 341-70. doi: 10.1007/978-1-60761-702-0\_22
- Mota MM, Braasch H, Bravo MA, Penas AC, Burgermeister W et al. (1999). First report of *Bursaphelenchus xylophilus* in Portugal and in Europe. *Nematology* 1: 727-734.
- Mota MM, Futai K, Vieira P (2009). Pine wilt disease and the pinewood nematode, *Bursaphelenchus xylophilus*. In: *Integrated Management of Fruit Crops Nematodes*, pp 253-274.
- Morkunas I, Ratajczak L (2014). The role of sugar signaling in plant defense responses against fungal pathogens. *Acta Physiologiae Plantarum* 36: 1607-1619.
- Morkunas I, Woźniak A, Formela M, Mai VC, Marczak L et al. (2015). Pea aphid infestation induces changes in flavonoids, antioxidative defence, soluble sugars and sugar transporter expression in leaves of pea seedlings. *Protoplasma* 253 (4): 10.1007/s00709-015-0865-7
- Nicol J, Turner D, Coyne L., den Nijs L, Hockland S et al. (2011). Current nematode threats to world agriculture. In: Jones J, Gheysen G, Fenoll C, editors. *Genomics and Molecular Genetics of Plant-Nematode Interactions*. Berlin: Springer Science Business Media 21-43. doi: 10.1007/978-94-007-0434-3\_2
- Öztürk N, Akbulut S, Baysal İ (2019). Determination of pathogenicity of *Bursaphelenchus* species on different pine species under natural conditions in Düzce. *Phytoparasitica* 47 (1): 89–97.
- Rhodes D, Nadolska-Orczyk A (2001). Plant stress physiology. *Encyclopaedia of Life Sciences*, doi: 10.1038/ngp.els.0001297.
- Rosa M, Prado C, Podazza G, Interdonato R, González JA et al. (2009). Soluble sugars—metabolism, sensing and abiotic stress. *Plant Signaling & Behavior* 4 (5): 388-393.
- Ruehle JL (2003). Nematodes and forest trees – types of damage to tree roots. *Annual Review of Phytopathology* 11 (1): 99-118.
- Salisbury FB, Ross CW (1994). *Fisiología vegetal*. Grupo Editorial Iberoamericana, México, DF. (in Spanish).
- Sami F, Yusuf M, Faizan M, Faraz A, Hayat S (2016). Role of sugars under abiotic stress. *Plant Physiology and Biochemistry* 109: 54-61.
- Sasser J, Freckman D (1987). A world perspective on nematology: The role of the society. In: Veech J, Dickson D, editors. *Vistas on Nematology*; Society of Nematologists, Inc.: Hyattsville, MD. pp.7-14
- Shibata M, Masaki T, Tanaka H, Niiyama K (2010). Effects of abiotic and biotic factors and stochasticity on tree regeneration in a temperate forest community. *Ecoscience* 17 (2): 137-145.
- Sikora EJ, Malek RB (1991). Influence of temperature on development of pine wilt in Scots pine. *Journal of Nematology* 23: 188-193.
- Singh S, Hodda M, Ash G (2013). Plant-parasitic nematodes of potential phytosanitary importance, their main hosts and reported yield losses. *Bulletin OEPP/EPPO Bulletin* 43 (2): 334-374.

- Singla J, Krattinger SG (2016). Biotic stress resistance genes in wheat encyclopedia of food grains (Second Edition), 4, 388-392.
- Stokes ME, Chattopadhyay A, Wilkins O, Nambara E, Campbell MM (2013). Interplay between sucrose and folate modulates auxin signalling in Arabidopsi. *Plant Physiology* 162: 1552-1565.
- Verma S, Nizam S, Verma PK (2013). Biotic and abiotic stress signalling in plants. In book: *Stress Signaling in Plants: Genomics and Proteomics Perspective* (pp. 25-49).
- Wang Y, Zwiazek JJ (1999). Effects of early spring photosynthesis on carbohydrate content, bud flushing and root and shoot growth of *Picea glauca* barerootseedlings. *Scandinavian Journal of Forest Research* 13: 295-302.
- Watschke TL, Schmidt RL, Carson EW, Blaser RE (1972). Some metabolic phenomena of Kentucky bluegrass under high temperature. *Crop Science* 12: 87-90.
- Watschke TL, Schmidt RL, Carson EW, Blaser RE (1973). Temperature influence on the physiology of selected cool season turfgrasses and bermudagrass. *Crop Science* 13: 591-594.
- Webster J, Mota M (2008). Pine wilt disease: global issues, trade and economic impact. In: Mota M, Vieira P (eds) *Pine wilt disease: a worldwide threat to forest ecosystems*, Springer, London, pp 1-3.
- Wilcox JR (2001). Sixty years of improvement in publicly developed elite soybean lines. *Crop Science* 41: 1711-1716.
- Wind J, Smeekens S, Hanson J (2010). Sucrose: metabolite and signaling molecule. *Phytochemistry* 71: 1610-1614.
- Wu XQ, Yuan WM, Tian XJ, Fan B, Fang X et al. (2013). Specific and functional diversity of endophytic bacteria from pine wood nematode *Bursaphelenchus xylophilus* with different virulence. *International Journal of Biological Science* 9 (1): 34-44.
- Yasseen BT, Al-Thani RE, Alhadi FA, Abbas RAA (2018). Soluble sugars in plants under stress at the arabian gulf region: Possible Roles of Microorganisms. *Journal of Plant Biochemistry & Physiology* 6: 4. doi: 10.4172/2329-9029.1000224
- Żebrowska E, Milewska M, Ciereszko I (2017). Mechanisms of oat (*Avena sativa* L.) acclimation to phosphate deficiency. *PeerJ* 5: e3989.