Research Article BEU Fen Bilimleri Dergisi 15(1): 169-176 (2026) DOI: 10.17798/bitlisfen.1784283

BİTLİS EREN UNIVERSITY
FEN BİLİMLERİ DERGİSİ
ISSN: 2147-3129 / e-ISSN: 2147-3188

MONTHLY DYNAMICS OF *NOSEMA CANBURENSIS* INFECTION ON *GRAPHOSOMA ITALICUM* MÜLLER (HEMIPTERA: PENTATOMIDAE) POPULATIONS

Çağrı BEKİRCAN [1*], Onur TOSUN [2], Hilal YILDIRIM [3]

[1] *Karadeniz Technical University, Maçka Vocational School, Department of the Chemistry and Chemical Processing Technology, Trabzon, Türkiye*
[2] *Karadeniz Technical University, Maçka Vocational School, Department of the Veterinary Medicine, Trabzon, Türkiye*
[3] *Giresun University, Espiye Vocational School, Department of the Crop and Animal Production, Giresun, Türkiye*
**** Corresponding Author:*** cagribekircan@hotmail.com

Article Info

Received: *September 15, 2025*
Revised: *December 19, 2025*
Accepted: *January 23, 2026*

Keywords
Climatic factors,
Entomopathogens,
Microsporidiosis,
Pests

ABSTRACT

Microsporidia are obligate intracellular parasites that predominantly cause chronic infections in their hosts, relying entirely on host cells for development and reproduction. Among entomopathogenic microsporidia, climatic variables such as temperature and humidity are pivotal in influencing infection dynamics. This study focuses on *Nosema canburensis*, a microsporidian pathogen of the Italian striped bug, *Graphosoma italicum*, a pest species that adversely affects Apiaceae crops. Field studies conducted between June and September from 2022 to 2024 in Trabzon Province, Türkiye, documented the prevalence of *N. canburensis* within populations of *G. italicum*. Microscopic analyses identified infection rates, with Ortahisar district showing significantly higher prevalence (16.67%) compared to Beşikdüzü (0%). Microscopic findings were supported by molecular confirmation via SSU rRNA-based PCR. The highest infection rate (22.5%) was recorded in 2023, while the lowest was observed in 2022 (13.33%). Seasonal fluctuations revealed peak infection rates in July and the lowest in September, consistently over three years. Statistical analyses indicated a significant positive correlation between temperature and microsporidiosis prevalence ($r = 0.462$, $P < 0.05$) and a weak negative correlation with humidity ($r = -0.077$, $P > 0.05$). The findings highlight the critical role of climatic factors in shaping microsporidiosis outbreaks and emphasize the potential of *N. canburensis* as a natural regulatory factor in pest insect management. Future research should focus on elucidating the environmental and biological interactions influencing the epidemiology of entomopathogenic microsporidia.

1. INTRODUCTION

Microsporidia, which are single-celled obligate intracellular pathogenic organisms, predominantly cause chronic infections rather than acute ones [1]. These microorganisms rely entirely on their host cells for development and reproduction. Consequently, any factors influencing the host organism—either directly or indirectly—also impact the microsporidia. This is particularly evident in the case of entomopathogenic microsporidia, which are significantly affected by both biotic and abiotic elements. Among these, abiotic factors, especially climatic conditions, play a critical role in regulating the metabolic processes of insects, including their growth and development [2, 3]. For example, a rise in temperature correlates with heightened insect activity, which in turn accelerates metabolic rates, a key factor contributing to insect growth [4]. Therefore, this concrete reality causes monthly and yearly fluctuations in the rates of microsporidiosis in insects.

Microsporidia, as obligate intracellular parasites, predominantly induce sublethal effects in their insect hosts, including reduced fitness, diminished fertility, and shortened lifespan [3, 5]. In the context of insect control strategies, microsporidia are often regarded as significant natural antagonists of various pest insects. However, perspectives on their efficacy in biological control remain divided. For instance, while some researchers argue that microsporidia lack sufficient potential for practical application in biological control programs, others highlight their utility in inoculative release strategies [6, 7]. Nevertheless, recent studies consistently demonstrate the suppressive impact of microsporidia on pest insect population dynamics. [8, 9]. These findings underscore the potential of microsporidia as a viable component of integrated pest management strategies. To optimize their application, it is crucial to thoroughly analyze factors influencing microsporidiosis in pest insects. Special attention should be directed toward climatic and environmental conditions, as these are pivotal in shaping the dynamics of insect disease outbreaks [10-12].

Nosema canburensis Bekircan, Tosun & Yıldırım, 2025 is a natural microsporidian pathogen of the Italian striped bug, *Graphosoma italicum* Müller (Hemiptera: Pentatomidae), commonly referred to as the minstrel bug [13]. These insects have been observed on various plant species, with a strong preference for members of the Apiaceae family, such as parsley and fennel [14, 15]. *G. italicum* and other species within the same genus inflict damage on the reproductive organs and seeds of their host plants, consequently reducing both the quality and yield of the seeds. Certain species are classified as pests due to the detrimental effects they exert on cultivated Apiaceae crops by feeding on their generative organs, which significantly impacts seed productivity and quality [16].

The present study aimed to investigate the distribution and prevalence of *N. canburensis* across various populations of *G. italicum*. This research sought to provide new insights into the potential role of *N. canburensis* as a natural suppressive factor influencing *G. italicum* populations. Furthermore, the study examined whether a correlation exists between *N. canburensis* infection rates and specific months.

2. MATERIAL AND METHOD

2.1. Sample Collections and Laboratory Studies

Adult specimens of *Graphosoma italicum* were collected from various locations cultivating parsley within the Ortahisar and Beşikdüzü districts of Trabzon province (Türkiye) during the period from June to September in 2022 and 2024. Identification of *G. italicum* was based on morphological features such as dorsal coloration, pronotal and scutellar stripe patterns, and antennal segmentation. Taxonomic confirmation followed standard keys [17]. The live samples were carefully transported to the laboratory using sterile plastic containers. The samples were dissected in Ringer's solution, and the dissected tissues were smeared onto microscopic slides. The slides were then examined under a Nikon Eclipse Ci light microscope equipped with a DS-Fi 2 digital camera at 400× magnification to identify the spore structure of *N. canburensis* [13]. All dissection data were systematically recorded for subsequent statistical analyses. Moreover, temperature and humidity data from the sampling sites were recorded using Trotec BL30 Data Loggers.

In addition, genomic DNA (gDNA) was extracted from ethanol-preserved infected tissue samples using the QIAGEN DNA Isolation Kit (Cat. No. 69504). Prior to extraction, the samples were rinsed three times with distilled water to eliminate residual ethanol and impurities. The washed tissues were then transferred to a sterile Eppendorf tube and mechanically homogenized using a micropestle. To facilitate disruption of the microsporidian spore wall, 0.5 mL of 0.5% hydrogen peroxide (H_2O_2) was added to the homogenized sample and incubated at room temperature for 30 minutes. Subsequent DNA isolation steps were performed following the manufacturer's protocol [13].

Following extraction, amplification of the target gene region—small subunit ribosomal RNA (SSU rRNA)—was carried out using the QIAGEN Multiplex PCR Kit (Cat. No. 206143). For initial screening of microsporidian infection, SSU rRNA-specific primers (18f and 1537r) were employed. The primer sequences were as follows:

18f: 5’-CAC CAG GTT GAT TCT GCC-3’

1537r: 5’-TTA TGA TCC TGC TAA TGG TTC-3’

PCR amplicons were resolved via electrophoresis on 1.5% agarose gels, subsequently stained with ethidium bromide for nucleic acid visualization, and imaged using a UV transilluminator.

2.2. Statistical Analysis

The Kolmogorov-Smirnov test was employed to assess the normality of the data sets and the statistical analysis of the collected data was performed through descriptive analysis, Spearman's rank correlation analysis utilizing the SPSS software version 23.0.0.0. [18, 19].

3. RESULTS AND DISCUSSION

3.1. Microsporidiosis Rates

Field studies conducted between June and September in the years 2022 to 2024 documented the collection of 570 adult individuals of *G. italicum* from the Beşikdüzü and Ortahisar districts of Trabzon province (Figure 1) (Table 1).

Figure 1. Adult of Graphosoma italicum Müller (Hemiptera: Pentatomidae)

Microsporidiosis was exclusively observed in samples taken from Ortahisar (Figure 2). All of the samples that were found to be positive by microscopic examination were also found to be positive by the PCR technique.

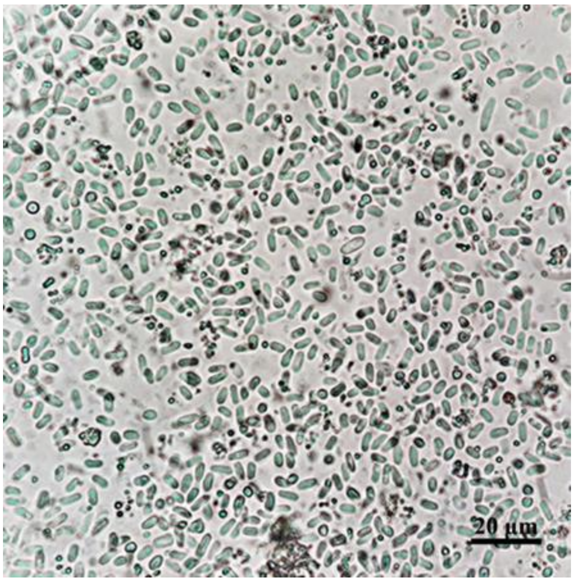

Figure 2. Light micrographs of the Nosema canburensis spores in the mid-gut of the Graphosoma italicum, 1000X magnification.

Following agarose gel electrophoresis and staining of the obtained products with ethidium bromide, 4 amplicons exhibited a length of 1000–1500 bp, which corresponds to *N. canburensis* (Figure 3). Field sampling was designed based on the presence of host insects and site accessibility within the region. However, due to the limited number of sampling events, it is not appropriate to generalize the infection prevalence across the entire province.

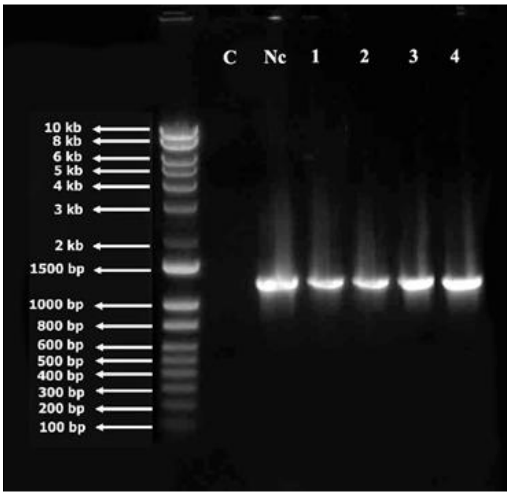

Figure 3. PCR amplification of genomic DNA extracted from Nosema canburensis (Nc) using microsporidian SSU rRNA-specific primers (18f/1537r). Amplicons are visible in lanes 1–4. Lane C corresponds to the negative control.

The average infection rate across all sites was determined to be 7.45%, with Ortahisar exhibiting a significantly higher rate of 16.67%. Notably, the highest infection rate was recorded in July 2023, whereas the lowest rate was observed in September 2022. Analyzing the data on an annual basis revealed that the year 2023 had the highest infection rate at 22.5%, in contrast to 2022, which exhibited the lowest rate of 13.33% (Figure 4). Moreover, September was consistently identified as the period with the lowest infection rates throughout the three-year duration of the study (Table 1). It is particularly noteworthy that, in all three years, July was the month with peak infection rates. Based on the monthly averages calculated over a three-year period, the mean infection rate was determined to be 30% in July and 6.78% in September. In the other months, the average rates were recorded as 9.54% in June and 22.45% in August.

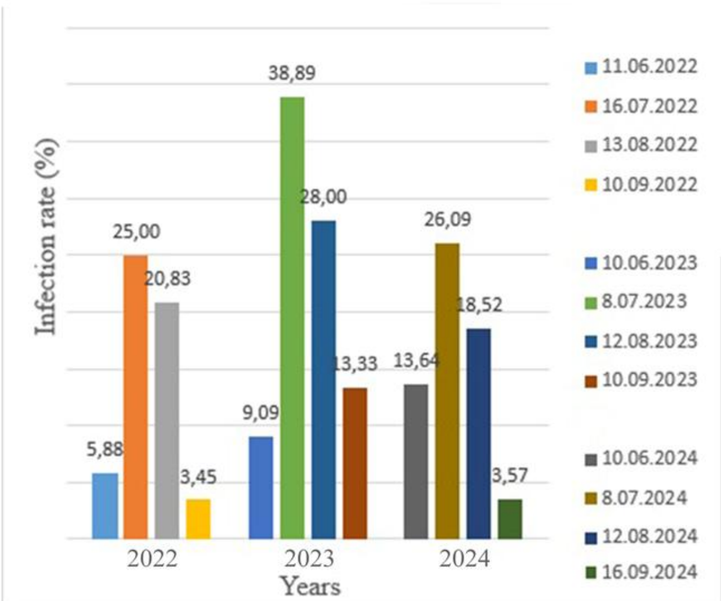

Figure 4. Microsporidiosis rates according to months and years

An evaluation of the statistical relationship between microsporidiosis rates and months revealed a weak negative correlation; however, this relationship was found to be statistically non-significant (r = -0.257, P > 0.05).

The detection of the infection exclusively in Ortahisar suggests that specific environmental or population-level conditions may be conducive to its transmission. Potential contributing factors include the distribution of infected carrier individuals, host density, environmental stressors, and the influence of microhabitat characteristics. Testing these hypotheses falls within the scope of advanced epidemiological investigations.

Table 1. Prevalence of microsporidiosis in Graphosoma italicum and climatic parameters.

	Locality	Date	Sample	Infected	Infection rate (%)	Temperature (ºC)	Humidity (%)
TRABZON	Beşikdüzü	11.06.2022	30	0	0	23.0	72.6
		16.07.2022	22	0	0	26.3	58.9
		13.08.2022	28	0	0	26.8	76.5
		10.09.2022	20	0	0	24.5	73.2
		10.06.2023	22	0	0	22.9	79.2
		8.07.2023	25	0	0	25.1	75.6
		12.08.2023	32	0	0	26.8	76.4
		10.09.2023	21	0	0	24.5	78.2
		10.06.2024	20	0	0	23.0	68.5
		8.07.2024	22	0	0	26.2	71.9
		12.08.2024	32	0	0	27.0	70.3
		16.09.2024	26	0	0	24.2	73.5
	Ortahisar	11.06.2022	17	1	5.88	18.0	74.2
		16.07.2022	20	5	25.00	24.0	70.9
		13.08.2022	24	5	20.83	23.8	74.7
		10.09.2022	29	1	3.45	22.9	72.8
		10.06.2023	22	2	9.09	20.0	82.0
		8.07.2023	18	7	38.89	22.6	73.7
		12.08.2023	25	7	28.00	24.0	74.3
		10.09.2023	15	2	13.33	22.8	77.3
		10.06.2024	22	3	13.64	21.0	68.9
		8.07.2024	23	6	26.09	24.8	72.7
		12.08.2024	27	5	18.52	24.2	69.4
		16.09.2024	28	1	3.57	23.0	72.3
Total			570	45	7.89		

3.2. Association Between Climatic Factors and Microsporidiosis

Statistical analyses revealed a significant relationship between climatic factors (temperature and humidity) and the prevalence of microsporidiosis in *G. italicum*. The correlation coefficient indicated a positive relationship between temperature and microsporidiosis prevalence (r = 0.462, P < 0.05). Conversely, humidity exhibited a weak negative effect on the prevalence of microsporidiosis (r = -0.077, P > 0.05). Given this weak correlation coefficient, partial correlation analysis was conducted to validate whether humidity influences microsporidiosis and temperature variables. The results confirmed that humidity does indeed have an effect on these variables (r = 0.456).

This research aimed to evaluate the monthly prevalence of microsporidiosis caused by *Nosema canburensis* in *G. italicum* and to determine the climatic conditions that promote the disease's development. The analysis conducted in this study identified a positive correlation between temperature and *N. canburensis* infection, a finding consistent with previous research. Temperature is recognized as a critical factor influencing the spread and persistence of microsporidiosis [2, 20, 21]. However, the role of temperature varies across microsporidia species. For instance, high temperatures promote spore formation and development in *Nosema lymantria*, whereas *Nosema pyrausta* exhibits no significant temperature dependency, with even lower temperatures fostering its development [22, 23]. A study by Bekircan et al. [2] further demonstrated that temperature serves as the primary climatic determinant for *Rugispora istanbulensis*, responsible for infections in the elm leaf beetle, *Xanthogaleruca luteola* Müller (Coleoptera: Chrysomelidae). Beyond this direct impact, temperature notably influences insect feeding behavior, with higher temperatures resulting in increased feeding activity [24]. Consequently, elevated feeding rates during warmer periods enhance horizontal transmission of microsporidia, leading to greater intensity and prevalence of microsporidiosis within insect populations. These findings align with the present study, which identified temperature as the primary factor driving microsporidiosis rates. Conversely, humidity, the second climatic variable investigated, exhibited a negative correlation with microsporidiosis prevalence, contrasting with findings from similar studies. For example, research by Rahmathulla et al. on silkworms (*Bombyx mori* L.) revealed that peak infection rates coincided with periods of maximum humidity [25]. Although this study identified a positive correlation between temperature and the rate of microsporidiosis, it is evident that temperature is not the sole determinant of infection rates. This conclusion is supported by the observation that the temperature (22.6 ºC) in July 2023, when the highest infection rate (38.89%) was recorded, was lower than the temperature (24 ºC) in August of the same year.

The findings of this study suggest that different climatic factors and variables may play a significant role in determining the severity of *Nosema canburensis* infections. Although such variables were not initially considered during the data collection phase, subsequent statistical analyses have underscored the importance of integrating climatic variables, particularly precipitation, in similar studies. Precipitation has been identified as a critical factor in the transmission of microsporidiosis among insect populations [26]. Feces of infected insects contain a large number of environmental microsporidian spores and contribute to horizontal transmission [27]. Raindrops facilitate the adhesion of fecal particles, leading to increased environmental contamination [28]. Furthermore, precipitation can effectively transport spores across different locations, thereby intensifying the prevalence and severity of microsporidiosis within insect populations. Apart from these, there are studies in the literature that declare that precipitation is the second climatic variable affecting the rate of microsporidiosis after temperature [2].

4. CONCLUSION AND SUGGESTIONS

This study elucidates the seasonal dynamics of *Nosema canburensis* infection in *Graphosoma italicum* populations, highlighting the pathogen's potential role as a natural regulatory agent in pest management. The observed peak infection rates during warmer months and the absence of infection in Beşikdüzü underscore the influence of localized climatic and ecological conditions on microsporidiosis prevalence. These findings support the hypothesis that temperature is a primary driver of infection intensity, while humidity exerts a more variable effect. Future research should prioritize long-term, multi-regional surveillance to better understand the epidemiological patterns of *N. canburensis*. Additionally, investigations into host-pathogen interactions at the physiological level, as well as the integration of molecular diagnostics into monitoring protocols, are recommended to enhance early detection and inform sustainable biocontrol strategies within integrated pest management frameworks.

Acknowledgements

A part of this study was supported by Scientific Research Committee of Karadeniz Technical University (FBA-2023-10689). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of Interest Statement

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The study is complied with research and publication ethics.

Artificial Intelligence (AI) Contribution Statement

This manuscript was entirely written, edited, analyzed, and prepared without the assistance of any artificial intelligence (AI) tools. All content, including text, data analysis, and figures, was solely generated by the authors.

Contributions of the Authors

ÇB: Writing – review & editing, Supervision, Resources, Investigation, Analysis.

OT: Resources, Investigation, Analysis.

HY: Resources, Investigation.

REFERENCES

[1] L. M. Weiss and A. W. Reinke, L. M. Weiss and A. W. Reinke, Eds. Microsporidia Current Advances in Biology (Experientia Supplementum). Switzerland: Springer, 2022.

[2] Ç. Bekircan, O. Tosun, and H. Baki, "Influence of climatic conditions on microsporidiosis that originated from Rugispora istanbulensis in Elm Leaf Beetle, Xanthogaleruca luteola Muller (Coleoptera: Chrysomelidae)," Kastamonu Üniversitesi Orman Fakültesi Dergisi, vol. 18, no. 2, pp. 230-235, 2018, doi: 10.17475/kastorman.344845.

[3] Y. Chen et al., "The microsporidian polar tube: origin, structure, composition, function, and application," Parasites & Vectors, vol. 16, no. 1, p. 305, 2023/08/30 2023, doi: 10.1186/s13071-023-05908-9.

[4] T. Miki, T. Shinohara, S. Chafino, S. Noji, and K. Tomioka, "Photoperiod and temperature separately regulate nymphal development through JH and insulin/TOR signaling pathways in an insect," (in eng), Proc Natl Acad Sci U S A, vol. 117, no. 10, pp. 5525-5531, Mar 10 2020, doi: 10.1073/pnas.1922747117.

[5] Ç. Bekircan, "Assignment of Vairimorpha leptinotarsae comb. nov. on the basis of molecular characterization of Nosema leptinotarsae Lipa, 1968 (Microsporidia: Nosematidae)," Parasitology, vol. 147, no. 9, pp. 1019-1025, 2020, doi: 10.1017/s0031182020000669.

[6] (1998). NE-247, Population Dynamics, Impacts, and Integrated Management of Forest Defoliating Insects.

[7] Y. S. Tokarev et al., "Ecological vs physiological host specificity the case of the microsporidium Nosema pyrausta (Paillot) Weiser, 1961," Acta Biologica Sibirica, vol. 8, pp. 297-316, 2022, doi: doi: 10.14258/abs.v8.e19.

[8] L. M. Weiss and J. J. Becnel, Microsporidia Pathogens of Opportunity. USA: John Wiley & Sons, Inc., 2014, p. 747.

[9] J. Vávra and J. Lukeš, "Microsporidia and 'The Art of Living Together'," in Advances in Parasitology, D. Rollinson Ed., (Advances in Parasitology: Academic Press, 2013, pp. 253-319.

[10] L. A. Lacey and H. K. Kaya, Field Manual of Techniques in Invertebrate Pathology_ Application and evaluation of pathogens for control of insects and other. USA: Springer-Science+Business Media, 2000.

[11] A. E. Hajek, T. R. Glare, and M. O'Callaghan, A. E. Hajek, T. R. Glare, and M. O'Callaghan, Eds. Use of Microbes for Control and Eradication of Invasive Arthropods (Progress in Biological Control). USA: Springer, 2006.

[12] L. I. Gilbert and S. S. Gill, Insect Control (BIOLOGICAL AND SYNTHETIC AGENTS). UK: Academic Press, 2010.

[13] Ç. Bekircan, O. Tosun, and H. Yıldırım, "Nosema canburensis sp. nov. (Microsporidia: Nosematidae): a novel microsporidium record infecting the Italian striped bug, Graphosoma italicum Müller (Hemiptera: Pentatomidae)," Systematic Parasitology, vol. 102, no. 2, p. 24, 2025/02/22 2025, doi: 10.1007/s11230-025-10220-4.

[14] M. Fent and A. Dursun, "An Up-to-Date Checklist of Turkish Pentatomidae (Hemiptera: Heteroptera) With Additional Records," Trakya University Journal of Natural Sciences, vol. 23, pp. 65-111, 2022, doi: 10.23902/trkjnat.1123152.

[15] M. Fent, A. Dursun, and S. Tezcan, "First record of Graphosoma inexpectatum (Hemiptera, Pentatomidae, Podopinae) from Turkey with description of the female," ZooKeys, vol. 319, pp. 51-57, 2013, doi: doi: 10.3897/zookeys.319.4298.

[16] Ş. Yiğit and İ. Saruhan, "Evaluation of biocontrol agents for Graphosoma lineatum (L.) (Hemiptera: Pentatomidae) in experimental conditions," International Journal of Tropical Insect Science, vol. 41, no. 2, pp. 1069-1074, 2021/06/01 2021, doi: 10.1007/s42690-020-00290-w.

[17] B. Aukema and C. Rieger, "Pentatomomorpha I," in Catalogue of the Heteroptera of the Palaearctic Region, vol. 4, P. Looijen Ed. Wageningen: Netherlands Entomological Society, 2001, p. 346.

[18] A. Justel, D. Peña, and R. Zamar, "A multivariate Kolmogorov-Smirnov test of goodness of fit," Statistics & Probability Letters, vol. 35, no. 3, pp. 251-259, 1997/10/15/ 1997, doi: https://doi.org/10.1016/S0167-7152(97)00020-5.

[19] C. Spearman, "The proof and measurement of association between two things," The American Journal of Psychology, vol. 15, no. 1, pp. 72-101, 1904.

[20] I. Fries, "Nosema ceranae in European honey bees (Apis mellifera)," Journal of Invertebrate Pathology, vol. 103, pp. S73-S79, 2010/01/01/ 2010, doi: https://doi.org/10.1016/j.jip.2009.06.017.

[21] L. A. Malone, H. S. Gatehouse, and E. L. Tregidga, "Effects of Time, Temperature, and Honey on Nosema apis (Microsporidia: Nosematidae), a Parasite of the Honeybee, Apis mellifera (Hymenoptera: Apidae)," Journal of Invertebrate Pathology, vol. 77, no. 4, pp. 258-268, 2001/05/01/ 2001, doi: https://doi.org/10.1006/jipa.2001.5028.

[22] S. Pollan, D. Goertz, and G. Hoch, "Effect of temperature on development of the microsporidium Nosema lymantriae and disease progress in the host Lymantria dispar (L. 1758)," Mitteilungen der Deutschen Gesellschaft für allgemeine und angewandte Entomologie, vol. 17, pp. 173-178, 2009.

[23] L. F. Solter, D. W. Onstad, and J. V. Maddox, "Timing of disease-influenced processes in the life cycle of Ostrinia nubilalis infected with Nosema pyrausta," Journal of Invertebrate Pathology, vol. 55, no. 3, pp. 337-341, 1990/05/01/ 1990, doi: https://doi.org/10.1016/0022-2011(90)90076-I.

[24] N. P. Lemoine, D. E. Burkepile, and J. D. Parker, "Variable effects of temperature on insect herbivory," Peer J, vol. 2, e376, 2014, doi: 10.7717/peerj.376.

[25] V. K. Rahmathulla, C. M. K. Kumar, B. S. Angadi, and V. Sivaprasad, "Influence of Weather Factors on Incidence and Intensity of Microsporidiosis in Silkworm (Bombyx mori L.)," Journal of Entomology, vol. 9, pp. 266-273, 2012, doi: 10.3923/je.2012.266.273.

[26] V. Neidel, "Effects of precipitation on fecal-oral transmission of the microsporidium Nosema lymantriae in experimental gypsy moth populations," Master, Institut für Forstentomologie, Forstpathologie und Forstschutz BOKU - Universität für Bodenkultur Wien, Austria, 2016.

[27] C. Steyer, "Die Rolle von Faeces und simuliertem Regen bei der Übertragung der Mikrosporidien Nosema lymantriae und Endoreticulatus schubergi bei Lymantria dispar," Master, BOKU - Universität für Bodenkultur Wien, Austria, 2010.

[28] D. Goertz and G. Hoch, "Horizontal transmission pathways of terrestrial microsporidia: A quantitative comparison of three pathogens infecting different organs in Lymantria dispar L. (Lep.: Lymantriidae) larvae," Biological Control, vol. 44, no. 2, pp. 196-206, 2008/02/01/ 2008, doi: https://doi.org/10.1016/j.biocontrol.2007.07.014.