REVIEW

on the dissertation for obtaining the educational and scientific degree "doctor" in: field of higher education 6. Agricultural Sciences and Veterinary Medicine, professional field 6.2. Plant Protection (Phytopathology); scientific specialty Plant Protection (Phytopathology)

Author of the dissertation: Martin Georgiev Marinov - PhD student (full-time) at the Department of Phytopathology at the Agricultural University, Plovdiv

Topic of the dissertation: Epidemiology and control of cherry leaf spot of cherry and sour cherry *(Blumeriella jaapii).*

Reviewer: Professor, Dsc Mariyana Borisova Nakova - Agricultural University Plovdiv, in the field of higher education 6. Agricultural Sciences and Veterinary Medicine, professional field 6.2. Plant protection (phytopathology), the scientific specialty Plant protection (phytopathology) was appointed as a member of the scientific jury by order No. RD-16-1330/22.11.2024 by the Rector of the Academy of Sciences.

1. Brief introduction of the candidate.

Martin Marinov was born in 1992 in the village of Mihailovtsi, Gabrovo municipality. In the period 2011 - 2015 he was a bachelor student in the Agricultural University of Plovdiv, majoring in Agronomy (Horticulture). He graduated as the top student of his class. Then he is a Master in the International Master's course in Plant Protection which he also graduated with honors. He developed a thesis related to the testing of fungicides for the control of powdery mildew on the vine. During his studies he worked as an agronomist at the Center for Integrated Disease Management at the University of Agriculture in Plovdiv. After the master's degree, he took part in a competition and became a full-time doctoral student at the Department of Phytopathology, in the period March 2018 - November 2021. As a doctoral student, he developed his dissertation topic, participated in courses as a part of his Individual plan for work; visited Norwegian Institute of Bioeconomy Research (NIBIO) for short trainings, during 2018 and 2020.

Doctoral student Martin Marinov took the exam and from September 2021 he has been an assistant professor at the department. As an assistant-professor, Martin Marinov leads practicals in Phytopathology for bachelor students in Agronomy and Horticulture, in the Disease forecasting and Signaling, and since 2023 on

General Phytopathology and Special Phytopathology, as well. He has good communication with students and explains the course material in a clear manner. In parallel, he also develops experimental activities by participating in scientific projects.

2. Relevance of the problem.

The topic of the dissertation is extremely important. Cherries are a major fruit crop and the interest in it worldwide is great. Cherry leaf spot/Cylindosporiosis is a disease that has long been known to science and practice. However, there are still details of the epidemiology of the disease and the possibilities for forecasting and control that need to be clarified.

In Europe, the disease was reported in 1884, and in Bulgaria in 1961. Due to its economic importance, it has been studied in depth by Velichkova, Borovinova and other researchers. Changes in technologies, varieties, climatic conditions and the population of the causative agent require new research to improve the applied methods and means for controlling the disease.

3. Purpose, tasks, hypotheses and research methods.

The dissertation has a classical generally accepted structure. The Introduction is related to the production of cherries in our country, to the new European strategies for disease control in the revision of spraying and the application of predictive models. The Literature review shows a very good knowledge of the problem to be studied. The symptoms of the disease in cherries and sour cherries, the morphological and biological features of the pathogen, the methods and means of control are discussed, including varieties, agricultural techniques, the application of forecasting methods and chemical control. The state of research on the problem in Bulgaria is analyzed. The literary sources are analyzed in order to establish patterns and trends. The list of literature have 47 Bulgarian publications and the rest are by foreign authors.

The aim of the dissertation is to study new aspects in the epidemiology of Cherry leaf spot, as a basis for the possibilities for forecasting and control of the disease. The tasks are precisely and clearly formulated and are related to the realization of the goal of the study.

The Materials and methods section is presented in detail, with the appropriate methodologies selected to achieve the tasks set. The methods for isolation and development of the pathogen on artificial nutrient media, for epidemiological studies and static data processing, for studying the dynamics of leaf decomposition, for validating a predictive model and a strategy for fungicide treatments are described in detail. The methodology is of a high level and guarantees obtaining reliable results. The methods are modern and allow

achieving the set goals. The data analysis reveals patterns useful for practice, some of which are new for phytopathological science.

4. Visuality and presentation of the results.

The dissertation is very well written and structured, with an analytical nature. It is easy to read and understand, the scientific terminology is correctly used, the interpretation of the data shows a thorough knowledge of the problem and is aimed at revealing trends. These, in turn, are the basis for scientifically substantiated conclusions and contributions.

The research focuses on the epidemiology of the pathogen using modern spore trap and meteorological data from each of the gardens. This allows for the analysis of the influence of rain, leaf moisture and relative humidity on the spread of spores (ascospores and spring macro-conidia) during the growing season. The dynamics of leaf decomposition under natural conditions, as well as the spread of the disease during the growing season (including leaf fall), were also studied. The Eisensmith and Jones model has been validated for the conditions of our country and on this basis, experiments have been conducted to control cherry leaf spot.

The dissertation is very well illustrated with about 28 author's photographs related to the symptoms of the disease, the morphology of the pathogen (colonies, spores), the equipment used and the design of the experiments, plus 23 figures showing trends from statistical analysis of the data obtained and 38 tables. They confirm the experiments conducted and the results obtained from them. In addition, there is an Appendix with three tables and 8 figures showing the decomposition of the leaf mass; climatic data for 2018 and 2019, during the period of exposure of control plants for the purpose of infection from overwintered leaves; annual dynamics of the spread of spores of the pathogen, related to the climatic factors temperature, precipitation and relative humidity.

5. Discussion of the results and used literature.

The dissertation work confirms the symptoms of Cherry leaf spot, describing them in two varieties of cherries and one sour cherry.

Symptoms on the stalks of sour cherries in a high infectious background and a sensitive variety are confirmed, and similar ones are reported in the cherry variety Cordia. Isolates of the pathogen from the Van variety were obtained, but unfortunately only from one location, which did not allow to comment on whether there is strain diversity in the population of the pathogen. The morphological features of the colonies, the sporulation of the pathogen from the nutrient medium and from naturally infected leaves are described. The data on the sizes of the spores and mycelia colonies are statistically processed and compared with those published by other authors.

The emphasis is on the study of epidemiology confirming that in the event of a lack of moisture, fewer micro-conidia are formed.

The dispersion of ascospores and spring macro-conidia in the study regions was monitored, as well as the diurnal dynamics of spore release. The influence of rain and leaf moisture on this process was analyzed. For ascospores and spring macro-conidia, the degree days required for their maturation were determined, 311 and 331, respectively. It was confirmed that at 22°C, which according to the author is optimal, this happens in 14 days. The data are comparable with those published by other authors.

The study on the decomposition of leaves in cherry and sour cherry, and the influence of climate on this process is new. A detailed study was conducted only in Hungary.

The spread of cherry leaf spot in the 4 regions was monitored, and the leaf fall of cherry and sour cherry from the disease. The meteorological data and the results were subjected to panel analysis.

The validation of the Eisensmith and Jones model has so far been done for summer macro-conidia. The author is investigating the possibility of its application in the analysis of the primary infection and has registered 32 infectious periods in 2018 and 33 infectious periods in 2019, respectively. Based on the model, treatments with contact and systemic fungicides were carried out in the garden of the Center for integrated disease management. This has allowed better control to be achieved through contact products (Delan) and the application of systemic fungicides, if necessary.

6. Contributions of the dissertation work.

The dissertation work investigates an economically significant disease of cherries in our country and worldwide. In Bulgaria, research on the problem was carried out by Dr. Velichkova and Prof. Borovinova in the period from the 1970s to the early 20th century. Considering the significance of the problem, it is necessary that research on the problem should not be interrupted, as new aspects of the biology and etiology of the causal agent, epidemiology and control measures are established.

Scientific contributions

The dissertation has contributions of both a scientific and a scientifically applied and practical nature. The main scientific contributions are:

1.To study the epidemiology of cherry leaf spot - and more precisely to track the dynamics of the dispersion of spores (ascospore and spring macro-conidia) in the air, for the first time in the world a 7-day spore trap of Burkard Manufacturing Co

Ltd. was used, with active air suction. An average concentration of spores was established.

2.The daily dynamics of spore dispersion was established. Ascospores are released between 01 and 07 a.m. Spring macro-conidia are released during the day, primarily between 14-16 hours. Differentiated reporting of the amount of ascospores and spring macro-conidia in the air, during the growing season and during the day, is new to the world literature.

3.Under laboratory conditions, the required amount of degree days for the maturation of ascospores in apothecia (AS – 311) and conidia in spring aservuli (SMC - 331) was calculated. The trends were also established: delayed development in apothecia up to 93 degree days, accelerated to 160 and again a slight slowdown to 311. Spring aservuli – up to 66 degree days rapid development, 66 – 133 degree days – slowdown, acceleration after that.

4.For the first time in the world, a study was conducted on the use of the Eisensmith and Jones predictive model with a built-in weather forecast, which allows a more flexible approach to decision-making and conducting protective, not just curative, treatments.

The dissertation has many contributions of a scientifically applied and confirmation nature:

1. For the first time in Europe, a study has been conducted to prove infectious events under field conditions, using control plants.

2. For the conditions of Bulgaria, symptoms of cherry leaf spot have been observed on stalks of sour cherry fruits. The doctoral student reports the development of symptoms also on the stalks of cherry fruits, in the Cordia variety. They are not a source of additional infection, because no spores of the pathogen were found there.

3. Synchronized release of ascospores has been established in *Blumeriella jaapii* - a phenomenon observed in *Sclerotinia sclerotiorum*. In this case the ascospores are carried into a higher air layer, remain in the atmosphere longer and travel greater distances with air currents.

4. Ascospores are the main source of infection for the conditions of Bulgaria. Spring conidia have a significant share in certain years, for example 2020, increasing the infectious pressure.

5. It is confirmed that the process of ascospore maturation under optimal, controlled conditions (22°C) is 14 days. This is the opinion of Velichkova (1983) and Garcia & Jones (1993).

6. In rain spore dispersion begins, and decreases after 4-5 hours, when the captured ascospores are few. In spring conidia the trend is similar, and after 4-5 hours there is no dispersion. This is important information for the forecast.

7. With insufficient moisture fewer microconidia are formed which is a confirming contribution.

8. Rainfall during the months of June/July has a key role in the spread of cherry leaf spot infection.

9. An important contribution to the practice is the fact that the release of ascospores in the second half of April correlates with a sensitive phenophase of the host, and the recommendation given to the practice at the moment for spraying after flowering, when the leaves are fully opened.

5.Critical notes and questions.

10 isolates of the pathogen from the Van variety were obtained from a garden of the Center for Integrated Disease Management. It would be good to have isolates from different varieties and regions. In this case, morphological differences in the population might be observed and strains could be identified. The literature talks about races. My recommendation is to study the pathogen population in our country in the future based on molecular methods. Research can also continue in the direction of fungicide-resistant populations.

On p. 96 (table 22) there is a comment about a faster development of cherry leaf spot in the Botevgrad region. This is explained by the influence of the climate which is indisputable. It should also be taken into consideration that the sour cherry variety Oblachinska is grown there which is sensitive to the pathogen.

The photographic material is generally very good. I have a remark only on Fig. 17, p. 65, which is not of a very good quality.

On p. 3 there are repetitions - of data from FAO and from Agrostatistics, in paragraphs 1 and 3, and 2 and 3 respectively.

I also have some questions for the doctoral student:

1. Why were only leaves with late infection used to isolate the pathogen? Does it matter that they are stored in the refrigerator for a while? (p. 31)

2. How is it proven that the pathogen does not survive in the buds on the shoots? (p. 128-130). Develop the hypothesis.

6. Published articles and citations.

The doctoral student Martin Marinov presents one publication on the dissertation work, namely: Marinov, M. (2022). Development of the cherry leaf spot epidemics in different regions of Bulgaria. Agricultural Sciences/Agrarni Nauki, 14(32), 47-55.

He is the only author and that brings him the required number of points.

The doctoral student has participated in two scientific conferences in Zlatibor (Serbia, VIII Congress on Plant Protection, 25.11.-29.11.2019) and Plovdiv (1-st International Symposium on Climate Change and Sustainable Agriculture, 14.11.-15.11.2019).

No information is provided about the citations known to date.

The presented abstract objectively reflects the structure and content of the dissertation work.

7.CONCLUSION:

Based on the dissertation presented by the doctoral student, I believe that Martin Marinov has mastered standard and modern research methods in the field of phytopathology and plant protection; he has conducted laboratory experiments, as well as in controlled and in field conditions at a very good level; he has made a correct analysis based on statistical processing of the results obtained; he has drawn correct conclusions and contributions.

The presented dissertation fully meets the requirements of the Law on Academic staff development in Republic of Bulgaria and the Regulations of the Agrarian University for its application, which gives me reason to evaluate it POSITIVELY.

I would like to suggest that the members of the Esteemed Scientific Jury also vote positively for awarding Martin Georgiev Marinov the educational and scientific degree of "Doctor" in the Scientific Specialty Plant Protection (phytopathology).

Date: 01.02.2025

Plovdiv

REVIEWER: .....

Prof. Drs. Mariyana B. Nakova