JOURNAL OF AGRICULTURE AND PLANT SCIENCES, JAPS, Vol 20, No. 1, 2022

Manuscript received: 31.05.2022
Accepted: 30.06.2022

In print: ISSN 2545-4447
On line: ISSN 2545-4455
doi: https://doi.org/10.46763/JAPS22201025b

Original scientific paper

FORECASTING MODEL BASED ON CUMULATIVE DEGREE DAYS FOR INCUBATION PERIOD OF *Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni

Gligor Bojkov[1*], Emilija Arsov[1] and Saša Mitrev[1]
[1]*Faculty of Agriculture, Department for Plant and Environmental Protection, Goce Delcev University, UNILAB laboratory, Stip, R. North Macedonia*
*Corresponding author: gligorbojkov@yahoo.com

Abstract

The overall development of *Plasmopara viticola* and its occurrence in time and space cause rapid disease increases. The incubation period is a part of the life cycle of *P. viticola,* between infection and the first appearance of symptoms. The forecasting based on cumulative degree days for determination of incubation allows the prediction of a small number of primary infections whose calculation is based on the temperature factor. This forecasting model, in essence, is a regression analysis that presents the relationship between average daily temperature and coefficient of incubation. The determination of the incubation allows precise management of the fungicides against *P. viticola* and gives the basic assumptions for the possible occurrence of the primary inoculum.

Keywords: *Plasmopara viticola, incubation period, average daily temperature, warning model, regression analysis, coefficient of incubation*

INTRODUCTION

The cause of grapevine downy mildew is the oomycete fungus *Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni and belongs to the order *Peronosporales*. The asexual multiplication cycles appear throughout grapevine vegetative growth while sexual structures serve as an overwintering phase. The asexual spore multiplications lead to secondary infections during grapevine growing seasons, originating from specialized gametogenic structures that cause primary infections at the beginning of vegetation of grapevine. These secondary cycles of infection, under favourable weather conditions, can occur repeatedly throughout the grapevine growing season (Gessler et al.,2011). The aim of this research is to predict the incubation or latent period so we could determine precisely the chemical treatments against *P. viticola*. In the Republic of North Macedonia, fungicide management strategies are focused mainly on routine chemical applications against *P. viticola* without taking into account the actual need for spraying. The number of fungicide treatments against secondary infections during grapevine growing season is usually 5 to 7. A properly timed spray programme is essential for managing downy mildew in the vineyard. (Caffi et al., 2009). In plant pathogens latent period is the time between host infection and the onset of pathogen sporulation from that infection (Pariaud et al., 2009). The importance of the latent period for the understanding and prediction of pathogen development has long been recognized in plant disease epidemiology (Vanderplank, 1963). It is important to note that *P.viticola* often has a short time of latency following infection leading to rapid disease increases over time. Since the disease spreads very fast during the secondary infection cycles, successful control depends on controlling the primary infections (Schwinn, 1981). There seems to be very little information about the variability of the latent periods of fungal pathogens on foliar that can be, used as warning models. Designing a forecasting model for the determination of incubation of *P.viticola* based on cumulative degree days mostly depends on the climatic conditions of the areas, which represents only one side of the disease triangle. A forecasting model for the determination of incubation of the *P.viticola* is a simplified approach to the incubation process, but in no way is it a replica of reality itself. Hence, a model serves as an assessment to help research and organize knowledge, enhance understanding of phenomena, and eventually become a tool for decision-making. The main aim of the forecasting model for the determination of incubation of the *P.viticola* is to provide a prediction for the end of the latency period and omit sprays when weather conditions are unfavourable for downy mildew. Primary inoculum plays a key role in epidemics not only at the beginning of the growing season but also in overlapping secondary infection cycles in late spring and summer (Gessler et al., 2003).

MATERIAL AND METHODS

The research was conducted in a vineyard located at Smilica, near Kavadarci, Republic of North Macedonia (41°42`71.4" N, 22°0`10.75" E). The area of the vineyard was 9 ha. A double Guyot pruning system was applied in the vineyard. The aim was to note the incubation of the *P. viticola*, according to the widely known 3/10 empiric role. This rule is based on the simultaneous occurrence of the following conditions: (i) air temperature equal to or greater than 10°C; (ii) vine shoots at least 10 cm in length; (iii) a minimum of 10 mm of rainfall in 24- 48 h (Baldacci, 1947). When the criteria of empirical rules 3/10 by realized in the field, an incubation period begins. Different grapevine varieties (Vranec, Smederevka, Zilavka and Merlot) are present in the vineyard where monitoring was performed during incubation. The incubation of the disease occurred in the following phenological stages of the development of grapevines, according to the BBCH-scale (Hack et al., 1992): BBCH 15/53 - Five leaves separated; Shoots about 10 cm long; Inflorescence visible, BBCH 17/55 - Seven leaves separated; Inflorescence closely pressed together, and BBCH 19/57 - Nine or more leaves separated; Inflorescences fully developed (Figure 1). The extended BBCH-scale is a system for a uniform coding of phenologically similar growth stages of all mono- and dicotyledonous plant species (Hack et al., 1992). The abbreviation BBCH derives from Biologische Bundesanstalt, Bundessortenamt and CHemical industry (Hack et al., 1992). BBCH- describes the phenological development of vines. The cumulative degree days-based model can be understood as an empirical model based upon statistical operation to calculate the incubation period. When the three elementary conditions of the above-mentioned empirical rule 3/10 for the occurrences of the primary infection from sexual structures (oospores), meet, the incubation period begins, which is the goal of observation and calculation (Table 1). The incubation was calculated as cumulative degree days, calculated as the sum of average daily temperatures. In other words, degree days can be understood as a period between infection and symptom detection, calculated based on temperature (°C). The incubation period was monitored every day from May 02 until May 12, 2022, when the first oil spot symptoms appeared on the upper side of the leaves.

Table 1. Observation of the incubation period at *P.viticola*

Varieties	ha	Observation of growth stage	Monitoring period	Incubation days	Σ of °C for cumulative degree days
Vranec	6,5	BBCH15/53-BBCH19/57	02.5.2022 -12.5.2022	10	181,1
Smederevka	1,5				
Zilavka	0,5				
Merlot	0,5				

Figure 1. Overview of phenological stages of the grapevines during the incubation period of *P. viticola:* A - BBCH 15/53 - Five leaves separated; shoots about 10 cm long; B - BBCH 17/55 - Seven leaves separated; Inflorescences closely pressed together; C - BBCH 19/57 - Nine or more leaves separated; Inflorescences fully developed. Smilica locality (authors photos)

MODEL DESCRIPTION

In explaining the model for predicting the length of incubation, we will start with the fact that the primary infection has already occurred. After that the incubation (latency) period is calculated according to the following parameters: average daily temperature, the effective temperature of incubation, and coefficient of incubation (table 2). Müller & Sleumer (1934) suggest that the minimum temperature of practical significance for the occurrence of an outbreak is 12 to 13°C. So, when calculating the incubation period, one should be based on average daily temperatures higher than 11 °C (Table 2). In order to estimate the duration of the incubation period of *P.viticola* the following formulas was used:

$$Ef = ADT - 11,$$

where: *Ef* - Effective temperature of incubation, *ADT* - Average daily temperature;

$$Coeff.In = Ef \div ADT,$$

where Coeff. In - Coefficient of incubation.

Table 2. Temperature parameters on a model for cumulative degree days

Dates	Average daily temperature (*ADT*)	Effective temperature of incubation (-11°C) (*Ef*)	Coefficient of incubation (*Coeff. In*)
02.5.2022	13,6	2,6	0,191176471
03.5.2022	12,9	1,9	0,147286822
04.5.2022	14,6	3,6	0,246575342
05.5.2022	17,8	6,8	0,382022472
06.5.2022	16,6	5,6	0,337349398
07.5.2022	16,6	5,6	0,337349398
08.5.2022	15,8	4,8	0,303797468
09.5.2022	16,7	5,7	0,341317365
10.5.2011	17,4	6,4	0,367816092
11.5.2022	18,3	7,3	0,398907104
12.5.2022	20,8	9,8	0,471153846
	∑=181,1	∑=60,1	

The results obtained from Table 2 were used for further calculation, using regression analysis $[y = \beta o + \beta 1(incubation\ days)]$ for investigating and modeling the relationship between variables. Where: βo *-intercept*, $\beta 1$ *- x variable* and $y = (incubation\ days)$ *- number of observations*

RESULT AND DISCUSSION

The development of disease happens in time and space, and the incubation period is a part of the life cycle of *P.viticola*. The grapevine downy mildew is an obligate parasite, which means when zoospores swim through stomata and enter leaves, consequently the incubation process begins. Figure 1 gives an overview of the phenological stages of the development of grapevines (A - BBCH 15/53, B - BBCH 17/55, C - BBCH 19/57) during the incubation period of *P. viticola* in the Smilica locality. That means that in the intensive vegetative growing stage from-BBCH 15/53 till BBCH 19/57th growth stage, vines are in a sensitive development period towards downy mildew. When the leaf is 1/3 its full size, it exports more food than it uses and begins to contribute to vine growth happening in the already mentioned phenological stages: BBCH 15/53, BBCH 17/55, and BBCH 19/57. Due to favourable conditions at the beginning of the growing season, the open stomata in the above-mentioned phenological stages allow for disease development. Further disease development depends on the impact of temperature because the zoospores already in the leaf tissue are encysted (zoospore which has shed its flagellum) then germinate and produce an appressorium while penetrating through the stoma. In this context linear regression is used to find the relationship between average daily temperature and coefficient of incubation (Table 2), which allows determining the incubation period, i.e., Its length expressed in a period of 24 hours.

Table 3. Statistical analysis of cumulative degree days-model

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0,98498
R Square	0,970186
Adjusted R Square	0,966459
Standard Error	0,016147
Observations	10

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95,0%*	*Upper 95,0%*
Intercept	-0,35047	0,042689	-8,20982	3,62E-05	-0,44891	-0,25203	-0,44891	-0,25203
X variable	0,040825	0,00253	16,13467	2,19E-07	0,034991	0,04666	0,034991	0,04666

The essential benefit of regression analysis is determining how changes in the independent variable x are associated with shifts in the dependent variable y.That means that the values of the independent variable x allow us to explain the variations of the dependent variable y. When setting up the forecasting model, the *x* variable represents the average daily temperatures on which the incubation coefficients represented by the *y* variable depends. The interdependence of variable quantities expressed by Multiple R shows how strong the linear relationship is. The Multiple R = 0,98498 is significant in this case and shows that *y* dependent variable is in interdependent connection with *x* independent variable. Frequently R-squared values range from 0 to 1 and are commonly stated as percentages from 0% to 100%. Further, the coefficient of determination (R^2) or R Square is an indication of how much changes in independent variable cause changes in the dependent variable and the convection is expressed in percentage, respectively R^2=0,970186 x 100= 97%. This means that the other 3% belong to the category of unknown factors. Adjusted R Square typically always lower than the R Square (R^2), respectively Adjusted R Square=0,966459. The adjusted R-squared can tell you how useful a model is. In essence, Adjusted R-squared is a modified version of R-squared and decreases when a predictor (dependent variable) improves the model by less than expected. The parameters Standard Error = 0,016147 (SE) and R Square = 0,970186 (R^2) are related because the lower coefficient for SE causes a more predictable R^2, which gives the model validity.

$y = \beta o + \beta 1(incubation\ days)$; $y = -0{,}35047 + 0{,}040825 \times (10)$; $y = 0{,}057$

The parameter (0,057) of calculations shows the end of the incubation process (Table 4). This result of the *y* variable (0,057) got on May 12, 2022, was confirmed in the field when the first oil spot symptoms on the upper side of leaves at grapevines were noticed (Figure 2). Until May 12, the vineyard was not fungicide sprayed meaning there was no obstacle to the development of incubation.

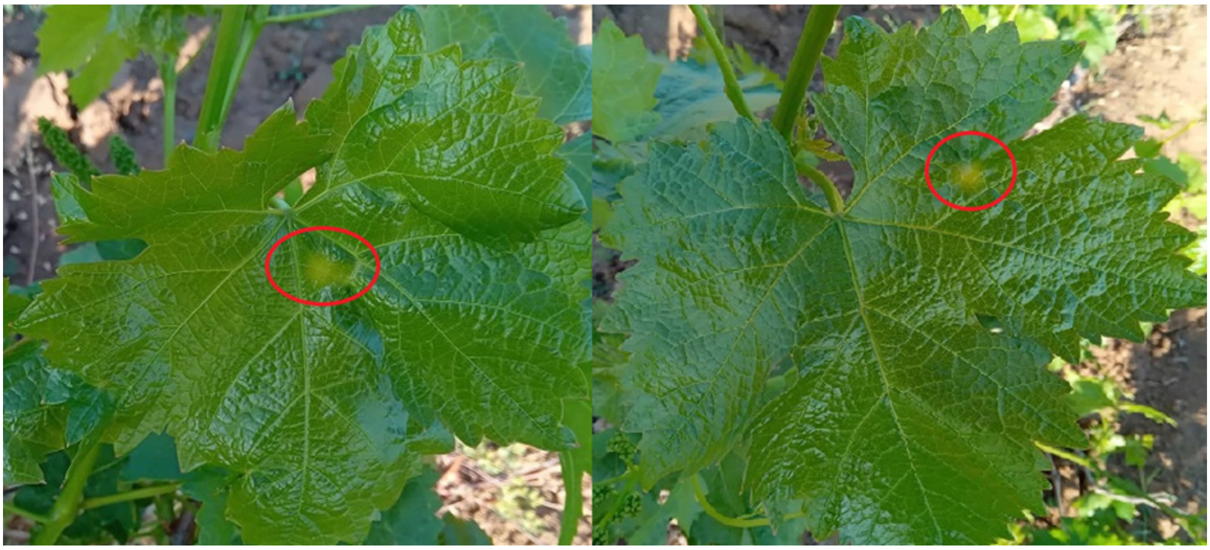

Figure 2. The occurrence of oil spot symptoms on the upper side of leaves indicates the end of incubation period of downy mildew at Smederevka variety. (photo of the author, 12.05.2022)

Any increase or decrease of disease intensity is in range of: $0<y\leq1$. The regression analysis result of the *y* variable in the days before May 12, 2022, have a negative sign and are not taken into account. The first day when the value of the *y* variable will have a positive coefficient marks the end of the incubation period and meets the expectations for predictions of the model (Table 4).

Table 4. Overview of values of the *y* prediction variable

Dates	Values of the *y* variable duration of incubation period $y = \beta o + \beta 1(incubation\ days)$
03.5.2022	-0,78694
04.5.2022	-0,42772
05.5.2022	-0,26144
06.5.2022	-0,21983
07.5.2022	-0,21983
08.5.2022	-0,17362
09.5.2022	-0,12661
10.5.2022	-0,07604
11.5.2022	-0,02118
12.5.2022	0,057-the end of incubation and occurrence of first symptoms

CONCLUSIONS

P.viticola the causal agent of downy mildew is very important fungal disease in grapevine production in the Republic of North Macedonia. The overall development of *P. viticola* and its occurrence in time and space cause rapid disease increases. The incubation period is a part of the life cycle of *P. viticola* between infection and the first appearance of symptoms. This simple model for determining the incubation period provides a quick prediction of the disease. According to Gobbin et al (2005), primary infections play a significant role in the downy mildew epidemic. This model serves to determine the end of the incubation period of the primary infection. Since the zoospores it's installed in plant tissue, which represents the invasive structure of *P.viticola*, obtain nutrients from the leaves, and the temperature remains the main driving factor for disease development, probably humidity of the environment has an indirect impact at this stage of the development of the disease Moreover, the inoculum is less present due to the delayed incubation period that depends on temperature conditions. At lower temperatures, the incubation is delayed and thus the appearance of the reproductive organs. The goal was to trace the incubation period through the temperature and discover when it ended. In practice, spraying is not applied during the first primary and the first secondary infection because the symptoms are visually less noticeable. Refraining from chemical treatments at the beginning of the vegetation is due to the lower temperatures that slow down the development of the disease in contrast to the period when the temperatures are higher and when we have an accelerated cycle of disease development that causes significant economic damages. The significance of determining the primary incubation lies in detecting the timing for the start of the first chemical treatments against the *P.viticola*. When we discover the end of incubation, if we have the appearance of favourable microclimatic conditions for the development of the disease, we must carry out spraying. If the weather is rainy, the chemical treatments can be carried out much earlier, i.e., for the duration of incubation. Due to the influence of lower temperatures at the beginning of the vegetation period, it often happens that the vine has a reduced intensity of growth, which does not exclude the possibility of the parasite carrying out infection and starting the incubation period. It is important to note that the prediction of the *P.viticola* should be based on temperature factors for disease development than on the grapevine's phenology.

REFERENCES

Baldacci E., (1947). Epifitie di *Plasmopara viticola* (1941-46) nell´Oltrepó Pavese ed adozione del calendario di incubazione come strumento di lotta. Atti Istituto Botanico, Laboratorio Crittogamico 8: 45-85

Caffi T., Rossi,V., Bugiani,R., Spanna, F., Flamini, L., Cossu, A., Nigro, C., (2009) A model predicting primary infections of *Plasmopara viticola* in different grapevine-growing areas of Italy. Journal of Plant Pathology, 91,535-548.

Gessler, C., Rumbou, A., Gobbin, D., Loskill, B., Pertot I., Raynal, M., Jermini M. (2003). A change in our conception of the life cycle of *Plasmopara viticola*: Oosporic infections versus asexual reproduction in epidemics. IOBC/WPRS Bulletin. 26, 13-16.

Gessler, C., Pertot, I., Perazzolli, M. (2011). Plasmopara viticola: a review of knowledge on downy mildew of grapevine and effective disease management. Phytopathologia Mediterranea, 50, 3-44

Gobbin, D., Jermini, M., Loskill, B., Pertot, I., Raynal, M., Gessler, C., (2005), Importance of secondary inoculum of *Plasmopara viticola* to epidemics of grapevine downy mildew. Plant Pathology, 54: 522-534.

Hack H., H. Bleiholder., L Buhr., U. Meier., U SchnockFricke., E. Weber., A. Witzenberger, (1992). Einheitliche Codierung der phanologischen Entwicklungsstadien mono- und dikotyler Pfl anzen - Erweiterte BBCH-Skala, Allgemein. Nachrichtenbl. Deut. Pfl anzenschutzd. 44: 265-270.

Muller K, Sleumer S, 1934. Biologische Untersuchungen uber die Peronosporakrankheit des Weinstockes, mit besonderer Berucksichtigung ihrer Bekampfung nach der Inkubationskalendermethode (Investigation of the biology and control of Peronospora disease of grapevines, with special consideration of the incubation calendar method). Landwirtschaftliche Jahrbucher, 79:509-576.

Pariaud, B., Robert, C., Goyeau, H., Lannou, C. (2009). Aggressiveness Components and Adaptation to a Host Cultivar in Wheat Leaf Rust. Phytopathology, 99, 869-878. https://apsjournals.apsnet.org/doi/full/10.1094/PHYTO-99-7-0869

Schwinn, F.J., (1981). Chemical control of downy mildews. In Spencer D.M. (ed.). The Downy Mildews, pp. 305-319 Academic Press, London, UK

Vanderplank, J.E. (1963) Plant Disease: Epidemics and Control. Academic Press, New York, 349 p.

ПРОГНОЗЕН МОДЕЛ ЗАСНОВАН НА АКУМУЛИРАНИ ДЕНОВИ СО ТЕМПЕРАТУРНА СУМА ЗА ИНКУБАЦИСКИОТ ПЕРИОД НА *Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni

Глигор Бојков[1*], Емилија Арсов[1], Саша Митрев[1]

[1]Земјоделски факултет, Катедра за заштита на растенијата и животната средина, Универзитет „Гоце Делчев", УНИЛАБ лабораторија, Штип, Република Северна Македонија

**Контакт-автор: gligorbojkov@yahoo.com*

Резиме

Појавата на *Plasmopara viticola* со нејзиниот комплетен развоен циклус во времето и просторот овозможува брзо интензивирање на болеста. Периодот на инкубацијата ја претставува фазата на развој од биолошкиот циклус на *P. viticola* помеѓу инфекцијата и првото појавување на симптомите на болеста. Прогнозата базирана на акумулирани денови со температурна сума за определување на инкубацијата овозможува предвидување на мал број примарни инфекции чиешто пресметување се врши врз основа на температурниот фактор. Овој модел за прогноза, во суштина, е регресивна анализа која ја прикажува врската помеѓу просечната дневна температура и коефициентот на инкубација. Одредувањето на инкубацијата овозможува правовремено користење на фунгицидите против *P. viticola* и ги дава основните претпоставки за можна појава на примарниот инокулум.

Клучни зборови: *Plasmopara viticola, инкубациски период, просечна дневна температура, модел за предупредување, регресиона анализа, коефициент на инкубација*