

## Forecasting model to predict the first occurrence of *Scaphoideus titanus* nymphal stages in selected locations in Austria

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### ABSTRACT

*Scaphoideus titanus* Ball, 1932 (Hemiptera: Cicadellidae) is the main vector of Grapevine flavescence dorée phytoplasma (FDp), causing the economically important grapevine yellows disease flavescence dorée in Europe. Effective management of this disease relies on vector control and the uprooting of infected grapevines. By controlling the *S. titanus* third nymphal stage (N3), the spread of FDp can be prevented. In Austria, yearly monitoring of *S. titanus* nymphs in different wine-growing regions is necessary to determine the best time for control. The recent phenology changes of *S. titanus* make monitoring scheduling more difficult and propose the use of an accurate forecasting model. The present study aimed to test existing forecasting models for their accuracy and applicability to predict the first seasonal occurrence of the first nymphal stage (N1) of *S. titanus* and to develop new prediction models for N1 and N3 in Austria for the first time. Monitoring data from 2013 to 2020 from six different wine-growing areas in Austria were analysed. The existing forecasting models examined in this study predicted the first seasonal occurrence of N1 on average 3.3 days too early or 5.8 days too late, whereas the newly developed multiple linear regression model (MLR) for N1 predicted the first seasonal occurrence on average 3.4 days too early. The newly developed model for N3 predicted the first occurrence on average 6.6 days too early. To continuously improve the multiple linear regression models additional datasets, in particular from years with extreme weather events, should be included in the analysis.

**Keywords:** American grapevine leafhopper, forecasting, multiple linear regression model, vector, Grapevine flavescence dorée, seasonal occurrence

### INTRODUCTION

The American grapevine leafhopper *Scaphoideus titanus* Ball, 1932 (Hemiptera: Cicadellidae) is an introduced leafhopper species in Europe, that became invasive and the main vector of the Grapevine flavescence dorée phytoplasma (FDp) on grapevines (Schvester et al., 1962; Caudwell, 1990; Chuche and Thiéry, 2014; EFSA PLH Panel 2014; Linder, 2016). FDp causes flavescence dorée (FD), a yellows disease that affects the vitality and yield of infected grapevines as well as the quality of the wine. FD can lead to the destruction of whole vineyards, if no appropriate measures are taken (Ge and Wen, 2006; Bellomo et al., 2007; Morone et al., 2007; EFSA PLH Panel et al., 2016). FDp is listed as Union quarantine pest

in the European Union, because of the severe economic damage it can cause to viticulture (EC, 2019). Because the phytoplasma itself cannot be controlled, it is important to uproot infected grapevines and to keep the population of *S. titanus* at a low level, to prevent the transmission of FDp from affected to healthy vines. *S. titanus* is an univoltine species that overwinters in the egg stage under the bark of grapevines. After completing five nymphal stages, the adult leafhoppers emerge in July (Schvester et al., 1962). Newly hatched first nymphal stages (N1) are always free of FDp. *S. titanus* mainly feeds on the genus *Vitis* sp. and gets infected by feeding on FDp-infected grapevines (Linder, 2016). After a latency period of one

to four weeks, *S. titanus* is able to transmit FDp (Alma et al., 2018). The greatest risk of FD spread arises from the adults, which are more mobile than the nymphs and capable of active dispersal (Lessio et al., 2014; Angelini et al., 2018). FD poses a major threat to viticulture because it can spread epidemically in vineyards in the presence of *S. titanus* (Morone et al. 2007; Chuche and Thiéry, 2014), which plays a key function in the epidemiology of FD as the main vector that rapidly spreads FDp. Its suppression is crucial for FD spread prevention or containment.

*S. titanus* was first recorded in France in the 1950s and since then spread to several other European wine-growing countries (Bonfils and Schvester, 1960). In 2021, *S. titanus* was reported from 19 European countries (EFSA et al., 2020; EPPO, 2022). In Austria, *S. titanus* was first ascertained in 2004 in vineyards in southeastern Styria, in 2010 in the South of Burgenland and in 2015 in the Northeast of Lower Austria (Rebschutzdienst, 2020; Strauss et al., 2014). FD-infected single grapevines were first detected in 2009 in Styria and in 2015 in Burgenland (Reisenzein and Steffek, 2011; Steffek et al., 2011; EPPO, 2018). Regional regulations to eradicate FD and to control the vector were adopted, following the first detection of FD in grapevines in Styria and Burgenland (LGBl, 2010; LGBl, 2016). Demarcated areas and buffer zones were delimited, in which systematic monitoring had to be performed every year to survey the occurrence of the vector and the disease. Obligatory measures are the uprooting of single-infested grapevines or the whole vineyard depending on the incidence of FD infection and the mandatory treatment against *S. titanus* in or also outside the demarcated areas, depending on the size of the vector population. To keep the vector population low and to prevent FDp transmission from grapevine to grapevine by *S. titanus*, the first treatment is recommended as soon as the first specimen belonging to the third nymphal stage (N3) appears. At this time, phytoplasma transmission can be prevented and most of the nymphs can be controlled (Falzoi et al., 2014).

A time-intensive monitoring is conducted every year for several weeks (4-6) in the different Austrian wine-growing regions to determine the occurrence of the target instar (N3) and the best timing for the plant protection product application. In Austria, the first N3 usually appear in the middle of June (grapevine BBCH 65-77) (Lorenz et al., 1994). However, egg hatching and nymphal development can vary considerably depending on the weather, latitude and altitude of the specific wine-growing region. Mild winters and high spring temperatures in recent years resulted in early egg-hatching. However, subsequent low temperatures and frost in May and June delayed the leafhopper development. This made monitoring more time-consuming and the appropriate timing of control measures more difficult. Both monitoring and the timing of insecticide applications could be optimised by the use of a model that accurately predicts the 1<sup>st</sup> seasonal occurrence of N1 and N3 (Maggi et al., 2013; Falzoi et al., 2014; Falzoi et al., 2016).

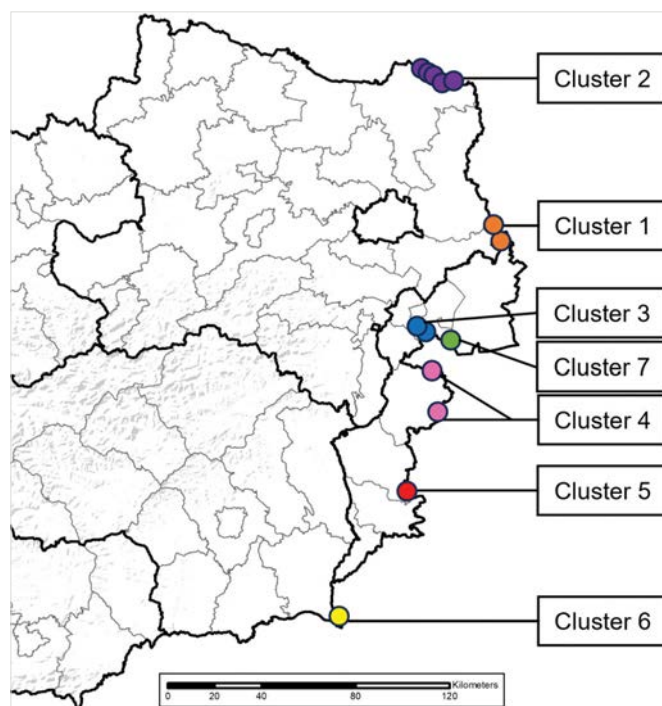
The aims of the present study were 1) to test if currently applied pest forecasting models from other countries can be used to accurately predict *S. titanus* egg hatching in Austria and 2) to develop new multiple linear regression (MLR) models with monitoring data for *S. titanus* in Austria to predict the first seasonal occurrence of N1 and N3. In this study, a linear model was preferred to a logistic model because the focus was on predicting the date of first occurrence and not the development dynamics. The linear regression analysis was selected because of its simplicity and ability to simultaneously consider various independent variables. A linear model allows an easy interpretation of the results for cross-checking their causal validity, regardless of the uncertainties associated with the applied monitoring data and weather data. Furthermore, MLR models developed for other pests displayed good results in predicting the date of first occurrence (Aswathi and Duraisamy, 2018; Balikai, et al., 2019; Blümel et al., 2020). The overall aim was to improve the monitoring and management of *S. titanus* in Austria.

## MATERIAL AND METHODS

### Monitoring data

Data from 13 monitoring sites in different wine-growing regions of Austria over several consecutive years were selected for the analysis. Monitoring sites were clustered according to their vicinity to reference weather stations (Table 1). Cluster 1 and 2 were located in the federal province of Lower Austria, clusters 3 to 5 in Burgenland and cluster 6 in Styria (Figure 1).

The observation data of site cluster 7 (Burgenland) was included as part of the model validation (Table 1, Figure 1). The occurrence of the different nymphal stages and the number of individuals was weekly assessed by visual examination of 400 random leaves (ten plants per four rows and ten leaves per plant) per monitoring site until N3 was detected in each cluster. The start of the monitoring differed from 16 May until 2 June depending on the monitoring year.



**Figure 1.** *S. titanus* monitoring sites (circles) per site cluster, section of Eastern Austria (Source: ©AGES 2019)

**Table 1.** Survey of *S. titanus* N1 / N3 monitoring sites and years

Cluster	Reference weather station (ZAMG)	Latitude	Monitoring sites	Sea level (m)	Monitoring years	Main grapevine variety/site
1	Bruckneudorf	~48°1'N	Hainburg an der Donau	117	2018 - 2020	Welschriesling
			Berg	168	2017 - 2020	Mixed varieties
2	Hohenau	~48°7'N	Drasenhofen	243	2020	White variety
			Steinebrunn <sup>1</sup>	260	2017 - 2018	White variety
			Schrattenberg	238	2016 - 2020	Zweigelt
			Katzelsdorf	232	2017 - 2018	White variety
			Reintal	202	2017 - 2020	Zweigelt
3	Eisenstadt-Nordost	~47°8'N	Siegendorf	175	2013 - 2018	Cabernet Sauvignon
			Wulkaprodersdorf	169	2013 - 2018	Welschriesling
4	Lutzmannsburg	~47°5'N	Deutschkreutz	179	2014 - 2015	Blaufränkisch
			Lutzmannsburg	219	2016 - 2020	Blaufränkisch
5	Kleinzicken	~47°2'N	Eisenberg an der Pinka <sup>1</sup>	372	2013 - 2020	Blaufränkisch
6	Bad Radkersburg	~46°7'N	Sicheldorf	202	2016 - 2020	Isabella
7	Neusiedl am See	~47°6'N	Mörbisch	113	2019 - 2020	Grüner Veltliner

<sup>1</sup> Sea level correction: Steinebrunn -0.42 °C & Eisenberg an der Pinka -0.535 °C.

The developmental stage of nymphs was determined according to the morphological characters described by Della Giustina et al. (1992). The 1<sup>st</sup> nymphal stage was identified with a magnifying glass. However, the 2<sup>nd</sup> and 3<sup>rd</sup> nymphal stages were determined using a field microscope.

Field data of the observed 1<sup>st</sup> occurrence of N1 and N3 at the clustered sites were used for the subsequent model calculations, as follows:

1. for clusters with multiple monitoring sites, the earliest observed dates of N1 or N3 occurrence were used for the calculations;
2. if the earliest appearance of N1 was detected at the same time as the 2<sup>nd</sup> nymphal stage (N2), the dates were not included in the calculation because the real onset of N1 was clearly undetected;
3. if the first appearance of N2 and N3 was determined at the same time, then this date was considered the first appearance of N3.

#### Meteorological data

Weather data (Table 2) were provided by GeoSphere Austria (former Austrian Central Institute for Meteorology and Geodynamics (ZAMG)) for the six reference weather

stations, which were closest to the monitoring site clusters.

The weather data were provided for the period from 15 November 2012 to 30 June 2020. The 15 November was the calculation starting point of the temperature sum model according to Cizej et al. (2017). This date is derived from the length of the diapause, which was reported to last about six months in southern and central European latitudes (Chuche and Thiéry, 2014).

#### Existing forecasting models

An extensive systematic literature search (ELS) (EFSA, 2010) was performed to identify existing prediction models for *S. titanus*. The search of scientific literature was carried out in the electronic database Ovid and on relevant websites (e.g. EPPO). The search terms covered the categories taxonomy, development, weather parameter, host plants and prediction models. The resulting records were evaluated for their relevance with a rating system, based on the forecasting models, weather parameters, phenology, life cycle, biology, occurrence and distribution. The ELS resulted in one temperature-driven phenological model developed by Rigamonti et al. (2011) for the prediction of egg-hatching (N1) and simulation of

**Table 2.** Weather parameters/variables considered in the MLR model development

Measured weather parameter (unit)	Extracted weather variable (unit)
Daily maximum air temperature (°C)	Mean daily maximum temperature (°C)
Daily minimum air temperature (°C)	Mean daily minimum temperature (°C)
Daily mean air temperature (°C)	Mean daily temperature (°C)
	Sum of days with a mean temperature < / > 8.7 °C <sup>1</sup> (No. days)
	Sum of daily mean temperature greater than 7.2 °C / ...± 0.5 °C... / 10.2 °C; 8.9 °C / ...± 0.5 °C... / 11.9 °C base (°C)
Daily precipitation (mm)	The sum of daily precipitation (mm)
	Sum of days with precipitation (No. days)
Daily global radiation (J/cm <sup>2</sup> )	Sum of daily global radiation (MJ/m <sup>2</sup> )
Daily wind speed (m/s)	Mean daily wind speed (m/s)
Daily relative humidity (%)	Mean daily relative humidity (%)

<sup>1</sup> Lower temperature threshold value for egg-hatching (Rigamonti et al., 2011)

the occurrence of *S. titanus* nymphal stages and adults. This model was parametrised and validated with field data collected in western and southern Switzerland. The model of Rigamonti et al. (2011) was not tested to its full extent, as it describes the phenology of *S. titanus* comprehensively from egg hatching to the adult stage. However, the present study exclusively aimed at the accurate prediction of the first seasonal appearance of N1 and N3 and therefore only used the model component for the prediction of N1. For the prediction of the 1<sup>st</sup> occurrence of N1, the sum of the positive differences between the measured daily mean temperature and the lower temperature threshold ( $\geq 8.7$  °C) is accumulated, starting from 15 April until 196.6 degree days (DD) are reached. Henceforth, this component of the phenological model according to Rigamonti et al. (2011) is abbreviated as TSM (temperature sum model) 196.6 DD model.

Additionally, one TSM used in Slovenia to predict the 1<sup>st</sup> occurrence of *S. titanus* N1 (Cizej et al., 2017) was included in the present work, because the climatic conditions in northern and central Slovenia are very similar to those in Austria, especially in Styrian wine-growing areas. Starting from 15 November, the sum of the positive differences between the measured daily mean temperature and the lower temperature threshold ( $\geq 5$  °C) is accumulated until 640 to 740 DD have been reached. Since the TSM according to Cizej et al. (2017) specified a temperature range for the prediction of the 1<sup>st</sup> occurrence of N1, this model was split into two sub-models. Henceforth, the models are abbreviated as TSM 640 DD and TSM 740 DD. More prediction models were found with the ELS but were not tested, either because of lack of applicability of the model variables applied or because the date of first occurrence of N1 and N3 was not predicted (Falzoi et al., 2014; Maggi et al., 2013). A review of the existing predictive models for *S. titanus* and other grapevine pests is given by Lessio and Alma (2021).

#### **Multiple linear regression analysis**

A new prediction model was developed applying MLR analysis (Microcal Origin version 6.0). For the generation of the equations to predict the 1<sup>st</sup> occurrence of *S. titanus* N1 and N3, the days of the year (DOY) with observed 1<sup>st</sup> occurrence of *S. titanus* N1/N3 at the six monitoring

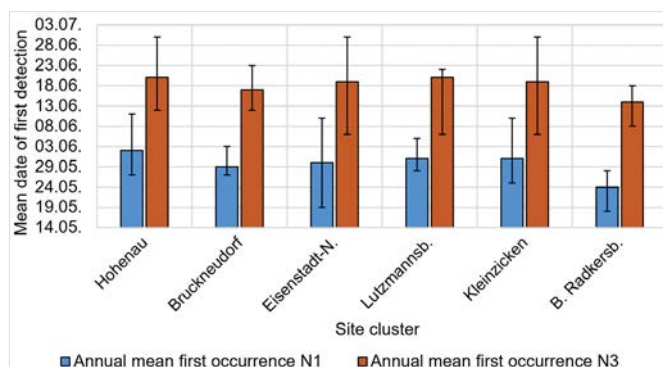
clusters from 2013 to 2019 and different weather parameters (e.g. mean daily maximum or minimum temperature, sum of precipitation, global radiation) were used for the calibration of the model. In addition, temperature sums with different temperature base values were considered. The adapted temperature base values for lower reported temperature threshold values for egg-hatching (8.7 °C) and for nymphal development (10.4 °C) (Rigamonti et al., 2011) were determined by performing a sensitivity analysis using deviations from - 1.5 °C to 1.5 °C in 0.5 °C steps (Table 2). An altitude correction for air temperature was applied for those monitoring sites where the sea level difference between the monitoring site and the weather station was  $> 50$  m; a decrease of 0.5 °C for each 100 m increase of altitude above the sea level was applied. Four different calculation periods were tested (April-June, March-June, February-June and February-May). A preliminary selection of relevant variables was carried out, using the coefficient of determination ( $R^2$ ). Initially, all variables with an  $R^2 \geq 0.30$  were included in the MLR analysis. The generated equations were optimised by gradually replacing and/or removing variables from the analysis by applying a stepwise manual procedure. Equations resulting in the smallest deviations of the calculated from the observed 1<sup>st</sup> occurrence (in DOY) were generated. A tolerance of up to four days for the prediction accuracy was applied, taking into consideration the developmental time between the different nymphal stages according to Falzoi et al. (2014).

The evaluation to determine the best generated equations was carried out with the coefficient of determination, the variance, the standard deviation, the Root-Mean-Square-Error and the mean bias (Table 3). The preliminary validation of the generated equations was carried out with monitoring data from the six site clusters surveyed in 2020 and additional monitoring data from a vineyard in Mörbisch am See (site cluster 7) that was monitored for the first time in 2019 and 2020. The validation of the two equations was carried out with the same statistical indicators as in the performance evaluation (Table 5). For comparison purposes, the TSM results are also provided in table 3 and 5.

## RESULTS

### *Influence of site characteristics on nymphal occurrence*

The overall earliest 1<sup>st</sup> detection of N1 occurred on 18 May 2020; the overall latest 1<sup>st</sup> detection was on 11 June 2019. On average, the earliest 1<sup>st</sup> occurrence of N1 was observed at the southernmost site cluster (Bad Radkersburg) and the latest at the northernmost (Hohenau) site cluster. The 2<sup>nd</sup> most southern, but highest situated monitoring site cluster (Kleinzicken, 372m a.s.l), was one of the two (site cluster Lutzmannsb.) locations with the mean penultimate date of N1 occurrence. Conversely, at the 2<sup>nd</sup> northernmost but 2<sup>nd</sup> lowest situated monitoring site cluster (Bruckneudorf, 143m a.s.l) the mean 2<sup>nd</sup> earliest N1 occurrence was found. For N3, on average the earliest 1<sup>st</sup> occurrence, was observed at the Bad Radkersburg site cluster and the latest 1<sup>st</sup> occurrence at the monitoring site Hohenau. The overall earliest 1<sup>st</sup> detection of N3 occurred on 6 June 2018, and the overall latest 1<sup>st</sup> detection on 30 June 2016 (Figure 2).

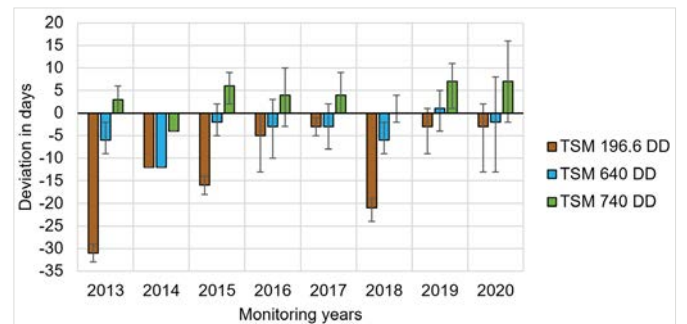


**Figure 2.** First detection of *S. titanus* N1 and N3 per site cluster 1 to 6 on average from 2013 to 2020. The whiskers display the earliest and latest day of first observed occurrence. The site clusters were arranged according to their geographical latitude (clusters from left to right: from the northernmost to the southernmost)

### *Prediction results existing forecasting models*

The comparison of field dates of the 1<sup>st</sup> N1 occurrence with predicted dates calculated with TSM 196.6 DD (Rigamonti et al., 2011) showed for all site clusters that 77% of the predicted dates were too early, 10% were exact and 13% were too late depending on the monitoring year. The TSMs according to Cizej et al. (2017), resulted

for all site clusters in 67% of the predictions too early and 33% too late for the threshold value of 640 DD. In contrast, for the threshold value of 740 DD 13% of the predictions were too early, 17% were exact and 70% were too late. On average, the TSM 640-740 DD displayed lower deviations between the observed and predicted 1<sup>st</sup> occurrence of N1 than the TSM 196.6 DD (Figure 3).



**Figure 3.** Comparison of the mean deviation of the observed and the predicted 1<sup>st</sup> occurrence of *S. titanus* N1 in days for the tested temperature sum-based models (TSM) at the calibration site cluster 1 to 6 from 2013 to 2020. The whiskers display the minimum and maximum deviation between the observed and predicted first occurrence (too early: -x days; too late: +x days)

### *Calibration and validation results of new MLR models*

The calibrated MLR models for predicting the 1<sup>st</sup> occurrence of N1 / N3 were considered to be of high accuracy if the following performance criteria were met:  $R^2 > 0.60$ ,  $RMSE < 5.00$ ,  $BIAS < 1.00$ . In addition, the calibrated MLR N1 model had to perform better in the evaluation than the TSMs (Table 3). For both *S. titanus* nymphal stages, the calculation period from 1 February to 31 May yielded the most accurate prediction results.

The most accurate generated equation for the prediction of the 1<sup>st</sup> occurrence of *S. titanus* N1 was:

$$DOY = 218.5765 + (-2.43865 \times \text{mean daily maximum temperature}) + (-0.01888 \times \text{sum of daily mean temperature above } 7.2 \text{ } ^\circ\text{C base}) + (-0.01556 \times \text{sum of daily global radiation}).$$

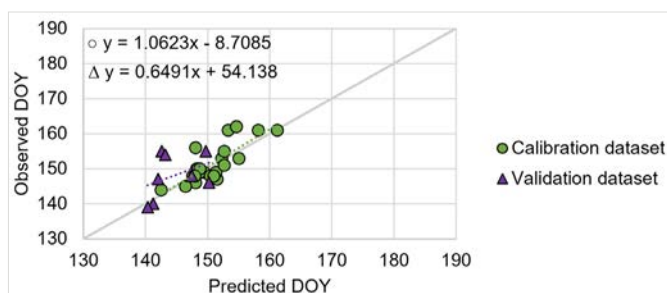
The influence of the weather variables “mean daily maximum air temperature”, “temperature sum above 7.2 °C base” and “sum of daily global radiation” on the 1<sup>st</sup> occurrence of *S. titanus* N1 showed different degrees of significance (Table 4) and no multicollinearity.

**Table 3.** Simulation performance of best-generated equations (MLR models) to predict the 1<sup>st</sup> seasonal N1 / N3 occurrence of *S. titanus* based on calibration datasets, in comparison to the three TSM

Monitoring years 2013-2019 (Cluster 1 to 6)			N dataset	Statistical indicators <sup>1</sup>				
Nymphal stage	Model type	1 <sup>st</sup> occurrence		R <sup>2</sup>	σ <sup>2</sup>	SD	RMSE	BIAS
N1	TSM 196.6 DD	observed	24	0.41	28.16	5.19	14.71	-10.71
		predicted						
	TSM 640 DD	observed	24	0.43	28.16	5.19	5.61	-3.17
		predicted						
	TSM 740 DD	observed	24	0.47	28.16	5.19	5.70	3.71
		predicted						
MLR N1 model	observed	24	0.63	28.16	5.19	3.26	-0.68	
	predicted							
N3	MLR N3 model	observed	25	0.62	57.07	7.40	4.60	0.05
		predicted						

<sup>1</sup> R<sup>2</sup> = coefficient of determination; σ<sup>2</sup> = variance; SD = standard deviation; RMSE = Root-Mean-Square-Error; BIAS = mean bias (negative/positive BIAS = forecast is biased xx days too early / too late)

The correspondence between the observed and predicted day of year (DOY) of the 1<sup>st</sup> N1 occurrence indicated a moderate accuracy with the validation dataset (Figure 4).



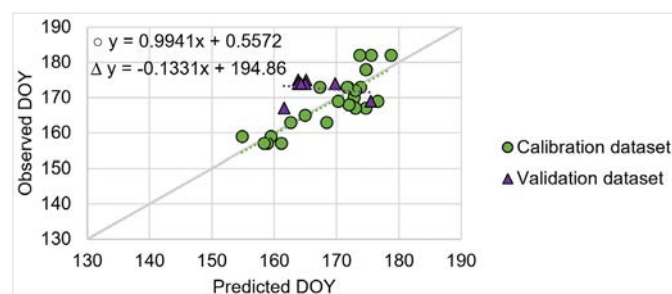
**Figure 4.** Linear regression between the observed and predicted (MLR model) days of the year (DOY) of 1<sup>st</sup> occurrence of *S. titanus* N1 at all site clusters, from 2013 - 2020

The most accurate generated formula for the prediction of the 1<sup>st</sup> occurrence of *S. titanus* N3 was:

$$\text{DOY} = 249.0398 + (2.56728 \times \text{mean daily minimum temperature}) + (-0.06048 \times \text{sum of daily mean temperature above } 7.2 \text{ } ^\circ\text{C base}) + (-0.03691 \times \text{sum of daily global radiation}) + (-0.0272 \times \text{sum of daily precipitation}).$$

The influence of the weather variables “temperature sum above 7.2 °C base”, “mean daily minimum air temperature”, “sum of daily precipitation” and “sum of

daily global radiation” on the 1<sup>st</sup> occurrence of *S. titanus* N3 showed different degrees of significance (Table 4) and no multicollinearity. The correspondence between the observed and predicted DOY of the 1<sup>st</sup> N3 occurrence indicated a moderate to poor prediction accuracy of the MLR N3 model with the validation dataset (Figure 5).



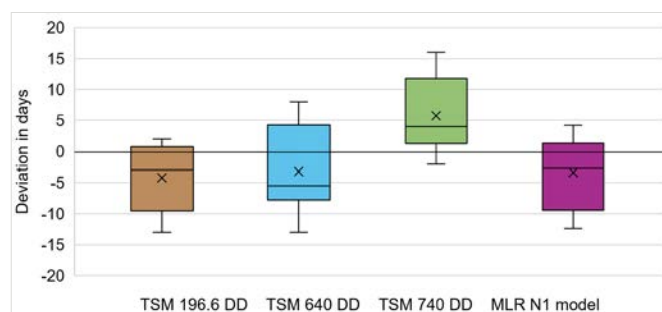
**Figure 5.** Linear regression between the observed and predicted (MLR model) days of year (DOY) of 1<sup>st</sup> occurrence of *S. titanus* N3 at all site clusters, from 2013 - 2020

The comparison of the prediction accuracy of the different models for the 1<sup>st</sup> seasonal N1 occurrence showed that the biggest deviations between observed and predicted dates were calculated with the TSM 740 DD. The TSM 196.6 DD and the MLR N1 model displayed the smallest deviations for the validation dataset (Figure 6).

**Table 4.** Significance (*P*) and test for multicollinearity (VIF: variance inflation factor) of the weather variables applied in the calibrated MLR models

Weather variable	MLR N1 model		MLR N3 model	
	<i>P</i>	VIF	<i>P</i>	VIF
Mean daily maximum air temperature	<0.0001	1.271	-	-
Mean daily minimum air temperature	-	-	0.041	1.794
Temperature sum above 7.2 °C base	0.004	1.431	0.002	2.603
The sum of daily global radiation	0.054	1.729	0.125	2.008
The sum of daily precipitation	-	-	0.062	1.246

The preliminary validation of the two generated best equations displayed a moderate prediction accuracy for the MLR N1 model and a poor accuracy for the MLR N3 model. The preliminary validation of the three TSMs displayed overall a moderate prediction accuracy. On average, the 1<sup>st</sup> occurrence of N1 was predicted 3.3 days too early by the TSM 640 DD, 3.4 days too early by the MLR N1 model, 4.3 days too early by the TSM 196.6 DD and 5.8 days too late by the TSM 740 DD for site clusters 1 to 6 in 2020 and site cluster 7 from 2019 to 2020. The 1<sup>st</sup> occurrence of N3 was predicted by the MLR N3 model on average 6.6 days too early for site clusters 1 to 6 and site cluster 7 (Table 5).

**Figure 6.** Comparison of the deviation of the observed and the predicted 1<sup>st</sup> occurrence of *S. titanus* N1 of the temperature sum based models (TSM) and the multiple linear regression (MLR) N1 model in days with the validation dataset (*N* = 8)**Table 5.** Statistical indicators for validating the best generated equations (MLR models) to predict the 1<sup>st</sup> N1 / N3 occurrence of *S. titanus*, in comparison to the three TSM

Monitoring years 2020 (Cluster 1 to 6) & 2019-2020 (Cluster 7)			<i>N</i> dataset	Statistical indicators <sup>1</sup>				
Nymphal stage	Model type	1 <sup>st</sup> occurrence		<i>R</i> <sup>2</sup>	$\sigma^2$	SD	RMSE	BIAS
N1	TSM 196.6 DD	observed	8	0.21	40.57	5.96	6.84	-4.25
		predicted			14.79	3.60		
	TSM 640 DD	observed	8	0.19	40.57	5.96	7.45	-3.25
		predicted			49.36	6.57		
	TSM 740 DD	observed	8	0.28	40.57	5.96	8.14	5.75
		predicted			39.93	5.91		
	MLR N1 model	observed	8	0.16	40.57	5.96	6.56	-3.39
		predicted			15.47	3.85		
N3	MLR N3 model	observed	8	0.04	9.07	2.82	8.58	-6.62
		predicted			19.69	4.15		

<sup>1</sup> *R*<sup>2</sup> = coefficient of determination;  $\sigma^2$  = variance; SD = standard deviation; RMSE = Root-Mean-Square-Error; BIAS = mean bias (negative/positive BIAS = forecast is biased xx days too early / too late)

## DISCUSSION

The aim of this study was to identify a suitable prediction model that accurately predicts the 1<sup>st</sup> seasonal occurrence of the 1<sup>st</sup> (N1) and 3<sup>rd</sup> (N3) nymphal stage of *S. titanus* in Austria. For this purpose, two existing temperature sum-based models (TSM) (Rigamonti et al., 2011; Cizej et al., 2017) were tested and new multiple linear regression models (MLR) were developed. The TSM 196.6 DD (Rigamonti et al., 2011) was developed for setting up monitoring activities and improving the timing of management measures primarily for the wine-growing areas in Switzerland. The model considers both, the development of *S. titanus* eggs from the end of post-dormancy and nymphal emergence. In the frame of the present study, predictions made with this model mainly resulted in a too-early prediction of the 1<sup>st</sup> occurrence of N1 in the considered Austrian wine-growing regions. One reason for the relatively large deviations between calculated and observed occurrences could be the climatic conditions prevailing in the region for which the forecasting model was developed. In the southern part of Ticino, where the wine-growing areas are located and from where the data for the model originated, the Mediterranean climate type is dominating with different seasonality of temperature and precipitation regimes, compared to the prevailing climatic conditions at the Austrian monitoring site clusters investigated in this study. Only the Bad Radkersburg site cluster showed more similar spring weather conditions, which could also be a reason why the prediction at this site corresponded best with the observed occurrences. Another influencing factor may be due to the fact that the model was calibrated with monitoring and weather data from 2006, 2008 and 2009. Since then, however, the average temperature has continuously increased, with possible implications for the phenology of *S. titanus*. The average temperature in 2013-2022 was 0.6 °C higher than in the period of 2003-2012 (MeteoSchweiz, 2023). Rigamonti et al. (2011) already pointed out that the model would need an improvement if the winters became warmer. Since the predicted and observed 1<sup>st</sup> N1 occurrence in Austrian wine-growing regions displayed larger deviations in some years, the

prediction of N3 with the model of Rigamonti et al. (2011) was no longer considered, as the prediction of the later developmental stage depends on the prediction of the previous one, which was already of low accuracy.

In the TSM by Cizej et al. (2017) the first N1 is predicted to occur between 640 DD to 740 DD and this was also the case for most N1 in this work. However, the actual aim of this study was to determine a specific date for the first occurrence and not a time period. The TSM 640 DD and 740 DD displayed for the calibration dataset smaller deviations between the predicted and the observed 1<sup>st</sup> occurrence of N1 than the TSM 196.6 DD. The reason for this is probably the similar climate of the considered wine-growing regions in Slovenia and Austria. The average temperatures from February to May (1991-2021) in south-eastern Styria (Graz = 7.0 °C) and eastern Slovenia (Maribor = 7.9 °C) are very similar which leads to a similar first occurrence of N1 (Climate-Data, 2023).

The TSM considers only one weather parameter (temperature), which is the most important abiotic factor influencing arthropod development (Briere et al., 1999). The impact of other weather parameters (relative humidity and photoperiod) besides temperature on *S. titanus* is often discussed but has not been thoroughly studied yet (Lessio and Alma, 2004b; Chuche et al., 2014). Apart from temperature, it is known that photoperiod also has an influence on the onset of diapause and duration of the succeeding dormancy (Tauber and Tauber, 1976; Danks, 2002; Chuche and Thiéry, 2009; Chuche and Thiéry, 2012). The relative humidity was found to affect the flight activity of the adults and therefore could also impact the nymphal stages in some way (Lessio and Alma, 2004a; Lessio and Alma, 2004b). Further studies will be required to test the impact of different weather variables on the development of *S. titanus*. The inclusion of additional weather parameters led to improved prediction accuracy with MLR models for the 1<sup>st</sup> seasonal occurrence of adult grapevine moth (*Lobesia botrana*) and grapeberry moth (*Eupoecilia ambiguella*) (Blümel et al., 2020). Another advantage of MLR models is the possibility to predict the 1<sup>st</sup> occurrences of the different developmental

stages independently from each other. Therefore, new MLR models (Aswathi and Duraisamy, 2018; Balikai, et al., 2019), including additional weather parameters were developed in the present study. The MLR analysis showed that temperature, precipitation and the global radiation are the most important weather parameters for the prediction of the 1<sup>st</sup> occurrence of *S. titanus* N1 and N3. The test for multicollinearity showed that the weather variables applied in the MLR models were only slightly correlated and therefore had an independent effect on the regression models.

The prediction accuracy of the newly generated MLR N1 model was higher or at least as good as the TSMs which could be explained by the following reasons. First, the calibration of the MLR N1 model with regional monitoring data have led to a higher accuracy in predicting the 1<sup>st</sup> occurrence of N1 at the Austrian site clusters. However, the risk for such a bias in the prediction accuracy should be rather small, as the MLR N1 model allowed the flexibility to incorporate site-specific conditions into the forecast. Another reason for the high accuracy of the predictions could be due to the fact that the monitoring data used were obtained recently and therefore also consider shifting in the 1<sup>st</sup> occurrence of *S. titanus* probably caused by climate change. The third reason for the improved forecast is due to the use of additional weather parameters. Several weather parameters were previously tested specifically for their influence on the development of N1 and N3, and only the parameters with a high impact were included in the calculations. Lastly, the defined calculation period (February to May) had a positive impact on the high prediction accuracy of the MLR N1 model. The incorporation of winter months in the calculation period has led to an increased prediction accuracy of the newly developed prediction models. This could also explain why the TSM that accounts for the temperatures already from 15 November (Cizej et al., 2017) shows a greater accuracy than the TSM that accumulates the degree days only from 15 April (Rigamonti et al., 2011).

Concerning the prediction quality for N3, the validation of the MLR model displayed a moderate to poor prediction quality, which could be due to the limited number of monitoring datasets. Although the validation results are not satisfactory, the generated MLR N3 model is an important first step to improving the N3 monitoring in Austria. The generated MLR N3 model will be further validated and subsequently adapted with ongoing monitoring data from several years. The MLR N3 model adaptation will be part of a further study. Both MLR models could be also improved with additional monitoring data considering ongoing climate change, especially of years with extreme weather events to verify whether the prediction accuracy is still given under such conditions. Furthermore, the influence of site-specific characteristics such as the slope or green cover which could affect the 1<sup>st</sup> occurrence of *S. titanus* N1 and N3, could be tested (Falzoi et al., 2016). Site-specific fluctuations in the occurrence of *S. titanus* nymphal stages and adults were also described by Decante and Van Helden (2006).

The MLR equations were calibrated with the observed dates of the first occurrence of N1 / N3 in the vineyards and the generated equations with the best conformity were selected. Although the predictions with the newly developed MLR models seem very promising, it is important to mention that there is some uncertainty in the data with which the equations were calibrated. The visual assessment of the nymphal stages in the field was carried out once a week, with a data gap regarding the absence or presence of nymphs for six days. Therefore, it cannot be excluded that N1 / N3 was detected too late on some days. In addition to the limitations mentioned regarding the monitoring data (e.g.: data quantity and frequency of observations), the weather data also displayed various limitations (e.g.: availability of different weather parameters, data gaps and in some cases far distance between the reference weather station and the monitoring site). These limitations were considered in the MLR analysis and the accuracy of the generated models was very satisfying. The newly generated MLR model

for predicting the 1<sup>st</sup> occurrence of N1 and an improved MLR N3 could facilitate the planning of monitoring activities and pest control in the future. In particular, a precise prediction model for *S. titanus* N3 could help the winegrower to optimise decision-making concerning pest control.

## CONCLUSION

New prediction models based on multiple linear regression analysis, incorporating three to four different weather variables, were developed to predict the 1<sup>st</sup> and 3<sup>rd</sup> nymphal stages of *S. titanus*. The newly developed forecasting model predicting the 1<sup>st</sup> occurrence of N1 in Austria displayed a slightly improved prediction accuracy in contrast to the existing temperature sum models. Although the N3 prediction model needs to be further improved it allows us to make first decisions regarding the timing of monitoring and management measures. Further refinement of both MLR models in the future seems reasonable, as it has already been shown in this work that the consideration of other weather parameters besides temperature improves the prediction accuracy.

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