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SPREAD PARAMETERS OF THE BORER *XYLOTRECHUS ARVICOLA* (OLIVIER) (COLEOPTERA: CERAMBYCIDAE) IN A 'TEMPRANILLO' VINEYARD IN LA RIOJA (SPAIN): A LONG-TERM STUDY

PARÂMETROS DE DISSEMINAÇÃO DA BROCA *XYLOTRECHUS ARVICOLA* (OLIVIER) (COLEOPTERA: CERAMBYCIDAE) EM VINHAS DE TEMPRANILLO NA RIOJA (ESPANHA): UM ESTUDO DE LONGO PRAZO

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SUMMARY

Xylotrechus arvicola (Olivier) (Coleoptera: Cerambycidae) is a polyphagous xylophagous beetle that is becoming a pest of increasing importance for vineyards in Spain, also because of the wood fungi developing in the galleries excavated by its larvae, which cause a progressive decline of the affected grapevines, until death. Between 1993 and 2015, a survey of the infestation caused by *X. arvicola* and the symptoms caused by pathogenic wood fungi was performed in a 'Tempranillo' variety vineyard in La Rioja region (Spain). Maps showing the overtime spread of the borer and the diffusion of symptoms of grapevine decline and *Eutypa* dieback were obtained. Results indicated that the borer colonization began in the centre of the plot, followed by the first symptoms caused by the wood fungi a few years later. The statistical analysis showed that the evolution of infestation is characterized by a linear increase of new holes whereas the pattern of their allocation in the vines follows a bimodal distribution which, to some extent, can be simulated by a Poisson's model. Based on these observations, a methodology to estimate the state of the infestation over time is proposed. The procedure - based on a linear regression of the average number of holes per vine over a set of years - can be applied in a relatively simple way and provides the probability for a grapevine to have a certain number of exit holes in a definite year with a mean error of around 5%.

RESUMO

Xylotrechus arvicola (Olivier) (Coleoptera: Cerambycidae) é um escaravelho xilófago polífago que está a tornar-se uma praga de importância crescente para as vinhas em Espanha, também devido aos fungos do lenho que se desenvolvem nas galerias escavadas pelas suas larvas, que provocam um declínio progressivo das videiras afectadas, até à sua morte. Entre 1993 e 2015, foi realizado um estudo da infestação causada por *X. arvicola* e dos sintomas causados por fungos do lenho em uma vinha da variedade 'Tempranillo' na região de La Rioja (Espanha). Os Mapas obtidos mostram o tempo de propagação da larva e o aparecimento dos sintomas do declínio da videira e posterior morte por Eutypa. Os resultados indicaram que a colonização pela broca começou no centro da parcela, surgindo alguns anos depois os primeiros sintomas causados pelos fungos do lenho. A análise estatística mostrou que o crescimento da infestação é caracterizado por um aumento linear de novas galerias, enquanto o padrão evolução nas videiras segue uma distribuição bimodal que, em certa medida, pode ser simulada por um modelo de Poisson. Com base nessas observações, é proposta uma metodologia para estimar o nível de infestação ao longo do tempo. O procedimento - baseado em uma regressão linear do número médio de galerias por videira durante um período de anos - pode ser aplicado de uma forma relativamente simples e indica a probabilidade de uma videira ter um certo número de orifícios de saída em um ano definido com um erro médio de cerca de 5%.

Key-words: Grapevine decline, long-horned beetles, wood fungi, xylophagous pest, infestation evolution.
Palavras Chave: Declínio da videira, escaravelhos de antenas longas, fungos do lenho, praga xilófaga, evolução da infestação.

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INTRODUCTION

Wood-boring long-horned beetles can damage tree crops and important economic losses are being reported from all over the world (Grebennikov *et al.*, 2010). Inside the Cerambycid family, the genus *Xylotrechus* has over 180 species (Özdikmen and Tezcan, 2011). One of them, *Xylotrechus arvicola* (Olivier, 1795) (Coleoptera: Cerambycidae) is becoming an important pest in several Spanish vine-growing areas, which include many Designations of Origin along with other delimited areas, such as La Rioja (Ocete and Del Tío, 1996), Castilla and León (Moreno *et al.*, 2004), Castilla-La Mancha, Valdepeñas, Navarra (Ocete *et al.*, 2004) and Murcia (Lucas Espada, 2008). Besides grapevines, infestations by this borer were also detected in plots of *Prunus spinosa* L., whose fruits are used in Navarra to produce a typical liqueur (Pacharán) (Biurrun *et al.*, 2007), as well as in other stone fruit tree species like *Prunus armeniaca* L. (apricot), *P. cerasifera cv. pisardii* (Carrière) Koehne (cherry plum), *P. dulcis* (Mill) DA Webb (sweet almond) and *P. domestica* subsp. *italica* (Borkh.) Gams ex Hegi (greengage) (Ocete *et al*., 2009).

The main hosts of *X. arvicola* are usually willows (*Salix* spp.) and poplars (*Populus* spp.) but it can occur also on plants of the genus *Quercus, Carpinus, Castanea, Fagus, Ficus, Tilia, Morus, Sorbus, Crataegus, Prunus, Malus* and *Cidonia* (Vives, 1984). In Spain, this borer is commonly found along rivers and creeks in the Northern-Central regions of the country, from the Cantabrian coast (Basque Country) to the Sierra Nevada mountain range (Eastern Andalusia) (Bahillo, 1995; Bahillo and Iturrondobeitia, 1996; Vives, 2001).

X. arvicola is a polyphagous species with a holomediterranean distribution, which usually inhabits dead wood and decaying trees of many wild/spontaneous and ornamental plants (Vives, 1984; Ocete *et al*., 2009), including those affected by wood fungal diseases. After mating, females lay about 200 eggs (García-Ruiz *et al*., 2012) in the cracks, crevices or wounds of the plant, in the proximity of the pruning cuts or underneath the crust rhytidome. The emerging larvae bore into the wood and make galleries inside the woody plant tissue. In field conditions, the larval development takes more than one year and a broader gallery connected to the outside is excavated where the larva metamorphose into pupa. The exit holes are round, about four millimeters in diameter. Adults emerge about 20-30 days after pupation. A major revision of the species biology was carried out by Armendáriz *et al*. (2016).

In the vineyards, detection of this borer usually occurs during pruning, when the exit holes of the adults can be observed in the trunk and the thicker branches of the vines. The damage is caused by the feeding activity of the larvae, known as "the grapevine screw" for their truncated conic shape. They excavate numerous large galleries inside the wood, resulting in progressive stunting and eventual death of the affected branches, which ultimately affects yield and wine quality (Ocete *et al*., 2004, 2008, 2009).

A pheromone is produced by males to attract females (Hall *et al*., 2007; Rodríguez-González *et al*., 2017) but it is not effective in traps (Armendáriz *et al*., 2016). There is no indication of how adults localize vines, although the use of visual clues, aggregation pheromones or specific kairomones might be involved, either alone or in combination.

Several fungal pathogens colonize the galleries excavated by the larvae in the arms of the vines (García-Benavides *et al*., 2013), further affecting productivity and vineyard longevity. Besides, affected vines become extremely fragile and are more susceptible to breakage following strong winds or the passage of machinery during crop management operations (Armendáriz *et al.*, 2008; Rodríguez-González *et al*., 2019).

The first infestation of *X. arvicola* in the vineyards was recorded in 1969-1970 in Tirgo, La Rioja territory (Ocete and Del Tío, 1996). Increasing reports of this borer as a grapevine pest are being recorded also in other Spanish wine producing areas, like Castilla and León (Moreno *et al*., 2004). However, to date, this cerambycid has never been recognized as a pest of vineyards in the nearby wine producing countries showing a Mediterranean climate, such as France, Italy, Portugal or Northern Africa countries, or in other Spanish wine-growing areas with similar climate, crop varieties and agricultural management, such as Extremadura and Andalucía.

Most specialists suggest multiple reasons for outbreaks of this borer in vineyards. Hypotheses include the intensification of grapevine cultivation, the age of plantations, the ban on the use of sodium arseniate as a winter biocide treatment (García-Calleja, 2004), the pruning system and the lack of wound seal practices (Peláez *et al*., 2006; Ocete *et al*., 2002a,b), as well as the absence of long periods of frost possibly associated with climate change (Álvarez and Villarías, 2002). The total area threatened by this cerambycid pest is about 695,000 hectares, which represents more than a half of the entire Spanish wine-growing area (1.1 million hectares) (Soria *et al*., 2013).

X. arvicola is able to complete its life cycle on the vines but there is a different susceptibility between the grape varieties (Ocete *et al*., 2002b), possibly related with timber composition (Rodríguez-González *et al*., 2016-b). Quality of food is

reflected in the female fecundity (García-Ruiz *et al*., 2012), as demonstrated by the choice of females for specific vine varieties. Ocete *et al*. (2017) found a higher infestation in the 'Tempranillo' variety, than in the 'Viura' variety.

The purpose of this article was to use a systematic multi-year survey to describe the process of attack and colonization by *X. arvicola* and the associated wooden fungi, resulting on the loss of an entire vineyard. The study also aimed to identify the parameters describing the expansion rate, in order to establish the distribution pattern and to create a model for predicting the evolution of the infestation over time.

MATERIAL AND METHODS

The analysed grapevine plot was a rectangle of 250 by 80 meters located in "Huerta La Rad" estate belonging to the municipality of Tirgo (La Rioja), with coordinates 42º31'56" N and 2º55'57" W. Its southern limit is only about 400 m from the plot where the first appearance was detected in 1969-70 at pruning time (Ocete, personal observations). Later, these symptoms were detected in several plots of La Rioja in the 1980s (Ocete and Del Tío, 1996).

The cultivar was Tempranillo grafted on Richter 110 rootstock with a traditional training *en vaso* (a kind of short pruning), with three arms. The total number of vines was 4,000, with plants every 1.25 m along rows separated 2.80 m.

The monitoring of *X. arvicola* exit holes started in 1993 on a two-year basis and the first occurrences were detected in 1997, ten years after the planting (1987). In the meantime, nearby areas were used also as vineyard ('Tempranillo' and 'Viura' varieties) or cultivated with herbaceous crops, such as cereals and beets.

As regards the chemical treatments applied yearly to the vineyard (Table I), the most important were to control the European grapevine moth *Lobesia botrana* (Dennis & Schiffermüller) (Lepidoptera: Tortricidae), with two applications; first around 20th June, after vines flowering, and the second around 20th July, when the berries are the size of a pea. For the control of the European red mite, *Tetranychus urticae* (Koch) (Acari: Tetranychidae), only one application was done at the end of April after sprouting.

Table I

Active ingredients used in the plot against *Lobesia botrana* and *Tetranychus urticae*

Substâncias ativas usadas para controle da Lobesia botrana e Tetranychus urticae

		Lobesia botrana		*Tetranychus urticae*
1995-2005	First applic.	Chlorpyrifos	1997-2005	Dimethoate
	Second applic	Fenitrothion	2006-2015	Abamectin
2006-2009	First applic.	Spinosad		
	Second applic.	Spinosad		
2010-2015	First applic.	Thiodicarb		
	Second applic.	Thiodicarb		

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The last winter treatment made with sodium arsenite was performed in 2003 and it did not stop the activity of the borer. Finally, the vineyard was uprooted in 2015.

All data were collected (Table II) between 1997 and 2015 (previous years did not show exit holes). Observations were performed as follows:

- On a two-year basis.
- Every five vines along four selected rows among the twenty rows existing in the plot (border rows were avoided).
- Observations of adult holes appearance were made in winter time, when plants were leafless and exit holes were more visible. In every occasion, the quantity of previous holes was subtracted to the observed one. In order to avoid imprecise counting, during the sampling of each vine, separated annotations were made regarding the number of holes in the trunk and for each of the three branches that were held for the next year; therefore, the values recorded in the Table present the sum of these differences.
- Selected rows were separated by four un-sampled rows (Figure 1).

Table II

Complete dataset

Conjunto de dados

Grapevine	Coordinates UTM-ETRS89 zone 30 X	Y	Total number of exit holes by year 1997	1999	2001	2003	2005	2007	2009	2011	2013	2015	Fungi detected in (year)
A005	505477.89	4708977.53	0	0	0	0	0	0	0	0	1	1	undetected
A010	505481.65	4708972.40	0	0	0	0	0	0	0	1	1	2	undetected
A015	505485.40	4708967.26	0	0	0	0	0	0	0	0	2	2	undetected
A020	505489.09	4708962.38	0	0	0	0	0	0	1	1	3	3	undetected
A025	505492.79	4708957.25	0	0	0	0	0	0	0	0	1	1	undetected
A030	505496.60	4708952.38	0	0	0	0	0	0	0	1	1	3	undetected
A035	505500.48	4708947.23	0	0	0	0	0	0	0	0	1	3	undetected
A040	505504.23	4708942.30	0	0	0	0	0	0	2	3	3	3	2015
A045	505507.99	4708937.12	0	0	0	0	0	1	3	4	4	6	undetected
A050	505511.53	4708932.22	1	1	1	1	1	2	2	3	3	4	undetected
A055	505515.23	4708927.05	1	1	2	2	2	2	2	4	4	6	undetected
A060	505519.08	4708922.00	0	0	0	0	1	2	2	3	3	5	undetected
A065	505522.79	4708916.90	0	0	2	2	3	3	5	5	5	7	2011
A070	505526.34	4708912.00	1	3	3	4	5	5	6	8	8	9	2009
A075	505530.17	4708906.89	1	2	4	4	4	5	5	6	7	7	2007
A080	505533.87	4708901.83	2	2	3	5	5	5	6	8	9	9	2009
A085	505537.67	4708896.86	1	1	3	3	4	4	4	6	7	7	2007
A090	505541.51	4708891.64	2	2	4	5	5	6	7	8	9	10	2009
A095	505545.25	4708886.59	2	2	3	3	3	4	6	6	6	8	2009
A100	505548.71	4708881.71	0	2	2	3	3	3	4	5	7	7	2009
A105	505552.63	4708876.59	1	1	3	3	4	4	5	5	5	7	2011
A110	505556.46	4708871.52	2	2	3	4	4	5	7	7	8	8	2009
A115	505559.93	4708866.50	1	2	2	2	2	3	5	5	6	6	2013
A120	505563.77	4708861.54	1	2	2	4	6	6	6	7	7	8	2013
A125	505567.57	4708856.34	2	3	3	5	5	5	6	6	8	9	undetected
A130	505571.26	4708851.25	1	1	3	3	3	3	5	5	5	5	undetected
A135	505574.96	4708846.39	2	2	3	3	4	4	5	5	7	8	undetected
A140	505578.64	4708841.14	1	1	1	2	2	3	3	5	5	7	2011
A145	505582.46	4708836.00	2	2	2	2	4	4	5	5	6	8	2007
A150	505586.23	4708831.13	2	2	3	4	4	5	5	6	6	9	undetected
A155	505589.89	4708826.11	1	1	1	1	2	2	3	5	5	5	undetected
A160	505593.50	4708821.07	1	1	1	2	2	2	2	3	3	4	2015
A165	505597.43	4708815.92	0	0	0	2	2	3	3	3	3	4	undetected
A170	505601.07	4708810.85	1	1	1	1	3	3	3	3	3	5	undetected
A175	505604.79	4708806.03	0	0	0	0	2	2	2	2	4	4	undetected
A180	505608.44	4708800.95	0	0	0	0	0	2	3	3	3	3	undetected
A185	505612.24	4708795.70	0	0	0	0	1	1	1	1	1	2	undetected
A190	505615.89	4708790.81	0	0	0	0	0	2	2	2	2	3	undetected
A195	505619.60	4708785.65	0	0	0	0	1	1	1	1	1	1	undetected
A200	505623.20	4708780.65	0	0	0	0	0	0	1	1	1	1	undetected
F005	505467.62	4708968.04	0	0	0	0	1	1	1	3	3	3	undetected
F010	505471.38	4708962.95	0	0	0	0	0	0	0	2	2	4	undetected
F015	505475.13	4708957.86	0	0	0	0	0	0	1	1	1	2	undetected

Table II

(continued)

Grapevine	Coordinates UTM-ETRS89 zone 30 X	Y	Total number of exit holes by year 1997	1999	2001	2003	2005	2007	2009	2011	2013	2015	Fungi detected in (year)
F020	505478.67	4708952.87	0	0	0	0	0	0	0	0	2	3	undetected
F025	505482.53	4708947.78	0	0	0	0	0	0	0	1	1	2	2013
F030	505486.25	4708942.77	0	0	0	0	1	1	1	2	3	4	2009
F035	505489.90	4708937.92	0	0	0	0	0	0	2	2	2	3	undetected
F040	505493.70	4708932.85	0	0	0	0	0	0	0	2	2	2	undetected
F045	505497.37	4708927.87	0	0	0	1	1	1	2	2	2	3	2013
F050	505501.14	4708922.61	0	0	0	2	2	2	4	4	4	6	undetected
F055	505504.65	4708917.60	0	1	1	1	3	3	3	5	5	5	undetected
F060	505508.47	4708912.62	1	2	2	2	3	3	5	5	5	6	2009
F065	505512.30	4708907.54	1	2	2	2	3	3	3	5	5	5	2009
F070	505516.01	4708902.53	2	2	3	3	4	4	4	5	6	6	2005
F075	505519.62	4708897.58	1	1	3	4	4	5	5	6	6	8	2003
F080	505523.44	4708892.45	2	2	2	3	3	5	5	5	7	7	2003
F085	505527.15	4708887.46	1	1	1	2	2	2	3	4	4	5	2003
F090	505530.91	4708882.51	1	1	3	3	3	3	4	4	5	7	2003
F095	505534.67	4708877.43	2	2	2	4	4	6	6	8	9	9	2005
F100	505538.34	4708872.34	1	1	3	3	3	5	5	6	6	8	2003
F105	505542.01	4708867.36	2	2	2	4	4	5	5	5	7	7	2005
F110	505545.84	4708862.31	1	1	1	3	3	4	4	5	7	10	2003
F115	505549.56	4708857.38	2	2	2	2	4	5	6	6	9	9	2003
F120	505553.36	4708852.23	2	2	3	3	3	3	4	6	8	8	2011
F125	505557.05	4708847.28	1	1	2	2	2	2	4	5	6	9	2005
F130	505560.73	4708842.19	1	1	2	2	3	3	5	5	7	7	undetected
F135	505564.56	4708837.21	2	1	2	3	3	3	5	7	7	8	2011
F140	505568.18	4708832.15	3	3	3	3	4	4	4	6	7	11	2009
F145	505572.03	4708827.20	1	1	1	1	1	3	4	4	5	8	undetected
F150	505575.52	4708822.25	0	0	0	1	1	1	3	3	4	5	undetected
F155	505579.20	4708817.15	0	0	0	0	0	2	3	3	5	6	2013
F160	505583.18	4708811.99	0	0	0	0	0	2	2	3	5	5	undetected
F165	505586.77	4708807.16	0	0	0	0	0	0	1	1	1	3	2015
F170	505590.71	4708802.06	0	0	0	1	1	1	3	3	3	4	2015
F175	505594.30	4708797.00	0	0	0	0	0	0	0	2	2	3	undetected
F180	505598.00	4708791.93	0	0	0	1	1	1	1	2	3	3	undetected
F185	505601.82	4708786.66	0	0	0	0	0	0	1	1	1	2	undetected
F190	505605.20	4708781.99	0	0	0	0	0	0	2	2	2	3	undetected
F195	505609.16	4708776.85	0	0	0	0	0	0	0	1	1	1	undetected
F200	505612.71	4708772.01	0	0	0	0	0	1	1	1	1	2	undetected
K005	505457.35	4708958.52	0	0	0	0	0	0	0	0	1	3	undetected
K010	505461.16	4708953.64	0	0	0	0	0	0	0	0	2	2	undetected
K015	505464.68	4708948.77	0	0	0	0	0	0	1	1	1	2	2015
K020	505468.48	4708943.54	0	0	0	0	0	0	2	2	2	2	2015
K025	505472.05	4708938.50	0	0	0	0	0	0	0	1	1	1	2013
K030	505475.66	4708933.58	0	0	0	0	0	0	0	0	0	2	undetected
K035	505479.59	4708928.68	0	0	0	0	0	1	1	2	2	3	undetected
K040	505482.97	4708923.65	0	0	0	0	0	0	0	0	0	1	undetected
K045	505486.85	4708918.54	0	0	0	1	2	2	2	2	3	3	2015
K050	505490.54	4708913.43	0	0	0	0	0	0	2	2	4	4	2015

Table II

(continued)

Grapevine	Coordinates UTM-ETRS89 zone 30 X	Y	Total number of exit holes by year 1997	1999	2001	2003	2005	2007	2009	2011	2013	2015	Fungi detected in (year)
K055	505494.27	4708908.47	0	0	0	0	0	1	1	3	4	5	2013
K060	505498.06	4708903.53	0	0	0	1	1	3	3	3	4	4	2011
K065	505501.84	4708898.38	0	0	2	2	2	2	4	5	5	5	2013
K070	505505.32	4708893.36	1	1	1	1	3	3	3	5	6	8	2005
K075	505509.22	4708888.14	2	2	4	4	5	5	5	6	6	9	2005
K080	505512.64	4708883.27	1	1	1	1	2	2	3	4	4	6	2005
K085	505516.63	4708878.48	1	1	1	3	3	4	4	4	6	7	2007
K090	505520.46	4708873.26	1	1	1	2	2	2	4	5	5	8	2007
K095	505524.18	4708868.32	2	2	3	3	3	5	5	6	6	10	2009
K100	505527.75	4708863.37	1	1	1	1	3	4	4	6	8	9	2007
K105	505531.57	4708858.33	1	2	2	4	5	5	6	8	9	9	2005
K110	505535.32	4708853.17	2	3	3	3	5	5	7	9	10	10	2005
K115	505538.90	4708848.29	2	2	3	3	4	5	5	7	8	8	2005
K120	505542.74	4708843.12	1	1	1	2	2	3	3	5	6	9	2011
K125	505546.44	4708838.10	2	2	3	3	4	5	5	7	8	10	2005
K130	505550.06	4708833.22	2	2	3	3	3	3	5	7	7	9	2009
K135	505553.69	4708828.12	2	2	2	4	5	5	7	8	8	11	2011
K140	505557.69	4708823.11	1	1	1	3	3	4	4	7	7	9	2013
K145	505561.23	4708818.04	2	2	3	3	3	3	5	6	6	8	2013
K150	505564.98	4708813.11	2	2	2	4	5	5	5	7	7	8	2013
K155	505568.78	4708808.18	3	3	3	5	5	6	7	7	10	10	undetected
K160	505572.53	4708802.98	2	2	2	2	2	3	5	6	6	8	2015
K165	505576.30	4708798.15	2	2	2	2	2	2	2	3	3	5	undetected
K170	505579.89	4708793.11	1	1	1	1	1	3	3	3	4	4	undetected
K175	505583.62	4708788.10	1	1	1	1	1	1	3	5	5	5	2015
K180	505587.38	4708783.04	0	0	0	1	1	3	3	3	3	4	undetected
K185	505591.05	4708777.98	0	0	0	0	0	0	0	1	1	3	undetected
K190	505595.08	4708772.79	0	0	0	0	0	1	1	1	2	3	undetected
K195	505598.66	4708768.07	0	0	0	0	0	0	0	2	2	4	undetected
K200	505602.26	4708763.19	0	0	0	0	0	0	1	1	2	2	undetected
O005	505447.67	4708948.06	0	0	0	0	0	0	0	0	0	0	undetected
O010	505451.12	4708943.22	0	0	0	0	0	0	0	0	0	0	undetected
O015	505454.93	4708938.17	0	0	0	0	0	0	1	1	1	2	undetected
O020	505458.54	4708933.11	0	0	0	0	0	0	0	0	2	2	undetected
O025	505462.27	4708928.30	0	0	0	0	0	0	0	0	1	1	undetected
O030	505465.97	4708923.23	0	0	0	0	0	0	1	2	2	2	2013
O035	505469.64	4708918.27	0	0	0	0	0	0	0	0	1	1	undetected
O040	505473.43	4708913.10	0	0	0	0	0	1	1	1	2	2	undetected
O045	505477.21	4708908.09	0	0	0	0	0	2	2	2	3	3	undetected
O050	505480.91	4708903.22	0	0	0	0	0	1	1	1	3	4	2013
O055	505484.73	4708898.03	0	0	0	2	2	2	3	4	4	6	undetected
O060	505488.36	4708893.10	1	1	1	1	3	3	3	5	5	7	undetected
O065	505491.94	4708888.11	1	1	1	3	3	3	4	4	6	6	2015
O070	505495.69	4708883.26	1	2	2	5	5	6	6	6	8	8	2015
O075	505499.45	4708878.06	2	2	2	3	3	5	5	7	7	9	2011
O080	505503.05	4708873.23	2	3	3	4	4	6	8	8	10	11	2005
O085	505506.87	4708868.17	1	2	2	4	5	5	5	6	8	12	undetected

Table II

(continued)

Grapevine	Coordinates UTM-ETRS89 zone 30 X	Y	Total number of exit holes by year 1997	1999	2001	2003	2005	2007	2009	2011	2013	2015	Fungi detected in (year)
O090	505510.53	4708863.28	2	2	2	2	2	5	5	7	8	9	2007
O095	505514.19	4708858.21	3	3	5	6	6	6	8	11	11	13	2007
O100	505517.95	4708853.10	2	2	2	5	5	7	7	7	9	10	2009
O105	505521.73	4708848.19	2	2	3	3	3	4	5	9	10	10	2007
O110	505525.38	4708843.16	2	2	2	4	4	4	5	7	8	9	2007
O115	505529.08	4708838.11	1	1	1	3	3	5	5	8	9	9	2005
O120	505532.79	4708833.17	2	2	2	4	4	5	5	8	11	14	2013
O125	505536.53	4708828.15	1	1	1	3	6	7	7	9	10	10	2007
O130	505540.32	4708823.23	3	3	4	4	5	5	7	8	8	9	2011
O135	505544.02	4708818.11	2	3	3	3	4	6	6	6	8	8	2013
O140	505547.73	4708813.10	2	2	2	4	5	5	7	7	7	10	2015
O145	505551.38	4708808.11	2	2	3	5	6	6	8	8	9	11	undetected
O150	505554.99	4708803.10	1	1	1	4	6	6	6	6	7	8	undetected
O155	505558.73	4708798.08	0	0	0	1	1	1	3	3	5	6	2013
O160	505562.51	4708793.29	0	0	1	1	1	3	3	3	4	6	undetected
O165	505566.31	4708788.14	1	1	1	1	2	2	2	2	3	3	undetected
O170	505570.10	4708783.16	1	1	1	1	1	1	1	1	2	2	2011
O175	505573.77	4708778.28	0	0	0	0	0	2	2	3	3	3	undetected
O180	505577.44	4708773.59	0	0	0	0	0	0	0	3	3	4	undetected
O185	505580.82	4708768.84	0	0	0	0	0	0	1	1	1	2	2015
O190	505584.69	4708763.78	0	0	0	0	1	1	1	1	3	3	undetected
O195	505587.93	4708758.99	1	1	3	3	3	3	3	4	4	4	undetected
O200	505591.54	4708754.42	0	0	1	1	2	2	3	3	3	4	undetected

The complete dataset includes: the code of each vine (see Figure 1 to identify them on a map), coordinates, number of holes year by year and the date when the fungi were first detected in every vine.

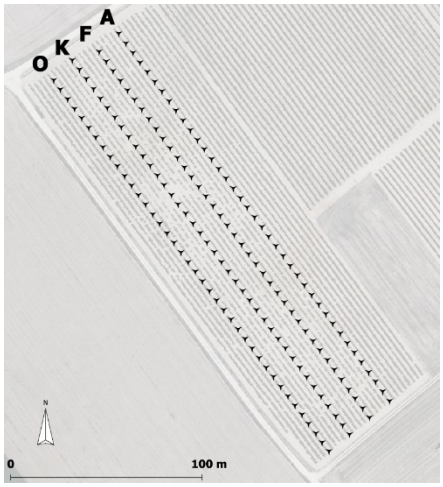

Figure 1. Arrangement of the surveyed vines within the plot. The vines naming is composed of the row (coded as: A, F, K and O) and the number of the grape vine in each row.

Posição das videiras avaliadas dentro da parcela. A nomenclatura das videiras é composta pela linha (codificada como: A, F, K e O) e pelo número da videira em cada linha.

The sampling for each of the 160 chosen vines (40 sampled vines by four rows) consisted of counting the exit holes of the borer both on the stock and the arms. Observations of the symptoms caused by wood fungal diseases (e.g. rachitic shoots) were recorded in the selected vines in springtime. It is assumed that the great majority of the holes remain in the wood between two successive samplings.

However, pruning actions could eliminate some holes, especially if there is a strong pruning to redirect a plant (Armendáriz *et al.*, 2008). The presence of galleries excavated by *X. arvicola* and clear symptoms caused by wooden fungi, observed mainly at pruning time, could be the main cause of the death of some vines.

In order to isolate the fungal species, five vines with new *X. arvicola* exit holes were randomly selected from the central sector of the plot in 2015. One wood sample from each pupation chamber was taken from an affected branch of each vine that had produced rachitic shoots in the previous spring. The isolation procedure followed the one described by García-Benavides *et al.* (2013) with splinters seeding and incubation at 25ºC, isolation and incubation of fungi, extraction of genomic DNA, PCR amplification and sequencing of the rDNA region, including ITS1 and ITS2 spacers and the 5.8S rDNA gene using ABI 377 Prism Sequencer (Applied Byosystems).

Isolated fungi were sequenced using primers ITS1-ITS3 and ITS2-ITS4 with T7 DNA Polymerase sequencing Kit (Pharmacia, Uppsala). The different chromatograms were later analysed with Chromas program and results were compared with those existing in the databases of the National Centre for Biotechnological Information and the European Bioinformatics Institute with the application of the Local Basic Alignment Search Tool (Altschul *et al.*, 1997).

The selected vines were georeferenced with centimetre accuracy by means of GNSS observations (GNSS, *Global Navigation Satellite System*) in order to allow a more accurate graphic representation of the samples and perform complementary geostatistical analyses.

Concerning the statistical tools applied to the dataset, the first step was to decide whether the number of holes located in all the vines from a particular year could be analysed together (as a single population) or if there were meaningful differences from row to row, suggesting that they need to be analysed separately. To that end, two-sample *t*-tests were conducted to check if the difference between the observed means of every pair of rows was significantly different from the ones that could be expected from a single population.

For instance, if it is considered that the samples for two rows come from the same population, the estimation of the standard deviation of the population (s') can be computed through Equation 1 (Mills, 1969):

$$s' = \sqrt{\frac{\sum d_1^2 + \sum d_2^2}{N_1 + N_2 - 2}} \qquad \textbf{Eq. 1}$$

Being (N_1) and (N_2) the number of samples of each row—in our case both are equal to 40—and (d_1) and (d_2) the differences between the number of holes of a particular vine and the average of holes of its respective row. Now, the standard error of the difference of means can be estimated by the expression shown in Equation 2.

$$s_{\bar{x}_1 - \bar{x}_2} = s' \sqrt{\frac{N_1 + N_2}{N_1 \cdot N_2}} \qquad \textbf{Eq. 2}$$

The quotient of the difference of means divided by the value obtained from the Equation 2 is distributed according to a Student's *t*-distribution (Equation 3).

$$t = \frac{\bar{x}_1 - \bar{x}_2}{s_{\bar{x}_1 - \bar{x}_2}} = \frac{\bar{x}_1 - \bar{x}_2}{s'} \sqrt{\frac{N_1 \cdot N_2}{N_1 + N_2}} \qquad \textbf{Eq. 3}$$

Therefore, the hypothesis that both means come from the same population will be rejected if the (t) value obtained in Equation 3 exceeds a limit value for the Student's distribution with (N_1+N_2-2) degrees of freedom. This critical value for (40+40-2=78) degrees of freedom and a confidence level of 95% (i.e. significance level: α = 0.05) is: $t_{0.05,78}$ = 1.99; and with a confidence of 99% (i.e. significance level: α = 0.01) is: $t_{0.01,78}$ = 2.64; in both cases, the test is considered two-tailed.

On the other hand, the dataset clearly showed a progressive increase of the number of holes over time. In order to determine whether the health status of the plot determines this rise or not, two different impact indexes were calculated: the number of new holes divided by the total amount of sampled vines (160) and, secondly, divided by the quantity of vines with at least one hole (either new or from previous years). Then, with the values obtained year after year a regression function was computed for each index (with "time" as the independent variable) and their significance in terms of the coefficient of determination (R^2) was obtained. A bigger level of significance of the first index would imply that the increase only depends on the size of the pest infestation (regardless the proportion between healthy and infested vines), while a higher value of the second index would suggest that the insect behaves differently in healthy and already infested vines.

A third impact index was also calculated. The sum of new holes for each survey was divided by the quantity of vines that showed and increased number of holes in the same period. A constant value of this index would denote that the amount of larvae that

simultaneously are feeding on the same plant does not change over the infestation.

This analysis might provide—if the obtained values are significant—some insight into the overall evolution of the pest, i.e. the average number of exit holes per vine for a particular year. To have a further description of the infestation process it would be also very useful to know the way in which the holes are allocated, that is to say, how many vines will get 1 new hole, how many 2, 3, 4 and so on. In other words, a suitable distribution of probability is searched.

A good initial candidate to be tested is the Poisson distribution, since its shape is similar to the ones obtained during the sampling work and, in addition, because this distribution depends only on one parameter (λ: the mean value of the phenomenon under study), which can be estimated by the regression function mentioned in the paragraphs above.

For example, Table III shows the number of vines with a definite quantity of observed new holes from the previous survey (the first column refers to 1999 and the last to 2015). The bottom row indicates the arithmetic mean, that is the average of new holes per vine (population size: 160 plants). Moreover, the Poisson distribution gives the probability associated to each value of the variable (x), in our case, the number of new holes (Equation 4).

$$P(\lambda: x) = \frac{\lambda^x}{x!} e^{-\lambda} \quad , x = 0,1,2 \qquad \textbf{Eq. 4}$$

If, for each occasion, the corresponding value for the parameter (λ) is taken from the respective arithmetic mean of Table III, then, the estimation of holes obtained by applying the Poisson distribution is presented in Table IV. As can be seen, the values in both Tables look similar (although this "similarity" will have to be analysed carefully before relying on it).

Table III

Number of grapevines with 0 to 5 new holes in each survey (observed values) and arithmetic mean of new holes per grapevine

Número de videiras com 0 a 5 novas galerias em cada amostragem (valores observados) e média aritmética de novas galerias por videira

Holes	1999	2001	2003	2005	2007	2009	2011	2013	2015
0	145	126	110	116	106	93	80	83	64
1	13	21	24	32	33	35	41	46	48
2	2	13	23	11	20	32	33	28	35
3	0	0	3	1	1	0	5	3	10
4	0	0	0	0	0	0	1	0	3
5	0	0	0	0	0	0	0	0	0
Mean	0.106	0.294	0.494	0.356	0.475	0.619	0.788	0.694	1.000

Table IV

Number of vines with 0 to 5 new holes in each survey (Poisson estimation taking as parameter λ the respective mean which is shown in the last row of Table II, considering a population of 160 grapevines)

Número de videiras com 0 a 5 novas galerias em cada amostragem (estimativa de Poisson tomando como parâmetro λ a respetiva média calculada na última linha da Tabela II, considerando uma população de 160 videiras)

Holes	*1999*	*2001*	*2003*	*2005*	*2007*	*2009*	*2011*	*2013*	*2015*
0	*144*	*119*	*98*	*112*	*100*	*86*	*73*	*80*	*59*
1	*15*	*35*	*48*	*40*	*47*	*53*	*57*	*55*	*59*
2	*1*	*5*	*12*	*7*	*11*	*16*	*23*	*19*	*29*
3	*0*	*1*	*2*	*1*	*2*	*3*	*6*	*4*	*10*
4	*0*	*0*	*0*	*0*	*0*	*1*	*1*	*1*	*2*
5	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*

The Poisson distribution is also additive, allowing the computation of the probabilities associated with each value (i.e. new holes per vine) after many consecutive occasions. Therefore, considering that a linear function for estimating the mean number of new holes per vine in a particular occasion is known, the development of a new model begins with the regression function, where time (t) is the independent variable (i.e. number of years from an initial moment, in our case 1997).

The linear equation that provides the mean value (λt) for the Poisson distribution is shown in Equation 5.

$$\lambda_t = a\,t + b \qquad \textbf{Eq. 5}$$

Where "a" and "b" are the known parameters of the regression line. The estimation of the number and the distribution of the new holes from the reference time will be obtained by a Poisson distribution, the parameter of which (λ) will be determined thanks to the additive property (Equation 6)

$$\lambda_{1999} = \lambda_{t=2} \qquad \textbf{Eq. 6}$$

$$\lambda_{2001} = \lambda_{t=2} + \lambda_{t=4}$$

$$\lambda_{2003} = \lambda_{t=2} + \lambda_{t=4} + \lambda_{t=6}$$

$$\lambda_{2005} = \lambda_{t=2} + \lambda_{t=4} + \lambda_{t=6} + \lambda_{t=8}$$

Summing up, the previous procedure will provide an estimation of the percentage of vines with 0, 1, 2, 3… new holes after a specific number of years. Nevertheless, before adopting the Poisson distribution as a suitable way to guess the allocation of the new holes it is necessary to check to which extent the estimations fit the real values well. This check will be carried out quantitatively (that is seeing the differences between the estimated and the real percentages) and statistically, by means of chi-square tests.

RESULTS AND DISCUSSION

Figure 2 shows the evolution overtime of the spread of *X. arvicola* in the considered area, highlighting the vines affected by the borer, together with the observed symptoms of grapevine decline/esca disease. Both the beetle (since the third occasion of sampling, in 1997) and the pathogenic fungi (since 2003) symptoms were initially detected in the central part of the plot and then spread out towards the borders until the almost complete infestation/infection of the vines. As the sequence of maps in Figure 2 suggests, the process became faster over time.

The first xylophagous infestation was detected in the central part of the plot and with a similar level in all four sampled lines. Nevertheless, in a comparable study, Ocete *et al*. (2010) did find a progressive spread of the borer from an infection source near to a neighbour infested vineyard. Similar to the present study, the infestation of that plot was rapid and, in 16 years, 98% of the vines were infested.

For the twenty different wood pathogenic fungi found in the galleries of *X. arvicola* in vine trunks in La Rioja Alta and Rioja Alavesa vineyards by García-Benavides et al. (2013), six were isolated during this survey: *Diplodia seriata* de Not., *Eutypa lata* (Pers.) Tull. & C. Tull, *Gibberella avenacea* R.J. Cook*, Fusarium acuminatum* Ellis & Everh., *Phomopsis vitícola* (Sacc.) Sacc. *and Phaeomoniella* chlamydospora (W.*Gams*, Crous, M. J. Wingf. & L. Mugnai) Crous & W. *Gams*.

The total number of adults' exit holes observed on the vines (Table V) shows the progressive increase in the signs of the borer's activity. The application of the *t*-test (Equation 3) to all the pairs of rows for every year did not show values exceeding the critical value of the test with confidence of 95% ($t_{0.05,78}$); therefore, for all years, it was deemed reasonable to consider all the vines as a single population. Figure 3 shows the percentage of vines without holes in each survey, revealing three well discernible phases: i) "phase 0: no infestation" (1993-1995) no holes were detected, although it is likely that some larvae were already inside the vine trunks; ii) "phase 1: start" (1995-1997) with a very fast increase of infestation almost half of the vines showed exit holes; iii) "phase 2: spread" (1997-2015) which has a logistic (S-shaped, inverted in this Figure) behaviour, that is with an initial very slow progression (1997-1999), followed by a progressive acceleration (1999-2007), then its growth slowed down until almost all vine are infested (2007-2015). Considering the fungal infection, the first symptoms were detected in 2001, in the spreading phase of the borer infestation, and it followed an almost linear pattern overtime until 50% of the vines showed infection in 2015 (end of observations).

As for the indexes defined to check the influence of the healthy status in the increase of the infestation, Figure 4 shows the applied linear regressions. Since the first index has a significant coefficient of determination ($R^2 = 0.89$), it means that the number of new holes grows steadily regardless of whether the plot is partially or totally infested; indeed, the regression adjusted to the second index, when only the already infested vines are considered, has a lower coefficient ($R^2 = 0.66$). These results suggest that the amount of new holes depends on the number of holes generated during the previous years, rather than the number of infested vines and that the rate of increase is constant over time. Therefore, once the regression line has been calculated, it would be possible to estimate the value of the first index for any other year in between the observations or for any future occasion

.

Figure 2. Spreading of the infestation by *Xylotrechus arvicola* (number of holes in each vine, represented by white dots of different sizes), as well as, the presence of symptoms of esca disease and grapevine decline (red dots).

Propagação da infestação por Xylotrechus arvicola (número de galerias em cada videira, representados por pontos brancos de diferentes tamanhos) assim como a presença de sintomas da doença da esca e do declínio da videira (pontos vermelhos).

Table V

Total number of adult exit holes of *Xylotrechus arvicola* recorded on each sampling in the selected lines of the vineyard (A, F, K and O).

Número total de galerias de saída de adultos de Xylotrechus arvicola registrados em cada amostragem nas linhas selecionadas na vinha (A, F, K e O)

Year →	**1997**	**1999**	**2001**	**2003**	**2005**	**2007**	**2009**	**2011**	**2013**	**2015**
A	29	37	55	70	87	102	128	152	174	210
F	27	29	40	56	67	84	112	143	171	212
K	35	37	46	63	77	96	121	160	184	233
O	36	40	49	80	95	120	140	172	209	243
Average per sampled vine	0.79	0.89	1.19	1.68	2.04	2.51	3.13	3.92	4.61	5.61

40 grapevines were analised per row.

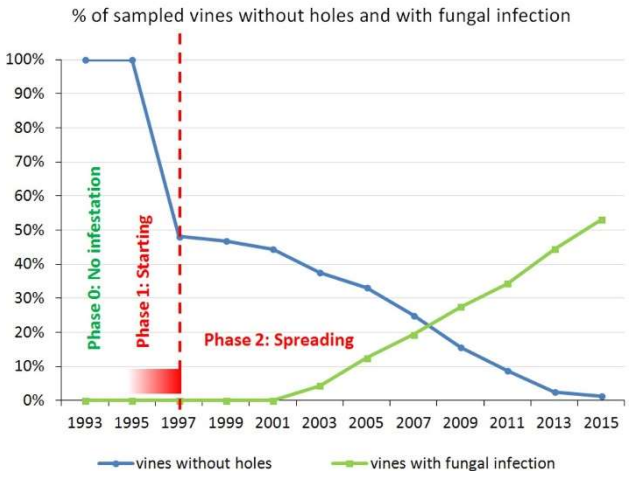

Figure 3. Percentage over time of sampled grapevines without emergence holes of *Xylotrechus arvicola* and vines with esca fungal infection.

Percentagem de videiras amostradas sem galerias de Xylotrechus arvicola e videiras infetadas pelo fungo da esca ao longo do tempo.

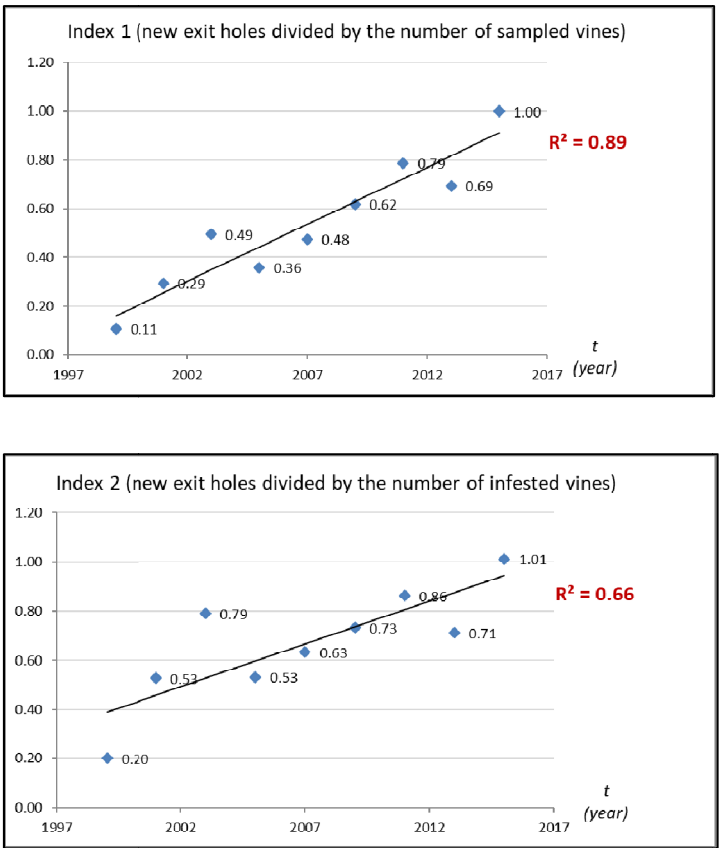

Figure 4. Graphical representation and adjusted linear regression of the time evolution of two indexes: (1) new holes divided by the total number of vines (with or without holes) and (2) new holes divided by the number of vines with holes.

Representação gráfica e regressão linear ajustada da evolução temporal de dois índices: (1) novas galerias dividido pelo número total de videiras (com ou sem galerias) e (2) novas galerias dividido pelo número de videiras com galerias.

The phase 2 of *X. arvicola* infestation is even better described plotting the accumulate average of holes year by year (bottom row in Table V) and adjusting a second order polynomial equation (the regression line that was obtained previously for index 1 is the first derivate of this polynomial multiplied by two—the time lapse between surveys) (Figure 5). The resulting parabolic function has a very high coefficient of determination ($R^2 > 0.99$) which means that it is consistent with the data. On the other hand, the minimum value of this parabola ($t \approx$ 1994, that is three years before the first detection of

exit holes) is positive (0.67), this result is relevant because it means that this function cannot describe the initial infestation pattern (phase 1). Therefore, the described function is coherent with the scheme of two different phases: one for the start and another for the spreading of the infestation.

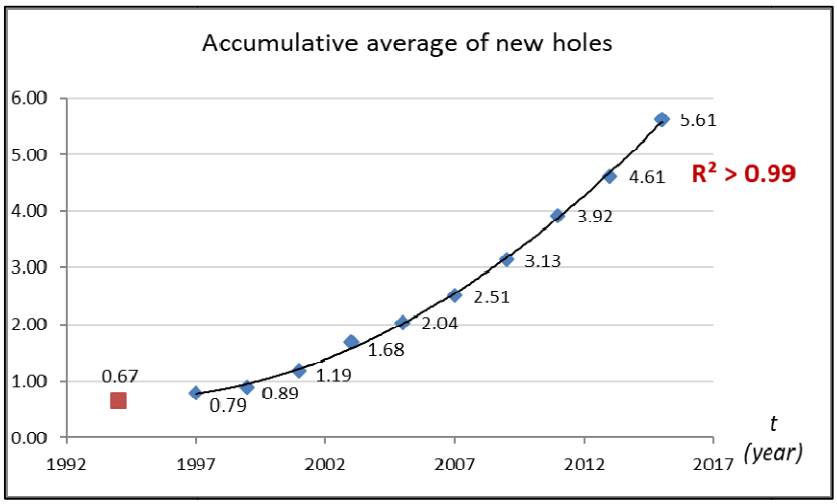

Figure 5. Number of new holes (accumulated yearly average per grapevine) and adjusted second order function. The rectangle in brown shows the estimated minimum value ($t \approx 1994$).

Número de galerias novas (média anual acumulada por videira) e função de segunda ordem ajustada. O retângulo em castanho representa o valor mínimo estimado ($t \approx 1994$).

A third index with the average number of new exit holes per vine infested in a particular period has been also calculated. The respective values from "1997-1999" to "2013-2015" are: 1.13, 1.38, 1.58, 1.30, 1.40, 1.41, 1.48, 1.58, 1.44 and 1.67. As abovementioned, the aim pursued with this index is to see whether the number of larvae per infested vine remains constant as the infestation develops. The values seem to support this idea; indeed, the mean value of the series is equal to 1.44 (standard deviation: 0.16), with all the values in the series in the range of two standard deviations to the mean. Moreover, the coefficient of variation is about 11%.

Hence, up to this point, the analysis of the dataset has provided a meaningful regression line which allows obtaining, for a particular year, a fair estimation regarding the probabilities of a vine to have a specific quantity of new holes from the initial year of the phase 2 (1997). More specifically, the parameters for Equation 5 (regression line) for the dataset were: $a = 0.0469$ and $b = 0.0674$; with the independent variable (t) as the number of years from 1997. The procedure to compute these probabilities is the following:

1. Firstly, the parameters ($\lambda_t = a\ t + b$) corresponding to the distribution of new holes for each two-year period were computed (Equation 5). These values are presented in the third row of the Table VI.
2. Secondly, the cumulative value of the parameter for a particular year (λ_{year}) is the sum of all the values of the upper row up to this column (Equation 6 and fourth row in Table VI).
3. Then, with each value of (λ_{year}) the probabilities were calculated by means of Equation 4 (lower part of the Table).

In order to avoid cells with very small probabilities the values at endpoints have been aggregated to be, at least, 0.05 (i.e. 5% of the population). This limit is a requirement of the chi-square test that will be calculated afterwards (the 5% of a population of 160 plants stands for 8 individuals, the specifications of the chi-square test state that all the analyzed classes need to have, at least, 5 individuals).

In addition, Table VII presents the real frequencies that were observed. These values were obtained from the dataset by subtracting—for each plant—the number of holes observed in the initial year of this phase 2 (1997) from the holes at the year under consideration (e.g. 2011). The Table shows relative frequencies, that is the number of times that each value—number of holes—occurs (i.e. the absolute frequency) divided by the population size (160 vines), hence, these values are comparable with the ones presented in Table VI (estimated probabilities).

The developed method will be analysed from two complementary perspectives:

a) On the one hand, the divergence between estimated and real occurrences will be quantified and summarized in a single value,

which will provide us a general measure of the success rate;

b) On the other hand, as the estimations were obtained under the hypothesis that the appearance of new holes followed a Poisson distribution, this assumption needs to be tested statistically so as to see whether it is consistent with the data.

Table VI

Probabilities (values between 0 and 1) regarding the number of vines with a total of 0, 1, 2… new holes betwen and 2015 (Poisson estimation)

Probabilidades (valores entre 0 e 1) em relação ao número de videiras com um total de 0, 1, 2 ... novas galerias de 1997 até ao ano 2015 (estimativa de Poisson)

Year →	**1999**	**2001**	**2003**	**2005**	**2007**	**2009**	**2011**	**2013**	**2015**
t = year-1997	2	4	6	8	10	12	14	16	18
$\lambda_t = a\ t + b$	0.161	0.255	0.349	0.443	0.536	0.630	0.724	0.818	0.912
λ_{year}	0.161	0.416	0.765	1.208	1.744	2.374	3.098	3.916	4.828
Holes (*x*)									
0	0.85	0.66	0.47	0.30	0.17	0.09	0.05		
1	0.15 *	0.27	0.36	0.36	0.30	0.22	0.14	0.10 *	0.05 *
2		0.07 *	0.18 *	0.22	0.27	0.26	0.22	0.15	0.09
3				0.12 *	0.15	0.21	0.22	0.20	0.15
4					0.10 *	0.12	0.17	0.20	0.18
5						0.09 *	0.11	0.15	0.17
6							0.09 *	0.10	0.14
7								0.06	0.10
8								0.05 *	0.06
9									0.06 *

Due to rounding, the sum of each column might slightly differ from "1.00". Values with asterisk (*) aggregate small percentages of the subsequent (or the previous) cells, so as the lower value shown by the Table is, at least, "0.05".

Table VII

Observed relative frequencies (values between 0 and 1) regarding the number of vines with a total of 0, 1, 2… new holes from 1997 to 2015

Frequências relativas observadas (valores entre 0 e 1) em relação ao número de vinhas com um total de 0, 1, 2 ... novas galerias de 1997 até ao ano 2015

Holes	**1999**	**2001**	**2003**	**2005**	**2007**	**2009**	**2011**	**2013**	**2015**
0	0.91	0.71	0.49	0.38	0.27	0.17	0.09		
1	0.09 *	0.20	0.24	0.22	0.20	0.17	0.15	0.18 *	0.08 *
2		0.09 *	0.28 *	0.24	0.22	0.17	0.13	0.14	0.12
3				0.16 *	0.21	0.26	0.19	0.14	0.17
4					0.11 *	0.13	0.18	0.18	0.12
5						0.10 *	0.15	0.14	0.08
6							0.10 *	0.10	0.16
7								0.08	0.13
8								0.05 *	0.09
9									0.05 *

Due to rounding, the sum of each column might slightly differ from "1.00". The cells with values in this Table are the same as in Table V, values with asterisk (*) aggregate small percentages of the subsequent (or the previous) cells.

Table VIII shows the discrepancies between estimations and observations, i.e. the differences between the values in Tables VI and VII (absolute values). The weighted mean of each column is presented as an assessment of the accuracy of each estimation. and the weights are the observed frequencies (Table VII). For instance, the corresponding weighted mean of 2005 is computed according to Equation 7.

$$0.08 \cdot 0.38 + 0.14 \cdot 0.22 + 0.03 \cdot 0.24 + 0.03 \cdot 0.16 = 0.07 \qquad \textbf{Eq. 7}$$

It can be seen that, on average, the estimation error goes from 0.03 to 0.07 (that is from 3% to 7%) being the arithmetic mean of these weighted values equal to 0.05 (5%).

On another note, Table VIII also includes the information necessary to perform the test of goodness of fit, which will state whether the theoretical distribution can statistically explain the observed frequencies. For this purpose, each column of the Table includes the cumulative test statistic (χ^2), which is computed as the population size multiplied by the sum of the squared

differences shown in Table VIII divided by the estimated frequencies (Table VI). For example, the statistic for 2005 is obtained according to Equation 8.

$$\chi^2 = 160 \cdot \left(\frac{0.08^2}{0.30} + \frac{0.14^2}{0.36} + \frac{0.03^2}{0.22} + \frac{0.03^2}{0.12}\right) = 14.604 \qquad \textbf{Eq. 8}$$

Table VIII

Difference between real frequencies and estimated probabilities (absolute values), calculated chi-squared test statistics and critical values (α = 0.05 and α = 0.01)

Diferenças entre frequências reais e probabilidades estimadas (valores absolutos), cálculo do teste do qui-quadrado e valores críticos (α = 0,05 e α = 0,01)

Holes	1999	2001	2003	2005	2007	2009	2011	2013	2015
0	0.06	0.05	0.02	0.08	0.09	0.08	0.05		
1	0.06	0.07	0.12	0.14	0.10	0.05	0.01	0.08	0.03
2		0.03	0.10	0.03	0.05	0.09	0.09	0.02	0.03
3				0.03	0.05	0.05	0.03	0.06	0.02
4					0.01	0.01	0.01	0.01	0.06
5						0.01	0.04	0.01	0.09
6							0.01	0.00	0.02
7								0.02	0.03
8								0.00	0.04
9									0.01
weighted mean	0.06	0.05	0.07	0.07	0.07	0.05	0.03	0.03	0.03
χ^2	3.837	5.640	14.785	14.604	18.016	19.657	17.349	14.371	21.684
$\chi^2_{0.05,k-1}$	3.841	7.815	7.815	7.815	9.488	11.070	12.592	14.067	15.507
$\chi^2_{0.01,k-1}$	6.635	11.341	11.341	11.341	13.277	15.086	16.812	18.475	20.090

(k) is the number of classes, i.e. the number of cells with data in each column. Greyed out cells indicate that the hypothesis cannot be accepted.

The null hypothesis (i.e. "observed values are compatible with the theoretical model") will be accepted with a certain level of confidence (usually, 95% → α = 0.05 or 99% → α = 0.01) if the cumulative statistic does not exceed a critical value that is obtained from a chi-square distribution with (k – 1) degrees of freedom, being (k) the number of classes analysed (that is, the number of cells with values in each column of the Table).

The Table includes the critical values for each column with the two commonest levels of confidence used. Three out of the nine cases could be accepted but the other six (greyed out cells) have to be rejected, although none of these latter are far away from the limit. This result shows that the theoretical distribution developed in the previous paragraphs may be an approximation of the behaviour of the pest, but it is not a fully accurate prediction. Indeed, this conclusion is in line with the quantitative analysis that was obtained from the weighted means of the discrepancies.

A cause of this lack of matching can be found in an unexpected feature of the observed frequencies (Table VII) that is not considered by the theoretical model: the bimodality of the histograms, a characteristic that is more pronounced as time goes by. For instance, Figure 6 shows the observed relative frequencies corresponding to the number of new holes from 1997 to 2015. Two experimental attempts were performed to simulate the bimodal behaviour by means of separate Poisson distributions that relate respectively to: (1) the already infested vines and the not infested ones; (2) the plants with and without wood fungi. However, none of these trials provided better results than the initial approach, so the issue is still open to further research. Perhaps, time differences in the emergence of male and female individuals that were reported by Soria *et al*. (2013) might play an important role is this bimodal behaviour. However, the analysed dataset does not include enough information for the assessment of this point.

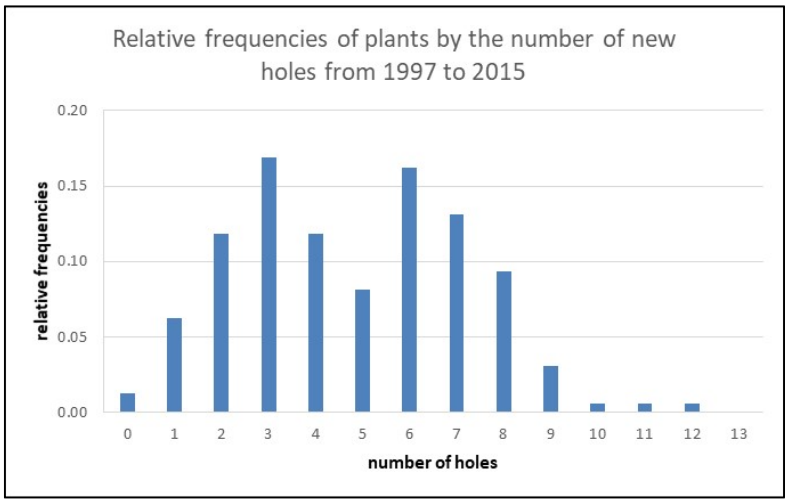

Figure 6. Example of the bimodal distribution of the observed frequencies of the number of new holes in surveyed grapevines.

Número de novas galerias nas videiras analisadas, exemplo da distribuição bimodal das frequências observadas.

There are other still unknown characteristics of this species linked with the infestation process, like the flight capacity. It is reasonable to assume that the first ovipositing females arrived from outside of vineyard, because plant material is cleaned and human facilitation is not supposed. But it is unclear if occurs a long or short dispersion of the reproductive adults.

Also, it is not known the oviposition behaviour. More than one larvae can feed in a single vine since several unconnected galleries can be found in dead wood (Ocete, personal observations). Larval cannibalism is avoided by the use of sound, as in other Cerambycidae like *Icosium tormentosum* (Kočárek, 2009). The larvae of *X. arvicola* produce stridulatory sounds when they are feeding inside the wood. These sounds are used to ward off possible parasites or predators, but also for their spatial location within the vine, thus avoiding the encounter between individuals (Moreno, 2004).

In Spanish vineyards (La Rioja, Navarra, Castilla y León y Castilla-La Mancha) no competition with other xylophagous insects was reported. Reinfestation is possible in dead vines (Armendáriz, personal observations) and is confirmed in this survey in living vines. Moreover, plant defence has not been reported in previous studies.

Colonisation and damage levels depend on dispersal and reproduction ratios. Longevity of adults in field conditions is unknown but, in laboratory conditions, it is close to a month, longer in adults reared on an artificial diet (37 ± 4 days) than in natural populations (24 ± 2 days). Females fed on artificial diet have bigger fecundity (244 eggs/female) than wild specimens (197 eggs/female) (García-Ruiz *et al*., 2012). In laboratory conditions there is a limitation concerning the larval mortality, but this fact is unknown in field conditions. With these characteristics *X. arvicola* has a moderate expansion ability (García-Ruiz *et al*., 2012). However, initial infestation in this study is remarkable and suggests a two-phase process instead of a single and continuous one.

The emergence of adults was detected in 1997, 10 years after the vineyard planting. However, Ocete *et al*. (2010) found that the activity of this borer started much earlier in a 'Tempranillo' vineyard in Tirgo, six years after the planting. This early incidence is similar to another vineyard pest species, *Coelosterna scabrator* Fabr. (Cerambycidae: Coleoptera) that has been observed in India in vine plots of only one year old (Kumari and Vijaya, 2015).

The Emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) has a similar behaviour in the infestation process and a model was developed using insect species variables (fertility, offspring, flying capacity) as well as host characteristics (age, diameter, distance for beetle emergence) (Anderson and Dragićević, 2015). However, the behaviour of *X. arvicola* is mostly unknown, as well as the clues to the choice of plants for oviposition. A field study on emergence and oviposition is needed to elucidate these unknown facts for a better management of the pest. In laboratory conditions females oviposite in rolled paper (García-Ruiz *et al*., 2012), but no field experimentation in oviposition has ever been carried out.

Many forest insects exploit trees that are physiologically weakened as a result of stress created by nutrient deficiencies, drought, flooding,

overcrowding, and other variables (Eisa and Roth, 2009). This behaviour is reported in *X. arvicola* inhabiting dead wood and decaying trees of many wild/spontaneous and ornamental plants (Vives, 1984). However, in this study infestation is homogenous.

The number of fungus species identified is quite scarce in comparison with the number found by García-Benavides *et al*. (2013) (6/20) but the number of samples is also unbalanced (5/32). On the other hand, the six fungal species identified from pupation chambers which cause esca disease and vine decline were also frequently isolated from other vineyards infested by this borer in different areas of La Rioja, as presented by García-Benavides *et al*. (2013). That article suggests the hypothesis that this borer could act as a vehicle for fungal pathogens, "inoculating" the grapevines during egg laying which occurs in cracks and pruning wounds. At the same time, the exit holes performed by the newly emerged adults represent easy points of entrance for spores. Zanzotto *et al*. (2013) found that esca disease fungi are propagated as a higher probability of infection along rows rather than between adjacent rows; this is similar to the initial fungi symptoms in the present study.

It is noteworthy that, forty years ago, only two fungal diseases affecting vine wood were cited in the specialized bibliography, Esca and Eutipiosis, in relation to three pathogenic fungi, *Phellinus ignarius*, *Stereum hirsutum* and *Eutypa lata*. With the development of molecular techniques, the number of fungi implicated have grown massively (Gramaje, 2017), ascending up to more than forty species, only in Spain (Armengol, 2017).

In the last years, wood diseases, such as grapevine decline, esca and Petri disease, became the first sanitary problem affecting Spanish nurseries and vineyards (Del Río-Conesa *et al*, 2002). Relationships between wood necrosis and grapevine decline and the presence of several fungi is well documented. On the other hand, the causes of the development of the typical foliar symptoms are still elusive (Bertsch *et al*., 2013).

These diseases drastically reduce the life of the vines due to the interactions among different pathogenic fungi, causing an important negative economic effect. They penetrate into vine wood through pruning wounds and the tools used in that activity. However, the galleries excavated by *X. arvicola* could also constitute an easy way of propagation along trunks and branches (García-Benavides *et al.*, 2013).

The control of the pest cannot be achieved due to the adult's long emergence period in La Rioja, from June to August (Soria *et al*., 2013*)* and the endophytic development of the larval and pupal stages (García-Benavides *et al.*, 2013). There is still no active substance allowed by the Ministry of Agriculture, Fisheries and Food (MAPA) for the treatment of this pest. Rodríguez-González *et al.* (2016-a) use different active substances in the laboratory to control the stages of egg, larva and adult, finding a good performance of natural insecticides such as *Beauveria bassiana* and spinosyns. Although no data is available on field applications. It is reasonable to assume that the applications of insecticides (Table I) can alter the dynamics of the xylophagous, but it is clear that, at least, they fail to control their development and expansion.

It is a present challenge to develop an efficient strategy that could help to reduce the impact of *X. arvicola* in vineyards. This strategy will surely include biological and microbiological control (Rodríguez-González *et al*., 2016-a), ecological management (e.g. use of riparian species as trap crops), agronomic practices (selection and use of less susceptible varieties, removal/destruction of wood remains), biotechnical (e.g. male sterilization) and semiochemical methods (identification and proper use of pheromones) as well as advances on modelling techniques (Armendáriz *et al*., 2016). Further research is needed on all these different fields.

It is reasonable to assume that climate is one of the factors that have facilitated the expansion of the beetle. Consequently, perspectives of climatic change for this pest should clarify future extension. Simulations performed using models such as ECHAM4 from IPPC (Roeckner *et al.,* 1996) characterized by an increase of summer temperatures and a moderate reduction of annual rainfall, result in a significative increase of the suitable area for *X. arvicola* (Felicísimo *et al.,* 2019), indicating that these circumstances could favour the extension of this borer in areas that presently are unsuitable. However, overall, the number of statistical models applied to pests or diseases is relatively small compared to other parameters related to climate change (Costa *et al*., 2015).

Finally, it is further possible that crop management may play a role, that is factors such as the way of ploughing and pruning, the distribution model of the vines into the plot, the chemical treatments and so on. A multimodal analysis using data on viticulture in Spain and surrounding countries could provide some explanation of this phenomenon and help as a forecast of expansion to new vine areas.

CONCLUSIONS

Forecasting the state of the vegetative and sanitary conditions of the crop is a very important management action because it allows estimating the

quality and quantity of the production in the coming years, evaluate the effectiveness of the corrective treatments and help to decide when the vineyard could be replanted. Moreover, having reference values is also useful for checking the influence of new factors such as chemical treatments or climatic changes. Additionally, if information from different areas were collected, the values could be compared and better assess the variables affecting the spread pattern of the beetle.

A procedure for explaining and foreseeing the spreading process of the pest in a plot has been developed considering only global values for each occasion (in particular, the total number of holes and the number of vines with new holes). The dataset collected showed a distinguishable behaviour during the initial phase of infestation, from year 1995 to 1997, in which, between these two consecutive sampled phases the number of vines without holes passed from the 100% (hence, the plot did not show any symptom of infestation) to a little less than 50%. As demonstrated, this initial colonization was drastic and fast. From 1997 onwards, data suggest a different phase of the infestation. Indeed, it is shown that the average number of new holes per vine increases linearly (R^2 = 0.89) and that this growth rate is very stable regardless of the number of infested vines. Matching is even better when fitting a second-degree polynomial to the average number of total holes (new and old ones) per vine.

Considering the probability of appearance of a definite number of new holes in a particular vine after certain years, a Poisson distribution was adjusted using as a unique parameter (λ), the aforementioned average of new holes for each epoch. Although the matching is not strong (only 3 of the 9 sets of data can be appropriately defined for a Poisson distribution), this relationship permits establishing a simple way for estimating the proportion of vines with a determinate number of new holes for a particular year. The procedure has been evaluated with the provided dataset, obtaining mean differences between the estimates and the real values at around 5%. The observed discrepancies are mainly due to the dataset's real frequencies bimodal distribution. This bimodal behaviour was unexpected, although, it may be a key factor that will deserve further attention.

Due to the availability of a comprehensive record of the infestation in this plot (12 observations from 1993 to 2015) it has been possible to distinguish two different phases. The described procedure estimates the so-called phase 2 of the infestation; by contrast, the information in the dataset does not allow analysing in detail how the phase 1 was produced. In any case, the research conducted offers suggestive clues that can be useful for further studies in this field and help to improve the management of this pest.

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