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RESEARCH PAPERS

Spatial distribution of esca symptomatic plants in Dão vineyards (Centre Portugal) and isolation of associated fungi

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Summary. During three years, from 2002 to 2004, a vineyard was surveyed for esca incidence and spatial distribution of deceased/symptomatic vines. Individual plants were recorded as diseased when characteristic esca symptoms were visible in summer surveys or in winter surveys. Some of the symptomatic vines were uprooted every year and fungi most commonly isolated from those plants were listed. Two dimensional maps referring the presence/absence of diseased plants were drawn every year and a cumulative map was obtained on the last year of the survey. Based on the former map, spatial distribution of esca symptomatic plants was analysed in order to confirm the null hypothesis of randomness on the disease spread. Our results showed that: *i)* internal lesions of uprooted vines were classifiable in two main categories, according to the observed decays; *ii)* with the exception of *Eutypa lata,* fungi commonly associated with esca were present in the uprooted vines*; iii)* the number of esca symptomatic plants increased every year of the survey; *iv)* esca foliar symptoms were discontinuous from year to year and *v)* the distribution of infected vines on the plot was random.

Key words: grapevine, esca, epidemiology, wood fungi, spatial pattern.

Introduction

Historically, economically, and culturally grapevine and wine growing represent one of the most important agricultural activities in Portugal.

The country is divided onto several wine regions, being the 'Região Demarcada do Dão (Dão Wine Region, *alias* Dão)', one of the most important, not only because of its 20,000 ha of intense grape production, but because of its exquisite and complex wines based mostly in Portuguese traditional varieties.

In recent years, the increase of esca on fully productive (less than 30 years) grapevines has become a major concern amongst viticulturists.

The present study is a contribution towards a more full understanding of esca behaviour in the Dão Wine Region, Portugal. The objectives of this

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study were to verify the expression of leaf symptoms from year to year, to evaluate the increase on esca-affected grapevines, the spatial distribution of symptomatic plants, and to investigate the fungi associated with diseased wood in this vineyard.

Materials and methods

Characterization of the plot

The experimental plot, planted in 1989 and grafted in place in 1990, has an area of ca. 4000 m^2 with 1296 grapevines cv. Touriga Nacional grafted on SO4 rootstock and it is located in a characteristic 11 ha vineyard, with a north-south orientation. The soil is of granite origin with a high proportion of sand, characterized by low pH, high level of potassium, low levels of phosphor and organic matter. The vines are trained in 'double Guyot' and the distance between the rows is of 2.50 m for 1.10 m spacing between plants in the row. Precipitation is limited except in the winter period.

Survey

During three years - 2002, 2003 and 2004 - the presence/absence of foliar symptoms of esca in each vine was recorded as well as the deceased plants. Surveys begun immediately after the first symptoms on herbaceous parts were noticed and took place during the months of June, July, August and September, at the average rate of one visit per month. Data were collected and analysed as in Surico *et al*., (2000). Two-dimensional occurrence maps were drawn concerning to each producing year (2002, 2003, and 2004) and a two-dimensional cumulative map was produced after the last survey (Fig.1). In order to evaluate the hypothesis of randomness in the expression of the disease the cumulative map was divided into quadrats 2×2, 3×2, 9×2 and 9×9 vines, laid out along the rows or columns, and the inside vines classified as healthy, diseased or missing value (no data available). Diseased plants inside the experimental plot could be scattered in one of three spatial patterns: aggregated, random or uniform. Two indices of dispersion - Variance-to-mean-ratio (VMR) and Morisita's index (I_d), (Krebs, 1989) - were applied, as well as the two dimensional distance class analysis program 2DCLASS (Nelson, 1995; Ferrandino, 1996; Nelson and Guzman, 1997) in order to typify the spatial pattern of plants with esca symptoms. Values of VMR and I_d not significantly different from 1 indicate a random distribution, values bigger than 1 are in favour of an aggregated pattern, and values tending to zero indicate a uniform pattern. For two-dimensional distance class analysis (2DCLASS) it was used the 2DCLASS software developed by Nelson and Guzman (1997), applicable to any culture where plants are regularly spaced, and where the binary presence/absence of symptoms or disease can be biologically accepted (Nelson and Campbell, 1993). This program using the X,Y coordinates of each plant as input data generates a matrix (Fig. 2), showing the position of standard count frequencies (SCF) less and greater than expected for each [X,Y] distance classes (1296). Data sets are interpreted as non-random if the total number of significant SCF is bigger than 5% of the total distance classes. The program performed 400 simulations.

Isolation

Every year, after the harvest, strongly symptomatic grapevines were uprooted. At a height of approximately 30 cm, the plants were sectioned and the wood examined for internal symptoms. All the observed lesions had originated on pruning wounds, as observed in preparatory longitudinal sections of the stems. The wood deterioration observed inside the uprooted vines was classified into two different categories adapted from Larignon and Dubos (1996) and Pollastro *et al*. (2000):

- category I, wood with a central area of pale coloration with three distinguishable zones: spongy white decay (SWD) bordered by a black line (BL) and a dark brown hard wood (BHW) lesion;
- category II, wood with a central dark brown coloration of hard consistency with included black streaks. Occasionally, an incipient presence of spongy white decay was observed.

Cross sections of the woody stem were cut off and 10 small portions (535 mm) of each of the different deteriorated wood tissues were plated in 2% malt extract agar (MEA, Difco®, Detroit, MI, USA), amended with 100 mg l^{-1} chloramphenicol (Panreac®, Barcelona, Spain) and incubated in the dark at 25±1ºC. Plates were observed every 7 days, and newly formed colonies were transferred to MEA for growing and identification. All observed colonies

Table 1. Fungi obtained from isolation of wood tissue from 23 esca affected grapevines during the years 2002, 2003 and 2004. Vines were collected from a single plot in the Dão wine region.

Lesion category[a]	Type of wood necrosis or decay	*Fomitiporia mediterranea*	*Phaeoacremonium* spp.	*Phaeomoniella chlamydospora*	*Botryosphaeria* spp.	Others
I (19 plants)	Spongy white decay (SWD)	60.52	5.26	1.05	13.68	8.94
	Black line (BL)	6.31	32.63	20	8.42	32.1
	Brown hard wood (BHW)	9.47	41.57	3.15	15.26	4.73
II (4 plants)	Brown hard wood	0	25	12.5	37.5	27.5
	Black line	0	15	47.5	0	22.5

[a] Adapted from Larignon and Dubos (1997).

were identified and their frequencies recorded. Twenty three grapevines were analysed.

Results

The results of the mycological analysis are presented on Table 1. It is evident that most of the uprooted plants were considered under category I of wood degradation.

On category I, *Fomitiporia mediterranea* (Fr.) Murrill colonies were obtained at a high frequency from spongy white decay (SWD) when compared with the number of *F. mediterranea* colonies obtained from black line (BL) and dark brown hard wood (BHW). *Phaeoacremonium* spp. and *Phaeomoniella chlamydospora* (W. Gams, Crous, M.J. Wingfield & L. Mugnai) Crous & W. Gams, pres-

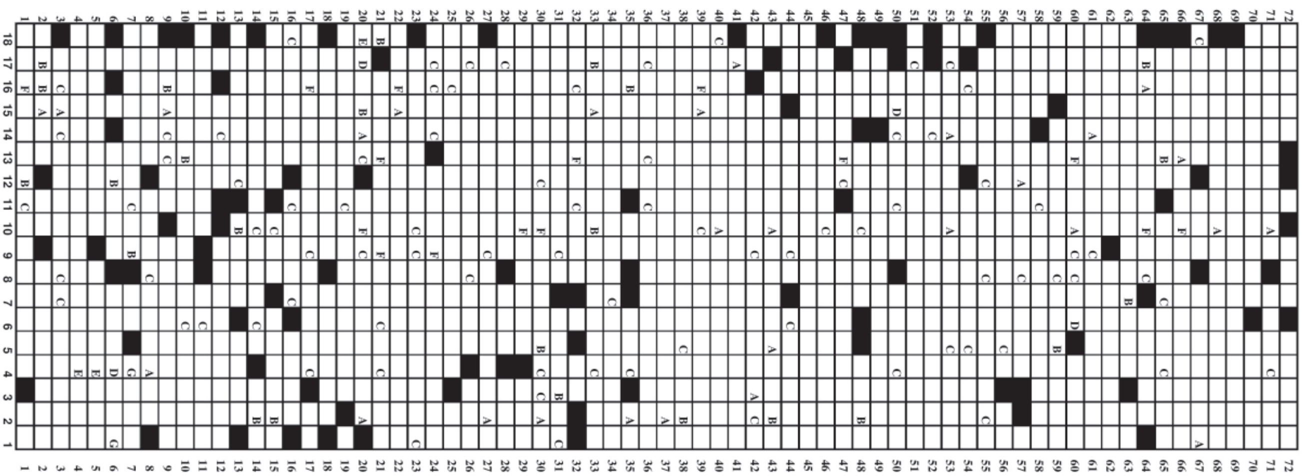

Fig. 1. Annual and cumulated spatial distribution of esca in the study plot. A. symptom expression in 2002; B, symptom expression in 2003; C, symptom expression in 2004; D, symptom expression in 2002 and 2003; E, symptom expression in 2002 and 2004; F, symptom expression in 2003 and 2004; G, symptom expression in all the three years. Black squares stand for missing value, white squares stand for non-symptomatic.

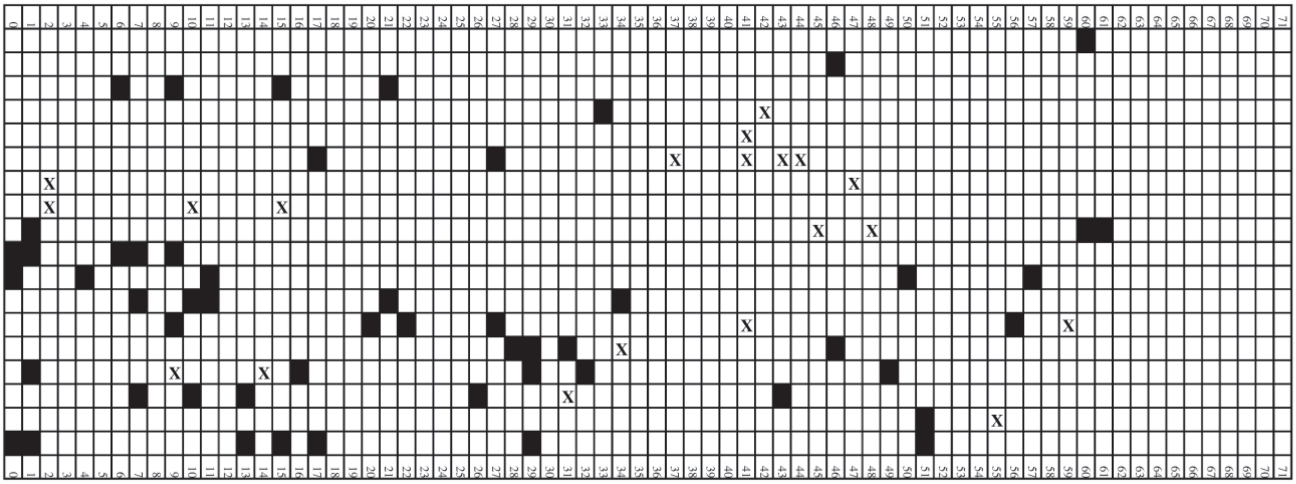

Fig. 2. Two-dimensional class analysis matrix for the cumulative values in the study plot. Black squares - SCFs (standard count frequencies) significantly greater than expected ($SCFs^+ = 53$); X signed squares - SCFs significantly less than expected ($SCFs^- = 20$); white squares indicate [X,Y] distance classes with SCFs as expected. As the proportion of significant SCFs is less than 0.05 this matrix represents a random data set.

ence was only residual on SWD, but their isolation frequencies were high on BL. On BHW *Phaeoacremonium* species were the most frequent, while *F. mediterranea* and *Pa. chlamydospora* had very low frequencies. In all the isolations on category I, there was a significant presence of *Botryosphaeria* spp. Under category II, *F. mediterranea* was never isolated. *Pa. chlamydospora* and *Phaeoacremonium* spp. were found on both types of necrosis, being *Phaeoacremonium* spp. prevalent in BHW while *Pa. chlamydospora* was more frequent on black streaks. *Botryosphaeria* spp. was also present in large numbers, with colonies obtained just from BHW. Other fungi, mostly saprophytic or weak pathogens, were common on plates from both categories.

The number of symptomatic vines increased from year to year during the survey (Fig. 1). Of an initial 1,296 plants, 37 plants (2.85% of the total) were recorded as symptomatic in 2002 and 45 (3.47% of the plot) in 2003. During 2004, the number of plants showing symptoms increased abruptly to 104 plants (8.02% of the plot). By the end of the 3 years survey 12.34% of the plot had

Table 2. Indices of dispersion (VMR and I_d) and Chi-square goodness-of-fit (χ^2) to Morisita's index (Id) and to VMR, for the study plot.

Quadrat size (vine × vine)	Diseased plants per quadrat (mean)	s^2 [a]	VMR [b]	I_d [c]	χ^2	D.F. [d]	*P*[e]
2×2	0.49	0.45	0.93	0.86	301.34	323	>0.05
3×2	0.73	0.76	1.03	1.04	222.05	215	>0.05
9×2	2.19	2.78	1.27	1.12	89.9	71	>0.05
9×9	9.88	12.92	1.31	1.03	19.62	15	>0.05

[a] Variance.
[b] Variance-to-mean ratio.
[c] Morisita's index.
[d] Degrees of freedom.
[e] *P*>0.05 indicates a random set of data.

clearly shown foliar symptoms in at least one year.

The distribution of esca affected vines was subject to two indices of dispersion -VMR and Morisita's index- using quadrats of different sizes (Table 2). The value of these indices were never significantly different from 1 (P>0.05) indicating that the spatial pattern of the symptomatic plants was indistinguishable from random.

Two distance class software analysis confirmed the randomness of the data set. The number of distance classes with significantly greater than expected SCF was 53 and the number of distance classes with fewer than expected SCF was 20, so the proportion of significant SCF was lower than 5% which meant a random data set (Nelson and Guzman, 1997).

Discussion

The mycological analysis of the affected vines pointed out that the main fungi associated with esca in this vineyard were those mentioned in other works on the same subject (Mugnai *et al*., 1996; Larignon and Dubos, 1997) for the same kind of decays and in similar proportions. Fungi from the genus *Botryosphaeria* were frequently isolated, a situation also reported by other researchers (Armengol *et al*., 2001; Oliveira *et al*., 2003) suggesting its role should not be neglected and deserves further investigation (Larignon *et al*., 2001; Oliveira *et al*., 2003. *Eutypa lata* (Pers.:Fr.)Tul. & C. Tul. was never detected. This fact is in accordance with the statement in the papers by Armengol *et al*. (2001) and Mugnai *et al*. (1999), that *E. lata* is not found at all or is rare in esca-infected vineyards in southern Europe. External symptoms similar to those considered typical of *E. lata* infection are not often found in this Portuguese wine region.

From the results it is possible to infer that there was a clear increase in the number of infected vines from year to year. In 2003 there was a small increase in respect to 2002, but in 2004 the number of symptomatic vines increased abruptly. This observation must be the object of further studies in order to determine if the 2004's sudden increase in symptom expression was due to an increase in contamination, or if the huge number of plants showing symptoms was a consequence of favourable environmental conditions. Of the 160 plants affected by esca, 46 died in the same year of symptoms manifestation, while 114 survived. A simple analysis of Figure 1 shows the erratic behaviour of symptom expression. Vines showing symptoms one year might not show them again in the following year, confirming the conclusions of other works in Italy and Spain (Mugnai *et al*., 1996; Surico *et al*. 2000; Redondo *et al*., 2001).

It is possible that during the 3 year survey, most of the esca infected vines have been recorded for the 15 year old vineyard. Considering the presence, in the close proximity, of an 18,000 m^2 esca-infected, 60 year old, non-pruned vineyard, the random distribution of the disease suggests that airborne spores carried out from the neighbouring vineyard may have played a role in disease spread in the vineyard surveyed. This hypothesis is supported by the regular spore-trap capture of *Pa. chlamydospora* and *Phaeoacremonium* spp. in other surveys (unpublished data) at the same location, as well as by the presence of *F. mediterranea* fruit bodies in the 60 year old vineyard. The hypothesis of secondary spread by pruning tools becomes less tenable in the absence of an aggregated pattern. Nevertheless, further surveys and the probable increase in disease incidence with consequent aggregation of symptomatic plants will clarify the role of pruning on the spread of the esca-pathogens.

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