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Epidemiological survey on esca disease in Umbria, central Italy

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Summary. Esca is a grapevine disease occurring in all vine-growing areas, including Italy. Multiple fungi that have been associated with esca spread by releasing aerial spores that infect pruning cuts and wounds. The aim of the study was to determine the occurrence of the most common fungi associated with esca in Umbria (central Italy) and to find under what weather conditions they release their spores. Disease incidence was also determined in the summers of 2006 and 2007 on ten grapevine cultivars (Cabernet Sauvignon, Grechetto, Sangiovese, Trebbiano toscano, Pinot bianco, Trebbiano spoletino, Ciliegiolo, Montepulciano, Chardonnay and Merlot) growing together in the same vineyard. Visual inspection of the vines showed that all cultivars were susceptible to esca, but that esca incidence differed in intensity between cultivars. In both years, esca incidence was higher with Cabernet Sauvignon, Sangiovese and Trebbiano toscano, and lower with Montepulciano and Merlot. Disease incidence went up in summer. *Phaeomoniella chlamydospora* was isolated from vine samples showing dark wood streaking, and *Fomitiporia mediterranea* from samples with white rot, confirming that these fungi are involved in esca. Spores of *P. chlamydospora* were trapped from March to December 2007. Spore release was affected by weather parameters (rain and temperature).

Key words: grapevine, airborne spores, isolation, *Phaeomoniella chlamydospora, Fomitiporia mediterranea*.

Introduction

Esca is a grapevine disease with a worldwide distribution (Fischer, 2006), which significantly reduces grape yield and quality and which shortens vine life. Esca is considered a complex of two distinct diseases (Surico *et al.*, 2006): a so-called phaeotracheomycosis caused mainly by *Phaeomoniella chlamydospora* (Pch) and/or *Phaeoacremonium aleophilum* (Pal), and a white rot caused by a wood-rotting basidiomycete such as *Fomitiporia mediterranea* (Fmed). The hyphomycetes cause dark wood streaking and gummosis, the typical tiger stripes on the leaves, and black measles on the berries. The incidence of the disease peaks in 25–35-year-old vineyards, but high incidences of infection are also common in young vineyards. Disease incidence varies over the years since symptoms on the leaves and berries do not appear every growing season on those vines that are infected (Surico *et al.*, 2006). The causal agents of esca typically spread by aerial spores released during and following rain events (Larignon and Dubos, 2000; Eskalen and Gubler, 2001). Spores infect the host vines mainly through pruning cuts and wounds (Surico *et al.*, 2006), even though the inoculum is also often transported to the vineyard by means of infected cuttings (Rooney and Gubler, 2001).

The aim of the present study was to verify the occurrence of the most common esca-associated fungi in Umbria (central Italy) and to determine, for the first time in Italy, when and under what weather conditions the fungi release the spores that then infect other vines. Surveys were also

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carried out to determine the incidence of esca on different grapevine cultivars growing under the same conditions.

Materials and methods

Esca incidence

Esca incidence was estimated during summers 2006 and 2007 in a 25-year-old experimental vineyard of the University of Perugia (Umbria, central Italy), trained to spur-pruned cordon with a 1.70 m trunk height, on ten cultivars: Chardonnay, Pinot bianco, Grechetto, Trebbiano spoletino, Trebbiano toscano, Cabernet Sauvignon, Montepulciano, Merlot, Ciliegiolo and Sangiovese. For each cultivar, 3 rows of about 65 plants each were surveyed, making a total of about 2000 plants.

Identification of esca associated fungi

Starting in autumn 2006, 4 vines with their rootstocks were sampled from each cultivar growing in the experimental vineyard of the University of Perugia, giving a total of 40 plants. For each cultivar, 2 vines were selected that in the summer of 2006 showed esca symptoms on the leaves and berries and 2 vines that during that season did not show any symptoms. Of the last 2 vines, one grew close to vines with esca symptoms, and one grew far away from vines with symptoms. Each vine-plant was cut into 4 portions (rootstock, bottom, centre and apex) and each portion was inspected for dark wood streaking and white rot. To detect Pch, 7 pieces of each section were surface-sterilised for 15 seconds in an aqueous solution of mercury chloride (1‰), rinsed in sterile deionised water and incubated on malt agar (MA) amended with 0.1 g l^{-1} tetracycline-HCl, pH 5.7, on 9-cm Petri dishes in the dark at 21±2°C. The isolation frequency was calculated as the percentage of pieces yielding Pch colonies. Data on isolation frequency were subjected to one-factor analysis of variance (ANOVA). Multiple comparison tests were carried out using Fisher's protected LSD.

Some samples showing white rot were dipped in 95% ethanol, sterilised in a flame and used for Fmed detection on the same medium and in the same conditions as above.

Spore capture

Spores were trapped on glass microscope slides according to the method of Eskalen and Gubler (2001). The slides were coated with silicone gel (900401 Silicone-Lanzoni srl) on both sides and affixed close to old pruning cuts and wounds on vine cordons which in the summer of 2006 showed esca symptoms (Fig. 1). Slides were replaced every week from January to December 2007. Collected slides were washed in 10 ml sterile deionised water that was then filtered through 5 μm and 0.45 μm Minisart microfilters (Minisart, Sartorius). The 0.45 μm filter was rinsed with 1 ml sterile deionised water and the rinsing water (250 μl) was placed on 9 cm Petri dishes containing MA amended with 0.1 g l^{-1} tetracycline-HCl, pH 5.7. Four Petri dishes per slide were obtained. The number of trapped spores was counted as Colony-Forming Units (CFU), which expresses the number of spores able to form new colonies. After 10 days of incubation at 21±2°C in the dark, colonies of the causal esca agents were counted on each of the four dishes obtained from every slide. Trapped spore data were related to weather data (rain and temperature), supplied from the weather network of the Umbria Regional Government.

Results

Esca incidence

In 2006, the first survey was carried out in mid-July, when the first esca symptoms appeared, and again at the end of August, a few days before the harvest, when the symptoms were at their height. The incidence of esca, estimated on the ten cultivars, increased from July (12.7% of vines infected) to August (24.3%) (Fig. 2a). In both months, the higher incidence of esca was recorded on Cabernet Sauvignon, Grechetto, Sangiovese and Trebbiano toscano, while the incidence was lower on the other cultivars (Fig. 2b).

In the summer of 2007, the vineyard was inspected three times during the season because symptoms appeared in June, earlier than in the previous year. As in 2006, esca incidence increased in the course of the summer, peaking at the end of August (Fig. 3a). The highest incidence again occurred in Cabernet Sauvignon, Grechetto, Sangiovese and Trebbiano toscano, confirming earlier findings (Fig. 3b).

Fungi associated with esca in a vineyard in Umbria

All the vines inspected showed dark wood streaking (Fig. 4a) and brown black spots (Fig. 4b) often associated with white rot (Fig.4a).

Fig. 1. Spore trap slide coated with silicone gel and affixed near an old pruning cut.

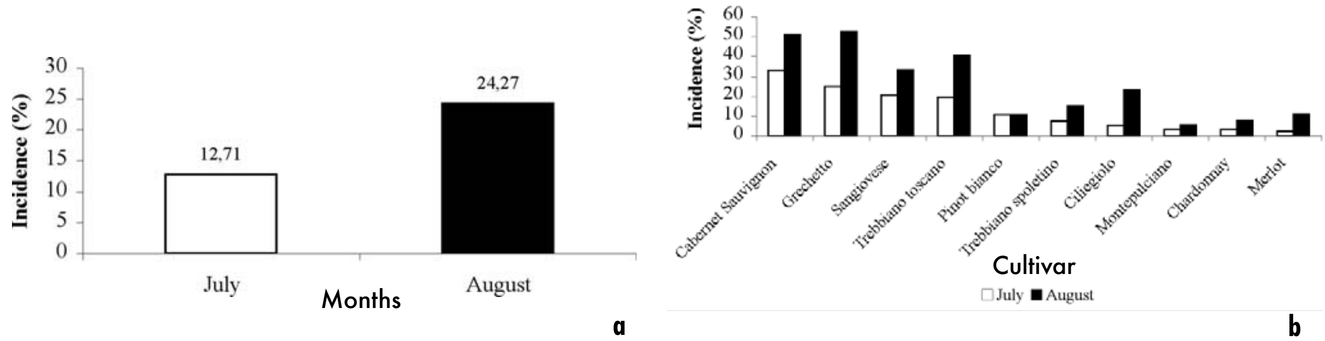

Fig. 2. (a) Incidence of esca recorded during the summer 2006 on the experimental vineyard of the University of Perugia (Umbria, central Italy). (b) Incidence of esca recorded on ten cultivars grown in the vineyard.

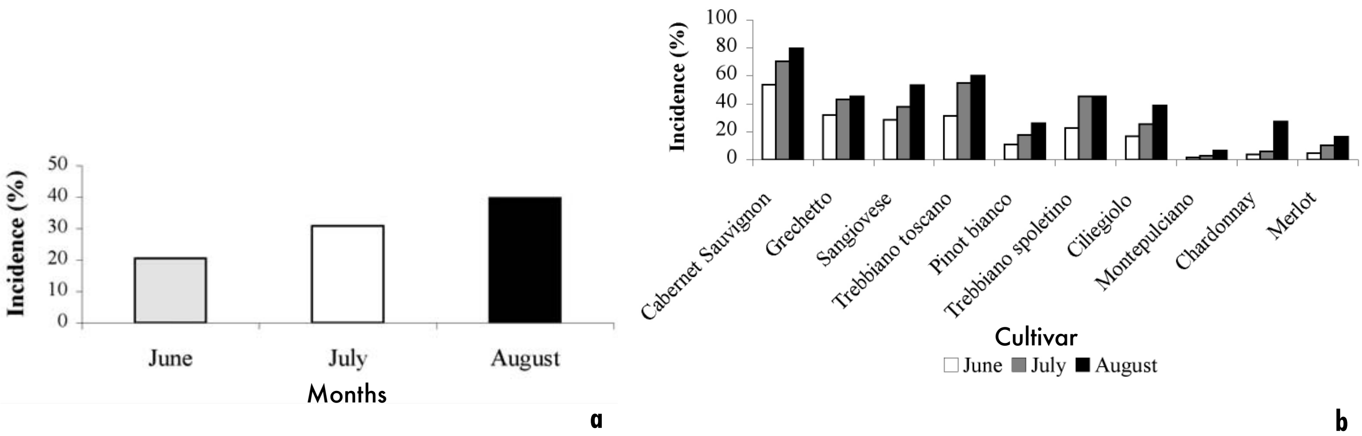

Fig. 3. (a) Incidence of esca recorded during the summer 2007 on the experimental vineyard of the University of Perugia (Umbria, central Italy). (b) Incidence of esca recorded on the ten cultivars grown in the vineyard.

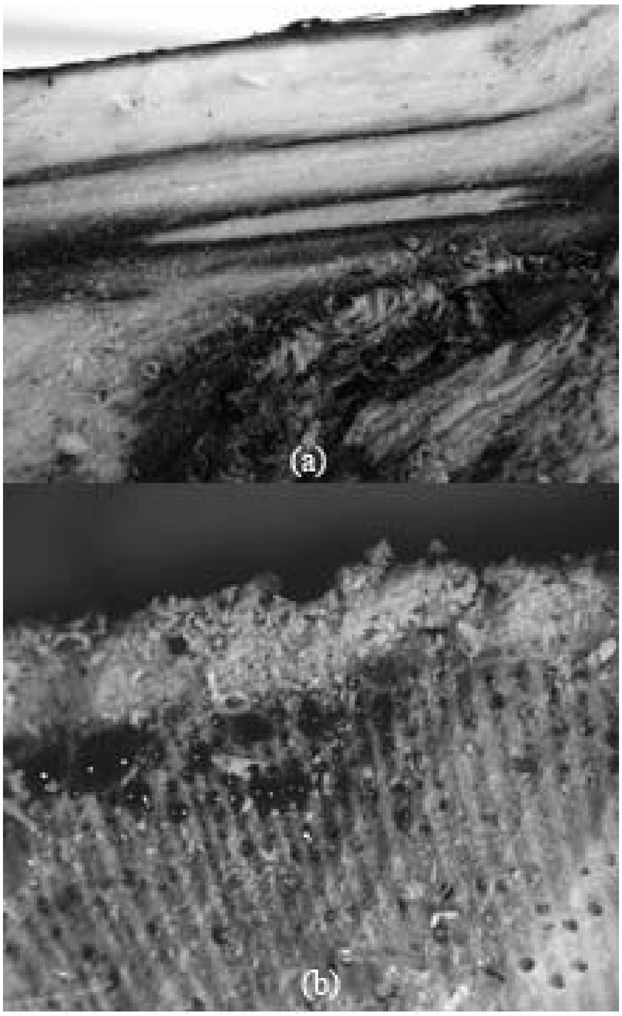

Fig. 4. (a) Dark wood streaking and white rot on longitudinal section of grapevine trunk. (b) Brown-black spots in cross sectioned arm.

Bright white, slow-growing colonies were obtained from the samples with dark streaks in the xylem. Over time, these colonies turned olive-grey, starting from their centre. Monophialidic conidiogenous cells producing subhyaline, oblong-ellipsoidal to obovate conidia (1.5-) 3–4×1–1.5 μm were seen. On the basis of the morphological features of the colonies and the conidia, the fungus was identified as Pch as described by Crous and Gams (2000). Identification was certified by the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands. Pch was isolated from all 40 vines. The Pch isolation frequency differed significantly between cultivars (Fig. 5a). It was significantly lower in Cabernet Sauvignon, Chardonnay and Grechetto, cultivars that also did not differ between each other, whereas it was significantly higher in Ciliegiolo and Trebbiano toscano, and these cultivars differed significantly between each other (Fig. 5a). There were no significant differences between the Pch isolation frequencies of the four plant portions (rootstock, bottom, centre and apex) (Fig. 5b). The Pch isolation frequency also did not differ significantly between vines with esca symptoms on the leaves and berries, and vines that did not show these symptoms (Fig. 5c). Among the vines that did not show esca symptoms on the leaves and berries, there was no significant difference in isolation frequency between vines that grew near vines with esca symptoms in summer 2006 and vines growing near vines that did not show such symptoms in the same period (Fig. 5d).

Colonies that were initially white and felted, then ochraceous and cottony, were obtained from vines that had white rot. The reverse of these colonies showed white-to-brown areas. On the basis of the morphological features of the colonies, the fungus was identified as *Fomitiporia mediterranea*, the most common agent of white rot in Europe (Fischer, 2006) and the only *Fomitiporia* species so far identified in Italian vineyards (Mugnai, personal communication).

Spore capture

Using microscope slides coated with silicone gel, spores of Pch were captured every month, except in January and February (Fig. 6 and 7a, b). Pch spore release occurred during and following rain events (Fig. 7a). Spore release was also affected by the temperature: the number of trapped spores was negligible in the hottest months of the year (January, February, November and December), rose in spring, reached a peak in May and June and became nil in the hottest weeks of the year (Fig. 7b). Spores of Fmed were never captured.

Discussion

Esca incidence is affected by a number of factors such as climate, vineyard age, the training system, agricultural practices, and the cultivar grown (Mugnai, 1999). In the present study, all the vines observed grew in the same environmental and cultural conditions and this allowed the susceptibility of the cultivars to be compared. In both years, Cabernet

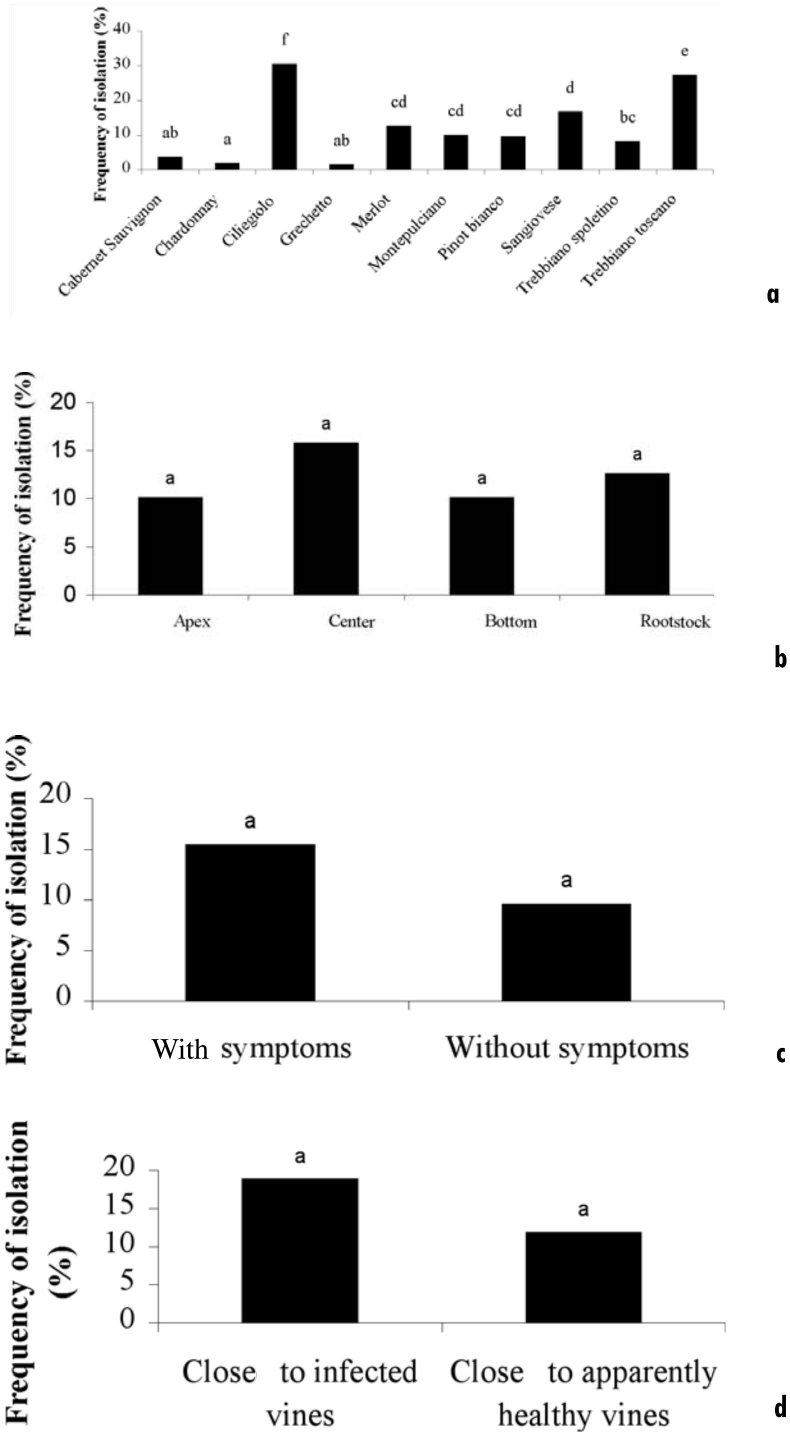

Fig. 5. Frequency of isolation of *Phaeomoniella chlamydospora* (a) on the ten cultivars grown in the vineyard of the University of Perugia (Umbria, central Italy), LSD (P=0.05) =15.45; (b) on the cross-section of the plants, LSD (P= 0.05)= 11.35; (c) on vines that showed esca symptoms on leaves and berries and on vines that did not show these symptoms, LSD (P= 0.05)=8.85; (d) on vines that did not show esca symptoms on leaves and berries and that were closed to infected or apparently healthy plants, LSD (P=0.05)= 15.48. S.E.

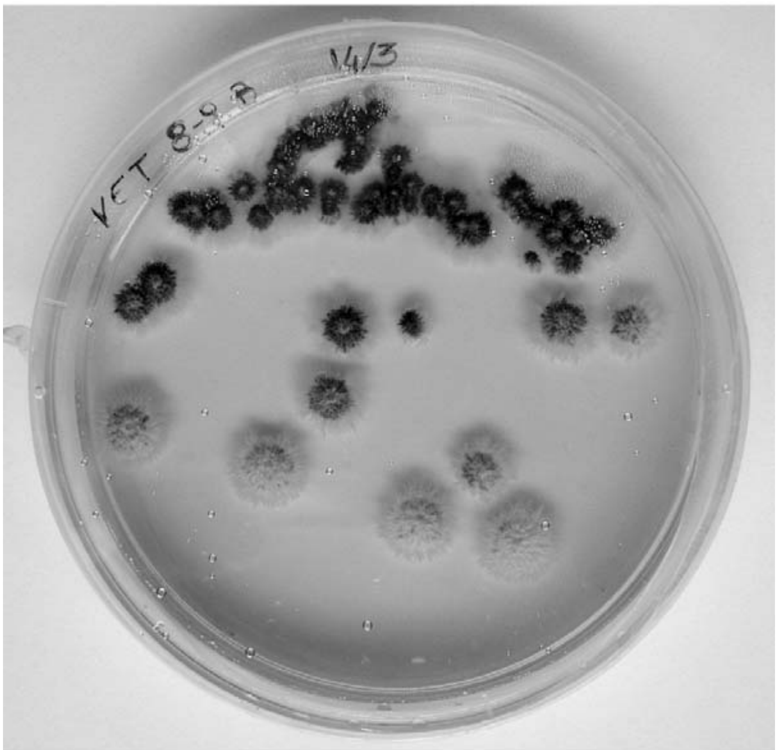

Fig. 6. Trapped *Phaeomoniella chlamydospora* growing on malt agar added with 0.1 g l^{-1} tetracycline-HCl.

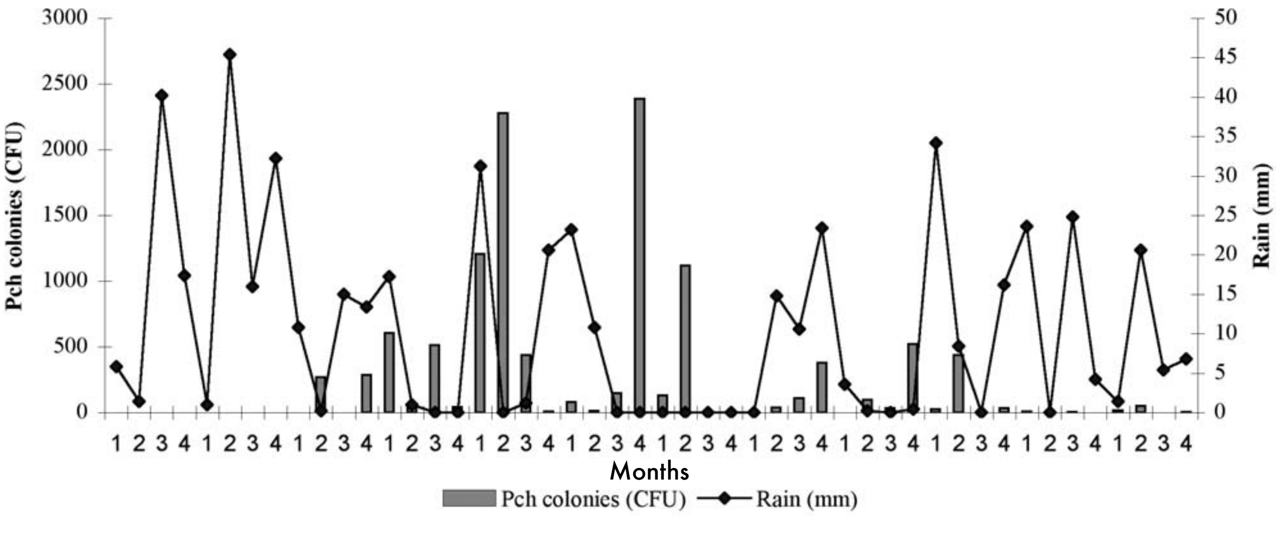

Fig. 7. (a) Number of spores of *Phaeomoniella chlamydospora* trapped per week and rainfall from January to December 2007.

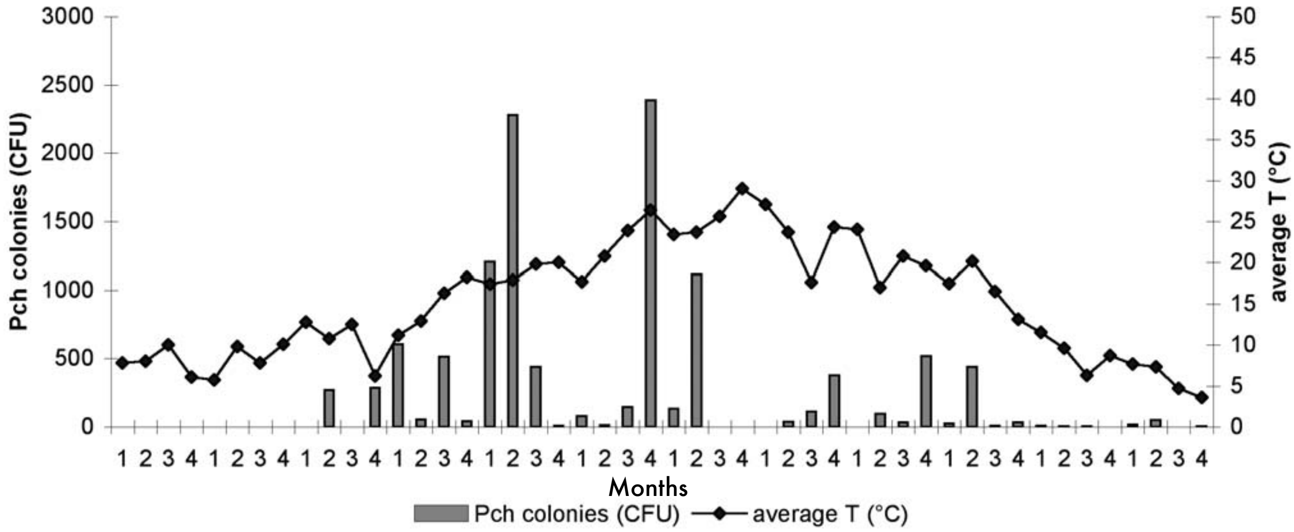

Fig. 7. (b) Number of spores of *Phaeomoniella chlamydospora* trapped per week and temperature, from January to December 2007. CFU= Colonies Forming Unit (number of spores able of giving origin to new colonies).

Sauvignon was the most susceptible cultivar, while Montepulciano and Merlot were less susceptible. This difference in susceptibility between Cabernet Sauvignon and Merlot was already reported by Borgo *et al*. (2008) in northern Italian vineyards.

Data from fungal isolation showed that Pch was the only hyphomycete associated with black wood streaking in the 25-year-old experimental vineyard of the University of Perugia, a finding that was already reported by Edwards *et al*. (2001). Moreover, Sparapano *et al*. (2001) reported that Pch caused dark wood streaking as well as the symptoms on the leaves and berries when it was inoculated singly in grapevine spurs. Consistent with the findings of other studies (Fischer, 2002, 2006; Surico, 2006), Fmed was found to be a white rot agent in esca-diseased grapevines in Umbria as well.

Pch was isolated from all the vines sampled, both those manifesting esca symptoms and those that were asymptomatic, confirming that the visible symptoms of the disease need not necessarily appear on diseased vines every year (Marchi *et al*., 2006). The discontinuity of esca symptoms on the leaves and berries that may occur for one or more growing seasons in succession makes it almost impossible to ascertain the real incidence of esca in a given vineyard at any time; the only way in which this can be done is by inspecting the vines annually for several years in the hope that all diseased vines, even those that are temporarily asymptomatic will eventually be found. Moreover, the incidence of visual esca symptoms was not correlated with the frequency of Pch. In both years, the highest incidence of esca symptoms was recorded in the cultivars Cabernet Sauvignon and Grechetto but these cultivars had a significantly lower Pch isolation frequency than the other cultivars that had a lower disease incidence. The Pch isolation frequency did not significantly differ between plant portions. The importance of aerial spores as infection agents in the epigeous parts of the plants is therefore confirmed (Eskalen and Gubler, 2001; Larignon and Dubos, 2001) while the infection of the rootstock can be attributed to the use of infected cuttings, since the infection rarely passes below the grafted portion (Mugnai, 1999).

As Larignon and Dubos (2000) found in France, so also in central Italy, aerial spores of Pch were trapped almost every month, even if the highest spore counts were found in spring: spores captured in May and June represented approximately 61% of the total amount of spores captured during the entire year. According to Larignon and Dubos (2000) and Eskalen and Gubler (2001), spores were released during and following rain events. The temperature also seemed to play a role since spores were rarely captured during the coldest and rainy weeks of the year. The role of temperature on the conservation and maturation of pathogen structures such as the oospores of *Plasmopara viticola* (Serra and Borgo, 1995) or the pycnidia of *Phomopsis viticola* (Prota, 1970) is well known. The fact that no spores were captured during the hottest period of the year could be explained by the absence of rain in the preceding weeks. Our data, even though they related to only one year of investigation, confirmed the trend in Pch spore release already found in France (Larignon and Dubos, 2000) and in California (Eskalen and Gubler, 2001).

By artificially inoculating Pch on canes, Serra *et al.* (2007) demonstrated that canes pruned in March were less susceptible to Pch infection than canes pruned in January and February, because the later cuts healed more rapidly. However, as is well known, pruning cuts remain susceptible to esca pathogens infection for about one month (Michelon *et al.*, 2005) so that the cuts made in March were exposed to infection when the airborne spread of Pch spores began to be significant. Therefore, the findings of the study confirm that the current trend to prune the vines in early winter, when airborne inoculum levels are low, is the correct one.

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Literature cited

Borgo M., D. Bellotto, G.L. Dal Cortivo, A. Zanzotto, E. Tosi and E. Marchesini, 2008. Sensibilità varietale al mal dell'esca della vite nel Veneto. *Atti Giornate Fitopatologiche 2008*, 2, 223–230.

Crous P.W. and W. Gams, 2000. *Phaeomoniella chlamydospora* gen. et comb. Nov., a causal organism of Petri grapevine decline and esca. *Phytopathologia Mediterranea* 39, 112–118.

Edwards J., G. Marchi and I.G. Pascoe, 2001. Young esca in Australia. *Phytopathologia Mediterranea* 40, Supplement, 303–310.

Eskalen A. and W.D. Gubler, 2001. Association of spores of *Phaeomoniella chlamydospora*, *Phaeoacremonium inflatipes*, and *Pm. aleophilum* with grapevine cordons in California. *Phytopathologia Mediterranea* 40, Supplement, 429–432.

Fischer M., 2002. A new wood-decaying basidiomycete species associated with esca of grapevine: *Fomitiporia mediterranea* (Hymenochaetales). *Mycological Progress* 1(3), 315–324.

Fischer M., 2006. Biodiversity and geographic distribution of basidiomycetes causing esca-associated white rot in grapevine: a worldwide perspective. *Phytopathologia Mediterranea* 45, Supplement, 30–42.

Larignon P. and B. Dubos, 2000. Preliminary studies on the biology of *Phaeoacremonium*. *Phytopathologia Mediterranea* 39, 184–189.

Larignon P. and B. Dubos, 2001. Il punto sul ciclo biologico dei funghi coinvolti nella sindrome dell'esca. *Informatore Fitopatologico* 5, 27–31.

Marchi G., F. Peduto, L. Mugnai, S. Di Marco, F. Calzarano and G. Surico, 2006. Some observations on the relationship of manifest and hidden esca to rainfall. *Phytopathologia Mediterranea* 45, Supplement, 117–126.

Michelon L., C. Pellegrini and I. Pertot, 2005. Il mal dell'esca della vite in Trentino. *Terra Trentina* 6, 35–41.

Mugnai L., 1999. Il mal dell'esca della vite. *Informatore agrario* 15, 77–81.

Prota U., 1970. Studies on the fruiting periods of *Phomopsis viticola* causing dead-arm disease in the vine, and on the effect of temperature and sodium arsenite treatment on the maturation of the pycnidia. *Studi Sassaresi* III, 18, 133–143.

Rooney S.N. and W.D. Gubler, 2001. Effect of hot water treatments on eradication of *Phaeomoniella chlamydospora* and *Phaeoacremonium inflatipes* from dormant grapevine wood. *Phytopathologia Mediterranea* 40, Supplement, 467–472.

Serra S. and M. Borgo, 1995. Investigations on maturation and germination of *Plasmopara viticola* oospores overwintered in natural conditions. *Petri* 5(2), 91–104.

Serra S., M.A. Mannoni and V. Ligios, 2007. Preliminary studies on the susceptibility of pruning wounds to contaminations with fungi involved in grapevine decline diseases in Italy. *Phytopathologia Mediterranea* 46, 115.

Sparapano L., G. Bruno and A. Graniti, 2001. Three-year observation of grapevine cross-inoculated with esca-associated fungi. *Phytopathologia Mediterranea* 40, Supplement, 376–386.

Surico G., L. Mugnai and G. Marchi, 2006. Older and more recent observations on esca: a critical overview. *Phytopathologia Mediterranea* 45, Supplement, 68–86.

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