

Studies on the susceptibility of pruning wounds to infection by fungi involved in grapevine wood diseases in Italy

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Summary. The susceptibility of grapevine annual pruning wounds to *Phaeoconiella chlamydospora*, *Phaeoacremonium aleophilum* and *Diplodia seriata* was investigated over three years (2005–2007) in a 15 year-old vineyard, cv. Sauvignon blanc. Vines were pruned each year in January, February and March and the wounds were inoculated weekly with conidial suspensions, and with sterile water as a control. Penetration of the fungi into the wood was assessed after 4 weeks by plating pieces of host tissue on agar medium. The susceptibility of annual pruning wounds, expressed as the infection percentages of inoculated spurs, varied with both the trial year and the fungus inoculated. Average infection percentages of inoculated spurs in the three years were respectively 14.7, 38.5 and 50.9% for *Pa. chlamydospora*, 31.7, 32.2 and 49.4% for *Pm. aleophilum* and 84.2, 43.8 and 40.9% for *D. seriata*. The period of pruning was significant for the infection percentages of all fungi in 2005, and for *D. seriata* in 2006. Natural infection of control spurs by *Pa. chlamydospora* (2, 4.4, and 11.7% of spurs in the three years respectively) and by *Pm. aleophilum* (0.3, 1.8, and 6.4%) began when average weekly temperatures stabilized around 10°C, while infection by *D. seriata* (12.2, 12 and 18.3% in the same period) occurred even below that threshold. Higher infection percentages of both artificially and naturally infected spurs in 2007 were probably due to the higher temperatures recorded in February and March (besides the use of a more efficient selective medium for the isolation of *Pa. chlamydospora* and *Pm. aleophilum*). Only artificial infections with *D. seriata* showed an opposite trend that cannot be explained by the weather data. Infection of one-year-old wood appeared to be an important factor in disease spread. Spurs remained liable to infections with any of the fungi for up to 4 months after pruning, and isolation percentages could be fairly high also in late spring. As a consequence, the planning of pruning does not seem to be an effective means to counteract the wood diseases caused by these fungi.

Key words: epidemiology, *Diplodia seriata*, *Phaeoacremonium aleophilum*, *Phaeoconiella chlamydospora*.

Introduction

Phaeoconiella chlamydospora and *Phaeoacremonium aleophilum* are associated with esca, a serious grapevine decline disease that occurs all over the world (Mugnai *et al.*, 1999; Mostert *et al.*, 2006; Surico *et al.*, 2006). Species in the family Botryosphaeriaceae are associated with a wide range of decline and dieback symptoms in grapevine (van Niekerk *et al.*, 2006). *Diplodia seriata* (anamorph

of “*Botryosphaeria*” *obtusa*, Phillips *et al.*, 2007), causes dieback and decline as well as cankers. In Italy (Cristinzio, 1978; Rovesti and Montermini, 1987) and France (Larignon *et al.*, 2001a, b), *D. seriata* (= *Sphaeropsis malorum*) has been associated with decline and dieback symptoms.

All these diseases can be spread by airborne inoculum getting in through fresh grapevine wounds and causing new infections. *Pa. chlamydospora* produces pycnidia or free conidiophores on old pruning wounds or in cracks of the wood (Edwards *et al.*, 2001; Eskalen *et al.*, 2002). Cracks and crevices provide a protected humid environment favorable for sporulation. Propagule release starts in winter

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and is closely linked to rainfall (Larignon and Dubos, 2000; Eskalen and Gubler, 2001). Moreover, Edwards *et al.* (2001) suggested that collembolans and mites could carry spores adhering to their body. *Pm. aleophilum* does not produce fruiting bodies, but its teleomorph, *Togninia minima*, produces perithecia in the same sites of *Pa. chlamydospora* fructifications (Rooney-Latham *et al.*, 2005). *Pm. aleophilum* spores are released mainly during the growing season and their release is not always correlated with rainfall (Larignon and Dubos, 2000; Eskalen and Gubler, 2001). Pycnidia of *D. seriata* are common on decorticated wood (Larignon *et al.*, 2001a). The release of *D. seriata* spores is related to periods of rain or high relative humidity (van Niekerk *et al.*, 2006).

Every year in winter, grapevines are subjected to more or less intense pruning depending on the training system. Consequently, wounds, through which fungi can penetrate and invade the wood, are always present; in particular, wounds on one-year-old wood to establish the bearing units are common to all training systems. Usually, these wounds are not protected with sealant because this treatment is too expensive and time-consuming. As a consequence, it is important to know when these cuts are receptive to penetration by fungi.

The receptivity of grapevine pruning wounds to *Pa. chlamydospora* and *Pm. aleophilum* has been studied in France and the USA. In the Bordeaux region of France, it was found that both fungi invaded the wood through wounds during winter and that infections were more serious and occurred for a longer time when grapevines were subjected to early pruning (Larignon and Dubos, 2000). In a study carried out in California, pruning wounds remained susceptible to both *Pa. chlamydospora* and *Pm. aleophilum* for up to four months even though infection incidence decreased after the second month (Eskalen *et al.*, 2007). Receptivity to *D. seriata* was investigated in France where it was found that infection of pruning wounds with this fungus decreased rapidly after 1–2 weeks (Larignon *et al.*, 2001a). By contrast, in South Africa, the susceptibility of pruning wounds to several trunk pathogens including *Pa. chlamydospora* and *Neofusicoccum australe*, did not decline over a 3-week period after inoculation, while wounds made and inoculated in late winter generally yielded higher pathogen levels than mid-winter wounds (van Niekerk *et al.*, 2007).

In Italy, while pycnidia and conidia of *D. seriata* are common (Serra *et al.*, unpublished data; Carlucci and Frisullo, unpublished data), *Pa. chlamydospora* and *Pm. aleophilum* (or *T. minima*) fruiting bodies have never been reported and spores have been trapped mainly in the south of the country (Michelon *et al.*, 2006; Carlucci and Frisullo, unpublished data). Nevertheless, infection through wounds on older wood (more than 2 years old) is generally thought to be the main method of dissemination of esca in the field (Petri, 1912; Graniti, 1960; Baldacci *et al.*, 1962; Mugnai *et al.*, 1999; Serra *et al.*, 2000). No information is available about wounds on one-year-old wood but it is commonly accepted by grapevine growers that late pruning minimizes the risk of infection, as wound healing is faster near bud break. The present study was undertaken to investigate the susceptibility of annual pruning wounds to *Pa. chlamydospora*, *Pm. aleophilum* and *D. seriata* in a Mediterranean environment.

Materials and methods

Experiments were carried out in a 15-year-old pergola-trained vineyard, cv. Sauvignon blanc grafted on SO4 rootstock, located in Alghero, northern Sardinia, Italy. In this vineyard many grapevines (over 60% in six years) showed esca symptoms (leaf chlorosis and necrosis, tiger-stripes, cane defoliation and wilt, cluster dehydration, apoplexy and death). Black spots, dark red-brown areas close to the pith and white rot as well as brown sectors and dark brown areas under the bark were often observed in the trunk or arm of the same grapevine.

In 2005, 2006 and 2007, one-year-old canes were pruned during the dormant season in the first ten days of January, February and March, leaving a 20–30-cm long spur on the vine. Every month, 1500–2000 canes, distributed on 470 vines along two rows, were cut. Wounds were not protected to avoid any influence on natural healing. Every week, from the date of pruning until April–May, five spurs on contiguous plants (1–2 spurs/plant) were artificially inoculated with each treatment (*Pa. chlamydospora*, *Pm. aleophilum*, *D. seriata* or control). Treatments were repeated four times (20 spurs per treatment).

Artificial inoculations were carried out using fungal strains isolated from Sardinian grapevines. The fungi were grown on malt extract agar (MEA,

2% malt extract, 1.5% agar). Conidial suspensions were produced as follows. *Pa. chlamydospora* and *Pm. aleophilum* were streaked on agar slants and incubated at 25°C for 10–15 days. Before inoculation, 10 ml of sterile distilled water was added to each slant, which was shaken to dislodge the conidia. *D. seriata* was grown on agar plates at room temperature under natural lighting for at least two weeks to promote formation of pycnidia. Before inoculation, about 50 pycnidia were harvested and crushed in 1 ml of sterile distilled water to release the conidia. The conidial concentration of *Pa. chlamydospora* and *Pm. aleophilum* was adjusted to about 1×10^5 conidia ml⁻¹, and the conidial concentration of *D. seriata* to about 6.25×10^4 conidia ml⁻¹. Concentrations were determined with a Thomas' haemocytometer. Forty μ l of each conidial suspension was inoculated on each wound (estimated inoculated conidia were 4000 each for *Pa. chlamydospora* and *Pm. aleophilum*, and 2500 for *D. seriata*). Control spurs were inoculated with 40 μ l of sterile distilled water. After inoculation, spurs were covered with sterilized wet cotton wool and aluminium foil to encourage infections and to prevent further natural infections.

Four weeks after inoculation, the spurs were excised and cut into ten 1-mm-thick slices sequentially from the top (Larignon and Dubos, 2000). Slices were placed on Petri plates (five slices per plate) containing MEA supplemented with tetracycline hydrochloride and streptomycin sulphate (50 mg l⁻¹ each). In 2006 and 2007, in addition to the antibiotics, the medium for the isolation of *Pa. chlamydospora* and *Pm. aleophilum* was supplemented with dichloran, hymexazol and tolclophos methyl (10, 5 and 0.1 mg l⁻¹ respectively) to limit fast-growing fungi. Plates were incubated at 25°C for up to 20 days and periodically inspected.

All fungi were identified by macroscopic features of the colonies and by examination of the reproductive structures under the light microscope. *Pa. chlamydospora*, *Pm. aleophilum* and *D. seriata* colonies isolated from the inoculated spurs were identified by comparing them with the inoculated strains. A spur was considered to be infected if at least one slice gave rise to a colony of the inoculated fungus.

All trials were established in a completely randomized design. Data were expressed as the percentage of spurs infected and were arcsin-square root transformed prior to analysis. Each treatment was

analyzed in function of the pruning period (weekly values being considered as repetitions of the same pruning period) and the year, by two-way analysis of variance (ANOVA). Differences among pruning periods within years were analyzed by one-way ANOVA. Means were separated using the Tukey HDS test at $P \leq 0.05$. All statistical analyses were performed with Statgraphics plus, 2000 (Standard ed. version 5, Manugistic Inc., Rochville, MD, USA). Temperature and rainfall data were obtained from a weather station (Prometeo, Micros, Castello Roganzuolo, Treviso, Italy) located on the farm.

Results

Isolation from spurs, regardless of the inoculation treatment, gave rise to numerous fungal colonies. Most of them were fast-growing fungi of the genera *Alternaria*, *Cladosporium*, *Botrytis*, *Epicoccum* and mycelia sterilia (isolation frequencies not recorded).

Analysis of variance on the percentage of artificially and naturally infected spurs showed significant F ratios ($P \leq 0.05$) for the year factor as concerns all fungi (Table 1). F ratios for the pruning period factor were not significant except for natural *Pa. chlamydospora* infections. Significant year \times pruning period interactions were observed with most treatments. For this reason pruning period data were analysed year by year (see below).

2005

Average temperatures were unusually low in the winter (Fig. 1). They remained below 9°C, more often near 5°C, and approached 10°C only at the end of March. The mean, minimum and maximum temperatures in the January–March period were 7.7, 2.5 and 12.9°C respectively. Rainfall was regularly distributed in the experimental period, but scarce in March. On Mar 30th profusely dripping sapwood from wounds (bleeding) was observed.

Phaeomoniella chlamydospora and *Pm. aleophilum* were inoculated at weekly intervals starting from the pruning dates (Jan 11st, Feb 10th, Mar 9th) until Apr 20th, while *D. seriata* was inoculated from the pruning dates until May 4th.

F ratios for the pruning period were significant with all inoculated fungi ($P=0.0016$, $P=0.0121$ and $P=0.0002$ for *Pa. chlamydospora*, *Pm. aleophilum* and *D. seriata* respectively) but not for natural infections (ANOVA Tables not shown).

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Table 1. Analysis of variance for year and pruning period on the percentage of infected spurs artificially inoculated or naturally infected with *Phaeoconiella chlamydospora*, *Phaeoacremonium aleophilum* or *Diplodia seriata*.

Treatment	Source of variation	<i>Phaeoconiella chlamydospora</i>				<i>Phaeoacremonium aleophilum</i>				<i>Diplodia seriata</i>			
		D.F. ^a	Mean square	F ratio ^b	P value	D.F.	Mean square	F ratio	P value	D.F.	Mean square	F ratio	P value
Artificially inoculated	Year	2	31987.2	64.0	<0.0001	2	9437.8	17.4	<0.0001	2	57698.6	100.5	<0.0001
	Pruning period	2	1153.2	2.3	0.1007	2	916.3	1.69	0.1853	2	676.3	1.2	0.3087
	Year × pruning period	4	2062.7	4.1	0.0027	4	1195.0	2.2	0.0674	4	5928.8	10.3	<0.0001
	Residual	451	499.8			451	541.6			479	574.0		
	Total (corrected)	459				459				487			
Naturally infected	Year	2	4616.4	28.4	<0.0001	2	2075.3	23.7	<0.0001	2	2193.8	7.0	0.0010
	Pruning period	2	528.1	3.3	0.0399	2	122.2	1.4	0.2495	2	114.3	0.4	0.6950
	Year × pruning period	4	529.0	3.3	0.0120	4	236.7	2.7	0.0304	4	306.6	1.0	0.4197
	Residual	451	162.7			451	87.8			479	313.9		
	Total (corrected)	459				459				487			

^a Degrees of freedom.

^b F ratio is significant at $P \leq 0.05$.

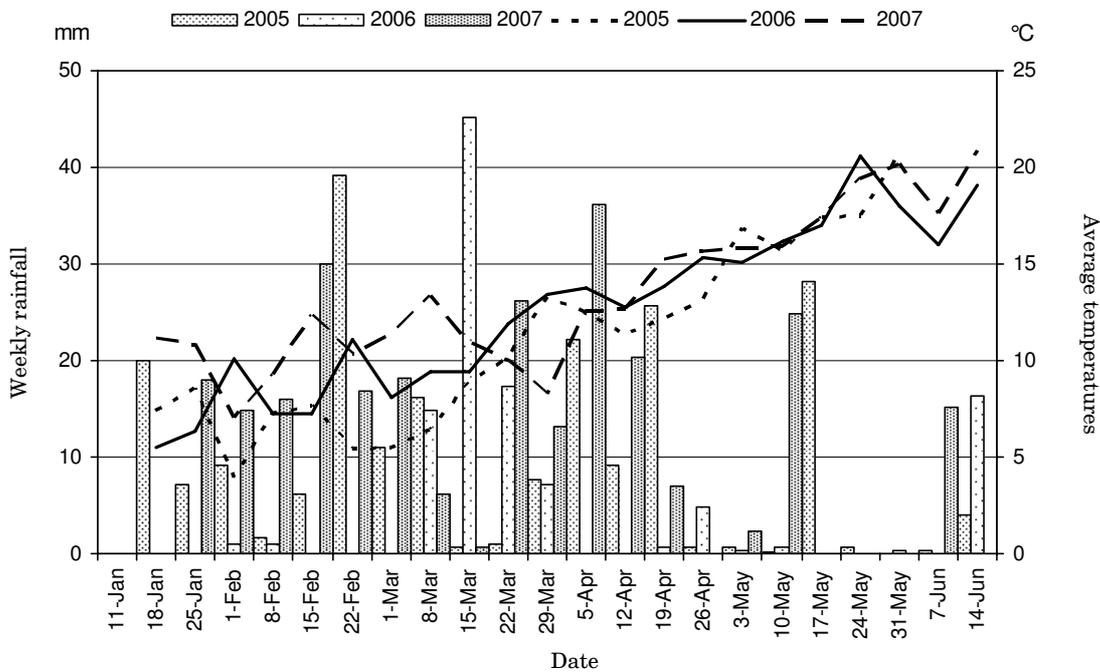


Fig. 1. Weekly rainfall (bars, mm) and average temperatures (lines, °C) recorded during the trials in the period 2005–2007.

Wound receptivity to *Pa. chlamydospora* decreased significantly from the first to the third pruning date (Fig. 2, Table 2). Spurs cut in January had the significantly highest percentage of *Pa. chlamydospora* infection. The frequency of infected spurs decreased to below 20% from the end of February and reached 0% on Mar 30th (bleeding), regardless of the pruning period. Very low percentages of infection or no infection at all were detected from the end of March until the last inoculation, when the isolation frequency of *Pa. chlamydospora* in spurs cut in January and March increased again. Infection of non-inoculated spurs by *Pa. chlamydospora* was fairly substantial only in the spurs cut in January and examined on Feb 9th and Apr 20th. Generally, natural infection was lower than 3%, without significant differences between pruning dates (Table 3). Crossed infections (*Pa. chlamydospora* isolated from spurs that had been inoculated with *Pm. aleophilum* and *D. seriata*) also occurred (1.5, 0.9 and 0.4% in the first, second and third pruning period respectively).

In contrast to *Pa. chlamydospora*, there were no significant differences in *Pm. aleophilum* infection

between the wounds of the first and second pruning period, but those of the third were the highest (Table 2). The isolation frequency was fairly high even in the last inoculated spurs. At the bleeding period, the infection percentage of *Pm. aleophilum* decreased but it remained at 30% as regards late wounds. No natural *Pm. aleophilum* infection of spurs pruned in February and March was recorded. Only spurs of the first pruning period were slightly infected (1%, Table 3). Crossed infections were 1, 1.1 and 1.1% in the three pruning periods.

Susceptibility to *D. seriata* was very high during most of the trial (Fig. 2), but the degree of infection of spurs cut in January was significantly lower (Table 1) because of low susceptibility during the first inoculations. From the fifth week on, there were no differences between the three pruning periods. When grapevines were bleeding, infection percentages decreased but they remained above 50% (Fig. 2). *D. seriata* infection events of non-inoculated spurs ranged from 10.6 to 14.4%, and were higher than those with *Pa. chlamydospora* and *Pm. aleophilum* (Table 3). The frequencies of crossed infection were 8, 8.6 and 6.1% in the three pruning period.

Table 2. Average infection percentages of spurs pruned in January, February and March and inoculated with *Phaeoconiella chlamydospora*, *Phaeoacremonium aleophilum* or *Diplodia seriata* in 2005–2007.

Fungal species	Pruning period	2005	2006	2007	Mean ^a
<i>Phaeoconiella chlamydospora</i>	Jan	23.67 a ^b	44.69 a	45.00 a	37.78 a
	Feb	12.73 b	37.86 a	55.36 a	35.31 a
	Mar	7.86 b	33.00 a	52.50 a	31.12 a
	Mean ^c	14.75 c ^d	38.51 b	50.95 a	
<i>Phaeoacremonium aleophilum</i>	Jan	27.50 ab ^b	34.69 a	45.00 a	35.73 a
	Feb	24.09 b	31.07 a	51.07 a	35.41 a
	Mar	43.57 a	31.00 a	52.00 a	42.19 a
	Mean ^c	31.72 b ^d	32.25 b	49.36 a	
<i>Diplodia seriata</i>	Jan	72.35 b ^b	57.35 a	33.89 a	54.53 a
	Feb	88.46 a	45.00 a	43.21 a	58.89 a
	Mar	91.67 a	29.00 b	45.50 a	55.39 a
	Mean ^c	84.16 a ^d	43.78 b	40.87 b	

^a Average of the three years.

^b Values followed by the same letter along the column do not differ statistically according to Tukey HSD test ($P \leq 0.05$).

^c Average of the three pruning periods.

^d Values followed by the same letter along the row do not differ statistically according to Tukey HSD test ($P \leq 0.05$).

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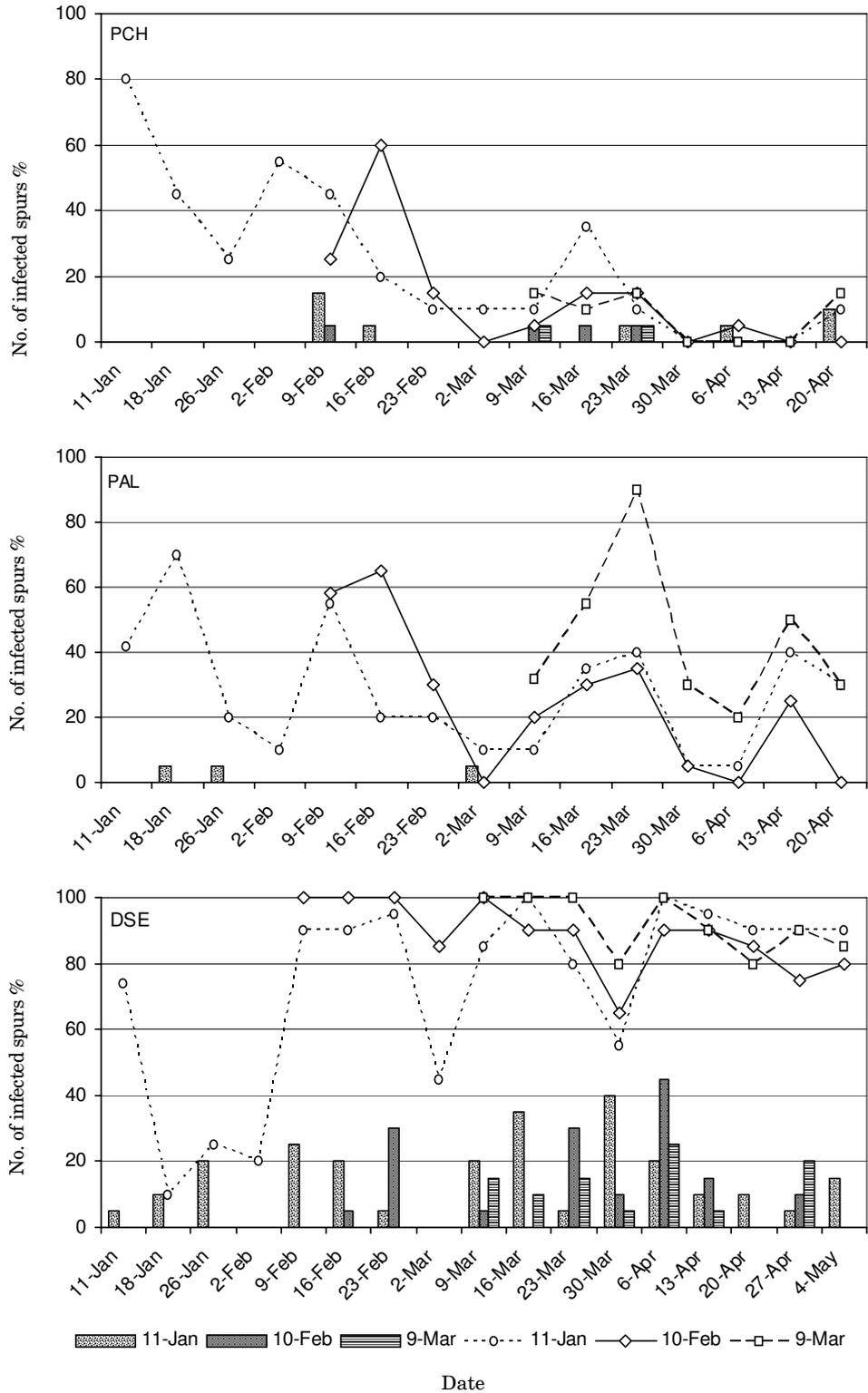


Fig. 2. Weekly infection percentages of spurs pruned in January, February and March and artificially inoculated (dotted lines) or naturally infected (bars) with *Phaeomoniella chlamydospora* (PCH), *Phaeoacremonium aleophilum* (PAL) or *Diplodia seriata* (DSE) in 2005.

2006

Average winter temperatures were higher than in 2005 (mean 9.1, minimum 3.8 and maximum 14.1°C) but spring temperatures were similar (Fig. 1). Temperatures stabilized at around 10°C by the end of February. Rainfall was concentrated in March and scarce in the other months. Grapevine bleeding extended from Mar 8th to Mar 22nd.

Phaeoconiella chlamydospora and *Pm. aleophilum* were inoculated weekly starting from the first pruning date (Jan 11th) until Apr 26th, while *D. seriata* was inoculated until May 3rd. All fungi were inoculated every week starting from the second and third pruning dates (Feb 8th and Mar 8th) until May 10th.

Only spurs inoculated with *D. seriata* showed a significant F ratio for the pruning period ($P=0.0003$, ANOVA Tables not shown).

Susceptibility to *Pa. chlamydospora* decreased from January to May but infection frequency was higher than in 2005 (Table 2). In the last part of the trial, isolation percentages were around 20%, sometimes over 30% (Fig. 3). Grapevine bleeding did not influence spur infection as it did in 2005. Natural infection of spurs by *Pa. chlamydospora* was more frequent than in the previous year (Table 3), starting with Feb 22nd, when the average temperature was always near 10°C (Fig. 1). Natural *Pa. chlamydospora* infection percentages were lower than artificial infections with this fungus, except for the last inoculations. Mean infection rates ranged from 3.4 to 5% while crossed infections were 1.9, 3 and 3% in the three pruning periods.

Phaeoacremonium aleophilum infection had a fluctuating trend but it definitely decreased from Apr 5th on (Fig. 3). There were no significant differences in the percentage of infected spurs between 2005 and 2006 (Table 2). Natural infection of spurs with *P. aleophilum* was detected starting from Feb 22nd. It was more frequent than in 2005, but was always lower than 3% (Table 3). The frequencies of crossed infection were 0.5, 1.6 and 2.8% in the three pruning periods.

The frequency of infection by *D. seriata* showed to be very high at the beginning of the trial, despite the low temperatures (Fig. 1). Infection of the inoculated spurs decreased substantially from Mar 8th on, reaching much lower values than during the first part of the trial. The infection percentage of spurs cut in March was therefore the lowest (Table 2). The

D. seriata infection percentage of non-inoculated spurs was sometimes higher than that of spurs inoculated from Mar 8th onwards. The mean values of naturally infected spurs ranged from 11 to 13.5% (Table 3), while those of crossed infections were 8, 3.4 and 10.3% in the three pruning period.

2007

In 2007 average winter temperatures were the highest in the three-year period (mean 10.4, minimum 5.3 and maximum 15.4°C), but later in the year they did not differ substantially from the other years (Fig. 1). Temperatures were above 10°C from the beginning of February. Rainfall was regularly distributed in the experimental period. Sapwood dripped profusely from spurs pruned on Mar 7th, but previously pruned spurs had already shown bleeding since Feb 14th.

All fungi were inoculated every week starting from the pruning dates (Jan 10th, Feb 7th, Mar 7th) until May 9th.

The F ratios for the pruning period were significant for spurs inoculated with *D. seriata* ($P=0.0481$) and for spurs naturally infected with *Pa. chlamydospora* and *Pm. aleophilum* ($P=0.004$ and $P=0.032$ respectively, ANOVA Tables not shown).

Wound susceptibility to *Pa. chlamydospora* was fairly throughout the trial reaching the highest percentage in the three-year period (Table 2). Susceptibility decreased on Mar 7th, coinciding with spur bleeding (Fig. 4). Generally, infection percentages decreased in April but remained around 40%. Natural infection of non-inoculated spurs by *Pa. chlamydospora* in 2007, particularly that of spurs pruned in February, was higher than in previous years (Table 3). Fifty percent of spurs cut on Feb 7th and examined on Apr 11st were infected with *Pa. chlamydospora* (Fig. 4). Natural infection started at the beginning of February, when average temperatures reached 10°C as in 2006 (Fig. 1). Also, crossed infection percentages were higher than in previous years (4.4, 10.9, 4.3 in the three pruning periods).

Wound susceptibility to *Pm. aleophilum*, like that of *Pa. chlamydospora*, was fairly high throughout the trial (Table 2), except for a pronounced decrease on Mar 7th, coinciding with spur bleeding (Fig. 4). Infection percentages decreased in May but remained around 40%. *Pm. aleophilum* infection of control spurs started from Feb 7th (Fig. 4)

and was higher than in previous years (Table 3). However, most natural infections showed lower percentages than the inoculated spurs, with mean values ranging from 4.4 to 9.3%. The frequencies of crossed infection were 1.7, 5.7 and 3% in the three pruning periods.

Spurs inoculated with *D. seriata* never reached infection rates of 100% as in previous years (Fig. 4). Inoculations carried out on the same day as the second and third pruning yielded the highest rates (75 and 95% respectively). As stated above, the F ratio was near the significance limit and the Tukey HDS test showed no significant differences among the pruning dates (Table 2). Natural infections with *D. seriata* occurred more frequently than in previous years (Table 3) and the isolation percentages of non-inoculated spurs were sometimes higher than those of the inoculated spurs (Fig. 4). Crossed infection percentages of spurs pruned in January, February and March were 11.5, 13.8 and 13.3% respectively.

Discussion

The susceptibility of pruning wounds to the pathogens on one-year-old wood varied with both

the fungus inoculated and the trial year. The period of pruning was significant for infection percentages of all fungi in 2005, and for *D. seriata* in 2006. Since pruning wounds were not protected until inoculation, in order to avoid any influence on natural healing, they were exposed to natural infection in previous months. Therefore infection frequencies of inoculated spurs could be a combination of natural and artificial infections. Natural infections prior to inoculation were detected by inspecting non-inoculated spurs. Infection percentages of control spurs were well below those of inoculated spurs, with few exceptions. Thus, in most cases, we can reasonably assume that the infection frequencies of inoculated spurs came mostly from the inoculated fungi, except for *D. seriata* in the second half of 2006 and in 2007. In these last periods, the infection percentages of inoculated spurs could be attributed mostly to natural infections.

Usually, *D. seriata* spur infection was very superficial: only the outer layer of the spur was colonized. This is consistent with Lecomte *et al.* (2005) who postulated that *Botryosphaeria* spp. invade only those vascular tissues that had already suffered mechanical damage. *Pa. chlamydospora* and *Pm. aleophilum* on the other hand were mostly isolated from the inner slices, indicating that they were able to move inside

Table 3. Average infection percentages of spurs pruned in January, February and March and naturally infected by *Phaeoconiella chlamydospora*, *Phaeoacremonium aleophilum* or *Diplodia seriata* in 2005–2007.

Fungal species	Pruning period	2005	2006	2007	Mean ^a
<i>Phaeoconiella chlamydospora</i>	Jan	2.67 a ^b	3.44 a	9.44 b	5.18 a
	Feb	1.82 a	4.64 a	18.21 a	8.23 a
	Mar	1.43 a	5.00 a	7.50 b	4.64 a
	Mean ^c	1.97 b ^d	4.36 b	11.72 a	
<i>Phaeoacremonium aleophilum</i>	Jan	1.00 a ^b	2.19 a	4.44 b	2.54 a
	Feb	0.00 a	1.79 a	9.29 a	3.69 a
	Mar	0.00 a	1.50 a	5.50 ab	2.33 a
	Mean ^c	0.33 b ^d	1.82 b	6.41 a	
<i>Diplodia seriata</i>	Jan	14.41 a ^b	13.53 a	16.39 a	14.78 a
	Feb	11.54 a	11.43 a	21.07 a	14.68 a
	Mar	10.56 a	11.00 a	17.50 a	13.02 a
	Mean ^c	12.17 b ^d	11.99 b	18.32 a	

^{a, b, c, d} See Table 1.

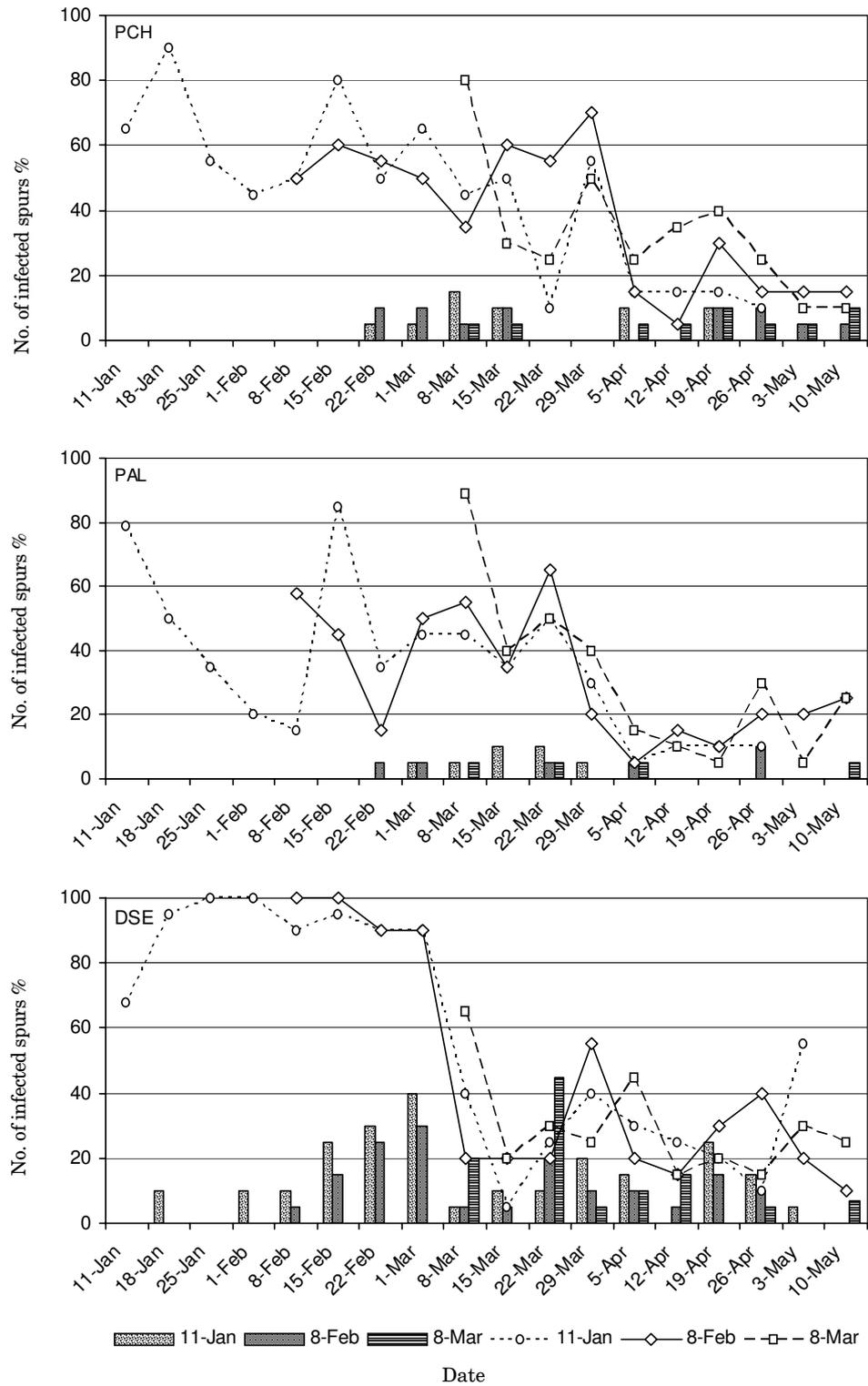


Fig. 3. Weekly infection percentages of spurs pruned in January, February and March and artificially inoculated (dotted lines) or naturally infected (bars) with *Phaeomoniella chlamydospora* (PCH), *Phaeoacremonium aleophilum* (PAL) or *Diplodia seriata* (DSE) in 2006.

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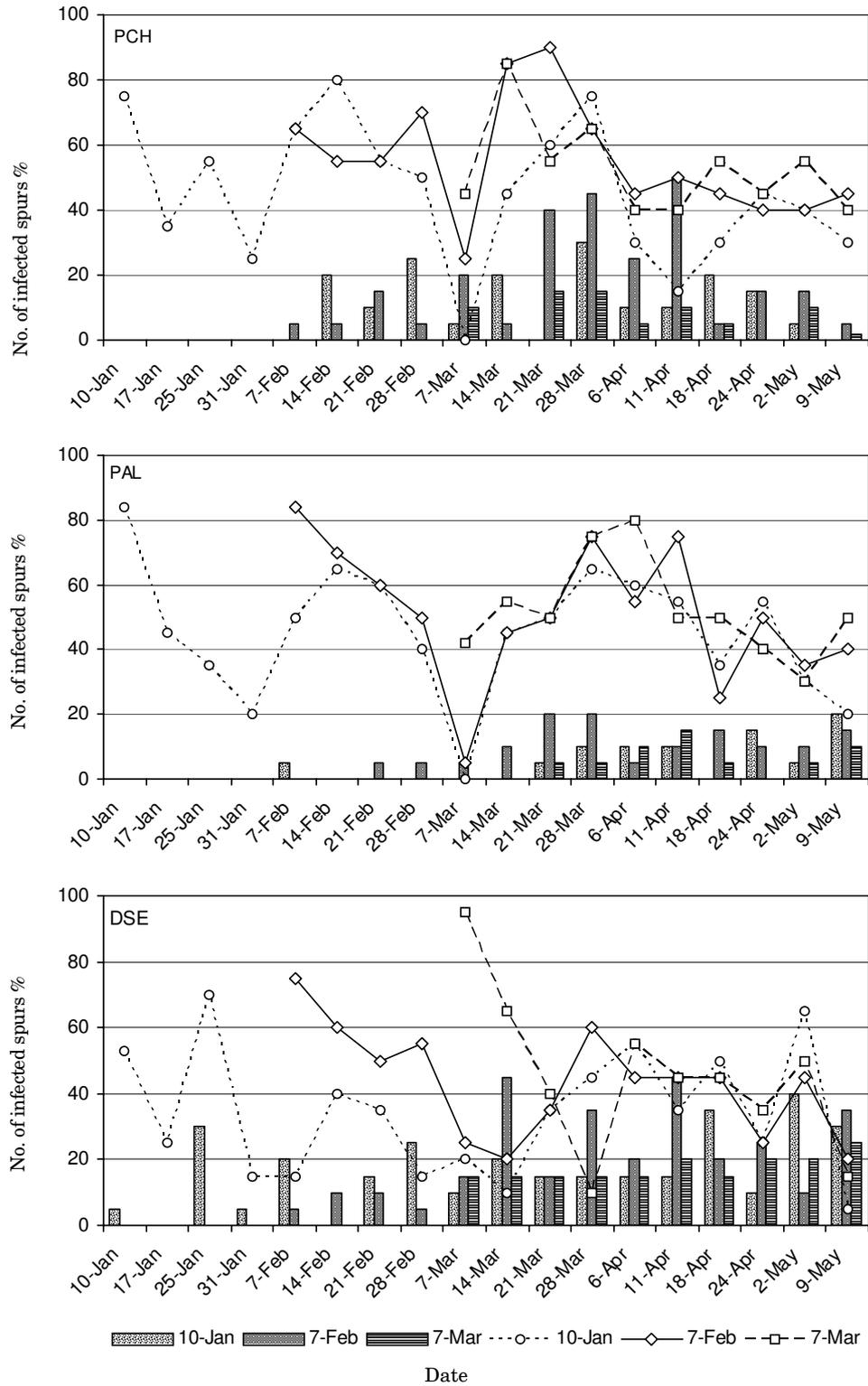


Fig. 4. Weekly infection percentages of spurs pruned in January, February and March and artificially inoculated (dotted lines) or naturally infected (bars) with *Phaeoconiella chlamydospora* (PCH), *Phaeoacremonium aleophilum* (PAL) or *Diplodia seriata* (DSE) in 2007.

active vessels (Pascoe and Cottral, 2000; Feliciano and Gubler, 2001; Troccoli *et al.*, 2001).

As regards *Pa. chlamydospora*, spurs cut in March were less receptive to infections with this fungus only in 2005. In 2005 and in 2006 receptivity decreased during the growing season, regardless of the pruning period. Nevertheless, only in 2005 did the isolation percentages go down to zero, while in 2006 spur infection was significant also in late spring. On the other hand, isolation percentages in 2007 were high throughout the season.

The susceptibility of grapevine to *Pm. aleophilum* fluctuated greatly, without substantial differences between pruning dates. Only in 2005 were spurs cut later more receptive to infections. The mean isolation percentages were similar in the first and second year; nevertheless, in the January–March period they increased from 32.6% in 2005 to 44.8% in 2006. As with *Pa. chlamydospora*, infection frequencies were high throughout the experimental period in 2007.

The higher infection percentages of *Pa. chlamydospora* and *Pm. aleophilum* in 2006 and 2007 are thought to be attributable in part to the fungicide with which the culture medium was amended. *Pa. chlamydospora* and *Pm. aleophilum* grow very slowly on agar media, particularly if they start from wood, and require at least 10–15 and 7–10 days respectively to form recognizable colonies. The addition of fungicides to the isolation media in 2006 and 2007 did not suppress but delayed the growth of fast-growing fungi, enabling for higher isolation percentages of the inoculated fungi. The influence of the culture medium is however not thought to be decisive so that the differences in *Pa. chlamydospora* and *Pm. aleophilum* infections between years could also be explained by a combination of other factors, such as the particular culture medium or mean temperature during the experimental period. Winter temperatures in 2006 and 2007 were higher than in 2005, and this would have favoured the isolation of *Pa. chlamydospora* and *Pm. aleophilum*. Optimum temperatures for the growth of *Pa. chlamydospora* and *Pm. aleophilum* are 25°C and 35°C respectively (Crous *et al.*, 1996; Rooney and Gubler, 2001; Whiteman *et al.*, 2001), so that higher infection percentages would be expected as the temperature increased.

Temperature trends in the three-year experimental period influenced grapevine development. Bud break of Sauvignon blanc began on Apr 8th in 2005, and one week earlier in 2006. In 2007 the

first open buds were already visible on Mar 14th followed by a stagnation in development until the end of that month. Temperature and rainfall also influenced the bleeding period. In 2005, moderate rainfall in March concentrated bleeding into a short period, while copious rainfall in March 2006 and February 2007 extended bleeding for a period of several weeks. However, it is very difficult to relate these phenological data to rates of fungal infection. In 2007, for example, the earlier bud break would have been expected to facilitate wound cicatrization, and the extended bleeding would have been expected to hamper penetration of the vessels by *Pa. chlamydospora*; but in fact *Pa. chlamydospora* infection percentages were higher than in previous years.

The susceptibility of pruning wounds to *D. seriata* in 2005 and 2006 showed an opposite pattern. In 2007 susceptibility to this fungus was irregular during the trial period, with infection percentages often lower than those in 2005 and in the winter of 2006. These trends cannot be explained by differences in the temperature (the *D. seriata* optimum growing temperature is 25°C, Úrbez-Torres *et al.*, 2006) or by the vegetative development of the grapevine in the three-year period. This topic requires further study.

The results presented here are inconsistent with those obtained in similar trials in France. Larignon and Dubos (2000) showed that pruning wounds were susceptible to *Pa. chlamydospora* and *Pm. aleophilum* for 9–12 and 7–9 weeks respectively during early pruning, but that susceptibility decreased to 1–2 weeks when the vines were pruned in March, during bleeding. Larignon *et al.* (2001a, b), found that pruning wounds were never susceptible to *D. seriata* for more than 1–2 weeks after pruning. In these trials, cane segments were however collected and examined 15 days after inoculation. It seems reasonable to assume that in our study a longer incubation period (4 weeks) as well as the mild Mediterranean climate favoured infection and prolonged the receptivity of pruning wounds. In California, Eskalen *et al.* (2007) found that susceptibility to *Pa. chlamydospora* and *Pm. aleophilum* regularly declined from February to late spring, but that 10–20% of spurs were susceptible to pathogens even 4 months after pruning; these findings are in accordance with our data.

Phaeoconiella chlamydospora and *Pm. aleophilum* infection of control spurs was scarce in 2005. It was impossible to know if natural spur infections were due to airborne spores or to movement of propagules

along the vessels from the trunks and cordons to the canes (Edwards *et al.*, 2004; Whiteman *et al.*, 2004). However, the isolation of *Pa. chlamydospora* and *Pm. aleophilum* from unpruned canes with traditional methods is possible but with low frequencies (Larignon and Dubos, 2000; Rego *et al.*, 2001; Fourie and Halleen, 2002; Giménez-Jaime *et al.*, 2006; Zanzotto *et al.*, 2007) so that, it is likely that the majority of natural infections in this trial were airborne. In 2006 and 2007, infection of control spurs with *Pa. chlamydospora* and *Pm. aleophilum* was higher, probably due to the use of a more efficient selective medium for isolation, and higher temperatures recorded in February and March, since in all the three years of the study, with some exceptions in 2005, infection by both the fungi began when the average temperature stabilized around 10°C. In 2007, this temperature was reached within the first ten days of February, and this was two weeks earlier than in 2006 and a good five weeks earlier than in 2005.

Natural infection by *D. seriata* was detected in all trials, even in winter. Apparently the temperature did not influence inoculum production and spread of this fungus. However, in 2007 *D. seriata* isolation from the control spurs was significantly higher than it has been in previous years. As has been mentioned, prolonged propitious temperature and regularly distributed rainfall in spring may have promoted the production and spread of *D. seriata* inoculum.

In conclusion, high temperature and regularly distributed rainfall promoted both grapevine growth and the pathogen infection process. Early vegetative development did not seem to influence wound receptivity, and bleeding hampered penetration of inoculated fungi only temporarily. On the other hand, propitious temperatures and rainfall favoured infections by all the fungi inoculated. The pruning period influenced susceptibility mostly in 2005. In that year, the highest mean infection percentage of spurs pruned in January and inoculated with *Pa. chlamydospora*, as well as the lowest average infection percentage of spurs inoculated with *D. seriata*, were presumably influenced by the isolation frequencies recorded at the beginning of the trial (January). From the second pruning date (Feb 10th) onwards, differences between the three pruning periods decreased to the point that their graphs overlapped with only small variations. Also in 2005, susceptibility to *Pm. aleophilum* was statistically and visually greater in spurs pruned in March than in spurs pruned in other months. Late

pruning, though not always, minimizes wound receptivity as commonly accepted by grapevine growers, and infection was possible during a long period of 3–4 months after pruning, regardless of pruning date. As a consequence, the planning of pruning does not seem to be an effective way to manage the spread of these decline diseases, and the penetration of airborne inoculum into wounds of one-year-old wood appears to be a very important means of producing infection.

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