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Preliminary studies on the biology of *Phaeoacremonium*

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Summary. Comparative microflora studies of one-year-old pruned and unpruned canes carried out during dormancy period over three consecutive years showed that *Phaeoacremonium chlamydosporum* was more frequently isolated from pruned than unpruned canes, while *Phaeoacremonium aleophilum* was found as much in pruned as in unpruned canes. Inoculations of pruning wounds made in two consecutive winters by suspensions of conidia of *P. chlamydosporum* and *P. aleophilum* showed that both fungi were able to invade through wounds during winter. Infections varied with pruning date and age of pruning wound, but were more serious and of longer duration with early pruning (December, January). Trapping studies showed that spores of *P. chlamydosporum* were captured throughout the whole year, while spores of *P. aleophilum* were mostly trapped during the vegetative period. Microscopic examination of adhesive tape applied to without bark zones of the canes showed the presence of conidia of *P. aleophilum*.
From these studies, it emerged that only *P. chlamydosporum* is able to contaminate pruning wounds during winter. *P. chlamydosporum* and *P. aleophilum* may be propagated by infected canes in the nursery. They can be considered airborne fungi during a period of their biological cycle.

Key words: biology, esca, *Phaeoacremonium*.

Introduction

Two species of *Phaeoacremonium* are mainly involved in esca in France: *Phaeoacremonium chlamydosporum* and *Phaeoacremonium aleophilum* W. Gams, P.W. Crous, M.J. Wingfield & Mugnai (Larignon and Dubos, 1997). These fungi may play an important role in the process leading to the wood rot characteristic of this disease and they are considered pioneers in bringing on esca (Larignon and Dubos, 1997). Moreover, these fungi have been reported from various countries including Italy (Mugnai *et al*., 1996), the United States of America (Scheck *et al*., 1998), South Africa (Ferreira, 1994) and Australia (Pascoe, 1999) where they are associated with young grapevine declines (Ferreira, 1994; Morton, 1997; Scheck *et al*., 1998), and with brown streaking in the wood of grafted rootstocks (Bertelli *et al*., 1998).

In order to control esca, it is necessary to know the biology of the fungi involved. The purpose of the present work was: i) to study the presence of *P. chlamydosporum and P. aleophilum* on pruning wounds and in cane wood during the dormancy period, ii) to ascertain if these fungi invade through wounds during winter and, iii) to determine their presence in the vineyard by spore trapping. This study is a step towards a greater understanding of their biology.

Materials and methods

Site of experimentation

Experiments were made in a vineyard close to Bordeaux at Naujan-et-Postiac (Entre-Deux-Mers). The cultivar was Cabernet Sauvignon grafted on

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SO4 rootstock, planted in 1963 and cordon-trained. The vineyard was not treated with sodium arsenite. Thirty-five percent of the vines showed characteristic symptoms of esca on the herbaceous parts in 1996, 29% in 1997 and 23% in 1998. Weather data were obtained from the meteorological station CIMEL La Sauve, located 12 km away.

Isolation of *Phaeoacremonium* from the canes

The experiments were made during three consecutive winters. On each pruning date in the winter 1996-97 (15 Jan. 1997, 12 Feb. 1997), 1997-98 (11 Dec. 1997, 21 Jan. 1998, 23 March 1998) and 1998-99 (6 Jan. 1999, 3 Feb. 1999, 17 March 1999), one-year-old canes were pruned leaving a piece 15 to 20 cm long on the vine. After each pruning date, 40 cane pieces of the ones left on the vine were collected randomly every week until 31 March in winter 1996-97, until 1 July in winter 1997-98 and until 28 April in winter 1998-99. The prunings made in March corresponded to the period of bleeding of the vine.

In the laboratory, the bark was removed over a length of 1 cm from the pruning wound. This excoriated end was dipped momentarily in alcohol, passed over a flame, and then cut into ten sections 1 mm thick with a guillotine (Paillassa, 1992). The sections were placed on malt agar (MA), 5 sections per plate, and incubated at room temperature (22°C) for up to 21 days. A cane was considered infected if one or more of the ten sections showed the presence of *Phaeoacremonium*.

From the unpruned vines, segments 10 to 15 cm long were collected from the base of each cane. They came from the same location on the plant as the cane pieces of the pruned vines. In the winter of 1996-97, the occurrence of *Phaeoacremonium* from unpruned canes was determined on only 80 canes (40 canes/pruning dates) but in the two following winters, it was determined on 1040 canes and 680 canes respectively (40 canes/week).

Susceptibility of pruning wounds to *Phaeoacremonium*

Experimentation was done during two years: winter 1997-98 and winter 1998-99 at the same pruning dates as before and on canes pruned in the same manner. For each pruning date, thirty pruning wounds were inoculated with 30 µl of a suspension containing 130-140 conidia μl^{-1} of *P. aleophilum* (Musée d'Histoire Naturelle de Paris, LCP 93 2940) or *P. chlamydosporum* (Musée d'Histoire Naturelle de Paris, LCP 93 2941) each week until 1 July in winter 1997-98, and each week until 28 April in winter 1998-99. Fifteen days after inoculation, cane segments were collected and examined according to the methods described above. The fungi used in this test had been isolated from diseased vines collected in 1985 from a Bordeaux vineyard.

Trapping of *Phaeoacremonium*.

Spores were trapped on glass microscope slides (76x26 mm) coated with white vaseline and placed 1 or 2 cm from the surface of the vine that was without bark. The face with vaseline was turned towards the wood. The experimentation began on 29 Jan. 1997 and ended on 6 Jan. 1999. Thirty vaseline coated slides were collected each week.

They were rinsed with one ml of sterile water at 50°C to remove vaseline and spores. The suspension obtained was stirred and 200 µl was put in Petri dishes containing MA with chloramphenicol (250 mg l^{-1}) and incubated at room temperature for 15 days. The number of colonies of each species of *Phaeoacremonium* was counted.

The effect of a temperature of 50°C on conidia viability of *Phaeoacremonium* was tested. The germination rate of *P. aleophilum* and *P. chlamydosporum* conidia from the rinsed slides was 100% and 99% respectively.

Results

Isolation of *Phaeoacremonium* from the canes

Microflora studies of pruning wounds on one-year-old canes in the dormant season showed that *P. chlamydosporum* was more frequently isolated from pruned than from unpruned canes (Table 1). It was not isolated from pruning wounds made in March.

After pruning on 15 Jan. 1997 (Fig. 1a) the fungus was isolated 3 weeks later while after pruning on 12 Feb. 1997 (Fig.1b) and 11 Dec. 1997 (Fig. 1c) it was isolated sooner. After pruning on 21 Jan. 1998 (Fig. 1d) it was detected 2, 4, 5 and 8 weeks later.

The percentage of isolation of *P. aleophilum* from pruned canes was similar to that from unpruned canes (Table 1).

Table 1. Percentage of isolation of *P. chlamydosporum* (*Pch*) and *P. aleophilum* (*Pal*) from pruned and unpruned canes during three winters.

Period of experimentation	Pruning date	No. of canes examined	% of canes with *Pch*	% of canes with *Pal*
Winter 96-97	15. 01. 1997	400	9.25	0.75
	12. 02. 1997	240	7.08	0.41
	Unpruned canes	80	0	0
Winter 97-98	11. 12. 1997	1,160	9.31	0.43
	21. 01. 1998	920	7.39	0.65
	23. 03. 1998	520	0	0.19
	Unpruned canes	1,040	0.38	2.5
Winter 98-99	06. 01. 1999	640	1.44	0.31
	03. 02. 1999	480	1.45	0.41
	17. 03. 1999	280	0	0
	Unpruned canes	680	0.15	0.44

Susceptibility of pruning wounds to *Phaeoacremonium* contamination

Susceptibility studies (Fig. 2a, b) showed that *P. chlamydosporum* contaminated pruning wounds in winter. Contamination varied with pruning date and the age of the pruning wound, and was more frequent and of longer duration with early pruning (December, January). The initial rate of contamination on fresh pruning wounds inoculated with *P. chlamydosporum* immediately after pruning was higher when the vines were pruned in December or January than when they were pruned later. Wounds remained susceptible for 9-12 weeks after early pruning. They had very low susceptibility when made in March, and the duration of susceptibility was then very short (2 weeks).

P. aleophilum contaminated pruned canes during winter. The initial rate of contamination on fresh pruning wounds inoculated with *P. aleophilum* immediately after pruning was higher in wounds made during early pruning (December, January) than when pruning occurred later (March). Susceptibility decreased to become very weak after 7-9 weeks with early pruning. The duration of susceptibility was very short (1-2 weeks) when the pruning was carried out during the period of bleeding of the vine.

Trapping of *Phaeoacremonium*

P. chlamydosporum spores were trapped throughout the year (Table 2). The total number of *P. aleophilum* spores trapped during 1997 and 1998 was 8,980 and 13,177 respectively. These spores were mostly trapped during the vegetative period (Fig. 3): from 4 to 18 June in 1997, and from 4 March to 10 April and from 13 to 27 May in 1998. The number of spores trapped on a single slide could be high (4,795 spores for example). Microscopic examination of adhesive tape applied to the zones without bark showed the presence of conidia of *P. aleophilum*.

Table 2. Number of spores of *P. chlamydosporum* trapped between 29 January 1997 and 6 January 1999.

Date of trapping	No. of spores / 30 slides
07/03/97 - 07/09/97	85
07/09/97 - 07/15/97	2.5
09/10/97 - 09/17/97	22.5
11/26/97 - 12/03/97	5
12/31/97 - 01/07/98	20
01/21/98 - 01/28/98	35
02/04/98 - 02/11/98	2.5
02/25/98 - 03/04/98	15
03/18/98 - 03/25/98	45
04/29/98 - 05/06/98	2.5
05/06/98 - 05/13/98	3,875.5
07/01/98 - 07/08/98	17.5
07/29/98 - 08/05/98	20
08/12/98 - 08/19/98	22.5
11/18/98 - 01/06/99	72.5

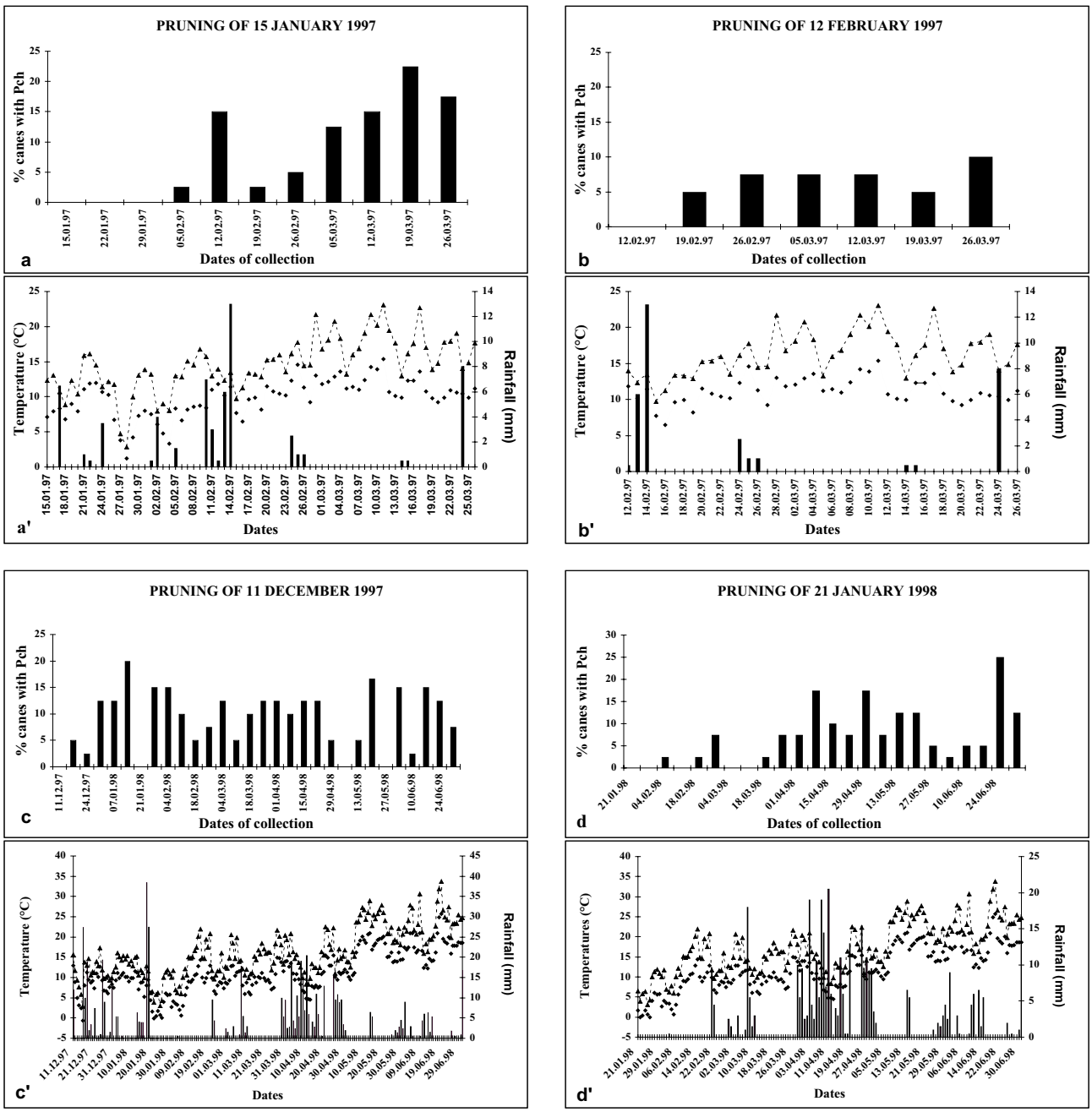

Fig. 1. Comparison of the periods of contamination of pruning wounds by *Phaeoacremonium chlamydosporum* (a, b, c, d) in relation to climatic data (a', b', c', d') at two pruning dates in winter 96-97 and in winter 97-98. Daily mean temperature (line), daily maximum temperature (dotted line) and daily rainfall (bar).

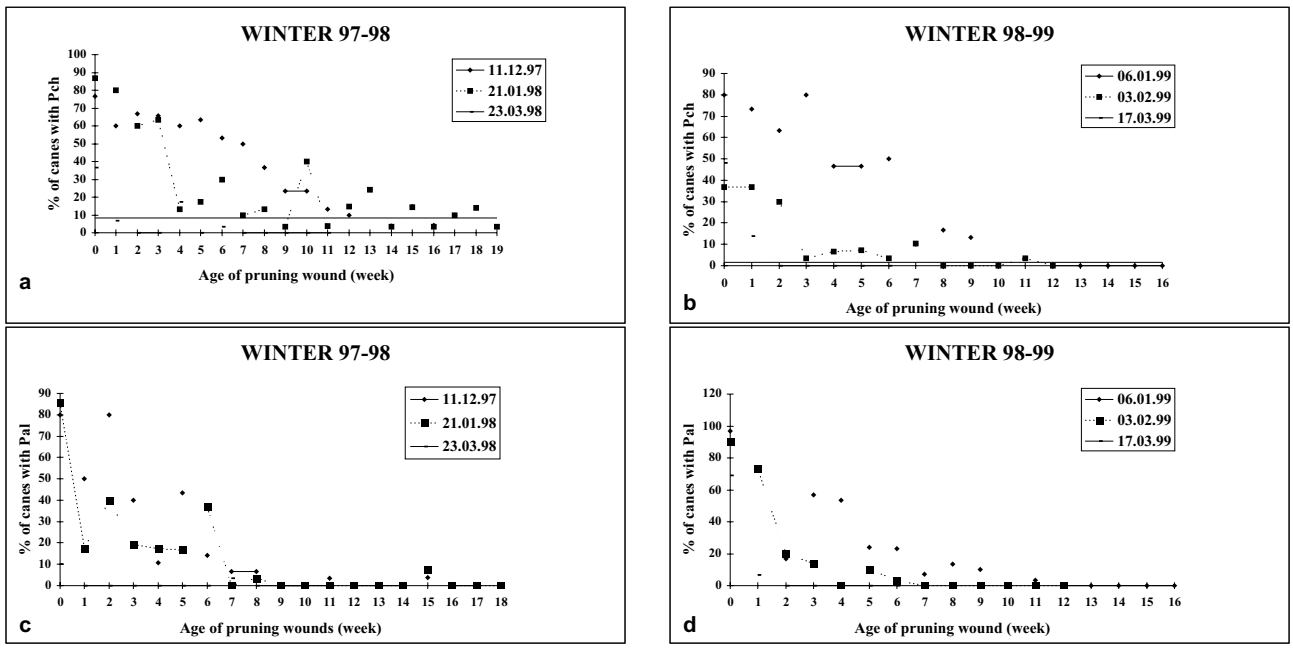

Fig. 2. Variation in the susceptibility of pruning wounds, expressed as percentage of wounds infected by *Phaeoacremonium chlamydosporum* (*Pch*) (a, b) and *P. aleophilum* (*Pal*) (c, d) after inoculation with conidia at various pruning dates in two winters of experimentation. The horizontal line corresponds to the mean natural contamination of pruning wounds by *P. chlamydosporum* (a, b).

Discussion

Microflora examination of pruning wounds in the dormant season indicated that *P. chlamydosporum* contaminated these wounds. Spore trapping of *P. chlamydosporum* during the winter months on vaseline-coated slides and its capacity to invade through wounds during winter corroborated this finding. Thus it seems that pruning wounds are one of the ways by which this fungus penetrates into the plant.

During this study, it was difficult to reach definite conclusions concerning optimal climatic conditions for contamination of pruning wounds because of wide variations in the percentage of pruned canes from which *P. chlamydosporum* was isolated. However, as the detection of this fungus in unpruned canes is a rare event (5 out of 1,800 canes examined over 3 years), it is possible to give some information on climatic conditions that favoured contamination of pruning wounds by *P. chlamydosporum* after pruning on 12 Feb. 1997 and 11 Dec. 1997. When examining the occurrence of *P. chlamydosporum* in relation to the climatic data (Fig. 1), it is seen that the rainfall plays an important role in the contamination of pruning wounds. The first detection of *P. chlamydosporum* in the

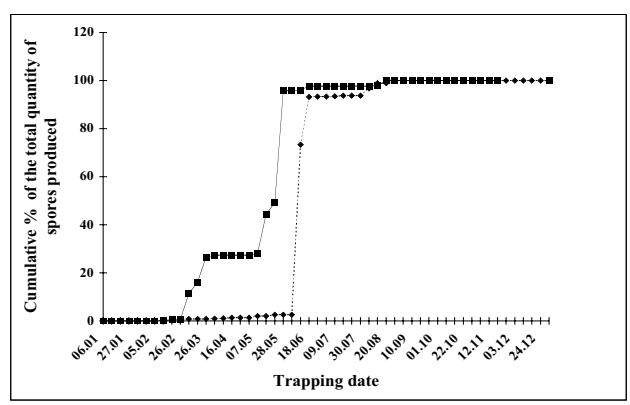

Fig. 3. Availability in 1997 (dotted line) and 1998 (solid line) of spores of *Phaeoacremonium aleophilum* expressed as the cumulative percentage of the total quantity of spores trapped.

canes was made one week after a rainfall when the temperatures were mild (mean temperature around 12°C). Further studies should be done in order to determine the suitable weather conditions for sporulation, propagation, contamination and infection.

The study showed that *P. chlamydosporum* was also isolated from unpruned canes. Its presence in unpruned canes suggests that this fungus can also be propagated by using infected canes from the nursery. Its occurrence in rooted grapevine cuttings, reported by Bertelli *et al*. (1998) in Italy, corroborates this hypothesis. As regards its presence within unpruned canes, it can be hypothesised that it was already present in the plant, and produced conidia which were carried towards the herbaceous parts in the sap stream. Several fungi causing vascular wilts of various plants (*Fusarium*, *Verticillium*, *Ceratocystis*) are known to spread internally through the xylem vessels by conidia (Agrios, 1978). Another possibility is that *P. chlamydosporum* infects branches through injuries caused by cultural practices during the entire vegetative period until leaf-fall. However, its low detection in unpruned canes suggests that this type of propagation by grafting is a rare event.

The source of fungal inoculum was not determinated in this study.

Microflora studies of pruning wounds in the dormant season showed that *P. aleophilum* did not infect pruning wounds during winter, even though wounds were susceptible during this period. The absence of infection was due to the absence of spores in the air during winter. It seems that pruning wounds are not the way of penetration of this fungus into the plant. Spores trapped, particularly during the vegetative period, raise many questions about the data of infection and the penetration of the fungus into the plant. The source of inoculum was at the surface of the excoriated wood, as shown by microscopic observation.

Studies showed that this fungus also occurred in unpruned canes. Its isolation from unpruned canes suggests that they may have been propagated by infected canes from the nursery. Its occurrence in unpruned canes may be explained in the same way as that for *P. chlamydosporum*.

This study has shed light on the biology of *Phaeoacremonium*. However, many questions remain. Further studies should explore the source of inoculum of *P. chlamydosporum*, determine the mode of penetration of *P. aleophilum* into the plant and confirm that the fungi are propagated by infected canes introduced in the nursery.

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