

Infection of grapevines by some fungi associated with esca.

I. *Fomitiporia punctata* as a wood-rot inducer

LORENZO SPARAPANO¹, GIOVANNI BRUNO¹, CLAUDIO CICCARONE² and ANTONIO GRANITI¹

¹Dipartimento di Biologia e Patologia Vegetale, Università, Via G. Amendola 165/A, 70126 Bari, Italy

²Istituto di Produzioni e Preparazioni Alimentari, Università, Via Napoli 25, 71100 Foggia, Italy

Summary. Inoculation experiments with three strains of *Fomitiporia punctata* on grapevine cv. Sangiovese and on grafted 'Italia' rootstocks were carried out in southern Italy in 1992-1993. Inoculations were performed on fresh wounds made on the spurs, branches and trunks of vines showing no symptoms of esca. The fungus developed in the discoloured wood around the inoculation site, and caused white rot within two years. No symptoms were induced on foliage or fruit of the infected vines, nor was there any significant difference in virulence of the strains of *F. punctata*. After 2 years, re-isolation of *F. punctata* from the diseased woody tissues was successful, whereas no other species of fungi suspected to act as a "precursor" of wood decay were isolated. In 1999, further experiments were carried out with one strain of *F. punctata* on standing vines cv. Italia and Matilde free of any sign of wood deterioration. The development of internal symptoms was recorded monthly. The results indicated that the cv. Matilde was less susceptible than the cv. Italia. The first signs of spongy wood decay appeared 6 months after inoculation on both cultivars. *F. punctata* was re-isolated from the infected vines, whereas no species of *Phaeoacremonium* or other wood-decaying fungi were isolated from either inoculated or non-inoculated vines. These findings suggest that *F. punctata* behaves as a primary pathogen, being able to cause wood deterioration and spongy decay both on adult and young grapevines in a relatively short time, without the prior or concurrent action of other fungi.

Key words: *Fomitiporia punctata*, wood-rot, esca, grapevine.

Introduction

Esca is a complex disease whose symptoms are possibly caused by the concomitant action of several factors (Mugnai *et al.*, 1999). These include the three species of fungi most commonly found in the woody tissue of esca-affected vines: the wood-rotting basidiomycete *Fomitiporia punctata* (Fr.) Murrill and two mitosporic fungi, *Phaeoacremonium chlamydosporum* W. Gams *et al.* (¹) and *P. aleophilum* W. Gams *et al.* The last two species have

been suspected to act as precursors of the wood decay (Larignon and Dubos, 1997).

The aim of the experiments carried out in southern Italy in the last seven years and reported here was to demonstrate that *F. punctata* invades, deteriorates, and rots the woody tissue of grapevines that show no sign of infection by other lignicolous fungi.

Materials and methods

1. - Inoculation of grapevines cv. Sangiovese

The inoculation trial was started in January 1992 in a 13-year-old vineyard cv. Sangiovese near Matera (Basilicata, southern Italy), with three strains of *F. punctata* (*Fop1*, *Fop2* and *Fop3*) isolated from esca-affected grapevines in the same

(¹) This species has been redisposed in a new genus *Phaeomoniella* Crous & W. Gams as *P. chlamydospora* (W. Gams *et al.*) Crous & W. Gams. See Crous and Gams in this issue.

Corresponding author: L. Sparapano

Fax: +39 080 5442906

E-mail: sparlor@agr.uniba.it

area. The vines selected for the trial were free of the foliar symptoms of esca in the previous two years and showed no sign of wood deterioration. Two experiments were performed.

Inoculation of spurs left by current-year pruning

The distal part of the spur of a branch was cut off, and a plug of each strain of *F. punctata* cultured on Petri dishes on malt-peptone-glucose-agar (MPGA) was placed on the newly-made cut as an inoculum. A total of 45 vines, 15 for each strain, were inoculated and an equal number of control vines received plugs of sterile MPGA. The wounds were protected with moist cotton and paper tape. At the end of the experiment (December 1994), all the branches bearing either inoculated or control spurs were sectioned, the length of wood discoloration was measured, and the presence of white rot was recorded. Re-isolation of the inoculated fungus, and isolation of concomitant fungi, was carried out at various distances from the inoculation site.

Inoculation of branches

A wooden tooth-pick colonised with *F. punctata* was inserted in a hole, 2 mm diam., 3 cm deep, drilled radially through a branch of each grapevine selected for this test. A sterile tooth-pick was inserted in control vines in the same way. The wounds were protected with moist cotton and paper tape. The experiment included 45 inoculated vines (15 with each strain of *F. punctata*) and an equal number of controls. At the end of the experiment the branches were sectioned and examined as above.

2. - Inoculation of grafted rootstocks

One deep V-shaped cut was made on each stem of potted, 2-year-old grapevine rootstocks 'Kober 5BB' grafted with 'Italia', some 20 cm above the roots. A plug from a culture of *F. punctata* was placed on the cut which was then protected with moist cotton and paper tape. A plug of sterile agar treated in the same way was used for the controls. Twenty-five vines were inoculated with each strain of fungus and placed in a greenhouse at 22±5°C and 70-90% RH together with the 25 control vines; 50 vines per strain and an equal number of controls were left outdoors. In total, the experiment comprised 225 inoculated and 75 control vines. At the end of the experiment (October 1994, 21 months after inoculation) the stem

of every vine was sectioned longitudinally and transversely, the length of discoloured wood was measured, the occurrence of white rot was recorded, and re-isolations were performed.

3. - Inoculation of grapevines 'Italia' and 'Matilde'

Starting from January 1999, further inoculation experiments were carried out in a vineyard near Andria (Apulia, southern Italy) with a single strain of *F. punctata* (*Fop1*) on 18 six-year-old 'Italia' vines (each inoculated on the trunk and on 4 spurs) and 18 nine-year-old 'Matilde' vines (each inoculated on 4 branches and 8 spurs), while an equal number of vines served as controls. The extent of wood deterioration and the development of white rot were recorded monthly through the year, as where any other wood-colonising fungi isolated.

Results

1. - Inoculation of grapevines cv. Sangiovese

Inoculation of spurs left by current-year pruning

The results of this experiment are shown in Table 1. All branches showed wood discoloration below the spurs, which extended up to 18 cm into the inoculated vines and up to 9.5 cm into the controls; however, the difference between the mean length of the discoloured woody tissue in inoculated vines and that in controls was not significant. One fourth of the inoculated vines developed white rot, whereas no wood decay occurred in the controls. The fungus was re-isolated at a distance from the inoculation site in 31% of inoculated vines but never from the controls. These findings indicated that *F. punctata* invaded the woody tissues of the pruning spurs, spread along the bearing branches, and induced development of white rot within 2 years.

Inoculation of branches

The results of this test are shown in Table 2. All the inoculated branches showed dark-brown wood discoloration (Fig. 1A) that extended up to 14 cm above and below the infection dowel, whereas discoloration was less long (up to 6-6.5 cm) in the controls; the mean extension of discoloured wood was also different (4.6 cm in the inoculated vines versus 3.7-3.4 cm in the controls). Thirteen per cent of the inoculated vines developed white

Table 1. Results of a two-year experiment with three strains of *F. punctata* inoculated on pruning spurs of 13-year-old grapevines cv. Sangiovese^a.

Fungal strain	Average length of discoloured wood (cm)	No. of vines showing white rot	No. of vines with successful re-isolation
<i>Fop1</i>	5.06	8	9
<i>Fop2</i>	7.93	2	2
<i>Fop3</i>	5.73	1	3
Range	2-18		
Mean ± SE	6.4±0.48		
Total		11	14
Controls ^b			
Range	2-9.5		
Mean ± SE	5.76±0.51		
Total		0	0

^a Inoculation with three strains (*Fop1*, *Fop2*, *Fop3*) of *F. punctata*, each on 15 vines.

^b Data refer to 45 vines.

rot starting from the innermost tissues, whereas no wood decay was found in the controls. The fungus was re-isolated at a distance from the inoculation site in 25% of the inoculated vines, but never in the controls.

No other species of fungi known to cause wood deterioration were recovered. These results indi-

cated that a deep inoculation of *F. punctata* into healthy grapevine branches produces wood decay and may result in the development of white rot within 2 years. During the same entire test period, the external symptoms of esca were not shown by either inoculated or non-inoculated vines.

Table 2. Results of a two-year experiment with three strains of *F. punctata* deep-inoculated in the branches of 13-year-old grapevines cv. Sangiovese^a.

Fungal strain	Average length of discoloured wood above /and below the dowel (cm)	No. of vines showing white rot	No. of vines with successful re-isolation
<i>Fop1</i>	6.00 / 6.53	1	6
<i>Fop2</i>	3.73 / 3.43	2	3
<i>Fop3</i>	4.10 / 3.83	3	2
Range	1.5-14 / 1.5-14		
Mean ± SE	4.6±0.4 / 4.6±0.4		
Total		6	11
Controls ^b			
Range	1.5-6.5 / 1.5-6		
Mean ± SE	3.7±0.4 / 3.4±0.3		
Total		0	0

^a Inoculation with three strains (*Fop1*, *Fop2*, *Fop3*) of *F. punctata*, each on 15 vines.

^b Data refer to 45 vines.



Fig. 1. A. Development of dark-brown wood discoloration in the wood of inoculated branches of grapevine cv. Sangiovese all around the infection dowel colonised by *F. punctata*, two years after inoculation. B. White rot developed in the pith and woody tissues of a rootstock 'Kobler 5BB' grafted with 'Italia', two years after wound-inoculation with *F. punctata*. C. Detail of decayed woody tissue all around the infection dowel (2 mm in diam., arrow) colonised by *F. punctata*, six months after branch-inoculation of a 9-year-old grapevine cv. Matilde.

2. - Inoculation of grafted rootstocks

The results are summarised in Table 3. In the greenhouse, the inoculated vines showed brown wood streaks extending to a maximum of 27 cm above, and 6.5 cm below the inoculation site, with a mean length of 9.28 above and 2.70 cm below the site.

The corresponding figures for the controls were: lightly discoloured wood extending to a maximum of 10 cm above and 4.5 cm below the inoculation site, with a mean length of 4.83 above and 2.08 cm below the site. In transverse section, the mean diameter of the discoloured area was 1 cm for inoculated vines and 0.88 cm for the controls. About 12% of the inoculated vines developed white rot (Fig. 1B), which was never shown by controls. *F. punctata* was re-isolated from 8% of inoculated vines, but never from controls, and no other species of fungi known to cause wood deterioration were recovered. The inoculated grapevines kept outdoors showed similar brown discoloration, but no white rot. Here too, no foliar symptoms of esca were shown by either inoculated or non-inoculated vines.

3. - Inoculation of grapevines 'Italia' and 'Matilde'

Cultivar Italia

In summer, five to six months after inoculation, the brown discoloration of the woody tissue above and below the inoculation site reached an extension significantly greater than that recorded for controls, e.g., in June, 95 mm (spurs) and 170 mm (trunks) versus 9 (spurs) and 60 mm (trunks) respectively (Fig. 2A). No foliar symptoms developed on either inoculated or the control vines. *F. punctata* was re-isolated at a distance from the site of inoculation, never from the controls (Fig. 2B). No species of *Phaeoacremonium* or other wood-degrading fungi were isolated from the discoloured tissue of either inoculated or control vines.

Cultivar Matilde

The results were similar to those obtained with cv. Italia (see above), but the length of wood discoloration in the inoculated vines was not as marked (Fig. 2A). Data recorded 6 and 8 months after inoculation were: spurs, 44 and 69 cm on inoculated

Table 3. Results of a 21-month experiment with three strains of *F. punctata* inoculated on the stem of 2-year-old grafted grapevines cv. Italia/Kober 5BB^a.

Fungal strain	Average length of discoloured wood above /and below the dowel (cm)	Average diameter of discoloured areas in transverse sections	No. of vines showing white rot	No. of vines with successful re-isolation
Greenhouse				
<i>Fop1</i>	10.80 / 2.16	0.90	1	1
<i>Fop2</i>	7.76 / 2.88	1.06	2	1
<i>Fop3</i>	9.02 / 3.12	1.04	6	4
Range	2.5-27 / 1-6.5	0.6-2.2		
Mean ± SE	9.28±0.63 / 2.70±0.15	1.00±0.37		
Total			9	6
Controls				
Range	1-10 / 1-4.5	0.70-1.10		
Mean ± SE	4.83±0.64 / 2.08±0.22	0.88±0.02		
Total			0	0
Outdoors				
<i>Fop1</i>	6.40 / 2.91	0.99		
<i>Fop2</i>	7.68 / 2.15	1.02		
<i>Fop3</i>	16.70 / 1.10	1.01		
Range	1-29 / 0.6-9	0.6-2.8		
Mean ± S.E.	10.2±0.72 / 3.19 ± 0.16	1.01±0.03		
Total			0	0
Controls				
range	1-21 / 0.5-4			
mean ± SE	5.57±0.85 / 1.16±0.17			
Total		0	0	0

^a Inoculation with three strains (*Fop1*, *Fop2*, *Fop3*) of *F. punctata*, each on 25 grapevines in the greenhouse and on 50 grapevines outdoors.

vines respectively versus 20 and 50 mm on control vines; branches, 41 and 57 mm on inoculated vines versus 45 and 49 mm on control vines. In summer, five to six months after inoculation, decay of the woody tissue was actively developing in the wood of the inoculated branches all around the infection dowels (Fig. 1C), whereas healing processes had sealed the inoculation wounds of the controls. *F. punctata* was re-isolated at a distance from the site of inoculation, never from the controls (Fig. 2B).

Discussion

The ability of *F. punctata* to cause spongy wood decay of grapevine has been demonstrated both in

the laboratory on surface-sterilised sections of grapevine wood (Chiarappa, 1959) and with field inoculations on standing vines (Chiarappa, 1997); those vines, however, were not tested for the presence of other lignicolous fungi, particularly species of *Phaeoacremonium*.

The results of the experiments carried out in the period 1992-1994 have been mentioned in articles or reviews elsewhere (Mugnai *et al.*, 1996, 1999; Graniti *et al.*, 1999). The results indicate that *F. punctata* is able by itself to colonise the woody tissue of grapevines cv. Sangiovese without other fungal infection, if it is inoculated on current-year pruning wounds, on fresh cuts made on the stems of young grapevines (2-year-old grafted rootstocks), or

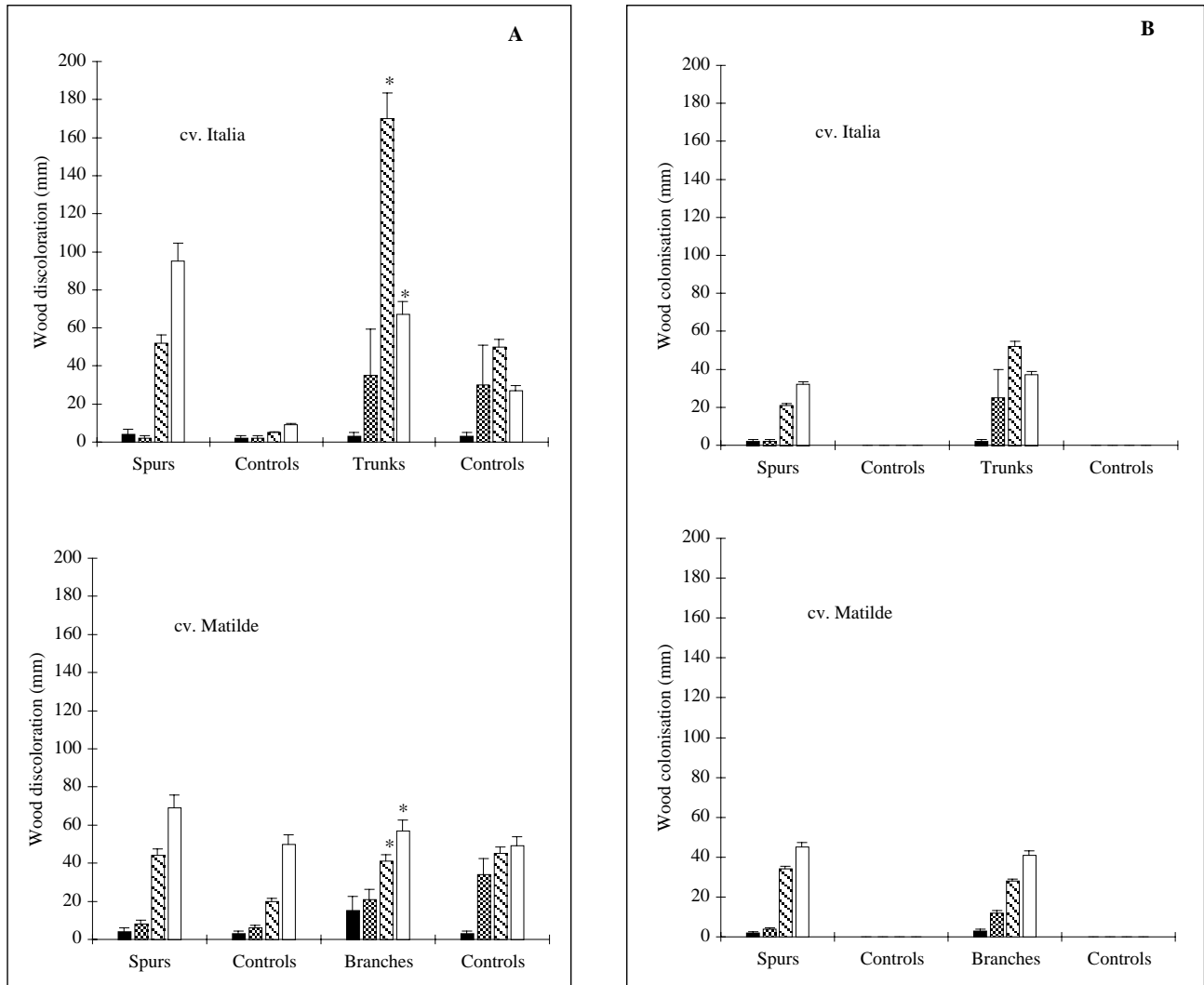


Fig. 2. A. Extent of wood discoloration in spurs and trunks of 6-year-old grapevines cv. Italia or in spurs and branches of 9-year-old grapevines cv. Matilde, 3 to 8 months after inoculation with *F. punctata*. B. Extent of wood colonisation by *F. punctata*, based on successful re-isolation of the pathogen. An asterisk (*) indicates the appearance of the first signs of spongy wood decay (white rot). Data are the means of 5 replicates \pm SE (April, June, July, September, 1999).

deeply into the branches of 12-year-old grapevines.

All three strains of *F. punctata* grew slowly in the woody tissue of the inoculated vines, where they caused relatively long columns of dark-brown wood extending above and below the inoculation site. The control vines also showed wood discoloration, though to a lesser extent and with reduced colour intensity, but no spongy decay (white rot).

At the end of the experiments, 2 years after

inoculation, clear symptoms of white rot were recorded from 12% (rootstocks), 25% (spurs) and 13% (branches) of all the inoculated vines, which however did not show any foliar or fruit symptoms during this period. *F. punctata* was re-isolated at a distance from the inoculation site in 31% (rootstocks), 25% (branches) and 8% (trunks) of inoculated vines, but not from the controls.

Considering the overall experimental data,

there were no significant differences in virulence among the three strains of *F. punctata* used for the inoculation trials.

The experiments on standing vines of the cultivars Matilde and Italia in 1999 also showed that *F. punctata* causes a brown discoloration of the woody tissue of inoculated plant parts (spurs, branches, trunks). Starting 6 months after inoculation, wood deterioration was significantly more severe in inoculated wood than in the controls. With regard to the length of wood discoloration, vines of the cv. Matilde were less susceptible than those of the cv. Italia. The first signs of white rot were recorded 6 months after inoculation. No symptoms appeared on the leaves of inoculated vines.

In conclusion, the findings indicate that *F. punctata* inoculated through wounds is able to cause wood deterioration and spongy decay on both adult and young grapevines that are not infected by species of *Phaeoacremonium* or other wood-colonising fungi. In other words, evidence is provided that *F. punctata* acts as a primary pathogen, inducing the internal symptoms of wood deterioration in a relatively short period of time (from 6 months onwards) without any previous or concurrent action of other, "precursor" fungi.

Acknowledgements

This work was supported by grants from the Italian Ministry of University and Scientific and Technological Research (MURST). Thanks are extended to Mr. L. Scarola, P. Basso, R. Mundo and G. Santorsola for technical assistance in performing the artificial inoculations and re-isolations.

Literature cited

- Chiarappa L., 1959. Wood decay of the grapevine and its relationship with black measles disease. *Phytopathology*, 49, 510-519.
- Chiarappa L., 1997. *Phellinus igniarius*: the cause of spongy wood decay of black measles ("esca") disease of grapevines. *Phytopathologia Mediterranea*, 36, 109-111.
- Graniti A., G. Surico and L. Mugnai, 1999. Considerazioni sul mal dell'esca e sulle venature brune del legno della vite. *Informatore Fitopatologico*, 49(5), 6-12.
- Larignon P. and B. Dubos, 1997. Fungi associated with esca disease in grapevine. *European Journal of Plant Pathology*, 103, 147-157.
- Mugnai L., A.M. Contesini, G. Surico, A. Graniti, R. Imbriani and N. Bianco, 1996. Recenti progressi nella conoscenza del "mal dell'esca" della vite in Italia. In: Convegno nazionale "Arsenico, Sì-No", 14 dicembre 1995, Codroipo, Udine, Forum Fitoiatrici, ERS, Udine, Italy, 115-122.
- Mugnai L., A. Graniti, and G. Surico, 1999. Esca (black measles) and brown wood-streaking: two old and elusive diseases of grapevines. *Plant Disease*, 83, 404-418.