Phytopathol. Mediterr. (2004) 43, 116–124

Benefit of fungicide treatment after trunk renewal of vines with different types of esca necrosis

Francesco Calzarano[1], Stefano Di Marco[2] and Augusto Cesari[3]

[1] Dipartimento di Scienze degli Alimenti, Università di Teramo, Via Spagna 1, 64023 Mosciano S.A., Teramo, Italy
[2] Istituto di Biometeorologia, Sez. di Bologna, CNR, Via Gobetti 101, 40129 Bologna, Italy
[3] DIPROVAL, Università di Bologna, Via Fanin 46, 40127 Bologna, Italy

Summary. Vines cv. Trebbiano d'Abruzzo grown in three vineyards located in Teramo Province, Abruzzi, Italy, severely affected by esca proper, were subjected to trunk renewal and thereafter treated with triazoles and fosetyl Al fungicides, applied either by trunk injection via syringe, or by ground injection via injector pole. Trunk renewal, by cutting the trunk, made it possible to determine the extent and type of wood deterioration on the residual trunk portion, where it was found that rotted and discoloured wood often still remained. Fungicide treatment was begun in 1995, when trunk renewal was performed, and was continued until 1998; after this the vines continued to be monitored for a further 4 years until 2002. In 2002, vines that had received trunk injections after trunk renewal were in better vegetative condition than vines without such injections, with full yield and lower mortality. Cyproconazole was particularly effective when trunk injected, but was not effective when injected into the ground. A possible explanation for this is given. Moreover the effect that the severity of the wood necrosis observed at the time of trunk renewal had on treatment effectiveness was evaluated. The current lack of similar trials and studies in other vineyards makes standardisation of control methods difficult; any positive effects may have been due to concurrent factors such as cultivar vigour, or peculiar environmental or cultural conditions.

Key words: trunk renewal, fungicides, discoloration, necrosis, trunk injection.

Introduction

The Abruzzi region in central Italy is an important vine-growing area where esca proper (Graniti *et al*., 2000) is widespread. This is possibly due to the age of the vineyards, commonly more than twenty years.

Some cv. Trebbiano d'Abruzzo vineyards, a common target of esca in this area, have been under investigation for a decade (1993–2002), providing new information on the aetiology, the epidemiology, and control of the disease (Calzarano *et al*., unpublished data).

Esca proper currently cannot be controlled except by trunk renewal, and even then the effect is only temporary (Di Marco *et al*., 2000).

This paper reports on a trial carried out in 1994–2002 on vines cv. Trebbiano d'Abruzzo in three different vineyards to study the effect of fungicide treatment after trunk renewal of vines affected with esca proper. Fungicides were applied by trunk injection or by ground injection with injector pole. Effects examined were: the growth condition of the

Corresponding author: F. Calzarano
Fax: +39 085 8071509
E-mail: calzar@tin.it

vines, the yield, and vine mortality. Comparison was with trunk-renewed vines that had not been fungicide-treated afterwards (control).

The effect that necrosis severity at the time of trunk renewal in 1995 had on subsequent fungicide effectiveness was also examined.

Materials and methods

Trunk renewal and treatments

Vines from three vineyards were identified as being infected with esca proper by direct inspection of foliar symptoms in summer 1994. In the following vegetative resting period in winter 1995, all these vines were trunk-renewed and the cut surfaces of the trunks were protected with healing varnish. In the spring of that same year the fungicide treatments were started. These treatments were repeated yearly for four years (1995–1998) during the vegetative season in the last week of May, by one of two methods: either by direct trunk injection with a syringe, or by ground injection with an injector pole. Both these application techniques were previously tested in studies on esca-infected vines that had not been trunk-renewed (Di Marco *et al*., 1993, 2000).

The fungicides applied were chosen on the basis of their demonstrated effectiveness in *in vitro* and *in vivo* studies (Di Marco *et al*., 1990, 1999; Bisiach *et al*., 1996) (Table 1).

Assessment

In order to determine the effectiveness of the various experimental schemes, the following variables were examined: extent and type of visible necrosis at the moment of trunk renewal, growth resumption, plant vigour, foliar symptoms (measured from 1994 to 2002), plant yield and mortality (determined at the end of the trial in 2002).

Necrosis severity

Necrosis severity was evaluated by examining: 1. the type of necrosis (discoloration and decay) occurring on the cut surface of the remaining part of the trunk, which became visible after trunk renewal, and 2. the extent (%) of necrosis covering the trunk cross section.

The type and extent of necrosis in each vine was classified in one of three categories:

1. extensive discoloration (>20% of trunk cross section) with wood decay;
2. extensive discoloration (>20% of trunk cross section) without wood decay;
3. moderate discoloration (<20% of trunk cross section) without wood decay.

Growth resumption

Growth resumption was determined in each test group, in March of each year from 1995 to 2002, by computation of the vines that did not show new shoots. Growth resumption is expressed as a percentage of the total number of vines in each test group.

Vigour

Starting after fungicide treatments were discontinued in 1998, plant vigour was visually evaluated annually until 2002. All vines were classified in one of three categories: 1. low vigour, <50% of the cultivar vegetative standard; 2. medium vigour, 50% of the cultivar vegetative standard or 3. normal vigour, 100% of the cultivar vegetative stand-

Table 1. Field-trial fungicide application details.

Vineyard No.	Application type	Active ingredient (a.i.) and commercial formula	Dose (g a.i. per plant)	Number of vines[a]
1	Trunk injection	Fosetyl Al Ca (Aliette Ca)	2	21
1	Trunk injection	Tetraconazole (M14360, 12.5 FL)	0.1	21
1	Untreated	-		21
2	Trunk injection	Cyproconazole (Atemi, 10 WG)	0.1	21
2	Untreated	-		21
3	Injector-pole	Cyproconazole (Atemi, 10 WG)	1	29
3	Untreated			29

[a] Each vine is considered a replication.

ard. Classification was started in 1998, after complete recovery from regeneration, when trunk-renewed vines had regained a vegetative capacity equal to that of adult vines of the same cultivar.

Vigour in 1995–1998, when vines were still in the regeneration stage, was also evaluated using the same classification as above. Trunk and canopy development of the vines were visually evaluated.

Yield

Yield was the weight of all bunches from all the vines of each test group in each vineyard in the last year of the experiment (2002).

Mortality

Mortality in the vineyards was determined in 2002, eight years after trunk renewal, taking into account the percentage of dead vines in each test group.

Foliar symptom appearance

Foliar symptoms on the vines with trunk renewal were detected by visual inspection. All vines were inspected and classified according to the following scheme: 1. moderate symptoms, on < 50% of canopy; 2. heavy symptoms, on > 50% of canopy.

Data analysis

Data on normal vigour and mortality in 2002 were statistically analysed using the Chi Square procedure (χ^2). For each test group in each vineyard the frequency of vines with normal vigour was compared using the statistics test 'set of two × r'. A similar test was performed for the variable "mortality".

In the case of vineyard 1, the statistical analysis was completed with Multiple Correspondence Analysis, using SAS System Rel 8.2 (SAS Institute, Cary, NC, USA). This test shows the effect of different treatments on plant vigour by associating each treatment with a different vigour category.

The 2002 yields of each test group in each vineyard were subjected to Duncan's Test using the SAS system.

Results

Trunk renewal and fungicide trunk injection

Growth resumption

Growth resumption in vines immediately after trunk renewal was high in all test groups (Table 2). Subsequently, fungicides favoured growth resumption in different ways. Cyproconazole was the most effective; its effect on growth resumption increased with time even after it had been discontinued and was most marked in 2002. Tetraconazole was also effective during treatment though somewhat less so in 2001 and 2002. Fosetyl Al led to a high vegetative resumption, decreasing during the years of treatment, which stabilised in 2001 and 2002, though still significantly higher than that shown by the renewed untreated vines.

Vigour, mortality and yield

In all test groups of both vineyards the pattern of plant vigour in 1995–1998 was similar: all trunk-renewed vines that resumed growth gradually increased in vigour until 1998.

After this initial period the benefit of trunk renewal was shown to be only temporary, unless it was combined with fungicide post-treatment, in which case it became permanent .

In vineyard 1 during 2001 and 2002, the proportion of vines treated with Tetraconazole and showing medium or normal vigour was drastically

Table 2. Growth resumption in trunk-renewed vines treated with fungicide by trunk injection.

Vineyard No.	Fungicide	1995	1996	1997	1998	2001	2002
		Vines (%)					
1	Fosetyl Al	85.7	85.7	81.0	76.2	76.2	76.2
1	Tetraconazole	100.0	100.0	100.0	100.0	71.4	71.4
1	Untreated	76.2	76.2	71.4	66.7	57.2	57.2
2	Cyproconazole	81.0	81.0	85.7	85.7	95.2	95.2
2	Untreated	61.9	57.2	57.2	57.2	57.2	57.2

reduced, though a positive 15% gap in vigour remained compared to the untreated (trunk-renewal only) vines (Table 3).

Nevertheless, statistical analysis of the normal-vigour vines detected no significant differences between untreated and tetraconazole-treated vines in that vineyard (Table 4).

However, statistical analysis detected significant differences between the fosetyl Al treated and the untreated group. Multiple Correspondence Analysis confirmed these results, linking vines of normal vigour to fosetyl Al, and vines of low and medium vigour to Tetraconazole (Fig. 1).

The effect of trunk injections on vigour was particularly strong in vineyard 2, where all cyproconazole-treated vines showed high vigour in the last two years, and indeed earlier, while a considerable portion of the control vines had low vigour. This result was significant, χ^2 <0.0001 (Table 4).

Mortality of the trunk-injected vines during the last year was much lower than in the controls. However, in the statistical analysis the result was significant only for cyproconazole (Table 5). Multi-

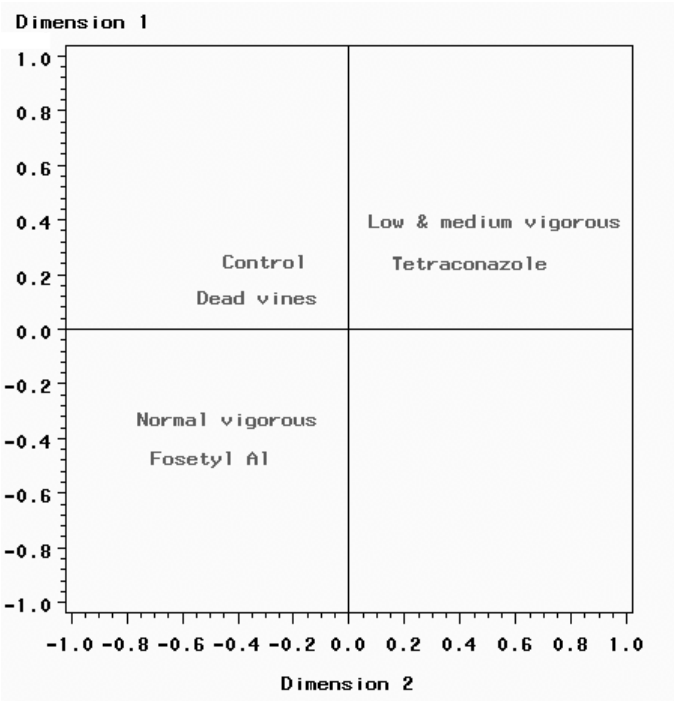

Fig. 1. Multiple Correspondence Analysis: correlation between vine vigour classes and fungicide treatment in vineyard 1.

Table 3. Trunk injection: vigour of trunk-renewed vines during and after trunk injection of fungicide, grouped by vine vigour level.

Vineyard No.	Fungicide	Vigour level	1995	1996	1997	1998	2001	2002
			Vines (%)					
1	Fosetyl Al	Low	0.0	9.5	4.8	0.0	0.0	0.0
		Medium	33.3	23.8	4.8	0.0	9.5	9.5
		Normal	52.4	52.4	71.4	76.2	66.7	66.7
1	Tetraconazole	Low	28.6	0.0	0.0	0.0	14.3	14.3
		Medium	42.8	28.6	0.0	0.0	14.3	14.3
		Normal	28.6	71.4	100.0	100.0	42.8	42.8
1	Untreated	Low	9.5	9.5	0.0	0.0	0.0	14.3
		Medium	42.8	9.5	0.0	0.0	23.8	9.5
		Normal	23.8	57.1	71.4	66.7	33.3	33.3
2	Cyproconazole	Low	23.8	4.8	0.0	0.0	0.0	0.0
		Medium	52.4	42.8	38.1	0.0	0.0	0.0
		Normal	4.8	33.3	47.6	85.7	95.2	95.2
2	Untreated	Low	23.8	9.5	9.5	0.0	4.8	0.0
		Medium	33.3	19.0	4.8	0.0	19.0	28.6
		Normal	4.8	23.8	38.1	52.3	28.6	28.6

Table 4. Statistical analysis using "set of two × r". The treated test groups in the trunk-injected vineyards were compared with the corresponding untreated test group (trunk-renewal only) according to the frequency of vines in the classes "normal vigour", as assessed in 2002.

Test group	Number of vines with normal vigour	No. of vines with normal vigour in the untreated test groups	
		Vineyard 1	Vineyard 2
		7	6
Fosetyl Al	14	0.0308[a]	
Tetraconazole	09	0.5251[a]	
Cyproconazole	20		<0.0001

[a] χ^2 probability value: the values shown were at the intersection between the two data of the compared test groups. The couple comparison was significantly different from χ^2 <0.05.

Table 5. Statistical analysis using "set of two × r". The treated test groups in the trunk-injected vineyards were compared with the corresponding untreated test group (trunk-renewal only) according to the frequency of vines in the class "mortality", as assessed in 2002.

Test group	Number of dead vines	No. of dead vines in the untreated test groups	
		Vineyard 1	Vineyard 2
		9	9
Fosetyl Al	5	0.1904[a]	
Tetraconazole	6	0.3340	
Cyproconazole	1		0.0038

[a] χ^2 probability value: the values shown were at the intersection between the two data of the compared test groups. The couple comparison was significantly different from χ^2 <0.05.

ple Correspondence Analysis was not used with vineyard 2, as the results with cyproconazole were highly significant in the χ^2 analysis.

In 2002 yield from vines treated with cyproconazole or fosetyl Al differed significantly from the yield of untreated vines. The yield from vines treated with Tetraconazole was greater than that of the untreated controls and lower than that of fosetyl Al, but the differences were not significant (Table 6). The statistical analysis on yield also included dead vines, counted as producing zero yield.

Table 6. Vines receiving trunk injection: average yield in 2002.

Vineyard No.	Fungicide	Yield (Kg/vine)
1	Fosetyl Al	14.3 a[a]
1	Tetraconazole	10.3 ab
1	Untreated	6.6 b
2	Cyproconazole	19.5 a
2	Untreated	13.4 b

[a] Values in column followed by the same letter do not differ according to Duncan's Test (P=0.05).

Foliar symptoms severity

Foliar symptoms in trunk-renewed vines were generally infrequent during the monitoring period, and no differences in foliar symptoms incidence were observed between fungicide-treated vines and the controls (Table 7).

Relation between necrosis severity and treatment effectiveness

Necrosis severity recorded at the time of trunk renewal in1995 did not affect subsequent fungicide effectiveness in vines with normal vigour and optimal yield in 2002. Many vines treated with cyproconazole and fosetyl Al, the most effective fungicides, had revealed severe necrosis in 1995; of these 95.2% of cyproconazole-treated vines showed optimal response to treatment, including 52.4% of vines in the necrosis category 'extensive discoloration and decay'; similarly, of the vines treated with fosetyl Al 66.7% showed optimal response to treatment, including 42.8% of vines that had had extensive discoloration and decay in 1995 (Fig. 2).

Table 7. Foliar symptom severity.

Vineyard No./fungicide	Symptom severity	No. of vines (%)					
		1995	1996	1997	1998	2001	2002
1/Fosetyl Al	Moderate	4.2	13.9	8.3	5.5	0.0	0.0
"	Great	0.0	0.0	0.0	0.0	0.0	0.0
1/Tetraconazole	Moderate	0.0	14.3	0.0	0.0	0.0	0.0
"	Great	0.0	0.0	14.3	14.3	0.0	28.6
1/Untreated	Moderate	0.0	15.3	4.2	0.0	0.0	0.0
"	Great	0.0	0.0	9.7	4.2	0.0	4.8
2/Cyproconazole	Moderate	0.0	0.0	12.5	0.0	0.0	0.0
"	Great	0.0	0.0	0.0	0.0	0.0	0.0
2/Untreated	Moderate	0.0	33.3	26.4	0.0	0.0	0.0
"	Great	0.0	0.0	0.0	0.0	0.0	0.0

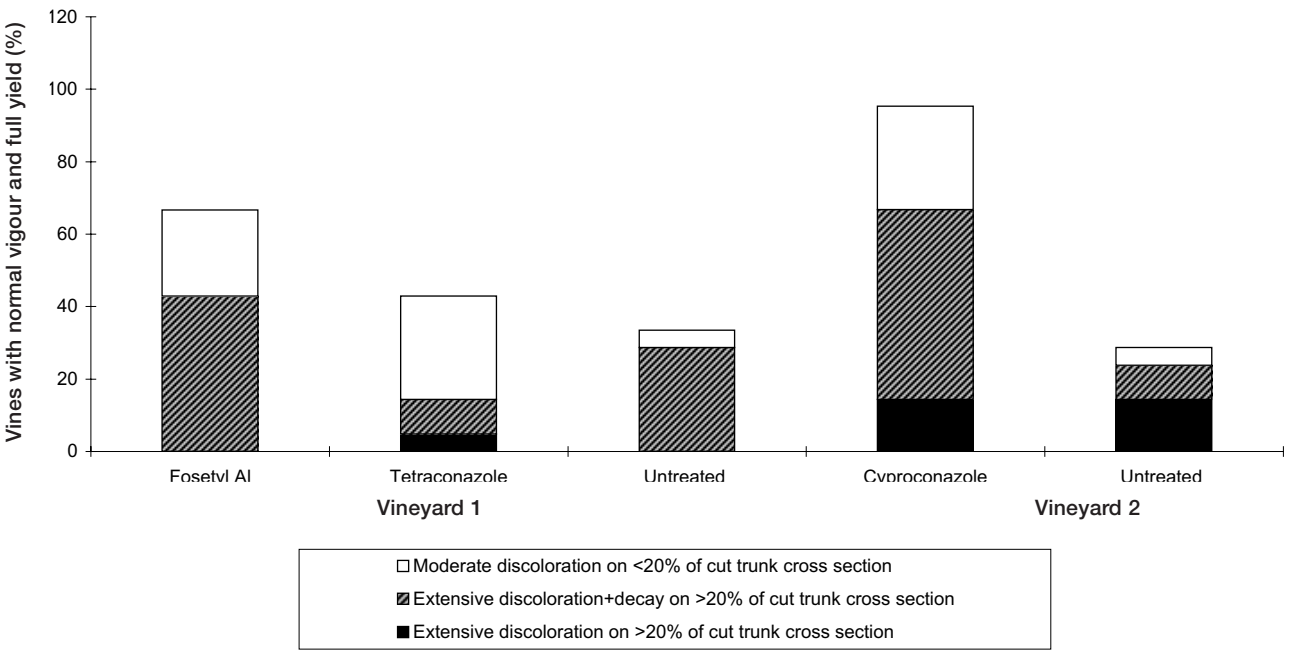

Fig. 2. Severity of wood necrosis (%) observed at the time of trunk renewal (1995) in vines showing normal vigour and full yield eight years later (2002).

Trunk renewal followed by fungicide application with injector pole

In vineyard 3, vines treated with ground applications of cyproconazole by injector pole did not differ from vines that had received trunk renewal alone; at the end of the test period in 2002, growth resumption, vigour, and hence yield were low both in the control vines (trunk renewal only) and in the vines treated with cyproconazole by injector pole after trunk renewal.

A limited resumption of growth occurred in this vineyard in both the control vines and the cyproconazole treated-vines, but the result was a high mortality in 2002 (Tables 8, 9 and 10).

Vines in vineyard 3 were also more seriously necrotic than vines in the other vineyards, with 91.4% of vines having extensive/discoloration+decay, and 8.6% extensive discoloration.

No foliar symptoms were noted in these vines after trunk renewal. The results (Tables 8, 9 and 10) did not allow any data analysis on the effect of necrosis severity on subsequent treatment efficacy. For this reason, data analysis was carried out only on the yield data, in which no significant differences were detected.

Discussion

The strong positive reaction of the vines to trunk renewal could be due to the characteristic vigour of the cv. Trebbiano d'Abruzzo. The immediate resumption of vine growth after trunk renewal on vigorous vines was also reported by Frausin e Spessotto (1996).

Anyway the growth resumption of trunk-renewed vines is a transient phenomenon, which

Table 8. Growth resumption of vines with trunk renewal and post-treated with ground applications by injector pole.

Vineyard No.	Fungicide	No. of vines (%)					
		1995	1996	1997	1998	2001	2002
3	Cyproconazole	55.2	31.0	34.5	34.5	31.0	31.0
3	Untreated	58.6	55.2	51.7	37.9	27.6	27.6

Table 9. Vines treated with ground applications of fungicide and grouped by vigour level.

Vineyard No.	Fungicide	Vigour level	No. of vines (%)					
			1995	1996	1997	1998	2001	2002
3	Cyproconazole	Low	10.3	17.2	17.2	10.3	13.8	13.8
"	"	Medium	44.8	13.8	0.0	0.0	0.0	0.0
"	"	Normal	0.0	0.0	17.2	24.1	17.2	17.2
3	Untreated	Low	6.9	17.2	17.2	0.0	17.2	17.2
"	"	Medium	0.0	0.0	10.3	0.0	0.0	0.0
"	"	Normal	51.7	37.9	24.1	37.9	13.8	13.8

Table 10. Ground application of fungicide by injector pole: percentage of dead plants and yield in 2002.

Vineyard No.	Fungicide	Dead vines (%)	Yield (Kg/vine)
3	Cyproconazole	69.0	2.9 a[a]
3	Untreated	69.0	2.5 a

[a] Values in column followed by same letter do not differ significantly according to Duncan's Test (P=0.05).

gradually fades over the years as more and more trunk renewed vines again lose their growth capability. This is possibly because trunk renewal does not excise all the necrotic wood, and hence cannot ensure the complete recovery of the vines, and indeed in the present study necrosis was still visible on the cut trunk surface of all trunk-renewed vines treated with fungicides; these necrotic processes presumably interfered with the growth processes of some of the trunk-renewed vines.

Nevertheless, in vineyard 1, growth resumption was low in far fewer vines that received trunk injections of fungicide than in trunk-renewed vines that were not so treated, particularly in 2001–2002, even though the fungicide applications had been discontinued at this time.

Moreover, in vineyard 2, the growth resumption of vines treated with cyproconazole increased steadily after trunk renewal until 2001–2002; in 2002 95.2% of vines showed growth resumption, whereas in both this vineyard and vineyard 1 most of the control vines (not receiving fungicide treatments) did not show growth resumption.

Starting in 2001 growth resumption appeared to stabilise in most cases. This suggested that given the operational conditions of the present study the length of time chosen to study growth resumption was sufficient to give meaningful results.

After trunk renewal the selected shoots grew very quickly, so that in the three years until 1998 the trunk-renewed vines appeared completely recovered, strongly vigorous and had a yield comparable to that of normal adult vines of the same cultivar trained to the same trellis system.

Monitoring vine growth in 1998–2002, i.e. in the four years after fungicide treatment had been discontinued, was crucial to assess the effectiveness of trunk renewal coupled with fungicide treatment. During this monitoring period the effect of cyproconazole and, to a less extent, fosetyl Al could be clearly identified by comparison with the control group, in which many vines at this time lost their vigour and became unproductive.

Trunk renewal combined with fungicide injection was particularly effective in restoring vigour. This was a very important finding because vigour was closely related to yield in all the vineyards.

At the end of the trials, in 2002, normal vigorous renewed vines had a yield comparable to that of healthy vines of the same cultivar and trained to the same trellis system (20 Kg/plant), the medium-vigorous vines had a yield approximately half that, and the yield of the low-vigorous plants was practically nil.

Statistical analysis detected significant differences in vigour and yield with both cyproconazole and fosetyl Al. Only cyproconazole had a significant effect on mortality. This suggested that though trunk injections of fosetyl Al improved the growth status and yield of trunk-renewed plants, they did not reduce mortality, which appeared to be dependent on cultivar vigour, or on some other, undefined parameter affecting growth resumption after trunk renewal.

The poor effect of Tetraconazole in this study was consistent with its scarce effect on esca fungi *in vitro* (Bisiach *et al*., 1996), although in the present study the formula used was specifically suited for trunk injections.

Vineyards 1 and 2 were characterised by an intrinsic growth resumption capability. Though a considerable number of vines showed extensive necrosis in 1995, yet response to treatment was high, suggesting that the treatment given could be suitable for adult vineyards with a high incidence and severity of esca.

In an earlier study (Di Marco *et al*., 2000), it was found that the fungicides used here were not effective on vines that had not been trunk renewed, possibly because in that case the necrotic wood could not be reached by the fungicides.

Fungicides injected by syringe showed good activity on vines with trunk renewal. This could be because the active ingredients of the fungicide interfered with wood colonisation by the esca pathogens, possibly by spreading through the vinewood and coming in direct contact with the wood-rotting fungi.

Fosetyl Al may operate by a mechanism based on the fungicide activity of phosphorous acid (Fenn and Coffey, 1984); it may also attack the esca fungi through the polyphenols, particularly resveratrol (Mazzullo *et al*., 2000).

The reappearance of esca symptoms on trunk-

renewed vines, though not extensive, was not related to the severity of the initial necrosis, nor, consequently, to the assumed activity of the fungicide formulations (Mugnai *et al*., 1999; Di Marco *et al*., 2000).

Cyproconazole treatment by ground pole injector was not more effective than trunk renewal alone, as already reported in other studies (Bisiach *et al*.,1996; Di Marco *et al*., 2000).

The ineffectiveness of ground-injected cyproconazole may have had a variety of causes: 1. the too great dispersal of the fungicide in the soil, 2. the fungicide is absorbed far from where it is needed (necrotic area) and must be transported by a long route via the roots and active parts of xylem (Di Marco *et al*., 2000).

The poor results in the vineyard where ground injection was tested could also be due, at least in part, to the fact that necrosis here was more severe than in the other vineyards.

Cyproconazole and to a less extent fosetyl Al applied by trunk injection to vines after trunk renewal had a beneficial effect on vine growth and yield; the combination of trunk renewal and treatment with the fungicides prolonged the benefit obtained with trunk renewal alone; and also improved and prolonged the vine life cycle. These findings make this combined approach a suitable control strategy for adult vineyards infected with esca proper.

The novel findings of this study must be verified on other sites to assess the extent to which other factors such as cultivar, rootstock and environment also contributed to the result.

Literature cited

Bisiach M., G. Minervini and S. Di Marco, 1996. Attualità nella difesa contro il mal dell'esca della vite. In: *Convegno Nazionale "Arsenico, Sì-No"*, 14 dicembre 1995, Codroipo, Udine, Forum fitoiatrici ERSA, Udine, Italy, 123–135.

Di Marco S., 1990b. Attività *in vitro* di fungicidi triazolici contro funghi lignicoli della vite e dei fruttiferi. *Atti Giornate Fitopatologiche* 2, 221–228.

Di Marco S., L. Draghetti, P. Larignon and A. Brunelli, 1993. Control of grapevine wood decay fungi by a syringe injection application technique. In: *A.N.P.P–B.C.P.C., Second International Symposium on Pesticide Application Techniques*, Strasbourg, 22–24 September, 1993, 595–602.

Di Marco S., A. Mazzullo, F. Calzarano and A. Cesari, 2000. The control of esca: status and perspective. *Phytopathologia Mediterranea* 39, 232–240.

Di Marco S., 2002. Recenti acquisizioni, stato attuale e prospettive nella lotta al mal dell'esca della vite. *Notiziario Tecnico* CRPV 63, 85–90.

Fenn M.E. and M.D. Coffey, 1984. Studies on the *in vitro* and *in vivo* antifungal activity of fosetyl Al and phosphorous acid. *Phytopathology* 74(5), 606–611.

Frausin C. and C. Spessotto, 1996. Verifica dell'efficacia di trattamenti chimici associati alla capitozzatura nel contenimento del mal dell'esca. *Atti Giornate Fitopatologiche* 2, 389–396.

Graniti A., G. Surico and L. Mugnai, 2000. Esca of grapevine: a disease complex or a complex of diseases? *Phytopathologia Mediterranea* 39, 16–20.

Mazzullo A., F. Calzarano, S. Di Marco and A. Cesari, 2000. Experiences on the control of esca disease fungi by fosetyl Al. In: *IOBC/wprs Bullettin, Working Group. "Integrated control in viticulture"*, 1–4 March, 2000, Florence, Italy.

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(5), 404–418.

Accepted for publication: March 8, 2004