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The control of esca: status and perspectives

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Summary. The development of an effective control strategy for esca greatly depends on major progress in understanding the disease biology and epidemiology. Experimental trials were carried out in vineyards and single infected vines in order to evaluate the effectiveness of different fungicides, application methods and cultural practices in controlling esca: experiences with sodium arsenite, dinitro-orthocresol, fosetyl Al, triazoles and first attempts with *Trichoderma* are discussed. Ongoing trials show promising results with fosetyl Al activity against *Phaeoacremonium* spp. under laboratory and greenhouse conditions, with the prospect of a favourable effect on esca control. Research is now being focused on the potential of a control strategy to prevent or reduce the establishment and development of the disease. The management of esca should be based on the age of the plant, the stage of the infection as well as the degree of spread of the disease.

Key words: grapevine, esca, control.

Introduction

In the last few years the incidence and the severity of esca have continuously increased making the disease a real problem in all grape-growing areas. Although great efforts have been made to understand the disease characteristics, the role of the microorganisms involved in the infection process and the epidemiological factors influencing the spread of esca are not yet clearly defined.

Esca is a complex disease characterized by several microorganisms producing wood discoloration and decay. Often different microorganisms coexist in the same plant. The mode of infection, the age of the vine, the fungi involved and the occurrence of different symptoms in the trunk and arms, should be considered in the development of control strategies.

Several esca characteristics make the effectiveness of control strategies still more difficult. The most important points are: i) the types of microorganisms involved, ii) the time required for foliar symptoms to appear, iii) the correlation of foliar symptoms with wood deterioration and, iv) the erratic nature of the disease.

With regard to the first point, several fungi have been associated with the disease: *Phellinus (Fomitiporia) punctatus* and, to a lesser extent, *Stereum hirsutum* have long been known as white-rot agents producing wood decay, whereas mitosporic fungi belonging to the *Phaeoacremonium* genus and, under certain climate conditions, the ascomycete *Eutypa lata*, are commonly isolated from discolored wood. The establishment and wood-tissue colonization as well as the role of these pathogens in the infection process overall

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should be determined in order to find proper treatments.

A very special point is also the varying lengths of the time required for foliar symptoms to appear once a plant becomes infected. In most cases the disease shows up in the vineyard when wood discoloration and decay inside the vine are already extensive; therefore, going by visible symptoms means that most control measures are taken too late.

Finally, the effectiveness of control measures has generally been assessed by the effect on foliar symptom appearance. However, the year-to-year fluctuation of symptoms, as well as the fact that the severity is not strictly correlated with the degree of vinewood infection require a large number of years to evaluate correctly the effectiveness of experiments to control esca.

The control of the disease

Treatments of the entire vineyard

Sodium arsenite

Chemical control is based on sodium arsenite sprayed on the trunk and arms. The chemical is applied at 1,250 g arsenite hl^{-1} according to a threshold of diseased vines ranging from 0.5 to 2.5% (depending on the number of plants per hectare). Treatments are applied for two years consecutively, 2 weeks after the end of pruning and not later than 3 weeks before sprouting; application can be omitted in the third and fourth year but must then be repeated in the following two years, and so on.

The arsenites, whose mode of action is not yet understood, are the only compounds that interfere with disease appearance so far (Geoffrion, 1982; Dubos *et al.*, 1983; Desaché *et al.*, 1992, 1995; Boubals, 1996). Because of their high toxicity, however, arsenites have been banned in several countries. The most important hazards are the carcinogenic effect on the human lung and skin, the impact on the environment and the accumulation in the food chain (IARC, 1987; Meneguz and Zaghi, 1995). For this reason, even countries where the sodium arsenite is still permitted, France, Portugal and Spain, have placed restrictions on its use in order to reduce potential worker exposure; application equipment must have recuperator panels providing a 60% reduction of the environmental waste and the chemical is combined with a wildlife repellent.

Dinitro-orthocresol

Dinitro-orthocresol (DNOC) compounds were tested to assess the caustic disinfecting action on vine wood. DNOC sprays (1-1.5%) were applied at least a few days after pruning (1,000 g a.i. hl^{-1}) and again 2 weeks before the start of budbreak (1,250 g a.i. hl^{-1}). After two years the percentage of plants showing spotting of berriers and a spread of esca was lower on treated vines than on untreated ones (Granata and Riva, 1980). Recently this kind of test has become less common because such tests, even when extended over a number of years, have failed to produce satisfactory results (Cavanni *et al.*, 1987; Bisiach *et al.*, 1996). Moreover, the high toxicity of the compound (acute oral LD_{50} for rats: 25-40 mg kg^{-1}) was not compatible with environmentally friendly approaches to crop disease control management.

Fosetyl Al foliar treatment

Promising results were obtained with fosetyl Al and its main metabolite, phosphorous acid, tested under controlled conditions against the pathogens involved in esca (Mazzullo *et al.*, 1996; Di Marco *et al.*, 1999). Therefore, further investigations were carried out on esca-infected vineyards (12-14 years old) in order to assess whether fosetyl Al applied against downy mildew had an effect on the incidence and severity of esca. Four treatments at 10-day intervals were carried out each year for 3 years at the end of blooming. These trials were then continued beyond the third year, but by the fifth year no reduction in either the severity of foliar symptoms or the incidence of the disease had been noticed (Di Marco, unpublished).

Moreover, foliar applications of fosetyl Al on mature French vines never provided any interesting indication about side effects on esca development (Dubos, personal communication).

Treatments of individual diseased vines

Several control trials were conducted on individual diseased vines to assess the effectiveness of treatments after the appearance of esca symptoms in a vineyard.

Triazolic fungicide applications

Most of these trials used triazolic fungicides because these compounds combine excellent systemic properties with a broad spectrum of fungicidal activity. Some of these chemicals proved to be effective *in vitro* at low concentrations against several fungi associated with esca (Di Marco, 1990; Bisiach *et al.*, 1996).

Secateurs fungicide sprayer

In order to prevent wound infection, the use of special pruning secateurs was tested. The secateurs were fitted with a capsule containing a mixture of a triazole (penconazole or cyproconazole) and carbendazim. Fungicides were sprayed on the wound surface under pressure at pruning; the fungicide mixture was made in order to improve the adhesion of the compounds to the cut wood surface. A three-year trial was carried out in northeast Italian vineyards: no difference in the severity of foliar symptoms or in incidence of the disease was noted between treated and normally pruned plants (Bisiach *et al.*, 1996).

Painting

Cyproconazole (Atemi, 10 WG), flusilazole (Nustar, 20 DF), penconazole (Topas, 10 EC) and tetraconazole (M 14360, 10 EC) were applied before blooming by painting the trunk surface of infected vines, taking care to remove the cork. Triazoles were mixed (1:1 vol.) with an organic polypeptide compound (Siapton 10L) to improve uptake and translocation inside the plant. The severity of foliar symptom appearance was assessed for 5 years. No positive results were ever observed (Bisiach *et al.,* 1996).

Injector-pole and syringe injection

This type of application was carried out on 10- to 17-year-old vineyards with different cultivars. Every year before harvest, the apparent disease incidence (percentage of visibly symptomatic plants) and the severity of foliar symptom appearance (using an arbitrary 0-5 scale) were recorded. At the beginning of the trial, in order to evaluate the existing infection conditions, several esca-infected plants were collected, cut crosswise and lengthwise and wood discoloration and decay were recorded.

A single application per year of triazolic fungicides was delivered by an injector pole equipped with a water-meter measuring the amount of fungicide solution applied. Two holes were made in the soil along the row of vines, one opposite the other at 1.5 m from the trunk and compounds were dispensed in 10 litres of water per plant.

For syringe injection, fungicides were applied to diseased grapevines by simple, specially designed syringes. Two syringes were placed in the trunk of each plant, one opposite the other, at a height of 25-40 cm from the ground. The holes were drilled in the wood with a cordless drill and a brass needle was driven into the hole. A PVC-plugged cylinder containing the desired amount of fungicide was then connected to the needle, so that the fungicide could be injected into the plant under pressure which was maintained by means of a plunger depressed and fixed by a nail (Di Marco *et al.*, 1993). The cylinder emptied in 24-36 h; it was then removed from the needle, which was covered with a plug. At the end of the 3-year treatment period, the hole in the trunk was healed with a waxy dressing.

Compounds tested were cyproconazole (Atemi, 10 WG), flusilazole (Nustar, 20 DF), penconazole (Topas, 10 EC) fosetyl Al Ca (Aliette Ca) and tetraconazole (M 14360, 10 EC for injector-pole and 12.5 FL for syringe). Triazoles were applied at 1 g of a.i. per plant by injector-pole and 100 mg of a.i. per plant by syringe; fosetyl Al Ca (specially formulated for wood injections) at 2 g of a.i. per plant. From 20 to 30 replicates per treatment were set up each consisting of one diseased vine. Treatments were started when the grapevine was at 10 cm shoots and continued for three consecutive years.

Fungicide applications by injector-pole and by syringe gave ambiguous results. In the many trials carried out two main kinds of outcome could be observed, depending on the type of wood deterioration.

Most of the trials had negative results. They were obtained operating in the oldest vineyards on diseased plants much affected with wood decay. Under these conditions, no difference was found in the severity of esca symptoms between treated and control plants (Fig. 1, 3).

However, treatments carried out at the first appearance of esca on vines, with only limited wood discoloration and moderate development of white rot – probably at the end stage of “young esca” (sensu Graniti *et al.*, this issue) – produced a statistically significant reduction in the severity of foliar symptom appearance on treated vines com-

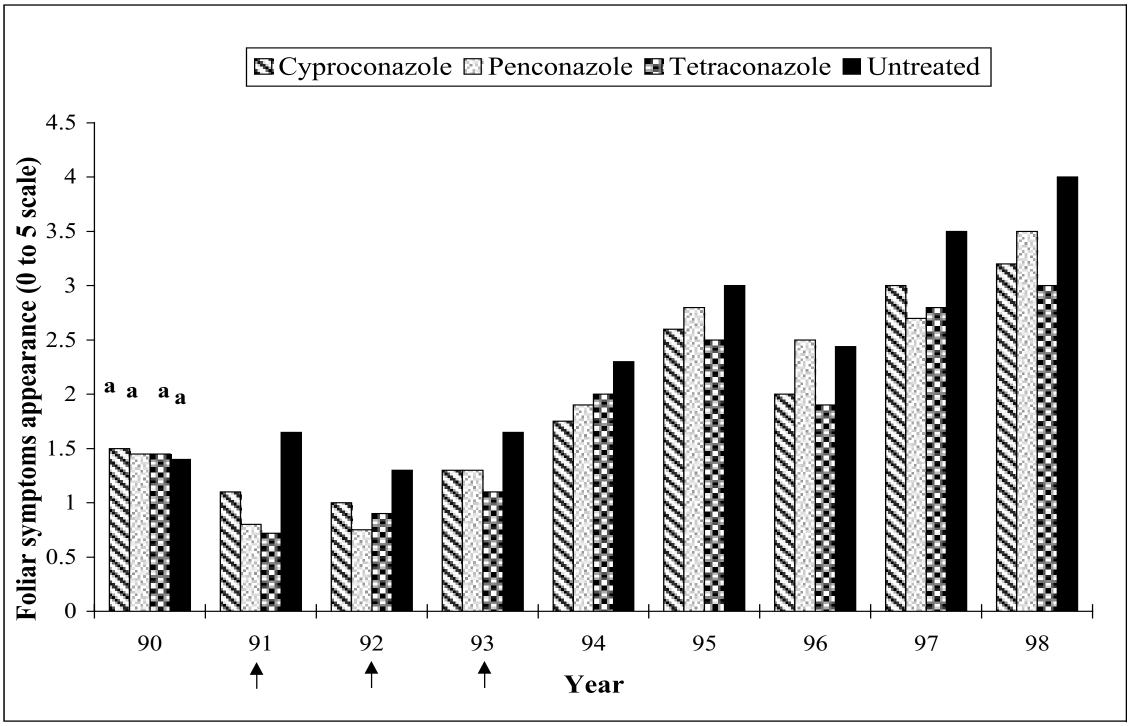

Fig. 1. Injector-pole field trial (cv. Cabernet). Black arrow means fungicide application. For each year values are not statistically different (P=0.05) according to Duncan's multiple range.

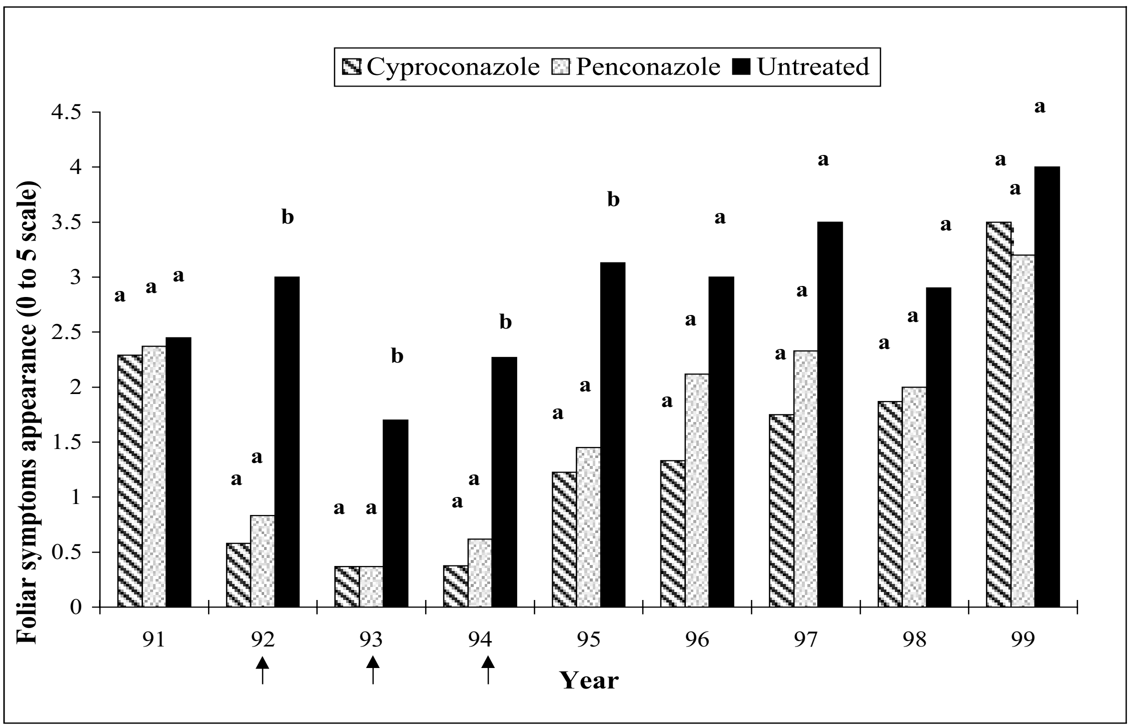

Fig. 2. Injector-pole field trial (cv. Sangiovese). Black arrow means fungicide application; values marked with the same letter are not statistically different (P=0.05) according to Duncan's multiple range.

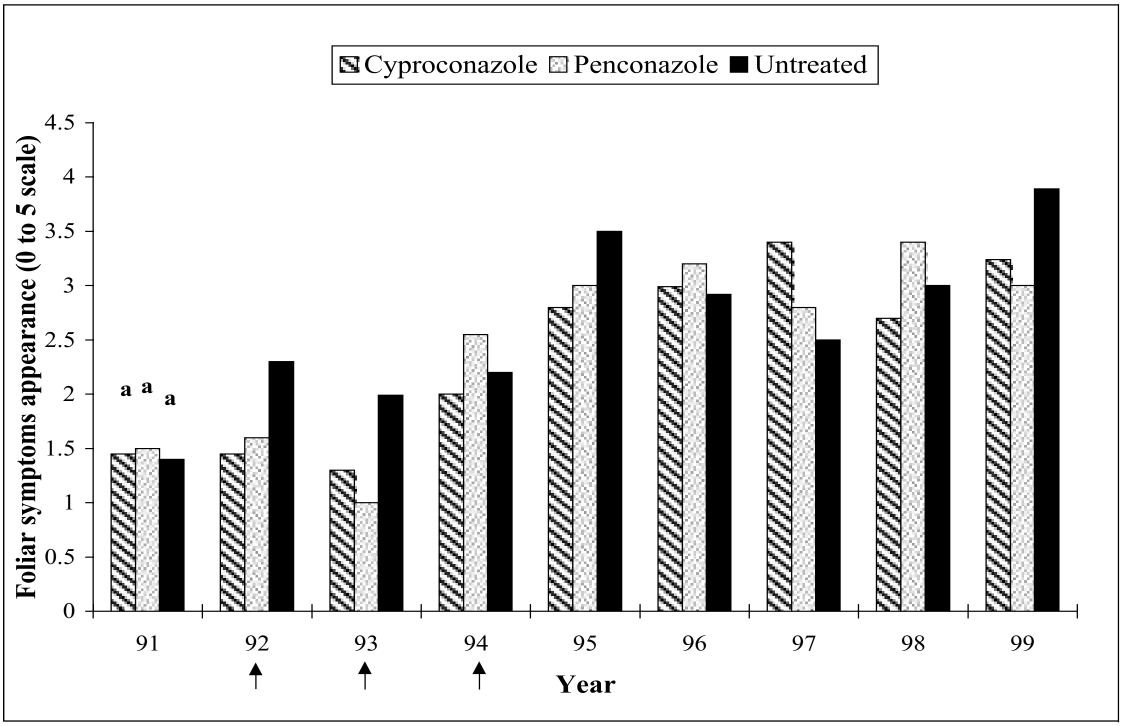

Fig. 3. Syringe field trial (cv. Cabernet-Sauvignon). Black arrow means fungicide application. For each year values are not statistically different (*P*=0.05) according to Duncan's multiple range.

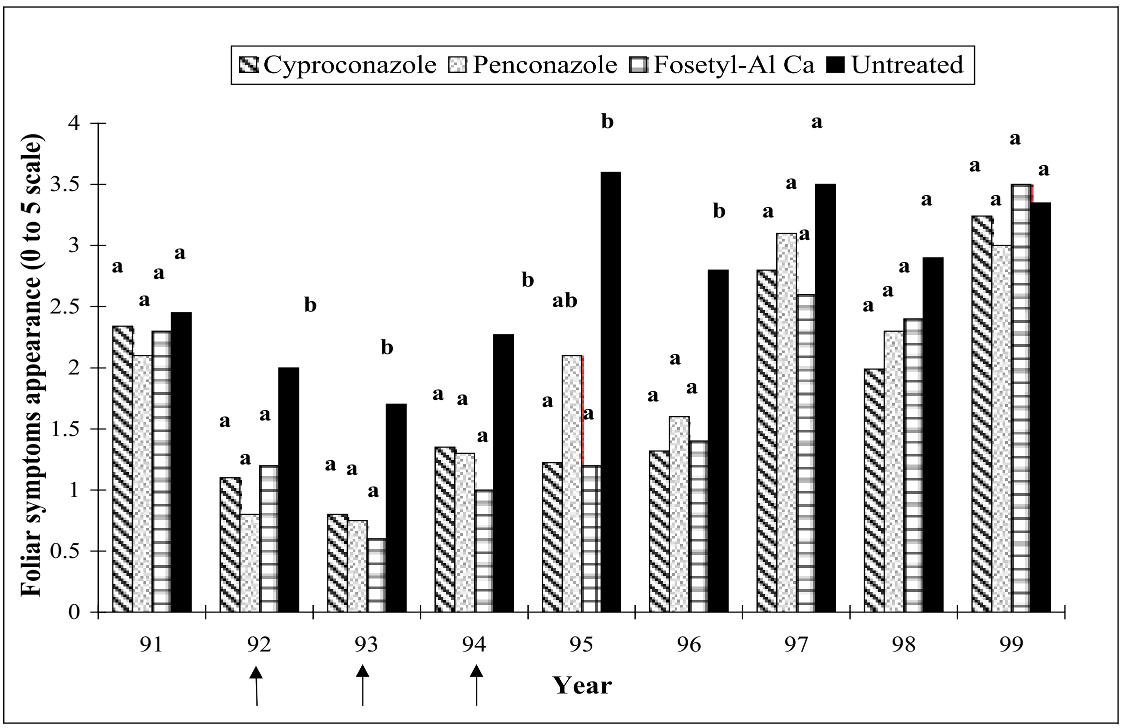

Fig. 4. Syringe field trial (cv. Sangiovese). Black arrow means fungicide application; values marked with the same letter are not statistically different (*P*=0.05) according to Duncan's multiple range.

pared with the control. This lowering effect lasted for 5 years (Fig. 2, 4). As a result the percentage of wilted shoots was lower and this led to a reduced need to prune cuttings, which normally creates favourable conditions for the spread of esca. Moreover, basic productive standards could be maintained without yield loss. In particular the quantity of grapes harvested per plant and the Brix reading values were similar to those for apparently healthy vines.

As regards symptom reduction obtained in the field trials, it is possible that chemicals translocated and distributed inside the vine, moving close to an infected wood area or spreading in it (when injected by syringe under pressure), somehow interfered with pathogen development. This condition then produced a delay in the unclear mechanism leading to foliar symptom occurrence, with a favourable effect on production, although no outright eradication of the disease was ever observed. However, triazole uptake, mobility and activity were evaluated in potted vines under greenhouse conditions by Di Marco and Draghetti (1993). They showed that there was root uptake and translocation of triazolic fungicides, whether applied by soil drenching or trunk injection. These fungicides then inhibited powdery mildew in the leaf. Moreover, *Sphaeropsis malorum*, the causative agent of black dead arm of grapevine, inoculated in the trunk, was also inhibited by fungicide application in the ways indicated.

The conditions which produced positive results against esca with injector pole and syringe applications of fungicide appeared to be at least correlated with a low disease incidence in the vineyards and with plants at an early stage of infection, when wood discoloration was still limited. Unfortunately, these conditions are difficult to ascertain in the vineyard without the actual examination of the wood, because they are never associated with foliar symptoms. For these reasons the injector-pole and syringe applications were generally carried out too late and achieved only limited success at field level.

Wound protection with *Trichoderma viride* applications

The use of biological agents as wound dressing showed promising results in other plant-pathogen system. *Trichoderma harzianum* controlled *Acer rubrum* rots caused by hymenomycetous fungi for up to 2 years (Pottle *et al.*, 1977). Smith *et al.* (1981) reported that when *T. harzianum* was used to treat red maple wounds it preempted *Phialophora melinii*.

Bisiach *et al.* (1996) reported experiments carried out on cv. Verdicchio in the 1980s; freshly cut pruning wounds were treated with three strains of *Trichoderma viride* at $1x10^6$ cfu ml^{-1} and 10% glycerol. The application was repeated for three consecutive years. Trials were carried out on apparently healthy vines and the effectiveness of the treatment was assessed by its ability to reduce the formation of external esca symptoms. Under these experimental conditions no difference was found between treated and untreated vines.

Cultural practices

The control of esca still relies on traditional cultural methods which reduce the losses of the disease and inhibit its spread. Although these methods do not ensure complete control, they are recommended as being associated with the health, productivity and longevity of vineyards (Bisiach *et al.*, 1996; Calzarano and Di Marco, 1997; Mugnai *et al.,* 1999).

Cultural practices before foliar symptom occurrence include:

– treating the vineyard with copper compounds soon after a severe frost in order to protect the wounds from pathogens;
– avoiding large wounds and protecting any wounds with healing varnish or a dressing containing a broad-spectrum fungicide;

After foliar symptom appearance:

– marking esca-infected vines;
– pruning all infected vines separately from the other, healthy looking vines;
– removing pruning residues of clearly diseased vines from the vineyard;
– uprooting and taking away all dead infected vines from the vineyard.

Finally, a sanitary measure can be carried out by cutting infected vines below the rotted and discoloured wood and protecting the wounds with healing varnish. This method generally works for 3-4 years, sometimes for longer if the affected trunk portion is removed completely.

Perspectives

Fosetyl Al has been reported to interact positively with the host resistance response towards diseases. Moreover, as phosphorous acid, to which fosetyl Al is rapidly degraded, it inhibits the pathogens *in vitro* (Raynal *et al.*, 1980; Fenn and Coffey, 1984).

In the field, a statistically significant reduction in esca symptom severity as a result of fosetyl Al trunk injections was noticed in vineyards that had a moderate incidence of the disease and vines not seriously affected by white rot (Di Marco *et al.*, 1997). This positive effect suggested a need for a further investigation into the esca pathogens, including the antifungal activity of phosphorous acid, resveratrol and pterostilbene.

Esca fungi were cultivated on potato-dextrose agar (PDA) and maintained at room temperature in the dark. Discs (6 mm in diameter) were cut from the margin of colonies and placed, mycelium downwards, in Petri dishes containing PDA. Treatments were added to the culture at the following concentrations of active ingredient: phosphorous acid (free acid, 99%, Aldrich, Milano, Italy) 300 mg ml^{-1}; resveratrol (99%, Sigma, Milano, Italy) 300 mg ml^{-1}; resveratrol + phosphorous acid: 300 + 300 mg ml^{-1}; pterostilbene (laboratory synthesis, 99%) 10 mg ml^{-1}; pterostilbene + phosphorous acid: 10 + 300 mg ml^{-1}. All plates were incubated at 25°C in the dark. Colony diameters were measured every day and results were expressed as percentage inhibition of radial growth (Abbott's formula). Five replicates per treatment were set up, each consisting of 1 plate.

Results are summarized in Table 1. Superior activity was shown by phosphorous acid in combination with *Vitis* stilbenes against *Libertella* sp. and, to a lesser extent, the same combination against *S. hirsutum, P. aleophilum* and *P. chlamydosporum*. Inhibition was smaller when the compounds were separately tested. No significant effect was noticed on *Phellinus igniarius* (Di Marco *et al.*, 1997).

Further investigations were conducted under greenhouse conditions on two-year-old grafted vines by spraying fosetyl Al on foliage (five applications at 10-day intervals and 20 g a.i. l^{-1}). Two months after the last chemical application, 2-year-old grafted vines were inoculated with *P. aleophilum* and *P. chlamydosporum* cultures. A hole (5 mm diameter, 5 mm deep) was drilled on the trunk and a plug (5 mm diameter) from a fresh PDA culture was inserted. The site of the inoculation was covered with a plastic film. A sterile plug of medium was used to assess the wound reaction. Six replicates per treatment, each consisting of 1 potted vine, were set up. Assessment was made by measuring the average length of wood discoloration after splitting the trunks longitudinally. Further *in vitro* tests assessed the viability of the fungi isolated from the plants inoculated with the pathogens.

The greenhouse results are summarized in Table 2. Values of necrotic area length (22 mm) on fosetyl Al treated plants inoculated with *P. aleophilum* were statistically different compared to

Table 1. *In vitro* average percentage of mycelial growth inhibition on esca fungi by phosphorous acid and stilbenes (from Di Marco *et al.*, 1997).

Fungi	Fungal growth inhibition average (%)				
	Resveratrol	Pterostilbene	Phosphorous acid	Ph. acid + Resveratrol	Ph. acid + Pterostilbene
P. aleophilum	-20[a]	40/20[b]	-40/40	80/40	80
P. chlamydosporum	20/20	40	40	60/40	40/60
Libertella sp.	20	40	80/60	100	100
S. hirsutum	n.e.[c]	40/20	n.e.	40/20	60/80
P. igniarius	n.e.	n.e.	n.e.	n.e.	n.e.

[a] The percentage of mycelial growth inhibition represents the average interval enclosing all values assessed during the tests (ranging from 5 to 25 evaluations depending on the pathogen considered). When the value is marked by the sign "-" a stimulation of mycelial growth was assessed.
[b] Two values percent of mycelial growth inhibition in the same column represent the assessment averages obtained in the first half test (left) and in the second half (right) respectively.
[c] n.e., no effect.

Table 2. Activity of fosetyl Al foliar applications against *Phaeoacremonium* spp.

Treatment	Average length of internal necrosis (mm)	
	Fosetyl Al	Untreated
P. aleophilum	22 a[a]	49 b
P. chlamydosporum	30 a	48 a
Sterile plug	-	19

[a] Data in the same line followed by the same letter do not differ significantly according to Duncan's MRT (*P*=0.05).

the untreated (49 mm). The average length of internal necrosis on fosetyl Al treated plants inoculated with *P. chlamydosporum* (30 mm) was less than the untreated (48 mm) but the difference was not significant.

No foliar symptoms were ever observed (Di Marco *et al*., 1999).

Basically, fosetyl Al activity can be linked with the localized interaction between phosphorous acid and *Vitis* stilbenes, through the enhancement of their antifungal activity. *In vitro* investigations clearly revealed that *P. aleophilum* and *P. chlamydosporum* could not metabolize resveratrol in the presence of phosphorous acid (Di Marco *et al*., 1999).

Further studies are under way to assess the potential of fosetyl Al applications, particularly on young vines, with favourable effects on developing an esca control strategy.

Research is now focusing on the effectiveness of strategies to prevent or reduce the establishment and development of esca. For this purpose, the evaluation of the activity of biocontrol agents, fungicide dipping and hot water treatment are underway on grape cuttings and rootlings in the vine nursery.

Conclusions

Research on the development of an effective control strategy against esca is far from over. This is in part a reflection of the lack of precise information about the complex interaction among fungi, vine and environmental factors.

The main effort is now being directed to evaluating the effectiveness of a preventive strategy. For this, research is underway on young vines concentrating on three main approaches:
i) spread of new forms of decline caused by *Phaeoacremonium* spp. in young grapevines and its possible effect on esca development (Ferreira *et al*., 1994; Morton, 1995, 1997; Bertelli *et al*., 1998; Pascoe, 1998; Scheck *et al*., 1998);
ii) positive early results in preventing and/or reducing *Phaeoacremonium* spp. infection occurrence and development by the use of fosetyl Al applications (Di Marco *et al*., 1999; Mazzullo *et al*., 1999);
iii) new assays for non-destructive detection methods of *Phaeoacremonium* spp. (Dupont *et al*., 1998; Tegli *et al*., 1999).

In conclusion, a correlation between proper vine management and the health, productivity and longevity of a vineyard can reasonably be supposed. For control of esca it is necessary to consider the age of the plant, the stage of the infection as well as the incidence of the disease in the vineyard, since if the incidence becomes too high the possibilities of control are correspondingly reduced.

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