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Evaluation of a trunk injection technique to control grapevine wood diseases

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Summary. Vineyard experiments were conducted over five years in the Bordeaux area to evaluate the effectiveness of trunk injections in controlling Eutypa dieback (4 trials) and esca (1 trial). Single treatments were applied in winter 2001 or 2002 using the tree injector StemJect®. Three compounds were tested: two triazole-derived fungicides, propiconazole and difenoconazole, and one elicitor, 2-hydroxybenzoïc acid. Symptomatic vines of two susceptible cultivars, Cabernet Sauvignon and Cabernet Franc, had first been identified in summer in the year before the treatments were started. A disease scale was used to rate the severity of the foliar symptoms. After treatment, disease development was recorded on the same vines in the following years, from 2002 to 2005. Analyses were based on the evolution of foliar symptoms and on the development of wood symptoms (% area of dead wood). This novel procedure made it possible to determine the sanitary status of each vine in terms of three classes of disease severity: remission of symptoms, stability or worsening. No treatment had a significantly durable effect on disease expression irrespective of the site, the compound or the disease studied. Some phytotoxic effects with the triazole fungicides were noticed. Prospects for trunk injections as a means to solve these insidious problems in viticulture are discussed.

Key words: *Vitis vinifera*, Eutypa dieback, esca, chemotherapy, disease assessment.

Introduction

Eutypa dieback and esca are widespread and major problems affecting grapevine production. These wood diseases lead to a gradual decline in vineyards. Foliar symptoms are associated with the development of endophytic fungi in the grapevine wood resulting in necroses and cankers whose importance varies depending on several factors. Of these, varietal susceptibility is generally considered a key-factor (Carter, 1991; Dubos, 2002). Control methods of these diseases, mainly achieved by protecting pruning wounds and by retraining or replacing infected vines, are still limited in effectiveness. One form of chemical control, sodium arsenite, traditionally used in France to limit esca development was banned in 2001 to protect human health and no acceptable alternative has been found. This situation has however stimulated research, and chemotherapy by trunk injections was proposed as a possible means to control the pathogens infecting grapevine tissues.

Trunk or stem injection of various compounds (fungicides, antibiotics, plant elicitors, insecticides) is a technique which has often been investigated for the control of pests or vascular parasites on many perennial plants (Perry *et al.*, 1991). For instance,

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as far as fungal diseases are concerned, the benzimidazoles used alone or in mixture have frequently been tested to control Dutch elm disease (e.g. Kondo, 1978; Pinon, 1981; Lanier, 1988; Marchetti *et al*., 1990). The development of sterol biosynthesis inhibitors or highly systemic phosphorous compounds have also led to attempts to apply them by intravascular injection in particular against Phytophthora diseases (e.g. Scheffer *et al.,* 1988; Wicks and Hall, 1990; Le Roux, 1991; Holderness, 1992). However, success has generally been variable and at present this method is not, to our knowledge, commercially used in Europe either on forest trees or on fruit crops. On grapevine, injection-based control procedures have been little studied (Di Marco *et al.,* 1993; Calzarano *et al*., 2004). This report describes some recent vineyard experiments to test the use of multivalent chemicals (two fungicides and one elicitor) injected into the trunk of mature vines for the control of grapevine wood diseases.

Materials and methods

Experimental design

Trials were performed from 2001 to 2005 in 5 vineyards located in the Bordeaux area. The main characteristics of the vineyards are given in Table 1, and their location on Fig. 1. Two cultivars, known to be very susceptible to Eutypa dieback and esca in this region (Dubos, 2002), were selected: Cabernet Franc and Cabernet Sauvignon. The training systems were representative of the local appellations. The vineyards were chosen because of their high level of disease expression.

Treatments were applied during the dormancy period before sap flow using a StemJect® (Chemcolour Industries Ltd., Auckland, New Zealand) tree injector adopted from fruit cultivation. This injection system delivered the compounds under high hydraulic pressure in a few minutes. Depending on the size of the vine, 2 to 4 holes, 8 mm in diameter, were drilled centrally into the healthy

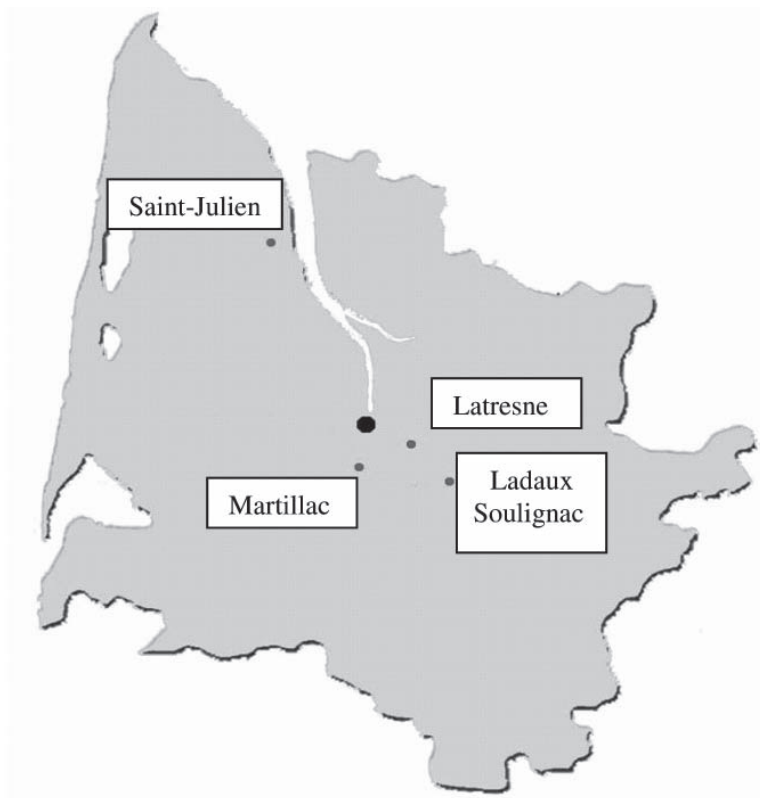

Fig. 1. Location of vineyards of the Bordeaux area in which the injection experiments were carried out

Table 1. Main characteristics of the Bordeaux vineyards in which the experiments were performed.

Vineyard number	Location	Cultivar	Age (in 2001)	Training system	No. of vines per treatment	Main decline observed	Years of observation
1	Cruzeau - Martillac Graves Pessac Léognan	Cabernet Sauvignon	28	Guyot Low "T" form	10	Esca	2001–2002
2	Cruzeau - Martillac Graves Pessac Léognan	"	21	Guyot Low "T" form	20	Eutypa dieback	2001–2004
3	La Mazerolle - Ladaux Entre-Deux-Mers	"	15	Guyot High "T" form	10	"	2001–2003
4	Léoville Las Cases - St Julien Médoc	"	16	Guyot Low "T" form	20	"	2002–2005
5	Experimental vineyard -Latresne 1ères Côtes de Bordeaux	Cabernet Franc	14	Lyra Cordon form	20	"	2002–2005

wood to a depth of about 80% of the diameter of the trunk. A fitting was screwed into the holes and about 50 ml of the formulated solutions was injected. As soon as the solution was delivered, plastic or rubber plugs were inserted to seal the hole and prevent the solution from running out.

Three or four treatments were tested at each test site, including a control with no injection. Individual plots consisted of at least 10 sampled vines. From 1 to 3 repeats were laid out per treatment depending on the experimental site.

Three compounds were tested: two fungicide solutions containing on a triazole-derived ingredient, either propiconazole or difenoconazole (20 g l^{-1}), and one plant-defence elicitor, a potassic salt of 2-hydroxy-benzoïc acid (18.5 g l^{-1}). All these formulations were provided by a private manufacturer and were applied once only.

Disease assessment

Symptomatic vines had been randomly identified in the summer of the year before treatment, either 2001 or 2002 according to the start of the trial. An index derived from disease scales previously developed for the assessment of Eutypa dieback symptoms (Boulay, 1991; Thanassopoulos *et al.*, 1996) was used to rate the incidence and severity of the grapevine wood diseases (Table 2). Disease development on the same vines was subsequently assessed in June (Eutypa dieback) or in September (esca) for 1 to 4 years (up to 2002 or 2005, depending on the trial). Additionally, as suggested by Péros (1995), the percentage of dead wood area was calculated when needed. For each vine, yearly disease severity data were compared with initial observations done before the injections (reference data). Each vine was then ranged in one of three classes, depending on whether: i) the disease had grown more severe (worsening), ii) the disease was stable or iii) the disease had regressed (remission). An example of analysis of field data is shown in Table 3. Differences in the progress of the disease were in the end analyzed using an χ^2 test comparing the results from each treated plot with those of the corresponding untreated plot (P=0.05). When the expected number of vines in one class was statistically insufficient (<5), only two classes of disease evolution were considered. For instance, in our study, when the number of vines in class ii) was low, classes i) and ii) were grouped in one class. An effect was considered positive when the number of vines exhibiting a more severe disease level was significantly lower in the treated plot than in the untreated plot. Because the effect of an injection may be delayed by up to several months following the injection, differences in disease progress were in some cases also analyzed, by comparing disease data obtained during the last year of observation

Table 2. Ranking index used for assessing the occurrence and severity of symptoms[a] associated with grapevine decline.

General description[a]		Description of foliar symptoms[a]	
Code	Meaning	Code	Meaning
E	Eutypa dieback		
Es	Esca		
DA	Dead arm	1	Mild form on 1 arm
U	Unic arm (after pruning)	2	Mild form on 2 arms
S	Low vigor (suffering vine)	3	Severe form on 1 arm
M	Dead vine	3+	Very severe symptom on 1 arm (dying arm, apoplectic form)
A	Absent	4	Severe form on 2 arms
CP	Replanted vine	5	Very severe form on 2 arms; no yield
J	Young vine	1/3	Intermediate symptom between mild or severe (1 arm)
R	Retrained vine	1+3	Mild symptom on one side and severe on the other side

[a] Example of data recorded: E1+E3 = Eutypa dieback, mild form on one arm and severe form on the other one.

Table 3. Example of the analysis carried out with some of the data recorded in trial 2 (Martillac). A. The health status of the vine was determined before treatment; each vine was then ranged in one of 3 classes: remission, worsening of disease, or stable. B. Total No. per final sanitary status was calculated and compared.

3A.

Vine location	Reference year	Observation year		Final sanitary status
	2001	2002	2003	
Line 4 vine 4	E1	Healthy	E1/E2 ?	Stable
Line 6 vine 16	E3	E3+	Dead Arm	Worsening of disease
Line 8 vine 2	E3	E4	DA + E1	Worsening of disease
Line 11 vine 48	E3	E3+	E1	Remission
Line 19 vine 19	E4	E2/E4 ?	DA 50%	Worsening of disease

3B.

Treatment	Total No. of vines treated	Health status (Eutypa dieback)		
		Sanitary status	No.	%
Propiconazole	32	Remission	6	19
		Stable	11	34
		Disease progress	15	47
2-hydroxybenzoïc acid	30	Remission	12	40
		Stable	9	30
		Disease progress	9	30
Control	32	Remission	12	38
		Stable	9	28
		Disease progress	11	34

with data recorded in the year after the injections (2002 or 2003 depending on the trial).

Results and discussion

Annual statistical analyses are summarized in Table 4. They remained generally unchanged over the years and showed that none of the treatments had a significant effect on disease expression irrespective of the experiment, the site, the compound tested or the reference year. Nevertheless there was a slight variability in some trials. In trial 3, plots treated with hydroxybenzoïc acid showed significant differences when compared with the control the following year after the injections (in 2002). This effect was however not confirmed thereafter and at the P=1% risk level in a complementary analysis. Such a result can be attributed to either compound efficacy, or to the year effect. Nevertheless, it suggests that the effect of repeated or long-term application of this compound, by injection or by some other way, should be studied further.

In trial 5, significant differences were also noticed between the size of the disease classes. But this effect was in fact negative because the number of vines exhibiting necrotic wood was higher among the vines treated with the triazoles than it was among the untreated controls. This result was clearly due to a phytotoxicity effect of the triazoles, particularly at the Latresne vineyard, which was established with the lyra training system. The high concentration of these chemicals, and the small diameter of the vine trunk, less than the diameter of vines trained on a low "T" form, may have contributed to this result. A high concentration of difenoconazole was probably also responsible for the unexpected appearance of a common basidiomycete (*Schizophillum* sp.) on the surface of the necrotic wood in vines injected with that fungicide. The basidiomycete appeared 2–3 years after treatment and its development may have been favoured by a selective effect of the fungicide on the microflora. No foliar phytotoxic effect was observed on grapevine, but such an effect was noticed in a similar experiment carried out on kiwifruit (unpublished), which produced a condition known as burnt leaves in the growing season after treatment.

Grapevine wood diseases are often characterized by yearly variations in symptom expression. This phenomenon has been noted with Eutypa dieback (Dubos, 1996; Creaser and Wicks, 2001) and with esca (Mugnai *et al.*, 1999). That being so, an assessment limited to only 1 or 2 years of observation can be criticised on the grounds that this particular yearly variation of symptoms may have caused the results obtained, and not the injections. This limitation was probably circumvented in our study by the duration of the experiments and by the disease assessment method. The observations were repeated annually for 3–4 years in some trials, and results were generally identical over all these years. The qualitative disease scale we used

Table 4. Results of statistical χ^2 tests (α=5%) comparing the disease evolution of treated vines with control vines.

Compound	Trial													
	1	2			3		4				5			
	2002[a]	2003[b]	2004[b]	2004[c]	2002[a]	2003[a]	2002[a]	2003[a]	2004[a]	2005[a]	2002[a]	2003[a]	2004[a]	2005[a]
Propiconazole	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	S^-	S^-
Difenoconazole	NS	n.t.	n.t.	n.t.	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
2-hydroxybenzoïc acid	NS	NS	NS	NS	S^+	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a] Reference year 2001.
[b] Reference year 2002.
[c] Reference year 2002.
n.t., not tested.
NS, Non-significant difference.
S, Significant difference :
S^-, disease expression became more severe in vines that were treated (this result was attributed to a phytotoxic effect).
S^+, disease expression became less severe in vines that were treated (this result could be attributed to a compound effect).

to rate the symptoms was completed by determining the percentage of dead wood area (if any) and in addition the type of disease development was simplified by having only two instead of three categories of decline expression. This procedure proved to be appropriate for a correct assessment of the disease progress and for the statistical analyses, although more accurate field data are obviously needed.

In our study, only one trial (No. 1) dealt with esca development. This was partly due to the difficulty of finding a site in our region that showed sufficient and regular disease expression of esca and in which experiments were possible.

In this trial, the injections did not cure the mature vines tested under the conditions of the experiments. However, the disappointing data recorded were quite similar to those from other experiments that have recently been reported (Loskill *et al.*, 2005; Sentenac *et al.*, 2005) and that were carried out in Europe during the same period to test the same compounds (Di Marco, personal communication). Another reason for the limited attention devoted to esca was that its etiology and the epidemiological factors that influence the development of the foliar symptoms remain unclear (Lecomte *et al.*, 2006; Marchi *et al*., 2006). By contrast, the relationship between the expression of Eutypa dieback and inoculum pressure (Carter, 1991), and the development of Eutypa wood infection is better known.

Because a significant curative or stabilizing effect was expected when the experiments were initiated, the total number of sampled vines per site was restricted and a preference was given to multiple-site experimentation. Nevertheless our sampling sizes were similar to those reported by others (Appel and Kurdyla, 1992; Larignon and Molot, 2004; Sentenac *et al.*, 2005). More than a hundred vines were treated in the Bordeaux area, and no significant improvement was observed after several years of experiment. This failure can be explained in a number of ways. One limitation was that only a single treatment was applied in the experimental design. Furthermore, injections were done in winter before bud break, during the dormancy period, and that no subsequent re-injection was undertaken during the following growing season or thereafter. Such a protocol was probably insufficient for the complete elimination of the pathogens as should have been made clear by Clifford *et al.* (1987). Even though the mode of injection employed was effective in delivering the compounds to a long distance from the injection site, it is probable that the compounds did not attain all the fungal targets inside the wood because there was an uneven distribution by the vascular system (Pinon, 1981). Despite this potential limitation of the test procedure, however, no assays were conducted to determine fungicide movement.

The discouraging results can also be attributed at least in part to the selection of the active ingredients, and this even though triazole-derivatives control a wide range of fungi. Nevertheless, other formulations, such as mixtures with benzimidazoles, should also have been tested for their ability to prevent disease development. The same reservation must also be made about the elicitor used in the study. Even so, however, as regards the choice of elicitor, in 2003–2005, we carried out some similar trials, in which a different elicitor was used, and this elicitor, a proprietary blend of nutrients provided by another manufacturer, was also ineffective (data not shown). Still using the same assessment method, other control experiments were also carried out in the same period with different nutrients including organic compounds supplemented with *Trichoderma* spores. These compounds were tested as alternatives for sodium arsenite, but they also gave unsatisfactory results (Lecomte *et al*., 2006).

In our study, all the sampled vines tested were diseased and showed severe or light foliar symptoms of decline during the growing season before the treatments were applied. Appel and Kurdyla (1992) found that tree injections of propiconazole at the presymptomatic or preventive stage gave a better control of oak wilt than injections of oak trees that already exhibited incipient symptoms. Injection of thiabendazole was also recommended at a very early stage of Dutch elm disease (Anonymous, 1990). There is currently no way to predict in which vines the wood diseases will develop. And injection is a relatively time-consuming and costly operation. Therefore, even if there is a lack of data on the efficiency of injections in asymptomatic vines, the recommendation on a preventive basis to deliver triazoles-derivative compound by injection may not seem to be an attractive option. Nevertheless this technique, if it could be made to

achieve successful results, could be restricted to individual vines in private gardens or high-value vineyards. In other respects, it seems more appropriate to concentrate research on plant stimulant or defence elicitors that can be applied by a more practical and cheaper mode of application (e. g. by spraying).

As suggested by Kondo (1978) for the control of Dutch elm disease, the trunk injection technique cannot be more than a small part of the whole spectrum of control of grapevine wood diseases. Consequently, it is imperative to maintain other practices as sanitation, prophylaxis and pruning wound management. The genetic approach must also be envisaged, in particular for the control of Eutypa dieback, a disease that seems more strongly to depend on inoculum pressure and on varietal susceptibility than esca. Concerning this latter syndrome, more research is needed to improve our knowledge of the agronomical factors that govern its development, and especially to understand the erratic nature of its foliar symptom expression.

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