Phytopathol. Mediterr. (2009) 48, 145–149

Suitability of an increment borer as a sampling device for grapevine trunk disease

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Summary. The sampling of wood from diseased grapevine trunks is usually a destructive process that involves cutting the arms or even total uprooting. As an alternative, an increment borer (Pressler borer) could allow the study of disease evolution over time for individual vines. A borer was evaluated on vines with and without Eutypa dieback, and covariance analyses conducted to determine the correlation between foliar symptoms and the relative incidence of *Eutypa lata* and *Diplodia seriata*. The variation of isolation frequencies was similar within and between vines. *D. seriata* was more frequently isolated than *E. lata*, but the two fungi followed different patterns in relation to foliar symptom intensity. *E. lata* was rare in asymptomatic material and was more frequently isolated as the foliar symptoms increased, stabilizing at the highest symptom intensities. For *D. seriata*, the isolation frequency was highest from asymptomatic and highly symptomatic vines, which could agree with the endophytic character of this fungus. The experimental error was high, probably due to the blind nature of the sampling.

Key words: non-destructive sampling, *Eutypa lata*, *Diplodia seriata*.

Grapevine trunk diseases are often a complex of diseases. In the last 20 years, new syndromes and new etiological agents have been described (Mugnai *et al.*, 1999). In spite of recent progress on rapid diagnostic methods (Retief *et al.*, 2005), fungal isolation remains vital, especially when further pathogenicity tests must be undertaken, for instance to test for Koch's postulates.

Although some vines with trunk diseases show foliar symptoms, the fungi causing those diseases grow within the wood. They can be isolated by removing chips of the wood and placing them on artificial culture media, but this sampling process typically involves cutting the arms or even the total uprooting of the vines, as described by Larignon and Dubos (1997). This destructive process precludes a follow-up of individual plants, as needed, for example, in epidemiological studies. The increment borer (Pressler borer) is a very common sampling device in forest research. It consists of a hollow metal cylinder threaded on the outside, that extracts a wood core when it is driven into the trunk (Grissino-Mayer, 2003). Its main applications are for dendrochronological studies, but it has also been used to search for wood-decaying fungi by Vasiliauskas *et al*. (1996). Its use in grapevine trunk disease studies has however been very limited. Bruno and Sparapano (2006, 2007) used it to assess internal esca symptoms but made no isolations from the wood cores. There are also some preliminary reports from New Zealand involving the use of an increment borer in vine trunk diseases (Manning *et al.*, 2007; Mundy *et al.*, 2009).

In this investigation, an increment borer was ap-

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plied to study the relative incidence of *Eutypa lata* and *Diplodia seriata* in relation to the expression of the foliar symptoms of Eutypa dieback. A double-thread increment borer, 15 cm long and with an inner diameter of 5 mm (Suunto, FIN-01510 Vantaa, Finland), was used to sample 14 plants, with different levels of the foliar symptoms of Eutypa dieback. Average symptom severity of the selected vines was calculated from measurements made over the three previous years following the 0 to 5 scale of Sosnowski *et al*. (2007). The vines were trained to the free-vase shape and were selected from different blocks in a 26 year-old vineyard of Tempranillo in Barriobusto (Rioja Alavesa, Basque Country, Spain) (Muruamendiaraz *et al*., 2007). Samples were obtained from every arm of every selected grapevine (29 arms in total, Fig. 1). They consisted of 5-mm diameter wood cores cut right through the arm. The height of sampling was midway between the arm separation and the base of the spurs. The direction of boring was from the outside of the vine, taken as a whole, towards its centre. The samples were surface-disinfected and each sample provided sufficient material for 4–5 chips that were placed onto four Petri dishes containing potato dextrose agar amended with streptomycin. After growing for five days at 21–23°C, the presence or absence of fungal colonies was recorded. *E. lata* and *D. seriata* were identified on the basis of their mycelium, spore morphology and their growth rate. *D. seriata* yields fast-growing coloured colonies that usually produce characteristic conidia after some 10 days. *E. lata* produces slower growing, completely white colonies that rarely sporulate (Carter, 1991). The data (percentage of dishes with presence of either fungus per sample) were subjected to covariance analysis (Sokal and Rohlf, 1995). The data were square root transformed because this procedure was empirically found to render the data homoscedastic with normally distributed errors. The data were modelled as a function of the symptom intensity (linear and quadratic covariates) for individual vines (random factor). Different arms were considered replicates within the same plant, and their effect relegated to the experimental error.

Eutypa lata and *D. seriata* were frequently isolated, in 23 and 56 dishes out of 116 respectively. Many saprophytic fungi were also isolated (for exam-

Fig. 1. Sampling of grapevine wood by means of an increment borer.

ple *Penicillium* spp., *Alternaria* spp., *Rhizopus* spp. and *Aspergillus* spp.). In spite of the differences in the mean isolation frequency, the experimental error was virtually the same for the two fungi, 0.0806 and 0.0801 respectively (Tables 1, 2). Variation between vines was similar to variation between arms within a vine (F test between vines / between arms P=0.07 for *E. lata* and 0.78 for *D. seriata*), indicating that each arm became independently infected. The isolation frequency of the two fungi however followed different patterns in vines with different disease severity scores (Fig. 2, 3). *E. lata* was rarely isolated from asymptomatic vines, being found in only one Petri dish out of 44 (5 plants × 2–3 arms × 4 dishes) from vines with a symptom score lower or equal to 1. In the 1–3.5 symptom range, the frequency of isolation varied greatly, between 0 and 100%, the fungus being recovered from 20 out of 64 dishes. The only vine with a symptom score greater than 4 provided positive isolation results for both arms, in one out of four dishes each. The model fitted showed a gradual increase from an average isolation frequency of 0.5% for a symptom score of 0, to 16% for the highest symptom score. The low level of significance for the covariates indicated that the goodness of fit was poor (P=0.028 for the linear component and P=0.54 for the quadratic component) and that almost any percent isolation could be expected over the whole range of symptom scores (Fig. 2). In contrast, *D. seriata* was very consistently isolated from asymptomatic plants (38 out of 44 dishes). In the mid-range of foliar symptom intensity, the isolation frequency was variable, but it never exceeded 50%, with the pathogen being recovered from 14 out of 64 dishes. The severely symptomatic vine had a greater frequency of isolation (7 out of 8 dishes). In the polynomial model fitted, a parabola clearly appeared (Fig. 3). Goodness of fit was better for this fungus (P=0.026 for the linear component and P=0.00036 for the quadratic component), with a lower isolation frequency in the-mid range of symptoms, and higher frequencies for vines with very low and very high symptom scores. Both fungi were found together in some of the samples, but statistical analysis detected no correlation between their isolation frequencies (r= -0.25, P=0.19). This indicated that the two fungi caused infection independently of each other.

In contrast to Bruno and Sparapano (2007), who found clear esca-associated symptoms such as brown streaking and soft rot in the wood cores, the different colours of the hard sectorial necrosis associated with dieback were not visible on these cores, nor were cortical cankers apparent in many instances at sampling time. The blind sampling inherent in the boring method could also partly explain the high experimental error. In contrast, destructive sampling specifically targets the dead

Table 1. Analysis of covariance of the proportion of Petri dishes showing *E. lata* growth (square root transform).

Source of variation	Degrees of freedom	Sum of squares	Mean square	F test[a]
Symptom	1	0.4733	0.4733	5.87*
Squared symptom	1	0.0309	0.0309	0.38 ns
Plant	11	2.0203	0.1837	2.28 ns
Error (arm within plant)	15	1.2096	0.0806	

[a] ns, not significant; *, 0.05>P>0.01; **, 0.01> P >0.001; ***, 0.001> P >0.0001.

Table 2. Analysis of covariance of the proportion of Petri dishes showing *D. seriata* growth (square root transform).

Source of variation	Degrees of freedom	Sum of squares	Mean square	F test[a]
Symptom	1	0.4884	0.4884	6.10*
Squared symptom	1	1.6804	1.6804	20.98***
Plant	11	0.5490	0.0499	0.62 ns
Error (arm within plant)	15	1.2017	0.0801	

[a] See Table 1.

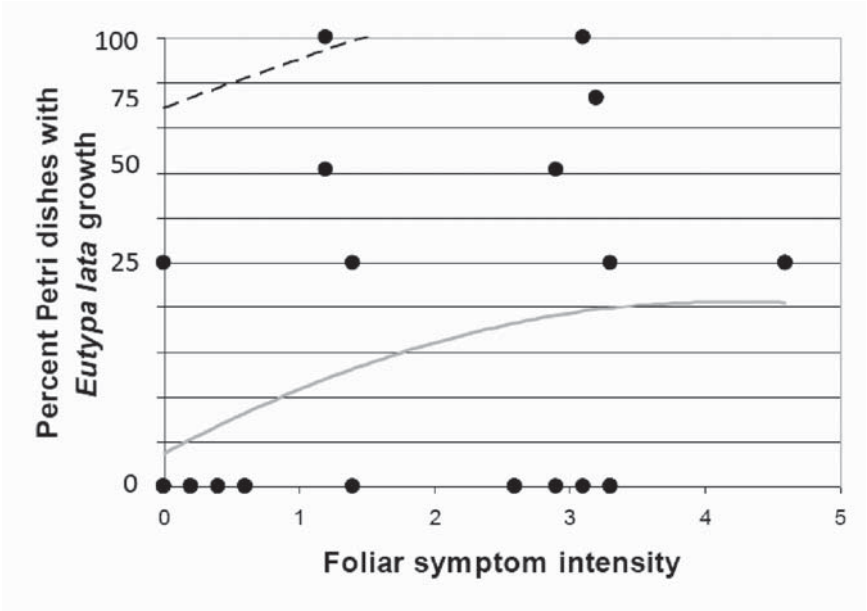

Fig. 2. Percent Petri dishes showing *E. lata* growth *vs.* foliar symptom intensity. Each dot represents at least one arm, and dots in the same vertical line represent the different arms from the same plant. The continuous line is the polynomial fitted to square root data (proportion of Petri dishes) = 0.0722+0.1615 Symptom – 0.0191 Symptom^2. The dotted line represents the 95% upper confidence limit.

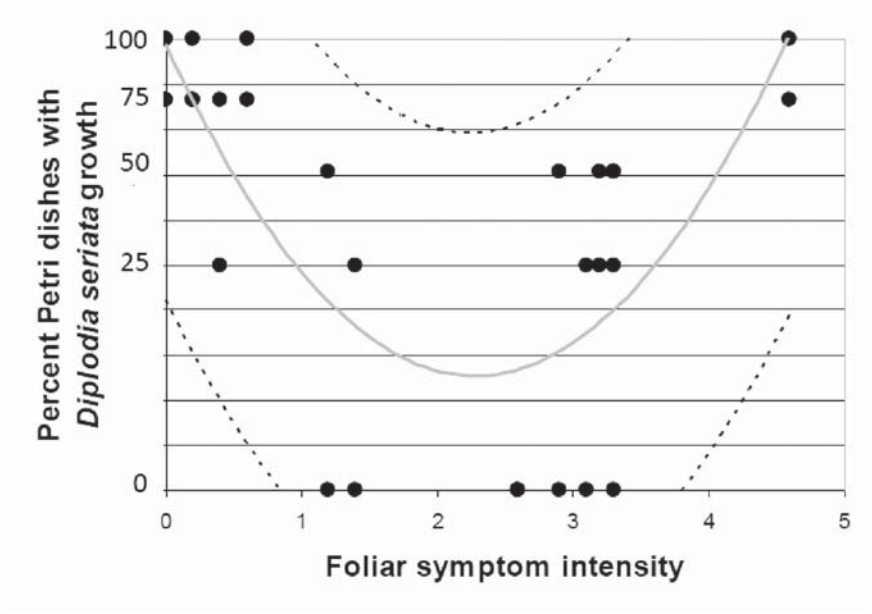

Fig. 3. Percent Petri dishes showing *D. seriata* growth *vs.* foliar symptom intensity. Each dot represents at least one arm, and dots in the same vertical line represent the different arms from the same plant. The continuous line is the polynomial fitted to square root data (proportion of Petri dishes) =0.9877–0.6444 Symptom +0.1411 Symptom^2. The dotted lines represent the 95% upper and lower confidence limits.

wood/live wood interface, where the fungi grow actively.

The sampling experiment indicated what were the relative roles of *E. lata* and *D. seriata* in grapevine dieback. *D. seriata* is commonly isolated from vines showing typical Eutypa dieback foliar symptoms (Úrbez-Torres *et al.*, 2006; Martín and Cobos, 2007), casting doubt on whether *E. lata* is really the causal agent. However, Eutypa foliar symptoms have never been reproduced by artificial inoculation with *D. seriata*. Overall, these results agree with previous reports since *D. seriata* might be better able to infect a vine when the pathogenic process of *E. lata* is almost over and the arm is dying. This is consistent with the primary endophytic character of the family Botryosphaeriaceae (Slippers and Wingfield, 2007). As researchers naturally tend to take samples from plants at the top end of the symptom scale, *D. seriata* is likely to show up strongly in such samples. However, at intermediate levels of symptom expression, which are likely to be in the middle stages of the pathogenic process, *E. lata* is likely to restrict *D. seriata*. In contrast, *E. lata* was only rarely isolated from asymptomatic vines, where *D. seriata* was common. Mundy *et al.* (2007) also reported similar isolation frequencies for Botryosphaeriaceae from both apparently healthy and diseased vines; while *E. lata* was more frequent, though with variations, in diseased vines.

Acknowledgements

Argiñe Muruamendiaraz is a PhD student funded by the Department of Agriculture, Fisheries and Food of the Basque Government. This work was supported by project RTA2007-00023-C04-02 from the Spanish National Institute for Agricultural and Food Research, INIA. The authors thank Manuel Mariño, Ernesto Ortiz and María-Eugenia Sanz for skilled technical assistance and the grape growers of the Alavese Farmer's Union (UAGA) for allowing free access to their vineyards. Stimulating comments from the journal referee are also acknowledged.

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Accepted for publication: March 11, 2009