

# Grapegrowing

## A rapid method of screening grapevine cultivars for susceptibility to eutypa dieback



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### Introduction

Eutypa dieback, caused by the fungus *Eutypa lata*, is one of the most serious diseases of grapevines worldwide. Symptoms include external cankers on infected wood and internal necrosis of woody tissue, visualised as a wedge-shaped area of stained tissue if a cross-section is made of an infected trunk or cordon. Foliar symptoms comprising stunted shoots with chlorotic leaves, often cupped and with tattered margins, are caused by compounds produced by the fungus in the wood and transported to the foliage (Moller and Kasimatis, 1981; Tey-Rulh *et al.* 1991). The disease causes yield decline and eventually kills infected vines. Management of eutypa dieback includes prevention by protection of wounds in the wood and control through remedial surgery (Sosnowski *et al.* 2004a&b).

Little is known about the epidemiology of eutypa dieback of grapevines, as much of the available information is based on research on eutypa dieback in apricots (see Carter 1991). Different cultivars display varying severity of foliar symptoms which may indicate degrees of resistance or tolerance to eutypa dieback. However, little is known about the growth of the fungus in grapevine wood, or of relative virulence of different isolates of *E. lata* towards grapevine. In the vineyard, delay between infection of wounds by ascospores and symptom expression can be between three and eight years (Tey-Rulh *et al.* 1991), hence there is a need for a bioassay which enables the rapid induction of symptoms to provide timely information on host-pathogen interactions. Moller and Kasimatis (1978) inoculated mature vines with mycelium of *E. lata* and observed symptoms one to three years later. Péros and Berger (1994) reported foliar symptom



Fig. 1. Grapevine plant with 5mm diameter hole filled with agar plug (arrow) and covered with Parafilm.

expression within 3-4 weeks of mycelial inoculation of unrooted cuttings in moist rockwool, however, recent attempts to repeat this have been unsuccessful (Creaser, M. and John, S., pers. com. 2002).

As part of CRCV project S2.2.4 "Diagnosis and management of eutypa dieback", we are examining pathogen variation, growth rate and the production of chemical markers by *E. lata* in infected vines. Here we report a bioassay that resulted in foliar symptoms within eight months of inoculation with *E. lata*. This technique will aid in the development of early diagnostic tools and management techniques for eutypa dieback.

### Methods

Rooted grapevine cuttings (cvs. Grenache, Cabernet Sauvignon and Merlot obtained from Kemps Nurseries, Barmera) were planted in plastic bags filled with potting soil in September 2003. Forty isolates of *E. lata* and eight isolates of other fungi commonly isolated from grapevine trunks were collected from wine regions in South Australia (SA) and Victoria and cultured on agar plates. In February 2004 agar plugs (5mm diameter) containing mycelium were inserted into 5mm diameter holes drilled in the main stem of the rootlings and sealed by wrapping with Parafilm (Figure 1). Two separate experiments were established using 768 grapevine plants. In the first experiment, Grenache vines were treated with 40 isolates of *E. lata* and eight isolates of other grapevine wood-inhabiting fungi. In the second experiment, all three cultivars were treated with 12 isolates of *E. lata* and eight non-*E. lata* isolates. Uninoculated control vines, either treated with a sterile agar



Fig. 2. Eutypa dieback foliar symptom scale developed by T. Wicks (SARDI).

plug or left untreated, were included in both experiments. There were eight replicates per treatment, including the controls. Following inoculation, vines were watered daily for seven days to ensure that the inoculation site remained moist. In March 2004, vines were pruned to two buds. Foliar symptoms were assessed in October 2004, when shoots were 50-70cm long, using a scale of 0 (no symptoms) to 4 (severe symptoms) (see Figure 2).

#### Results

Foliar symptoms typical of eutypa dieback were evident on some treated vines eight months after inoculation (see Figures 3A&B). In experiment one, 26 of the 40 *E. lata* isolates induced symptoms on at least one of the eight replicate Grenache vines. Six isolates, from Eden Valley, Clare, Loxton, Barossa Valley in SA and Strathbogie Ranges in Victoria, induced foliar symptoms on more than 50% of the inoculated vines. The mean foliar symptom rating on Grenache varied from 0 to 1.25 for all isolates. In experiment two, foliar symptoms were induced by two *E. lata* isolates in all three cultivars, by four isolates in Cabernet and five isolates in Grenache. Statistical analysis revealed that the mean foliar rating for Grenache was significantly greater than that for Cabernet Sauvignon or Merlot. Symptoms did not develop in vines inoculated with other wood fungi or in uninoculated controls in either



Fig 3. Left and below: Eutypa dieback symptoms on Grenache with characteristic chlorotic, cupped leaves with tattered margins.



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Fig. 4. Uninoculated control Grenache vine (left) compared with the symptomatic shoots of vines inoculated with *E. lata* isolates which originated from Loxton (A) and Clare (B) in South Australia.

experiment (see Figure 4). Foliar symptoms were observed on shoots both above and below the site of inoculation on three vines (two Grenache and one Cabernet Sauvignon, Figure 5).

### Conclusions

This study shows that foliar symptoms of eutypa dieback can be induced on rooted vines within eight months of inoculation. The initial results confirm reports by Péros and Berger (1994) suggesting that isolates of *E. lata* vary in their ability to induce foliar symptoms of eutypa dieback on grapevines. The preliminary observation that Grenache was more susceptible than the other cultivars tested, also confirms previous research findings (Carter, 1991; Highet and Wicks, 1998). There was no apparent correlation between geographic origin and virulence of isolates. The appearance of symptoms on shoots arising from the stem below the inoculation site suggested that toxic compounds may be translocated in both directions in the wood, or that mycelium has colonised the wood below the inoculation point much more rapidly than expected. This observation may be an artefact of the experimental conditions, however, if this is not the case, there may be implications for disease management.

Some of the fungal isolates used in this study have previously been characterised for toxin production in artificial culture (Mahoney *et al.* 2003). The ability to induce foliar symptoms using these isolates will aid in developing an early diagnostic test for eutypa dieback based upon the detection of chemical markers in the foliage of infected vines. Extracts from leaves of inoculated plants will be analysed using HPLC and results compared with

those obtained by Mahoney *et al.* (2003) in order to identify chemical markers which are indicative of infection by *E. lata*.

Following the second foliar assessment of these experiments in spring 2005, wood will be destructively sampled to determine the extent to which mycelium has colonised the stems and whether variation exists between cultivars and fungal isolates. This will be assessed using DNA markers developed as part of CRCV project 2.2.1 (Lardner *et al.* 2004).

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Fig. 5. Grenache vine inoculated with an *E. lata* isolate from Coonawarra, South Australia, displaying foliar symptoms on shoots extending from both above and below the inoculation site (arrow).



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