THE POTENTIAL OF RAMAN SPECTROSCOPY FOR EARLY DETECTION OF EUTYPA DIEBACK IN GRAPEVINES

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INTRODUCTION

Eutypa lata (Pers.:Fr.) Tul et C. Tul. is the causal agent of Eutypa dieback in grapevines. Eutypa dieback is a chronic disease that significantly reduces grapevine yield. *E. lata* infects through pruning wounds and grows slowly in the vascular tissue not producing symptoms for several years (1). Stunted shoots with chlorotic leaves that may indicate Eutypa infection are most easily seen in early spring. (Figure 1). These foliar symptoms are a result of a phytotoxin, eutypine, produced by the fungus that inhabits the xylem (1) (Figure 2). Eutypine is a well-characterised phytotoxin that could be used as the basis of a rapid diagnosis system. Changes in tissue biochemistry must precede any symptomatic manifestation associated with the disease so spectroscopic diagnosis is a logical alternative for the early detection of disease. Raman spectroscopy has been widely used to study changes in biological systems. Raman spectroscopy that directly monitors molecular structure and changes in cellular chemistry has been shown by numerous studies to have potential in the diagnosis of cancer. The technique is rapid, amenable to onsite monitoring non-destructively and requires only small amounts of sample.

Figure 1. Eutypa affected vine.

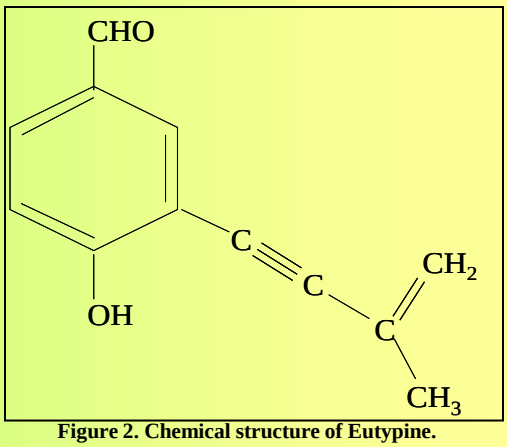

Figure 2. Chemical structure of Eutypine.

The aim of this study is to determine the potential of Raman spectroscopy as a rapid diagnostic technique to detect Eutypa dieback in grapevine.

MATERIALS AND METHODS

Sample collection Shoots with characteristic foliar symptoms of Eutypa dieback were collected from the Mt Mary in the Yarra Valley region of Victoria and Dookie in February 2001.

Raman spectroscopy of pure eutypine Raman spectra were recorded on a "Renishaw model 2000 Ramanscope", a dispersive spectrograph, with microscope sampling and a 780nm. laser. The spectrum was obtained easily in a single 10 second scan.

Raman spectroscopy of tissue samples The NIR laser used to record the spectrum of eutypine was used to analyse the following tissues: leaf surface; bevelled cut edge of a leaf; LS of the petiole; longitudinal and transverse sections of the stem; and sap.

RESULTS

The Raman profile of pure eutypine has a very strong peak at 2204cm^{-1}, which is assigned to the eutypine triple bond (Figure 3). This an ideal peak to monitor eutypine because of its intensity and the fact that very few functional groups give Raman signals in this spectrally clear region.

We were unable to detect the eutypine profile in any of the tissue samples analysed with Raman.

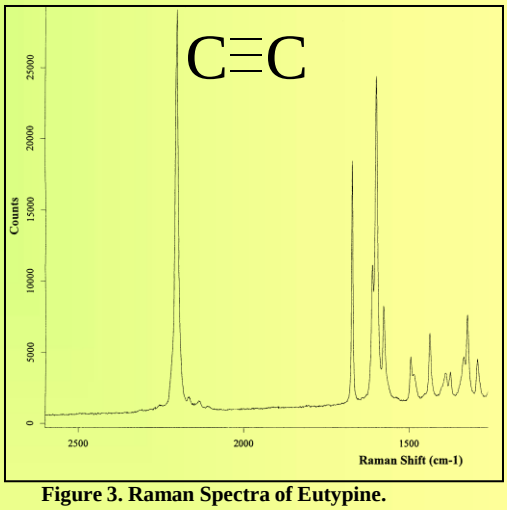

Figure 3. Raman Spectra of Eutypine.

DISCUSSION

There are numerous possible reasons why we were unable to detect eutypine in the tissue samples analysed. Firstly, there may be no eutypine present in the shoots in autumn, perhaps it is only present in the tissue in the early growth stages. A French study has reported that eutypine can be converted to a non-toxic compound, eutypinol, by *V. vinifera* cell suspension cultures (2). The conversion of eutypine to eutypinol occurs in cells from both tolerant and susceptible cultivars. However, the rate of conversion is lower in the highly susceptible cultivar than the tolerant cultivar. As a result, eutypine may be present in the tissues but in such low levels that it cannot be detected by Raman spectroscopy. Future research will focus on young shoots taken in spring when eutypine may be present in tissues at higher concentrations.

Secondly, as Raman measurement is essentially limited to surface analysis of opaque materials, it is necessary to ensure the analyte is present in the laser illuminated surface layer. Sample fluorescence under laser illumination can also obscure the often-weaker Raman signals. Longer wavelength lasers usually reduce this effect.

ACKNOWLEDGEMENTS

This project was supported by the Commonwealth Cooperative Research Centres Program and conducted by the CRC for Viticulture with GWRDC support. We would like to thank: Prof. R. Tabacchi, University of Neuchatel, Switzerland for providing a pure sample of eutypine and the growers who provided eutypa affected vine material.

REFERENCES

1.Tey-Rulh, P., et al., (1991). Eutypine, a phytotoxin produced by *Eutypa lata* the causal agent of dying-arm disease of grapevine. *Phytochemistry* 30:471-473.

2.Colrat, S., et al., (1999). Enzymatic detoxification of eutypine, a toxin from *Eutypa lata*, by *Vitis vinifera* cells: partial purification of an NADPH-dependent aldehyde reductase. *Planta* 207:544-550.