THE DEVELOPMENT OF DIAGNOSTIC TOOLS FOR THE GRAPEVINE PATHOGEN *EUTYPA LATA*.

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ABSTRACT.

Eutypa lata is the causal agent of Eutypa dieback on grapevines. The fungus invades the vine and grows there unnoticed, possibly for several years, causing discolouration and deformation of the vine shoots and leaves. Most berries fail to establish on these shoots and the fungus eventually kills the vine. The damaging effects of this fungus have had a notable financial impact on the grape and wine industry world wide and *E. lata* is at present the primary constraint on vineyard longevity in many places including California and Australia. Little is known about the occurrence and distribution of Eutypa dieback within New Zealand. This is due mainly to difficulties associated with identification of the disease in grapevines.

To develop a molecular probe for the identification of *E. lata* from grapevine wood the Polymerase Chain Reaction (PCR) amplified the Internal Transcribed Spacers (ITS1 and ITS2) and the intervening 5.8S gene of ribosomal DNA (rDNA) from representative isolates. The sequences of the *E. lata* ITS regions were used to design two pairs of primers, each of which was subsequently shown to be specific for the amplification of predicted-size fragments from genomic DNA of *E. lata*. The primer pairs were further tested using template DNA extracted from healthy grapevines and from other fungi commonly isolated from dieback diseased grapevines but no PCR amplification was observed. Simple DNA extraction protocols, leading to the rapid release of DNA, were tested to enable identification of *E. lata* from pure culture and grapevine wood; however, a suitable DNA extraction method from these materials was not found.

Currently the only known source of inoculum is ascospores, which are released from perithecia during and immediately after rainfall. However, few perithecia have been found in New Zealand vineyards. This has prompted the study of the mating habits of *E. lata*. As the sexual stage of *E. lata* cannot be obtained in culture at present, the analysis of its mating system must be performed in natural populations. Molecular characterisation of the mating type at the outset of a mating project allows significant savings in time and effort as it drastically reduces the number of crosses that must be set up. So far, cloning of mating type (*MAT*) genes from fungi has been hampered by low conservation among them. Most ascomycete fungi have one mating type gene with two alternative forms or idiomorphs (*MAT1-1* and *MAT1-2*). One of the pair of *MAT* genes, *MAT1-2*, encodes a protein with a conserved DNA binding motif called the high mobility group (HMG) box. There is sufficient sequence conservation at the borders of the HMG box to allow PCR amplification. New Zealand isolates of *E. lata*, including sixteen single ascospore isolates from one perithecium, were tested for the presence of a *MAT1-2* idiomorph using this PCR based approach. Five different sets of primers were used which were designed to anneal at different target sites with different specificities. PCR products of the expected size were obtained and sequenced, but despite exhaustive attempts to optimise PCR specificity, none of these had convincing homology to fungal mating type genes.

Progress on the basic aspects of the genetics of *E. lata* will continue to be hampered until the organism is induced to complete its life cycle in culture. Molecular studies into the mating type genes which regulate sexual compatibility and sexual reproduction in

the fungus should lead to a deeper understanding of the life-cycle of *E. lata* and the critical influence of sex on population genetics. In addition, it will provide a scientific basis for a management program urgently needed to minimise the impact of this disease.

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