INTRODUCTION
In recent years, vineyards throughout New Zealand have been affected by a range of grapevine trunk diseases. The Botryosphaeria species found in symptomatic vines were mostly associated with dieback of canes and trunks, stunted growth, delayed bud burst and necrosis of green tissues, all leading to a general loss of vine vigour. Elsewhere in the world, these fungi have been isolated from areas of wood-rot, dieback and cankers in trunks of grapevines, although severe dieback symptoms generally became visible only when vines were 8 or more years old, or had been subjected to stress (1).

The incidence of Botryosphaeria dieback is likely to increase with national vineyard expansion and as they become more susceptible with age. The aim of this study was to establish the prevalence and identity of the Botryosphaeria species associated with dieback in grapevines and common woody hosts that surround vineyards in New Zealand, to determine the nature of the potential problem.

MATERIALS AND METHODS
In November 2006, 20 vineyards in the grape-growing regions of Canterbury, Marlborough, Nelson, Gisborne and Auckland were sampled for tissues with symptoms characteristic of Botryosphaeria infection. In addition, twigs and stems of other surrounding woody plants that showed symptoms of dieback were also collected.

Sections of symptomatic tissues as well as plant debris were surface-sterilised with 70% alcohol for 30 s, 1% NaOCl for 1 min and then 70% alcohol for 30 s (2), rinsed in sterilised water and air-dried within a laminar flow cabinet. The healthy tissues on the margin of necrotic areas were excised for isolation and placed cut surface down onto half-strength potato dextrose agar (PDA) amended with 0.1 μg/ml tetracycline.

After 3 days incubation at room temperature, all fast-growing fungal colonies characteristic of Botryosphaeria spp. were subcultured onto half strength PDA and incubated at 23°C in continuous darkness. Colour and morphology of colonies were observed after 3-4 days and then at 2 and 4 weeks. After 4-5 weeks, conidia were extracted from the pycnidia that occurred for some isolates and the isolates identified.

RESULTS
A total of 43 Botryosphaeria-type isolates were obtained from grapevines and 10 from non-grapevine woody hosts. Of the seven different colony types that grew on half strength PDA, five types were identified as being B. lutea (Fusicoecum luteum), B. parva (Fusicoecum parva), B. stevensii (Diplodia mutila), D. samentorum and B. obtusa (Diplodia seriae) according to published descriptions (3, 4). Later sequence analysis of some isolates confirmed the identity of B. stevensii but those initially identified as B. lutea (they caused the PDA to become yellow) as being either B. lutea or B. australis. The identities of other isolates will also be confirmed with molecular methods. Most of the Botryosphaeria species isolated were associated with woody material, although a small percentage of symptomatic leaves, flowers, weak buds and green shoots also contained these species (Figure 1).

DISTRIBUTION, IDENTIFICATION AND INOCULUM SOURCES OF BOTRYOSPHAERIA SPECIES FOUND IN NEW ZEALAND VINEYARDS
N.T. Ampornsah, E.E. Jones, H.J. Ridgway and M.V. Jaspers
Bio-Protection & Ecology Division, Box 84 Lincoln University, Christchurch, New Zealand

DISCUSSION
This is the first report of Botryosphaeria spp. isolated from grapevines in New Zealand. Other reports on Botryosphaeria isolates were from hosts such as Actinidia deliciosa, Populus nigra, Malus sp. and Pinus nigra. In addition to these reported hosts, this survey identified other host plants from which Botryosphaeria spp. were isolated. B. lutea and D. mutila were recovered on grapevines from all the five regions whereas B. parva and B. obtusa were found only in the Nelson and Marlborough regions. The age range of all the grapevines from which the fungus was isolated was 2-33 years, with the greater frequency of recovery from older plants. Preliminary pathogenicity studies conducted showed that all the species isolated from the non-grapevine hosts were able to infect grapevine shoots, indicating their potential as inoculum sources for vineyards.

ACKNOWLEDGEMENTS
We gratefully acknowledge funding from the New Zealand Winegrowers and the support of grapegrowers who allowed sample collection from their vineyards.

REFERENCES
4. CMI Description of Pathogenic Fungi & Bacteria.