MATERIALS AND METHODS

Spore counts. A slit-type volumetric spore trap was established at a commercial Boronia plantation in Mt Barker, Western Australia from Jan-Sep 2004. A length of Melinex tape was coated with TangleFoot adhesive and placed in the drum set to rotate once every 7 days. Tapes were sectioned in 24 h strips, stained and total basidiospores counted in 1 cm² of Melinex tape was coated with TangleFoot adhesive and placed in the drum set to rotate once every 7 days. Tapes were sectioned in 24 h strips, stained and total basidiospores counted in 1 cm² of TangleFoot adhesive. Basidiospores were observed on forty-four. Thirty-one were captured from half the tape using a modified method of Calderon et al. (1). The ITS region of P. boroniae was amplified using a nested PCR protocol, with species-specific primers designed for the experiment. Nested PCR products were digested with DraI and HaellI or TaqI, and analysed by gel electrophoresis.

RESULTS

Spore counts. Basidiospores morphologically resembling those of P. boroniae were captured from Feb-Aug 2004, peaking in number and daily occurrence in Apr 2004 (autumn). Daily basidiospore numbers were significantly (p < 0.05) correlated with minimum temperature and total daily rainfall.

A diurnal periodicity of basidiospore release was observed, peaking on average between 02:00 and 05:00 hrs (Fig 1). Hourly basidiospore numbers were significantly (p < 0.01) correlated with relative humidity, air temperature, solar radiation and evaporation.

PCR-RFLP analysis. Samples from 13th Feb-8th Apr were further sectioned horizontally, and DNA extracted from half the tape using a modified method of Calderon et al. (1). The ITS region of P. boroniae was amplified using a nested PCR protocol, with species-specific primers designed for the experiment. Nested PCR products were digested with DraI and HaellI or TaqI, and analysed by gel electrophoresis.

DISCUSSION

The data from this study showed that in the mild climate of the southwest of Western Australia, formation and dispersal of basidiospores of P. boroniae was possible over a large portion of the year. Similar to many rust fungi, basidiospores were released periodically at night. Further investigations into disease incidence and severity, and their relationship with weather conditions are still needed.

The data showed that P. boroniae could be identified from air samples containing high numbers (> 100 spores per field of view) of different fungal spores using the nested PCR-RFLP protocol. Further optimisation of the primer specificity together with Q-PCR would provide a valuable quantitative alternative to the traditional method of spore counting; a time consuming and often highly subjective method.

ACKNOWLEDGEMENTS
Western Australian Department of Agriculture (industry partner for ARC LINKAGE APAI grant C00107300)

REFERENCES