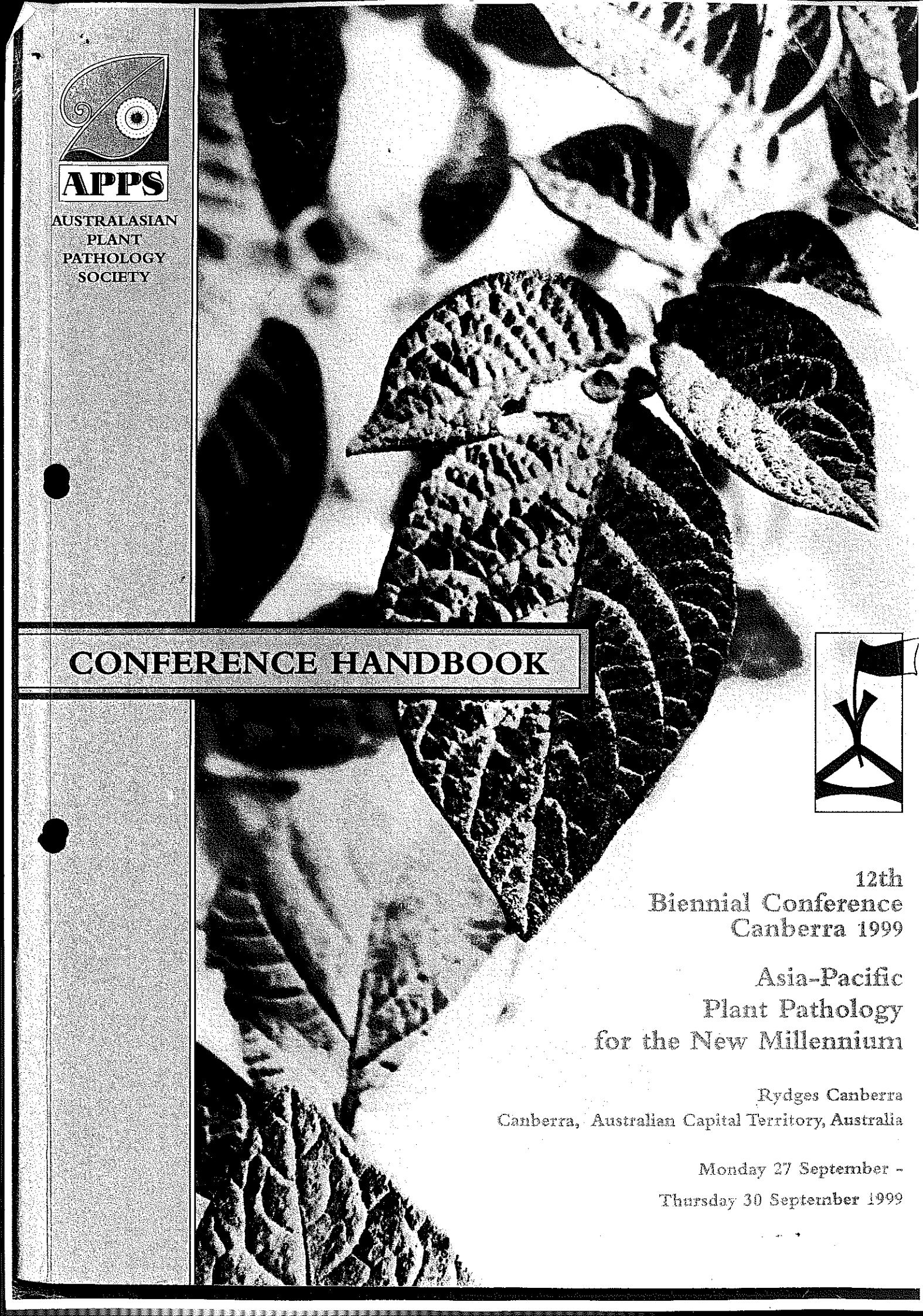

APPS

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# PSEUDOHOMOTHALLISM IN *ARMILLARIA LUTEOBUBALINA* ISOLATES FROM SOUTH-WESTERN AUSTRALIA

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## INTRODUCTION

The majority of *Armillaria* species have a bifactorial heterothallic mating system. Some primary homothallic and secondary homothallic behaviour has been observed in a small number of different *A. mellea* and *A. heimii* strains from Africa and Asia. In a typical bifactorial heterothallic *Armillaria* species four haploid nuclei are produced in the basidia during meiosis and the subsequent migration of each nucleus produces haploid basidiospores.

Although it is known that *A. luteobubalina* is a bifactorial heterothallic species the nuclear life cycle is not thoroughly described (1). This present study aimed to investigate the cytology and nuclear arrangement within the different basidiome tissues and basidiospores in *A. luteobubalina*.

## MATERIALS AND METHODS

**Field sampling** During June/July 1998 66 basidiomes of *A. luteobubalina* were collected from two sites in the South-Western Australia. All the collected basidiomes were utilised in two ways, firstly gill tissue sections were fixed in a solution of ethanol, lactic acid and acetic acid (3:2:2) for 24 hrs before being preserved in 70% ethanol. Secondly, the caps of the collected basidiomes were placed gill-side down on brown paper for 24 hrs to obtain a spore print.

**Staining techniques** The preserved gill sections were stained with 1% phloxine or 0.9% natural orecein in lactic-propionic acid. The stained sections were transferred to a humidifier box for 24 hrs before counting nuclei in mature spores, young spores, basidia hymenial and tramal tissue at 400 X magnification. Basidiospores from the spore prints and other gill sections were stained with 4', 6-diamindino-phenylindol (DAPI) without Triton X (2), mounted in 45% propionic acid. Nuclear arrangement within the stained material was observed using ultraviolet fluorescence (365nm) @ 800 X. In all cases the relative basidium stage of development was estimated as in (4) from measurements of the shape and size of the basidium. The stage of development of the sterigmata as basidial development was asynchronous in any field of view.

## RESULTS

The tramal and hymenial tissues were predominantly uninucleate. In contrast, the subhymenium were often binucleate. Clamps were not observed in either the subhymenium, the hymenium nor the tramal tissue. Four different putative sequential stages (shown within the parentheses) within the basidia were observed: binucleate (prekaryogamy), uninucleate (diploid) and nuclear patterns consistent with two different stages of meiosis (Metaphase I and Telophase I). There was no evidence of any extra mitotic divisions in basidia. Basidia have a variable number of sterigmata (between 2-4). Basidiospores were 96.5% uninucleate, 2.65% binucleate and 0.78% enucleate.

## DISCUSSION

We hypothesize that *A. luteobubalina* is amphithallic ie has heterothallic and homothallic behaviour. Due to the absence of a post-meiotic mitosis within the basidia, it appears that the homothallic behaviour arises from pseudohomothallism (explanatory phrase) rather than secondary homothallism. Variation within the number of sterigmata per basidiome is the likely cause two nuclei migrating into the mature basidiospores. The variation in the number of sterigmata has been previously reported in other *A. luteobubalina* isolates (3).

The observations support the hypothesis that a vegetative segregation of a vegetative diploid nucleus occurs within the subhymenium resulting in the binucleate state. This is similar to the behaviour of *A. mellea* (4). A binucleate state in the subhymenium or young basidium would be a prerequisite for karyogamy and meiosis. This behaviour may be usual in many *Armillaria* species, due to the predominance of the vegetative diploid stage for most of the life history of the pathogen. The vegetative diploid stage would arise from the mating of two monokaryons, persist throughout the vegetative phase during tree and shrub colonisation and for most of the basidiome development.

The two nuclei within the subhymenium subsequently migrate into the basidia, fuse and undergo meiosis to produce four putative haploid nuclei. Nuclei may be parental or recombinant for mating-types. The mating behaviour of some *A. luteobubalina* single spore isolates provided evidence for two nuclei mating-type compatible nuclei having migrated into a basidiospore. Although the frequency of this was 5.65% given the vast numbers of spores produced by basidiome at a population this may have an important influence on population structure and disease development.

Accurate measurements of DNA content will be required to confirm the hypotheses constructed during this investigation. Previous investigations have indicated that the nuclear patterns within the basidiome can be variable between different species and strains. The occurrence of homothallism and heterothallism in *A. luteobubalina* allows for the balance of inbreeding and outbreeding and thus is an evolutionary advantage.

## ACKNOWLEDGEMENTS

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## REFERENCES

1. Kile, G.A. (1983). Identification of genotypes and clonal development of *Armillaria luteobubalina* Watling & Kile in eucalypt forests. *Australian Journal of Botany* **31**: 657-671.
2. Vergne, P., Delavallee, I., and Dumas, C. (1987). Rapid assessment of microspore and pollen development stage in wheat and maize using DAPI and membrane permeabilization. *Stain Technology* **62**:5 299-304.
3. Bougher, N.L., and Syme, K. (1998). *The Fungi of Southern Australia.* UWA Press, Western Australia
4. Tommerup, I.C., and Broadbent, D (1975). Nuclear fusion, meiosis and the origin of dikaryotic hyphae in *Armillaria mellea. Archives of Microbiology* **103**: 279-272.