Confocal and light microscopy of infection by, and resistance to, *Ciborinia camelliae* in *Camellia* species and the potential for biocontrol

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To my wife, Malliha and children, Neelujah and Keerthanan:
With my deepest gratitude and love, forever.

Abstract

In this thesis, factors affecting *C. camelliae* ascospore germination, germ tube penetration, infection and subsequent disease development and resistance in C*amellia* species were studied in detached camellia petals by microscopy. Potential biocontrol agents were also isolated and evaluated against *C. camelliae* in the laboratory and in the field.

Trypan blue in lactophenol was used to stain for fungal structure in bright field microscopic investigations of factors affecting ascospore germination, germ tube penetration and infection of *C. camelliae*. Relative humidity affected ascospore germination and germ tube penetration, but temperature had little or no effect although low temperature restricted the growth of penetration hyphae in the tissue and disease development. Absence of free water affected ascospore germination and germ tube penetration when ascospores were inoculated as suspensions using a pipette or atomizer. Ascospores released from apothecia and allowed to settle on petals by gravity germinated and the germ tube penetrated petal tissue in both the absence and presence of free water. A possible reason for high spore germination and germ tube penetration in the absence of free water could be the association of liquid drops adhering to freshly released ascospores that could have acted as a reservoir of moisture. Multiple germ tubes and swollen hyphae were common and many germ tubes grew across the petal surface without penetration in brush inoculated (ascospores directly transferred from apothecia using a paint brush) petals under free water conditions. Production of antifungal materials over the petal surface due to petal damage by brush bristles during inoculation may be the reason for the malfunction of some germ tubes.

A glutaraldehyde technique was developed to increase image contrast by reduction of background fluorescence from plant tissue in confocal microcopy. Ascospores were found to germinate and produce a short germ tube that directly penetrated the petal cuticle within 6 h. During next 12 h, the penetrated germ tube transformed into a subcuticular swelling underneath the cuticle. From this, a narrow tube enlarged into a

subcuticular hypha that grew underneath the cuticle to the junction between two cells. The mycelium continued to develop intercellularly for the next 60 hours but without causing extensive destruction of the cell walls. The fungus appears to follow the infection process strategy of subcuticular intramural pathogen such as *Colletotrichum capsici*.

Resistance mechanisms of *Camellia* species against *C. camelliae* were investigated by light and confocal microscopy. *C. cuspidata* expresses resistance to *C. camelliae* by formation of papillae and a hypersensitive reaction. *C. lutchuensis* and *C. transnokoensis* express their resistance by papillae and production of antifungal metabolites such as PR proteins respectively. Excess microorganisms were observed in *C. polyodonta* with distorted ascospores and in *C. tricocarpa* with abnormal swollen hyphae which suggests biocontrol activities of the organisms.

A total of 13 bacterial isolates and 6 yeast biocontrol agent (BCA) candidates were obtained from an attachment assay. Bacterial isolates, 07L1B and 04S2B, gave maximum disease control when tested *in vivo* on intact petals and in the field. Direct contact of these bacterial isolates with ascospores totally inhibited germination. Biocontrol was not effective if germ tube penetration occurred before BCA arrival. Biocontrol efficacy was observed for at least 72 h both in the laboratory and in the field condition although the bacterial populations declined. Antibiotic production was observed from these two isolates and was the prime mode of action against *C. camelliae*.

The main goal of this thesis was achieved by understanding some basic biology of *C. camelliae* and these principles were used in the biocontrol strategy and study of resistance mechanism of *Camellia* species.

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