Phytophthora root rot of chestnut

by S. M. Chambers

Thesis submitted for the degree of

Doctor of Philosophy

in the

Department of Crop Protection

The University of Adelaide

December 1993

TABLE OF CONTENTS

ABSTRACT

Aspects of *Phytophthora* root rot of chestnut were investigated in this study. The modified excised shoot assay (using micropropagated material) was used, for the first time, to screen micropropagated chestnut cvs, used as rootstocks, for resistance to *Phytophthora* spp. *in vitro*. The cvs "Buffalo Queen" and "Goldsworthy" were susceptible to infection by *P. cinnamomi*, *P. citricola*, *P. cryptogea* and *P. cambivora*. Plantlets of the cvs "Buffalo Queen" and "Goldsworthy" growing *ex vitro* in vermiculite, which were inoculated with zoospores, were also susceptible to these *Phytophthora* spp.. The assay allowed comparison of pathogenicity of *Phytophthora* species on chestnut and indicated that genotypes of "Buffalo Queen" may vary in susceptibility to *Phytophthora* root rot.

Whole plant inoculation methods were also used to screen seedlings of a number of chestnut cvs for resistance to *P. cinnamomi*, *P. citricola* and *P. cryptogea*. Plants were inoculated either by dipping roots in a zoospore suspension or by placing mycelium in a wound made on the tap root. Seedlings of all cvs were susceptible to *P. cinnamomi*, *P. citricola* and *P. cryptogea* when inoculated using either of these methods. This is the first report of *P. citricola* causing root and crown rot of chestnut. Disease symptoms observed on infected plants included wilting, leaf chlorosis, lesion formation on stems and root rot.

Three fungi, isolated from soil from which *Phytophthora* was not obtained, were evaluated for potential biocontrol of *Phytophthora* root rot of chestnut. *Trichoderma hamatum* and *T. pseudokoningii* inhibited *P. cinnamomi* by mycoparasitism, with evidence of parallel growth and coiling, and all three antagonists grew over *P. cinnamomi in vitro*, preventing further growth of this pathogen. Antibiotics produced by young *T. hamatum* cultures and *Gliocladium virens* in cellophane overlay and culture filtrate experiments inhibited growth of *P. cinnamomi* and *P. citricola*, with filtrate from 4 d-old cultures of *G. virens* showing the greatest potential for biocontrol. Volatile antibiotics produced by *T. pseudokoningii* significantly inhibited growth of *P. cinnamomi* and *P. citricola*. All three antagonists

prevented *P. cinnamomi* and *P. citricola* from infecting micropropagated shoots of chestnut cvs "Goldsworthy" and "Buffalo Queen" in a biocontrol excised shoot bioassay *in vitro*. In pot experiments, plant root and shoot weights were generally higher in the presence of the antagonists than in plants inoculated with the antagonists and *P. cinnamomi*.

Infection of micropropagated plantlets of a susceptible cv. of chestnut "Goldsworthy" resulting from zoospore inoculum of *P. cinnamomi* was also followed. Hyphae grew quickly over the roots of plantlets, killing them within 4 d of inoculation. Chlamydospore and sporangium production occurred from 1-2 d after inoculation. Peripheral vesicles, intracellular organelles previously identified in *P. cinnamomi* zoospores, were labelled using monoclonal antibodies, for the first time, in hyphae infecting these chestnut roots from 1 d after inoculation, corresponding with the induction of asexual sporogenesis. Such vesicles have been found previously in nutrient-stressed hyphae *in vitro*.

Oospores of *P. cinnamomi* produced by A1 x A2 mating type crosses, as well as by A1 and A2 isolates alone, were induced in micropropagated chestnut roots for the first time. Large peripheral vesicles were labelled, using monoclonal antibodies, in these oospores as well as in oospores produced axenically in carrot agar culture.