EFFECTS OF ROOT-INVADING FUNGI ON THE GROWTH OF RED CLOVER (*Trifolium pratense* L.)

A thesis presented in partial fulfilment
of the requirements for the degree
of
Doctor of Philosophy
in Plant Pathology
at Massey University

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1989

ABSTRACT

Methods to monitor invasion of red clover roots by soilborne fungi, and assess effects on clover growth and persistence, were developed and tested using soil from a plant breeder's red clover evaluation block at DSIR Grasslands Division, Palmerston North, which was known to contain several fungal species pathogenic to red clover. A quantitative method employing tissue maceration and plating was used to determine the internal microflora of red clover roots from the evaluation block. Effects of environmental factors, and of application of fungicide drenches to the soil, on root invasion under controlled environment and field conditions were also studied using the root maceration method. Fungi isolated from roots of red clover from the evaluation block were tested for their effects on establishment, growth, and persistence of red clover in the glasshouse and in field microplots. Light microscopy and transmission electron microscopy were used to study invasion of red clover roots by Trichocladium basicola, and the effects of the fungicides benomyl and prochloraz on this fungus in vitro and in vivo.

The root maceration method detected a similar range of fungi to that found by plating 1 - 2 mm long segments, but yielded more colonies and showed less variation. Using standardised amounts of tissue and blending times (2 g and 60 or 120 sec.) differences in fungal populations in roots subjected to different treatments were readily detected.

Verticillium dahliae, Trichocladium basicola, and Cylindrocladium scoparium were the major components of the root-invading mycoflora of red clover in the evaluation block, which consisted of 40 fungal species. Other major invaders were Fusarium solani, F. oxysporum, Cylindrocarpon destructans, and Gliocladium roseum, which are the fungi most commonly isolated from roots of red clover and other forage legumes worldwide.

Fungal invasion of red clover roots was affected by plant age, soil temperature and moisture. Generally, numbers of fungal colonies isolated progressively increased from the seedling stage onwards and more colonies were

isolated from roots of plants grown at 20 and 25°C than at 10 and 15°C, and from 60 and 80% WHC than at 40% WHC.

Prochloraz was the most suitable of 11 fungicides tested for use as a soil drench to study effects of root-invading fungi on red clover growth in the field. It showed a broad spectrum of antifungal activity, controlled the major root-invading fungi encountered in the experimental soil, was not toxic to Rhizobium trifolii, and was least retardant to red clover growth.

The numbers of fungal colonies recovered per gram of roots was 60 - 80% lower from plants from field plots receiving a single application of prochloraz drench at 3.46 g/m^2 than from plants from untreated plots. Yields from treated plots harvested 4 times over a period of 45 weeks were 28 - 95% higher than those from untreated plots.

The major root-invading fungi isolated from red clover plants grown in the plant breeder's evaluation block, Cy. scoparium, T. basicola, F. solani, and F. oxysporum, reduced survival of red clover plants in field plots and microplots by 20 -75%, and dry matter yield by 20 -60%, over a periods of 62 and 76 weeks. V. dahliae, C. destructans, and G. roseum, also reduced plant growth in field plots but to a lesser extent. Seedling establishment, and nitrogen fixation and nodulation, were affected adversely by some fungal isolates.

T. basicola was found to penetrate roots of red clover directly and colonise tissue by means of "beaded hyphae" (intracellular hyphae which were constricted at their septa) then "straight hyphae" (unconstricted hyphae growing parallel to the root axis). The fungus is hemibiotrophic. Invaded host cells in the epidermis and cortex of the root are apparently unaffected at first then degenerate and die. Papillae are often found at sites of penetration through cell walls but these rarely obstruct fungal development. Pre-treatment of seedlings with benomyl or prochloraz reduced fungal penetration and growth within tissues. Changes in fungal ultrastructure resulting from benomyl treatment were an increased frequency of lomasome production and occasionally a disorganisation of cell contents. Changes resulting from prochloraz treatment included thickening and fragmentation of cell walls, and necrosis of hyphal cells.

ACKNOWLEDGEMENTS

It has been a privilege for me to study at Massey University and DSIR Plant Protection. I wish to express my sincere thanks to: -

my supervisors, Dr P.G. Long of the Plant Health Department, Massey University, and Dr R.A. Skipp of DSIR Plant Protection, Palmerston North, for their understanding and willingness to provide supervision during the developmental phase of this study, for their stimulation and wise counsel during the experimental work, for their constructive criticism and correction of my English during the writing of the thesis, and for their looking after my personal welfare during my stay in New Zealand. In particular, I am deeply indebted to Dr R.A. Skipp for his persistent efforts to obtain the financial support which made this study possible, for his untiring guidance throughout, especially during the last three months of the writing when Dr Long was on sabbatical leave overseas. To me, the three year study has been an invaluable educational experience. I am proud of my relationship with them both which is not only as supervisor and student, but also as intimate friends.

Prof. K.S. Milne of the Plant Health Department, Massey University, for acting as chief supervisor when Dr Long was away, and for his encouragement and interest during the study.

This study was funded by Department of Scientific and Industrial Research (DSIR) of New Zealand. The receipt of a DSIR post graduate research fellowship is greatly appreciated. Travelling allowance and study leave given by Gansu Grassland Ecological Research Institute of China is also acknowledged. I am most grateful to: -

Dr P.J. Brook of DSIR Plant Protection, and Prof. Ren Jizhou of Gansu Grassland Ecological Research Institute, China, for their encouragement throughout and for their major role in obtaining and administering financial support for this study;

Dr G.C.M. Latch for his permission to use the DSIR Plant Protection laboratory and facilities at the Palmerston North sub-station, for his encouragement, forbearance, and help.

I sincerely acknowledge the helpful support from the staff of DSIR Plant Protection, Palmerston North. Their co-operative manner, willingness to help, and friendly attitude have made a substantial contribution to the success of this study. In particular, I wish express my thanks to:-

Mr M.J. Christensen for his personal interest and providing support and assistance whenever it was needed.

Dr R.E. Falloon for his efforts to obtain funds and for his encouragement when he worked at Palmerston North.

Mr B.J. Tidball and Mr R.E. Hickson for field assistance.

I also wish to thank the following at DSIR Palmerston North Centre:

Mr R.H. Fletcher for statistical help.

Mr D. Hopcroft and Mr R.J. Bennett for their skilful processing of experimental material for electron microscopy studies, and for valuable comments on some TEM photographs.

Mr P. Spring for photographic services.

Mr S.A. Northover, Miss K. Jones, and Mrs A. Ainscough for their prompt and efficient library service.

Mr J.P.M. Evans, Dr J.R. Crush, Dr J.R. Caradus, Dr R.J.M. Hay, Dr M.P. Rolston and Mr M.J. Hare for their help at different stages of the study.

I acknowledge the assistance given to me by the following at Massey University: -

Dr D.R. Scotter for measuring soil water potential.

Mr H.F. Nelson and Mrs L.K. Davis for field assistance.

Grateful thanks is extended to our friends, Nitayaporn and Michael Hare, for their genuine concern, care and understanding.

My heartfelt thanks also goes to my parents and the family, and my parents in law for their enthusiasm and sacrifices made to look after our daughter.

Finally, I thank my wife, Yan Rong, for her continual moral support during my first year study, for her invaluable company during the last two years while she was also studying at Massey University for a masterate in the Seed Technology Centre, her presence has made life in a foreign land much easier and most interesting.

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