

## MYCOPHAGOUS AMOEBAE IN A SUPPRESSIVE PASTURE SOIL IN RELATION TO THE TAKE-ALL DISEASE OF WHEAT

bу

Sukumar Chakraborty

B.Sc.(Ag.) Honours, M.Sc.

Department of Plant Pathology Waite Agricultural Research Institute The University of Adelaide South Australia

Thesis submitted to The University of Adelaide
in fulfilment of the requirements for the
degree of Doctor of Philosophy

March, 1983.

## TABLE OF CONTENTS

				Page No
		SUL	MMARY	υ
		STA	ATEMENT	vii
		ACI	KNOWLEDGEMENTS	viii
Ι	INT	RODUCT	ION	1
II.	MAT	ERIALS	AND METHODS	9
	ORI TES	GIN AND T FOR SU	ND CHEMICAL PROPERTIES OF EXPERIMENTAL SOILS MAINTENANCE OF FUNGI AND BACTERIA UPPRESSIVENESS OF SOILS ENESS OF EXPERIMENTAL SOILS	9 9 13 14
III.	STU	DIES 0	N SOIL AMOEBAE	19
	Α.	ISOLAT	ION, MAINTENANCE AND CHARACTERIZATION OF AMOEBA	E 19
		Isolat	ion of amoebae	20
	·	(ii)	The soil plate method Isolation from soil suspension Soil enrichment method Membrane filter burial method	20 21 22 22
		Purifi	cation and Maintenance .	25
		Cultur	al characters of Amoebae	26
		(i) (ii) (iii) (iv)	Growth responses of amoebae to different temperatures Thermal death points of amoebae Effect of antibiotics on the growth of amoebae Suitability of various brands of agar for the cultivation of amoebae	26 28 30 32
	В.		PTION AND IDENTIFICATION OF AMOEBAE ISOLATED HE WAITE PERMANENT PASTURE	36
		Method	s ·	36
		The am	oebae studied	37
			Acanthamoeba polyphaga Echinamoeba sp. Gephyramoeba sp. Unidentified leptomyxid amoeba Mayorella sp.	37 39 41 44 47

			Page No
		Platyamoeba stenopodia Platyamoeba sp. Saccamoeba sp.	49 51 53
		The came et a quadrilineata	55
		Thecamoeba sphaeronucleolus Thecamoeba granifera sub-species minor	57 58
		Unidentified Vampyrellid amoeba	62
	C.	IN VITRO INTERACTION OF SOIL AMOEBAE WITH $Ggt$ AND OTHER SOIL FUNGI	66
		The feeding trials	67
		Feeding activities of the amoebae	68
		The unidentified leptomyxid amoeba Saccamoeba sp	70 79
		Gephyramoeba sp. Thecamoeba granifera sub-species minor	81 83
		Mayorella sp.	85
		Feeding of mycophagous amoebae on other soil fungi	87
		Discussion	89
IV.	ROLE OF SOIL AMOEBAE IN SUPPRESSION OF GGT		
	Α.	SOIL AMOEBAE AND SAPROPHYTIC SURVIVAL OF ${\it Ggt}$	91
		The method used	94
		Amoebae and saprophytic survival of $\mathit{Ggt}$ hyphae in suppressive and non-suppressive soil	95
		Amoebae and saprophytic survival of ${\it Ggt}$ hyphae in WPP, CW, MB and CS	101
		Saprophytic survival of $Ggt$ in sterile soils amended with suspensions of mycophagous amoebae	106
•		Ability of mycophagous amoebae to invade infected wheat roots	109
		Discussion	112
	В.	POPULATIONS OF AMOEBAE IN SUPPRESSIVE AND NON-	·
		SUPPRESSIVE SOILS	114
		Soils	115
		Consideration of methods	116
		Populations of amoebae in the soils	121
		Populations of <i>Ggt</i> in the survey soils	123
		Effect of soil moisture on amoebal activity	125
		Survival of amoebae under extremes of moisture stress	128

			Page No
		Populations of amoebae in suppressive and non- suppressive soils with similar soil texture	129
		Suitability of the survey soils as a medium for amoebal growth	132
		Discussion	132
	С.	ASSOCIATION OF AMOEBAE IN WHEAT RHIZOSPHERES IN SUPPRESSIVE AND NON-SUPPRESSIVE SOILS	139
		Association of soil amoebae with wheat roots - a survey	141
		Population dynamics of amoebae in suppressive and non-suppressive soils	143
		Selection of soils and sampling	143
		Populations of mycophagous and other amoebae in the PPW, PeW and CW soils	146
		Amoebae in wheat rhizoplane in suppressive and non-suppressive soils	148
		Correlation between soil moisture, population of <i>Ggt</i> and amoebae in the suppressive and non-suppressive soils	150
	D.	REDUCTION OF TAKE-ALL BY MYCOPHAGOUS AMOEBAE IN POT BIOASSAYS	155
		Effect of mycophagous amoebae on the severity of take-all	157
		Reduction of take-all severity by soil cultures of mycophagous amoebae	158
		Soil cultures of mycophagous amoebae	<b>1</b> 60
		Effect of soil cultures of mycophagous amoebae on the severity of take-all	160
		Effect of initial population size of mycophagous amoebae on the severity of take-all	164
		The accompanying organisms of Thecamoeba granifera sub-species minor	164
		Discussion	167
V.	GEI	NERAL DISCUSSION	169

		Page No.
VI.	APPENDICES	
	APPENDIX I - Formulae for media and saline solutions	175
	APPENDIX IIA - Generic populations of amoebae in samples of the Waite permanent pasture, Avon and Murray Bridge soil	179
	APPENDIX IIB - Population (no, g <sup>-1</sup> dry wt) of soil amoebae during two wheat crop and inter-crop fallow in suppressive (PPW and PeW) and non-suppressive (CW) soil	180
	APPENDIX IIC - Wheat rhizoplane amoebae (percentage root plated) during two crops in suppressive (PPW and PeW) and non-suppressive (CW) soil	181
	APPENDIX III - Publications	183
VII.	BIBLIOGRAPHY	184

## SUMMARY

Amoebae were isolated, characterised and identified from soil of the Waite Institute permanent pasture plot which is suppressive in pot bioassays to the take-all disease of wheat. Nine species of amoebae belonging to eight genera were tested for their mycophagy against three plant pathogenic fungi including Gaeumannomyces graminis tritici.

Members of the genera, Gephyramoeba, Mayorella, Saccamoeba, Thecamoeba and an unidentified species of the order Leptomyxida were mycophagous. All mycophagous amoebae, except the unidentified leptomyxid were able to feed on pigmented (melanised) fungal cells.

Populations of the various genera of soil amoebae were assessed from samples of the naturally suppressive pasture soil, a suppressive wheat-pasture rotation and three non-suppressive wheat-field soils. The suppressive soils showed higher populations of both mycophagous and other amoebae and a higher frequency of occurrence of mycophagous genera. Soil texture and water holding capacity were not related with population levels of amoebae in these soils.

Saprophytic survival of the take-all fungus was studied by burying fungal hyphae in suppressive and non-suppressive soils. Hyphal density and survival of pigmented hyphae declined at a faster rate in the permanent pasture soil than in the non-suppressive soils. Hyphae recovered from the suppressive soil showed a higher association of mycophagous and other amoebae and scanning electron microscopy of these hyphae showed extensive erosion and discrete perforations in their walls.

The decline in survival of the fungus was related to the rate of decline in the density of pigmented hyphae in suppressive soil, irrespective of the soil type.

Studies on the population dynamics of amoebae showed a higher rhizosphere population and a higher rhizoplane association of mycophagous and other amoebae in suppressive soils. Populations in the suppressive pasture-wheat soil did not correlate with soil moisture during two wheat crops and one inter-crop fallow.

Three mycophagous amoebae, Gephyramoeba, Saccamoeba and Thecamoeba granifera sub-species minor, alone or mixed effectively reduced take-all severity in pot bioassays. The reduction in disease rating and the increase in height and dry weight of plants were comparable to that obtained with the suppressive pasture soil. Higher populations of these amoebae in combination were able to further reduce disease severity and improve plant growth.

Mycophagous amoebae are proposed as a component of the suppressive factors in the permanent pasture soil. The observations that mycophagous amoebae associate themselves with the fungus in soils, can lyse both pigmented and hyaline hyphae during the pre-colonisation and parasitic phase of the fungus, and reduce take-all severity in pot bioassays substantiate this proposal.