

**EFFECT OF HOST PLANT
ON
MEALYBUGS AND THEIR
PARASITIDS**

BY

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DEDICATION

**This work is dedicated to my parents
and teachers
who guided me to the
Scientific World**

ABSTRACT

Six host plant species were tested as food plants for various life history parameters of two species of mealybug Planococcus citri (Risso) and Pseudococcus affinis (Maskell). The range of acceptability suggested that plants can be divided into host, acceptable non-host and unacceptable hosts. Mealybugs reared on soy bean plants developed significantly faster and reached a higher body weight in comparison to insects reared on Gesneriaceae. Passiflora plant appeared to be completely resistant to P. citri. Possible reasons for this are discussed. The fecundity level of mealybugs reared on leguminous plants showed a two fold increase in comparison to Gesneriaceae plants. The relationship between fecundity, fresh weight and mean body length of adults showed differences between mealybugs reared on different plant species.

The influence of host plant factors on the third trophic level was investigated. Anagyrus pseudococci (Girault) and Leptomastix dactylopii Howard were allowed to oviposit on P. citri reared on various host plants and the fitness parameters were measured. Mealybugs fed on leguminous plants appear to be a better source of nutrient for the developing parasitoid larvae since the larger adults with longer life expectancy emerged from those hosts. More females emerged when the parasitoids oviposit on mealybugs reared on leguminous plants.

The influence of host plant factors on the efficiency of encapsulation of eggs of A. pseudococci and L. dactylopii was studied under solitary and superparasitized conditions. Mealybugs reared on high phenolic plants such as Aeschynanthus ellipticus and Passiflora coccinea showed a significantly greater ability to encapsulate parasitoids eggs.

Over 90% of the eggs of L. dactylopii were encapsulated by P. affinis but 40% of the eggs of A. pseudococci developed within the host body cavity. A proper immunity reaction was observed when parasitoids oviposit on P. citri but no clear response was noted in P. affinis.

The impact of plant water stress on the life history parameters of mealybugs was investigated. A significant reduction in mean body size of mealybugs was recorded when they were reared on high stressed plants and as a consequence the fecundity level of the insect was drastically reduced. The parasitoids developed from smaller host showed reduced fitness parameters.

Population parameters of mealybugs were compared on potato and coleus plants fertilized with different levels of nitrogen. Mealybugs fed on low nitrogen plants showed a significant reduction in fecundity and this may be the main reason for the low rm value of mealybugs on low nitrogen plants. Some fitness parameters of parasitoids were not influenced by the level of nitrogen in the host plants.

A study of searching behaviour of L. dactylopii on various plant species showed that trichomes of Streptocarpus and soy bean leaves can change the normal searching behaviour of the insect. Parasitoids walked short distances on Streptocarpus plants while the speed of movement and area covered during searching was higher on winged bean plants.

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CONTENTS

<u>DEDICATION</u>	2
<u>ABSTRACT</u>	3
<u>ACKNOWLEDGEMENTS</u>	5
<u>CONTENTS</u>	7
<u>LIST OF TABLES</u>	26
<u>LIST OF FIGURES</u>	30
<u>LIST OF PLATES</u>	32

SECTION 1

EFFECT OF HOST PLANTS ON LIFE HISTORY PARAMETERS OF MEALYBUGS

1.1	INTRODUCTION.....	33
1.2	REVIEW OF LITERATURE.....	35
1.2.1	Varietal differences of host plants and insect performance.....	35
1.2.2	Host plant morphological features and insects.....	35
1.2.3	Influence of host plants on the developmental period of insects.....	37
1.2.4	Influence of host plant on the fecundity of herbivorous insects.....	38
1.2.5	Secondary plant substances and insect performance.....	39
1.2.6	The experimental plants.....	41
1.2.6.1	<u>Passiflora caerulea</u>	41
1.2.6.2	<u>Passiflora coccinea</u>	41
1.2.6.3	<u>Psophocarpus tetragonolobus</u> Winged bean	41
1.2.6.4	<u>Glycine max</u> Soy bean	43

CONTENTS (cont.)

1.2.6.5	<u>Streptocarpus hybridus</u>	43
1.2.6.6	<u>Aeschynanthus ellipticus</u>	45
1.2.7	The pest.....	45
1.2.7.1	<u>Planococcus citri</u> (Risso):common mealybug	45
1.2.7.2	<u>Pseudococcus affinis</u> (Maskell). The obscure mealybug	47
1.3	MATERIALS METHODS.....	49
1.3.0.1	Preparation of plant materials.....	49
1.3.0.2	Physical Environment.....	49
1.3.0.3	Method of obtaining crawlers.....	50
1.3.0.4	Incubation of mealybugs eggs.....	50
1.3.1	Influence of host plant on the developmental period of mealybug.....	51
1.3.2	Influence of the host plant on the pre-ovipositional period of mealybugs.....	52
1.3.3	Influence of plant nutrient on mealybug fecundity.....	52
1.3.4	Influence of host plant on mealybug egg viability.....	53
1.3.5	Influence of host plant on mealybug egg incubation period.....	53
1.3.6	Influence of host plant on mealybug body weight.....	54
1.3.7	Correlation between weight, length and fecundity of mealybug.....	54
1.3.8	Influence of host plants on the growth parameters of mealybug.....	55
1.3.9	Analysis of host plants phenolics.....	55
1.3.10	Analysis of host plants total nitrogen content.....	56

CONTENTS (cont.)

1.4	RESULTS.....	57
1.4.1	Influence of host plant on the developmental period of mealybug.....	57
1.4.2	Influence of host plant on the pre-ovipositional period of mealybug.....	59
1.4.3	Influence of host plant on mealybug fecundity.....	60
1.4.4	Influence of host plant on mealybug egg viability.....	61
1.4.5	Influence of host plants on mealybug egg incubation period.....	62
1.4.6	Influence of host plant on mealybug body weight.....	63
1.4.7	Correlation between weight, length and fecundity of mealybug.....	64
1.4.8	Influence of host plant on the growth parameters of mealybug.....	70
1.4.9	Analysis of host plant soluble phenols.....	71
1.4.10	Analysis of host plant total nitrogen content.....	75
1.5	DISCUSSION.....	79
1.5.1	Influence of host plant on the developmental period of mealybug.....	79
1.5.2	Influence of host plants on the pre-ovipositional period of mealybug.....	82
1.5.3	Influence of host plant on mealybug fecundity.....	83
1.5.4	Influence of host plants on mealybug egg viability.....	85
1.5.5	Influence of host plant on mealybug egg incubation period.....	85
1.5.6	Influence of host plant on mealybug body weight.....	85

CONTENTS (cont.)

1.5.7	Correlation between weight, length and fecundity of mealybugs.....	86
1.5.8	Influence of host plant on the growth parameters of mealybug.....	87
1.6	CONCLUSIONS.....	90

SECTION 2

EFFECT OF HOST PLANT FACTORS ON MEALYBUG PARASITOIDS

2.1	INTRODUCTION.....	92
2.2	REVIEW OF LITERATURE.....	94
2.2.1	Influence of host plant nutritional value on parasitoids.....	94
2.2.2	Effect of plant allelochemicals on the third trophic level.....	95
2.2.3	Effect of toxic secondary plant metabolites on the third trophic level.....	96
2.2.4	Host plant morphology and parasitoid interactions.....	97
2.2.5	The Parasitoids.....	98
2.3	MATERIALS AND METHODS.....	101
2.3.1	Influence of host plant on successful parasitism by <u>A. pseudococci</u> and <u>L. dactylopii</u>	101
2.3.2	Influence of host plants on the developmental period of <u>A. pseudococci</u> and <u>L. dactylopii</u>	103
2.3.3	Influence of host plant on the sex allocation by <u>A. pseudococci</u> and <u>L. dactylopii</u>	103

CONTENTS (cont.)

2.3.4	Influence of host plant on the longevity of female <u>A. pseudococci</u> and <u>L. dactylopii</u>	104
2.3.5	Influence of host plant on the size of the emerging parasitoid.....	105
2.4	RESULTS.....	106
2.4.1	Influence of host plant on successful parasitism by <u>A. pseudococci</u> and <u>L. dactylopii</u>	106
2.4.2	Influence of host plant on the developmental periods of <u>A. pseudococci</u> and <u>L. dactylopii</u>	107
2.4.3	Influence of host plant on sex allocation by <u>A. pseudococci</u> and <u>L. dactylopii</u>	108
2.4.4	Influence of host plant on the longevity of female <u>A. pseudococci</u> and <u>L. dactylopii</u>	111
2.4.5	Influence of host plant on the size of the emerging parasitoid.....	112
2.5	DISCUSSION.....	114
2.5.1	Influence of host plant on successful parasitism by <u>A. pseudococci</u> and <u>L. dactylopii</u>	114
2.5.2	Influence of host plant on the developmental period of <u>A. pseudococci</u> and <u>L. dactylopii</u>	115
2.5.3	Influence of host plant on sex allocation by <u>A. pseudococci</u> and <u>L. dactylopii</u>	116
2.5.4	Influence of host plant on the longevity of female <u>A. pseudococci</u> and <u>L. dactylopii</u>	117
2.5.5	Influence of host plant on the size of the emerging parasitoid.....	118
2.6	CONCLUSIONS.....	119

CONTENTS (cont.)

SECTION 3

PLANT FACTORS AND ENCAPSULATION

3.1	INTRODUCTION.....	120
3.2	REVIEW OF LITERATURE.....	122
3.2.1	Insect cellular defence mechanisms and encapsulation.....	122
3.2.2	Mechanism of encapsulation.....	122
3.2.3	Inhibitors of encapsulation.....	124
3.2.4	Encapsulation of inorganic substances.....	124
3.2.5	Factors affecting encapsulation.....	124
3.2.6	Immunity aspect of host defence.....	125
3.2.7	Electrophoretic analysis of insect haemolymph proteins.....	126
3.2.8	Role of parasitoids in overcoming host defence mechanism.....	127
3.2.9	Inhibition of host immune system.....	127
3.2.10	Effect of calyx fluid of parasitoids to overcome the host defence system.....	128
3.2.11	Influence of superparasitism on encapsulation.....	128
3.2.12	Encapsulation in mealybugs.....	129
3.3	MATERIALS AND METHODS.....	130
3.3.0.	Effect of host plants on the encapsulation.....	130
3.3.1	Effect of host plant on the frequency of encapsulation of solitary eggs of <u>A. pseudococci</u> and <u>L. dactylopii</u>	131

CONTENTS (cont.)

3.3.2	Effect of host plant on oviposition rejection by <u>A. pseudococci</u>	132
3.3.3	Effect of host plant on the development of a capsule along the larval body of <u>A. pseudococci</u>	132
3.3.4	Influence of superparasitism on frequency of encapsulation.	133
3.3.5	Effect of host plant on the ability of mealybugs to produce a melanin cover over the developing larvae under superparasitized condition.....	134
3.3.6.1	Immunity response of mealybugs to parasitoids.....	134
3.3.6.2	Gradient gel electrophoresis.....	136
3.3.7	Inert foreign material and mealybug haemolymph reaction.....	136
3.4	RESULTS.....	138
3.4.1	Effect of host plant on the frequency of encapsulation of solitary eggs of <u>A. pseudococci</u> and <u>L. dactylopii</u>	138
3.4.2	Effect of host plant on oviposition rejection by <u>A. pseudococci</u>	140
3.4.3	Effect of host plant on the development of a capsule along the larval body of <u>A. pseudococci</u>	141
3.4.4	Influence of superparasitism on the frequency of encapsulation.....	142
3.4.5	Effect of host plant on the ability of mealybugs to produce a melanin cover over the developing larvae under superparasitism.....	144
3.4.6.1	Immunity responses of mealybugs to parasitoids.....	145

CONTENTS (cont.)

3.4.7	Inert foreign material and mealybugs haemolymph reaction.....	148
3.5	DISCUSSION.....	150
3.5.1.1	Involvement of host plant factors in the encapsulation process.....	150
3.5.1.2	Importance of parasitoid species on the frequency of encapsulation.....	152
3.5.2	Effect of host plant on oviposition rejection by <u>A. pseudococci</u>	154
3.5.3	Effect of host plant on the development of a capsule along the larval body of <u>A. pseudococci</u>	155
3.5.4	Influence of superparasitism on frequency of encapsulation.....	157
3.5.5	Immunity responses of mealybugs to parasitoids.....	157
3.5.6	Inert foreign material and mealybugs haemolymph reaction.....	159
3.5.7	Implication of encapsulation on biological control.....	160
3.6	CONCLUSIONS.....	161

SECTION 4

INFLUENCE OF WATER STRESS ON MEALYBUGS AND ITS PARASITIDS.

4.1	INTRODUCTION.....	162
4.2	REVIEW OF LITRATURE.....	164
4.2.1.0	Plant water stress and techniques of assessing the stress levels.....	164

CONTENTS (cont.)

4.2.1.1	Measurement of plant water potential.....	164
4.2.2	Relative water content (RWC).....	164
4.2.3	Effect of water shortage on plant nutrient balance.....	165
4.2.3.1	Free amino acid.....	165
4.2.3.2	Influence of water stress on total nitrogen of the plant.....	165
4.2.3.3	Influence of water stress on some physical characteristics of the plant.....	166
4.2.4	Water stress and insect interactions.....	167
4.2.4.1	Host plant turgor pressure and feeding efficiency of insects.....	167
4.2.4.2	Water stress and nutrient balance in herbivores.....	167
4.2.4.3	Influence of water stress on herbivorous insects with various feeding habits.....	168
4.2.4.4	Influence of water stress on the developmental period of herbivorous insects.....	169
4.2.4.5	Influence of water stress on survival rates of herbivore insects.....	170
4.2.4.6	Effect of plant water stress on reproductive capacity of herbivorous insects.....	170
4.2.4.7	Influence of water stress on life table statistics of herbivorous insect.....	171
4.2.5.1	Plant induced water stress as a factor in parasitoid efficacy.....	172
4.3	MATERIALS and METHODS.....	173
4.3.0	Experimental procedure to achieve required water stress levels.....	173

CONTENTS (cont.)

4.3.0.1	Percentage water at field capacity.....	173
4.3.0.2	Percentage water at permanent wilting point.....	174
4.3.0.3	Available water.....	174
4.3.0.4	Unstressed treatment.....	174
4.3.0.5	Medium stress treatment.....	175
4.3.0.6	High stress treatment.....	175
4.3.0.7	Introduction of crawlers on to the host plants.....	175
4.3.1.1	Measurement of relative water content.....	176
4.3.1.2	Analysis of total soluble amino acid.....	177
4.3.1.3	Analysis of total leaf nitrogen.....	178
4.3.2.1	Influence of water stress on the developmental period of mealybug.....	178
4.3.2.2	Influence of water stress on the fecundity of mealybug.....	179
4.3.2.3	Influence of water stress on pre-ovipositional ovipositional and post-ovipositional periods of mealybug.....	180
4.3.2.4	Influence of water stress on intrinsic rate of population growth of mealybug.....	180
4.3.2.5	Influence of water stress on body size of adult mealybug.....	182
4.3.3.1	Influence of water stress on successful parasitism.....	182
4.3.3.2	Influence of water stress on the developmental period of parasitoids.....	182
4.3.3.3	Influence of water stress on the longevity of female parasitoids.....	183

CONTENTS (cont.)

4.3.3.4	Influence of water stress on the size of emerging parasitoids.....	183
4.4	RESULTS.....	184
4.4.1.1	Relative water content.....	184
4.4.1.2	Analysis of total soluble amino acid.....	184
4.4.1.3	Analysis of total leaf nitrogen.....	186
4.4.2.1	Influence of water stress on the developmental period of mealybugs.....	186
4.4.2.2	Influence of water stress on the fecundity of mealybug.....	190
4.4.2.3	Influence of water stress on ovipositional and post-ovipositional periods of mealybug.....	191
4.4.2.4	Influence of water stress on intrinsic rate of population growth of mealybug.....	193
4.4.2.5	Influence of water stress on the body size of adult mealybug.....	197
4.4.3.1	Influence of water stress on successful parasitism.....	199
4.4.3.2	Influence of water stress on the developmental period of parasitoids.....	200
4.4.3.3	Influence of water stress on the longevity of parasitoids.....	201
4.4.3.4	Influence of water stress on the size of the emerging parasitoids.....	202
4.5	DISCUSSION.....	204
4.5.1.1	Relative water content.....	204

CONTENTS (cont.)

4.5.1.2	Analysis of soluble amino acid content.	204
4.5.1.3	Water stress and total leaf nitrogen	205
4.5.2.1	Influence of water stress on the developmental period of mealybug	205
4.5.2.2	Effect of water stress on the fecundity of mealybug	206
4.5.2.3	Influence of water stress on ovipositional periods of mealybug	207
4.5.2.4	Influence of water stress on intrinsic rate of population growth of mealybug.	208
4.5.3.1	Influence of water stress on successful parasitism	210
4.5.3.2	Influence of water stress on the development period of parasitoids	210
4.5.3.3	Influence of water stress on the longevity of parasitoids	211
4.5.3.4	Influence of water stress on the size of emerging parasitoids	211
4.5.3.5	Influence of water stress on biological control	211
4.6	CONCLUSIONS	213

SECTION 5

INFLUENCE OF HOST PLANT NITROGEN LEVELS ON LIFE HISTORY

PARAMETERS OF MEALYBUGS AND THEIR NATURAL ENEMIES

5.1	INTRODUCTION	214
5.2	REVIEW OF LITERATURE	216

CONTENTS (cont.)

5.2.1.1	Nitrogen requirement of plants	216
5.2.1.2	Nitrogen translocation in the plants	216
5.2.1.3	Influence of nitrogen on chemical changes in the plant	217
5.2.1.4	Effect of nitrogen fertilizer on plant total amino acid levels	217
5.2.1.5	Influence of soil nitrogen on total nitrogen content of plants	218
5.2.2.1	Requirement of nitrogen in insects	218
5.2.2.2	Influence of host plant nitrogen levels on the developmental period of herbivorous insects	219
5.2.2.3	Influence of host plant nitrogen levels on the mortality rate of herbivorous insects	220
5.2.2.4	Influence of plant nitrogen on the fecundity of herbivorous insects	221
5.2.2.5	Influence of host plant nitrogen on life table statistics of herbivorous insects	222
5.2.3.1	Influence of plant nitrogen on some aspects of parasitoid biology	222
5.3	MATERIALS AND METHODS	224
5.3.0.1	Host plant species	224
5.3.0.2	Experimental arrangement of Coleus plants	224
5.3.0.3	Nutrient treatments	225
5.3.0.4	Experimental arrangement of potato plants	227
5.3.1.1	Chemical analysis of plant material	227
5.3.1.2	Effect of host plant nitrogen on the developmental period of mealybugs	229

CONTENTS (cont.)

5.3.1.3	Effect of host plant nitrogen on survival rate of mealybug	229
5.3.1.4	Effect of host plant nitrogen on the fecundity of mealybug	229
5.3.1.5	Effect of host plant nitrogen on life table parameters of mealybug	230
5.3.1.6	Effect of host plant nitrogen on the body size of mealybug	230
5.3.1.7	SDS-PAGE analysis of mealybug protein	230
5.3.2.1	Effect of host plant nitrogen on successful parasitism by <u>A. pseudococci</u>	230
5.3.2.2	Effect of host plant nitrogen on the developmental period of <u>A. pseudococci</u>	231
5.3.2.3	Effect of host plant nitrogen on the longevity of <u>A. pseudococci</u>	231
5.3.2.4	Effect of host plant nitrogen on the body size of the emerging parasitoids	231
5.4	RESULTS.....	232
5.4.1.1.1	Analysis of foliar concentrations of total nitrogen	232
5.4.1.1.2	Analysis of soluble amino acids.....	232
5.4.1.2	Effect of host plant nitrogen on the developmental period of mealybug	235
5.4.1.3	Effect of host plant nitrogen on the survival rate of mealybug	236
5.4.1.4	Effect of host plant nitrogen on the fecundity of mealybug	237

CONTENTS (cont.)

5.4.1.5.1	Effect of host plant nitrogen on life table parameters of mealybug	238
5.4.1.5.2	Intrinsic rate of population growth of <u>P. citri</u> under the influence of host plant nitrogen levels	238
5.4.1.6	Effect of host plant nitrogen on the body size of mealybug	241
5.4.1.7	SDS-PAGE analysis of mealybug protein	243
5.4.2.1	Effect of host plant nitrogen on successful parasitism by <u>A. pseudococci</u>	243
5.4.2.2	Effect of host plant nitrogen on the development period of <u>A. pseudococci</u>	245
5.4.2.3	Effect of host plant nitrogen on the longevity of <u>A. pseudococci</u>	246
5.4.2.4	Effect of host plant nitrogen on the body size of the emerging parasitoids	247
5.5	DISCUSSION	249
5.5.1.1.1	The effect of applied nitrogen on total leaf nitrogen content	249
5.5.1.1.2	Effect of applied nitrogen on soluble amino acid content	249
5.5.1.2	Effect of host plant nitrogen on the development period of mealybug	250
5.5.1.3	Effect of host plant nitrogen on mortality rate of mealybug	251
5.5.1.4	Effect of host plant nitrogen on the fecundity of mealybug	252

CONTENTS (cont.)

5.5.1.5	Effect of host plant nitrogen on life table parameters of mealybug	253
5.5.1.6	Effect of host plant nitrogen on the body size of mealybug	254
5.5.1.7	SDS-PAGE analysis of mealybug protein	255
5.5.2.1	Effect of host plant nitrogen on successful parasitism	255
5.5.2.2	Effect of host plant nitrogen on the development period of parasitoids	256
5.5.2.3	Effect of host plant nitrogen on the longevity of parasitoids	257
5.5.2.4	Effect of host plant nitrogen on the size of emerging parasitoids	257
5.6	CONCLUSIONS	259

SECTION 6

BEHAVIOURAL RESPONSES OF PARASITIDS TOWARDS FOUR PLANT SPECIES

6.1	INTRODUCTION	261
6.2	REVIEW OF LITERATURE	262
6.2.1	Importance of host location.	262
6.2.2	Factors affecting habitat location by natural enemies	262
6.2.2.1	Host density	262
6.2.2.2	Environmental factors	263

CONTENTS (cont.)

6.2.3	Influence of host plants on searching behaviour of parasitoids	263
6.3	MATERIALS AND METHODS	265
6.3.0.1	Preparation of leaf samples	265
6.3.0.2	Arrangement of apparatus and arena	265
6.3.0.3	Selections of suitable parasitoids	266
6.3.0.4	Recording procedure	266
6.3.1	Walking	266
6.3.2	Palpating	267
6.3.3	Preening	267
6.3.4	Turning	267
6.3.5	Jumps or flights	267
6.3.6	Resting	267
6.3.5	Electron microscopic studies on plant surface structures	268
6.4	RESULTS	271
6.4.1.1	Total time spent walking by <u>L. dactylopii</u> while searching on various host plant surfaces	271
6.4.1.2	Total distance walked by <u>L. dactylopii</u> during searching on various leaf surfaces	272
6.4.1.3	Effect of leaf surfaces on speed of movement of <u>L. dactylopii</u>	273
6.4.2	Effect of leaf surfaces on time allocation for palpation by <u>L. dactylopii</u>	274

CONTENTS (cont.)

6.4.3	Effect of leaf surfaces on time allocation for preening by <u>L. dactylopii</u>	275
6.4.4	Effect of leaf surfaces on rate of turns (over 90°) made by <u>L. dactylopii</u>	276
6.4.5	Effect of leaf surfaces on rate of jumps made by <u>L. dactylopii</u>	277
6.4.6	Effect of leaf surfaces on time allocation for resting by <u>L. dactylopii</u>	278
6.4.7	Effect of leaf surfaces on total time allocation for searching by <u>L. dactylopii</u>	279
6.4.8	Time apportioned to various activities	280
6.4.9	Searching pattern of parasitoids on various leaf surfaces.....	280
6.5	DISCUSSION	285
6.6	CONCLUSIONS	289

SECTION 7

CONCLUSIONS	290
REFERENCES	297
APPENDICES	321

LIST OF TABLES

<u>Table 1.1.1</u>	Influence of host plant on the developmental period of larval stages of <u>P. citri</u>	57
<u>Table 1.1.2</u>	Influence of host plant on the developmental period of larval stages of <u>P. affinis</u>	58
<u>Table 1.2</u>	Influence of host plant on the pre-ovipositional periods of mealybug	59
<u>Table 1.3</u>	Influence of host plant on mealybug fecundity	60
<u>Table 1.4</u>	Influence of host plant on egg viability	61
<u>Table 1.5</u>	Influence of host plant on mealybug egg incubation period	62
<u>Table 1.6</u>	Influence of host plant on mealybug body weight	63
<u>Table 1.7.1</u>	Correlation between weight, length and fecundity of <u>P. citri</u>	64
<u>Table 1.7.2</u>	Correlation between weight, length and fecundity of <u>P. affinis</u>	70
<u>Table 2.1</u>	Influence of host plant species on successful parasitism by <u>A. pseudococci</u> and <u>L. dactylopii</u>	106
<u>Table 2.2.1</u>	Influence of host plant on the developmental period of male parasitoids	107
<u>Table 2.2.2</u>	Influence of host plant on the developmental period of female parasitoids	108
<u>Table 2.3.1</u>	Influence of host plant on sex allocation by <u>A. pseudococci</u>	109
<u>Table 2.3.2</u>	Influence of host plant on sex allocation by <u>L. dactylopii</u>	110
<u>Table 2.4</u>	Influence of host plant on the longevity of female <u>A. pseudococci</u> and <u>L. dactylopii</u>	111

LIST OF TABLES (cont.)

<u>Table 3.1.1</u>	Effect of host plant on the frequency of encapsulation of solitary eggs of <u>A. pseudococci</u>	138
<u>Table 3.1.2</u>	Effect of host plant on the frequency of encapsulation of solitary eggs of <u>L. dactylopii</u>	139
<u>Table 3.2</u>	Effect of host plant on oviposition rejection by <u>A. pseudococci</u>	140
<u>Table 3.3</u>	Effect of host plant on the development of capsule along the larval body of <u>A. pseudococci</u>	141
<u>Table 3.4</u>	Influence of superparasitism on the frequency of encapsulation	142
<u>Table 3.5</u>	Effect of host plant on the ability of mealybugs to produce a melanin cover over the developing larvae under superparasitism	144
<u>Table 4.1.1</u>	Influence of water stress on the developmental period of larval stages of mealybug	188
<u>Table 4.1.2</u>	Influence of water stress on the developmental period of adult mealybug	189
<u>Table 4.1.3</u>	Effect of water stress on the developmental period of mealybugs (species pooled)	190
<u>Table 4.2</u>	Influence of water stress on the fecundity of two species of mealybug	191
<u>Table 4.3</u>	Influence of water stress on ovipositional and post-ovipositional period of mealybug	192
<u>Table 4.4</u>	Influence of water stress on age specific life tables and related parameters of <u>P. citri</u> and <u>P. affinis</u>	196
<u>Table 4.5</u>	Effect of water stress on the body size of the adult mealybug	197

LIST OF TABLES (cont.)

<u>Table 4.6</u>	Influence of water stress on successful emergence of parasitoids	199
<u>Table 4.7.1</u>	Influence of water stress on the developmental period of <u>A. pseudococci</u>	200
<u>Table 4.7.2</u>	Influence of water stress on the developmental period of <u>L. dactylopii</u>	201
<u>Table 4.8</u>	Influence of water stress on the longevity of female parasitoids	202
<u>Table 5.1</u>	Effect of host plant nitrogen (200, 100 and 10 ppm) on the developmental period of <u>P. citri</u>	235
<u>Table 5.2</u>	Effect of host plant nitrogen (200, 100 and 10 ppm) on percentage survival of different stages of mealybug.....	236
<u>Table 5.3</u>	Effect of host plant nitrogen (200, 100 and 10 ppm) on the fecundity of <u>P. citri</u>	237
<u>Table 5.4</u>	Effect of host plant nitrogen (200, 100 and 10 ppm) on life table parameters of <u>P. citri</u>	238
<u>Table 5.5</u>	Effect of host plant nitrogen (200, 100 and 10 ppm) on successful emergences of <u>A. pseudococci</u>	243
<u>Table 5.6</u>	Effect of host plant nitrogen (200, 100 and 10 ppm) on the developmental period of male <u>A. pseudococci</u>	245
<u>Table 5.7</u>	Effect of host plant nitrogen (200, 100 and 10 ppm) on the development period of female <u>A. pseudococci</u>	245
<u>Table 5.8</u>	Effect of host plant nitrogen (200, 100 and 10 ppm) on the longevity of <u>A. pseudococci</u>	246
<u>Table 5.9.</u>	Effect of host plant nitrogen (200, 100 and 10 ppm) on the head width of male <u>A. pseudococci</u>	247
<u>Table 5.10</u>	Effect of host plant nitrogen (200, 100 and 10 ppm) on the head width of female <u>A. pseudococci</u>	248

LIST OF TABLES (cont.)

<u>Table 6.1</u>	Total time spent walking in seconds by <u>L. dactylopii</u> while searching on various leaf surfaces	271
<u>Table 6.2</u>	Total distance walked in centimeters by <u>L. dactylopii</u> during searching on various leaf surfaces	272
<u>Table 6.3</u>	Effect of leaf surfaces on speed of movement (cm/s) of <u>L. dactylopii</u>	273
<u>Table 6.4</u>	Effect of leaf surfaces on time allocation in seconds for palpation by <u>L. dactylopii</u>	274
<u>Table 6.5</u>	Effect of leaf surfaces on time allocation for preening in seconds by <u>L. dactylopii</u>	275
<u>Table 6.6</u>	Effect of leaf surfaces on number of turns made per cm walked by <u>L. dactylopii</u>	276
<u>Table 6.7</u>	Effect of leaf surfaces on number of jumps made per cm walked by <u>L. dactylopii</u>	277
<u>Table 6.8</u>	Effect of leaf surfaces on time allocation for resting in seconds by <u>L. dactylopii</u>	278
<u>Table 6.9</u>	Effect of leaf surfaces on total time allocation for searching in seconds by <u>L. dactylopii</u>	279

LIST OF TABLES (cont.)

<u>Table 4.6</u>	Influence of water stress on successful emergence of parasitoids	199
<u>Table 4.7.1</u>	Influence of water stress on the developmental period of <u>A. pseudococci</u>	200
<u>Table 4.7.2</u>	Influence of water stress on the developmental period of <u>L. dactylopii</u>	201
<u>Table 4.8</u>	Influence of water stress on the longevity of female parasitoids	202
<u>Table 5.1</u>	Effect of host plant nitrogen (200, 100 and 10 ppm) on the developmental period of <u>P. citri</u>	235
<u>Table 5.2</u>	Effect of host plant nitrogen (200, 100 and 10 ppm) on percentage survival of different stages of mealybug.....	236
<u>Table 5.3</u>	Effect of host plant nitrogen (200, 100 and 10 ppm) on the fecundity of <u>P. citri</u>	237
<u>Table 5.4</u>	Effect of host plant nitrogen (200, 100 and 10 ppm) on life table parameters of <u>P. citri</u>	238
<u>Table 5.5</u>	Effect of host plant nitrogen (200, 100 and 10 ppm) on successful emergences of <u>A. pseudococci</u>	243
<u>Table 5.6</u>	Effect of host plant nitrogen (200, 100 and 10 ppm) on the developmental period of male <u>A. pseudococci</u>	245
<u>Table 5.7</u>	Effect of host plant nitrogen (200, 100 and 10 ppm) on the development period of female <u>A. pseudococci</u>	245
<u>Table 5.8</u>	Effect of host plant nitrogen (200, 100 and 10 ppm) on the longevity of <u>A. pseudococci</u>	246
<u>Table 5.9.</u>	Effect of host plant nitrogen (200, 100 and 10 ppm) on the head width of male <u>A. pseudococci</u>	247
<u>Table 5.10</u>	Effect of host plant nitrogen (200, 100 and 10 ppm) on the head width of female <u>A. pseudococci</u>	248

LIST OF FIGURES

Fig. 1.1	The relationship between mean body length vs mean fecundity and mean fresh weight vs mean fecundity of <u>P. citri</u> reared on various host plant species.....	.65
Fig. 1.2	The relationship between mean body length vs mean fecundity and mean fresh weight vs mean fecundity of <u>P. affinis</u> reared on various host plant species	.67
Fig. 1.3	Growth curves of <u>P. citri</u> and <u>P. affinis</u> reared on various host plant species.....	72
Fig. 1.4	Growth curves of <u>P. citri</u> and <u>P. affinis</u> reared on various host plant species.....	73
Fig. 1.5	Chromatogram of the acid hydrolysed soluble phenolic compounds of various host plant species of mealybugs.....	74
Fig. 1.6	Histograms showing acid hydrolysed total phenolic concentrations of various host plant species of mealybugs.....	76
Fig. 1.7	Total nitrogen content of the leaf samples of various host plant species.....	78
Fig. 2.1	Histograms showing mean head width of <u>A. pseudococci</u> and <u>L. dactylopii</u> emerged from <u>P. citri</u> reared on various host plant species.....	113
Fig. 3.1	Histogram showing frequency of encapsulation of solitary and superparasitized eggs of <u>A. pseudococci</u> in <u>P. affinis</u>	143
Fig. 3.2	Histogram showing relationship between frequency of encapsulation and total phenol concentration of various host plant species where mealybugs were reared.....	153
Fig. 4.1	Effect of intermittent water status in the growing media on relative water content of <u>Streptocarpus</u> plants.....	185

LIST OF FIGURES (cont.)

Fig. 4.2	Effect of intermittent water stress condition on (A) percent soluble amino acid content and (B) percent total nitrogen content of <u>Streptocarpus</u> plants.....	187
Fig. 4.3	Effect of host plant water stress condition on age specific fecundity of <u>P. citri</u> and <u>P. affinis</u>	195
Fig. 4.4	Histograms showing mean head width of <u>A. pseudococci</u> and <u>L. dactylopii</u> emerged from <u>P. citri</u> reared on water stressed plants.....	203
Fig. 5.1	Effect of various concentrations of nitrogen applied to coleus and potato plants on the total nitrogen content of leaf samples.....	233
Fig. 5.2	Effect of various concentrations of nitrogen applied to coleus and potato plants on the soluble amino acid content of leaf samples.....	234
Fig. 5.3	Effect of host plant nitrogen levels on age specific fecundity of <u>P. citri</u> reared on coleus and potato plants.....	240
Fig. 5.4	Histograms showing mean body length of <u>P. citri</u> reared on coleus and potato plants maintained under 3 nitrogen levels.....	242
Fig. 6.1	Pie-diagrams illustrating the proportions of time spent in various activities by female <u>L. dactylopii</u> whilst searching on various leaf surfaces	282
Fig. 6.2	Searching pattern of female <u>L. dactylopii</u> on leaves of various plant species during its entire search bout	283

LIST OF PLATES

PLATE 1.1	<u>Passiflora coccinea</u> plants after heavy infestation with <u>P. affinis</u>	42
PLATE 1.2	<u>Streptocarpus hybridus</u> plant infested with mealybugs.....	44
PLATE 1.3	<u>Pseudococcus affinis</u> and <u>Planococcus citri</u>	46
PLATE 1.4	Apparatus to monitor sedentary feeding behaviour of mealybug through honeydew droplets	48
PLATE 1.5	SDS-PAGE (12.5% resolving gel) electrophoretic pattern of leaf extract of 6 host plant species.....	77
PLATE 2.1	Female <u>Anagyrus pseudococci</u>	100
PLATE 2.2	Female <u>Leptomastix dactylopii</u>	100
PLATE 3.1	SDS-PAGE (12.5% resolving gel) electrophoretic pattern of <u>P. citri</u> and <u>P. affinis</u> . Extraction of host protein was carried out after oviposition by <u>A. pseudococci</u> and <u>L. dactylopii</u>	146
PLATE 3.2	SDS-PAGE (7.5%-17.5% gradient resolving gel) electrophoretic pattern of <u>P. citri</u> and <u>P. affinis</u> . Extraction of host protein was carried out after oviposition by <u>A. pseudococci</u> and <u>L. dactylopii</u>	147
PLATE 3.3	SDS-PAGE (7.5%-17.5% gradient resolving gel) electrophoretic pattern of <u>P. citri</u> and <u>P. affinis</u> . Mealybug protein analysis was carried out after wound treatment or stone insertion.....	149
PLATE 3.4	Partially and fully melanized larvae of <u>A. pseudococci</u> within the body cavity of mealybug.....	156
PLATE 4.1	The size of mealybugs become extremely small when they develop on high stressed plants.....	198
PLATE 4.2	Effect of water stress on body size of mealybugs and its parasitoids.....	198
PLATE 5.1	Experimental setup for nutrient film technique to grow coleus plants under 3 nitrogen levels.....	226
PLATE 5.2	Potato plants grown on 3 nitrogen levels.....	228
PLATE 5.3	SDS-PAGE (12.5% resolving gel) electrophoretic pattern of protein extract of <u>P. citri</u> . Insects were fed on potato plants grown under 3 nitrogen levels	244
PLATE 6.1	Scanning Electronmicrographs of various leaf samples.....	269

SECTION: 1

EFFECT OF HOST PLANTS ON LIFE HISTORY PARAMETERS OF MEALYBUGS.

INTRODUCTION

Any attempt to understand the factors involved in population changes of an insect pest would be incomplete if the relationship between plant and insect is excluded. Host plants are an essential biotic factor in insect life, providing food, shelter, protection, site for attachment and in transporting the inhabiting fauna. The plant itself is a complex structure with many variations in internal and external morphology, spatial arrangement, architecture and chemical constituents (Dethier, 1966). These variations may have a positive, negative or neutral effect on herbivorous insects.

There is general acceptance that the physiological status and the species of host plant is of significance in the population dynamics of phytophagous insects. However, there are few instances in which the role of the plant in the population dynamics of mealybugs species has been qualitatively assessed in the field or laboratory condition (Tingle, 1985). At Wye College a large conservatory has a wide range of tropical crops and ornamental plants and often these plants are infested with two species of mealybugs. It was noticed that the obscure mealybug Pseudococcus affinis (Mask.), became more abundant than the common glasshouse mealybug Planococcus citri (Risso) when biological control of the latter species was applied.

A survey carried out on distribution of these two species within various plant varieties, showed that P. affinis has a wider range of hosts than P. citri. This was seen particularly in Passiflora plants where P. citri was unable to develop but P. affinis showed a remarkable success on those plants. The present study was to elucidate the possible influence of plant varieties on some biological characters such as reproduction, growth, mortality, and developmental period of two species of mealybugs. The selection of experimental plants was primarily aimed to highlight the influences of morphological and biochemical characters of host plants on the biology of these mealybug species.

Tingle and Copland (1988) studied the population dynamics of mealybugs on various host plant species with special attention to temperature changes in this conservatory. They showed that mealybug populations were reduced during the summer, but winter temperatures impaired the activities of natural enemies allowing mealybug levels to increase beyond the capacity of the natural enemy population. They highlighted the importance of ambient temperature for better performance of parasitoids. However, the potential deleterious effect of secondary plant substances such as high phenolics of some plant varieties on development, fecundity and egg viability of these insects remains unknown. The present study is an attempt to fill these gaps.

1.2 REVIEW OF LITERATURE

1.2.1 Varietal differences of host plants and insect performance.

The relationship between a herbivorous insect and its host plant primarily depends upon the species of food plant and also behaviour and biology of the insect involved (Southwood, 1987). Host trees exhibit inter-specific, inter-tree and within-tree variability in phenology, architecture or nutrition and in secondary metabolites (Faeth, 1987). Schultz, (1983) remarked that the temporal and spatial diversity of host plants represent a variable micro habitat setting a selective pressure to the inhabiting fauna on the plant. Undoubtedly these morphological and chemical variations between plant species can bring about changes in the insect populations (Southwood & Reader, 1976; Edmunds & Alstad, 1978).

The species differences among host plants have a significant effect on various aspects of herbivorous insects. Although living plant material supplies the necessary water to the herbivorous insects, air movement often causes a saturation deficit creating a drier atmosphere around the insect. However, plants with a dense foliage and higher transpiration capacity can provide a humid micro-environment for a successful colonization by most insects (Fennah, 1963).

1.2.2 Host plant morphological features and insects.

The aerial surfaces of plants display great diversity; they may be smooth or textured, covered in simple or complex hairs and of different colourations (Jeffree, 1986).

The thickness and texture of the bark of the plant is particularly important to some Hemiptera and can be a formidable barrier for the insect to pierce and locate the vascular bundles. Undoubtedly the surface structures of plants play a vital role in plant sap sucking insects (Moran & Southwood, 1982; Southwood, 1987). van Emden (1972) claimed that aphids tend to withdraw their stylets as a response to physical characteristics of the substrate, and they probably reject plants following difficulties in reaching the phloem. Further more most phloem feeders tend to aggregate in places where vascular bundles are easily accessible, particularly on more succulent plant parts (Gibson, 1972; Kidd, 1976).

Hairs on the plant parts can affect the attachment of insects, their free movement and feeding. In both natural and agro-ecosystems, the trichomes of several plant species are known to contribute to defence against many pests. Thus trichomes can be an important factor in plant resistance to insects (Gregory et al., 1986). Many authors have highlighted the importance of glandular trichomes in the resistance mechanism of various commercially grown crops such as tomato, potato and alfalfa. Gibson and Turner (1977) showed the resistance mechanism of glandular trichomes of Solanum berthaultii Hawkes towards Myzus persicae (Sulzer). Further they found polyphenoloxidase and peroxidase activities of substances secreted by the glandular trichomes of potato. Therefore this evidence suggests that trichomes can influence not only the movement of insects on the surface of plants, but their ability to reach to the real surface to feed on it (Levin, 1973; Lyman & Cardona, 1982).

1.2.3 Influence of host plants on the developmental period of insects.

There are many factors that determine resistance and these often involve more than nutritional factors (Onuf, 1978). The toxicity of plants to insects is one example (Way & Murdie, 1965) while morphology, anatomy, phenology and autoecology may often be involved. The problem of obtaining adequate nitrogen from the plant is one of the major limitations in insect development (Strong et al., 1984). Comparison of the composition of various plant materials indicate that the protein content can vary to a great degree depending on the plant variety. For instance, the quantity of protein on a dry weight basis is 4% on reedmace (Typha) and 38% on peanut (Arachis) (Southwood, 1972). Researchers have identified within plant diversity of various nutritional compounds and the importance of such variations on the distribution and abundance of herbivorous insects. For example, Heliconius larvae feed only on young growing Passiflora shoots and some tropical Satyrines, Mycalesis perseus select young and nutrient rich grass shoots for oviposition. Thus the availability of particular parts of plants has a profound influence on the behaviour and life history of herbivorous insects (Southwood, 1987).

Hsieh et al., (1974) reported that the developmental rate of alfalfa weevil larvae, Hypera postica (Gyllenhal) varied with the host plant species. The larvae took nearly 212 degree days to develop on an eastern strain variety of alfalfa while on the western strain variety they developed very slowly and required 270 degree days. The influence of host plant species on the developmental period of the coccid Icerya seychellarum (Westw) was studied by McNewbery et al., (1983). They found that on different host plants it took between 20 and 54 days.

Similarly the developmental period of the 3rd instar ranged from 35-102 days. McNewbery (1980) demonstrated that it is not the level of leaf nitrogen that is important to herbivorous insect but the rate at which nitrogen becomes available to them. The larval developmental time of Douglas-fir tussock moth Orgyia pseudotsugata McDunough, ranged from 27-36 days depending on the host plant species (Beckwith, 1976).

1.2.4 Influence of host plant on the fecundity of herbivorous insects.

Miller (1957) found that the pupal size and fecundity of spruce budworm decreases when the larvae were forced to complete development on old-growth foliage. However, Beckwith (1976) experimented on three species of fir plants and found that plant varieties have no influence on the fecundity level of Douglas-fir Tussock moth. The influence of leaf quality on fecundity was discussed by Phillips (1976) who found low fecundity of Haltica lythri Aube when they were forced to feed on senescent leaves of Epilobium hirsutum. Goker and Finch (1973) highlighted the importance of proteinaceous diet for oviposition and fecundity of cabbage root fly Erioischia brassicae (Bouche). Under normal diet flies generally get more carbohydrate and relatively less protein. To overcome protein deficiencies the flies feed on pollen from various plants and acquire sufficient amino acids to trigger the oviposition process.

Further evidence on the influence of food plant on the rate of oviposition was discussed by Schotzko and O'Keeffe (1988). The egg laying capacity of pea leaf weevil Sitona lineatus (L.), was significantly higher when the weevils were fed on pea foliage instead of alfalfa or lentil leaves.

This reduced ovipositional rate was attributed to poor quality of host plant and this demonstrates the importance of food plant on the early development of the reproductive system. Many insects exhibit retarded reproductive development when they were placed in adverse environments in which reproductive success is low. The influence of host plant factors on the fecundity level can be easily demonstrated by switching the host plant during the developmental period of a given insect.

Al-Munshi et al., (1982) reported that Lygus hesperus Knight produced significantly more nymphs on green beans and wheat than on mustard and tomato. Further it was observed that when the insects were transferred to green bean from broad bean they produced significantly more nymphs than those transferred from alfalfa.

1.2.5 Secondary plant substances and insect performance.

Plants are very rich in chemicals which are apparently not directly involved with the normal metabolic processes. Most of these secondary metabolites are connected with defence mechanisms of the plant (Harborne, 1978). Insects obtain most of these chemicals through their normal diet but some of them have evolved to deal with these chemicals either by metabolizing them into nontoxic compounds or eliminating them from their bodies (Bell, 1987). Beck (1965) considered the host plant resistance to insect attack as the collective heritable phenotypic features by which a plant species may overcome the possible threat of insect attack. Some plants store nitrogen as non-protein amino acids many of which are poisonous to insects due to antimetabolic activity. Canavanine is a toxic non-protein amino acid, stored in many legumes and is a poisonous to some insects (Rosenthal & Bell, 1979; Makita et al., 1976).

Tannins are polymeric phenolic compounds that have strong protein binding properties (Brewer et al., 1984). Tannin can adversely affect digestibility by inactivating protease enzymes such as trypsin. These plant phenolic derivatives can therefore play a vital role in the host plant resistance mechanism. Digestion-reducing substances act in proportion to their concentrations. Thus high concentrations favour the resistant properties of the plant. Feeding deterrent properties of phenolic derivatives were discussed by Burnett et al., (1974) and Picman et al., (1978). They reported that the leaf resin of Hymenaea courbaril is composed of a mixture of sesquiterpenes which are toxic and have feeding deterrent properties against the generalist herbivore, such as beet armyworm, Spodoptera exigua (Hubner). Some insects can resist hydrolysable tannins by possessing high pH (average of 8.8) in the midgut which eliminate the digestion-inhibiting properties of these compounds (Berenbaum, 1981).

Many plants have specialist phytophages that are resistant to noxious phytochemicals and some have evolved to sequester these toxic compounds to render themselves unsuitable for their natural enemies. Alkaloids are complex nitrogenous bases of diverse molecular structures occurring in many plants. Involvement of alkaloid derivatives in insect defence was discussed by Spencer (1988). He carried out a survey for cyanogenesis in Passifloraceae and found two-thirds of the species were cyanogenic positive. Cyanogenic glycosides are known toxins that have been demonstrated to be important plant defence chemicals against herbivores (Angseesing & Angseesing, 1973; Cooper-Drive & Swain, 1976). However, some animals are capable of rapidly and effectively detoxifying hydrogen cyanide (Brattsten et al., 1983). It has also been reported that cyanides below the lethal concentrations can retard larval growth of some insects (Brattsten et al., 1983).

1.2.6 The experimental plants.

1.2.6.1 Passiflora caerulea.

Glabrous, often glaucous climber of great vigour with palmately 5-7 lobed, 100-180 cm across, oblong-lanceolate leaves. Leaves and stems are smooth with few trichomes. Flowers are solitary, slightly fragrant and white in middle, purple at base. This species has cyanogenic compounds as secondary plant metabolites. Generally distributed in central and western South America (Seigler, 1982).

1.2.6.2 Passiflora coccinea.

Climber with grooved downy stems. Leaves 70-150 cm long and 30-70 cm wide rounded to subcorate at base, irregularly toothed, 2-6 glanded (Plate 1.1). Leaves and tender branches have a reasonably dense trichome cover. Flowers yellowish outside, scarlet within and with a deep purple corona. This species has Passicoccin and Epipassicoccin as the cyanogenic compounds. It has been reported that Passiflora tannins are effective antifeedants even at low concentrations. This species is generally distributed in tropic and South American countries (Spencer, 1988).

1.2.6.3 Psophocarpus tetragonolobus (L.), Winged bean .

The winged bean is a climbing, herbaceous perennial. Stems are usually green and flowers are blue, purple or white. Pods are four-cornered and rectangular or square in cross section with wings at each corner. Leaves and stems are relatively free from trichomes.



PLATE 1.1 Passiflora coccinea plants after heavy infestation with P. affinis. (Note the effect of infestation on defoliation)

The winged bean contains about as much protein and amino acids as the soy bean. Like most of the other legumes, the winged bean is relatively deficient in lysine (Anon 1981). Seeds are reported to contain trypsin inhibitors, chymotrypsin inhibitors and haemagglutinins. Hydrogen cyanide has been reported from the stems. Winged bean appears to be indigenous to Africa (Duke, 1981).

1.2.6.4 Glycine max (L.), Soy bean .

Cultivated species are usually variable because of artificial selection under diverse environments. Leaves pinnately 3 foliolate and grow to varying size depending on the environmental conditions. The leaves and tender branches are covered with bushy rather coarse trichomes. Soy beans have relatively high protein content and their amino acid profile, is both superior to and complementary to that of other leguminous plants (Allen & Allen, 1981).

1.2.6.5 Streptocarpus hybridus.

Streptocarpus belongs to the family Gesneriaceae and tribe Didymocarpeae. The leaves do not have a basal meristem and they do not all subtend inflorescences. Streptocarpus has well developed rhizomes. The leaf is often oblique, upper surface dark green, pilose on the veins, pubescent between them, lower surface pale green and full of densely packed trichomes (Plate 1.2 & 6.1). The cultivated variety is a hybrid of wild varieties (Hilliard & Burtt 1971).



PLATE 1.2 Streptocarpus hybridus plant infested with mealybugs. The distribution of mealybug is always confined to senescing, fully matured or damaged leaves.
(Note clumps of white, cottony egg mass)

1.2.6.6 Aeschynanthus ellipticus.

These epiphytes, of which more than 170 species are known, are found in the humid forests of Asia. They are also members of Gesneriaceae. These herbaceous plants produce scarlet, orange or yellow flowers and grow either terminally or in the axils of the leaves. The shortened calyx consist of five sepals. The four stamens nearly always extend beyond the petals. The leaves are opposite, fleshy or leathery, green or marbled and without trichomes (Herwig, 1984).

1.2.7 The pest.

1.2.7.1 Planococcus citri (Risso): The citrus or common mealybug.

This species occurs very commonly in glasshouses (Tingle & Copland, 1988) in Europe and North America, and is distributed around the world and can attack almost any flowering plant. The adult female is oval, flattened with a thickly dusted powdery wax cover over the body. All lateral wax tassels are very short and can be used to distinguish the species from others. The 17 pairs of cerarii are positioned around the body margin, with a uniform distance. The anal filaments are slightly longer than the others but comparatively shorter than those of P. affinis (Smith & Armitage, 1931) (Plate 1.3). The body length of an adult female is 2.5-4.0 mm and 2.0-3.0 mm in width and the antennae are 8-segmented. A fluffy ovisac is formed at maturity and within which straw-yellow eggs which range from 0.25-0.40 mm in size are laid (Sutherland, 1932).



PLATE 1.3 Pseudococcus affinis (Maskell), obscure mealybug (above) and Planococcus citri (Risso), citrus or common mealybug (below). (Note, the long caudal filaments of P. affinis. (Magnification x10 approx.)

The first instars (crawlers) are oval in shape, white, with well developed antennae. The rostral filaments are well developed and have no lateral wax tassels. This is the dispersive phase of the life cycle and they move about to find new feeding sites. The second instars are active feeding stages and a less mobile phase. After the second moult the sexes become apparent as males become dark brown pre-pupae, which produce a cottony cocoon around the body. The 3rd instar female has 7 antennal segments with 7 pairs of lateral cerarii and an incomplete mid-dorsal line (Sutherland, 1932). P. citri has been identified as the vector of cacao and tobacco virus diseases (Entwistle, 1972).

1.2.6.6 Pseudococcus affinis (Maskell). The obscure mealybug.

P. affinis (= P. obscurus Essig) can feed on almost any flowering plant and is widely distributed around the world. It occurs on any part of the plant from the crown to the leaves and fruit. The length of the adult female ranges from 4.5-5.0 mm and has an elongated, convex shape. The female is reddish brown in colour with powdery wax and quite long (half of the body length) caudal filaments. The lateral filaments are relatively shorter and become progressively shorter toward the head (Panis, 1979). There is no visible mid-dorsal line but the inter segmental demarcations are very prominent. At maturity females produce an ovisac and may lay more than 500 brownish yellow eggs during a 5-18 days ovipositional period. The first instar is the mobile phase and can be distinguished from other stages because of two prominent rostral filaments. At the end of the 2nd instar the males form a white cocoon while females continue to feed until moulting to the 3rd instar. The 3rd instars are similar to that of adults except for the half developed caudal and lateral filaments (Panis, 1979). Mealybugs are sedentary feeders, and rarely change their feeding position (Plate 1.4).



PLATE 1.4 Apparatus to monitor sedentary feeding behaviour of mealybug through honeydew droplets. The honeydew droplets are being collected to a filter paper attached to a rotating device (one revolution per 24 hours). The collected droplets are treated with 1% ninhydrin solution followed by oven drying at 80°C for 4 hours. The filter paper was replaced every 24 hours. Note stained droplets in a circular arrangement indicating sedentary feeding behaviour of mealybug. No further detail is given in the thesis.

1.3 MATERIALS AND METHODS

1.3.0.1 Preparation of plant materials.

Rooted cuttings of Passiflora caerulea and Passiflora coccinea were potted in 10 cm plastic pots containing 4:1 ratio peat/grit compost. These plants were prepared 5 months prior to the experiment and kept in a heated glasshouse. During this period all the plants were given 500 ml liquid fertilizer, (standard Cooper solution, see Appendix 2) twice a week and every other day plants were adequately watered. Growing plants were supported with 2 m sticks to facilitate climbing.

Gesneriaceae, Streptocarpus hybridus and Aeschynanthus ellipticus were planted in similar sized pots and nutrient and watering carried out as described above.

Leguminous plants, Winged bean Psophocarpus tetragonolobus and Soy bean Glycine max, seeds were potted 2 months before the experiment, treated similarly to the above. The climbers were supported with vertical sticks.

1.3.0.2 Physical Environment.

The glasshouse cubicle was kept heated to a minimum temperature of 26°C throughout the experimental periods with a relative humidity of ca. 70 - 80% by regularly wetting the capillary matting. Supplementary lighting was provided by 400W mercury bulbs (1 bulb per table), to avoid short daylength in the latter part of the experiment. All the host plants were kept under similar environmental conditions up to the flowering stages to obtain uniformly matured plants.

They were free from mealybug infestation until they were experimentally infested with different numbers of crawlers.

1.3.0.3 Method of obtaining crawlers.

Egg masses of P. citri and P. affinis were obtained from pure cultures maintained at Wye College. These egg masses were introduced to 10 plants of each of the six species and maintained until oviposition. The two species of mealybugs were separated by keeping these plants in separate glasshouse cubicles to avoid possible mixing of the two progenies. The aim of this was to obtain a cohort of F₁ generation, reared on respective host plant species. However, this was not possible for Passiflora species. The first and second day collection of egg masses were removed and transferred to ELYSA plates (13 x 3 x 1 cm) for incubation.

1.3.0.4 Incubation of mealybugs eggs.

The holes of the ELYSA plates were half filled with wet absorbent cotton wool to keep the micro-environment at a high moisture level. After placing egg masses in each hole, the plates were covered by a glass plate, fastened with rubber bands and then placed in an incubator at 28±1°C. Three plates were used for each species of plant and mealybug. These ELYSA plates were observed daily under a stereo-microscope to check the emergence of crawlers. The total number of crawlers emerged on the first day were introduced to the experimental plants.

1.3.1 Influence of host plant on the developmental period of mealybugs.

The primary objective was to study the influence of various host plant species on the developmental period of different stages of mealybugs. Insect cages were used to confine the insects to the host plants.

Insect cages.

The cages consisted of "Stewart" plastic boxes (17.3 x 11.5 x 6.3 cm), with ventilation supplied through upper and lower side openings 150 mm x 100 mm and covered with nylon gauze (40 mesh/cm). Two holes were made on the sides to enable the branch to pass through the box. Cotton wool was used to plug any hole to prevent crawlers escaping

Five plants with insect cages of each plant species were randomly used for each species of mealybug and each plant species. Fifty, one day old crawlers were taken from the ELYSA plates (1.3.0.4) and transferred to each experimental branch in the insect cage. The total number of insects and their developmental stages were recorded every 24 hours.

Developmental stages were recognized by external morphological features and cast skins were counted and removed at the end of every count.

The experiment was continued until most of the insects had reached the adult stage. The developmental period for each instar was calculated separately. The time to develop into a successive instar was considered with the number of surviving insects in the respective instar stage for the analysis. Due to natural mortality factors the number of insects in each replicate diminished with time. Therefore a uniform number of insects was taken from each replicate for analysis of variance. One way analysis of variance was performed to obtain the mean values and significant differences were tested using Student Newman Keul test (SNK).

1.3.2 Influence of the host plant on the pre-ovipositional period of mealybugs.

Relevant information for the present study was obtained from the experiment described in 1.3.1. Five, late third instar mealybugs per replicate and mealybug species, were separated from the batch of insects in the insect cages and removed. These insects were recaged using leaf cages and were examined daily until ovisac formation.

The pre-ovipositional period of individual adults was considered as the duration from eclosion of the adult to the first day of egg laying. The data were used in one way analysis of variance to obtain treatment means.

1.3.3 Influence of plant nutrient on mealybug fecundity.

Crawlers of P. citri and P. affinis were obtained from the ELYSA plates (see experiment 1.3.0.3) and were introduced to selected host plant species maintained in a glasshouse cubicle. Five host plants per plant species and mealybugs species were used. These plants were maintained as described above until most of the insects reached the adult stage. Insects were observed until they formed an ovisac and 5 insects per plant were removed to determine daily egg production. The mealybugs were transferred to ELYSA plates and each day the ovisacs were removed for counting eggs. The same batch of eggs were used for further studies on percentage viability and incubation period. One way analysis of variance was carried out to obtain mean values and the means were separated using the SNK test.

1.3.4 Influence of host plant on mealybug egg viability.

The relevant information for the present study was taken from the experiment described in 1.3.3. The egg masses were placed in labelled ELYSA plates and a similar method as in experiment 1.3.0.4 was followed to obtain developing larvae. Daily emergence of crawlers were recorded for up to 15 days and the unhatched eggs were considered as non-viable. At the end of the incubation period, the percentage viability of eggs were calculated on an individual insect basis. Finally the percentage values were subjected to a square root transformation before using analysis of variance.

1.3.5 Influence of host plant on mealybug egg incubation period.

The necessary information for this analysis was obtained from experiment 1.3.3 and the egg masses in the ELYSA plates were checked every 24 hours, for emerging crawlers. Observations were carried out for upto 15 days allowing most of the eggs to hatch.

The mean incubation period of an individual insect was calculated as follows:

- (1) Daily proportional emergence value was multiplied by the time taken to hatch the eggs (E). This was carried out for total hatching period of the particular egg mass.
- (2) The cumulative value of E was divided by the total number of eggs hatched in the particular egg mass.

The mean values were used in one way analysis of variance.

1.3.6 Influence of host plant on mealybug body weight.

The insects for this study were collected from the experiment 1.3.3, where they had fed throughout their life on the respective plant species. A total of 10 fully mature mealybugs per plant and eight plants (replicate) per treatment were used, to collect fully matured adult mealybugs. The insects were kept in labelled vials to avoid any desiccation and were weighed by using a Sartorius electronic microbalance (model 4503 MP6 accuracy 1 μ g). One way analysis of variance was performed to obtain treatment means and mean values were separated using SNK test.

1.3.7 Correlation between weight, length and fecundity of mealybug.

The intention of this experiment was to investigate the possible relationship between adult body length, and weight on the fecundity levels of 2 species of mealybug fed on different plant species. Insects for the present study were collected from the experiment 1.3.3. Five fully mature mealybugs per species and 5 plants per variety (treatment) were allocated to collect insects. These insects were kept in labelled vials then their body lengths measured and weighed and transferred to labelled ELYSA plates and kept in an incubator $26 \pm 1^\circ\text{C}$. The eggs produced by these insects were removed daily to obtain their total fecundity. Linear regression analysis was carried out for each independent variable.

1.3.8 Influence of host plants on the growth parameters of mealybug.

A sample of 25 mealybugs of each species were taken from 5 plants of each plant species at weekly intervals. Insects were transferred to labelled vials with 1 ml of saline solution and their length measured as described in experiment 1.3.7. The mean body lengths of insects were used in a polynomial equation to obtain estimated values to construct growth curves.

1.3.9 Analysis of host plants phenolics

There are several methods available for measuring plant phenolics namely, ultraviolet (uv) absorbance of the extract, high pressure liquid chromatography (hplc) and gas liquid chromatography (glc) of silylated derivatives (van de Castele et al., 1976).

Acid hydrolysis, silylated derivatives method

Leaf discs were taken using a 14 mm diameter cork borer. The discs were immediately weighed and shredded into labelled conical flasks (50 ml) containing 15 ml of 70% methanol. The samples were boiled for 10-15 minutes until all the methanol had been removed. Subsequently another 15% distilled water was added and boiled until a volume of approximately 1 ml was obtained. This was then filtered through 9 cm Whatman No 1 filter papers to labelled test tubes. The shredded plant material was then ground in a pestle and mortar and the whole process repeated once again. The same labelled test tube was used to collect the filtrate of the second time extract.

The filtrate was acidified to pH 2 with 1N HCl, extracted 3 times with equal volumes of diethyl ether. The ether extracts were evaporated to dryness in a rotavapor under vacuum at 40°C. The phenolic substances were then redissolved in 20 ml of 100% methanol and transferred to small stoppered test tubes and evaporated to dryness under vacuum at room temperature. The samples were then dissolved in 0.1 ml of pyridine and 0.1 ml of Bis trimethylsilyl trifluoroacetamide (BSTFA) mixture was kept at 80°C for 3 hours to produce trisilyliated derivatives.

Gas chromatography was carried out under the following conditions.

Columns	- 5% OV-17 on chromosorb WHP mesh range 100-120
Carrier gas	- Nitrogen
Injector temp:	- 200°C
Detector temp:	- 350°C
Column initial	- 150°C
Column final	- 300°C (12 min temp gradient)

The graphic recorder was adjusted before the recording started.

The working standard, (0.1 mg sample dissolved in a μ l N teracosane and pyridine was added to v/v equal volume of BSTFA) was injected to obtain a respective peak area. Using this peak area, the concentrations of different phenols in the samples were calculated. The experiment was repeated with fresh leaf samples 3 times to obtain the means and the s.d.

1.3.10 Analysis of host plants total nitrogen content

Fully matured leaves were removed from each host plant species at weekly intervals. The leaf samples were immediately freeze dried for 24 h and a 0.1 g sub-sample was taken from each sample for micro Kjeldahl analysis. Three sub-samples were analysed per plant variety to obtain weekly mean values and s.d.

1.4 RESULTS

1.4.1 Influence of host plant on the developmental period of mealybug.

The mean number of days required for each instar to complete development is shown in Table 1.1.1. P. citri was unable to grow on Passiflora plants. The crawlers developed slowest on winged bean, then Streptocarpus and fastest on A. ellipticus and soy bean plants. The 2nd instar developed faster on soy bean and A. ellipticus than on winged bean and were slowest on Streptocarpus ($P < 0.05$). However, the 3rd instar larvae of P. citri fed on winged bean plants completed their development within 9.5 days which is faster ($P < 0.05$) development than all the other treatments. The longest development of 3rd instar was on Streptocarpus plants.

Table 1.1.1 Influence of host plant on the developmental period of larval stages of P. citri.

MEAN DEVELOPMENTAL PERIOD (DAYS)									
HOST PLANTS	1st instar			2nd instar			3rd instar		
	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.
<u>S.hybridus</u>	75	14.9b	0.23	50	19.5d	2.3	25	23.7p	0.7
<u>A.ellipticus</u>	75	11.8c	0.13	50	11.6f	1.1	25	16.4q	1.2
<u>G.max</u>	75	11.8c	0.66	50	10.8f	1.4	25	13.4r	1.9
<u>P.tetragonolobus</u>	75	19.8a	1.60	50	15.0e	1.4	25	9.5s	2.2
SNK test	(16,5) $S\bar{x}=0.407$ MS=0.830			(16,5) $S\bar{x}=0.74$ MS=2.74			(16,5) $S\bar{x}=0.729$ MS=2.66		

n = number of insects. Each mean is an average of 5 replicates. Figures with the same letters within column are not significantly different at the 5% level.

Table 1.1.2 illustrates the influence of host plants on the developmental period of P. affinis. The crawlers fed on soy bean plants, showed a faster development than the insects grown on other plant species ($P < 0.05$). Development took twice as long as on A. ellipticus. The second and third instars grew significantly faster on the leguminous hosts than on the other species.

Table 1.1.2 Influence of host plant on the developmental period of larval stages of P. affinis.

MEAN DEVELOPMENTAL PERIOD (DAYS)									
HOST PLANTS	1st instar			2nd instar			3rd instar		
	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.
<u>S. hybridus</u>	75	15.3b	0.6	50	22.2d	2.3	25	22.7f	2.0
<u>A. ellipticus</u>	75	21.8a	2.5	50	17.1d	6.5	25	18.4g	1.9
<u>G. max</u>	75	11.9c	0.4	50	13.2e	1.1	25	10.0h	0.7
<u>P. tetragonolobus</u>	75	19.3a	2.4	50	12.6e	1.3	25	11.3h	1.4
<u>P. coccinea</u>	75	20.5a	3.1	50	17.4d	2.2	25	16.7g	2.4
<u>P. caerulea</u>	75	19.5a	1.5	50	18.4d	1.8	25	20.0g	3.2
SNK test	(24,5) $S\bar{x}=0.9$ MS=4.0			(24,5) $S\bar{x}=1.4$ MS=10.0			(24,5) $S\bar{x}=0.94$ MS=4.4		

n = number of insect. Each mean is an average of 5 replicates. Figures with the same letters within column are not significantly different at the 5% level.

1.4.2 Influence of host plant on the pre-ovipositional period of mealybug.

Table 1.2 shows the results of the study. The pre-ovipositional periods on soy and winged bean reared insects were similar ($P > 0.05$), but took longer on Gesneriaceae host plants. P. affinis showed a relatively faster egg maturation on leguminous hosts, moderate on Passiflora and slowest on the Gesneriaceae.

Table 1.2 Influence of host plant on the pre-ovipositional periods of mealybug.

MEAN PREOVIPOSITIONAL PERIOD (DAYS)						
HOST PLANTS	<u>P. citri</u>			<u>P. affinis</u>		
	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.
<u>S. hybridus</u>	25	15.0a	1.0	25	17.6p	2.6
<u>A. ellipticus</u>	25	12.1b	1.8	25	18.2p	0.4
<u>G. max</u>	25	9.8c	1.6	25	9.5r	0.8
<u>P. tetragonolobus</u>	25	9.7c	0.2	25	8.4r	1.0
<u>P. coccinea</u>	-	-	-	25	12.5q	1.8
<u>P. caerulea</u>	-	-	-	25	14.6q	2.3
SNK test	(16,5)	$\bar{Sx}= 0.48$ MS=1.16		(24,5)	$\bar{Sx}=0.76$ MS=2.92	

n = number of insects. Each mean is an average of 5 replicates. Figures with the same letters within column are not significantly different at the 5% level.

1.4.3 Influence of host plant on mealybug fecundity.

The mean fecundity of P. citri was least on A. ellipticus and highest on the leguminous plants while they were unable to reproduce on Passiflora. P. affinis reproduced well on leguminous and on P. caerulea plants and least well on the Gesneriaceae.

Table 1.3 Influence of host plant on mealybug fecundity.

MEAN NUMBER OF EGGS PER FEMALE						
HOST PLANTS	<u>P. citri</u>			<u>P. affinis</u>		
	n	$\bar{x}$	s.e.	n	$\bar{x}$	s.e.
<u>S. hybridus</u>	25	268.8e	7.90	25	220.3f	9.7
<u>A. ellipticus</u>	25	195.1f	7.30	25	157.6g	7.2
<u>G. max</u>	25	474.0a	11.70	25	432.0bc	11.3
<u>P. tetragonolobus</u>	25	463.0ab	18.50	25	416.0c	13.3
<u>P. coccinea</u>	-	-	-	25	341.0d	14.0
<u>P. caerulea</u>	-	-	-	25	436.0abc	15.2
SNK test	(240, 25) $S\bar{x} = 12.2$ MS = 3701.8					

n = number of insects, comparisons can be made between any means. Figures with the same letters are not significantly different at the 5% level.

1.4.4 Influence of host plant on mealybug egg viability.

Table 1.4 shows there is no significance influence of host plant species on the percentage viability of mealybug eggs ($P > 0.05$).

Table 1.4 Influence of host plant on egg viability.

PERCENTAGE VIABILITY						
HOST PLANTS	<u>P. citri</u>			<u>P. affinis</u>		
	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.
<u>S. hybridus</u>	25	97.7a	3.9	25	93.9a	12.6
<u>A. ellipticus</u>	25	97.7a	6.4	25	98.3a	03.4
<u>G. max</u>	25	98.1a	2.3	25	98.2a	11.5
<u>P. tetragonolobus</u>	25	93.7a	8.0	25	98.2a	02.5
<u>P. coccinea</u>	-	-	-	25	98.1a	05.3
<u>P. caerulea</u>	-	-	-	25	96.0a	08.6
SNK test	(240,25) $S\bar{x} = 0.089$, $MS = 0.1985$					

Calculations were based on square root transformed data. Untransformed means are presented in the Table. n = number of insects. Figures with the same letters are not significantly different at the 5% level.

1.4.5 Influence of host plants on mealybug egg incubation period.

The results shown in Table 1.5, indicated that the variety of host plant has no influence on the incubation period of mealybug eggs. However, the incubation period of P. citri was faster than that of P. affinis, under identical conditions

Table 1.5 Influence of host plant on mealybug egg incubation period.

MEAN INCUBATION PERIOD (DAYS)						
HOST PLANTS	<u>P. citri</u>			<u>P. affinis</u>		
	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.
<u>S. hybridus</u>	25	5.0b	0.2	25	8.4a	0.3
<u>A. ellipticus</u>	25	5.1b	0.3	25	8.5a	0.3
<u>G. max</u>	25	5.2b	0.1	25	8.3a	0.1
<u>P. tetragonolobus</u>	25	4.7b	0.1	25	8.8a	0.1
<u>P. coccinea</u>	-	-	-	25	8.9a	0.1
<u>P. caerulea</u>	-	-	-	25	8.6a	0.1
SNK test	(240,25) $\bar{S}_x = 0.20$, MS = 1.02					

n = number of insects. Comparison may be made between any mean. Means with identical letters are not significantly different at the 5% level.

1.4.6 Influence of host plant on mealybug body weight.

The results are shown in Table 1.6. The mean body weight of P. citri reared on leguminous plants was greater than those developed on Gesnereaceae plants ($P < 0.05$). Similarly the mean body weight of P. affinis was greatest on leguminous and P. caerulea and least on Gesneriaceae host plants. Generally P. affinis is a larger mealybug than P. citri.

Table 1.6 Influence of host plant on mealybug body weight.

MEAN BODY WEIGHT OF FEMALE IN mg.						
HOST PLANTS	<u>P. citri</u>			<u>P. affinis</u>		
	n	$\bar{x}$	s.e.	n	$\bar{x}$	s.e.
<u>S. hybridus</u>	80	2.0e	0.1	80	2.6d	0.1
<u>A. ellipticus</u>	80	1.9e	0.2	80	2.0e	0.1
<u>G. max</u>	80	3.5c	0.2	80	5.8a	0.09
<u>P. tetragonolobus</u>	80	3.4c	0.09	80	5.0b	0.2
<u>P. coccinea</u>	-	-	-	80	3.7c	0.1
<u>P. caerulea</u>	-	-	-	80	5.3b	0.2
SNK test	(70,80) $S\bar{x} = 0.12$, $MS = 0.107$					

n = number of insects. Each mean is an average of 8 replicates. Figures with the same letters are not significantly different at the 5% level.

1.4.7 Correlation between weight, length and fecundity of mealybug.

Table 1.7.1 Correlation of weight and length with fecundity of P. citri.

HOST PLANTS	MEAN FECUNDITY			MEAN BODY WEIGHT mg			MEAN BODY LENGTH mm		
	n	$\bar{x}$	s.e.	$\bar{x}$	s.e.	r^2	$\bar{x}$	s.e.	r^2
<u>S. hybridus</u>	25	261	18.6	2.1	0.32	0.38*	2.6	0.12	0.39*
<u>A. ellipticus</u>	25	201	9.6	2.0	0.18	0.82**	2.5	0.10	0.92**
<u>G. max</u>	25	465	14.9	3.6	0.19	0.58*	3.4	0.08	0.63*
<u>P. tetragonolobus</u>	25	457	12.6	3.3	0.20	0.83**	3.3	0.06	0.67*

Significance was tested using t-test, * = 5%, ** = 1% level significant.

The linear correlations (Tables 1.7.1 and 1.7.2) and linear regressions (Fig. 1.1 - 1.2) between mealybug fecundity and body weight and fecundity and body length indicate positive correlations and linear relationship between the above variables. The correlation coefficient values for body weight vs fecundity ranged between 0.38-0.83 for P. citri and 0.64-0.83 for P. affinis, indicating a high positive correlation. Similar correlations for length and fecundity ranged from 0.39-0.92 for P. citri and 0.57-0.92 for P. affinis. The weight or length of the adult mealybugs were equally related to the fecundity level of the insect.

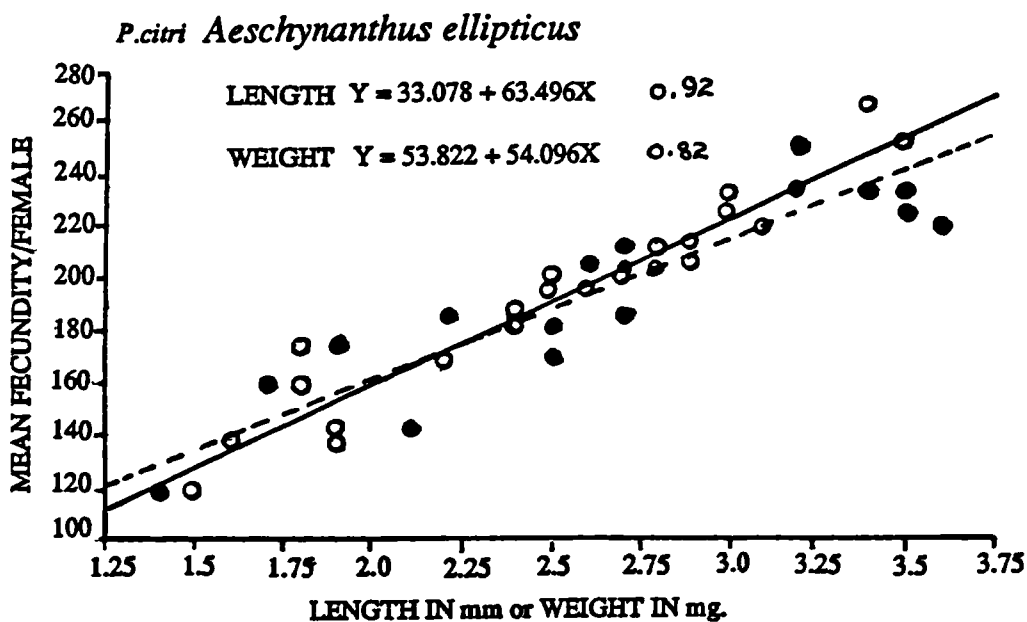
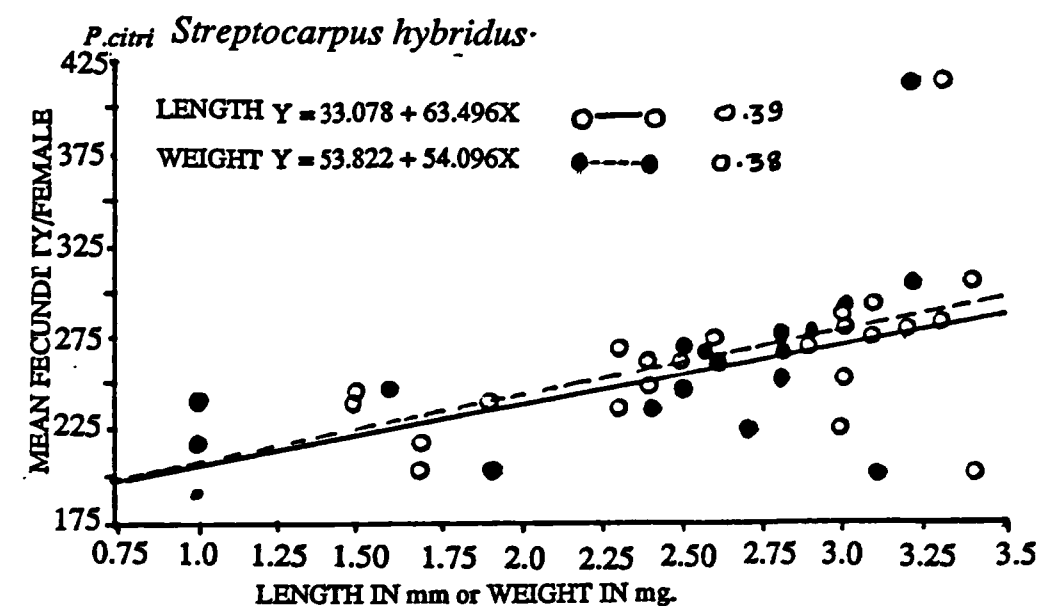


Fig. 1.1 The relationship between mean body length vs mean fecundity and mean fresh weight vs mean fecundity of *P. citri* reared on various host plant species. The regression lines were fitted using calculated values. Both independent variables were marked on the x axis.

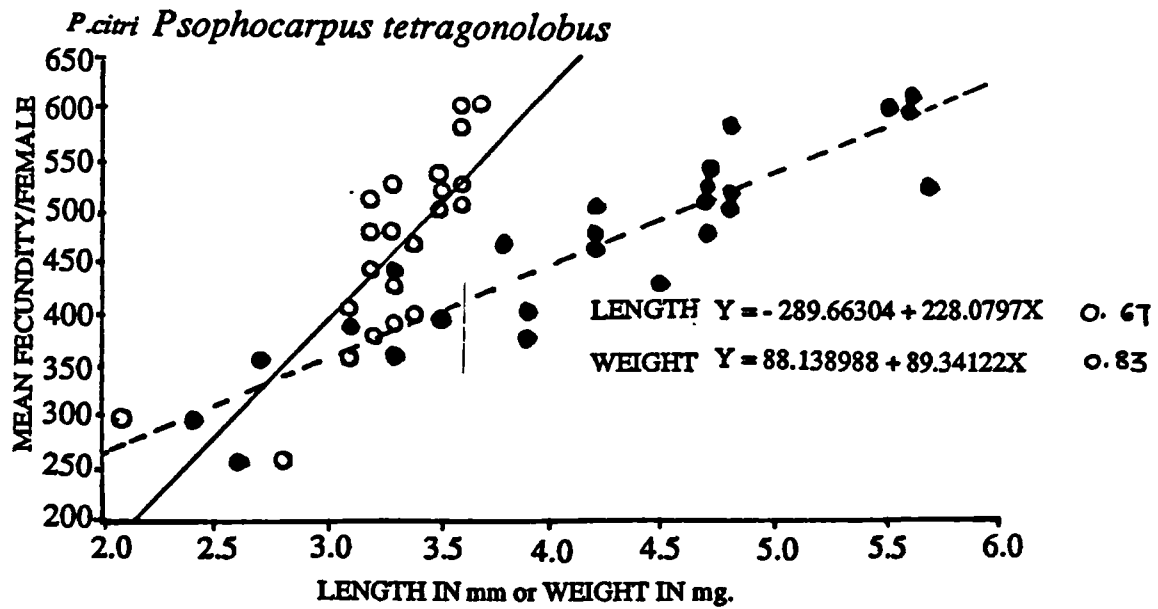
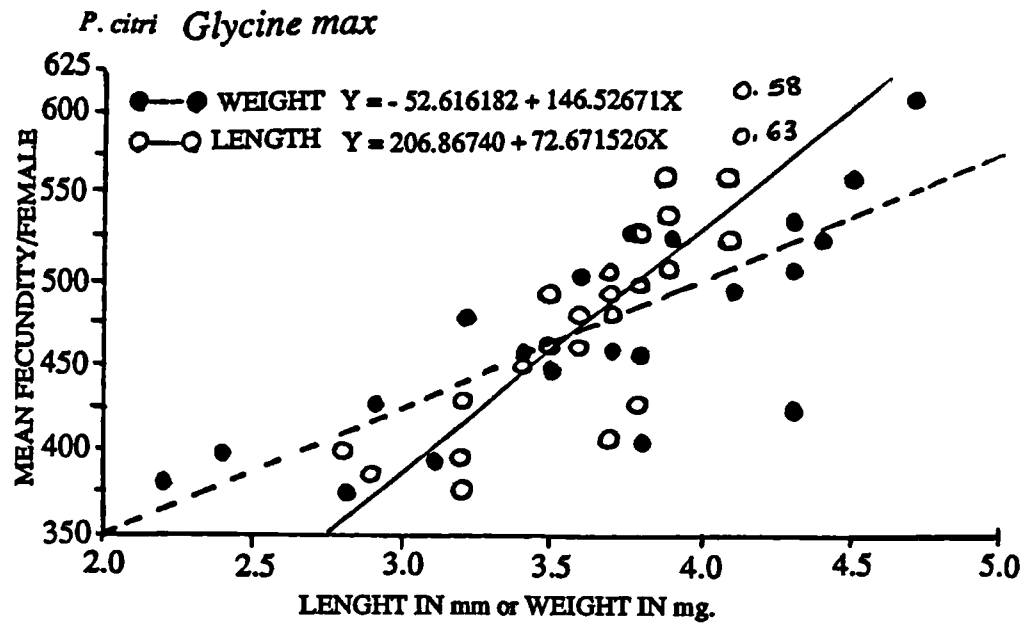


Fig. 1.1 contd.

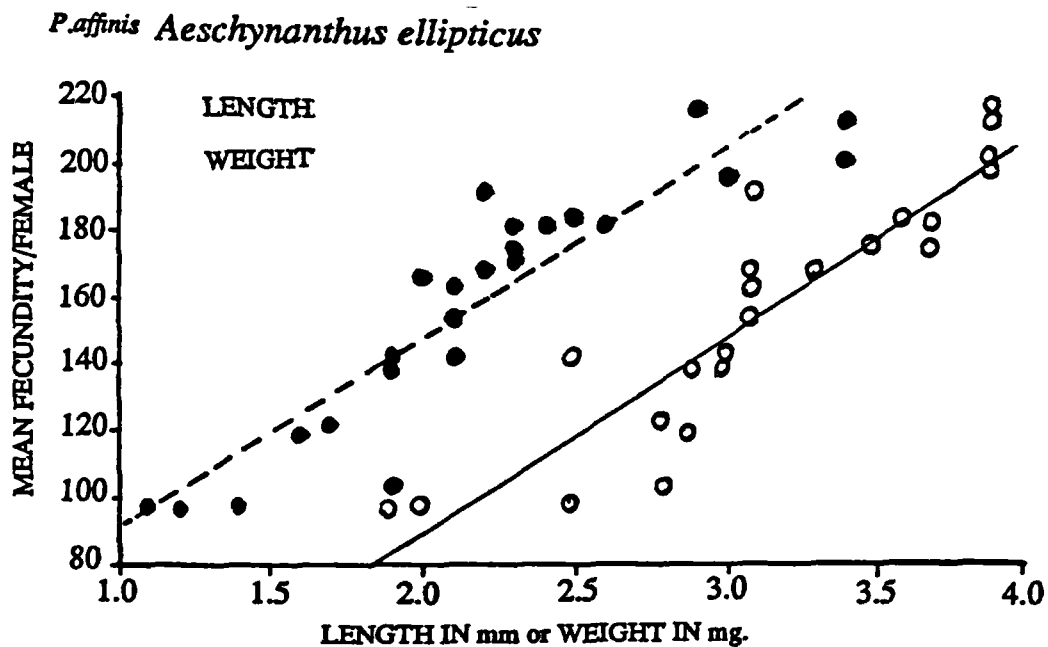
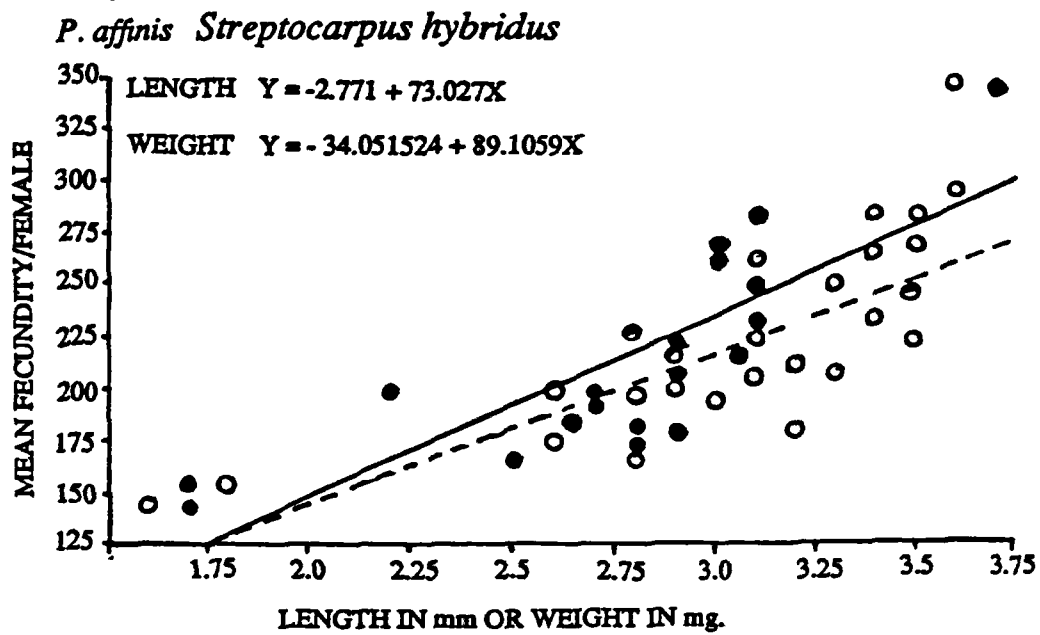


Fig. 1.2 The relationship between mean body length vs mean fecundity and mean fresh weight vs mean fecundity of *P. affinis* reared on various host plant species. The regression lines were fitted using calculated values. Both independent variables were marked on the x axis.

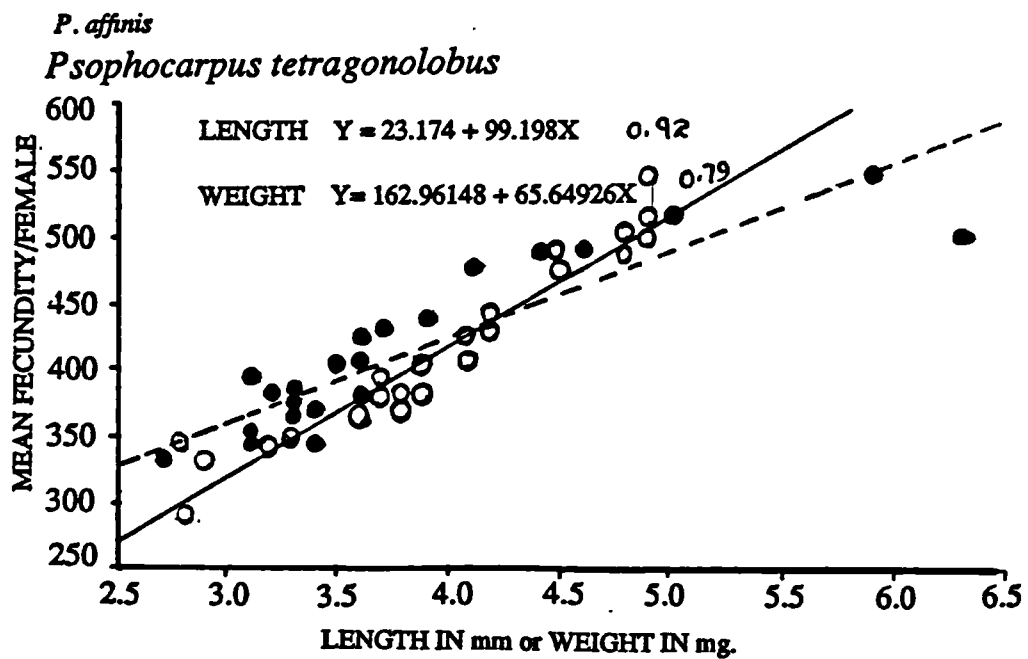
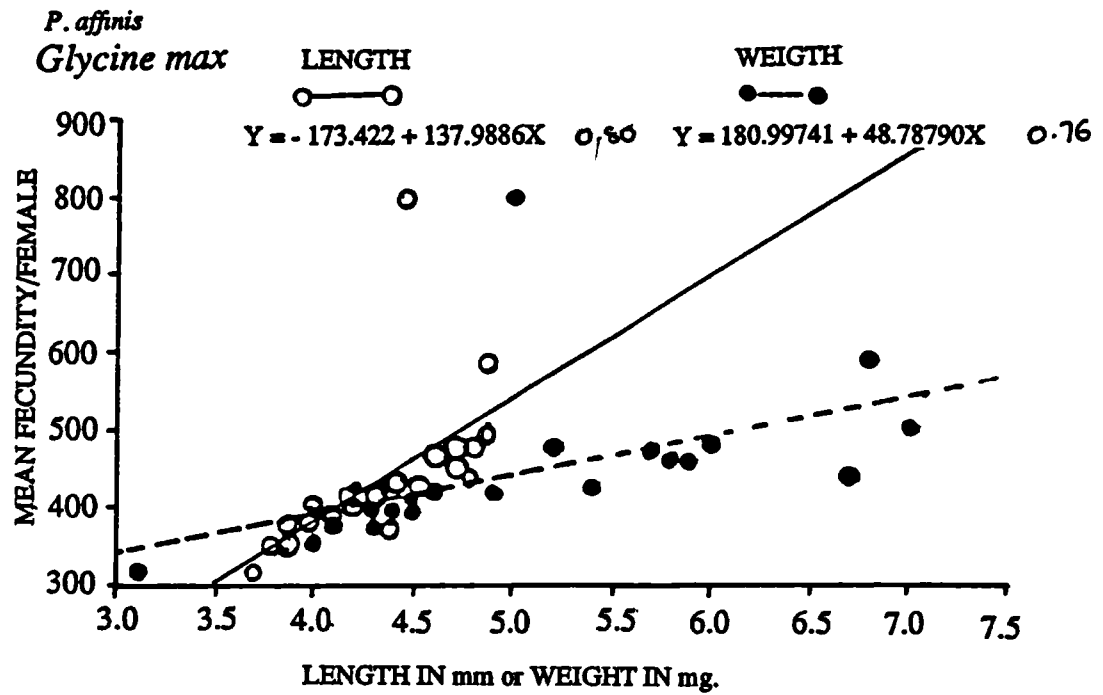


Fig. 1.2 contd.

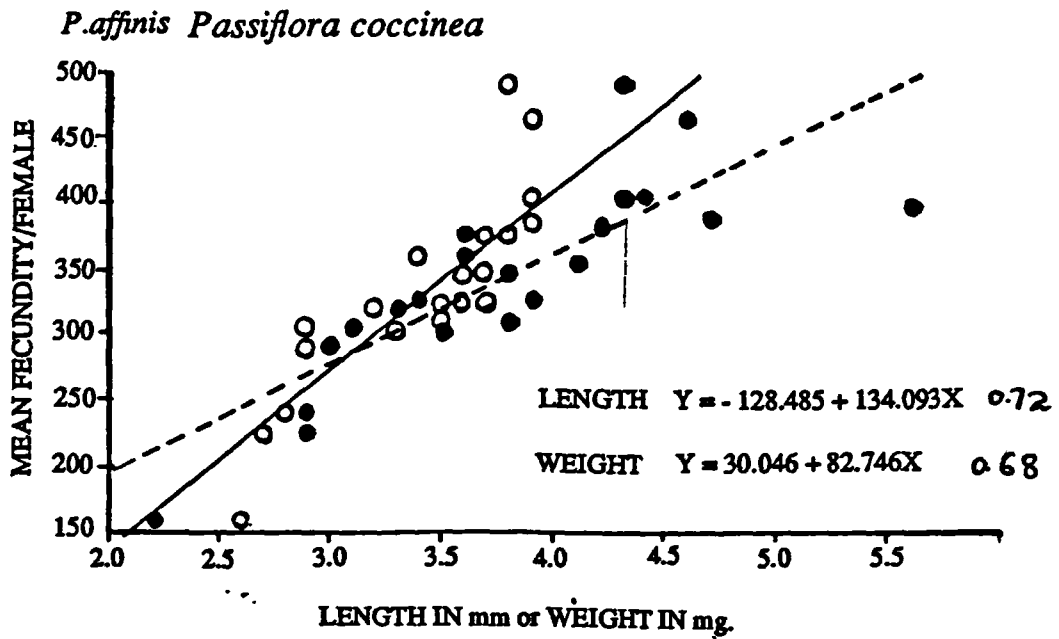
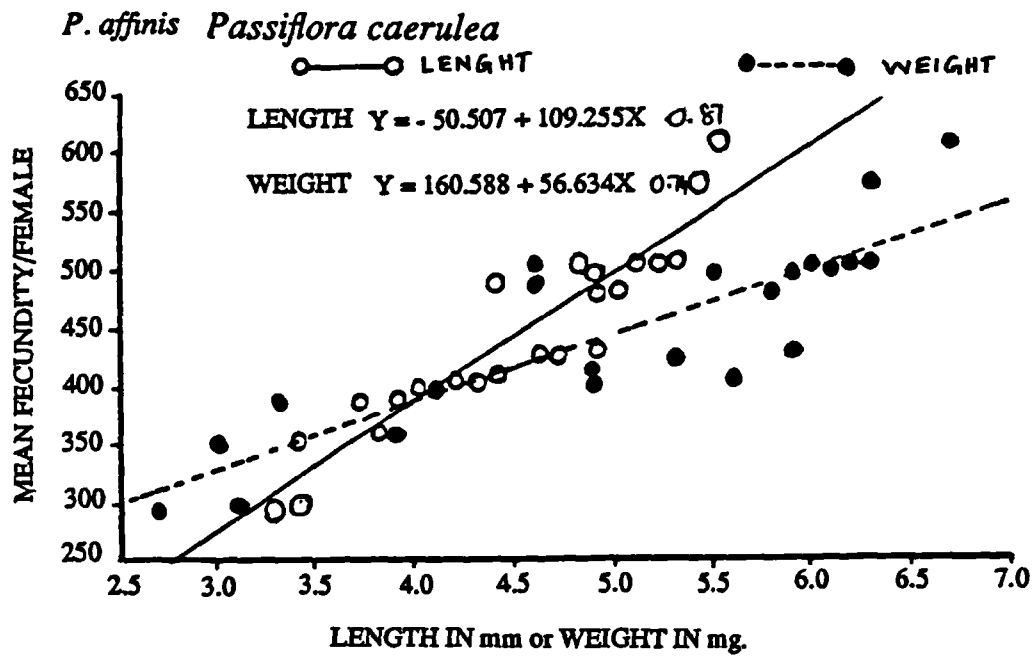


Fig. 1.2 contd.

Table 1.7.2 Correlation of weight and length with fecundity of P. affinis.

HOST PLANTS	MEAN FECUNDITY			MEAN BODY WEIGHT mg			MEAN BODY LENGTH mm		
	n	$\bar{x}$	s.e.	$\bar{x}$	s.e.	r^2	$\bar{x}$	s.e.	r^2
<u>S. hybridus</u>	25	121	10.2	2.3	0.12	0.64*	3.0	0.10	0.57*
<u>A. ellipticus</u>	25	181	9.6	2.1	0.12	0.83**	3.2	0.11	0.82**
<u>G. max</u>	25	451	13.2	5.6	0.20	0.76*	4.5	0.13	0.80**
<u>P. tetragonolobus</u>	25	423	11.2	5.3	0.10	0.79**	3.9	0.13	0.92**
<u>P. coccinea</u>	25	386	16.3	4.0	0.12	0.68**	3.8	0.09	0.72*
<u>P. caerulea</u>	25	428	12.3	5.4	0.23	0.74**	4.5	0.12	0.87**

Significance was tested using t-test, * = 5%, ** = 1% level significant.

1.4.8 Influence of host plant on the growth parameters of mealybugs.

Influence of host plant morphological or nutritional factors on the growth parameters of P. citri are shown in Figure 1.3 and 1.4. The mean body length of P. citri remained unchanged throughout the experimental period when they were reared on Passiflora plants. At the end of the crawler phase the mean body length of P. citri reached a length of 0.8 mm when they were fed on Gesneriaceae plants while the value on leguminous plants was 1.0 mm.

Lengthening of the crawler phase was noticed when the nymphal stages of P. citri were reared on winged bean plants. The second and third instar stages of P. citri showed a relatively faster growth on soy bean plants whilst in Streptocarpus plants insects showed slower growth. At the end of the third instar stage P. citri reached to a mean length of ca. 3.0 mm when they were reared on leguminous plants whilst the value for Gesneriaceae was ca. 2.0 mm.

The mean body length of P. affinis reared on various host plant species are shown in Figures 1.3 and 1.4. It was observed that the growth of P. affinis on most of the host plant varieties was consistent except on Gesneriaceae plants. In most cases P. affinis showed better growth performances than P. citri and the mean body length of adults reached almost 4.0 mm where as in P. citri the maximum mean body length was 3.5 mm in soy bean plants. In general both insect species performed better on soy bean plants and P. affinis was successful on Passiflora plants and reached a mean length of 4.0 mm while P. citri was unable to complete development.

1.4.9 Analysis of host plant soluble phenols.

Fig 1.5 shows the range of phenolic compounds that appeared in various temperature regimes. Each peak reveals a different phenolic compound and the peak area represents the concentrations of such compounds.

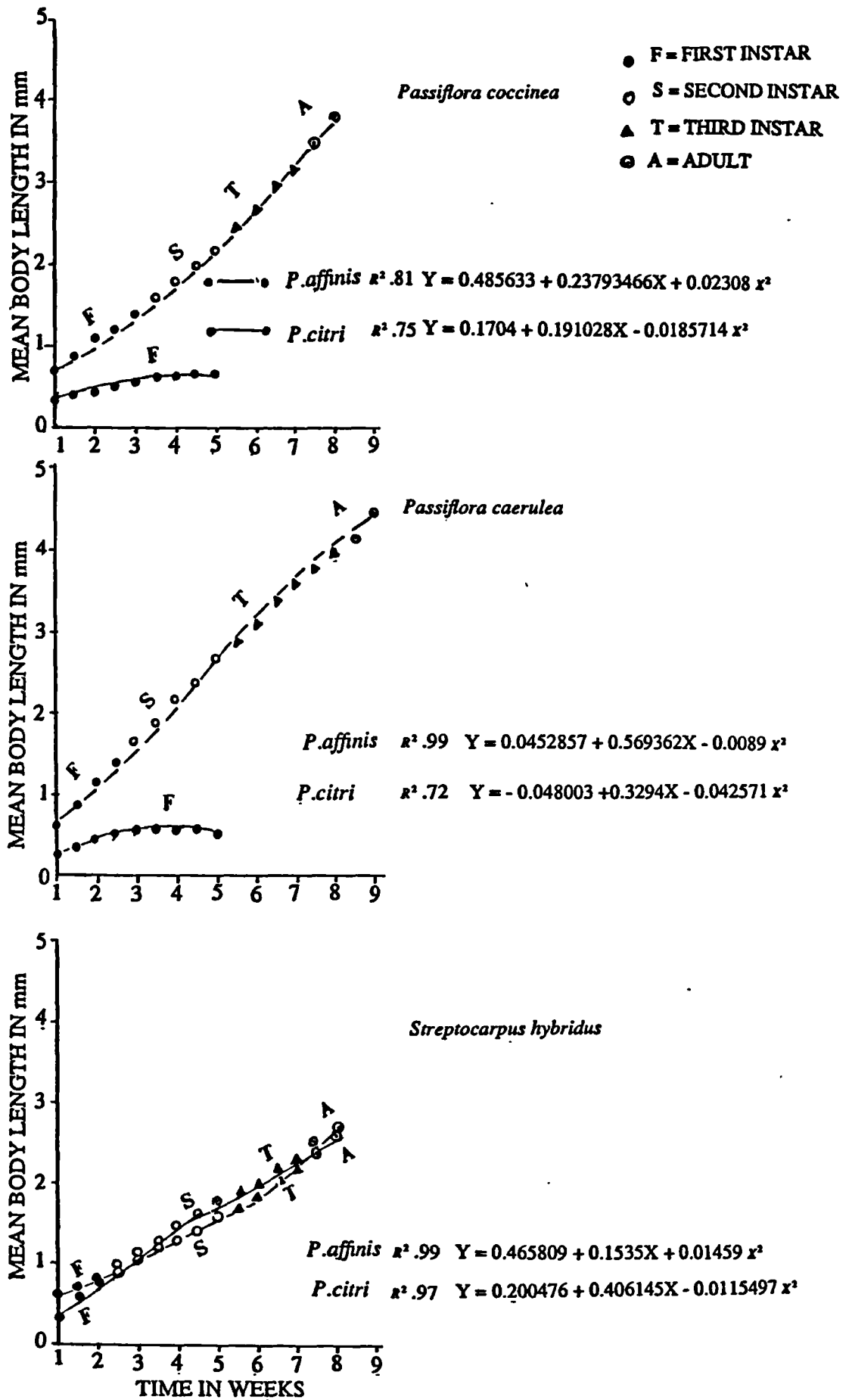


Fig. 1.3 Growth curves of *P. citri* and *P. affinis* reared on various host plant species. Mean body length of various stages of mealybugs were taken in fitting curves and each point represent a mean of 25 insects. Curves are fitted by estimated values obtained from second order polynomial equations.

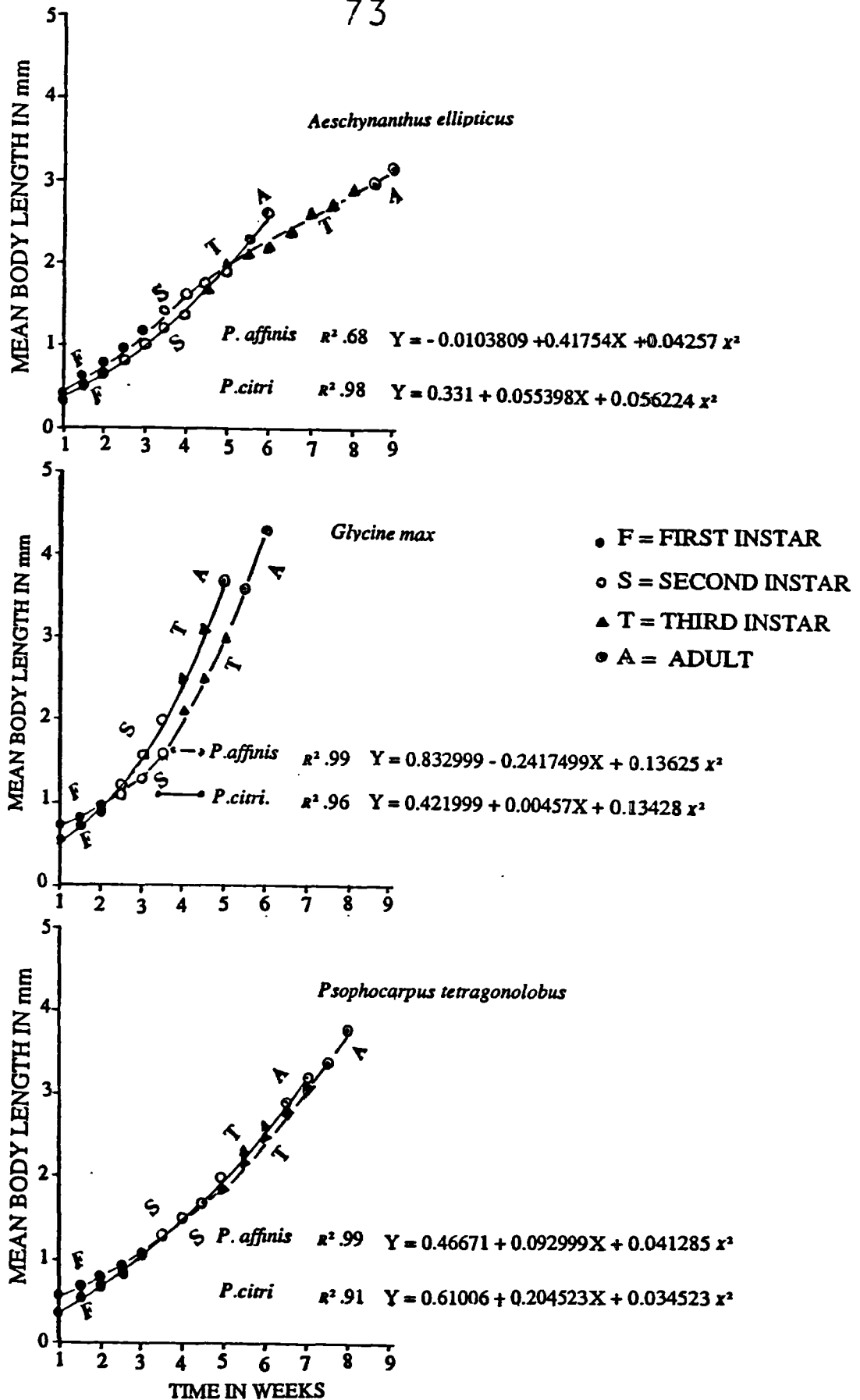


Fig. 1.4 Growth curves of *P. citri* and *P. affinis* reared on various host plant species. Mean body length of various stages of mealybugs were taken in fitting curves and each point represent a mean of 25 insects. Curves are fitted by estimated values obtained from second order polynomial equations.

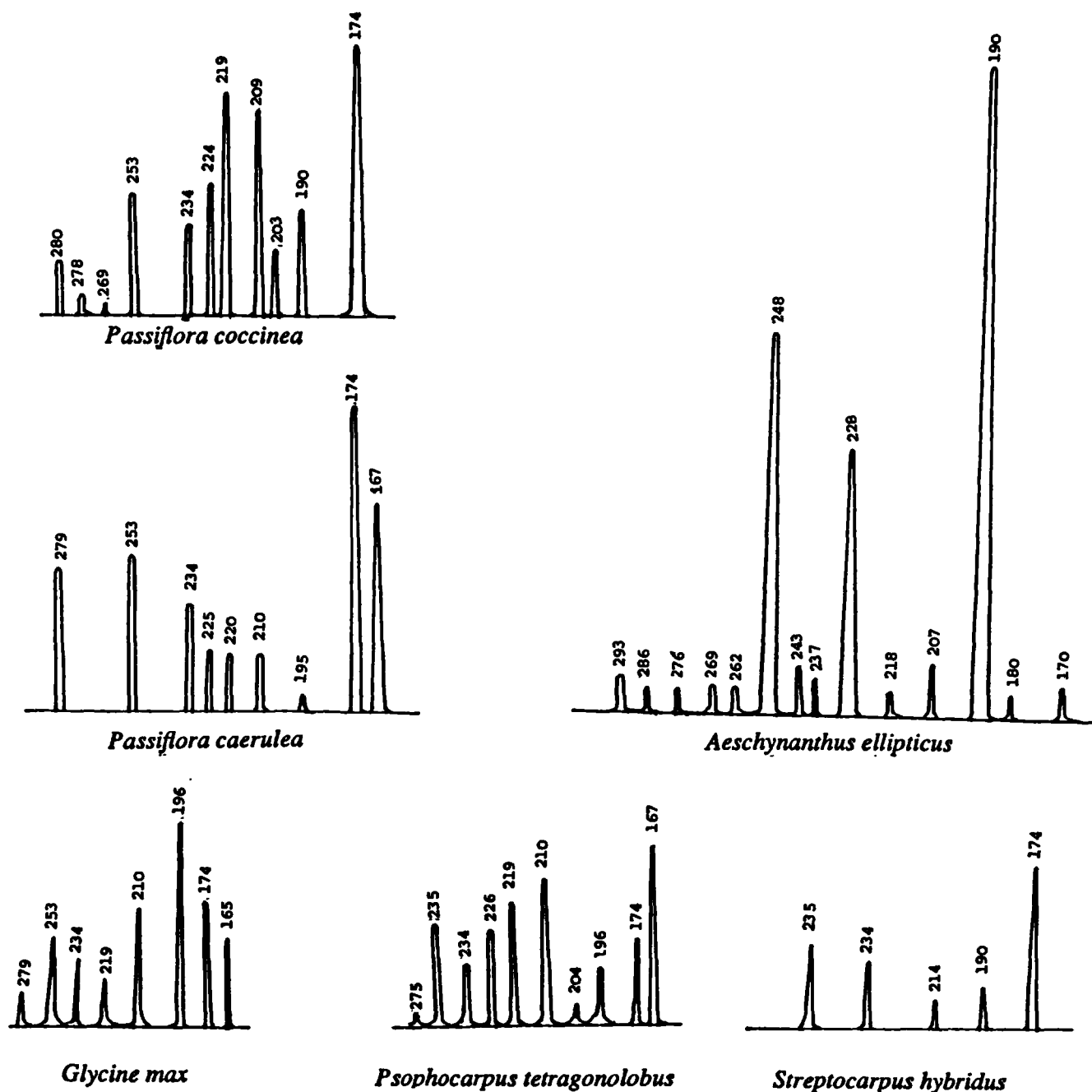


Fig. 1.5 Chromatogram of the acid hydrolysed soluble phenolic compounds of various host plant species of mealybugs. Each peak represent various phenolic compounds and the peak area gives the relative concentrations. Columns 5% OV-17 on chromosorb WHP mesh range 100-120, carrier gas nitrogen, injector temperature 200°C, detector temperature 350°C. Column initial 150°C and column final 300°C. Programmed at a rate of 12 min: temperature gradient.

Identification of phenols may be made by comparing the similar peak detected in the standard samples run under identical experimental conditions (see Appendix 1). It can be seen from Fig. 1.5 that A. ellipticus has 14 different peaks whilst only 5 peaks could be detected in Streptocarpus plants. A prominent peak appeared at 190 (could be vanilin, see Appendix 1) was remarkably developed in A. ellipticus. The total concentrations of phenols in various host plant species are shown in Fig 1.6. A. ellipticus showed high levels of soluble phenols followed by P. coccinea and they were lowest in Streptocarpus plants.

1.4.10 Analysis of host plant total nitrogen content.

Total nitrogen content of host plant leaf samples are presented in Figure 1.7. Gesneriaceae plants had relatively low levels of total nitrogen throughout the experiment, but leguminous and P. caerulea showed high level of nitrogen during experimentation.

Host plant protein was qualitatively analysed through SDS-PAG electrophoresis. However, no protein bands were detected in Gesneriaceae plants (Plate 1.5).

S.h = *S.hybridus*
 P.t = *P.tetragonolobus*
 P.ce = *P.caerulea*
 G.m = *G.max*
 P.co = *P.coccinea*
 A.e = *A.ellipticus*

| s.d.

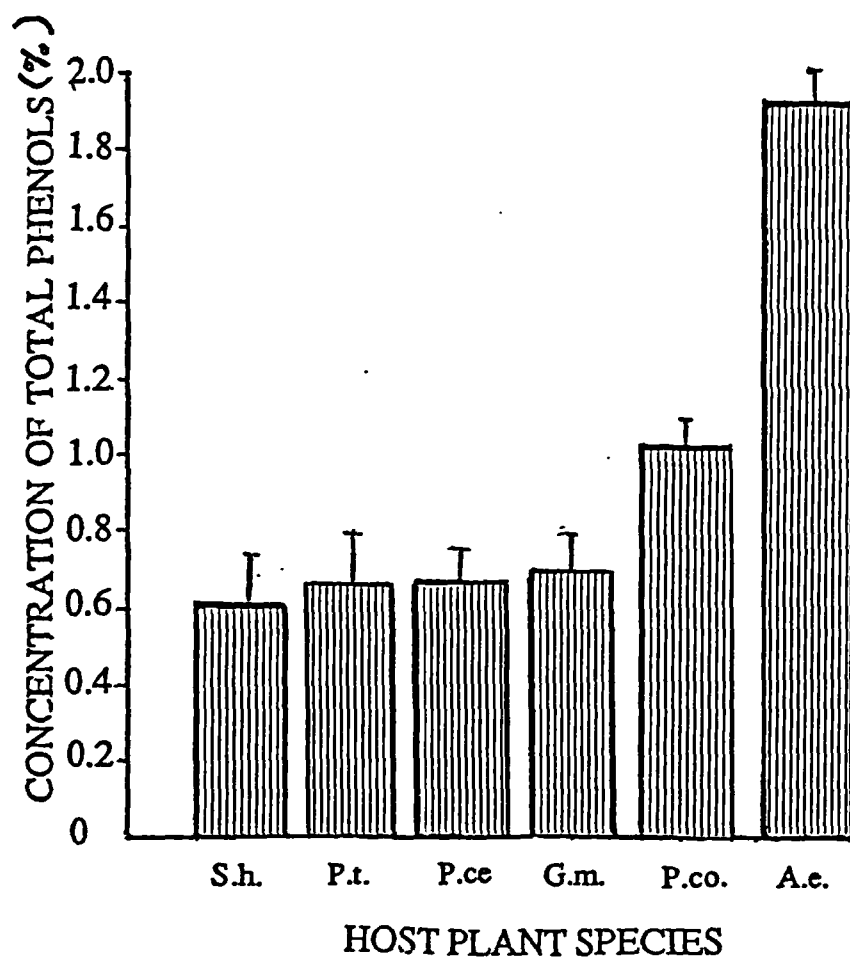


Fig. 1.6 Histograms showing acid hydrolysed total phenolic concentrations of various host plant species of mealybugs. Each mean value is an average of 3 samples. Analysis was based on the fresh weight of the samples.

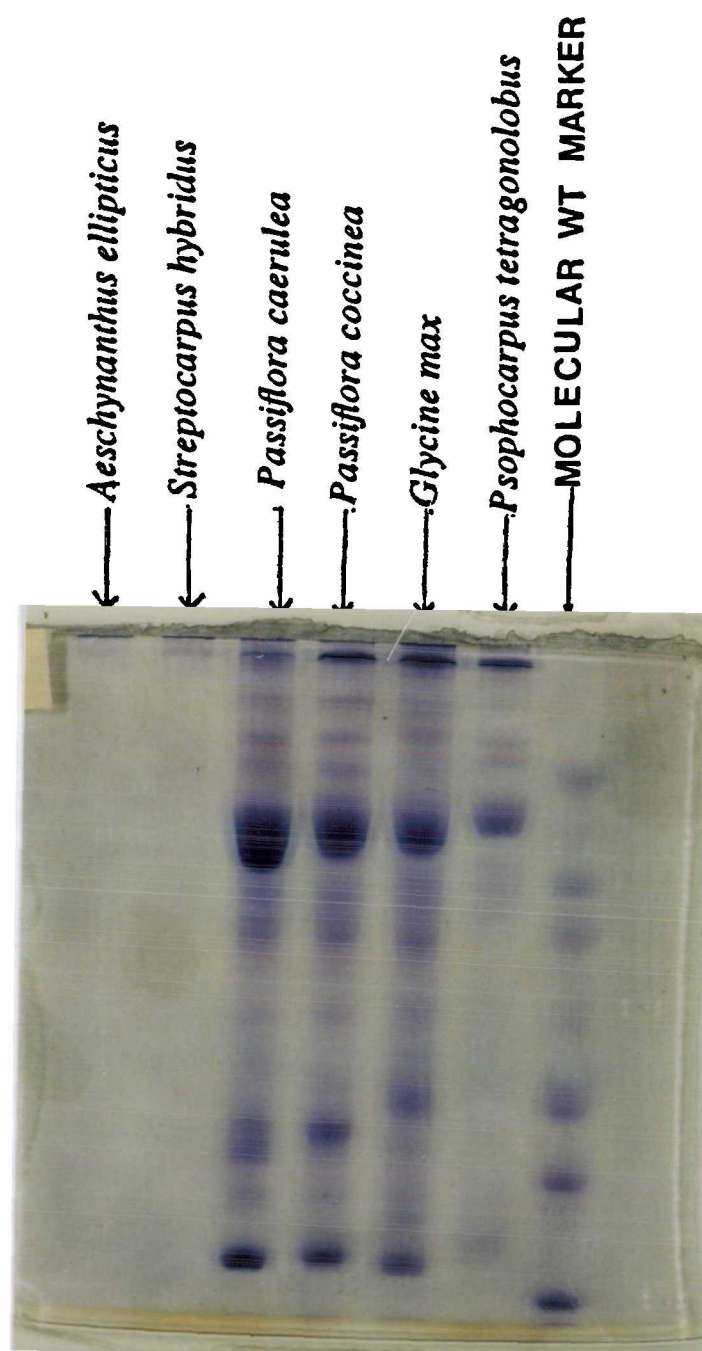


PLATE 1.5 SDS-PAGE (12.5% resolving gel) electrophoretic pattern of leaf extract of 6 host plant species. Each column represent a different plant species. Extration was done after adding PVP to separate proteins from phenolic compounds. (Note invisible banding pattern of A. ellipticus and S. hybridus plants)

HOST PLANT SPECIES

- *A. ellipticus*
 ○—○ *S. hybridus*
 ●—● *P. tetragonolobus*
 ⊖—⊖ *P. coccinea*
 ▲---▲ *G. max*
 --- *P. caerulea*

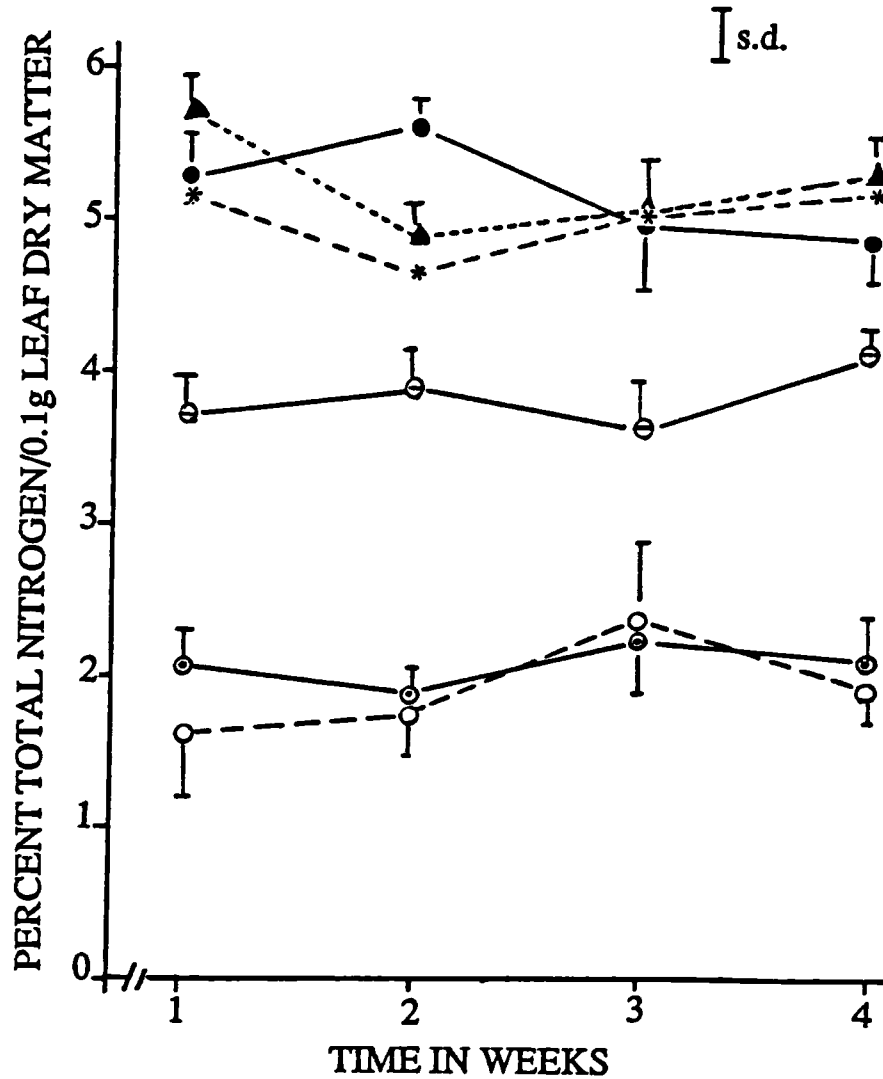


Fig. 1.7 Total nitrogen content of the leaf samples of various host plant species. Each point represents an average of 3 samples. Analysis was carried out for 4 consecutive weeks.

1.5 DISCUSSION

1.5.1 Influence of host plant on the developmental period of mealybugs.

The early larval stages of P. citri reared on winged bean plants showed remarkably slow development. Leaf nutrient, particularly total nitrogen, appears to play a less important role than any other plant factors in determining the developmental period of crawlers on the winged bean plants. The total leaf nitrogen content of 5.4% (dry weight basis) closely resembles the values observed in soy bean and P. caerulea plants where mealybugs showed a faster development (Fig. 1.7)

The retarded development perhaps could be attributed to plant morphological features such as dense trichomes (Gibson & Turner, 1977) and tough cuticle (Jeffree, 1986). However, winged bean plants are smooth and would not provide a morphological barrier. A phytochemical substance(s) in the winged bean, might be involved as a growth retardant for P. citri.

The concentration of tripsin inhibitor enzymes in winged bean plants are higher than that of soy bean plants (Duke, 1981). In general tripsin inhibitors are digestion-reducing substances and early nymphal stages may be more vulnerable to such chemicals than to the late stages. However, the 3rd instar of P. citri reared on winged bean showed a significantly faster development in comparison to other plant varieties. Thus the contrasting difference in crawlers and the 3rd instar's rate of development in winged bean plant may be attributed to different reasons. Perhaps the adult mealybugs can tolerate or detoxify plant toxic substances more efficiently than the nymphs or the surviving larvae become adapted to cope with plant toxic substances.

Experiments carried out by Gatehouse et al., (1985), on protease activity in the gut of larval bruchid beetle, Callosobruchus maculatus revealed that cowpea protease inhibitors can be toxic to the developing larvae. The protein trypsin inhibitors from legume seeds which are not hosts to bruchid larvae were not as effective inhibitors of larval proteolytic activities. Thus this proves that insects can sometimes tolerate trypsin inhibitor activities.

Another possibility is that the selection pressure results in the early elimination of susceptible individuals. Many insects use the same process to build resistance against pesticides (Devonshire & Needham, 1974). More work is needed to investigate this problem.

Mealybugs reared on soy bean plants showed relatively faster development throughout the larval period. This indicates that plant defence, such as physical, nutritional or noxious phytochemicals have little or no effect on mealybug growth and development. The level of total leaf nitrogen was highest in soy bean (5.7%) and the phenolic concentrations were relatively low (Fig. 1.6.). Presumably these factors might have contributed to faster development of mealybugs.

Mealybugs reared on S. hybridus showed a slower nymphal development. This plant has dense trichomes on both upper and lower sides of the leaves. It is generally believed that the mechanical effect of trichomes depend on four main characteristics of, density, erectness, length and shape (Brewer et al., 1984). The trichomes of S. hybridus plants are certainly long, erect, sharp and dense with little space for mealybug nymphs. It appears that mealybugs simply cannot feed on young or immature leaves of S. hybridus because of the densely packed trichomes.

The distribution of mealybugs on S. hybridus plants were strictly confined to fully mature or senescent leaves, in which there is a relatively wider space between trichomes (see Plate 1.2). Apart from the physical barrier, the leaves of S. hybridus were relatively poor in nitrogen. Hence these two factors might have contributed to delayed development of mealybugs on S. hybridus plants.

The prolonged development of nymphal stages of P. affinis fed on A. ellipticus plants may be attributed to the low level of nitrogen in the plant or high concentrations of phenolic derivatives (Figures 1.5 & 1.6). Feeny (1970) reported an identical situation when the winter moth Operoptera brumata L. was allowed to develop on oak. The larvae of winter moth normally feed on the oak leaf when the tannin content is low but, during hot months tannin levels increase and form complex compounds with protein, rendering the diet less suitable as a source of protein nitrogen. Feeny also showed that 1% tannin is sufficient to reduce the larval growth. Thus it is clear that the host plant varieties can exert their effect on growth and development of the insect in three basic ways.

- (1) Quality and quantity of food supply by the plants.
- (2) The content of substances with growth regulatory effects such as secondary plant metabolites.
- (3) Specificity of volatile compounds (Osborne, 1972).

1.5.2 Influence of host plants on the pre-ovipositional period of mealybugs.

The rapid reproductive development of mealybugs reared on leguminous plants suggest that some plant species can provide more favourable conditions for faster development and maturation of eggs. The importance of a balanced diet on egg production has been discussed by many authors (Hill, 1976; Mattson; 1980; Scriber & Slansky, 1981; Brodbeck & Strong, 1987). Dietary deficiencies may restrict the laying down of sufficient food reserves in the form of fat body to carry the insect through the larval and adult stages. Many insects acquire more food during the pre-ovipositional period to provide sufficient fat reserve for ovarian function. Even the accepted food plant may not always provide a perfect diet if the condition of the plant is not favourable (Brewer et al., 1984).

The pre-ovipositional periods of P. citri and P. affinis on S. hybridus and A. ellipticus plants were significantly longer than those developing on leguminous plants. The possible reason for the delay in egg maturation could be due to low fat accumulation in the insect body as a result of inadequate nutrient supply from the host plants. This suggests that the nutritive value of Gesneriaceae plants are relatively low.

An attempt to resolve host plant protein through SDS PAG Electrophoresis was abandoned due to poor and invisible banding pattern in Gesneriaceae plants (see Plate 1.5). This situation may be attributed to either high phenols or less nitrogenous substances in those plants (pers. comm. Dr. J. Mansfield and Dr. V. Koritsas). The available proteins might have been involved in the formation of complex compounds with phenols thus making the plant less favourable for the insect.

The low nitrogen concentrations of those plants could also aggravate the problem for insects. Thus it may be that the longer pre-ovipositional period of mealybugs on some plant varieties could be due to a combination of plant morphological and nutritional factors. However, further studies will be necessary to assess the relative importance of plant factors on the reproductive development of mealybugs

1.5.3 Influence of host plant on mealybug fecundity.

Compared to Gesneriaceae reared individuals, females reared on leguminous plants of both mealybug species under identical experimental conditions and genetical origin showed great variability in egg production. The reduction of egg laying capacity was most obvious when the mealybugs fed on A. ellipticus. The likely explanation for differences in fecundity is that some insects have not acquired sufficient nutrients from the respective host plants to build the fat reserves for egg production. There is a possible involvement of plant morphological, physiological and biochemical factors on the fecundity level of mealybugs (van Emden, 1966b; House, 1969; Southwood, 1972).

Although the involvement of plant phenolic substances on the reproductive capacity of mealybugs has not been fully investigated the present study suggests no relationship between total phenols and fecundity. Despite the controversial arguments against the involvements of plant phenolic derivatives to sap sucking insects, Zucker (1982) showed a strong negative correlation between total phenols and the feeding of the gall aphid, Pemphigus betae, although this is not shown with the present data with mealybugs.

San Gupta and Miles (1975) related differences in susceptibility of apple tree varieties to the wooly aphids, Eriosoma langerum to the concentrations of leaf phenolic substances.

Banerjee and Haque (1985) showed that ovarian biomass of Diacrisia casignetum Kollar. was much influenced by the host plants species and the ovary of an insect reared on low phenolic Sunflower was always heavier than those in Hempweed with high phenols. Further they suggested that the synthesis of yolk proteins may be under the control of different hormonal regimes, but the level of foliar phenols incorporated into the female reproductive system may alter ovarian physiology.

Results given by Hough and Pimentel (1978) and Barbosa and Greenblatt (1979) showed that the fecundity of the gypsy moth Lymantria dispar L. was strongly influenced by the various host plants on which it was reared. The present finding is also in close agreement with similar work reported by Banerjee and Haque (1985) and Hough and Pimentel (1978).

However, there are limitations of the acid hydrolysed phenol extraction method where only soluble phenolic derivatives can be detected.

Nevertheless in natural physiological processes plants may be involved with different forms of phenols and the effect of such compounds may be different from what we have reported. Thus further research is required for a more complete understanding of the relationship between plant factors particularly phenolic derivatives on fecundity.

1.5.4 Influence of host plants on mealybug egg viability.

Percentage egg viability was not affected by the food material ingested by the insect but depends on fertilization of females. It has been suggested that P. citri and P. affinis need fertilization for progeny production (Sutherland, 1932), and the sexual behaviour of mealybugs is independent of the food type. However, Banerjee and Haque (1985) found low viability of eggs of D. casignetum when they were reared on high phenolic plants. Furthermore plant morphological characters also can affect the egg viability. Viability was reduced when the eggs of Oulema melanopus (L.), were laid on pubescent leaves, apparently owing to desiccation (Brewer et al., 1984).

1.5.5 Influence of host plant on mealybug egg incubation period.

There was no host plant involvement in the incubation period of mealybug eggs. Incubation period largely depends on the environmental condition such as ambient temperature (Tingle, 1985). The longer incubation period recorded in P. affinis could be attributed to inherent characters of the species.

1.5.6 Influence of host plant on mealybug body weight.

There appears to be a good correlation between the nitrogen level in the plant and the body weight of mealybugs. Perhaps nutritionally rich host plants allow the full expression of the genetical potential of mealybugs. Scriber (1979a) found that when Papilio larvae were fed on nutritionally rich leaves, the biomass assimilation and conversion efficiencies were enhanced resulting in bigger pupae. Larvae fed on poor quality plants had a lower average weight gain and resulted in smaller pupae.

Here A. ellipticus and S. hybridus plants, do not appear to be a good food plant as judged by adult weight gain. Thus the present finding is in agreement with Beckwith (1976) who found mean pupal weight of Orgyia pseudotsugata was significantly affected by plant species. The influence of host plant factors on M. persicae was studied by Gladder and Peters (1986) who found that when the aphids were transferred to rape, they produced heavier adults than when transferred to beet irrespective of whether they had been reared on rape or beet. Thus the varietal differences of the host plant can affect the growth performances of an insect.

1.5.7 Correlation between weight, length and fecundity of mealybugs.

A positive correlation has been shown in many studies between insect weight and fecundity (Dixon & Wratten, 1971; Dewar, 1977) but the use of body length to show these correlations has only been used on a few occasions (Hard, 1976). The present finding provides strong positive correlation between weight and fecundity and length and fecundity of both the species of mealybugs. Once the relation between size and fecundity or body length and fecundity has been established, one can estimate the natality of mealybugs in a given population, using either weight or length of the insect. Miller (1957) used the length of empty pupal cases of spruce bud worm as a suitable criterion to estimate fecundity of a natural population. However, the potential fecundity of an insect may be influenced by various abiotic factors such as temperatures or biotic factors like variety of host plants. Effects of these factors on the potential fecundity can be evaluated under laboratory conditions and the findings can be incorporated into the regression equation.

Single regression equations cannot be applied to estimate population since the parameters vary with the variety of host plant. In practice it is necessary to have different equations for different host plant species (Miller, 1957). However, the present finding provides an overview about the possible use of regression equations to estimate mealybug populations under varying conditions using measurements such as total body length. Kempton et al., (1980) suggested the use of the negative exponential curve to obtain a better fit than a linear regressions. Further study is needed to find out the most suitable regression equation to predict the potential fecundity of mealybugs under various conditions.

1.5.8 Influence of host plant on the growth parameters of mealybugs.

P. affinis reared on A. ellipticus plants had a smaller body size and longer development. However, no explanation is obvious why P. citri developed faster than P. affinis. Perhaps different mealybug species have different mechanisms to overcome the effect of plant toxic substances. P. affinis appears to have a full resistance to cynogenic glycosides of Passiflora whereas P. citri is fully susceptible to the host plant toxicity. P. citri fails to grow beyond the size of a crawler even though some insects managed to survive for up to 5 weeks. Over 95% of the crawlers of P. citri died within 5 days of introduction to Passiflora plants.

Some plant factors in Passiflora obviously affect the growth and development of P. citri. However, the present findings seem to conflict with the report of Murray (1978) who found P. citri successful on Passiflora plants.

The observed difference in the present investigation may be due to two factors. Spencer (1988) showed the vast majority of Passiflora species have 6 major groups of toxic chemical compounds and the nature of the compound varies with the plant species concerned. P. caerulea has Tetraphyllin B sulfate and Epitetraphyllin B sulfates as cyanogenic compounds. The chemical compounds of P. coccinea are Passicoccin and Epipassicoccin (Spencer, 1988). Thus the varietal differences could be a possible reason for Murray's (1978) observations. The second possibility is that because the Passiflora are tropical plants, the chemical constituent might vary when grown under temperate climates. However, further research is required for a proper understanding of the host plant specificity towards mealybugs species.

The soy bean plant is a superior host compared to other experimental plants for P. citri's growth rate, survival, adult size, and fecundity. The early larval stages of P. citri were retarded when they were reared on winged bean plants. However, the 3rd and adults showed relatively faster growth and finally they reached a mean length of 3.2 mm a reasonable size for a healthy grown P. citri (Sutherland, 1932). Therefore these observations show that the host plant resistance towards mealybugs cannot be judged only by slow developmental rate of early stages.

The growth curve studies are more informative than the developmental period observations to assess the host plant suitability towards mealybugs. This is because the growth curves in the present study were constructed using the mean length of the developing insect, to monitor the real growth under varying environmental conditions. On Passiflora plants, where some P. citri lived up to 5 weeks as crawlers, the size of the insects remained constant.

If the developmental period of the insect is taken as the criterion to assess the host plant quality one would mistakenly identify the Passiflora as suitable host plants for P. citri.

It appears that P. affinis has a greater ability to withstand plant secondary metabolites than P. citri. Survival and reproductive success of P. citri was completely arrested when they were forced to feed on Passiflora plants. The polyphagous nature of P. affinis may be attributed to a heritable character where they might have evolved to detoxify or eliminate noxious phytochemical obtain during food ingestion. Nevertheless some plant species such as A. ellipticus showed partial resistance to both mealybug species affecting growth and reproductive success. Thus plant resistance factors should be incorporated into biocontrol programmes to maximize the effects of natural enemies.

1.6 CONCLUSIONS

1. The crawler phase of P. citri took a considerably longer period to develop winged bean but the third instars showed fastest development on the same plant species. Thus the resistance mechanism of host plants cannot be judged only by the delayed development of early stages of mealybug.
2. P. caerulea and P. coccinea plants appeared to be completely resistant to P. citri but P. affinis showed a better growth and reproductive success on the same plant variety. This infers that P. affinis has evolved to cope with cyanogenic compounds of Passiflora plants.
3. The egg maturation period of mealybug was considerably delayed when they were reared on Gesneriaceae plants and this may be attributed to insufficient nutrient to build fat reserves.
4. Viability and incubation periods of mealybug eggs appeared to be independent of host plant factors.

5. Mealybug reared on leguminous plants produced a significantly greater number of eggs and a remarkable reduction was observed when they were reared on Gesneriaceae plants.
6. A significant reduction in body size was noted when the mealybugs were fed on Gesneriaceae plants. Invariably nutritional quality of host plants ~~is~~ involved in reduced growth of insects.
7. The body weight and length of mealybugs were positively correlated ~~with~~ the total fecundity of insects.
8. A. ellipticus and P. coccinea plants showed high levels of soluble phenolic compounds.
9. Gesneriaceae plants had relatively low concentrations of total nitrogen in comparison to other experimental plant species.

SECTION 2

EFFECT OF HOST PLANT FACTORS ON MEALYBUG PARASITIDS.

2.1 INTRODUCTION

Plants provide food, shelter and diverse micro-habitats for herbivores and other trophic levels. Most studies on plant-herbivore interactions have focused primarily on the direct effects that plant and primary consumers have upon one another. Natural enemies indirectly affect plant growth by controlling harmful insects feeding on plant parts. In 1980, Price et al., highlighted the importance of third trophic levels and plant resistant factors in controlling phytophagous insects. Host plant resistance factors such as non-preference, antibiosis or morphological characters could affect natural enemy population either due to low host numbers or poor searching ability resulting from host plant morphological barriers.

Biological control programmes for glasshouses differ from those applied in field crops particularly because natural enemies have to be introduced regularly and in large numbers. The inundative release method can be applied when parasitoid occurrence is not synchronized with the host, due to low temperatures or the parasitoid population does not develop sufficiently fast (van Lenteren, 1986). The slower development of parasitoids may be attributed to host plant factors such as secondary metabolites or poor nutritive quality.

Tingle (1985) reported that the two mealybug parasitoids, L. dactylopii and A. pseudococci were unable to control mealybugs fed on Passiflora plants. He suggested that Passiflora plant has repellent chemicals where parasitoids may not be recognised as a host plant or mealybugs develop an ability to sequester toxins from the plant rendering themselves unsuitable to natural enemies. However, in the present study, the direct involvement of allelochemicals to parasitoids was not investigated. Instead we studied the indirect involvement acting through the herbivore on the parasitoids.

The same plant species described in section 1 were used for the present investigation and P. citri were reared on those plants before offering them to the parasitoids. Thus the investigation was aimed at providing information on trophic interaction on parasitoid parameters such as developmental period, longevity, survival rate, sex ratio and the body size of the emerging insects.

2.2 REVIEW OF LITERATURE

2.2.1 Influence of host plant nutritional value on parasitoids.

Feeding behaviour and the variety of food are primarily controlled by genetical factors supplemented by ecological adaptations (Mattson & Scriber, 1987). Food utilization budgets have been extensively used to assess suitability of host plants for primary consumers. However, relatively little work has been done on the efficiency of conversion of food ingested by the consumer to the parasitoids (Greenblatt, et al., 1982). Nonetheless some authors have used nitrogen utilization efficiency as a measurement to evaluate host suitability (Greenblatt & Barbosa, 1980).

Activities of certain biocontrol agents may be enhanced or retarded by the type of host plant upon which their host develops. Natural enemies of herbivores may be stressed due to lack of quality food, when their potential host develops on unsuitable host plants (Duffey, 1980). Host plant influence on weight gain of predaceous mites

Phytoseiulus persimilis Athias-Henriot was reported by Moraes and McMurty (1987). The predators fed on adult females of Tetranychus urticae Koch reared on nightshade plants. The view of Weis and Abrahamson (1985) was that the third trophic level may push a plant herbivore interaction in either direction, depending mainly on the selection pressure on each of the interacting species. Orr and Boethel (1986) reported that soy bean looper Pseudoplusia includens (Walker) was affected by antibiosis of the host plant and reduced weight gain was observed in the predator, P. maculiventris.

Therefore it is clear that the nutritional aspect of host plants primarily influence the growth and behavioural parameters of the herbivores and no doubt these changes would certainly influence their enemies to a lesser magnitude.

2.2.2 Effect of plant allelochemicals on the third trophic level.

Plants synthesize not only nutritionally important compounds such as carbohydrates, fats and proteins but also chemical substances of little or no nutritive value to give resistance to pests. However, some insects have succeeded in dealing with the secondary substances either by breaking them into nontoxic compounds or excreting them as a waste product (van Emden, 1978; Bell, 1987).

Insects and plants interact with each other in many ways. Chemical mediation through allelochemicals is one possible way where plants, herbivores and parasitoids/predators interact (Scriber, 1984b; Whitman, 1988). Smith (1963) and Wood (1982) reported that pine trees use terpenoids to attract certain bark beetle predators benefiting both the host plant and the predators. Floral volatiles or scents also play a vital role in attracting or arresting potential natural enemies of herbivores.

Adult parasitoids generally obtain nutrition from flower nectaries and pollen to sustain their lives. Moreover these nutrients enhance most of the fitness parameters like life span, fecundity and searching capacity of parasitoids (Leius, 1967; Sundby, 1967; Streams, 1968; Foster & Ruesink, 1984). It has even been suggested that plant extrafloral nectaries have evolved as supplementary nutrients for natural enemies.

Another possibility is that these nutrients may contain volatile substances such as synomones to attract natural enemies towards the host plant (Tilman, 1978; Boecklen, 1984).

2.2.3 Effect of toxic secondary plant metabolites on the third trophic level.

Many secondary substances such as sesquiterpene, lactones and alkaloids may have evolved in the plants as anti-feedants to avoid being eaten by herbivores (Burnett & Jones, 1978). Certain plant compounds or the plant's nutritional qualities can affect the quality of a host as a resource for parasitoids. Some toxic substances in plants which repel, reduce vigour or kill susceptible herbivores may poison beneficial insects or affect the physiological or metabolic process leading to retarded growth (Campbell & Duffey, 1979).

Nevertheless some herbivores may cope with toxic plant metabolites by sequestration, tolerance, or metabolic detoxification. Such herbivorous insects may not be such a suitable source of food for the developing parasitoid larvae (Williams et al., 1988). Chan et al., (1978) found reduced growth rate of Heliothis virescens (Fab.) when "gossypol" (Extract from cotton plant) was incorporated in the diet. Further they proved that the concentration of "gossypol" needed to reduce the growth rate of the host by 50% is sufficient to reduce the body weight of emerging parasitoids, Campoletis sonorensis, from those hosts. Host acceptance of the parasitoid Hyposoter exiguae (Viereck) was reduced when the host Heliothis zea (Boddie) was grown on an artificial diet with alpha tomatine. This behaviour was clearly visible when the host was grown on tomato plants with low total sterol: alpha tomatine ratios (Campbell & Duffey, 1979).

Impairment of Adalia bipunctata L. and Coccinella 7-punctata L., the predators of aphids by the host plant was discussed by Blackman, (1967). The predators were adversely affected probably because of sequestered secondary compounds gained from their host. Therefore it is evident that host plant allelochemicals sometimes impair rather than enhance the activities of natural enemies. Further evidence of the ability of the host to sequester toxins from the host plants was discussed by Smith (1957). He reported that oviposition by the parasitoid Comperiella bifasciata was retarded when its host the California red scale, Aonidiella aurantii (Mark.) was fed on sago palm rather than Citrus spp.

2.2.4 Host plant morphology and parasitoid interactions.

Many species of parasitoids are affected by morphological characters of the plant which their host inhabits. The structural diversity of plants generally includes various morphological differences in surface structures, architectural diversity and spatial diversity. The richness of the insect fauna associated with plants invariably depends on the structural diversity of plants (Neuvonen & Niemela, 1981; Moran & Southwood, 1982). Plant architecture provides a greater niche diversification on tree species than on low-growing plants. The arboreal concentration of herbivorous insects is apparently related to the greater structural diversity of the host plant.

Abundance of parasitoids generally follows the population density of herbivorous insects (Lawton, 1983). Apart from the plant architectural diversity, smaller morphological variation can alter parasitoid behaviour to a greater extent.

Bracon mellitor (Say), a parasitoid of the boll weevil. Anthonomus grandis (Boh), was able to parasitise ca. 50% of host larvae with frego bract fruit, compared to only ca. 7% in cotton without bract fruit (McGovern & Cross, 1976).

Activities of parasitoids are sometimes greatly influenced by the type of plant the herbivore host inhabits. A tachinid parasitoid Gonia capitata preferentially searches for the potential host Perosagrotis spp, on native grasses, even though the host is found in greater numbers on cultivated grains (Shepard & Dahlman, 1988). Katanyukul and Thurston, (1979) produced further evidence of the influence of host plant type on behavioural responses of parasitoids. Eggs of Manduca sexta (L.), were highly parasitized when they were laid on Jimson weeds than on tobacco plants. Denser canopy sometimes hampers the normal searching behaviour of parasitoids resulting in poor parasitism. This situation was clearly visible in transplanted rice and direct seeded rice fields. The eggs of the rice yellow stem borer, Scirpophaga incertulas (Walker), were highly parasitized in the transplanted field due to the greater searching capacity of parasitoids (Shepard & Arida, 1986).

2.2.5 The Parasitoids.

Anagyrus pseudococci (Girault)

A. pseudococci (Hymenoptera: Chalcidoidea) is a solitary internal parasitoid of the family Encyrtidae. The female is about 1.5 - 2 mm in length and brown, and with characteristic black and white banded antennae, whilst the male is smaller (0.8 - 0.9 mm in length) and black in colour (Plate 2.1). The adults show sexual dimorphism, and virgin females produce male offspring only (arrhenotokous).

The female generally lays a single egg in each host, preferably the 3rd stage or adult mealybugs. Sex allocation often depends on the size class of the host and smaller hosts always favour male parasitoids (Chandler et al., 1980). The larvae develop within the host body consuming the contents and pass 5 instar stages before pupating within the mummified host (Rosen & Rossler, 1966). It was reported to be the dominant parasitoid of P. citri but can be developed on Pseudococcus citriculus Green, and Pseudococcus adonidum (L.), (Rosen & Rossler, 1966).

Leptomastix dactylopii Howard

L. dactylopii is a solitary internal parasitoid, belonging to the family Encyrtidae. The female is relatively larger and yellowish in colour (Plate 2.2). The adult shows marked sexual dimorphism. The male can be easily distinguished because of curved "hairy" antennae. The females are arrhenotokous, and in general, the life history and biology of the two species are very similar. The female generally lays a solitary egg in preferably the 3rd instar larvae or adult mealybugs. In the field females are highly monophagous, with P. citri as its only host. Oviposition success is extremely poor when they oviposit on other species of mealybugs such as P. adonidum and P. maritimus (Lloyd, 1958).



PLATE 2.1 Female Anagyrus pseudococci (Magnification x 50 approx.)



PLATE 2.2 Female Leptomastix dactylopii (Magnification x 50 approx.)

2.3 MATERIALS AND METHODS

Unless otherwise mentioned in this section, the same batch of parasitoids, emerging from each treatment was used for the following studies.

2.3.1 Influence of host plant on successful parasitism by

A. pseudococci and L. dactylopii.

Four host plant species i.e. S. hybridus, A. ellipticus, G. max and P. tetragonolobus, were identified as the experimental plants. Larvae of P. citri were introduced to the selected host plants and were maintained until a sufficient number of adults developed on each host plant. The host plant species were used as treatments and 5 plants per treatment were considered as replicates. Groups of forty adult mealybugs per plant, were collected and transferred to labelled vials with sufficient moisture provided from a wet cotton plug.

Uniform size female A. pseudococci and L. dactylopii, 5 days old, mated and well fed (50:50 honey and water) were obtained from laboratory maintained cultures.

The experimental arena was a petri-dish (5 cm diam, 1 cm height) with a clean filter paper in the bottom of the plate. Three arenas were used at any given time to expedite the oviposition process.

Twenty mealybugs and a parasitoid were introduced into the arena and the parasitoid was given a chance to select any host and to oviposit an egg. The parasitized host was immediately removed and kept in a vial with an appropriate label. The number of mealybugs in the arena was kept constant by adding new ones, while the attacked insects were taken out. The purpose of this was to ensure uniform patch density throughout the experiment to avoid behavioural changes of the parasitoid. Thus out of 40 insects originally allocated for a replicate, 20 mealybugs were attacked by the wasp. After oviposition, parasitoids were kept away from the arena and were used again after 2 hours so ensuring enough mature eggs for the subsequent ovipositions.

For each parasitoid species and plant variety, 100 mealybugs were parasitized and the oviposition was performed between 0800 and 1400 hours, with artificial lighting provided by overhead fluorescent bulbs. After oviposition the mealybugs were transferred to properly labelled and enclosed vials and were kept in a constantly illuminated incubator at $28 \pm 1^{\circ}\text{C}$ and 55% r.h.. These mealybugs were checked daily for developing parasitoids and the sex of the emerging adults was recorded. This was continued for 25 days allowing all the parasitoids to emerge from the mummies. Some days later the remaining mummies were dissected and the parasitoids were classified as dead or diapausing. The emerging parasitoids were kept alive for further studies on sex ratio, longevity and head width measurements.

The proportion of parasitoids emerging from each replicate was computed as a fraction of the original sample size. These frequency values were used for factorial analysis. Parasitoid species were considered as the two levels of factor one, and the four plant species as factor two.

Two way analysis of variance was carried out using arc-sine transformed data and the resulting treatment means were separated at 5% level applying Student-Newman-Keul test.

2.3.2 Influence of host plants on the developmental period of

A. pseudococci and L. dactylopii.

Relevant information for the present study was obtained from the experiment reported in 2.3.1. The developmental period of parasitoids was defined as the time (days) from oviposition to time of eclosion. In general male parasitoids have a shorter developmental period than the females. Hence the two sexes were considered separately, to find out the influence of host plant factors on the developmental periods of the wasps.

The developmental periods of twenty parasitoids of each sex and species in each treatment were calculated. Statistical analysis was similar to the method used in experiment 2.3.1. However, no data transformation was used in the two way analysis of variance because of the smaller variation between replicates in each treatment. Treatment means were separated using SNK at 5% level.

2.3.3 Influence of host plant on the sex allocation by

A. pseudococci and L. dactylopii.

The parasitoids emerged from experiment 2.3.1 were used in the present study. The sex ratios of the parasitoids were expressed as the proportion of males out of the total emergence in each treatment (male/total).

The total number of males and females in each treatment was used to prepare contingency tables where host plant varieties represent the columns and the sexes denote the rows. Two such tables were prepared for both species and subjected to Chi-square analysis. Comparisons were done between host plant varieties under one degree of freedom. All possible combinations were compared to find out the influence of host plants on sex allocation behaviour of parasitoids.

2.3.4 Influence of host plant on the longevity of female

A. pseudococci and L. dactylopii.

It was decided to use only female parasitoids for the present investigation and they were obtained from the experiment described in 2.3.1. The emerging females were isolated individually into labelled vials just after eclosion. Thirty insects of each species were allocated per treatment. These vials (50 mm height x 25 mm diam.) were streaked with a 50% honey solution. A smaller glass vial containing water and with a cotton wool plug was inverted within the lid of the first vial, to ensure high humidity condition in the insect chamber. Every other day honey and the cotton plugs were replaced to ensure a sufficient fresh food and a clean growth chamber without fungal contamination. The labelled vials were kept at $28 \pm 1^\circ\text{C}$ in a constantly illuminated incubator and were examined every 24 hours until the natural death of the insects.

Insects dying other than from natural causes, were eliminated from the replications, leaving a total of 20 insects per treatment. Means were compared using factorial analysis followed by SNK test at 5% level.

2.3.5 Influence of host plant on the size of the emerging parasitoid.

Relevant information for the present investigation was obtained from experiment 2.3.1. The emerging insects were isolated individually into transparent gelatine capsules and a drop of water was inserted into the capsule with the help of a small glass rod. When the insect stood still the head width was measured using a stereo microscope with an eyepiece graticule. The two sexes were treated as independent variables in the analysis of head width data, because of inherent size variation in male and female parasitoids. Fifteen insects of each sex and species were taken from each treatment to measure the head capsule width. The treatment differences were assessed using factorial analysis followed by SNK test at 5% level.

2.4 RESULTS

2.4.1 Influence of host plant on successful parasitism by

A. pseudococci and L. dactylopii.

The proportional emergence of adult parasitoids from P. citri reared on various host plant species are shown in Table 2.1. Analysis of variance shows that the host plant species has no influence on the frequency of successful emergence of either parasitoid species.

Table 2.1 Influence of host plant species on successful parasitism by A. pseudococci and L. dactylopii.

MEAN PERCENTAGE EMERGENCE				
HOST PLANTS	<u>A. pseudococci</u>		<u>L. dactylopii</u>	
	$\bar{x}$	s.d.	$\bar{x}$	s.d.
<u>S. hybridus</u>	58b	15.2	68ab	11.5
<u>A. ellipticus</u>	71ab	4.2	78a	5.7
<u>G. max</u>	75ab	3.5	73ab	11.5
<u>P. tetragonolobus</u>	73ab	6.7	79a	9.7
SNK test	(32, 5) $\bar{S}_x = 2.606$ MS = 33.96			

Calculations were based on Arc-sine transformation. Untransformed means are presented in the Table. Each mean is an average of 100 observations with 5 replicates. Figures with the same letters are not significantly different at the 5% level.

2.4.2 Influence of host plant on the developmental periods of

A. pseudococci and L. dactylopii.

It is evident from Table 2.2.1 that the developmental period of male parasitoids shows no significant difference between treatments.

Similarly there was no significant difference in the developmental period between two species of parasitoids.

Table 2.2.1 Influence of host plant on the developmental period of male parasitoids at $28 \pm 1^\circ\text{C}$.

MEAN DEVELOPMENTAL PERIOD (DAYS)				
HOST PLANTS	<u>A. pseudococci</u>		<u>L. dactylopii</u>	
	$\bar{x}$	s.d.	$\bar{x}$	s.d.
<u>S. hybridus</u>	14.8ab	0.9	15.6a	1.1
<u>A. ellipticus</u>	15.2a	0.6	15.2a	1.2
<u>G. max</u>	14.2b	0.9	14.9ab	1.7
<u>P. tetragonolobus</u>	14.7ab	1.2	15.2a	1.3
SNK test	(152, 20)	$S\bar{x} = 0.24$	MS = 1.156	

Each mean is an average of 20 replicates. Figures with the same letters are not significantly different at the 5% level.

Mean developmental period of female parasitoids is shown in Table 2.2.2.

Female A. pseudococci grew faster on P. citri reared on soy bean and winged bean plants ($P < 0.05$) than on the Gesneriaceae plants. There were no effect of host plants on the development of L. dactylopii.

Table 2.2.2 Influence of host plant on the developmental period of female parasitoids at $28 \pm 1^\circ\text{C}$.

MEAN DEVELOPMENTAL PERIOD (DAYS)				
HOST PLANTS	<u>A. pseudococci</u>		<u>L. dactylopii</u>	
	$\bar{x}$	s.d.	$\bar{x}$	s.d.
<u>S. hybridus</u>	16.0a	0.9	16.2a	0.8
<u>A. ellipticus</u>	15.9a	1.1	16.2a	0.8
<u>G. max</u>	15.1b	0.7	15.9a	0.6
<u>P. tetragonolobus</u>	15.3b	0.7	15.8a	0.5
SNK test	(152, 20) $\bar{Sx} = 0.173$ MS = 0.597			

Each mean is an average of 20 replicates. Figures with the same letters are not significantly different at the 5% level.

2.4.3 Influence of host plant on sex allocation by A. pseudococci and L. dactylopii.

The sex ratios were expressed as frequencies of male in each treatment.

The relevant chi-square analysis is shown in Tables 2.3.1 and 2.3.2.

Chi-square analysis shows that the host plant varieties have little influence on sex allocation behaviour of A. pseudococci.

However, the frequency of males emerged from soy bean host was significantly lower than that of A. ellipticus reared hosts ($P < 0.05$). In general the sex ratio became more female biased when they developed on leguminous reared mealybugs.

Table 2.3.1 Influence of host plant on sex allocation by
A. pseudococci.

HOST PLANTS	SEX RATIO	<u>S. hybridus</u>	<u>A. ellipticus</u>	<u>G. max</u>
<u>S. hybridus</u>	0.45			
<u>A. ellipticus</u>	0.51	0.44 ns		
<u>G. max</u>	0.32	2.3 ns	5.2 *	
<u>P.tetragonolobus</u>	0.36	1.1 ns	3.3 ns	0.22 ns

Two plants were taken at a time for the analysis of contingency tables. The respective Chi-square values are given in the table. The tabulated Chi-square value = 3.8, (1 d.f.). * significant at 5% level.

Table 2.3.2 Influence of host plant on sex allocation by

L. dactylopii.

HOST PLANTS	SEX RATIO	<u>S. hybridus</u>	<u>A. ellipticus</u>	<u>G. max</u>
<u>S. hybridus</u>	0.51			
<u>A. ellipticus</u>	0.58	0.57 ns		
<u>G. max</u>	0.31	5.7 *	10.4 *	
<u>P.tetragonolobus</u>	0.38	2.8 ns	6.2 *	0.63 ns

Two plants were taken at a time for the analysis of contingency tables. The respective Chi-square values are given in the table. The tabulated Chi-square value = 3.8, (1 d.f.). * significant at 5% level.

The sex ratio of parasitoids shifted towards females when they develop on mealybugs fed on both the leguminous plants. Particularly on soy bean reared hosts.

2.4.4 Influence of host plant on the longevity of female

A. pseudococci and L. dactylopii.

The results are summarized in the table 2.4. Longevity of individual insects was taken as the day from eclosion to the natural death of the insect. From the results it would appear that the parasitoid longevity had been greatly influenced by the plants on which their host developed.

Table 2.4 Influence of host plant on the longevity of female A. pseudococci and L. dactylopii at $25 \pm 1^\circ\text{C}$.

MEAN LONGEVITY (DAYS)				
HOST PLANTS	<u>A. pseudococci</u>		<u>L. dactylopii</u>	
	$\bar{x}$	s.d.	$\bar{x}$	s.d.
<u>S. hybridus</u>	29.7c	4.0	35.1b	4.6
<u>A. ellipticus</u>	29.6c	2.1	36.1b	3.6
<u>G. max</u>	33.5b	4.2	39.4a	2.9
<u>P. tetragonolobus</u>	33.2b	3.9	38.4a	2.7
SNK test	(152, 20) $\bar{S}_x = 0.809$		MS = 13.07	

Each mean is an average of 20 replicates. Figures with the same letters are not significantly different at the 5% level.

Both parasitoids from S. hybridus and A. ellipticus reared hosts, showed a significant reduction in the life expectancy ($P < 0.05$). There were also significant differences in the longevity between the two parasitoids reared on any given host.

2.4.5 Influence of host plant on the size of the emerging parasitoid.

Body size of the emerging parasitoid reflected in the mean head capsule width of male and female is shown in Fig 2.1. The male and female A. pseudococci and female L. dactylopii emerging from P. citri on soy bean and winged bean hosts, had a greater head width in comparison with other host plant treatments ($P < 0.05$). Male L. dactylopii also indicated a significantly greater head width from hosts reared on soy bean.

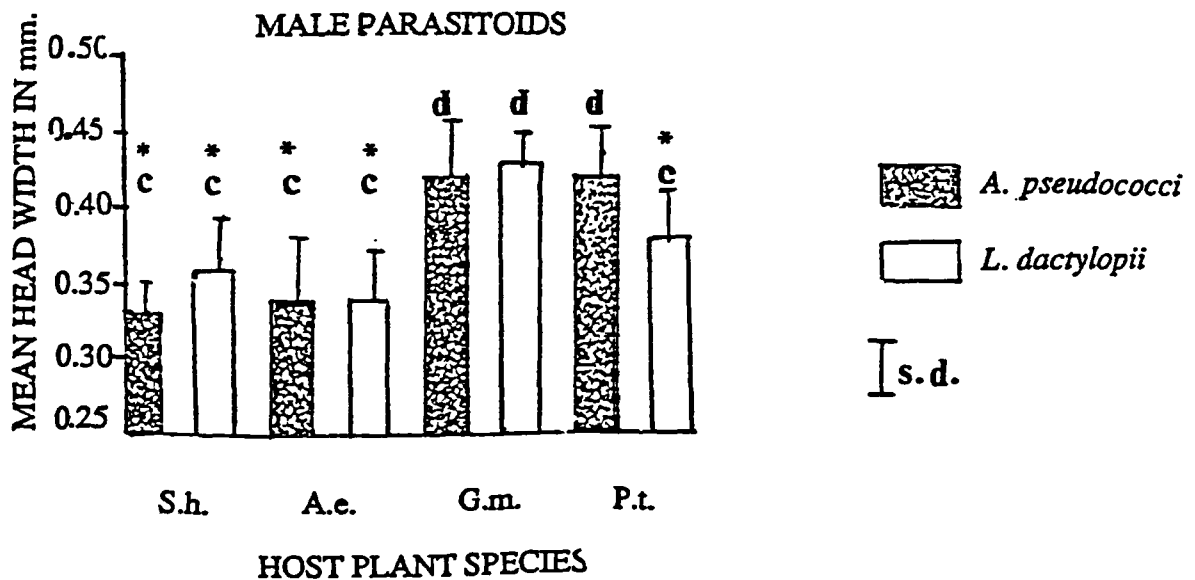
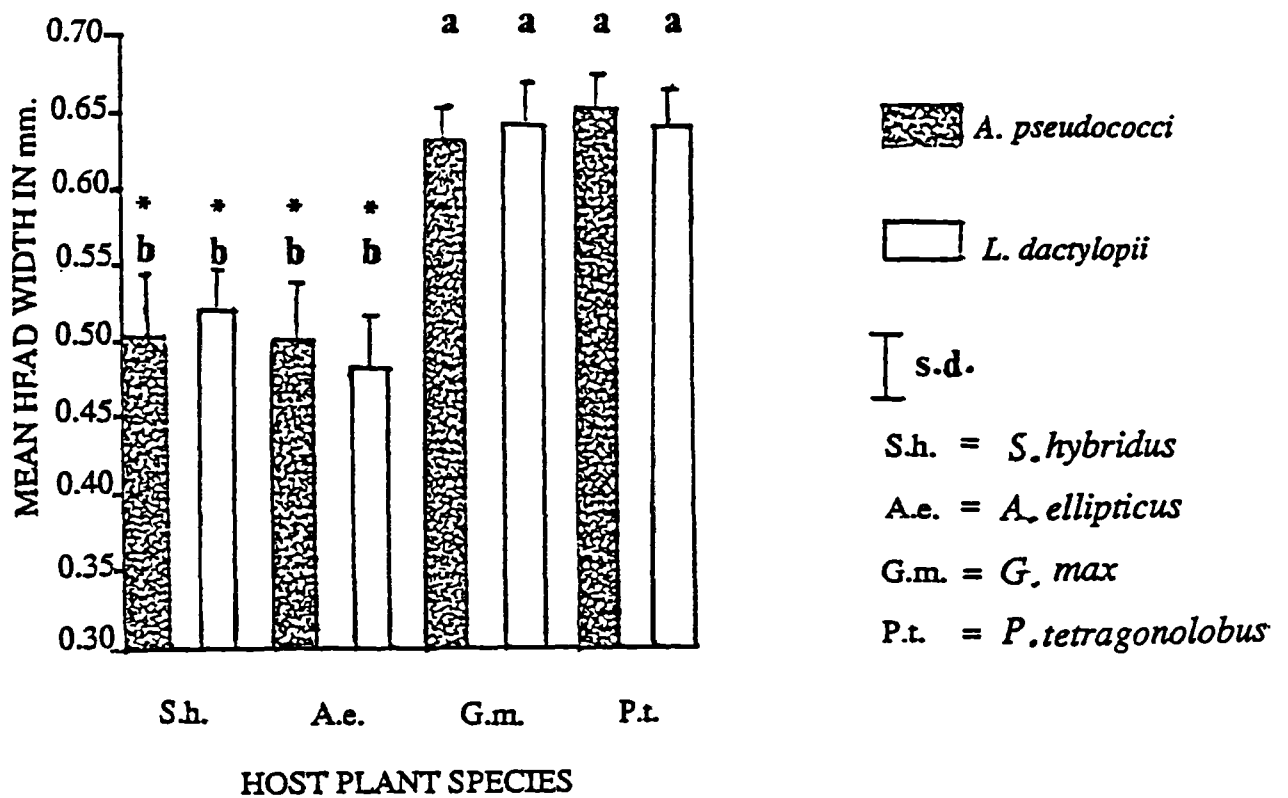


Fig. 2.1 Histograms showing mean head width $\pm$ s.d. of *A. pseudococci* and *L. dactylopii* emerged from *P. ctri* reared on various host plant species. Each mean value is an average of 15 insects. Means with the identical letters are not significantly different at the 5% level, SNK test.

2.5 DISCUSSION

2.5.1 Influence of host plant on successful parasitism by

A. pseudococci and L. dactylopii.

The inability of the host plant varieties to induce substantial changes in percentage parasitism of A. pseudococci or L. dactylopii may be due to a number of factors. However, two important points can be deduced from these observations. The parasitoids have not shown any discrimination between various groups of mealybugs reared on different host plants. The developing larvae seem unaffected either by nutritional quality of the hosts or existence of secondary plant substances.

As discussed in the review of literature, the direct interaction of plants in the third trophic level through chemical mediation such as synomones was not involved in the present investigation. In this study the parasitoids were released into an artificial arena with no direct interaction with the host plants. Thus any reason for changes in host acceptance could be — due to indirect involvement of the plants through the host. Neither factor seems to be involved in the present investigation, particularly as no difference in parasitism was observed between various host plant species. This result seems to conflict with the findings of Sato and Ohsaki (1987). They reported the level of parasitism of Pieris larvae was changed due to host plant effects. In our study the parasitoids were not exposed to field conditions but were confined to an arena to eliminate the direct effect of the host plants.

2.5.2 Influence of host plant on the developmental period of

A. pseudococci and L. dactylopii.

The mean developmental periods of male and female A. pseudococci and L. dactylopii are in agreement with those of Tingle (1985), who determined the influence of temperature on developmental periods of the same insects. Our findings showed that the males develop generally faster than the females, but showed no significant differences in developmental periods as a result of host plant intervention. However, there was an indication of faster development of female parasitoids grown on soy bean and winged bean reared host. There are two possible reasons for this. Firstly, the females are larger than the males and may require more food to develop within the host.

In section 1, we showed that mealybugs grown on A. ellipticus and S. hybridus plants were smaller than when reared on soy bean and winged bean. The poor nutritional status of the smaller mealybugs may have affected the slower developmental rate of female parasitoids in A. ellipticus and S. hybridus plants. Secondly, the male being smaller in size may require less food. Thus the smaller quantity of food may not be an important limitation for growth parameters of male parasitoids.

A similar explanation was given by Charnov et al., (1981) who showed that the shorter developmental period of males is primarily due to the smaller size of the insect. The present findings agree with Greenblatt and Barbosa (1981) who found that the mean development time of Brachymeria intermedia (Nees) was significantly reduced when the larvae of gypsy moth were fed on white oak reared hosts compared with other host plants.

The amount of nitrogen consumed by the parasitoids was greater when the parasitoid larvae were allowed to develop on grey-birch and synthetic diet reared host, than on oak leaf reared hosts (Greenblatt, et al., 1982). They attributed the longer development time to inadequate food in the host.

This theory was supported by Zhody (1976) who reported delayed development of Aphelinus asychis Walker when the potential host was reared on nutritionally poor diet. In accordance with these factors, the slower development of female parasitoids on S. hybridus and A. ellipticus grown hosts, could be due to poor nutrition in the host. More detailed work would be necessary to demonstrate the cause.

2.5.3 Influence of host plant on sex allocation by A. pseudococci and L. dactylopii.

The sex allocation mechanism of parasitoids largely depends on the size factor of the host. In general larger hosts favour female parasitoids (Waage, 1982; Waage & Stookming, 1984). Presumably a similar mechanism is adopted by A. pseudococci and L. dactylopii in allocating fertilized eggs on mealybug hosts. The frequency of females emerging from leguminous reared hosts was significantly greater than those developed from Streptocarpus reared hosts. Table 2.3.1. and 2.3.2 shows that the body length of P. citri is greater when they develop on leguminous plants. However, parasitoids offered only one size class of host eventually begin to produce both sexes (Sandlan, 1979). This accounts for the emergence of females from smaller mealybugs reared on Streptocarpus plants.

The polyphagous insects developing in a natural ecosystem invariably have to obtain their food from diverse resources. Diversity may be attributed to either morphological features or to the chemical constituents of the plants. Host plants are heterogeneous systems which provide varying quality and quantity of food for the herbivores. Population density, patch size and unit size of an individual insect greatly depend on the availability and exploitation efficiency of food by the insect (Letourneau, 1988).

In the present study we found higher quantities of phenolic compounds in A. ellipticus plant (see section 1) and low quantities of nitrogen on both Streptocarpus plants. Mealybugs reared on those poor host plants remained small and inferior. Parasitoids associating with various size classes of hosts also tend to produce diverse progeny in both sex ratios and body sizes. This variation could be equalized to a certain degree through mixing with various size classes. However, with crops the diversity of plants and herbivores is small resulting in a comparatively homogeneous host population. Under such a situation any adverse effect of host plants may affect the hosts growth and result in smaller parasitoids and male biased progenies. This will invariably lead to a poor establishment of natural enemies in a given ecosystem.

2.5.4 Influence of host plant on the longevity of female

A. pseudococci and L. dactylopii.

We have seen that the size and the longevity of parasitoids are positively correlated. Therefore the longer life span of parasitoids developed from leguminous reared mealybugs may be attributed to the larger hosts.

Similar effects have been recorded by various authors. Reduced life span was observed in Aphelinus asychis, a parasitoid of Myzus persicae, when the level of iron and sucrose was reduced in the aphids diet. Sandlan (1979) found positive correlations between the longevity of Coccygomimus turionellae L. and the size of the host. It appears therefore that the host plant factors can influence the life expectancy of parasitoids. This increased longevity may provide a greater reproductive capacity, since they have a longer ovipositional period.

2.5.5 Influence of host plant on the size of the emerging parasitoid.

The influence of plant factors on the third trophic level is particularly apparent in the size of parasitoids that emerged from soy and winged bean reared hosts (Fig 2.1). The fact that one group of parasitoids grew better than the other, may be attributed to the size of the host they fed upon. Other authors have shown that the size of the parasitoid positively correlates with the body size of the host (Charnov, et al., 1981).

In section 1 we showed that the length and weight of mealybugs, increased when they were reared on soy and winged bean plants. The reasons for size variations of mealybugs reared on various host plant species has been discussed in section 1. Similarly parasitoids developed on larger hosts should obtain sufficient food or balance of nutrients to grow into normal sized individuals. Hence the diversity of host plants can cause changes on the host population (van Emden & Williams, 1974; Murdoch, 1975) and such variations will be reflected in the parasitoids developing on those hosts (Powell, 1986).

2.6 CONCLUSIONS

1. There was no involvement of host plant factors on successful emergence rate of A. pseudococci and L. dactylopii.
2. Female A. pseudococci showed significantly faster development on soy bean and winged bean reared hosts than S. hybridus and A. ellipticus hosts.
3. Significantly larger parasitoids emerged from soy bean and winged bean reared hosts than from S. hybridus and A. ellipticus hosts.
4. Life span of adult parasitoids emerged from soy bean and winged bean reared hosts were significantly longer than from S. hybridus and A. ellipticus grown parasitoids.
5. More females emerged from soy bean and winged bean reared mealybugs but the sex ratio was male biased in S. hybridus and A. ellipticus reared hosts. Most of the fitness parameters of parasitoids were related to the size of the emerging insects.

SECTION 3

PLANT FACTORS AND ENCAPSULATION

3.1 INTRODUCTION

In order to achieve any substantial control of a given pest, the potential natural enemy should establish their progeny effectively. However, encapsulation may prevent or interrupt such establishment making biological control of some indigenous pests impossible. Encapsulation is a cellular defence reaction in which an insect's blood cells surround and isolate any foreign body that invades the haemocoel. This defence mechanism is widely adopted by hosts to overcome invasion of endophagous insect parasitoids. Generally this reaction is most effective in bringing about the immediate death of the parasite. Thus, the ability of haemocytes to encapsulate these foreign bodies has a significant value for the survival of the host. The efficiency of the host's defence system may be measured by the proportion of eggs or early larval stages encapsulated by the host haemocytes. It must be emphasized, however, that the ability of a host to encapsulate a parasitoid's eggs depends on many factors, such as the number of eggs in the haemocoel, parasitoid immuno-depression activities and the immune response of the host.

However, involvement of host plant factors such as nutrients and secondary plant metabolites on the physiological process or frequency of encapsulation are not fully understood. The aim of carrying out investigations on the effect of host plant factors on the third trophic level was to determine the relationship between host plants and mealybugs and the developmental stages of parasitoids within the host's body. If the host can sequester a host plant's secondary metabolites and make use of these substances to inhibit the development of parasitoid egg's then the success of a biocontrol agent may be reduced.

Involvement of phenolic derivatives such as polyphenol-oxidases and tyrosine in the encapsulation process are well documented in many insects. The present study deals with the additional data for the hypothesis that plant phenolic compounds are directly or indirectly involved in the encapsulation process. It was assumed that high phenolic plants provide extra phenolic compounds and hosts may sequester these phenols to enhance the melanization process of the parasitoid's eggs.

L. dactylopii proved to be an unacceptable parasitoid of P. affinis but A. pseudococci seems to be somewhat effective in suppressing the host population. Thus the present study was extended to find out the encapsulation frequencies of L. dactylopii and A. pseudococci under the influence of six host plant species. Further it was assumed that different parasitoid species have varying capacities to suppress the host immunity, either by injecting substances or coating the egg with resistant materials (Nappi & Streams, 1969).

Thus comparative studies were carried out to assess the intensity of the immunity reactions of P. citri and P. affinis when L. dactylopii and A. pseudococci oviposited on them. Two main hypothesis have been put forward to explain how parasitoids evade host defence systems. One hypothesis states that parasitoids secrete some unknown substances to initiate an immunity reaction in the host (Vinson, 1980). The alternative hypothesis suggests that an immunity reaction may be attributed to a wound reaction at the time of oviposition. In the present study these two hypothesis were tested by inserting inert materials into the haemocoel and wounding the host body to stimulate an immunity reaction.

3.2 REVIEW OF LITERATURE

3.2.1 Insect cellular defence mechanisms and encapsulation.

In most cases the formation of the capsule is carried out by blood cells adhering to each other and covering the foreign body to form a thick cellular mass (Salt, 1970). Fully developed cellular capsules are made up of 3 layers. The middle layer is the thickest and consists of flattened cells, while the outer and innermost layers are thinner and made up of somewhat spherical cells. All the cells in the capsule bind together with an intercellular cementing substance. The structure of the sheath capsule is somewhat different from cellular capsules as it has few layers of cells. The sheath starts at the micropyle end of the parasite egg, and spreads as a thin layer over the whole surface, eventually converting into a tough, brown sheath. However, the classification of capsules into distinctive categories is rather a difficult task (Salt, 1970).

3.2.2 Mechanism of encapsulation.

Many authors have produced evidence to prove that the encapsulation process is the product of the blood cells of insects (Rotherham, 1967; Kitano, 1969; Salt, 1970). However, Griffith (1961) highlighted two theories about insect defence systems against parasite invasion. He contended that the parasitoid was subjected to chemical activities of the host's body fluid, or it succumbed to phagocytosis or encapsulation. Researchers have shown that insect blood cells react after contact with a foreign body rather than to a stimulus. In the second phase the blood cells tend to adhere to the foreign object and the cells that adhere first pass the stimulus to the rest and form a mass of cells.

The aggregating process of blood cells seems to be enhanced by chemotaxis activities of haemocytes. Reik (1968) suggested that the on-going process of cellular aggregation finally ends in the formation of distinctive layers with intertwined cells bonded by cementing substances secreted by the cells. Dunn (1986) emphasized the role of haemocytes in insect defence against foreign bodies. He highlighted haemocytes in defence, particularly granulocytes and plasmatocytes.

More frequently the capsules under go a special chemical process to make them more tough and resistant (Salt 1970). This hardening mechanism is quite common in arthropods and is generally known as melanization. The melanization process is initiated by tyrosine under catalytic oxidation by phenol-oxidase. Phenol-oxidase occurs as an inactive precursor and is activated by limited proteolysis (Ashida & Dohke, 1980). However, the melanization process has been observed in the presence of phenol-oxidase inhibitors. This suggests that the activity of phenol-oxidase does not appear to be certain in the melanization process (Ratner & Vinson, 1983).

Nappi and Streams (1969) explained the function of crystalline cells in the melanization process. They observed a reduction in the number of crystal cells after 33 hours of oviposition by the parasitoid. The involvement of phenols in the formation of melanin is well documented in almost all the arthropods (Hackman, 1964; Wigglesworth, 1974). Catechol tyrosine. controls the blackening process and 3,4-dihydroxy benzoic acid and dopa only cause an intensification of the darkening of the melanin. Therefore it is quite evident that phenolic substances play a vital role in the melanization mechanism in insects (Hackman, 1964; Wigglesworth, 1974).

3.2.3 Inhibitors of encapsulation.

Tyrosine is a prerequisite for encapsulation and is readily available in the insect blood cells. However, Salt, (1970) stated that the addition of phenyl thiourea can inhibit the tyrosine-melanin reaction. A high percentage of nonencapsulated parasite eggs were observed in the larvae of saturniid moths after injection of 2,4-dinitrophenol, which is a respiratory inhibitor (Brewer & Vinson, 1971).

3.2.4 Encapsulation of inorganic substances.

Insects can recognize any foreign body in their haemocoel. Aggregation of blood cells towards inorganic substances in the haemocoel of insects is an indication of haemocytic reaction. A piece of nylon thread implanted into the haemocoel of Ephestia was successfully encapsulated 48 hours after implantation (Salt, 1970). This behaviour of an insect argues strongly against the possibility of having any stimulus in the foreign body to initiate encapsulation.

3.2.5 Factors affecting encapsulation.

Environmental and host factors, both can affect the rate of encapsulation. Many authors have shown that the ambient temperature can change the rate of encapsulation inside the host's body. Blumberg and DeBach, (1981), working on soft scale Coccus hesperidum L., reported that the rate of encapsulation increases with rising temperature. The encapsulation rate of P. manihoti Mat.-Ferr. under the parasitism of Epidinocarsis lopezi (De Santis) showed an increasing trend up to 35°C and the rate declined with a further rise in temperature (van Driesche et al., 1986).

The influence of temperature on the encapsulation rate of Metaphycus swirskii eggs in Saissetia oleae (Olivier) was studied by Blumberg (1982) who found a greater increase in the rate of encapsulation from 20% - 96% as the temperature increase from 24°C-32°C.

Further it was reported that the age of the host has a potential influence to alter the encapsulation rate. Blumberg (1982) reported that the rubber stage of S. oleae is more efficient in encapsulating than young females. Berberet (1982) observed a relatively higher percentage encapsulation by 3rd stage larvae in comparison with 2nd stage and 4th stage larvae of Hypera postica (Gyllenhal). However, relatively little information has been found on the influence of diet composition, plant metabolites, starvation or health and vigour of the host on the rate of encapsulation. Nonetheless Salt (1962) highlighted the importance of these factors in the success of biological control.

3.2.6 Immunity aspect of host defence.

The immunity mechanisms of insects are not fully understood due to the complexity of haemocytic reactions in the haemocoel (Dunn, 1986). An in depth study carried out by Kitano (1974) revealed that the percentage of haemocytes in haemocoel steadily increased after oviposition by Apanteles glomeratus L. on Pieris rapae (L.). He explained that the lack of encapsulative capacity of fourth instar larva of P. rapae might have been attributed to a low population of plasmatocytes. Some insects are capable of synthesizing new haemolymph protein in response to infection. The newly synthesized protein may differ between developmental stages within a single species (Nappi, 1970; Hughes et al., 1983).

Some haemocytes such as granulocytes and plasmatocytes are capable of identifying the foreignness through surface receptors and special proteins called lectins (Dunn, 1986). Brewer et al., (1972) found the lowest percentage of non-encapsulated eggs after incubation of Cardiochiles nigriceps in parasite egg-serum. They speculated this situation was due to the presence of antigen or a lack of an opsonin in host haemocoel. Brewer et al., (1972) found extra antigen bands in the gel electrophoretic plate of Heliothis zea (Boddie) haemolymph and they related this situation to the higher capacity of the host to encapsulate the eggs of Cardiochiles nigriceps Viereck.

3.2.7 Electrophoretic analysis of insect haemolymph proteins.

Oviposition may change many physical and chemical properties of the haemolymph of the host. Specific gravity and dry weight have been observed to be changed due to parasitism. This observation suggests that the parasitoids are capable of regulating the host haemolymph enabling them to grow within the host body (Vinson & Iwantsch, 1980).

Changes in the haemolymph constituents basically reflect the activities or physiological status of the insect. Variations in the protein composition of the haemolymph of insects have been observed after injection of bacteria or viruses (Arthur et al., 1969; Dunn, 1986). Gotz and Boman (1985) demonstrated by N-Lauroylsarcosine Sodium salt (SDS) Polyacrylamide gel electrophoresis that some insects can synthesise new protein as an immune response to foreign substances. Using isotopes they identified 2 major groups of immune proteins in the haemolymph of Hyalophora cecropia.

3.2.8 Role of parasitoids in overcoming host defence mechanism.

If all foreign objects are recognised as invaders by the host, the success of a parasitoid may depend on the failure of the host to recognise the parasitoid as a foreign object or the ability of the parasite to suppress the defence reaction of the host (Nappi & Streams, 1969). This phenomenon was strongly supported by Gotz and Boman (1985) who stated that through an effective natural selection process, parasitoids have evolved to overcome immunity enforced by the host. Salt (1968) highlighted some mechanisms such as rapid growth and development to evade cellular encapsulation. A typical example for this is Phaegenes nigridens which completes the first 3 larval stages within a day. The developing parasitoid larvae possess a trophic membrane with special cells known as teratocytes. These cells can dilute the host's haemocytes resulting in its ineffective defence (Gotz & Boman, 1985).

3.2.9 Inhibition of host immune system.

Some authors have identified symbiotic viruses possessed by certain braconid and ichneumonid parasitoids. These parasitoids use these virus particles as the immune depressing substances to overcome host immunity (Edson et al., 1981). These foreign particles have the capability to reduce host lysosomal activities. Moreover these particles can release proteases and lipases which can counter attack the host immunity system (Gotz & Boman, 1985).

3.2.10 Effect of calyx fluid of parasitoids to overcome the host defence system

Parasitoid's calyx fluids have a special mechanism for immuno depression in the host haemocoel (Dahlman & Vinson, 1977; Vinson, 1984; Gotz & Boman, 1985). Rotherham (1967) working on the ultrastructure of the calyx of adult Nemeritis found densely staining particles outside the calyx cells in the ducts. The surface of the egg was also covered with similar particles, evidently proving that parasitoids coat their eggs with special substances while they pass through the oviduct.

3.2.11 Influence of superparasitism on encapsulation.

The frequency of occurrence of encapsulation is affected by several variables. Kitano (1969) carried out extensive experiments on the influence of superparasitism on Pieris rapae. He found 98% encapsulation when parasitoid's eggs were introduced to non-parasitized larvae but the frequency reduced to 38% when the eggs were introduced to already parasitized larvae.

Kitano and Nakatsuji, (1977) hypothesized that some substances secreted by the parasitoid's eggs into the haemolymph prevent the encapsulation. Furthermore the amount of such a substance(s) presumably increases with a higher number of eggs deposited into the haemocoel, resulting in a reduction in the defence system of the host.

3.2.12 Encapsulation in mealybugs.

The defence mechanism of the order Hemiptera was extensively reviewed by Bess (1939), who studied the encapsulation behaviour of six mealybug species with two species of parasitoid, Coccophagus gurneyi Compere and L. dactylopii. He reported that when P. citri was offered to L. dactylopii only supernumerary eggs and larvae were encapsulated indicating a lower encapsulation rate in the normal host. But in contrast P. maritimus Ehr. was able to encapsulate most of the eggs of C. gurneyi revealing that P. maritimus is a non-host of C. gurneyi. Nenon et al., (1988) using the same host and parasitoid combination, found that superparasitism favours the frequency of encapsulation which seems contradictory to the other findings.

3.3 MATERIALS AND METHODS

3.3.0. Effect of host plants on the encapsulation.

Six host plant species were used in the current investigation and the details of the plants were given in section 1. P. affinis was used as the experimental insect and was fed throughout their life on the respective host plants prior to use in the experiment. The host plant species represented the treatments and each treatment consisted of 10 plants. Groups of twenty early fourth instar mealybugs were collected from each host plant for each parasitoid species and were transferred to labelled vials. Five-day old female adult parasitoids, A. pseudococci and L. dactylopii were collected from a culture maintained in laboratory conditions. The parasitoids were isolated and fed with 50% honey for two days prior to being used as experimental insects.

A replicate consisting of 20 mealybugs were placed into an experimental arena with one gravid parasitoid. Oviposition behaviour of the parasitoid was carefully observed and after just a single sting the mealybug was removed from the arena and kept in a labelled vial. The parasitoid was also removed after a single oviposition and was used subsequently at 2 hour intervals. Four such parasitization chambers were used at a time to expedite the oviposition process. Shortage of time to handle all the treatments together made it impossible to run the full replications concurrently so they were run on six consecutive days. The oviposited mealybugs were transferred to mature detached leaves in a covered petridish with a sufficient amount of moisture, and were kept in a constantly illuminated heated incubator ($28 \pm 1^\circ\text{C}$) for 5 days. At the end of the period, insects were transferred to 80% ethanol and dissected under a stereo-microscope to obtain the following measurements and counts.

For A. pseudococci

1. Number of mealybugs with encapsulated eggs.
2. Number of mealybugs with developing larvae.
3. Total length of the developing larvae.
4. Total length of the capsule.

For L. dactylopii

1. Number of mealybugs with encapsulated eggs.
2. Number of mealybugs with developing larvae.

3.3.1 Effect of host plant on the frequency of encapsulation of solitary eggs of A. pseudococci and L. dactylopii.

The frequency of encapsulation was calculated on the basis of the total number of mealybugs successfully parasitised by the parasitoids. Mealybugs without parasitoid larvae or eggs were not taken for the frequency calculations. Thus it was decided to use weighted analysis of variance to overcome the effect of unequal replication. The proportions of mealybugs with encapsulated eggs were calculated on a replicate basis and subsequently these values were used in weighted analysis of variance. Each parasitoid species was taken separately for the analysis of variance and comparisons of treatment means were based on calculated l.s.d. values on a pair basis.

3.3.2 Effect of host plant on oviposition rejection by

A. pseudococci.

Relevant information for the present analysis was obtained from the experiment described above. The proportions of mealybugs without parasitoid eggs or larvae was obtained on a replicate basis, as a fraction of the sample size. These frequencies were then subjected to one way analysis of variance. The treatment means were separated using Student Newman Keuls test at 5% level.

3.3.3 Effect of host plant on the development of a capsule along the larval body of A. pseudococci.

Relevant data for the present analysis was obtained from the section described in 3.3.0. Parasitized mealybugs were dissected 5 days after oviposition and thirty parasitoid larvae per host plant type were examined. The specimens were immediately transferred to 80% alcohol in a watch-glass. The length of the larvae and the capsule length which covered the body of the larvae were measured using a stereo microscope with an eye piece graticule. The proportional length of the capsule was calculated on an insect basis and 30 replicates per treatment were used in a one way analysis of variance. In a mixed larval population, the length of the capsule ranges from 0-100. Therefore the proportions were transformed to arc-sine after first substituting 0 values by $1/4n$, where n is the number of observations in a given treatment and the 100 by $100 - 1/4n$ (Gomez & Gomez, 1983). Analysis was based on transformed data and the treatment means were separated using Student-Newman-Keuls test at 5% level.

3.3.4 Influence of superparasitism on frequency of encapsulation.

The experimental procedure was similar to that of section 3.3.1. However, it was decided to use only A. pseudococci in the present investigation. Adult hosts were isolated in a parasitization chamber with a gravid female parasitoid. Similarly 3 such chambers were arranged and once parasitized mealybugs were repeatedly oviposited another 3 times. After the first oviposition all 3 parasitoids were kept away for 2 hours before using them again. Twenty mealybugs per plant and 10 plants per treatment were used in oviposition studies.

The following observations were recorded.

1. Number of mealybugs having 2 or more encapsulated eggs.
2. Number of mealybugs with partially encapsulated larvae.
3. Number of mealybugs with larvae and encapsulated eggs.
4. Total number of mealybugs with at least a single larvae.

The proportions of superparasitised mealybugs were calculated as a fraction of total mealybugs successfully parasitised by the wasp. The number of mealybugs with more than 2 encapsulated eggs were taken on a replicate basis for the frequency values. Weighted analysis of variance was carried out to obtain treatment means. The means were separated using calculated l.s.d. values on a pair basis.

3.3.5 Effect of host plant on the ability of mealybugs to produce a melanin cover over the developing larvae under superparasitized condition.

Relevant information and analysis were as in experiment 3.3.4. The proportions of partially encapsulated larvae were calculated as a fraction of total developing larvae in each treatment.

3.3.6.1 Immunity response of mealybugs to parasitoids.

The objective of this study was to find out the immunity reaction of mealybug species to various parasitoid species. Immunity responses will be measured as synthesis of new polypeptides or the increase of existing proteins in the mealybugs as a result of oviposition. Mealybugs were collected from uniform cultures maintained in the laboratory, grown on potato sprouts and they were used in the following treatments.

TREATMENT	HOST	PARASITOIDS	TIME AFTER OVIPOSSITION HOURS
1	<u>P. citri</u>	Control	
2	<u>P. citri</u>	<u>A. pseudococci</u>	24
3	<u>P. citri</u>	"	48
4	<u>P. citri</u>	<u>L dactylopii</u>	24
5	<u>P. citri</u>	"	48
6	<u>P. affinis</u>	control	
7	<u>P. affinis</u>	<u>A pseudococci</u>	24
8	<u>P. affinis</u>	"	48
9	<u>P. affinis</u>	<u>L dactylopii</u>	24
10	<u>P. affinis</u>	"	48

Approximately 25 mealybugs were used in each treatment. Five-day old gravid parasitoids were used in the oviposition and each parasitoid was used only once for this purpose. The oviposited mealybugs were kept in labelled vials with sufficient moisture until used in experiments. At the end of the prescribed periods, 20 mg of mealybugs were weighed and dissected under a stereo microscope to remove the parasitoid's eggs. These insects were immediately transferred to an ice cooled tissue grinder. The grinder was immersed in a beaker with sufficient ice cubes to cool the apparatus. Insect buffer (IB) (consisting of 20 mM Alistidine - HCl, pH 6.1, 2 mM EDTA and allowing it to stand for 60 min at 4°C) was added at a rate of 0.1 ml to the insect sample and was macerated until all the particles disappeared. The resulting liquid was then transferred to a labelled "Eppendorf" tube. Before the next batch of insects were ground the apparatus was thoroughly washed with distilled water. After macerating, the insect samples were kept in a refrigerator.

The macerated samples were clarified by centrifugation at 13,000 G for 15 minutes at 1°C. The supernatant was pipetted out to another set of labelled Eppendorf tubes and 0.1 ml of SDS was added and boiled for 3 minutes in a water bath. SDS PAGE gel (12.5%) was prepared (Laemmli, 1970) and 30 µl of insect samples were loaded to each well. The molecular weight markers (Bio-Rad) (30 µl) were also run simultaneously with other samples. The gels were stained overnight at room temperature with 0.25% (w/v) Coomassie brilliant blue. Destaining was done using 15% (v/v) methanol and 10% (v/v) acetic acid at 40°C. The destained gels were kept on a glass plate with adequate destained mixture until photographing.

3.3.6.2 Gradient gel electrophoresis.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was used to separate the proteins and the procedure was carried out as described by Koritsas (1987). The resolving gel contained a gradient of 7.5 - 17.5% (w/v) acrylamide. The stacking phase contained 3.75% acrylamide and 0.15% (w/v) bis acrylamide. Molecular weight markers (Bio-Rad) were run concurrently with the 10 samples. The quantity of samples used in each track was 30 μ l. The staining and photographic procedures were similar to those in experiment 3.3.6.1. The experiment was repeated 3 times with a new set of insects.

3.3.7 Inert foreign material and mealybug haemolymph reaction.

Approximately 0.1 mm diameter sand granules were selected as foreign inert material. These granules were washed and dried for 30 minutes under a flame and transferred into absolute alcohol to sterilize. Sterilized dissecting needles and a stereo microscope were arranged in a lamina flow sterile cabinet. Fifty adult P. affinis and P. citri were collected from potato sprouts to insert sterilized stone granules. A single stone bead per insect was inserted into the haemocoel. After each dissection needles were sterilized. Similarly the body of another batch of mealybugs were pierced for wound reaction.

Thereafter the dissected insects were moved into labelled vials with a moist filter paper until needed. After the prescribed period the mealybugs were taken for protein analysis according to the format shown below.

TREATMENT	HOST	OPERATION	DURATION IN HOURS
1	<u>P. citri</u>	control	
2	<u>P. citri</u>	wounded	24
3	<u>P. citri</u>	wounded	48
4	<u>P. citri</u>	stone inserted	24
5	<u>P. citri</u>	stone inserted	48
6	<u>P. affinis</u>	control	
7	<u>P. affinis</u>	wounded	24
8	<u>P. affinis</u>	wounded	48
9	<u>P. affinis</u>	stone inserted	24
10	<u>P. affinis</u>	stone inserted	48

The rest of the analysis was precisely similar to the method described in experiment 3.3.6.2. However, before grinding, the stone particles were removed from the insects with melanized capsules.

3.4 RESULTS

3.4.1 Effect of host plant on the frequency of encapsulation of solitary eggs of A. pseudococci and L. dactylopii.

The effect of host plant factors on the frequency of encapsulation of A. pseudococci are shown in table 3.1.1. The highest frequency of encapsulation was observed when P. affinis was reared on A. ellipticus and P. coccinea and least on S. hybridus plants.

Table 3.1.1 Effect of host plant on the frequency of encapsulation of solitary eggs of A. pseudococci.

HOST PLANT	No.OF OVIPOSITED MEALYBUGS	No.OF PARASITIZED MEALYBUGS	FREQUENCY OF ENCAPSULATION		
			UNWEIGHTED s.d.		WEIGHTED
<u>S. hybridus</u>	200	165	0.47	0.05	0.47c
<u>A. ellipticus</u>	200	116	0.71	0.13	0.69a
<u>G. max</u>	200	132	0.55	0.09	0.55b
<u>P. tetragonolobus</u>	200	137	0.54	0.06	0.55b
<u>P. coccinea</u>	200	125	0.63	0.14	0.62a
<u>P. caerulea</u>	200	146	0.55	0.11	0.55b
5% l.s.d. max-min 0.0889					

Analysis was based on unequal replication. The mean values were compared using calculated l.s.d. values on a pair basis. Figures with the same letters are not significantly different at the 5% level.

Table 3.1.2 summarises the influence of various host plant species on the ability of P. affinis to encapsulate the eggs of L. dactylopii. The analysis of variance shows that host plant species have no significant effect on the frequency of encapsulation of L. dactylopii. P. affinis has an ability to encapsulate a greater proportion of eggs of L. dactylopii than of A. pseudococci.

Table 3.1.2 Effect of host plant on the frequency of encapsulation of solitary eggs of L. dactylopii.

PLANT SPECIES	No.OF OVIPOSITED MEALYBUGS	No.OF PARASITISED MEALYBUGS	FREQUENCY OF ENCAPSULATION		
			UNWEIGHTED	s.d.	WEIGHTED
<u>S. hybridus</u>	200	167	0.94	0.05	0.94a
<u>A. ellipticus</u>	200	162	0.96	0.04	0.95a
<u>G. max</u>	200	166	0.95	0.03	0.95a
<u>P. tetragonolobus</u>	200	180	0.94	0.04	0.93a
<u>P. coccinea</u>	200	143	0.93	0.06	0.93a
<u>P. caerulea</u>	200	157	0.94	0.04	0.94a
5% l.s.d. Max - Min = 0.045					

Analysis was based on unequal replication. The mean values were compared using calculated l.s.d. values on a pair basis. Figures with the same letters are not significantly different at the 5% level.

3.4.2 Effect of host plant on oviposition rejection by A. pseudococci.

Table 3.2 summarises the frequency of unparasitized mealybugs after ovipositor piercing by the parasitoids. The highest but not significant percentage oviposition rejection was observed in A. ellipticus reared mealybugs and least rejection occurred on S. hybridus.

Table 3.2 Effect of host plant on oviposition rejection by A. pseudococci

HOST PLANTS	No.OF OVIPOSITED MEALYBUGS	No.OF MEALYBUGS WITH PARASITISED EGGS	FREQUENCY OF UNPARASITISED MEALYBUGS	s.d.
<u>S. hybridus</u>	200	165	0.175b	0.08
<u>A. ellipticus</u>	200	116	0.420a	0.12
<u>G. max</u>	200	132	0.340ab	0.12
<u>P. tetragonolobus</u>	200	137	0.315ab	0.17
<u>P. coccinea</u>	200	125	0.375a	0.19
<u>P. caerulea</u>	200	146	0.270ab	0.11
5% l.s.d. Max - Min = 0.124				

Analysis was based on unequal replication. Figures with the same letters are not significantly different at the 5% level.

3.4.3 Effect of host plant on the development of a capsule along
the larval body of A. pseudococci.

Table 3.3 summarises the results and the analysis of variance shows that the host species have a significant influence on the length of the capsule of A. pseudococci. The length of the capsule ranged from 35% - 63% of body length of the developing larvae. The capsule length was greatest on Gesneriaceae and leguminous plants and least on the Passiflora ($P < 0.05$).

Table 3.3 Effect of host plant on the development of a capsule along the larval body of A. pseudococci.

HOST PLANTS	MEAN CAPSULE LENGTH IN mm		MEAN LARVAL LENGTH IN mm		MEAN PROPORTIONAL LENGTH OF CAPSULE	
	$\bar{x}$	s.e.	$\bar{x}$	s.e.	$\bar{x}$	s.e.
<u>S. hybridus</u>	0.68	0.05	0.56	0.06	47.6ab	3.5
<u>A. ellipticus</u>	0.77	0.08	0.82	0.05	52.4ab	2.5
<u>G. max</u>	0.37	0.02	0.22	0.01	62.9a	1.7
<u>P. tetragonolobus</u>	0.69	0.05	0.75	0.07	50.6ab	4.2
<u>P. coccinea</u>	0.53	0.07	0.84	0.04	38.6bc	3.0
<u>P. caerulea</u>	0.55	0.09	1.15	0.09	35.8c	5.3
SNK test	(174, 30)		$S\bar{x} = 2.65$		MS = 216.2	

Analysis was based on arc-sine transformed proportions. Untransformed means are presented in the table. Figures with the same letters are not significantly different at the 5% level.

3.4.4 Influence of superparasitism on the frequency of encapsulation.

Table 3.4 shows that the proportion of mealybugs having more than two encapsulated eggs is unaffected by the host plant species on which mealybugs were reared. Nearly 60% mealybugs had more than two eggs and the recorded frequency of encapsulation were closely similar to those reported for the solitary condition (see Table 3.1.1). The influence of superparasitism on encapsulation is shown in Fig. 3.1. and no significant reduction was noticed in most of the host plant varieties except in S. hybridus plants.

Table 3.4 Influence of superparasitism on the frequency of encapsulation.

HOST PLANTS	No.OF OVIPOSITED MEALYBUGS	No.OF PARASITISED MEALYBUGS	FREQUENCY OF ENCAPSULATION WEIGHTED s.d	PROPORTION OF INSECTS WITH MORE THAN 2 EGGS WEIGHTED
<u>S. hybridus</u>	200	141	0.397e 0.06	0.67y
<u>A. ellipticus</u>	200	137	0.620a 0.06	0.58y
<u>G. max</u>	200	163	0.479cb 0.03	0.70y
<u>P. tetragonolobus</u>	200	141	0.518b 0.06	0.63y
<u>P. coccinea</u>	200	149	0.550ab 0.05	0.61y
<u>P. caerulea</u>	200	146	0.521ab 0.06	0.60y

5% l.s.d. Max - Min =0.143

Analysis was based on unequal replication. The means were compared using calculated l.s.d. value on a pair basis. Figures with the same letters within columns are not significantly different at the 5% level.

S.h. = *S. hybridus*
 A.e. = *A. ellipticus*
 P.co. = *P. coccinea*
 P.ce = *P. caerulea*
 G.m. = *G. max*
 P.t. = *P. tetragonolobus*

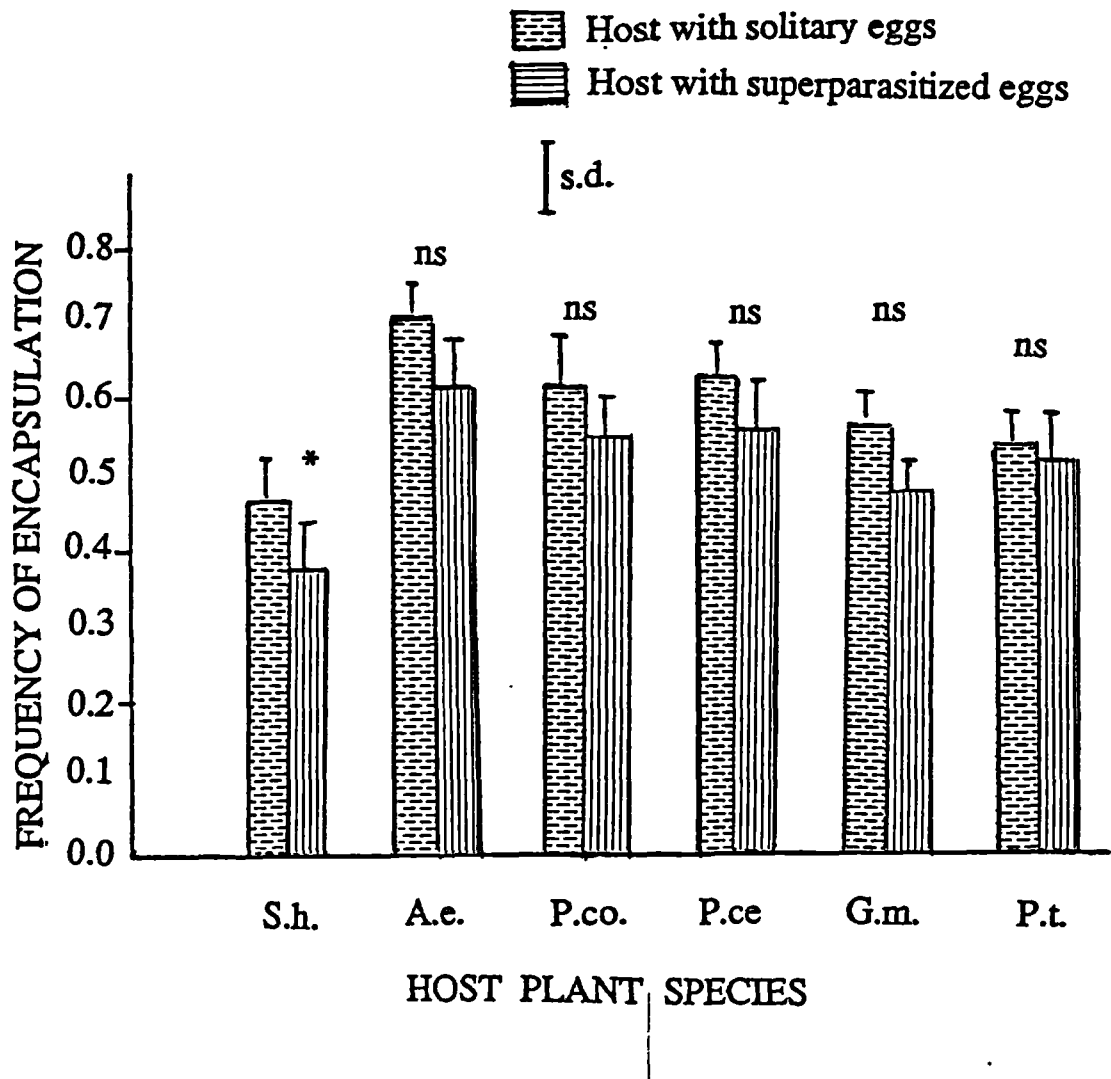


Fig. 3.1 Histogram showing frequency of encapsulation of solitary and superparasitized eggs of *A. pseudococci* in *P. affinis*. The host was reared on various host plant species and the effect of superparasitism on the frequency of encapsulation was compared using two-sample t-test at 5% level.

3.4.5 Effect of host plant on the ability of mealybugs to produce
a melanin cover over the developing larvae under superparasitism.

Partial melanization was observed in the greater majority of developing larvae in all the host plant combinations (Table 3.5). There was no significant difference between host plant species but obviously a higher percentage (ca. 90%) of larvae showed a melanin cover around the body. Often P. affinis showed higher potential to encapsulate A. pseudococci eggs even with developing larvae inside the body of the mealybugs.

Table 3.5 Effect of host plant on the ability of mealybugs to produce a melanin cover over the developing larvae under superparasitism.

HOST PLANTS	No.OF OVIPOSITED MEALYBUGS	No.OF MEALYBUGS WITH DEVELOPING LARVAE	FREQUENCY OF MEALYBUGS WITH PARTIALLY ENCAPSULATED LARVAE	FREQUENCY OF MEALYBUGS WITH DEVELOPING LARVAE AND ENCAPSULATED EGGS
<u>S. hybridus</u>	200	78	0.91 a	0.71 y
<u>A. ellipticus</u>	200	53	0.89 a	0.71 y
<u>G. max</u>	200	75	0.98 a	0.66 y
<u>P. tetragonolobus</u>	200	78	0.88 a	0.73 y
<u>P. coccinea</u>	200	67	0.88 a	0.64 y
<u>P. caerulea</u>	200	69	0.88 a	0.63 y

5% l.s.d. Max - Min = 0.196

Analysis was based on unequal replication. The mean values were compared using calculated l.s.d. values on a pair basis. Figures with the same letters within columns are not significantly different at the 5% level.

3.4.6.1 Immunity responses of mealybugs to parasitoids.

Attempts to separate various protein bands in the mealybugs using 12.5% resolving gel was not successful due to poor separation of low molecular weight proteins (plate 3.1).

Therefore a gradient gel was set to resolve low molecular protein in the haemolymph. Plate 3.2 shows an average of 9 visible bands in P. citri out of which 3 bands were distinctive from the others and the appropriate molecular weight of these bands were approximately MWr 54,000, 51,000 and 49,000.

Protein bands in unparasitized P. citri.

The prominent protein bands were in the range of MWr 54,000, 51,000 and 49,500 and are presumably associated with the immunity reaction of the insect. The low molecular weight band MWr 20,000 also appears to be a prominent protein band.

Protein bands in parasitized P. citri.

As a result of parasitism the concentration of some proteins, notably MWr 54,000, 51,000, 49,500 and 20,000 have increased to a detectable level. However, any new bands resulting from parasitism were not detected in the present experiment. In general, the protein banding pattern of non-parasitized host were similar to those parasitized ones except some selective bands become more dense in the latter.

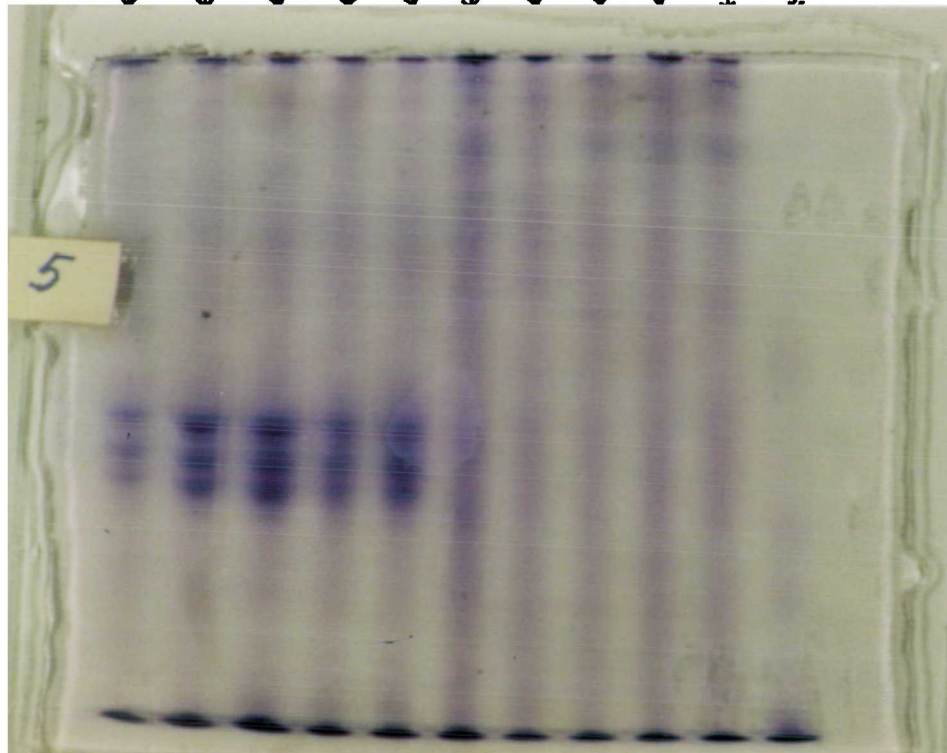
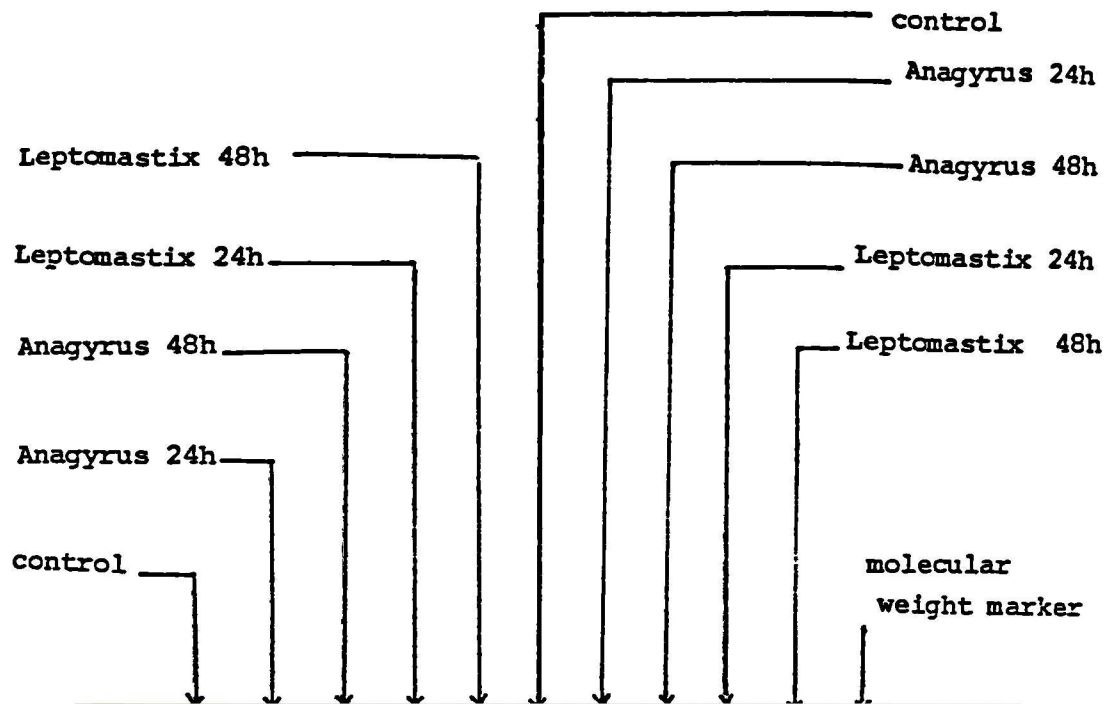
*P. citri**P. affinis*

PLATE 3.1 SDS-PAGE (12.5% resolving gel) electrophoretic pattern of *P. citri* and *P. affinis*. Extraction of host protein was carried out after oviposition by *A. pseudococci* and *L. dactylopii* (see text). Each column represent a different treatment.
(Note increasing of some protein bands with time in *P. citri*)

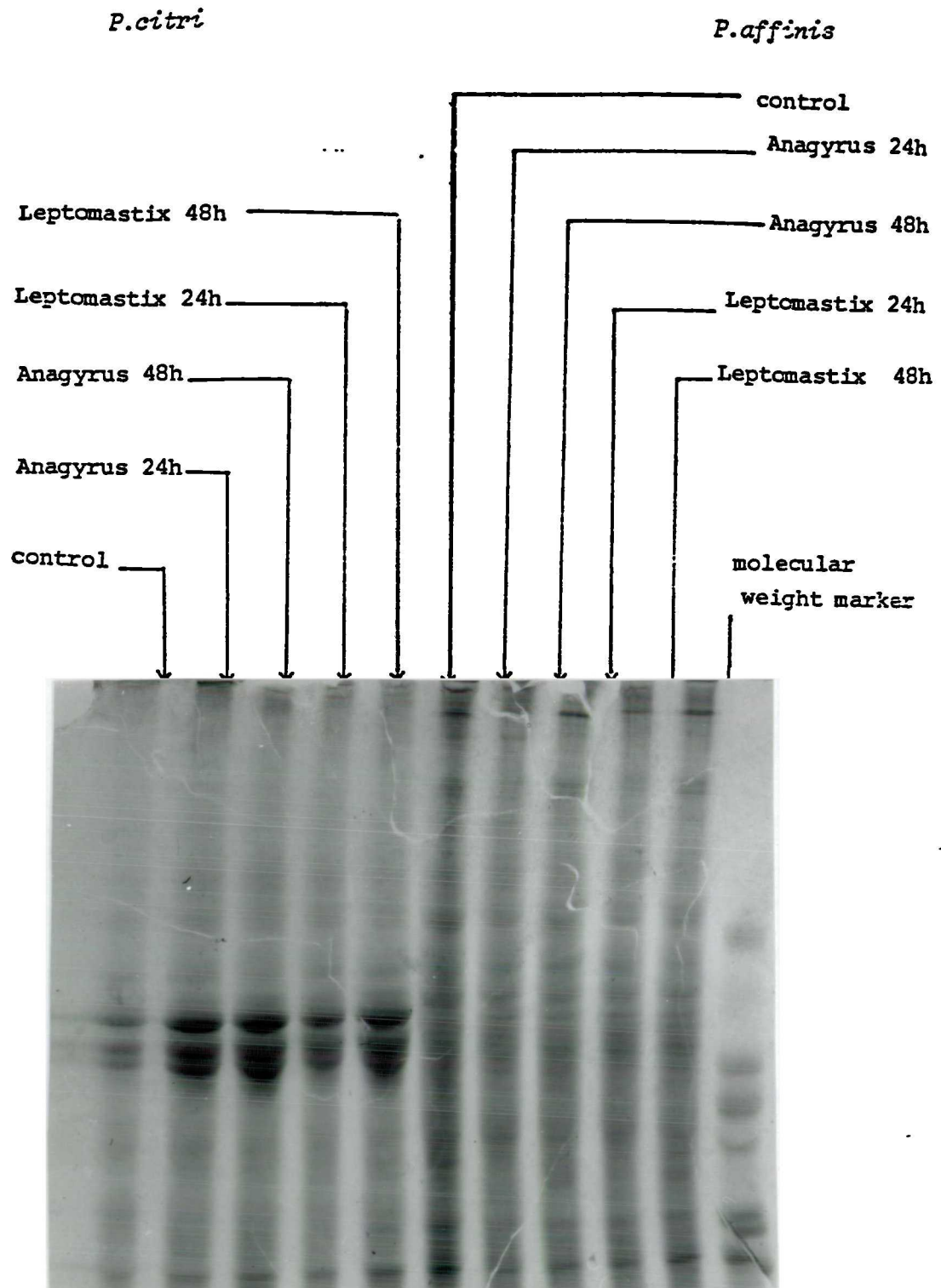


PLATE 3.2 SDS-PAGE (7.5%-17.5% gradient resolving gel) electrophoretic pattern of *P. citri* and *P. affinis*. Extraction of host protein was carried out after oviposition by *A. pseudococci* and *L. dactylopii* (see text). Each column represent a different treatment.
(Note better separation of low molecular weight proteins).

The immunity response to A. pseudococci was more obvious than L. dactylopii, as the latter induces relatively less protein in the host (see Plate 3.2 band thickness). The band intensities were visually estimated, and some increasing trends of concentration of proteins (i.e. 54,000, 51,000, 49,500, and 20,000) were observed in 48h treatment in comparison with 24h.

Protein bands in P. affinis

However, the immunity response of P. affinis was undetectable. The appearance of new polypeptide bands or thickening of existing bands were not evident in P. affinis. Nevertheless, there were about 15 visible bands in every treatment including the non-parasitized P. affinis (see Plate 3.1 & 3. 2).

3.4.7 Inert foreign material and mealybugs haemolymph reaction.

Plate 3.3 shows the polypeptide banding pattern of P. citri and P. affinis in response to wound reaction and artificial introduction of inorganic substances to the haemocoel. The most obvious dense 3 bands ranged between MWr 54,000 - 49,500 and another thicker band was visible at ca. MWr 20,000. This banding pattern was common for all the treatments including the control. Response to injury reactions was not visible either as formation of extra bands or increased density of the existing bands. There was not any apparent change in the polypeptide banding pattern in response to stone insertion to the haemolymph of either the insects (Plate 3.3).

*P. citri**P. affinis*

stone inserted 48h
 stone inserted 24h
 wounded 48h
 wounded 24h
 control

control
 wounded 24h
 wounded 48h
 stone inserted 24h
 stone inserted 48h

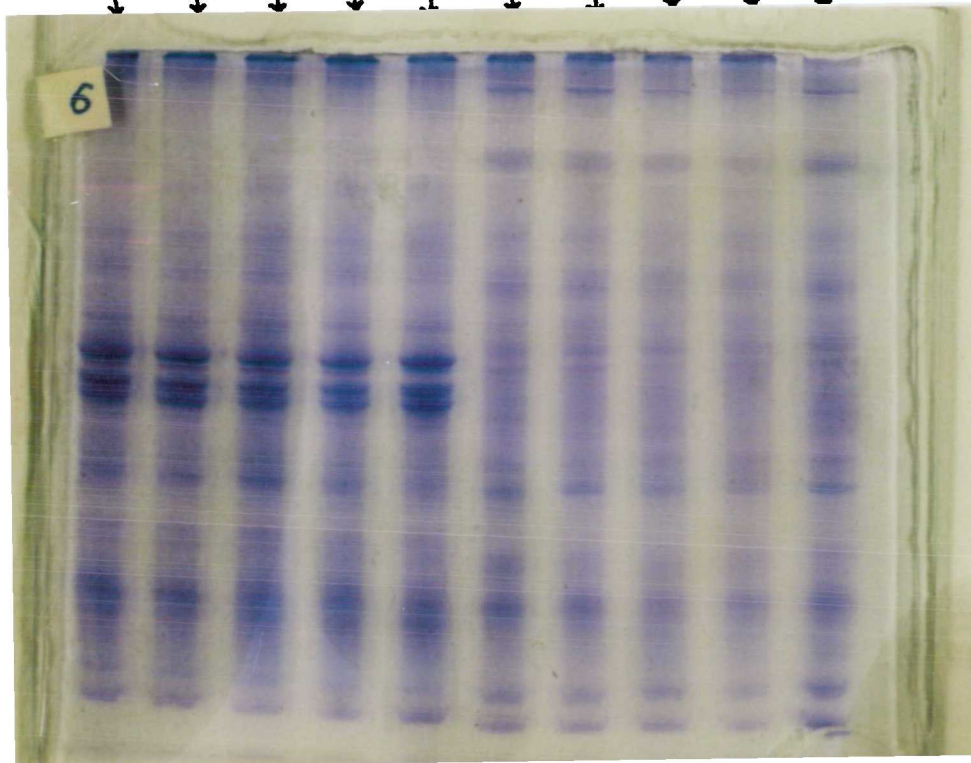


PLATE 3.3 SDS-PAGE (7.5%-17.5% gradient resolving gel) electrophoretic pattern of *P. citri* and *P. affinis*. Mealybug protein analysis was carried out after wound treatment or stone insertion. Each column represent a different treatment. (Note the similarity in banding pattern in all the treatments)

3.5 DISCUSSION

3.5.1.1 Involvement of host plant factors in the encapsulation process

It is apparent from the results that P. affinis is extremely effective in encapsulating eggs of A. pseudococci, when the hosts were fed on A. ellipticus and P. coccinea plants. The fact that some groups of mealybugs were more efficient in encapsulating parasitoid eggs than others suggests that there is a host plant intervention in this process. Streams (1968) showed the importance of the host's vigour to combat foreign invasion. Blumberg (1982) reported that the scale insect Coccus hesperidum was able to encapsulate eggs of Metaphycus swirskii Annecke & Mynhardt more efficiently when the hosts were on the host plant than when detached. He attributed the reduction in encapsulation on detached leaves to the weakened vigour of the host. Thus it was suggested that the observed differences in encapsulation frequencies in the present study could be due to the vigour of the mealybug acquired from their respective host plants.

Section 1, showed that the mealybugs fed on soy bean plants were much bigger than those fed on A. ellipticus plants. If insect vigour is needed for efficient encapsulation, one would expect a high frequency of encapsulation in soy bean, winged bean and Passiflora grown mealybugs. But the observed frequency values indicated that mealybugs reared on A. ellipticus plants had the greatest capacity to encapsulate eggs of A. pseudococci. Hence the available evidence does not support the hypothesis that the bigger hosts are more efficient in encapsulating parasitoid's eggs.

The present findings of host plant involvement in encapsulation seem to conflict with the findings by Blumberg (1977), who reported that the efficiency of encapsulation of Saissetia oleae grown on 3 different host plant species did not vary as a result of host plant varieties. Nevertheless the reported encapsulation frequencies in his study ranged between 3.3% - 11.5% indicating relatively low encapsulation rates. However, in the present study P. affinis were able to encapsulate ca. 60% of A. pseudococci's eggs when the hosts were grown on A. ellipticus plants but the value diminished to ca. 45% when the hosts were allowed to feed on S. hybridus plants indicating a greater difference than that reported by Blumberg (1977).

Another possible reason for higher encapsulation rates in A. ellipticus and P. coccinea plants could be the involvement of plant phenolic compounds in the encapsulation process. We reported in the literature review that insects in general make use of phenolic derivatives in the melanization process (Hackman, 1964). Despite the controversial evidence on the involvement of polyphenol oxidases in the encapsulation mechanism, Taylor (1969) highlighted the existence of more than one polyphenol oxidase system in insect haemolymph. It may be that the mealybugs make use of phenolic derivatives sequestered from host plants in the melanization.

However, involvement of host plant phenolic derivatives in the encapsulation has not been extensively investigated. Nevertheless, the present study indicated that mealybugs grown on high phenolic plants have a greater capacity to encapsulate the parasitoids eggs.

Further it was noticed that the relative concentration of vanillin was nearly 10 times greater in A. ellipticus plants as compared to S. hybridus plants. Interestingly the respective encapsulation frequencies also indicated a positive trend towards the total phenolic concentration in the plants (Fig. 3.2). Thus it can be suggested that mealybugs feeding on high phenolic plants may be obtaining relatively larger quantities of plant phenolic precursors and they may be utilizing them for defensive purposes. Phenolic derivatives such as tyrosine have a major role in the formation of the melanin cover over the encapsulated egg (Salt, 1970). Polyphenol-oxidases play an important role in the entire melanization process as an enzyme catalyst. Thus an inadequate supply of the above phenolic derivatives may deprive the normal melanization process, hampering the encapsulation process. However, more experimental evidence is required to prove this hypothesis, but it is one possible explanation.

3.5.1.2 Importance of parasitoid species on the frequency of encapsulation

Bess (1939) reported that P. gahani and P. maritimus were able to encapsulate all the eggs of L. dactylopii. This situation is essentially similar to the present investigation where nearly 95% of the eggs of L. dactylopii were successfully encapsulated by P. affinis. In P. citri, the normal host of L. dactylopii, only supernumerary eggs and larvae were encapsulated.

S.h = *S. hybridus*

P.t = *P. tetragonolobus*

P.ce = *P. caerulea*

G.m = *G. max*

P.co = *P. coccinea*

A.e = *A. ellipticus*

| s.d.



FREQUENCY OF ENCAPSULATION



CONCENTRATION OF TOTAL PHENOLS

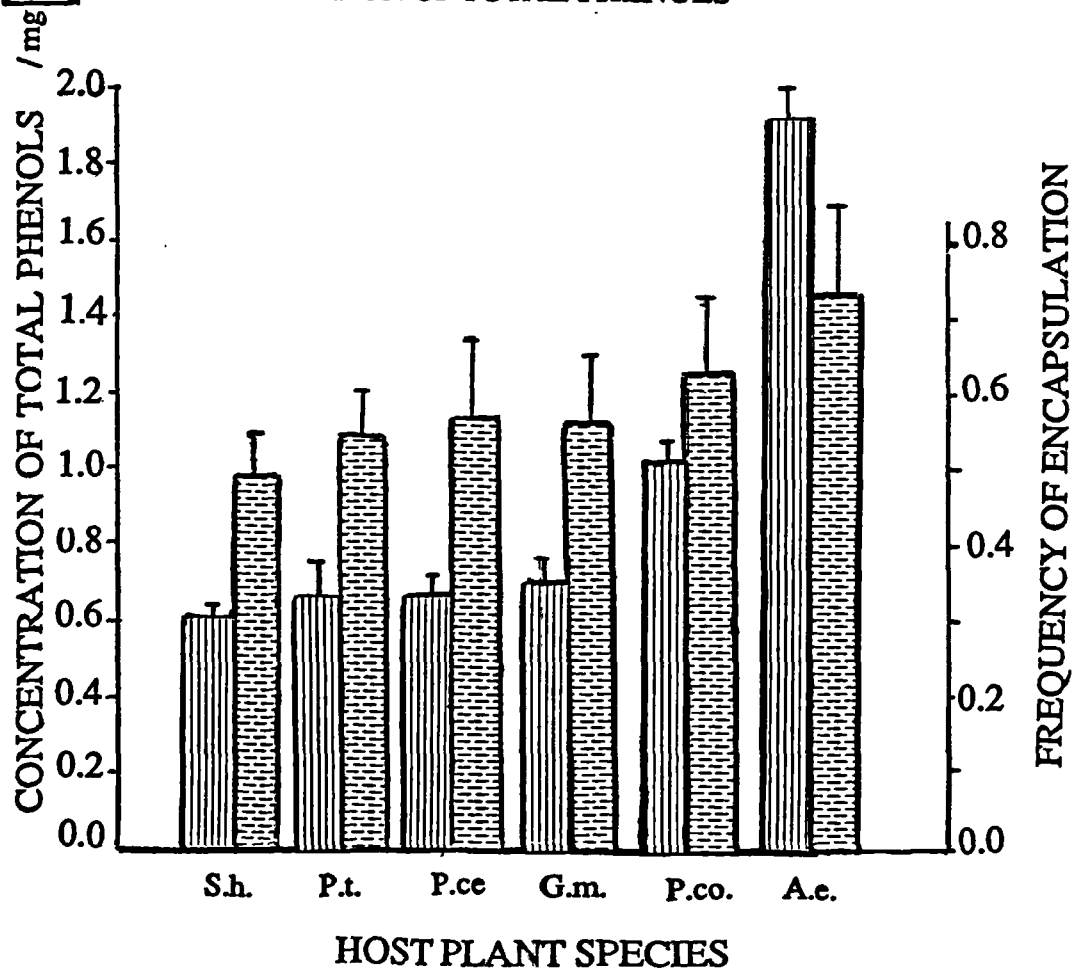


Fig. 3.2 Histogram showing relationship between frequency of encapsulation and total phenol concentration of various host plant species where mealybugs were reared. *A. pseudococci* was served as the parasitoid and *P. affinis* as the host.

In the light of these observations one can classify the host into 3 major categories depending on the ability of the host to encapsulate eggs of parasitoids:

- (A) Non acceptance where almost all the eggs are encapsulated.
- (B) Partial acceptance, where certain parasitoids will eventually develop.
- (C) Acceptance by the host where there is usually no encapsulation.

In the present investigation P. affinis could be considered as a non-acceptance host for L. dactylopii (ca. 90% encapsulation) and as a partial acceptance host for A. pseudococci (ca. 60% encapsulation).

3.5.2 Effect of host plant on oviposition rejection by A. pseudococci.

Vinson and Iwantsch (1980) revealed that some parasitoids are capable of using the ovipositor to detect the suitability of the host's internal condition before egg laying. Presumably a similar mechanism might have been adopted by A. pseudococci in detecting the internal conditions of mealybugs. It was noticed that the frequencies of oviposition rejection were significantly higher when the parasitoids were allowed to oviposit in A. ellipticus and P. coccinea reared hosts. In fact the same groups of mealybugs showed the highest encapsulation rates. This will lead to speculation as to whether some host plant factors are involved in changing the host's internal composition. Again further work would be necessary to confirm the host plant intervention in oviposition rejection by the parasitoids.

3.5.3 Effect of host plant on the development of a capsule along the larval body of A. pseudococci.

Salt (1962) reported that most of the eggs of L. dactylopii were able to hatch in P. gossypii but very few of the larvae succeeded in developing. The larvae living or dead became partly or wholly covered in a melanized sheath. A precisely similar observation was recorded in the present investigation. In most of the parasitoid larvae, the melanin cover was confined to the distal end of the larvae but progressive development of a melanin sheath was observed. It was noticed that the mealybugs reared on soy bean plant showed the greatest ability to form a melanin cover over the larval body. However, the recorded frequency of encapsulation was not as high as A. ellipticus reared mealybugs. This may be an indication that the efficiency of formation of the melanin cover is an independent event from the encapsulation process in the insect body. However, more work should be carried out to prove the suggestions.

This finding infers that, for encapsulation to be effective, two basic requirements should be fulfilled. Firstly, the host should recognize the parasitoid egg as a foreign organism and immediately should enclose it with a melanin capsule. Secondly, any larvae which do hatch should be enclosed completely from the distal to the proximal end of the larvae. Any melanin cover enclosing less than 100% of the larvae would be a fruitless endeavour of the host to combat against the foreign body (see Plate 3.4). In the light of these facts the group of mealybugs collected from A. ellipticus plants can be classified as most efficient in encapsulating A. pseudococci's eggs.

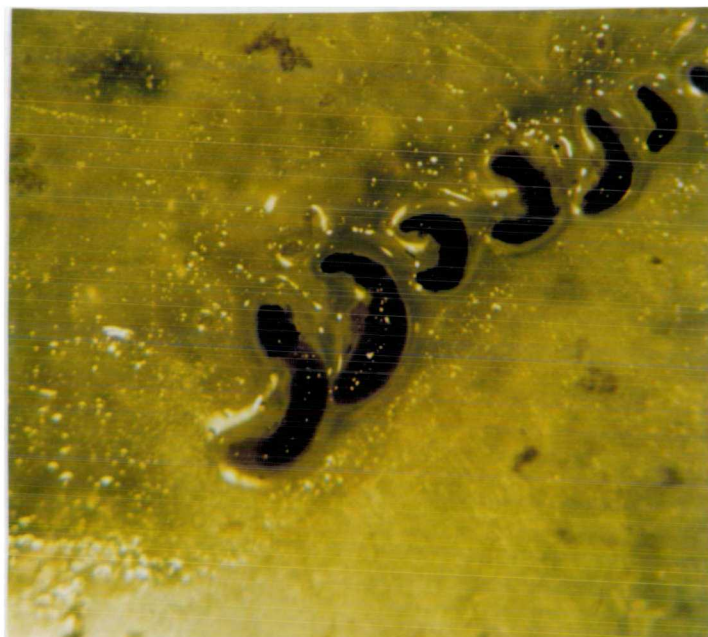


PLATE 3.4 Partially and fully melanized larvae of A. pseudococci within the body cavity of mealybug. Partially melanized larvae always have a clear anterior end. (Magnification x 40 approx)

3.5.4 Influence of superparasitism on frequency of encapsulation

Several authors have reviewed superparasitism in various host-parasitoid combinations and suggest that a greater number of eggs or larvae in a single host may deprive the host of its defence system. Under such conditions, parasitoids have a greater chance of evading the host defence mechanism (Streams, 1968; Blumberg, 1977; Kitano & Nakatsuji, 1978). The result of the present investigation indicated a pattern of reduction in the frequency of encapsulation due to superparasitism. However, there was no any indication of host plant intervention in the frequency of encapsulation under a superparasitized situation.

The possible reason for low encapsulation under superparasitized conditions could be due to parasitoid immune depression as a result of more eggs in the haemolymph (Kitano & Nakatsuji, 1978). A similar response to superparasitism was observed by Sullivan and Neuenschwander (1984) who reported ca. 10% reduction in the encapsulation frequency of Epidinocarsis lopezi on Phenacoccus manihoti. Thus the present findings are in agreement with several other workers.

3.5.5 Immunity responses of mealybugs to parasitoids.

The differences in the electrophoretic patterns obtained in the oviposition experiment clearly indicated that P. citri has synthesized extra protein as a response to parasitoid oviposition. The prominent bands appeared around 54,000, 51,000 and 49,500 MWr and could be the haemolymph protein of P. citri. Wyatt and Pan (1978) described haemolymph protein as slow migrating with a high molecular weight and the ability to form distinct bands in the gel.

However, a prominent banding pattern was not visible in P. affinis leading to speculation about the pH range in the SDS-PAGE system. Levenbook, (1985) used polyacrylamide gel electrophoresis to analyse storage protein of selected species of insects. He emphasized the importance of purity of the extract and the relevant pH values in the buffer, according to the molecular weights. Thus one possible explanation for the poor banding pattern of P. affinis could be due to an inappropriate pH value in the dissociation buffer. However, more experiments should be carried out to show this.

The density of newly synthesized proteins showed an increasing trend with time. The changes in the quantity of protein reported in this study agree in part with those reported by Vinson and Iwantsch (1980). However, Smilowitz (1973) found totally new protein bands in the electrophoretic plates resulting from oviposition by the parasitoid. The results of the present study seem to conflict with these findings of Smilowitz (1973) as there were no new bands in the gels. But the same author reported, denser protein bands in the gels with time which is identical to the present observations. It was observed that P. citri start premature egg production after oviposition by A. pseudococci and L. dactylopii. Thus the thickening of protein bands in P. citri could be attributed to enhanced egg production as a result of the oviposition by the parasitoids. This suggestion may be supported by the detection of similar positioned protein bands in SDS-PAGE of P. citri fed on host plants maintained under 3 nitrogen levels, where high fecundity of insects was associated with denser protein bands in the gel (see Plate 5.3).

3.5.6 Inert foreign material and mealybugs haemolymph reaction.

Visible new bands, or thickening of existing bands were not detected in the gels of stone-inserted and wounding treatments. This observation was explained by Gotz and Boman (1985).

Hence the results from these studies provide a positive indication that the synthesis of new protein (immunity response) in the host's body is induced by the parasitoid, presumably due to some substances injected by the insect (Srodaka & Vinson, 1978).

Most of the inserted stone particles were covered with a thin black layer presumably the melanin cover. This indicates the ability of insects to identify foreign particles in their haemocoel and respond to enclose them. The results resemble the insertion of pieces of nylon threads in Ephestia, where the entire piece was encapsulated after 48 hours (Salt, 1970). However, no proper explanation can be suggested for the encapsulation of stone particles without additional protein synthesis in mealybug host.

The parasitoids calyx fluid may be involved in an immunity reaction either by secreting fluid during oviposition or covering the egg while it is passing through the oviduct. However, it can be suggested that the high level of protein detected in SDS-PAGE of A. pseudococci could be the reason for the enhanced ability of this parasitoid to overcome the mealybug defence system.

Lower frequency of encapsulation, i.e. ca. 55% in A. pseudococci may be an indication of an enhanced immunity depression ability of the insect. However, more detailed work would be necessary to demonstrate the above unequivocally.

3.5.7 Implication of encapsulation on biological control

Encapsulation may considerably influence the degree of biological control or it can completely prevent or retard the establishment of parasitoids imported against indigenous hosts (van den Bosch, 1964). The efficiency of a parasitoid may be curtailed due to a greater number of eggs being encapsulated in the host's body. Thus mass rearing of parasitoids may be hampered as a result of the host defence system to overcome foreign body invasion.

Through superparasitism, parasitoids can overcome the host defence system to a certain degree. However, superparasitism has a limited role in field conditions as parasitoids tend to discriminate parasitized hosts. Therefore a clear understanding of host plant involvement in the encapsulation process is essential to achieve success in biological control.

3.6 CONCLUSIONS

1. P. affinis showed a high ability to encapsulate eggs of A. pseudococci when the mealybugs were reared on high phenolic plants such as A. ellipticus and P. coccinea.
2. Frequency of encapsulation was extremely high when L. dactylopii oviposited on P. affinis.
3. Frequency of oviposition rejection was greatest when A. pseudococci oviposited on mealybugs fed on A. ellipticus plants.
4. Mealybugs were able to encapsulate more than one egg in their body but no significant reduction in frequency of encapsulation occurred due to superparasitism.
5. The immunity reaction of P. citri was more prominent than P. affinis and the density of newly synthesized proteins showed an increasing trend with time.
6. Visible new bands or thickening of existing bands were not detected in the gels as a result of stone-inserting and wounding. This suggests that inert materials are incapable of inducing immunity reaction in mealybugs.

SECTION 4

INFLUENCE OF WATER STRESS ON MEALYBUGS AND ITS PARASITOIDS

4.1 INTRODUCTION

Water stress may be attributed either to a deficit or an excess of water in the growing medium. However, the deficit stress condition is usually known as water stress or drought stress (Levitt, 1972). It has been observed on many occasions that when plants are stressed they become a better source of nutrient for herbivores due to increases in the amount of soluble amino acids (Miles et al., 1982). Nevertheless under extreme water deficit condition, the level of nutrient in the plant might deplete through lack of water in the root zone.

Manipulation of the water status of the host plant could be a useful cultural method of controlling aphids. This idea was discussed by Kennedy, Lamb and Booth (1958) who found a deleterious effect of water stress on Aphis fabae. Moreover most of the work on water stress on host plant relationships have been done on a two trophic level system. This suggests that in-depth studies of natural populations and communities on host plants under various conditions is a prerequisite to obtain a clear understanding of the interaction in biological control. In spite of the fact that biological control has been practised in nearly every country for many years, relatively little basic information has been gathered on the effect of host plant water stress on herbivores and their natural enemies.

The present investigation was designed to obtain information on the influence of host plant water stress on mealybug population growth rates, fecundity, mortality and development rate. Cornwell (1956) reported that mealybugs tend to migrate in large numbers under wilting conditions. The results have also shown that reproduction of mealybugs is stimulated as the host plant starts to wilt. Therefore wilting of the host plant may change the host population densities and may affect the parasitoid populations. Severe drought stress is frequently associated with high ambient temperatures and low humidity levels around the plant. This situation may be detrimental to most of the arthropods associating with the plant, as they face problems of desiccation. Such a situation may adversely affect the ability of natural enemies to regulate their host (Mattson and Haack 1987). Hence the present study was extended to find out the influence of host plant water stress on aspects of the parasitoid's biology.

4.2 REVIEW OF LITERATURE

4.2.1.0 Plant water stress and techniques of assessing the stress levels.

Plant internal water deficits depend at any given time on the equilibrium between the water lost from the shoot and that absorbed by the roots (Etherington, 1975). Water deficit can be measured as a soil factor or more directly as a plant factor, where internal water relations are monitored by various techniques.

4.2.1.1 Measurement of plant water potential.

Leaf water potential is the accepted method of estimating water level in the plant, which is regulated by stomatal control. Accuracy of the measurement depends on various environmental factors like temperature, sunlight and wind speed (Etherington, 1975).

4.2.2 Relative water content (RWC).

Relative water content is a plant water characteristic which is simple to measure under field conditions. Plant characteristics such as waxiness and environmental factors generally do not affect relative water content (Slatyer and Barrs, 1965). Miles *et al.*, (1982) observed a decreasing trend of relative water content from 87% to 50% over a 10 day period after watering ceased in Brassica napus L. plants.

Youngman and Barnes (1986) reported similar variation in mean leaf water potential during experiments on almond trees. However, Wearing (1972) obtained 3 water stress levels in experimental plants by controlling soil water level between field capacity and permanent wilting point.

4.2.3 Effect of water shortage on plant nutrient balance.

4.2.3.1 Free amino acid.

Hsiao et al., (1976), Turner and Johnes, (1980) and Fukuntoku and Yamada, (1981) showed the osmoregulatory responses of plants to water stress involve many changes including increases in amino acids and soluble sugars. van Emden and Bashford (1971) recorded a higher level of proline in the stressed plants in comparison to unstressed ones. Barnett and Naylor, (1966) recognized the process of proline synthesis during drought stress as one way of storing nitrogen and carbon for future use by the plant itself.

4.2.3.2 Influence of water stress on total nitrogen of the plant.

The effect of water deficit on the nitrogen economy of the plant depends on the species and duration of the water stress relative to the other events in the plant's growth (Hanson & Hitz, 1983). Under high stress conditions physiological activities of plants become weaker, resulting in poor assimilation rates of nitrogen. Effects of water stress on nitrogen economy have been reported in various crops, such as corn, (Morilla et al., 1973) and barley, (Huffaker et al., (1970) under drought stress situations.

However, Mattson and Haack, (1987) reported that during mild to moderate stress the total nitrogen concentrations decline in roots and old above ground portions but increases in young above ground tissues. Scriber and Slansky (1981) have pointed out that the plant nitrogen and water levels are positively correlated not only to each other but also with growth and food utilization efficiency of insects. This idea was supported by Mattson (1980) and Bryant et al., (1983) who showed that plants poor in nitrogen and water tend to be rich in carbon based secondary compounds such as phenolics and terpenoids, whereas nitrogen and water rich plants are rich in nitrogen based compounds which favour insect growth.

4.2.3.3 Influence of water stress on some physical characteristics of the plant.

Curtis and Clark, (1950) reported that the cell walls and the cuticle thickness tend to increase with water stress. When stress becomes moderate to severe, cell division and enlargement is reduced, resulting in smaller plant parts e.g. leaves, buds, and xylem growth rings. Hsiao et al., (1976) highlighted the changes of micro-climate around the plant, due to water stress. He emphasized that during severe water deficit the stomata close to reduce transpiration. The reduced transpiration rate, increases leaf temperature and decreases vascular turgor pressure and humidity near the leaf surface.

4.2.4 Water stress and insect interactions.

4.2.4.1 Host plant turgor pressure and feeding efficiency of insects.

The role of turgor pressure and the rate of sap flow in the sieve tubes on the feeding efficiency of sap sucking insects is a controversial subject. This concept was positively argued by Kennedy *et al.*, (1958) who demonstrated that the aphid's feeding efficiency depend on the host plant turgor pressure. Weatherley *et al.*, (1959) showed poor larviposition by aphids, grown on stressed plants. They identified a reduction in the rate of sap flow and suggested that the low pressure caused a lower uptake of food by the aphids. This finding was supported by Wearing (1967), who found that Brevicoryne brassicae (L.), is more dependent on turgor pressure for feeding than Myzus persicae (Sulz). Growth and survival of aphids and mites has been shown to be influenced by severe water stress, and poor growth rates are due to lowered turgor pressure or increased cell-sap viscosity. A similar explanation was proposed by Auclair (1963) working with aphids where he stated that plant turgor pressure is one of the major limiting factors to feeding by sap-sucking arthropods.

4.2.4.2 Water stress and nutrient balance in herbivores.

Herbivorous insects possess sensory structures to detect common plant osmolytes which are generally phago-stimulating compounds. Sugar, free amino acids, salts and allelochemicals play a vital role as phago-stimulant osmolytes. Apparently most of these chemicals increase during drought stress (Visser, 1986). Proline and inositol are key compounds in stressed plants, and increase with the onset of drought.

Mattson and Haack (1987) identified these two chemicals as important feeding stimulants, though these chemicals are not essential nutrients for insects.

They hypothesized that since proline is a common plant osmolyte synthesized during drought stress, insects may use these cues to detect stressed plants. The importance of nutritional factors on insect outbreaks caused by weather induced plant water stress has been reviewed by White (1969, 1974 & 1984). He highlighted the greater survival of larval herbivores under stress conditions, due to improved plant nutritional quality. Physiological variations such as changes in the nutrient constituents are often not sufficient to change the performance of herbivorous insects. Scriber (1979b) demonstrated ^{the} survival of first instar larvae of Lepidoptera feeding on severely stressed plants, depend~~ed~~ on the available water in the plant rather than the increased nutrients due to stress. Thus it is certain that both, nutritional factors and water level of the host plant can affect the growth parameters of herbivores.

4.2.4.3 Influence of water stress on herbivorous insects with various feeding habits.

The influence of plant water stress on herbivorous insects depends upon the feeding habits of the insect (Mattson & Scriber, 1987; Scriber, 1977, 1984a; Miles et al., 1982). External leaf feeders such as lepidopteran larvae showed poor performance on severe drought stressed plants (Scriber, 1977 & 1979b). However, Orthoptera show a better performance under water deficits (Lewis, 1982; Lewis & Bernays, 1985).

Insects that feed by piercing and sucking plant tissues often perform better on plants grown under water deficits. However Wearing and van Emden, (1967) suggested that this aphid behaviour is not always true. Under severe stress condition aphid performances were poor as a result of low turgor pressure in the phloem vessels.

4.2.4.4 Influence of water stress on the developmental period of herbivorous insects.

Scriber (1977) experimented with Hyalophora cecropia L. (Lepidoptera: Saturniidae) and found, ^{the} that duration of both fourth and fifth larval instars as significantly greater when insects were fed with stressed leaves. He related the longer larval developmental period in the stressed treatment to poor utilization (or assimilation) of nitrogen due to reduced water for metabolic activities. Connor (1988), in supporting that argument, showed the poor performance of larvae of Corythucha arcuata (Say) (Heteroptera: Tingidae) in water stressed leaves. He suggested that the poor growth of larvae fed on high stressed leaves arose because of increased costs of extraction of water from the gut content or for the production of metabolic water. The animals which are forced to feed on a nutritionally poor diet consume less with reduced body metabolism. This will eventually result in lower relative growth rates and longer periods of development than organisms adapted to rich diets (Mattson & Scriber, 1987).

4.2.4.5 Influence of water stress on survival rates of herbivoreous insects.

The impact of water stress on survival of the Psyllid Cardiaspina densitexta was studied by White (1970). He observed a greater survival of the psyllid on the sun leaves of Eucaliptus (leaves which are facing sunlight) than on the shade leaves. This difference was mainly due to availability of richer food in the stressed leaves (those directly facing sunlight) than unstressed shaded leaves.

White (1974, & 1984) suggested that high early larval mortality resulting from starvation mainly checks the population at a low density level. He further explained that insect outbreaks are caused by weather-induced plant water stress that provides more balanced nutrient supply and hence increases early larval survival.

4.2.4.6 Effect of plant water stress on reproductive capacity of herbivorous insects.

Wearing (1972) produced experimental evidence for a lower percentage alate production of Myzus persicae and Brevicoryne brassicae in stressed plants. He found lower progeny production of aphids in stressed plants and attributed this to a combined effect of stress and leaf age. In contrast to this finding, Miles et al., (1982) reported no significant difference in the number of young produced by B. brassicae under varying stress conditions. English-Loeb (1989) studied the influence of water stress on population responses of spider mites and found that the mite did as well or better on well-watered plants compared to drought stressed ones.

4.2.4.7 Influence of water stress on life table statistics of herbivorous insect.

The r_m value of poikilothermic organisms largely depends upon the temperature and moisture, since those factors are closely related to longevity and fecundity. Moreover in nature many other biotic and abiotic factors affect the essential lifetable parameters influencing changes in r_m value (Andrewartha & Birch, 1954).

The relationship between many factors such as body size of the animal (Fenchel, 1974; Gaston, 1988), temperature effect (McMurty et al., 1970) host plant water stress level (Schulthess et al., 1987; Sumner et al., 1986) with innate capacity of increase of different animals have been discussed by various authors. Schulthess et al., (1987) performed extensive studies on the effect of various factors on the r_m , R_0 , and T , of cassava mealybug Phenacoccus manihoti and found that drought stress mainly influenced the net reproductive rate and had a mild influence on r_m and T in the low temperature regimes. However, at higher temperatures of 32°C the r_m value decreased in comparison with temperatures of about 20°C. They concluded that temperature changes during drought are not sufficient to change the r_m value in any significant shift to the level of an insect outbreak.

4.2.5.1 Plant induced water stress as a factor in parasitoid efficacy.

Stress induced allelochemicals may be sequestered by the host and passed on to the third trophic level influencing their life (Shepard & Dahlman, 1988). Similarly stress induces the production of several volatile compounds like ethylene, ethanol, ethane, and acetaldehyde (Mattson & Haack, 1987). Some parasitoids have evolved to use these volatiles as cues to find suitable hosts (Nordlund, 1987). Size factor of the host may be one of the parameters for a parasitoid to select a suitable host for oviposition. Hosts of different sizes may differ in their suitability for a parasitoid. Host handling behaviour, success of oviposition, development period, size of the emerging parasitoid, sex of the offspring, largely depend on the size factor of the host (see section 2). Thus water stress can enforce changes in most of the fitness parameters of the parasitoid.

4.3 MATERIALS AND METHODS

4.3.0. Experimental procedure to achieve required water stress levels.

Streptocarpus hybridus were selected as the test plants. Those showing good growth were potted into 10 cm diam. plastic pots with a peat based potting media. They were allowed to grow over 2 months to obtain a uniform and healthy appearance prior to the experiment. A preliminary experiment was carried out to determine the amount of water required to achieve 3 water stress levels.

4.3.0.1 Percentage water at field capacity

Initially it was necessary to calculate the available water which was held by the potting mixture at field capacity. Four soil samples were taken from the mixture and kept separately in a pressure plate apparatus for 7 days under 1/3 of a bar continuous pressure applied to the apparatus. At the end of the period the pressure was released and the sample weights were noted prior and after oven drying at 105°C. The calculation of field capacity value was based on the following formula

$$\frac{\text{wet wt} - \text{dry wt}}{\text{dry wt}} \times 100$$

4.3.0.2 Percentage water at permanent wilting point

The calculation of permanent wilting point was based on a similar procedure to field capacity but a higher pressure of 15 bar was set in the pressure plate apparatus over a 7 day period.

4.3.0.3 Available water

The difference between field capacity and permanent wilting point is known as the available water for plants. Plants have to obtain the required water within the range of field capacity and permanent wilting point and this value varies with both soil and plant types.

Field capacity of the potting mixture = $33.6 \pm 1.1\%$ s.d.

Permanent wilting point = $22.7 \pm 0.7\%$

Available water = $11.1 \pm 0.5\%$

4.3.0.4 Unstressed treatment

Plants in this treatment were given surplus water every 3 days and extra water was allowed to drain off. Under such a situation the soil reaches the field capacity level in a two days interval.

To achieve medium and high stress levels in the experimental plants, the potting media should be maintained in between field capacity and permanent wilting point values.

Therefore, two approximate values were identified of 28% for medium water, which is close to the field capacity and about 24% for high stress treatments, which is near to the permanent wilting point.

The potting media was oven dried at 105°C for two days prior to use in the experimental pots. Each pot contained 260 g of the dried potting mixture with 10 g of NPK fertilizer mixture.

4.3.0.5 Medium stress treatment

Healthy plants were removed from the growing media after 2 months and repotted with 260 g of dry potting media in each pot. In order to achieve 28% saturation, the potting mixture was given 72 ml of water. Subsequently the total weight of the individual pot was recorded and this weight was maintained throughout the experimental period by adding water every other day.

4.3.0.6 High stress treatment

A similar procedure was followed to maintain 24% saturation by adding and maintaining the required amount of water on a weight basis.

4.3.0.7 Introduction of crawlers on to the host plants.

For each treatment 20 uniform plants were arranged in a complete randomized design in a heated glasshouse cubicle. The experimental plants were kept for 30 days under the above conditions prior to the introduction of mealybug crawlers. This ensured the experimental plants reached appropriate stress condition before the insects started to feed.

Five plants per treatment for each species were selected to introduce mealybug crawlers to study the effect of stress conditions. A similar set of 5 plants per treatment were selected to grow mealybugs for further experiments with parasitoids. The rest of the plants were kept to collect leaves for the analysis of relative water content, total soluble amino acid and total nitrogen content.

4.3.1.1 Measurement of relative water content

An attempt to use the pressure chamber apparatus to measure leaf water content was abandoned, due to the high water content in the Streptocarpus leaf petioles. Instead the relative water content of leaf discs were measured by adopting the following method.

A mature leaf from each plant allocated for this purpose, was removed to obtain leaf discs at weekly intervals. Two leaf discs from each leaf were removed using a (2 cm diam.) cork borer. A total of 10 discs were taken on a weekly basis from each treatment to measure the relative water content. Immediately after excision these discs were weighed and transferred individually into labelled vials (2.5 cm diam.) with 5 ml of distilled water.

Vials were covered with a parafilm membrane and kept at $20 \pm 1^\circ\text{C}$, in an incubator for 24 hours. During the saturation period leaf discs take up water depending on the water deficit and become fully turgid. These turgid discs were removed and extra water droplets were removed without pressing the disc, and the turgid weight of the respective discs were recorded. Finally all the discs were transferred to labelled glass vials and oven dried at 110°C for 24 hours until constant weight was obtained.

The following formula was used to calculate the relative water content value of individual disc separately.

$$\frac{(\text{fresh wt} - \text{dry wt})}{(\text{turgid wt} - \text{dry weight})} \times 100$$

Using 10 replicates the mean relative water content and the s.d. were obtained for each treatment separately on a weekly basis. The weekly data for 3 treatments were presented in graphical form for total developmental period of the mealybugs.

4.3.1.2 Analysis of total soluble amino acid.

Three mature leaves were removed on a weekly basis from selected plants of each treatment to measure soluble amino acids. These leaves were immediately freeze dried for 24 hours and 50 mg of samples were taken for further analysis. These samples were transferred to 50 ml conical flasks with 25 ml of hot water and boiled for 20 minutes and filtered while still hot. The filtrates were made up to 25 ml by adding further hot water.

Ninhydrin reagent, Potassium Iodide (diluent) and Glycine standard were prepared in separate bottles (Appendix 3). A series of test tubes containing 0.5 ml of diluted plant extract and 0.5 ml ninhydrin reagent were prepared. A similar set of test tubes were prepared to which was added 0, 5, 10, 15, 20, 30 and 40 ml of glycine and 0.5 ml of ninhydrin. These tubes were boiled in a water bath for 15 minutes allowed to cool ^{then} for a few minutes and 5 ml of KI solution (diluent) added to each tube.

The spectrophotometer was adjusted to 570 nm, optical density value and standardized with a zero glycine working standard. Subsequently the respective optical density values of other glycine working standards were obtained using zero glycine as the reference sample. Treated leaf extracts were also subjected to spectrophotometric analysis to obtain relevant optical density values. The concentration of glycine standards and the respective absorbent values were plotted on to a graph and used to measure the leaf sample concentration. Total soluble amino acid concentrations were calculated by using a formula shown in Appendix 3.

4.3.1.3 Analysis of total leaf nitrogen.

Three mature leaves were removed from each treatment on a weekly basis and 0.1 g dried samples were taken to use in micro kjeldahl and automatic nitrogen analyser.

4.3.2.1 Influence of water stress on the developmental period of mealybug.

Five plants for each mealybug species and stress level were allocated and arranged in a completely randomised design. A fully mature healthy leaf was chosen from each and 200, one-day old crawlers were introduced to the upper surface of the selected leaves. The introduction of crawlers of the two mealybug species was done at 20 day intervals to facilitate accurate counting of the early developmental stages. The number of insects and their developmental stages were recorded every 24 h until all the surviving adults started egg laying.

As a result of natural mortality factors, the number of insects in each replicate diminished during the development of the insects. Therefore a fixed number of individuals per replicate and for each developmental stage were chosen. The number of insects chosen per replicate was 20, 12, 6 and 5 for first, second, third and fourth instars respectively. In the first instant the mean developmental period of a given stage was calculated on a replicate basis for all the treatments. Each stage was considered for factorial analysis separately, considering water stress (3 levels) and mealybug species (2 levels) as the variables. The mean values resulting from two way analysis of variance were separated using the SNK test.

4.3.2.2 Influence of water stress on the fecundity of mealybug.

Five host plants per treatment were identified and five egg laying mealybugs per plant for each mealybug species were collected. The mealybugs were kept in labelled gelatine capsules. Every 24 hours the total number of eggs produced by individual insects was separated and counts were made under a stereo-microscope. This was carried out during an entire egg laying period. The observations were carried out until natural death of the individuals. Total fecundity was obtained at the end of the ovipositional period. Factorial analysis was carried out as described in experiment 3.3.2.1.

4.3.2.3 Influence of water stress on pre-ovipositional, ovipositional and post-ovipositional periods of mealybug.

The pre-ovipositional period of the experimental insects were obtained from the experiment described in 4.3.2.1. Similarly ovipositional and post- ovipositional periods were taken from the experiment 4.3.2.2. Five adults per replicate and 5 host plants per treatment and for each mealybug species were used to obtain all the above values. Analysis was carried out as described in experiment 4.3.2.2.

4.3.2.4 Influence of water stress on intrinsic rate of population growth of mealybug

Life tables were constructed for each species from the daily glasshouse observation and laboratory maintained P. citri and P. affinis cultures. Some parameters, such as developmental period, total adult longevity, daily fecundity, and adult survival rate were obtained from experiments already described. However, the calculation of sex ratio was not based on daily progeny production. Instead three values were obtained at the beginning, middle and end of oviposition.

Life tables were constructed using the method of Andrewartha and Birch, (1954) using daily survival, daily progeny production, progeny sex ratio and adult longevity of two species of mealybugs, to allow comparison of the effect of water stress.

The following basic information was used to calculate r_m and related parameters.

x Age of individuals in days (age interval column).

l_x Proportion of individuals still alive at age x .

m_x Age specific fecundity: (daily eggs per female) $\times$ (female sex ratio).

GRR The Gross Reproductive Rate: the total number of females produced by an average female with full survival capacity.

R_0 The Net Reproductive Rate: the capacity of a species to multiply in each generation.

r_m Intrinsic rate of increase of a population: the increase under constant environmental conditions without any constraint of space and food.

T Generation time: total duration from birth of parents to that of their offspring. The following formula was used to obtain the T value ($\log_e R_0 / r_m$).

D Doubling time: time required for a population to double. The accepted formula to obtain the D value is $D = \log_e 2 / r_m$

A computer program written for this purpose was used to obtain GRR, R_0 and the r_m values (Appendix 4).

4.3.2.5 Influence of water stress on body size of adult mealybug.

Five plants per treatment were selected and five fully matured mealybugs were collected from each plant and kept in labelled vials. The length of individuals were measured under a stereo-microscope as described in experiment 1.3.7. The treatment difference was tested using factorial analysis.

4.3.3.1 Influence of water stress on successful parasitism.

The efficiency of parasitoids were estimated through the percentage successful parasitism by A. pseudococci and L. dactylopii on fully mature P. citri grown on 3 water stressed plants. Five plants per treatment were selected at random to collect P. citri for this study. Twenty early 4th instar per plant were collected for ovipositional studies. The experimental procedure and the statistical analysis was carried out as described in experiment 2.3.1.

4.3.3.2 Influence of water stress on the developmental period of parasitoids.

Relevant information for the present study was obtained from the experiment 4.3.3.1. Experimental procedure and the analysis of the data was performed as described in 2.3.2.

4.3.3.3 Influence of water stress on the longevity of female parasitoids.

The female parasitoids used in the experiment 4.3.3.1 were kept in separate vials for longevity studies. Thirty parasitoids per treatment and per species were identified and each insect was isolated individually in 50 mm x 25 mm glass vials streaked with 50% honey. Experimental detail and the statistical analysis was carried out as described in the experiment 2.3.4.

4.3.3.4 Influence of water stress on the size of emerging parasitoids.

Required information for the present investigation was obtained from the experiment 4.3.3.1. Experimental procedure and the statistical analysis was carried out as shown in the experiment 2.3.5.

4.4 RESULTS

4.4.1.1 Relative water content.

The mean relative water content of experimental plants are presented on a weekly basis in Fig 4.1. The standard error bars indicate the magnitude of fluctuation of stress levels with time. The plants kept under field capacity had the highest relative water content and the value ranged between 80% - 92% whilst the high stressed plants had low water content, ranging between 53% - 63%. The highest and lowest values for the medium water treatment was 81% and 69% respectively. During 6 - 9 weeks of stress treatment, the fluctuation of relative water content became relatively low indicating steady stress levels in the experimental plants. However, in the early part of the experiment greater changes in relative water content values were observed, presumably attributed to initial stress adjustments.

4.4.1.2 Analysis of total soluble amino acid.

The total soluble amino acid content of the leaf samples rose with increases in the water stress level in the host plants. For three weeks of stress treatment the level of amino acids in the experimental plants showed a relatively lower value but a steady increase was then observed up to the end of the experimental period. Further it is evident from the Figure 4.2 that the high stressed plants generally have a higher content of soluble amino acids than the other treatments.

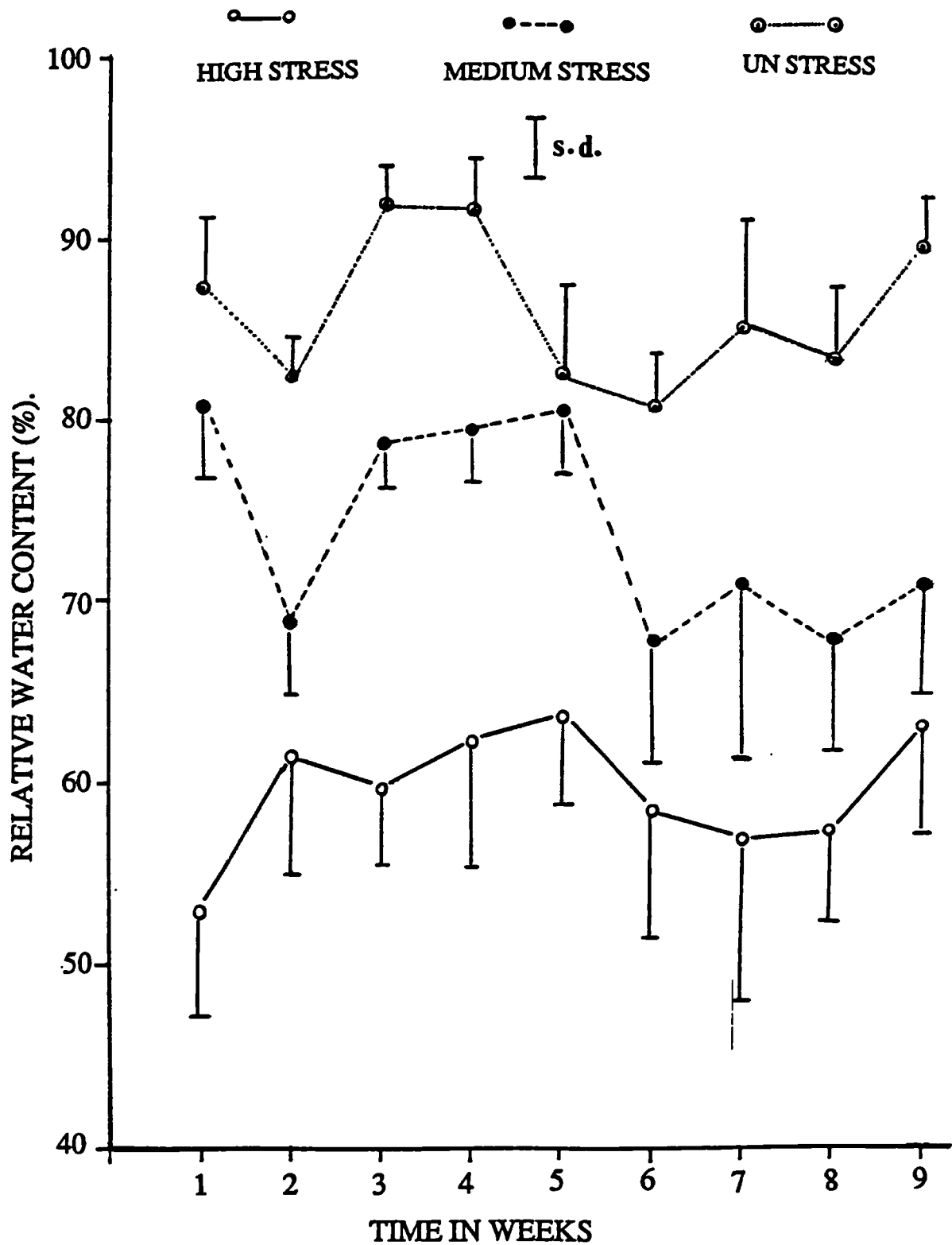


Fig. 4.1 Effect of intermittent water status in the growing media on relative water content of Streptocarpus plants. Plants were grown on 3 water regimes. Each mean value represents an average of 10 samples.

4.4.1.3 Analysis of total leaf nitrogen.

The effect of water stress on the total nitrogen level of the host plants was less consistent up to five weeks of stress treatment. The treatment means showed relatively higher values in the medium stressed plants followed by unstressed and lower concentrations in the high stressed treatments (Fig. 4.2).

4.4.2.1 Influence of water stress on the developmental period of mealybugs.

The influence of water stress on the developmental period of three larval stages and adult P. citri and P. affinis are shown in Table 4.1.1 and 4.1.2. The developmental period of the crawlers showed no difference between stress levels in either species ($P > 0.05$). The 2nd instar P. citri showed no significant difference between the stress treatments. However, the 2nd instar of P. affinis took significantly longer than P. citri although the medium stress treatment was significantly faster than the other treatments ($P < 0.05$). The 3rd instar P. citri fed on high stressed plants showed significantly longer developmental period ($P < 0.05$). Nevertheless the 3rd instar of P. affinis showed no response to water stress, although the time taken by the 3rd instar was considerably shorter than that of the 2nd instar.

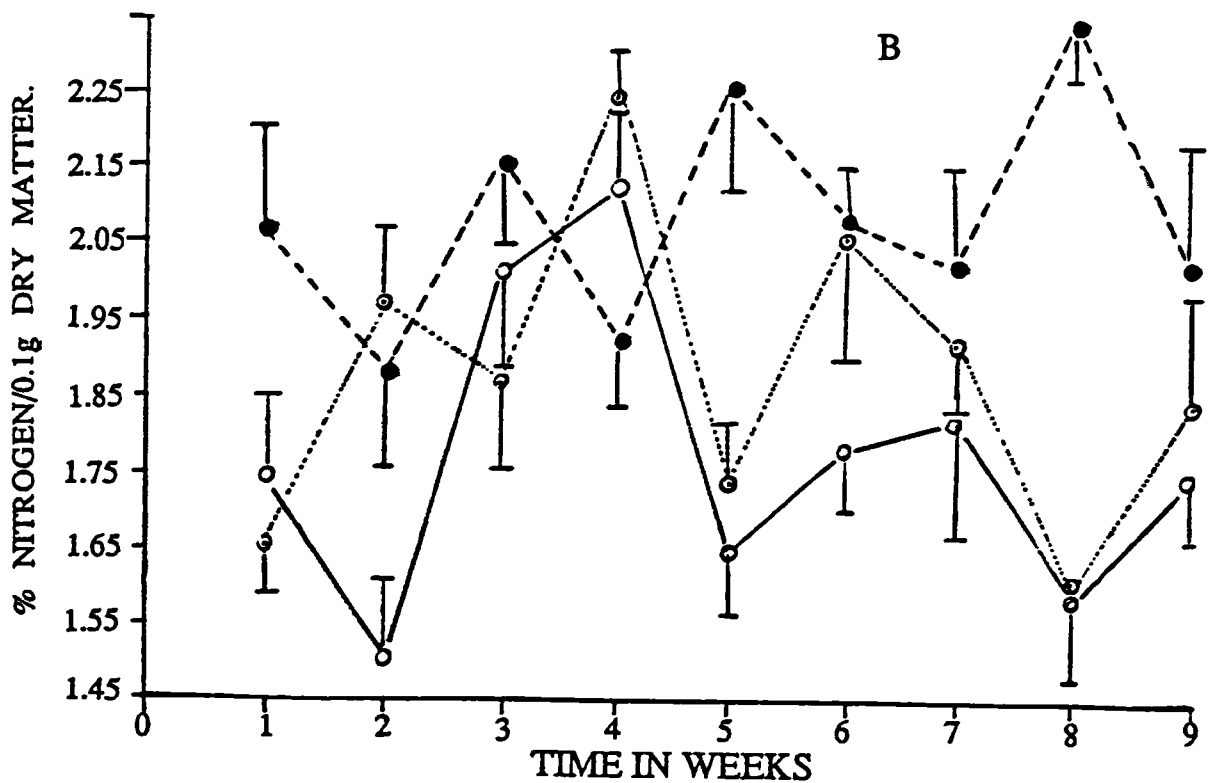
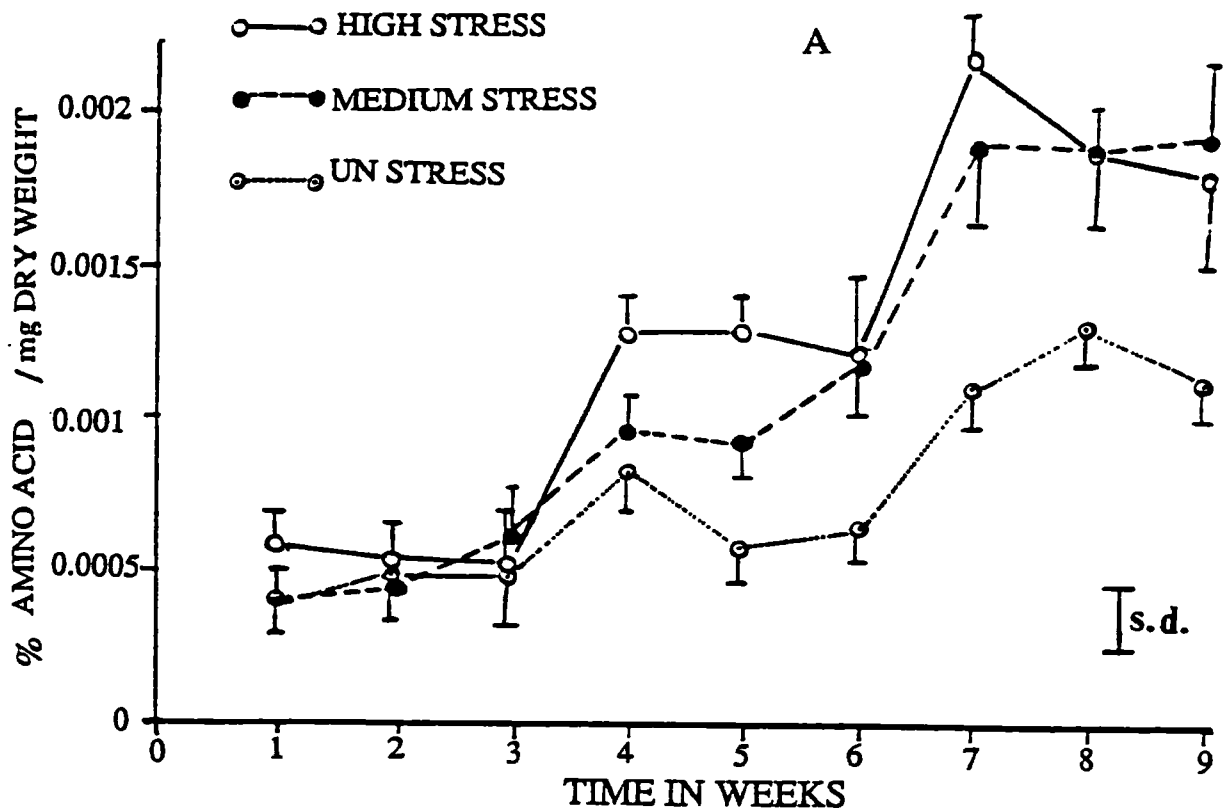


Fig. 4.2 Effect of intermittent water stress condition on (A) percent soluble amino acid content and (B) percent total nitrogen content of *Streptocarpus* plants. Each mean value is an average of 3 samples. Plants were maintained at 3 stress levels.

Table 4.1.1 Influence of water stress on the developmental period of larval stages of mealybug.

MEAN DEVELOPMENTAL PERIOD (DAYS)							
HOST	STRESS LEVEL	CRAWLERS		2ND INSTAR		3RD INSTAR	
		$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.
<u>P. citri</u>	Unstress	11.5c	0.7	16.4r	1.7	15.1v	2.2
	Medium	11.7bc	0.7	15.6r	1.5	14.1v	0.9
	High	10.7c	1.0	15.1r	2.7	21.8t	5.2
<u>P. affinis</u>	Unstress	13.7a	0.6	25.9p	1.5	15.4uv	2.4
	Medium	12.8ab	0.8	20.9q	1.9	18.5tu	1.1
	High	13.6a	1.4	26.2p	1.4	15.8uv	1.4
SNK test (24, 5)	n	100		60		30	
	$S\bar{x}$	0.377		0.83		1.18	
	MS	0.712		3.44		6.98	

Figures with the same letters within columns are not significantly different at the 5% level.

The developmental period of adult mealybugs was taken from the moult of the 4th instar to the formation of the ovisac (Table 4.1.2). The adults of both the species reared on unstressed plants have shown significantly faster development in comparison with high stress treatments ($P < 0.05$). Mealybugs fed on medium and high stressed host plants showed no significant difference between each other. However, the total developmental period of both the species of mealybugs have clearly shown that high stress condition is capable of inducing sufficient changes in the developmental period of mealybugs.

Table 4.1.2 Influence of water stress on the developmental period of adult mealybug.

MEAN DEVELOPMENTAL PERIOD (DAYS)					
HOST	STRESS LEVEL	ADULTS		TOTAL DEV.PERIOD	
		$\bar{x}$	s.d.	$\bar{x}$	s.d.
<u>P. citri</u>	Unstress	13.8d	0.4	45.1r	3.5
	Medium	14.8cd	0.54	47.9r	7.3
	High	15.9bc	1.0	56.9q	6.7
<u>P. affinis</u>	Unstress	13.6d	0.4	57.8q	3.6
	Medium	17.1ab	0.6	55.9q	6.2
	High	18.4a	1.2	63.1p	5.3
SNK test	n	25		15	
	$S\bar{x}$	1.21		1.29	
	MS	22.3		25.8	

Figures with the same letters within columns are not significantly different at the 5% level.

Table 4.1.3 summarises the overall effect of water stress on the developmental period of various stages of mealybugs derived by pooling both mealybug species data together. The results show that the stress condition has little or no response on the early developmental stages of mealybugs. The developmental period of 3rd instar and the adults is significantly longer in the high stress treatment compared with mealybugs fed on unstressed plants.

Table 4.1.3 Effect of water stress on the developmental period of mealybugs (species pooled).

MEAN DEVELOPMENTAL PERIOD (DAYS)				
STRESS LEVEL	CRAWLERS	2ND INSTARS	3RD INSTARS	ADULTS
	$\bar{x}$	$\bar{x}$	$\bar{x}$	$\bar{x}$
Unstress	12.5a	21.1d	15.3g	13.7q
Medium	12.2a	18.2e	16.3g	15.8p
High	12.1a	20.7d	18.8f	16.9p
n	200	120	60	50
5% l.s.d.	0.78	1.7	2.4	1.3

Figures with the same letters within columns are not significantly different at the 5% level.

4.4.2.2 Influence of water stress on the fecundity of mealybug.

Table 4.2 shows that the total fecundity rate of both the species is significantly reduced in the high stress treatment ($P < 0.001$). However, no such difference was noted between medium and unstressed treatments. The reproductive capacity of P. citri under high stress condition was greater than P. affinis, showing a greater ability to tolerate stress situations.

Table 4.2 Influence of water stress on the fecundity of two species of mealybug.

MEAN FECUNDITY PER FEMALE				
STRESS LEVEL	<u>P. citri</u>		<u>P. affinis</u>	
	$\bar{x}$	s.e.	$\bar{x}$	s.e.
Unstress	284.5a	14.0	248.7ab	13.5
Medium	275.2a	16.9	244.8b	13.3
High	112.9c	9.8	52.5d	3.8
SNK test	(144, 25)	$S\bar{x} = 12.6$	$MS = 3962.0$	

Each mean value is an average of 25 insects with 5 replicates. Figures with the same letters are not significantly different at the 5% level.

4.4.2.3 Influence of water stress on ovipositional and post-ovipositional periods of mealybug.

The ovipositional period of both the species showed a descending trend with the increase in water stress levels in the host plants. Table 4.3 and 4.4 shows that those insects which had a longer adult developmental period showed a shorter ovipositional period. That is under high stress conditions mealybugs have taken a longer period to reach sexual maturity and spend a comparatively shorter period in egg laying. The post-ovipositional periods of both the species are significantly longer under high stress conditions ($P < 0.05$).

Table 4.3 Influence of water stress on ovipositional and post-ovipositional period of mealybug.

MEAN DURATION OF VARIOUS STAGES (DAYS)							
HOST	STRESS LEVEL	OVIPOSITIONAL PERIOD		POST-OVIPOSIT PERIOD		TOTAL ADULT PERIOD	
		$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.
<u>P. citri</u>	Unstress	12.0a	2.8	2.3q	2.2	27.8m	3.2
	Medium	11.0ab	2.8	2.4q	2.3	27.8m	3.7
	High	8.6bc	2.5	5.0p	2.0	29.6lm	4.4
<u>P. affinis</u>	Unstress	11.0ab	2.3	2.6q	2.1	27.2m	3.6
	Medium	11.2ab	2.5	2.8q	2.5	31.2kl	4.2
	High	9.8bc	2.7	7.2p	1.7	33.6k	4.3
	n	25		25		25	
	Sx	0.25		0.38		1.32	
	MS	6.98		3.6		43.63	

Figures with the same letters within columns are not significantly different at the 5% level. (SNK test 144, 25).

Table 4.3 shows that the total adult life span of P. citri was unaffected by the level of water in the host plants where they fed throughout their life. However, P. affinis had a significantly longer life span when they were reared on medium and high stressed plants ($P < 0.05$).

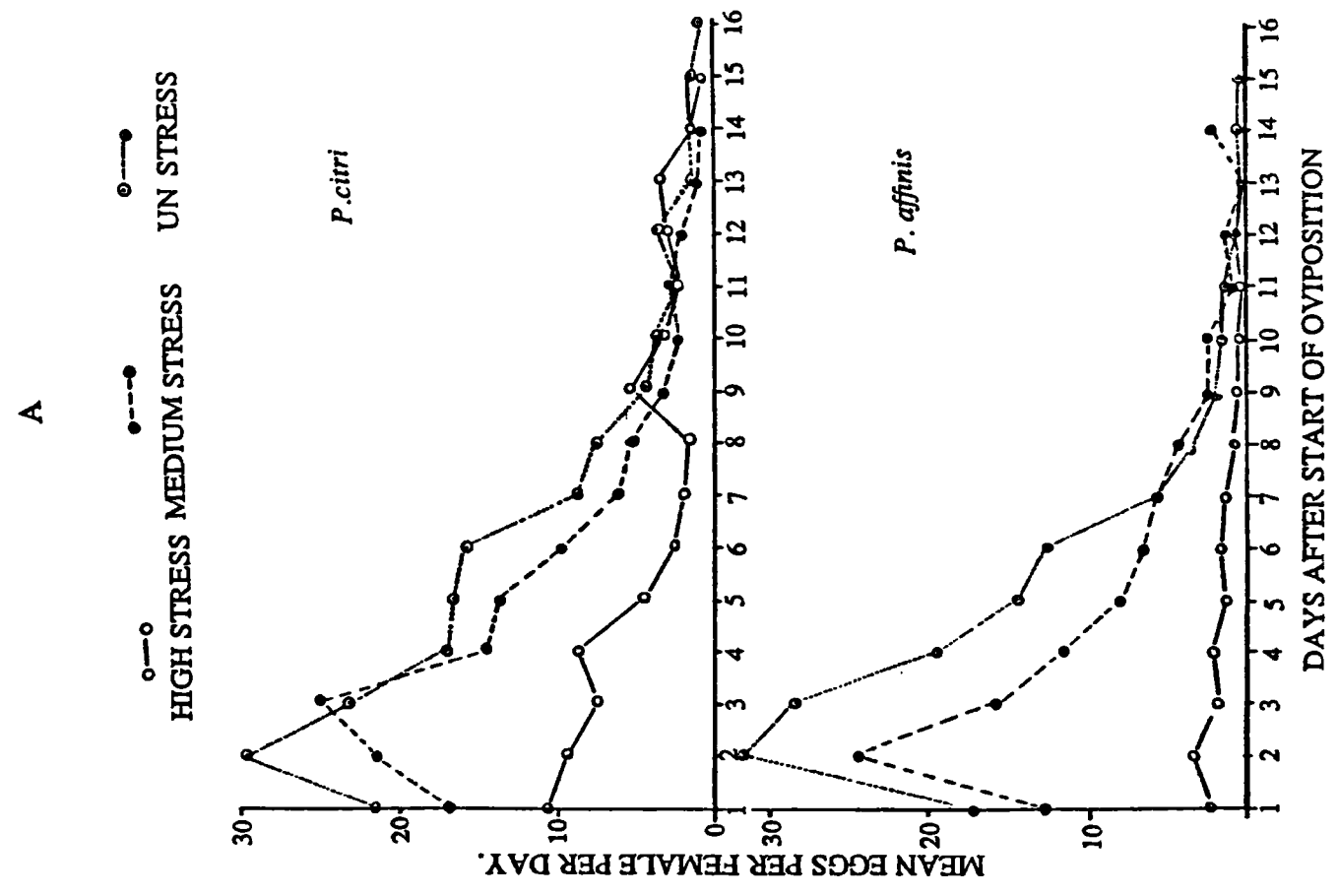
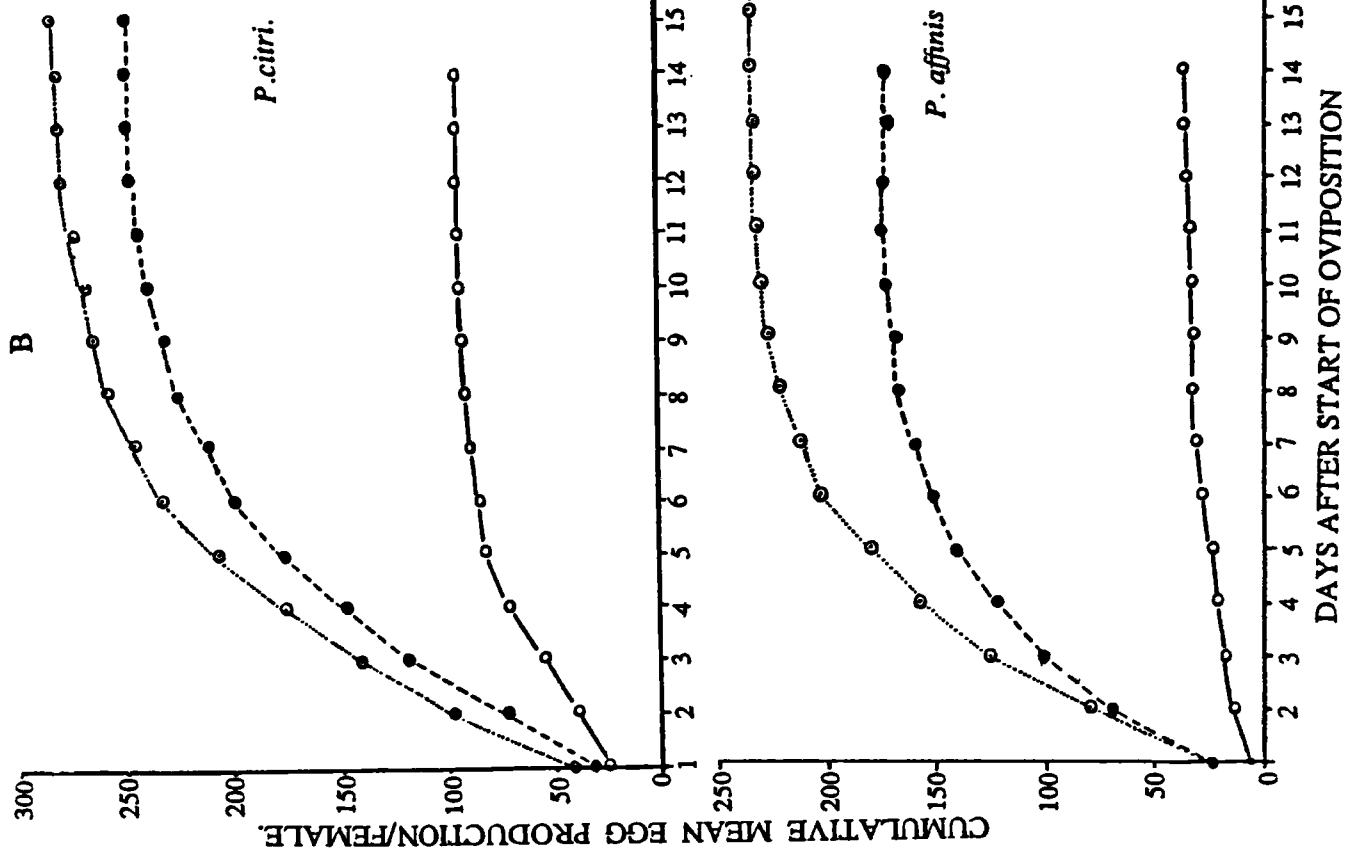
4.4.2.4 Influence of water stress on intrinsic rate of population growth of mealybug.

Age specific fecundity

The age specific fecundity is an essential parameter in calculating life table statistics. The graphical presentation of age specific fecundity of two species of mealybugs under 3 water regimes are shown in Fig.4.3. In most cases a general pattern was followed i.e. a rapid increase to a peak within 2-4 days, followed by a small fluctuation and finally a sharp decline until the end of the ovipositional period. The peak egg production of P. citri in unstressed plants, was reached on the second day of oviposition and approximately 30 eggs per day/female was observed. A rapid decline of oviposition was noted at the end of the 6th day when over 80% of the total eggs had been laid. This is further illustrated in Fig. 4.3 B.

P. citri on medium stressed plants had peak egg production on the 3rd day of oviposition when about 25 eggs per day/female was observed. After 5 days of oviposition females have laid over 65% of their total egg production. The egg laying behaviour of P. citri on high stressed treatments was some what different and peaks of 10 eggs per female were observed on the first day of oviposition.

The egg laying behaviour of P. affinis also showed a similar trend to P. citri. The maximum oviposition rates 31.8, 24.5 and 3.7 eggs per day/female were observed in unstressed, medium stressed and high stressed treatments respectively. The cumulative daily mean fecundity figures indicated that the effective ovipositional period in mealybugs lasts only for 5-6 days during which period the insects deposit more than 80% of the total egg production.



Construction of life table

Table 4.4 Influence of water stress on age specific life tables and related parameters of P. citri and P. affinis.

STRESS LEVEL	<u>P. citri</u>			<u>P. affinis</u>		
	NONE	MEDIUM	HIGH	NONE	MEDIUM	HIGH
Sex ratio (F/F+M)	000.55	000.51	00.51	000.60	00.60	00.51
GRR	162.7	129.7	50.6	143.3	95.9	17.9
Ro	160.6	124.2	48.8	142.4	91.6	17.7
rm	000.092	000.086	00.069	000.08	00.074	00.042
T	055.2	055.8	55.6	061.9	60.4	69.1
D	007.53	008.03	09.92	008.66	09.28	16.60

GRR = Potential reproductive rate, Ro = Net reproductive rate,
rm = Intrinsic rate of increase, T = Generation time, D = Doubling time.

Fewer females were produced on dry regime plants than on wet ones. The potential reproductive rate (GRR) decreased with rising stress levels. The GRR and Ro values for P. citri in unstressed treatments was 3 times greater than under high stress. The same value for P. affinis showed a nearly 7 fold reduction indicating the greater influence of stress on P. affinis.

The rm values were in decreasing order with rising stress levels of the host plant. The highest rm value was recorded in the unstressed treatment for both insect species. The rm values of P. citri under various stress treatments were shown to be higher than the respective values of P. affinis. The generation time was little influenced by the stress factors.

However, the "doubling" time for both species increased with a rise in the stress condition of the plant. High stress treatment caused a two-fold increase in the "doubling" time of P. affinis.

4.4.2.5 Influence of water stress on the body size of adult mealybug.

As shown in Table 4.5 the mean body length of mealybugs was inversely proportional with the increase of water stress levels of the host plants.

Table 4.5 Effect of water stress on the body size of the adult mealybug.

MEAN BODY LENGTH IN mm					
STRESS LEVEL	<u>P. citri</u>			<u>P. affinis</u>	
	n	$\bar{x}$	s.e.	$\bar{x}$	s.e.
Unstress	25	3.3b	0.12	3.6a	0.09
Medium	25	3.0b	0.13	2.9b	0.12
High	25	2.1c***	0.07	2.3c***	0.09
SNK test	(144, 25)	$S\bar{x} = 0.25$		MS = 1.672	

Figures with the same letters are not significantly different at the 5% level. (***) = significantly different at 0.1% level).

Analysis of variance failed to indicate significant differences in the mean body length of P. citri fed on unstressed and medium stressed plants. However, insects reared on high stressed plants showed a significant reduction in the body length ($P < 0.001$) (Plate 4.1).

P. affinis showed a reduction in body size with rising stress ($P < 0.05$).



PLATE 4.1 The size of mealybugs become extremely small when they develop on high stressed plants. (Note smaller size mealybugs fed on high stressed plants). (Magnification x 15 approx).



PLATE 4.2 Parasitoids developed on smaller hosts were smaller in size. The larger females L. dactylopii emerged from unstressed or medium stressed treatments and the smaller one from a high stress treatment

4.4.3.1 Influence of water stress on successful parasitism.

Table 4.6 summarises the percentage successful parasitism by A. pseudococci and L. dactylopii when ovipositing on P. citri reared on water stressed host plants. It is evident from Table 4.6 that both the parasitoid species have a similar behavioural pattern towards the stress treatment. There was no significant different in successful parasitism between unstressed and medium stress treatments.

Table 4.6 Influence of water stress on successful emergence of parasitoids.

PERCENTAGE EMERGENCE OF PARASITIDS				
STRESS LEVEL	<u>A. pseudococci</u>		<u>L. dactylopii</u>	
	$\bar{x}$	s.e.	$\bar{x}$	s.e.
Unstress	64.0a	5.3	65.0a	5.9
Medium	59.0a	2.9	68.0a	4.6
High	14.0b***	1.0	22.0b***	4.6
SNK test	(24,25)	$S\bar{x} = 2.75$	MS = 37.8	

The analysis was based on arc-sine transformed percentage values. Untransformed means are presented in the Table. Each mean value is an average of 100 observations. Figures with the same letters are not significantly different at the 5% level.

There was a marked reduction in successful parasitism on high stressed plants ($P < 0.001$). Greater number of oviposited mealybugs in the high stress treatment died before the emergence of parasitoids. Under high stress conditions the efficiency of both the parasitoid species was reduced so affecting the optimal progeny allocation by the parasitoids.

4.4.3.2. Influence of water stress on the developmental period of parasitoids.

Parasitoids developing on smaller mealybugs collected from high stressed host plants showed a significantly longer developmental period in comparison with other treatments ($P < .05$).

Table 4.7.1 Influence of water stress on the developmental period of A. pseudococci reared at $28 \pm 1^\circ\text{C}$.

MEAN DEVELOPMENTAL PERIOD (DAYS)						
STRESS LEVEL	MALES			FEMALES		
	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.
unstress	20	12.7d	0.85	20	13.2c	0.77
Medium	20	13.2cd	1.18	20	13.6cb	1.05
High	20	14.2ba	1.15	20	14.4a	1.05
SNK test	(114, 20) $S\bar{x} = 0.225$ MS = 1.009					

n = number of individuals. Figures with the same letters are not significantly different at the 5% level.

Table 4.7.2 Influence of water stress on the developmental period of L. dactylopii reared at $28 \pm 1^\circ\text{C}$.

MEAN DEVELOPMENTAL PERIOD (DAYS)						
STRESS LEVEL	MALES			FEMALES		
	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.
unstress	20	13.4b	0.59	20	13.7b	0.73
Medium	20	13.5b	0.61	20	13.8b	0.87
High	20	15.0a	0.88	20	15.3a	0.91
SNK test	(114, 20)		$S\bar{x} = 0.1747$	MS = 0.610		

n = number of individuals. Figures with the same letters are not significantly different at the 5% level.

The responses of L. dactylopii in various stress treatments were similar to that of A. pseudococci. Both the sexes showed a significantly longer developmental period when developing on P. citri fed on high stressed host plants ($P < 0.05$).

4.4.3.3 Influence of water stress on the longevity of parasitoids.

Analysis of variance shows that host plant water stress condition had no influence on the adult life span of A. pseudococci. However, the trend was different for L. dactylopii where parasitoid longevity was significantly reduced as a result of stress conditions in the host plant. Table 4.8 also shows that the life span of L. dactylopii under similar conditions was longer than A. pseudococci.

Table 4.8 Influence of water stress on the longevity of female parasitoids.

MEAN LONGEVITY OF ADULTS (DAYS)						
STRESS LEVEL	<u>A. pseudococci</u>			<u>L. dactylopii</u>		
	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.
Unstress	20	29.9b	4.8	20	37.3a	5.1
Medium	20	28.5b	5.2	20	38.8a	9.8
High	20	28.4b	4.7	20	31.2b	3.2
SNK test	(114, 20)		$S\bar{x} = 1.084$	MS = 23.5026		

n = number of individuals. Figures with the same letters are not significantly different at the 5% level.

4.4.3.4 Influence of water stress on the size of the emerging parasitoids.

Both parasitoid species developing on smaller mealybugs collected from high water stress plants had significantly smaller head widths in both male and female ($P < 0.05$) (Fig. 4.4 & Plate 2). Nevertheless females in any treatment were larger than the males even without treatment effects.

FEMALE PARASITOIDS

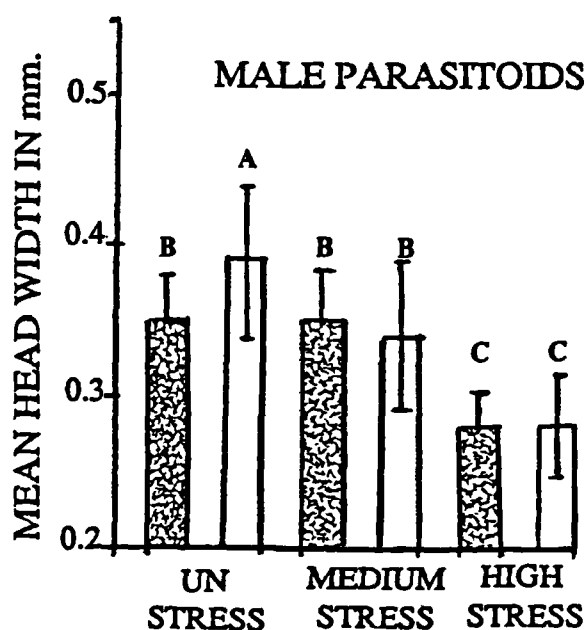
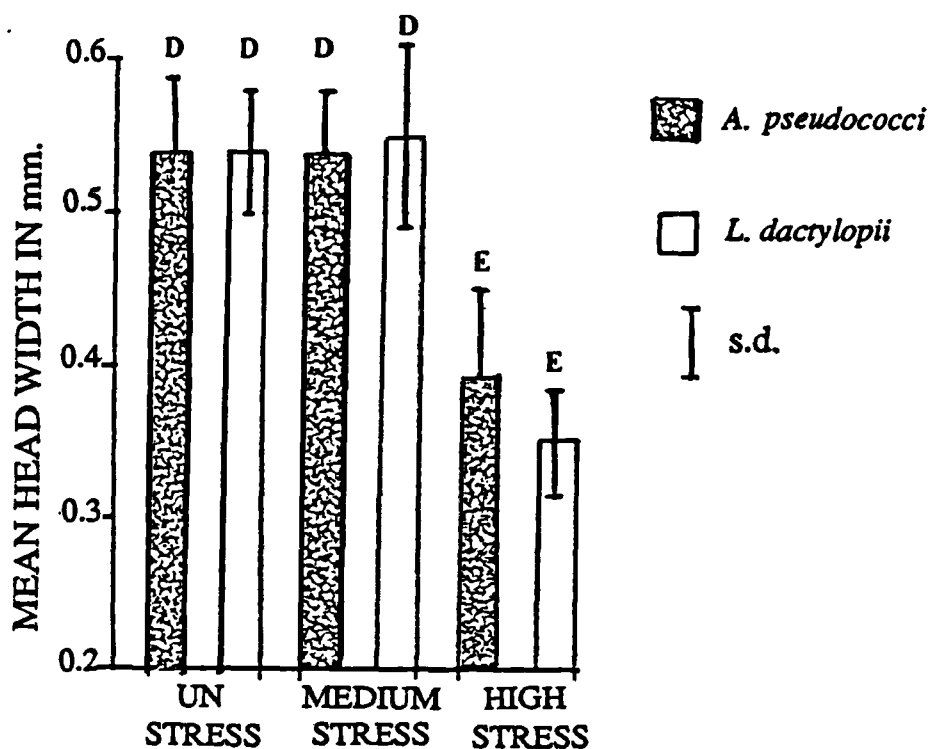


Fig. 4.4 Histograms showing mean head width mm $\pm$ s.d. of *A. pseudococci* and *L. dactylopii* emerged from *P. citri* reared on water stressed plants. Each mean value is an average of 15 insects. Means with the identical letters are not significantly different at the 5% level. SNK test

4.5 DISCUSSION

4.5.1.1 Relative water content

Some authors have discussed the difficulty of achieving a constant water stress level in a long term water stress experiment (Slatyer & Barrs 1965; Wearing 1972). The fluctuations of stress levels in the experimental plants are primarily attributed to changes in water level in the growing media at the time of watering. The same reason could be suggested to explain the observed variations in the relative water content of Streptocarpus plants. As a result of watering the available water content in the potting media increased allowing plants to absorb more water. This situation leads to intermittent stress conditions in the plants.

4.5.1.2 Analysis of soluble amino acid content.

As a result of intermittent water stress conditions, the free amino acid content fluctuated within a limited range during experimentation. Nevertheless the mean amino acid content produced is greatest in the high stressed plants. This agrees with many authors who have recorded high levels of amino acid production during drought stress and revealed that in addition to the morphological and physiological responses, important biochemical changes occur at the cellular level (Turner & Johnes, 1980). Nitrogen metabolism is affected resulting in poor protein synthesis and enhanced proteolysis. The end product of most of these chemical processes are higher free amino acids especially proline and amides (see review of literature).

4.5.1.3 Water stress and total leaf nitrogen.

The experimental plants in the medium stressed treatment had the highest nitrogen level followed by high stressed plants and finally a low level in the unstressed plants. However, the concentration of total nitrogen did not show any increasing trend with the time, presumably due to the intermittent stress condition in the plant. Hanson and Hitz (1983) found similar results and they identified that if water stress is set during the acquisition period of nitrogen, the concentration increases, provided soil water level is adequate for roots to absorb more nitrate.

The reason for higher nitrogen levels in the medium stress plants, could be attributed to stress factors supplemented by sufficient soil moisture for the active uptake of nitrogen from the soil. However, the soil moisture level in the high stressed plants was close to the wilting point. Therefore limited supplies of water in the root zone might be the reason for reduced uptake of nitrate in these plants. Although unstressed plants had no limitation for water, there was no external force to enhance metabolic activities and to assimilate more nitrogen in the dry matter. Our finding is in agreement with other workers (Morilla et al., 1973; Scriber & Slansky, 1981).

4.5.2.1 Influence of water stress on the developmental period of mealybug.

The crawlers stage is shorter than other stages and the stress factors did not influence the developmental period. The food requirement of these early stages of mealybugs are in minute quantities and crawlers are a dispersive phase rather than a feeding one.

They may have food reserve or perhaps are epidermal cell feeders. Therefore stress induced food limitations may not affect this stage.

Sutherland, (1932) stated that the crawler phase of mealybugs is important for dispersion to select a suitable habitat, the second instar are more sedentary but occasionally move. The 3rd instar and adult are more sedentary and active feeders. Therefore lengthening of the developmental period of 3rd and 4th instars of mealybugs reared on high stress plants may be attributed to the limited supply of nutrient from the host plants. Scriber and Feeny, (1979) and Slansky and Scriber, (1985) reported that organisms with access to a poor diet will have reduced body metabolism and lower relative growth rates resulting in a longer period of development.

4.5.2.2 Effect of water stress on the fecundity of mealybug.

The total fecundity levels of both mealybugs were reduced as a result of high water stress in the experimental plants. *P. affinis* showed a 5 fold reduction in egg numbers. The reduced fecundity of both species is primarily attributed to their smaller body size. Mealybugs, as phloem feeders are like aphids and presumably require a certain turgor pressure in the vessels for ingestion of nutrients (Kennedy et al., 1958). When the plants reach the permanent wilting point the pressure level reduces while viscosity of the solute increases due to accumulation of different compounds in the phloem vessels (Kramer, 1983). Although the level of amino acid in phloem sap of stressed plants increases, this is off-set by the reduced pressure and increased viscosity.

The influence of stress on fecundity levels depend not only on the severity and duration of the stress but also the age class of the host plant (Wearing, 1972). The age of the leaves in the present experiment was approximately uniform and the duration of stress was constant for all the treatments. Therefore the low fecundity of mealybugs, fed on high stressed plants is likely due to poor growth resulting from insufficient food. Connor, (1988) suggested that environmental factors such as low ambient humidity, and high temperature around the plant could induce an unsuitable micro-environment hampering normal functions of the insects. High stressed plants in the present study were maintained close to the permanent wilting point except at the time of watering. This situation might have aggravated the moisture problem around the plant. The combined effects of all these factors may reduce food uptake by mealybugs resulting in poor reproductive performance.

4.5.2.3 Influence of water stress on ovipositional periods of mealybug.

Wearing, (1967) observed a longer pre-ovipositional period in M. persicae when insects were grown on young leaves of stressed host plants. Similar responses to water stress were recorded in both the species of mealybugs in the present investigation. The lengthening of this period may be attributed to an inadequate food supply. However, Cornwell, (1956) found a shorter preovipositional period of cacao mealybugs Pseudococcus njalensis Laing under water stress condition of cacao plants. The differences between these observations could be attributed either to different stress levels in the experimental plants or to species variation.

Mealybugs fed on high water stress plants showed a significantly shorter reproductive period, perhaps due to the low fecundity level of mealybugs reared on high stressed plants (Table 4.3).

Longer reproductive periods of M. persicae and B. brassicae were observed when they feed on young stressed leaves of brussels sprouts but a reduction of larviposition period was observed when the insects were exposed to old leaves. The shorter reproductive period was attributed to the poor larviposition by the aphids (Wearing, 1972). Thus the present finding is in agreement with other authors. However, the effective egg laying period of both mealybug species was unaffected by the stress levels in the host plants. Thus the egg laying behaviour of mealybugs seems to be a heritable character independent of stress levels in the host plants.

4.5.2.4 Influence of water stress on intrinsic rate of population growth of mealybug.

Innate capacity of population increase

The innate capacity of population growth is an appropriate method to estimate population growth of an organism under known environmental conditions. Sumner et al., (1986) found no significant differences in the r_m value of Schizaphis graminum when they were grown on water stressed plants. However, there was a steady decline of the r_m value with increase of water stress levels in the experimental plants. The same author reported no significant difference in generation length (T) under different stress levels but the doubling time (D) and the mean number of eggs per day/female increased linearly with increasing stress levels.

The present finding is in good agreement with above report and we observed an inverse relationship between rm values and the stress levels of the host plants. Further it was noticed that the rm value of P. affinis in the high stressed treatment was 50% lower than that of P. citri. It appears that some insect species can tolerate stress factors more effectively than the others. The consequence of such a drop in rm value is a threat to population stability.

Yaninek, (1985) reported that the rm value of cassava green mite (CGM) tends to increase during the wet season. He attributed the greater biotic potential of CGM during this season to a higher net reproduction (R_0), lower mortality and a greater rm value. Similarly the greater rm of mealybug species in unstressed plants may be due to cumulative effects of the following contributory factors.

- (a) Higher R_0 in unstressed plants.
- (b) Higher fecundity in the early ovipositional period.
- (c) Female biased sex ratio in unstressed plants.
- (d) Higher survival rate in unstressed plants.
- (e) Shorter developmental period in unstressed treatments.

Birch and Wratten, (1984) highlighted the importance of higher larval mortality for reduced rm value. In the present study we observed a greater larval mortality in the high stressed treatments. Therefore these factors might have contributed to the greater rm in both species of mealybugs on unstressed plants. The mealybugs generation length (T) was not affected by the stress factors in the host plants.

As the "doubling" time increases with the stress levels, so the population growth rate decreases. Therefore the unfavourable conditions by reducing the r_m affects the biotic potential of the insects.

4.5.3.1 Influence of water stress on successful parasitism.

Both parasitoid species had remarkable success when they oviposited on P. citri grown on unstressed and medium stressed plants. The lower emergence rate of parasitoids under high stress conditions may be attributed to the host size factor. The importance of host size for successful parasitism was emphasized by many authors (Richerson & Borden, 1972; Vinson, 1976; Vinson & Iwantsch, 1980). There are two possible reasons for the lower frequency emergence from high stress treatments. Either the parasites rejected the small host or there was insufficient food reserves to complete development (Duffey, 1980).

4.5.3.2 Influence of water stress on the development period of parasitoids.

The developmental periods of both male and female parasitoids were significantly increased when they were reared on mealybugs fed on high stressed plants (Tables 4.7.1 and 4.7.2). The prolonged parasitoid development could be the result of limited food resources in the smaller mealybugs (Vinson and Iwantsch, 1980). The physiological or biochemical changes in the host resulting from stress, might have adversely affected the parasitoid larvae causing retarded development. Further work is required to settle this point.

4.5.3.3 Influence of water stress on the longevity of parasitoids.

The life span of an insect is primarily determined by genetical characters but external factors can influence longevity to a greater extent (see section 2). In the present investigation we observed that the relatively larger parasitoids from unstressed and medium stressed treatments had a longer life expectancy. The shorter life expectancy of the small parasitoids is attributed to the reduced fitness parameters gained from their hosts.

4.5.3.4 Influence of water stress on the size of emerging parasitoids.

Smaller body size of the parasitoid in the high stress treatment is related to the smaller size of the mealybugs reared on high stressed plants. Most of the fitness parameters of parasitoids depend on the size. Hence large parasitoids are always favoured in biological control as they possess higher survival rate, fecundity, longevity and searching capacity.

4.5.3.5 Influence of water stress on biological control.

The lack of field data, on the influence of water stress on parasitoids make^s it hard to design laboratory experiments with a realistic pattern of host and parasitoid abundance and distribution.

Plants undergo many ecological adaptations to overcome severe stress by adopting different methods such as shedding leaves, poor vegetative growth, wilting symptoms and in some extreme conditions losing all the leaves until favourable conditions return (Baskin & Baskin, 1974). Such morphological changes may alter the micro-climate around the plant affecting not only the herbivores but also their natural enemies. Water stress can alter morphology, physiology and behaviour of herbivorous insects resulting in poor and weaker individuals. Inferior and weak hosts deprive the parasitoids of normal growth, affecting many fitness parameters such as longevity, sex ratio, reproductive capacity and searching behaviour. Thus the end result of water stress is the poor establishment of natural enemies and ineffective Biological Control.

4.6 CONCLUSIONS

1. High concentrations of soluble amino acids were found in high water stressed plants.
2. The total nitrogen content was greater in the medium stressed plants in comparison with unstressed and high stressed plants.
3. A prolonged development was observed when 3rd and 4th instar mealybugs were reared on high stressed plants.
4. The fecundity levels of P. citri and P. affinis were significantly reduced as a result of high stress in the host plants.
5. Innate capacity of population growth of mealybugs was inversely related to the stress level in the host plants.
6. Mealybugs remained small when reared on high water stressed host plants.
7. Percentage successful emergence of L. dactylopii and A. pseudococci was significantly reduced as a result of high stress condition in the host plants.
8. The life span of parasitoids was significantly curtailed when developing on P. citri fed on high stressed plants.
9. A significant reduction in the body size of parasitoids was observed when they develop on mealybugs grown on high stressed plants.

SECTION 5

INFLUENCE OF HOST PLANT NITROGEN LEVELS ON LIFE HISTORY
PARAMETERS OF MEALYBUGS AND THEIR NATURAL ENEMIES.

5.1 INTRODUCTION.

As nitrogen is a constituent of both structural and nonstructural components of the cell it is required to maintain growth. Nitrogen deficiencies cause much damage to the plant and in the most critical lower levels of nitrogen, plants may die back. Scriber, (1984a) listed over 115 different studies in which insect damage, growth, fecundity, or populations increased with enhanced nitrogen concentrations. A further 44 studies with insects indicated a decrease in herbivore populations or damage to plants with high nitrogen concentrations. Smaller differences in nitrogen level can enhance the rate of increase of some insect herbivores to such an extent that they outgrow the numerical response abilities of their natural enemies (White, 1978). Hence enrichment of a crop system with nitrogen may destabilize the population dynamics, affecting the populations of some insect species.

Fertilization of plants will affect the host selection, survival, growth rates, and reproduction of insect herbivores and may also alter plant micro-climate due to morphological changes in the canopy. Mealybug has an enormously wide host range making it a problem in many cultivated crops.

It is rated as a serious pest in Citrus and Cacao. Often these commercially grown crops are fertilized with nitrogen but there has been little effort to investigate the consequence of such host plant enrichment on mealybugs and their natural enemies. Therefore this is an attempt to find the effect of applied nitrogen on life history parameters of P. citri reared on two host plant species under various nitrogen levels.

There is a growing realization about the importance of considerations of changes in numbers or composition of the insect natural enemies under nitrogen fertilization. Higher nitrogen levels in the plant, influences the population growth rate of phytophagous insects which in turn may enhance the population of natural enemies. The present investigation was aimed to elucidate the possible effect of host plant nitrogen levels on mealybug growth parameters and assessment of host suitability using various fitness parameters of parasitoids. Success of a parasitoid primarily depends upon the quality, particularly the size, of the host. Host size is often affected by the host plant nitrogen level and is one of the most important factors for determining longevity, fecundity, sex ratio and developmental period of parasitoids.

5.2 REVIEW OF LITERATURE

5.2.1.1 Nitrogen requirement of plants.

Plants assimilate mineral elements into food substances such as carbohydrates, proteins, fats and vitamins (Lewis, 1986). Dry plant material contains only 2-4% nitrogen which is much lower than that of carbon. Plants are continuously involved in the conversion of inorganic NO_3 and NH_4 to organic derivatives. In green plant material, protein nitrogen is the largest fraction exceeding 80-85% of the total nitrogen in the plant (Greenwood 1976).

5.2.1.2 Nitrogen translocation in the plants.

Nitrogen compounds are translocated primarily as nitrates and amino acids in the phloem (Pate et al., 1979), young leaves receiving more amino acids than the older leaves (Mengel & Kirkby, 1978). The distribution of nitrogen in the various plant parts are primarily dependent on the rate of protein metabolism in these areas (Lewis, 1986). When the supply of nitrogen from the root is inadequate, plants tend to mobilize nitrogen from the older leaves to those parts requiring it. In such a situation the deficiency symptoms are more prominent in older leaves as proteolysis tends to degenerate the chloroplasts (Davies, 1979). Even under nitrogen deprived conditions, the growing plant parts may possess relatively high concentrations of nitrogen.

5.2.1.3 Influence of nitrogen on chemical changes in the plant.

Experimental evidence shows that a high level of nitrogen in the growing media causes a considerable increase in the content of soluble amino compounds, particularly free amino acids, amines and amides whilst the content of protein is only raised to a limited extent (Mengel & Helal, 1970). Glutamate and aspartate as well as their amide derivatives also accumulate when high levels of inorganic nitrogen are applied to the plant. Nitrogen deficiencies often cause chemical imbalances in the plant and the concentration of starch and sucrose tend to increase, while the concentration of protein and amino acids tends to reduce (Oparka et al., 1987). Similar observations have been recorded in low nitrogen treatment of brussels sprout (Koritsas & Garsed, 1985).

5.2.1.4 Effect of nitrogen fertilizer on plant total amino acid levels.

Foliar nitrogen content is often considered to be a reliable indicator of nutritional quality of host plants for phytophagous insects (McNeil & Southwood, 1978). van Emden (1966a), experimenting on the effect of high nitrogen (840 ppm), medium nitrogen (105 ppm) and low nitrogen (0 ppm) on brussel sprout plants, showed soluble nitrogen ranged from 225, 161 and 100 ($\mu\text{g} / \text{mg}$ dry weight) respectively. Field experiments with potato plants under various nitrogen levels revealed that the concentration of free amino acids were consistently higher in high nitrogen treatment than under low nitrogen treatment (Jansson & Smilowitz, 1986).

However, Koritsas and Garsed (1985) found that total amino acid concentration of brussels sprout plants was not positively correlated with applied nitrogen levels in the media.

5.2.1.5 Influence of soil nitrogen on total nitrogen content of plants.

Morris (1961) used nutrient film techniques to study the influence of N, P and K on clover mites and found a significant increase of total nitrogen content in the leaf samples of high nitrogen treatment. The influence of applied nitrogen fertilizer to grassland flora was investigated by Prestidge, (1982) who found significant increases of total nitrogen in fertilized fields. Moore & Clements, (1984) claimed a similar situation in ryegrass land where high nitrogen treatments produced significantly higher total nitrogen content than the low nitrogen treatments. The effect of nitrogen fertilizer on the growth and distribution of nitrogen within the potato canopy was studied by Millard and Mac Kerron, (1986) who found high nitrogen always increased total nitrogen content of leaves, stems and tubers.

5.2.2.1 Requirement of nitrogen in insects.

Tsiropoulos, (1978) showed that dietary deficiencies of insects result from insufficient concentrations of essential amino acids rather than a lack of total nitrogen. Where essential amino acids are rare in the diet, no insect development occurs regardless of the amount of total nitrogen in the diet. Experiments carried out by various authors on the dietary requirements of insects have shown that if the concentrations of essential amino acids are adequate, dietary supplementation even of essential amino acids does not improve insect performance (Vanderzart, 1958; Tsiropoulos, 1978; Dadd, 1985).

Supporting the above theory Brodbeck and Strong (1987) showed that, any amino acids in excess of the normal threshold value may lead to toxicity, as ammonia accumulates when specific metabolic pathways are overloaded.

Therefore inadequate supplies of amino acid may lead to poor growth of the insect while excess may also be detrimental. Immature stages of insects require more nitrogenous substances since they utilize most of these compounds to build the body structure. However, the demand for nitrogen diminishes when the insect becomes adult, except for egg production in the female (Dadd, 1985). Certain larvae have been shown to increase their food consumption on plants with low nitrogen to maintain physiological activities.

5.2.2.2 Influence of host plant nitrogen levels on the developmental period of herbivorous insects.

Insects exhibit differences in the duration of larval instars when feeding on varying quality of food. These differences are more visible at low temperatures (Dale 1988). Growth and nitrogen accumulation rates are likely to vary with the species of insect and the type and position of the host plant they feed upon (Fox & Macauley, 1977). Mattson (1980) stated that herbivores in nitrogen poor environments, exhibit slower growth and prolonged generation times, unless they have faster and efficient consumption rates. Herbivores exposed to high nitrogen diets presumably possess efficient mechanisms to utilize energy and influence the biomass production. The rice stem borer, Chilo suppressalis (Walker) larvae require a relatively high concentration of protein for optimal growth and development (Ishii & Hirano, 1957).

The effect of host plant nitrogen fertilizer on the biology of two spotted spider mite Tetranychus urticae was studied by Wermelinger et al., (1985). They found, a reduction of nitrogen content to one half was associated with a 2.6 days more development time. Furthermore the larval developmental time was significantly lengthened on plants treated with nitrogen free nutrient solution.

5.2.2.3 Influence of host plant nitrogen levels on the mortality rate of herbivorous insects.

The probability of survival of insects depends to a great extent on the quantity and quality of food they obtain during the developmental period (Slansky & Scriber, 1985). Protein or amino acids are a prime dietary requirements when the whole life cycle is considered. Therefore a limited supply of any essential amino acid may lead to retarded growth or cessation of life. Myers & Post (1981) found a positive correlation ($r = 0.82$) with average percentage protein in the ragwort, Senecio jacobaea and the average larval survival of the cinnabar moth, Tyria jacobaeae. Higher survival (82%) of elongate hemlock scale, Fiorinia externa was observed when they were reared on plants with higher applied nitrogen but the corresponding value for low nitrogen plant was 68.5 (McClure, 1980).

Similar behaviour was recorded in geometrid larvae, Epirrita autumnata (Bkh.), where high mortality rates were recorded on poor quality trees (Neuvonen & Haukioja, 1984).

5.2.2.4 Influence of plant nitrogen on the fecundity of herbivorous insects

The ovariole number, structure, size and life span of the female plays a vital role in the fecundity of an insect. When insects feed on more protein either as late larva or young adult or both stages, they produce more offspring than those that take little. The majority of sap-feeding insects respond to host plant quality, rather than quantity. Hinckley (1963) reported that higher levels of applied nitrogen fertilizer increase the fecundity level of rice plant hoppers. Metcalfe, (1970) correlated the increased fecundity of Saccharosydne saccharivora to an increase in plant nitrogen level in the sugar cane plant.

The reproductive capacity of beet army worm fed on high nitrogen sugar beet plants showed a significantly higher fecundity level than those fed on low nitrogen plants (Al-Zubaidi & Capinera, 1984). Harrewijn (1970) studied the fecundity and reproductive rate of caged M. persicae on leaves of potato plants growing on solutions with different nitrogen levels, and found a positive correlation between the total soluble nitrogen in the leaves and reproductive rate of aphids. Further evidence for positive correlation between foliar concentrations of amino acids and population growth rate of M. persicae was discussed by Jansson and Smilowitz, (1986).

5.2.2.5 Influence of host plant nitrogen on life table statistics of herbivorous insects.

The quality and quantity of food utilized by insects have a direct influence on the key factors of rm (Andrewartha & Birch, 1954). Taylor (1955) has suggested that variation in the amino nitrogen concentrations in the plant might influence the population changes of sap-feeding insects. Population parameters of green peach aphid, M. persicae, also indicated a positive correlation with foliar concentrations of nitrogen (van Emden, 1966a; van Emden & Bashford, 1969). The population growth rate of M. persicae was increased with an increase in the level of nitrogen applied to the experimental potato plants. Similarly the daily fecundity of Tetranychus urticae reached a peak after 4 days of oviposition with a significant increase in the high nitrogen treatment ($R_o = 40.3$ and $rm = 0.22$) in comparison to low nitrogen treatment ($R_o = 4.7$ and $rm = 0.06$) (Wermelinger et al., 1985).

5.2.3.1 Influence of plant nitrogen on some aspects of parasitoid biology.

Host nutrition can affect parasitoid survival and development. When the soil is rich in nitrogen and it is the growing season of the plant, the vegetative parts remain succulent with a high nitrogen: carbon ratio. This situation may lead to rapid build up of herbivorous insects and their natural enemies (Dale, 1988).

Adkisson (1958) studied the influence of fertilizer applications on the population growth of H. zea and their natural enemies and found considerably higher populations of natural enemies on fertilized fields.

The importance of a healthy host for the success of natural enemies was discussed by Marti and Hamm, (1985). When Geocoris punctipes were fed with diseased armyworm, the survival of the predator was significantly reduced due to nutritional deficiencies. Stiling et al., (1982) found a positive correlation between leafminer Hydrellia valida densities and total leaf nitrogen content. The higher densities of leaf miners were always associated with a high percentage of two species of parasitoids. Nitrogen utilization efficiency of two species of parasitoid, Brachymeria intermedia and Coccygomimus turionellae (L.) was studied under laboratory condition using Lymantria dispar as the host. The observed differences in the weights of parasitoids was found to be associated with differences in host diet. The availability of nitrogen to parasitoids varied with the diet of the host (Greenblatt et al., 1982).

van Alphen and Janssen (1982) showed that Asobara tabida Nees is capable of ranking five Drosophila species according to the suitability of the host for greater survival rate of the parasitoid. Percentage parasitism depends on many factors such as density of host (van Lenteren et al., 1978), innate preference for the host and host size where parasitoid larvae can obtain more resources (Sato & Tanaka, 1984). The haemolymph of Galleria mellonella was found to contain hexose and amino acid compounds that induce oviposition by Itoplectis conquisitor (Arthur et al., 1969).

5.3 MATERIALS AND METHODS

5.3.0.1 Host plant species.

Coleus (Coleus blumei hybrids) and Potato (Solanum tuberosum L. cv Maris piper) were used as experimental plants.

5.3.0.2 Experimental arrangement of Coleus plants.

Single node 4 cm length Coleus cuttings were obtained from mother plants. Approximately 50 such cuttings were transplanted on to 4 x 4 x 4 cm "rock-wool" blocks and placed in a wet container. These plantlets were kept in a humid chamber for 3 weeks prior to use in the experiment. The experimental setup consisted of six N.F.T. channels, width 9.5 cm and length 180 cm arranged on a bench with a flow gradient of 3 cm. The channels were covered with black polythene to prevent light penetration to the roots. At the lower end of the channels buckets were placed to collect the nutrient to recirculate back to the main channels. Two channels were allocated per treatment and the three nitrogen levels were high nitrogen 200 ppm, medium nitrogen 100 ppm and low nitrogen 10 ppm.

5.3.0.3 Nutrient treatments.

The nutrient solution was based on the standard Cooper formula (Cooper, 1979). The stock solutions were prepared 200 times concentrated and kept in separate amber bottles. Except for nitrogen, the cations and anions were kept approximately constant in all 3 treatments (see Appendix 2 for nutrient balance sheet).

Ferrous as EDTA was kept in a separate bottle. The rooted cuttings with the "rock-wool" blocks were placed at a distance of 25 cm, on the channels through incisions made in the polythene cover.

The buckets were filled with appropriate concentrations of nutrient solution and the pH value was adjusted to 5.5-6.0 by adding acids or hydroxides. The conductivity (CF) of the solutions were maintained at 20-23 by adding sufficient nutrients. The pH and CF of the nutrient solutions were kept constant through out the experiment. The solutions in the buckets were replenished every 10 days to avoid possible contamination with fungal growth (Plate 5.1). Plants were allowed to grow for 6 weeks, prior to the introduction of mealybugs crawlers. Eight plants per treatment and 4 plants per channel were selected at random and 200, one-day old crawlers of P. citri were introduced to each selected plant. The rest of the plants in each treatment were used to collect weekly leaf samples to analyse total amino acid and total leaf nitrogen.



PLATE 5.1 Experimental setup for nutrient film technique to grow coleus plants under 3 nitrogen levels. (Note better growth performances of plants grown under 200 ppm nitrogen)

5.3.0.4 Experimental arrangement of potato plants.

The rooted plantlets of potato were obtained from tubers grown for this purpose. These plantlets were immediately transplanted into 9 cm plastic pots containing coarse sand and gravel. Plants were arranged on a bench and kept in a glasshouse cubicle under the condition described in the experiment 1.3.2. (Plate 5.2). Fifteen plants per treatment were allocated and each pot was given a saucer to hold extra nutrient. These plants were positioned 25 cm apart on a capillary matting. The matting was soaked with water daily to keep the plant and insects under a high ambient relative humidity.

The 3 treatments of high (200 ppm) medium (100 ppm) and low nitrogen (10 ppm) were randomly assigned to previously selected pots. Cooper solution (experiment 5.3.0.3 and Appendix 2), was used as the nutrient media. Individual plants in each treatment was given 150 ml of nutrient solution twice a week and the rest of the days, plants were given distilled water to avoid desiccation. Yellow sticky traps were setup to reduce the potential outbreak of whitefly and thrips on the test plants. The treated plants were kept for 6 weeks under the above condition prior to the introduction of mealybug crawlers. Eight plants were selected out of the 15 in a treatment, for the introduction the crawlers of P. citri. The rest of the plants in a treatment were used to remove leaf samples for the analysis of soluble amino acids, and total leaf nitrogen content.

5.3.1.1 Chemical analysis of plant material.

Leaf samples for analysis of total soluble amino acids and nitrogen were taken at weekly intervals from the coleus and potato plants.



PLATE 5.2 Potato plants grown on 3 nitrogen levels. (Note better growth performances of plants grown under 200 ppm nitrogen)

Mature leaves close to the terminal bud were removed, two leaves per plants and three plants/treatment and these leaves were kept in labelled paper bags and stored at -20°C. Leaf samples were taken throughout the development period of mealybugs and the analysis was carried out as described in experiment 4.2.3.

5.3.1.2 Effect of host plant nitrogen on the developmental period of mealybugs.

Crawlers were introduced to the selected plants and the number of insects and their developmental stages were recorded on a daily basis. The rest of the methodology and the analysis of data were similar to the method described in experiment 1.4.1.

5.3.1.3 Effect of host plant nitrogen on survival rate of mealybug.

Relevant information for the present investigation was obtained from the experiment described in 5.3.1.2. The total number of individuals surviving up to 2nd, 3rd and 4th instar and egg laying stages were obtained. The percentage survival values were calculated considering the initial introduction as 200 individuals. The data were then used in a factorial analysis.

5.3.1.4 Effect of host plant nitrogen on the fecundity of mealybug.

The experimental procedure and the statistical analysis of the data were similar to that of experiment 1.4.3.1.

5.3.1.5 Effect of host plant nitrogen on life table parameters of mealybug.

Relevant data for life table statistics were obtained from the experiment 5.3.1.2 and the sex ratio of the F1 generation was obtained as described in 4.3.2.4. The analysis of data and presentation were carried out following the method described in section 4.

5.3.1.6 Effect of host plant nitrogen on the body size of mealybug.

Methodology and the statistical analysis was similar to that of experiment 1.3.6.

5.3.1.7 SDS-PAGE analysis of mealybug protein.

P. citri was used as the experimental insects and were fed throughout their life on the host plants maintained under 3 nitrogen levels. SDS-PAGE was carried out as described in experiment 3.3.6.1.

5.3.2.1 Effect of host plant nitrogen on successful parasitism by A. pseudococci.

Experimental procedure and the analysis of treatment difference were carried out as described in 2.3.1.

5.3.2.2 Effect of host plant nitrogen on the developmental period of
A. pseudococci.

Out of a total of male and female parasitoids developed from the experiment 5.3.2.1, 20 males and 20 females were collected at random per treatment/ host plant species. The developmental period of these insects were taken for the analysis. Experimental procedure and the analysis of data were carried out as described in experiment 2.3.2.

5.3.2.3 Effect of host plant nitrogen on the longevity of
A. pseudococci.

Female parasitoids emerging from various nitrogen treatments were used to investigate longevity. The parasitoids were individually isolated into prepared labelled vials. The rest of the procedure and the analysis of data was carried out as described in experiment 2.3.4.

5.3.2.4 Effect of host plant nitrogen on the body size of the emerging
parasitoids.

The parasitoids that emerged from the experiment 5.3.2.1 were used, to measure the head width. Measurements were taken as described in experiment 2.3.5 and fifteen insects from each sex were taken for one way analysis of variance.

5.4 RESULTS

5.4.1.1.1 Analysis of foliar concentrations of total nitrogen.

The concentrations of total nitrogen in the leaf samples of potato and coleus plants are shown in Fig. 5.1. Leaf nitrogen content increases with a rise in the level of nitrogen applied to the host plants. Concentrations of total nitrogen were consistently high in high nitrogen treatment throughout the experiment. Potato plants showed high concentrations of total nitrogen, in the high nitrogen treatment compared with coleus plants. In coleus plants the level of nitrogen increased with time but potato plants showed an initial peak followed by a decline and then an increase towards the end.

5.4.1.1.2 Analysis of soluble amino acids.

The total soluble amino acid levels increased with a rise in the level of nitrogen in the growing media (Fig. 5.2). Generally potato plants showed higher levels of soluble amino acid accumulation than coleus in the high nitrogen treatment. High nitrogen treatments of both the plant species increased soluble amino acids concentrations in comparison to low nitrogen treatments.

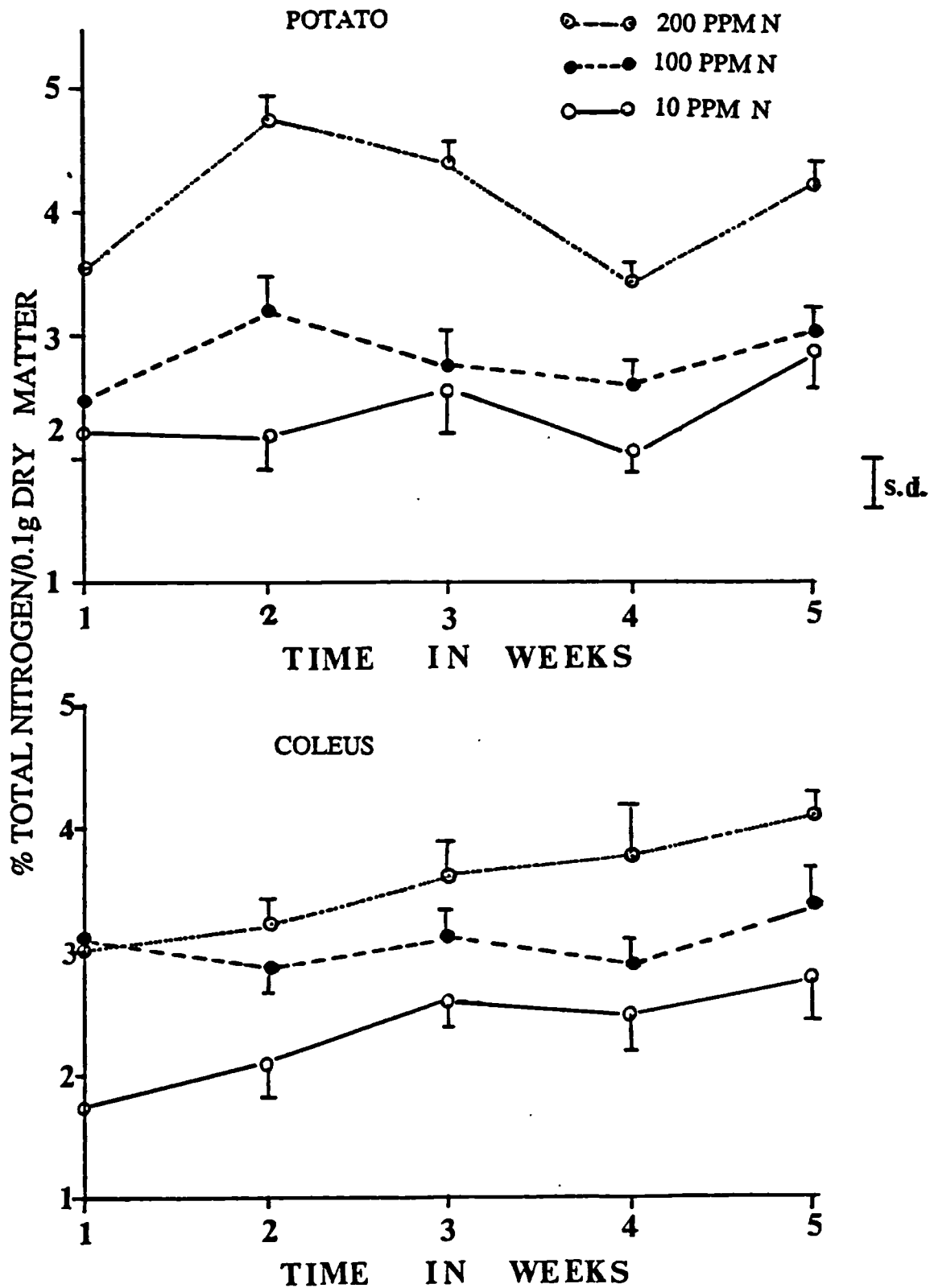


Fig. 5.1 Effect of various concentrations of nitrogen applied to coleus and potato plants on the total nitrogen content of leaf samples. Each mean value is an average of 3 samples. Nutrient film technique was used on host plants.

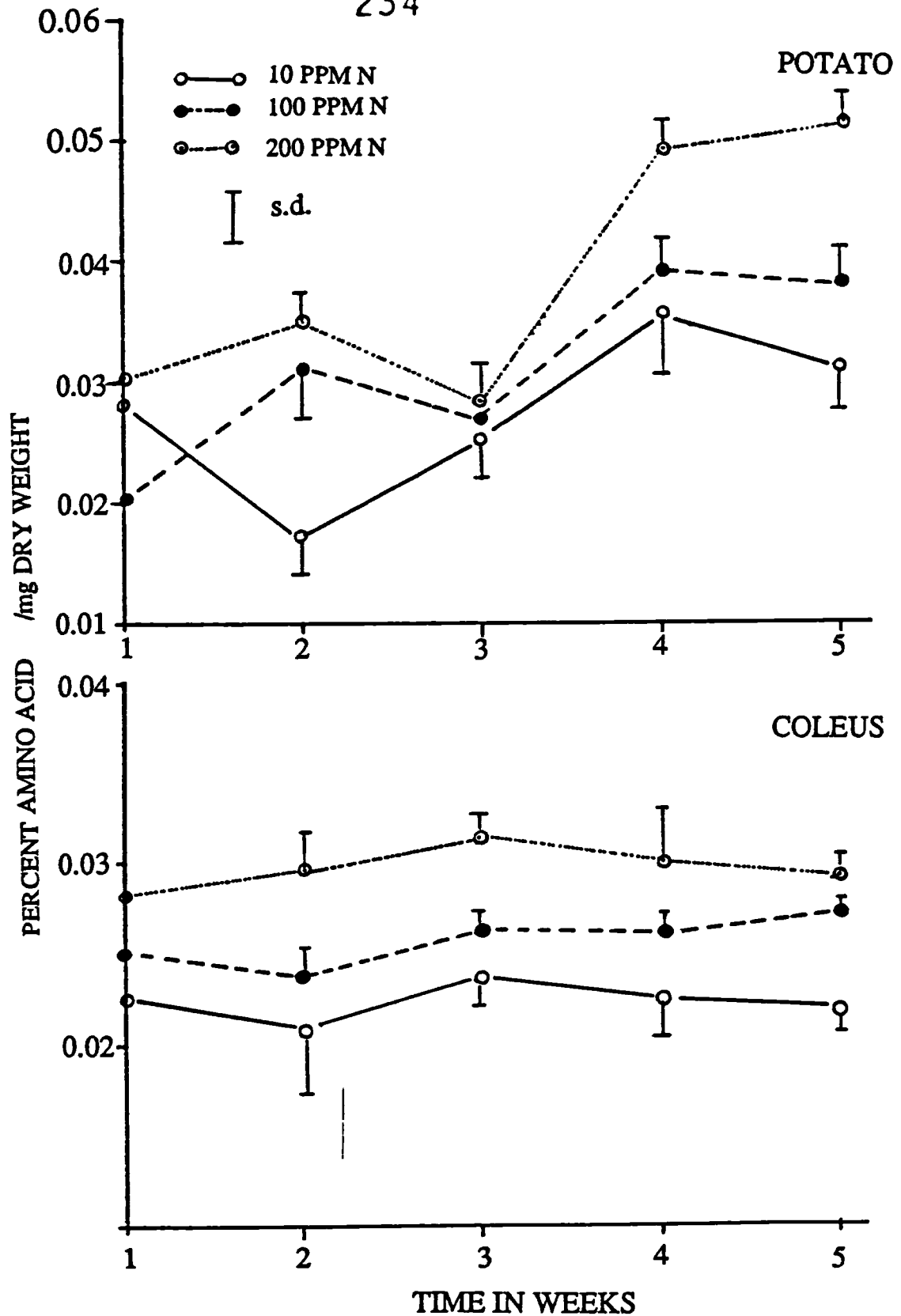


Fig. 5.2 Effect of various concentrations of nitrogen applied to coleus and potato plants on the soluble amino acid content of leaf samples. Each mean value is an average of 3 samples. Nutrient film technique was used to grow the host plants

5.4.1.2 Effect of host plant nitrogen on the developmental period of mealybug.

The developmental periods of various stages of mealybugs are shown in Table 5.1. The developmental period of crawlers grown on both the host plant species and at various nitrogen levels showed no significant difference ($P > 0.05$).

Table 5.1 Effect of host plant nitrogen (200, 100 and 10 ppm) on the developmental period of P. citri.

MEAN DEVELOPMENTAL PERIOD (DAYS)										
PLANTS N LEVEL ppm			crawlers		2nd instar		3rd instar		adult	
			$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.
Coleus	High	200	9.5a	0.5	13.5b	1.0	11.0d	1.1	9.1fg	1.0
	Medium	100	10.1a	1.8	13.8b	0.2	11.1d	0.7	9.9f	1.5
	low	10	9.5a	1.1	14.6b	1.8	11.2d	1.0	10.0f	0.7
Potato	High	200	8.2a	0.8	9.9c	1.2	8.1e	0.5	8.1g	0.7
	Medium	100	8.9a	0.3	10.2c	1.6	9.2e	0.7	8.8fg	0.8
	Low	10	8.7a	0.4	10.4c	0.5	8.4e	0.6	10.4f	0.6
n			100		100		50		25	
SNK test, $S\bar{x}$			0.45		0.55		0.37		0.37	
MS			0.99		1.53		0.71		0.90	

Figures with the same letters within columns are not significantly different at the 5% level.

The mean developmental periods of 2nd and the 3rd instar mealybugs were not influenced by the level of nitrogen in the growing media of either potato or coleus plants ($P > 0.05$). However, 2nd and 3rd instars reared on potato plants developed faster than on coleus plants ($P < 0.05$). Adult *P. citri* grew faster on high nitrogen potato than with low nitrogen treatment ($P < 0.05$).

5.4.1.3 Effect of host plant nitrogen on the survival rate of mealybug.

Table 5.2 shows that the crawlers are the most vulnerable stage for natural mortality. Nearly 70% of the original cohort died before reaching the 2nd instar.

Table 5.2 Effect of host plant nitrogen (200, 100 and 10 ppm) on percentage survival of different stages of mealybug.

MEAN PERCENTAGE SURVIVAL						
HOST PLANTS	NITROGEN ppm		CRAWLER to SECOND	SECOND to THIRD	THIRD to ADULT	ADULT to EGG LAYING
Coleus	High	200	38.2a	25.9b	8.6d	6.6ef
	Medium	100	40.9a	22.5b	18.6c	8.1e
	Low	10	34.7a	24.1b	16.5c	7.2ef
Potato	High	200	30.1a	17.4b	17.4c	5.6ef
	Medium	100	31.3a	20.4b	9.8d	4.8f
	Low	10	36.4a	18.3b	10.8d	5.6ef
SNK test	$\bar{Sx}$		2.9	2.0	1.5	0.7
	MS		42.5	20.7	11.7	2.7

Figures with the same letters within columns are not significantly different at the 5% level. SNK test (24, 5).

The survival of crawlers and the 2nd instars were not affected by the host plant nitrogen levels. Only high nitrogen levels affected the survival of 3rd instar mealybugs and in opposite directions in the different host plants. There was no difference in adult survival.

5.4.1.4 Effect of host plant nitrogen on the fecundity of mealybug.

Table 5.3 shows a significant reduction in fecundity associated with low nitrogen in both the host plant species ($P < 0.05$). However, there was no significant difference between fecundity at medium and high levels of nitrogen ($P > 0.05$).

Table 5.3 Effect of host plant nitrogen (200, 100 and 10 ppm) on the fecundity of P. citri.

MEAN NUMBER OF EGGS/ FEMALE						
NITROGEN ppm	COLEUS			POTATO		
	n	$\bar{x}$	s.e.	n	$\bar{x}$	s.e.
High 200	25	369.9ab	22.1	25	393.8a	13.3
Medium 100	25	311.3b	13.2	25	349.6ab	19.0
Low 10	25	214.0c	10.3	25	208.9c	11.4
SNK test	(29, 25) $S\bar{x} = 17.5$ MS = 7697					

Figures with the same letters within columns are not significantly different at the 5% level.

5.4.1.5.1 Effect of host plant nitrogen on life table parameters of mealybug.

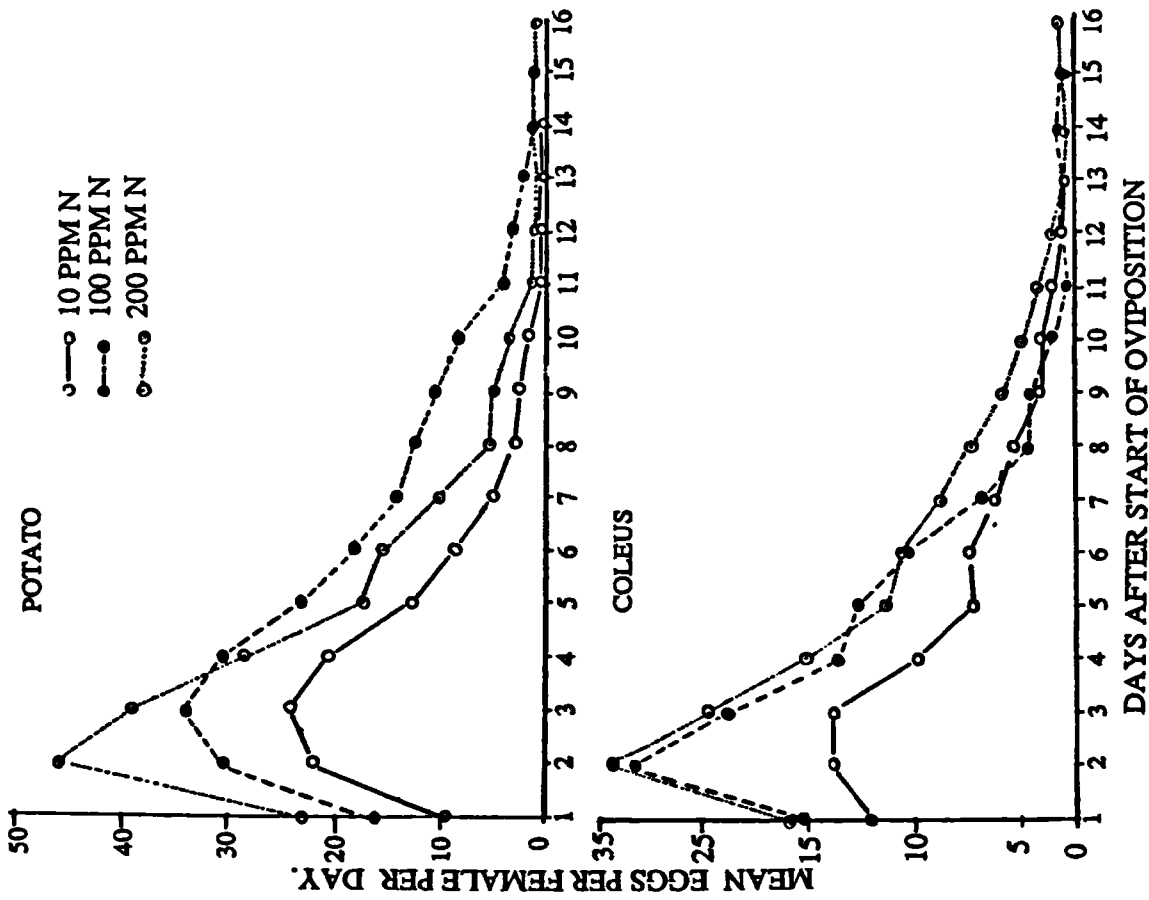
In Fig. 5.3A the progeny of each day are presented separately while in Fig. 5.3B they are shown cumulatively. The general trend was a rapid rise to a peak egg production within 24 - 48 hours, followed by a slow decline and rapid reduction towards the end of oviposition. In the high nitrogen treatment, the oviposition rate reached the peak (ca. 45 eggs/female/day) on the second day. The cumulative Figure showed that mealybug deposit almost 80% of their total egg production during the first 7 days.

5.4.1.5.2 Intrinsic rate of population growth of P. citri under the influence of host plant nitrogen levels.

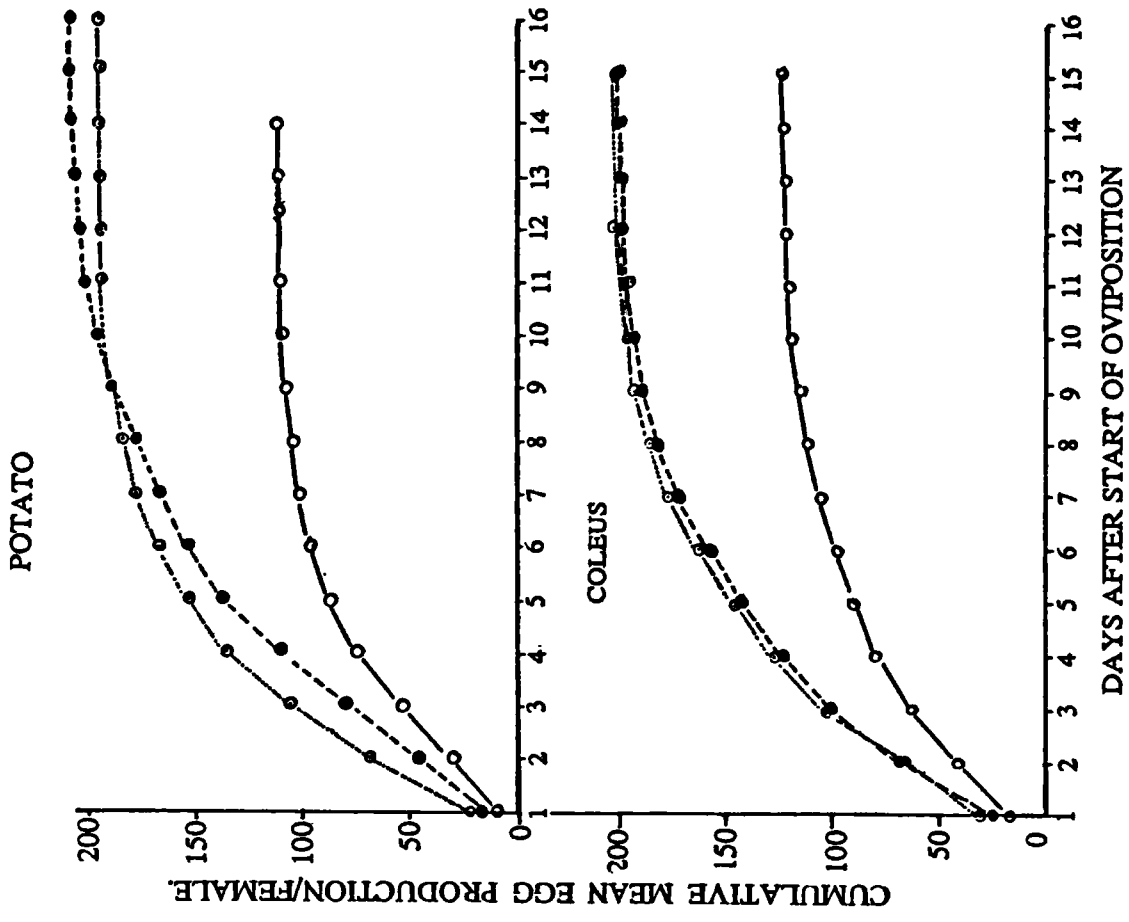
Table 5.4 Effect of host plant nitrogen (200, 100 and 10 ppm) on life table parameters of P. citri.

	COLEUS			POTATO		
NITROGEN ppm	HIGH 200	MEDIUM 100	LOW 10	HIGH 200	MEDIUM 100	LOW 10
sex ratio (F/F+M)	000.57	000.60	000.58	000.53	000.53	000.52
GRR	204.2	198.5	125.8	214.5	198.0	113.6
Ro	202.7	197.4	122.6	213.2	197.9	112.5
rm	000.128	000.126	000.121	000.141	000.139	000.128
T	041.49	041.94	039.74	038.03	038.04	036.89
D	005.41	005.50	005.73	004.91	004.98	005.41

GRR = gross reproductive rate, Ro = net reproductive rate, rm = innate capacity of population growth, D = doubling time, T = generation time.



A



B

The sex ratio of P. citri reared on coleus plants was slightly female biased but there was no indication of an influence of host plant nitrogen level on sex determination of mealybugs.

The gross reproductive rate (GRR) of P. citri is proportional to the plant's nitrogen levels and a similar trend was noted in net reproductive rate. The population growth rate (r_m) of P. citri increased with an increase in the level of nitrogen applied to the host plants. Mealybugs reared on potato plants had a higher r_m value than the corresponding value for coleus plants. Shorter generation times were recorded in low nitrogen treatments while the doubling times (D) were proportional with increases in the nitrogen level in the plants.

5.4.1.6 Effect of host plant nitrogen on the body size of mealybug.

Figure 5.4 shows that the mean body length of the adult mealybugs reared on high and medium nitrogen were not affected by the level of nitrogen in the growing media of either potato or coleus ($P > 0.05$). However, mealybugs reared on low nitrogen coleus plants showed a significant reduction in body length in comparison to high nitrogen plants ($P < 0.05$). In general there was an increasing but not significant, trend in the mean body lengths of mealybugs with an increase in the applied nitrogen to the host plants.

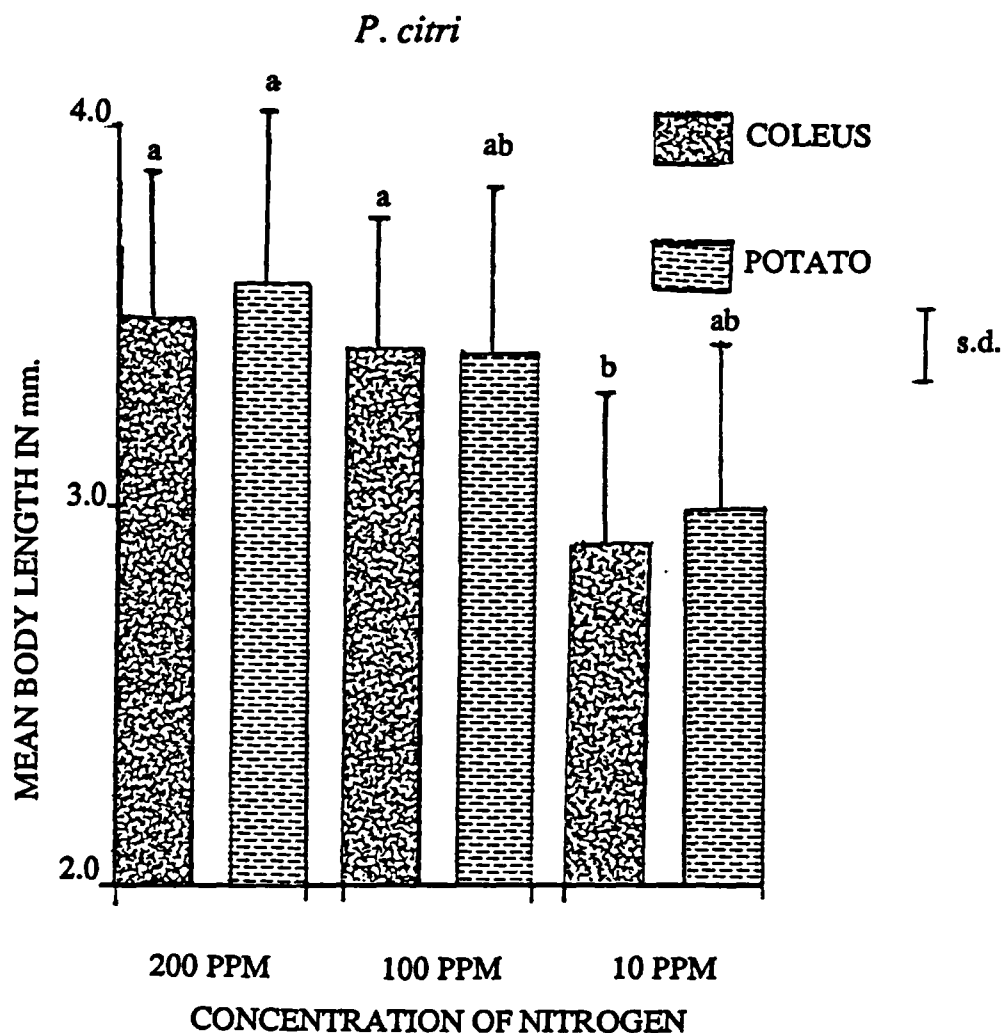


Fig. 5.4 Histograms showing mean body length mm $\pm$ s.d. of *P. citri* reared on coleus and potato plants maintained under 3 nitrogen levels. Each mean value is an average of 25 insects. Means with the identical letters are not significantly different at 5% level. SNK test

5.4.1.7 SDS-PAGE analysis of mealybug protein

The prominent protein bands were detected in mealybugs fed on 200 and 100 ppm nitrogen levels (Plate 5.3).

5.4.2.1 Effect of host plant nitrogen on successful parasitism by A. pseudococci.

The percentage successful parasitism of A. pseudococci under the influence of host plant nitrogen level is illustrated in Table 5.5. Analysis of variance shows there is no significant influence of host plant nitrogen levels on the success of parasitism ($P > 0.05$).

Table 5.5 Effect of host plant nitrogen (200, 100 and 10 ppm) on successful emergence of A. pseudococci.

MEAN PERCENTAGE EMERGENCE					
NITROGEN ppm		COLEUS		POTATO	
		$\bar{x}$	s.d.	$\bar{x}$	s.d.
High	200	79a	6.5	84a	4.1
Medium	100	67a	7.5	82a	5.7
Low	10	69a	7.4	75a	5.0
SNK test		(24, 5)	$S\bar{x} = 2.62$	MS = 34.3	

Calculations were based on Arc-sine transformation. Untransformed means are presented in the Table. Figures with the same letters are not significantly different at the 5% level.

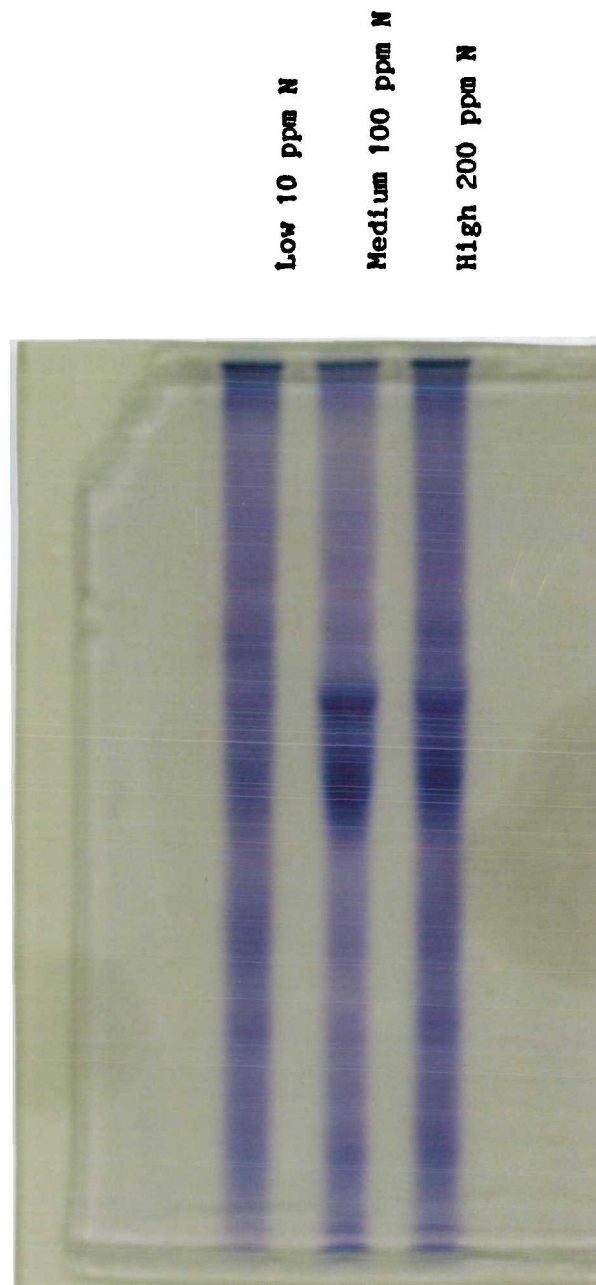


PLATE 5.3 SDS-PAGE (12.5% resolving gel) electrophoretic pattern of protein extract of *P. citri*. Insects were fed on potato plants grown under 3 nitrogen levels before use in protein analysis. Each column represents a different treatment. (Note denser protein bands in 200 and 100 ppm nitrogen treatments.)

5.4.2.2 Effect of host plant nitrogen on the development period of

A. pseudococci.

Neither host plant species nor the level of nitrogen in the host plants had a significant effect on the developmental period of male parasitoids ($P > 0.05$).

Table 5.6 Effect of host plant nitrogen (200, 100 and 10 ppm) on the developmental period of male A. pseudococci.

MEAN DEVELOPMENTAL PERIOD (DAYS)						
NITROGEN ppm		COLEUS			POTATO	
		n	$\bar{x}$	s.d.	n	$\bar{x}$ s.d.
High	200	20	14.2a	1.1	20	13.6a 0.6
Medium	100	20	14.3a	1.3	20	13.5a 0.8
Low	10	20	13.8a	0.6	20	14.5a 1.0

No significant difference. SNK test (144, 6) $S\bar{x} = 0.402$,

Table 5.7 Effect of host plant nitrogen (200, 100 and 10 ppm) on the development period of female A. pseudococci.

MEAN DEVELOPMENTAL PERIOD (DAYS)						
NITROGEN ppm		COLEUS			POTATO	
		n	$\bar{x}$	s.d.	n	$\bar{x}$ s.d.
High	200	20	14.3a	1.4	20	14.1a 0.7
Medium	100	20	14.7a	1.5	20	14.6a 0.9
Low	10	20	14.2a	0.9	20	14.5a 0.9

No significant difference. SNK test, (114, 6) $S\bar{x} 0.208$

5.4.2.3 Effect of host plant nitrogen on the longevity
of A. pseudococci.

The longevity of the emerging parasitoid from eclosion to the natural death of the insect is shown in Table 5.8. Adults for high nitrogen grown hosts live significantly longer than from low nitrogen plants ($P < 0.05$).

Table 5.8 Effect of host plant nitrogen (200, 100 and 10 ppm) on the longevity of A. pseudococci at $25 \pm 1^\circ\text{C}$.

MEAN LONGEVITY OF ADULTS (DAYS)						
NITROGEN ppm	COLEUS			POTATO		
	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.
High 200	20	34.5a	4.3	20	34.6a	5.1
Medium 100	20	33.6b	3.6	20	34.2a	4.6
Low 10	20	28.5c	2.7	20	28.9c	4.2
SNK test	(114, 5)		$S\bar{x} = 0.159$	MS = 0.5083		

Figures with the same letters are not significantly different at the 5% level.

5.4.2.4 Effect of host plant nitrogen on the body size of the emerging parasitoids.

Table 5.9. Effect of host plant nitrogen (200, 100 and 10 ppm) on the head width of male A. pseudococci.

MEAN HEAD WIDTH IN mm.						
NITROGEN ppm	COLEUS			POTATO		
	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.
High 200	15	0.42ab	0.06	15	0.44a	0.02
Medium 100	15	0.41ab	0.06	15	0.41ab	0.07
Low 10	15	0.37b	0.03	15	0.36b	0.06
SNK test	(84, 15)	$S\bar{x} = 0.017$		MS = 0.0036		

Figures with the same letters are not significantly different at the 5% level.

Body size of the emerging parasitoids were represented as head width and the analysis was carried out separately for males and females since males are generally smaller than the females (Tables 5.9 and 5.10).

Analysis of variance showed no significant difference in head width at different nitrogen levels when they were reared on coleus plants ($P > 0.05$). However, the males emerging from high nitrogen potato plants were larger than those from the low nitrogen treatment ($P < 0.05$). The pooled mean values showed that low nitrogen level can affect the mean head width of the parasitoid.

Table 5.10 Effect of host plant nitrogen (200, 100 and 10 ppm) on the head width of female A. pseudococci.

MEAN HEAD WIDTH IN mm.						
NITROGEN ppm	COLEUS			POTATO		
	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.
High 200	15	0.59a	0.02	15	0.59a	0.02
Medium 100	15	0.56a	0.04	15	0.56a	0.04
Low 10	15	0.52b	0.04	15	0.49b	0.05
SNK test	(84, 15)		$S\bar{x} = 0.017$	MS = 0.0022		

Figures with the same letters are not significantly different at the 5% level.

The analysis of variance shows a reduced female size as a result of low nitrogen in the host plant ($P < 0.05$).

5.5 DISCUSSION

5.5.1.1.1 The effect of applied nitrogen on total leaf nitrogen content.

Potato and coleus plants responded in a similar way with a higher total leaf nitrogen in high nitrogen treatments but with higher concentrations in the potato plants. This difference highlights the ability of potato to assimilate nitrogen from the growing media more efficiently than coleus. Better performance of mealybugs observed on these potato plants may be attributed to the higher nitrogen levels. Harrewijn (1970) analysed the total leaf nitrogen of potato plants grown on liquid fertilizer media (NFT) and found 4.76 and 2.14 percent nitrogen in the high and low nitrogen treatment respectively. The relative concentrations of total nitrogen was in broad agreement with other workers.

5.5.1.1.2 Effect of applied nitrogen on soluble amino acid content.

Although the total nitrogen content of the host plants gives incomplete information about plant suitability for herbivorous insects the nitrogen concentration is likely to be broadly correlated with the amino acid composition of the plant (McNeil & Southwood, 1978). In our experiment the total amino acid concentrations were closely related to the total nitrogen content of the host plants (Figures 5.1 & 5.2).

The concentrations of total amino acid in the high nitrogen treatments could be attributed to the physiological status of the host plants. Positive correlations between total amino acids and the level of nitrogen applied to the host plants have been discussed by various authors, using different concentrations of nitrogen in the growing media and plant varieties, (see review of literature). Insects absorb nitrogen through the gut primarily in the form of free amino acids other than proteins. Therefore plants which have higher concentrations of free amino acids seem to be preferred by the sap sucking insects (White, 1984).

5.5.1.2 Effect of host plant nitrogen on the development period of mealybug.

Some studies have shown that high nitrogen content in the host plants can induce changes in the physiological activities of insects leading to a faster development. However, in the present study no significant difference was found in the developmental period of larval stages of mealybugs reared on host plants with various nitrogen levels. The concentrations of total nitrogen and total amino acids on the low nitrogen plants were insufficient to induce changes in the mealybug development periods. As mealybugs are phloem feeders, the concentrations of total leaf nitrogen or amino acid may not directly correspond with the biological activities of the insect. The concentration of nitrogenous compounds in the phloem might not have reached the critical lower level to initiate physiological changes in mealybugs leading to a longer development period.

Plants grown on low nitrogen treatments had more senescing leaves presumably attributed to low nutrient in the plants. Proteins on such leaves tend to break down into soluble amino acids and translocate via the phloem to the growing areas of the plant (Hill, 1980).

Thus the phloem sap of low nitrogen plants might have been enriched with amino acids to support the normal growth of mealybugs. Such conditions may mask the changes in the nitrogen levels between high and low nitrogen treatments. Other biotic or abiotic factors such as genetic control, maternal physiology (Andrewartha & Birch 1954), temperature and (Tingle, 1985) might also be involved in the rate of development of mealybugs. Therefore it is difficult to single out one possible reason for the present observation.

5.5.1.3 Effect of host plant nitrogen on mortality rate of mealybug.

It appears that there was no relationship between mortality and nitrogen treatments. An inverse relationship between foliar nitrogen level and mortality rate of insects has been reported by various authors (Myers & Post 1981; Moore & Clements, 1984). Perhaps in our study the level of amino acid available in the low nitrogen plants had not reached the critical lower level to affect the mortality rate.

5.5.1.4 Effect of host plant nitrogen on the fecundity of mealybug.

The reproductive capacity of P. citri reared on low nitrogen treatment was certainly impaired. The reproductive performance reached a maximum at the intermediate (100 ppm) nitrogen levels and further nitrogen appeared unimportant. Most of the fitness parameters of mealybugs measured showed no significant difference between high and medium nitrogen treatments. van Emden (1966a) found increases in the fecundity of B. brassicae with increased Nitrogen or decreased Potassium supply to the plants. The maximum fecundity of aphids was reached in the medium nitrogen treatment and a declining trend was noted due to a further increase in applied nitrogen.

Thus the present finding is in good agreement with van Emden (1966a). In general, insects have a greater nitrogen demand during egg maturation. Inadequate supplies of nitrogen during this period would result in a depletion of the egg production. However, the egg production need not to be greatest at the highest level of available nitrogen (Dale, 1988). A possible reason for low fecundity of our mealybugs recorded in the low nitrogen plants might be attributed to an inadequate supply of essential amino acids. However, the real mechanisms underlining the direct involvement of nitrogenous compounds are not well understood, mainly because of numerous abiotic and biotic factors affecting egg production (Slansky & Scriber, 1985).

Food consumed by the larval instars has some impact on the fecundity and egg quality. Therefore the lower fecundity level of P. citri grown on low nitrogen plants could be attributed to an inadequate supply of nitrogenous food during the larval and pre-ovipositional periods.

The potential fecundity of P. citri, recorded in other experiments (see section 1) was greater than the highest value observed in this study. Hence the lower fecundity level of P. citri in the present study strongly suggest that other factors might also be involved in the reproductive capacity of mealybugs.

The daily mean fecundity of mealybugs was not affected by nitrogen levels in the host plant apart from a reduction in the number of eggs produced at low nitrogen treatments. The duration of oviposition was not affected by the nitrogen levels in the host plants. Similar observations were recorded in spider mites under high, medium and low nitrogen concentrations (Wermelinger et al., 1985).

The pattern of oviposition of the mites was similar in all the nitrogen treatments but the number of eggs produced by females in each treatment varied according to the level of amino acid in the host plants.

We reported a similar behaviour of mealybugs in water stress conditions where daily egg production was independent of stress level in the host plants.

5.5.1.5 Effect of host plant nitrogen on life table parameters of mealybug.

The rm value observed in the present study, indicated a positive relationship with the level of nitrogen in the host plants. The fecundity of an insect plays a major role in determining the rm value.

Thus the higher fecundity levels observed in mealybugs fed on high nitrogen plants might have contributed to higher rm values recorded in the present investigation. Mealybugs fed on high nitrogen potato plants reached sexual maturity in a shorter time than those developing on low nitrogen plants and this will also contribute to the rm value.

The rm values of mealybugs fed on potato plants were greater than the corresponding values in coleus plants. This difference could be attributed to a higher fecundity and faster development rate on potato plants. A similar situation was reported by Al-Zubaidi and Capinera (1983) who showed that food and nitrogen utilization efficiencies of insects varied with the type of host plant they fed upon. The rm value is derived from development period, fecundity, survival rate and sex ratio and is an appropriate measurement to evaluate the host plant quality. Overall we may conclude that high nitrogen levels in the host plants may contribute to a higher rm value and a greater potential to increase the population.

5.5.1.6 Effect of host plant nitrogen on the body size of mealybug.

Host plant nitrogen levels appear to have no significant influence on the body length of the mealybugs. Although the observed differences were not significant, the body lengths of mealybugs were in a descending order with the reduction of host plant nitrogen levels. Experiments carried out on feeding behaviour of aphids in low nitrogen conditions indicate that aphids compensate for a decrease in nutrient value, by an increase food uptake (Mittler, 1958).

Although the total nitrogen content and amino acid levels showed a considerable reduction in the low nitrogen potato plants, the biomass reduction of mealybugs was not significantly affected. A faster feeding rate of the mealybugs reared on low nitrogen plants or an availability of sufficient nutrients in the low nitrogen plants might account for the similarity of the body length. On coleus it appears that mealybugs suffered with an amino acid deficiency leading to poor growth. Nevertheless more work should be carried out to test the above hypothesis.

5.5.1.7 SDS-PAGE analysis of mealybug protein

We reported that mealybugs fed on low nitrogen plants laid significantly fewer eggs than those fed on medium and high nitrogen plants. Thus the thicker protein bands detected in mealybugs fed on high and medium nitrogen treatments could be attributed to the larger number of eggs on those insects. However, we cannot rule out the possibility of involvement of certain essential amino acids and proteins in high egg laying capacity of mealybugs fed on high and medium nitrogen plants. Further work is needed to prove this suggestion.

5.5.2.1 Effect of host plant nitrogen on successful parasitism.

Nitrogen utilization efficiency of natural enemies is not fully understood. A parasitoid's progeny grows and develops at the expense of the host, utilizing the host as a food reserve. Thus morphological, physiological, or biochemical changes in the host may directly influence the parasitoid morphological or behavioural characters (Duffey, 1980).

The size and fitness parameters of the parasitoid largely dependent on the size of the host (Sandlan, 1979). However, in the present study there was no relationship between host plant nitrogen and the frequency of emergence of parasitoids.

Mealybugs fed on low nitrogen plants were equally acceptable to the parasitoids for progeny production. Our finding is in contradiction with Duffy (1980) who found that poor quality host plant can enforce changes in parasitoids resulting in poor fitness parameters. Two possible suggestions can be put forward to explain the similarity in frequency of parasitism in the present study.

Firstly the parasitoids may not discriminate mealybug hosts due to the size or internal quality. Alternatively the larval stages of the parasitoids were not subjected to differential mortality due to inadequacies of the host. Since the size of mealybugs was not significantly affected by host plant nutrient quality the parasites would not be affected.

5.5.2.2. Effect of host plant nitrogen on the development period of parasitoids

The developmental periods of male and female parasitoids were not affected by the level of host plant nitrogen. Nevertheless the effect of host plant food quality on the development of various parasitoids has been discussed by many authors.

Thompson, (1976) worked on the importance of free amino acids and carbohydrate level for the larval development of Exeristes roborator and found that an inadequate supply of these compounds would result in a slower development of the parasitoids. In the light of the above findings, the similarity of the developmental period of A. pseudococci between the treatments, could be attributed to the uniformity in the quality and biomass of the hosts.

5.5.2.3 Effect of host plant nitrogen on the longevity of parasitoids.

The results revealed that parasitoids developing in high and medium nitrogen treatments survived a significantly longer period than those developed on low nitrogen treatments. As discussed in the preceding sections, the size of parasitoid is one of the important factors for most of the fitness parameters. Larger parasitoids have better searching capacity, reproductive ability and longer life expectancy (Weseloh, 1974; Sandlan, 1979). A similar situation was observed in the present investigation where larger parasitoids showed a longer life expectancy.

5.5.2.4 Effect of host plant nitrogen on the size of emerging parasitoids

The size of the emerging parasitoid depends mainly on the quantity of food obtained during larval development (Wylie, 1967; Shepard & Dahlman, 1988). The present findings indicate that the host plant's nitrogen levels ^{did} have some effect on the size of the emerging parasitoids particularly those developed on mealybugs, fed on low nitrogen host plants.

Although the variations in the host size have not influenced some fitness parameters such as developmental period and frequency of parasitism, the size of the parasitoid appears to be depend on the host size. A similar explanation was given by Wylie (1967) who found that the size of the host Musca domestica L. had a great influence on the size and fitness parameters of parasitoid Nasonia vitripennis (Walk.). Slansky and Scriber (1985) showed that the development of a parasitoid within its host is a compromise between the feeding and growth rates and efficiencities in the rate of utilizing the host tissues.

Hence the differences in size of the emerging parasitoids in the low nitrogen treatment could be attributed to the limited supply of food or inadequacy of essential amino acids.

5.6 CONCLUSIONS

1. The concentrations of total leaf nitrogen and total amino acids in the potato and coleus plants showed a positive relationship with applied nitrogen in the media.
2. The potato plants developed a higher level of total nitrogen and amino acids than the coleus plants.
3. The larval developmental period of P. citri was not influenced by the plant nitrogen levels. However, adult mealybugs in low nitrogen treatment had a significantly longer developmental period and they remained smaller.
4. The mortality rate of mealybugs was independent of the host plant nitrogen level.
5. The reproductive capacity of mealybugs was significantly reduced due to low nitrogen levels in the host plants.
6. The innate capacity of population increase was positively correlated to the host plants nitrogen levels.
7. No relationship was found between host plant nitrogen level and percentage parasitism.
8. The developmental periods of parasitoids was not influenced by the host plant nitrogen level.

9. There was a significant reduction in parasitoid size when they were reared on smaller mealybugs from low nitrogen plants.
10. The small parasitoids emerging from small mealybugs fed on low nitrogen plants had a relatively shorter life expectancy.

SECTION 6

BEHAVIOURAL RESPONSES OF PARASITIDS TOWARDS FOUR PLANT SPECIES

6.1 INTRODUCTION

The searching behaviour of the mealybug parasitoid Leptomastix dactylopii was studied on 4 different host plant species with varying degrees of trichome densities. Unsuitable surface properties of the host plant where host insects are feeding might have a direct influence on the searching efficiency of parasitoids. Such a situation may lead to a virtual exclusion of parasitoids or predators from a part of the host population.

A parasitoid's chance of encountering prey increases with the time spent searching preferred prey habitat.

Parasitoid searching time, pattern and efficiency are governed by the host plant characteristics and its innate behavioral pattern. The searching efficiency is hampered by the length, density and alignment (erect, procumbent, etc) of the leaf pubescence. Insects may be trapped by the sticky exudates of glandular trichomes, leading to slower walking speed and poor encounter rates. Vertically oriented long trichomes impair normal searching pattern and sometimes irritate the insect leading to it abandoning the surface and flying away from the prey. Secondary plant chemicals may also influence the searching behaviour of natural enemies.

The Wye College conservatory has many different species of plant and it was observed that some plant species were heavily infested with mealybugs apparently without parasitoid attacks. It was also noticed that the degree of parasitism varies with the plant species (Tingle and Copland, 1988). In the previous sections we discussed various possibilities for the difference in parasitism on various host plant species. This section outlines experiments carried out under laboratory conditions, designed to obtain information on host plant surface properties on the searching behaviour of L. dactylopii.

6.2 REVIEW OF LITERATURE

6.2.1 Importance of host location.

Parasitic Hymenoptera are most often used in biological control programmes and more than 80% of the successful natural enemies used in biocontrol are parasitoids (DeBach, 1964). It has been suggested that high host searching capacity is an essential characteristic for reaching self-sustaining economic pest control. Parasitoids usually successfully parasitize only a limited number of host species. The process required for successful parasitism has been divided into a sequence of steps namely (1) host habitat location, (2) host location, (3) host acceptance and (4) host suitability (Vinson, 1976). However, in a natural ecosystem parasitoids may not follow all these events and a great deal of overlap may occur depending on environmental factors (Lewis et al., 1976).

6.2.2 Factors affecting habitat location by natural enemies.

6.2.2.1 Host density.

The presence of unsuitable hosts or the lack of hosts in a habitat may affect the normal searching behaviour of a parasitoid. A female parasitoid has to spend energy to search an unproductive habitat without any gain, thus high host densities favours progeny production by parasitoids (Price, 1975).

At high host densities the frequency of encounter with unparasitized hosts increases with less movement within patches.

6.2.2.2 Environmental factors

Environmental factors can enforce changes in searching patterns of a parasitoid and often lead to the dispersal of the female. High humidity can affect insect mobility and insects may face problems if the substrate is too wet. The importance of intensity, colour and duration of light on the searching behaviour of female parasitoids have been discussed by various authors (Mathews & Mathews, 1978). The walking speed of the parasitoid Trichogramma is highly dependent on temperature, and low temperatures are unfavourable resulting in unproductive activities (Biever, 1972).

6.2.3 Influence of host plants on searching behaviour of parasitoids

The plant itself is an important factor that may affect searching behaviour of natural enemies. Chemical and physical characters of plants are known to affect searching behaviour of parasitoids. Eggs of Manduca sexta were readily parasitized by Telenomus sphingis and Trichogramma minutum when they occurred on several species of plants but were poorly parasitized when they occurred on tobacco (Vinson, 1976). A similar observation was reported by Treacy et al., (1984) who found that Trichogramma pretiosum efficiency was reduced when they searched on cotton leaves with a high density of trichomes.

The larvae of Propylea quatuordecimpunctata (L.) move more slowly on hairy potato leaves than on glabrous bean leaves (Banks, 1957).

Carter et al., (1984) reported that Coccinella septempunctata L. found the smooth leaves of pea difficult to grasp and frequently fell from pea plants resulting in a poor searching capacity compared with bean plants.

Dowell et al., (1981) studied searching behaviour of Amitus hesperidum (Silvestri) and Encarsia opulenta (Silvestri) and found that female parasitoids start random searching and follow to the mid-vein at either end of the leaf, where hosts generally congregate. Keller (1987) reported that the walking behaviour, rates of turning and flight propensity of Trichogramma exiguum varies among plant species due to morphological differences in leaf structures, especially the type and the density of trichomes. A similar behaviour was reported by Evans (1976) who showed that Anthocoris confusus (Reuter) is greatly influenced by the topography of the plant, and most of the activities of the insect were found along the mid-rib veins and leaf edges.

6.3 MATERIALS AND METHODS

6.3.0.1 Preparation of leaf samples.

Four plant species, Streptocarpus hybridus, Aeschynanthus ellipticus, Glycine max and Psophocarpus tetragonolobus which bear varying degrees of trichome densities and surface structures were used in this experiment. Uniformity of the experimental leaves were maintained by taking the sixth leaf from the terminal bud. The leaves were uninfested and samples were collected just before the experiment and were used without rubbing or soaking in water which may alter natural surface characters of the leaves. Preliminary studies with leaf portions, gave unsatisfactory results with abnormal behaviour of parasitoid on the cut surfaces. Thus only complete leaves were taken to avoid possible effect of damage surfaces. Since A. ellipticus and winged bean leaves are small in size, 2-3 leaves were joined by placing gum tape beneath the joints.

6.3.0.2 Arrangement of apparatus and arena

A cylinder, made out of acetate papers with a diameter of 8.5 cm and height of 7 cm, was used as the arena. A lid was fixed with the same material and the bottom was kept open to place on the leaf surface. For each replicate freshly excised leaves were positioned over a 3 mm thick piece of glass to get an even surface. This set up was placed under a uniformly illuminated platform where lighting was provided by overhead fluorescent lamps (cool white, 18 W, 60 cm two twin bulbs) placed 30 cm above the arena which provided illumination of 40 W/m². A video camera (JVC) was fixed on a vertical stand and focussed onto the arena.

The camera had a stop watch device which displayed the time on the video signal. The camera was connected to a video cassette (JVC VHS) recorder (HR-S10EK). A test run was carried out to obtain a suitable magnification and appropriate colour to distinguish leaf surface from the test insect in the arena. After standardization the position of the camera was fixed throughout the experimental period.

6.3.0.3 Selections of suitable parasitoids

Mated female L. dactylopii parasitoids, 3-5 days old, were fed on 50% honey for 2 days before using them in the experiment. Twelve hours prior to the experiment the test insects were taken away from the food source and kept under the illuminated platform to get acclimatized. Parasitoids were individually introduced into the arena with the leaf to be searched. Each female parasitoid and each leaf surface was used only once. Twenty fresh leaves per plant species served as replicates.

6.3.0.4 Recording procedure

The introduced insect was allowed to settle down onto the leaf surface and then the timer and the video recorder were started simultaneously and the following activities of the insect were recorded through direct observations.

6.3.1 Walking

Where the parasitoid moved over the leaf surface.

6.3.2 Palpating

Where the searching parasitoid was stationary but sensed the leaf surface with its mouth parts.

6.3.3 Preening

Where the insect was motionless but grooms itself or cleans mouth part, legs or wings.

6.3.4 Turning

A turn through an angle of 90° or more.

6.3.5 Jumps or flights

This activity was recorded when the insect jumps from one place to another place on the leaf surface or abandons the surface.

6.3.6 Resting

Motionless stage without any other activities.

Any insects which stayed more than 15 seconds on the experimental leaf surface were considered as replicates. The number of turns and jumps were counted in situ and the time taken for preening, palpating and resting were measured using a stopwatch. The other activities such as walking time, searching pattern and total distance walked were obtained from the video tape once the recording was terminated. Recording was carried out between 0900-1400 hours to avoid any diurnal variation. A piece of acetate paper was fixed to the screen of the colour video monitor to trace the searching paths of the insect already recorded in the video tape. For every replicate one such acetate paper was used and the total time taken was obtained from the records.

The paths were measured using a piece of thread. The enlargement factor was taken into consideration in the calculation of walking speed.

Walking speed, total distance covered, total time spent in searching, total number of turns, time taken for palpating, preening and resting were obtained from every replicate and analysed by two factorial analysis of variance and comparisons were made using l.s.d. at 5% levels.

6.3.5 Electron microscopic studies on plant surface structures

Uniform aged leaf samples were prepared as described below for scanning electron microscope studies (SEM). Small plant samples were treated to a standard dehydration procedure. The dried samples were placed on a circular aluminium stub with an adhesive. The stub was mounted on a slightly larger diameter cork, labelled and placed in an Edwards Model E12-E3 vacuum coating unit and coated with Carbon-Gold at a pressure of 10^{-4} mm Hg . After the vacuum was released each stub was examined under the SEM and the sections were photographed with polaroid film (Plate 6.1).

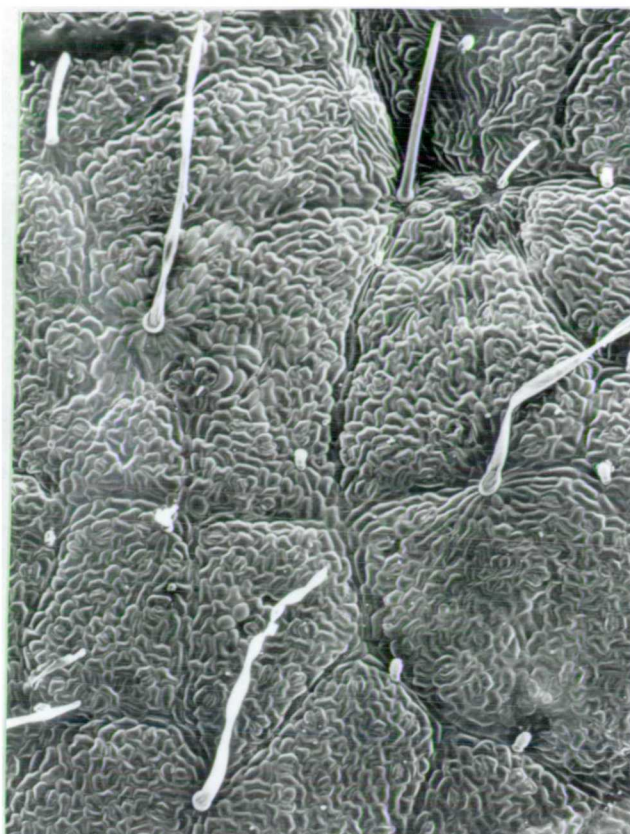
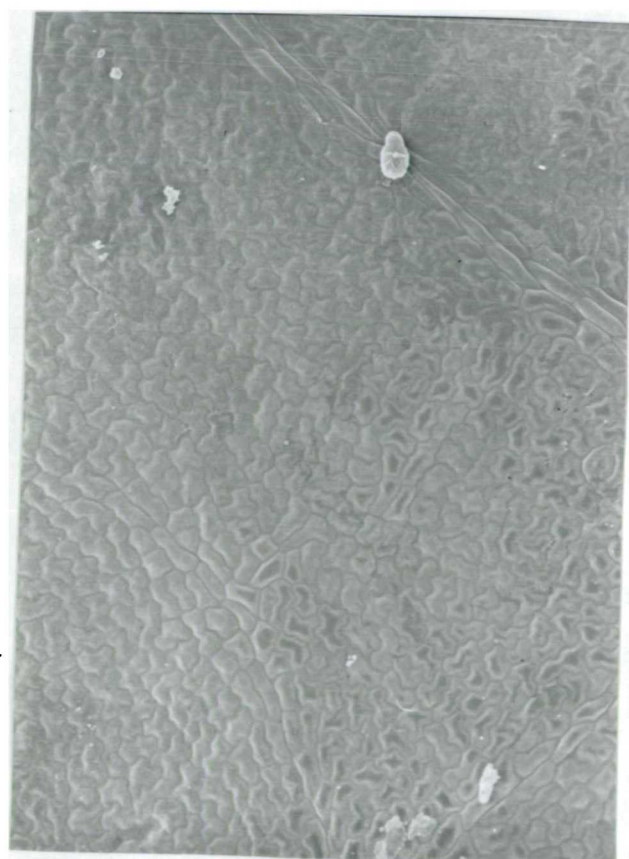
Glycine maxMagnification 500 μ *Psophocarpus tetragonolobus*Magnification 50 μ 

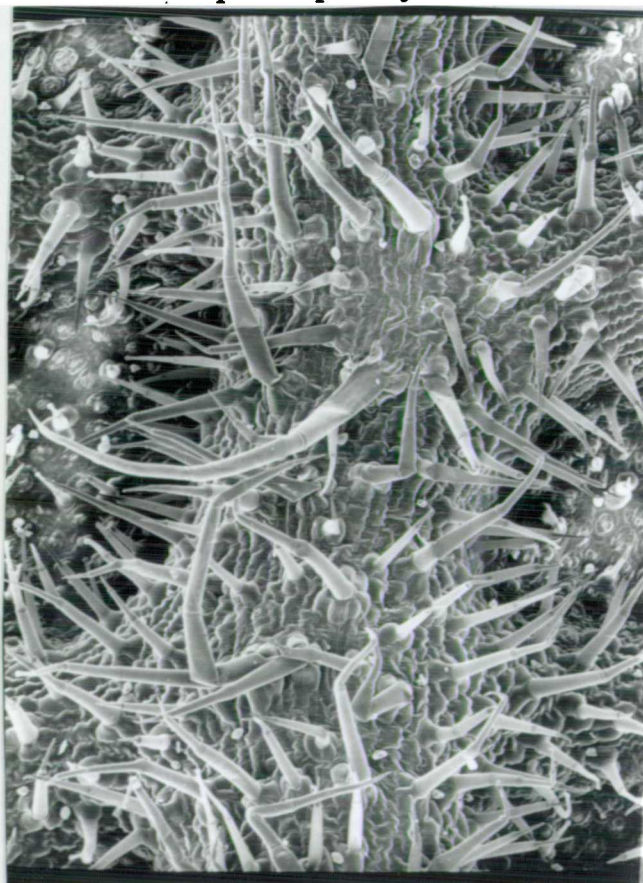
PLATE 6.1 Scanning Electronmicrographs of lower surfaces of various leaf samples,



Aeschynanthus ellipticus

Magnification 500 μ

Streptocarpus hybridus



Magnification 500 μ

6.4 RESULTS

Results of various searching activities of L. dactylopii on different leaf surfaces are presented in Tables 6.1 - 6.9.

6.4.1.1 Total time spent walking by L. dactylopii while searching on various host plant surfaces

L. dactylopii spent the longest time on S. hybridus when searching on the upper leaf surface ($P < 0.05$). However, no significant difference was observed on the lower side of any plant species. Parasitoids spent a relatively shorter time walking on the upper surface of leguminous plants. The plant species-surface interaction showed that parasitoids walk for a longer period on the lower surface of the leaves than on the upper side (Table 6.1).

Table 6.1 Total time spent walking in seconds by L. dactylopii while searching on various leaf surfaces.

Plant species	Upper surface		Lower surface		Means for Pooled surface effect
	Interactions l.s.d. 5% = 20.4				l.s.d. 5% 14.4
	$\bar{x}$	s.e.	$\bar{x}$	s.e.	$\bar{x}$
<u>A. ellipticus</u>	91.9 cd	7.5	108.7 bc	8.3	100.3 q
<u>S. hybridus</u>	133.0 a	13.1	112.9 ab	7.8	123.0 p
<u>G. max</u>	71.6 de	5.6	100.0 bc	6.9	86.3 qr
<u>P. tetragonolobus</u>	63.3 e	4.2	97.3 bc	6.4	80.3 r
Means (Species pooled)	90.0 n		105.1 m		
	l.s.d. 5% 10.2				

Each mean is an average of 20 replicates. Figures with the same letters are not significantly different at the 5% level.

6.4.1.2 Total distance walked by L. dactylopii during searching on various leaf surfaces.

The results are summarised in Table 6.2 and the analysis of variance showed that the distance covered by the parasitoid differed depending on the plant species and the surface. Parasitoids walked the longest distance (60.7 cm) on winged bean plant and the shortest distance (8.2) on S. hybridus plants ($P < 0.05$). There was no significant difference between the distance walked on upper and lower surfaces of the plants except on soy bean where the insects covered a shorter distance on the upper side of the leaf ($P < 0.05$). Analysis of the pooled surface mean values highlighted the importance of plant species on parasitoid movements.

Table 6.2 Total distance walked in centimeters by L. dactylopii during searching on various leaf surfaces.

Plant species	Upper surface		Lower surface		Means for Pooled surface effect
	Interactions 1.s.d. 5% = 6.4				1.s.d. 5% = 4.6
	$\bar{x}$	s.e.	$\bar{x}$	s.e.	$\bar{x}$
<u>A. ellipticus</u>	25.1 c	3.9	29.4 c	3.8	27.5 r
<u>S. hybridus</u>	8.2 e	0.9	9.1 e	1.0	8.6 s
<u>G. max</u>	28.1 c	1.5	38.2 b	2.5	33.1 q
<u>P. tetragonolobus</u>	60.7 a	2.6	54.7 a	5.0	57.7 p
Means (Species pooled)	30.5		32.8		
1.s.d. 5% = 3.2, ns					

Each mean is an average of 20 replicates. Figures with the same letters are not significantly different at the 5% level.

6.4.1.3 Effect of leaf surfaces on speed of movement of L. dactylopii

The data are presented in Table 6.3. Parasitoids walked fastest (0.59 cm/s) on the upper surface of winged bean leaves and slowest (0.03 cm/s) on S. hybridus ($P < 0.05$). Analysis of pooled surface mean values showed that speed of movement can be highly influenced by the plant species surface properties. In general parasitoids moved faster on the upper surface than on lower surface of the leaves.

Table 6.3 Effect of leaf surfaces on speed of movement (cm/s) of L. dactylopii

Plant species	Upper surface		Lower surface		Means for Pooled surface effect
	Interactions l.s.d. 5% = 0.05				l.s.d. 5% = 0.038
	$\bar{x}$	s.e.	$\bar{x}$	s.e.	$\bar{x}$
<u>A. ellipticus</u>	0.20 c	0.02	0.19 c	0.03	0.19 r
<u>S. hybridus</u>	0.03 d	0.02	0.04 d	0.10	0.03 s
<u>G. max</u>	0.28 b	0.10	0.27 b	0.02	0.27 q
<u>P. tetragonolobus</u>	0.59 a	0.04	0.29 b	0.09	0.44 p
Means (Species pooled)	0.27 m		0.19 n		
l.s.d. 5% = 0.026					

Each mean is an average of 20 replicates. Figures with the same letters are not significantly different at the 5% level.

6.4.2 Effect of leaf surfaces on time allocation for palpation

by L. dactylopii

Analysis of data is shown in Table 6.4. Insects spent the longest time for palpation when they search on Streptocarpus and the lower surface of winged bean leaves. Pooled surface data showed a significant difference between plant species with shortest palpation on soy bean.

Table 6.4 Effect of leaf surfaces on time allocation in seconds for palpation by L. dactylopii.

Plant species	Upper surface		Lower surface		Means for Pooled surface effect
	Interactions l.s.d. 5% = 5.1				l.s.d. 5% = 3.6
	$\bar{x}$	s.e.	$\bar{x}$	s.e.	$\bar{x}$
<u>A. ellipticus</u>	8.7 bc	1.9	4.7 cd	1.2	6.7 q
<u>S. hybridus</u>	14.1 a	1.6	16.5 a	3.3	15.3 p
<u>G. max</u>	2.2 d	0.34	2.5 d	0.6	2.4 r
<u>P. tetragonolobus</u>	6.5 cd	1.4	13.2 ab	2.2	9.8 q
Means (Species pooled)	7.91		9.25		
l.s.d. 5% = 2.5 ns					

Each mean is an average of 20 replicates. Figures with the same letters are not significantly different at the 5% level.

6.4.3 Effect of leaf surfaces on time allocation for preening

by L. dactylopii

The pooled surface mean values show that insects spent a longer period on preening when they search on S. hybridus and the upper surface of soy bean ($P < 0.05$). However, there was no significant difference in time allocation for preening when they search on upper and lower surfaces of leaves on various plant species (Table 6.5).

Table 6.5 Effect of leaf surfaces on time allocation for preening in seconds by L. dactylopii

Plant species	Upper surface		Lower surface		Means for Pooled surface effect
	Interactions l.s.d. 5% = 11.6				l.s.d. 5% = 8.17
	$\bar{x}$	s.e.	$\bar{x}$	s.e.	$\bar{x}$
<u>A. ellipticus</u>	7.7 c	2.9	15.9 bc	4.9	11.8 q
<u>S. hybridus</u>	32.7 a	4.4	31.8 a	5.2	32.3 p
<u>G. max</u>	22.5 ab	3.8	14.9 bc	2.5	18.7 q
<u>P. tetragonolobus</u>	11.2 bc	2.2	15.8 bc	2.6	13.5 q
Means (Species pooled)	18.5		19.6		
l.s.d. 5% = 5.8	ns				

Each mean is an average of 20 replicates. Figures with the same letters are not significantly different at the 5% level.

6.4.4 Effect of leaf surfaces on rate of turns (over 90°) made
by L. dactylopii

The effect of plant topography on the number of major turns made per unit distance is illustrated in Table 6.6. Analysis of variance shows that plant species surface properties have no significant effect on the number of turns made per centimeter distance searched by the parasitoid.

However, the pooled means for surfaces showed that parasitoids made a significantly higher number of turns when searching on A. ellipticus in comparison to winged bean plants ($P < 0.05$).

Table 6.6 Effect of leaf surfaces on number of turns made
per cm walked by L. dactylopii

Plant species	Upper surface		Lower surface		Means for Pooled surface effect
	Interactions l.s.d. 5% = 0.0571(ns)				l.s.d. 5% = 0.0404
	$\bar{x}$	s.e.	$\bar{x}$	s.e.	$\bar{x}$
<u>A. ellipticus</u>	0.0052	0.0002	0.0065	0.0002	0.0585a
<u>S. hybridus</u>	0.0573	0.0049	0.0471	0.0058	0.0522ab
<u>G. max</u>	0.038	0.0038	0.0330	0.0038	0.0354ab
<u>P. tetragonolobus</u>	0.0145	0.0026	0.0100	0.0057	0.0122b
Means (Species pooled)	0.0287		0.046764		
l.s.d. 5% = 0.0286	ns				

Each mean is an average of 20 replicates. Figures with the same letters are not significantly different at the 5% level. ns not significant.

6.4.5 Effect of leaf surfaces on rate of jumps made by

L. dactylopii

The results are shown in Table 6.7. The analysis of variance showed that the parasitoid made significantly more jumps when they search on the upper surface of soy bean leaves and lower surface of Streptocarpus leaves. The pooled means for surfaces also highlighted that the rate of jumps made by the parasitoids was significantly greater when they search on soy bean and Streptocarpus surfaces ($P < 0.05$).

Table 6.7 Effect of leaf surfaces on number of jumps made per cm walked by L. dactylopii

Plant species	Upper surface		Lower surface		Means for Pooled surface effect
	Interactions l.s.d. 5% = 0.1651				l.s.d. 5% = 0.1167
	$\bar{x}$	s.e.	$\bar{x}$	s.e.	$\bar{x}$
<u>A. ellipticus</u>	0.152 b	0.0026	0.075 b	0.008	0.113897q
<u>S. hybridus</u>	0.152 b	0.043	0.443 a	0.095	0.2975p
<u>G. max</u>	0.334 a	0.086	0.162 b	0.051	0.2479p
<u>P. tetragonolobus</u>	0.008 b	0.002	0.095 b	0.007	0.0518q
Means (Species pooled)	0.16195		0.19364		
l.s.d. 5% = 0.08254	ns				

Each mean is an average of 20 replicates. Figures with the same letters are not significantly different at the 5% level.

6.4.6 Effect of leaf surfaces on time allocation for resting

by L. dactylopii

The results are summarised in Table 6.8. Analysis of variance showed that parasitoids stayed a significantly longer period motionless on Streptocarpus and the lower side of winged bean leaves while they spent a shorter period resting on A. ellipticus leaves and the upper surface of soy bean leaves.

Table 6.8 Effect of leaf surfaces on time allocation for resting in seconds by L. dactylopii.

Plant species	Upper surface		Lower surface		Means for Pooled surface effect
	Interactions l.s.d. 5% = 13.2				l.s.d. 5% = 9.4
	$\bar{x}$	s.e.	$\bar{x}$	s.e.	$\bar{x}$
<u>A. ellipticus</u>	13.1 c	4.4	14.7 c	4.0	13.7 s
<u>S. hybridus</u>	53.3 a	5.5	54.8 a	1.5	54.1 p
<u>G. max</u>	18.4 bc	4.8	30.2 b	4.2	24.3 r
<u>P. tetragonolobus</u>	27.9 b	5.8	60.3 a	4.7	44.1 q
Means (Species pooled)	28.2n		39.9m		
l.s.d. 5% = 6.7					

Each mean is an average of 20 replicates. Figures with the same letters are not significantly different at the 5% level.

6.4.7 Effect of leaf surfaces on total time allocation for searching
by L. dactylopii

The results are presented on Table 6.9 and a clear variety-surface interaction was observed in the analysis of variance. Parasitoids spent a significantly longer period when they search on Streptocarpus plants. In general the insect spent a longer period on the lower side of the leaf.

Table 6.9 Effect of leaf surfaces on total time allocation for searching in seconds by L. dactylopii

Plant species	Upper surface		Lower surface		Means for Pooled surface effect
	Interactions l.s.d. 5% = 32.4				l.s.d. 5% = 23.0
	$\bar{x}$	s.e.	$\bar{x}$	s.e.	$\bar{x}$
<u>A. ellipticus</u>	121.5cde	3.3	143.7 c	4.1	132.6 q
<u>S. hybridus</u>	232.6 a	8.7	215.4 ab	8.3	224.0 p
<u>G. max</u>	114.8de	7.3	148.2 cd	5.0	131.5 q
<u>P. tetragonolobus</u>	109.0 e	7.9	188.1 b	6.3	148.5 q
Means (Species pooled)	144.5 n		173.9 m		
l.s.d. 5%	16.2				

Each mean is an average of 20 replicates. Figures with the same letters are not significantly different at the 5% level.

6.4.8. Time apportioned to various activities

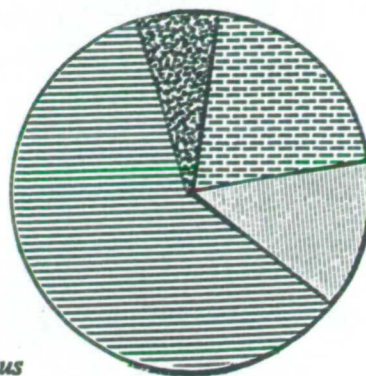
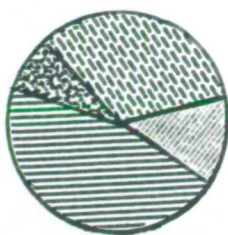
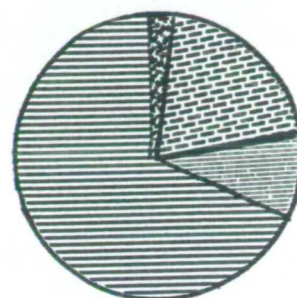
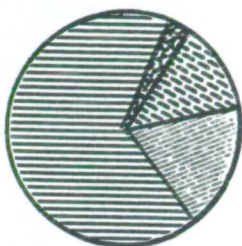
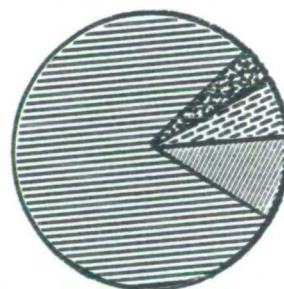
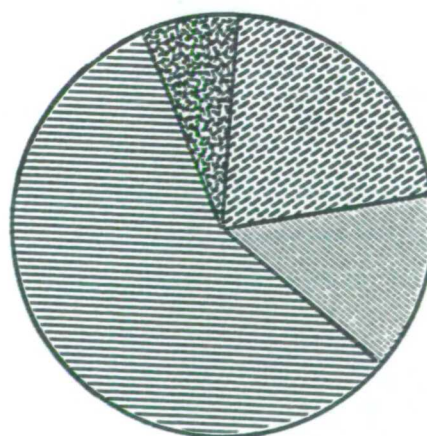
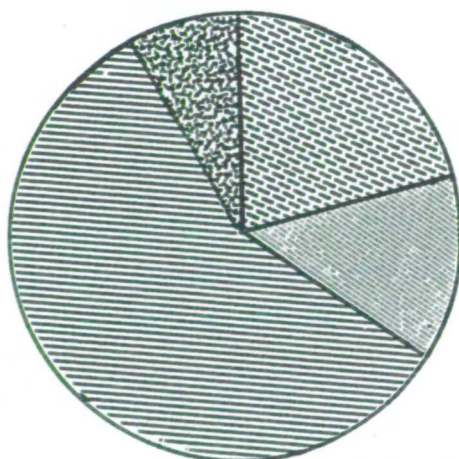
The data on the time spent on various activities on different surfaces, was taken for calculating the proportion of time given to each of the activities, preening, palpating, walking and resting, whilst searching on leaf surfaces. The data is presented as a series of pie-diagrams (Fig. 6.1). The area of each circle represents the total time spent by the parasitoid while searching on the substrate.

6.4.9. Searching pattern of parasitoids on various leaf surfaces

The searching patterns of parasitoid are presented in a series of diagrams (Fig 6.2 and 6.3).

UPPER

LOWER

*Psophocarpus tetragonolobus**Glycine max**Aeschynanthus ellipticus**Streptocarpus hybridus*

Walking



Resting



Palpating



Preening

UPPER

LOWER

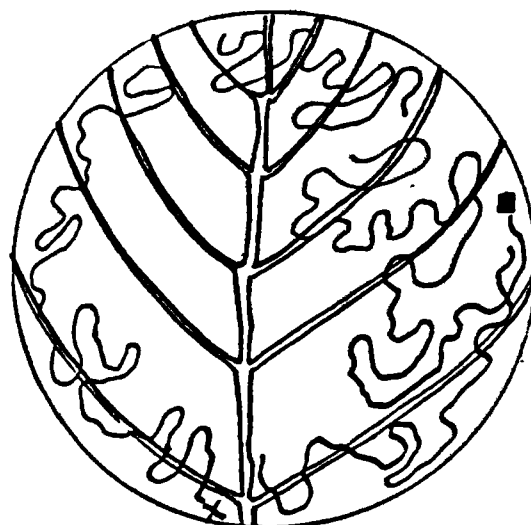
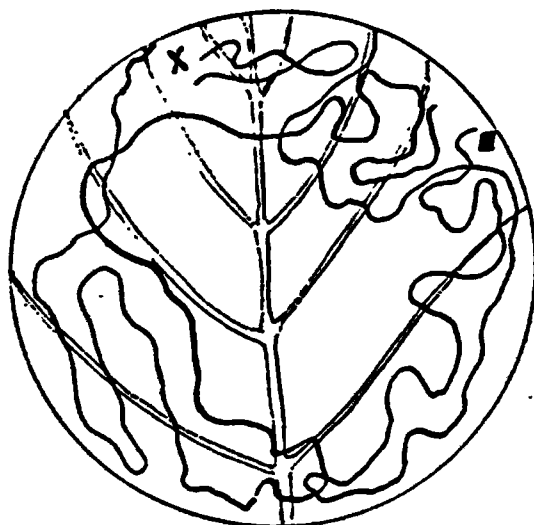
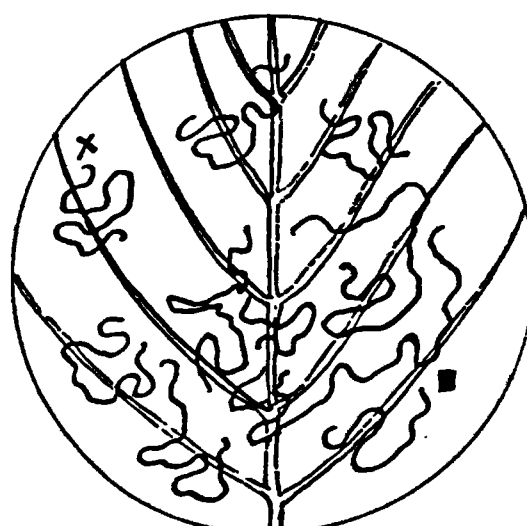
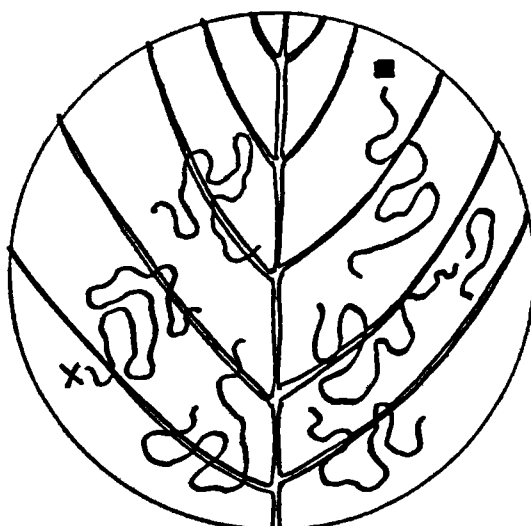
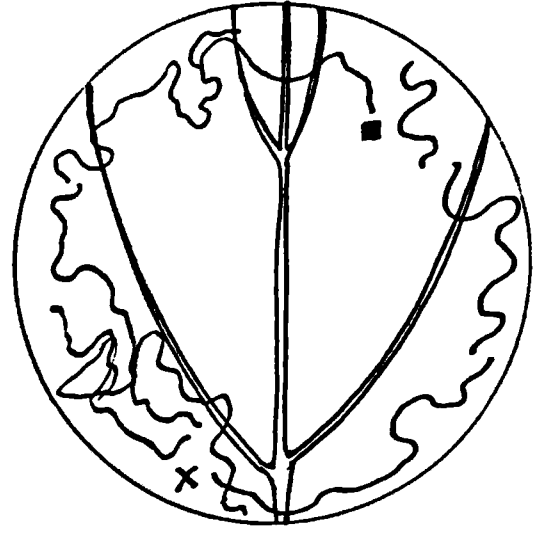
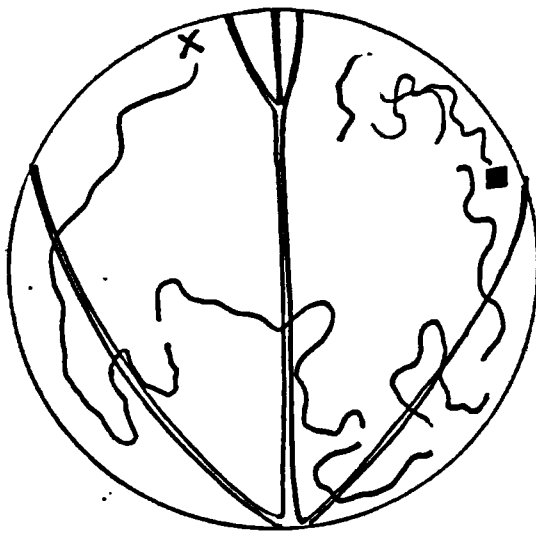
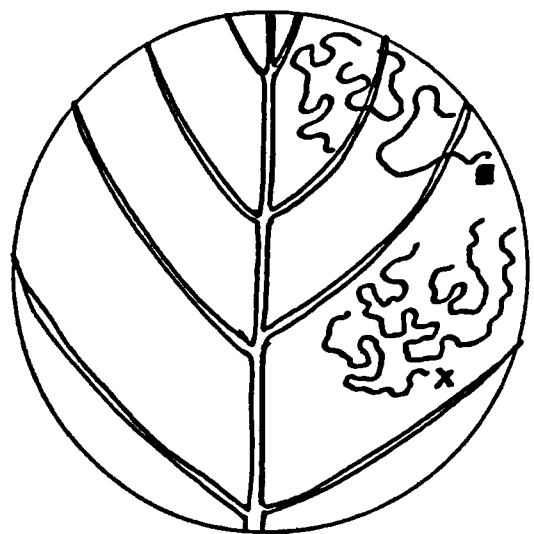
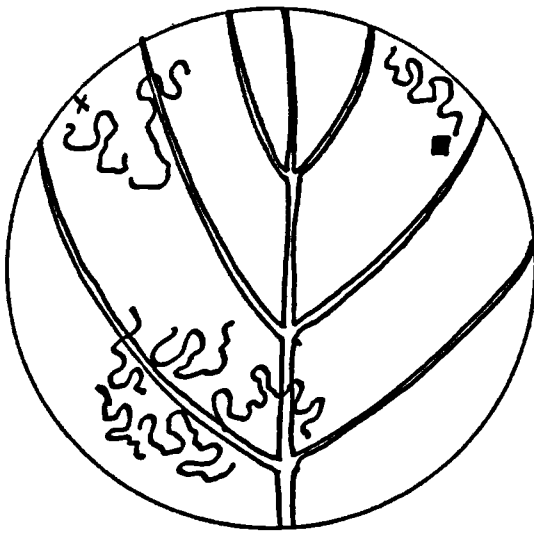
*Psophocarpus tetragonolobus**Glycine max*

Fig. 6.2 Searching pattern of female *L. dactylopii* on leaves of various plant species during its entire search bout.
 X - Starting point. ■ - Position at end of searching activities.

UPPER

LOWER

*Aeschynanthus ellipticus**Streptocarpus hybridus*

6.5 DISCUSSION

It is evident from these results that the overall effect of plant surface properties on searching behaviour is the cumulative effect of various activities of the parasitoid. Some activities are more prominent over the others depending on the plant characters and this leads to differences in searching pattern. The importance of leaf topography on searching behaviour of natural enemies has been discussed by various authors (Flanders, 1953; Gardner & Dixon, 1985). The optimal foraging theory emphasises the importance of time allocation by the parasitoids to enhance the searching efficiency.

The searching efficiency has been defined as the proportion of hosts encountered/parasitoid/unit of search time (Waage & Hassell, 1982). Adult female parasitoids allocate their total time to various activities namely feeding, foraging for host and resting. Foraging is a series of locomotory activities which require energy and time. Thus efficiency of a parasitoid depends on the ability to find a host in the shortest possible time with least energy expenditure. However, plant topography may be a formidable barrier for locomotory activities of a parasitoid.

Parasitoids spent a longer period walking on Streptocarpus plants but the distance covered during the search was remarkably low in comparison to other plant species. A considerable reduction in the speed of movement was noticed thus affecting the potential area that a parasitoid can search. Streptocarpus has densely packed, erect and long trichomes on the entire leaf surface (see plate 6.1).

Thus the speed of movement might have been adversely affected due to the resistance caused by this thick layer of trichomes. Moreover the exudates from glandular trichomes of Streptocarpus might aggravate the problems of parasitoids. Johnson (1956) showed that Aphis craccivora and Myzus persicae failed to colonize on tomato plants because of the gummy exudate of trichomes.

The parasitoid spent less time in walking on A. ellipticus in comparison to leguminous plants. Walking speed and area searched while walking was relatively lower than one would expect from a glabrous leaf. The leaf surface of this plant is covered with a thick slippery wax-layer, without any visible trichomes on the surface (Plate 6.1). One possible reason for slow movement and low searching ability of the parasitoid may be attributed to the slippery nature of the leaf where the insect was unable to clasp with their legs. Insects moved relatively slowly on interveinal areas affecting the searching efficiency. A similar situation was reported by Shah (1982) who found that the larvae of Adalia bipunctata could move only along the edge or protruding veins, when they were released on to glabrous leaves.

A possible reason for the slow speed and low searching ability of parasitoid on soy bean leaves is perhaps attributed to the hairy nature of the leaves. Soy bean has sharp and erect trichomes which can hinder the normal behaviour of the parasitoid leading to unproductive activities (Plate 6.2). However, the parasitoid's walking speed reached its maximum when they search on winged bean leaves. They searched relatively larger areas during a shorter period of time on winged bean indicating the importance of quality of substrate for efficient searching behaviour.

The leaf surface of winged bean is free of trichomes and without slippery wax-layers. Thus the parasitoids have apparently no resistance to walking on this substrate.

Increased turning movements are one of the important characters that leads to more thorough coverage of patches (Yano, 1978). Further it has been suggested that the frequency of turns generally increases when parasitoids walk in the presence of hosts than in their absence. Intensive searching with enhanced number of turns per unit distance generally occurs when they search within patches (Murdie & Hassell, 1973). However in the present study, the parasitoids were released to a host free habitat, thus one cannot expect a substantial increase in the number of turns per unit distance. Nevertheless the high number of turns recorded on A. ellipticus plants may be attributed to the slippery nature of the plant surface.

It is evident from the results that the parasitoids made more jumps when they search on Streptocarpus and soy bean substrates. Parasitoid jumping indicates a desire to leave the substrate either for dispersion or to avoid unsuitable substrates. The more likely explanation for the high jumping frequency on Streptocarpus and soy bean plant may be caused by the type and density of trichomes on those plants.

Preening appears to be a non-productive searching event that can reduce the efficiency of a parasitoids. However, the frequency of preening appears to be related to the presence of trichomes on the substrate.

The purpose of preening is to clean the body parts or to rearrange the disorganized wings, a problem which is likely to occur on a hairy surface.

All four plant species studied, caused marked differences in parasitoid searching behaviour. These behavioural changes in response to plant topography are well documented and many parasitoids have shown similar behavioural responses to plant characters.

We cannot rule out the possibility of some effect of secondary plant chemicals. Certainly the phenol level appears to have no influence but further work would be required to see if other volatile chemicals have any influence.

Many factors may affect the searching of L. dactylopii but the present finding has emphasized the importance of plant surface characters on the efficiency of L. dactylopii to search for the potential host in a mixed cropping system. In section 1 we showed that soy bean is a superior host plant for P. citri and the population of mealybugs increased to a greater degree within a shorter period when they develop on this plant. However, the present finding reveals that the parasitoids are relatively inefficient when they search on soy bean. This may lead to a destabilization of the population dynamics of the host/parasitoid system.

6.6 CONCLUSIONS

1. Walking speed and distance covered by L. dactylopii was significantly reduced when they search on Streptocarpus plants. The thick trichome layer of the plant surface might have interrupted the normal searching behaviour of the insect.
2. Walking time was shorter when parasitoids search on A. ellipticus plants. This may be attributed to the slippery wax-layer of the leaf surface.
3. Winged bean appears to be the preferred plant where parasitoids showed faster movement resulting in larger areas searched during a shorter period of time. The leaf surface of this plant is relatively rough without any visible trichomes and the insect might have experienced least resistance to movement on this substrate.
4. The number of turns appears to be independent of plant surface characters.
5. The parasitoid made a significantly greater number of jumps per unit distance walked on Streptocarpus and soy bean plants indicating a desire to leave. A likely reason may be the type or density of trichomes.

SECTION 7

CONCLUSIONS

The present investigation emphasized the importance of host plant species on life history parameters of P. citri, P. affinis and their parasitoids. The developmental period of mealybugs was retarded as a result of host plant factors particularly due to low nutritional value of some host plants species or the intervention of secondary plant metabolites.

A. ellipticus appears to be an acceptable non-host plant for both mealybug species but where mealybugs showed poor performances. High phenolic content and low nitrogen in these plants might be the possible reasons for low nutritive quality. Passiflora plants showed complete resistance to P. citri where no development or growth of the insect was detected. However, the crawlers of P. citri settled on Passiflora plants as they normally do on accepted host plants such as soy bean. However, within 3-4 days over 90% of crawlers feeding on Passiflora plants died and the rest remained as crawlers for few weeks. There may be a potential benefit to use these two Passiflora plants as protective barriers to reduce the spread of P. citri onto more susceptible host plant species. Nevertheless, more work would be needed to support this suggestion.

Many insect pests are not continuously present in crops, particularly in annuals, so their parasitoids must survive on other resources during their absence. Thus availability of alternative hosts in or around the crop may help to ensure survival of parasitoids (van den Bosch & Telford, 1964). Pests on different crops may act as alternative host for the natural enemies. However, the present investigation showed that the host plant species can influence the life history parameters of mealybugs and their parasitoids.

Parasitoids remained relatively small when they are reared on mealybugs fed on Gesneriaceae plants and their life span was significantly reduced. On the contrary parasitoids reared on leguminous plants showed better performance with a female biased sex ratio and long life expectancy. The amount of resources available for the developing parasitoid larvae determines survival and fitness of the emerging adults.

Large female parasitoids require more nutrient and a nutrient deficit may lead to differential mortality and a shift in the sex ratio. Host size is one of the important factors for sex allocation by female parasitoids at the time of oviposition. In general small mealybugs resulting from poor nutritive quality of the host plant favoured a male biased sex ratio. Increased fitness parameters of parasitoids always favours biological control resulting in better establishment of parasitoids.

Defence reactions of insects to foreign organisms are well known but little is known about this in mealybugs. The haemolymph of mealybugs appears to have defence mechanisms against parasitoids and the rate of encapsulation seems to depend upon the host parasite relationship.

P. citri was susceptible to attack by A. pseudococci and L. dactylopii and no encapsulation was detected. However, a protein synthesis reaction was observed. When an inert material was inserted, encapsulation took place but no protein synthesis was detected. This suggests that the immunity mechanism and or protein synthesis is an integrated reaction between host and parasite, initiated by the parasitoid. The main theory that has been proposed to explain the success of some parasitoids within the host body suggest that the host is unable to recognize the parasitoid's eggs as foreign. The host immunity reaction may be evaded by an immunodepressive effect of the parasitoids caused by injecting toxic substances or coating the eggs with specific substances. The present investigation suggested that P. affinis can successfully overcome the immunodepressive mechanism of L. dactylopii but shows only partial resistance to A. pseudococci.

Host plant phenolic compounds appear to be related to the frequency of encapsulation. Mealybugs fed on high phenolic plants showed a greater capacity to encapsulate eggs of A. pseudococci, making parasitoids less efficient. However, the real mechanism involved in this process has yet to be elucidated. Nevertheless the involvement of host plant phenolic compounds in the frequency of encapsulation may be a limiting factor in the establishment of natural enemies in some crops.

Environmental factors such as water stress may be an important factor affecting plant growth and a development. Moisture deficits cause morphological and physiological changes in plants. Morphological changes in plants can alter the micro-environment through leaf shedding, leaf rolling or changes in the canopy structures. These changes often affect herbivores and their natural enemies.

Physiological changes by water stress includes protein breakdown, increases in proline content, reduced translocation and changes in mineral nutrition in the plant parts.

These factors may have an impact on insects with a limited supply of nutrient or water. Reduced phloem pressure or lack of sufficient nutrient could both account for poor performances of mealybugs in the present investigation. The body size and the fecundity level of mealybugs were greatly reduced when they were reared on high stressed plants. The prolonged developmental period and low fecundity level of mealybugs on these plants inturn leads to a low rm value. Thus reduced rm value is an indication of poor habitat quality (eg. low nutrient, less water, and low relative humidity) and the low population levels of mealybugs will inturn affect the population growth of parasitoids.

Parasitoids developed on smaller mealybugs fed on high stressed plants remained small with low successful emergence rate and reduced life expectancy. These poor quality parasitoids may affect the efficiency of biological control. The effect of nitrogen fertilizer on insect populations, varies depending on the circumstances, feeding habits of the insect and available water level around the plant (Scriber, 1977; Connor, 1988;). The results from the present investigation suggested that nitrogen levels in the growing media were unable to induce substantial changes in some life history parameters such as developmental and mortality rate and adult body size of mealybugs. The normal growth and development of mealybug under low nitrogen level could be attributed to the similarity in nutritional levels of host plants maintained under various nitrogen levels. However, some researchers have managed to grow the experimental plants under "zero" N level in order to achieve better treatment differences.

However, in the present study, we were unable to use "zero" N ppm in the growing media because the plants would not survive for the duration of the mealybug life cycle and attempts to remove the mealybugs resulted in death through broken stylets or premature egg production.

The results did indicate that low nitrogen mainly affected the fecundity level of the insect. Low fecundity level contribute to the low rm value and was a principal factor for reduced population growth rate of mealybugs. The low rm value of mealybugs affected the population growth of parasitoids and the stability of biological control programme. On the contrary, enrichment of crops with high levels of nitrogen may lead to rapid increase of mealybugs reaching outbreak populations. However, if natural enemies show good density responsiveness, and great reproductive potential, then a stable pest-enemy equilibrium may still be achieved.

Searching efficiency of parasitoid is an important behavioural response for successful parasitism (Messenger & van den Bosch, 1971). However, plant surface properties may impair the normal searching behaviour of natural enemies and lead to less effective biocontrol. Trichomes of Streptocarpus and soy bean plants appear to be formidable barriers for normal searching behaviour of L. dactylopii. The walking speed and area searched were greatly reduced when they searched on Streptocarpus and soy bean plants. A similar behaviour was recorded on A. ellipticus leaves owing to the smooth, waxy and slippery nature of the surface. However, walking speed and area searched were highest on winged bean plants apparently due to smooth surface property of the leaves.

A large population of P. affinis exists in the Wye College glasshouse. This may be attributed to its wide host range perhaps because it has a better capacity to cope with plant secondary metabolites than P. citri. In addition the parasitoids which control P. citri are ineffective through encapsulation process, and biocontrol relies upon the predator Cryptolaemus montrouzieri. Thus the present finding highlights the importance of finding and introducing more efficient parasitoids to control P. affinis.

At this point in the thesis it may be useful to speculate on the value of this work to tropical field crops. Agricultural productivity is determined by various biotic and abiotic factors among which climate plays a vital role. Rainfall appears to be the major limiting factor and frequent crop failures have been reported in the tropics due to drought conditions. Since water stress can affect growth and the chemical constituents of plants, these changes invariably influence the other trophic levels either to enhance or retard the population growth of associating fauna. Moderate stress levels often increase the total plant so lead to sudden outbreak of herbivorous insects. However, severe stress conditions adversely affect the nutritional value affecting the herbivores and their natural enemies and can alter the self-sustaining pattern of the host-parasitoid system.

Nitrogen fertilizer is a major component of agricultural systems and often farmers in the developing countries face problems of obtaining adequate fertilizer for their cropping lands. Thus most soils are deficient in nitrogen. We have shown that the low level of nitrogen in the growing media will adversely affect the population growth of mealybugs and their parasitoids.

Adequate soil nitrogen levels lead to healthy pests, better establishment of natural enemies and strong plants capable of withstanding pest attack.

The concept of mix-cropping or multiple-cropping is popular in the developing countries because of the limitation of cultivable lands. The present study shows the effect of various host plant species on growth and development of mealybugs and their parasitoids. The high phenolic plants and those with long trichomes are unsuitable for mealybugs but in turn produce parasitoids with poor growth and fitness or searching parameters. Thus plant resistance factors should be incorporated into plant breeding programmes, they may also be detrimental to natural enemies and further work is needed to assess the overall effect.

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APPENDIX 1

The list of samples injected simultaneously with the plant phenolic extracts to obtain respective peak values.

STANDARD SAPMLESTEMPERATURE AT PEAK APPEARANCE

Catechol	159
Vanillin	190
trans.Cinnamic acid	185
Benzoic acids	142
Quinic acid	205
Protocatechuic acid	207
Gallic acid	225
Caffeic acid	243
Ferulic acid	245
Coumaric acid	227

APPENDIX 2

Weights of various chemicals dissolved to obtain 200 ppm, 100 ppm and 10 ppm Nitrogen in the NFT media. Concentrations were based on standard Cooper (200 ppm nitrogen) solution.

COMPOUNDS	WEIGHTS IN g		
	200 ppm	100 ppm	10 ppm
KH_2PO_4	52.600	52.600	26.300
KNO_3	116.600	116.600	-----
K_2SO_4	-----	-----	117.340
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	200.600	32.300	16.900
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	-----	105.000	71.800
$\text{Ca}_3(\text{PO}_4)_2$	-----	-----	30.000
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	102.600	102.600	102.600
EDTA	15.800	15.800	15.800
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	1.220	1.220	1.220
H_3BO_3	0.340	0.340	0.340
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.078	0.078	0.078
$(\text{NH}_4)_5\text{Mo}_7\text{H}_3\text{O}$	0.074	0.074	0.074
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.088	0.088	0.088

Solutions were prepared by dissolving appropriate weights in 1 litre of distilled water to obtain 200 time concentrated stocks

APPENDIX 3

Reagents for amino acid analysis

NINHYDRIN REAGENT

Anhydrous KH_2PO_4	9.5g
Anhydrous Na_2HPO_4	4.3g
Ninhydrin	0.5g
Fructose	0.3g

Dissolved in 100ml of H_2O

DILUENT

KI 2.0g

Dissolved in 600ml of warm distilled water, allowed to cool and added 400ml of absolute ethanol.

GLYCINE STANDARD

Glycine 100mg dissolved in 1 litre of water and stored at 4°C .

Formula used to calculate amino acid content.

$$\frac{\text{Wt. glycine dissolved } (\mu\text{g}) \times \text{Total volume of the extract ml.} \times 100.}{10 \times \text{aliquot volume ml} \times \text{sample dry weight.}}$$

APPENDIX 4

Functional part of programme written in "BASIC" to calculate r_m , R_o and GRR.

```

5 I=100
10 DIM X(I),L(I),M(I)
30 READ N
40 READ R
50 FOR I =1 TO N
60 READ X(I),L(I),M(I)
110 A=L(I)*M(I)
120 B=X(I)*R
130 C=5-B
140 D=EXP(C)
150 E=A*D
159 W=W+M(I)
160 M=M+A
170 T=T+E
171 REM * "PRINT"DISPLAY (Y OR N)
172 GETA$:IFA$=""THEN172
173 IFA$="Y"THEN225 :PRINT
225 PRINT"-----"
226 PRINT X(I),L(I),M(I):PRINT
227 PRINT A,B:PRINT
228 PRINT C,D,E:PRINT
229 REM * PRINT"-----"
230 NEXTI
250 PRINT"GRR ="W,"RO ="M,"E5-RMXLXMX ="T
260 REM * X-----L-----M *
270 REM *-----*
271 LIST280-281
280 DATA 20 :REM ** INPUT SAMPLE SIZE **
281 DATA .126:REM ** INPUT PROVISIONAL RM **

```