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**A study of root aphid
Aploneura lentisci Pass. biology
and root aphid-host interactions
with perennial ryegrass/endophyte
associations in New Zealand**

A thesis presented in partial fulfilment of the
requirements for the degree of

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ABSTRACT

The root aphid *Aploneura lentisci* Pass. is an underestimated, under-researched pasture pest likely to become more problematic in New Zealand if the environmental temperature and the frequency of water deficit stress increase, as predicted. The research presented here aimed at gaining first insights into its biology and interaction with plants and endophytes to promote future pest management research. For this purpose, root aphids were observed in model systems (in climate chambers, glasshouse or insectary; in empty microcentrifuge tubes or on diploid perennial ryegrass *Lolium perenne* L. plants grown on nutrient-enriched agar, with or without endophyte *Epichloë festucae* var. *lolii* [Latch, M.J. Chr. & Samuels] C.W. Bacon & Schard of the AR1, AR37 or common-toxic CT strains).

Apterous neonate offspring, the presumed main dispersal stage of *A. lentisci*, survived up to four weeks without food (median survival: 8 days). On endophyte-free, mature ryegrass kept at 17 to 21 °C, neonates developed to adults within three to four weeks and lived about two months, feeding mainly on young roots of first and second branching order. Taking into account lower outdoor temperatures, root aphids are thus likely to complete six to nine generations per year in the field. Adults produced 39 to 70 offspring over their lifetime. Presuming a similar nymphal mortality in the field as in the experiments, outdoor root aphid populations could theoretically multiply 23- to 45-fold at each generation.

Root aphids raised on endophyte-infected, mature plants were shorter-lived than peers raised on endophyte-free plants. Most aphids on AR37-infected plants did not even reach reproductive maturity. The response to CT-infection was dependent on the plant genotype. Why AR1-infected plants frequently support larger root aphid populations than endophyte-free plants in the field could not be explained by the data collected, however.

Root aphid feeding affected the root biomass but not the shoot biomass of perennial ryegrass in the experimental environment. This finding differed from previous reports. Furthermore, colour analyses suggested root aphid feeding could modify some leaf properties. More research will be required to confirm these findings and assess whether irrigation or fertilisation could mitigate root aphid yield losses in the field.

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TABLE OF CONTENTS

ABSTRACT	I
ACKNOWLEDGMENT	II
TABLE OF CONTENTS	III
LIST OF ILLUSTRATIONS.....	IX
LIST OF TABLES	XVI
LIST OF FORMULAE	XXIII
LIST OF ABBREVIATIONS	XXVII
CHAPTER 1: LITERATURE REVIEW.....	1
1.1. Introduction.....	1
1.2. Biology and ecology of <i>A. lentisci</i>	3
1.2.1. <i>A. lentisci</i> in New Zealand: history and significance.....	3
1.2.2. Identification.....	4
1.2.3. Life cycle and ontogeny.....	6
1.2.4. Anatomy and physiology.....	8
1.2.4.1. Feeding and nutrition	8
1.2.4.2. Growth	11
1.2.4.3. Reproduction	12
1.2.4.4. Sensory organs	16
1.2.4.5. Wings	17
1.2.4.6. Wax	19
1.2.5. Influences of the abiotic environment on root aphids	20
1.2.6. Aphid-host relations.....	21
1.2.6.1. Host plants	21
1.2.6.2. Host plant finding	22
1.2.6.3. Feeding site choice	24
1.2.6.4. Food quality	24
1.2.6.5. Effect on host plant	25

1.2.6.6. Plant resistance to aphids	27
1.2.7. Colonies and population dynamics	28
1.3. Biology and ecology of <i>Lolium perenne</i>.....	29
1.3.1. Anatomy and physiology	29
1.3.2. Environmental requirements and biotic influences	35
1.4. Biology and ecology of endophyte <i>E. festucae</i> var. <i>lolii</i>.....	36
1.4.1. Characteristics and life cycle.....	36
1.4.2. Impact on plants	39
1.5. Interactions between <i>A. lentisci</i>, <i>L. perenne</i> and <i>E. festucae</i> var. <i>lolii</i>	42
1.6. Interactions with other biological grassland elements	45
1.7. Knowledge gaps and thesis overview	46
CHAPTER 2: GENERAL MATERIALS AND METHODS.....	49
2.1. Experimental overview.....	49
2.2. Facilities	52
2.2.1. Outdoor area	52
2.2.2. Insectary	54
2.2.3. Glasshouses	54
2.2.4. Climate Chambers.....	55
2.3. Origin and handling of plants, aphids and endophytes	56
2.3.1. Plants.....	56
2.3.1.1. Plant material.....	56
2.3.1.2. General plant handling.....	58
2.3.1.3. Plant mounting.....	59
2.3.2. Aphids.....	63
2.3.3. Endophytes.....	67
2.4. Data collection and processing.....	70
2.4.1. Environmental conditions recording	70
2.4.2. Plant parameters.....	73
2.4.3. Aphid and aphid feeding-related parameters.....	79
2.5. Statistics	83

CHAPTER 3: BIOLOGY OF APTEROUS MORPHS OF <i>APLONEURA LENTISCI</i>	85
3.1. Introduction	85
3.2. Materials and methods	86
3.2.1. Experimental designs.....	86
3.2.1.1. Biology I experiment.....	86
3.2.1.2. Biology II experiment.....	87
3.2.1.3. Mature plant experiment.....	91
3.2.1.4. Follow-up experiments: offspring survival <i>ex planta</i>	91
3.2.2. Data processing and statistical analysis.....	92
3.2.2.1. Biology I experiment.....	93
3.2.2.2. Biology II experiment.....	98
3.2.2.3. Mature plant experiment.....	101
3.2.2.4. Follow-up experiments on offspring survival.....	102
3.3. Results	104
3.3.1. Biology I experiment.....	104
3.3.1.1. Instars.....	104
3.3.1.2. Aphid body size.....	106
3.3.1.3. Aphid establishment, development and longevity.....	108
3.3.1.4. Aphid fecundity.....	110
3.3.2. Biology II experiment.....	111
3.3.2.1. Aphid size.....	111
3.3.2.2. Aphid establishment, colonisation success, development and longevity.....	114
3.3.2.3. Aphid fecundity.....	117
3.3.3. Mature plant experiment.....	119
3.3.4. Offspring survival in follow-up experiments.....	121
3.4. Discussion	125
3.5. Conclusion	131
CHAPTER 4: EFFECTS OF ROOT APHIDS ON PERENNIAL RYEGRASS PLANTS	133
4.1. Introduction	133

4.2. Materials and methods.....	135
4.2.1. Seedling experiment.....	135
4.2.2. Mature plant experiment.....	136
4.2.3. Data analysis and statistics.....	139
4.3. Results.....	143
4.3.1. Seedling experiment.....	143
4.3.2. Mature plant experiment.....	146
4.4. Discussion	152
4.5. Conclusion	154
CHAPTER 5: ESTABLISHMENT, COLONISATION, AND POPULATION ECOLOGY OF ROOT APHIDS.....	155
5.1. Introduction.....	155
5.2. Materials and methods.....	157
5.2.1. Biology II experiment.....	157
5.2.2. Colony wax observations.....	160
5.2.3. Population experiment.....	164
5.3. Results.....	167
5.3.1. Biology II experiment, behaviour.....	167
5.3.2. Biology II experiment, feeding site characterisation.....	168
5.3.3. Wax colony observations.....	174
5.3.4. Population experiment.....	176
5.4. Discussion	184
5.5. Conclusion	189
CHAPTER 6: GENERAL DISCUSSION AND CONCLUSIONS	191
6.1. Root aphid biology, behaviour and life cycle	191
6.2. Effects of root aphids on perennial ryegrass plants	195
6.3. Root aphid-<i>Epichloë</i> endophyte interactions	196
6.4. Gaps in knowledge and future research	198
6.5. Prospective pest management options for <i>A. lentisci</i>	203
6.6. Conclusions	205
REFERENCES.....	207

APPENDICES

APPENDIX 1: Declaration of content.....	A- 1
APPENDIX 2: Glossary.....	A- 3
APPENDIX 3: Meteorological data for Palmerston North and the research site	A- 9
APPENDIX 4: Light measurements at the experimental locations	A- 15
APPENDIX 5: Nursery media and chemicals.....	A- 21
APPENDIX 6: Agar preparation	A- 23
APPENDIX 7: Viviposition trial.....	A- 25
APPENDIX 8: Image analysis steps.....	A- 29
APPENDIX 9: Root aphid live size measurements.....	A- 31
A9.1. Instar measurements (Live size of various instars).....	A- 31
A9.2. Calibration trial I (Preserved vs. live size calibration)	A- 38
A9.3. Calibration trial II (Weight vs. live size calibration)	A- 43
APPENDIX 10: Chapter 3 addendum	A- 47
A10.1. Biology I experiment, viviposition plants	A- 47
A10.2. Biology I experiment, size	A- 48
A10.2.1. Neonate size.....	A- 48
A10.2.2. Growth.....	A- 49
A10.2.3. Size	A- 52
A10.3. Biology I experiment, reproduction	A- 54
A10.4. Biology II experiment, size statistics	A- 55
A10.4.1. Neonate size.....	A- 55
A10.4.2. Growth curves.....	A- 56
A10.4.3. Canonical discriminant analysis (CDA) for size groups .	A- 58
A10.4.4. Adult size	A- 60
A10.5. Biology II experiment, establishment and development	A- 61
A10.6. Biology II experiment, reproduction	A- 65
A10.7. Mature plant experiment, colonisation success	A- 66
APPENDIX 11: Chapter 4 addendum	A- 69
A11.1. Seedling experiment statistics	A- 69
A11.1.1. Aphid numbers.....	A- 69
A11.1.2. Plant morphology criteria	A- 69

A11.1.3. Dry matter content.....	A- 71
A11.1.4. Biomasses.....	A- 73
A11.2. Mature plant experiment statistics	A- 77
A11.2.1. Aphid loadings during the Mature plant experiment	A- 77
A11.2.2. Tillers, green leaves, dead leaves and root count at final harvest	A- 79
A11.2.3. Tiller diameter at start and initial tiller weight.....	A- 80
A11.2.4. Dry matter content of shoot.....	A- 82
A11.2.5. Regrowth 24h	A- 83
A11.2.6. Green shoot area.....	A- 84
A11.2.7. Green shoot colour.....	A- 85
A11.2.8. Root/shoot ratio.....	A- 89
A11.2.9. Biomass analyses.....	A- 91
APPENDIX 12: Chapter 5 addendum	A- 92
A12.1. Biology II experiment, behaviour	A- 92
A12.2. Biology II experiment, site characteristics	A- 94
A12.2.1. Branching order.....	A- 94
A12.2.2. Position on root.....	A-99
A12.2.3. Colour of root.....	A-104
A12.2.4. Root diameter at feeding sites.....	A-109
A12.3. Wax	A-111
A12.4. Population experiment	A-115

LIST OF ILLUSTRATIONS

Figure 1.1. Plots of tetraploid ryegrass with AR1, AR37 or Endo5 (AR5) endophyte strains at Ballarat (Australia).....	3
Figure 1.2. Aphid terminology, illustrated on an example of an apterous vivipara of <i>Macrosiphum rosae</i> seen from above (left side, yellow) vs. below (right side, blue).....	4
Figure 1.3. Stylised life cycle of <i>A. lentisci</i> , for a given aphid genotype.....	7
Figure 1.4. Diagram of a root aphid feeding on root phloem.....	9
Figure 1.5. Aphid reproductive system.....	15
Figure 1.6. Flight apparatus of aphids.....	18
Figure 1.7. Vegetative and reproductive characteristics of perennial ryegrass.....	32
Figure 1.8. Phytomer development diagram for perennial ryegrass.....	33
Figure 1.9. <i>Epichloë</i> endophyte in perennial ryegrass.....	38
Figure 1.10. Diagram of the interactions between root aphids, perennial ryegrass, endophyte, and environment.....	42
Figure 2.1. Research locations.....	53
Figure 2.2. Petri dish (PD) embedding.....	61
Figure 2.3. Tube embedding.....	62
Figure 2.4. Trimming perennial ryegrass tillers.....	62
Figure 2.5. Root aphid handling.....	66
Figure 2.6. Root aphid handling issues and solutions.....	66
Figure 2.7. Detecting endophyte presence by direct microscopy.....	68
Figure 2.8. Revealing endophyte presence by immunodetection, blotting results.....	70
Figure 2.9. Digitech data logger.....	71
Figure 2.10. Watchdog weather station.....	72
Figure 2.11. LI-COR measuring device, mounted as for light measurements.....	72

Figure 2.12. Measuring of tiller diameter and regrowth 24 h after the initial trim (Regrowth 24 h), on a photograph taken 24 h after the shoot had been trimmed.....	74
Figure 2.13. Refining raw photographs into a suitable form for computerized image analysis.....	78
Figure 2.14. Size measurements on root aphids.....	81
Figure 3.1. Diagram of experimental set up for Population experiment, Biology II experiment and Biology II follow-up experiment.....	90
Figure 3.2. Development of <i>A. lentisci</i> on ryegrass roots.....	105
Figure 3.3. Aphid EP size development over the Biology I experiment.....	107
Figure 3.4. Aphid age at ecdyses 1 to 4 (E1, E2, E3 and E4), reproductive maturity (Reproduction) and death during the Biology I experiment.....	109
Figure 3.5. Size development of individual root aphids living on endophyte-free perennial ryegrass tillers of two different genotypes N (a) and S (b).....	112
Figure 3.6. Growth of aphid size of large and small groups on two perennial ryegrass plant genotypes (N, S) infected with endophyte <i>E. festucae</i> var. <i>lolii</i> strain AR1 or CT, or without endophyte (NIL).....	112
Figure 3.7. Probability of reproduction and mortality of root aphids on perennial ryegrass of two genotypes (N and S) with AR1 (a), AR37 (b), or common-toxic CT (c) endophyte symbiont, or without any endophyte [NIL(d)].....	116
Figure 3.8. Colonisation success (mean + SD) of <i>A. lentisci</i> neonates on perennial ryegrass plants with different plant genotype (N or S) and endophyte status (AR1, AR37, CT or NIL) in the Mature plant experiment, as assessed 9 to 12 weeks after aphid placement.....	120
Figure 3.9. Offspring survival in the Biology II follow-up experiment (a) and the Mature plant follow-up experiment (b).....	122
Figure 4.1. Seedling experiment.....	136
Figure 4.2. Schematic representation, diverse data collection time points and water stress in the Mature plant experiment.....	138
Figure 4.3. Biomasses of green shoot (a), senescing and dead shoot (b), and root (c) at the end of the Mature plant experiment, as determined by the MANCOVA model by average covariate values (86 mg initial tiller weight after trim, harvest 116 to 117 days after the initial trim), in the presence or absence of root aphids, for roots ($p = 0.052$).....	151

Figure 5.1.	Position terminology for a perennial ryegrass root of 3 rd branching order (BO).....	158
Figure 5.2.	<i>A. lentisci</i> colony, unprocessed photograph (left) and photograph with wax measurements (root length, wax area and root diameters D1 and D2; right).....	163
Figure 5.3.	Effects of aphid age and endophyte status on aphid restlessness (back-transformed means with 95% confidence intervals).....	168
Figure 5.4.	An aphid at the same site on its (a) 2nd, (b) 4th and (c) 13th day of life..	169
Figure 5.5.	Root branching pattern of <i>L. perenne</i> plants of genotype N at final harvest (bars; means \pm SD) for endophyte-free plants (a) or symbioses with AR1 (b), AR37 (c) or common-toxic CT endophyte (d), and usage frequency of each root type by <i>A. lentisci</i> (dots; percent of all records on the respective BO).....	170
Figure 5.6.	Root branching pattern of <i>L. perenne</i> plants of genotype S at final harvest (bars; means \pm SD) for endophyte-free plants (a) or symbioses with AR1 (b), AR37 (c) or common-toxic CT endophyte (d), and usage frequency of each root type by <i>A. lentisci</i> (dots; percent of all records on the respective BO).....	171
Figure 5.7.	Colour of roots used by immature nymphs and adult <i>A. lentisci</i> aphids on perennial ryegrass of increasing plant age [plant age period] (a, b), and plants of various genotypes (N or S) and endophyte statuses (AR1, AR37, CT or NIL) (c, d).....	173
Figure 5.8.	Number of exuviae in a colony, represented as boxplot.....	175
Figure 5.9.	Separation achieved by the canonical discriminant functions (CDF) of Table 5.3.....	178
Figure 5.10.	Relationship between duration of aphid occupation, aphid population and plant parameters at harvest in the Population experiment, on plants supplied with one starter aphid.....	181
Figure 5.11.	Relationship between duration of aphid occupation, aphid population and plant parameters at harvest in the Population experiment, on plants supplied with five starter aphids.....	182
Figure 5.12.	Population and plant parameters in relation to the total number of feeding aphids, as recorded at the harvest of the Population experiment.....	183
Figure 5.13.	Graphical representation of root use in root aphids on perennial ryegrass grown in nutrient-enriched agar.....	186

Figure A3.1.	Air temperature in the outdoor nursery plot of the Grasslands Research Centre, AgResearch Ltd, Palmerston North.....	A-13
Figure A3.2.	Daily rainfall at the research site from July 2015 to June 2016, as estimated, by the average of records from two independent Palmerston North weather stations.....	A-13
Figure A3.3.	Average solar radiation at the research site during daylight in Palmerston North, as measured from July 2015 to September 2016 by a Watchdog weather station at the site of the Grasslands Research Centre, AgResearch Ltd.....	A-14
Figure A4.1.	Diagram of data collection and processing in one location.....	A-18
Figure A5.1.	Properties of the C.A.N. bark produced by Daltons Ltd..	A-21
Figure A7.1.	Reproductive performance of mothers kept in vials with low vs. high humidity.....	A-27
Figure A7.2.	Survival in vials.....	A-27
Figure A7.3.	Survival of aphids born during the first two days of maternal captivity in a glass vial, by dry and moist conditions.....	A-28
Figure A8.1.	Photographic chamber used for image acquisition, from (a) outside and (b) inside with mounted camera and colour referential (red box with grey rectangle).....	A-29
Figure A9.1.1.	First instar root aphids marked with magenta powder dye for live size measurements by instar (a) just after marking and (b) several days later.....	A-33
Figure A9.1.2.	Dorsal (a) and ventral (b) photographs of an adult aphid (5 th instar), as used for size measurements.....	A-33
Figure A9.1.3.	Size measurements by instar, measurements in mean \pm standard deviation (a) and derived parameter ellipsoid body projection, calculated as $EP = \text{Length}/2 \cdot \text{abdominal width}/2 \cdot \pi$ (b).....	A-35
Figure A9.1.4.	Instar discrimination by canonical discriminant analysis.....	A-37
Figure A9.2.1.	Relationship between live and dead size measurements, for (a) body length and (b) abdominal width, in early	

	immature (< 1 mm length) and older instars (> 1 mm length) of root aphid <i>A. lentisci</i> , before and after being killed by and preserved in 70%, 80%, or 90% ethanol solution for two days.....	A-41
Figure A9.2.2.	Relationship between live and dead size measurements, for (a) thoracic width and (b) ellipsoid body projection EP, in early immature (< 1 mm length) and older instars (> 1 mm length) of root aphid <i>A. lentisci</i> , before and after being killed by and preserved in 70%, 80%, or 90% ethanol solution for two days.....	A-42
Figure A9.3.1.	Relationship between adult weight and (a) length, body width at the thorax, (c) body weight at the abdomen, or (d) ellipsoid body projection (EP).....	A-44
Figure A10.1.1	Root germination cardboard (a), used for embedding plants (b).....	A-47
Figure A10.2.2.1.	Aphid length development during the Biology I experiment.....	A-49
Figure A10.2.2.2.	Size development of <i>A. lentisci</i> during Biology I experiment, abdominal width.....	A-50
Figure A10.2.2.3	Maternal effects in aphid size development during the Biology I experiment, with information on reproductive maturity (pink circles).....	A-51
Figure A10.2.3.1.	Aphid- and plant-related characteristics associated with large vs. small size development during the Biology I experiment.....	A-52
Figure A10.2.3.2.	Plant-related characteristics associated with large vs. small size development during the Biology I experiment.....	A-53
Figure A10.3.1.	Maternal influences on cumulative offspring production in the Biology I experiment, with heat stress exposure information.....	A-54
Figure A10.4.1.	Size and size trends for neonate starter aphids placed onto plants of genotype N or S with one of four possible endophyte statuses (with endophytes AR1, AR37 or CT, or without endophyte, i.e. NIL), with information of the plant on which the mother (Ancestor; circles) lived.....	A-55

Figure A10.4.2.1.	Size development of root aphids living on symbionts of endophyte AR1-perennial ryegrass of two different genotypes N (a) and S (b).....	A-57
Figure A10.4.2.2.	Size development of root aphids living on symbionts of endophyte CT-perennial ryegrass of two different genotypes N (a) and S (b).....	A-57
Figure A10.4.2.3.	Size development of root aphids living on symbionts of endophyte AR37-perennial ryegrass of two different genotypes N (a) and S (b).....	A-58
Figure A10.4.3.1.	Canonical discriminant analysis plot (CDF: canonical discriminant function) for adult ellipsoid body projection (EP) size group.....	A-59
Figure A10.6.1.	Cumulative offspring production of root aphids raised on plants with various genotypes (N or S) and endophyte statuses [in symbiosis with strain AR37, AR1, or CT or without any symbiont (NIL)].....	A-65
Figure A10.7.1.	Canonical discriminant analysis functions (CDF) for establishment success during the Mature plant experiment.....	A-67
Figure A12.1.1.	Behaviour of root aphids during the Biology II experiment, for specimen living on plants of N-AR37 (a), N-AR1 (b), N-CT (c) and N-NIL (d) plant genotype-endophyte status.....	A-92
Figure A12.1.2.	Behaviour of root aphids during the Biology II experiment, for specimens living on plants of S-AR37 (a), S-AR1 (b), S-CT (c), S-NIL (d) plant genotype-endophyte status.....	A-93
Figure A12.2.1.1.	<i>L. perenne</i> root branching (BO) pattern at final dissection (barplots) and <i>A. lentisci</i> root use (blue points) on plants of genotype N in absence (d) or presence of the endophyte strains AR1 (a), AR37 (b) or common-toxic (c).....	A-94
Figure A12.2.1.2.	<i>L. perenne</i> root branching (BO) pattern at final dissection (barplots) and <i>A. lentisci</i> root use (yellow points) on plants of genotype S in absence (d) or presence of the endophyte strains AR1 (a), AR37 (b) or common-toxic (c).....	A-95
Figure A12.2.2.1.	Position of <i>A. lentisci</i> on the roots of perennial ryegrass of plant genotype N or S with AR1, AR37 or common-	

	toxic (CT) endophyte, or without endophyte (NIL), at various plant ages.....	A-99
Figure A12.4.1.	Colony development during the Population experiment, by aphid loads on roots [number of live older immature or adult aphids per g dry root biomass 38 to 111 days after aphid placement].....	A-116
Figure A12.4.2.	Colony development during the Population experiment, row data by aphid loads per green shoot weight [number of live feeding aphids/g green shoot dry matter 38 to 111 days after aphid placement].....	A-117
Figure A12.4.3.	Colony development during the Population experiment, raw data by aphid loads on green shoot area [Feeding aphids by mm ² green shoot at the final harvest, 38 to 111 days after aphid placement].....	A-118

LIST OF TABLES

Table 1.1.	Identification and anatomic description of adult apterous and alate <i>A. lentisci</i> morphs.....	5
Table 1.2.	Inheritance of the ability to reproduce sexually, as reported for <i>Myzus persicae</i> Sulzer (Dixon, 1985) and <i>Rhopalosiphum padi</i> L. (Simon et al., 1994).....	13
Table 1.3.	Root development and characteristics by root branching order (BO) [Robin 2011].....	34
Table 1.4.	Characteristics of three important <i>E. festucae</i> var. <i>lolii</i> endophyte strains (AR1, AR37 and common-toxic CT) and summary of their impact on perennial ryegrass plants and herbivores.....	40
Table 1.5.	Endophytic alkaloid toxins and their properties, with focus on perennial ryegrass as host plant.....	41
Table 1.6.	Thesis structure.....	48
Table 2.1.	Overview of the pre-trials reported in the appendices.....	50
Table 2.2.	Overview of the experiments reported in Chapters 3, 4, 5.....	51
Table 2.3.	Clone-plant nomenclature.....	57
Table 2.4.	Perennial ryegrass accessions used for the Biology I and Seedling experiments.....	58
Table 2.5.	Aphid-related parameters collected, by experiment.....	80
Table 3.1.	Covariates recorded and used in analyses during the Biology I experiment.....	96
Table 3.2.	Plant and aphid parameters recorded and used as covariates during the Biology II experiment.....	101
Table 3.3.	Parameters initially (all) and finally (grey) included in the Biology II and mature plant follow-up experiments to analyse offspring survival.....	103
Table 3.4.	Exuviae collection in Petri Dishes (PD).....	104

Table 3.5.	Mean body length [mm], abdominal width [mm] and ellipsoid body projection EP [mm ²] of different instars (\pm standard deviation).....	106
Table 3.6.	Estimated parameters (\pm standard error) of the log-logistic EP size growth models for aphids of the ‘Small’ and ‘Large’ size group in the Biology I experiment.....	107
Table 3.7.	Influence of aphid and plant characteristics on age at moults (ecdysis 1 to 4) and reproduction (Repro) in the Biology I experiment.....	110
Table 3.8.	Coefficients and log-likelihood of factors affecting the log-transformed neonate offspring size.....	113
Table 3.9.	Maximum reproductive performance (Max), average lifetime fecundity of adult root aphids (biological fecundity) and average lifetime fecundity of a root aphid populations (ecological fecundity), as estimated by various methods for the Biology I and Biology II experiments.....	118
Table 3.10.	Analysis of covariance table for the log-transformed reproductive rate.....	119
Table 3.11.	<i>Ex planta</i> reproductive rate of starter aphids from the Mature plant experiment raised on two plant genotypes (N or S) without endophyte [NIL] or with AR1, AR37 or CT endophytic partner.....	121
Table 3.12.	Factors affecting offspring survival in the Biology II follow-up experiment.....	123
Table 3.13.	Factors affecting offspring survival in the Mature plant follow-up experiment.....	124
Table 4.1.	Variables used for analyses in the Mature plant experiment.....	142
Table 4.2.	Seedling mortality in <i>L. perenne</i> by root aphid treatment (‘No aphids’ vs. ‘Aphids’) and further characteristics of the accessions.....	143
Table 4.3.	Seedling characteristics at final harvest (means \pm SD) and significance of selected variables (<i>p</i> -values).....	145
Table 4.4.	Morphological characteristics of two perennial ryegrass genotypes (N, S) with endophyte <i>E. festucae</i> var. <i>lolii</i> symbiont	

	(AR1, AR37 or common-toxic CT strain) or without endophyte (NIL), and influence of various statistical parameters	148
Table 4.5.	Green shoot and biomass-related characteristics of two distinct perennial ryegrass genotypes (N, S) living either in symbiosis with one of three strains of the endophyte <i>E. festucae</i> var. <i>lolii</i> (AR1, AR37 or common-toxic CT) or without any symbiotic partner (NIL).....	149
Table 4.6.	Multivariate analysis of covariance and parameter estimates for plant biomass [g dry matter] in mature perennial ryegrass tillers of two distinct genotypes (N, S) in symbiosis with one of three endophyte <i>E. festucae</i> var. <i>lolii</i> strains (AR1, AR37 or common-toxic CT) or without any symbiotic partner (NIL), 112 to 121 days after the initial trim.....	150
Table 5.1.	Explanatory variables used for colony wax measurement analyses.....	163
Table 5.2.	Spearman's rank correlation coefficient (Spearman's ρ) for the relationship between the average root diameter and various aphid traits in the Biology II experiment.....	173
Table 5.3.	Coefficients of linear mixed-effects model describing the wax area.....	176
Table 5.4.	Effect of plant genotype (N, S)-endophyte status (AR1, AR37, CT and SNIL) combinations and number of root aphids placed (1 vs. 5 first instar nymphs) on the colonisation success	178
Table 5.5.	Standardised canonical discriminant analysis coefficients for colonisation success analyses in plants of various plant genotype (N, S) and endophyte status (AR37 or a common-toxic CT endophyte).....	179
Table 6.1	Summary of life-history and biological traits observed in <i>A. lentisci</i> root aphids reared on endophyte-free perennial ryegrass (<i>L. perenne</i>).....	194
Table A3.1.	Temperatures in Palmerston North (average of monthly means for years 1987 to 2016, as recorded by the PN reference weather station).....	A-9
Table A3.2.	Astronomical day length calculated by the U.S. Naval Observatory (2013) (daylight hours), and sunshine, rainfall,	

	rain days, pan evaporation, and wind run in Palmerston North (average of monthly means for years 1987 to 2016, as recorded by the PN reference weather station).....	A-10
Table A3.3.	Wind in the outdoor nursery area of the Grasslands Research Centre, AgResearch Ltd, Palmerston North.....	A-11
Table A3.4.	Wind gusts (short episodes of wind increase that lasted only for a few seconds) in the outdoor nursery area of the Grasslands Research Centre, AgResearch Ltd, Palmerston North.....	A-12
Table A4.1.	Summary of light measurements, variance analysis and estimated correction factors by experimental location.....	A-19
Table A5.1.	Chemicals used for pest control in the glasshouses.....	A-22
Table A6.1.	Nutrient mix.....	A-24
Table A6.2.	Microelement solution recipe, as prepared in July 2012 with RO water.....	A-24
Table A9.1.1.	Size measurements of living apterous root aphids of known instar (clear section) and derived parameters (shaded section)..	A-36
Table A9.1.2.	Canonical discriminant analysis results: coefficients of linear discriminants and proportion of variance explained by each linear dimension (trace).....	A-36
Table A9.2.1	Pearson's correlation coefficients and <i>p</i> -values for live vs. dead measurement.....	A-40
Table A10.1.1.	Viviposition plants statistics, number of offspring collected (N) and size of offspring on their first day of life.....	A-47
Table A10.2.2.1.	Estimated parameters (\pm standard error) for the log-logistic length growth models for aphids of different size groups in the Biology I experiment.....	A-49
Table A10.2.2.2.	Estimated parameters (\pm standard error) for the log-logistic abdominal width growth models for aphids of different size groups in the Biology I experiment.....	A-50
Table A10.4.2.1.	Gompertz growth models parameters for aphids of the 'Large' and 'Small' groups, and comparison between 'Small' and 'Large' for model parameters by plant genotype-endophyte status group (PG-E).....	A-56
Table A10.4.4.1.	Mean and standard deviation (SD) of adult size measurements for <i>Aploneura lentisci</i> individuals raised on plants of N or S	

	genotype without (NIL) or with endophyte <i>Epichloë festucae</i> var. <i>lolii</i> of strain AR1 or common/toxic (CT).....	A-60
Table A10.5.1.	Number and proportion of aphids that established and achieved maturity during the Biology II experiment on plants of two distinct genotypes (N, S) without endophyte (NIL), or with AR1, AR37 or common-toxic (CT) endophyte.....	A-61
Table A11.1.1.	Root aphid numbers and loads per g dry matter on the seedlings successfully colonised in the Seedling experiment (mean \pm standard deviation).....	A-69
Table A11.2.1.	Specifics for aphid loading calculations.....	A-77
Table A11.2.2.	Total number of dead and live aphids at final harvest (98 to 108 days after initial plant trim, i.e. 63 to 79 days after aphid placement; means \pm standard deviation) and estimated plant loadings on two distinct perennial ryegrass plant genotypes (N, S) living in symbiosis with one of three endophyte <i>E. festucae</i> var. <i>lolii</i> strains (AR1, AR37 or common-toxic CT) or without endophyte (NIL).....	A-78
Table A11.2.7.1.	Average colour measurements (R: red, G: green, B: blue) during the Mature plant experiment and correlation factors (Spearman's ρ) between the various R, G and B measurements	A-85
Table A11.2.9.1.	Plant biomass at harvest (raw means, with vs. without aphids pooled).....	A-91
Table A12.2.1.1.	Number of records collected for branching order use, by plant genotype (N, S)-endophyte (endophyte-free NIL, with AR1, AR37 or common-toxic CT endophyte) and plant age group (plant age period).....	A-96
Table A12.2.1.2.	Plant age effect on branching order selection in <i>A. lentisci</i> of all ages on various plant genotype (N, S)-endophyte (endophyte-free NIL, with AR1, AR37 or common-toxic CT endophyte) symbionts.....	A-96
Table A12.2.1.3.	Effect of plant genotype (N, S)-endophyte status (with AR1, AR37 or common-toxic CT endophyte, or without endophyte NIL) combination on root branching order use by <i>A. lentisci</i> of all ages.....	A-97
Table A12.2.1.4.	Effect of aphid age (immature vs. adult) on root branching order use, by observations.....	A-98
Table A12.2.2.1.	Plant age effect on position used by root aphids of all ages when feeding on various plant genotype (N, S)-endophyte	

	(endophyte-free NIL, with AR1, AR37 or common-toxic CT endophyte) symbioses.....	A-100
Table A12.2.2.2.	Effect of plant genotype (N, S)-endophyte status (with AR1, AR37 or common-toxic CT endophyte, or without endophyte NIL) combination on position used by mature <i>A. lentisci</i>	A-101
Table A12.2.2.3.	Effect of aphid age (immature vs. adult <i>A. lentisci</i>) on root position when feeding on various plant genotype (N, S)-endophyte (endophyte-free NIL, with AR1, AR37 or common-toxic CT endophyte) symbioses.....	A-103
Table A12.2.3.1.	Plant age effect on root colour (3 colour categories: dark brown, pale brown and white roots).....	A-104
Table A12.2.3.2.	Effect of plant genotype (N, S)-endophyte status (with AR1, AR37 or common-toxic CT endophyte, or without endophyte NIL) combination on the colour of roots used by mature <i>A. lentisci</i> (3 colour categories: dark brown, pale brown and white roots).....	A-106
Table A12.2.3.3.	Effect of plant genotype (N, S)-endophyte status (with AR1, AR37 or common-toxic CT endophyte, or without endophyte NIL) combination on the colour of the roots used by immature <i>A. lentisci</i> (3 colour categories: dark brown, pale brown and white roots).....	A-107
Table A12.2.3.4.	Effect of aphid age (immature vs. adult <i>A. lentisci</i>) on root colour (frequency of dark brown, pale brown and white roots)..	A-108
Table A12.2.4.1.	Root diameter used by immature and mature root aphids on plants of various genotype (N, S) and endophyte status (in symbiosis with an AR1, AR37, common-toxic CT endophyte strain or without endophyte [NIL]) (raw means \pm standard deviation [mm]).....	A-109
Table A12.2.4.2.	Root diameter of feeding sites used by aphids, by plant genotype (N, S)-endophyte status (in symbiosis with an AR1, AR37, common-toxic CT endophyte strain or without endophyte [NIL]) combination (raw means \pm standard deviation [mm]).....	A-110
Table A12.3.1.	Properties of the colonies collected during the colony wax observations.....	A-111
Table A12.3.2.	Spearman's rank correlation coefficient (Spearman's ρ) for the relationship between number of exuviae, aphids of both age categories and root or colony properties.....	A-112

Table A12.3.3. Spearman's rank correlation coefficient (Spearman's ρ) for the relationship between proportion of feeding aphids of both age categories and root or colony properties..... A-112

Table A12.4.1. Aphid population structures and loads at the end of the Population experiment 60 to 140 days after initial plant trim, i.e. 38 to 111 days after aphid placement, on two distinct perennial ryegrass plant genotypes (N, S) living in symbiosis with one of three strains of the endophyte *E. festucae* var. *lolii* (AR1, AR37 or common-toxic CT) or without endophyte (NIL)..... A-115

LIST OF FORMULAE

Equation 2.1 Initial dry tiller weight

$$\text{Tiller DM}_{\text{Start } i} [\text{g}] = \text{Tiller FW}_{\text{Start } i} [\text{g}] \cdot \text{DM}_{\text{refStart}}$$

Equation 2.2 Initial root/shoot ratio

$$\text{Root/Shoot}_{\text{Start } i} [\%] = \frac{\text{Root DM}_{\text{Start } j} [\text{g}]}{\text{Shoot DM}_{\text{Start } j} [\text{g}]} \cdot 100$$

Equation 2.3 Initial dry root (or shoot) biomass

$$\text{Root DM}_{\text{Start } i} [\text{g}] = \frac{\text{Root DM}_{\text{Start } j} [\text{g}] \cdot \text{Tiller FW}_{\text{Start } i} [\text{g}]}{\text{Tiller FW}_{\text{Start } j} [\text{g}]}$$

Equation 2.4 Green shoot area 24 h after initial trim

$$\text{GSA 24h} [\text{cm}^2] = \sum_{i=1}^n \text{length}_i [\text{cm}] \cdot \frac{(\text{width}_i \text{ at base} [\text{cm}] + \text{width}_i \text{ at top} [\text{cm}])}{2}$$

Equation 2.5 Green ratio

$$\text{G ratio} = \frac{\text{G}}{(\text{R} + \text{G} + \text{B})}$$

Equation 2.6 Normalised red-blue difference

$$\text{nRBd} = \frac{(\text{R} - \text{B})}{(\text{R} + \text{B})}$$

Equation 2.7 Average branching order

$$\text{ABO} = \frac{(1 \cdot \text{nR}_{0^\circ} + 2 \cdot \text{nR}_{1^\circ} + 3 \cdot \text{nR}_{2^\circ} + 4 \cdot \text{nR}_{3^\circ} + 5 \cdot \text{nR}_{4^\circ})}{\text{Total number of roots}}$$

Equation 2.8 Dry tiller weight at harvest

$$\text{Tiller DM}_{\text{Harv}} [\text{g}] = \text{Root DM}_{\text{Harv}} [\text{g}] + \text{Green shoot DM}_{\text{Harv}} [\text{g}] + \text{S\&D shoot DM}_{\text{Harv}} [\text{g}]$$

Equation 2.9 Root/shoot ratio at harvest

$$\text{Root/Shoot}_{\text{Harv}} = \frac{\text{Root DM}_{\text{Harv}} [\text{g}]}{\text{Green shoot DM}_{\text{Harv}} [\text{g}]}$$

Equation 2.10 Net growth of plant part x

$$\text{Net growth of plant part } x [\text{g}] = x \text{ DM}_{\text{Harv}} [\text{g}] - x \text{ DM}_{\text{Start}} [\text{g}]$$

Equation 2.11 Dry matter content of the green shoot at harvest

$$\text{DM content green shoot} [\%] = \frac{\text{Green shoot DM}_{\text{Harv}} [\text{g}]}{\text{Green shoot FW}_{\text{Harv}} [\text{g}]} \cdot 100$$

Equation 2.12 Ellipsoid body projection

$$EP [mm^2] = \frac{L [mm]}{2} \cdot \frac{W [mm]}{2} \cdot \pi$$

Equation 2.13 Establishment success

$$\text{Establishment success [\%]} = \frac{\text{Number of established aphids}}{\text{Number of aphids placed}} \cdot 100$$

Equation 2.14 Colonisation success

$$\text{Colonisation success [\%]} = \frac{\text{Number mature aphids (dead and alive)}}{\text{Number of aphids placed}} \cdot 100$$

Equation 2.15 Age of aphid at event x

$$\text{Age of aphid at event } x \text{ [days]} = \text{Date of event } x - \text{date of day 1} + 1$$

Equation 2.16 Duration of aphid occupation

$$\text{Aphid occupation [days]} = \text{Date of aphid harvest} - \text{date of aphid placement} + 1$$

Equation 2.17 Reproductive rate

$$RR [\text{offspring/day}] = \frac{\text{Number of offspring collected}}{\text{Reproductive time [days]}}$$

Equation 3.1 Instar on day i

$$\text{Instar } (i) = \text{Number of exuviae removed up to day } i + 1$$

Equation 3.2 Instar on day i (mature aphids only)

$$\text{Instar } (i) = 5 - \text{Number of exuviae found after day } i$$

Equation 3.3 Aphid size at a given age

$$\text{Size (Age) [mm or mm}^2] = \frac{d - c}{(1 + e^{(b \cdot (\log_e(\text{Age}[\text{days}]) - \log_e(f)))})^B}$$

Equation 3.4 Reproductive lifespan

$$RLS_i [\text{days}] = \text{Age at exit [days]}_i - (\text{Age at reproduction [days]}_i - 3)$$

Equation 3.5 Average general reproductive rate

$$aRR [\text{offspring/day}] = \frac{1}{n} \cdot \sum_{i=1}^n \left(\frac{\text{Total number of offspring produced by } i}{RLS_i} \right)$$

Equation 3.6	Average lifetime fecundity	Average lifetime fecundity = $\text{aRR} \cdot \frac{1}{m} \cdot \sum_{k=1}^m (\text{RLSk})$ [offspring/established aphid]
Equation 3.7	Net reproduction rate	R_0 [offspring/established aphid] = $\sum_{x=1}^q (\text{lx} \cdot \text{mx})$
Equation 3.8	Size of aphid at a given age	Size (Age) [mm or mm ²] = $\mathbf{d} \cdot \mathbf{e}^{(-b \cdot (\text{Age}[\text{days}] - c))}$
Equation 5.1	Theoretical branching order use	Theoretical BO _x use (<i>PG-Ei</i> , <i>t</i>) = $n(\text{PG-Ei}, t) \cdot \text{pBO}_x(\text{PG-Ei}, t)$
Equation A4.1	ANOVA model for light intensity measurements	$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$
Equation A4.2	Variance within a position (model for light intensity)	$\text{VAR}_{\text{Measures}} = \text{MS}_{\text{Error}}$
Equation A4.3	Variance between positions (model for light intensity)	$\text{VAR}_{\text{Position}} = \frac{(\text{MS}_{\beta} - \text{MS}_{\alpha\beta})}{(3 \cdot n)}$
Equation A9.2.1	Model to analyse preserved vs. live size measurements in older instars	Size MX _{Dij} = $\mu + \gamma_i + \beta_i \cdot [\text{Size MX}_{Lij} - \text{mean}(\text{Size MX}_{L.i.})] + \varepsilon_{ij}$
Equation A9.2.2	Model to analyse preserved vs. live size measurements in early immatures	Size MX _{Dij} = $\mu + \gamma_i + \beta \cdot [\text{Size MX}_{Lij} - \text{mean}(\text{Size MX}_{L.i.})] + \varepsilon_{ij}$
Equation A11.2.1	Growth rate of plant <i>i</i>	$G_i = \frac{(\text{Parameter}_{T_x i} - \text{Parameter}_{T_1 i})}{\dots}$

$$\frac{(\text{AgePlant}_{\text{Tx } i} - \text{AgePlant}_{\text{T1 } i})}{}$$

Equation A11.2.2 Correction factor for plant i

$$\text{CF}_i = G_i \cdot (\text{AgePlant}_{\text{AphidCollection } i} - \text{AgePlant}_{\text{Tx } i})$$

Equation A11.2.3 Time-adjusted parameter

$$\text{Parameter}_{\text{Adj } i} = \text{Parameter}_{\text{Tx } i} + \text{CF}_i$$

LIST OF ABBREVIATIONS

ABO	Average branching order
aE	Endophyte status of the plant the ancestor (i.e. the mother of the observed root aphid) lived upon
AgeM	Age of root aphid at maturity (when it has produced its first offspring)
AgePAP	Age of plant at aphid placement
AIC	Akaike information criterion
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
aPG	Plant genotype of the plant the ancestor (i.e. the mother of the observed root aphid) lived upon
AphP	Aphid placement
BO	Branching order of a root
BS	Blocking solution
CT	Common-toxic strain of endophyte (sometimes referred to as "wild-type" or "standard" endophyte; AR93 in clone-plants)
DM	Dry matter, i.e. dry weight
DM_{Harv}	Dry matter (i.e. dry biomass) at harvest
DM_{refStart}	Dry matter content of one or several reference tiller(s) at the beginning of an experiment.
DM_{Start}	Dry matter (i.e. dry biomass) at the beginning of an experiment (after tiller trim)
E⁻	Endophyte-free plant
E⁺	Endophyte-containing plant

ELISA	Enzyme-linked immunosorbent assay
EP	Ellipsoid body projection
EPadult	Ellipsoid body projection of a root aphid as adult (size as adult [mm ²], estimated as body length/2 · abdominal width/2· π)
EPneo	Ellipsoid body projection of a root aphid as neonate (size as neonate[mm ²], estimated as body length/2 · abdominal width/2· π)
FW_{Harv}	Fresh weight at harvest
FW_{Start}	Fresh weight at start, just after a tiller has been trimmed for experimental purposes
G ratio	Green ratio; Colour measurement coefficient, calculated from reflectance measurements in the RGB colour model space as follows: $G/(R+G+B)$, with R, G and B being the red, green and blue reflectance measurements in the RGB colour space.
GL	Number of green leaves (GLi: number of green leaves at aphid placement; GLf: number of green leaves at final harvest)
GSA (T1, T2)	Green shoot area [mm ²]; green blade and sheath surface visible on a two-dimensional projection of a plant (i.e. on a photograph taken at a first time point T1 or a later time point T2; Section 2.4.2)
HRM	High resolution melting
KW	Kruskal-Wallis test; non-parametrical test for groups with > 2 levels
L	Body length of root aphids [mm]
Ladult	Body length of a root aphid as adult [mm]
Lneo	Body length of a root aphid as neonate [mm]
LFA	Long-term feeding aphids. Number of adult and older immature aphids found on a plant at harvest; all aphids minus first instars.
LME	Linear mixed-effects models
L_{Ref}	Light reference value, i.e. the average light intensity measured outside during daylight hours next to the Watchdog weather station;

	This value was used as 100%, to compare the differences in light intensity between various locations (glasshouses, insectary, climate chambers; Appendix 2)
MANCOVA	Multivariate analysis of covariance
MANOVA	Multivariate analysis of variance
MBM agar	Modified Bollard's medium [(Bollard, 1966); Appendix 7]
MC tube	Microcentrifuge tube (1.5 to 2.0 mL tube with lid)
MWU	Mann-Whitney-U non-parametrical test for groups with exactly 2 levels
N-AR1	A clone of a perennial ryegrass genotype (named N) of the cultivar 'Grasslands Nui', hosting the AR1 endophyte strain
N-AR37	A clone of a perennial ryegrass genotype (named N) of the cultivar 'Grasslands Nui', hosting the AR37 endophyte strain
NCM	Nitrocellulose membrane
N-CT	A clone of a perennial ryegrass genotype (named N) of the cultivar 'Grasslands Nui', hosting the common-toxic endophyte strain AR93
Nil or NIL	Endophyte-free plant; synonym: E ⁻
NIRS	Near infrared reflectance spectroscopy
nm	Nanometer
N-NIL	An endophyte-free clone of a perennial ryegrass genotype (named N) of the cultivar 'Grasslands Nui'
nRBd	Normalised red (R)-blue (B) difference; colour measurement coefficient, calculated from reflectance measurements in the RGB colour model space as follows: $nRBd = (R-B)/(R+B)$
PA	Plant age period (see Table A12.2.1.1)
PAR	Photosynthetically active radiation ($\mu\text{mol of photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)
PD	Petri dish

PG-E	Plant genotype-endophyte status combination; describes groups
R²c	Conditional R ² ; Variance explained by the fixed and random factors in a mixed-effects model, as calculated by the ‘MuMIn’ R package (Barton, 2018)
relAgePAP	Age of plant at aphid placement, in relation to the first day an aphid was placed during the Biology II experiment (date of placement - 14/11/2013)
RO-water	Water purified by reverse osmosis
S&D	Senescing and dead
S&DL	Number of senescing and dead leaves (S&DLi: number of senescing and dead leaves at aphid placement; S&DLf; number of senescing and dead leaves at the final harvest)
S-AR1	A clone of a perennial ryegrass genotype (named S) of the cultivar ‘Grasslands Samson’, hosting the AR1 endophyte strain
S-AR37	A clone of a perennial ryegrass genotype (named S) of the cultivar ‘Grasslands Samson’, hosting the AR37 endophyte strain
S-CT	A clone of a perennial ryegrass genotype (named S) of the cultivar ‘Grasslands Samson’, hosting the common-toxic endophyte strain AR93
SD	Standard deviation
S-NIL	An endophyte-free clone of a perennial ryegrass genotype (named S) of the cultivar ‘Grasslands Samson’
W	Abdominal width measurement, dimension of aphid at the widest point of the abdomen [mm]
Wadult	Abdominal width measurement of a root aphid as adult [mm]
Wneo	Abdominal width measurement of a root aphid as neonate [mm]