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

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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 endophy *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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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

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

Equation 2.6 Normalised red-blue difference

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

Equation 2.7 Average branching order

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

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

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

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

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

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

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

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*

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

Equation 2.16 Duration of aphid occupation

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

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)

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

Equation 3.3 Aphid size at a given age

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

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

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

Equation 3.6	Average lifetime fecundity	Average lifetime fecundity [offspring/established aphid] = $\mathbf{aRR} \cdot \frac{\mathbf{1}}{\boldsymbol{m}} \cdot \sum_{\boldsymbol{k=1}}^{\boldsymbol{m}} (\mathbf{RLS}\boldsymbol{k})$
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Equation 3.8	Size of aphid at a given age	Size (Age) [mm or mm^2] = $\mathbf{d} \cdot \mathbf{e}^{(\mathbf{e}^{-\mathbf{b}\cdot(\mathbf{Age[days]}-\mathbf{c})})}$
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Equation A4.2	Variance within a position (model for light intensity)	$VAR_{Measures} = MS_{Error}$
Equation A4.3	Variance between positions (model for light intensity)	$VAR_{Position} = \frac{(MS_{\beta} - MS_{\alpha\beta})}{(3 \cdot n)}$
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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*	$Gi = \frac{(\text{Parameter}_{Tx}\ i - \text{Parameter}_{T1}\ i)}{}$

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

Equation A11.2.2 Correction factor for plant *i*

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

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
$\mathbf{DM_{Harv}}$	Dry matter (i.e. dry biomass) at harvest
$\mathbf{DMref_{Start}}$	Dry matter content of one or several reference tiller(s) at the beginning of an experiment.
$\mathbf{DM_{Start}}$	Dry matter (i.e. dry biomass) at the beginning of an experiment (after tiller trim)
$\mathbf{E^-}$	Endophyte-free plant
$\mathbf{E^+}$	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 (μmol of photons$\cdot m^{-2}\cdot s^{-1}$)
PD	Petri dish

PG-E	Plant genotype-endophyte status combination; describes groups
R^2c	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]