Plant-endophyte-herbivore interactions

More than just alkaloids?

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A recent paper by Rasmussen et al., (New Phytol 2007; 173:787–97) describes the interactions between Lolium perenne cultivars with contrasting carbohydrate content and the symbiotic fungal endophyte Neotyphodium lolii at different levels of nitrogen supply. In a subsequent study undertaken by Rasmussen et al., (Plant Physiol 2008; 146:1440–53) 66 metabolic variables were analysed in the same material, revealing widespread effects of endophyte infection, N supply and cultivar carbohydrate content on both primary and secondary metabolites. Here, we link insect numerical responses to these metabolic responses using multiple regression analysis.

Pasture grasses are often infected with symbiotic fungal endophytes and benefits for host plants arising out of these associations are generally ascribed to endophyte produced anti-herbivorous alkaloids. We tested the effects of (i) infection with three strains of endophytes differing in their alkaloid profiles, (ii) high vs. low nitrogen (N) supply, and (iii) ryegrass cultivars with high vs. control levels of water soluble carbohydrates (WSCs) on numerical insect responses (aphids, thrips, mites). A difference in WSC content is generally ascribed to endophyte produced anti-herbivorous alkaloids, but decreased apterous Rhopalosiphum padi, high sugar grasses, metabolomics, insect herbivores

Insights into Molecular Grass-Endophyte Interactions

In a previous study13,14 we infected two ryegrass cultivars differing in their water soluble carbohydrate (WSC) content with three N. lolii strains (common strain CS—produces peramine, lolitrem B and ergovaline; AR1—produces peramine only; AR37—produces janthitrems only), and grew them at two nitrogen (N) levels. We quantified endophyte concentrations and 66 metabolic variables in the symbiotic tissue. Major findings were: Both, high N supply and high WSC content reduced fungal endophyte and alkaloid concentrations by approx. half, resulting in a 75% reduction in the high sugar cultivar at high N. A principal component analysis with subsequent factor rotation of the metabolic variables showed that (i) at high N proteins, major amino acids, organic acids and lipids were increased; WSCs, chlorogenic acid and fibers were decreased; (ii) the high sugar cultivar AberDove had reduced levels of nitrate, most minor amino acids, sulfur and fibers, whereas WSCs, CGA, and methionine were increased; (iii) plants infected with endophytes had increased levels of WSCs, some organic acids, lipids and CGA, whereas nitrate and several amino acids, esp., L-asparagine, were decreased.

Several of these metabolites have been linked to plant quality for various herbivores.15-20 We were interested in whether such changes in plant quality could be linked directly to herbivore performance (rather than indirectly via a treatment effect).

Insect Herbivore Performance and Plant Metabolic Quality

After harvesting the blades, plants were enclosed in plastic boxes and two alate adults of Rhopalosiphum padi L. were added to each plant. In addition to these aphids, two other aphid species, Sitobion nr fragariae and Aploneura lentisci, a variety of thrips (Frankliniella
and an unidentified mite species were already present on
the plants prior to enclosure. We also found some *R. insertum* aphids
on the plants, but analysed those together with *R. padi* numbers.

After four weeks, we recorded the number of individual
*Rhopalosiphum* spp., (apterous, alate), *S. nr frageriae* (apterous) and *A. lentisci* aphids. Alate *S. nr frageriae* were absent from most plants and
were not included in the statistical analysis. We did not differentiate
between *Rhopalosiphum* and *Sitobion* nymphs and, due to the fact that
the adult aphid species responded differently to the treatments, we
did not include total nymphs in our statistical analysis but report the
numbers. Only young nymphs of *A. lentisci* were found on the foliage.
This species lives primarily on roots of grasses which were not sampled
for aphids, so we also excluded *A. lentisci* found on the foliage from our
statistical analysis. For the majority of these response variables, both N
supply and endophyte status significantly altered herbivore numbers
(Figs. 1 and 2); however, differences in the WSC content of the two
cultivars did not have any significant effect on insect responses.

To link insect responses to metabolite profiles we used multiple
regression analysis and Akaike’s Information Criterion to select the best
model of herbivore numerical responses from among the measured
metabolites. After finding the best fitting models, we standardized all
the response and predicting variables, so that they each had a mean
of 0 and a standard deviation of 1. We then re-fitted the best regres-
sion models. This method means that the magnitude of the slopes can
be directly compared across metabolites, despite their very different
concentrations. These regression equations are shown below.

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\begin{align*}
\text{Total Nymphs} & = 4.27 + 0.42 \times \text{Nitrogen} + 0.34 \times \text{Endophyte} - 0.02 \times \text{Low N} + 0.01 \times \text{High N} \\
\text{Rhopalosiphum spp. (apt.)} & = 0.42 \times \text{Nitrogen} + 0.35 \times \text{Endophyte} - 0.26 \times \text{Low N} + 0.20 \times \text{High N} \\
\text{Rhopalosiphum spp. (ala.)} & = 0.41 \times \text{Peramine} - 0.32 \times \text{L-Ser} - 0.28 \times \text{Lolitrem B} - 0.21 \times \text{ADF} + 0.18 \times \text{Malonate} \\
\text{Rhahogalosiphum spp. (ala.)} & = 0.32 \times \text{Peramine} - 0.27 \times \text{Lolitrem B} + 0.18 \times \text{L-Ser} \\
\end{align*}
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**Conclusion and Outlook**

Arguably, these results do not present a clear response of insect
herbivores to endophytes and endophyte related alkaloid production,
but Figures 1 and 2 and equations (1–5) permit several interesting observations. First, different groups of herbivores respond differently to the different endophyte strains: *Rhopalosiphum* spp., responded positively to AR1, and *S. nfrageriae* and mites positively to AR37. Second, different herbivores responded differently to specific alkaloids, e.g., *Rhopalosiphum* spp., responded positively to peramine, but negatively to lolitrem B. In contrast, *S. nfrageriae* responded negatively to peramine. Third, for responses of thrips and mites, alkaloids seemed to be unimportant. Although these results cannot be interpreted to mean there are direct positive or negative effects of these alkaloids they clearly show that population sizes of all herbivores are predicted by concentrations of metabolites other than alkaloids. Since we know that a large range of metabolites varies with endophyte infection, strain and concentration, the overriding conclusion from these results is that alkaloids are certainly not the only factors, and may not even be the most important factor in the endophyte-grass-herbivore story. Furthermore, we also know that in addition to the known alkaloids, there are a range of other endophyte specific metabolites that have just recently been discovered and which may have biological activity as well, but were not part of our analysis. A note of caution should be added here as well, as we have not included possible insect species interactions into our analysis. Thus, where populations of an insect species, particularly an opportunistic one, are reduced by an endophyte, that niche may be filled opportunistically by another species that is not negatively affected by that endophyte.

An additional complicating issue for our analysis is certainly that aphids are phloem feeders, and that the degree to which metabolite and alkaloid concentrations in whole blade extracts covary with those in phloem sap is, as yet, unknown. There is clearly much more work to be done to fully understand this interaction, and a combination of transcriptomics and metabolomics approaches in an ecological context seems likely to eventually point the way toward a mechanistic explanation of these interactions. This type of study might also help reconcile conflicting evidence found in the literature regarding the overall nature of the grass-*Neotyphodium* symbiosis, and especially to unravel the mechanisms underlying the dynamics of the mutualism-parasitism continuum of this association.

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References


Figure 2. Numbers of (A) total nymphs, (B) apterous, (C) alate *Rhopalosiphum* spp., (D) apterous *S. frageriae*, (E) mites and (F) thrips on foliage of *L. perenne* plants infected with different endophyte strains or uninfected (different letters denote means that are significantly different as determined by Tukey’s Honestly Significant Difference test).


