

Temperature-driven variations in *Epichloë* endophyte-mediated porina resistance in perennial ryegrass

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New Zealand's pastoral systems rely heavily on perennial ryegrass (*Lolium perenne*), which are intentionally associated with selected *Epichloë* fungal endophytes that form a mutualistic symbiosis that protects the host against herbivorous insects through the production of alkaloids. The commercially available endophyte *Epichloë* LpTG-3 strain AR37 is a perennial ryegrass endophyte that confers resistance against the larvae of porina (*Wiseana* spp.), a significant pest in cooler regions of New Zealand. To expand pest management options, a newly identified strain of *Epichloë*, designated AR128 has been identified. This study investigated how temperature influences alkaloid concentrations in AR37 and AR128-infected perennial ryegrass plants and the subsequent effects on porina fitness, plant damage, and plant size.

Perennial ryegrass plants, both with and without AR37 or AR128, were grown at three temperature regimes: 8°C, 13°C, and 18°C. After 4 weeks of growth, one porina larva was added to each plant, and left to feed for 8 weeks. Following this feeding period, the tillers were assessed for feeding damage using a scoring system from 0-5. Porina survival and weight gain were also measured. Generalised linear mixed models were used to analyse the effect of perennial ryegrass and/or temperature on porina fitness, plant damage, and plant growth. There was no significant interaction between temperature and endophyte strain; however, both factors significantly affected the proportion of severely damaged tillers. Endophyte infection with AR37 or AR128 significantly reduced the proportion of

severely damaged tillers (score 5) by 1.5% and 1.3%, respectively, compared to endophyte-free plants with 4.4%. The highest proportion of severely damaged tillers was observed at 8°C, while the lowest occurred at 18°C. The reduction in tiller damage at 18°C was associated with lower porina survival. Although there was no significant difference in porina survival between AR37 (41%) and AR128 (29%), both strains significantly reduced porina survival compared to endophyte-free plants (73%). Across all treatments, temperature influenced porina survival, which was lowest at 18°C with 21%. However, temperature had no significant effect on the weight gain of surviving larvae. The temperature-dependent trend in damage corresponded with epoxyjanthitrem concentrations in endophyte-infected plants, an alkaloid compound highly deterrent/toxic to porina. Although there was no significant difference in total epoxyjanthitrem levels between AR37 and AR128, concentrations were lowest at 8°C (5.9 µg/mL) and increased with temperature, reaching 29.4 µg/mL at 18°C.

Across all treatments, this research demonstrated that porina causes greater plant damage at lower temperatures. Although endophyte infection with AR37 or AR128 consistently reduced plant damage, their efficacy declined at lower temperatures, which was associated with a reduction in alkaloid concentrations. These findings highlight the importance of temperature in modulating endophyte-derived insect protection and suggests that the efficacy of this endophyte technology may be compromised under cooler growing conditions.