CARBON ISOTOPE DISCRIMINATION IN TALL FESCUE–ENDOPHYTE ASSOCIATIONS AS A FUNCTION OF DEFOLIATION INTENSITY AND LIGHT AVAILABILITY

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INTRODUCTION

Tall fescue [*Lolium arundinaceum*, Schreb., S. J. Darbyshire] thrives in agro-ecosystems when infected with *Neotyphodium coenophialum* (Morgan-Jones and Gams, Glenn, Bacon, and Hanlin) endophyte. This is attributed in part to drought stress avoidance or tolerance (Malinowski and Belesky, 2000), and improved nitrogen-use efficiency (Lyons *et al.*, 1990). Both features are likely to influence photosynthetic capacity of plants harboring endophytes.

Ecophysiological experiments with plants clipped to simulate grazing, are not common because of the confounding influences of environment and management. Collecting numerous and often destructive measurements from plants at various times is difficult. Consequently, conclusions are based on instantaneous measurements made at fixed points.

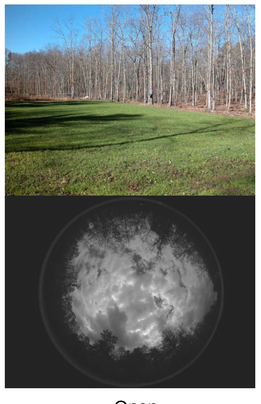
Stable carbon isotope discrimination (Δ) can provide an integration of responses to management and environment (Jumpponen *et al*., 2005) and could link responses with agro-ecosystem function. Significant determinants of Δ include stomatal function and ribulose-1,5-bisphosphate carboxylase /oxygenase (RuBP) activity (Farquhar *et al*., 1989). Since the integrative measure of ambient relative to internal CO_2 concentration is associated with water and nitrogen use efficiency, and endophyte seems to benefit tall fescue in this respect, Δ might be a way to distinguish infected and non-infected plant responses.

OBJECTIVE

Determine if host-endophyte associations interacting with microsite and management influence Δ.

H_0: Carbon isotope discrimination does not vary with host-endophyte association

Results could help explain how endophytes influence plant productivity and spatial distribution.



Open
full sunlight

North Edge
~ 60% of full sunlight

South Edge
~ 20% of full sunlight

MATERIALS & METHODS

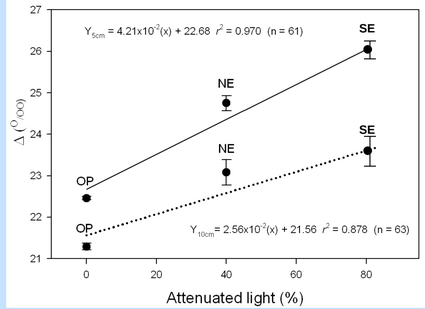
Plant resources & microsite characteristics
Plant material – cv. Jesup wild-type (J+) or novel AR542 (MaxQ™)† endophyte, or devoid of endophyte (J-)
Microsites – Sites at 81º 7′ W; 37º 45′ N; 770m above sea level included open (OP), full sunlight; north edge (NE) and south edge (SE) of a *Quercus* spp. dominant forest opening, parallel to the sun path. Edge sites were about 35 m apart and the OP midway between.
Microclimate – Data collected from 1 April through 31 October including temperature, precipitation, relative humidity, light (PPFD; mol m^{-2} s^{-1}; 400-700 nm), and wind speed were used to compute potential evapotranspiration (ET_o).
Defoliation – Plants were clipped to 5- or 10-cm residual plant height in early May (staging) and were clipped to the residual heights each time mean extended canopy height reached 20 cm.

Sample collection and analysis
Leaf dry matter content – LDMC (g kg^{-1}) = 100 - (g wet mass – g dry mass) (g wet mass x 100)$^{-1}$.
Photosynthetic nitrogen use efficiency – PNUE [g DM (mol leaf N)$^{-1}$ d^{-1}] = g whole plant DM mol^{-1} leaf N (Th_1 – Th_0)$^{-1}$; DM (dry matter), T_n (days between harvests)
Natural abundance carbon isotopes – ^{13}C isotope natural abundance determined at U C Davis (Stable Isotope Facility, Davis, California, USA). ($\Delta^{13}C$) = [$\delta^{13}C_a$ - ΔPDB] [1 + (ΔPDB (1000)$^{-1}$)]$^{-1}$; $\delta^{13}C_a$ = atmospheric composition (δ_a) of -8‰; ΔPDB = change in ^{12}C and ^{13}C from standard.
Estimates of herbage nutritive value –
- N (as crude protein, CP; N g 100g^{-1} x 6.25) by tissue combustion (Carlo Erba EA 1108 CHNSO. Fisons Instruments, Beverly, MA, USA).
- *In vitro* organic matter disappearance (IVOMD) by a two-stage digestion procedure using rumen fluid obtained from rumen-cannulated steers (*Bos taurus*) offered orchardgrass (*Dactylis glomerata* L.) – alfalfa (*Medicago sativa* L.) hay.
- Metabolizable energy of feed (ME); ME (MJ kg^{-1} DM) = 0.0157 (IVOMD).
- Total digestible nutrients (TDN) calculated from ME data to assess energy to protein quotient (Neel *et al*., 2008).

Statistical procedures
Mixed model procedures (SAS Inst. Cary, North Carolina, USA) identify treatment (host-endophyte association, microsite, defoliation) effects. Regression analysis models Δ relative to selected environmental, ecophysiological, and nutritive value parameters.

† Trade names are used for the convenience of the reader and do not imply endorsement by USDA over comparable products and services.

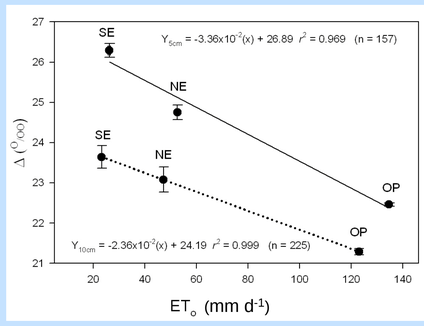
RESULTS
Environment & Management



Microsite (environment) and defoliation (management) influenced Δ. Season-long mean Δ increased from OP (full sun) to SE (most shade) as available light decreased.

Δ reflects resource availability and canopy management.

Tall fescue - endophyte associations did not differ in Δ as a function of microsite, residual height, or time.



ET_o was about six times greater at OP compared to the SE site, irrespective of residual clipping height.

This occurred in part because growth interval duration differed for each microsite and residual clipping height.

Δ decreased as ET_o increased, which suggests possible decreased water use efficiency at OP.

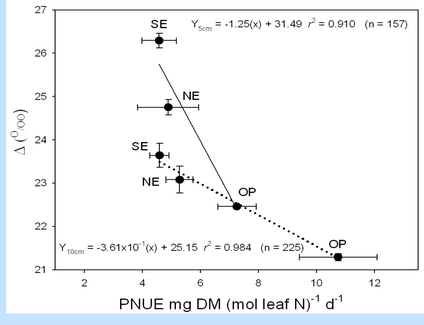
We expected that host-endophyte associations would differ in Δ and were surprised when no differences in Δ were observed.

The similarity in Δ might be attributable, in part, to benign growing conditions at the site.

The similarity in Δ might also reflect a minimal cost of harboring endophyte when resources are plentiful.

Equally surprising was the wide range in Δ occurring as a function of microsite.

Ecophysiological Response

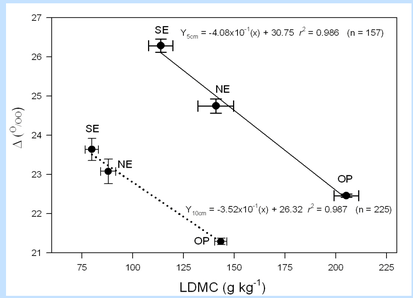


PNUE increased as available light increased, and did so in an inverse manner relative to Δ.

PNUE links physiology with composition and productivity. Factors contributing to PNUE include the proportion of leaf N as RuBP, specific activity of the enzyme, and intercellular CO_2 concentrations that influence Δ.

Photosynthetic efficiency drives agro-ecosystem productivity in the form of herbage mass. Herbage composition is N-rich and energy-depleted in plants growing in partially shaded sites.

RESULTS
Agro-ecosystem Products



Herbage Dry Mass

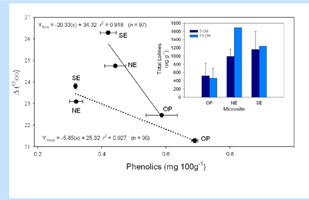
LDMC was inversely related to Δ.

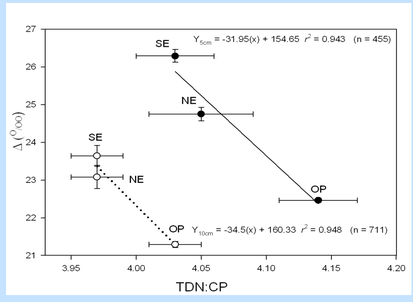
Trends indicate that Δ can serve as an indicator of dry matter production, which is an important agro-ecosystem service of forage plants.

LDMC integrates leaf composition, leaf longevity and nutritive value, which are associated with herbage composition and ultimately palatability.

An Interesting Relationship

Phenolics were detected in each of the host-endophyte associations and declined as shading increased. Loline alkaloids increased in J+ and MaxQ (not present in J-) as shading increased (insert). Ergoalkaloids were present in J+ only. Phenolics might function as photosystem protectants in full sun, and alkaloids as herbivory deterrents in shade.





Energy : Protein

Herbage nutritive value suggests relative efficiencies that can represent plant adaptive strategies or functional traits with ecological meaning at a much broader scale.

The TDN:CP of tall fescue herbage was related inversely to Δ.

Trends indicate that Δ can serve as an indicator of nutritive value; an agro-ecosystem product that is as important as herbage dry matter production in pasture.

SUMMARY

- Endophyte helps tall fescue tolerate abiotic stresses. A means to assess this is needed to understand how host-endophyte associations function across landscapes and with management practices.
- A field experiment to determine if a tall fescue cultivar that was devoid of or infected with a native or a novel non-ergoalkaloid producing endophyte was influenced by environment and forage management practices. Stable carbon isotope discrimination was used to determine responses of associations to environment and management.
- The positive relationship of Δ with LDMC and herbage energy – protein (TDN:CP) links physiological efficiency with system function.
- Host-endophyte associations did not differ in Δ. Similar Δ for host-endophyte associations might be attributable to benign growing conditions at the site.
- Patterns of Δ could help identify microsite conditions and management practices that sustain herbage productivity and desirable nutritive value in agro-ecosystems.

LITERATURE CITED

Farquhar GD, Ehleringer JR, Hubick KT. 1989. Carbon isotope discrimination and photosynthesis. Ann. Rev. Plant Physiol. Plant Mol. Biol. 40, 503-537.

Jumpponen A, Mulder CPH, Huss-Daniel K, Hogberg P. 2005. Winners and losers in herbaceous plant communities: insights from foliar carbon isotope composition in monocultures and mixtures. J. Ecol. 93, 1136-1147.

Lyons PC, Evans JJ, Bacon CW, 1990. Effects of the fungal endophyte *Acremonium coenophialum* on nitrogen accumulation and metabolism in tall fescue. Plant Physiol. 92, 726-732.

Malinowski DP, Belesky DP. 2000. Adaptations of endophyte-infected cool-season grasses to environmental stresses: Mechanisms of drought and mineral stress tolerance. Crop Sci. 40, 923-940.

Neel JPS, Feldhake CM, Belesky DP. 2008. Solar radiation and season influence botanical composition and nutritive value of cool-season forage swards growing in a conifer woodlot. Grass Forage Sci. 63, 38-47.