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Impact of guttation fluid from perennial ryegrass infected with different strains of *Epichloe festucae* var. *lolii* endophyte on *Microctonus aethiopoides* adult longevity

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Abstract Perennial ryegrass (*Lolium perenne* L.) grows in association with a fungal endophyte *Epichloe festucae* var. *lolii* (Latch, Christensen & Samuels) Bacon & Schardl, which produces alkaloids that protect the grass against grazing by mammals and insects. These alkaloids are found in guttation fluid (xylem sap exuded from leaves through special structures known as hydathodes) and have the potential to impact on beneficial invertebrates in pastoral ecosystems. Newly emerged adults of the parasitoid *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) were supplied with guttation fluid from pot-grown ryegrasses infected with three different strains of endophyte (standard, AR37, AR1) or no endophyte collected at different times of the year, or water, sucrose solution or no liquid. Longevity was compared when individuals were held in separate vials in controlled environment room at 20°C with 16:8 h light:dark photoperiod. An enzymatic method was used to measure sugars in guttation fluid samples collected on three dates. Guttation fluid from endophyte-infected grasses was found to have no detrimental effect on *M. aethiopoides* longevity and to contain glucose and fructose. Guttation fluid from AR37-infected ryegrass collected in autumn increased insect longevity compared to water and fluid from standard-type infected ryegrass by 26% and 24% respectively. The lack of available food sources in New Zealand ryegrass-dominant pastures means that guttation fluid from AR37-infected ryegrass in autumn may contribute to *M. aethiopoides* efficacy as a biocontrol agent through enhanced longevity.

Keywords Parasitoid, guttation, alkaloid, fructose, glucose, peramine

INTRODUCTION

Perennial ryegrass (*Lolium perenne* L.) can grow in association with a fungal endophyte *Epichloë festucae* var. *lolii* (Latch, Christensen & Samuels) Bacon & Schardl, which makes it less vulnerable to herbivore grazing (Barker et al. 1990; Prestidge et al. 1994) and improves both pasture production and persistence (Hume et al. 2009; Kerr et al. 2012). The standard endophyte strain (also known as wild type), produces two mammalian toxins, the alkaloids lolitrem B and ergovaline, that are detrimental to grazing animals (di Menna et al. and references therein). However, other endophyte strains with reduced or no toxicity in grazing animals, such as AR1 and AR37, have been identified and commercialised. These strains have different alkaloid profiles that still provide protection against attack by a wide range of herbivorous insect pests of ryegrass, (Thom et al. 2012; Ruppert et al. 2017).

While the endophytic association significantly benefits the ryegrass, it also impacts on other non-target organisms in the pasture environment. Such impacts may be especially important in New Zealand where pastures are dominated by perennial ryegrass, averaging just over 70% of total dry matter (Tozer et al. 2014). Some effects may be beneficial: white clover (*Trifolium repens* L.) growth is enhanced when plants are grown in soil in which endophyte-infected ryegrass was grown previously and the size of the response varies with endophyte strain (Cripps et al. 2013). However, endophyte presence can have negative multitrophic interactions. The feeding preference of the aphid *Rhopalosiphum padi* L. (Homoptera: Aphididae) to ryegrass was unaffected by the presence of endophytes (Latch et al. 1985). However, the aphid takes up the alkaloids peramine and lolitrem B present in standard endophyte-infected grass when reared on this material and, in turn, cascade up the food chain to

aphid predators resulting in fitness disadvantages (Fuchs et al. 2013). Survival of the parasitic chalcid wasp *Euplectrus comstockii* Howard (Hymenoptera: Eulophidae: Euplectrini) was reduced when provided with fall armyworm (*Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae: Amphipyrini)) hosts that had been fed tall fescue *Festuca arundinacea* Schreb. infected with standard or the experimental AR542 endophyte, which produces three loline alkaloid derivatives. However, wasp survival was not affected when their hosts had been fed the experimental AR502-endophyte-infected foliage, which produces only *N*-acetyl noroline (Bultman et al. 2009). Larval development of the parasitoid *Microctonus hyperodae* Loan (Hymentoptera: Braconidae) was retarded when its host *Listronotus bonariensis* (Kuschel) (Coleoptera: Curculionidae) fed on ryegrass infected with the standard endophyte compared to endophyte-free ryegrass (Barker & Addison 1997) and percent parasitism in the field by this species has been found to be inversely related to the amount of the alkaloid peramine in pasture ryegrass samples (Goldson et al. 2000). However, this effect was not consistent across endophyte strains (Urrutia et al. 2007).

One pathway in which grass endophytes may have a direct effect on beneficial organisms is through guttation fluid (i.e., xylem sap exuded from leaves through special structures known as hydathodes). Guttation fluid occurs most noticeably in early morning or at night, and is a means of disposal of solutes, improvement of nutrient acquisition, maintenance of water balance for proper growth, and defence and attraction of other organisms (Singh 2013 and references therein). For example, guttation fluid from barley (*Hordeum vulgare* L.) has been shown to contain pathogenesis-related proteins, the composition of which changes in response to stimuli, and can protect the plant against invasion by motile bacteria (Grunwald et al. 2003). This ability of plants to exude bioactive metabolites is seen as a mechanism that could be exploited to improve resistance to pest insects and microbes (Shepherd & Wagner 2007). Furthermore, the nutrient content in guttation fluid from blueberries (*Vaccinium corymbosum* L.) has been found to enhance beneficial insect survival and fecundity compared to water, and increase the numbers of predators and parasitoids visiting the plants (Urbaneja-Bernat et al. 2020).

Two introduced biocontrol agents can be found in pastures throughout New Zealand; namely the braconid parasitoids *Microctonus hyperodae* introduced to control Argentine stem weevil *Listronotus bonariensis* (Kuschel) (Coleoptera: Curculoinidae) and *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) introduced to control clover root weevil (*Sitona obsoletus* (Gmelin) (Coleoptera: Curculoinidae). The parasitoids emerge in a fully reproductive state and their only limitation to laying all their eggs is if they can live long enough to find sufficient hosts. The availability of water and food sources, such as nectar, improves adult *Microctonus* spp. longevity and, in turn, oviposition (Phillips 1998). However, this genus cannot access clover nectar (Vattala et al. 2006) and there are few other fluid sources in high-quality mixed ryegrass and white clover pastures so parasitoid adults may ingest guttation fluid and any endophyte metabolites present in the fluid. Koulman et al. (2007) found that guttation fluid collected from ryegrass infected with *E. festucae* var. *lolii* endophyte strain AR93 contained 0.554 µg/mL of the alkaloid peramine, a similar level as found in cut leaf fluid. Differences among endophyte strains can also be detected in guttation fluid for minor compounds such as ferriepichloënin A (Koulman et al. 2012).

Experiments were undertaken to compare longevity of *M. aethiopoides* adults supplied with seasonal guttation fluid from ryegrasses infected with either of three *E. festucae* var. *lolii* endophyte strains (standard, AR37, or AR1) or no endophyte. These three endophyte strains vary in the alkaloids they produce. The standard strain produces lolitrem B, ergovaline and peramine, the AR1 strain produces peramine and the AR37 strain produces epoxy-janthitrems (Meale et al. 2013). The hypothesis was that guttation fluid produced by ryegrasses infected with endophyte, especially those strains producing peramine, would have a detrimental effect on parasitoid longevity.

MATERIALS AND METHODS

Parasitoid longevity

The experiments were run at the Ruakura Research Centre in Hamilton, New Zealand between 2012 and 2014. Prior to the experiments, seedlings of the diploid perennial ryegrass cv. GA66 (an experimental line related to 'Grasslands Samson') infected with either standard, AR1, AR37 or no endophyte (nil) were tested when at least six weeks old using a tissue print immunoblot assay (Hahn et al. 2003) to confirm endophyte presence. Twenty mature plants of each endophyte line were established in pots containing commercial potting mix in summer 2012. The plants were held in an outdoor shade house with a translucent roof, bird-netting walls, and automatic overhead watering, and were trimmed and fertilised as required to maintain plant vigour. A shade house was selected, rather than a glasshouse, so that the plants would be exposed to normal diurnal temperatures and photoperiod and would be likely to have similar alkaloid expression to ryegrasses in the field. Guttation fluid was collected from plants in early morning using glass Pasteur pipettes from the same plants over a period of two years. The ability to collect any fluid, let alone a workable amount from all endophyte lines, was highly dependent on the weather, especially wind and humidity. Fluid from plants with the same endophyte was pooled in labelled 1.5 mL Eppendorf microcentrifuge tubes for each collection date and stored immediately in a freezer at -20°C. For most replicates, sufficient guttation fluid was collected only a few days prior to, or during, the assay to complete the experimental run. However, poor collecting conditions during late summer 2012 necessitated the use of some samples of up to 10 days old.

Sitona obsoletus adults were collected by suction using a modified Dolmar PB250 blower-vac from pastures known to have an abundance of both the weevil and its parasitoid at multiple times throughout the experimental period, except in spring when parasitism levels are extremely low. The weevils were placed in mesh-bottomed cages and supplied with fresh white clover. Each cage was fitted over

a second container which had several layers of paper towel in the base. The cages were held in either a laboratory or, if parasitoid larval diapause needed to be prevented or broken, a 20°C controlled environment room with 16:8 h light:dark photoperiod. Parasitoid prepupae emerging from weevils dropped through the mesh base and made cocoons under or between the paper towel layers. Once the larvae had fully pupated, segments bearing cocoons were cut from the paper towels, placed in labelled Petri dishes lined with slightly damp filter paper and incubated at varying temperatures to synchronise adult emergence as much as possible. *M. aethiopoides* adults do not differ physiologically with season.

To test guttation fluid, parasitoids cocoons were inspected daily in the late morning and each newly emerged parasitoid was placed in an individually-labelled 5 mL screw top plastic vial, the base of each having been replaced with a fine mesh. Whatman 42 filter paper, cut into approximately 5 mm squares, was dipped into the test fluid, and a single wet square was placed on the inside wall of the appropriate test vial. Water and blank (empty) vials were used as controls. Parasitoid emergence occurred over several days so care was made to set up the same number of replicates for each test fluid each day. The vials were placed in an open plastic container and held in a 20°C controlled environment room with 16:8 h light:dark photoperiod. Humidity was not controlled and was generally in the range of 45-60%. The test squares were replaced every 24 h, and the date of parasitoid death recorded. After death, head capsule width was measured as parasitoid size was likely to be a confounding factor with the facultative gregarious genotype of *M. aethiopoides* used in this assay. Six runs were made of the assay as parasitoids came available to assess if any responses observed changed with season and plant age. A 10% sucrose solution was included in the final run, which only had eight replicates due to low concurrent availability of parasitoids and guttation fluid. The runs for the six collection periods were as follows:

1. Late February 2012 (late summer): 20 replicates of each of the three endophytes (AR1, AR37 and standard), nil endophyte and a water control.
2. Mid-April 2012 (mid-autumn): 25 replicates of each of the three endophytes, nil endophyte and a water control, and 14 replicates of a blank control.
3. Mid-late June 2012 (early winter): 20 replicates of each of the three endophytes, nil endophyte and a water control.
4. Late July 2012 (mid-winter): 17 replicates of each of the three endophytes, nil endophyte and a water control.
5. February 2013 (late summer): 14 replicates of each of the three endophyte treatments plus nil endophyte and water and blank controls.
6. Mid-April 2013 (mid-autumn): 8 replicates of each of the three endophytes, nil endophyte and three controls (water, blank and 10% sucrose solution).

Statistical analyses

The data were analysed with Genstat, 16th edition (VSN International 2013) using Residual Maximum Likelihood (REML) (Patterson and Thompson 1971) to produce means allowing for the unbalanced design. The model fitted had head capsule width as a co-variate; season (summer (December-February), autumn (March-May), winter (June-August)) x treatment as fixed effects and assay run and replicates within runs as random effects. For analyses of individual runs, a simpler model was used with treatment as fixed and replicate as random effects.

Sugar analysis

In March 2015, three sets of guttation samples from the very limited number remaining in the freezer were retrospectively analysed using HPLC for "nectar-feeder" sugars D-fructose, D-glucose, sucrose, and trehalose. A full set of samples collected on 14 February 2013 had been used during the experiment (Run 5) so had been previously thawed for use then re-frozen. These samples were re-thawed then were diluted with distilled water by a factor of 10. The two other sets collected outside the run periods (March 2013 and February 2014), consisted only of two endophyte guttation samples each, and had not been opened since collection: These were diluted by a factor of 5.

Analysis was undertaken using a Shimadzu HPLC (Shimadzu Corporation, Kyoto, Japan) with ELSD detector and an injection volume of 25 μL. The system was equipped with a LC-20 AD pump with a DGU-20A5 online degasser, Prevail™ Carbohydrate ES Columns (250 x 4.6 mm) with guard column and Alltech 3300-ELS Detector. The gradient mobile phase was A) % acetonitrile, and B) % distilled water (0-5 min, 20% B; 5-10 min 20%-50% B; 10-11 min 50%-20% B; 11-15min 20% B) at a flow rate of 1.0 mL/min. The column temperature was maintained at 20°C during analysis. The detector flow rate was 1.4 L/min and temperature 38°C.

The sugars were identified by comparing retention time of standards and sample quantification was determined from the peak area of chromatograms using the external calibration standard curve; all data were processed using LC solution software. The sugar concentrations (expressed as ppm) in the samples were then calculated taking into consideration the dilution factors.

The February 2013 samples were also analysed during the development and as a means of testing a colourimetric method later published by Phillips et al. (2018). This method exploits a short series of enzymatic reactions followed by reduction of thiazolyl blue tetrazolium bromide to produce a visible purple formazan in order to quantify nectar sugars in very small samples, such as available in this study.

Samples of guttation fluid were measured either with no dilution or were diluted 2 or 4 times. 20 μL of each sample was added to the microplate wall in duplicates and was mixed with 20 μL of stain (Phillips et al. 2018). The colour was developed for 20 minutes at room temperature in the dark, and reactions were stopped by adding stop solution (10% SDS in 0.001 M HCl). Reagent blanks were included as zero points for standard curves. Optical densities of solutions in each well were measured with a FLUOstar®Omega microplate reader (BMG Labtech, Germany) fitted with a 570 nm filter. Optical densities were recorded as the mean of two replicate wells.

RESULTS

Parasitoid longevity

Table 1 summarises the adult longevity data from the six assay runs. Overall, there was no evidence that guttation fluid from ryegrass plants containing endophytes had any negative effect on parasitoid survival compared to that from the nil-endophyte ryegrass. Parasitoid size was a significant covariate only in the early winter run (P=0.030).

There was a significant interaction between endophyte and the season in which the guttation fluid was collected (P = 0.003). While there was no significant difference between water or guttation fluids collected in summer and winter when seasonal data were pooled, parasitoids provided with autumn-collected guttation fluid from AR37-infected ryegrass had an overall mean longevity of 6.7 days across both years, which was significantly higher than that for standard endophyte (5.4 days) and water (5.3 days) (P=0.017).

Supplying 10% sucrose solution in the Autumn 2013 assay increased parasitoid longevity substantially compared with guttation fluid samples and water (P>0.05), whereas the blank control had no significant effect on longevity compared with these samples (Table 1).

Sugar analysis

Sugar analysis by HPLC of guttation fluid samples obtained at different times to the longevity assays (Table 2) showed that all samples contained D-fructose and D-glucose, and with the exception of AR37 in 2014, mostly in ratios between 1:1 and 2:1. None had detectable levels of sucrose and nil-endophyte fluid had a trace of trehalose on one occasion.

Comparison of the HPLC and colourimetric methods for the February 2013 samples (Table 3) showed similar results for the nil, AR1 and AR37 samples with the exception of the detection of small amounts of trehalose using the colourimetric method. The analysed concentrations of both fructose and glucose in guttation fluid from standard endophyte-infected ryegrass were higher using the HPLC method than the colourimetric one but the colourimetric assay detected sucrose that was not observed by HPLC.

DISCUSSION

Parasitoid longevity

In this experiment, guttation fluid from endophyte-infected grasses was found to have no detrimental effect on *M. aethiopoides* longevity. The similarity in parasitoid response to the blank controls, water and the guttation fluids could have been due to the parasitoids not ingesting the fluids, irrespective of whether any feeding deterrent was present. However, feeding was observed at times when the old dry filter paper square was replaced by a fresh square wetted with a test guttation fluid. Also, the overall mean longevity of 5.9 ± 1.3 days on water at 20°C is similar to that observed previously (Gerard et al. 2013) and for *M. hyperodae* (Phillips 1998). In the final run, parasitoids provided with sucrose solution had a marked increase in longevity (2.4 days or greater) compared to all other treatments (Table 1) so it is postulated that lack of nutrients, rather than dehydration, was the cause of earlier mortality. The longevity of *Microctonus* spp. adults has been shown previously to double or triple when supplied with sucrose-dominant nectars (Vattala et al. 2006).

Table 1 Comparison of mean longevity (days) of *Microctonus aethiopoides* adults held at 20°C and supplied with seasonal guttation fluid from perennial ryegrass cv. GA66 infected with one of three different endophyte strains or no endophyte (nil); or a control treatment (water, blank or sucrose solution).

Fluid type	Endophyte	2012				2013	
		1	2	3	4	5	6
		Summer	Autumn	Early winter	Mid-winter	Summer	Autumn
Ryegrass guttation fluid	AR1	6.0	5.7	6.1	5.6	4.9	6.3
	AR37	6.2	6.8	6.0	5.8	5.1	6.5
	Nil	6.0	5.5	5.9	4.9	5.5	6.1
	Standard	5.3	5.3	6.1	6.0	5.3	5.6
Water		6.1	5.4	6.5	6.1	5.2	5.3
None	Blank		6.2			4.3	5.4
10% sucrose							8.9
LSD 5%		1.0	1.1	1.1	0.9	1.4	2.1
P value		0.248	0.039	0.796	0.088	0.605	0.030

Table 2 Sugars detected by HPLC in guttation fluid collected and frozen on three dates from perennial ryegrass cv. GA66 with each of three different endophyte strains or no endophyte

Date	Endophyte	Sugar (ppm)			
		D-Fructose	D-Glucose	Sucrose	Trehalose
14/2/13[1]	Nil	67	40	-	7
	AR1	26	28	-	-
	AR37	66	65	-	-
	Standard	88	83	-	-
28/3/13	AR37	58	50	-	-
	Standard	35	20	-	-
5/2/14	Nil	19	12	-	-
	AR37	49	20	-	-

[1] Previously thawed for use during February 2013 assay then re-frozen

The lack of any difference in longevity between parasitoids exposed to fluids from the nil-endophyte and the peramine-producing endophytes (standard and AR1) suggests *M. aethiopoides* adults may not be sensitive to peramine, contrary to our hypothesis. Peramine is a feeding deterrent (Rowan 1993) that is secreted by ryegrass hydathodes into guttation fluid at a similar level as found in cut leaf fluid (Koulman et al. 2007). By depositing peramine onto the leaf surface, guttation fluid provides a first line of defence against some herbivores.

The largest run (25 replicates) conducted in autumn 2012 showed increased longevity in parasitoids provided with guttation fluid from ryegrass infected with endophyte strain AR37 compared with water or the standard endophyte, and this was echoed in the smaller run (8 replicates) the following autumn to give the significant interaction between the season when guttation fluid was collected and endophyte strain.

Sugar analysis

Analysis of sugars by either HPLC or colourimetric methods showed that ryegrass guttation fluid contains fructose and glucose, sugars commonly utilised by insects. The levels of sugars in the guttation fluid samples detected in the collected samples were present in concentrations of parts per million but, in the field, guttation fluid would evaporate so the nutrient concentration would become correspondingly concentrated. This source of nutrients would be of value to parasitoids and other sugar -feeding invertebrates in grassland ecosystems lacking nectar sources.

The HPLC data set presented in Table 2 is very small and only one set of insect-experiment samples (from February 2013) was used in the sugar analysis. Where comparisons could be made, there was considerable difference in fructose:glucose ratio and concentration for AR37 samples from February 2013 and the same time a year later i.e., February 2014. There was also considerable difference

Table 3 Comparison of HPLC and colourimetric assay results for guttation fluid collected on 14 February 2013 from perennial ryegrass cv. GA66 with each of three different endophyte strains or no endophyte (nil) then and frozen and thawed prior to analysis.

Sample	Sugar (ppm)							
	D-Fructose		D-Glucose		Sucrose		Trehalose	
	HPLC	Colorimetric	HPLC	Colorimetric	HPLC	Colorimetric	HPLC	Colorimetric
Nil	67	60	40	35	-	-	7	0.6
AR1	26	21	28	24	-	-	-	3.7
AR37	66	61	65	66	-	-	-	0.5
Standard	88	67	83	67	-	135	-	0

in fructose:glucose ratio and concentration of standard samples analysed one month apart. However, the guttation fluid results align with previously published data on sugar content in ryegrass foliage. Thom et al. (1989) showed that non-structural carbohydrates (starch and soluble sugars) in perennial ryegrass vary with season. A strong effect of endophyte strain on low molecular weight carbohydrates has been observed in the low carbohydrate ryegrass cultivar 'Fennema', with the AR37 strain causing higher sugar levels than the AR1 or standard strains or in the absence of endophyte (nil) (Rasmussen et al. 2007). However, more comprehensive studies are required to determine if the results suggesting potential beneficial effects of autumn guttation fluid from AR37-infected ryegrass on parasitoid longevity are reproducible and linked to sugar concentration and/or composition, and to explore if this effect can enhance the efficacy of these parasitoids in the field.

The colourimetric method generally produced similar sugar identification and concentration results to HPLC, and is quicker and less expensive to undertake than HPLC (Phillips et al. 2018). It can be successfully used for general studies of detecting sugar sources for parasitoids.

CONCLUSIONS

In summary, guttation fluid from perennial ryegrass infected with different strains of endophyte has been shown to have no detrimental effect on *M. aethiopoides* adult longevity. Instead, fluid from plants infected with the AR37 strain endophyte may contribute to parasitoid efficacy as a biocontrol agent by enhancing longevity in autumn.

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REFERENCES

Barker GM, Prestidge RA, Pottinger RP 1990. Utilization of an endophytic fungus for insect resistance in New Zealand ryegrass. Aspects of Applied Biology 24: 279-281.

Barker GM, Addison PJ 1997. Clavicipitaceous endophytic infection in ryegrass influences attack rate of the parasitoid *Microctonus hyperodae* (Hymenoptera: Braconidae, Euphorinae) in *Listronotus bonariensis* (Coleoptera: Curculionidae). Environmental Entomology 26: 416-420. https://doi.org/10.1093/ee/26.2.416

Bultman TL, Rodstrom JL, Radabaugh KR, VanDop JD, Librizzi JM, Longwell LL, Pulas C, Grant L, Sullivan TJ 2009. Influence of genetic variation in the fungal endophyte of a grass on an herbivore and its parasitoid. Entomologia Experimentalis et Applicata 130: 173-180. https://doi.org/10.1111/j.1570-7458.2008.00802.x

Cripps MG, Edwards GR, McKenzie SL 2013. Grass species and their fungal symbionts affect subsequent forage growth. Basic and Applied Ecology 14: 225-234. https://doi.org/10.1016/j.baae.2013.01.008

di Menna ME, Finch SC, Popay AJ, Smith BL. 2012. A review of the *Neotyphodium lolii/Lolium perenne* symbiosis and its associated effects on animal and plant health, with particular emphasis on ryegrass staggers. New Zealand Veterinary Journal.60:315-328. https://doi.org/10.1080/00480169.2012.697429

Fuchs B, Krischke M, Mueller MJ, Krauss J 2013. Peramine and lolitrem B from endophyte-grass associations cascade up the food chain. Journal of Chemical Ecology 39: 1385-1389. https://doi.org/10.1007/s10886-013-0364-2

Gerard PJ, Carswell KR, Eden TM 2013. Effect of black legume aphid *Aphis craccivora* honeydew on survival of the parasitoid *Microctonus aethiopoides*. New Zealand Plant Protection 66: 153-156. https://doi.org/10.30843/nzpp.2013.66.5578

Goldson SL, Proffitt JR, Fletcher LR, Baird DB 2000. Multitrophic interaction between the ryegrass *Lolium perenne*, its endophyte *Neotyphodium lolii*, the weevil pest *Listronotus bonariensis*, and its parasitoid *Microctonus hyperodae*. New Zealand Journal of Agricultural Research 43: 227-233. https://doi.org/10.1080/00288233.2000.9513423

Grunwald I, Rupprecht I, Schuster G, Kloppstech K 2003. Identification of guttation fluid proteins: the presence of pathogenesis-related proteins in non-infected barley plants. Physiologia Plantarum 119: 192-202. https://doi.org/10.1034/j.1399-3054.2003.00202.x

Hahn H, Huth W, Schoberlein W, Diepenbrock W 2003. Detection of endophytic fungi in *Festuca* spp. by means of tissue print immunoassay. Plant Breeding 122: 217-222. https://doi.org/10.1046/j.1439-0523.2003.00855.x

Hume DE, Cooper BM, Panckhurst KA 2009. The role of endophyte in determining the persistence and productivity of ryegrass, tall fescue and meadow fescue in Northland. Proceedings of the New Zealand Grassland Association 71: 145-150. https://doi.org/10.33584/jnzg.2009.71.2754

Kerr GA, Chapman DF, Thom ER, Matthew C, Linden Avd, Baird DB, Johnston E, Corkran JR 2012. Evaluating perennial ryegrass cultivars: improving testing. Proceedings of the New Zealand Grassland Association 74: 127-136. https://doi.org/10.33584/jnzg.2012.74.2875

Koulman A, Lane GA, Christensen MJ, Fraser K, Tapper BA 2007. Peramine and other fungal alkaloids are exuded in the guttation fluid of endophyte-infected grasses. Phytochemistry 68: 355-360. https://doi.org/10.1016/j.phytochem.2006.10.012

Koulman A, Lee TV, Fraser K, Johnson L, Arcus V, Lott JS, Rasmussen S, Lane G 2012. Identification of extracellular

siderophores and a related peptide from the endophytic fungus *Epichloë festucae* in culture and endophyte-infected *Lolium perenne*. Phytochemistry 75: 128-139. https://doi.org/10.1016/j.phytochem.2011.11.020

Latch GCM, Christensen MJ, Gaynor DL 1985. Aphid detection of endophyte infection in tall fescue. New Zealand Journal of Agricultural Research 28:129-132. https://doi.org/10.1080/00288233.1985.10427006

Meale SJ, Chaves AV, Hannah MC, Williams SRO, Hume DE, Mace WJ, Moate PJ 2013. Comparison of wild type, AR1 and AR37 endophyte infected perennial ryegrass on in vitro methanogenesis. Animal Feed Science and Technology 180: 10-17. https://doi.org/10.1016/j.anifeedsci.2012.12.003

Phillips CB 1998. Influence of liquid food on fecundity and longevity of *Microctonus hyperodae* Loan. Proceedings of the 51st New Zealand Plant Protection Conference: 23-27. https://doi.org/10.30843/nzpp.1998.51.11672

Phillips CB, Hiszczynska-Sawicka E, Iline II, Novoselov M, Jiao J, Richards NK, Hardwick S 2018. A modified enzymatic method for measuring insect sugars and the effect of storing samples in ethanol on subsequent trehalose measurements. Biological Control 126: 127-135. https://doi.org/10.1016/j.biocontrol.2018.08.008

Prestidge RA, Popay AJ, Ball OJP 1994. Biological control of pastoral pests using *Acremonium* spp. endophytes. Proceedings of the New Zealand Grassland Association 56: 33-38. https://doi.org/10.33584/jnzg.1994.56.2130

Rasmussen S, Parsons AJ, Bassett S, Christensen MJ, Hume DE, Johnson LJ, Johnson RD, Simpson WR, Stacke C, Voisey CR, Xue H, Newman JA 2007. High nitrogen supply and carbohydrate content reduce fungal endophyte and alkaloid concentration in *Lolium perenne*. New Phytologist 173: 787-797. https://doi.org/10.1111/j.1469-8137.2006.01960.x

Rowan DD 1993. Lolitrems, peramine and paxilline: mycotoxins of the ryegrass/endophyte interaction. Agriculture, Ecosystems & Environment 44: 103-122. https://doi.org/10.1016/0167-8809(93)90041-M

Ruppert KG, Matthew C, McKenzie CM, Popay AJ 2017. Impact of *Epichloë* endophytes on adult Argentine stem weevil damage to perennial ryegrass seedlings. Entomologia Experimentalis et Applicata 163: 328-337. https://doi.org/10.1111/eea.12584

Shepherd RW, Wagner GJ 2007. Phylloplane proteins: emerging defenses at the aerial frontline? Trends in Plant Science 12: 51-56. https://doi.org/10.1016/j.tplants.2006.12.003

Singh S 2013. Guttation: Path, principles and functions. Australian Journal of Botany 61: 497-515. https://doi.org/10.1071/BT12308

Thom ER, Sheath GW, Bryant AM 1989. Seasonal variations in total nonstructural carbohydrate and major element levels in perennial ryegrass and paspalum in a mixed pasture. New Zealand Journal of Agricultural Research 32: 157-165. https://doi.org/10.1080/00288233.1989.10423450

Thom ER, Popay AJ, Hume DE, Fletcher LR 2012. Evaluating the performance of endophytes in farm systems to improve farmer outcomes - a review. Crop & Pasture Science 63: 927-943. https://doi.org/10.1071/CP12152

Tozer KN, Chapman DF, Bell NL, Crush JR, King WM, Rennie GM, Wilson DJ, Mapp NR, Rossi L, Aalders LT, Cameron CA 2014. Botanical survey of perennial ryegrass-based dairy pastures in three regions of New Zealand: implications for ryegrass persistence. New Zealand Journal of Agricultural Research 57: 14-29. https://doi.org/10.1080/00288233.2013.863785

Urbaneja-Bernat P, Tena A, González-Cabrera J, Rodriguez-Saona C 2020. Plant guttation provides nutrient-rich food for insects: Guttation: a reliable insect food source. Proceedings of the Royal Society B: Biological Sciences 287. https://doi.org/10.1098/rspb.2020.1080

Urrutia C, M. A., Wade MR, Phillips CB, Wratten SD 2007. Influence of host diet on parasitoid fitness: Unravelling the complexity of a temperate pastoral agroecosystem. Entomologia Experimentalis et Applicata 123: 63-71. https://doi.org/10.1111/j.1570-7458.2007.00526.x

Vattala HD, Wratten SD, Phillips CB, Wäckers FL 2006. The influence of flower morphology and nectar quality on the longevity of a parasitoid biological control agent. Biological Control 39: 179-185. https://doi.org/10.1016/j.biocontrol.2006.06.003