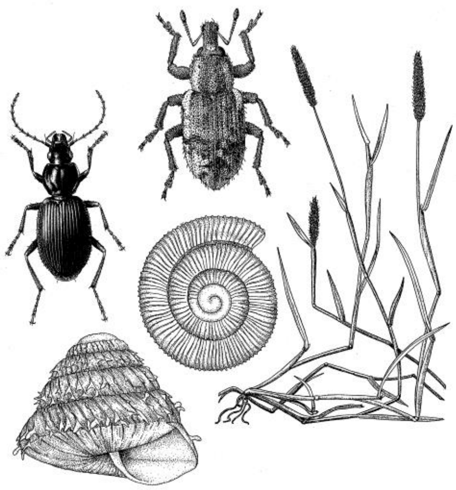
Evaluation of *Epichloë uncinata*-infected *Festulolium* for management of wireworms (Elateridae): 2. Response of wireworms to *Epichloë*-infected swards and soil amendments varying in loline concentration

Report prepared for Cropmark Seeds Ltd

by Gary M. Barker



G. M. Barker & Research Associates

The Invertebrate Biodiversity Specialists
Working in production agriculture and its interface with biodiversity conservation

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SUMMARY

This report describes two greenhouse pot experiments to examine the role of lolines in providing plant protection from wireworms.

Experiment 1 examined the response in two wireworm species (Potato wireworm *Hapatesus hirtus* Candeze, and Pasture wireworm *Conoderus excel* (Sharp)) to a gradient of loline concentration in soil amendments. The amendment of soil with loline-containing organic matter did not influence wheat plant establishment and foliage DM yields in the absence of wireworm infestation, suggesting neither the addition of high rates of organic matter to soil (20 t DM/ha equivalent in the upper 80 mm of the profile) nor the high concentrations of lolines (up to 25,000 µg/g) were detrimental to plant growth.

The addition of organic amendment improved wheat establishment and yield in the presence of wireworms when that amendment contained high concentrations of lolines. The results indicated that, in the presence of wireworms, a concentration of 25,000 µg/g lolines in the amendment (the maximum concentration evaluated) was necessary to achieve wheat establishment similar to that attained in the absence of wireworms. For wheat yields, the response was asymptotic, with little yield advantage above 12,500 µg/g lolines.

There were differential rates of wireworm survival in the pots (treatment means 68 to 98%) and varying numbers of wireworms may in part accounted for differences in wheat establishment and yield across amendment treatments.

The results suggest that amendment of soils with organic matter containing high concentrations of lolines prior to sowing crops such as wheat may enhance crop establishment and yield in the presence of wireworm infestation.

Experiment 2 examined Pasture wireworm survivorship and live-weight changes under swards of six plant x endophyte genotypes over a 26 week period. Survivorship and live-weights of Pasture wireworms were generally similar under swards of the six plant x endophyte genotypes. The only plant x endophyte genotypes effect was lower final live-weights in wireworms recovered from soil under Barrier *E. uncinata* U2, suggesting a possible role of root lolines in reducing larval growth.

The presence of wireworms did not influence herbage DM yield over the experimental period.

The loline concentrations in the roots of Barrier *E. uncinata* U2 during the course of the experiment was not recorded. Nonetheless, the results suggest that *E. uncinata* infection in grasses may influence the ecology of wireworms.

Conclusions. The results of these two experiments, coupled with an earlier assay with loline-containing semi-artificial diets, suggest that *Epichloë uncinata*-infected forage grasses may have a role in management of pestiferous wireworms in arable cropping systems, albeit possibly conditional on the wireworm species present. Specific R&D in cropping systems infested with wireworms is warranted.

INTRODUCTION

Because of their natural role in biological protection of the grass hosts, *Epichloë* endophytes are widely recognised as beneficial mycosymbionts in pastoral and turf systems. There is also considerable interest internationally in *Epichloë* endophytes in pest management in arable cropping systems.

Wireworms – the larvae of elatrid beetles – are important crop pests in many regions (see summary by Barker 2017). The influence of *Epichloë* infection in grasses on wireworm feeding ecology and the potential of *Epichloë*-infected grasses in the management of wireworm infestations are presently poorly understood. This report describes two greenhouse pot experiments to examine the role of lolines in providing plant protection from wireworms. These experiments build on an earlier assay that demonstrated that lolines in the diet may influence feeding in some wireworm species (Barker 2017).

MATERIALS AND METHODS

Experiment 1 - Response to a gradient of loline concentration in soil amendments

Experimental design

The experiment was performed using amendments containing a range of loline concentrations to simulate the incorporation of loline-containing organic matter into the soil at the rate of 20 t DM/ha. The amendments were prepared by mixing different ratios of loline-containing (*Epichloë uncinata*-infected) and loline-free (endophyte-free) *Festulolium* seed and incorporating into Koonwarra loam in 100 mm diameter x 100 mm deep plastic pots. Loline-containing seed (*Festulolium* FHCF0802U2, 2348M, 26,514 µg/g lolines) and loline-free seed (Ultra *Festulolium* URL10L1) were each finely ground in a domestic coffee grinder and added in varying ratios to yield five amendment treatments varying in loline concentration (Table 1). An additional treatment comprised soil with no amendment (total soil weight in the pots was equivalent to the amendment + soil in the amendment treatments). All pots received NPKS fertilizer at the kg/ha rate equivalent to 80 N, 36 P, 50 K, 25 S, mixed through the soil at the time the amendments were incorporated.

Two species of wireworms were collected in sufficient numbers during February 2016 at Thropdale, Victoria, Australia to be included in the experiment, namely the Potato wireworm (*Hapatesus hirtus* Candeze) (Neboiss 1962; Horne and Horne 1991), and the Pasture wireworm (*Conoderus exsul* (Sharp)) (Stone 1980; Stone and Wilcox 1979; Doane et al. 1985; Robertson et al. 1981; Robertson 1987; Williams and Galbreath 1987). Wireworms were collected using bait traps placed in potato and weedy fallow fields (following potato crops) for 10-11 days. Traps consisted of 650 ml plastic pots filled with moistened vermiculite and a 60 g mix pre-germinated maize and wheat seeds (1:1) as baits, buried in the ground, covered with soil and a plastic lid covered with a second soil layer; the plastic pots had ten 15mm diameter holes cut in the sides to allow wireworm entry. Wireworms were identified principally by the shape of last abdominal segment and its arrangement of setae (van

Zwaltjwenburg 1939; Neboiss 1962; LN Robertson and GM Barker, unpubl.). Only healthy wireworms with head capsule width above 1.0mm were utilized.

Two days after incorporation of the organic matter amendment treatments, five Potato wireworm were add to each of 10 replicate pots, five Pasture wireworms were added to a further 10 replicate pots, and a further 10 pots received no wireworms. Only five wireworms were added to individual pots to minimise mortality to combat and cannibalism. The pots were arranged in a randomised block layout on the greenhouse bench.

After 30 days, 10 seeds of Harper (Intergrain) APW wheat were sown in each pot. A germination test with 100 seeds on moistened filter paper in Petri dishes indicated 99% seed viability.

After a further 30 days, the pots were destructively harvested. The numbers of wireworms surviving were counted, the numbers of established wheat plants were counted, and wheat foliage harvested by cutting at soil level with a scalpel and dried at 60 °C for DM yields.

Table 1. Experiment 1: Organic amendments added to soil, made from mixing different ratios of finely ground loline-containing (*Neotyphodium uncinatum*-infected) (26,514 µg/g total lolines) and loline-free (endophyte-free) Festulolium seed.

Treatment	Loline concentration in amendment	Weight of amendment added to soil	
	µg/g	g DM/pot	kg DM/ha
1	25,000	15	20
2	12,500	15	20
3	6,250	15	20
4	3,125	15	20
5	0	15	20
6	0	0	0

Statistical analyses

Numbers of wireworm, numbers of wheat plants, and wheat foliage yields at close of the experiment were analysed by ANOVA in S-Plus version 4. In cases of non-homogeneity in variances, square root transformations were applied to insect count and plant data prior to analyses.

Experiment 2 – Wireworm survivorship and live-weight change under swards of six plant x endophyte genotypes

Experimental design

Seed was received from Cropmark Seeds Ltd in August 2015 and held at 4°C in small air-tight plastic drums. Seed was sown into root trainers in steam-sterilised Osmocote-amended Koonwarra loam soil-sand (1:1) potting medium, and maintained in the greenhouse at Stony Creek, Gippsland,

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Victoria. Because endophyte infection of sown seed was not expected to be 100%, *Epichloë* infection status in individual plants was determined at 6 weeks of age by microscopic examination of leaf sheaths from two tillers per plant stained in Aniline blue and mounted temporarily on glass slides. Any plant not conforming to the expected endophyte status was discarded. In early March 2016 these plants were transplanted on a 60 x 50 mm grid into Osmocote-amended Koonwarra loam soil-sand (1:1) potting medium in 300 x 250 mm x 150 deep plastic nursery trays to give monocultures of each of six plant x endophyte genotypes (Table 2). Each of the 10 replicates were maintained in the greenhouse with watering and trimming, and received periodic application of nutrient solution (Yates Thrive All Purpose Liquid Plant Food, 12.4 : 2.7 : 6.2 NPK) as required to produce vigorous micro-swards.

Wireworms were obtained in March 2016 from cropping areas in Thorpdale, Victoria using bait traps as described in Experiment 1. Only one species of wireworm was collected in sufficient numbers for the experiment, namely Pasture wireworm (*Conoderus exsul* (Sharp)). The collected wireworms were weighed, and 10 randomly-allocated insects added to five replicates of each plant x endophyte genotype treatment. To avoid the results being influenced by possible pupation of larger larvae during the experiment, only wireworms with head capsule width between 0.85 and 1.60 mm were used. The remaining five replicates of each treatment were maintained as insect-free (no wireworm) controls. The

Table 2. Seedlines used in Experiment 2, with details of *Epichloë*-infection and alkaloid status.

Treatment	Plant and endophyte genotypes	Seed infection with *Epichloë* (%)	Alkaloid spectra
1. Barrier Endophyte-free	Barrier *Festulolium* (FhCF0802)[a] (Bc142), endophyte free	0	Nil
2. Barrier *E. uncinata* U2	Barrier *Festulolium* (FhCF0802)[b] (Bc142B), *E. uncinata* strain U2	95	Lolines
3. Samson Endophyte-free	Samson *Lolium perenne*, endophyte free	0	Nil
4. Samson *E. festucae* var. *lolii* AR37	Samson *Lolium perenne*, *E. festucae* var. *lolii* strain AR37	95	Epoxy-Janthitrem
5. Samson *E. festucae* var. *lolii* SE	Samson *Lolium perenne*, *E. festucae* var. *lolii* strain Wild Type	75	Ergovaline, Peramine
6. Matrix *E. festucae* var. *lolii* SE	Matrix *Festulolium*[c] , *E. festucae* var. *lolii* strain Wild Type	98	Ergovaline, Peramine

Seedlines: a = Bc142, b = Bc142B, c = MTX1301

The micro-swards were periodically trimmed to ~40 mm height and all clippings dried in an oven at ~60 and weighed for estimates of foliage yields. At completion of the experiment on 28 August 2016, all plants were destructively harvested, with foliage yield measurements taken by again trimming to ~40 mm height. The potting medium was then thoroughly searched to recover all surviving wireworms, which then weighed.

Statistical analyses
Foliage yields, numbers of wireworms, and wireworm weights were analysed by ANOVA in S-Plus version 4. Homogeneity in variances meant that transformation of raw data was not required prior to analyses.

RESULTS

Experiment 1 - Response to a gradient of loline concentration in soil amendments

Emergence of the sown wheat was complete by Day 16, and by that stage amendment treatment effects were already evident with pots amended with organic material containing high concentrations of lolines exhibiting higher and more uniform numbers of seedling established, and those seedling present exhibiting higher vigour.

The expected numbers of wheat plants was 10/pot given the ~100% seed viability. In the absence of wireworms, the realised number of wheat plants established averaged 9.9/pot. Numbers of wheat plants established in the pots varied among amendment treatments ($F_{5, 179} = 45.064$, $P < 0.001$), with differences depending on wireworm addition treatments ($F_{2, 179} = 477.930$, $P < 0.001$), with a significant amendment treatment x wireworm treatment interaction ($F_{10, 179} = 15.653$, $P < 0.001$).

In the presence of either Potato wireworm (*Hapatesus hirtus*) or Pasture wireworm (*Conoderus excel*), numbers of wheat plants increased with increase in the loline concentration of the organic amendment treatment (Table 3, Figure 1). In pots with Potato wireworm, numbers of wheat plants established was 5.9/pot where the amendment contained no lolines to 9.2/pot at 25,000 µg/g lolines. In pots with Pasture wireworm, numbers of wheat plants established averaged 4.1/pot where the amendment contained no lolines, and increased to 7.2/pot where the amendment contained 25,000 µg/g lolines.

Wheat yields were strongly influenced by amendment treatment ($F_{5, 179} = 14.963$, $P < 0.001$), with the wireworm-addition treatments ($F_{2, 179} = 191.632$, $P = 0.001$) and interactions between amendment and wireworm-addition treatments ($F_{10, 179} = 5.223$, $P < 0.001$). In the absence of wireworm infestation, yields did not vary about the amendment treatments (Table 3). However, in the presence of wireworms, higher foliage DM yields occurred in pots treated with amendment containing high loline concentration. In the presence of either Potato wireworm (*Hapatesus hirtus*) or Pasture wireworm (*Conoderus excel*) there were increases in wheat yields with increasing loline concentration of the amendment.

Numbers of live wireworms remaining in the pots at the close of the experiment varied among amendment treatments ($F_{5, 119} = 12.240$, $P < 0.001$) with lower numbers in pots amended with organic material containing the higher loline concentrations. This effect was more pronounced for the Potato wireworm than the Pasture wireworm (amendment treatment x wireworm species interaction: $F_{1, 119} = 2.459$, $P = 0.038$), with similar numbers of wireworms surviving in the pots treated with amendment without loline content (96 and 94% respectively) but declining to 68 and 86% of initial numbers wireworms, respectively, in the pots treated with the amendment containing 25,000 µg/g lolines (Table 3).

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Table 3. Experiment 1: Influence of loline-containing organic amendments, added to soil at 20t/ha equivalent, on numbers of wheat plants established, wheat foliage yields, and numbers of wireworms at close of the experiment.

Lolines in the amendment (µg/g)	Wheat plants (no./pot)			Wheat foliage yields (g DM/pot)			Wireworms (no./pot)	
	Without wireworms	Potato wireworm [2]	Pasture wireworm [2]	Without wireworms	Potato wireworm [2]	Pasture wireworm [2]	Potato wireworm [2]	Pasture wireworm [2]
25,000	9.9	9.2	7.2	10.46	8.55	6.73	3.4	4.3
12,500	10.0	8.9	6.2	10.43	8.12	6.49	3.6	4.4
6,250	9.9	6.9	6.1	10.24	6.55	6.10	4.3	4.7
3,125	9.8	5.8	5.9	10.49	4.53	5.41	4.9	4.8
0	9.9	5.9	4.1	10.17	3.66	4.48	4.8	4.7
0 [1]	9.9	6.1	4.0	10.45	3.64	4.57	4.9	4.6
Mean	9.9	7.1	5.6	10.37	5.79	5.61	4.3	4.6
$F_{5, 59}$	0.415	32.150	21.384	0.0716	18.567	4.650	11.099	3.236
P value	0.836	<0.001	<0.001	0.996	<0.001	0.001	<0.001	0.012
$LSD_{0.05}$	0.39	0.84	0.72	1.48	1.41	1.35	0.69	0.262

1. No amendment added
2. Statistical analyses within these wireworm-addition treatments based on square root transformation of raw data. Means and LSDs presented are back transformed values.

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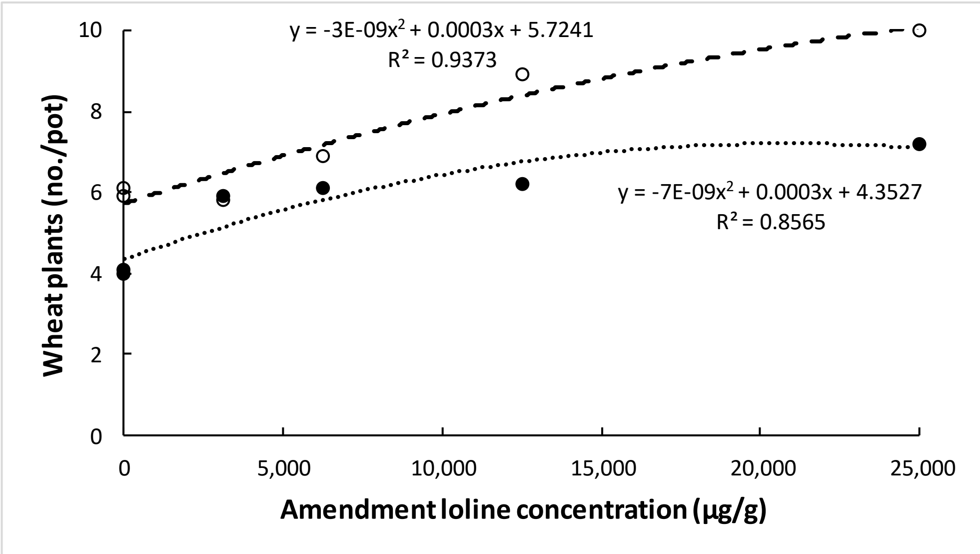

Figure 1. Numbers of wheat plants established in wireworm-infested pots amended with organic matter (at 20t DM/ha equivalent) containing a range of loline concentrations. O - pots infested with Potato wireworm (*Hapatesus hirtus*); ● - pots infested with pasture wireworm (*Conoderus excel*).

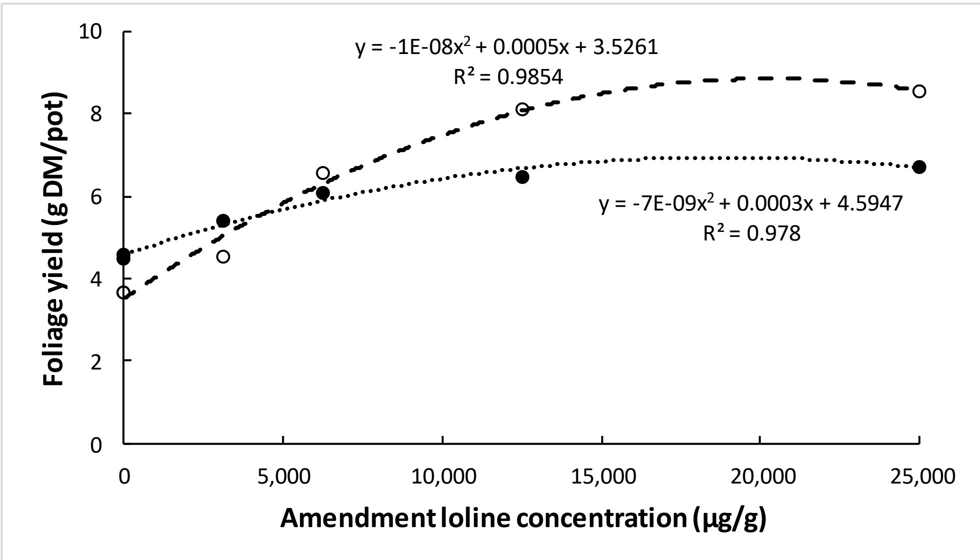

Figure 2. Foliage yields of wheat established in wireworm-infested pots amended with organic matter (at 20t DM/ha equivalent) containing a range of loline concentrations. O - pots infested with Potato wireworm (*Hapatesus hirtus*); ● - pots infested with pasture wireworm (*Conoderus excel*).

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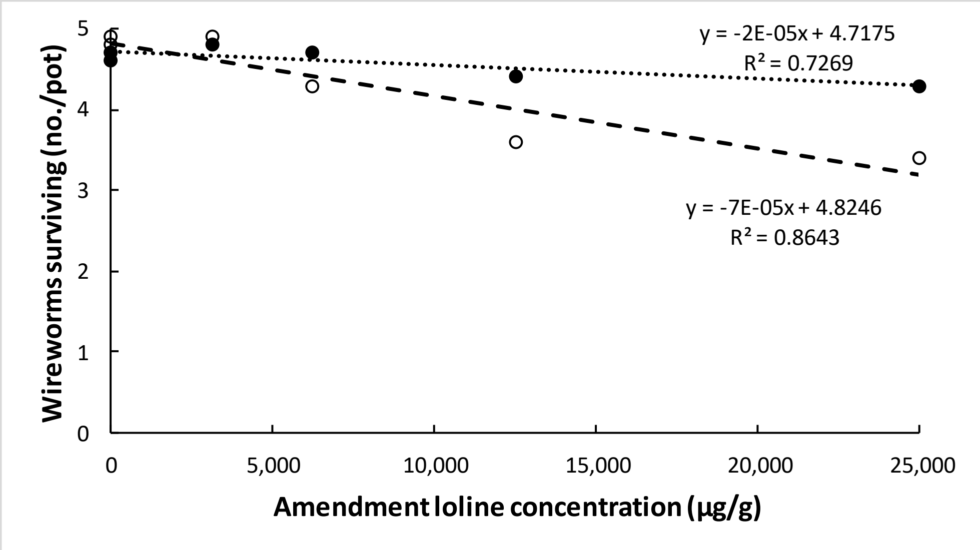

Figure 3. Numbers of wireworms surviving pots amended with organic matter (at 20t DM/ha equivalent) containing a range of loline concentrations. O - pots infested with Potato wireworm (*Hapatesus hirtus*); ● - pots infested with pasture wireworm (*Conoderus excel*).

Experiment 2 – Wireworm survivorship and live-weight change under swards of six plant x endophyte genotypes

Numbers of Pasture wireworms at the close of the experiment did not differ among plant x endophyte genotype treatments ($P>0.05$). Survival rate varied 83-88% across treatments. Wireworm live-weights were similar across all treatments at the beginning of the experiment but differed among plant x endophyte genotype treatments at the close of the experiment (Table 4). This effect was due solely to wireworms in the soil under Barrier *E. uncinata* U2 having 23% lower live-weight than wireworms in all other treatments.

Six harvests taken for foliage DM yields over the 26 week period from 17 March 2016 and 28 August 2016 showed no effects of plant x endophyte genotype treatments and wireworm infestation (Table 5).

Table 4. Abundance and live-weights of Pasture wireworm (*Conoderus excel*) recovered from monoculture micro-swards of different plant x endophyte genotypes.

Plant x endophyte genotype	Final wireworm density no./micro-sward	Wireworm live-weight (mg)	
		Initial	Final
1. Barrier Endophyte-free	8.3	13.42	42.26
2. Barrier *E. uncinata* U2	8.5	13.12	32.04
3. Samson Endophyte-free	8.8	14.30	41.50
4. Samson *E. festucae* var. *lolii* AR37	8.7	13.64	42.86
5. Samson *E. festucae* var. *lolii* SE	8.5	13.02	39.14
6. Matrix *E. festucae* var. *lolii* SE	8.6	13.60	42.16
$F_{5, 29}$	0.425	0.532	15.602
P-values	0.826	0.749	P<0.001
$LSD_{0.05}$	0.60	1.31	2.68

Table 5. Accumulative foliage yields of micro-swards of different plant x endophyte genotypes in presence and absence of Pasture wireworm (*Conoderus excel*).

	Foliage DM yield (g/micro-sward)[1]	
Plant x endophyte genotype	**Insect-free**	**With wireworms**
1. Barrier Endophyte-free	125.2	122.8
2. Barrier *E. uncinata* U2	126.6	123.8
3. Samson Endophyte-free	125.9	125.4
4. Samson *E. festucae* var. *lolii* AR37	126.7	125.1
5. Samson *E. festucae* var. *lolii* SE	125.6	123.3
6. Matrix *E. festucae* var. *lolii* SE	129.2	126.5
$F_{5, 59}$ plant x endophyte genotype	0.964	
P-value	0.448	
$F_{1, 59}$ wireworm treatment	3.240	
P-value	0.078	
$LSD_{0.05}$	4.09	

1. Total yield over six harvests between 17 March 2016 and 28 August 2016

DISCUSSION AND CONCLUSIONS

Experiment 1 - Response to a gradient of loline concentration in soil amendments

The amendment of soil with loline-containing organic matter did not influence wheat plant establishment and foliage DM yields in the absence of wireworm infestation, suggesting neither the addition of high rates of organic matter addition to the soil (20 t DM/ha equivalent in the upper 80 mm of the profile) nor the high concentrations of lolines (up to 25,000 µg/g) were detrimental to plant growth.

The addition of organic amendment improved wheat establishment and yield in the presence of wireworms when that amendment contained high concentrations of lolines. The results indicated that, in the presence of wireworms, a concentration of 25,000 µg/g lolines in the amendment (the maximum concentration evaluated) was necessary to achieve wheat establishment similar to that attained in the absence of wireworms. For wheat yields, the response was asymptotic, with little yield advantage above 12,500 µg/g lolines.

There was differential rates of wireworm survival in the pots (treatment means 68 to 98%) and varying numbers of wireworms may in part accounted for differences in wheat establishment and yield across amendment treatments. The experimental design did not enable assessment of when wireworm mortalities occurred relative to the 30 day pre-sowing and 30-day post-sowing periods of the experiment. Nonetheless, the results suggest that amendment of soils with organic matter containing high concentrations of lolines prior to sowing crops such as wheat may enhance crop establishment and yield in the presence of wireworm infestation.

Experiment 2 - Wireworm survivorship and live-weight change under swards of six plant x endophyte genotypes

Survivorship and live-weights of Pasture wireworms (*Conoderus excel*) were similar under swards of six plant x endophyte genotypes. The only plant x endophyte genotypes effect was lower final live-weights in wireworms recovered from soil under Barrier *E. uncinata* U2, suggesting a possible role of root lolines. The loline concentrations in the roots of Barrier *E. uncinata* U2 during the course of the experiment was not recorded and therefore cannot be directly compared to the loline concentrations of the soil amendments in Experiment 1 or with those in the semi-artificial diet assay reported by Barker (2017). The concentrations of lolines documented in roots of meadow fescue (*Festuca pratensis*) and *Festulolium* infected with *E. uncinata* (Patchett et al. 2008, 2011, Barker et al. 2015) are within the range of concentrations used in the semi-artificial diet study of Barker (2017), but the diet study failed to detect a feeding response in Pasture wireworm to loline concentration.

Conclusions. The results of these two experiments, coupled with an earlier assay with loline-containing semi-artificial diets, suggest that *Epichloë uncinata*-infected forage grasses may have a role in management of pestiferous wireworms in arable cropping systems, albeit possibly conditional on the wireworm species present. Specific R&D in cropping systems infested with wireworms is warranted.

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