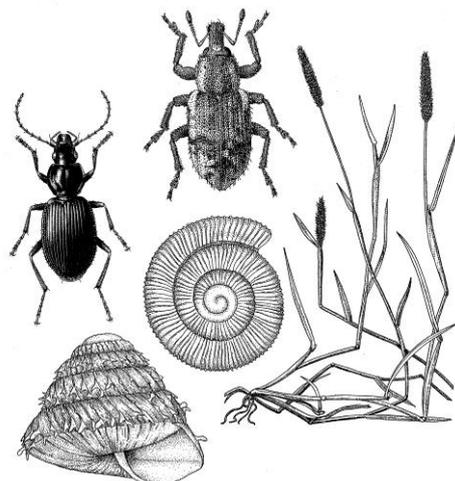


**Evaluation of *Epichloë uncinata*-infected *Festulolium* for  
management of wireworms (Elateridae):  
1. Response of wireworms to loline concentration in the diet**

**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  
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## SUMMARY

A laboratory experiment was conducted with loline-containing artificial diets to determine the response of three species of wireworms (click beetle larvae: Elatridae) to a gradient of loline alkaloids in their diet.

Potato wireworm *Hapatesus hirtus*, was highly responsive to the concentration of lolines in the diet, with decline in feeding with increasing loline concentration. The extent of feeding was linearly related to the square root of loline concentration in the diet, suggesting that the loline suppressive effect on feeding developed rapidly at the lower end of the loline concentration gradient and additional suppression at the higher end of the gradient was modest: at loline concentrations of 2800 and 5600 µg/g, quantities of diet consumed by Potato wireworm was reduced by 52% relative to diet without lolines.

Quantities of diet consumed by Pasture wireworm *Conoderus exsul* and Variable wireworm *Agrypnus variabilis* were not influenced by presence of lolines, irrespective of the alkaloid concentration.

These data indicate that forage grasses infected with *Epichloë* endophytes and supporting lolines in the roots may offer potential for management of wireworm pests in cropping systems, although utility may be dependent on the identity of the wireworms in specific cropping situations. While use of grasses as cover crops in cropping rotations has been discouraged, the results indicate that *Epichloë*-infected, loline-bearing forage grasses should be investigated as a component of arable rotations as a strategy to suppress wireworm infestations in susceptible crops can be suppressed. Lolines in live roots, and lolines in soil organic matter after incorporating the cover crop by cultivation, may for example have important roles in the feeding ecology and population dynamics of wireworms and warrant investigation.

## 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 considerable interest internationally in the development of forage and turf grasses infected with *Epichloë* endophytes. Understanding the role of different alkaloids in protecting plants against various herbivorous pests is critical to development of endophyte-containing grasses for commercial use.

Cropmark Seeds Ltd. has been developing forage grasses based on Meadow fescue (*Festuca pratensis* Hudson) and its loline-producing endophyte *Epichloë uncinata* (W. Gams, Petrini & D. Schmidt) Leucht. & Schardl. because of potential agronomic advantages, not least pest resistance and tolerance.

Wireworms are the larvae of click beetles (Coleoptera: Elateridae). More than 9,000 species of click beetles are known and the wireworms are important pests in a wide variety of crops, such as potato, sweet potato, cereals, corn/maize, carrot, sugar beet, sugarcane, sunflower, soft fruits including strawberries, and seedlings of brassicas, pulses etc. Reviews of wireworms in agricultural cropping systems have been provided by Jary (1942), Miles (1942), Vernon et al. (2004), Barsics et al. (2013), Traugott et al. (2015) and others. The species of importance in agriculture varies regionally. More than 200 species of genus *Agriotes* Eschscholtz occur worldwide and are important pests principally in Europe, and as introduced species in North America. Wireworms of the genus *Melanotus* Eschscholtz are well studied pests in Japan and in the USA. The “Pacific coast” wireworm, *Limonius canus* Leconte, is responsible, along with other species of the genus, for crop damage along the western coast of North America. Other genera important for their impact on agriculture are *Athous* Eschscholtz, *Conoderus* Eschscholtz, *Ctenicera* Latreille and *Hypolithus* Eschscholtz. The main effects of wireworm feeding on basal hypocotyl/coleoptile and belowground plant organs are seedling mortality, and all implied yield losses, and damage to tubers and consequent losses in tuber quality.

Despite the numerous studies conducted to improve their management, wireworms remain important pests. The mainstay of wireworm management has been soil-applied pesticides. Due to often poor performance of pesticides, and increasing concerns about their environmental safety, multiple alternatives to broadcast application of pesticides have been examined worldwide. Nonetheless, no satisfactory non-pesticidal control is currently available.

Wireworms are predominantly phytophagous, with an influence of the type of vegetable food on growth rates (Evans and Gough 1942). The youngest larvae need live vegetable material to grow (Evans and Gough 1942, Furlan 1998, 2004), while older larvae may persist on soil organic matter. Besides crops, weeds and general soil organic matter, wireworms also feed on animal prey and in some agricultural systems are important predators of other crop pests.

Various volatile chemicals emitted by food plants or germinating seeds affect wireworm movements (Thorpe et al. 1946; Klingler 1957; Doane et al. 1975; Horton and Landolt 2002; Johnson and Gregory 2006; Barsics et al. 2014, 2016), but perception of natural chemicals associated with the host plant are known to vary accordingly to physiological condition of the plant, physiological differences between wireworm species (Johnson and Gregory 2006) and the nutritional state of individual insects. There is some evidence that food location by wireworms has a high element of chance encounter (e.g. Chaton et al. 2003). Furthermore, the high density of roots question efficiency of CO<sub>2</sub> and some common volatiles as a local host-locator, particularly for specialized herbivores in mixed plant communities (Thorpe et al. 1946). The perception of host specific semiochemical(s), such as

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volatiles, is thought to elicit recognition of different host species (Johnson and Gregory 2006) but there are insufficient data to make generalizations across the great diversity of wireworm species. In the belowground interactions, it should be noted that there is a trend for low molecular weight compounds (e.g., alcohols, esters, aldehydes) to have 'attractant' properties, while hydrocarbons tend to be 'repellent' (Johnson and Nielsen 2012). The final orientation step involves chemosensory cues at the root surface inducing acceptance or rejection.

Wireworms are very common in grasslands worldwide. Varietal differences in susceptibility to wireworms are known in crop species (e.g. Johnson et al. 2008) but there has been very limited studies of forage grasses. Hemerik and de Fluiter (1999) were unable to find preferences among four grass species by *Agriotes lineatus* (L.), but Hemerik et al. (2003) documented preference, as indicated by their location in the respective root zones in a choice experiment, of *Agriotes obscurus* (L.) and *Agriotes haemorrhoidalis* (Fab.) for two nutrient-rich grassland plant species over to two nutrient-poor ones. Among cultural practices to reduce wireworm damage in crops, it is generally recommended that inclusion of grass swards in the crop rotation be avoided, since it promotes oviposition, egg and larval survival and is generally correlated with further crop damage (Balachowsky and Mesnil 1936; Gough and Evans 1942; Jary 1942; Miles 1942; Parker and Seeney 1997; Keiser et al. 2012). The influence of *Epichloë* infection in grasses on wireworm feeding ecology is presently not unknown.

This report describes an assay to examine the role of lolines in providing plant protection from wireworms.

## MATERIALS AND METHODS

### *Experimental design*

An assay was performed using artificial diets containing a range of loline concentrations. In the absence of the availability of analytical grade lolines, the diets were prepared by incorporating different ratios of loline-containing (*Epichloë uncinata*-infected) and loline-free (endophyte-free) grass seed into the agar-based diets. Loline-containing seed (*Festulolium* FHCF0802U2, 2348M, 26514 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 molten (50 °C) 4% agar in tap water to yield seven treatments varying in loline concentration (Table 1). The molten diet thus prepared was poured into Petri dishes and held at 4 °C to solidify.

As wireworms may orient towards carbon dioxide and volatile plant root exudates by klinotaxis, this behaviour was utilized in the experiment to enhance insect feeding on the diets. In 20 replicate diet plugs of each loline treatment, a single *Festulolium* seedling was grown in each diet plug to enhance likelihood of insect feeding on the diet plugs [seedling- augmented]; the remaining 20 replicates were not seeded [non-augmented]. Endophyte-free seeds (Ultra *Festulolium* URL10L1), lacking loline content, were surface sterilised by soaking in 2.5% sodium hypochlorite solution for 15 min followed by 5 rinses in sterilised distilled water, germinated in Petri dishes on filter paper moistened with sterile tap water, and then placed singly in a small cavity made in prepared diet plugs using a sterilized scalpel. Seedling-augmented and non-augmented diet plugs were placed in a 50mm deep bed of moistened vermiculite in 200ml screw-top plastic jars. As the lolines may influence seedling development, 20 additional replicates of each diet were set up without wireworms.

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Three species of wireworms were collected in sufficient numbers during November 2015 at Thropdale, Victoria, Australia to be included in the experiment, namely the Potato wireworm (*Hapatesus hirtus* Candeze) (Neboiss 1962; Horne and Horne 1991), 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), and Variable wireworm (*Agrypnus variabilis* (Candeze)) (Robertson et al. 1981; Doane et al. 1985; 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.). The collected wireworms were held for 24 hours without food before use in the experiment; only healthy wireworms with head capsule width above 1.0mm were utilized. Each replicate comprised a single wireworm placed in a 200ml plastic jar, randomly assigned to treatment, and provided with a single pre-weighted plug of diet taken from the appropriate stock diet with a 15mm cork borer. For each wireworm species, each loline treatment had 40 replicates, one half of which the diet plugs supported a germinating *Festulolium* seed [seedling-augmented], arranged on the laboratory bench in a randomised block design.

Twenty additional diet plugs were held in jars as described above, but without wireworms, to determine weight loss in the absence of insect feeding. This weight loss was used as a correction factor in estimates of amount of diet consumed by wireworms during the experiment. Prepared diets had 25% dry matter content.

**Table 1. Experiment 1: Artificial diets made from mixing into 4% agar different ratios of finely ground loline-containing (*Neotyphodium uncinatum*-infected) (26514 µg/g total lolines) and loline-free (endophyte-free) grass seed.**

Treatment	Loline concentration in diet (µg/g)	Weight of loline-containing seed (g/100g diet)	Weight of loline-free seed (g/100g diet)
1	5600	21.20	0
2	2800	10.60	10.60
3	1400	5.30	15.90
4	700	2.65	18.55
5	350	1.32	19.88
6	175	0.66	20.54
7	0	0.00	21.20

After 96 hours the amount of diet consumed was estimated as 'initial diet weight' minus 'final diet weight' corrected for diet weight loss in the absence of wireworms.

#### *Statistical analyses*

Quantities of diet consumed by wireworms were analysed by ANOVA in S-Plus version 4. Homogeneity in variances meant no transformations of data were required prior to analyses.

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## RESULTS

Feeding by Potato wireworm *Hapatesus hirtus* on the diet plugs was influenced strongly by both loline concentration and seedling-augmentation, i.e. presence/absence of a germinating *Festulolium* seed upon the diet plug. Seedling-augmentation increased feeding on the diet plugs on average by 27% relative to diet plugs without seedling augmentation ( $F_{1, 269} = 68.742$ ,  $P < 0.001$ ). Nonetheless, the loline concentration effect was similar irrespective of this diet plug treatment (loline x diet plug treatment interaction:  $F_{6, 269} = 2.188$ ,  $P = 0.044$ ). Thus the loline concentration results presented in Table 2 are the averages across the seedling-augmented and non-augmented plug treatments.

**Table 2. Quantities of on agar-based diet, containing varying concentrations of loline alkaloids, consumed by three species of wireworms.**

Treatment	Loline concentration ( $\mu\text{g/g}$ )	Diet consumed (mg) <sup>1</sup>		
		<i>Hapatesus hirtus</i>	<i>Conoderus exsul</i>	<i>Agrypnus variabilis</i>
1	5600	695	1102	828
2	2800	723	1032	847
3	1400	885	1094	787
4	700	1058	1152	823
5	350	1098	1038	871
6	175	1344	1143	878
7	0	1477	1176	811
<b>Mean</b>		1040	1105	836
<b>Treatment effects</b>				
	$F_{6, 279}$	57.452	1.512	0.432
	P value	<0.001	<0.731	0.857
	Fisher's $\text{LSD}_{0.05}$	116.4	146.0	145.2

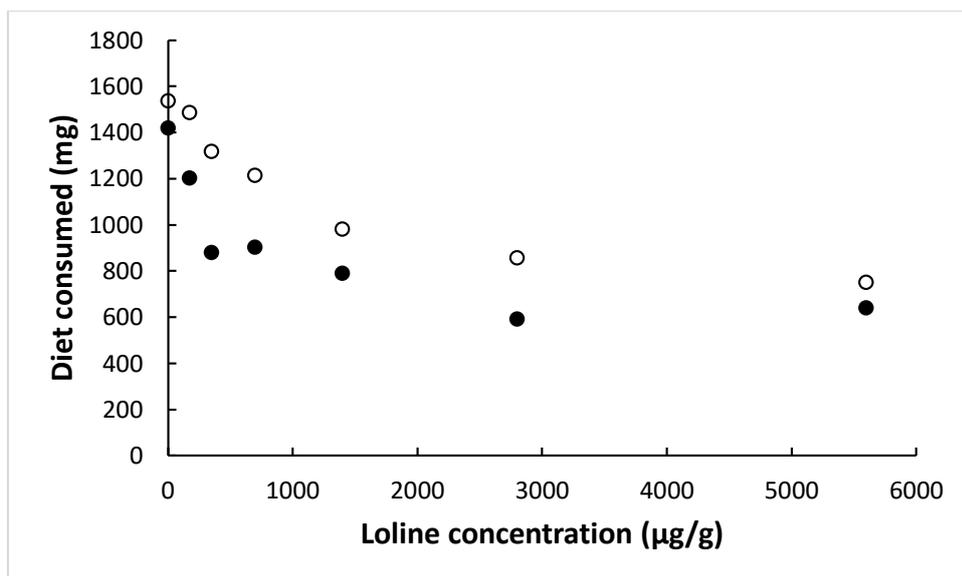
<sup>1</sup>. Values presented are the averages of diet plugs with and without seedling augmentation.

Pooling diet plug treatments, the relationship between quantity of diet consumed by Potato wireworm and loline concentration (Figure 1) was best described as:

$$\text{Diet consumed (mg)} = -11.082(\text{square root loline concentration}) + 1395.7 \quad R^2 = 0.8852$$

In the case of Pasture wireworm *Conoderus exsul*, feeding as influenced neither by seedling augmentation of the diet plugs ( $F_{1, 269} = 2.169$ ,  $P = 0.142$ ) nor influenced by loline concentration (Table 2).

Variable wireworm *Agrypnus variabilis* exhibited similar levels of feeding on the diet plugs irrespective of the seedling augmentation of the diet plugs ( $t_{(F_{1, 269} = 0.461, P = 0.498)}$ ) and loline concentration ( $F_{6, 279} = 0.431$ ,  $P = 0.857$ ) (Table 2).



**Figure 1.** Graphic of quantities of diet consumed by Potato wireworm *Hapatesus hirtus* in relation to loline concentration in the diet. ● diet plugs without germinating *Festulolium* seed; ○ diet plugs augmented with germinating *Festulolium* seed.

## DISCUSSION AND CONCLUSIONS

The assay with semi-artificial diet showed that one of three of the included wireworm species, namely Potato wireworm *Hapatesus hirtus*, was highly responsive to the concentration of lolines in the diet, with decline in feeding with increasing loline concentration. At loline concentrations of 2800 and 5600 µg/g, quantities of diet consumed by Potato wireworm was reduced by 52% relative to diet without lolines. Quantities of diet consumed by Pasture wireworm *Conoderus exsul* and Variable wireworm *Agrypnus variabilis* were not influenced by presence of lolines, irrespective of the alkaloid concentration.

These data indicate that forage grasses infected with *Epichloë* endophytes and supporting lolines in the roots may offer potential for management of at least some wireworm pests in cropping systems. For example, *Epichloë*-infected, loline-bearing forage grasses should be investigated as a component of arable rotations with a view to reducing levels of wireworm infestation in susceptible crops. Lolines in live roots, and lolines in soil organic matter after incorporating the cover crop by cultivation, may have important roles in the feeding ecology and population dynamics of wireworms and warrant investigation as potential mechanisms by which wireworm numbers might be suppressed in cropping systems.

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