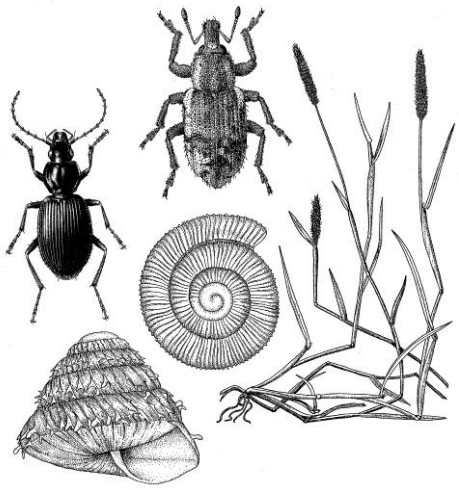
Effects *of Neotyphodium uncinatum* infected, loline-containing, meadow fescue–ryegrass hybrid grasses on the feeding behaviour of black beetle and red-headed pasture cockchafer.

2. Laboratory assays with adult black beetle.

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

Laboratory assay experiments were conducted to provide data on the role of *Neotyphodium* infection in the feeding behaviour in adult black beetle (*Heteronychus arator*). Assays examined beetle damage to excised and intact tillers from mature plants, and to seedlings. The incidence of feeding on tillers and seedlings was too low to yield useful information. The nature of the damage that did occur to tillers and seedlings was consistent with that observed in the field.

In view of the uncertainties about requisite experimental conditions needed to obtain useful data on black beetle adult feeding on tillers and seedlings under laboratory conditions, it was decided not to repeat the experiments. Rather, it is proposed that the incidence of adult black beetle feeding damage to seedlings be assessed in field plot trials. To this end, assessments will be made in trials established in autumn 2011 by Cropmark Seeds at Silverdale Road, Hamilton.

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INTRODUCTION

Because of their natural role in biological protection of the grass hosts, *Neotyphodium* 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 *Neotyphodium* 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*) and its loline-producing endophyte *Neotyphodium uncinatum* because of potential agronomic advantages, not least pest resistance and tolerance. Meadow fescue (*Festuca pratensis*) infected with *N. uncinatum* has been shown to deter attack from several pasture insects, as summarised in the first part of this report series to Cropmark Seeds Ltd (Barker, 2011). Among other attributes, Cropmark Seeds is particularly interested in the agronomic advantage of meadow fescue and hybrid cultivars in regions prone to the scarabaeids Black beetle (*Heteronychus arator*) and Red-headed pasture cockchafer (*Adoryphorus couloni*). Black beetle is a major pest of warm temperate pastures throughout southern Australia and northern New Zealand, while Red-headed pasture cockchafer is a major pasture pest in southeastern Australia.

This report describes laboratory assay experiments aimed at evaluating the role of *Neotyphodium uncinatum* in reducing feeding by the adult stage of black beetle on grass tillers and seedlings.

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MATERIALS AND METHODS

Experiments 1 and 2 examined feeding of black beetle adults on tillers of mature vegetative plants, using excised and intact tillers, respectively. These experiments were based on clonal plant material provided by Cropmark Seeds and initially sourced from a field plot trial at Morrinsville, Waikato, established to evaluate under commercial farming conditions the agronomic performance of loline-producing *Neotyphodium uncinatum*-infected forage grasses developed by plant breeders at Cropmark Seeds. Individual plants were shipped to Darfield, Canterbury, subdivided into clonal ramets and propagated in a gravelly sand (Osmocote amended) soil, before being shipped to Hamilton on 30 November and 6 December 2010 in polybags (no. 5). The plant material received from Cropmark Seeds comprises 50 individual plants of 8 *Neotyphodium uncinatum*-infected meadow fescue–ryegrass hybrids (each comprising 1 to 8 clonal lines) and 30 individual plants of a *Neotyphodium*-free clonal line of one of these meadow fescue–ryegrass hybrids (Table 1).

Experiment 3 examined feeding of black beetle on seedlings. Seed used in this experiment was provided by Cropmark Seeds (Table 2).

Adult black beetle for all experiments were collected from paspalum plants in a dairy pasture at Ruakura Agricultural Centre, Hamilton on 10 April 2011. These insects were maintained in the laboratory in 5 litre pots partially filled with Horotiu sandy loam soil and provided with carrot root as a food source.

Table 1. Indicative root and pseudostem loline concentrations (µg/g DM) in endophyte-free and presumitive *Neotyphodium uncinatum* U2 infected meadow fescue–ryegrass hybrid genotypes used in Experiments 1 and 2 as determined earlier during the plant propagation cycle (see Barker, 2011)

	Roots					Pseudostems				
	NFL	NAL	NANL	NML	Total	NFL	NAL	NANL	NML	Total
FhCF0802NIL	0	0	0	0	0	0	0	0	0	0
FhD25	0	0	0	0	0	0	0	0	0	0
FhCF0802	498	0	0	0	498	2657	288	382	243	3570
FhA106	0	0	0	0	0	0	0	0	0	0
FhB15	0	0	0	0	0	2843	416	451	225	3934
FhAB0802	1839	0	158	0	1997	13062	2834	1802	575	18273
FhC2	1303	0	0	0	1303	13000	2399	1784	629	17812
FhC148	865	0	0	0	865	12052	2869	1788	740	17449
LpE0802	801	0	0	0	801	12918	2690	1548	811	17967

Table 2. Loline concentrations (µg/g DM) and percent incidence of viable *Neotyphodium* endophyte in seed of meadow fescue–ryegrass hybrid genotypes used in Experiment 3.

	Loline concentrations in seed					% Viable
	NFL	NAL	NANL	NML	Total	endophyte
FHCF0802 U2	17521	5330	2743	919	26514	96
LPE0802 U2	15641	6423	2177	778	25020	100
FHCD0802 U10	12828	4752	2301	652	20532	100
FHAC0802 U5	15641	5164	9199	514	30518	96
FHAB0802 U2	21058	7341	3213	786	32398	100
FHAB0802 U8	16731	5846	5103	704	28385	92
FHCD0802 U6	16578	3942	4355	467	25343	93
LPAC0802 U10A	0	0	0	0	0	0
LPB0802 U5A	0	0	0	0	0	0
Tama NIL	NA	NA	NA	NA	NA	NA

NA = not analyzed

Experiment 1. Assay of excised tillers – adult black beetle

After several months maintenance in the glasshouse at The University of Waikato, tillers were harvested by cutting at soil level with a scalpel, trimmed to provide pseudstems of 35 to 40 mm length (tiller base with leaves trimmed to ~ 5 mm) and 20 placed in each of 20 replicate 500 ml plastic pots for each treatment. Two black beetle adults (1 male, 1 female) were added to ten replicate pots of each treatment, while a further 10 replicates served as insect free controls. The pots were placed in a randomised block design within a growth chamber, covered with moistened paper towels, and incubated at 20° C.

At Days 5, 10 and 15, the residual tiller material remaining was removed and replaced with freshly excised tillers. The residual material was examined for beetle damage and numbers of damaged tillers counted.

Experiment 2. Assay with intact tillers – adult black beetle

After several months maintenance in the glasshouse at The University of Waikato, a number of plants of each clonal line were broken up into ramets of 3-4 tillers and planted into individual cells in plastic propagation trays with Horotiu sandy loam potting medium.

After 4 weeks, for use in the experiment, the plants were lifted from the propagation trays, the root ball wrapped in 'OzEzy plastic warp' and presented to beetles in 500 ml plastic honey pots – the wrapping of the root ball prevented the beetles from feeding on the roots and thus potentially confounding the results, and allowed plants to remain in a live, healthy condition. Two black beetle adults (1 male, 1 female) were added to ten replicate pots of each grass genotype, while a further 10 replicates served as insect free controls.

The pots were covered with covered with a perforated plastic lid, and placed in a randomised block design on a laboratory bench at 18-20°.

At Days 2, 5, 10 and 15, the incidence and extent of black beetle feeding on the tillers was assessed visually.

Experiment 3. Potted seedling assay – adult black beetle

For each treatment, fifteen seeds were sown on 11 April 2011 in the central area of 20 replicate 5 litre pots filled with Horotiu sandy loam soil and the pots maintained the glasshouse at the University of Waikato in a randomized block design. Following emergence, the seedlings were thinned to 10 per pot. When the seedlings reached 30 mm in height, 2 adult black beetle (1 male, 1 female) were enclosed with the seedlings in one half of the replicates by pressing an inverted, perforated plastic vessel into the soil to a depth of 20 mm. Because of differences in seedling vigour among treatments, beetles were added to the plots for a particular treatment when the seedlings reached the requisite 30 mm height.

Lolium multiflorum cv. Grasslands Tama was included as the control treatment due the known high susceptibility of this ryegrass cultivar to black beetle feeding.

The pots were held in a randomised block on the glasshouse bench.

At Days 2, 5, 10, 15 and 25, the incidence and extent of black beetle feeding on the seedling was assessed visually.

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RESULTS

Experiments 1 and 2 – black beetle feeding on excised and intact tillers

The incidences of black beetle feeding on tillers in Experiments 1 and 2 are summarized in Table 3. So few tillers sustained feeding by beetles that the data obtained on incidence of damage were not analysed statistically.

Table 3. Experiments 1 and 2. Incidence of black beetle feeding on excised and intact tillers.			
	Total number of tillers damaged across 10 replicates		
	Expt. 1 Excised tillers		Expt. 2 Intact tillers
FhCF0802NIL	4		3
FhD25	0		0
FhCF0802	0		0
FhA106	6		3
FhB15	0		0
FhAB0802	1		0
FhC2	0		1
FhC148	0		0
LpE0802	0		1

Experiments 1 and 2 – black beetle feeding on excised and intact tillers

The incidence of black beetle feeding on seedlings in Experiment 3 is summarized in Table 4. Damage was sustained only in the endophyte-free LPAC0802 U10A and Tama. Nonetheless, the incidence of damage in seedlings was low and the data did not warrant statistical analyses

Table 4. Experiment 3. Incidence and extent of black beetle feeding on seedlings.	
	Mean number of seedlings damaged
FHCF0802 U2	0
LPE0802 U2	0
FHCD0802 U10	0
FHAC0802 U5	0
FHAB0802 U2	0
FHAB0802 U8	0
FHCD0802 U6	0
LPAC0802 U10A	0.2
LPB0802 U5A	0
Tama NIL	0.2

DISCUSSION

The experiments conducted failed to provide data on the role of *Neotyphodium* infection in the feeding behaviour of adult black beetle. Simply, the incidence of feeding on tillers and seedlings was too low to yield useful information.

Experiments 1 and 3, using excised tillers and seedlings respectively, were conducted as agreed with Cropmark Seeds, but failed to yield useful data. Experiment 2 was added as a repeat of Experiment 1 but using intact rather than excised tillers. This experiment too yielded inconclusive results.

The intent in Experiments 1-3 was to not only count the damaged tillers/seedlings, but also to score visually the extent of damage and then determine the dry weight differences between replicates with and without black beetle infestation. However, the incidence of beetle feeding was so slight as not to warrant visual scoring or weighing.

The nature of the damage that did occur to tillers and seedlings was consistent with that observed in the field.

It is unclear why black beetle adults used in these experiments exhibited little interest in feeding on tillers or seedlings. This was contrary to expectations based on field observations and previous experience in laboratory assays. The beetles feed readily on carrot, so there was no evidence for cessation of feeding in these overwintering insects. It is possible that the beetles are highly selective of what tillers or seedlings they will feed on and requisite tiller/seedling quality may not have been achieved in these experiments. Further, it is possible that individual beetles vary greatly in their propensity to feed on tillers or seedlings and much higher beetle numbers may be needed in experiments to ensure at least some data on incidence of damage is obtained.

In view of the uncertainties about requisite experimental conditions needed to obtain useful data on black beetle adult feeding on tillers and seedlings under laboratory conditions, it was decided not to repeat the experiments. Rather, it is proposed that the incidence of adult black beetle feeding damage to seedlings be assessed in field plot trials. To this end, assessments will be made in trials established in autumn 2011 by Cropmark Seeds at Silverdale Road, Hamilton.

REFERENCE

Barker, G.M. 2011. Effects of *Neotyphodium uncinatum* infected, loline-containing, meadow fescue–ryegrass hybrid grasses on the feeding behaviour of black beetle and red-headed pasture cockchafer larvae. 1. Assays with excised roots and potted plants. Report to Cropmark Seeds Ltd. G. M. Barker & Research Associates, March 2011, 20 p.