EFFECT OF TALL FESCUE AND RYEGRASS ENDOPHYTES ON ARGENTINE STEM WEEVIL

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SUMMARY

The effect of endophyte infection in tall fescue *(Festuca arundinacea)* and perennial ryegrass *(Lolium perenne)* on feeding and oviposition by adult Argentine stem weevil *(Listronotus bonariensis)* was studied in laboratory experiments. Weevils were responsive to endophyte infection in both host plant species. In choice situations weevils preferred endophyte-free plants for feeding and oviposition. In non-choice situations feeding and oviposition were reduced in weevils confined on endophyte-infected plants compared to those confined on endophyte-free plants.

INTRODUCTION

Argentine stem weevil is a serious pest of pasture grasses in New Zealand. There are considerable differences between grass species and cultivars in oviposition preferences by adult weevils and tolerances to larval damage (Kain *et al* 1981, 1982; Goldson 1982). In recent years much effort has been directed toward identification and breeding of ryegrass ecotypes and cultivars of superior resistance to Argentine stem weevil. Recently, an association has been noted between endophytic fungal infection and field resistance in ryegrasses to Argentine stem weevil (Mortimer *et al* 1982; Prestidge *et al* 1982). This association was confirmed in laboratory experiments (Barker *et al* 1983).

Experiments reported in this paper were conducted to determine if the endophyte of tall fescue confers resistance to Argentine stem weevil similar to that in ryegrasses.

MATERIALS AND METHODS

Plant material

Seed of tall fescue was collected from a single endophyte-infected plant on a roadside in March 1982. Two seed lines of 'Ellett' perennial ryegrass were used: a 1982 harvest line of high endophyte infection (fresh Ellett), and a 1977 harvest line of non-viable endophyte infection (aged Ellett). The latter served as controls. Seeds of each were sown following treatment with captan (Yates Captan) fungicide (1.5 g ai/kg seed) or treatment with captan (same rate) and prochloraz (Sportak) fungicide (0.5 g ai/kg seed). Seed treatment with prochloraz has been shown to eliminate viable endophyte infection (Latch and Christensen 1982; Harvey *et al* 1982).

Plastic pots (200 ml) filled to capacity with sieved Horotiu sandy loam soil were sown with individual germinated seeds in April 1982. The pots were maintained in a screenhouse with natural light and temperature and frequent overhead and capillary bed watering, including nutrient solution. The plants were regularly trimmed to maintain vegetative growth.

All plants were examined for endophyte infection in December 1982. Tiller sheath tissue from each plant was stained in lactophenol cotton blue overnight, rinsed briefly in tap water, and mounted on microscope slide in glycerol jelly. Mounts were examined at 250X magnification on a compound microscope.

Insect material

Weevils from two widely separated localities were used in bioassays to determine if biotypes exist with regard to responses to endophyte. Weevils were swept from ryegrass/white clover pastures near Hamilton and Christchurch in January 1983. Dissections of weevil subsamples confirmed the reproductive status of both the Waikato and Canterbury weevils.

Feeding bioassay

Choice and non-choice feeding experiments were set up on 14 January 1983. Three centimeter long leaf segments were offered to adult weevils in 8 cm dia. petri dishes provided with a moistened filter paper floor. For the choice experiments, two leaf segments from each of an endophyte-free (prochloraz treated) and an endophyte-infected (untreated) plant were presented to five weevils. For the non-choice experiments, two leaf segments from a single plant, either endophyte-free or infected were offered to five weevils. Ten replicates were used for each bioassay. Weevil feeding scars were counted and scored (0-10 scale) after holding for 96 h at room temperature (18-22 °C).

Feeding and oviposition bioassay

Choice and non-choice experiments were set up on 13 January 1983. For choice experiments, 15 weevils were caged with two plants, one endophyte-infected, one uninfected. For non-choice experiments, 15 weevils were caged with a single plant, either endophyte-infected or uninfected. Ten replicate cages were held in a screenhouse for 96 h before being transferred to a cool store at 4 °C to arrest weevil activity. Tillers from individual plants were severed at their base and eggs and weevil feeding scars counted. At completion of the bioassay (96 h) weevils were dissected and the reproductive state of the females determined.

RESULTS

Prochloraz seed treatment effected 96% control of endophyte fungus. Reproductive weevils were responsive to endophyte infection in both ryegrass and tall fescue. Weevils were able to distinguish between endophyte-infected and uninfected plants when provided with leaf segments (Table 1). When offered a choice of leaf segments, weevils fed more extensively on leaves of uninfected plants. Most weevils sampled the endophyte-infected leaves but moved to the uninfected leaves to feed. In non-choice experiments, weevils provided with endophyte-infected leaf segments fed less than those provided with leaf segments from uninfected plants (Table 2). Although Canterbury weevils showed higher initial feeding there was no difference between Waikato and Canterbury weevils in their overall response to endophyte-infected host plants.

TABLE 1: Feeding by weevils when offered a choice of endophyte-infected and endophyte-free leaf tissue.

	No. feeding scars/ weevil at 96 h			Visual scoring of feeding (0-10)* at 96 h		
	untreated	prochloraz	SED	untreated	prochloraz	SED
Waikato weevils						
Tall fescue	0.87	3.39	0.46	1.80	4.10	0.50
Fresh Ellett ryegrass	0.32	4.04	0.61	1.20	5.00	0.57
Aged Ellett ryegrass	6.18	6.20	0.36	7.80	7.80	0.30
Canterbury weevils						
Fresh Ellett ryegrass	2.12	4.18	0.31	4.80	7.70	0.46
Aged Ellett ryegrass	4.58	4.50	0.27	7.30	7.20	0.41

SED (standard error of difference of means). df (degrees of freedom) 10

* 0 = no feeding, 10 = maximum feeding for the bioassay

Reproductive weevils caged with whole plants showed similar responses to endophyte. In preference tests weevils fed more extensively and deposited more eggs on the non-endophyte plants (Table 3). Similarly weevils caged on endophyte infected or uninfected plants in a non-choice situation fed more and deposited more eggs on the

TABLE 2: Feeding by weevils in a non-choice situation when provided with endophyte-infected or endophyte-free leaf tissue.

	No. feeding scars/ weevil at 96 h			Visual scoring of feeding (0-10)* at 96 h		
	untreated	prochloraz	SED	untreated	prochloraz	SED
Waikato weevils						
Tall fescue	1.76	4.07	0.57	2.3	4.7	0.40
Fresh Ellett ryegrass	1.42	6.72	0.87	2.3	7.1	0.74
Aged Ellett ryegrass	7.66	6.14	1.15	8.4	7.2	0.52
Canterbury weevils						
Fresh Ellett ryegrass	2.06	5.80	0.80	4.6	7.5	0.75
Aged Ellett ryegrass	5.90	5.06	1.00	6.8	6.8	0.78

df 10 * see Table 1

uninfected plants with the exception that egg laying was unaffected on tall fescue plants (Table 4). The endophytes of tall fescue and ryegrass had the same effects on adult weevil behaviour. After 96 h weevils confined to endophyte-infected plants contained less eggs in their calyces than those confined to uninfected plants (tall fescue 0.20 ± 0.18 vs 1.81 ± 0.23 eggs/female, Ellett ryegrass 0 vs 2.38 ± 0.36 eggs/female, $P < 0.05$).

TABLE 3: Feeding and oviposition by weevils when offered a choice of endophyte-infected and endophyte-free plants.

	No. feeding scars/ weevil at 96 h			No eggs deposited/ ♀ weevil at 96 h		
	untreated	prochloraz	SED	untreated	prochloraz	SED
Tall fescue	1.26	2.71	0.50	0.93	2.07	0.24
Fresh Ellett ryegrass	1.99	9.19	1.52	0.97	2.63	0.33

df 19

TABLE 4: Feeding and oviposition by weevils in a non-choice situation when provided with endophyte-infected or endophyte-free plants.

	No. feeding scars/ weevil at 96 h			No. eggs deposited/ ♀ weevil at 96 h		
	untreated	prochloraz	SED	untreated	prochloraz	SED
Tall fescue	1.96	3.73	0.98	1.18	1.31	0.66
Fresh Ellett ryegrass	2.43	7.35	1.33	0.21	2.22	0.49

df 19

DISCUSSION

Fescues and ryegrasses are closely related, belonging to the family Festucoidae. The endophytic fungi in question are graminicolous members of the Clavipitaceae, specific to these grasses. The endophyte of tall fescue has recently been named *Acremonium coenophialum* (Morgan - Jones and Gams 1982). The *Lolium* endophyte is clearly related or identical to that of the tall fescue.

The experiments reported in this paper show the endophytes of tall fescue and ryegrass have the same adverse effects on Argentine stem weevil adults. Weevils are clearly able to distinguish between endophyte-infected and uninfected plants and concentrate their feeding and reproductive activity on those grasses lacking the fungus. In choice experiments many weevils fed extensively on non-endophyte infected material. Other weevils made initial feeding on endophyte infected material before moving to uninfected material. Endophyte hyphae are concentrated in the stem of the plant but weevils are able to detect endophytic fungal presence during feeding on leaves of low hyphal content. The site of oviposition is largely a reflection of feeding preference though the act of oviposition involves a further sampling of the plant in the region where endophyte fungus is most abundant.

The mechanism of Argentine stem weevil resistance in endophyte-infected ryegrasses and tall fescue has not been determined. This resistance might involve toxins produced by the endophyte or by the host plant as a result of host-fungal interactions (phytoalexins).

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REFERENCES

Barker, G.M., Pottinger, R.P., Addison, P.J. and Prestidge, R.A., 1983. Population biology and pest status of Argentine stem weevil in northern New Zealand pastures. I. Effect of *Lolium* infection of endophyte fungus on feeding and oviposition by weevils. *N.Z. J. agr. Res.* (in press).

Goldson, S.L., 1982. An examination of the relationship between Argentine stem weevil *(Listronotus bonariensis* (Kuschel)*)* and several of its host grasses. *N.Z. J. agr. Res. 25:* 295-403

Harvey, I.C., Fletcher, L.R. and Emms, L.M., 1982. Effects of several fungicides on the *Lolium* endophyte in ryegrass plants, seeds and in culture. *N.Z. J. agr. Res. 25:* 601-606

Kain, W.M., Slay, M.W. and Wyeth, T.K., 1981. Occurrence and development of Argentine stem weevil *(Hyperodes bonariensis* Kuschel*)* resistance in perennial ryegrass *(Lolium perenne). Proc. 3rd Aust. conf. Grassland Invert. Ecology* K.E. Lee, ed., South Australian Govt. Printer, p. 265-271

Kain, W.M., Wyeth, T.K., Gaynor, D.L. and Slay, M.W., 1982. Argentine stem weevil *(Hyperodes bonariensis* Kuschel*)* resistance in perennial ryegrass *(Lolium perenne* L.*). N.Z. J. agr. Res. 25:* 255-259

Latch, G.C.M. and Christensen, M.J., 1982. Ryegrass endophyte, incidence and control. *N.Z. J. agr. Res. 25:* 443-448

Morgan-Jones, G. and Gams, W., 1982. Notes on Hyphomycetes. XLI. An endophyte of *Festuca arundinacea* and the anamorph of *Epichloe typhina,* new taxa in one of two new sections of *Acremonium. Mycotaxon 15:* 311-318

Mortimer, P.H., Fletcher, L.R., di Menna, M.E., Harvey, I.C., Smith, G.S., Barker, G.M., Gallagher, R.T. and White, G.P., 1982. Recent advances in ryegrass staggers. *Proc. Ruakura Farmers' Conf. 24:* 71-74

Prestidge, R.A., Pottinger, R.P. and Barker, G.M., 1982. As association of *Lolium* endophyte with ryegrass resistance to Argentine stem weevil. *Proc. N.Z. Weed and Pest Control Conf. 35:* 119-122