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EVALUATING A NOVEL ENDOPHYTIC GRASS FOR ITS POTENTIAL TO REDUCE INVERTEBRATE POPULATIONS AND ASSOCIATED BIRD STRIKE RISK AT AIRPORTS

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EVALUATING A NOVEL ENDOPHYTIC GRASS FOR ITS POTENTIAL TO REDUCE INVERTEBRATE POPULATIONS AND ASSOCIATED BIRD STRIKE RISK AT AIRPORTS

THESIS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in the College of Agriculture, Food and Environment at the University of Kentucky

By
Diana Marietta Miller
Lexington, Kentucky

Director: Dr. Daniel A. Potter, Professor of Entomology
Lexington, Kentucky
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ABSTRACT OF THESIS

EVALUATING A NOVEL ENDOPHYTIC GRASS FOR ITS POTENTIAL TO REDUCE INVERTEBRATE POPULATIONS AND ASSOCIATED BIRD STRIKE RISK AT AIRPORTS

Aircraft strikes are a significant safety hazard on airports worldwide. Wildlife management at airfields is the most effective tactic to reduce airstrike risk – to modify the habitat to be undesirable to animals. Tall fescue grasses containing a fungal symbiont may serve that purpose. They produce alkaloids that convey resistance to some grass-feeding invertebrates, which might in turn reduce incidence of insectivorous birds. A commercial endophytic grass (Avanex™) consisting of ‘Jackal’ tall fescue infected with a unique endophyte (AR 601) is purported to contain especially high levels of alkaloids and to reduce bird populations if planted at airports. I evaluated it against the common KY31 tall fescue with its wild-type endophyte for invertebrate and vertebrate deterrence. Invertebrate abundance, survival, growth, and development were generally similar on Jackal E+ or KY31 E+. Spanish goats and wild birds showed no avoidance of Jackal E+, nor did Jackal E+ contain significantly higher levels of alkaloids than did KY31 E+. The Avanex™ tall fescue was not any better than KY31 in deterring herbivores but the concept is sound. However, better understanding of the relationship between grass, endophyte, alkaloid, and herbivore is needed to inform how such grasses might be used to reduce bird strike hazard.

KEYWORDS: Tall fescue, Festuca arundineacea, endophyte, bird strike, herbivory

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May 9, 2015
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INTRODUCTION

Wildlife Airplane Strikes

Wildlife airplane strikes cost the United States aviation industry roughly $682 million annually (Washburn 2012). There have been 25 fatalities and 279 injuries reported since the FAA’s Wildlife Strike Database creation in 1990 (FAA 2014). In 2013, there were 11,000 reported strikes at 650 airports in the United States (FAA 2014). Most strikes occurred during takeoff or landing, which is when aircraft experience their highest risk of substantial damage after colliding with birds (Marra et al. 2009). A notable bird airstrike occurred January 15, 2009. A US Airways passenger plane was hit by a flock of geese, damaging its engines and causing the pilot to make an emergency landing in the Hudson River. Fortunately, all passengers and crew survived the water landing (Marra et al. 2009).

Wildlife airplane strikes are not only caused by birds but by small mammals, deer, and reptiles (FAA 2014). However, 97% of reported air strikes are caused by birds (FAA 2014). These birds can include gulls, Canada geese, ducks, raptors, blackbirds, starlings, and others (Washburn 2012). Canada geese are more likely to cause serious damage to aircraft due to their size and flocking nature (FAA 2014). To reduce air strike risk, airfields need to reduce their wildlife presence.

The most effective way to reduce wildlife at airfields is to change the existing habitat. If airfields can maintain unattractive habitats to wildlife it can reduce their populations (Washburn and Seamans 2004). The type of vegetation chosen is important. The plants need to be aesthetically pleasing to the public eye, relatively inflammable, tolerant of traffic and drought, and not require many additional inputs (Washburn and
To be unattractive to wildlife, airport vegetation should provide minimal food resources for birds (seeds, berries, and insects), little cover for the small mammals that may attract raptors, and resist invasion by plants that are attractive to wildlife (Washburn and Seamans 2004). One such plant that might be unattractive to most wildlife is tall fescue.

**Tall Fescue and its Endophytic Fungus**

Tall fescue, *Festuca arundinacea* Schreb., contains a symbiotic fungus, the endophyte *Epichloë coenophiala* (formerly *Neotyphodium coenophialum*) (Leuchtmann et al. 2014). The endophyte is transmitted by seed from one generation to the next and spreads within the grass plant by mycelia (Ball 1993, Schardl et al. 2004). The endophytic fungus does not harm the grass plant – it does not penetrate or physically alter the cells but rather appears to grow around the cells (Ball 1993). This is a symbiotic relationship.

The endophyte was not known when this grass was planted widely across the United States in the 1940s as the Kentucky 31 (KY31) cultivar (Ball 1993). However, in the 1960s concerns were raised when farmers were having trouble with livestock and research into the endophyte began (Ball 1993). The ergot alkaloids produced by the endophyte cause “fescue toxicosis” in livestock (Porter and Thompson 1992, Ball 1993, Schardl et al. 2004). Cattle, when grazed on Kentucky 31, have poor weight gain, rough hair coats, and an intolerance to heat called the “summer slump”, while mares have devastating reproduction issues – abortion of foals and stillbirths (Porter and Thompson
1992, Ball 1993). Goats are also affected by “fescue toxicosis” with vasoconstriction of their main arteries causing a disruption in thermoregulation (Aiken and Flythe 2014).

The endophytic fungus provides multiple benefits to tall fescue. When KY31 was discovered by E.N. Fergus, he noticed that this grass resisted pests and adapted well to a wide range of environmental conditions, such as drought, poor soils, and variable soil pH (Ball 1993). The endophytic fungus stimulates growth in tall fescue and increases the survival rate of the grass when under stressful conditions (Arachevaleta et al. 1989). The endophyte also releases a suite of alkaloids that make the grass relatively unpalatable to some insects and wildlife (Ball 1993, Bush et al. 1997, Breen 1994). Ergot alkaloids, most notably ergovaline, are the main cause of fescue toxicosis (Porter and Thompson 1992). Endophytic tall fescue also contains loline alkaloids, which have insecticidal properties, and peramine, which deters insect feeding (Bush et al. 1997, Malinowski and Belesky 2000, Schardl et al. 2004). Thus the endophyte provides tall fescue with protection from not only abiotic stresses but from biotic factors as well.

**Invertebrate Resistance**

Many studies have explored the relationship between endophytic grasses and invertebrate herbivores (Breen 1994). The accumulated research indicates that endophytic grasses are sometimes, although not always, less suitable than their endophyte-free counterparts as a resource for grass-feeding insects. The aphids *Rhipalosiphum padi* (L.) and *Schizaphis graminum* (Rondani), for example, preferred endophyte free (E-) tall fescue when given a choice between E- and E+ (endophyte present) tall fescue, and had poor or no survival when confined to E+ tall fescue (Johnson

Conclusions drawn from trials with potted plants or clippings in controlled environments with highly endophyte-sensitive species (e.g., *R. padi*) may not reflect responses of adapted grass-feeding invertebrates in the field. Keathley and Potter (2011), for example, observed relatively weak or no endophyte effects on native grass-feeding insects in tall fescue grasslands and pastures. Rudgers and Clay (2008) found in a field study that several invertebrate guilds were more abundant and diverse in E- tall fescue, but those differences were more closely associated with greater plant species diversity rather than direct effects of alkaloid toxicity. Clearly, insect response to E+ grasses varies depending on the particular combination of insect and grass-endophyte association, as well as extrinsic factors such as grass age or plant stress (Scharld et al. 2004).

Endophytic grass does not seem to adversely affect earthworms. Humphries et al. (2001) fed either E+ or E- tall fescue leaf and root tissue to *Eisenia fetida* (Savigny). For all treatments there was 100% survival but the earthworms that were fed E+ leaf tissue
had increased growth compared to those fed E- leaf tissue. Rattray et al. (2010) showed that *E. fetida* has microbes in its gut that appear to detoxify ergovaline.

**Bird Deterrence**

Tall fescue with endophyte has the potential to deter grass- or seed-feeding birds from undesirable locations. These birds gain a learned response that, through post-ingestion feedback, influences them to avoid unpalatable grasses. Madej and Clay (1991) showed that seed-eating birds significantly preferred E- over E+ seeds in choice tests. In no-choice tests, the birds fed on E+ seeds had significant weight loss. Conover and Messmer (1996) studied effects of E+ tall fescue on Canada geese, *Branta canadensis* (L.), a bird that grazes on submerged aquatic vegetation as well as many land plants, including grasses, and that is particularly attracted to mowed lawns around homes, golf courses, parks, and similar areas next to open water (Link 2005). The geese were caged in tall fescue field plots containing either E+ or E- grass for two years. The geese that foraged on E+ tall fescue had significant weight loss compared to geese fed E- tall fescue. Furthermore, at the end of the two years, when the geese were placed in larger cages that contained both E+ and E- tall fescue, they spent significantly more time foraging in the E- plots, supporting the theory of food aversion learning.

**Avanex™**

The turf type tall fescue cultivar ‘Jackal’, bred by PGG Wrightson Seeds, New Zealand, was inoculated with the endophyte strain AR601, further selected for high alkaloid expression, and trademarked Avanex™ (Pennell et al. 2010, Pennell and Rolston
Avanex™ Jackal reportedly produces higher levels of alkaloids (>1100 parts per million (ppm) lolines; >3.4 ppm ergovaline) than do “off the shelf cultivars” with wild-type endophytes commonly used in lawns and road-sides (Pennell and Rolston 2013). A similar program was completed for a perennial ryegrass cv. ‘Colosseum’ inoculated with the AR95 endophyte. Both Jackal and Colosseum are being marketed in New Zealand and Australia under the Avanex™ unique endophyte technology brand for the recreational turf and aviation industries (Figure 1). They reportedly withstand high traffic, low fertility, drought, and compacted soils (Pennell and Rolston 2013).

The Avanex™ grasses are purported to deter insects and other invertebrates enough to reduce attractiveness of the grass to insectivorous birds (Pennell and Rolston 2013). These grasses are reported to directly deter vertebrate grazers, most notably Canada geese and rabbits, via “post-digestion feedback” or food aversion learning (Pennell and Rolston 2003, 2011, 2013). Those characteristics, if reliable, could make them useful as ground covers for wildlife management at airports, and also for golf courses, parks, and other public areas where geese and their droppings can be a significant nuisance. Both grasses are the subject of international patent applications, but, until the work summarized in this thesis, neither grass had been evaluated in independent scientific research or in replicated trials beyond New Zealand.

Essentially all of the support for the hypothesis, that the Avanex™ grasses with their novel endophytes deter insects, birds, and other wildlife enough to have potential value for mitigating air strike risk, comes from in-house studies done by scientists affiliated with the companies having commercial interest in the technology. The data are reported in semi-technical conference proceedings (e.g., Pennell and Rolston 2010, 2011, 2011).
2012), short communications (Pennell et al. 2010), or are cited as “unpublished”. Some of the reported trials reflect non-realistic scenarios, whereas others were apparently non-randomized and non-replicated. Pennell and Rolston (2011), for example, report that ‘Jackal’ AR601 planted at three airports in New Zealand reduced bird presence and insect populations compared to existing mixed vegetation; the latter arguably an “apples to oranges” type comparison. There was no true replication and their data suggesting reductions in total insect biomass in the Avanex™ stands did not indicate what types of insects were present or being affected.

Although the published evidence for effectiveness of a bird-deterrent airport grass is mainly from pilot or preliminary trials, the concept is credible. There is precedence for endophytic grasses to reduce insect populations in field settings (e.g., Breen 1994, Richmond et al. 2000, Popay and Hume 2011). Endophytic tall fescue may repel Canada geese after a period of post-digestion feedback and learning (e.g., Conover and Messmer 1996), and such geese may avoid foraging on tall fescue compared to perennial ryegrass or mixed stands of perennial ryegrass and white clover, Trifolium repens L. (Washburn et al. 2007); although in the latter study it was unclear if the avoidance of tall fescue was endophyte-related. Those questions were compelling and sparked my interest to research this grass-endophyte relationship; to find out whether Avanex™ has promise for use in insect and wildlife management to reduce bird strike hazard at airports and if so, if the benefit is any greater than that provided by the often-used KY 31 tall fescue with its wild type endophyte.
Figure 1. AVANEX™ Brochure.
Objectives


2. Compare the efficacy of Jackal E+ and KY 31+ tall fescue for reducing invertebrate populations relative to endophyte-free plots, and in relation to seasonal changes in alkaloid content in the field.

3. Evaluate and compare efficacy of the endophytic grasses for reducing bird presence, and for deterring a mammalian grazer in the field.
RESEARCH METHODS

This section describes a combination of laboratory assays, field trials, and phytochemical analyses to evaluate if either commercial ‘Jackal’ tall fescue with the novel AR601 endophyte (AVANEX™; PGG Wrightson and AgResearch, New Zealand) or endemic KY31 tall fescue infected with a wild-type endophyte have strong enough anti-herbivore effects to hold promise for use in mitigating wildlife strike hazard at airports. The working hypotheses were that both E+ tall fescues will reduce invertebrate growth, survival, and population densities compared to the same E- cultivars, and that because of its purportedly high alkaloid levels, the anti-herbivore effects of Jackal E+ will be stronger than those of KY31 E+.

Experimental Design – Greenhouse

Four types of tall fescue, cvs. ‘Jackal’ or ‘KY31” either infected (E+) or without (E-) their symbiotic fungal endophytes (AR601 or wild-type, respectively), were seeded and grown in the UK Research Greenhouses. The first batch of grass seed (Batch 1) was planted in early October 2011 for use in a preliminary trial conducted by Dr. Carl Redmond (University of Kentucky). Each pot (10.2 × 10.2 cm) was filled with a 3:1 ratio of Pro-Mix potting medium (Pro-Mix, Quakertown, PA) to sterilized Maury silt loam topsoil from The University of Kentucky (UK) Spindletop Research Farm (Lexington, KY) and seeded with about 50 seeds. The flats were randomly placed on the greenhouse bench to mitigate confounding environmental factors. My trials, which used similar growing conditions, started with different, newly-shipped batches of seed (Batches 2 and
3). Batch 2 was planted 1 October 2013 in which sixteen flats of eighteen pots each were seeded (four flats for each grass type). Batch 3 was planted 1 October 2014 in which ten pots were seeded with each of the four grass types. Those pots (10.2 cm diameter) were filled with the same ratio of soil as used for Batch 1.

The greenhouse-grown grasses were watered every 2-3 days with fertilizer applications applied once a month. The pots were placed in solid gardening flats that allowed for watering from the base-up. The fertilizer used was Scotts Peters Professional Water Soluble Fertilizer 20-10-20 (Scott’s, Marysville, OH) and was applied with a watering can at the rate of ½ tsp (2.7 g) per gallon of water (approx. 142 ppm N). The grass was clipped about weekly at 10.2 cm height. The greenhouse-grown grasses grown from Seed Batches 1, 2, and 3 ranged from 10-12, 10-26, and 17-19 weeks old, respectively, when harvested for use in the trials described below.

Model Insects

The model insects used in the following feeding assays were the black cutworm Agrotis ipsilon (Hufnagel), the fall armyworm Spodoptera frugiperda (J.E. Smith), and the bird cherry-oat aphid Rhopalosiphum padi (L.). Black cutworms and fall armyworms were purchased and shipped as eggs from a commercial insectary (Benzon Research, Carlisle, PA). The bird cherry-oat aphids came from an existing colony at UK Department of Entomology that had been started with field-collected aphids from western Kentucky that were maintained on caged wheat plants in the greenhouse.

Several types of assays were designed in which survival, growth, or feeding preference of different species and instars of insects were compared using clippings,
rooted tillers, or whole pots of grasses. Although some assays were redundant insofar as insect species and treatments, using several types of assays was intended to help safeguard against possible bias due to assay conditions (e.g., Risch 1985) while collectively giving a more accurate assessment of grass suitability than if only one type of assay had been used. All assays were conducted in incubators set at 24°C with a 13:11 h (light:dark) schedule.

**Neonate Caterpillar Feeding Assays with Clippings in Petri Dishes**

Petri dishes, with a moistened filter paper, were each provisioned with one type of grass for the insects to feed on. The dishes were sealed with parafilm (Parafilm M; Bemis; Oshkosh, WI) to prevent escapes and to maintain humidity levels.

*Growth and Survival of Black Cutworms.* Two trials, starting with black cutworm neonates, were completed in December 2012 and February 2015. The first trial was conducted by Dr. Carl Redmond (University of Kentucky) as a preliminary experiment to determine if the AVANEX grass showed promise for suppressing grass-feeding caterpillars. In that trial, the four types of grass grown from Batch 1 seed were compared using 5 neonates per dish, 6 replicates per grass type, for 7 days. The second trial compared three types of grass from Batch 3 seed (Jackal E- was excluded due to a lack of germination) using 10 neonates per dish, 6 replicates per grass, for 10 days. In each trial, the surviving caterpillars were weighed and their instar determined.

*Growth and Survival of Fall Armyworms.* One trial using fall armyworm neonates was completed February 2015. In this trial, the same three grass types used in the third cutworm trial, grown from Batch 3 seed, were compared (10 neonates per dish,
6 replicates, 10 days). The surviving caterpillars were weighed and their instar determined.

**Insect Feeding Assays on Whole Grass Tillers**

Tapered planting tubes (Cone-tainers, 4 cm top diameter, 15 cm deep; Stuewe & Sons; Tangent, OR) were filled with Pro-Mix potting medium and planted with 2-5 grass tillers each. The tubes were topped with acrylic tubing (4 cm diameter, 15 cm height; Interstate Plastics; Sacramento, CA), to provide an enclosed arena for the insects to move around in (Figure 2A). A paper towel was affixed over the top of the tubing to allow for some air and water movement while preventing insect escape. During the experiments, the tubes with grass tillers and larvae were arranged in racks, by replicate.

*Growth and Survival of Black Cutworms.* The trial was carried out as two 10-day runs, each with 12 replicates of the four grass types from Batch 2, which ran from 9-19 December and 16-26 December 2013. Each replicate contained two grass tillers and one black cutworm neonate. However, after 7 days the caterpillars had eaten most of the rooted tillers, so several freshly cut grass blades of the same grass were added to prevent food limitation. Surviving caterpillars were weighed and their instar determined after 10 days.

*Growth and Survival of Fall Armyworms.* The trial ran for 7 days (to prevent food limitation), from 17-24 January 2014, with 24 replicates for each of the four types of grass grown from Batch 2 seed. Each replicate contained five grass tillers and one fall armyworm neonate. The surviving caterpillars were weighed and their instar determined.
Bird Cherry-Oat Aphid Population Growth. This trial ran for 14 days starting 4 April 2014, with 24 replicates of the four grasses grown from Batch 2 seed. Each replicate contained a single grass tiller and started with five full-sized aphids. After 2 weeks the aphid populations (nymphs and adults) were counted. A corresponding immunoblot assay was done on the grass tillers from this trial, after the aphids were removed, to confirm absence or presence of the endophyte.

Insect Feeding Assays with Grasses in Pots

Large, cylindrical clear plastic containers (20 cm diameter, 18.5 cm deep) with lids were filled with sand. A single pot (10.2 × 10.2 cm) of greenhouse-grass (Batch 2) was nestled into the sand, with the sand serving as an extended walking surface for the insects (Figure 2B).

Growth and Survival of Neonate Caterpillars. Two trials were conducted starting with 10 neonate black cutworms or fall armyworms per container and 6 replicates for each grass type. The cutworm trial started on 9 December 2013 and was evaluated after 10 days. The armyworm trial started 17 January 2014 and ran for 7 days. Surviving caterpillars were weighed and their instar was determined at the end of each trial.

Performance of Individual Mid-sized Black Cutworms. Pots of greenhouse-grown grass (Batch 2) were individually topped with an inverted transparent plastic cup to create an enclosed arena (Figure 2C). There were 24 replicates starting with one 4th instar caterpillar per pot. Larvae were individually weighed at the beginning and end of the trial when their instar, too, was determined. The trial started on 10 March 2014 and ran for 6 days.
Field Studies

Site Preparation and Planting. A field site (approx. 25 × 50 m) at the UK A.J. Powell Turfgrass Research Center was prepared by Dr. David Williams and associates (UK Department of Plant and Soil Sciences) who treated it with herbicides to kill existing grasses and then cultivated to provide a suitable seedbed. Plots of the four tall fescues, Jackal and KY 31 with or without their respective endophytes, were seeded on 12 September 2013 using a Gandy drop spreader (0.914 m [3 ft] wide) calibrated to apply 2.27 kg of seed per 92.9 m² (5 lbs/1000 ft²) in one pass. The KY 31 E+ and E- seed was supplied by Dr. Tim Phillips (UK Plant and Soil Sciences). The Avanex™ Jackal E+ and E- tall fescue (Batch 2) was supplied by PGG Wrightson/AgResearch, New Zealand. There were 24 total plots with six replicates for each of the four grass types in a randomized complete block design. Each plot measured 5.5 × 5.5 m (18 ft × 18 ft). Plots were separated by 0.91 m (3 ft) borders of perennial ryegrass to ensure clear delineation between grass types (Figure 3).

Once seeded, the field site was covered with Reemay fabric, set up with external irrigation lines, and irrigated as needed for 3 weeks (13 September to 4 October 2013) to ensure germination. The Reemay was removed once grass seedlings appeared. After this establishment phase the irrigation system was removed. The field site was mowed at 10.2 cm height. Fertilizer and herbicides were applied according to UK recommendations for low maintenance tall fescue turf (http://www.uky.edu/Ag/ukturf/lawns.html). No chemical applications were made once the grass was well established, or during the period during which data were collected (early May to late September 2014).
**Pitfall Traps.** Activity-density of surface-dwelling invertebrates was assessed with pitfall traps made from a pair of nested plastic cups (473 mL, 9.53 cm top diameter; Solo, Lake Forest, IL) set into the ground with the lip of the cups level with the soil surface. There were three, 1-week collection periods: 20-27 May, 25 July-1 August, and 22-29 September 2014. There were 5 traps per plot, a central trap and one half-way along a diagonal toward each corner (in the shape of a 5-marked die). About 2 cm of antifreeze (ethylene glycol) was added to each cup to kill and preserve insects that fell inside. After 7 days, the cups were removed from the field and the antifreeze filtered out. Collections from the five traps from each plot were pooled and stored in sealed containers in 90-proof ethanol (total: 24 pooled samples per date). The captured arthropods were separated from debris, and predominant taxa were identified to order (e.g., Araneae) or family (most insects).

**Vacuum Sampling.** The vacuum sampling coincided with the pitfall trapping with samples taken on 27 May, 25 July, and 22 September. A reversed gasoline-powered leaf blower (Troy-Bilt, Cleveland, OH) with a paint strainer bag clamped inside the intake tube was used to vacuum insects. Each replicate was vacuumed separately, walking a zigzag pattern within each plot, for 30 seconds, after which the sample was placed into a paper bag that was folded, stapled, and placed in a cooler (total: 24 samples per date). Samples were transferred to and stored in a chest freezer until they could be processed. At sorting, the arthropods were separated from grass debris, stored, and identified as described for the pitfall samples.

**Earthworm and White Grub Abundance.** Earthworm (Lumbricidae) and white grub (Scarabaeidae) populations were sampled on 8 October 2014 to test the hypothesis
that feeding on roots, ingesting associated soil, or leaching of alkaloids from clippings in
the E+ plots throughout the growing season would adversely affect those subterranean
invertebrates. Sampling was by cutting, removing, and examining five 18 × 18 × 10.2 cm
deep slabs of turf with soil from each plot (120 total slabs). Samples were taken in
consistent locations between and equidistant from the pitfall traps. The sampling was
done on 8 October 2014 when resident grub populations, mostly masked chafer
(Cyclocephala spp.) and Japanese beetle (Popillia japonica) larvae, were predominantly
third instars. The earthworms and white grubs were collected in separate containers,
weighed, and identified to species. Grub species were distinguished by their distinctive
rastral patterns (Potter 1998) and earthworms were identified using published keys and
descriptions of species found in central Kentucky turfgrass settings (Redmond et al.
2014).

Grass-Weed Counts. A survey of grass coverage in the field plots was conducted
11 July 2014 to test the hypothesis that plots of the E+ grasses, due to the enhanced vigor
conveyed by the endophyte, would have fewer weeds and bare spots than plots with E-
grasses. A PVC frame (1 m²) with string every 12.7 cm in a grid pattern was placed in
two randomly chosen locations within each plot and recorded whether there was tall
fescue, annual bluegrass, clover, other weeds, or bare ground at each spot where two
strings crossed. Data from the two samples per plot were pooled for analysis.

Caterpillar Choice Test with Field-Grown Tillers. The hypothesis that mid-sized
fall armyworms foraging in a mixed stand will actively avoid E+ grass plants was tested
in a choice test. The arenas were greenhouse flats (28 × 54 × 6 cm deep) filled with
potting soil. There were two grass types in each flat (10 tillers per grass type) with five
replicates of each of the following pairings (total: 15 tests): Jackal E+ versus KY31 E+, Jackal E+ versus KY31 E-, and KY31 E+ versus KY31 E-. Apparently healthy tillers with roots were collected from the field plots on 21 August 2014 and transplanted to the flats in an alternating array. The edges of the flat were coated with petroleum jelly to discourage caterpillar escape (Figure 2D). Ten 4th instar fall armyworms were placed on the surface of each flat between the rows of tillers. The flats were kept in an incubator at 24°C with a 13:11 h (light:dark) schedule for 48 h. Every six hours the locations of the fall armyworms were recorded (8 observation times). The amount of feeding on each grass tiller was rated for damage by two independent observers (0 being completely eaten to 10 being untouched) at the end of the trial.

Short-term Choice Tests with a Vertebrate Grazer. Spanish goats (Capra hircus L.), were used as a surrogate for more airport-relevant vertebrate grazers such as deer or rabbits to test if one or both E+ grasses are unpalatable enough to deter such animals and cause them to move off the infected grass. Dr. Michael Flythe (USDA-ARS Forage-Animal Production Research Unit, UK, Lexington) who uses goats to study the animals’ physiological responses to endophytic forage grasses, collaborated on this trial. The protocol was submitted to and approved by the UK Institutional Animal Care and Use Committee before doing the trial (IACUC Identification Number: 2014-1267).

Five pens (5.5 × 6.1 m) made from 1.52 m high goat fencing supported by steel fence posts were installed on the field plots. Each pen overlapped two grass types for the goats to choose between Jackal E+ versus KY31 E-, or KY 31+ versus KY 31 E-. The non-infected KY 31 served as the control. The trial was conducted on 8 and 15 September 2014, with five replicates of each choice over the two days (3:2, 2:3). The
five goats were randomly assigned to their individual pens which they occupied during the feeding sessions on a given day. Goats were randomly reassigned to pens on the second day. Plywood boards were placed over the perennial ryegrass borders, and water dishes were provided in each pen. One goat was placed in each pen for a 2-h session in the morning and another 2-h session in the afternoon on each day. All goats were moved to a communal enclosure (5 × 5 m) for 2 h between the morning and afternoon sessions, the purpose of which was to allow time for any short-term malaise that might result in food-aversion learning manifest as a change in preference between the morning and afternoon sessions. I observed and recorded the activity of each goat every 5 min, including feeding, lying down, or standing in one or the other of the grass plots, or other behaviors such as drinking water or standing on the plywood board. Moultrie mini-game cameras (EBSCO Industries, Birmingham, AL) were also set up beside each pen to take pictures of the goats every minute.

Bird Counts. Moultrie mini-game cameras were set up at the field site to record bird presence. There were three 3-day long sessions over the 2014 summer (24-26 June, 9-11 July, and 6-8 August). Six game cameras were placed around the field site, one to observe each replicate, which took pictures every 5 minutes. The total number of pictures containing birds was recorded for each plot and grass type.
Quantification of Endophyte Infection and Alkaloid Levels

*Immunoblot Assays.* Fungal endophyte (*N. coenophialum*) infection was determined by an enzyme-linked, endophyte-specific immunosorbent (immunoblot) assay (Hill et al. 2002) on both the greenhouse-grown grass (Batch 2) and for the grasses in the field plots. The greenhouse grass immunoblot assays were done with assistance from Walter Hollin (Dept. of Plant and Soil Sciences, UK). The field grass immunoblot assays were done by Agrinostics Ltd. Co. (Watkinsville, GA) and AgResearch USA Ltd. (Ashville, NC). Endophyte infection frequency was calculated as the percentage of infected tillers based on samples of 20 tillers from each plot or replicate.

*Alkaloid and Nitrogen Analyses.* Loline (pyrrolizidine) and ergot (ergopeptide) alkaloids, which have been shown to affect insect and mammalian herbivores (Bush et al. 1997), were quantified in the greenhouse-grown grass (Batch 2) and in the grasses from the field plots collected on three dates (13 May, 24 July, and 24 September) during the 2014 growing season. Fresh grass clippings were collected, by replicate, frozen at -80°C, freeze-dried, and ground through a 40-mesh screen using a Wiley Mill (see Appendix A for sampling protocol). Loline alkaloids, namely *N*-formyl loline (NFL), *N*-acetyl norloline (NANL) and *N*-acetyl loline (NAL), were extracted and quantified using a gas chromatograph equipped with a flame ionization detector by the protocol of Blankenship et al. (2001). Ergot alkaloids (ergovaline and ergovalinine) were analyzed by a high-performance liquid chromatography (HPLC) procedure developed by Yates and Powell (1988). Alkaloid analyses were performed by Dr. Lowell Bush and associates (Dept. of Plant and Soil Sciences, UK).
Nitrogen is a limiting element in the diet of grass-feeding insects (Mattson 1980, Davidson and Potter 1995), so nitrogen analyses were done for the Batch 2 and 3 greenhouse-grown grasses, and for the field-grown grasses, to test the hypothesis that nitrogen levels were different among the grass types. Grass samples were frozen at -80°C, freeze-dried, and ground through a 40-mesh screen. Nitrogen content was determined by using a Flash EA1112 elemental analyser (ThermoFisher Scientific Inc., Waltham, MA, USA).

**Statistical Methods**

Most of the experiments deployed a randomized complete block design so the data were analyzed for main effects of treatment and replicate by two-way analysis of variance (ANOVA) followed by Fisher’s LSD test for mean separation if the F-statistic indicated a significant treatment effect. Most of the data sets conformed to ANOVA assumptions for homogeneity of variance; in the few cases where they did not, square root or log transformations were used. Chi square tests for equality of proportions between two samples were used to analyze binary choice data from trials comparing numbers of armyworms or goats feeding on one or the other of two grass types at a point in time or over a particular interval. Analyses were done with Statistix 9.0 (Analytical Software 2009). All data are reported as original (non-transformed) means ± standard error (SE).
Figure 2: Designs of selected insect feeding assays; (A) assay on whole grass tillers; (B) assay with neonates and grasses in pots; (C) assay with individual mid-sized caterpillars and grasses in pots; (D) caterpillar choice test with field-grown tillers.
Figure 3: Pictures of field plot establishment and the plot map.
RESULTS

Neonate Caterpillar Feeding Assays with Clippings in Petri Dishes

*Growth and Survival of Black Cutworms.* The caterpillars fed on Jackal E+ grass blades in Trial 1 (Batch 1 seed) were significantly smaller in size, had slower development, and had lower survival than the black cutworms fed on the other three grasses (Table 1). In Trial 2, however, the caterpillars fed on Jackal E+ grass blades (Batch 3 seed) were significantly larger in size, had faster development, and had greater survival than the black cutworms fed on KY31 E+ and KY31 E-.

*Growth and Survival of Fall Armyworms.* Fall armyworms fed on Jackal E+ grass blades (Batch 3 seed) were significantly larger in size, had faster development, and had greater survival than the fall armyworms fed on KY31 E+ (Table 2). Furthermore, there were no significant differences between the KY31 E+ and KY31 E- fed caterpillars in that trial.

Insect Feeding Assays on Whole Grass Tillers

*Growth and Survival of Black Cutworms.* The caterpillars grown on Jackal E- tillers grown from Batch 2 seed weighed more, had greater survival, and faster development than the neonates grown on the other three grasses (Table 3). Also, there were no significant differences between the Jackal E+ and KY31 E+ fed caterpillars.

*Growth and Survival of Fall Armyworms.* The weight, but not the survival or development, of the neonate fall armyworms fed on living grass tillers (Batch 2 seed) was
statistically different among the grasses (Table 4). The caterpillars fed on Jackal E+ tillers weighed, on average, significantly less than those fed on KY31 E+ tillers.

*Bird Cherry-Oat Aphid Population Growth.* Bird cherry-oat aphids feeding on single living tillers of Jackal E- (Batch 2 seed) had significantly higher population growth than those aphids fed on single living tillers of Jackal E+, KY31 E+, and KY31 E- (Table 5). Both E+ grasses reduced aphid population growth relative to their E- counterparts, but there was no difference between the aphids fed on Jackal E+ and KY31 E+ grass. The immunoblot assay confirmed absence of endophyte in both E- grasses with 63% or 71% infection rates in the E+ tillers (Table 5).

**Insect Feeding Assays with Grasses in Pots**

*Growth and Survival of Neonate Caterpillars.* The neonate black cutworms had similar survival on all four types of potted grasses grown from Batch 2 seed, and they were all second instars when the trial was evaluated after 10 days. However, cohorts fed Jackal E- grass had significantly greater weight gain than did caterpillars fed on the other three grasses (Table 6).

The neonate fall armyworms had no statistically significant differences in survival, weight gain, and development regardless of which of the four grasses they fed upon (Table 7).

*Performance of Individual Mid-sized Black Cutworms.* There were no statistically significant differences between the four grasses for 4th instar black cutworm weight gain, survival, or development (Table 8).
Field Studies

Pitfall Traps. The invertebrates collected by pitfall trapping during the 2014 summer were separated into 11 groups – Carabidae (ground beetles), Staphylinidae (rove beetles), other Coleoptera (beetles), Formicidae (ants), other Hymenoptera (wasps), Diptera (flies), Hemiptera (true bugs, leafhoppers, froghoppers), Lepidoptera (caterpillars), Orthoptera (crickets and grasshoppers), Araneae (spiders), and Gastropoda (slugs). Carabidae had significantly higher abundance in Jackal E+ grass in May (Figure 4). Staphylinidae had lower abundance in KY31 E+ grass in September (Figure 4). Hemiptera had higher abundance in Jackal E- grass in May (Figure 6). Lepidoptera had significantly lower abundance in KY31 E- and KY31 E+ grasses in September (Figure 6). The other invertebrate groups had no significant differences among grass types.

Vacuum Sampling. The invertebrates collected by vacuum sampling during the 2014 summer were separated into 12 groups – Cicadellidae (leafhoppers), Cercopidae (froghoppers), other Hemiptera, Formicidae, other Hymenoptera, Diptera, Staphylinidae, other Coleoptera, Orthoptera, lady beetle larvae (Coccinellidae), Lepidoptera, and Araneae. Cercopidae had significantly lower abundance in KY31 E+ grass in May (Figure 8). Diptera had significantly higher abundance in Jackal E- grass in May (Figure 9). Coleoptera (excluding Staphylinidae and lady beetle larvae) had significantly lower abundance in KY31 E+ grass in July (Figure 10). Lepidoptera had higher abundance in KY31 E+ grass in September (Figure 11). Araneae had lower abundance in KY31 E+ grass and higher abundance in Jackal E- grass in May (Figure 11). The other invertebrate groups had no significant differences among grass types.
Earthworm and White Grub Abundance. There were no statistically significant differences between the four grasses for earthworm (*Aporrectodea* spp.) or white grub abundance and biomass (Table 9). Most (77%) of the white grubs were Japanese beetles (*Popillia japonica* Newman) and May beetles (*Phyllophaga* spp.); the remainder were masked chafer (*Cyclocephala* spp.) grubs. Of the grubs sampled, 21% were 2\textsuperscript{nd} instars and 79% were 3\textsuperscript{rd} instars.

Grass-Weed Counts. There were no significant differences in weed abundance between field plots of the four grasses (Table 10). There was similar grass coverage, as opposed to bare spots, regardless of cultivar and presence or absence of endophyte (Table 10).

Caterpillar Choice Test with Field-Grown Tillers. Fourth instar fall armyworms showed no significant preference between living tillers of KY 31 E- and either of the E+ grasses in choice tests. Also, as many larvae were seen feeding on the Jackal E+ as on the KY 31 E+ grass (Figure 12). Most of the larvae in each flat were observed feeding on grass tillers at both 24 and 48 h after their release.

Short-term Choice Tests with a Vertebrate Grazer. There were significantly more observations of the Spanish goats feeding in plots of Jackal E+ than in plots of KY31 E+ grass for both the morning and afternoon sessions (Table 11). For choice test 2, the goats spent more time feeding on KY31 E- than on KY31 E+ during the morning session, but they showed no significant preference in the afternoon session (Table 11). Figures 13 and 14 show the proportion of total observations, in which goats were engaged in various behaviors, during the morning and afternoon sessions.
**Bird Counts.** Mean total number of birds observed in photos of the field plots taken every 5 minutes during three, 72-h sessions at different times showed no statistically significant differences between the four grass types (Table 12). Most of the birds in the photos appeared to be starlings, grackles, or other medium-sized bird species. Only 98 of the 4,940 photos contained images of birds, and none of them showed presence of Canada geese on the plots.

**Quantification of Endophyte Infection and Alkaloid Levels**

Immuno blot Assay. The immunoblot assays confirmed relatively high percentage of infected tillers in the field plots of both endophytic grasses, with no significant difference between the Jackal E+ and KY31 E+ (Table 13). The assay detected very low or no incidence of infection in the field plots of Jackal E- and KY31 E-, confirming their endophyte-free status (Table 13).

Alkaloid and Nitrogen Analyses. Neither of the non-endophytic grasses (Batch 2 greenhouse-grown grass) contained detectable levels of alkaloids (Table 14). Compared to Jackal E+, the endemic KY31 E+ had higher levels of ergot alkaloids and similar levels of lolines (Table 14). Nitrogen content was slightly higher in both of the endophytic grasses than in KY31 E- (Table 14).

Neither of the non-endophytic grasses (Batch 2 field-grown grass) contained detectable levels of alkaloids (Figure 15). In May, KY31 E+ contained significantly higher levels of ergot alkaloids than Jackal E+ but had similar levels for the rest of the season (Figure 15). Nitrogen content was significantly higher in both Jackal E+ and E- in July and September (Figure 15).
Table 1. Survival and growth of neonate black cutworm, *Agrotis ipsilon*, on greenhouse-grown KY 31 or Jackal tall fescue with or without their endophytes (wild-type or AR 601, respectively) using grass grown from two different allotments of seed.

<table>
<thead>
<tr>
<th>Grass</th>
<th>Endo</th>
<th>Instar attained$^1$</th>
<th>Survival (%)</th>
<th>Mean wt (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial 1$^2$</td>
<td>KY31 + 2.7 ± 0.2 a</td>
<td>97 ± 3 a</td>
<td>37 ± 6 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Jackal + 1.2 ± 0.3 b</td>
<td>67 ± 18 b</td>
<td>9 ± 3 b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>KY31 - 2.5 ± 0.1 a</td>
<td>100 ± 0 a</td>
<td>44 ± 3 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Jackal - 2.8 ± 0.1 a</td>
<td>100 ± 0 a</td>
<td>45 ± 3 a</td>
<td></td>
</tr>
<tr>
<td>$F_{3,20}$ =</td>
<td>18.1</td>
<td>3.0</td>
<td>18.0</td>
<td></td>
</tr>
<tr>
<td>$P \leq$</td>
<td>0.001</td>
<td>0.05</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

Trial 2$^3$

<table>
<thead>
<tr>
<th>Grass</th>
<th>Endo</th>
<th>Instar attained$^1$</th>
<th>Survival (%)</th>
<th>Mean wt (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KY31 + 2.0 ± 0.0 c</td>
<td>42 ± 6 c</td>
<td>7 ± 1 c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jackal + 2.9 ± 0.0 a</td>
<td>98 ± 2 a</td>
<td>250 ± 30 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KY31 - 2.2 ± 0.1 b</td>
<td>82 ± 3 b</td>
<td>50 ± 7 b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$F_{2,15}$ =</td>
<td>129</td>
<td>52.6</td>
<td>69.0</td>
<td></td>
</tr>
<tr>
<td>$P \leq$</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

$^1$Data are means (± SE) per cohort of 5 (Trial 1) or 10 (Trial 2) neonate larvae per replicate.

$^2$Trial 1: Batch 1 grass, 6 reps, 7 days, 5 neonates per rep.

$^3$Trial 2: Batch 3 grass, 6 reps, 10 days, 10 neonates per rep.

Within trials, means within columns not followed by the same letter differ significantly (ANOVA, LSD, $P < 0.05$).

Table 2. Survival and growth of neonate fall armyworm, *Spodoptera frugiperda*, on greenhouse-grown KY 31 or Jackal tall fescue with or without their endophytes (wild-type or AR 601, respectively).

<table>
<thead>
<tr>
<th>Grass</th>
<th>Endo</th>
<th>Instar attained$^1$</th>
<th>Survival (%)</th>
<th>Mean wt (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial 1$^2$</td>
<td>KY31 + 1.9 ± 0.1 a</td>
<td>32 ± 3 a</td>
<td>10 ± 2.0 b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Jackal + 2.2 ± 0.1 b</td>
<td>65 ± 9 b</td>
<td>119 ± 30 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>KY31 - 2.0 ± 0.0 ab</td>
<td>43 ± 7 b</td>
<td>23 ± 5.0 b</td>
<td></td>
</tr>
<tr>
<td>$F_{2,15}$ =</td>
<td>3.8</td>
<td>6.5</td>
<td>9.7</td>
<td></td>
</tr>
<tr>
<td>$P \leq$</td>
<td>0.05</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

$^1$Data are means (± SE) per cohort of 10 neonate larvae per replicate.

$^2$Trial 1: Batch 3 grass, 6 reps, 9 days, 10 neonates per rep.

Within trials, means within columns not followed by the same letter differ significantly (ANOVA, LSD, $P < 0.05$).
Table 3. Survival and growth of neonate black cutworm, *Agrotis ipsilon*, on greenhouse-grown KY31 or Jackal tall fescue tillers with or without their endophytes (wild-type or AR601, respectively).

<table>
<thead>
<tr>
<th>Grass</th>
<th>Endo</th>
<th>Instar attained</th>
<th>Survival (%)</th>
<th>Mean wt (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KY31</td>
<td>+</td>
<td>1.2 ± 0.3 b</td>
<td>42 ± 10 b</td>
<td>6.1 ± 1.9 b</td>
</tr>
<tr>
<td>Jackal</td>
<td>+</td>
<td>1.4 ± 0.3 b</td>
<td>50 ± 10 b</td>
<td>6.7 ± 1.8 b</td>
</tr>
<tr>
<td>KY31</td>
<td>-</td>
<td>1.5 ± 0.4 b</td>
<td>46 ± 10 b</td>
<td>7.7 ± 2.2 b</td>
</tr>
<tr>
<td>Jackal</td>
<td>-</td>
<td>2.7 ± 0.3 a</td>
<td>80 ± 8 a</td>
<td>16.1 ± 2.3 a</td>
</tr>
</tbody>
</table>

$F_{3,69} =$ 4.52 3.01 5.19  
P ≤ 0.006 0.04 0.003

1 Grass is from Batch 2 seed.  
2 Data are means (± SE) per 24 replicates.  
Means within columns not followed by the same letter differ significantly (ANOVA, LSD, $P<0.05$).

Table 4. Survival of neonate fall armyworm, *Spodoptera frugiperda*, on greenhouse-grown KY31 or Jackal tall fescue tillers with or without their endophytes (wild-type or AR601, respectively).

<table>
<thead>
<tr>
<th>Grass</th>
<th>Endo</th>
<th>Instar Attained</th>
<th>Survival (%)</th>
<th>Mean wt (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KY31</td>
<td>+</td>
<td>2.3 ± 0.2</td>
<td>83 ± 8</td>
<td>12.2 ± 1.8 a</td>
</tr>
<tr>
<td>Jackal</td>
<td>+</td>
<td>1.4 ± 0.2</td>
<td>67 ± 10</td>
<td>4.7 ± 0.8 c</td>
</tr>
<tr>
<td>KY31</td>
<td>-</td>
<td>1.7 ± 0.3</td>
<td>63 ± 10</td>
<td>7.1 ± 1.4 bc</td>
</tr>
<tr>
<td>Jackal</td>
<td>-</td>
<td>2.0 ± 0.3</td>
<td>71 ± 9</td>
<td>10.8 ± 1.9 ab</td>
</tr>
</tbody>
</table>

$F_{3,69} =$ 2.28 0.96 4.95  
P ≤ 0.09 0.5 0.004

1 Grass is from Batch 2 seed.  
2 Data are means (± SE) per 24 replicates.  
Means within columns not followed by the same letter differ significantly (ANOVA, LSD, $P<0.05$).
Table 5. Population growth of bird cherry-oat aphid, *Rhopalosiphum padi*, on greenhouse-grown KY31 or Jackal tall fescue tillers with or without their endophytes (wild-type or AR601, respectively), coinciding with the percent infection of the grass tillers.

<table>
<thead>
<tr>
<th>Grass</th>
<th>Endo</th>
<th>Population after 14 d$^{1}$</th>
<th>% infected tillers$^{2}$</th>
<th>Population on infected tillers after 14 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>KY31</td>
<td>+</td>
<td>4.5 ± 1.7 c</td>
<td>70.8 ± 9.5 a</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Jackal</td>
<td>+</td>
<td>12.8 ± 3.3 c</td>
<td>62.5 ± 10.1 a</td>
<td>2.5 ± 1.4</td>
</tr>
<tr>
<td>KY31</td>
<td>-</td>
<td>29.3 ± 3.1 b</td>
<td>0.0 ± 0.0 b</td>
<td>n/a</td>
</tr>
<tr>
<td>Jackal</td>
<td>-</td>
<td>47.9 ± 5.1 a</td>
<td>0.0 ± 0.0 b</td>
<td>n/a</td>
</tr>
</tbody>
</table>

$^{1}$Data are means (±SE) per cohort of 5 aphids on single tillers per replicate (24 reps, 14 days).

$^{2}$Endophyte infection of tillers was determined by immunoblot tests (2 blots per replicate).

Means within columns not followed by the same letter differ significantly (ANOVA, LSD, $P<0.05$).
Table 6. Survival and growth of neonate black cutworm, *Agrotis ipsilon*, on greenhouse-grown KY31 or Jackal tall fescue grass in a large contained arena for 10 days.

<table>
<thead>
<tr>
<th>Grass</th>
<th>Endo</th>
<th>Instar attained</th>
<th>Survival (%)</th>
<th>Mean wt (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KY31</td>
<td>+</td>
<td>2.0 ± 0.0</td>
<td>32 ± 8</td>
<td>6.3 ± 1.6 b</td>
</tr>
<tr>
<td>Jackal</td>
<td>+</td>
<td>2.0 ± 0.0</td>
<td>35 ± 11</td>
<td>12.4 ± 4.3 b</td>
</tr>
<tr>
<td>KY31</td>
<td>-</td>
<td>2.0 ± 0.0</td>
<td>45 ± 12</td>
<td>16.2 ± 4.7 b</td>
</tr>
<tr>
<td>Jackal</td>
<td>-</td>
<td>2.0 ± 0.0</td>
<td>67 ± 12</td>
<td>43.0 ± 8.9 a</td>
</tr>
<tr>
<td>F_{3,20} =</td>
<td>1.0</td>
<td>2.06</td>
<td>8.51</td>
<td></td>
</tr>
<tr>
<td>P ≤</td>
<td>0.41</td>
<td>0.14</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

*Grass is from Batch 2 seed.  
Data are means (± SE) per cohort of 10 neonate larvae per replicate (6 reps). Means within columns not followed by the same letter differ significantly (ANOVA, LSD, *P* < 0.05).

Table 7. Survival and growth of neonate fall armyworm, *Spodoptera frugiperda*, on greenhouse-grown KY31 or Jackal tall fescue grass in a large contained arena for 7 days.

<table>
<thead>
<tr>
<th>Grass</th>
<th>Endo</th>
<th>Instar attained</th>
<th>Survival (%)</th>
<th>Mean wt (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KY31</td>
<td>+</td>
<td>2.2 ± 0.5</td>
<td>57 ± 17</td>
<td>104.3 ± 37.5</td>
</tr>
<tr>
<td>Jackal</td>
<td>+</td>
<td>2.4 ± 0.5</td>
<td>62 ± 16</td>
<td>114.7 ± 39.1</td>
</tr>
<tr>
<td>KY31</td>
<td>-</td>
<td>2.5 ± 0.5</td>
<td>65 ± 16</td>
<td>134.3 ± 30.6</td>
</tr>
<tr>
<td>Jackal</td>
<td>-</td>
<td>2.1 ± 0.5</td>
<td>37 ± 15</td>
<td>91.0 ± 40.9</td>
</tr>
<tr>
<td>F_{3,20} =</td>
<td>0.11</td>
<td>0.61</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>P ≤</td>
<td>0.95</td>
<td>0.62</td>
<td>0.87</td>
<td></td>
</tr>
</tbody>
</table>

*Grass is from Batch 2 seed.  
Data are means (± SE) per cohort of 10 neonate larvae per replicate (6 reps). Means within columns did not differ significantly (ANOVA, LSD, *P* < 0.05).
Table 8. Growth of 4th instar black cutworm, *Agrotis ipsilon*, on greenhouse-grown KY31 or Jackal tall fescue grass in a single pot.

<table>
<thead>
<tr>
<th>Grass</th>
<th>Larval weight (mg) at:</th>
<th>No. surviving (out of 24)</th>
<th>Mean instar attained</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Endo</td>
<td>Start</td>
<td>End</td>
</tr>
<tr>
<td>KY31</td>
<td>+</td>
<td>290.7 ± 8.3</td>
<td>619.2 ± 44.0</td>
</tr>
<tr>
<td>Jackal</td>
<td>+</td>
<td>289.1 ± 9.4</td>
<td>654.0 ± 49.1</td>
</tr>
<tr>
<td>KY31</td>
<td>-</td>
<td>294.4 ± 11.1</td>
<td>655.0 ± 30.5</td>
</tr>
<tr>
<td>Jackal</td>
<td>-</td>
<td>292.0 ± 9.9</td>
<td>654.6 ± 37.5</td>
</tr>
</tbody>
</table>

1Grass is from Batch 2 seed.
2\(F = 0.30, P = 0.83\); 3\(F = 1.05, P = 0.38\); 4\(F = 1.03, P = 0.39\) (ANOVA, \(P < 0.05\)).
5Length of feeding assay was 6 days.

Table 9. Population size and collective biomass of earthworms (*Aporrectodea* spp.) and white grubs (*Cyclocephala* spp., *Phyllophaga* spp., and *P. japonica*) sampled from field plots on 8 October 2014.

<table>
<thead>
<tr>
<th>Grass</th>
<th>Endo</th>
<th>Mean per sample</th>
<th>Biomass (g)</th>
<th>Mean per sample</th>
<th>Biomass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KY31</td>
<td>+</td>
<td>25.8 ± 4.4</td>
<td>8.6 ± 2.0</td>
<td>0.8 ± 0.3</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>Jackal</td>
<td>+</td>
<td>25.3 ± 5.0</td>
<td>8.0 ± 1.7</td>
<td>1.5 ± 0.7</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>KY31</td>
<td>-</td>
<td>32.0 ± 4.7</td>
<td>8.2 ± 0.9</td>
<td>1.3 ± 0.6</td>
<td>0.4 ± 0.3</td>
</tr>
<tr>
<td>Jackal</td>
<td>-</td>
<td>31.2 ± 6.5</td>
<td>9.4 ± 1.9</td>
<td>2.8 ± 1.3</td>
<td>0.9 ± 0.5</td>
</tr>
<tr>
<td>(F_{3,15} = 042)</td>
<td>0.74</td>
<td>0.13</td>
<td>1.15</td>
<td>1.17</td>
<td></td>
</tr>
</tbody>
</table>

1Sample size is five slabs of turf and soil (each 18 × 18 × 10.2 cm deep) per replicate (6 reps).
Means ± SE within columns did not differ significantly (ANOVA, LSD, \(P < 0.05\)).
Table 10. Coverage of field plots by assorted grasses and weeds; 11 July 2014.

Percent Coverage by:

<table>
<thead>
<tr>
<th>Grass</th>
<th>Endo</th>
<th>Tall fescue</th>
<th>Annual</th>
<th>Clover</th>
<th>Unidentified broadleaf weeds</th>
<th>Bare ground</th>
</tr>
</thead>
<tbody>
<tr>
<td>KY31</td>
<td>+</td>
<td>87.7 ± 1.6</td>
<td>3.3 ± 1.4</td>
<td>0.1 ± 0.1</td>
<td>0.9 ± 0.4</td>
<td>8.0 ± 1.6</td>
</tr>
<tr>
<td>Jackal</td>
<td>+</td>
<td>89.8 ± 1.8</td>
<td>2.4 ± 0.8</td>
<td>0.2 ± 0.2</td>
<td>0.3 ± 0.2</td>
<td>7.3 ± 1.4</td>
</tr>
<tr>
<td>KY31</td>
<td>-</td>
<td>86.9 ± 1.6</td>
<td>3.7 ± 0.9</td>
<td>0.0 ± 0.0</td>
<td>0.6 ± 0.3</td>
<td>8.8 ± 1.1</td>
</tr>
<tr>
<td>Jackal</td>
<td>-</td>
<td>86.2 ± 3.7</td>
<td>5.1 ± 2.3</td>
<td>0.0 ± 0.0</td>
<td>0.8 ± 0.4</td>
<td>8.0 ± 1.3</td>
</tr>
<tr>
<td>$F_{3,15} =$</td>
<td>0.62</td>
<td>1.25</td>
<td>0.70</td>
<td>0.66</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>$P \leq$</td>
<td>0.61</td>
<td>0.33</td>
<td>0.57</td>
<td>0.59</td>
<td>0.83</td>
<td></td>
</tr>
</tbody>
</table>

Means within columns did not differ significantly (ANOVA, LSD, $P < 0.05$). Sample based on plants touched by a grid of 100 points within a 1 m$^2$ frame. Two such samples were counted within each plot.

Table 11. Comparison of goat eating preference between the two choice tests at AM and PM feeding times.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>AM$^1$</th>
<th>PM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jackal +</td>
<td>58</td>
<td>142</td>
</tr>
<tr>
<td>KY31 -</td>
<td>24</td>
<td>78</td>
</tr>
<tr>
<td>$\chi^2 =$</td>
<td>14.1</td>
<td>18.6</td>
</tr>
<tr>
<td>$P \leq$</td>
<td>$\leq 0.001$</td>
<td>$\leq 0.001$</td>
</tr>
</tbody>
</table>

| KY31 +     | 24     | 61 |
| KY31 -     | 58     | 51 |
| $\chi^2 =$ | 14.1   | 0.89|
| $P \leq$   | $\leq 0.001$ | $\geq 0.20$ |

$^1$Total observations of 5 goats. Within comparisons and time periods, the Chi-Square test evaluates the null hypothesis of equal feeding (number of observations) in each choice; df = 1.
Table 12. Mean total number of birds observed in photos of the field plots taken every 5 minutes during three, 72-h sessions starting 24 June, 9 July, and 6 August during the 2014 growing season.

<table>
<thead>
<tr>
<th>Grass</th>
<th>Endo</th>
<th>Mean (± SE) number of birds$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>KY31</td>
<td>+</td>
<td>6.2 ± 2.1</td>
</tr>
<tr>
<td>Jackal</td>
<td>+</td>
<td>6.3 ± 1.9</td>
</tr>
<tr>
<td>KY31</td>
<td>-</td>
<td>4.0 ± 0.9</td>
</tr>
<tr>
<td>Jackal</td>
<td>-</td>
<td>5.7 ± 1.1</td>
</tr>
</tbody>
</table>

$^1$: Photos were taken with separate mini-game cameras set up beside each replicate to record bird presence both during daylight and at night.

Table 13. Percentage infection of Jackal and KY31 tall fescue tillers collected from the field plots in July 2014.

<table>
<thead>
<tr>
<th>Grass</th>
<th>Endo</th>
<th>Mean % Infection$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>KY31</td>
<td>+</td>
<td>77.0 ± 4.8 a</td>
</tr>
<tr>
<td>Jackal</td>
<td>+</td>
<td>68.5 ± 4.5 a</td>
</tr>
<tr>
<td>KY31</td>
<td>-</td>
<td>0.0 ± 0.0 b</td>
</tr>
<tr>
<td>Jackal</td>
<td>-</td>
<td>1.2 ± 1.2 b</td>
</tr>
</tbody>
</table>

$^1$: Infection rates were determined using an immunoblot; based on a sample of 12 tillers per plot. Means not followed by the same letter are significantly different (LSD test).

Table 14. Alkaloid and nitrogen content of Jackal and KY31 tall fescue (with and without endophyte) grown in the greenhouse from Batch 2 seed.

<table>
<thead>
<tr>
<th>Grass</th>
<th>Endo</th>
<th>Ergot alkaloids (ppm)</th>
<th>Loxine alkaloids (ppm)</th>
<th>Nitrogen (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KY31</td>
<td>+</td>
<td>0.8 ± 0.05 a</td>
<td>409.8 ± 49.8 a</td>
<td>5.1 ± 0.2 a</td>
</tr>
<tr>
<td>Jackal</td>
<td>+</td>
<td>0.5 ± 0.03 b</td>
<td>487.8 ± 27.4 a</td>
<td>5.2 ± 0.1 a</td>
</tr>
<tr>
<td>KY31</td>
<td>-</td>
<td>0.0 ± 0.0 c</td>
<td>0.0 ± 0.0 b</td>
<td>4.0 ± 0.4 b</td>
</tr>
<tr>
<td>Jackal</td>
<td>-</td>
<td>0.0 ± 0.0 c</td>
<td>0.0 ± 0.0 b</td>
<td>4.6 ± 0.2 ab</td>
</tr>
</tbody>
</table>

$F_{3,9} = 151.42$, $P \leq 0.001$  

Within columns, means not followed by the same letter are significantly different (LSD, $P < 0.05$).
Figure 4. Pitfall trap abundance of Carabidae, Staphylinidae, and of other Coleoptera.

Carabid abundance was significantly higher in Jackal E- grasses in May and is lower in KY31 E+ grasses in September (May: $F_{3,15} = 17.2, P < 0.001$; July: $F_{3,15} = 0.49, P = 0.7$; September: $F_{3,15} = 2.87, P = 0.07$). Staphylinid abundance was lower in KY31 E+ grasses in September (May: $F_{3,15} = 1.18, P = 0.35$; July: $F_{3,15} = 0.44, P = 0.73$; September: $F_{3,15} = 3.27, P = 0.05$). Abundance of other Coleoptera did not differ across the four grasses (May: $F_{3,15} = 1.15, P = 0.36$; July: $F_{3,15} = 1.62, P = 0.23$; September: $F_{3,15} = 1.44, P = 0.27$).
Figure 5. Pitfall trap abundance of Formicidae and of the orders Hymenoptera and Diptera. The abundance of ants did not differ across the four grasses (May: $F_{3,15} = 0.63$, $P = 0.61$; July: $F_{3,15} = 0.23$, $P = 0.87$; September: $F_{3,15} = 0.83$, $P = 0.50$). Abundance of other Hymenoptera did not differ across the four grasses (May: $F_{3,15} = 0.70$, $P = 0.57$; July: $F_{3,15} = 0.60$, $P = 0.62$; September: $F_{3,15} = 0.56$, $P = 0.65$). Abundance of Diptera did not significantly differ across the four grasses (May: $F_{3,15} = 1.60$, $P = 0.23$; July: $F_{3,15} = 0.61$, $P = 0.62$; September: $F_{3,15} = 3.02$, $P = 0.06$).
Figure 6. Pitfall trap abundance of the insect orders Hemiptera, Lepidoptera, and Orthoptera. Hemiptera abundance was significantly higher in Jackal E- grass in the month of May (May: $F_{3,15} = 3.19, P = 0.05$; July: $F_{3,15} = 1.61, P = 0.23$; September: $F_{3,15} = 0.74, P = 0.54$). Lepidoptera abundance was lower in KY31 E+ grass in May and September and lower in KY31 E- grass in September (May: $F_{3,15} = 2.31, P = 0.12$; July: $F_{3,15} = 0.62, P = 0.61$; September: $F_{3,15} = 4.51, P = 0.02$). The abundance of orthoptera did not differ across the four grasses (May: $F_{3,15} = 0.36, P = 0.78$; July: $F_{3,15} = 1.40, P = 0.28$; September: $F_{3,15} = 1.43, P = 0.27$).
Figure 7. Pitfall trap abundance of the invertebrate groups Araneae and Gastropoda. The abundance of spiders (Araneae) did not significantly differ across the four grasses (May: $F_{3,15} = 1.36, P = 0.29$; July: $F_{3,15} = 0.44, P = 0.73$; September: $F_{3,15} = 2.00, P = 0.16$).

Slug abundance (Gastropoda) did not differ significantly across the four grasses (May: $F_{3,15} = 0.04, P = 0.99$; July: $F_{3,15} = 1.17, P = 0.36$; September: $F_{3,15} = 0.23, P = 0.89$).
Figure 8. Vacuum sample abundance of the insect families Cicadellidae and Cercopidae and of other Hemiptera. Cicadellid abundance did not differ significantly across the four grasses (May: $F_{3,15} = 1.15, P = 0.36$; July: $F_{3,15} = 1.62, P = 0.23$; September: $F_{3,15} = 1.59, P = 0.23$). The abundance of cercopids was significantly lower on KY31 E+ grass in May (May: $F_{3,15} = 4.42, P = 0.02$; July: $F_{3,15} = 1.15, P = 0.36$; September: $F_{3,15} = 1.17, P = 0.35$). Abundance of other Hemiptera did not differ significantly across the four grasses (May: $F_{3,15} = 1.54, P = 0.25$; July: $F_{3,15} = 0.60, P = 0.63$; September: $F_{3,15} = 1.08, P = 0.39$).
Figure 9. Vacuum sample abundance of the insect family Formicidae and the orders Hymenoptera and Diptera. The abundance of ants (Formicidae) did not significantly differ across the four grasses (May: $F_{3,15} = 0.58, P = 0.64$; July: $F_{3,15} = 0.60, P = 0.63$; September: $F_{3,15} = 0.73, P = 0.55$). The abundance of other Hymenoptera did not differ significantly across the four grasses (May: $F_{3,15} = 0.06, P = 0.98$; July: $F_{3,15} = 0.16, P = 0.92$; September: $F_{3,15} = 0.55, P = 0.66$). Fly abundance (Diptera) was significantly higher in Jackal E- grass in May (May: $F_{3,15} = 6.77, P = 0.004$; July: $F_{3,15} = 1.24, P = 0.33$; September: $F_{3,15} = 0.18, P = 0.91$).
Figure 10. Vacuum sample abundance of the insect family Staphylinidae and the orders Coleoptera and Orthoptera. Staphylinid abundance did not significantly differ across the four grasses (May: $F_{3,15} = 0.56$, $P = 0.65$; July: $F_{3,15} = 1.30$, $P = 0.31$; September: $F_{3,15} = 0.97$, $P = 0.43$). The abundance of other coleoptera was significantly lower in KY31 E+ in July (May: $F_{3,15} = 0.83$, $P = 0.50$; July: $F_{3,15} = 3.66$, $P = 0.04$; September: $F_{3,15} = 1.71$, $P = 0.21$). The abundance of orthoptera did not differ significantly across the four grasses (May: $F_{3,15} = 1.00$, $P = 0.42$; July: $F_{3,15} = 1.28$, $P = 0.32$; September: $F_{3,15} = 0.81$, $P = 0.51$).
Figure 11. Vacuum sample abundances of the insect group lady beetle larvae, of the order Lepidoptera, and of Araneae. The abundance of lady beetle larvae did not significantly differ across the four grasses (May: $F_{3,15} = 0.45$, $P = 0.72$; July: $F_{3,15} =$ N/A, $P =$ N/A; September: $F_{3,15} =$ N/A, $P =$ N/A). Lepidoptera abundance was higher in KY31 E+ grass in September (May: $F_{3,15} = 0.38$, $P = 0.77$; July: $F_{3,15} = 0.82$, $P = 0.50$; September: $F_{3,15} = 3.14$, $P = 0.06$). Spider abundance (Araneae) was lower in KY31 E+ grass and higher in Jackal E- grass in May (May: $F_{3,15} = 2.40$, $P = 0.11$; July: $F_{3,15} = 0.87$, $P = 0.48$; September: $F_{3,15} = 0.51$, $P = 0.68$).
Figure 12. Three caterpillar choice tests with field-grown grass tillers; Choice test 1: Jackal E+ vs. KY31 E-; Choice test 2: Jackal E+ vs. KY31 E+; Choice test 3: KY31 E+ vs. KY31 E-. Fall armyworm caterpillar location was observed at time 24 and 48-h after initial inoculation.
Figure 13. Goat preference choice test of Jackal E+ vs. KY31 E- comparing the behaviors of the goats for the morning and afternoon sessions. The values refer to the total number of observations of the five goats and the corresponding percentage.
Figure 14. Goat preference choice test of KY31 E+ vs. KY31 E- comparing the behaviors of the goats for the morning and afternoon sessions. The values refer to the total number of observations of the five goats and the corresponding percentage.

**KY31 E+ vs. KY31 E- AM**

- Eat K+: 24, 5%
- In K+: 122, 26%
- In K-: 97, 21%
- other: 171, 36%
- Eat K-: 58, 12%

**KY31 E+ vs. KY31 E- PM**

- Eat K+: 61, 10%
- In K+: 122, 20%
- In K-: 153, 25%
- other: 213, 36%
- Eat K-: 51, 9%
Figure 15. Alkaloid, ergots and lolines, and nitrogen content of field grass from three
dates during the 2014 growing season; 13 May, 24 July, 24 September. Ergot alkaloids
were significantly higher in KY31 E+ grass than Jackal E+ in May (May: $F_{3,15} = 81.5,
P < 0.001$; July: $F_{3,15} = 31.4, P < 0.001$; September: $F_{3,15} = 11.4, P < 0.001$). Both
endophytic grasses contained similar levels of loline alkaloids (May: $F_{3,15} = 54.5, P <
0.001$; July: $F_{3,15} = 69.0, P < 0.001$; September: $F_{3,15} = 143.8, P < 0.001$). Nitrogen
content was significantly higher in the Jackal grasses (E+ and E-) in July and September
(May: $F_{3,15} = 0.49, P= 0.69$; July: $F_{3,15} = 11.8, P < 0.001$; September: $F_{3,15} = 10.6, P <
0.001$).
DISCUSSION

Endophytic grasses have been shown to have activity against invertebrates and vertebrate herbivores, but little is known about whether or not these grasses could be effective in reducing wildlife airstrike risk at airports. I addressed this question—whether a New Zealand-bred novel or endemic wild-type endophytic tall fescue grass would repel or reduce abundance of invertebrates, birds, and goats (a surrogate for grazers such as deer) to an extent at which airfields would have less risk of airstrike—in this thesis. This concept was drawn to my attention by Avanex™, a unique endophyte technology grass marketed for reducing bird airstrike. Avanex™, cultivar Jackal, is an endophytic tall fescue grass that is purported to contain high levels of alkaloids when compared to the standard wild-type KY31 (> 1100 ppm loline; > 3.4 ppm ergovaline) (Pennell and Rolston 2013). These alkaloids, at high enough concentrations, are known to cause invertebrate and vertebrate herbivores to avoid feeding on endophytic grasses (Porter and Thompson 1992, Bush et al. 1997). However, my research into this concept, specifically this unique grass, has resulted in my accepting the null hypothesis – that Jackal is not any better than the wild-type KY31. Jackal E+ does not contain significantly more alkaloids than KY31 E+ (in some cases KY31 E+ contained more) and does not produce a strong, effective deterrence on herbivores.

Multiple insect feeding assays were conducted with both Jackal and KY31 (with and without endophyte) tall fescue grasses. The first feeding assay showed promising results: Jackal E+ fed caterpillars had significantly lower survival rates, lower weight gain, and slower development. But all subsequent feeding assays did not indicate
significant differences between Jackal E+ and KY31 E+. The caterpillars (black cutworms and fall armyworms) and bird cherry-oat aphids responded to Jackal E+ similarly to KY31 E+. This strong change between the first feeding assay and all the rest may be due to variation in the different batches of Jackal seed sent to us by PGG Wrightson, the supplier. The first batch of Jackal seed was only used in the first feeding assay which showed significant results. The remainder of the feeding assays used different batches of Jackal seed, which, with supporting alkaloid analyses, were found to be not significantly different from KY31 E+. This potential for batch to batch variability is concerning from the standpoint of quality control. If a research scientist is unable to depend on receiving a consistently active supply of Jackal E+ seed, would an airport grounds manager be able to do so?

The model insects used in these feeding assays are known to respond to endophytic grasses differently. Black cutworms and fall armyworms, which are native grass feeding caterpillars, have likely adapted to endophytic tall fescue, especially KY31, and are no longer greatly affected by such grasses. Bird cherry-oat aphids, which normally feed on grain crops, have apparently not adapted to endophytic tall fescue and therefore show great sensitivity to the presence of endophytes. These aphids, due to this sensitivity, have been widely used in endophytic research (e.g., Johnson et al. 1985, Bush et al. 1997, Breen 1994, Davidson and Potter 1995) but this cannot be directly related to a field setting where stands of tall fescue would be inhabited by a diverse community of adapted grass- and litter-feeding insects and earthworms along with their invertebrate predators. Moreover, the prevailing use of selected alkaloid-sensitive test species such as R. padi in evaluating activity of endophytic grasses has probably biased the literature as
far as the extent to which such grasses can suppress the whole community of
invertebrates in the field. The aphid feeding assays were run to assuage concerns that the
endophytic grasses weren’t really endophytic (due to the consistent non-difference
between Jackal E+ and KY31 E+ when fed on by black cutworms and fall armyworms);
along with a corresponding immunoblot assay of the grass tillers used in the bioassay.
When the tillers were infected with the endophyte, the aphid populations struggled, and
vice versa, which confirmed the integrity of my grass treatments.

During many of the feeding assays, the caterpillars and aphids seemed to do
particularly well on Jackal E- than on the other three grass types. While not always
statistically significant, the insects appeared to develop quicker and gain more weight
when fed on Jackal E-. This surprising result led me to evaluate the nitrogen content of
all the grasses. Interestingly, the batch 2 greenhouse-grown grass did not show higher
nitrogen contents in Jackal E- but this may be due to the regular fertilization of said grass.
The batch 2 grass from my field plots, however, did show higher nitrogen contents in
Jackal E- as well as Jackal E+ compared to their KY 31 counterparts. Nitrogen is a
limiting macronutrient for insect herbivores (Matson 1980) which often develop faster,
grow larger, and have increased fecundity on plant tissues having relatively higher
nitrogen content (Huberty and Denno 2006). This could have a potentially disastrous
effect if the Jackal E+ grass planted at an airport had low endophyte infection rates,
effectively making it Jackal E-. Insects would be able to grow faster and reproduce more
on such a grass and the risk of bird airstrike could be increased.

Insect populations are sometimes reduced in field stands of endophytic grasses
(Breen 1994, Richmond et al. 2000, Popay and Hume 2011). However, my research did
not show this result. Both the pitfall traps and vacuum samples showed no consistent differences or trends among the invertebrate groups and the grasses across the growing season. I expected there to be more of an endophyte effect, with fewer insects in Jackal E+ and KY31 E+ than Jackal E- and KY31 E-, but that was not the case. The endophyte infection rates were acceptable and within normal ranges for the field, and the alkaloid analyses and immunoblot assays confirmed the integrity of my grass treatments. Still, the endophytic and non-endophytic grasses all had similar abundance of insects.

Were there potentially confounding factors that prevented seeing stronger endophyte effects? The endemic insect populations at Spindletop farm where my field study was conducted presumably have long encountered endophytic tall fescue in Kentucky grassland habitats, so it is possible that they are less sensitive to the alkaloids than are the test insects and pests that were used in so much of the past work on endophyte effects on insects. Perhaps my field plots were too small for the sampling methods; e.g., insects might jump, fly, or crawl between plots if disturbed by vacuum sampling or while emptying the pitfall traps. There was a perennial ryegrass border surrounding each plot which, while useful for visual delineation between plots, might have served as a refuge for some insects, diluting the time they spent in the tall fescue. Still, my individual plots were of reasonably large size (5.5 × 5.5 m), especially for a field study with 24 plots, and certainly large enough to support localized populations of ground dwelling and subterranean invertebrates while still allowing those organisms to disperse and resettle in other plots if they had been inclined to do so.

Soil-dwelling invertebrates, such as earthworms and white grubs, also did not demonstrate a response to endophytes. This is most likely due to negligible amounts of
alkaloids found in the roots of endophytic grasses, making the fungus non-effective against such invertebrates (Bush et al. 1997, Potter et al. 1992). It is possible that leaching of alkaloids from the thatch layer could affect earthworms and white grubs but there is no evidence yet of such a phenomenon.

The preference test using Spanish goats was inconclusive. As expected, the goats showed some preference for KY31 E- over KY31 E+, but the opposite effect was observed with Jackal E+ which they somewhat preferred over KY31 E-. Considering that Jackal and KY31 tall fescue are two different cultivars, the taste and texture of the Jackal grass blades might be more desirable than KY31 to the goats, or perhaps they detected the former’s relatively higher nitrogen content. Also, the trials may not have been long enough for the goats to either feel or learn from negative effects (i.e., food aversion learning) or to learn the differences between each grass based on palatability. Also, the goats were not used to being in individual pens, separated from their counterparts, so their behavior might have been modified; e.g., they might have been inclined to remain on the side of the enclosure closest to the nearest neighboring goat. With all these variables possibly affecting the results, it is hard to extrapolate my results to deer and rabbits that would have more time to associate and learn from potential post-ingestion feedback from grazing on an endophytic grass that is planted at an airport. Even if an animal is deterred by the endophytic grass, who’s to say that the animal won’t just cross a runway to sample a potentially more palatable grass on the opposite side, becoming an even bigger hazard than before? Clearly much more needs to be learned about wildlife behavior in the context of airport landscapes before we can assume that planting a less palatable grass would have the desired benefits for reducing strike hazard.
Bird presence on my field plots was low, and there was no noticeable preference for, or avoidance of, one grass over the others. Granted, the observation periods may not have been frequent enough to detect such a difference, but given the similarity in invertebrate populations between the different grasses there is no reason to expect one. I observed mostly black birds (starlings and grackles) visiting my plots with the occasional robin; these types of birds eat earthworms and insects. Canada geese, on the other hand, eat grass. Unfortunately, no Canada geese were seen or photographed on my plots, and it was not practical to obtain the permits or collect the birds needed to do cage trials to test for learning and avoidance of the endophytic grasses. Further investigation into whether endophytic grasses will deter Canada geese away from airfields is needed.
CONCLUSION

Bird and other wildlife airstrike is an important safety issue for airports and airplane passengers and for that reason, wildlife should be repelled from airfields. The most sustainable method is to have plants that are undesirable to wildlife adjacent to the runways, and in some cases more attractive habitat in outlying areas to lure wildlife to where it does not pose a hazard to incoming or outgoing planes. If invertebrate populations could be substantially decreased by an endophytic grass, it stands to reason that birds which feed on invertebrates follow. Animals that also feed on grass would be deterred by the endophyte due to non-palatability and post-digestion feedback and learning. Unfortunately, the “novel endophyte technology” grass, Jackal E+, touted to be more effective in reducing insect and bird populations than are conventional endophytic grasses, was largely ineffective in my trials. Indeed, even the common wild-type KY31 E+ had weak, inconsistent, or no effect on insects other than aphids, a result consistent with Keathley and Potter (2012) who also saw weak or no reduction in wild invertebrate populations in pastures containing live-stock “safe” endophytes that lacked ergot alkaloids but retained the rest of the wild-type alkaloids. Insectivorous birds are not likely to be deterred when the invertebrate abundance is unchanged.

Although the concept of using endophytic grasses in airport wildlife management seems sound, better understanding of the strengths and limitations of that approach is needed before it can be assumed that use of such grasses will provide the desired benefits. Also, endophyte-containing grasses are a living symbiota, maternally transmitted, and subject to decline in viability and seasonal variability in alkaloid expression (Ball 1993,
Schardl et al. 2004). The apparent batch to batch variability of the seed lots used in my trials suggests quality control issues which would have to be solved for a reliable and effective commercial product. With this research, I hope questions have been posed that will initiate a more widespread investigation into the value of using endophytic grasses for managing nuisance wildlife.
APPENDIX A

Alkaloid Measurements in Small Plot Trials of Continental-type Tall Fescue
Authors: David Hume, Wade Mace, Brian Tapper, Tony Stratton

1. **Frequency of endophyte infection** (a one-off sampling between May - November):
   a. Create an Excel spreadsheet with details of:
   b. Location, Tall fescue TILLERS, Variety / Endophyte name, Replicate #, Plot #, Sample date
   c. and email to Agrinostics = services@agrinostics.com
   d. For each plot, label a Ziploc plastic bag with:
      Location, Tall fescue, Variety / Endophyte name, Replicate #, Plot #, Sample date.
   e. Take 10-15 independent tillers from each plot (6 replicates), or 15-20 independent tillers from each plot (4 replicates)
   f. Courier the tiller samples to Agrinostics laboratory for immunoblot analyses of % endophyte-infected tillers, and for ELISA analyses of % ergot alkaloids-infected tillers.
   g. These tests are needed to normalize data of alkaloid concentrations if % endophyte infection differs between treatments, and also identify any errors and contamination by the endemic strain of KY31 endophyte.

2. **Sampling herbage for alkaloids**:
   a. Label a Ziploc plastic bag (1 per plot) with: Location, Tall fescue HERBAGE, Variety / Endophyte name, Replicate #, Plot #, Sample date
   b. From each plot, take samples selected randomly from 20 positions (3 to 5 tillers at each position) and bulk
   c. At each position cut only tall fescue with a knife or scissors to ground level or to the crown of the plant if this is higher than the ground level
   d. Retain leaves, stems and seed heads of tall fescue plants.
   e. Avoid soil contamination. Remove any soil that is with the sampled herbage
   f. Discard any dead material, weeds, and non-tall fescue plant material
   g. Place sample (bulk of herbage from 20 positions) in bag, seal bag and place in a Cooler box ASAP, or at 4°C.
   h. Freeze samples ASAP
   i. Freeze-dry samples
   j. Mill samples through a 1 mm sieve (UDY or Wiley cyclonic mill), and
   k. Store samples in air tight bags in a freezer until shipping to the laboratory.

3. **Time of herbage sampling**:
   a. Early Spring, before reproductive tillers appear, say mid April.
   b. Late Spring, including reproductive tillers, say mid May
   c. Summer, say early-mid June, or earlier at first signs of plant stress (leaf rolling)
d. Early Autumn, say early-mid September when new growth permits sampling

e. Late Autumn, say early-mid October

f. Winter, say early November prior to cessation of growth.

4. **Photographs**

   a. A digital photo to be taken of herbage samples from each treatment (1 replicate) to document stage of growth, plant height, plant health, ratio of stem to leaf lamina.

   b. Prepare typical examples of individual tillers (samples from 5 above),

   c. Lay tillers on a blue cardboard background with metric grid (1 cm) and metric ruler scale on the side

   d. Label each photo with details of:

      Location, Tall fescue, Variety / Endophyte name, Replicate #, Plot #, Sample date

   e. Aim is to capture colour, height and relative proportions of leaf and pseudostem
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