

Avanex Unique Endophyte Technology: Reduced Insect Food Source at Airports

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Environ. Entomol. 1–10 (2015); DOI: 10.1093/ee/nvv145

ABSTRACT Birds and other forms of wildlife are a major issue for airport authorities worldwide, as they can create hazards to operating aircraft. Wildlife “strikes,” the majority caused by birds, can cause damage to operating aircraft and in severe cases lead to a loss of human life. Many airfields contain large areas of ground cover herbage alongside their runways that consist of mixtures of grasses, legumes, and weeds that can harbor many invertebrates. Many airfields use insecticides to control insect populations; however, mounting pressure from regional councils and water boards aim to reduce this practice due to ground water runoff and contamination concerns. Avanex Unique Endophyte Technology, a product specifically developed to reduce the attractiveness of airports and surrounding areas to birds, is based on a novel association between a selected strain of *Epichloë* endophyte and a turf-type tall fescue cultivar. This grass–endophyte association acts through a direct mechanism whereby a negative response in birds is created through taste aversion and postingestion feedback as well as an indirect mechanism by deterring many invertebrates, a food source of many bird species.

KEY WORDS *Epichloë*, invertebrate, tall fescue, wildlife

Bird strike is strictly defined as a collision between a bird and an aircraft which is in flight or on a take-off or landing roll. Bird strike is an increasing problem worldwide as aircraft movements increase, manufacturers introduce much quieter engines, and ecological changes occur to bird habitats including the urban sprawl of many cities. These collisions cost the US civilian aviation industry up to US\$957 million annually in direct and other monetary losses (Dolbeer et al. 2011) and a conservative estimate of US\$1.2 billion per annum globally (Allan 2002). Furthermore, these strikes put the lives of aircraft crew and their passengers at risk, with >255 people killed and >243 aircraft destroyed since 1988 (Dolbeer et al. 2011). Birds are attracted to airfields, as the large open spaces provide an abundance of feeding opportunities in the form of herbage, seeds, and insects, together with an unobstructed range of view that decreases the threat from potential predators (Solman 1981). Many airfields contain large areas of ground cover herbage alongside their runways that consist of mixtures of grasses, legumes, and weeds that can harbor many invertebrates.

Grass areas in close proximity to runways may harbor high populations of insects by comparison with those further away due to runoff from runways creating areas of higher fertility and lighting of these areas

attracting insects. Insectivorous birds feeding in these areas may pose a particular risk to planes. Many airfields use insecticides to control insect populations, but mounting pressure from regional councils and water management boards aim to reduce this practice due to ground water runoff and contamination concerns close to cities.

Tall fescue (*Lolium arundinaceum* (Schreb.) S.J. Darbyshire (= *Festuca arundinacea* Schreb)) is an important perennial forage grass utilized throughout the moderate to high rainfall temperate zones of the world (Young et al. 2013). This grass forms mutualistic symbiotic associations with fungal endophytes of the genus *Epichloë* (Johnson et al. 2013, Leuchtman et al. 2014). These endophyte–grass associations are thought to have coevolved ca. 40 million years ago and probably evolved at the same time as the subfamily Poöideae (family Poaceae), with suggestions that these endosymbionts supported early shifts for their grass hosts from shady to sunny habitats (Schardl et al. 2008). This hypothesis is supported by the large number of journal articles that document the beneficial traits imposed on grass hosts by these fungi. Such traits include herbivore deterrence from mammals (Bacon et al. 1977, Fletcher and Harvey 1981, Schmidt et al. 1982), invertebrates (Kuldau and Bacon 2008), birds (Pennell and Rolston 2003), and biochemical and physiological adaptations for drought tolerance (Malinowski and Belesky 2000, He et al. 2013).

AgResearch Limited, a Crown Research Institute (CRI) based in New Zealand, is a world leader in the research and development of novel, or artificial, grass–endophyte associations. This CRI has a large collection

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of *Epichloë* strains, characterized by way of their genetic diversity, secondary metabolite profiles, and bioactivity, for research and development purposes (Johnson et al. 2013). These strains were originally isolated from grasses collected from around the world to benefit New Zealand agriculture, particularly for deterring major pasture pests (Rowan and Gaynor 1986, Rowan et al. 1986, Pennell et al. 2005, Popay and Thom 2009). Based on their secondary metabolite profiles, many of these endophyte strains were determined unsuitable for pastoral use, specifically due to their high production of ergovaline, a powerful animal toxin (Schardl et al. 2012). Ergovaline, however, has also been implicated in the induction of an avoidance behavior observed in a number of mammals and birds (Coley et al. 1995, Conover and Messmer 1996, Durham and Tannenbaum 1998, Jones et al. 2000, Watson 2000, Pennell and Rolston 2003, Washburn et al. 2005, Panaccione et al. 2006). Coupled with their capability to produce loline alkaloids, potent insecticidal and insect-deterrent compounds, selected endophyte strains were deemed suitable from a wildlife deterrent perspective (Pennell et al. 2010, Pennell and Rolston 2010).

In 2003, a research project was initiated by Christchurch International Airport Limited (CIAL) and AgResearch to develop an endophyte-infected grass to deter birds and insects (a food source of many bird species) at airport localities with the objective of decreasing the risk of bird strike. The final product, marketed as Avanex® (a registered trademark of PGG Wrightson Seeds Limited), was based on a novel association between a selected strain of *Epichloë coenophiala* Morgan-Jones and Gams (Leuchtman et al. 2014) and a turf-type tall fescue cultivar. Although this area of endophyte science is largely unresearched, many believe cool-season grass endophytes are the way forward in many wildlife management scenarios being more eco-friendly and sustainable than alternative less humane methods of control (Coley et al. 1995). Previous research by Pennell et al. (2010) identified a particular strain of *Epichloë* endophyte (designated AR601) that when associated with the tall fescue cultivar 'Jackal' expressed a high amount of bioactivity toward insects and birds in preliminary experiments. This novel grass–fungal association was later marketed, by PGG Wrightson Seeds Ltd., as a wildlife deterrent product under the trade name Avanex (Pennell et al. 2010, Johnson et al. 2013). The research reported in this manuscript was aimed at investigating the invertebrate deterrence aspect of Avanex.

Materials and Methods

Proximity of Invertebrates Relative to Airport Runways. An invertebrate sampling exercise was undertaken at CIAL, New Zealand, from September 2004 to July 2005 to investigate invertebrate populations at different distances from the runway. Invertebrates were collected from eight sampling points along the bitumen runway verge and at two distances adjacent to the verge (10 and 20 m) using a modified,

handheld, petrol-driven, blower-vac (Husqvarna New Zealand Ltd.) set to collection mode with a fine gauze sleeve recessed into the blower nozzle. Collections were made six times over an 11-mo period by dragging the mouth of the blower-vac through the grass plots for a 30-m distance at a slow walking pace and the contents of the collection sleeve emptied into a labeled plastic container. The container contents were then emptied and invertebrates separated from the plant and soil debris using a Berlese-Tullgren funnel (Peterson 1964, Southwood 1994). In summary, the sample was placed on a filter paper above mesh under a low-intensity lamp; the invertebrates in avoiding the heat and light of the lamp fall off the filter paper, through the mesh into a collecting vial containing 95% ethanol. The sample was then poured through a funnel fitted with a filter paper (Whatman Grade 1, GE Healthcare Ltd.). The filter paper containing the invertebrates was air dried in a cupboard at ambient temperature to allow the ethanol to evaporate. After 7 d, a dry weight was obtained for all the samples. A second weight was also obtained after the removal of larger insects (>3 mm long). Representative samples of invertebrates were stored for classification. Soil temperatures and the type of vegetation cover were recorded each time invertebrate samples were collected. Data were log transformed before being analyzed by repeated measurements ANOVA with distance (0, 10, and 20 m from verge) and time (sampling month) as factors followed by Fisher's Least Significant Difference (LSD) test to determine significant ($P \leq 0.05$) differences between treatment means, conducted using GenStat, 17th edition (VSN International 2014).

Efficacy of Endophyte-Infected Tall Fescue in Detering Invertebrates. The artificial association created by infecting the tall fescue cultivar 'Jackal' with the endophyte strain AR601 was assessed in two trials for its invertebrate deterrent properties and compared to an endophyte-free line of 'Jackal' and a turf-type tall fescue (cv. 'Currawong II', PGG Wrightson Turf) infected with a common-toxic endophyte strain known for its high level of insect and disease resistance.

Trial 1—Above- and Below-Ground Invertebrates. The trial was established in February 2008 at PGG Wrightson Seeds Ltd., Kimihia Research Centre, New Zealand. Seed from the two tall fescue–endophyte associations and endophyte-free 'Jackal' mentioned previously, were sown at a rate of 150 kg/ha into plots 1.5 by 2 m². There were six replicate plots per treatment arranged in a complete randomized block design. Six weeks after establishment endophyte infection frequency was assessed for 'Jackal' AR601 and 'Currawong II' using the tissue immunoblot technique (Simpson et al. 2012). Invertebrates were collected above and below ground from each plot. Aboveground sampling was achieved using a Vortis suction sampler (Burkard Manufacturing Co. Ltd., Hertfordshire, United Kingdom), which extracts invertebrates from herbage or debris from the plant and ground surface (Arnold 1994). The machine was operated at five randomly chosen points within each plot for 5 s at full throttle to maximize the centrifuge and improve the

invertebrate collection. The total catch was then pooled to constitute one sample. This was repeated across each plot. Invertebrates were separated from plant and soil debris using a Berlese-Tullgren funnel as described previously. Below ground sampling was achieved by collecting insects present from soil cores (two obtained per plot at each sampling date) measuring 15 cm deep and 10 cm diameter. Cores were longitudinally sliced into quarters with invertebrates collected from each quarter. Data were log transformed, analyzed by ANOVA and followed by Fisher's LSD test to determine significant ($P \leq 0.05$) differences between treatment means (conducted using GenStat).

Trial 2—Effect of Grass Height on Above-Ground Invertebrates. The plots in Trial 1 with the same layout were maintained for Trial 2. However, in September 2009 the plots were divided into two groups; one group was cut to a height of 300 mm (tall) while the second was cut to a height of between 3–5 mm (short). The height of the sward in both groups was measured using a falling plate meter (custom made) where the operator lowered a Perspex plate down a calibrated measuring rod and took a measurement of the average height of the sward. The swards were maintained at the two cutting heights (short and tall) using a ride on mower. On three separate summer dates over a period of 3 yr (8th December 2009, 30th March 2010, and 10th January 2011) invertebrates were suctioned from each plot using the method described for Trial 1. There were three replicates per treatment. Data were log transformed before being analyzed by repeated measurements ANOVA with grass height (short or tall) and sampling time as factors followed by Fisher's LSD test to determine significant ($P \leq 0.05$) differences between treatment means (conducted using GenStat).

Effect of a Novel Grass-Endophyte Association on Invertebrates at CIAL. In each of two separate trials in April 2010 and April 2015, sticky insect traps were established at CIAL in two separate sites, one composed of the existing airport vegetation and the second sown with AvaneX ('Jackal' infected with the *Epicloë* endophyte strain designated AR601). The viable endophyte infection frequency was determined as mentioned above. It was not possible to establish a true control plot on airport grounds for comparative purposes, such as endophyte-free lines of 'Jackal,' as this would breach the airport's wildlife hazard management action plan due to the possible increase in bird numbers and increased risk of bird strike. The existing vegetation was therefore used for comparison and was composed of an uncultivated mix of plant species typical of that found in low fertility sites in New Zealand, dominated by browntop (*Agrostis capillaris* L.), Chewings fescue (*F. rubra* ssp. *commutata* Gaudin), cocksfoot (*Dactylis glomerata* L.), couch grass (*Elytrigia repens* L.), Yorkshire fog (*Holcus lanatus* L.), sweet vernal (*Anthoxanthum odoratum* L.) and the herbs—narrowleaf plantain (*Plantago lanceolata* L.) and yarrow (*Achillea millefolium* L.). The soil at CIAL consisted of a low fertility compacted Templeton silt loam.

Insect traps were composed of yellow sheets with nondrying adhesive glue on one side, wrapped around

an orange plastic pipe (8 cm diameter by 21 cm height) with the glue facing outwards to trap insects. Each sticky trap had a total surface area of 408 cm² in 2010 (with four replicate traps) and 204 cm² in 2015 (with eight replicate traps). Traps were placed on each plot (existing airport vegetation or AvaneX) 100 m apart. Traps were left in the field for 4 d before being removed. Total invertebrate numbers were then assessed per trap and the dominant taxonomic groups identified. Data were log transformed before being analyzed using a two sample *t*-test (conducted using GenStat).

Results

Proximity of Invertebrates Relative to Airport Runways. The dry weight of invertebrates, obtained from samples collected at the verge (average of 0.044 g), was significantly ($F = 20.30$; $df = 2, 4$; $P = 0.008$) higher than the total dry weight collected at a distance of 10 and 20 m from the verge (0.014 and 0.009 g, respectively) over all the months (Fig. 1). There were no significant differences between mean invertebrate weights collected at 10 and 20 m from the verge or any significant interaction with respect to the month of assessment. The majority of invertebrates collected were identified as plant feeders with plant-sucking Hemiptera including shield bugs (family Pentatomidae), wheat bugs (*Nysius huttoni* White, family Lygaeidae), capsid bugs (family Miridae), leaf hoppers (family Cicadellidae), and plant-feeding springtails (Collembola), particularly common. When large arachnids and insects, mostly spiders and beetles (*Listronotus bonariensis* Kusche and *Atrichonotus taeniatus* Berg, family Curculionidae; *Conoderus exsul* Sharp, family Elateridae; *Pyronota* spp. family Scarabaeidae) were removed from the samples there was still a significantly

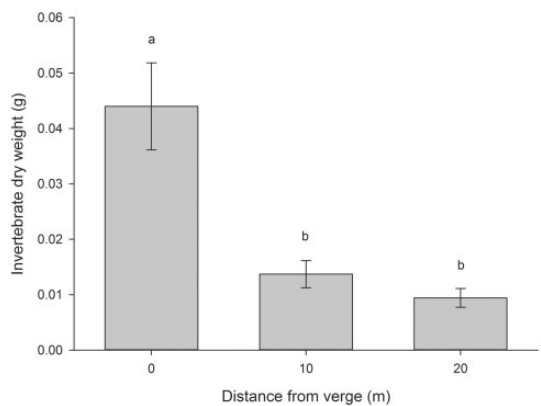


Fig. 1. Mean total dry weight of invertebrates collected at three distances from the bitumen runway verge (0, 10, and 20 m) over eight sampling dates from September 2004. Bars followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's LSD test analyzed with transformed data. Standard error bars were calculated from back-transformed data.

($P < 0.001$) greater dry weight of invertebrates present in the samples collected from the verge compared to the other distances. There were no apparent effects of vegetation or soil temperature on invertebrate dry weight or their distribution (data not shown).

Efficacy of Endophyte-Infected Tall Fescue in Detering Invertebrates. All grass–endophyte associations had viable infection frequencies greater than 70%. There was no significant differences in the below ground weight of insects collected across the three grass–endophyte associations (Fig. 2). However, a significantly greater ($F = 8.13$; $df = 2, 13$; $P = 0.005$) weight of invertebrates was collected from the above-ground traps located on plots sown with an endophyte-free line of ‘Jackal’ and ‘Currawong II’ infected with a common-toxic endophyte strain compared to ‘Jackal’ infected with AR601 (Fig. 2).

Longer grass harbored significantly ($F = 24.37$; $df = 1, 10$; $P < 0.001$) more invertebrates than short and significantly ($F = 24.16$; $df = 2, 24$; $P < 0.001$) more insects were collected in 2011 than in the other years. Overall, there were marginally significant ($F = 2.48$; $df = 2, 24$; $P = 0.077$) differences between the grass–endophyte associations, with the greatest weight of invertebrates recorded on ‘Jackal’ endophyte-free, followed by ‘Currawong II’ and then by ‘Jackal’ AR601 for each grass height at each sampling (Fig. 3). The only exception was for short grass height in 2009 when all the grass–endophyte associations recorded a similar weight of invertebrates (Fig. 3A).

Comparison of Insect Numbers at Avanex and Existing Vegetation Plots at CIAL. In 2010 and 2015, a significantly greater ($t = 13.03$; $df = 8$;

$P < 0.001$ for 2010 and $t = 13.22$; $df = 11.33$; $P < 0.001$ for 2015) number of invertebrates were captured on the sticky traps positioned in plots containing the existing airport vegetation (598 on average for 2010 and 144 for 2015) compared to those on plots containing Avanex (93 on average for 2010 and 23 for 2015; Fig. 4A and B). For both years the insect types observed on the traps taken from the existing vegetation was dominated by *Diptera* spp. such as fungus gnats (family Sciaridae) and striped dung flies (*Oxysarcodexia varia* Townsend), small parasitic wasps (order Hymenoptera), and true bugs (family Miridae). The traps taken from the Avanex plots were devoid of leaf miners, fungus gnats, and leaf and grass bugs. However, observations of large proportions of plant feeding springtails (Collembola) and parasitic wasps were made on the Avanex plots (data not shown).

Discussion

The majority of bird strikes occur up to 152 m (500 ft) above ground level, generally within the vicinity of the airport runways, taxiways, and ramps during the take-off or landing phase (Cleary et al. 2005, Dolbeer 2006). Airport authorities should therefore focus their wildlife management action plans on these specific locations to reduce bird populations. As Allan (2002) states “the most effective combination of techniques depends on the environmental conditions that prevail at the airport concerned and on the bird species that are causing the hazard”. The majority of action plans are therefore composed of two main elements, namely, modification of the habitat (reducing availability of

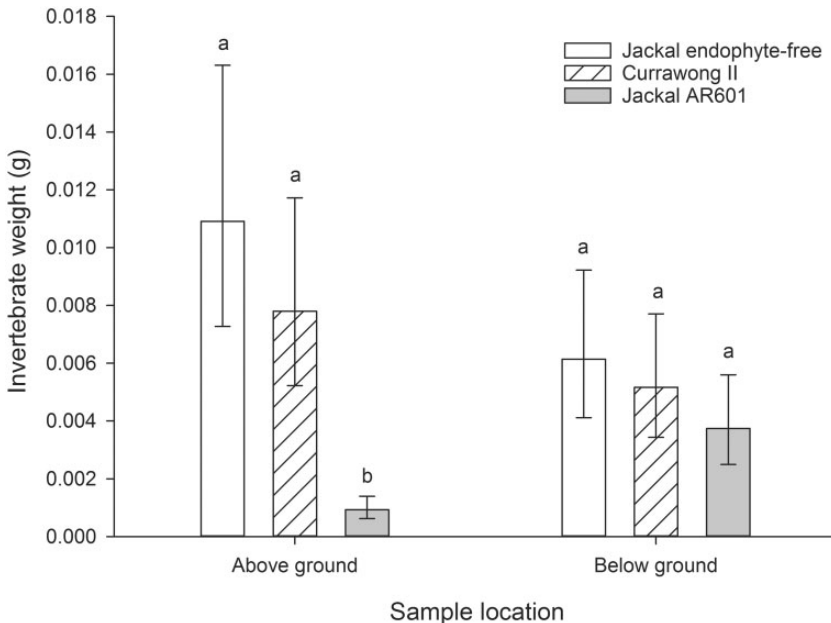


Fig. 2. Mean weight of invertebrates collected above and below ground on plots sown with three different endophyte-tall fescue associations (‘Jackal’ infected with AR601, an endophyte-free line of ‘Jackal’, and ‘Currawong II’ infected with a common-toxic endophyte strain). Bars followed by the same letter are not significantly different ($P > 0.05$) according to Fisher’s LSD test, analyzed with transformed data. Standard error bars were calculated from back-transformed data.

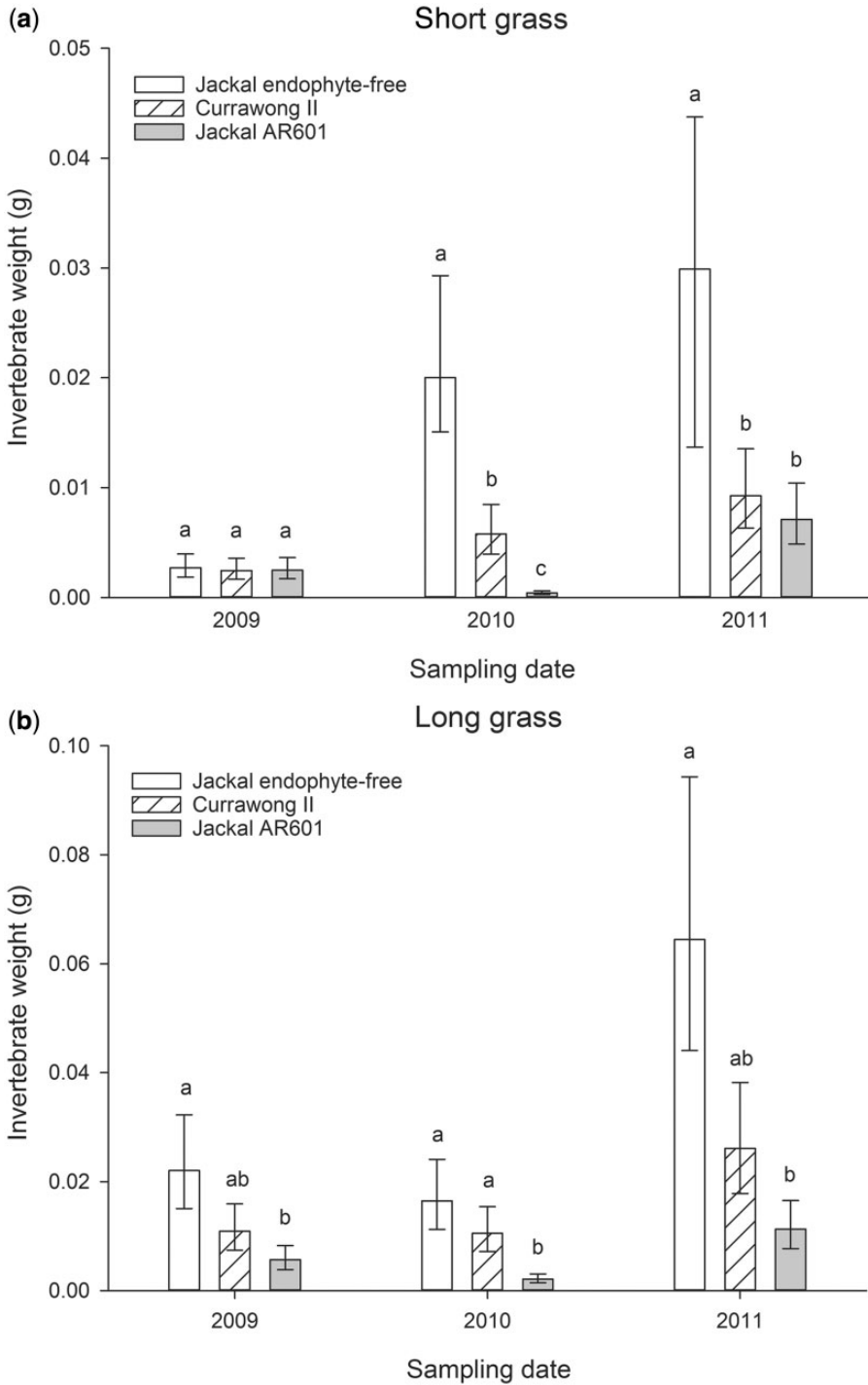


Fig. 3. (A, B) Mean number of invertebrates collected above ground by suctioning on plots sown with three different endophyte-tall fescue associations ('Jackal' infected with AR601, an endophyte-free line of 'Jackal', and 'Currawong II') and mown at two heights. Bars followed by the same letter, for the same sampling year, are not significantly different ($P > 0.05$) according to Fisher's LSD test, analyzed with log-transformed data. Standard error bars were calculated from back-transformed data. Note that the scale on the y axis differs between figures.

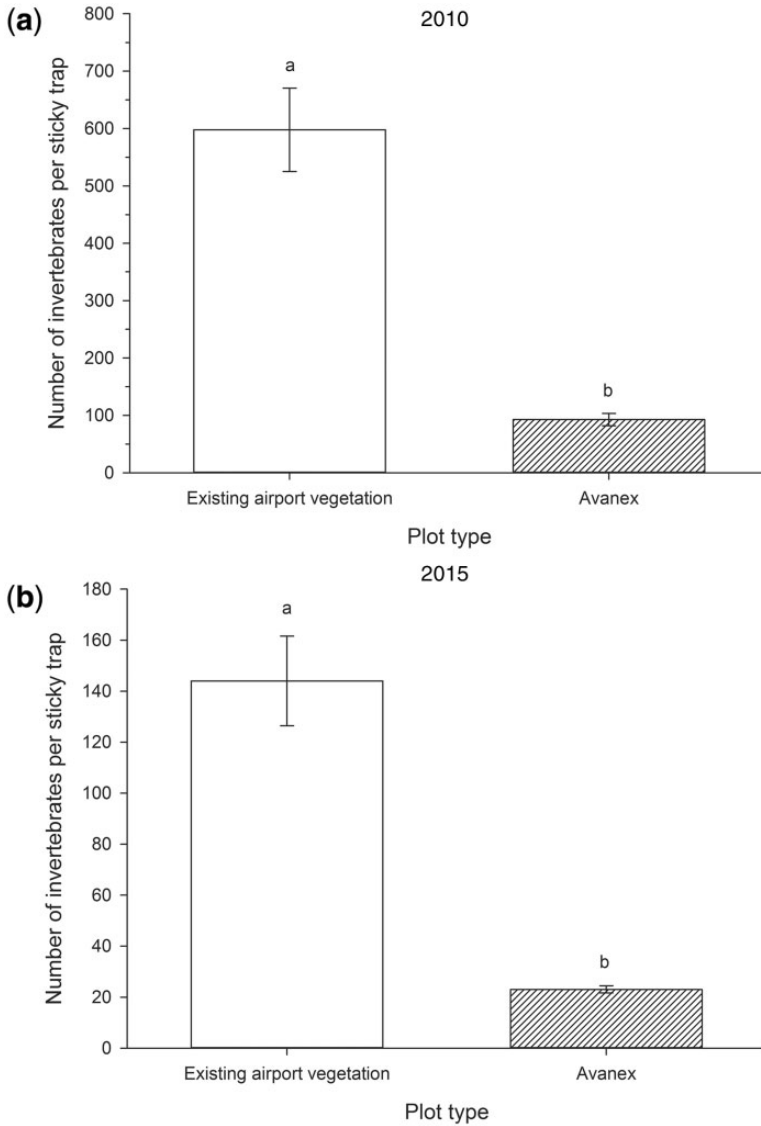


Fig. 4. (A, B) Mean number (\pm SE) of invertebrates collected on sticky traps (each trap had a surface area of 408 cm² or 204 cm² in 2010 and 2015, respectively) located on two different plots (existing airport vegetation and Avanex). For each year bars followed by the same letter are not significantly different ($P > 0.05$) according to two sample *t*-test. Note that the y axis differs between trials.

food and water) and an active bird deterrence whereby scaring devices or patrols are utilized (Allan 2002). The first trial outlined in this manuscript identifies that the grass sward closest to the bitumen runway harbors a far greater amount of invertebrates (more than double) than grass swards further away from the runway. In addition a number of spur-winged plovers were shot at CIAL in 2003 by wildlife management staff and their gut contents examined. For the 10 birds examined, 93% of the invertebrates identified were plant feeders (C.G.L.P., unpublished data). The invertebrate community sampled was very diverse as might be expected and therefore total mass representing that which is available to the birds was a justifiable method to assess

that community, rather than identifying and comparing particular invertebrate groups (i.e., genera). Because size of invertebrates can considerably influence the weight, especially if they are numerous, it is pertinent that when large invertebrates were removed from the collection closest to the runway there was still a difference between weight of that collection and weight at greater distances from the runway. Airport managers, particularly those with budget constraints, should first consider sowing Avanex in areas closest to the runway verge before developing their complete airfield. It is proposed that this would decrease the amount of invertebrates on these areas and subsequently decrease the amount of insectivorous birds.

Subsequent trials evaluated Avanex (tall fescue 'Jackal' infected with *E. coenophiala* strain AR601) for its invertebrate deterrent properties. Results from small demonstration plots showed that Avanex was substantially more effective at deterring above-ground invertebrates than an endophyte-free line of 'Jackal' and an endophyte-infected turf-type tall fescue 'Currawong II', chosen for its similarity to Avanex in terms of its alkaloid profile and grass phenotype. Avanex reduced the numbers of fungus gnats (family Sciaridae), striped dung flies (*Oxysarcodexia varia* Townsend), and true bugs (family Miridae). However, Avanex had no noticeable effect on beneficial invertebrates including springtails (Collembola) and parasitic wasps. The observation of Collembola was interesting, as these invertebrates do not fly; however, they can get distributed by wind. Reducing the diet of the common starling (*Sturnus vulgaris* L.) and spur-winged plovers (*Vanellus miles* Boddaert), which feed mainly on grassland invertebrates (New Zealand Birds Online 2013) and are recognized as the most problematic species at New Zealand airport localities (Pennell, unpublished data), has the potential to reduce bird strike. However, as these particular grass-endophyte associations are also known to deter certain bird species through other mechanisms (Pennell and Rolston 2003), we did not attempt to determine how much of the invertebrate deterrence was responsible for reducing bird communities at airports. Avanex is known to produce high levels of ergovaline and loline alkaloids (Pennell et al. 2010). Peramine, also a common alkaloid that is deterrent to Argentine stem weevil (*L. bonariensis*), a common pasture pest, is produced by many endophyte-grass associations (Rowan and Gaynor 1986, Rowan et al. 1986, Rowan 1993) with *E. coenophiala* AR601 known to possess the gene responsible for its production (R.D. Johnson personal communication). However, concentrations have been low to negligible in many artificial AR601-grass cultivar associations (W. J. Mace unpublished data). Although ergovaline, and possibly some intermediate compounds, have been implicated in some invertebrate bioactivity (Ball et al. 1997, Johnson et al. 2013) it is believed lolines are largely responsible for the invertebrate deterrence exhibited by Avanex. This grass-endophyte association typically produces >600 ppm of total lolines in the above-ground plant tissues, sometimes exceeding 1,200 ppm, with dominant loline species being *N*-formylolone (NFL; ~75%), then *N*-acetylloine (NAL; ~20%) and *N*-acetylolorlone (NANL; ~5%) (W. J. Mace unpublished data). Loline alkaloids exhibit a particularly broad spectrum of bioactivity (reviewed by Schardl et al. 2007) boasting both insecticidal and feeding-deterrent properties, with activity also shown to influence the epiphytic bacterial microflora (Roberts and Lindow 2014). In 2009, no differences were observed between treatments managed at a short grass height; however, invertebrate weights were low for all grass-endophyte associations evaluated. This may have been attributed to a less than optimal environment for certain invertebrate lifecycles, a management regime that the researchers were unaware of at the airport (such as an insecticidal

agricultural application or spray drift from a neighboring area), or some other reason. In terms of differences between treatments, there are also appreciable differences in alkaloid concentrations produced by different endophyte-grass associations between months, seasons, and plant lines (Patchett et al. 2011). Different loline alkaloids also vary in their specificity to different insect species and on certain development stages (Schardl et al. 2013). For instance, Patchett et al. (2008) showed that there were considerable changes in the different loline alkaloid concentrations over time produced by certain meadow fescue-endophyte associations and suggested that this may be advantageous to the host plant and be correlated to the maximum risk period of herbivory by third-instar grass grub larvae.

In the experiments conducted below ground, Avanex did not significantly deter invertebrates compared to the other grass-endophyte associations evaluated. This may be attributed to the lack of alkaloids found in the root tissue of these plants, with further work required to determine this. Loline alkaloids have, however, been shown to inhibit the feeding behavior of root feeding beetle grubs at concentrations similar to that found in endophyte-infected tall fescue roots (Patterson et al. 1991) and grass grubs fed endophyte-free meadow fescue roots gained more weight compared with those offered roots from endophyte-infected plants (Popay et al. 2003).

Grass height was also investigated with respect to above- and below-ground invertebrate dry weights with similar trends observed for the three assessed grass-endophyte associations over 3yr of trials from 2009 to 2011. In the United Kingdom, it was observed that grass cover managed to a taller height was more favorable than short grass, with a marked reduction in bird numbers occurring after the RAF adopted a specially designed maintenance regime in 1974 (Deacon and Rochard 2000). Later, Brough and Bridgman (1980) observed fewer birds on tall grass (15–20 cm) than short grass (5–10 cm) areas situated on 13 airfields in Britain. However, although many of the most hazardous bird species were reduced, such as many species of gull (*Larus* spp.), lapwings (*Vanellus vanellus* L.), common wood pigeons (*Columba palumbus* L.), and starlings (*S. vulgaris*), many raptors such as the common kestrel (*Falco tinnunculus* L.) were not. This is not surprising, as this tall grass would have been a highly suitable habitat for many small mammals, especially voles, a food source of many birds of prey. Another advantage for airfields of tall grass can be the reduced cost for management. Keeping grass short requires high input with regard to regular mowing and agricultural applications. On this basis a program of tall-grass management was recommended for Kennedy International Airport, New York City, where their main hazard is laughing gulls (*Larus atricilla* L.) (Buckley and McCarthy 1994). However, tall grass can be prone to thatch which can lead to an increase in disease and insect pest problems (Murray and Juska 1977) with both long and short grass management regimes requiring regular maintenance in the form of agriculturals and mowing regimes. An alternative management practice has

involved reducing the nutrient status of the soil to reduce grass growth and hence lower cutting frequency as well as encouraging a diverse flora by eliminating the use of herbicides and insecticides. For example, in desert environments, where cultivating grass swards is impossible, the airfield is simply rolled flat and no vegetation is permitted to grow. This results in little or no bird attraction (Allan 2002). The results described in this manuscript allow for a novel management regime that can be applied to temperate areas with the aim of reducing numbers of graminaceous and insectivorous birds, depending on the specific bird species that are viewed as the primary hazards.

For any particular airport, multiple wildlife management techniques should be applied to minimize the potential of bird strike and reliance on just one method should be avoided. Many airports will have particular hazards unique to their location, such as areas of water (ponds, lakes, streams, oceans) that make them particularly suitable for waterfowl or nearby meadows or forests particularly suitable for small mammals such as voles, mice, and rabbits providing suitable hunting grounds for raptors. Habitat modification is seen as the most effective long-term measure to reduce bird strike (Blackwell et al. 2009, DeVault et al. 2011) with AvaneX, a biological control based on a grass–endophyte association proven to be effective at multiple airport localities (Pennell, unpublished data). In the AvaneX development process, it was necessary to achieve a considerable understanding of bird behavior at and near New Zealand airports. As it became apparent that many bird species can be responsible for bird strike and that there was great diversity in these species, in their feeding and flying habits, it was evident that a product with multiple mechanisms of action was required. The AvaneX grass–endophyte association addresses this situation by the production of secondary metabolites, predominantly ergovaline and lolines (Pennell et al. 2010; Pennell and Rolston 2011, 2013; Johnson et al. 2013). The alkaloid ergovaline is an ergopeptine and is well known as a potent vasoconstrictor in mammals (Lyons et al. 1986, Klotz et al. 2007) and although a serious problem in agriculture, especially in certain parts of the USA (Bacon et al. 1977, Schmidt et al. 1982), it is particularly suited as a wild-life deterrent compound. This is twofold, where the compound not only deters a wide range of invertebrates and small mammals (Finch et al. 2015) but also induces post-ingestion feedback in birds whereby illness can alter long-term feeding preferences and aversion to certain localities associated with that illness (Pennell and Rolston 2003, Pennell and Rolston 2011). Loline is a potent insecticidal and invertebrate feeding deterrent alkaloids produced by AvaneX that have been shown to reduce a wide range of invertebrates that would make up the food source of potentially hazardous bird species. AvaneX is a unique biological control option; many species of invertebrates and birds are not harmed but deterred away from areas where these grass swards are established, making this option more socially acceptable than other management practices that kill or permanently harm birds. A second product, also marketed

under the AvaneX Unique Endophyte Technology brand in turf ryegrass (*Lolium perenne* L.) associated with the fungal endophyte *E. festucae* var. *lolii* strain AR95, is also available for the sport and recreation industry for areas such as sports fields and parks (Johnson et al. 2013, Pennell and Rolston 2013).

Acknowledgments

We thank Helen Townsend and Craig Phillips of AgResearch Limited for sorting and identifying invertebrates. Funding was provided by Christchurch International Airport Limited, the Foundation for Arable Research, Grasslands Technology Limited, and PGG Wrightson Seeds Limited. AvaneX® Unique Endophyte Technology is a registered trademark of PGG Wrightson Seeds Limited.

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Received 24 May 2015; accepted 20 August 2015.
