

Avanex™ Unique Endophyte Technology - Bird deterrent endophytic grass for amenity turf and airports

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Abstract. Bird strike is recognised throughout the civil and military aviation industries as a significant cost with more than \$US 2 billion/year attributed to this problem globally. To manage this risk the aviation industries have in the past adopted many practices to frighten wildlife from airports rather than reducing the attractiveness of the area by grass habitat management. There has been little proven research on ground cover species evaluation using fungal endophyte to reduce wildlife. Our research has shown that the adoption of a specialist *Neotyphodium* fungal endophyte (strain AR601) placed in a turf type tall fescue cv. Jackal (*Festuca arundinacea* Schreb.), is reducing bird numbers and strike rates on the test airfields under study. In the recreational industry, large birds such as Canada geese (*Branta canadensis*), which forage on tender new shoots and stems, can cause severe damage to turf with the added issue of faecal contamination and associated heath issues. The inoculation of another specialist endophyte (strain AR95) into a turf type ryegrass (*Lolium perenne* L.) cv. Colosseum offers another tool to reduce this problem. This paper reviews the results from trials of these selected turf type grasses containing the unique fungal endophytes and shows effects on herbivorous, insectivorous and omnivorous birds that visit airfields and surrounding parklands for foraging. Specialty endophyte grasses may also have a place in the viticulture and horticulture industries to reduce the use of insecticides. Extracts and mulches offer a new opportunity to use grass endophyte associations.

Keywords: Novel endophyte, Avanex™, habitat modification, extracts.

Introduction and background

Air traffic and passengers travelling have increased substantially since 1980. Worldwide the total cost of collisions with wildlife has been estimated at \$US 2 billion/year by the World Bird Strike Association (WBA) an international forum and association of bird strike committees and wildlife experts in aviation safety around the globe. International Civil Aviation Organisation (ICAO) International Bird strike Information System (IBIS) (Pinos 1996) data base have recorded that the majority of bird strikes occurs during the takeoff and landing phases. A grass that reduces the attractiveness of airports to bird activity by habitat modification may be an important part of the long term solution. This threat from wildlife to both the aviation and recreational industries is increasing with birds such as Canada geese (*Branta canadensis*) that are becoming non-migratory. This is due to short cut manicured parks and golf courses offering security, a reliable food source, and a clear view to monitor predators. Insectivorous birds such as the starling (*Sturnus vulgaris*), a common flocking species, are attracted to airfields and open spaces, and are a known threat to aircraft along with gulls (*Larus dominicus*). Seed feeding birds such as green finches (*Carduelis chloris*) are attracted to flowering annual grasses (e.g. *Poa annua* L.) also common on airfields.

Potential and current solutions

Reducing birds in these areas has relied in the past on

‘primary response mechanisms’ such as acoustical distress calls, gas guns, lights, lasers, dogs, falconry, kites, balloons, sticky pastes, spikes, repellent sprays, wires, and netting, which all attempt to frighten the birds from an area. These ‘primary repellents’ fail due to habituation, cost and maintenance. Other tools described as ‘secondary repellents’ can include a chemical irritant such as methiocarb carbamate (Mesuro 50 HBT) or methyl anthranilate (MA) a taste aversion spray described as conditioned taste aversion (CTA) products. Lowel *et al.* (1989) describe the use of carbachol to induce CTA in ravens. Products like these are the basis of many repellents that may also use colour and smells, as well as taste to create aversion, but have to be regularly applied to the food source. Secondary repellents, which act as feeding deterrents, are known to be more effective in the long term and have been described as causing ‘post digestion feedback’ (PDF) (Conover and Messmer 1996). PDF develops from eating something not associated initially with taste but causing a malaise/sickness over time. Conover and Messmer (1996) looked at the feeding preferences and changes in mass of Canada geese consuming endophyte infected tall fescue. They found that the geese lost body weight, first suggesting a bitter taste, but also they proposed PDF where illness alters long term food preference and induces avoidance to that food by memory.

The potential of grasses infected with selected fungal endophytes (*Neotyphodium* spp.) that contain an endophyte-induced metabolite that acts as a secondary repellent and induces PDF was first demonstrated with

Canada geese (Pennell and Rolston 2003). They described and recognised the action of the metabolite ergovaline for inducing PDF, and this was patented as 'Grass based avian deterrent' (USA 8101400, NZ 579801). Seed feeding finches and omnivorous feeding gulls also showed similar responses (Pennell, unpubl. data). Washburn *et al.* (2007) evaluated commercially available tall fescue varieties for airfields at a number of sites in the USA and found considerable variation in the wild-type endophyte content and cultivar persistence. This work showed that considerable effort was needed to produce a viable purpose built product for this industry.

Development of Avanex™

AgResearch a leading Crown Research Institute (CRI) in New Zealand has a large database of alkaloid profiles of different endophyte strains from many parts of the globe. The advantages of some grass endophyte mutualism are expressed by less insect predation through the presence of loline and peramine which acts on a number of insect pests (Schardl *et al.* 2007; Popay and Bonos 2005). Improved livestock performance through the use of selected endophytes in elite cultivars of tall fescue and perennial ryegrass, is described as novel associations (Fletcher 2012). The metabolites produced in some wild-type endophyte grass associations are not used in the pastoral industry as they induce animal avoidance and ill thrift. However these metabolites have been the key to developing the Avanex™ brand for wildlife management using two grass hosts, cv. Jackal tall fescue and cv. Colosseum perennial ryegrass, both bred by PGG Wrightson Seeds, Lincoln, New Zealand. The development of Avanex™ is the result of over 12 years of selecting and testing prototypes of grass-endophytes to produce a unique product for airports and recreation areas. The resultant grass endophyte interaction was chosen as they are tough, wear-resistant cultivars with a stable endophyte. Pennell *et al.* (2010) describe the process of the development and selection of this material. A selection of these wild-type endophytes that produce high levels of ergovaline (a vasoconstrictor in animals) and loline (a natural insecticides), are present in the fungal endophyte (*N. coenophialum*, strain AR601). This strain was inoculated into a continental turf type tall fescue cv. Jackal in 2004 using a method described by Latch and Christensen (1985). Endophyte transmission into the seed was confirmed by the seed squash method (Latch *et al.* 1987). These plants were then further selected for high levels of alkaloid expression (lolines for insect repellence >1100 parts per million (ppm), and ergovaline > 3.4 ppm to induce PDF). A similar programme was completed for a perennial ryegrass cv. Colosseum inoculated with the AR95 endophyte. Both these products are now marketed under the Avanex™ unique endophyte technology brand for the recreation turf and aviation industries.

Field testing

The potential of these selected endophyte-infected grasses to act as a secondary repellent to induce PDF has been tested with captive finches (*Carduelis choris*) which showed a 30 to 40% reduction in feeding. Wild gulls (*Larus dominicus*) initially consumed 60% endophyte-

infected feed, but by the end of the trial were consuming only 30%, confirming learned avoidance behaviour. Wild Canada geese were used to test the effect on herbivorous birds for associations of endophyte-containing grass in a field trial where these birds visited regularly. This trial showed a significant avoidance of the Avanex™ endophyte-infected tall fescue and ryegrass test plants, with a strong negative correlation between ergovaline levels and grazing scores, reconfirming that high levels of ergovaline will deter geese. Pennell and Rolston (2010, 2012) recorded significantly lower numbers of above and below ground insects for the two Avanex™ grasses when compared with wild-types and nil endophytes in the same cultivars. Data collected over 12 months from the two test airports have shown fivefold more bird visitations on the existing airport vegetation when compared with areas sown with Avanex™ (Pennell and Rolston 2011). At one site, the bird strike data over 3 years shows an 18 month downward trend associated with increasing areas established in Avanex™ tall fescue, now occupying 90% of the active runway surrounds ($R^2 = 0.91$, $P < 0.01$).

Other possible opportunities for endophyte technology

Unpublished data on the effect of Jackal AR601 seed fed to mice has shown a significant reduction in consumption of seed that contained high ergovaline. The result suggests that mice did not find the Jackal AR601 palatable and, if given a choice, may choose to eat an alternative diet. Evidence that ergot alkaloids in grass reduce the appeal of grazing by rabbits is described by Panaccione *et al.* (2006). Pennell and Rolston (unpublished data) have shown a significant reduction in feeding by domestic rabbits when fed grass derived from the ergovaline producing Avanex™ grasses when compared with endophyte-free plant material in the same cultivars. Rabbits refused approximately 10% of their total diet when offered the endophyte-free grass. This changed considerably when the endophyte-infected grass was offered, with refusals reaching close to 40%. This data may offer airports that have a risk from raptor strikes some control options and provides further opportunities for the Avanex™ product for airfields in temperate climatic zones where field mice, rabbits and voles may be a problem.

Recent data by the authors on the uses of mulch and leachate from mown loline-producing endophytic grasses has shown that there are opportunities outside the pastoral, aviation and recreational industries for this metabolite. Results of a trial set up to test the hypotheses that it is possible to deliver pest protection via loline to another plant that does not have the advantage of a symbiotic relationship with a fungus is described by Pennell and Rolston (2012). The bio-actives obtained by leachate extraction from the loline producing endophyte seed process was absorbed through the roots into endophyte-free plants of tall fescue and ryegrass, reducing the numbers of bird cherry oat aphid (*Rhopalosiphum padi*) colonising leaves. The extraction process of taking endophyte leachate from endophyte-infected plant material to make an insecticide derived from endophyte is patented ('pesticidal plant extract containing loline derivatives' (2008 NZ

553892)). The invention relates to a product and methods of providing endophyte-free plants with improved pest protection using naturally produced pyrrolizidine alkaloids obtained from endophyte-infected grasses. Further work confirmed that this bio-active effect could be transferred to plants other than grasses from mulches.

There is also some evidence from USA that some endophyte-infected tall fescue planted as a ground cover before planting peach trees has improved the vigour of the trees by root-knot nematode (*Meloidogyne incognita*) suppression (ARS 2011).

Conclusion

Pilot studies indicate fewer birds on areas sown with cv. Jackal infected with AR601 endophyte and cv. Colosseum infected with AR95 endophyte now branded as Avanex™. This newly developed endophytic technology for specialist turf grasses bred for wildlife management, offers airport managers a better option than “off the shelf cultivars” with wild-type endophytes commonly used in lawns and roadsides. Some of these wild-type endophytes are known to have poor endophyte viability in the seed, most likely the result of poor storage, and therefore have low alkaloid expression and poor plant persistence.

Any re-grassing is an expensive exercise, especially at an airport where the soils are commonly compacted, of low fertility, have high buried seed counts, and have limitations on machinery access and irrigation. When re-grassing is undertaken, the use of grass-endophyte associations branded as Avanex™ Unique Endophyte Technology developed specifically for this purpose will provide an option for better bird control.

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