THE POTENTIAL OF SPECIALTY ENDOPHYTE-INFECTED GRASSES FOR THE AVIATION INDUSTRY

Chris Pennell[1] Phil Rolston[2]

[1] AgResearch Ltd, Private Bag 4749, Christchurch 8140. chris.pennell@agresearch.co.nz

[2] AgResearch Ltd, Private Bag 4749, Christchurch 8140. phil.rolston@agresearch.co.nz

ABSTRACT

Endophytic fungi (*Neotyphodium sp*.) co-evolved with grasses and are known to protect plants from overgrazing by animals and insect predation by the production of toxins. This has led to the development of endophytes that are animal safe but reduce insect pests and the discovery of other chemicals that deter grazing. For airports and recreational areas turf grasses were inoculated with novel endophytes that produce insect and animal/bird avoidance behaviour. Bird strikes mostly occur during the takeoff and landing phases, so a grass that reduces the attractiveness of airports and surrounding areas to bird activity by habitat modification may be an important part of the long term solution.

This paper will discuss results from trials of the selected turf type grasses containing the unique fungal endophytes and show effects on herbivorous, insectivorous and omnivorous birds that visit airfields and surrounding parklands for foraging. Preliminary data on a short versus tall grass trial using these test endophytes are presented.

A large plot trial of a selected endophyte (AR601) was inoculated into a turf type tall fescue Jackal, sown at Christchurch International Airport (New Zealand) has shown a significant reduction in bird number visits in its first year. Earlier trials with caged finches (*Carduelis choris)* showed a 30 to 40% reduction in feeding when exposed to selected endophytic seed. Gulls (*Larus dominicus)* were initially consuming 60% endophyte treated feed but by the end of the trial were consuming only 30% confirming learned avoidance behaviour or post digestional feedback (PDF) to treated endophyte feed. Wild Canada geese (*Branta canadensis)* were used to test the effect on herbivorous birds on associations of endophyte-containing grass in a field trial where birds visited regularly. Results of this trial have shown a significant avoidance of the novel endophyte fescue and ryegrass test plants.
Novel endophyte enhanced grasses for bird management at airports will soon become available in commercial quantities. This material has been established in large areas at Auckland, Hamilton and Christchurch International Airports.

Data on establishment methodology, insect presence, bird behaviour, bird numbers and strike rate will be monitored over these new large areas.

Key Words: AR601, AR95, bird numbers, Colosseum, endophyte, habitat modification, Jackal.

INTRODUCTION

Bird strike on airfields and surrounding lands commonly involves birds that feed on herbage cover or insects living there. *Neotyphodium* endophytic fungi that live in mutualistic associations with grasses such as ryegrass (*Lolium*) and fescues (*Festuca*) produce a number of alkaloids that reduce insect pest numbers and herbivore feeding (Conover *et al.*, 1996; Howard *et al.,* 1992; Parish *et al.,* 2003). This knowledge has been exploited to produce a novel product as one of many tools to combat the increasing problem of bird strike on or near airfields. 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 wildtype (WT) endophyte content and cultivar persistence. It is known that the alkaloid ergovaline produced by endophytes in both perennial ryegrass (*L. perenne*) and tall fescue (*F. arundinacea)* not only reduces the number of insects but can also reduce feeding of Canada geese (*Branta canadensis*) (Pennell & Rolston 2003; Pennell *et al.* 2010) by inducing avoidance behaviour known as "post-digestion feedback" (Mason & Reidinger 1983). Another major group of alkaloids, called lolines, produced by the fescue endophyte *N. coenophialum* but not in ryegrass associations, acts on a number of insect pests (Schardl *et al.* 2007; Popay *et al*. 2009).

Initial concept testing of wild type endophytes was carried out in 2000 with captive Canada geese contained in separate fenced plots over two years with two separate gaggles of Canada geese to evaluate learned avoidance response to selected grass/endophyte associations that contain high levels of ergot alkaloids. This early work identified the active alkaloid responsible for the feeding avoidance behaviour (Pennell & Rolston 2003).

The management of mowing height to maintain a tall grass cover at airports has been under discussion since 1949. There have been papers at IBSC conferences at Amsterdam (Deacon & Rochard 2000), and at Athens (Morgenroth 2005) for and against the implementation of tall grass management. Seamens & Dolbeer (1999) suggested in three studies that tall grass may not result in fewer birds and that more work is needed to answer this question for airport managers.

This paper reports on the development of a high alkaloid endophyte association in turf tall fescue and turf perennial ryegrass and the research findings of trials on and off airfields and its adoption at three international airports in New Zealand.

Identification of specialty endophytes for wildlife management:

AgResearch has collected a large database of alkaloid profiles of different endophyte strains from many parts of the globe to improve animal and forage plant performance. Some of these endophytes have been have been shown to produce high levels of ergovaline, a powerful animal toxin, and so are unsuitable for the forage industry. These unwanted strains were inoculated into a continental turf type tall fescue Jackal in 2004 using a method described by Latch & Christensen (1985). The transmission of the selected endophyte into the Jackal seed was confirmed by seed squash method (Latch *et al*. 1987).

Jackal was bred by PGG Wrightson Seeds, Lincoln New Zealand and was chosen as a tough, wear-resistant cultivar that should stand up to aircraft movement, jet blasting and braking.

Methods and Results of trial I:

Fifty plants of Jackal tall fescue were inoculated with AR601 endophyte and tested for alkaloid concentration and those which showed the best expression for endophyte presence and alkaloid expression (> 3.4 ppm, parts per million, ergovaline and >1100 ppm lolines) were chosen for seed multiplication to create Jackal "AR601". Further testing compared the levels of ergovaline and lolines of these inoculated Jackal plants with Kentucky 31, a tall fescue infected with a wild-type endophyte (Table1).

The average alkaloid levels of ergovaline (known to provoke avoidance behaviour) and loline (offering insect protection) were 5 and 3.5 times higher respectively in Jackal AR601 single plant selections compared to the average Kentucky 31 with wild-type endophyte taken from literature publications (Table 1).

Table 1: Alkaloid profile of Jackal AR601 leaf material compared with average Kentucky 31 wildtype endophyte from two different trial sites

	Ergovaline (ppm)		Lolines (ppm)	
Endophyte	Mean	range	mean	Range
Jackal AR601	8.3	3.4 - 15.9	1820	1160 – 3150
Kentucky 31 Wild-type	1.4	1.3 - 1.5	517	338 - 750

In other trials the ergovaline levels for Jackal AR601 were 1.2 ppm (Table 2) and 15.1 ppm (Table 3), suggesting seasonal variation may occur. The ergovaline levels in Jackal AR601 were consistently higher than values known to affect livestock; e.g. thresholds of ergovaline in straw as an undiluted ration in livestock feed are 0.5 ppm (Young et al. 2006).

A turf perennial ryegrass ("Colosseum" bred by PGG Wrightson) was inoculated with a high alkaloid (ergovaline and lolitrem) producing endophyte AR95. Seed was multiplied and evaluated as Colosseum 'AR95' using the same methodology.

Trials to test concept at airports

Methods and Results Trial 2:

In autumn 2006 a small plot trial was established at Christchurch International Airport, comparing Jackal AR601 with commercially available tall fescue, "Currawong," containing wild-type endophyte. Insect samples, endophyte infection, alkaloid concentration and plant density measurements were taken seasonally. There were less insects and higher plant density recorded for Jackal AR601 in the trial (Table 2). There were also higher levels of the desirable alkaloids present in Jackal AR601 compared with a commercial cultivar Currawong with the wildtype endophyte (Table 2).

This result could be associated with a lower percentage of infected plants in the Currawong tall fescue and emphasises the importance of having high viable endophyte in the seed. This lower level of endophyte may be due to seed age or mortality associated with poor seed storage (Hill and Roach 2009).

Table 2: Comparison of Jackal AR601 with a commercially available tall fescue "Currawong" with wild type endophyte

		6 Sep 06	2006/07	12 Sep 07	2007	2007
Cultivar	Endophyte	(%) Endophyte from blots	Insect weight as % of control	Plant density (%)	Loline (ppm)	Ergovaline (ppm)
Jackal	Nil	0	100	33	-	-
Jackal	AR601	95	41	87	1490	1.2
Currawong	Wild-type	75	95	59	270	0.7

Methods and Results Trial 3:

In 2007 larger 0.25 ha plots were sown at Christchurch International Airport and bird numbers, species and behaviour were recorded comparing Jackal AR601 with existing airport low fertility grass species. Bird numbers were counted on each plot at 0900 and 1700 hours daily for a year.

There was a significant reduction in the number of birds recorded on the Jackal AR601 compared to the resident airport grasses during eight of the twelve months recorded (Figure 1).

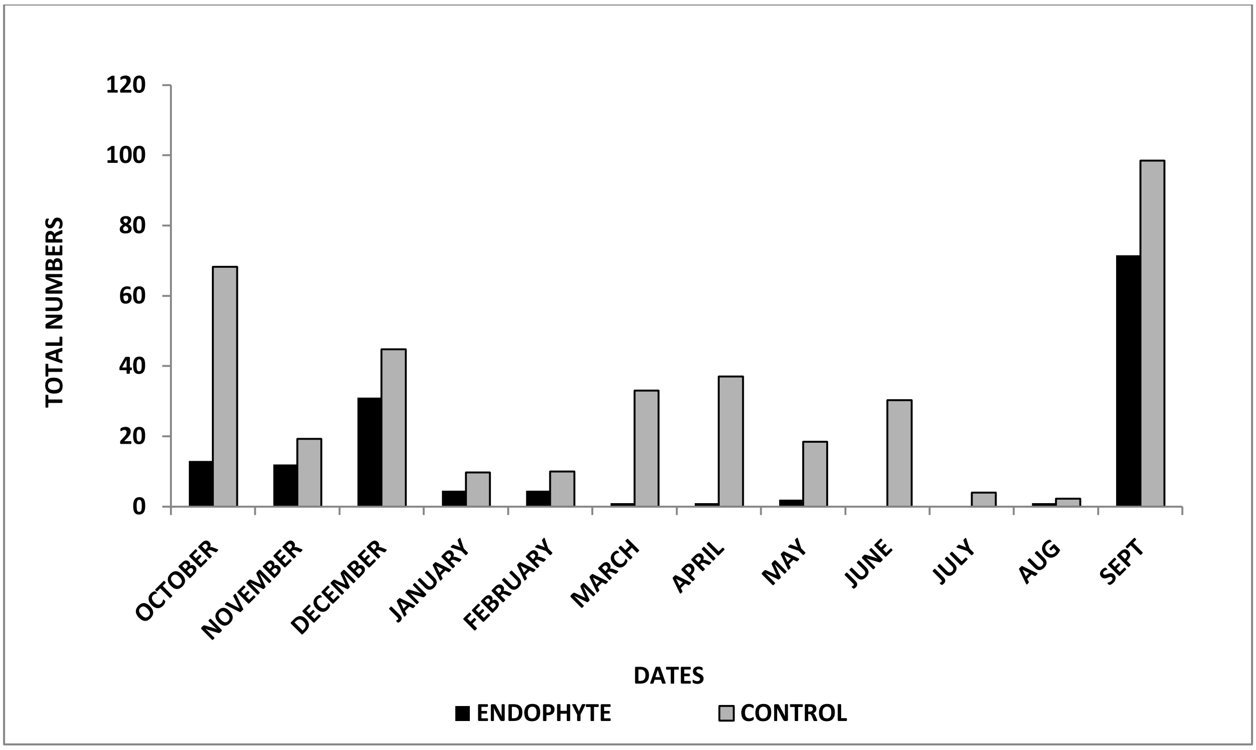

Figure 1: Total monthly bird visits to 0.25 ha plots of Jackal AR601 endophyte compared with existing CIAL control grasses (Trial 3).

These are the first known data showing a reduction in bird numbers at an airport using endophyte technology.

Methods and Results Trial 4:

A new 12ha area sown in autumn 2010 at Christchurch International Airport is being monitored for birds morning and evening by the resident wildlife officer. Observations are taken morning and evening on comparable areas of airport threshold land and also on an adjacent 12ha farm land property.

Bird numbers on seventy observation days recorded to date showed for every bird on the Jackal AR601 there were 7 and 14 times more birds respectively on the airport threshold lands and adjacent farmland.

Methods and Results Trial 5:

A large area of Jackal AR601 was also sown in (2008) for the Auckland International Airport domestic runway development. This area is being monitored for birds. Interim results of 5 observations between February and July 2010 have shown a considerable reduction in bird numbers (Figure2).

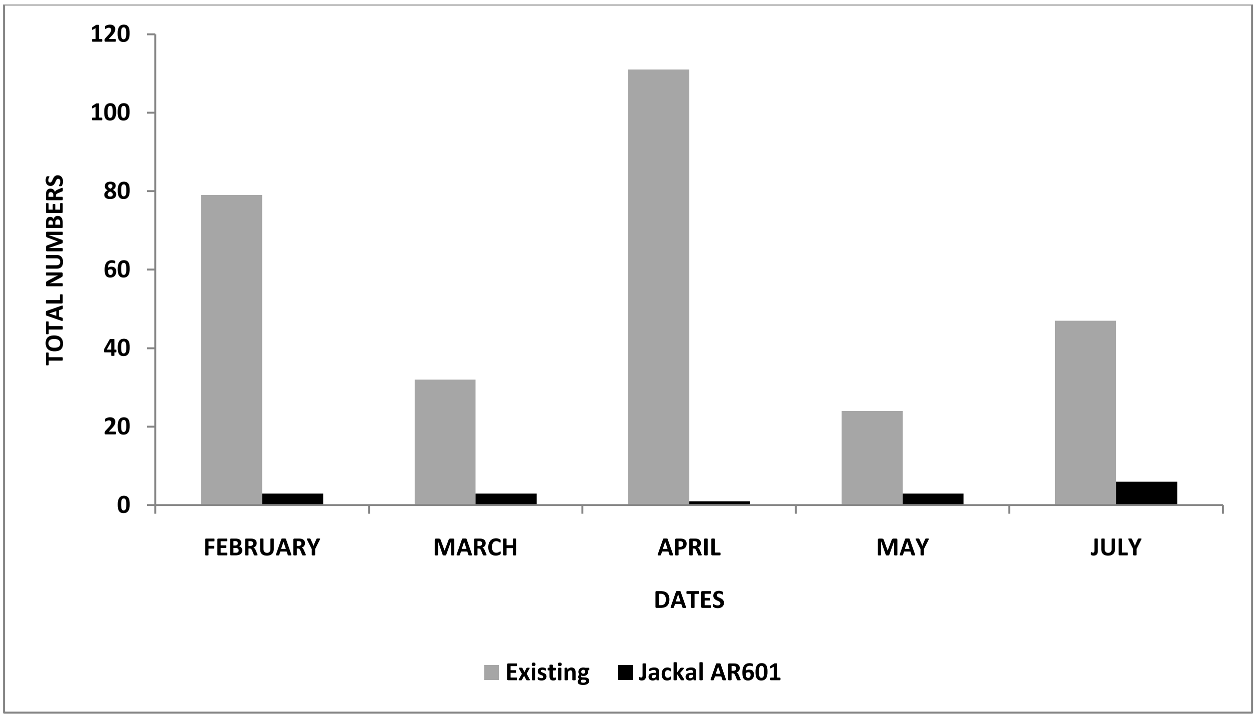

Figure 2: Total bird visits to plots of Jackal AR601 endophyte compared with existing control grasses at Auckland Airport (Trial 5).

Seasonal insect data were captured using a purpose built centrifuge sampler over the same area at Auckland International Airport. Results have shown a significant reduction in insect numbers on the Jackal AR601 sowings compared with samples taken from resident grasses on the airfield (Figure3).

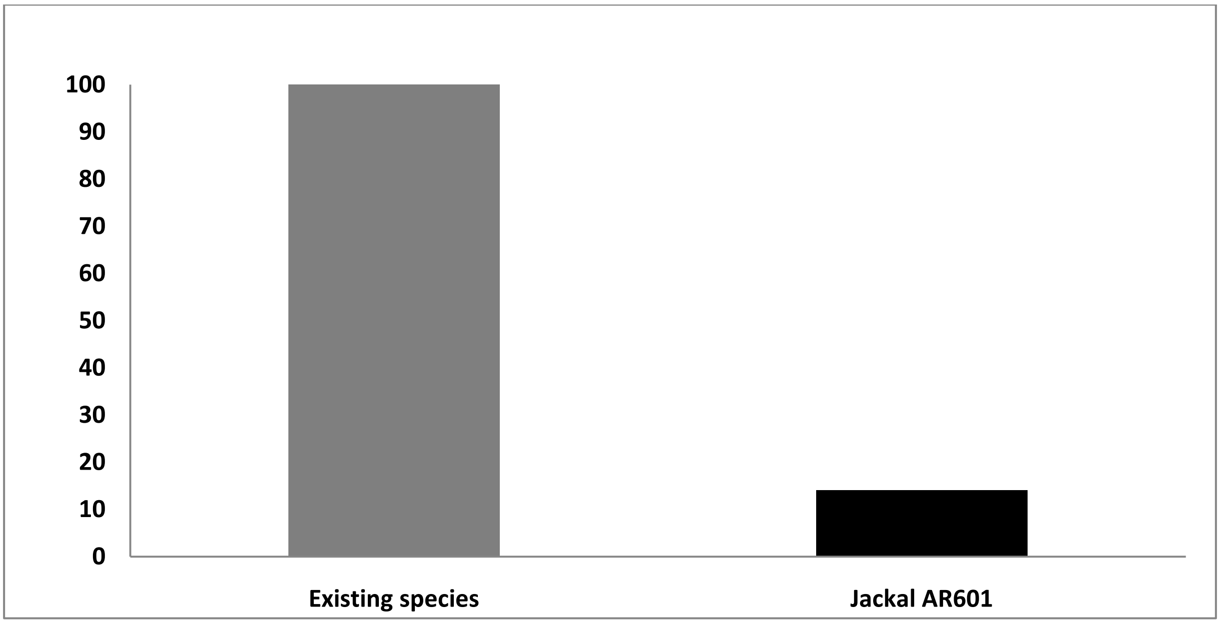

Figure 3: The relative average of insect dry weights taken from Auckland Airport over three seasons (Trial 5).

Off-airfield testing with wild Canada geese and herbivorous birds

Methods and Results Trial 6:

Another trial was established in September 2007 in an area known to be frequently grazed by wild (herbivorous) Canada geese and other birds and examined the levels of ergovaline in ryegrass and tall fescue necessary to induce avoidance behaviour (Trial 6). Results have shown a significant negative association between endophyte ergovaline levels and mean grazing score (Table 3).

Table 3: Mean ergovaline levels of test plants and geese grazing scores for Jackal tall fescue (TF) AR601 and Colosseum ryegrass (RG) AR95 with nil endophyte (Trial 6).

Description	Endophyte	Mean ergovaline level (ppm) †	Mean grazing score over the season
Jackal TF	AR601	15.1	1.3 *
Jackal TF-high	AR601	8.7	1.5 *
Jackal TF-low	AR601	2.8	1.5 *
Jackal TF	NIL	0	2.0
Colosseum RG	AR95	2.8	1.6 *

* = significantly different from nil endophyte control P<0.05; † ppm = parts per million (mg/kg)

Methods and Results Trial 7:

In early autumn of 2008, plots of Jackal AR601 and 'Colosseum' AR95 were established at a recreation/sports field in Christchurch New Zealand that was frequently grazed by Canada geese and other herbivorous birds notably paradise shellducks (*Tadorna variegata*). These test plots were compared with existing species (mainly browntop, *Agrostis capillaris*, and *Vulpia* hair grass) and some commercially available ryegrass and tall fescue infected with wild-type endophyte. Faecal pellets were counted in mid autumn 2009 as a measure of grazing behaviour of the plots.

Goose grazing was less on Jackal AR601 than nil endophyte and this was associated with ergovaline levels ≥ 2.8 ppm.

The mean faecal counts averaged 24 on the test endophyte plots compared with 213 per plot on the existing species.

Methods and Results Trial 8:

Insects in tall grass / short grass

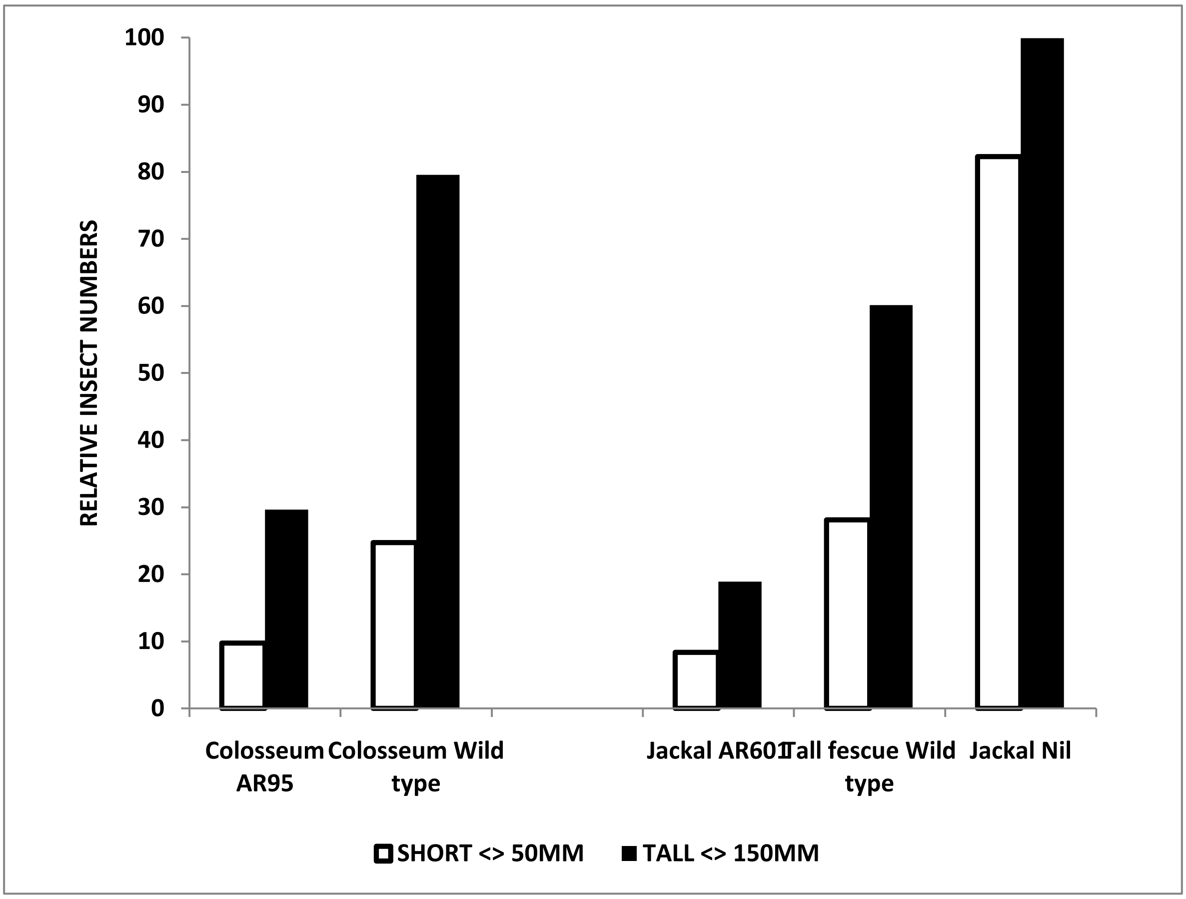

Figure 4: Comparison of short and tall grass and the AR95endophyte in ryegrass and AR601 in tall fescue for relative insect dry weight over summer 2009 and autumn 2010 (Trial 7).

With the controversy and debate between tall grass and short grass at airports a trial has been set up to examine insect populations in Colosseum ryegrass AR95 and Jackal tall fescue AR601 to compare these with existing commercially available material with wildtype endophytes under short (50 mm) and tall (150 mm) mowing regimes over two seasons, summer and autumn 2010 (Trial 8). Insect samples for these two seasons were collected and total dry weights of insects recorded.

Insect mass was higher on long grass than short grass for all endophyte-grass associations (Figure 4). The selected endophytes plots (ryegrass AR95 and tall fescue AR601) had less insects compared to either nil endophyte or wild type in the same cultivar. Higher insect numbers could increase the likelihood of bird feeding in the area. It is hoped to establish a tall grass short grass comparison on one of the larger trials on a working airport that has an established stand of the Jackal AR601 over the next year. Counts of bird activity will then commence.

DISCUSSION

Pilot studies have indicated that bird numbers are reduced when Jackal AR601 is used at airports and some of the surrounding habitats. As more airports take up this technology and more bird data obtained we may be able to compare actual bird strike numbers pre and post the establishment of the Jackal AR601 and Colosseum AR95 grasses. There were also less Canada geese feeding at recreational turf sites where this new technology is used and further work is underway. Insect numbers are lower when these endophytes are used, reducing one feed source for birds.

The debate on tall versus short grass is still ongoing, but two seasons of insect capture have shown fewer insects under short grass than tall (Figure 4). Many airports that have tall grass management do not maintain them as good upright stands and in so doing negate their objective of keeping birds away. Jackal tall fescue is hardy, stiff and upright in nature, making it suitable for either short or long grass management.

Starlings are a common cause of birdstrike. Chafers (scarabs) are a known food source for this species. New work in the use of endophytes to combat chafers (Bryant *et al.* 2010) in press is encouraging as endophytes that have loline alkaloids are known to reduce this below ground food source.

Tall fescue is slow to establish. 'Colosseum' ryegrass was developed to be used either in areas suitable for ryegrass or as a nursery plant for Jackal tall fescue to provide early ground cover and reduce dust nuisance during establishment and also provide off airfield wildlife management on golf courses and recreational parks.

Seed production is now being undertaken to supply seed during 2011 year. During 2009/10 three international airports in New Zealand have established areas with Jackal and there are encouraging signs that they have fewer birds. The sites are:

- Christchurch -12ha are sown at the 02 threshold,
- Hamilton has reduced its farming operation on the airfield by replacing lucerne (alfalfa) with Jackal AR601 with 2.5 ha sown autumn 2010 and more planned for 2010/11 years.
- Auckland Airport has sown its domestic runway extension preparation area with Jackal AR601. More sowings are planned.

CONCLUSION

Pilot studies indicate fewer birds on areas sown with Jackal AR601 and Colosseum AR95 endophytes The development of this new endophytic technology associated with specialist turf grasses bred for wildlife management offers airport managers a better option than "off the shelf cultivars" with wildtype endophytes commonly used in lawns and roadsides. Some of these wildtype endophytes are known to have poor endophyte viability in the seed, through poor storage and therefore have low alkaloid expression and poor plant persistence.

Any regrassing is an expensive exercise especially at an airport where the soils are commonly compacted, of low fertility, have high buried seed counts and limitations for machinery access and irrigation. When re-grassing is undertaken, the use of grass-endophyte associations developed for the purpose will provide better bird control.

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