Environmental Protection Authority
Te Mana Rauhī Taiao

APPLICATION FORM
RELEASE

Application to import for release or to release from containment new organisms

under the Hazardous Substances and New Organisms Act 1996

Send by post to: Environmental Protection Authority, Private Bag 63002, Wellington 6140 OR email to: noinfo@epa.govt.nz

Application number

APP201519

Applicant

DLF Seeds Ltd

Key contact

Tom Bruynel

General Manager

Important

This application form is to seek approval to import for release or release from containment new organisms (including genetically modified organisms).

The application form is also to be used when applying to import for release or release from containment new organisms that are or are contained within a human or veterinary medicine.

Applications may undergo rapid assessment at the Authority's discretion if they fulfil specific criteria.

This application will be publicly notified unless the Authority undertakes a rapid assessment of the application.

This application form will be made publicly available so any confidential information must be collated in a separate labelled appendix.

The fee for this application can be found on our website at www.epa.govt.nz.

If you need help to complete this form, please look at our website (www.epa.govt.nz) or email us at noinfo@epa.govt.nz.

This form was approved on 1 May 2012.

1. Brief application description

Provide a short description (approximately 30 words) of what you are applying to do.

To import and release *Neotyphodium siegelii*, an endophytic fungus that is safe to animals and the environment, and contributes to ryegrass and fescue persistence, protecting the plants from invertebrate pests and drought.

2. Summary of application

Provide a plain English, non-technical description of what you are applying to do and why you want to do it.

Endophytes are fungi that exist in grass and are transferred via the seed. They exist in a normal symbiotic relationship with grass, protecting it from insect attack and offering other advantages such as drought tolerance.

Most ryegrass and fescue pastures in New Zealand contain endophytes. These include Neotyphodium species present in lolium (*ryegrass)*. In association with the grass, endophytes produce a wide range of bio-active alkaloid compounds, including peramine, ergovaline, lolitrem B and lolines. All four alkaloids deter insect pests, protecting the plant against common pasture pests such as Argentinian stem weevil, black beetle, root aphid, porina and mealy bug. However, at high concentrations ergovaline and lolitrem B are toxic to cattle and sheep. The associations of ryegrass and toxic endophyte species which exist in New Zealand cause livestock illness including ryegrass staggers, which presents a significant problem to the agricultural industry. The benefit of introducing *Neotyphodium siegelii* is that it only produces peramine and loline, which protect the plant from insect attack but do not affect animals.

Neotyphodium endophytes, unlike other fungi, are asexual and do not produce spores, meaning they cannot spread from one plant to another and they cannot change as sexual recombination does not occur. The symbiotic relationship between endophytes and their host plants almost always benefits the host plant. Since novel or 'safe' grass endophytes were introduced into NZ in the early 90's they have only been perceived as beneficial to the economy and the environment.

Some endophytes found in tall and meadow fescue produce only loline alkaloids. These are non-toxic and completely safe for animals, but still have the capacity to deter insect feeding. Until 2001, there were only two fungal endophytes known to produce this compound in New Zealand, namely, *Neotyphodium coenophialum* and *N. uncinatum.* These two organisms are widespread in the New Zealand environment in association with fescues.

In 2001 a scientific paper (Craven et al., 2001) reported that within meadow fescue there was a previously un-described endophyte species which was named *Neotyphodium siegelii*. The paper suggested this species shares an ancestry with *N. uncinatum*, and observed that from a biological point of view, *N. siegelii* behaves in the same way as *N. uncinatum* and produces the same safe alkaloid, loline.

We are applying to introduce the *N. siegelii* endophyte to the New Zealand environment for use in grass pastures to improve forage and animal production, pasture persistence and tolerance to drought and reduce pesticide use.

3. Describe the background and aims of the application

This section is intended to put the new organism(s) in perspective of how they will be used. You may use more technical language but please make sure that any technical words used are included in a glossary.

3.1 Background

DLF Seeds Ltd is a wholly-owned subsidiary of DLF-Trifolium, a Danish farmers' co-operative that began in 1906 and is now the world's largest producer of grass and clover seed for cool and temperate climates. DLF Seeds Ltd was established in 2004 to serve the NZ and Australian farming and amenity (turf) markets and operates from Christchurch where it also has a breeding station developing new cultivars for farming applications in this part of the world.

Pasture related outputs in New Zealand for beef, sheep, dairy and deer were worth $16 billion at the farm gate for the 2010-2011 production season. The total GDP contribution of pasture-based products to the NZ economy equals around $24.5 billion.

The extensive pastoral environment from which much of the wealth in New Zealand has been generated has been established almost entirely from plants and animals introduced from the northern hemisphere. Other introduced components of this ecosystem are the Argentine Stem Weevil (*Listronotus bonariensis*) and the African black beetle (*Heteronychus arator*), both of which are devastating pests affecting grasses and in particular perennial and other ryegrasses. Added to this mix are endemic insect pests such as the grass grub (*Costelytra zealandica*). Unknown until 1941 was the presence within perennial ryegrass and tall fescue of fungal endophytes (Neill, 1941).

3.2 Grass and Endophytes

Perennial ryegrass (PRG) is the most widely sown species in NZ pastures and has been intensely researched since the 1920's (Hunt and Easton, 1989). As late as the 1980's, the discoveries that the endophytic fungus *Neotyphodium lolii* caused ryegrass staggers (Fletcher and Harvey, 1981) and protected the plant from Argentinian Stem Weevil (ASW) (Prestidge et al., 1982) required a complete re-examination of what was known about ryegrass. Endophyte research also improved understanding of tall fescue.

When the endophyte of PRG was first discovered it was named *Acremonium lolii*, however it was later reclassified as *Neotyphodium lolii* on the basis of the alkaloids produced (Glenn et al., 1996).

Considerable variability has been recognised within *Neotyphodium* endophytes within a single grass species (Christensen et al., 1991a, b, 1993; de Jong et al., 2008). The variability involves cultural characteristics including growth rate, appearance and conidial morphology, and genetic variability as indicated by sequence analysis of non-coding regions of genes such as β-tubulin (*tubβ*) and the translation elongation factor 1-α (*tefA*), SSR variability, and isozyme analysis.

Functionally, the most important variability between species is in the range of bioactive alkaloids produced. It is these alkaloids that enhance the persistence of host grasses within ecosystems.

Neotyphodium endophytes, with just two rare exceptions, are essentially indistinguishable in the nature of their association with host grasses.

The functional variability within *Neotyphodium* endophytes is related to the bioactive alkaloids that are produced within host grasses. This is clearly shown with the *Neotyphodium* endophytes of perennial ryegrass. Within strains considered to be *N. lolii* three bioactive alkaloids were identified in host plants (Christensen *et al.,* 1993) - ergovaline, lolitrem B and peramine. Plants that host *N. lolii* strains collected outside New Zealand have been found to produce janthitrems. Ergovaline causes ill-thrift of warm blooded animals, including birds, by disrupting body temperature regulation. Lolitrem B is a neurotoxin that causes the disorder of grazing animals termed 'ryegrass staggers'. Peramine is a feeding deterrent of insect pests and affects ovipositioning of insect pests. The key example of this effect is the Argentine Stem Weevil. Janthitrems protect host ryegrass plants from a wider range of insects including the black beetle, however, they can also sometimes produce a mild form of ryegrass staggers in livestock (see http://www.grasslanz.com/UnderstandingtheScience/AR37Endophyte.aspx).

3.3 The Search for Better Endophytes

The search for endophyte strains in plants grown from ryegrass seeds collected in Europe that didn't contain lolitrem B but had peramine, lead to the original selection of an endophyte strain, termed 'Endosafe'. This strain was used in perennial ryegrass cultivars to prevent ryegrass staggers (Fletcher et al., 1991). At that stage it was thought that if an endophyte was in perennial ryegrass and had hyphae that were orientated parallel to the longitudinal leaf axis and were seldom branched, it was called *Acremonium lolii*. Subsequently, it was observed that sheep grazing 'Endosafe' ryegrass pastures sometimes developed health problems and investigations revealed that the host plants also contained ergovaline, leading to the removal of most cultivars with 'Endosafe' from the market. Strains (also collected in Europe) within the *Acremonium lolii* functional grouping that contained neither lolitrem B or ergovaline were then subjected to studies to select a replacement strain which produced peramine only, giving rise to the AR1 strain being used today in perennial ryegrass cultivars.

Since that time another characterised endophyte strain called AR37, not known to have existed in New Zealand previously and selected from European seed collections, has become available for use in perennial ryegrass cultivars; this produces just janthitrems in host grasses. At this stage both AR1 and AR37 are termed *N. lolii.* The introduction to New Zealand of the AR1 and AR37 strains (in 2000 and 2007 respectively) has received rapid endorsement from farmers, and the majority of ryegrass seed currently sold contains either of these introduced endophyte strains. The benefits to the country have been a large reduction in animal health disorders, improved animal performance, reduced insecticide use and greater pasture persistence and production. There has been no incidence of any negative effect on the environment from these endophytes.

Three other endophytes are available in perennial ryegrass cultivars in New Zealand, AR6, NEA2, and Endo 5. AR6, present in New Zealand prior to 1998, was originally referred to as being *N. lolii* but recently, as part of a taxonomic study involving genetic tests, it was found to be an interspecific hybrid species referred to as LpTG-2 (de Jong et al., 2008). Little information is readily accessible regarding the taxonomic status of NEA2 and Endo 5 and the alkaloid profile of host plants. However, on-line information states that NEA2 has recently been shown to be a combination of two endophyte strains.

In late 2012 Cropmark Seeds (a New Zealand company) announced the 2013 release of its Grubout U2 endophyte in a festulolium grass mixture. The U2 endophyte is stated to be a strain of *N.uncinatum* and is claimed to provide a degree of protection against grass grub.

3.4 The Symbiosis between Grasses and Endophytes

Many species of grasses within the sub-family Pooideae (which includes ryegrass and fescue) are host to fungal endophytes belonging to the genus *Neotyphodium* (Moon et al., 2004). **In nature this is the only place where these fungi have been found**. Host plants are symptomless; the presence of these fungi in grasses was only found by microscopic examination of leaf tissue. In leaf sheaths, the easiest place to observe these fungi, the fungus is typically revealed as filamentous seldom-branched hyphae that are orientated parallel to the longitudinal leaf axis. The hyphae are found growing between leaf cells, which are never invaded.

All above-ground parts of the plant are colonised by the *Neotyphodium* endophytes while few if any hyphae are present within roots. **Transmission in nature is *solely* within seeds produced by the host grass**. *Neotyphodium* endophytes obtain all of their nutrients from the host plants, almost certainly by direct absorption from the walls of the host cells to which they are firmly attached. This association between fungal endophyte and host grass is mutualistic with both partners receiving benefits with no adverse consequences.

A key feature of the association between the fungal endophyte and the host grass is that the growth of both partners of the symbiosis is synchronised with plant tissues only becoming colonised while they are developing. To achieve this synchronised state the endophytic hyphae grow as if they were a plant tissue. Production of new hyphae occurs within the meristematic tissues of the plant. When hyphae are amongst enlarging plant tissue, notably the leaf expansion zone and the internodal zone of reproductive tillers, they extend not at their tips as with those of all other filamentous fungi but along their length (Christensen et al., 2008). By this means the hyphae elongate at exactly the same speed as the leaves or reproductive tillers are elongating. When hyphae are no longer amongst dividing or enlarging plant tissue they cease branching and elongating but remain metabolically active (Tan et al., 2001), functioning as a tissue that carries out chemical synthesis, notably producing biologically active alkaloids that protect the host grass from invertebrate grazers. See Lane, Christensen and Miles (2000) for a review of endophyte-associated alkaloids.

When leaf tissue host to these fungal endophytes senesce and die the hyphae retain their synchronised regulation, and die with the leaf (Christensen & Voisey, 2009). (This reference provides a detailed well-illustrated review of the basic biology of the fungal endophyte/grass host mutualistic symbiosis.)

In many parts of New Zealand most perennial ryegrass and tall fescue plants are host to a *Neotyphodium* endophyte (Hume & Barker, 2005, Neill, 1940). Predominant endophyte species are *N.lolii* and *N.uncinatum*. Perennial ryegrass plants are rapidly killed by the feeding of insects, in particular the Argentine Stem Weevil, if they are not a host of a *Neotyphodium* endophyte.

A European survey using phylogenetic analysis revealed that most *Neotyphodium* endophytes of meadow fescue could be grouped as belonging to *N. uncinatum* but based on the phylogenetic criteria and supported by conidial characteristics, another grouping was detected, comprising just one strain, and this was designated *N. siegelii* (Craven et al., 2001).

We aim to introduce this endophyte for use in New Zealand pastures.

4. Information about the new organism(s)

- Provide a taxonomic description of the new organism(s) (if the organism is a genetically modified organism, provide a taxonomic description of the host organism(s) and details of the genetic modification).

Before 2001 the name *Neotyphodium siegelii* did not exist and the organism would have been considered a member of *Neotyphodium uncinatum*. *N.siegelii* was described by Craven el al. (2001) when studying endophytes symbiotic with the grass *Lolium pratense* (syn *Festuca pratensis*) using morphological and molecular phylogenetic characteristics. Their results suggested that *N. uncinatum* is an interspecific hybrid of two Epichloe species (a closely-related family of endophytes). A second hybrid sharing one of the two Epichloe ancestors with *N. uncinatum* was the previously undescribed *N. siegelii*.

N.siegelii only differs from *N.uncinatum* in the shape of its conidia (spores) – and this can only be detected under a microscope when the organism is grown in vitro. Other authors have observed similar differences when working with other endophyte species but have deemed these to be insufficient grounds to warrant naming of a new species (Christensen, pers. comm., Feb. 2012).

The *N.siegelii* strain that is the subject of this application was isolated several decades ago from meadow fescue growing in Europe. This organism has also been found in Turkey.

In many parts of New Zealand most perennial ryegrass and tall fescue plants are host to a *Neotyphodium* endophyte (Hume & Barker, 2005, Neill, 1940), and many meadow fescue plants also host *Neotyphodium* endophytes including *N.uncinatum* and *N.coenophialum*. Endophyte-infected and uninfected plants are indistinguishable when growing in clean glasshouse conditions. However, in some grasslands environments meadow fescue plants host to a *Neotyphodium* endophyte are more robust and persistent due to the presence of loline alkaloids that give protection against invertebrate pests (Hume et al., 2009; Fletcher et al., 2001).

The naming of the species *N.uncinatum* dates from 1990 (Gams et al., 1990). The first documented report of a *Neotyphodium* endophyte in meadow fescue in New Zealand was in 1993 (Christensen et al., 1993) **although it seems certain that this grass/endophyte association had been long present but unrecognised.**

Within representative isolates from meadow fescue, including two isolates from North Auckland, examined using colony characteristics and isozyme analysis in the 1993 study, two groupings were identified. The most common type, including the two strains from North Auckland, had curved conidia while the other grouping had straight conidia. Applying morphology-based taxonomy principles such a difference in conidial morphology was arguably justification for the splitting of this group into two species, as Craven et al. (2001) did later, but Christensen considered both groupings as belonging to *N. uncinatum*. Seed from this North Auckland meadow fescue was sown in Autumn 1998 in the field at Lincoln to test herbage and sheep production of the material with and without endophyte (Fletcher et al., 2001) and its effect on grass grub (Popay et al., 2003).

- Describe the biology and main features of the organism including if it has inseparable organisms.

The application of phylogenetic and other genetic tests has given rise to an increased recognition of diversity within this genus, and has formed the basis for the establishment of new species (Craven et al., 2001, de Jong et al., 2008). The definition of how much genetic diversity constitutes a distinct species is a matter of debate. However, what is more relevant in distinguishing *Neotyphodium* endophytes are the functional groupings based on characteristics of the endophyte/plant symbiosis, the most important being spectrum of bioactive alkaloids. These groupings overlap between multiple *Neotyphodium* species within a single grass species. *N. siegelii* when growing in host meadow fescue plants is **functionally identical** in alkaloid spectrum and hyphal characteristics to the more common species, *N. uncinatum*. Where it differs from strains of *N. uncinatum* is the ability to form compatible associations with perennial ryegrass and tall fescue as well as meadow fescue. Some perennial ryegrass (*Lolium perenne*) plants hosting this *N. siegelii* strain have high expression of loline alkaloids, sufficient to give protection against major insect pests including the grass grub, unaffected by *Neotyphodium* endophyte strains currently available in cultivars of this most important pastoral grass. This is an opportunity to apply technology and expertise to enhance pastoral productivity in New Zealand in a risk-free way.

Loline alkaloids are non-toxic to sheep when administered at maximum exposures during grazing (Patchett, 2007).

- Describe if the organism has affinities (e.g. close taxonomic relationships) with other organisms in New Zealand.

The organism is part of the genus *Neotyphodium* of which at least three species have existed in New Zealand for potentially more than 100 years, corresponding with importation of the host grasses from Europe. They are: *N. lolii, N. coenophialum* and *N. uncinatum*. *Neotyphodium siegelii* may have shared one of its ancestors with *N. uncinatum* according to Craven et al. (2001). It has to be noted that *N. siegelii* may very likely already have been present in New Zealand via import and growing of seeds of meadow fescue. As reported by Craven et al. (2001) *N. siegelii* originates from Germany, and grass seeds have been imported to New Zealand from Germany and other European countries during the past 150 years without any consideration given to their endophyte status. It is believed that only two native grass endophyte species have been found (one in poa and one in hedgehog grass (Christensen, pers. comm., Feb. 2013).

- Could the organism form an undesirable self-sustaining population? If not, why not?

The organism cannot form a self-sustaining population. However in symbiosis with host grasses it will be sustainable.

Its beneficial properties cannot change to undesirable properties. The organism is asexual and can only live inside the plant and it is only transmitted in nature by seed coming from that plant. As it is asexual it cannot change by sexual recombination, so its transmission by seed will never result in different types. *Neotyphodium* infections do not appear to spread from infected to uninfected plants even though such plants are often in direct contact and their frequent clipping creates wound sites for potential infections (Craven et al., 2001). This paper mentioned that *N. siegelii* infected plants were in very close proximity to uninfected plants, yet the endophyte was never observed in the original uninfected plants.

This endophyte cannot spread to, or be hosted by, any other plant that is not subfamily Pooideae. If a plant is subfamily Pooideae, the endophyte has to be artificially infected in the laboratory. Fruit, vegetable crops and forestry species are not Pooideae and cannot be infected at all.

- What is the ease with which the organism could be eradicated if it established an undesirable self-sustaining population?

To eradicate the organism the grass crop can be sprayed with glyphosate or a grass selective herbicide. This procedure can be followed by a cropping phase such as peas and cereals, before returning to a clean paddock for perennial ryegrass after two/three years. Not having ergovaline, peramine or lolitrem B in its alkaloid make-up would make a grass hosting *N. siegelii* very susceptible to grazing animals. Preferential grazing may be the reason this endophyte has not been found in the country as it may only occur in isolated pockets if already in our environment.

The organism that is the subject of this application is also the subject of:

a. an innovative medicine application as defined in section 23A of the Medicines Act 1981. X☐ No

b. an innovative agricultural compound application as defined in Part 6 of the Agricultural Compounds and Veterinary Medicines Act 1997. X☐ No

5. Detail of Māori engagement (if any)

Discuss any engagement or consultation with Māori undertaken and summarise the outcomes.

DLF Seeds believes that this endophyte will be of long term benefit to Maori farming enterprises throughout New Zealand, and especially so in the northern parts of the North Island where insect pressure is high.

In October 2012 DLF Seeds Ltd wrote to over 120 Maori groups listed on the Maori National Network. By the middle of December 2012, three responses had been received and these are summarised below.

A spokesperson for Maniapoto/Ngati Kaputuhi/Ngati Rora requested the complete bibliographic references (which were duly provided); clarification on the genetically modified status of the endophyte (it is non-GMO), clarification of the patent status relating to the endophyte (it is owned by DLF-Trifolium) and clarification of the likely impact on native grasses or the ecosystem (being asexual it cannot spread to other organisms and will only be grown in man-made pastures). A spokesperson for Ngai Tahu commented that the application appeared 'relatively benign' and that the letter was very self-explanatory. A spokesperson for Tiakina te Taiao Limited invited DLF Seeds to meet with manawhenua iwi in Tasman Nelson to discuss the proposal (the visit has not been possible as yet).

6. Identification and assessment of beneficial (positive) and adverse effects of the new organism(s)

Adverse effects include risks and costs. Beneficial or positive effects are benefits.

- Identification involves describing the potential effects that you are aware of (what might happen and how it might happen).
- Assessment involves considering the magnitude of the effect and the likelihood or probability of the effect being realised.

Consider the adverse or positive effects in the context of this application on the environment (e.g. could the organism cause any significant displacement of any native species within its natural habitat, cause any significant deterioration of natural habitats or cause significant adverse effect to New Zealand's inherent genetic diversity, or is the organism likely to cause disease, be parasitic, or become a vector for animal or plant disease?), human health and safety, the relationship of Māori to the environment, the principles of the Treaty of Waitangi, society and the community, the market economy and New Zealand's international obligations.

6.1 Potential hazards

The designated strain does not present an actual or potential hazard to vertebrate species as it produces only one type of recognised bioactive alkaloid, the lolines, and these are active only against invertebrate pests. Beneficial invertebrates, including earthworms, are not affected by grasses containing only loline alkaloids. Prestidge et al (1997) found no negative relationship between earthworms and endophyte-infected ryegrass in the Waikato. Bees are certainly not affected as these grasses are wind pollinated. Invertebrate pests that may be affected by the bioactive alkaloids produced by *N. siegelii* are Argentinian stem weevil, black beetle, root aphid, porina and mealy bug.

There are very toxic *Neotyphodium* endophytes currently available in the New Zealand market and also some naturally occurring in our environment. For instance, AR601 (*N.coenophialum*) and AR95 (species unknown) are used as a bird deterrent for airports and these could potentially cause severe illness in animals. These endophytes are used in fescue and ryegrass respectively and are available commercially under the brand name Avanex™ (see www.agresearch.co.nz/our-science/plant-forage/endophytes). **The effect of these endophytes on birds and farm animals is due to alkaloids which are not produced by *N. siegelii.***

The *N.siegelii* endophyte may have an effect on grass grub *Costelytra zealandica*, an insect native to New Zealand. If this proves to be the case, where this endophyte is used as planned, the size and population (due to reduced reproductive performance) of grass grub could be reduced, but it would never be eradicated. The presence of this endophyte discourages insects from eating the host plant, but does not act to kill the insect. The density of the grass grub population would hopefully be decreased to a level where economically significant damage to pasture does not occur. Currently there are few practical pesticide options available for grass grub control. Diazinon is one example however its use is constrained by the need for significant rainfall at or shortly after the time of spraying. Also it is likely that the list of agrichemicals available to farmers will decrease in the face of increasing environmental concerns. Thus, an endophyte that provides a natural solution to grass grub would be widely accepted.

Neotyphodium siegelii can only be introduced to grassland by seeding ryegrass seeds that host *N. siegelii*, restricting its use to arable land. Grass grub is present in tussock and scrub land (its natural habitat), so the vast majority of NZ will remain a healthy environment for the species. Birds are the only predators for grass grub, with starlings and rooks the most common. Both are introduced species and they will always have a good source of grass grub and other food. All other insects likely to be affected by *N. siegelii* are introduced species and are considered only as pests with no benefits to farming or natural ecosystems.

There are no known or potential hazards of plants associated with *N. siegelii* strain. As with *N. uncinatum* the only known bioactive alkaloids present in host plants, regardless of plant species, are lolines and there have been no adverse effects reported in extensive trials with livestock ingesting grasses that contain only loline alkaloids (Bush et al. 1997; Patchett, 2007).

No effects on beneficial invertebrates including earthworms were detected in field trials using tall fescues with two *Neotyphodium* strains that contained either just lolines or lolines and peramine (Popay and Jensen 2005). The trials (at Palmerston North and Lincoln) used two Neotyphodium endophyte strains AR542 and AR501 but did not name the actual species.

Any native species that could be displaced by ryegrass or fescue with endophyte would have already been displaced in the last 160 years as these introduced grasses are used all over New Zealand.

The *N. siegelii* endophyte cannot displace native or valued endophyte species as it cannot spread from infected to uninfected plants. The organism may be under high grazing pressure, as it is grazed by animals in preference to those grasses with the "wild" endophyte which contains toxic alkaloids.

6.2 Benefits

Over 80% of the ryegrass seed sold in New Zealand is infected with endophytes (Fletcher, 2009), all of which have been introduced into the country. In addition to the widely known effect on insect pests, endophyte infection has been reported to confer drought tolerance (Kane, 2011; Malinowski and Beleski, 2006). According to AgResearch, new endophyte strains contribute at least $200 million each year to the New Zealand economy (see www.agresearch.co.nz/our-science/plant-forage/endophytes).

New Zealand is the world leader when it comes to utilizing the beneficial properties of selected *Neotyphodium* strains to alleviate disorders caused by alkaloids produced in some *Neotyphodium*/grass associations and to prevent severe damage to forage grasses, in particular perennial ryegrass, from insect pests. It is here that much of the research involving the identification and effects of *Neotyphodium*-produced alkaloids and the ways to inoculate plants with selected strains (Latch and Christensen, 1985) was carried out. It is New Zealand-based research that has lead to the production and acceptance of cultivars of perennial ryegrass and tall fescue infected with selected strains of *Neotyphodium* endophytes to enhance pastoral-based agriculture productivity. This technological development is widely regarded as a major success of New Zealand research (Easton et al., 2001).

It is important to note that this research was only possible by bringing into New Zealand many collections of seed of perennial ryegrass and tall fescue from Europe and northern Africa. At the time when much of the collecting of this seed was undertaken the taxonomy of these endophytic fungi was essentially based on the host species.

The selection of endophyte strains with the appropriate alkaloid profile is just the first step in the process of producing endophyte-infected agronomically-useful cultivars of forage grasses. Several requirements of strains additional to alkaloid profile are essential to produce useful cultivars:

- The first is to have strains that can be isolated from infected plants and grown in culture to produce inocula to infect seedlings via an inoculation process.
- The second requirement is that cultures of strains will prove to be infectious. To achieve this endpoint the endophyte has to switch from the synchronised biotrophic regulation in the host plant to unrestricted saprotrophic growth to grow in culture. To successfully colonise an inoculated seedling the endophyte must again switch back to synchronised biotrophic growth. The second switching must occur not just in seedlings of several cultivars of the original host species but sometimes in cultivars of another host species.

- The third requirement is that the strains form stable compatible associations with artificially infected grasses. There are several malfunctions of associations that can occur including the host plants becoming stunted, premature death of hyphae, and instability of associations (Christensen 1995; Koga et al., 1993).
- The fourth is that the endophyte must have high infection of seeds and viability during storage.
- The last requirement is that the desired alkaloids must be expressed in bioactive concentrations in host grasses. With loline alkaloids, in particular the concentrations produced in cultivars of artificially infected grasses of a different species to that where the endophyte originated, may be too low to provide effective protection against insect pests.

The meadow fescue strain designated as *N. siegelii* (registered as "Happe") has proved to meet the five requirements for selection as an endophyte strain used to produce agronomically-useful endophyte-infected grass cultivars, and thus has great potential to increase productivity of a range of pastoral grasses in New Zealand.

What research approach would we take with a new endophyte?

To determine if the *Neotyphodium siegelii* endophyte will be agriculturally advantageous, the following steps would be followed once it is approved for release (as with any new endophyte):

1) Production and persistence trials against all known commercial local endophytes
2) Testing of best grass/endophyte combinations against insects in controlled environment
3) Test the storing capacity in seed of the new endophytes against commercial controls
4) Animal safety trials with the best endophytes to determine improvements in animal production (on the basis of its known alkaloid profile, no health problems are expected)
5) Commercial seed multiplication of the new grass/endophyte combinations.

Summary of benefits

We believe that the Happe endophyte (*Neotyphodium siegelii*) has the potential to provide significant economic benefits for the NZ farmer. Immediate benefits will accrue from less pasture damage from insects (and thus reduced re-sowing costs), greater drought resistance, improved animal health, higher meat, milk and wool production, and less expenditure on pesticide application. Ultimately the organism could also lead to grass seed exports to Australia and South America, thus providing greater earning opportunities for NZ farmers.

7. Could your organism(s) undergo rapid assessment?

If your application involves a new organism that is or is contained within a veterinary or human medicine, could your organism undergo rapid assessment (s38I of the HSNO Act)?

Describe the controls you propose to mitigate potential risks (if any). Discuss what controls may be imposed under the ACVM Act (for veterinary medicines) or the Medicines Act (for human medicines).

Discuss if it is highly improbable (after taking into account controls if any):

- the doses and routes of administration of the medicine would have significant adverse effects on the health of the public or any valued species; and
- the organism could form an undesirable self-sustaining population and have significant adverse effects on the health and safety of the public, any valued species, natural habitats or the environment.

Do not include effects of the medicine or new organism on the person or animal being treated with the medicine.

We have elected to proceed with the full assessment. Although *N.siegelli* cannot form self-sustaining populations, in conjunction with its grass host it can do so.

8. Other information

Add here any further information you wish to include in this application including if there are any ethical considerations that you are aware of in relation to your application.

The organism is not an unwanted organism as defined by the biosecurity act 1993.

9. Appendices(s) and referenced material (if any) and glossary (if required)

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10. Signature of applicant or person authorised to sign on behalf of applicant

X☐ I request the Authority to waive any legislative information requirements (i.e. concerning the information that shall be supplied in my application) that my application does not meet (tick if applicable).

I have completed this application to the best of my ability and, as far as I am aware, the information I have provided in this application form is correct.

	21 February 2013
Signature	**Date**