Environmental Protection Authority
Te Mana Rauhī Taiao

EPA staff report

Import and release of *Neotyphodium siegelii*

June 2013

Advice to the Decision Making Committee on application APP201519: – To import and release *Neotyphodium siegelii*, an endophytic fungus, under section 34 of the Hazardous Substances and New Organisms Act 1996

Executive Summary and Recommendation

In February 2013, DLF Seeds made an application to the Environmental Protection Authority (EPA) seeking to import and release *Neotyphodium siegelii*, an endophytic fungus that is safe to animals and the environment, and contributes to ryegrass and fescue persistence by protecting the plants from invertebrate pests and drought.

Evidence in the application shows that no native and/or taonga plants will be adversely affected, and no organisms will be displaced by this fungus. We recommend that it be approved for release.

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1. The application process

Purpose of this document

1.1. This document has been prepared by EPA staff; Asela Atapattu (Manager, New Organisms), Amy Rowe (Advisor, New Organisms), Kate Bromfield (Senior Advisor, New Organisms) and Manu Graham (Senior Advisor, Māori Policy and Operations), to advise the HSNO Decision Making Committee on the results of our risk assessment of this application to import and release *Neotyphodium siegelii*, an endophytic fungus that contributes to ryegrass and fescue persistence by protecting the plants from invertebrate pests and drought. The document discusses information provided in the application and other readily available sources.

Submission process

1.2. DLF Seeds Ltd lodged an application with the EPA on 22 February 2013 to import and release *N. siegelii* under section 34 of the Hazardous Substances and New Organisms (HSNO) Act (the Act).

1.3. Application APP201519 was publicly notified as required by section 53(1)(b) of the Act. The 30 working day notification period began on 7 March 2013 and closed on 22 April 2013.

1.4. Submitters were asked to provide information, make comments and raise issues, particularly with regard to, but not limited to the following matters:

- adverse effects[1], especially adverse effects not identified in the application, and
- positive effects[2], especially positive effects not identified in the application.

Submissions

1.5. Six submissions were received in response to public notification of the application. The submissions are summarised in Appendix 1. Five submitters; Grasslanz Technology Ltd, the New Zealand Plant Breeding and Research Association, Beef + Lamb New Zealand, Federated Farmers of New Zealand, and Dairy NZ supported the application to import and release *N. siegelii*. One submitter, Bevan Weir neither supported nor opposed the application. Grasslanz Technology and the New Zealand

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[1] Adverse effects can include any risks and costs associated with approving the release of these organisms.
[2] Positive effects can include any benefits associated with approving the release of these organisms.

Plant Breeding and Research Association requested to be heard in support of their submissions.

Submissions from MPI and DOC

1.6. As required by the Act and the Hazardous Substances and New Organisms (Methodology) Order 1998 (the Methodology), the Ministry for Primary Industries (MPI) and the Department of Conservation (DOC) were advised of the application and provided with the opportunity to comment. We gave particular regard to the comments provided by DOC, and these full comments are provided in Appendix 2. MPI provided no comment on the application.

Application summary

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

1.8. *Neotyphodium* endophytes show a high degree of host specificity. The natural host for *N. siegelii* is meadow fescue (*Lolium pratense*); however, this endophyte can also be artificially and stably inoculated into perennial ryegrass (*Lolium perenne*) and tall fescue (*Lolium arundinaceum*). The applicant seeks to import and release *N. siegelii* with the aim of improving the pest resistance and drought tolerance of perennial ryegrass, an important forage species in New Zealand agriculture.

Background

1.9. *Neotyphodium* endophytes are asexual fungal symbionts of cool season grasses that form long term associations with their grass hosts. They live in a symbiotic[3] relationship with their hosts, growing in the intercellular spaces between the plant's cells in all above-ground tissues of the grass. Through this association, the endophyte gains access to nutrients and an exclusive biological niche, while producing bioactive alkaloid compounds which can protect the grass host from insect and mammal herbivory, drought stress, and other abiotic factors (Kuldau et al 2008). Host grasses infected with *Neotyphodium* are asymptomatic, and most pasture grasses in New Zealand are host to one of several *Neotyphodium* endophytes species (Fletcher 2009).

[3] A close, prolonged, and usually obligatory association of two organisms of different species that live together, often to their mutual benefit.

1.10. *Neotyphodium* endophytes are not passed between grass species, or between individual plants, but transmitted vertically. That is, the fungus grows into the reproductive tissues and seeds of the host grass, and is transmitted asexually to the next generation through the seeds produced. Endophyte growth is synchronized with the host plant tissues, and when the leaf tissues hosting the endophyte senesce and die, the endophyte dies with them.

1.11. Six species of *Neotyphodium* endophytes are known to be present in New Zealand, including the native *N. aotearoae* which lives within the endemic grass species *Echinopogon ovatus* (Forest hedgehog grass) (Moon et al 2002). The other introduced species *N. coenophialum*, *N. lolii*, *N. occultans*, *N. typhinum*, and *N. uncinatum*, are found in the pasture grasses tall fescue; perennial ryegrass; Persian, Italian, and Wimmera ryegrasses; Canary Islands ryegrass; and meadow fescue, respectively (see Table 1) (Clay & Schardl 2002, Moon et al 2000, Moon et al 2002) .

1.12. In association with grasses, these endophytes produce a wide range of bio-active alkaloid compounds, including peramine, ergovaline, lolitrems and lolines. All of these compounds deter insect feeding, protecting the plant against a wide range of insect pests including Argentinean stem weevil, black beetle, root aphid, and grass grub. However, at high concentrations ergovaline and lolitrem B, produced by *N. lolii* and *N. coenophialum* in ryegrass and tall fescue respectively, are toxic to cattle and sheep, causing the neurological disorders known as ryegrass staggers and fescue toxicosis. Ryegrass toxicity and other livestock illness caused by some endophyte-grass associations present a significant problem to New Zealand's agricultural industry.

1.13. *Neotyphodium* endophytes are derived from the closely related *Epichloë* endophytes. *Epichloë* are sexual fungal endophytes that form associations in host grasses ranging from mutualism[4] to parasitism[5], with some species causing 'choke' disease[6]. A number of *Epichloë* species are known to be present in New Zealand.

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[4] A symbiotic relationship between individuals of different species in which both individuals benefit from the association.

[5] A form of symbiosis in which one organism benefits at the expense of another organism of a different species.

[6] 'Choke' arises when some *Epichloe* endophytes enter the sexual stage of their lifecycle, and the rapidly growing fungal stroma engulfs the grass inflorescence, 'choking' seed production of the grass.

2. The organisms proposed for release

Background on *Neotyphodium siegelii.*

2.1. This organism has the following taxonomy:

Kingdom = Fungi

Phylum = Ascomycota

Class = Ascomycetes

Order = Hypocreales

Family = Clavicipitaceae

Genus = Neotyphodium

Species = *N. siegelii*

2.2. *Neotyphodium siegelii* was first described as an asexual symbiont of meadow fescue in 2001 (Craven et al. 2001), and determined to be a distinct species based on phylogenetic analysis. Prior to this, *N. siegelii* was thought to be another *Neotyphodium* endophyte found in meadow fescue – namely *N. uncinatum*. This assumption was on the basis that both *N. siegelii* and *N. uncinatum* inhabit meadow fescue, and are functionally similar.

2.3. Like all *Neotyphodium* endophytes, *N. siegelii* and *N. uncinatum* form asymptomatic mutualistic symbioses with their grass host. They reproduce asexually, by infecting the seeds of the host plant, and are transmitted vertically.

2.4. Both *N. uncinatum* and *N. siegelii* deter insect herbivory of their host grass by producing only non-toxic loline alkaloids (Zhang et al 2009). Lolines are non-toxic to vertebrates, but still have the capacity to deter invertebrate feeding. Because they produce only lolines, grass species hosting *N. uncinatum* and *N. siegelii* are completely harmless to grazing animals and birds, but show resistance to insect pests and drought stress (Kuldau et al 2008).

2.5. In contrast to *N. uncinatum* which cannot inhabit grass species other than meadow fescue, *N. siegelii* is capable of forming stable endophytic associations with tall fescue and perennial ryegrass. This is one of the characteristics that the applicant seeks to exploit if this application to import and release *N. siegelii* is approved.

2.6. Phylogenetic analysis shows *N. siegelii* is likely to be an asexual hybrid of the sexually reproducing endophytes *Epichloë festucae* and *E. bromicola*. *Epichloë festucae* is present in New Zealand. There is no evidence that *E. bromicola* is present in New Zealand, however other *Neotyphodium* endophytes that share *E. bromicola* as a hybrid parent are widespread in New Zealand pastures (i.e.: *N. uncinatum,* and *N. occultans* (see Table 1).

2.7. There are no inseparable organisms associated with *Neotyphodium siegelii*.

Table 1: Host grass, parent species, and bioactive alkaloid production of *N. siegelii* and other *Neotyphodium* endophytes present in New Zealand.

Endophyte	Host grass	Parent species	Bioactive alkaloids produced			
			Ergovaline	Lolines	Peramine	Lolitrems
Neotyphodium siegelii	*Lolium pratense* (meadow fescue)	*E. bromicola x E. festucae*	-	+++	-	-
Epichloë festucae	*Festuca rubra, F. gigantean,* and *F. longifolia*	-	+	+	+	+
N. aotearoae	*Echinopogon ovatus*	-	-	+++	-	-
N. coenophialum	*Lolium arundinaceum* (tall fescue)	*E. baconii, E. festucae, E. typhina*	++	+++	+++	-
N. lolii	*Lolium perenne* (perennial ryegrass)	*E. festucae*	++	-	+++	++
N. occultans	*Lolium canariense, L.multiflorum, L. persicum, L. remotum,* and *L. rigidum*	*E. baconii x E. bromicola*	-	+++	nt	nt
N. typhinum	*Lolium canariense* and *Poa sylvestris*	*E. typhina*	nt	nt	nt	nt
N. uncinatum	*Lolium pratense* (meadow fescue)	*E. bromicola x E. typhina*	-	+++	-	-

This table compares the host grass, hybrid parental species, and bioactive alkaloid profiles of *N. siegelii*, and other Neotyphodium species present in New Zealand.

Epichloë festucae is included here as it is present in New Zealand, and is thought to be a hybrid parent of *N. siegelii*.

Key: - = undetected from any isolates, + = detected in some isolates, ++ = detected in most isolates, +++ = detected in all isolates, nt = not tested.

Data compiled from Bush et al 1997, Clay & Schardl, 2002, and Kuldau et al 2008.

3. Risk and benefit assessment

3.1. EPA staff have conducted a risk benefit assessment for the import and release of *N. siegelii*. This includes risks and benefits to the environment, human health and safety, Māori culture and spiritual values, society and community, and the market economy.

3.2. In performing this assessment EPA staff conducted a literature review in addition to reviewing the information provided by the applicant and submitters. We have also consulted other EPA staff with skills and knowledge specific to the application, including Dr Geoff Ridley, Principal Scientist, EPA – a qualified and experienced fungal taxonomist. EPA staff also consulted independent expert Professor Dr Adrian Leuchtmann, Institute of Integrative Biology, Plant Ecological Genetics, ETH Zurich. Professor Dr Leuchtmann is a distinguished fungal ecologist, and was among the researchers who first characterised and described *N. siegelii* in 2001.

4. Minimum standards

4.1. Prior to approving any new organism the EPA is required to ensure that if the organism were to be released, it would meet the following minimum standards set out in section 36 of Act.

Section 36 (a): whether *Neotyphodium siegelii* is likely to cause any significant displacement of any native species within its natural habitat

4.2. The applicant has provided evidence that *N. siegelii* is an asexual endophyte and is vertically transmitted. It can only pass between plants by colonization of seeds produced by the host.

4.3. Similarly to other species of *Neotyphodium* (e.g. *N. aotearoae*, and *N. typhinum* (Moon et al 2002, Tadych et al 2007)) *N. siegelii* produces extensive mycelia[7] on the undersides of host plant leaf surfaces, and produces conidia[8] *in planta*, in association with *L. pratense* and *L. perenne* (Craven et al 2001).

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[7] Masses of fine branching tubes that make up the body of a fungus.

[8] An asexually produced fungal spore.

4.4. When artificially cultured on potato dextrose agar, *N. siegelii* is also known to produce conidia abundantly. In mating tests, these conidia were not interfertile with either of its hybrid parents *E. festucae*, and *E. bromicola*, and did not reproduce (Craven et al 2001). This suggests that *N. siegelii* is not likely to hybridise with other fungi.

4.5. Professor Dr Adrian Leuchtmann notes asexual conidia produced by *N. siegelii in vitro* and *in planta* are functional in the sense that they are able to germinate and establish new colonies *in vitro*, but it has never been experimentally demonstrated that *N. siegelii* can spread to uninfected plants via these conidia (pers comms.).

4.6. The only known grass endophyte native to New Zealand is *N. aotearoae*, which colonises only *Echinopogos ovatus* (Hedgehog grass). Neotyphodium species are host specific, meaning *Neotyphodium aotearoae* inhabits only *E. ovatus*, and *N. siegelii* naturally inhabits only *L. pratense.* Because of this, and because *N. siegelii* cannot be passed between plants, *N. siegelii* will not displace *N. aotearoae* in its natural habitat.

4.7. EPA staff consider that the potential for *N. siegelii* to colonise another host species is extremely unlikely.

Section 36 (b): whether *Neotyphodium siegelii* is likely to cause any significant deterioration of natural habitats

4.8. *Neotyphodium siegelii* is not a free-living organism. It lives only within the tissues of its host, *L. pratense* (meadow fescue)(or perennial ryegrass), and is not exposed to the environment. The organism itself cannot cause any significant deterioration of natural habitats.

4.9. DOC notes that there is the potential for native species to be displaced by ryegrass or fescue carrying the endophyte due to increased fitness and tolerance of the host.

4.10. It is possible that the increased drought tolerance and pest resistance conferred by *N. siegelii* may make ryegrass/meadow fescue more persistent in the environment. However, the applicant claims that when associated with *N. siegelii* (in contrast to the toxic *N. coenophialum* and *N. lolii*) grasses are more palatable to grazing animals, and thus more heavily grazed.

4.11. EPA staff note that most forage grass species in New Zealand contain endophytes. Ryegrass and tall fescue host other *Neotyphodium* species that produce ergovaline and lolitrem B, which are toxic to grazing vertebrates. In contrast, *N. siegelii* produces only loline alkaloids which are non-toxic to cattle and other vertebrates. Grasses associated with non-toxic endophytes like *N. siegelii* are likely to be more palatable and heavily grazed than grasses carrying toxic endophyte.

4.12. Studies have demonstrated an increase in the population of plants carrying toxic endophytes in heavily grazed pastures. This is due to grazing vertebrates avoiding plants containing the toxic endophytes in favour of those that don’t. This results in a decline in the population of more palatable plants as they are more heavily grazed than others (G. Ridley, Appendix 3; Clay et al 2005, Hume et al 2009).

4.13. Therefore, any increase in host fitness or persistence conferred to ryegrass or meadow fescue as a result of *N. siegelii* infection is likely to be balanced by an increase in palatability and grazing by vertebrates, including cattle, sheep, and rabbits.

4.14. EPA staff consider it unlikely that increasing the drought and pest tolerance of grasses already present in New Zealand will result in grasses expanding their established range into native environments, displacing native plants.

Section 36 (c): whether *Neotyphodium siegelii* is likely to cause any significant adverse effects on human health and safety

4.15. EPA staff have searched the literature, and there are no examples of *N. siegelii* acting as a human pathogen or posing a threat of any kind to human health or safety. We therefore consider that *N. siegelii* is not likely to cause significant adverse effects on human health and safety.

Section 36 (d): whether *Neotyphodium siegelii* is likely to cause any significant adverse effect to New Zealand’s inherent genetic diversity

4.16. Bevan Weir commented in his submission that *N. siegelii* is a hybrid of two *Epichloë* species: *E. festucae* and *E. bromicola*. He noted that there is a possibility that *N. siegelii* could hybridise with indigenous *Neotyphodium* species, and spread into native grasses.

4.17. EPA staff note that the hybrid species described to date, including *N. siegelii*, are *“believed to be the result of very ancient hybridization events (probably having taken place before the last glaciation or beyond). As such, the hybrids represent old and persistent phylogenetic lineages that can be considered distinct species, both taxonomically and genetically, defined as reproductively isolated units”* (A. Leuchtmann[9], pers. coms.).

[9] Prof. Dr. Adrian Leuchtmann, ETH Zurich, Institute of Integrative Biology, Plant Ecological Genetics, Zurich, Switzerland.

4.18. Professor Dr Adrian Leuchtmann notes that there is no information to suggest that *N. siegelii* can hybridize with other endophytes. (pers. comms.) He explains *"hybridizations are assumed to occur by somatic fusion and parasexual processes, which require co-infection of a host plant by two endophytes, and then survival of the host strain".* This is considered to be an extremely rare event.

4.19. As previously noted the only known *Neotyphodium* endophyte endemic to New Zealand is *N. aotearoae*. The natural host for *N. aotearoae* is *Echinopogon ovatus* (Forest hedgehog grass), a woodland perennial grass species endemic to Australia and New Zealand (Miles et al 1997). For *N. siegelii* and *N. aotearoae* to hybridise, both species would need to be present in a single plant. Since *N. siegelii* is not known to infect *E. ovatus*; *N. aotearoae* is not known to infect perennial ryegrass, tall fescue, or meadow fescue; and as the grass species are unlikely to grow in the same habitat, it is not thought possible for *N. aotearoae* and *N. siegelii* to hybridize.

4.20. *Neotyphodium siegelii* is an asexual fungal endophyte, and does not undergo sexual recombination, and there is no evidence to suggest that *N. siegelii*, or any other *Neotyphodium* endophyte is capable of hybridization. We consider that the evolutionary changes required for *N. siegelii* to hybridise with another endophyte would happen over hundreds or thousands of generations, even under positive selective pressure. Therefore, EPA staff consider that there is no possibility that *N. siegelii* would hybridise with an endemic fungus like *N. aotearoae* or any other.

4.21. The introduction of any new organism to New Zealand has the potential to cause harm to New Zealand's genetic diversity. However, the taxonomic classification and characterised biology of *N. siegelii* indicates that this potential is highly unlikely to eventuate. We therefore consider that *N. siegelii* is unlikely to cause any significant adverse effects to New Zealand's inherent genetic diversity.

Section 36 (e): whether *Neotyphodium siegelii* is likely to cause disease, be parasitic, or become a vector for human, animal, or plant disease

4.22. EPA staff have searched the literature, and there are no examples of *N. siegelii* acting as a pathogen, parasite, or vector of human, animal, or plant disease. We therefore consider that *N. siegelii* is not likely to cause disease, be parasitic, or become a vector for human, animal, or plant disease.

Conclusion on the minimum standards

4.23. We consider that *Neotyphodium siegelii* is unlikely to cause significant displacement of other organisms, cause significant deterioration of natural habitats, have any significant adverse effects on human health and safety, or have significant adverse effects on New Zealand's inherent genetic diversity. It is unlikely to cause disease, be parasitic, or become a vector for human, animal, or plant disease.

4.24. We therefore consider that *N. siegelii* meets the minimum standards as stated in the Act.

5. Adverse effects

5.1. The applicant has identified potential adverse effects associated with the release of *N. siegelii* (see pages 10-11 of the application). In particular, the applicant identifies the possible impact of *N. siegelii* on grass grub (*Costelytra zealandica*), an insect pest native to New Zealand.

5.2. *Neotyphodium siegelii* is known to deter feeding and placement of grass grub (Popay et al 2003, Patchett et al 2011), and so it is possible that grass grub may be displaced from pasture grasses. However they will not be displaced tussock and scrub, which is their natural habitat. Additionally, grass grub is a significant agricultural pest species, and currently millions of dollars are spent every year in efforts to control this pest. In their submission, Grasslanz Technology Ltd describes grass grub as a major limiting factor in New Zealand pastures that greatly affects the persistence of the grass.

5.3. DOC commented that the applicant has stated that all insects likely to be affected by *N. siegelii* are introduced species considered only as pests with no benefits to farming or natural ecosystems. They have questioned what specific host testing has been done to support this claim, particularly with respect to New Zealand's endemic grass moth species (family Crambidae), which are known to feed on a range of grass species, including pasture grasses.

5.4. EPA staff acknowledge that no New Zealand-specific host range testing has been conducted for *N. siegelii* in meadow fescue or ryegrass. Research indicates that some species of sod webworms (genus *Crambus*), an invertebrate pasture pest, are deterred by *Neotyphodium* endophytes in tall fescue and perennial ryegrass (Funk et al 1983, Richmond 2007), so it is possible that other related Crambid species may be affected by the introduction of *N. siegelii*. However, the endophytes shown to deter *Crambus* spp., *N. coenophialum* and *N. lolii*, do so through their production of bioactive alkaloids. *Neotyphodium coenophialum* produces ergovaline, peramine, and loline alkaloids, while *N. lolii* produces ergovaline and peramine. As discussed, *N. siegelii* produces only loline alkaloids, similarly to *N. uncinatum*. Since *N. coenophialum*, *N. lolii*, and *N. uncinatum* are all present in New Zealand's pasture grasses, any Crambid or other insect species likely to be affected by *Neotyphodium* alkaloids is already exposed to these compounds.

5.5. Federated Farmers of New Zealand noted in their submission that bees will not be affected by *N. siegelii* or the alkaloids it produces, since honey bees do not collect pollen from ryegrass or other pasture grasses.

5.6. The alkaloids produced by *Neotyphodium* endophytes accumulate in host grass tissues, but do not leak into the environment. Any insect that feeds on infected tissues or depends on insects that feed on the grasses may be potentially affected by these compounds (Professor Dr A. Leuchtmann, pers. comms.).

5.7. However, as previously noted *N. siegelii* produces only loline alkaloids. *Neotyphodium uncinatum*, *N. aotearoae*, and *N. occultans* also produce only loline alkaloids, and have been present in the New Zealand environment for some time. No adverse effects on beneficial or native invertebrates as a result of loline exposure or feeding on these host grasses has been reported.

5.8. A study comparing loline expression in *N. uncinatum* and *N. siegelii* reported that *N. siegelii* showed a slightly lower expression of loline alkaloids in the leaf blades of infected meadow fescue, though the difference was not significant (Zhang et al 2009). This study also demonstrated that these alkaloids accumulate to similar levels in *N. siegelii* to those in *N. uncinatum*.

5.9. Therefore, New Zealand insects are already exposed to loline compounds produced by *N. uncinatum* in meadow fescue in similar concentrations to those produced by *N. siegelii*. EPA staff consider it very unlikely that the introduction of *N. siegelii* will cause any new or significant adverse effects on beneficial or native insect species as a result of feeding.

5.10. EPA staff note that there is a risk that *N. siegelii* may confer such a competitive advantage to pasture grasses through enhanced resistance to drought and insect pests that they become a pest species. However, Professor Dr Adrian Leuchtmann notes that "*all available evidence suggests that the benefits conferred are moderate, and it is unlikely that endophytes [will] turn grass into an invasive species*" (pers. comms.). Wayne Simpson, Forage Improvement, AgResearch agrees with Prof. Dr Leuchtmann, noting there is no evidence that a *Neotyphodium* endophyte could confer host fitness benefits to the extent the host grass become a weed species.

5.11. Furthermore, as discussed in section 4, the host fitness advantages conferred through infection with *N. siegelii* will be balanced with an increase in palatability and grazing by vertebrates, including cattle, sheep, and rabbits. Given the moderate host fitness benefits conferred by this endophyte, and the increased grazing pressure, EPA staff consider it unlikely that *N. siegelii* would cause any pasture grass to become invasive.

5.12. If this application to import and release *N. siegelii* is approved, the organism can be imported, unrestricted. There is a risk that *N. siegelii* may be artificially inoculated into other plant species besides perennial ryegrass where its behaviour *in planta* may differ.

5.13. EPA staff note that to date *N. siegelii* has only been reported to have compatible associations with meadow fescue, tall fescue, and perennial ryegrass, though the possibility exists that future research efforts may try to artificially inoculate other species with *N. siegelii*. However, *Neotyphodium* endophytes are host specific, having evolved in symbiosis with their grass hosts. It is considered very unlikely that *N. siegelii* could form a compatible association with any plant outside of the true grass (Poacae) sub-family Pooidae, which the *Lolium* species belong to, and *N. siegelii* is expected to display greater incompatibility issues with species not closely related to meadow fescue (W. Simpson[10], pers. comms.).

5.14. Professor Dr Adrian Leuchtmann and Wayne Simpson both note that artificial inoculation and association of endophytes can be difficult, and even if they are successful initially they may not be persistent in the long term.

5.15. Therefore, EPA staff consider that it is possible that *N. siegelii* can be inoculated into other hosts besides meadow fescue, perennial ryegrass, or tall fescue, but consider it unlikely that the organism could establish and form stable associations with other hosts.

5.16. EPA staff consider that the introduction of *N. siegelii* in association with pasture grasses is very unlikely to have any adverse effects on beneficial insects. We also consider that *N. siegelii* is very unlikely to confer such host advantages that a pasture grass species could become an invasive pest. Therefore, EPA staff consider that the adverse effects associated with the release of *Neotyphodium siegelii* are **negligible**.

[10] Wayne Simpson, Forage Improvement, AgResearch Palmerston North.

7. Positive effects

7.1. The applicant has identified potential positive effects on the environment, on society and communities, and on the market economy, associated with the release of *N. siegelii* (see pages 11-12 of the application). They consider such effects have the potential to provide significant economic benefits for the New Zealand farmer, through less pasture damage from insects and greater drought resistance. In turn, this will lead to reduced re-sowing costs, improved animal health, and less expenditure on pesticide application.

7.2. The applicant states that "*since other 'safe' grass endophytes were introduced into NZ in the early 1990's they have only been perceived as beneficial to the economy and to the environment*".

7.3. Grasslanz Technology, Beef + Lamb New Zealand, Bevan Weir, and Dairy NZ all noted in their submissions that the use of *N. siegelii* is likely to improve pasture resistance to pests and drought, and bring benefits to New Zealand farmers and the New Zealand economy. In their submission Dairy NZ noted that the importance of perennial ryegrass as a pasture species outweighs meadow fescue and tall fescue, and novel endophyte associations that enhance the tolerance and productivity of ryegrass are likely to be significantly beneficial.

7.4. The New Zealand Plant Breeding and Research Association notes that the use of *N. siegelii* is permitted in Australia, South America, and the US, and believes New Zealand could be at a competitive disadvantage if the import and release of this endophyte is declined. As well as offering benefits to New Zealand farmers, the import and release of *N. siegelii* will allow New Zealand's agricultural researchers the ability to continue their world leading research into the development and use of beneficial endophytes in grass species.

7.5. Beef + Lamb New Zealand note in their submission that they are encouraged by the possibility that the use of *N. siegelii* may reduce or alleviate animal health disorders such as ryegrass staggers and fescue toxicosis.

7.6. Dairy NZ support the use of endophytes such as *N. siegelii* as an alternative to chemical pesticides. In their submission, Grasslanz Technology notes pests like grass grub are a major limiting factor in New Zealand pastures and greatly affects the persistence of the grass. Grasslanz explains that currently the only option for control of this major below ground pest is the chemical Diazinon, which is of limited use and effect. Dairy NZ and Grasslanz both noted in their submissions that currently available grass endophytes show only limited resistance to grass grub, though the recently released GrubOUT U2 endophyte by Cropmark Seeds confers some resistance. Both Dairy NZ and Grasslanz Technology speculate that use of endophytes that confer pest resistance to grass species has the potential to reduce the use of chemical pesticides.

7.7. The EPA is currently evaluating a reassessment of the approval and use of organophosphate and carbamate pesticides in New Zealand, including Diazinon. While this reassessment has not yet been decided, the EPA has recommended a ten-year phase out of the use of Diazinon. If this change is approved, New Zealand farmers will no longer have access to Diazinon to control grass grub. Neotyphodium siegelii association with pasture grasses is a potential pesticide-free way of controlling grass grub.

7.8. EPA staff note that for the stated positive effects from *N. siegelii* to accrue New Zealand farmers will need to be receptive to new ryegrass-endophyte associations, and to plant them in their pastures.

7.9. New Zealand is considered the world leader when it comes to the production and acceptance of cultivars of perennial ryegrass and tall fescue associated with *Neotyphodium* endophytes to enhance pastoral–based agricultural productivity. New endophyte strains contribute at least $200 million each year to the New Zealand economy, and this development is regarded as a major success of New Zealand research (Easton et al 2001). Therefore, EPA staff consider that the positive effects stated by the applicant are likely to accrue.

7.10. Having evaluated the information, we consider that the beneficial effects that can be accredited to the release of *Neotyphodium siegelii* are **non-negligible**.

8. Effects on Māori and their culture and traditions and the principles of the Treaty of Waitangi (Te Tiriti o Waitangi)

Relationship of Māori to the environment

8.1. The potential effects of the import and release of *Neotyphodium siegelii* on the relationship of Māori to the environment have been assessed in accordance sections 6(d) and 8 of the HSNO Act. Under these sections all persons exercising functions, powers, and duties under this Act shall take into account the relationship of Māori and their culture and traditions with their ancestral lands, water, taonga and the principles of the Treaty of Waitangi (te Tiriti o Waitangi).

8.2. In consideration of these functions and duties, this section of the report will provide an overall evaluation of the consultation process with Māori that was undertaken by the applicant and their response to issues that were raised from this. Finally an assessment of the impact this application may have on the principles of te Tiriti o Waitangi has been provided.

Consultation

8.3. The EPA policy on consultation with Māori requires that consultation be undertaken by the applicant in the first instance, and should lead to the effective exchange of information between the applicant and iwi/Māori as appropriate. In addition, another purpose of consultation in this context is to lead to the provision of information to the decision makers to enable them to evaluate risks, costs and benefits and make informed decisions in accordance with their legal duty under the Act.

8.4. To fulfil this requirement the applicant sent out over 300 letters and emails to members of the EPA Māori National Network which outlined the purpose of the application, proposed research they would undertake and made an invitation for parties to make comments/queries. The applicant received three responses which:

- requested a bibliographic reference list and ecological reports;
- queried whether the applicant had the rights to the patent for this organism;
- queried if any testing on native species had been undertaken;
- encouraged more containment trials to investigate if the second generation of grass infected by the endophyte are not toxic to animal/native species through ingestion;
- sought confirmation that *N. siegelii* was not invasive by way of competition to native species, for rot, and toxicity; and
- required certainty that the endophyte was not genetically modified.

8.5. In response the applicant:

- provided the bibliography;
- confirmed that they hold the patent for *N. siegelii*;
- confirmed that the endophyte is not genetically modified;

- explained that the endophyte is only spread by vertical means so it cannot spread to any other plants;
- stated that the endophyte is not a pathogen and does not cause rot; and
- stated that the only alkaloid compound produced by *N. siegelii* (loline) is widely reported to be non-toxic to mammalian species.

8.6. Ngā Kaihautū Tikanga Taiao (the EPA's Māori advisory committee) have also reviewed the application and advised that they will not be making a submission. Their rationale for this is based on three premises: *Neotyphodium siegelii* only produces loline, which protects the plant from insect attack but does not affect animals; the endophyte is asexual so does not produce spores; and the whakapapa of this endophytic fungus have been present in New Zealand for some time. Therefore, this signifies that *Neotyphodium siegelii* does not pose a significant risk so therefore no further comment is needed.

Impact on the principles of the Treaty of Waitangi (Te Tiriti o Waitangi)

8.7. Under section 8 of the Act, all persons exercising powers and functions under the Act are to take into account the principles of the Treaty of Waitangi (te Tiriti o Waitangi).

8.8. In reference to the "principles" of the Treaty of Waitangi, as currently accepted by the Courts and Waitangi Tribunal, they are stated to be that of partnership, participation and protection.

8.9. The principles of partnership and participation refer to the shared obligation on both the Crown and iwi/Maori to act reasonably, honourably and in good faith towards each other to ensure the making of informed decisions on matters affecting the interests of Māori. In fulfilment of these principles, as previously stated, the applicant has completed a consultation program for the application and responded to queries.

8.10. The principle of active protection refers to the Crown's obligation to take positive steps to ensure that Māori interests are protected. Taking into account this principle requires this application to provide sufficient evidence to show that the introduction of *N. siegelii* does not pose a significant risk to native or taonga species, ecosystems and traditional Māori values, practices, health and well-being.

8.11. As outlined in more detail in section 4, staff consider that *N. siegelii* is unlikely to cause significant displacement of other organisms, cause significant deterioration of natural habitats, have any significant adverse effects on human health and safety, or have significant adverse effects on New Zealand's inherent genetic diversity. It is unlikely to cause disease, be parasitic, or become a vector for human, animal, or plant disease. We therefore consider that *N. siegelii* meets the minimum standards as stated in the Act.

8.12. Consequently, it is considered that the application provides sufficient information to take into account the principle of "active protection" and is considered to be consistent with the principles of the Treaty of Waitangi. Given this assessment we anticipate a **minimal** effect on the principles of the Treaty of Waitangi and therefore the level of effect is therefore deemed to be **negligible**.

9. Conclusion on adverse and positive effects

9.1. After completing our risk assessment and reviewing the relevant information, we consider that the adverse effects of releasing *N. siegelii* are negligible and the positive effects are non-negligible. Therefore the positive effects from the import and release of *N. siegelii* outweigh the adverse effects.

10. Recommendation

10.1. After weighing the adverse and positive effects, the EPA recommends that the import and release of *Neotyphodium siegelii* be approved.

Asela Atapattu	Manu Graham	Dr Amy Rowe
Manager	**Senior Advisor**	**Advisor**
New Organsims	**Māori and Policy**	**New Organisms**

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Appendix 1

Submission	Submitter/ organisation	Support/ Oppose	Submitter comments
102778	Grasslanz Technology Ltd	Support	• Members of the *Neotyphodium* genus have been present in New Zealand for over 100 years; • *Neotyphodium siegelii* produces loline alkaloids which may make the grass tolerant to grass grub, but has no impact on animal performance or welfare; • Sheep show no effects of lolines administered at the maximum exposure level; • Grass endophytes currently used show only limited impact on grass grub, a major agronomic pest. Only the U2 endophyte from Cropmark Seeds confers any resistance to grassgrub, but the success of this endophyte in the market is still unknown.
102789	New Zealand Plant Breeding and Research Association	Support	• The importation and release of *N. siegelii* could confer important advantages for some grass types against insect pests and drought; • Endophytes in the *Neotyphodium* genus have proven economic benefits to New Zealand farmers through enhanced pasture productivity and animal performance; • The use of *N. siegelii* has the potential to reduce pesticide use. • It is extremely unlikely that *Neotyphodium* endophytes will transfer to other plants in the natural environment; • *Neotyphodium siegelii* is permitted for use in Australia, South America, and the US, and New Zealand farmers could be at a competitive disadvantage if this application is declined.
102790	Beef + Lamb New Zealand	Support	• B+LNZ is supportive of the application, provided the information presented by the applicant is substantively correct. • They are encouraged by the suggestion that *N. siegelii* may contribute to alleviating animal health disorders associated with toxic alkaloids, improve pasture resistance to pests, and afford drought tolerance.
102793	Federated Farmers of New Zealand	Support	• Endophytes are proven to significantly increase the potential of ryegrass by providing pest deterrence and aiding persistence in dry conditions; • New Zealand is a pioneer of endophyte technology; • The characteristics and behaviour of *N. siegelii* are sufficiently similar to existing endophytes in New Zealand, and no adverse

Submission	Submitter/ organisation	Support/ Oppose	Submitter comments
			effects are likely; • Endophytes cannot transfer between species or plants; • The Bee Industry Group of Federated Farmers is satisfied that there is no risk to bees, non-target insects, or other pollinators by *N. siegelii* because honey bees do not collect pollen from ryegrass plants.
102794	Department of Conservation	Neither support nor oppose	• There is a concern that if the EPA does not receive feedback from experts after consultation, then the default perception is that the organism poses no risk. They recommend that the EPA seeks input from an independent pathologist to provide qualified feedback. • The applicant states that all insects likely to be affected by *N. siegelii* are introduced species considered pests with no benefits to farming or natural ecosystems. What host range testing has been done to support this claim, particularly for New Zealand's endemic *Crambid* pasture moths? • We accept that any affects to endemic *Crambid* species and non-pest species of Porina that live in bog habitats are likely to be minor. • We agree that *N. siegelii* will not displace native endophytes because it cannot spread from infected to uninfected plants. • We agree that *N. siegelii* can only be introduced to the environment by seeding grass seeds hosting the endophyte, restricting its use to arable land. Since the native New Zealand grass grub (*Costelytra zealandica*) habitat is tussock and scrub, we agree most of its habitat will be unaffected. • There is the potential for native species to be displaced by ryegrass or fescue with endophyte due to increased host fitness.
102797	Bevan Weir	Neither support nor oppose	• The positive effects of *Neotyphodium* species in grasses are well documented, and it's likely this species will have positive effects on agriculture. • This organism is a hybrid (*Neotyphodium x siegelii*) between *Epichloë festucae* and *Epichloë bromicola*. • The applicant should address the possibility of *N. siegelii* hybridising with indigenous *Neotyphodium* species. If any such hybrids spread into native grasses this would impact on

Submission	Submitter/ organisation	Support/ Oppose	Submitter comments
			indigenous insect communities; • A specimen should be lodged in a national collection.
102798	Dairy NZ	Support	• We support the development of new means to enhance pasture resistance to drought and pests, and support the use of endophytes as an alternative to chemical pest control. • Current novel endophytes (eg. AR1, AR37, NEA2) and the wild-type endophyte have minimal or no effect on grass grub feeding. *Neotyphodium siegelii* may reduce attack by grass grub on the roots of grasses, and other major insect pests such as Argentine Stem Weevil and African black beetle will also potentially be controlled. • Our understanding is that *N. siegelii* has been successfully introduced into perennial ryegrass and tall fescue where the alkaloid production is the same as in meadow fescue, that is, the production of lolines. In New Zealand perennial ryegrass is an important forage species. • We are supportive of DLF Seeds carrying out research trials to ensure animal safety. • The new endophyte should be enrolled in the National Forage Variety Trials and Forage Value Index, and results of research should be supplied to the Industry Endophyte Committee. • DairyNZ notes the application's reference to *N. siegelii* producing peramine and lolines is factually incorrect, citing the US Patent No. 6,815591 B1 (dated 9 November 2004). *Neotyphodium siegelii* in the non-native host perennial ryegrass produces only loline alkaloids.

Appendix 2

DOC comments on EPA new organism for release application

22 April 2013

Application number: APP201519

Applicant: DLF Seeds Limited

Application purpose: to import and release *Neotyphodium siegelii*, an endophytic fungus that lives within ryegrass, which contributes to ryegrass and fescue persistence by protecting the plants from invertebrate pests and drought.

Thank you for the opportunity to comment on this application. Please note we do **not** wish to be heard at a public hearing in support of our comments.

Assessment of risk to conservation values

DOC'S knowledge limitations

1. Unfortunately the Department does not have internal endophytic capability specifically, and as such we can only offer very limited appraisal and comment to this application. We note the EPA's adverse effects assessment relies solely on the evidence provided by the applicant, references cited within the application, and additional information raised through public engagement, and as such we ask that the Authority takes our knowledge precincts into account when deciding whether suitable assessment has been done on this new organism's potential adverse effects to the environment. There is a concern that if EPA publicly notify such applications but do not receive any feedback from experts the default perception is that the application poses little or no risk. Within this context we recommend the Authority seeks input from an independent pathologist who could provide more qualified feedback than what we can offer. This approach would enable an appropriately informed, objective and independent decision on this application.

Comments on potential adverse effects

2. The applicant advises (in 6.1 of the full application) that the designated endophyte strain of *N. siegelii* is active only against invertebrate pests (specifically, Argentinian stem weevil, black beetle, root aphid, porina and mealy bug). This logic seems to assume that all phytophagous insects on ryegrass are pests, but this is not necessarily the case. New Zealand has a wide range of grass moth species (Crambidae) that feed on a range of grass species, including pasture grasses. In addition we have some rare, non-pest, species of porina (Hepialiade) which occur in more specialized bog habitats. However, it is accepted that any affects to these endemic species are likely to be minor.

3. The applicant states that the *N. siegelii* endophyte may adversely affect the native New Zealand grass grub, *Costelytra zealandica*, by reducing its reproductive performance in areas where this endophyte is used. However, this affect appears reasonably constrained given *N. siegelii* can only be introduced to grassland by seeding ryegrass seeds that host *N. siegelii*, restricting its use to arable land. As the grass grub's natural habitat is tussock and scrub, we agree that most of its habitat will remain unaffected.

4. We agree with the applicant that the *N. siegelii* endophyte will not displace native endophyte species given it cannot spread from infected to uninfected plants. However, even after 160 years ryegrass is unlikely to have reached the full extent of its prospective range, and as such there is potential for native species to still be displaced by ryegrass or fescue with endophyte due to host fitness.

Questions the Authority may wish to seek answers to prior to decision

5. After reading the full application the following area is still not clear:
    a. The applicant states that 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. What specific host testing has been done to support this claim, for example on endemic crambid species?

Comments co-ordinated on behalf of the Department of Conservation by:

Verity Forbes

Technical Advisor (Biosecurity), Science and Technical Group, Wellington

Contributors:

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Chris Green (Technical Advisor – Threats), S & T Group, Hamilton (based in Auckland)

Appendix 3

File Ref: APP201519

To: Amy Rowe

Copy To:

From: Geoff Ridley, Principal Scientist

Date: 24 May 2013

Subject: Science Advice

Evaluation of the science content of Application APP201519

Recommendation

1. I have reviewed the application and the cited literature and it is my opinion that the application is a fair statement and evaluation of the literature and information on the biology of *Neotyphodium* × *siegelii*. There is no indication in the application or scientific literature to indicate that *N.* × *siegelii* would not meet the minimum standards.

Background

2. The applicant APP201519 is to release Neotyphodium × siegelii, an endophytic fungus of meadow fescue and rye grass, into the New Zealand environment without controls.

What is Neotyphodium?

3. *Neotyphodium* is an asexual/anamorphic form-genus[11], i.e. not forming any sexual reproductive propagules (ascospores), thought to have developed from species of *Epichloë*, a sexual or teleomorphic genus, either through the loss of the ability to sexually sporulate or through hybridisation of different *Epichloë* species resulting in the loss of sexual sporulation. *Neotyphodium* are endophytic, living in the tissue of their host plant and spread by vertical transmission. Vertical transmission is where the fungus grows into the tissue of the plant's seed and is dispersed with the seed and not independently of it. Asexual spores are produced in in vitro culture but probably only play a very minor role, if any, in the spread of the organism in nature (Tadych et al. 2007; Leuchtmann in litt. 21 May 2013).

[11] Form-genus: (chiefly mycology and palaeontology) a collective group of (parts of) organisms showing morphological similarities but not necessarily a genetic relationship. (OED)

4. *Neotyphodium* taxa are endophytic symbionts of cool-season grasses and occur in all above ground plant tissues of the host. As noted above these taxa are either the result of the fusion of vegetative hyphae and subsequent fusion of nuclei between two *Epichloë* species or aberrant chromosomal segregation when sexual mating occurs between two *Epichloë* species. The outcome in both cases is a sterile hybrid that can only be transmitted vertically via the host's seeds. The lack of horizontal transmission, i.e. by spores, tends to isolate the hybrid to a particular host and geographic region. Further hybridisation can occur between the hybrids and other *Epichloë* species that inhabit the host thus allowing some genetic change. Further change occurs due to the isolation of the *Neotyphodium* individual and either the accumulation of marginally deleterious mutations (Muller's rachet) or the loss of redundant chromosomes or segments.
5. *Neotyphodium siegelii* conforms to the biology of the genus.

Propagules

6. The applicant states that Neotyphodium endophytes 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. This statement is slightly confused and I interpret the applicant to mean that Neotyphodium spp. do not produce sexual (ascospores) but may produce asexual (conidiospores). The lack of ascospores prevents the spread of the fungus from plant to plant (horizontal transmission). Transmission has to be vertical with the fungus colonising the developing seed of the host plant and be dispersed with the seed. The conidiospores are not frequent in nature and are not thought to play a significant if any role in horizontal transmission (see point 7).
7. Tadych et al. 2007 report the production of conidiospores on the surface of the host leaves by a Neotyphodium sp. They hypothesis that these conidiospores might play a role in horizontal transmissions however the authors have not demonstrated that the conidiospores are a significant pathway of transmission in nature. This point was also noted by Leuchtmann (in litt. 21 May 2013).

Alkaloids

8. *Neotyphodium* spp. produce a large range of alkaloids[12] that can have a deleterious effect on both vertebrates and invertebrates that feed upon the host plant. The focus of the applicant's research is to find *Neotyphodium* spp. that have the alkaloids that affect invertebrates, and thus control pest insects, and do not have the alkaloids that affect mammal, i.e. staggers in cattle and sheep.

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[12] Alkaloid are a class of naturally occurring organic nitrogen-containing bases which have a range of physiological effects on animals e.g. morphine, quinine and nicotine.

9. The species *N.* × *siegelii* has the ability to produce alkaloids that suppress grass grub, an important pasture pest, and also has the ability to colonise perennial ryegrass, tall fescue and meadow fescue which are not readily colonised by the *Neotyphodium* spp already present in New Zealand.

Undesirable self-sustaining population

10. The applicant did not address this question adequately, focussing on the fungus rather than on the dual organism of host/fungus. The question is whether the combination of host and *N.* × *siegelii* will make the host- *N.* × *siegelii* association more invasive than the host without *N.* × *siegelii*.

11. This was addressed under *ease of eradication of an undesirable self-sustaining population* section of the application. It would appear that the trade-off for grass species carrying *N. siegelii* being less palatable to invertebrates makes them more palatable to vertebrates. The consequence is that the desired plants are selectively grazed out of the pasture and struggle to persist, and are replaced by plants with less desirable endophyte partners (Bush et al. 1997; Clay et al. 2005; Koh and Hik 2007) (eg. plants containing *N coenophialum* produce alkaloids which vertebrates avoid therefore these plants increase in the population at the expense of individuals that do not contain *N coenophialum*). This will also occur beyond the pasture system where these palatable plants will be grazed out by pest mammal species, e.g. rabbits and hares.

Conclusion

12. Examining each of the minimum standards (s36):

13. *Cause any significant displacement of any native species within its natural habitat:* There is no evidence to suggest *N.* × *siegelii* will displace any native species within its natural habitat as it is limited to its introduced grass hosts.

14. *Cause any significant deterioration of natural habitats:* There is no evidence that the introduction of *N.* × *siegelii* will increase the invasiveness of its host species and make them more invasive and thus cause a significant deterioration of natural habitats. The evidence however suggests that *N.* × *siegelii* will be more palatable to grazing animals and therefore sought out and selectively grazed, thus reducing its ability to persist in the environment.

15. *Cause any significant adverse effects on human health and safety*: *N.* × *siegelii* is not known to have any effect on human health and safety.

16. *Cause any significant adverse effect to New Zealand's inherent genetic diversity:* *N.* × *siegelii* is a non-sexual reproducing fungus that is confined to the tissue of its host plant and therefore cannot reduce New Zealand's inherent genetic diversity.

17. *Cause disease, be parasitic, or become a vector for human, animal, or plant disease, unless the purpose of that importation or release is to import or release an organism to cause disease, be a parasite, or a vector for disease:* the purpose of this application is to release an organism that does have a detrimental on insects that feed on the host plant.

18. Thus *N.* × *siegelii* does not have any significant effect with respect to the minimum standards.

19. In reviewing this application and the cited literature it is my opinion that it is a fair statement and evaluation of the literature and information on the biology of *Neotyphodium* × *siegelii*.

References

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