

# Delivering quality seed to specification – the USA and NZ novel endophyte experience

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## Abstract

Seed Certification and Seed Testing Schemes provide the rules and guidelines to maintain both varietal genetic purity and physical purity during field production and seed processing. However the Seed Certification agencies are not involved in ensuring that the quality of the endophytic component of seed is maintained. The delivery of quality endophytic seed to end users therefore requires that the commercial seed entity that is producing and marketing a novel endophyte product develops and maintains strict production and quality assurance guidelines to ensure that the benefits of novel endophyte technology are realised. The paper uses the New Zealand (NZ) experience with AR1 endophyte in ryegrass and the USA experience with MaxQ® Jesup tall fescue to highlight the quality control steps required to deliver a quality product to end users.

**Keywords:** novel endophyte, tall fescue, ryegrass, *Neotyphodium*, MaxQ, AR1, seed production

## Introduction

Development of new forage varieties typically involves some genetic improvement for traits such as yield or disease resistance. Commercial seed production of this improved variety in the field focuses upon maintaining the new, unique attributes through cropping histories, field isolation and field inspections by the breeder and official Seed Certifying agencies. After seed harvest, certification standards for purity and germination<sup>1</sup> further guarantee the delivery of a quality seed product to the end user. The end user can be reasonably assured that the commercial seed purchased will deliver the varietal improvement that the breeder intended. Often one seed production harvest may be carried over into subsequent years for commercial sale if adequate germination is maintained or if the seed is blended off with current year's production to produce an end product with acceptable germination. The introduction of novel endophytes into commercial seed production adds a layer of complexity and quality assurance.

In both the USA and NZ, no officially regulated seed production protocol exists to maintain the integrity of grass variety/endophyte combinations. Therefore it is imperative that a commercial seed entity marketing a novel endophyte product develops and maintains strict production and quality assurance guidelines to ensure that the benefits of novel endophyte technology are realised by the end user.

The commercial release of the tall fescue (*Festuca arundinacea*) /novel endophyte (*Neotyphodium coenophialum*) combination cv. Jesup MaxQ®, in the USA represented one of the largest forage improvements by a single product within a species to date. Research results showed dramatic animal performance improvements and none of the plant persistence issues previously experienced with endophyte-free tall fescue varieties. In NZ the

release of a range of forage perennial and hybrid ryegrasses (*Lolium perenne* and *L. boucheanum*) /novel endophyte AR1 (*Neotyphodium lolii*) was similarly a major improvement.

To ensure that the benefits of novel endophytes are realised by the end user, the production and marketing companies of Jesup MaxQ and AR1 ryegrass re-worked their seed production protocols to maximise the survival of the novel endophyte from the seed production phase to pasture planting. Quality control approaches have evolved from those described by Rolston (1993) for the first commercially released novel endophyte (Endosafe™).

## What is Quality?

A high quality endophytic seed product can be defined as having the following attributes:

- High level of endophyte viability (high proportion of seeds infected with viable endophyte).
- An alkaloid profile that is true-to-type.
- A genetically verifiable endophyte selection that can be identified using simple sequence repeats (SSR) or other technology.

## What affects endophyte viability

Endophyte viability generally declines faster than seed viability, leaving seeds that can germinate but without endophyte. The main factors resulting in seed viability loss are storage temperature and seed moisture content (SMC) which is in equilibrium with atmospheric moisture as measured by the relative humidity (RH%) (Rolston et al 1986; Welty *et al.* 1987). Endophyte viability is determined using “grow-out” tests that identify endophyte in a recently germinated seedling.

## Alkaloid verification

Novel endophytes were selected and released commercially because they had a specific alkaloid profile (e.g. AR1 produced peramine but did not produce lolitrem or ergovaline). To maintain the selected alkaloid profile and ensure contamination has not occurred requires constant monitoring during each generation of the seed production cycle from the breeder to commercial certified seed. In NZ all ryegrass seed with AR1 endophyte is tested for presence of lolitrem for the AgriQuality National Seed Laboratory by AgResearch using an ELISA analysis (Briggs *et al.* 2007) to confirm that there has been no contamination by lolitrem-producing “wild-type” endophytes. The lolitrem results and viable endophyte results are printed as an endorsement on the Official Seed Testing certificate.

## Genetic verification

As new endophytes enter commercial production, separation and identification on the basis of alkaloid profile may not be adequate. The classical morphological descriptors of type traditionally used to identify and classify endophytes are being replaced by genetic

<sup>1</sup> Not all Seed Certification Schemes have germination standards; e.g. New Zealand Seed Certification Scheme. Consumer protection laws may be a substitute law in some countries.

finger-printing using SSRs (van Zijll de Jong *et al.* 2005). The use of SSRs is now an important tool used by AgResearch to identify the successful incorporation of a novel endophyte into new plant selections and for Intellectual Property (IP) monitoring.

### Production Pathway

In managing quality control of novel endophytes, four phases in the production to end-user pathway are recognised and in each phase quality checks and careful management are required. These phases are: (1) breeding new cultivars-Foundation (Breeders) seed production; (2) commercial seed production; (3) harvest, cleaning and storage management; and (4) retail chain to end-user, the farmer's paddock.

### Breeding new cultivars - Foundation (Breeders) seed production

Traditionally new endophytes have been inoculated into a small number of seedlings to produce between 50 and 100 endophytic plants (Bouton & Easton 2005). Before entering seed production, these plants are verified as having viable endophyte checked for the expected alkaloid profile or genetically finger-printed by SSRs. Often seedlines submitted for inoculation contain wild-type endophytes that need to be killed off before the new selected endophytic strain is introduced. Occasionally "dead" endophyte is dormant rather than dead, and contaminated plants must be identified and removed before seed multiplication occurs. These plants are then grown on for seed using border rows or a larger surrounding area of the cultivar as a pollen source, to ensure that the genetic integrity of the grass cultivar has not been compromised during the inoculation step. Host-endophyte incompatibility may not be immediately apparent. We have observed plants where the endophyte "drops-out" of the host plant months after inoculation or fails to fully colonise reproductive tillers during seed production (Wilson & Easton 1998). Many plant breeders have now moved selected endophytes into their breeding pools enabling selection for compatibility between host and endophyte and avoiding the need for costly inoculations with endophyte at the end of the breeding programme.

### Commercial seed production

In most seed production systems a "generation production system" is used by the Seed Certification agency with four generations of seed grown from the small handful produced by the breeder to the large quantities of seed used by consumers. The following NZ/USA nomenclature is used to describe successive generations (i) Nucleus/breeders; (ii) Breeders/Foundation; (iii) Basic/Registered; (iv) 1<sup>st</sup> Generation/Certified.

Production and distribution points identified as critical include maintaining high levels of novel endophyte in Foundation and Registered seed fields, grower selection, grower training, field selection, fungicide use for disease control, ergot control to avoid ergot alkaloids (tall fescue) and indole-diterpene contamination, endophyte tiller testing, seed moisture at harvest and swathing, farm equipment inspection, cleaning facility inspection, seed field grazing management, seed lot endophyte testing, post-harvest handling and storage, retail bag composition, mid-season retail endophyte testing, and dealer product return management.

### Region and grower selection: the MaxQ approach

Due to climate and soils, the state of Oregon produces virtually all of the cool season grass seed sold in the USA. Pennington Seed is one of the largest seed production contractors in Oregon and uses its extensive contacts with premier seed producers to

recruit potential top quality growers to produce novel endophyte-enhanced tall fescue seed. Once these proven growers of high quality seed are identified and express an interest, they are required to attend orientation and training workshops prior to sowing. Topics covered included endophyte biology, record keeping, equipment cleaning and harvesting guidelines. Due to past experience in seed production of endophyte-free varieties, many growers are partially familiar with endophyte biology and how it relates to seed production. Specialised logbooks are provided to growers to document all aspects of novel endophyte tall fescue production, harvesting, seed conditioning (cleaning), and delivery. Setting and maintaining strict production guidelines and working with quality seed producers from the beginning has protected the value of novel endophyte seed technology and ultimately contributed to the success of the Jesup MaxQ product.

### Field selection and certification requirements for MaxQ

Oregon seed production fields are selected that have a proven history of producing good seed yields. These fields have deep, fertile and moisture retentive soil without weed or other crop contamination, minimal water sheeting or flooding, a good crop rotation history and good drainage. Also, to meet Oregon Seed Certification requirements land must not have grown or been seeded to any tall fescue or ryegrass during the previous 5 years to be eligible to produce Foundation seed. Land must not have grown or been seeded to these grasses during the previous 18 months to produce Registered or Certified seed unless the previous crop was of the same variety and class, and certified. Tall fescue must be planted in distinct rows. Isolation requirements are the minimum distances required between other tall fescue cultivars by a Seed Certification agency to ensure a high level of genetic purity is maintained. Soil samples are also taken to determine buried weed and contaminant crop levels; results from each field are evaluated individually as to impact on the tall fescue seed crop.

### Establishment of Max Q

Time of sowing is critical as late autumn sown tall fescue often does not produce an economically viable seed crop in the first year. Companion cropping is not recommended for tall fescue establishment as tall fescue seedlings are not competitive, and companion crops often delay plant development and reduce first-year seed yields. Weed contamination may also be higher when using a companion crop.

Tiller sampling and testing are used to monitor seed fields' viable endophyte percentage and determine the presence of ergot alkaloid producing contaminants. Pennington Seed maintains the right to downgrade any field with excessive contaminants.

### Disease management

Stem rust (*Puccinia graminis* subspecies *graminicola*) can reduce seed yields by up to half. Labelled fungicides approved for rust control have the potential to affect the viability of the novel endophyte. Fungicide trial plots were established to identify which products, application rates and dates would control stem rust without compromising endophyte viability and/or endophyte storage life. Trials in Oregon and NZ have demonstrated that not all stem rust fungicides are safe to use on tall fescue with novel endophytes. In contrast the ARI endophyte in ryegrass is tolerant to a range of stem rust fungicides (Rolston *et al.* 2002).

Straw shortening plant growth regulators, commonly used by seed growers to increase seed yields were also tested and

appropriate application rates and dates were established. The fungicide and growth regulator data have proven invaluable in developing a seed crop management protocol that does not compromise endophyte development and survival.

### Harvest, cleaning and storage management

Seed moisture content at harvest and swathage is carefully monitored to reduce negative effects on endophyte viability and long term storage properties. Seed movement from field to cleaning facility is expedited to reduce exposure of harvested seed to direct sunlight. Cleaning facilities can be major sources for the introduction of contaminant seed and those facilities used by Pennington either clean Jesup MaxQ as their first tall fescue crop of the season or after several weeks of cleaning perennial ryegrass seed. After cleaning, Jesup MaxQ seed is assigned final lot numbers and sampled and tested for endophyte viability and ergot alkaloid contamination in addition to the standard tests for purity and germination. The time from seed harvest to cleaning and packaging is approximately 4 to 6 weeks.

### Retail chain to farmer's paddock

#### Packaging and retail outlet product management

Most variables that might affect endophyte viability during the seed production phase are largely under production companies' control. Once the packaged seed enters the distribution network, factors negatively affecting endophyte viability are not as easy to control. It is well known that high temperatures and relative humidity will rapidly degrade endophyte viability within ryegrass tall fescue seed (Rolston *et al.* 1986; Hare *et al.* 1990; Welty *et al.* 1987). Being able to monitor temperature and relative humidity during shipments of novel endophyte seed will identify the amount of time that seed is exposed to deteriorating conditions and ensure action is taken to reduce problems in future shipments. Also, inferior packaging could result in early demise of the endophyte and compromise product integrity.

Since approximately 85% of Jesup MaxQ seed is fall planted in the USA tall fescue belt within 3 months of harvest, the percent viable endophyte in newly established fields is nearly identical to the seed fields from which the seed originated. However, for spring planted seed, 7 to 9 months elapse before planting, and endophyte viability may have been compromised. A second seedling growout is required to test endophyte viability and identify any seed lots that may have fallen below acceptable levels for a successful spring planting. Nine months after seed harvest at the end of the spring planting season, all seed is removed from distribution centres and seed dealers and returned to Pennington Seeds. Returned seed is no longer sold as a novel endophyte product and the entire process is repeated beginning with the next novel endophyte seed harvest. To economically market novel endophytes it is critical to have variety not stated (VNS) markets for "out-of-specification" seed and a return seed policy that ensures retailers are not left to dispose of this seed.

### Future Issues

Experience in NZ and the USA has demonstrated that unless a comprehensive quality assurance programme is developed to cover all phases from plant breeding, seed production, storage and retailer to the end-user, quality failures in endophyte viability and endophyte genetics will occur. As new endophytes in ryegrass and tall fescue enter the market, increased levels of quality control will be required to avoid contamination.

## Conclusion

1. Unless quality control measures are taken to ensure high levels of viable endophyte are present at every stage of seed production, harvest, and distribution, no claims should be made regarding the benefits of a novel endophyte and plant combination.
2. Production and storage protocols are needed by seed companies to ensure seedlines have high viable endophyte that is true-to-type and not contaminated with a toxic endophyte.
3. The release of a new endophyte to the market without appropriate in-house-controls and a quality management system will result in product failure.
4. Seed companies need to treat seed infected with selected endophyte as a high-value perishable product; an approach that is foreign to most seed companies.

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