Seed Quality Testing for Novel Endophyte Technology

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Abstract

Fungal endophyte (*Epichloë coenophiala* (*Morgan-Jones & W.Gams*)) in Kentucky-31 tall fescue (*Lolium arundinaceum (Schreb.) Darbyshire*) produces ergot alkaloids that adversely affect livestock. Novel endophyte-infected tall fescue cultivars, with agronomic enhancing properties without livestock toxicity, are commercially available but producer perception and adoption of the technology has been apprehensive. Educational programs by the Alliance for Grassland Renewal were developed to promote use of the novel endophyte technology. Standards of endophyte quality in seed for existing and new novel technologies were established and used to educate producers and ancillary professionals in Alliance-sponsored schools. Surveys of the presentation on seed quality were highly favorable and considered "useful." The number of seed lots tested for novel endophyte technology increased steadily once the Alliance-sponsored schools were implemented. Attempts to develop new technologies to provide alternatives to immunochemical testing for novel endophyte technologies are in progress.

Introduction

Livestock production problems associated with Kentucky-31 tall fescue were documented almost as soon as its utilization as a soil conservation practice began in the USA during the 1950's (Pratt and Hayes, 1950). In the early 1970's, a fungal endophyte, of the genus *Epichloë*, was isolated from Kentucky-31 tall fescue and hypothesized to be the causal agent of the livestock syndrome (Bacon et al., 1975). Soon after, livestock grazing studies provided direct evidence that endophytic fungi producing ergot alkaloids were associated with the livestock disorder (Hoveland et al., 1980; Robbins, 1993; Hill et al., 1994). Surveys of pastures found that nearly all tall fescue in the fescue growing region of the US contained this endophyte.A proposed solution to remediating the fescue toxicosis syndrome was to use endophyte-free tall fescue. However, it was soon found that endophyte-free tall fescue resulted in poor plant performance when confronted by drought and insect pest pressure. This led to novel, non-toxic *Epichloë* endophyte strains being identified and inserted into modern tall fescue cultivars (Bouton et al., 2002). These novel endophyte-infected tall fescue cultivars have been available since the early 2000's, but acceptance by producers has been lackluster at best.

The Alliance for Grassland Renewal was formed in 2012 with the goal of promoting the use of novel endophyte tall fescue cultivars. The Alliance members include competing companies interested in both promoting their products and self-regulating testing protocols to maintain their product's integrity. Seed laws in the USA are predicated on the Seed Act of 1939 with modifications to include information for transgenic crops (Torrence, 2006). However, there is no law governing labeling endophyte presence in seed. Therefore, a "Quality Assurance" sub-committee was established within the Alliance with the objectives of establishing: 1) standardized seed testing protocols for novel endophyte-infected commercial seed, 2) testing protocols for new novel endophyte-tall fescue cultivar combinations, and 3) information on endophyte purity and viability in seed to educate producers.

Materials and Methods

Standardized seed testing:

The Quality Assurance sub-committee first aim was to determine which testing protocols would be used for documentation. The committee opted for the immunoblot procedure to test for endophyte presence in seed because it is an accepted international testing standard among seed agencies (Hill et al., 2002) An

ELISA procedure is used to test ergot alkaloid presence in seed because the antibody provides a direct measurement of the toxic component of endophyte-infected tall fescue (Hill et al., 1994; 2000). Immunblot testing of 6-week old seedling plants was used to test for viable endophyte transmission. The committee directed that 100 seeds in each seed lot be tested for endophyte and ergot alkaloids, and 100 seedling plants tested to determine endophyte viability. Threshold levels of 70% viable endophyte and not more than 5% of seeds containing toxic endophyte are permissible in the novel endophyte tall fescue seed. Those seed lots meeting the threshold standards received the Alliance logo to brand the seed as passing the Alliance mandated standards of quality.

***New endophyte-cultivar products*:**

Testing protocols for new combinations of novel endophyte and tall fescue cultivars are predicated upon whether the endophyte is present in existing tall fescue cultivars, or whether the endophyte is unique with no previous commercial history. There are six endophytes currently in commercial tall fescue seed production. Utilizing an existing endophyte in a new cultivar must be tested in at least two field locations with sufficient replication and environmental (years) observations. Response data must include yield, maturity, persistence, endophyte, and alkaloid analyses to document the value-added attributes of the new endophyte-cultivar combination. Once the value-added attributes have been established, breeders, registered, foundation and certified seed must meet all parameters established for standardized seed testing. Cultivars with endophytes that have no previous commercial history must be tested for the same parameters but must also have livestock health and welfare studies undertaken to confirm no adverse effects caused by unknown alkaloids.

***Seed Quality Education*:**

Beginning in 2014 the Alliance organized a series of in-person and virtual schools to educate stakeholders about fescue toxicosis. A section of this program aims to educate stakeholders on how endophyte quality in seed is verified and maintained during the seed production process. The significance of the endophyte on livestock performance, the identity of the toxic agent, the life cycle of the endophyte, and a description of how novel endophyte tall fescue seed lots are tested for presence, viability, and toxicity are reviewed. Microscopic and immunoblot demonstrations of endophyte presence in the plant were set up at the schools for participants to view. Surveys were disseminated to participants to determine the effectiveness of each subject on the program.

Results and Discussion

***Standardized seed testing*:**

Six novel endophyte tall fescue products were commercially available at the time when the Alliance was established. Two additional products were in late-stage development, complete with animal testing, and have since been added to the Alliance list. Since spring 2013, there have been 256 seed lots tested for seed endophyte, viable endophyte, and toxicity in the United States. Of the 256 lots, only three seed lots were rejected because they did not meet the Alliance threshold standards. All three seed lots were rejected within the first year of testing due to contamination by toxic types. The sources of contamination are unknown but were likely associated with remnant toxic seed in the seed production field or cleaning equipment. Thus, seed testing procedures were sufficient to discover lots that did not meet Alliance standards and force changes in production practices to meet the Alliance standards.

New endophyte-cultivar products:

Two new novel endophyte tall fescue products that were scheduled to be released were rejected by the Alliance because of excessive contamination by toxic endophyte in the breeder's seed. The contamination occurred as a result of neglecting endophyte tests of breeder seed fields. This illustrates

the need for vigilant testing during all phases of novel endophyte product development and seed production to maintain product integrity.

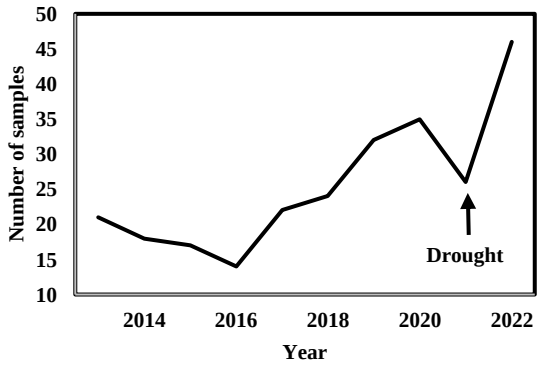

Figure 1. Number of novel endophyte tall fescue products tested (2013-2022).

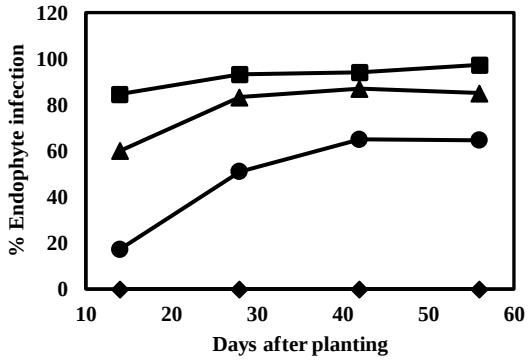

Figure 2. Effect of seedling age on infection rate of three novel endophyte and one E- tall fescue cultivars.

Seed Quality Education:

Survey results from 30 Alliance sponsored schools suggest that those who attended the Alliance schools were highly receptive to the information provided in the presentation on seed quality (Table 1). Over 90% of 497 respondents surveyed said the explanation of seed quality was either extremely or somewhat useful. The number of seed lots tested for quality is an indication of seed demand and sales volume. Following an initial lag in sales, seed sales increased steadily over time once the Alliance-sponsored schools were implemented in 2014 (Figure 1). There was an exception in 2021 when drought conditions prevailed and Oregon seed production experienced significant reductions in seed yield.

Table 1. Survey results on usefulness of the seed quality presentations at Alliance sponsored schools.

	Extremely useful	Somewhat useful	Neutral	Not Useful
Mean	60.3	30.1	3.8	5.0
CI ($P<0.05$)†	5.3	5.0	2.0	0.2

Confidence interval at the 5% level of probability.

Conclusions:

Providing testing protocols to establish quality assurance guidelines for novel endophyte tall fescue products prevented contaminated seed lots from commercialization and, therefore, maintained the integrity of novel endophyte technology. Stakeholders accepted that testing protocols were sufficient in maintaining seed quality which partially explains increased seed sales with time. Seed company feedback indicates that seed is often marketed prior to availability of endophyte viability of data. An effort was made to test seedling plants at earlier stages development, but not all endophytes infect seedling plants at the same rate (Figure 2). This presents risk of having to recall marketed seed. High resolution melt DNA (Johnson and Voisey, 2017) and hyperspectral imaging techniques to detect endophyte viability in juvenile seedling plants are being tested in an effort to reduce the six-week grow out process.

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