From Forest Nursery Notes Winter 2013

161. © Development and evaluation of a real-time PCR seed lot screening method for *Fusarium circinatum*, causal agent of pitch canker disease. Dreaden, T. J., Smith, J. A., Barnard, E. L., and Blakeslee, G. Forest Pathology 42:405-411. 2012.

Forest Pathology WILEY-BLACKWELL

For. Path. 42 (2012) 405–411 doi: 10.1111/j.1439-0329.2012.00774.x

Development and evaluation of a real-time PCR seed lot screening method for *Fusarium circinatum*, causal agent of pitch canker disease

By T. J. Dreaden[1,3], J. A. Smith[1], E. L. Barnard[2] and G. Blakeslee[1]

[1]School of Forest Resources and Conservation, University of Florida, PO Box 110410, Gainesville, FL 32611-0410, USA; [2]Florida Division of Forestry, Division of Plant Industry, Florida Division of Agricultural and Consumer Services, Gainesville, FL 32608, USA;
[3]E-mail: tdreaden@ufl.edu (for correspondence)

Summary

Fusarium circinatum is a serious pathogen of *Pinus* spp. worldwide, causing pitch canker disease. *F. circinatum* can contaminate seeds both internally and externally and is readily disseminated via contaminated seed. Many countries require screening of pine seeds for *F. circinatum* before they can be imported. The currently accepted screening method is based on culturing the pathogen on a semi-selective medium and identifying it using morphological traits. This method is time-consuming and does not allow for accurate identification of the pathogen to the species level. A bulk DNA extraction and real-time PCR procedure to screen seeds for the presence of *F. circinatum* were developed in this study. The real-time PCR method resulted in the detection of *F. circinatum* in 5 of 6 commercial seed lots tested and has a lower detection limit of 1×10^{-5} ng of *F. circinatum* DNA per PCR. The culture-based method detected *Fusarium* spp. in four of six of the same seed lots. The real-time PCR method can be used to screen multiple seed lots in 2 days, whereas the culture-based method requires a minimum of 1–2 weeks. This new real-time PCR seed screening method allows for fast, sensitive and accurate screening and can be adapted to handle larger volumes of seeds.

1 Introduction

Pitch canker, caused by *Fusarium circinatum* Nirenberg & O'Donnell [*F. subglutinans* (Wollenweb and Reinking) Nelson, Toussoun, and Marasas f. sp. *pini* (teleomorph: *Gibberella circinata* Nirenberg and O'Donnell)], is a serious disease of *Pinus* spp. in many parts of the world (Brasier 2008; Stenlid et al. 2011). Because *F. circinatum* is readily transported in and on *Pinus* seeds, many countries require *Pinus* seeds to be screened for the presence of the pathogen before they can be imported (Anonymous 2009). Currently, the International Seed Testing Association (ISTA) seed screening method, referred to as blotter paper method in this paper, as applied at the United States Department of Agriculture Forest Service Resistance Screening Center relies on culturing the pathogen from seed on blotter paper infused with PCNB broth medium and identifying suspect colonies morphologically (Don 2002). This method does not allow for reliable identification of suspect colonies to the species level because observation of coiled hyphae, which are needed to identify colonies as *F. circinatum* (Nirenberg and O'Donnell 1998), is not required by this method and was not always observed during this study. This lack of certainty in identification at species level can result in false-positive results because of misidentification of suspect colonies. This method can also lead to false-negative results because many fungi can grow out of the seeds intermingling with or covering *F. circinatum* colonies. Additionally, this method is time-consuming; thus, quick screening of large numbers of seed lots is difficult because of the 1–2 or more weeks needed for fungal growth and hours of laboratory time needed to screen a seed lot. Because of these limitations, a new method of screening pine seeds for *F. circinatum* is needed that can provide high throughput at low cost with accurate results. Real-time PCR has the potential to improve the seed screening methodology allowing for more rapid, lower cost and more accurate seed screening (Schaad and Frederick 2002).

Two real-time PCR methods for detecting *F. circinatum* have been published. The first, by Schweigkofler et al. (2004), was used to measure the airborne conidia and not tested on seeds. The second method, developed by Ioos et al. (2009), was used to detect *F. circinatum* in seeds using a bio-enrichment step and extracting DNA from a subsample of the seeds; however, this method was not compared to the currently used screening method.

The objectives of this study were to (i) develop an improved real-time PCR screening method for *F. circinatum* in seeds; (ii) compare the detection results with the blotter paper–based method; and (iii) compare the cost and time needed for screening commercial slash pine (*P. elliottii* var. *elliottii*) seed lots with the blotter paper–based method and the new real-time PCR-based method.

2 Materials and methods

2.1 Seed lots

Samples from six commercially available seed lots of *Pinus elliottii* Engelm. var. *elliottii* consisting of approximately 5000 seeds each were used in the study. Six seed lots were used because this is the maximum number that the USDA Forest Service Resistance Screening Center (RSC) can process at one time during commercial screening (J. Bronson, personal Communication). The test seed lots were collected between 2003 and 2007 from three different orchard locations (3 from southern

Received: 28. 2. 2012; accepted: 28. 2. 2012; editor: S. Woodward