Developing a DNA barcoding pipeline for the identification and prevention of invasive plant propagules entering the Port of Savannah, Georgia, USA

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Abstract

Background: Over 90% of global trade involves seaports that can also serve as important points of entry for invasive plant species. The Port of Savannah, is the fourth largest and fastest growing container terminal in the USA. The goal of this research is to increase the speed and reliability in the identification of plant propagules intercepted from shipping containers at seaports. Here, we 1) develop and evaluate the efficacy of a DNA barcoding pipeline to identify plant propagules based on their morphology and genetic identity; 2) identify alternative sources of invasive propagules through the development of a local DNA barcode library; and 3) evaluate the fitness of seeds recovered from shipping containers to determine potential invasiveness.

Results: We collected 5,582 seeds from the intake grills of 331 refrigerated shipping containers coming into the Port of Savannah between Aug. 2015 and Feb. 2016. Preliminary DNA barcoding and germination trials indicate that barcoding tissue from potentially invasive germinates is possible. Based on our DNA barcode library of plants growing on the port – 208 species, we found that 32% were non-native and were not representative of the seeds collected from containers at the port.

Significance: Our results have broad implications for assisting regulatory agencies in the prevention of invasive propagules entering ports. Firstly, it is possible to identify seeds collected from containers based on their DNA barcodes; BLASTn analyses can confirm the identity of Federal Noxious Weeds present at ports. Secondly, ports appear to be a critical, yet cryptic point of entry for invasive species; the percentage of non-native flora at ports may be higher than averages for similarly disturbed areas. Finally, seeds intercepted at ports have the potential to become invasive; preliminary germination trials reveal that introduced seeds are viable.

Introduction

Invasive Species

Invasive species negatively influence a nation through environmental, economic, and agricultural damage. Propagule pressure is an important factor for predicting the potential of an invader to become established (Allendorf & Luikart 2007). This includes the influences of size and frequency of introduction events as well as the geography of the introduction area (Lockwood et al. 2005).

Ports

Places where geography can influence propagule pressure are ports. Since they receive shipments from all over the world, they are potential places of entry for invasive species. Currently US Customs and Border Protection and USDA-APPHIS (Animal and Plant Health Inspection Service) make decisions regarding intercepted plant material.

DNA barcoding

This project focuses on using DNA barcoding to identify seeds intercepted on refrigerated shipping containers entering the Port of Savannah, GA. One of the most important features of DNA barcoding is its ability to provide rapid and accurate identifications of unknown organisms (Hollingsworth et al. 2011). The rbcL + matK gene regions of the chloroplast genome have proven effective in the discernment of plant species (CBOL Plant Working Group et al. 2009). Through the use of DNA barcoding, botanical interceptions can be identified genetically.

Objectives

1. Develop and evaluate the efficacy of a DNA barcoding pipeline to identify propagules based on their morphological vs. genetic identity.
2. Conduct floristic surveys of the local flora to identify alternative sources of invasive propagules through the development of a DNA barcode library.
3. Determine the fitness of seeds recovered from shipping containers.

Methods

1. Shipping containers were sampled within 24 hours upon arrival at the port using backpack vacuums to remove seeds from intake grills on refrigerated containers.

2. Four floristic surveys of the Port of Savannah were conducted between 2015-2017, representing the flora of all four seasons. Vouchers are being used to develop a local DNA barcode library of the flora.

3. Seeds of different morphotypes were selected for germination trials to assess their viability.

Results

During the first collection season, Aug. 2015 - Feb. 2016, 331 containers were sampled, resulting in 5,582 seeds collected. Preliminary DNA barcoding analysis of seedlings have detected the presence of invasive species (ex: Cogongrass, Imperata cylindrical) that are on the Federal Noxious Weed list. Floristic surveys based on morphological identifications indicate that 32% (of 208 species) of the plants present at the port are introduced species. To confirm all morphological identifications, a DNA barcode library is currently being developed for the flora. Preliminary germination trials indicate that seeds of invasive species intercepted from containers are viable.

Discussion

1. It is possible to identify seeds collected from containers based on their DNA barcodes.
2. The Port of Savannah serves as a significant point of entry for invasive species since a relatively high percentage of the flora is composed of introduced species.
3. Seeds intercepted at the Port of Savannah have the potential to become invasive as preliminary germination trials reveal that seeds are viable.

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