



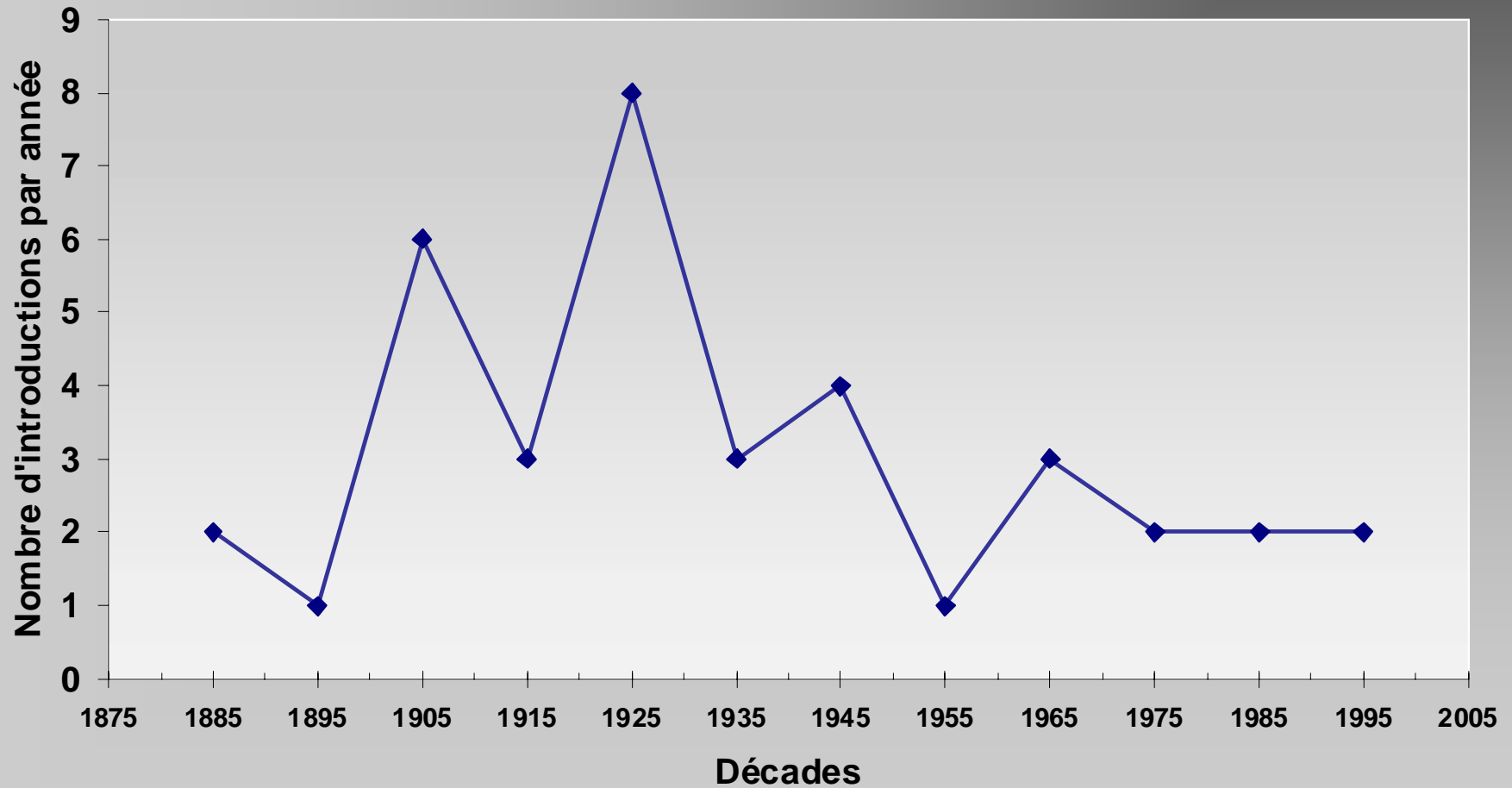
Early Warning System Against Forest Invasive Alien Fungal Species Entering Canada

CFS-CFIA Collaboration



Historic perspective

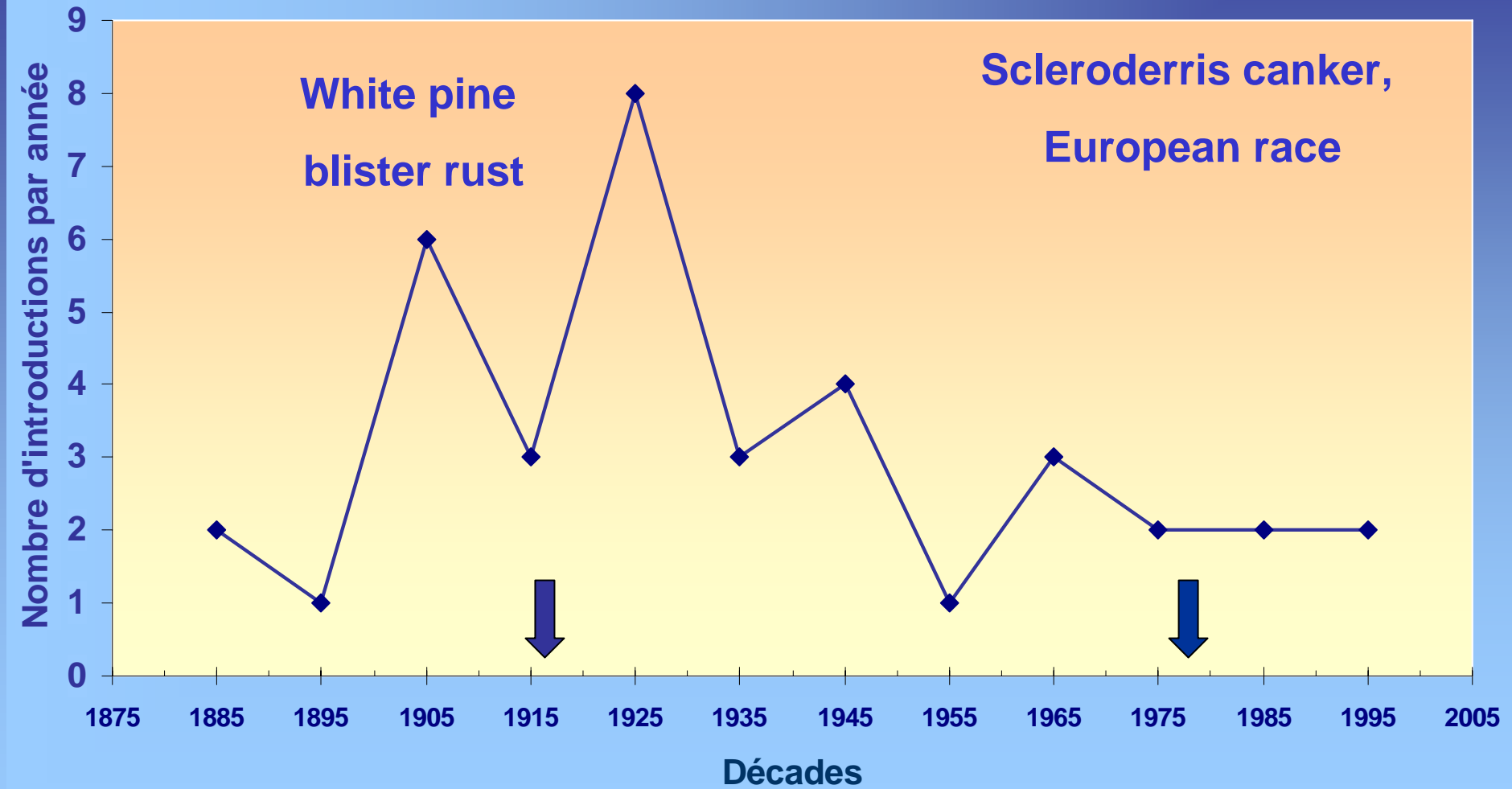
Introductions of exotic forest pests in Canada



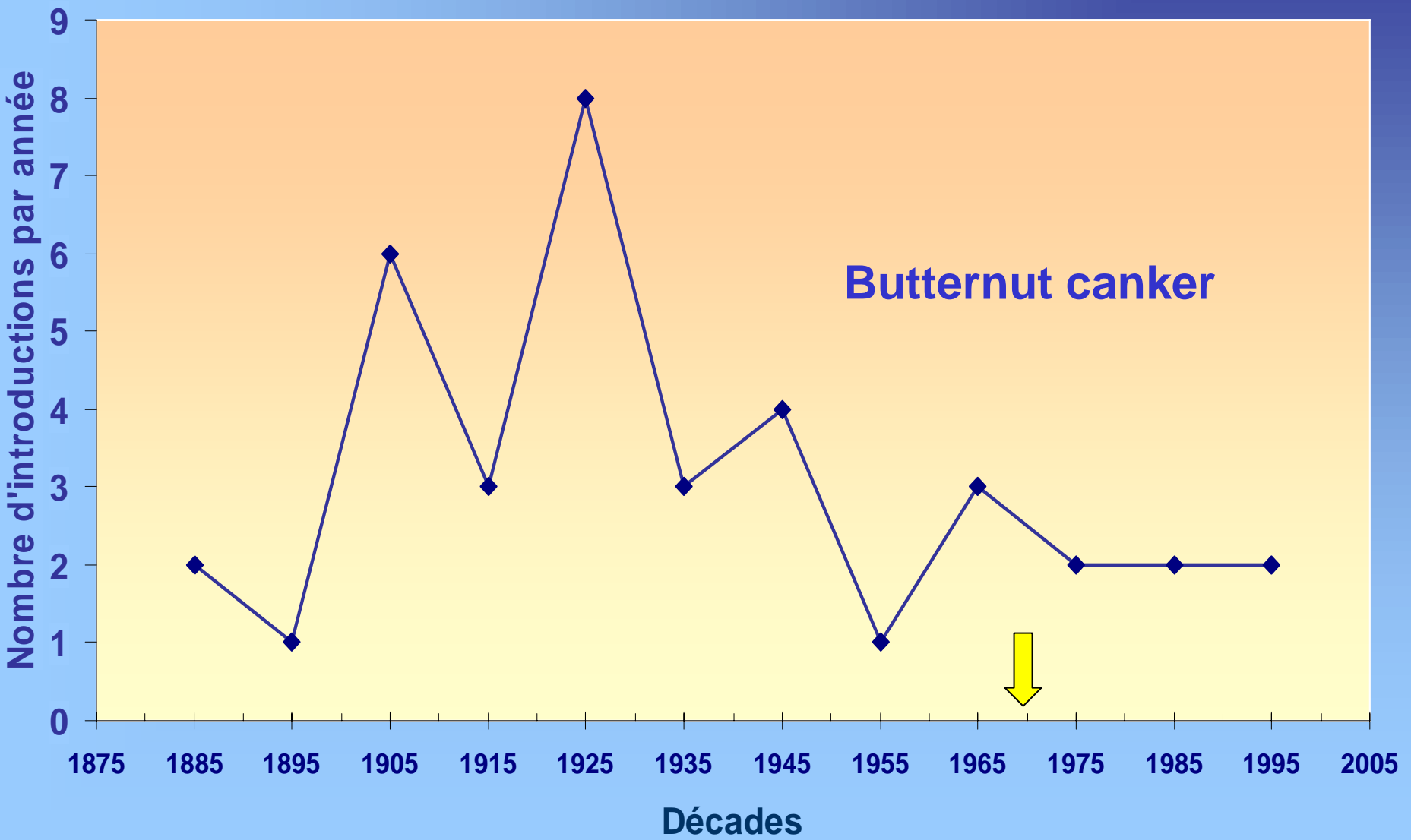
ISPM #15 Standard

- 56C for 30 minutes or fumigation
- Wood dunnage and wood packaging
- Effective for insects and fungal pathogens found in dry wood
 - Dutch elm disease
- Limited efficacy of fumigation on fungi

Introductions of exotic forest pests in Canada



Introductions of exotic forest pests in Canada



Other diseases

- Chestnut blight (*Cryphonectria parasitica*)
- Sudden oak death (*Phytophthora ramorum*)



Introduction pathway

- Live hosts, plants for planting
- Problem: fungi are inconspicuous



Rhizosphaera kahlkoffii
www.ag.ndsu.nodak.edu

Introduction pathway

- Living hosts
- Problem: fungi are 'cloaked'
- Latent phase pathogens
- Asymptomatic pathogens
-endophytes



Introduction pathway

- Living hosts
- not a disease in the country of origin
- not yet a regulated disease in Canada
- you cannot treat living tissues by ISPM # 15
- ISPM #15 is ineffective for this type of material
- Inspectors can not intercept what is not visible

Introduction pathway

- 7 millions containers arriving in Canada each year
- % inspected by CFIA
- Some with live plant material

Probability of new Introductions

- Based on the past 120 years data
- One new devastating forest disease introduction every 20 years on average
- Is likely to increase with increasing imports from China
 - Chinese forests have many genetically related tree species with North America

Project objectives

- Provide CFIA with an early warning (~radar image) of new fungal invasive pathogens
 - introduction frequencies
 - genus and families of these pathogens
 - most affected hosts
 - countries, exporters, points of entry
- Allowing CFIA inspectors to focus their interventions
- Discover new and unknown pathogens to achieve better interceptions

Project objectives

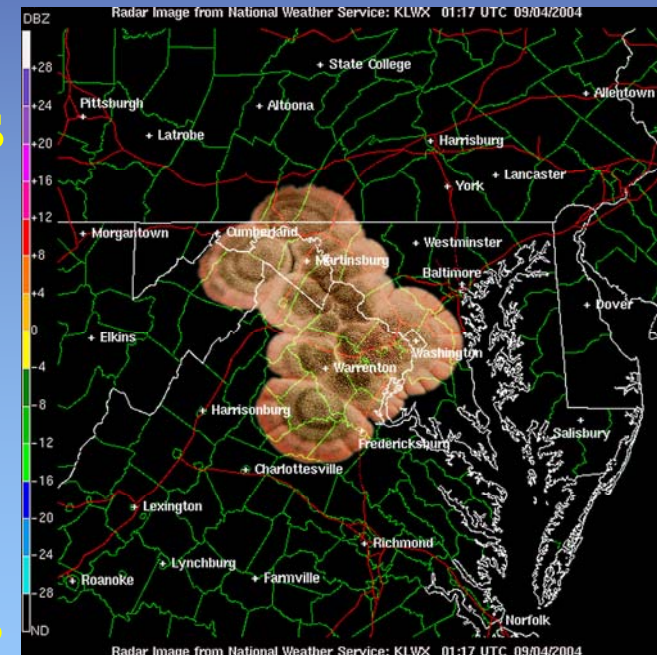


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Project objectives

- Collaboration with CFIA inspectors
- In the context of routine inspections
- Will not substitute the need of CFIA phytosanitary diagnostic labs

Materials and Methods

- Materials
 - leaves/needles, branches, twigs, asymptomatic
 - woody plants
 - no seeds or soil (for the moment)
 - northern hemispheres
- Methods
 - direct DNA cloning, no petri cultures
 - access to ‘cloaked’ fungi
 - ribosomal ITS-LSU region
 - cloning singles out individuals in a DNA mixture
 - cloning yielded new genera, families, classes and orders
- Comparison with Genbank sequences

Materials and Methods

- What are we looking for?
- -clones with very high sequence similarity (98%) to known exotic fungal pathogen species
 - clones with high sequence similarity (94%) with genera having virulent pathogenic species
 - Ophiostoma*, *Sirococcus*, *Phytophthora*, *Nectria*...
 - species not found in Genbank
 - clones with low sequence similarity (85%) to any Genbank entries
 - closest match is an order or class with pathogens

Materials and Methods

- Pilot project in Québec, 2008
 - 60 sample lots
 - lot: samples from one host, of the same origin/shipper
- Canadian projet 2008-2009
 - 400 sample lots
 - N.S., Québec, Ontario, B.C.
- Random but representative sampling of the hundreds live woody material shipments

Field Protocol

- Collect samples
- Fill in CFIA-CFS form
- Put in the envelope
- Mail

Results

- 32 sample lots received, 6 treated
- Buds, bark and wood extracted separately and then PCR amplified
- All amplified DNA mixed together then cloned (single out individual species)
- 48 clones/lot are sent for sequencing

Results

Buds: *Quercus rubra* USA, 7 fungal species

Phoma sojiicola

Phoma herbarum

Microbotryomycetidae sp.

Tremella sp.

Cryptococcus victoriae

Epicoccum nigrum

Taphrina carpini

Common fungi except *Tremella sp.*

Results

Buds: Toba Hawthorn USA, 6 fungal species

A. pullulans

Phoma sojiicola

Cryptococcus victoriae

Tetracladium maxilliformis

Entrophospora sp.

Cryptococcus wieringae

Common fungi

Results

Bark: *Quercus rubra* USA, 9 fungal species

Cladosporium cladosporioides

Cladosporium sphaerospermum

Coniothyrium aleuritis

Phoma glomerata

Phoma herbarum

Epicoccum nigrum

Massarina corticola

Diaporthe viticola

Black spruce foliar endophyte 27.37 Sokolski

Common bark fungi, except the foliar endophyte

Results

- Wood : Weeping birch USA, 3 fungal species

Coniothyrium fuckelii

Cladosporium subtilissimum

Cladosporium cladosporioides

- Common fungi

Results

- Mixed DNA lots
- All three DNAs: 2 *Chaenomeles* sp. and *Kerria japonica* from France
- 12 fungal species, 10 not found in previous lots
- *Aureobasidium pullulans*, *Botrytis fabae*, *Cryptococcus chernovii*, *Davidiella macrospora*, *Gibberella avenacea*
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- *Clathrosphaerina* sp. 89%, *Kabatina thujae* 94%, *Leptosphaeria doliolum* 91%, *Myriosclerotinia ciborium* 94%, *Tetracladium furcatum* 89%
- 5 possibly new species, probably unknown to science, belonging to low-risk genera/orders

Results

- 6 hosts species
 - 30 fungal species
 - 23 genera
 - 14 orders
 - 11 classes, very diverse
 - 3 phyla
-
- Few new species, no known exotic pathogens
 - Unknown taxa belonging to low risk genera/orders
 - By comparison, cloned foliar endophyte from black spruce needles give a large variety of new fungal species and genera
 - Problem: Are new species found by cloning; native, introduced, cosmopolitan?

Outputs

- List of fungal species identified sent to CFIA every 3 month
- Scientific publications and posters
- Pest alert to CFIA if potential exotic pathogens are found

Conclusions

- Early warning system provides a diverse view of which fungal species are introduced in Canada
- Few new species: good news or a bias caused by molecular methods?
- Positive controls will have to be included in the protocol: calibration

Future

- Canada is in a continental continuum with USA
- Need for a continental approach
- APHIS, USDA Forest Service

Acknowledgments

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