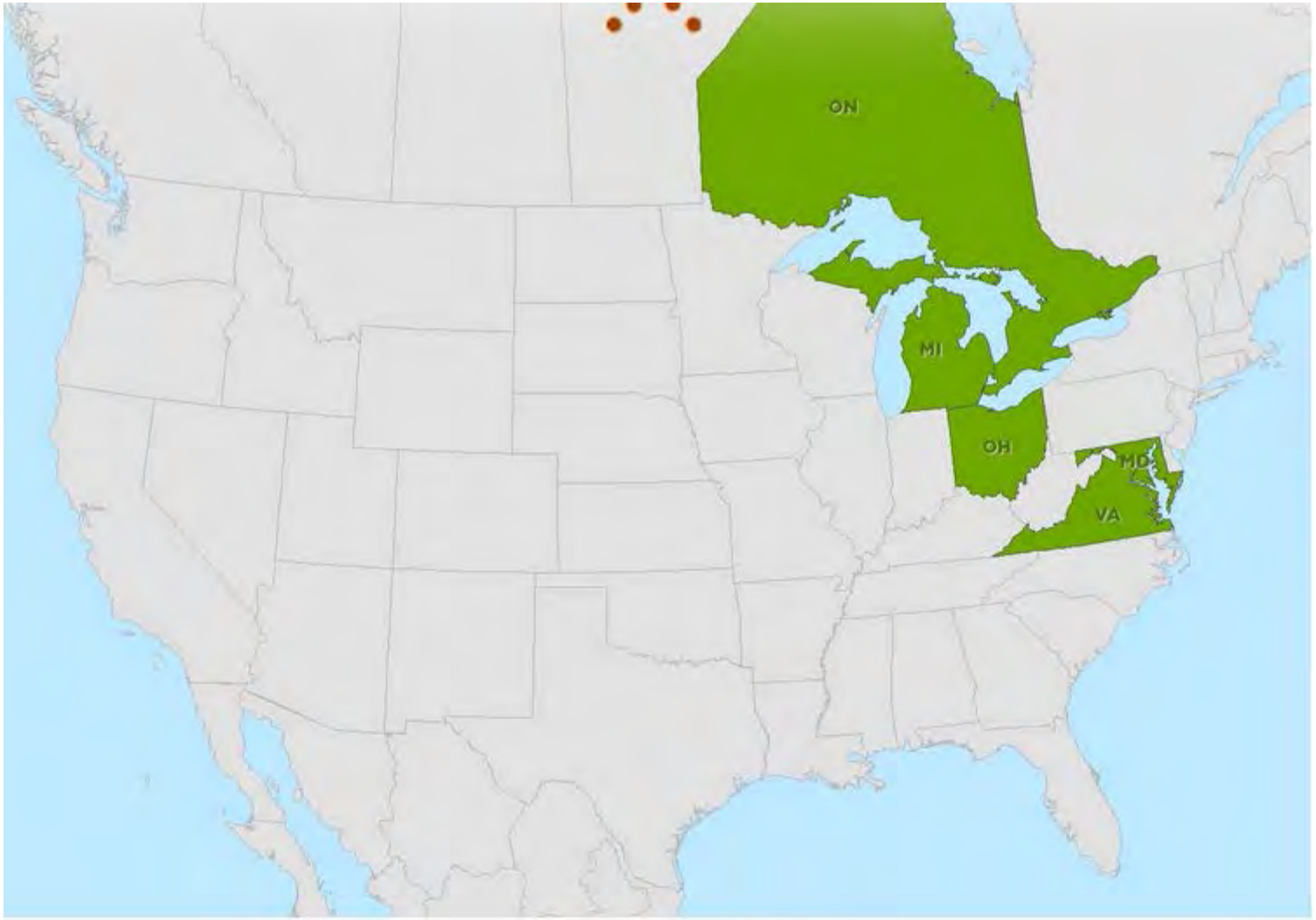
Integration of **Molecular Biotechnologies** for Developing **Insect Resistance** and **Flowering Control** in Black Ash

Oct. 27th 2015

HTIRC Advisory Committee Meeting

Jun Hyung Lee and Paula M. Pijut

2002



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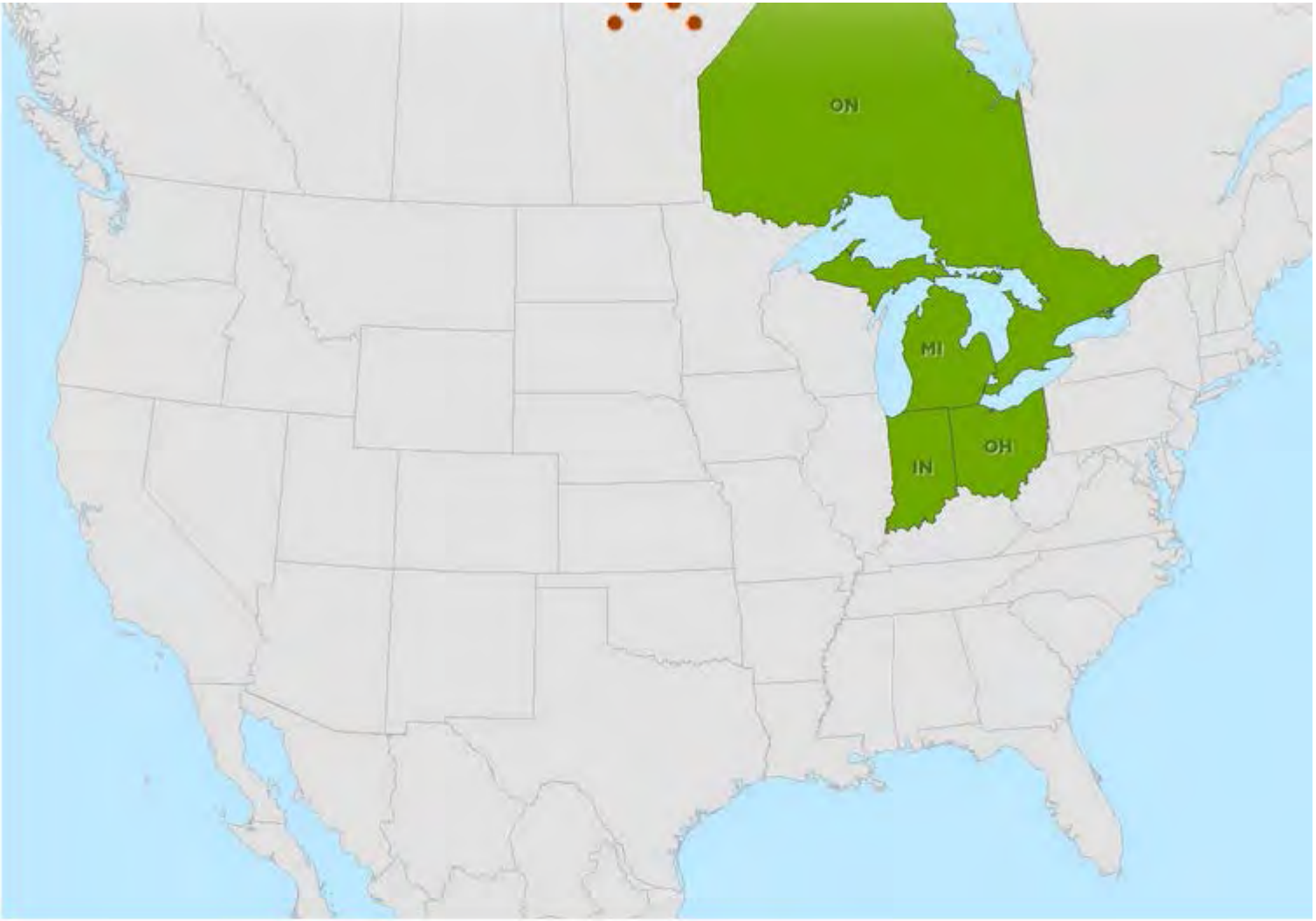

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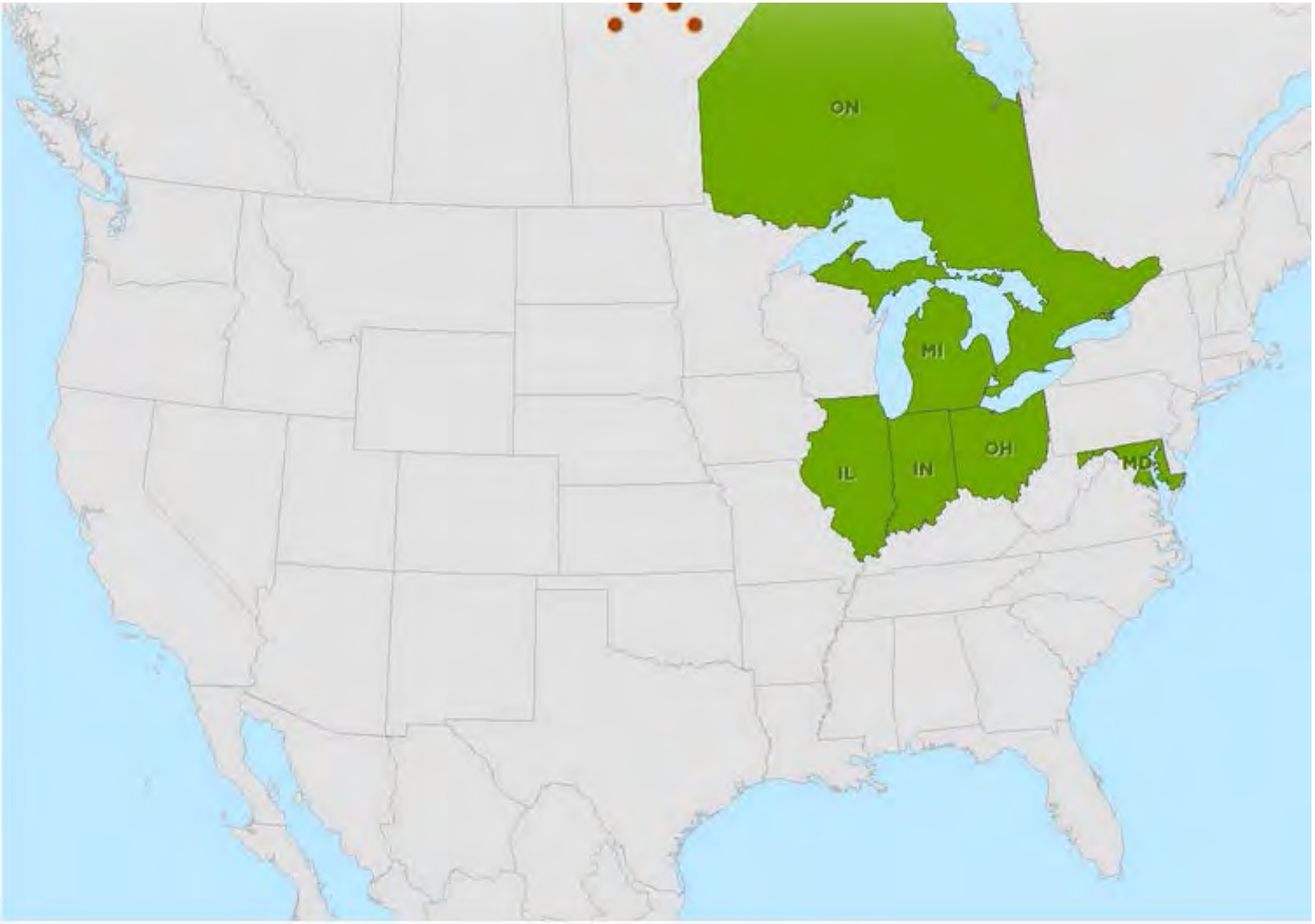

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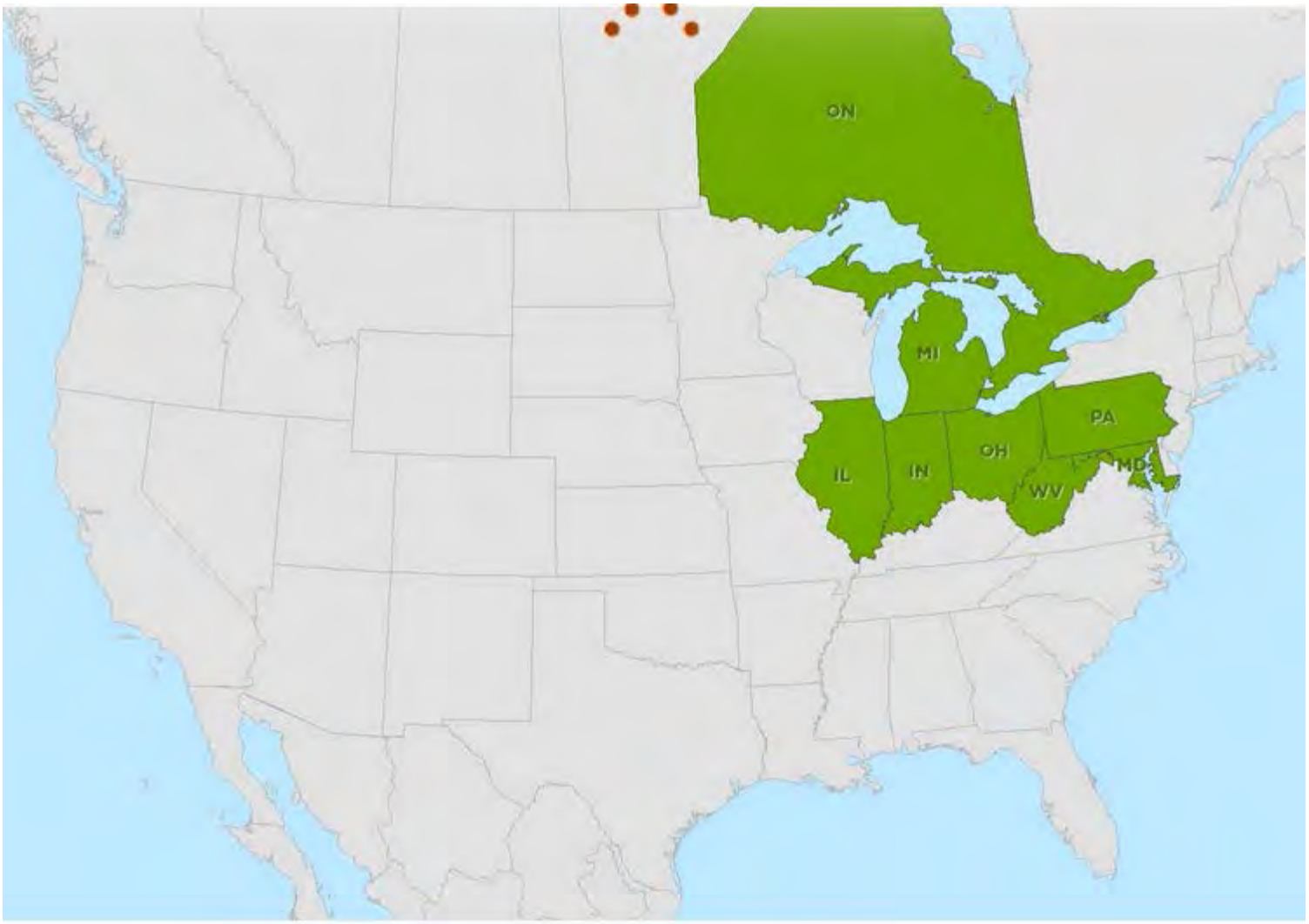

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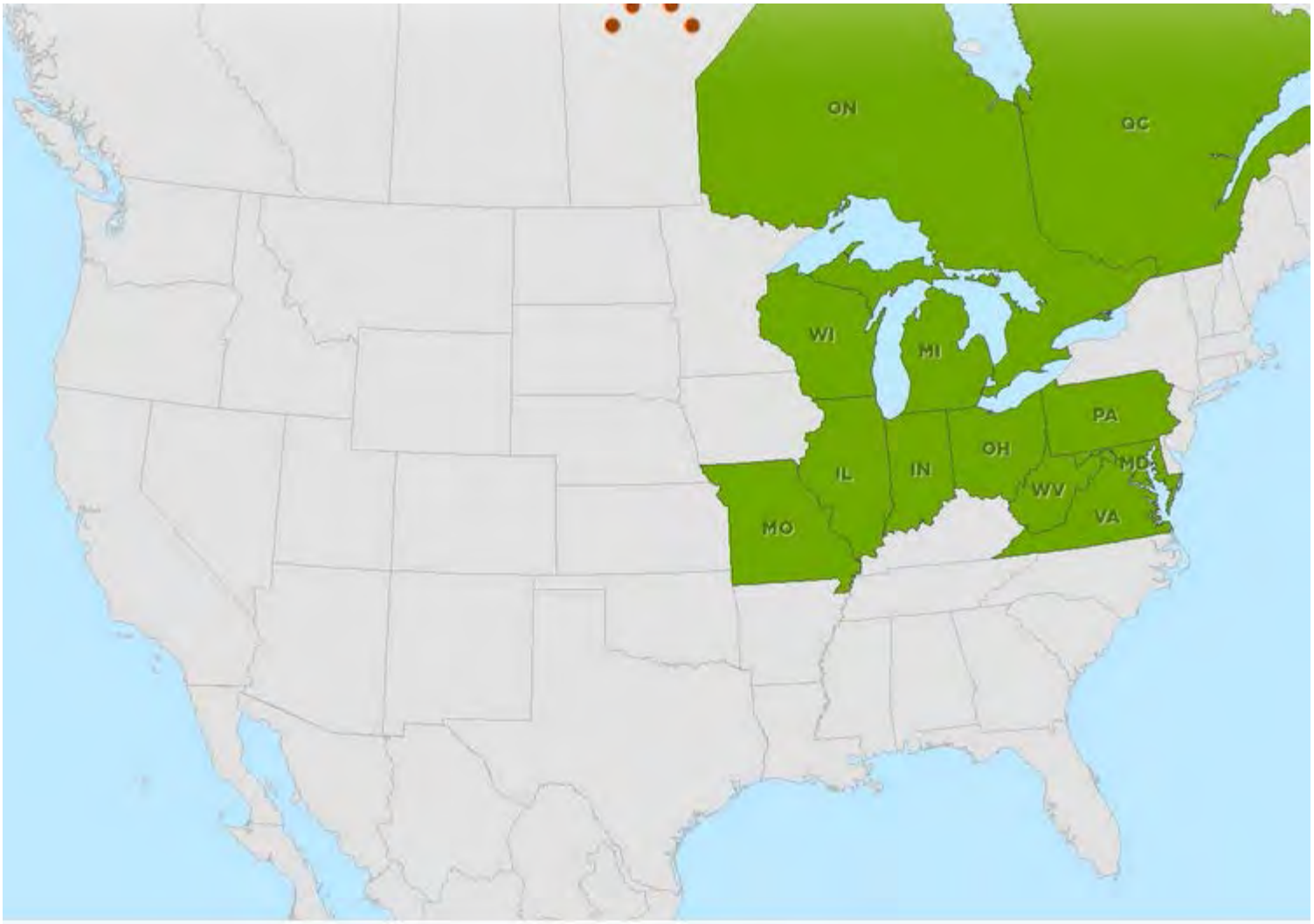

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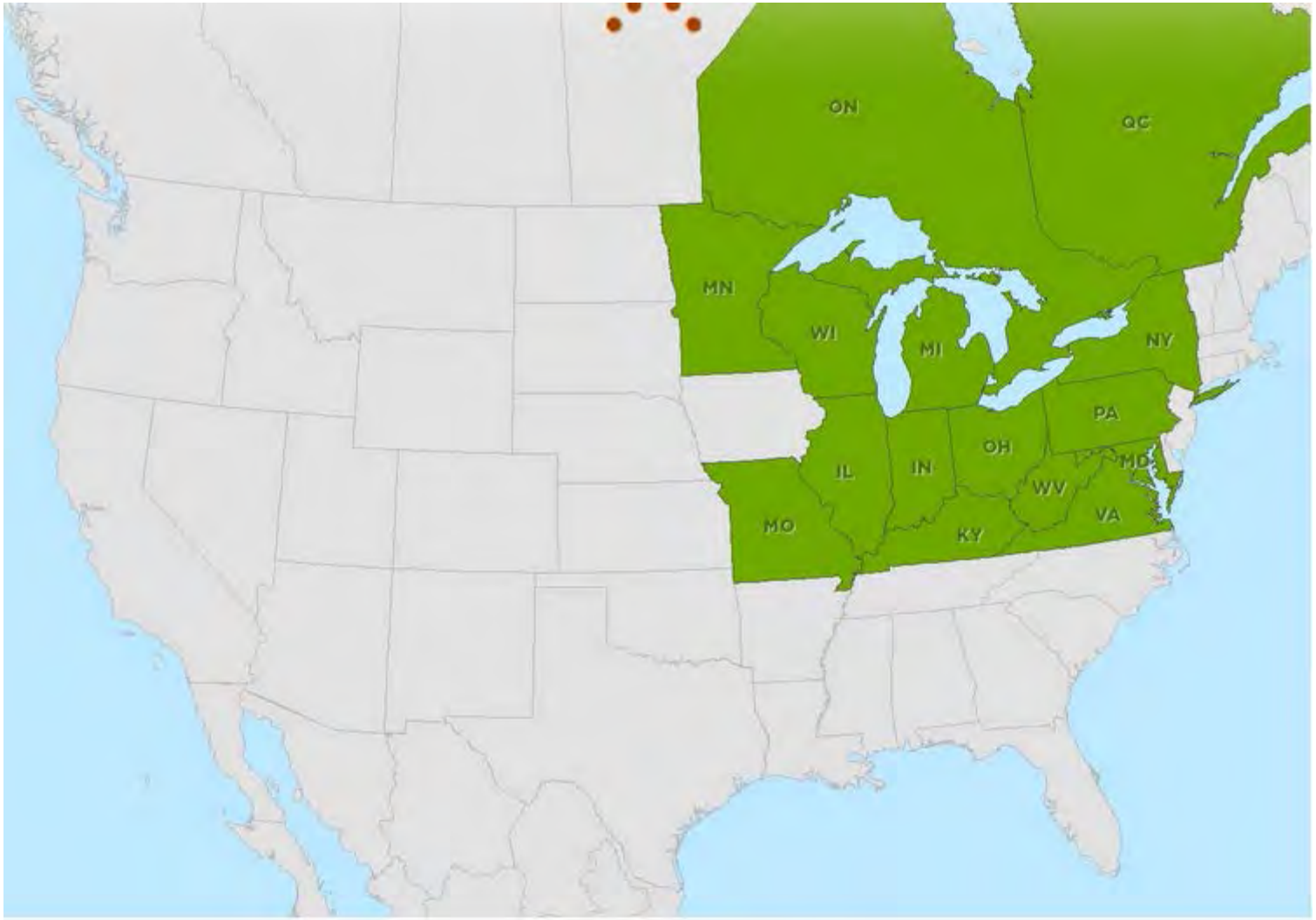

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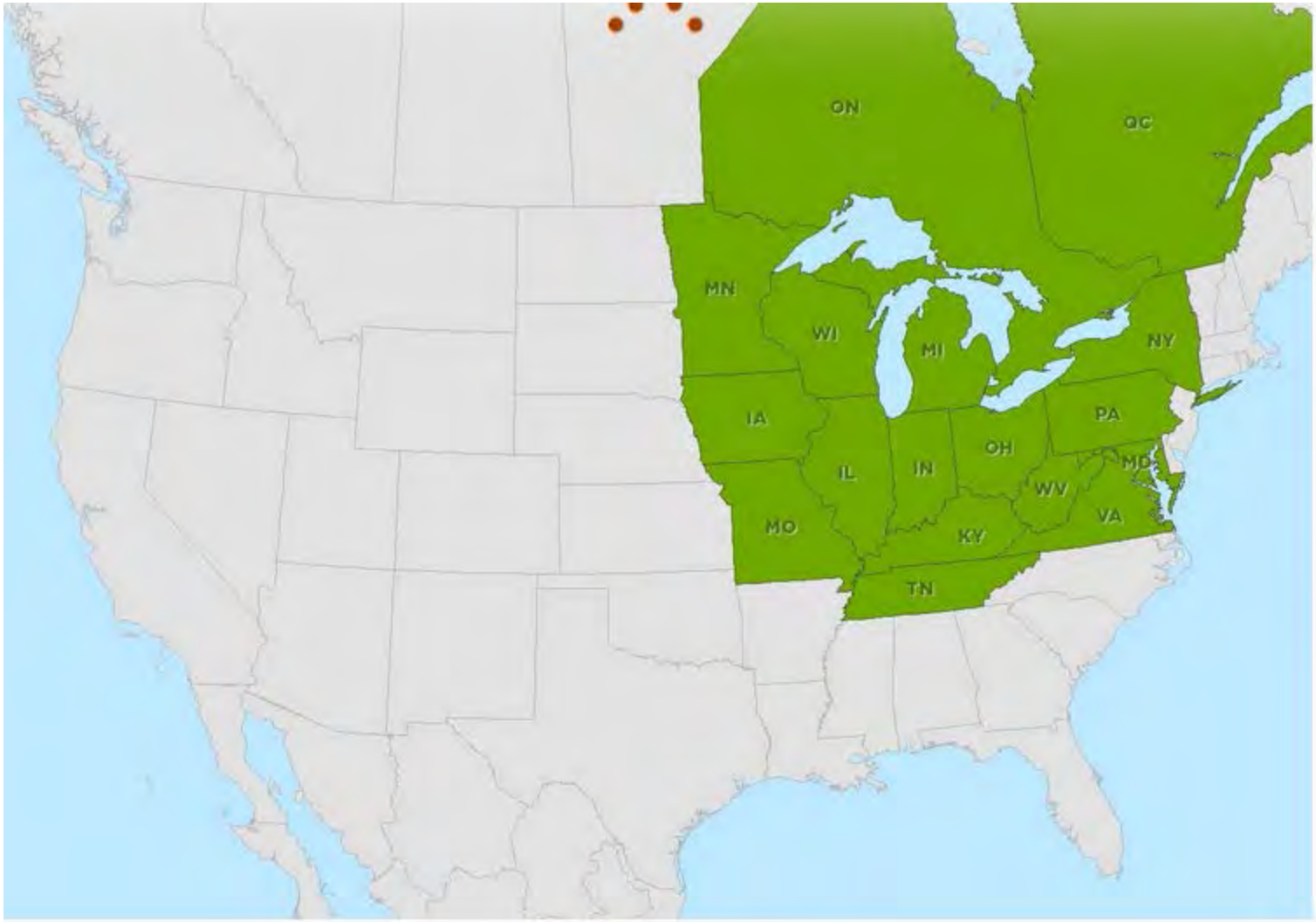

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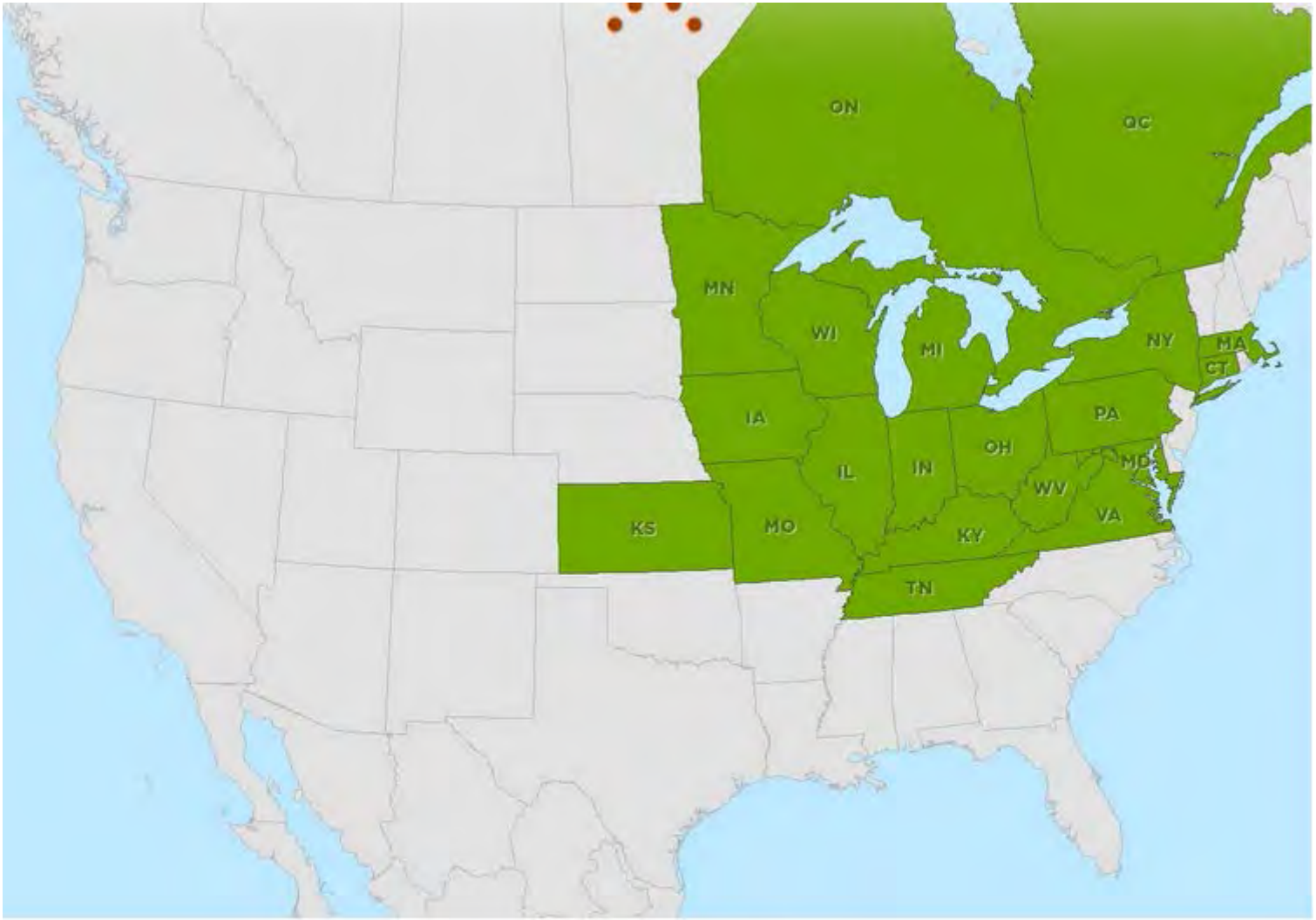

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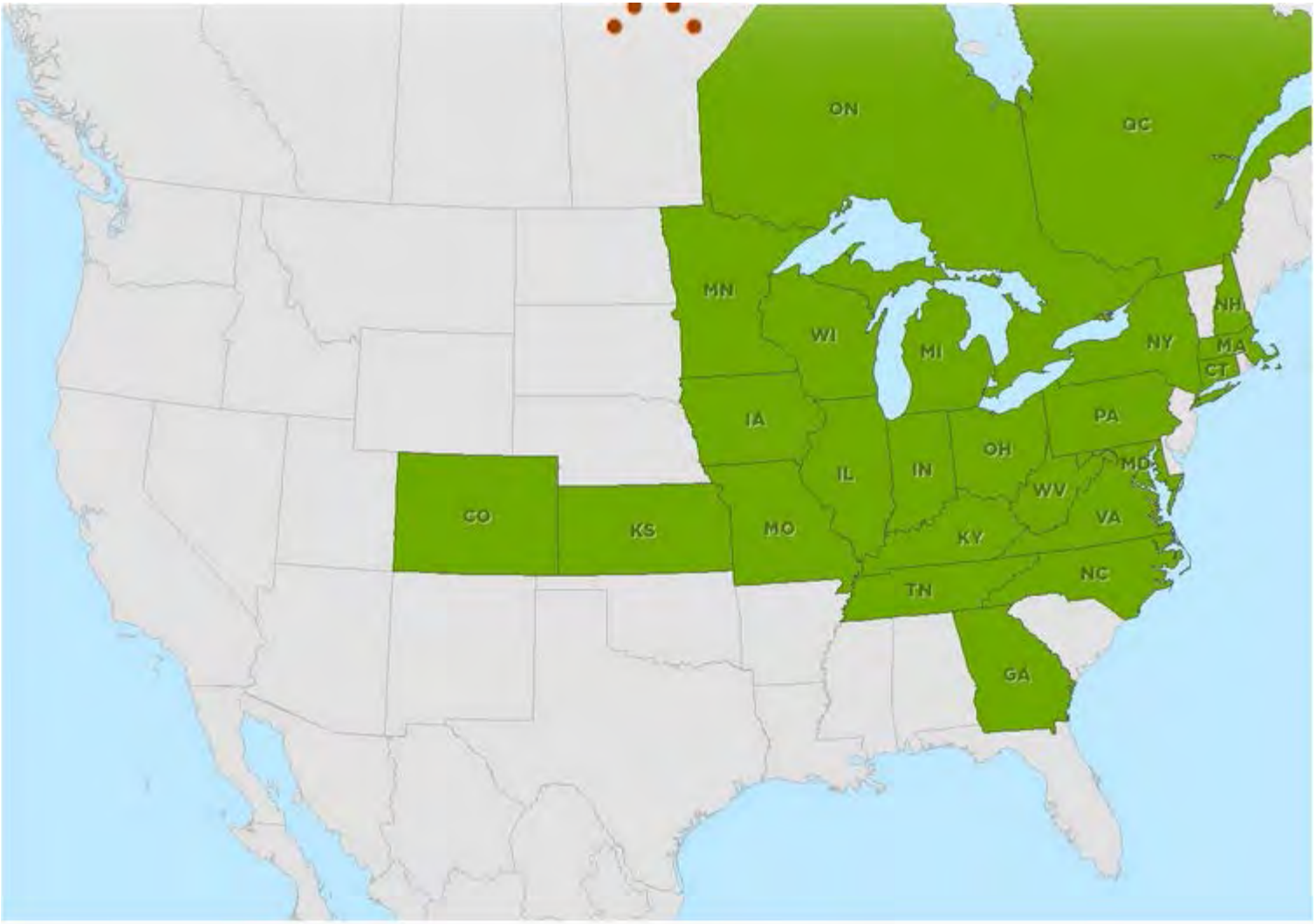

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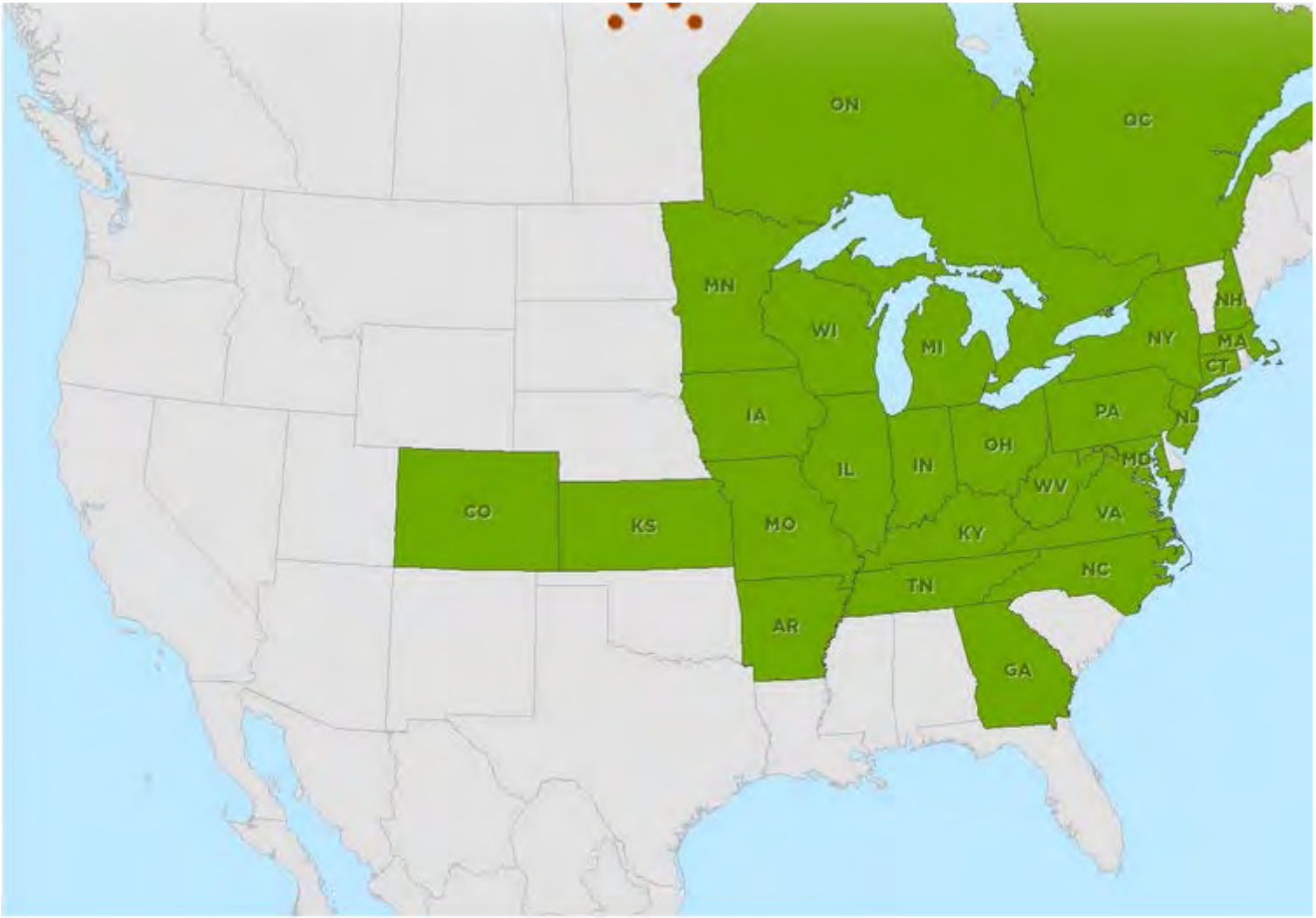
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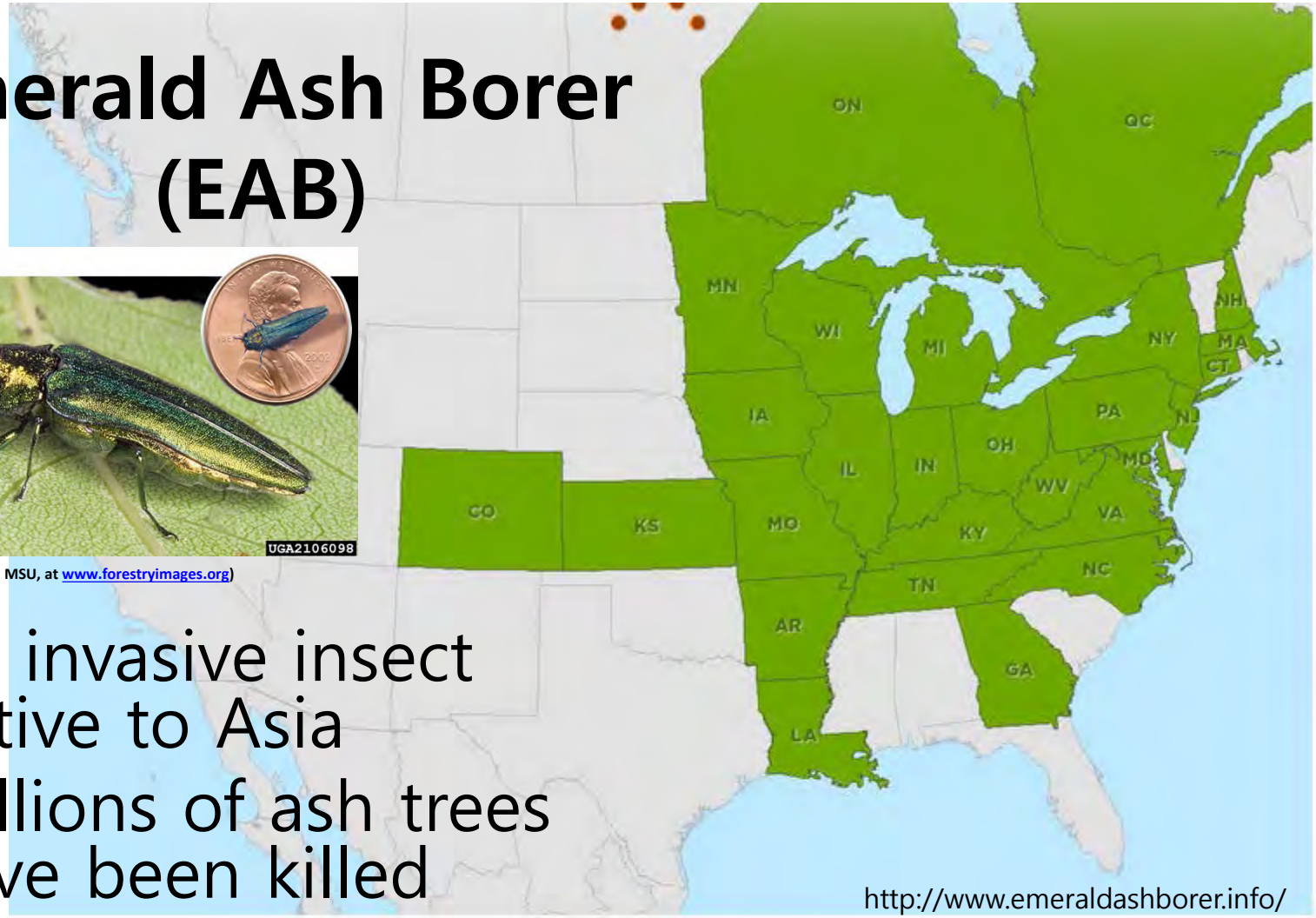
2014

Emerald Ash Borer (EAB)

(D. Cappaert, MSU, at www.forestryimages.org)

- An invasive insect native to Asia
- Millions of ash trees have been killed



http://www.emeraldashborer.info/

2015

http://www.emeraldashborer.info/lifecycle.cfm#sthash.lVLdmgpI.dpbs

http://www.bauermeistertree.com/emerald-ash-borer.html

June 2006

August 2009

Toledo, Ohio

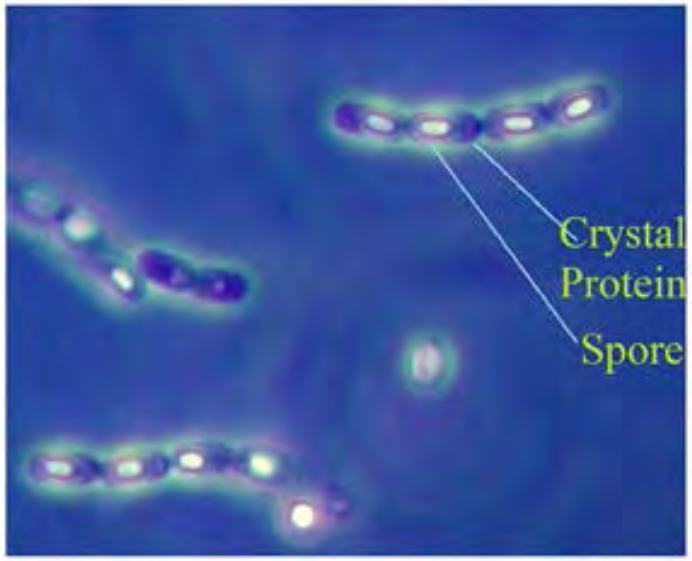
http://www.fs.fed.us/pnw/news/2013/01/tree-human-health.shtml

Objective 1

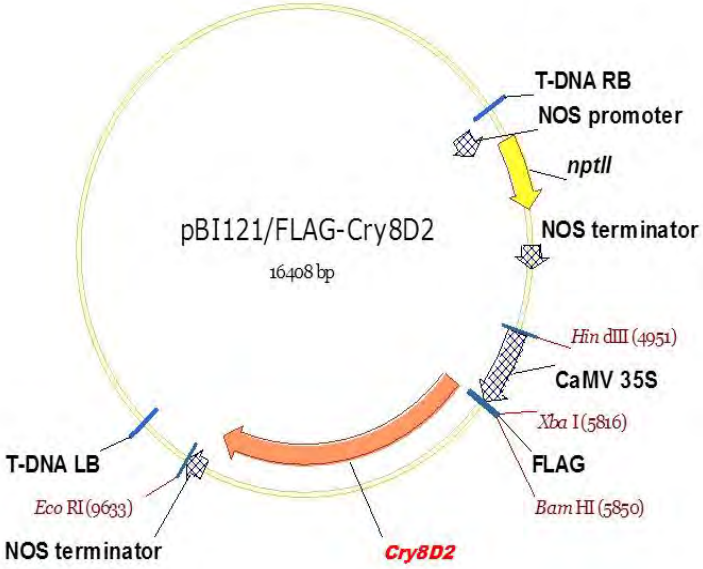
- Develop **genetically modified** black ash trees expressing a *Bt* toxic protein for management of the EAB

Insertion of bacterial gene into black ash

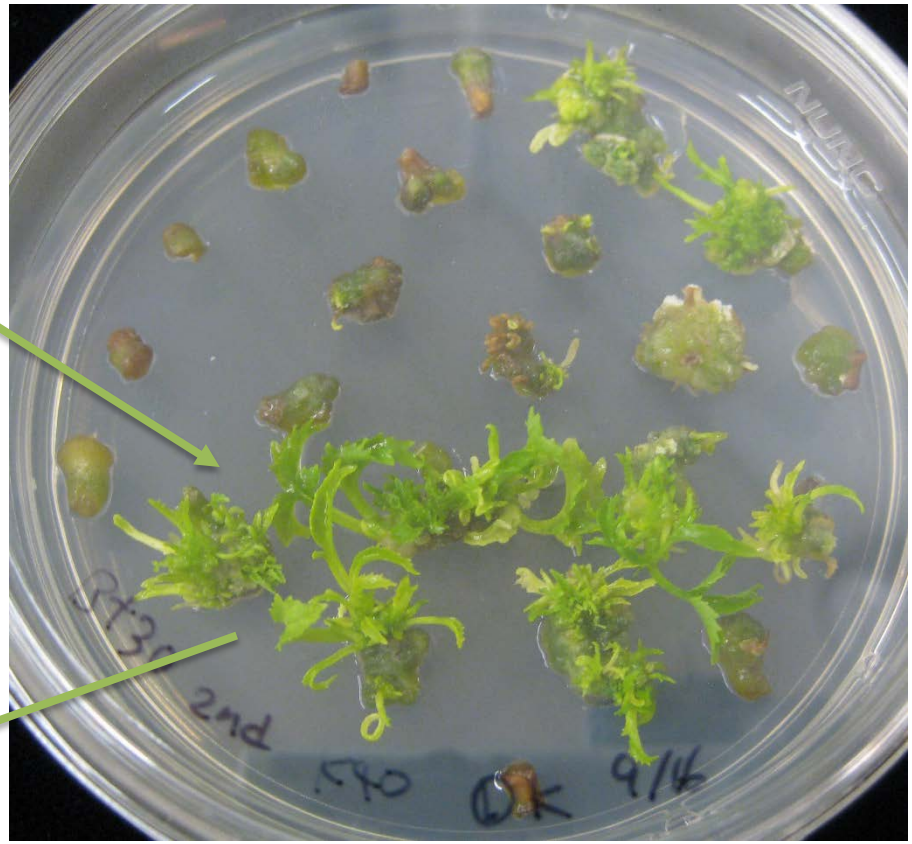
- *Bacillus thuringiensis (Bt)*
  - A naturally occurring bacterium
  - Produces crystal proteins that bind to insect midgut, creating perforations

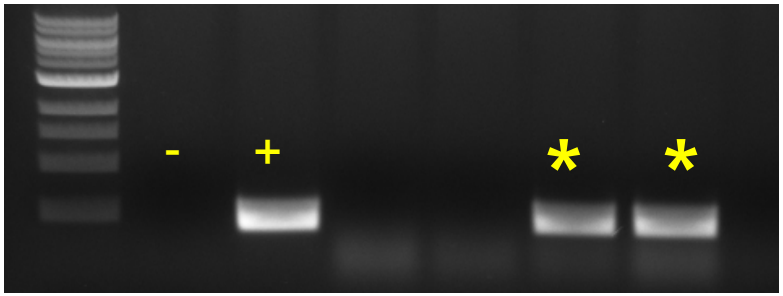


(Shishir et al., 2014)



Regeneration of transformed cells





Rooting, acclimatization, and bioassay

- Regulation on genetically modified trees
  - Forest ‘green’ certification
  - Need gene containment strategies

Objective 2

- Develop **reproductively sterile** black ash trees for gene containment
  - Disrupt gene involved in flower development
  - Reduce the spread of transgene to wild populations through pollen dispersal

A gene involved in flower development

- *AGAMOUS*
  - C-class floral organ identity gene responsible for stamens and carpels.

http://www.adonline.id.au/flowers/floral-identity/

A gene involved in flower development

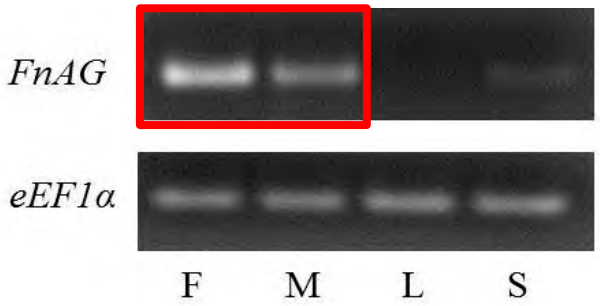
– In flowers of *agamous* mutant plants, stamens and carpels are replaced by petals and a new flower, respectively, resulting in sterility.

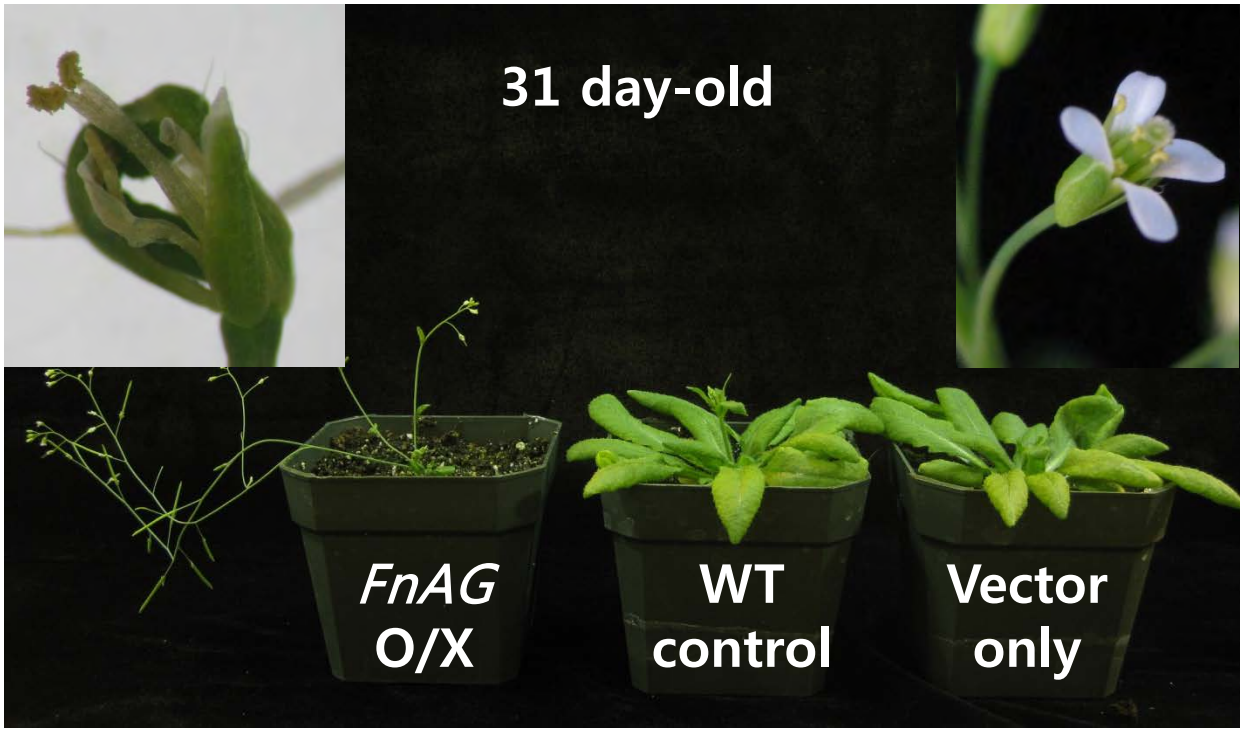
Normal flowers

agamous mutant flower

Cloning an *AGAMOUS* homolog in black ash

- Isolated and sequenced
- Confirmed the gene expression and function

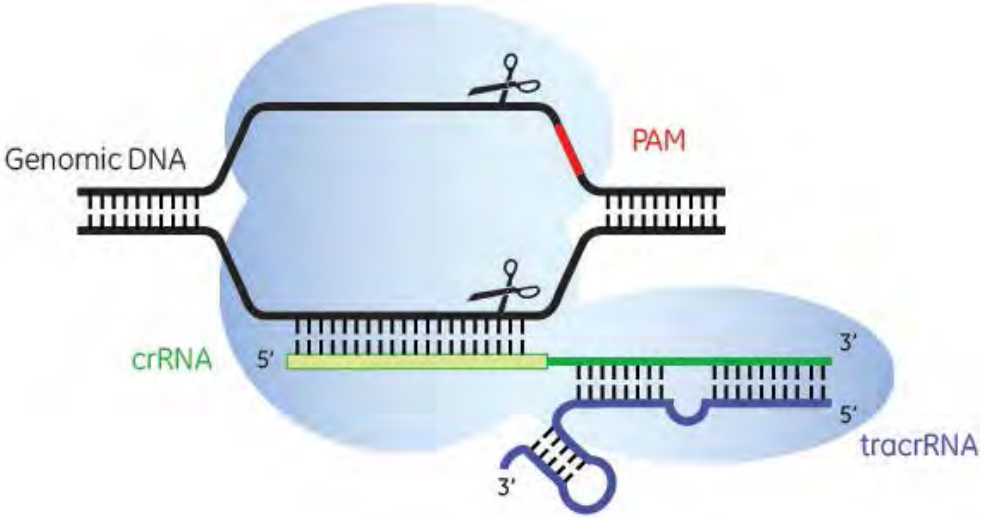




Targeted genome modification

Nature, 2014 March

- CRISPR/Cas9 system
  - Precise targeted genome modification technology
  - Introduce permanent gene knockout

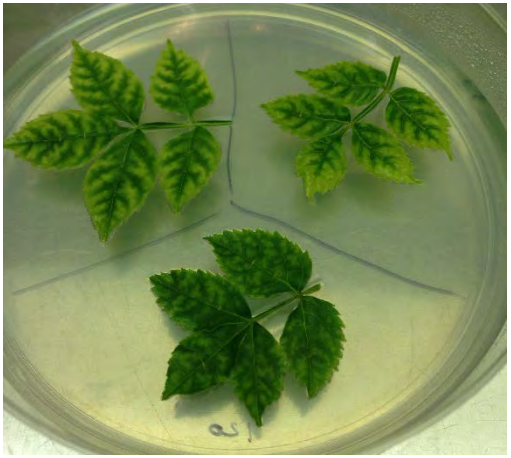


http://dharmacon.gelifesciences.com/applications/gene-editing/

Objective 3

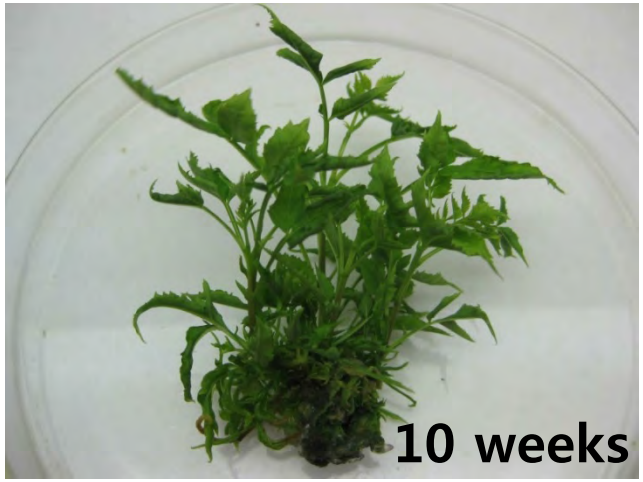
- Develop a **regeneration protocol** using **leaf explants**
  - Prerequisite technique for gene stacking (e.g.) biotic and abiotic stress resistance
  - Recalcitrant

Succeed in regeneration using leaves



4 weeks

7 weeks



10 weeks

Summary

- Using molecular biotechnologies,
  - Introduce genes of interest to improve tree traits,
  - Propagate clones of genetically enhanced tree lines,
  - Precisely modify tree genomes, especially to control flowering and prevent spread of transgenes.

Acknowledgements

- Dr. Pijut's lab.
  - Micah
  - Melissa
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Thanks for your attention!

References

- Riechmann, J.L., Ito, T., and Meyerowitz, E.M. (1999) Non-AUG initiation of *AGAMOUS* mRNA translation in *Arabidopsis thaliana*. Mol. Cell. Biol. 19:8505-8512.
- Shishir, A., Roy, A., Islam, N., Rahman, A., Khan, S.N., and Hoq, M.M. (2014) Abundance and diversity of *Bacillus thuringiensis* in Bangladesh and their *cry* genes profile. Front. Environ. Sci. 2:1-10.