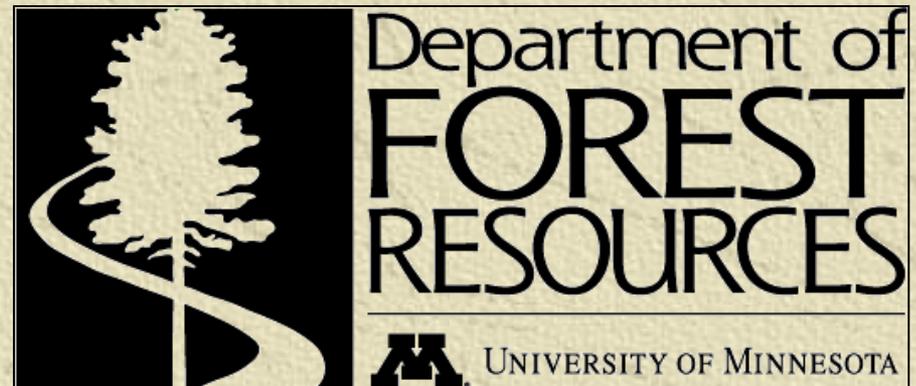


# A Model System for the Genetic Conservation of Ash (*Fraxinus spp.*)

J. D. Zeleznik, A. J. David and J. Hendrickson



# Ash loss in native ecosystems



# Ash loss in urban ecosystems



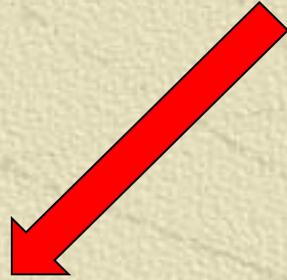
Photo courtesy of the City of Toledo, OH



Photo courtesy of the City of Toledo, OH

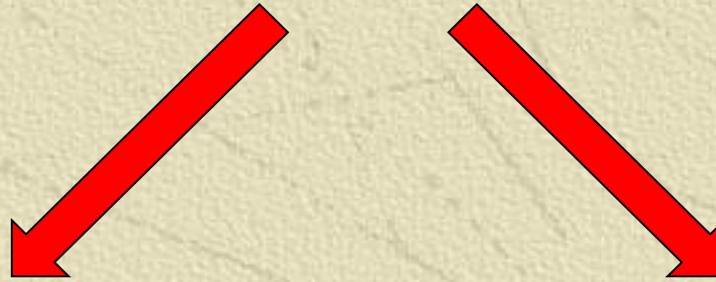
**Save the genetic diversity of ash**

Save the genetic diversity of ash



Insecticide  
injections

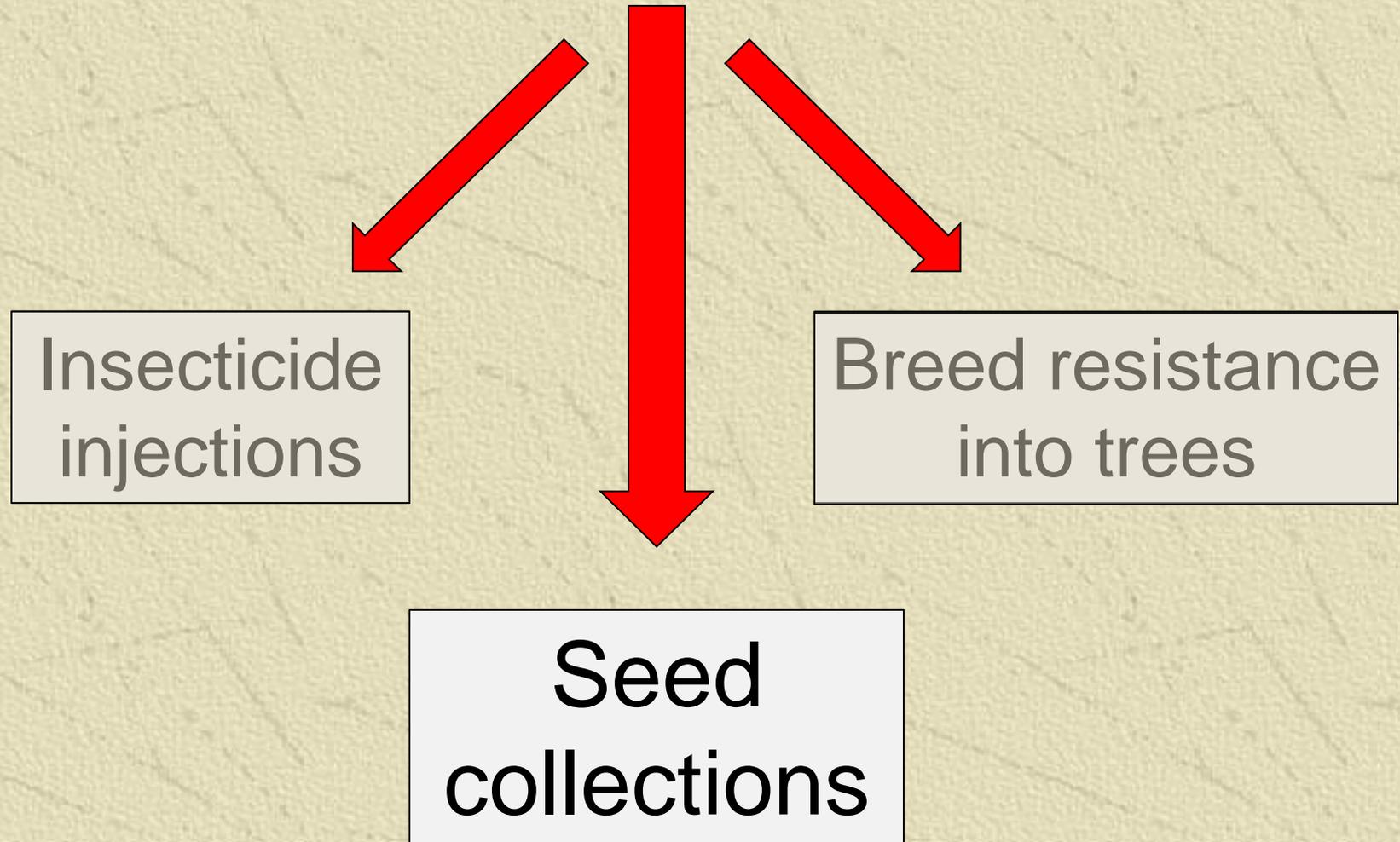
# Save the genetic diversity of ash



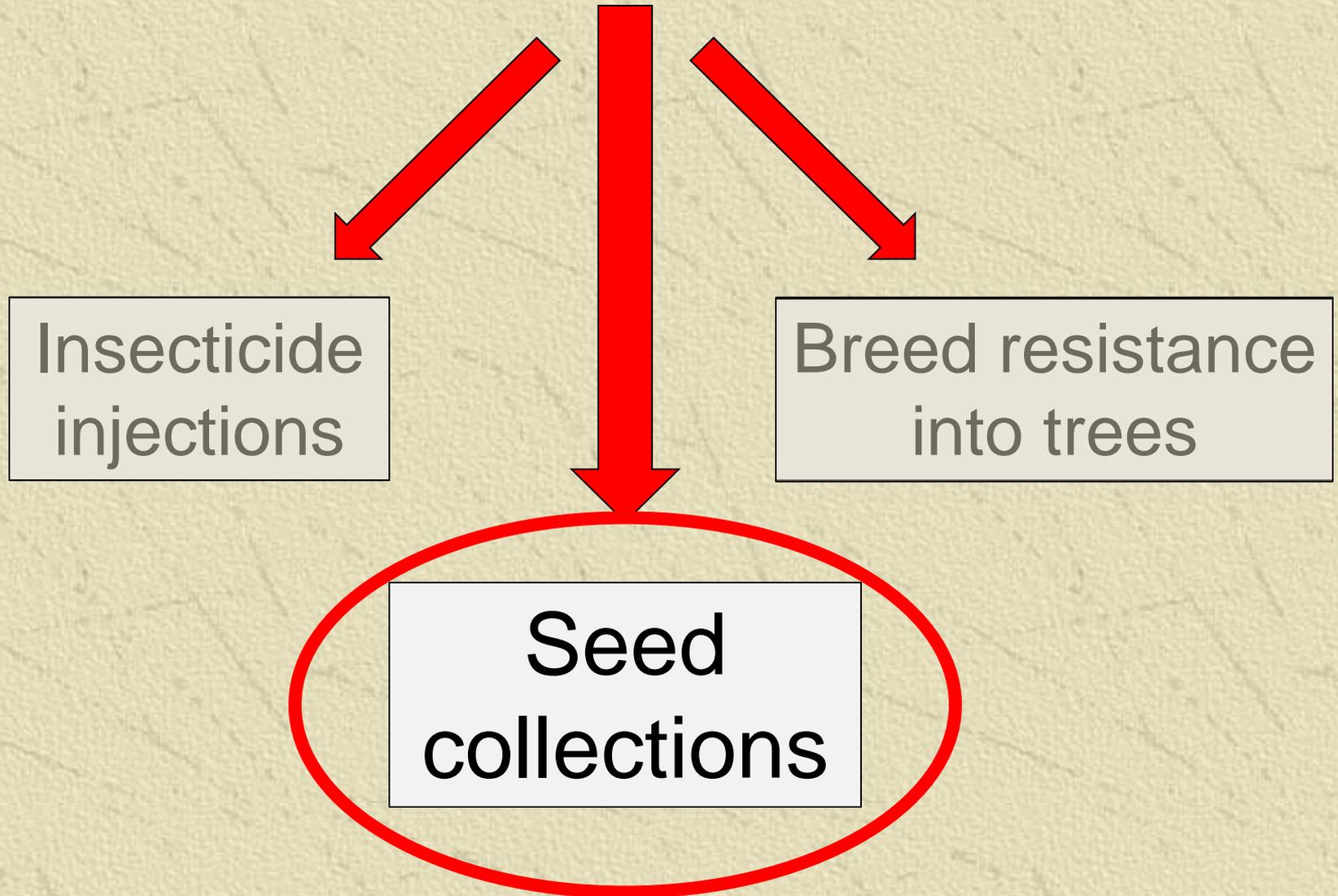
Insecticide  
injections

Breed resistance  
into trees

# Save the genetic diversity of ash



# Save the genetic diversity of ash



# Save the genetic diversity of ash



**Agricultural  
Research  
Service**



Seed  
collections



# 3 Goals

1. Physical collections.
2. Basic research into seed collection.
3. Education.

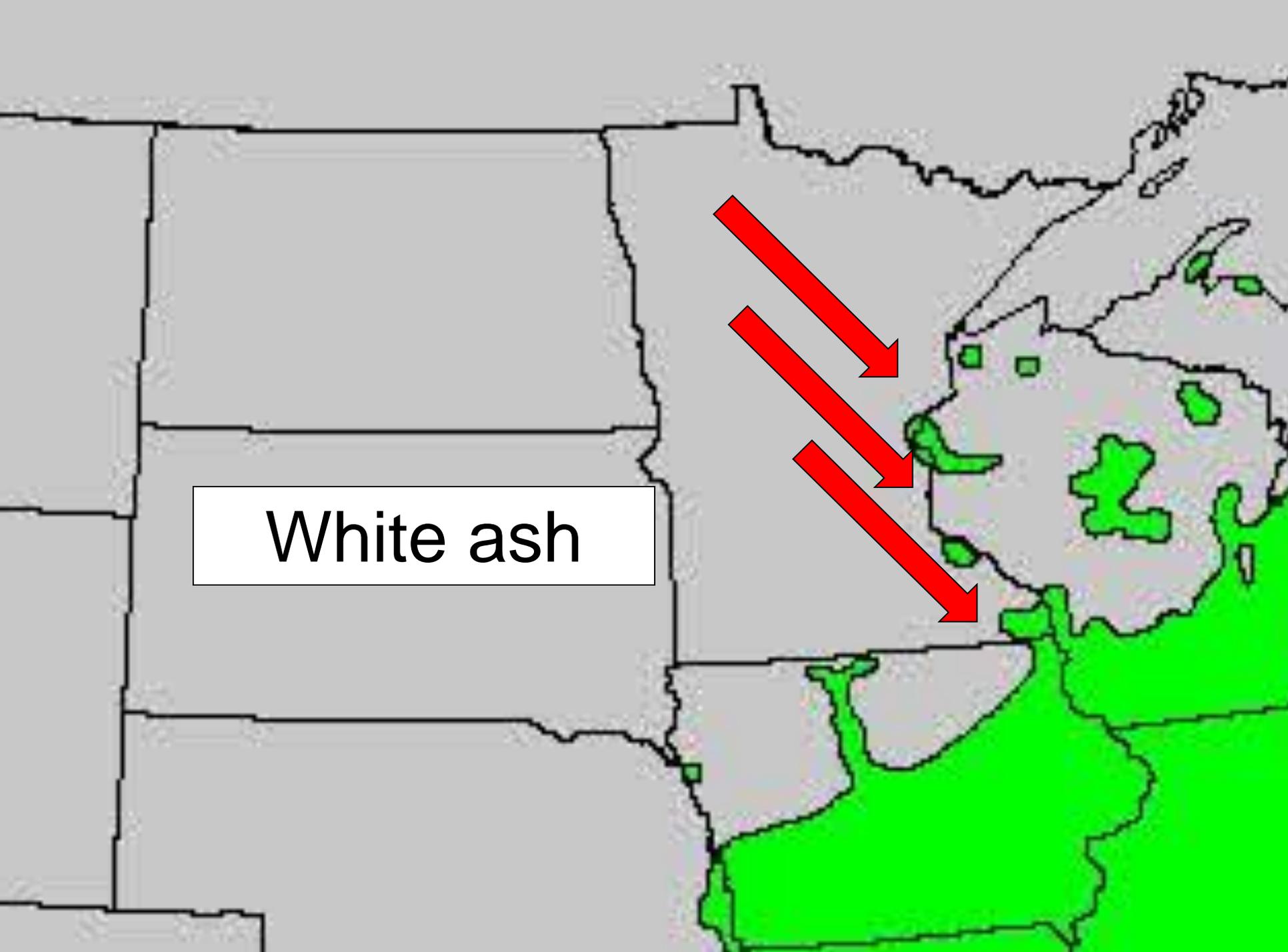
# 3 Goals

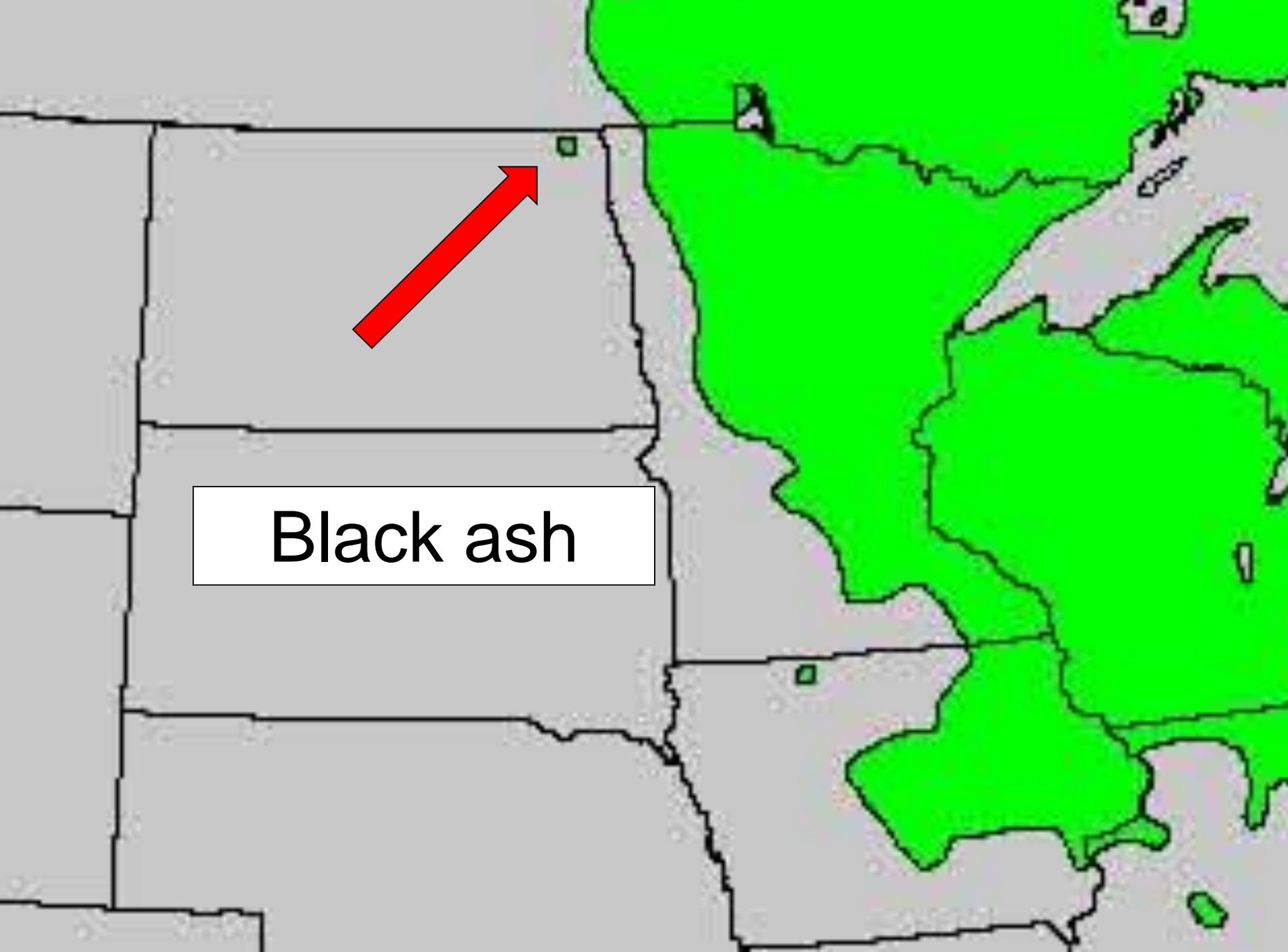
**1. Physical collections.**

2. Basic research into seed collection.

3. Education.

White ash





Black ash

A map of the United States with a green shaded region covering the Northeast and Midwest. The shaded area includes parts of New York, Pennsylvania, Ohio, Michigan, Indiana, Wisconsin, Illinois, and Missouri. A white text box with a black border is centered over the Midwest region, containing the text "Green ash".

Green ash







# Seed collections – MN goals

- ✦ 8-10 populations across state, each spp.
- ✦  $\geq 20$  trees per population
- ✦  $\geq 50$  viable seeds per tree
- ✦ Trees separated by  $\geq 150'$



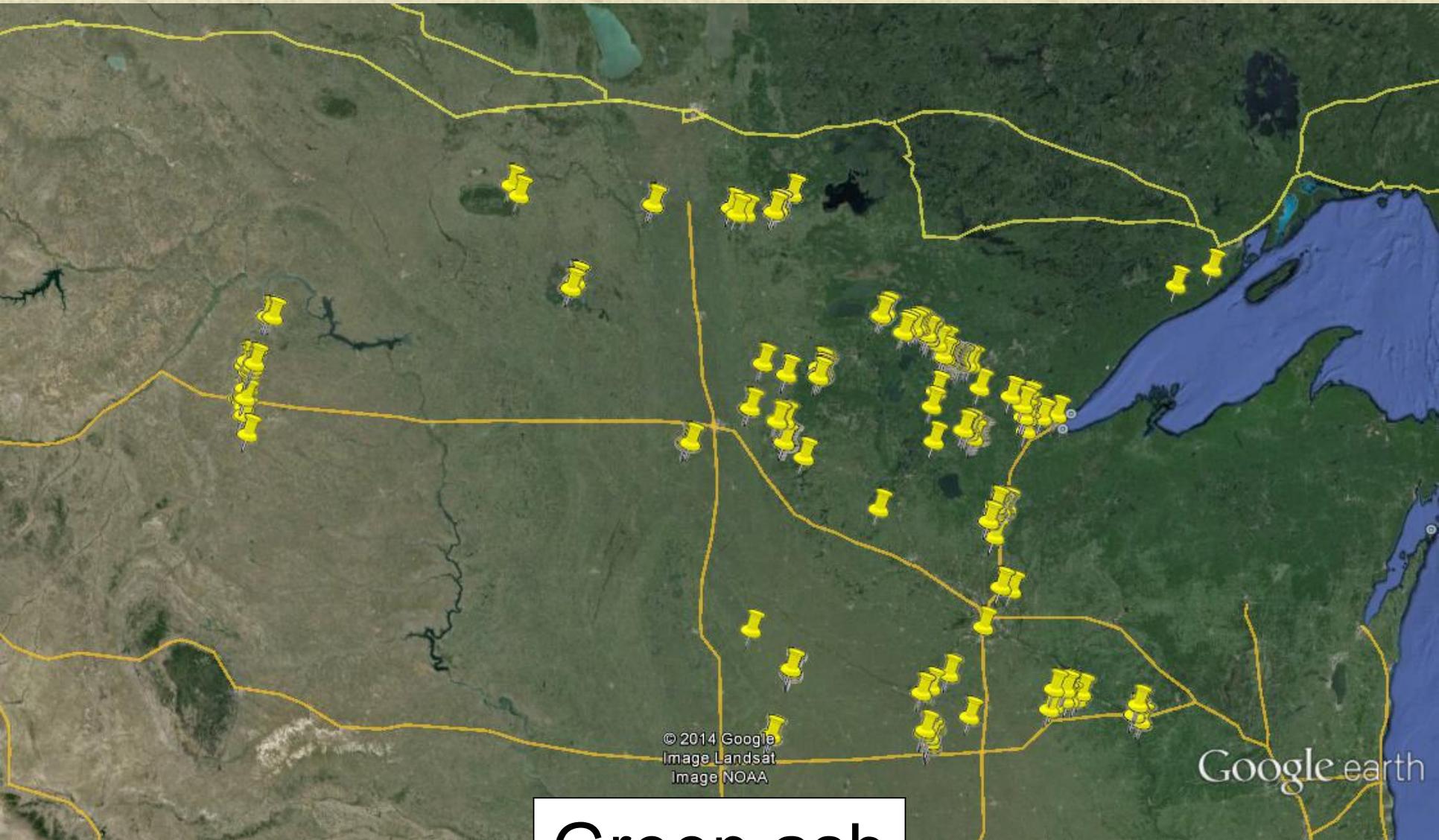
# Collections to date

## UMN

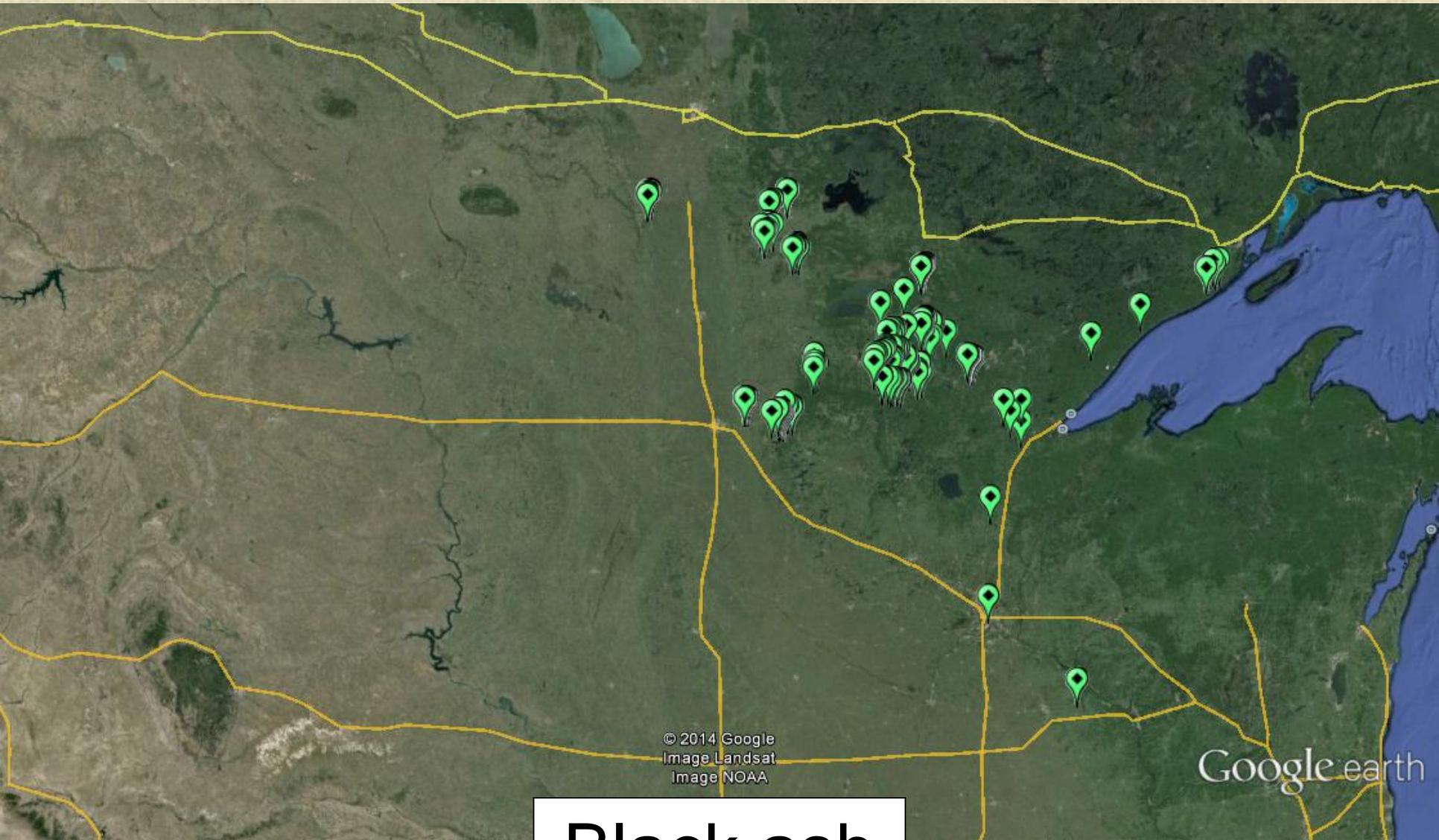
- ✱ 259 black ash
- ✱ 422 green ash
- ✱ 2 white ash
- ✱ 683 trees

## NDSU

- ✱ 131 black ash
- ✱ 137 green ash
- ✱ 0 white ash
- ✱ 268 trees



**Green ash**  
UMN through 2011  
NDSU through 2014



# Black ash

UMN through 2011  
NDSU through 2014



Pelican Rapids, MN

Luverne, MN



Nov 24, 2009 11 pm

N

MNGA0374

A0375

MNGA0377

MNGA0373

MNGA0371

MNGA0384

MNGA0382

MNGA0389

MNGA0370

MNGA0385

MNGA0387

MNGA0388

MNGA0390

MNGA0397

MNGA0398

MNGA0399

MNGA0395

MNGA0392

MNGA0394

# 3 Goals

**1. Physical collections.**

2. Basic research into seed collection.

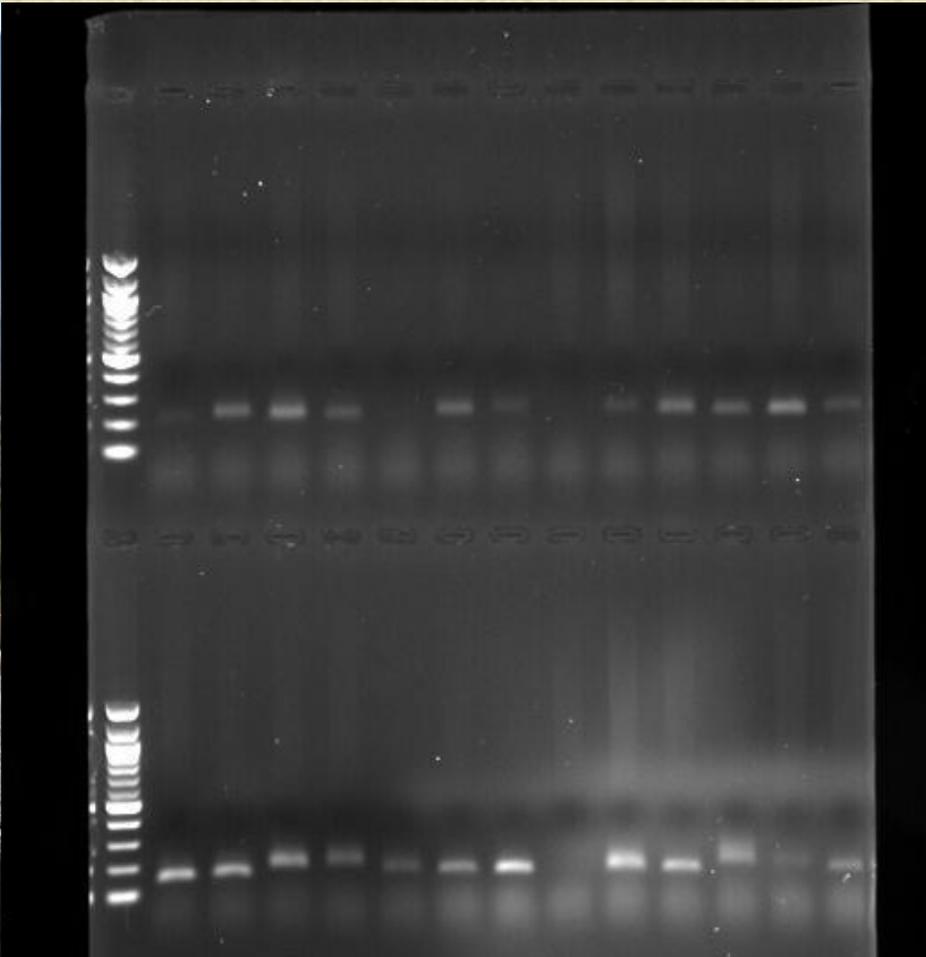
3. Education.

# 3 Goals

1. Physical collections.

**2. Basic research into seed collection.**

3. Education.



- 
- 
- ✦ How much seed needed for 95% of the genetic variation from individual tree?
  - ✦ Are high and low seed production years different?
  - ✦ Are microsatellite markers (SSRs) inherited in a Mendelian fashion?
- 
- 

- 
- 
- ✦ How much seed needed for 95% of the genetic variation from individual tree?
  - ✦ Are high and low seed production years different?
  - ✦ Are microsatellite markers (SSRs) inherited in a Mendelian fashion?
- 
- 



✦ How much seed needed for 95% of the genetic variation from individual tree?

✦ Are high and low altitude populations different?

+/- YES

✦ Are microsatellite markers (SSRs) inherited in a Mendelian fashion?





✦ How much seed needed for 95% of the genetic variation from individual tree?

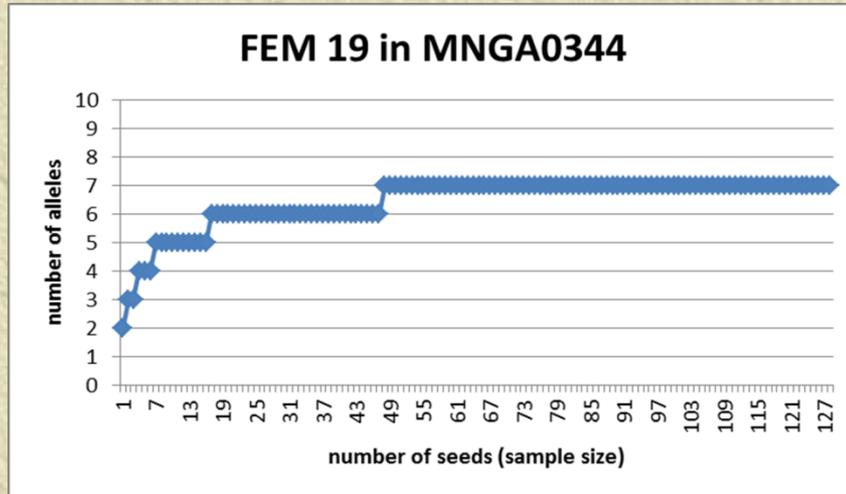
✦ Are high and low seed production years different?

✦ Are microsatellite markers (SSRs) inherited in a Mendelian fashion?



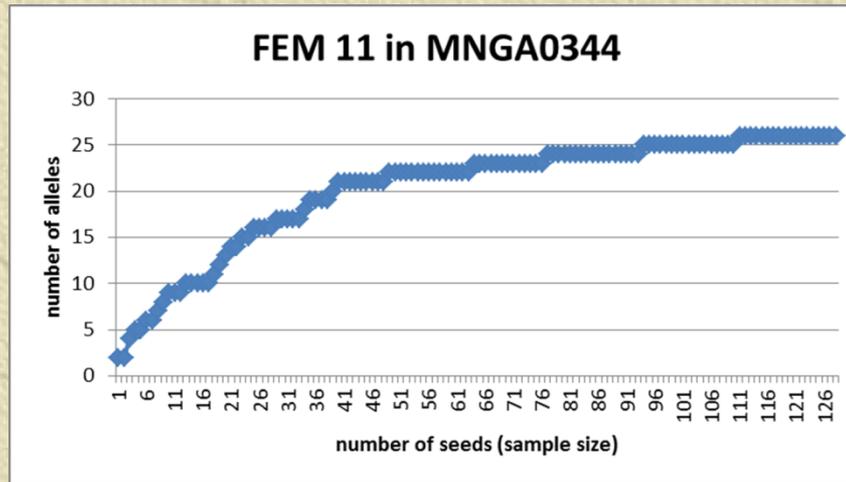
# How much genetic variation collected?

$$A_o = 7$$



✦ Genetic variation increases with increased number of seeds sampled

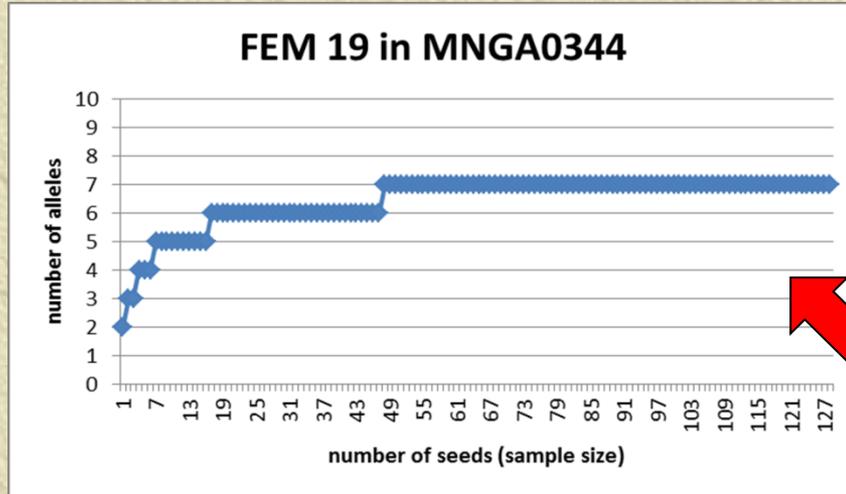
$$A_o = 26$$



✦ Low variation plateaus sooner

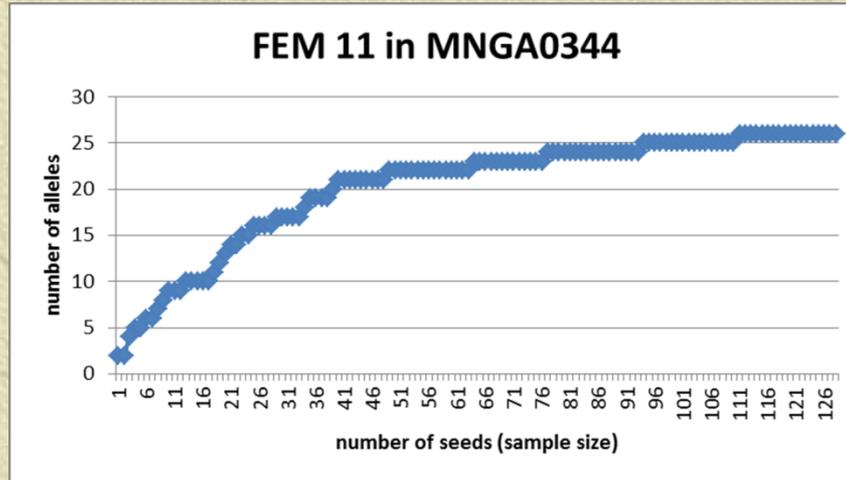
# How much genetic variation collected?

$$A_o = 7$$



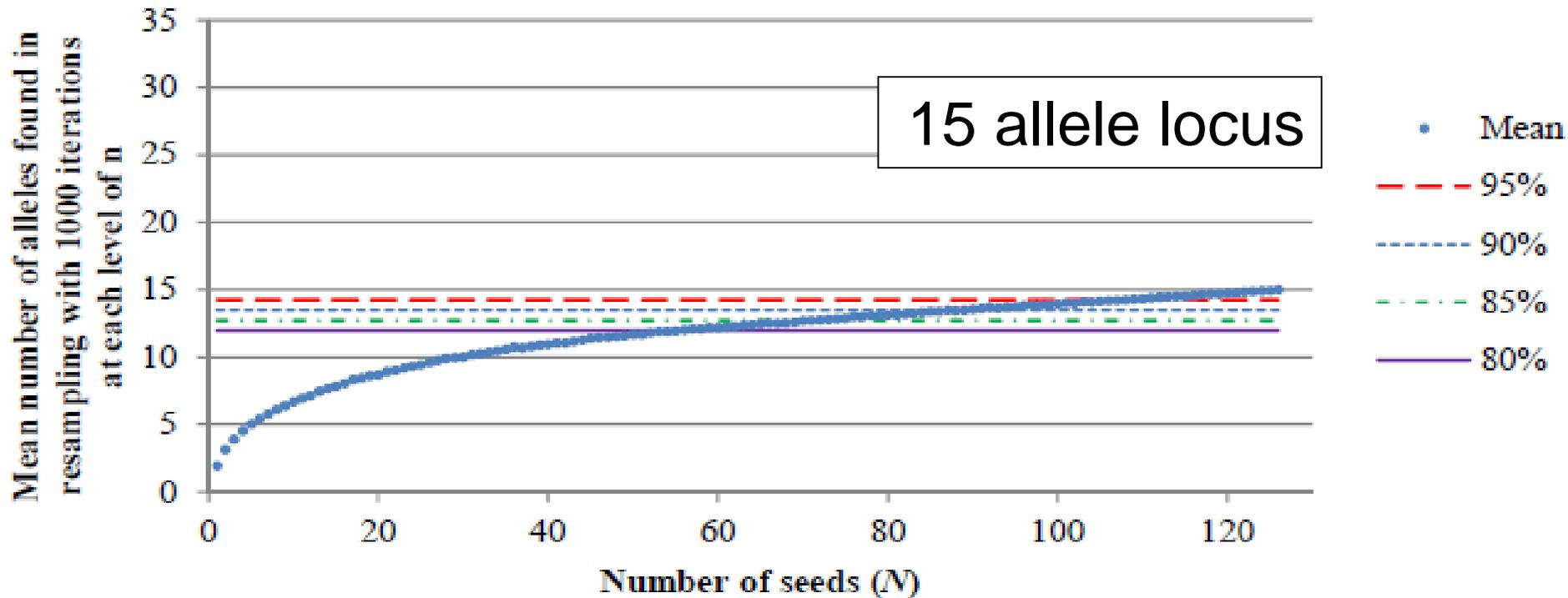
✦ Genetic variation increases with increased number of seeds sampled

$$A_o = 26$$



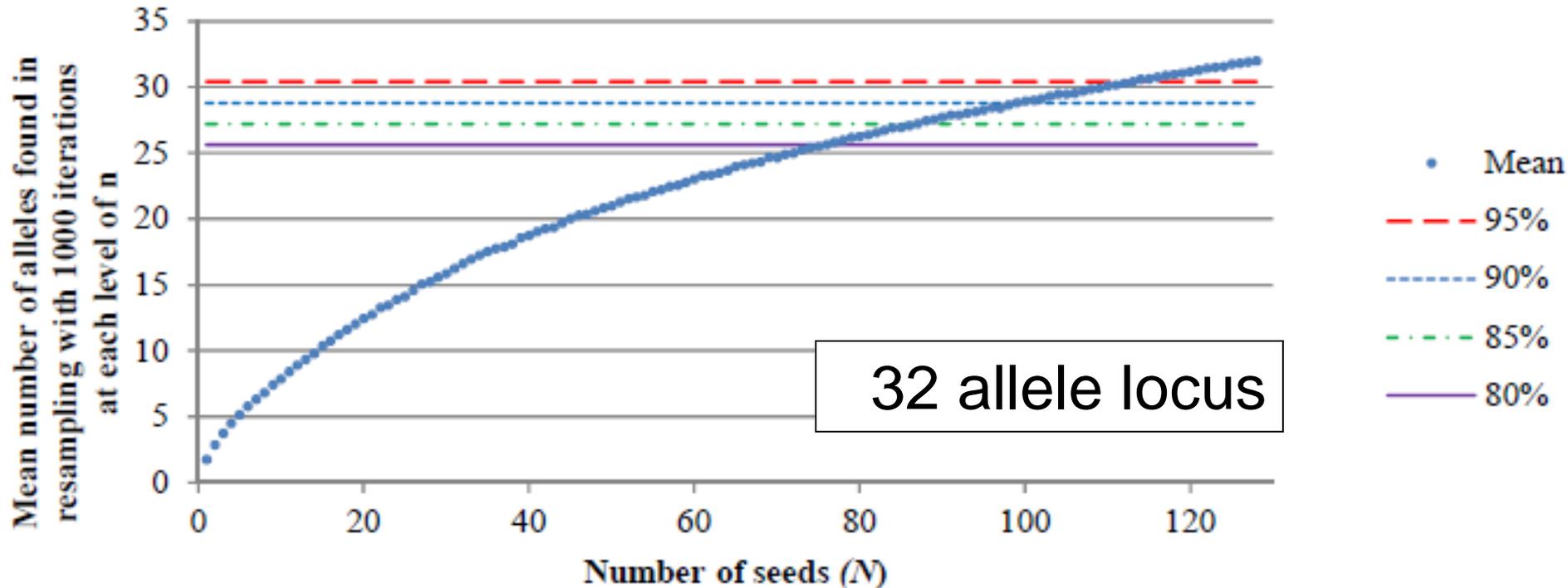
✦ Low variation plateaus sooner

How much seed needed for 95% of the genetic variation from individual tree?



**15 allele locus  $\rightarrow$  ~ 105 seeds**

How much seed needed for 95% of the genetic variation from individual tree?



**32 allele locus → ~ 115 seeds**



✦ How much seed needed for 95% of the genetic variation from individual tree?

✦ Are high and low seed production years different?

✦ Are microsatellite markers (SSRs) inherited in a Mendelian fashion?



- 
- 
- ✦ How much seed needed for 95% of the genetic variation from individual tree?
  - ✦ Are high and low seed production years different?
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- 
- 

# Are high and low seed production years different?

	MNGA0027		MNGA0147		MNGA0143		MNGA0365		MNBA0144		MNBA0007		MNBA0001	
Collection date	Sept 2008	Oct 2011	Oct 2009	Oct 2011	Oct 2009	Oct 2011	Oct 2009	Oct 2011	Sept 2008	Oct 2011	Sept 2008	Sept 2011	Sept 2008	Oct 2011
Seed production year	high	low	high	low	high	low	high	low	high	low	high	low	high	low
<i>N</i>	126	127	126	124	127	127	126	125	127	127	127	127	127	127
Total alleles, all markers	73	64	77	87	67	78	88	79	67	58	53	50	51	42
Mean number of alleles per marker	10.4	9.1	11.0	12.4	9.6	11.1	12.6	11.3	13.4	11.6	10.6	10.0	10.2	8.4
Family mean $H_O$	0.643	0.590	0.617	0.661	0.665	0.704	0.781	0.783	0.635	0.649	0.613	0.639	0.682	0.723
Family mean $H_E$	0.582	0.562	0.560	0.583	0.586	0.644	0.671	0.669	0.595	0.601	0.562	0.583	0.564	0.573
Shannon index	3.159	3.057	3.244	3.301	3.155	3.390	3.506	3.460	2.880	2.863	2.803	2.828	2.850	2.744
p-value for t-test	0.492		0.744		0.127		0.774		0.912		0.872		0.426	

- Negligible differences for  $A_T$ , and  $A_{\bar{X}}$ , some are counterintuitive.
- No significant difference in genetic variation (Shannon's Index).

# Are high and low seed production years different?

Mostly no.

	MNGA0027	MNGA0147	MNGA0143	MNGA0365	MNBA0144	MNBA0007	MNBA0001	Oct 2011							
Collecti date															
Seed product year	high	low	high	low	high	low	high	low							
<i>N</i>	126	127	126	124	127	127	126	125	127	127	127	127	127	127	127
Total alleles, all markers	73	64	77	87	67	78	88	79	67	58	53	50	51	42	
Mean number of alleles per marker	10.4	9.1	11.0	12.4	9.6	11.1	12.6	11.3	13.4	11.6	10.6	10.0	10.2	8.4	
Family mean $H_O$	0.643	0.590	0.617	0.661	0.665	0.704	0.781	0.783	0.635	0.649	0.613	0.639	0.682	0.723	
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1. Physical collections.

**2. Basic research into seed collection.**

3. Education.

# 3 Goals

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## Rapid Agriculture Response Fund Projects

- ✦ Andrew David – UMN
- ✦ Shanelle Dube – Itasca Community College/UMN
- ✦ Mike Benedict – BIA
- ✦ David Heinzen – MN DNR Resource Assessment
- ✦ Julie Hendrickson – UMN
- ✦ Egon Humenberger – UMN
- ✦ Rick Klevorn – MN DNR Forestry
- ✦ Sam Krueger – Itasca Community College
- ✦ Mike Reichenbach – UMN Extension
- ✦ Keith Webb – Itasca Community College

# Minnesota's Ash Tree Seed Collection Program

## Ash Seed Collection Instructions:

1. **Tree Identification** – Tree identification is critical to successful seed collection. Ash trees can be identified by leaf, bark, stem, bud and general structure characteristics. Refer to <http://forest.nrri.umn.edu/ash> for resources on how to identify ash species.

2. **Seed Collection** – Seeds should be collected from a single native wild ash tree and kept separate from other collections. GPS receivers may be used to determine and record the latitude and longitude of specific trees. Be sure to record your map datum on the form if you are using a GPS receiver. (WGS 84 map datum is preferred.)

3. **Harvest Time**- Harvest when the seed is mature in early-autumn. The inner seed coat will be tan and the seed will easily fall from the tree. Fill a large paper grocery bag with 1 to 2" of seed per tree. At a minimum collect at least 2 cups of seed per tree.

4. **Ash Seed Collection Form.** Complete a separate form for each tree. Ship the seed with the form to:

Andrew David  
University of Minnesota  
North Central Research and Outreach Center  
1861 Hwy 169 East  
Grand Rapids, MN 55744  
[adavid@umn.edu](mailto:adavid@umn.edu)  
office: 218.327.4521  
fax: 218.327.4126

5. **Seed Storage and Shipment** – Place harvested seeds in a paper bag and label the bag to accurately identify the sample. Be sure to label each collection as it is made. Do not store seeds in a plastic bag. Keep seed of each sample in a separate paper bag. Store seed under cool, dry conditions until shipped. Please ship early in the week so the seed does not sit over a weekend.

6. **Questions** contact Mike Reichenbach: [reich027@umn.edu](mailto:reich027@umn.edu) or 218-726-6470



Lowland hardwood forest with black ash. Photo courtesy of Michael Benedict

Emerald ash borer, an insect from Asia has been found in Minnesota. This insect kills ash trees of all sizes. The insect has spread rapidly from the Detroit, Michigan area through the movement of ash firewood, ash tree debris, and nursery stock. The loss of all of Minnesota's ash trees is a possibility.

Protecting the genetic resource of ash trees in Minnesota by collecting ash seed from native wild grown trees will provide options for research into resistance and re-establishment. You can help with this effort. For more information visit, <http://forest.nrri.umn.edu/ash>



University of Minnesota Extension is an equal opportunity educator and employer. This material is available in alternative formats upon request. Direct requests to 218-726-6464 or 888-241-0724

# Ash Seed Collection Form

Accession Number \_\_\_\_\_

## PLANT INFORMATION

Check species enclosed

Black Ash

Green Ash

White Ash

Single tree collection? Yes or No

How collected?

Hand picked

Clipped

Shook onto tarp/catcher

Set out tarp/catcher  
and returned to later

## COLLECTION INFORMATION

Date Collected \_\_\_\_\_

Collector's Name \_\_\_\_\_

Collection's Affiliation \_\_\_\_\_

Address \_\_\_\_\_

City, State ZIP \_\_\_\_\_

Phone \_\_\_\_\_

Email \_\_\_\_\_

Is this tribal seed? Yes or No

If yes, what tribe? \_\_\_\_\_

## COLLECTION SITE INFORMATION

State \_\_\_\_\_

County \_\_\_\_\_

Township \_\_\_\_\_

Range \_\_\_\_\_

Latitude \_\_\_\_\_

Longitude \_\_\_\_\_

Map Datum \_\_\_\_\_

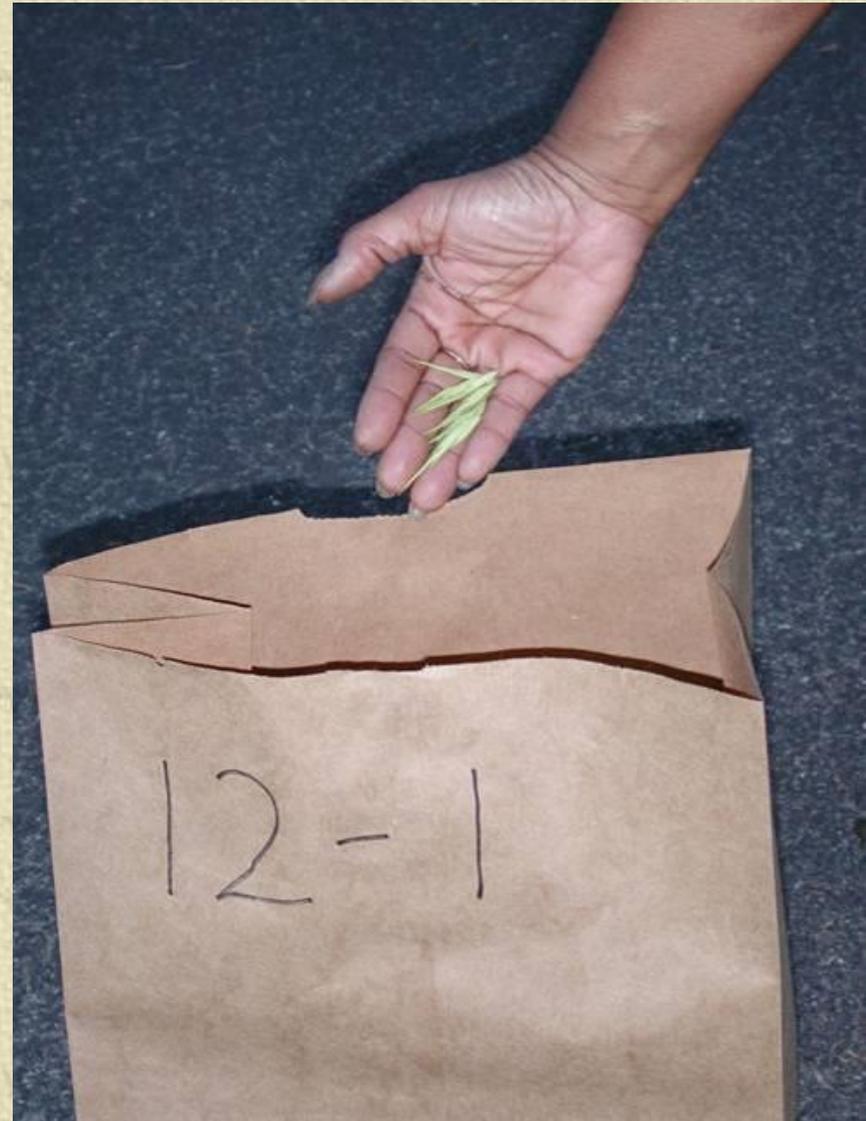
(WGS 84 preferred)

Other information about the sample or collection site.

- Please complete this form for each tree
- Enclose the completed form with the seed shipment
- Any questions visit [www.ashseed.org](http://www.ashseed.org) or email Mike Reichenbach, [reich027@umn.edu](mailto:reich027@umn.edu).

# Volunteer collections

- ✦ Sources – families, boy scouts, individuals, farmers
- ✦ Not reliable (< 2% of collections)
- ✦ Primarily single tree collections



# Student collections

- ✦ Special-topics course – NDSU
- ✦ Real-world conservation
- ✦ Students collect, process and submit samples





# Student collections

- ✦ Experiential learning
- ✦ Genetic analysis – not available
- ✦ Collaborations, mapping and communication emphasized



# Student collections

- ✦ Distance learning technology
- ✦ Timing – fall semester ...
- ✦ Travel – a cost, and a challenge with time



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# Questions?

