Plant invasion genetics; what DNA can tell us about some of Washington's worst plants

John Gaskin

Botanist

USDA Agricultural Research Service, Montana

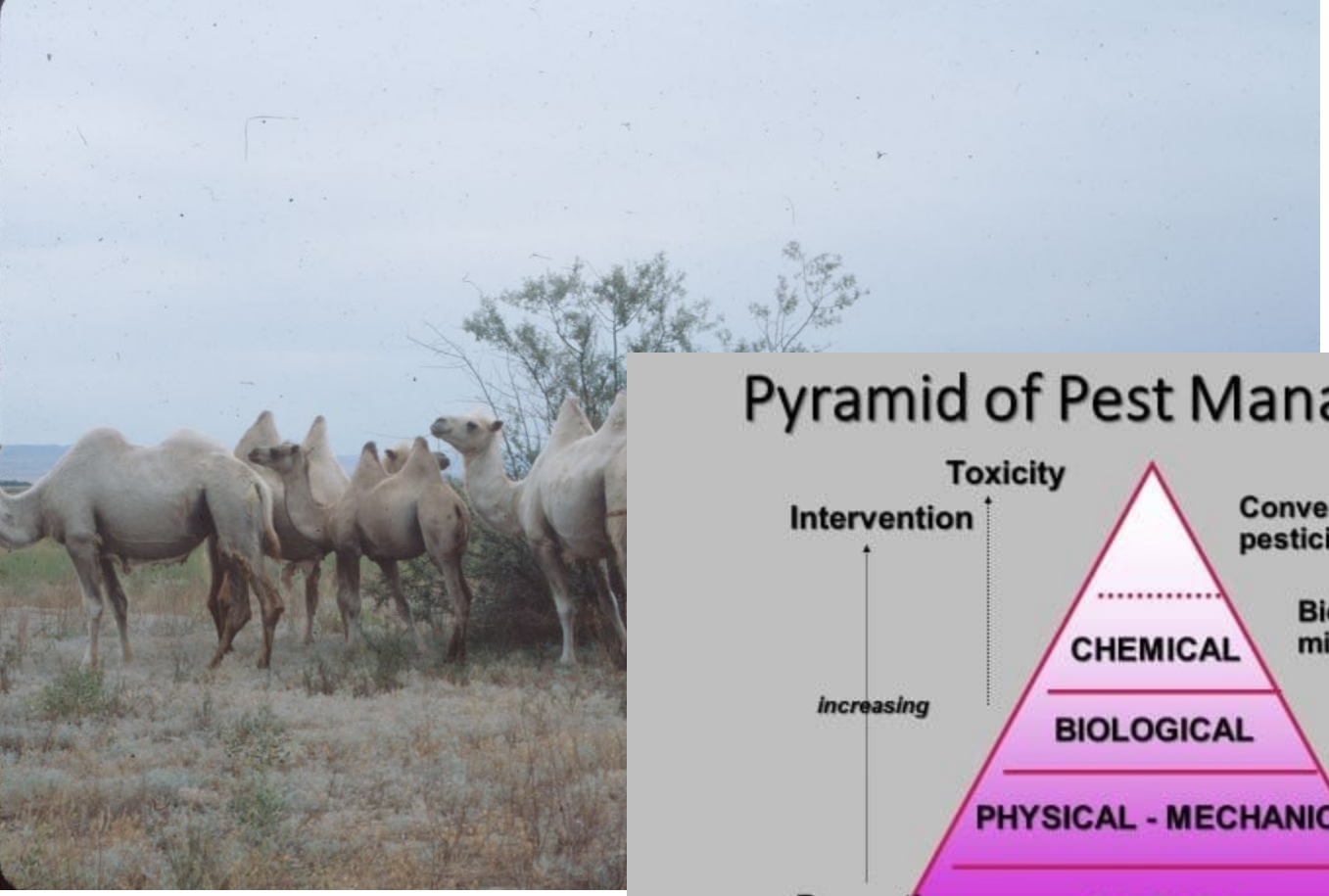
USDA Agricultural Research Service

Snake River

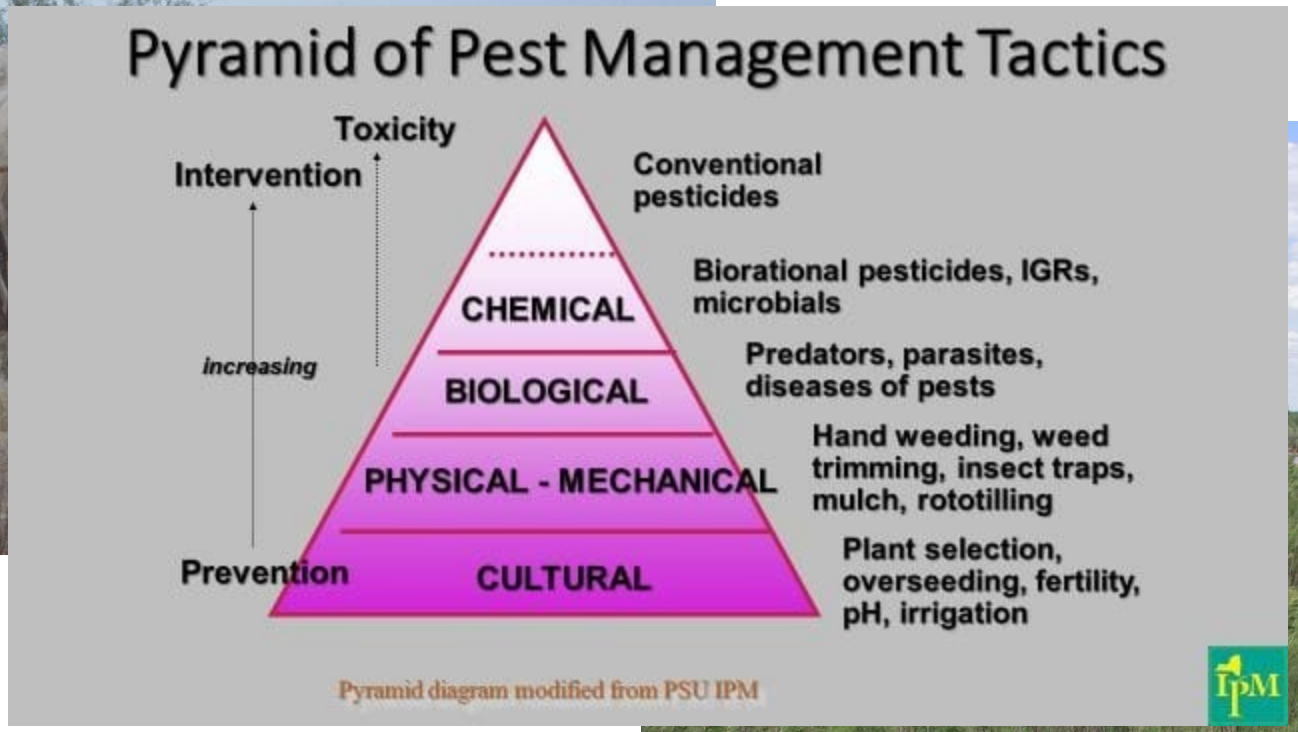
How do plants spread??

Tamarix ramosissima
saltcedar
Kazakhstan

Tamarix ramosissima
saltcedar
USA



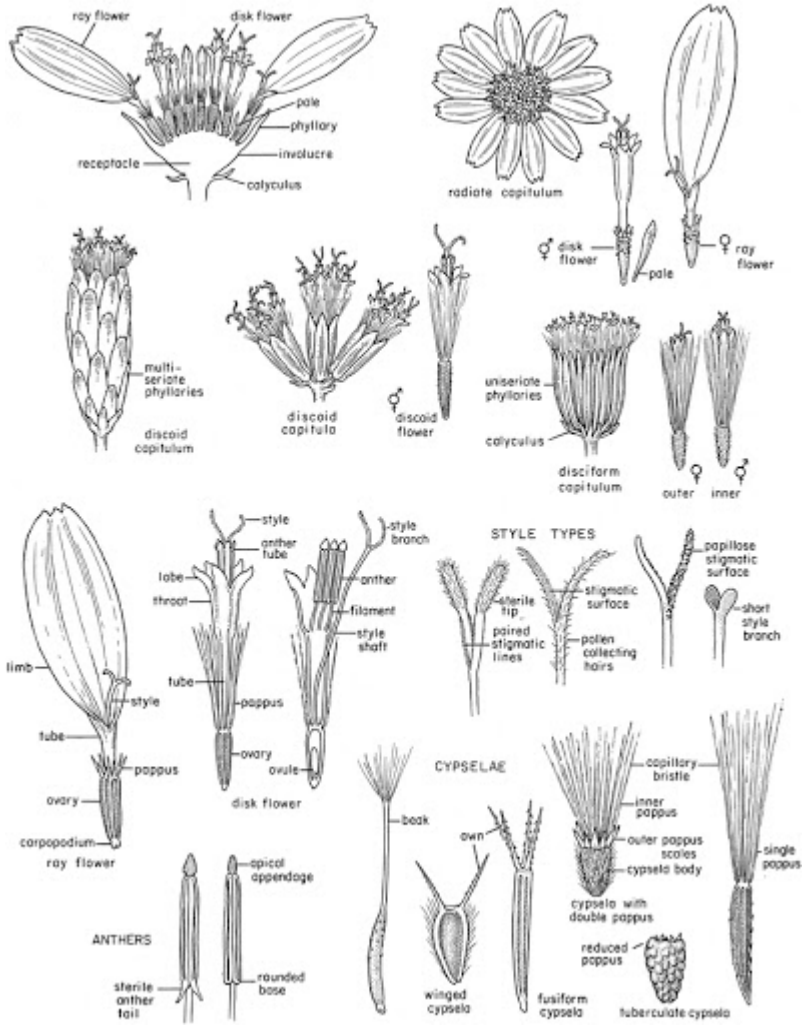
Tamarix ramosissima
saltcedar
Kazakhstan

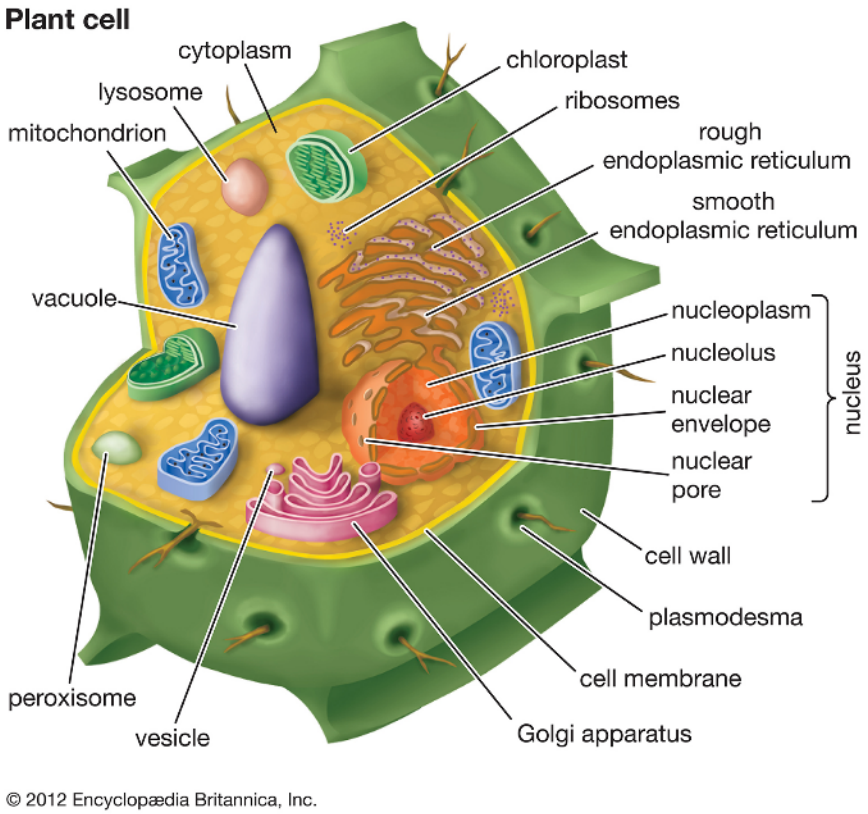


Tamarix ramosissima
saltcedar
USA

Snake River

Morphology can tell us a lot about weeds; DNA can tell us some hidden secrets





Questions that DNA can help answer

- What species is this weed?
  - Know your enemy

Euphorbia spp.
leafy spurge

Questions that DNA can help answer

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- How does it reproduce?
  - Helps us design control options

Rhaponticum repens
Russian knapweed

Questions that DNA can help answer

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- Are there different genotypes? Hybrids?
  - Differences in resistance or tolerance to control

Phragmites australis
common reed

Questions that DNA can help answer

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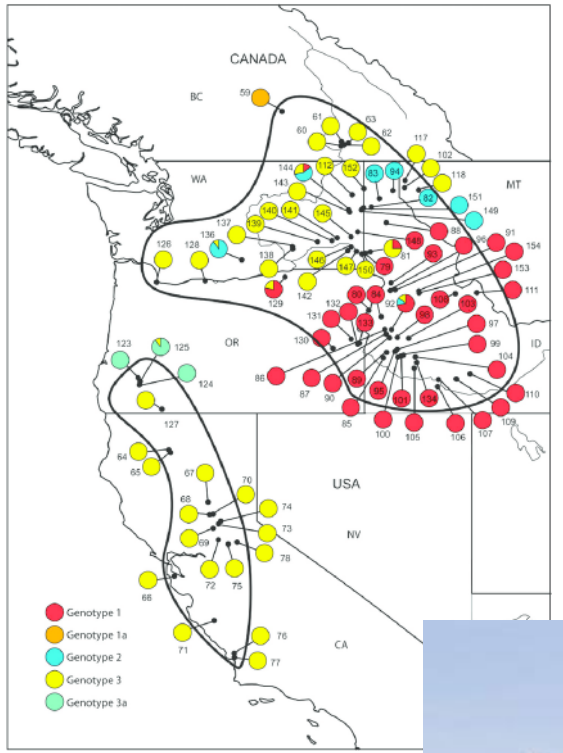

Chondrilla juncea
rush skeletonweed

Questions that DNA can help answer

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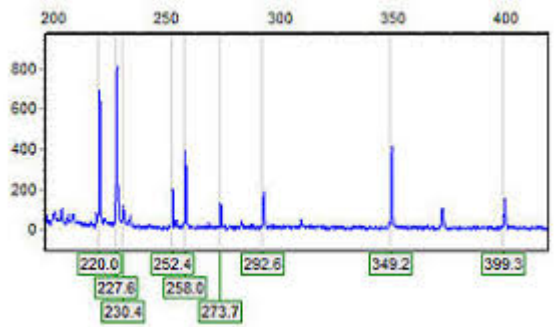
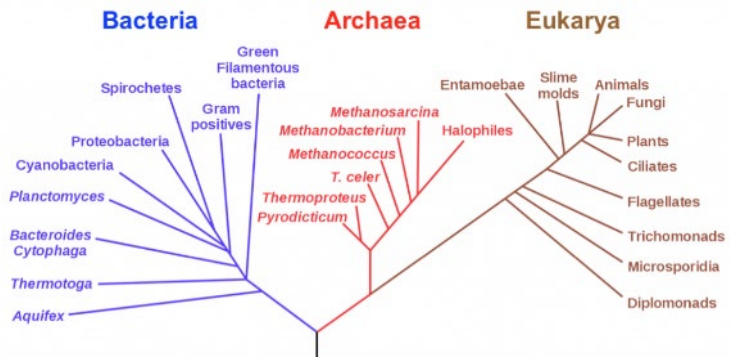
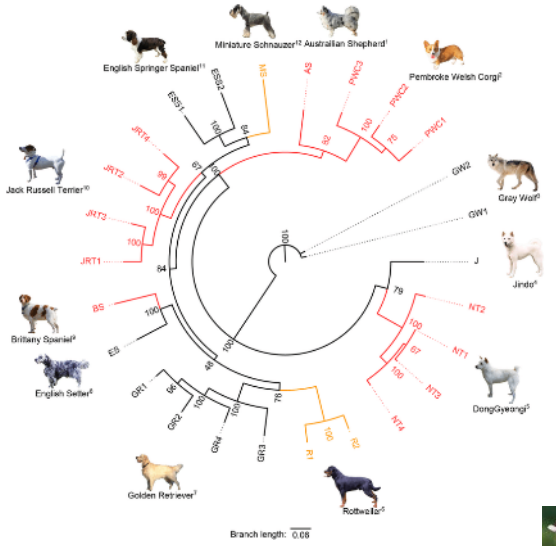
- Where did the invasion come from?
  - Finding host-specific enemies

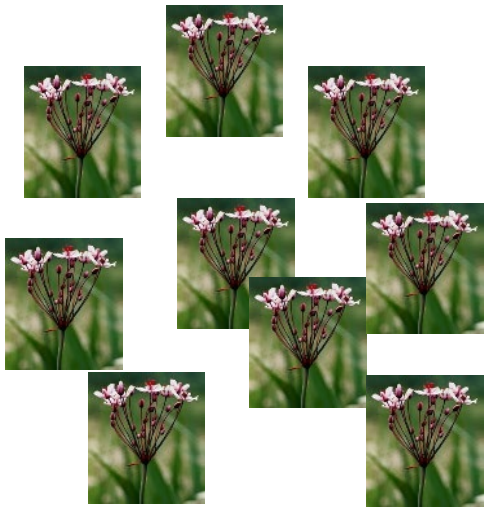
Which DNA method/marker?



- DNA sequencing
- AFLPs
- RAPDs
- microsatellites
- RAD-seq
- …







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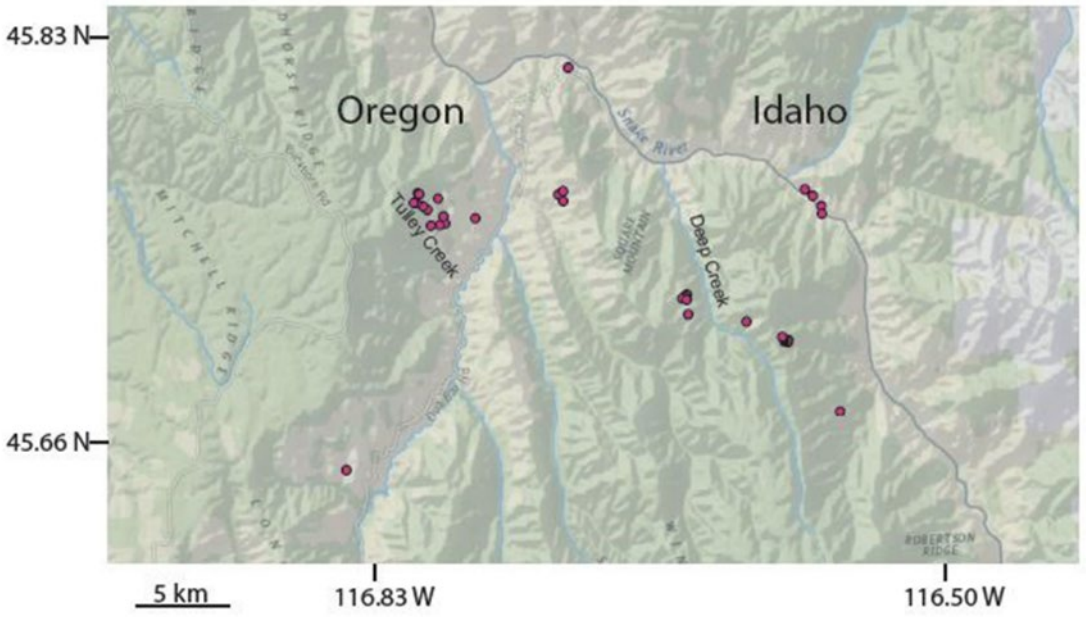
What thistle is this?



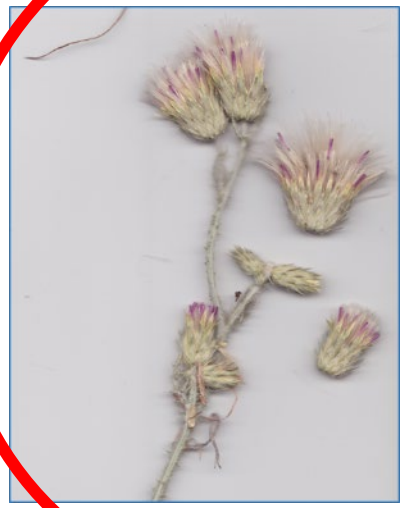
Hells Canyon
40km upstream from WA

Refresher course: *Carduus* in N. America

All non-native, state noxious

- *Carduus nutans*
- *Carduus acanthoides*
- *Carduus crispus*
- *Carduus tenuiflorus*
- *Carduus pycnocephalus*

- Carduus nutans
- Carduus acanthoides
- Carduus crispus
- *Carduus tenuiflorus*
- *Carduus pycnocephalus?*
- *Carduus? sp.???*



DNA sequencing

…GATCAGACGTCTC…
…GATCCGACGTCTC…
…GATCTGACGTCTC…
…GATCGGACGTCTC…

Carduus pycnocephalus 15219.01 Oregon USA
Carduus pycnocephalus KY242484.1 Italy
Carduus pycnocephalus 13960 Oregon USA
Carduus pycnocephalus 15219.03 Oregon USA
Carduus pycnocephalus EF123105 California USA
Carduus tenuiflorus 15218.02 Oregon USA
Carduus pycnocephalus? 14362.01 Oregon USA
Carduus tenuiflorus HM921408 USA
Carduus tenuiflorus AF443679 USA
Carduus acanthoides JX867641 USA
Carduus nutans HQ540426 USA
Carduus nutans EF543521 USA
Carduus acanthoides subsp. sintenisii KT013086.1 Turkey
Carduus tmoleus KT013085.1 Turkey
Carduus defloratus AY826241 Spain
Carduus personata KM262846 Austria
Carduus crispus GU188570 South Korea
Carduus crispus AY914813 China
Carduus carlinoides AY826240
Carduus nervosus KT013070.1 Turkey
13961
15221.02
15217.02
15220.03
15221.03
14363.01
15217.03
15220.01
14364.02
15220.02
15217.01
15221.01
Carduus cinereus MK253665 USA
"Carduus pycnocephalus subsp. arabicus" KT013082 Turkey
Carduus pycnocephalus subsp. albidus KT013081.1 Turkey
Cirsium vulgare JX867638
Cirsium mohavense AF443700
Cirsium douglasii AF443686
Onopordum illyricum FJ007887
Centaurea solstitialis DQ319163
80
97
99
99
95
92
90
68
64
99
47
99
46
99
87
71
0.020

- *Carduus pycnocephalus*??
  - Garbage can… No.
- Hybrid?
  - Nuclear DNA. No.
- Eastern Euro *C. pycnocephalus?*
- *Revise the genus!*
- ***Carduus cinereus!!***
- Is it invasive?
  - *Carduus* a notorious genus

MADROÑO, Vol. 66, No. 4, pp. 142–147, 2019

CARDUUS CINEREUS (ASTERACEAE) – NEW TO NORTH AMERICA

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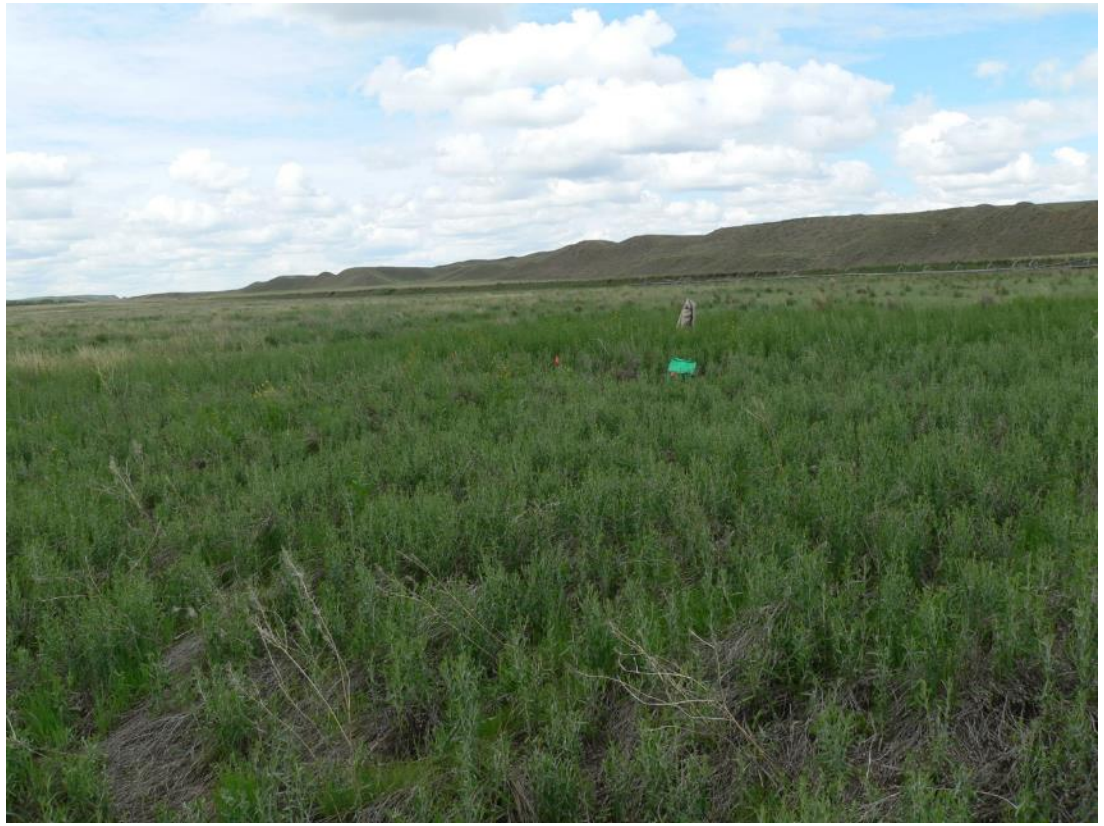
Reproductive mode of Russian knapweed



MSU

USDA

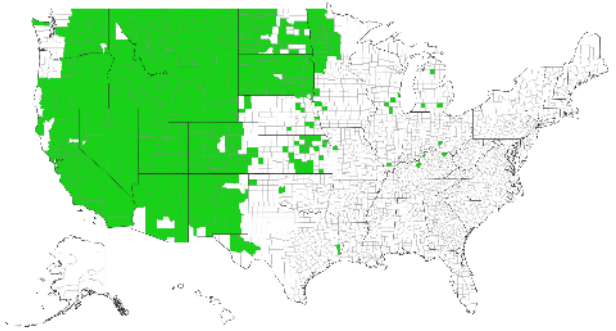
Invasive Plant Science and Management 2017 10:119–124

WSSA

Invasive Russian Knapweed (*Acroptilon repens*) Creates Large Patches Almost Entirely by Rhizomic Growth

John F. Gaskin and Jeffrey L. Littlefield*

Outcrossing
Selfing
Apomictic
Clonal (vegetative)



How is this plant spreading?

- Locally
- Long distance

Why do this research?

- Mode of reproduction at times unknowable without genetic analysis
- Effective management can require knowledge of reproductive mode
  - Classical biological control
  - Timing of chemical or manual control
    - E.g. Fall is better spray time maybe but too late for seed control?
- Sometimes results are not what you would predict…

Goal: Distinguish ramets from genets to test hypotheses:

- If clonal spread, plants genetically identical
- If spread by seed (and self-incompatible), all plants genetically different.
- Some combination of two above.

Russian knapweed

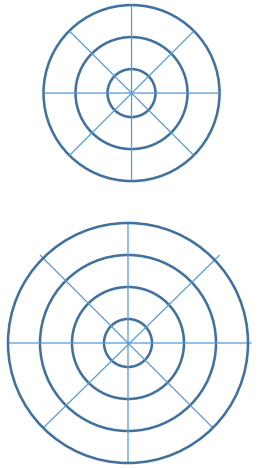
- Known to reproduce clonally from rhizomes (Watson 1980)
- Also produces seed (50-1200/plant; Beck 2001 Ivanova 1966)
- Self-incompatible (Young & Clements 2003; Harrod & Taylor 1995)
- How much local spread is from vegetative vs. seed propagation? How big can clones get?

Aulacidea acroptilonica

Jaapiella ivannikovi gall

Methods

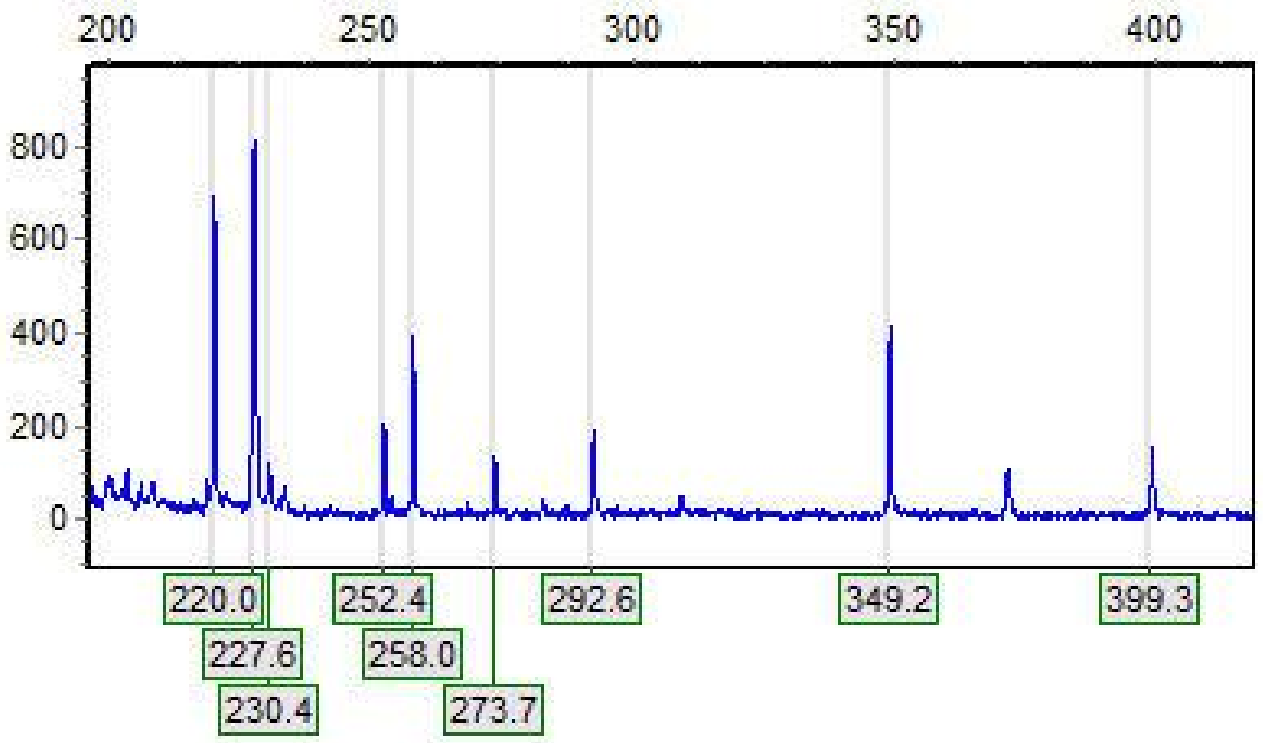
- Collected leaf tissue from 174 shoots from 6 dense patches, 10-56m diameter
- 25-33 samples per patch
- AFLPs (54 loci)

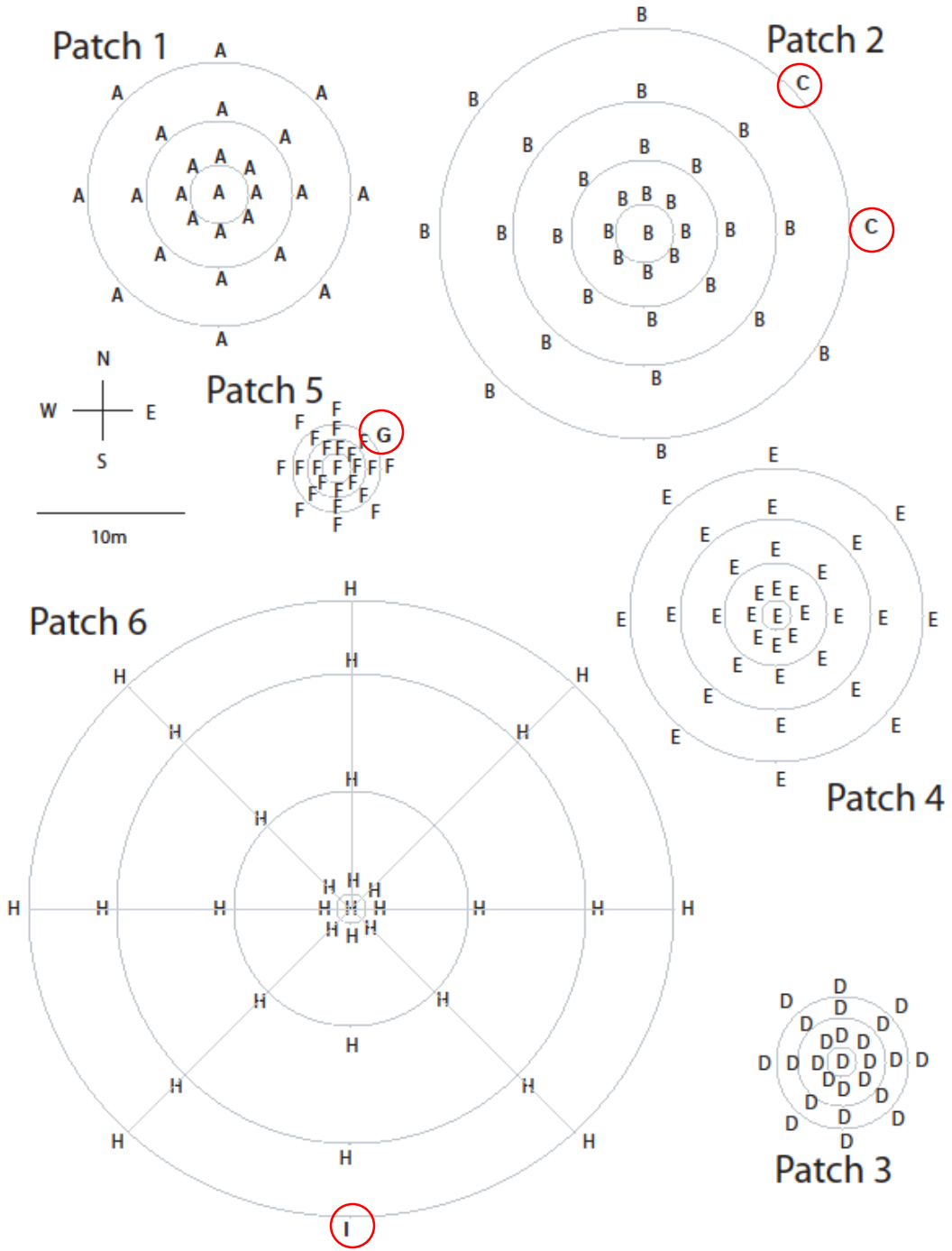


AFLPs
Amplified Fragment Length Polymorphisms (DNA fingerprint)

- Extract DNA from leaves
- Cut at restriction sites
  TCGT cut TCCGTA
- Make multiple copies of fragments so signal is strong enough
- Run on fragment analyzer (sequencing machine)
- 1, 0 data

- AFLPs are repeatable
- AFLPs are high resolution




Patch 1
Patch 2
C
C
N
W
E
S
10m
Patch 5
G
Patch 6
I
Patch 4
Patch 3

Conclusions

- Patches are typically one individual plant
- Largest genet size 56m, as big as our biggest patch (unexpected result)
- There are likely even larger individuals

Conclusions

- New establishment of patches (n=6) likely by seed
- Control of existing patches will have to focus on methods that are effective against root mass
- Control of establishment of patches relies on destruction of seed in non-tilled/harvested environment

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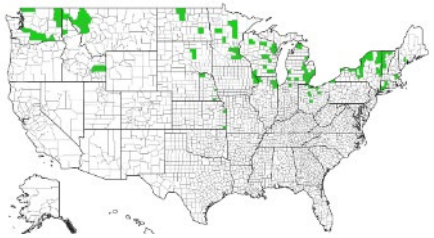
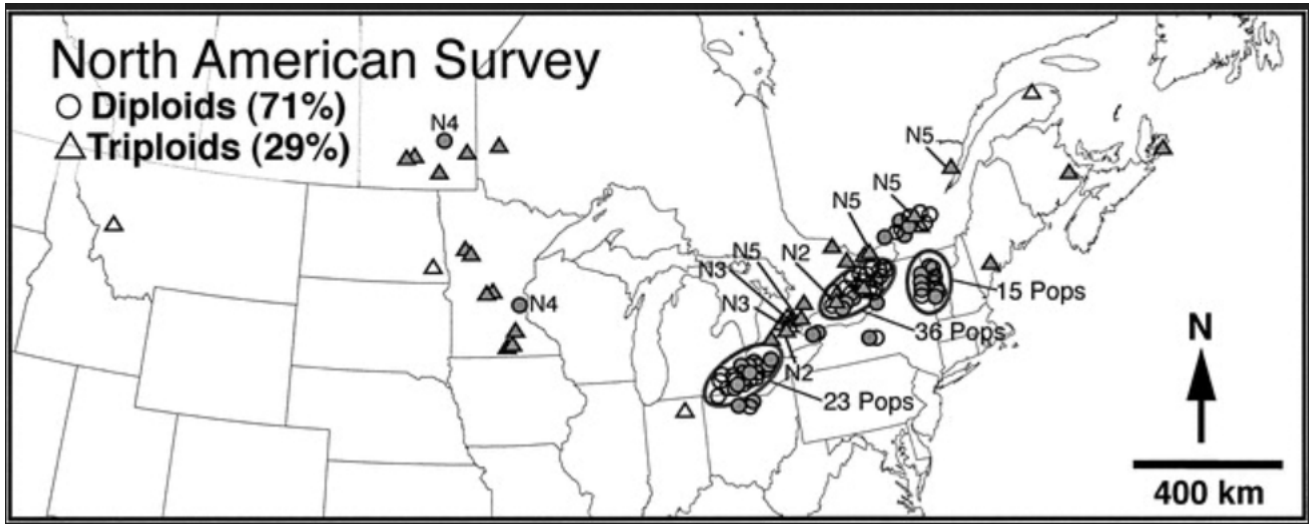
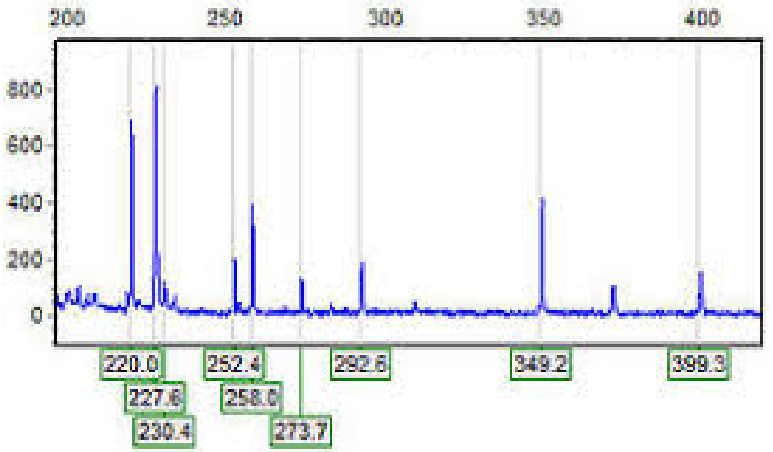
Flowering rush
Butomus umbellatus



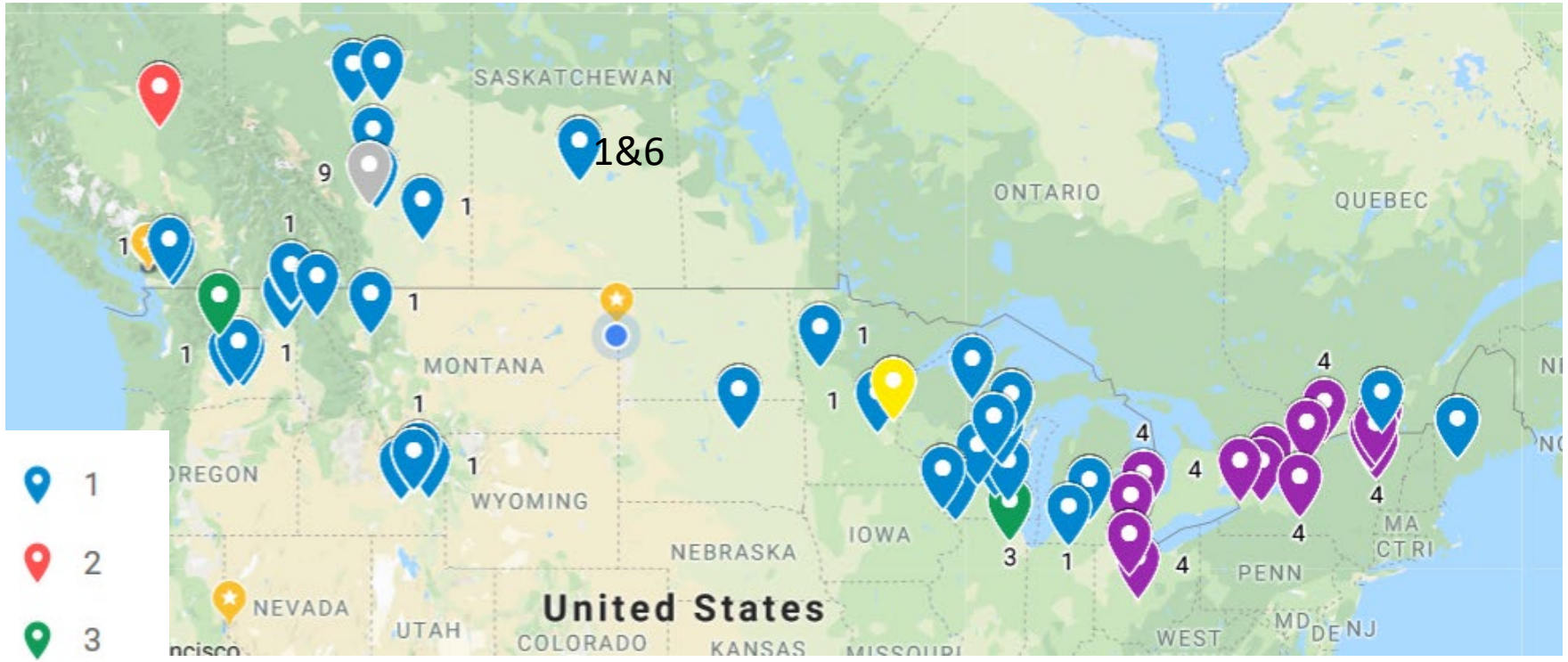


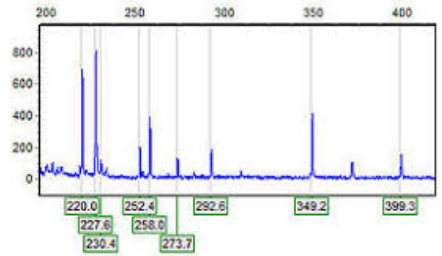
Kliber and Eckert 2005.



AFLPs

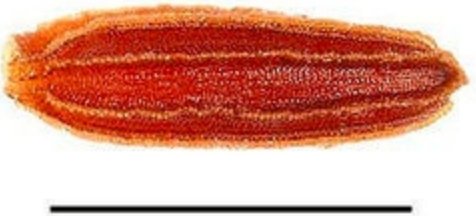
How are they distributed?





Who cares about ploidy!?

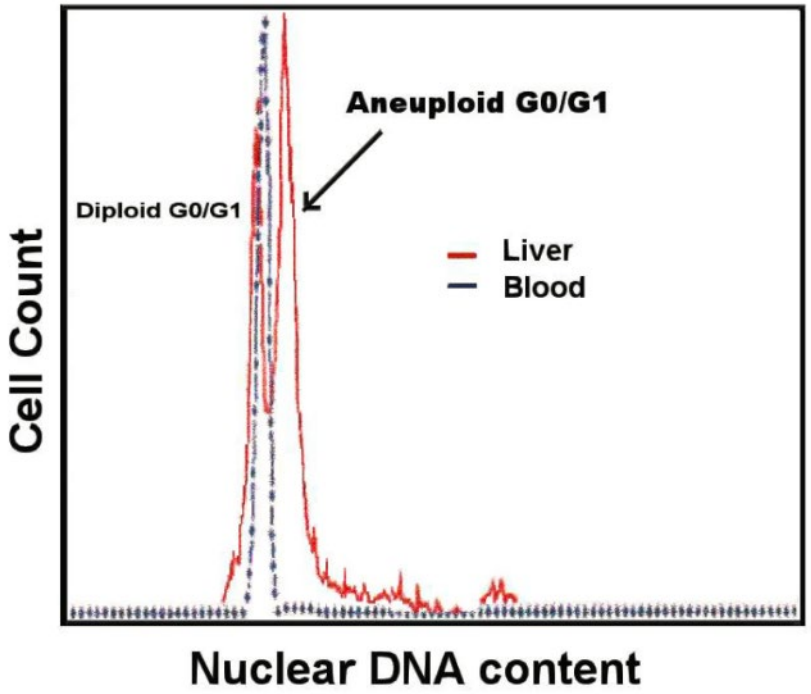
- Reproduction and spread
  - 2x (diploid): seed and bulbil and fragments
  - 3x (triploid): only root fragments?
- Invasiveness?
- Control?
  - Chemical
  - Biological



Diploid or triploid?

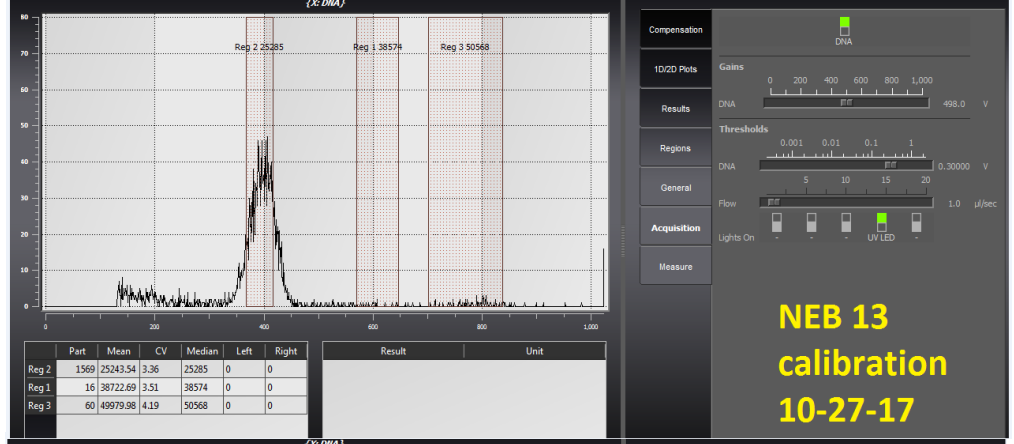
Fresh tissue

Can also tell by stomata

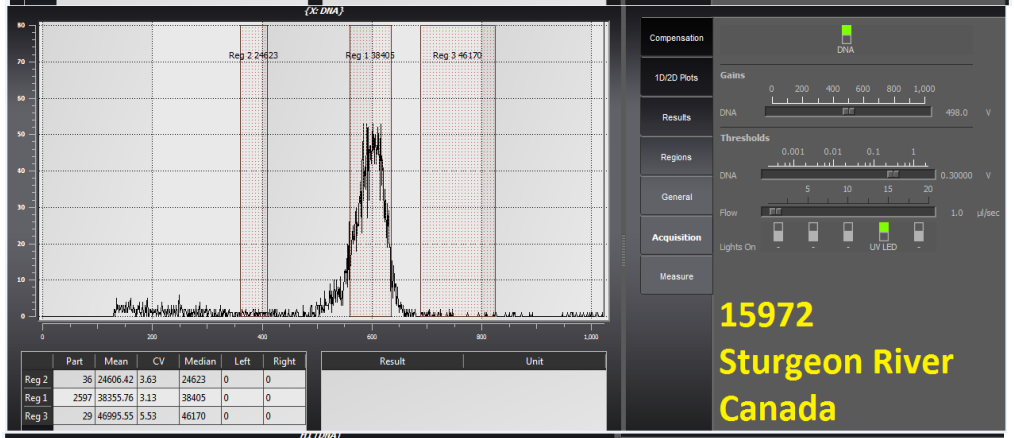


2x= 400
3x=600

Fresh leaf material

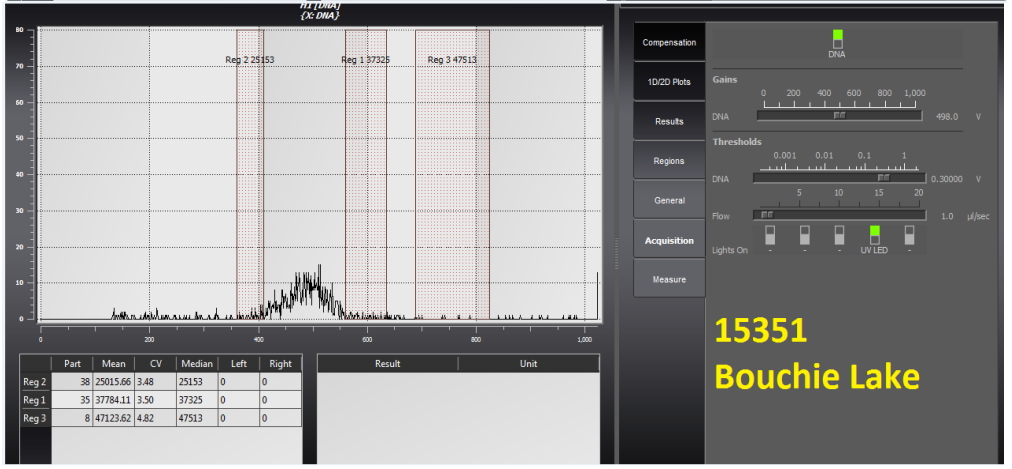


Genotype 4 VT

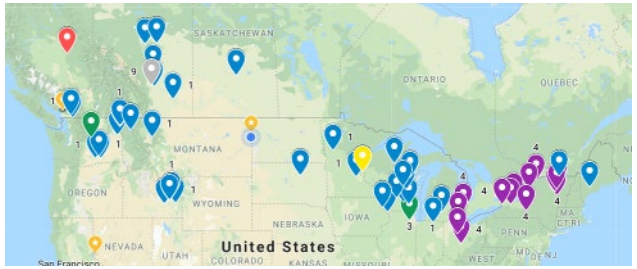


Genotype 1

Grrr. Stringy tissue?



Genotype 2



Species Code	ARS PMRU DNA Sample #		# Root Tip Evaluations	# Cell Evaluations	Chromosome Count (*2n*=)
BUUM	15348.00	Yak-Pross	2	15	26
BUUM	15349.00	Yak-Pross	2	15	26
BUUM	15350.00	Yak-Pross	2	15	26
BUUM	15351.00	Bouchie	2	16	39

3-D analysis

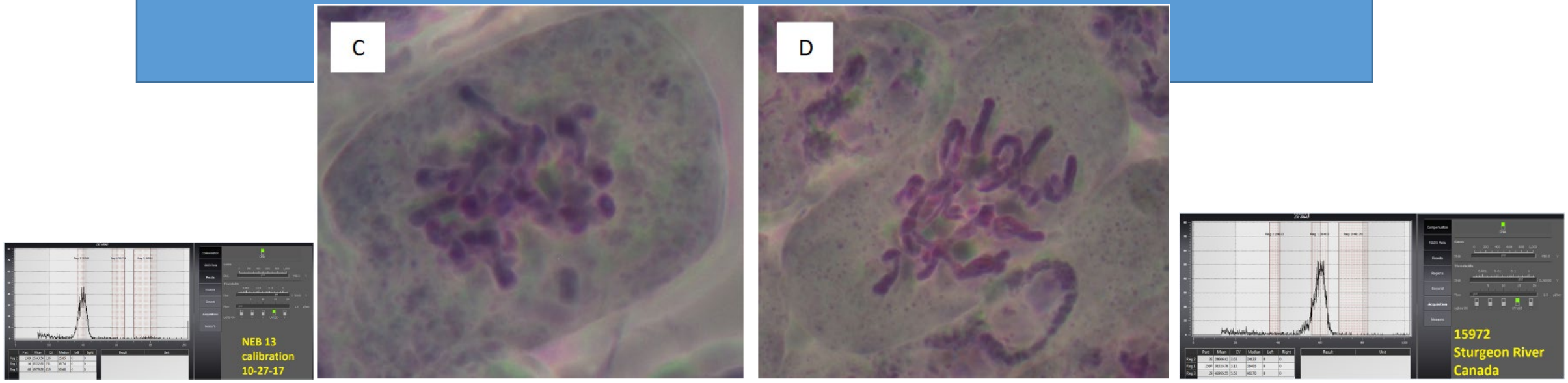
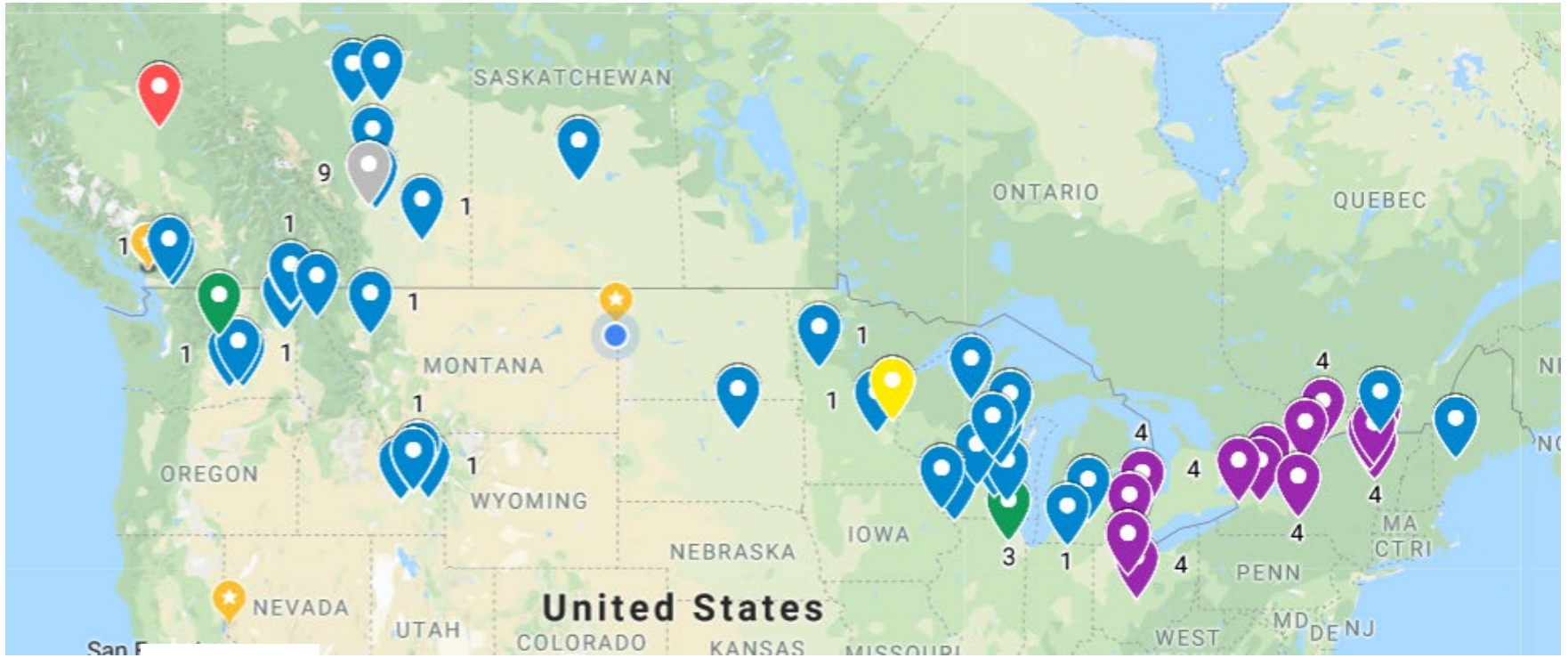


Figure 1. Composite cell images from *Butomus umbellatus* with A-C) 2*n*=26 chromosomes, D) 2*n*=39 chromosomes.

USDA ARS UC Davis Brenda Grewell and Joy Furtrell



1	3x	(2x at Prosser Diversion dam) [Jennifer Parsons]
2	3x	
3	2x	
4	2x	
5	2x	
6	?	
9	?	

yellow star=favorite restaurants

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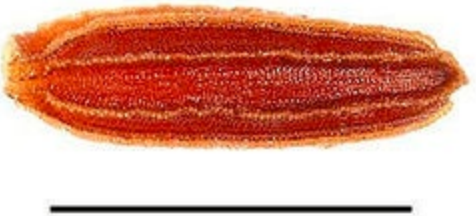
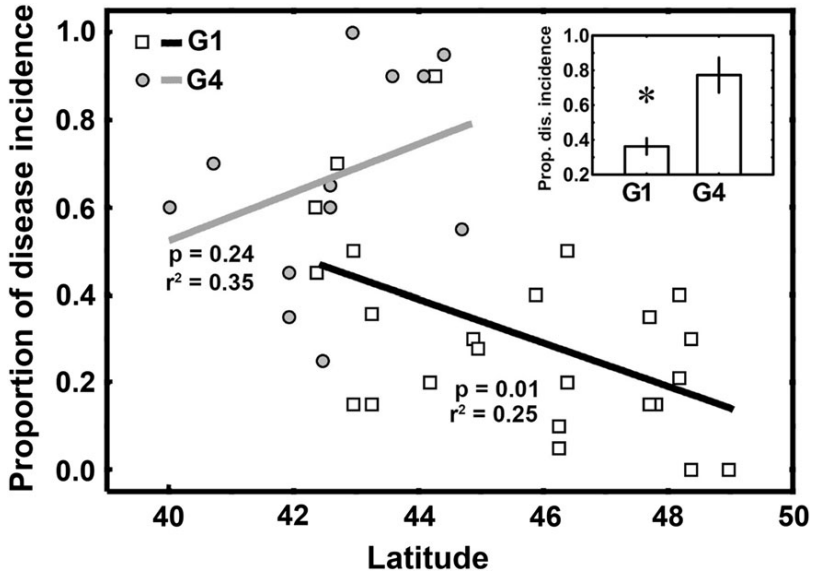


Flowering rush NA diseases. Does genotype matter?



Harms, N., Shearer, J., Cronin, J.T. and Gaskin, J.F., 2019. Geographic and genetic variation in susceptibility of Butomus umbellatus to foliar fungal pathogens. Biological Invasions, pp.1-14.

US Army Corps of Engineers

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- What species is this weed?
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Genetics of Knotweeds

Biol Invasions
DOI 10.1007/s10530-014-0652-y

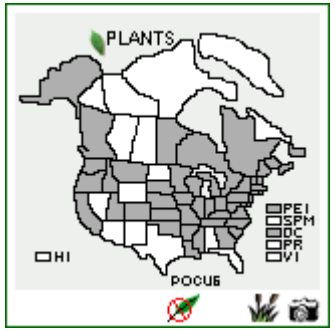
ORIGINAL PAPER

Extreme differences in population structure and genetic diversity for three invasive congeners: knotweeds in western North America

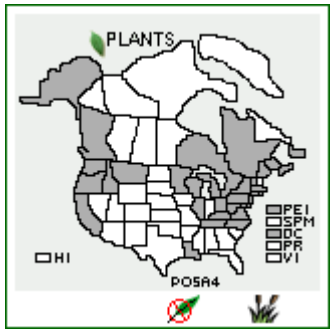
John F. Gaskin · Mark Schwarzländer · Fritzi S. Grevstad · Marijka A. Haverhals · Robert S. Bourchier · Timothy W. Miller

Background info: Three knotweed taxa

- Japanese
  - *Fallopia japonica*



- Giant
  - *Fallopia sachalinensis*



- Bohemian (hybrid of the two above)
  - *Fallopia x bohemica*

Knotweeds in western North America

1. How are species distributed in western North America?
2. Can managers distinguish the different species in the field?

Biocontrol update

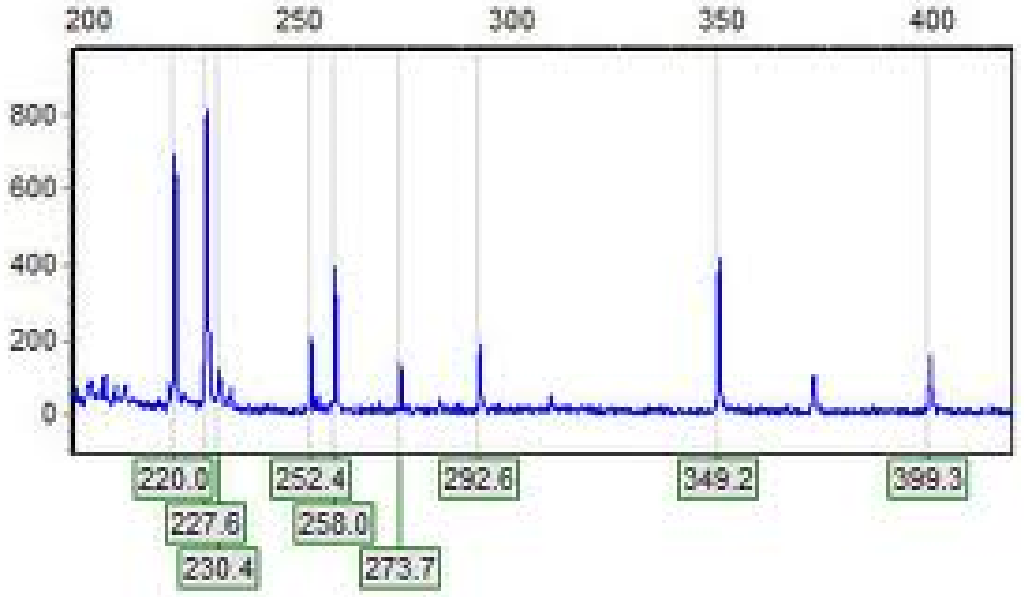
Aphalara itadori

- A psyllid biological control agent for Japanese knotweed has been released in the U.K. (Shaw et al. 2009) and Canada (and USA)
- Two strains of the psyllid that are being considered for use in Canada/USA have contrasting preferences for Japanese, giant and Bohemian knotweed (Grevstad et al. 2013).
- The psyllid also shows differential development on different Bohemian knotweed collections (Bourchier and Grevstad pers. obs.)

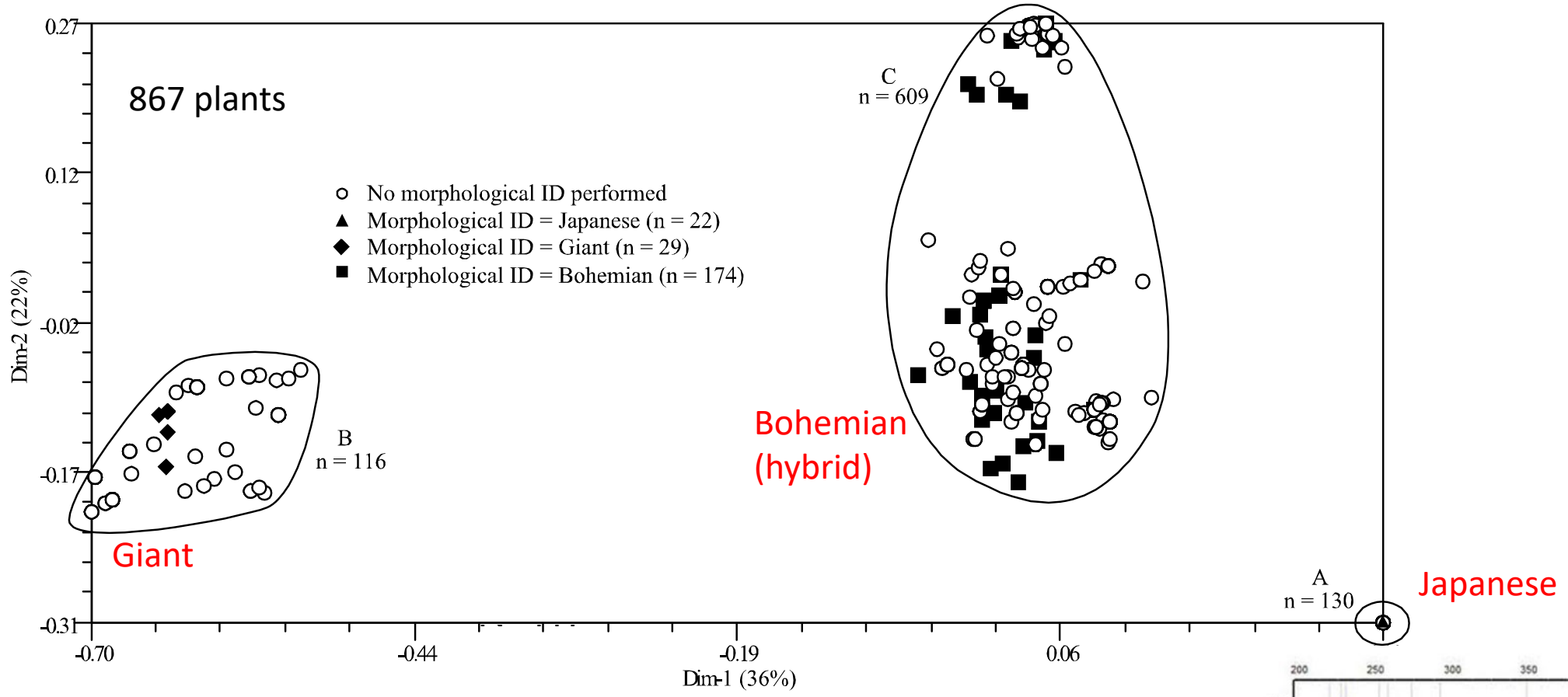
Key to Identification of Invasive Knotweeds in British Columbia

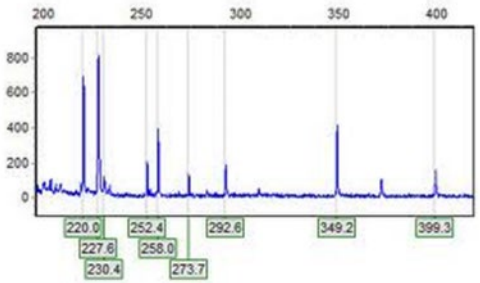
DNA Method

- Amplified Fragment Length Polymorphisms (AFLPs)
  - 135 repeatable, variable loci, presence/absence (1/0)



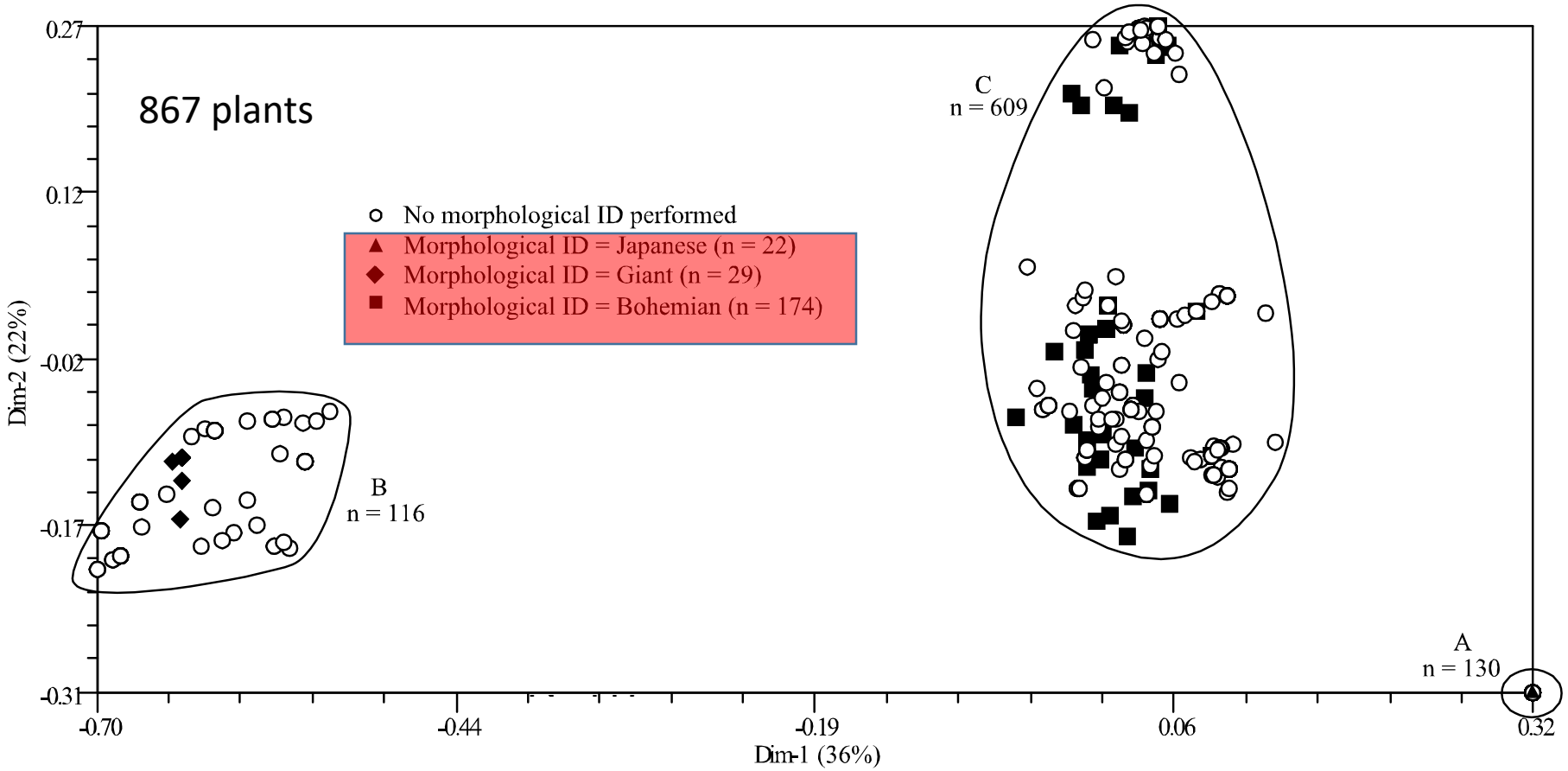
Principle coordinates analysis of AFLPs





Thanks to Jennifer Andreas for collections!

Principle coordinates analysis of AFLPs



Can managers identify species?

N=222 identified in field, even in mixed pops.

- How are the taxa distributed?

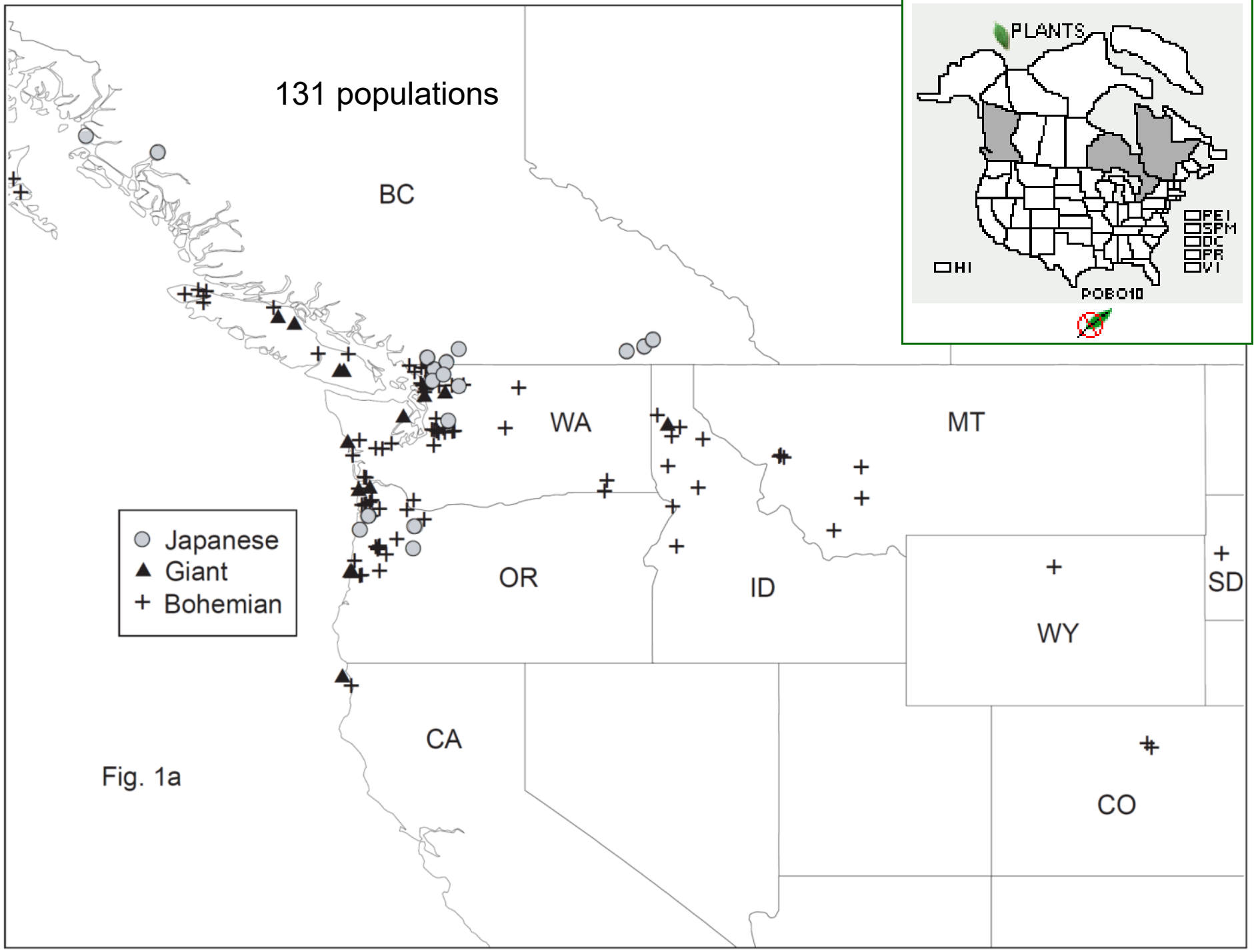
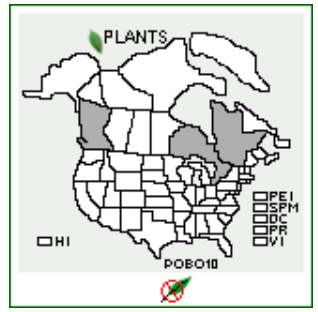

131 populations
BC
WA
MT
OR
ID
SD
WY
CA
CO
Japanese
Giant
Bohemian
PLANTS
PEI
SPM
DC
PR
VI
HI
POBO10

Fig. 1a

Implications for biocontrol in the Northwest?

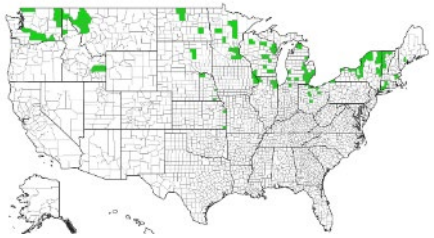
- Plants can be identified with morphology.
- Host-specificity test lineages are genotyped.
- Bohemian most common, most widespread, and most genetically diverse in PNW. Priority target??
- Japanese knotweed is one genotype (Same genotype as in UK).



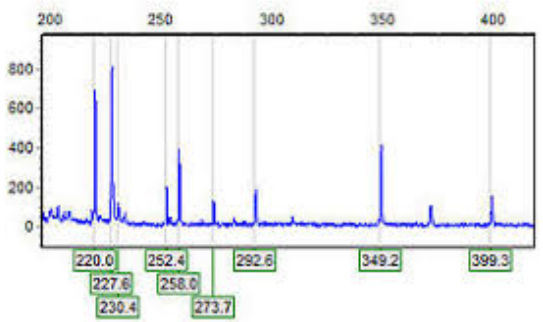
Questions that DNA can help answer

- What species is this weed?
  - Know your enemy
- How does it reproduce?
  - Helps us design control options
- Are there different genotypes? Hybrids?
  - Differences in resistance or tolerance to control
- How are genotypes distributed?
  - What control to use where
- Where did the invasion come from?
  - Finding host-specific enemies

Flowering rush
Butomus umbellatus



Butomus (flowering rush) AFLP genotype similarity - PCOA

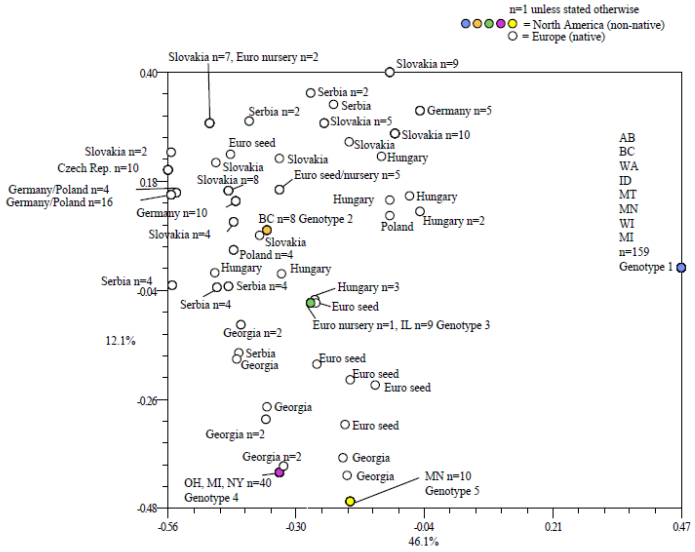


Slovakia n=7, Euro nursery n=2
Slovakia n=9
0.40
Serbia n=2
Serbia
Serbia n=2
Germany n=5
Slovakia n=5
Slovakia n=10
Euro seed
Slovakia
Slovakia n=2
Slovakia
Hungary
Czech Rep. n=10
Slovakia
Euro seed/nursery n=5
Slovakia n=8
0.18
Germany/Poland n=4
Germany/Poland n=16
Hungary
Hungary
Germany n=10
Poland
Hungary n=2
BC n=8
Slovakia n=4
Slovakia
Poland n=4
Hungary
Hungary
Serbia n=4
-0.04
Serbia n=4
Hungary n=3
Serbia n=4
Euro seed
Euro nursery n=1, IL n=9
Georgia n=2
12.1%
Serbia
Georgia
Euro seed
Euro seed
Euro seed
-0.26
Georgia
Georgia n=2
Euro seed
Georgia n=2
Georgia
Georgia
OH, MI, NY n=30
MI n=10
MN n=10
-0.48
-0.56
-0.30
-0.04
0.21
0.47
46.1%

AB
BC
WA
ID
MT
MN
WI
MI
n=159

n=1 unless stated otherwise
= North America (non-native)
= Europe (native)

Biocontrol with foreign disease (CABI UK)
Will a disease attack all of our *Butomus*?



No close genetic match in Eurasia for our genotype 1…yet.

- *Doassansia niesslii*
- Smut
- Does not attack our genotype 1…need to find 1 in Eurasia…collect disease from it

•Questions?

- John Gaskin
- USDA Agricultural Research Service, Sidney, Montana
- Population genetics, origins, and hybridization of invasive plants.

Saltcedar

Perennial pepperweed

Rush skeletonweed

Casuarina