Root-based Testing for Sweetpotato Viruses

Chris Clark
Department of Plant Pathology & Crop Physiology

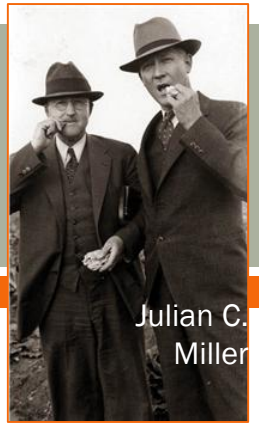
SPHI Sweetpotato Seed Systems and Crop Management Community of Practice
Kigali, Rwanda - May, 2018

Propagation is vegetative – from roots in temperate zones

Storage roots ‘seed’ are bedded in fields to sprout and cuttings are used to transplant. (Photos by Gerald Holmes)

Why Do We Do It?

- 1940's – to eliminate mutations
- 1999 – to manage viruses
- 2015 – joined the National Clean Plant Network



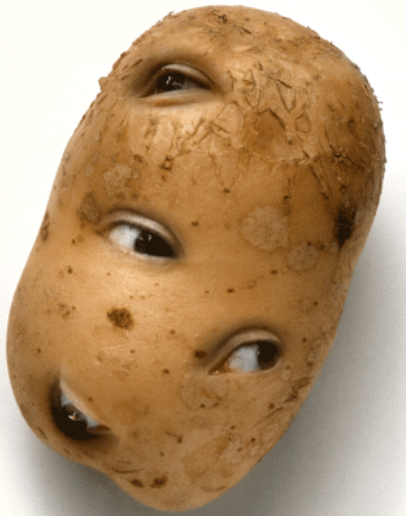
Prior to ~ 1930

Post breeding

A ‘Louisiana yam’ that is not a yam.

Miller, J. C. 1937. Inducing the sweet potato to bloom and set seed. J. Hered. 28:347-349.

Propagating from storage roots is an exercise in somaclonal variation.

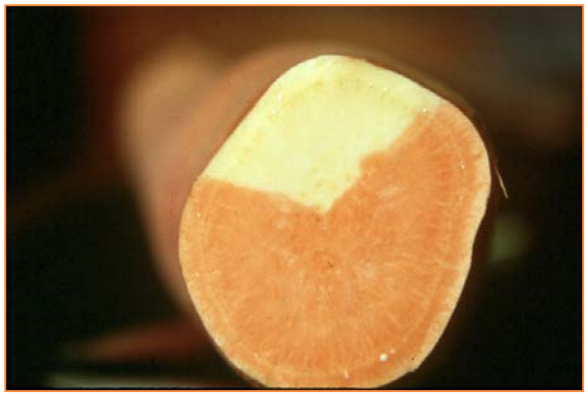


Dennys Diner - https://66.media.tumblr.com/4d9b74c64410af20b7e7b5ea8af340fb/tumblr_o6lwhazD9J1qez3nzo1_500.gif

The "eyes" on this Yukon Gold seed potato have already begun to sprout. Source: Charlotte Glen, NCSU, CES

Potatoes make tubers that have 'eyes'. Sweetpotatoes make storage roots, **not tubers**! and do not have eyes with preformed buds.

Mutations were the main worry…



Genetic Variation among Sweetpotatoes Propagated through Nodal and Adventitious Sprouts

Arthur Q. Villordon[1] and Don R. LaBonte[2]
Department of Horticulture, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, LA 70803

Additional index words. *Ipomoea batatas*, arbitrary primers, RAPD/AP-PCR, clonal variability, tissue culture

Abstract. **Genetic uniformity was assessed among sweetpotato (*Ipomoea batatas*) clones propagated through adventitious and nodal procedures. A single sprout each of 'Jewel,' 'Sumor,' and L87-95 was used as source of clonal plants that were simultaneously propagated through conventional adventitious procedures and a tissue culture-based nodal culture technique. A sample of 15 decamer primers generated 64 scorable amplified fragments in a PCR-based assay, 29 of which were putatively polymorphic across n = 60 samples (10 each of nodal and adventitiously derived plants/genotype). Within adventitiously derived materials, putative polymorphisms ranged from 4.7% to 31.3% depending on the genotypic class. In contrast, putative polymorphisms ranged from 0.0% to 3.1% among nodally derived samples. Marker loci differentiated genotypes as well as putative marker phenotype variants through a multidimensional scaling analysis of the genetic similarity matrix. An 'analysis of molecular variance' shows that genotypic effects accounted for 88.7% of the total molecular marker variability, while propagation effects (within genotypic groups) accounted for 11.3%. Results confirm that clonal plants derived from preexisting meristematic regions are more genetically uniform than plants propagated from adventitious origins.**

Sweetpotato 'Beauregard' Mericlones Vary in Yield, Vine Characteristics, and Storage Root Size and Shape Attributes

A.Q. Villordon[1], J.M. Cannon[2], H.L. Carroll[3], and J.W. Franklin[3]
Sweet Potato Research Station, LSU AgCenter, Chase, LA 71324

C.A. Clark[4]
Department of Plant Pathology and Crop Physiology, LSU AgCenter, Baton Rouge, LA 70803

D.R. LaBonte[4]
Department of Horticulture, LSU AgCenter, Baton Rouge, LA 70803

Additional index words. virus-tested, foundation seed program

Abstract. **Yield tests and evaluation of selected storage root and vine characters were conducted among 12 'Beauregard' sweetpotato [*Ipomoea batatas* (L.) Lam.] mericlones. Maximum yield differences were 43%, 48%, 79%, and 40% for U.S. #1, canners, jumbos, and total marketable yield, respectively. Additive main effect and multiplicative interaction (AMMI) biplot analysis was useful in graphically presenting the yield differences and stability patterns of mericlones. Differences were also detected in vine length, internode diameter, and internode length. Digital image analysis of U.S. #1 storage roots also revealed differences in storage root minor axis length, roundness, and elongation attributes. The results provide valuable information for enhancing current methods of evaluation and selection of mericlones for inclusion in sweetpotato foundation seed programs.**

...and foundation seed programs started in 1940's to manage mutations.

Louisiana's Sweetpotato Foundation Seed Program

W.A. Mulkey and
J.H. Hernandez

Additional index words. breeder seed, mutation, variety purity, flesh color, rogue, vine cuttings

Summary. Foundation sweetpotato [*Ipomoea batatas* (L.) Lam] seedstock has been produced annually at the Sweet Potato Research Station since 1949. Breeder seedstock is selected from superior hills and used for the following year's foundation seedstock. Fields are intensely monitored after planting until harvest to remove off-type plants, mutations, etc. Seedstock is harvested from August through October, stored, graded, and repacked beginning in late January, and then made available to the growers during the early spring.

Fig. 1. Breeder seedstock hill selection.

Fig. 2. Slicing hill selections to check for internal disorders and internal color.

Vegetative propagation leads to Cultivar Decline

Beauregard

Virus-tested

Naturally Infected

Potyviruses reduce yield in LA

- Four potyviruses are common:
  - SPFMV
  - SPVC
  - SPVG
  - SPV2
- Cleaning up Beauregard:
  - Yield reductions up to 42%, mean = 23%

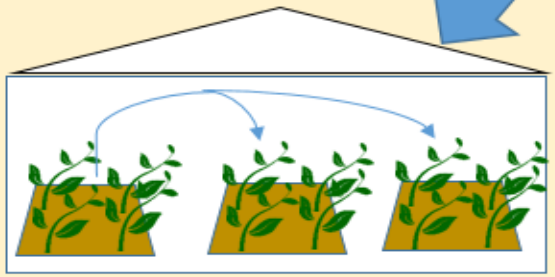
Clean Plant Production in Louisiana

Increased capacity is currently possible at this step

Phase 1

1. Virus tested meristem produced

***2. In vitro* Increase** – Production is 2,000 - 4,000 *in vitro* plants/ meristem



3. Greenhouse Increase –Plant cuttings in protected greenhouse; G0; Production is 200 – 1000 plants/ *in vitro* plant (400,000 – 4,000,000 cuttings/ meristem)

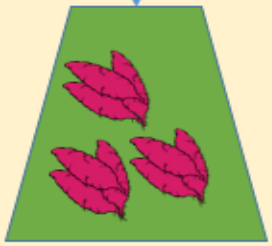
Phase 2

4. Field Increase at LA Sweet Potato Research Station; G1;Plant cuttings

5. Harvest roots, inspect and sell to growers

Phase 3

6. Grower Increase - Grower plants potatoes in early spring in field, G2; harvests sprouts in late spring; Each potato makes 5 sprouts



7. Production Planting - Grower plants sprouts in spring of year 3, harvest potatoes in fall

8. Potatoes sold to consumer

LSU AgCenter Sweetpotato Foundation Seed Program - 1999

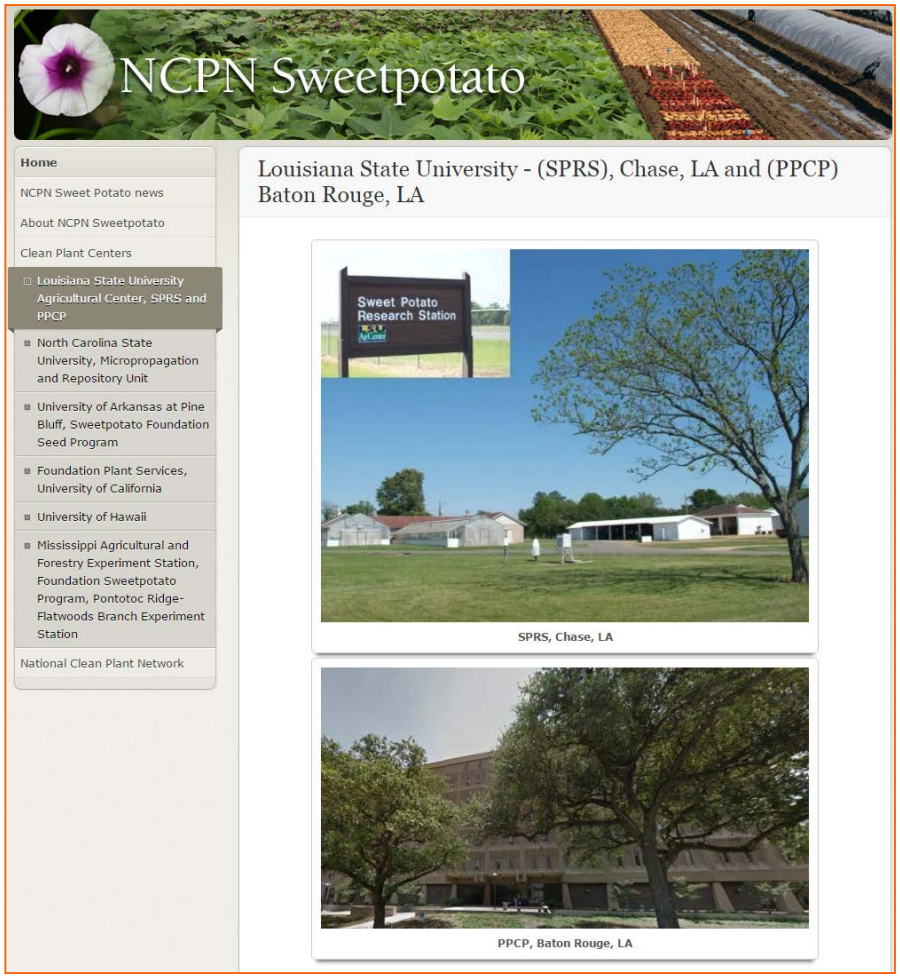
Mission and Foundation Seed Programs

more... > Mission and Foundation Seed Programs >

Mission and Foundation Seed Program

The Louisiana State University Foundation Seed Program began in 1934. The LSU AgCenter Sweet Potato Research Station, located in Chase, La., was established in 1948 through a direct appropriation of the Louisiana legislature. The mission of the station was to produce top-quality planting seed to serve the commercial sweet potato industry in our state. In 1999 the foundation seed program was upgraded to a virus-tested foundation seed program.

Sweetpotato added to NCPN in 2015

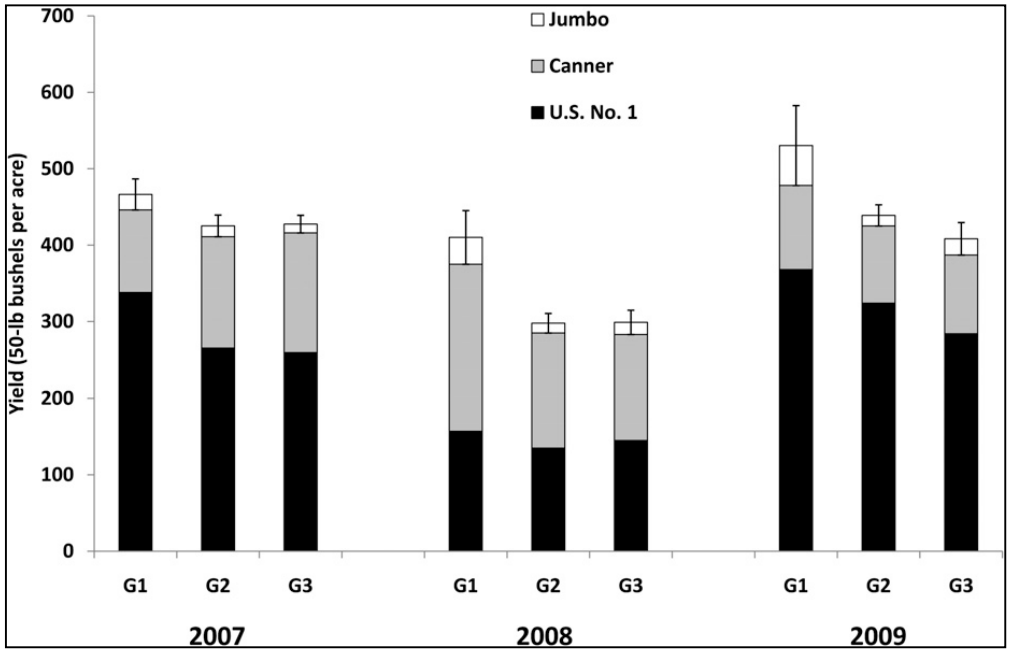


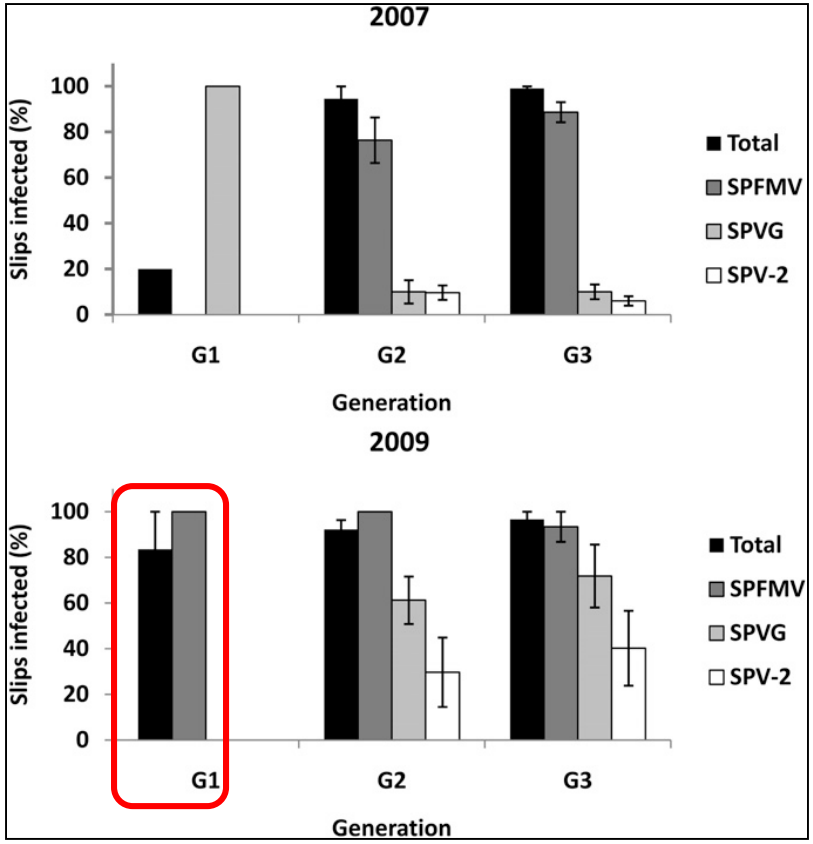
http://ucanr.edu/sites/ncpnsweetpotato/Clean_Plant_Centers/Louisiana_State_University/

Moving past the easy part

We have been able to produce virus-tested sweetpotatoes since at least the 1960's, but how do we deliver planting materials to farmers that remain clean and how do we know whether what they are receiving is clean?

But delivering on farm is not so easy.





Performance of Sweetpotato Foundation Seed after Incorporation into Commercial Operations in Louisiana

Christopher A. Clark[1,3], Tara P. Smith[2], Donald M. Ferrin[1], and Arthur Q. Villordon[2]

Bottlenecks to epidemiology studies

False negatives are common.
Individual plants are hard to assess.
Grafting is very cumbersome.

4-5 main vines, 20 feet long, >600 leaves

How can we evaluate outcomes?

Graft indexing is +/-, but which viruses are present?

SPFMV

Healthy

Simultaneous detection and differentiation of four closely related sweet potato potyviruses by a multiplex one-step RT-PCR

Fan Li[a,b], Ruijuan Zuo[b], Jorge Abad[c], Donglin Xu[a], Gaili Bao[b], Ruhui Li[a,*]

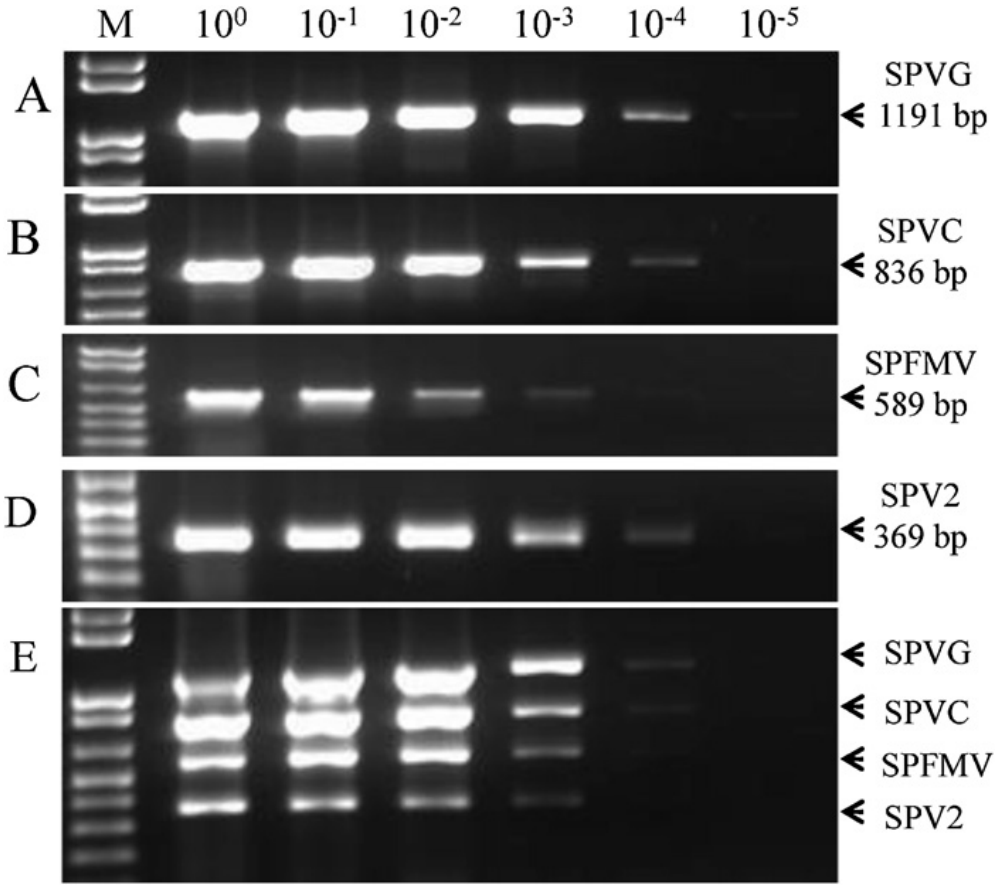
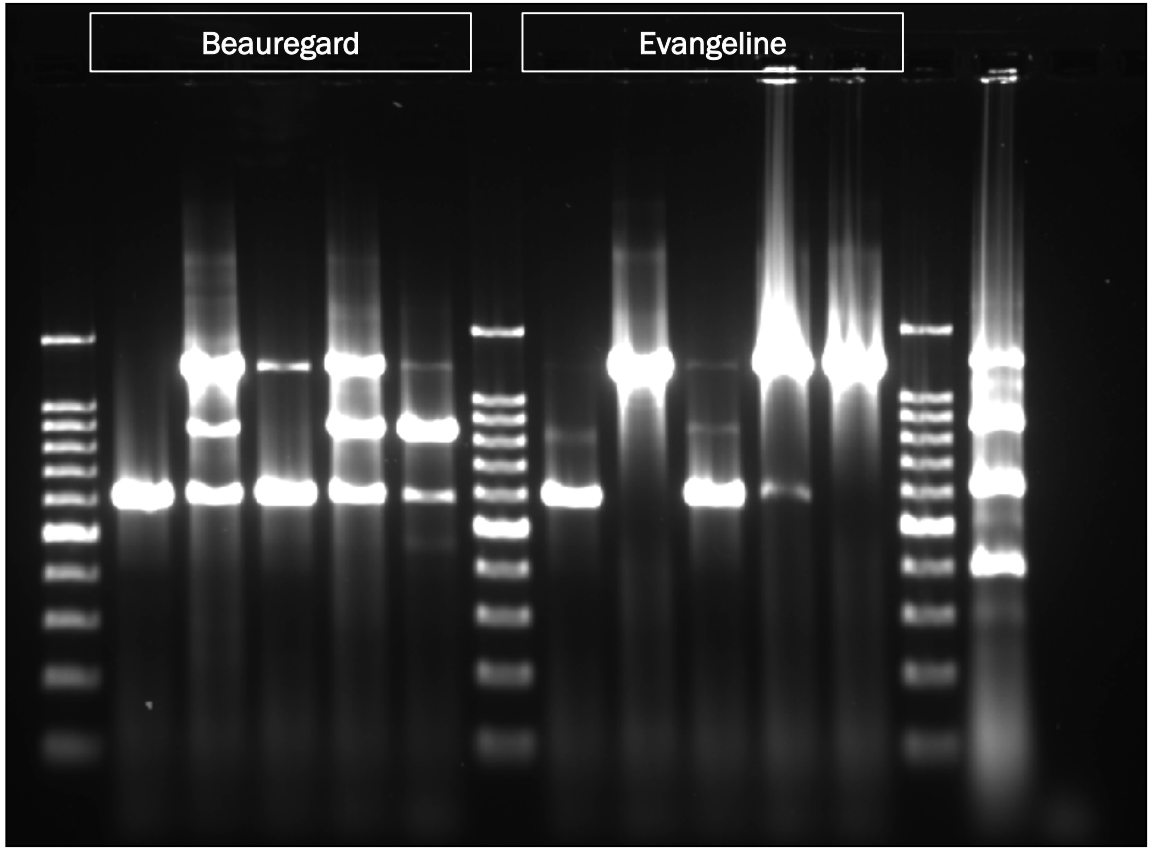


Fig. 2. Comparison of sensitivities of the uniplex and multiplex RT-PCR assays for the detection of Sweet potato feathery mottle virus (SPFMV), Sweet potato virus C (SPVC), Sweet potato virus G (SPVG) and Sweet potato virus 2 (SPV2). The target virus DNA fragment was amplified from 10-fold serial dilutions of extract from a sweet potato infected by all four target viruses by the uniplex RT-PCR for SPVG (A), SPVC (B), SPFMV (C) and SPV2 (D). The four target viruses were simultaneously amplified by the multiplex RT-PCR (E). Lane M was 1 kb plus DNA ladder.

Journal of Virological Methods 186 (2012) 161– 166

Seed roots can be assayed directly by PCR



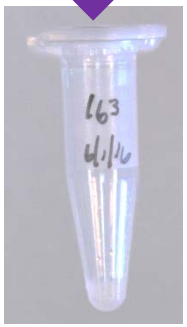
- SPVG
- SPVC
- SPFMV
- SPV2

LSU AgCenter Storage Root Indexing

Roots randomly collected during harvest, cured, stored.

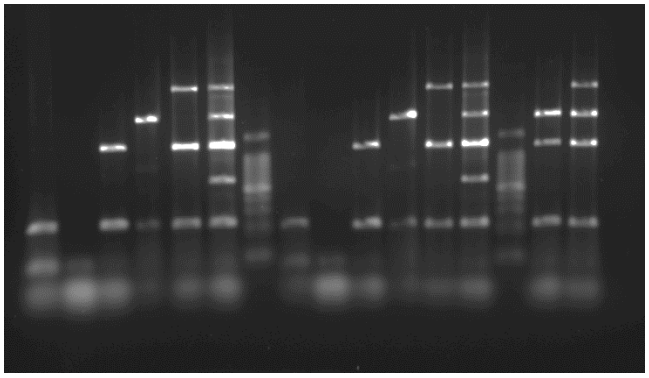
Cross section slice cut from proximal end with mandolin.

Slice placed in bag with CTAB buffer, homogenized.



Total nucleic acid extracted.

Potyvirus multiplex PCR performed.



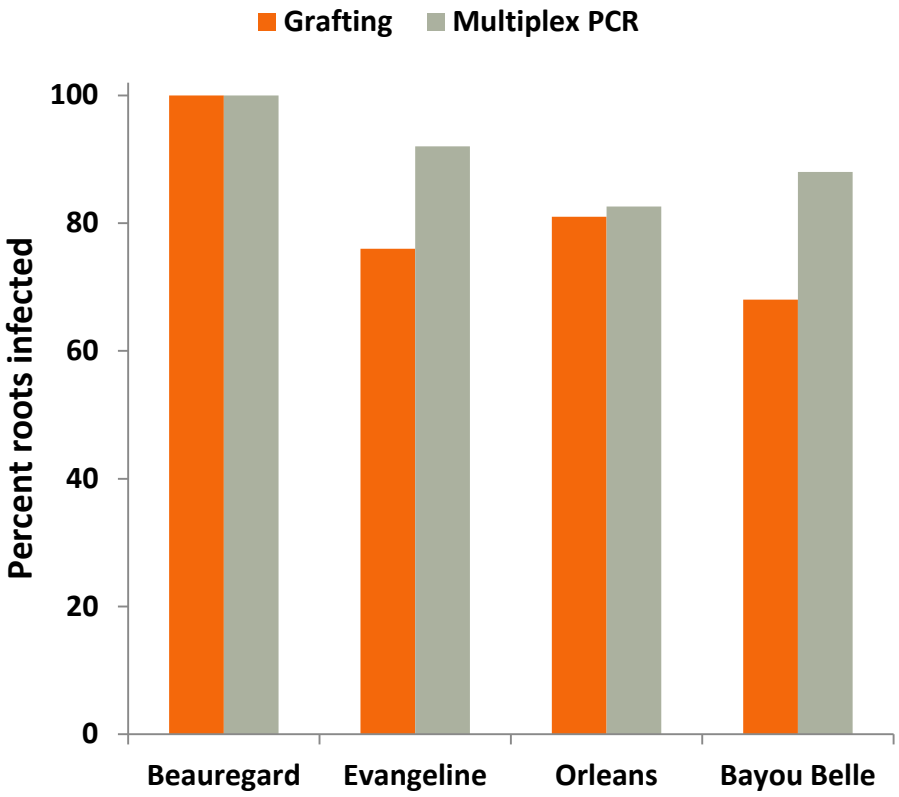
Products of PCR revealed by electrophoresis.

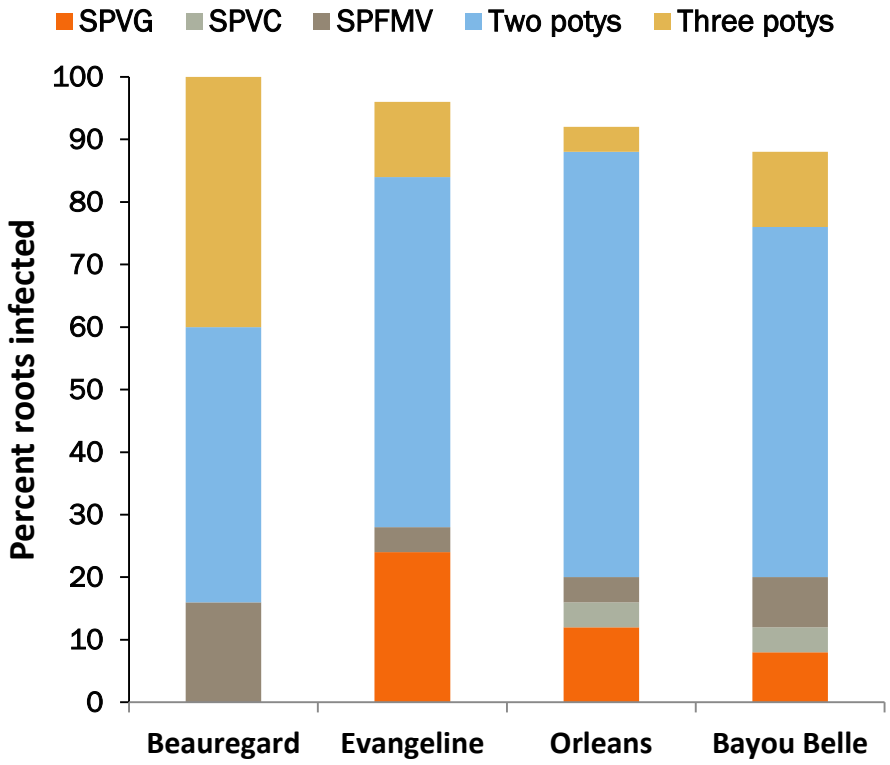
Small plot evaluations

Foundation seed tested as apparently virus-free were inoculated with SPFMV + SPVG +SPVC by core grafting (Inf) and compared with virus-tested (VT) in replicated plots at Burden.

PCR appears more sensitive than grafting

2014





Using Storage Roots

- **Advantages**
  - Higher titers? = fewer false negatives
  - Sampling strategy is less complicated
  - They are the product we sell
  - It allows procrastination

- **On the other hand:**
  - Not as convenient during growing season

- **Costs**
  - Time and supply costs should be similar for leaves vs. storage roots

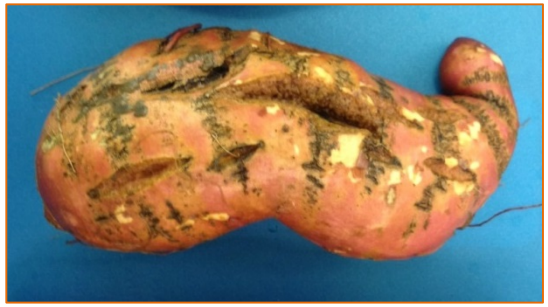
Making progress

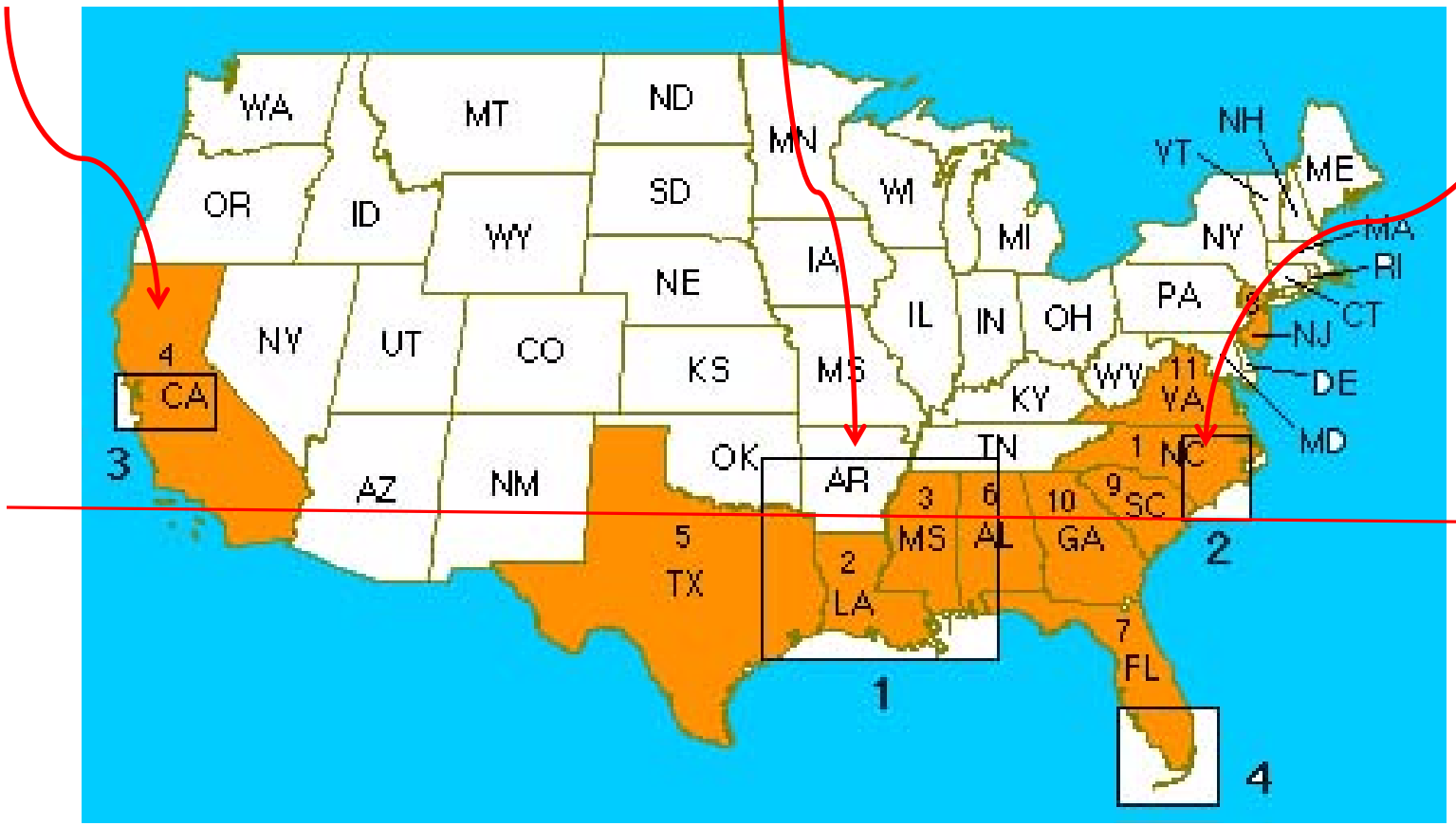
Steps taken:

- Producing research seed at separate location
- War on morning glories
- Rogueing
- Increased quality testing – move to PCR testing of seed roots, and beginning in 2016, testing of foundation plants in greenhouses.

	2012	2013	2014	2015	2016	2017
Number of seed lots tested	2	18	18	16	21	26
Number of seed lots with infected roots	2	10	3	1	4	2
Number of seed roots tested	125	523	745	380	499	622
Range (%) of seed roots infected	7-21	0-42	0-2	0-4	0-12.5	0-12.5
Overall mean % of seed infected	14	9	0.8	0.3	1.2	0.6

Questions?





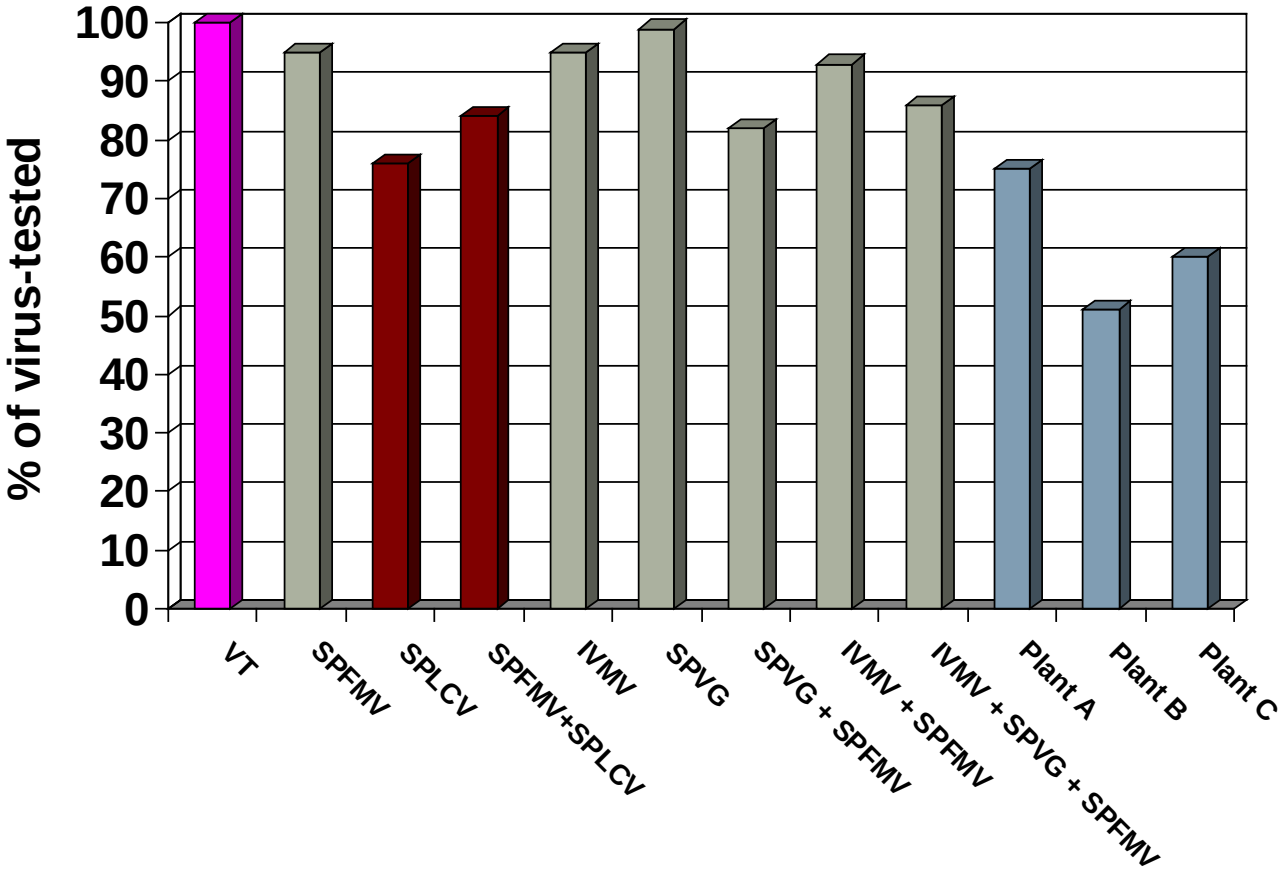
Map from Barry Duell's website - The ABCs of Sweetpotatoes in the USA

Virus reservoirs are abundant

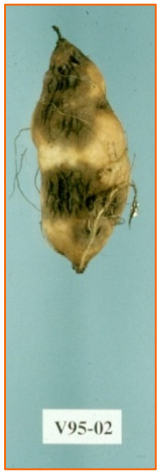
Morning glory hosts:
annuals
perennial

...but incidence on Beauregard and other lines is negligible in Louisiana.

16 replicated tests over 8 years

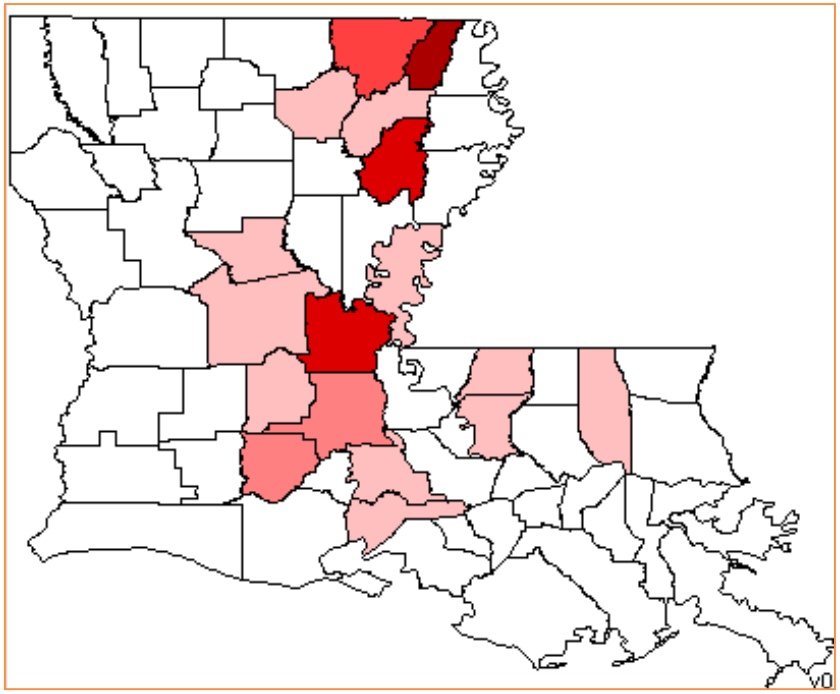


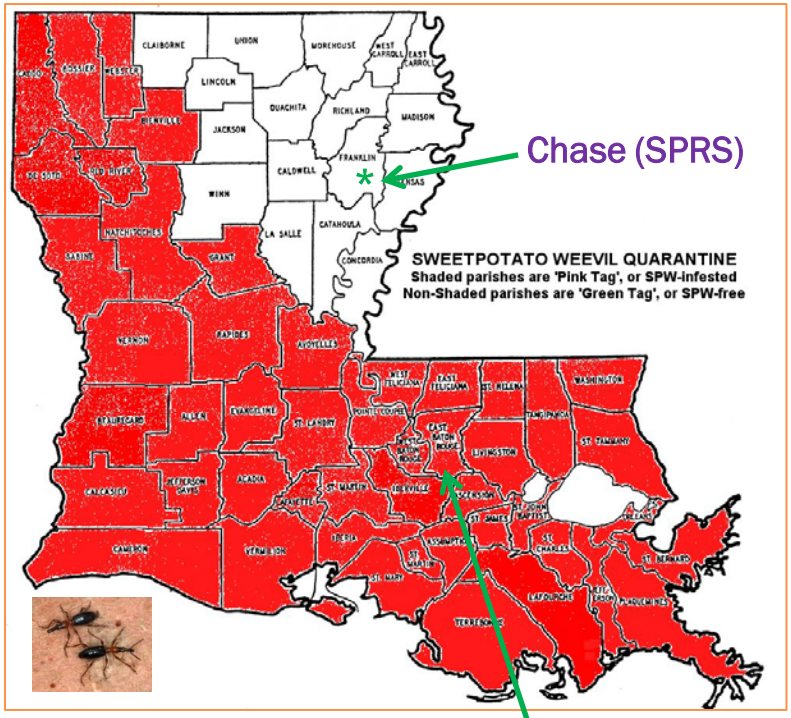
SPFMV = 95-2
No russet crack observed.



Clark, C. A., and Hoy, M. W. 2006. Effects of common viruses on yield and quality of Beauregard sweetpotato in Louisiana. Plant Dis. 90:83-88.

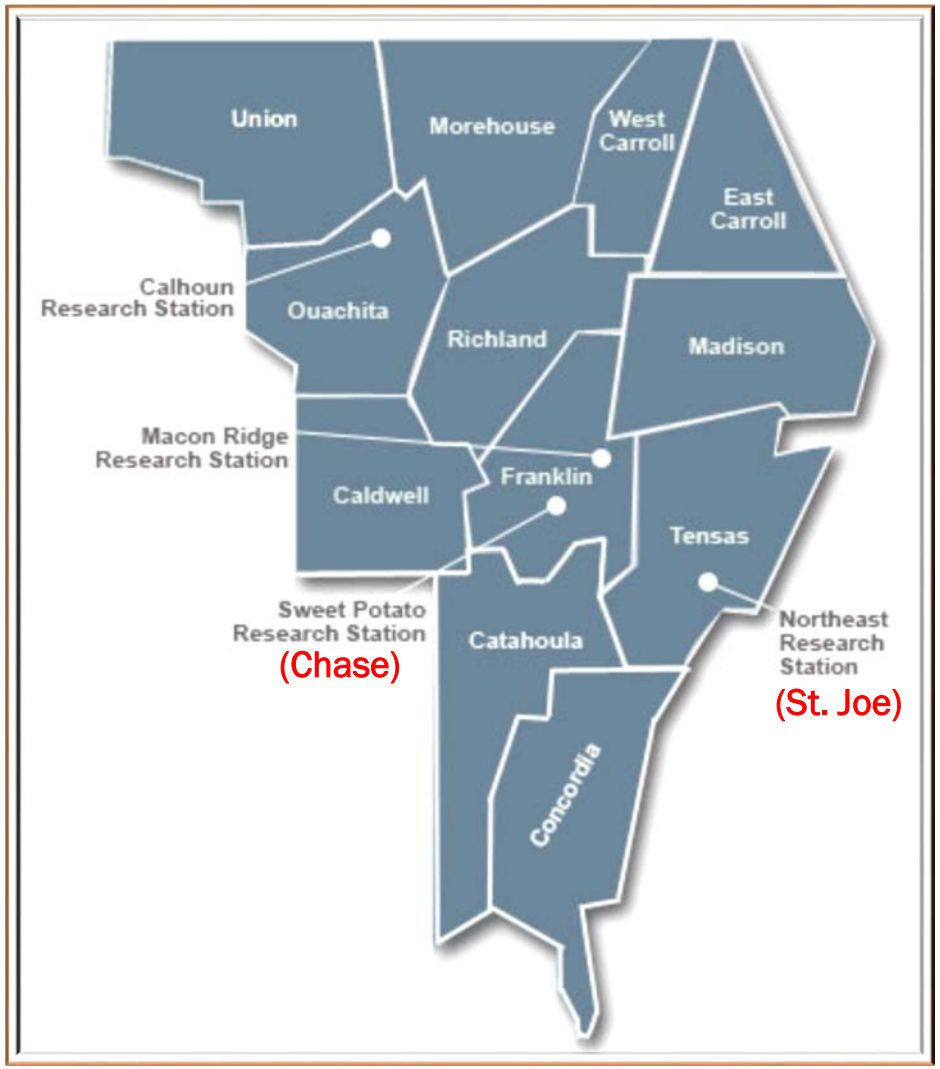
Seed is produced in weevil-free area

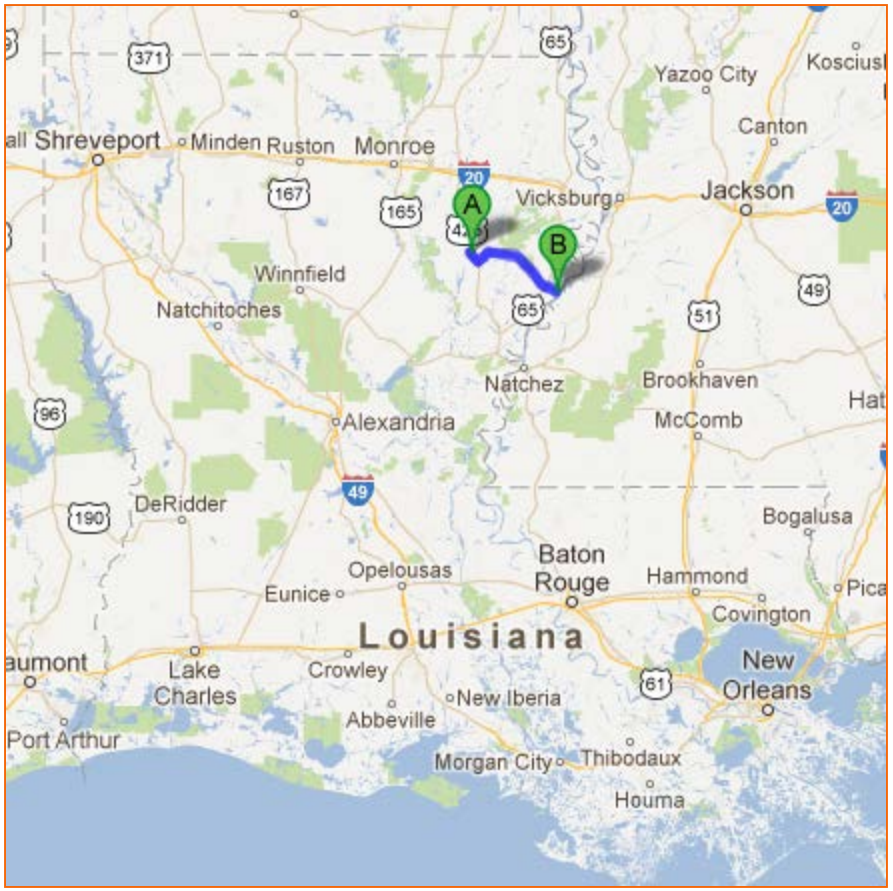




Maps courtesy Louisiana Dept. Agric. & Forestry

Producing 'seed' for research in isolation

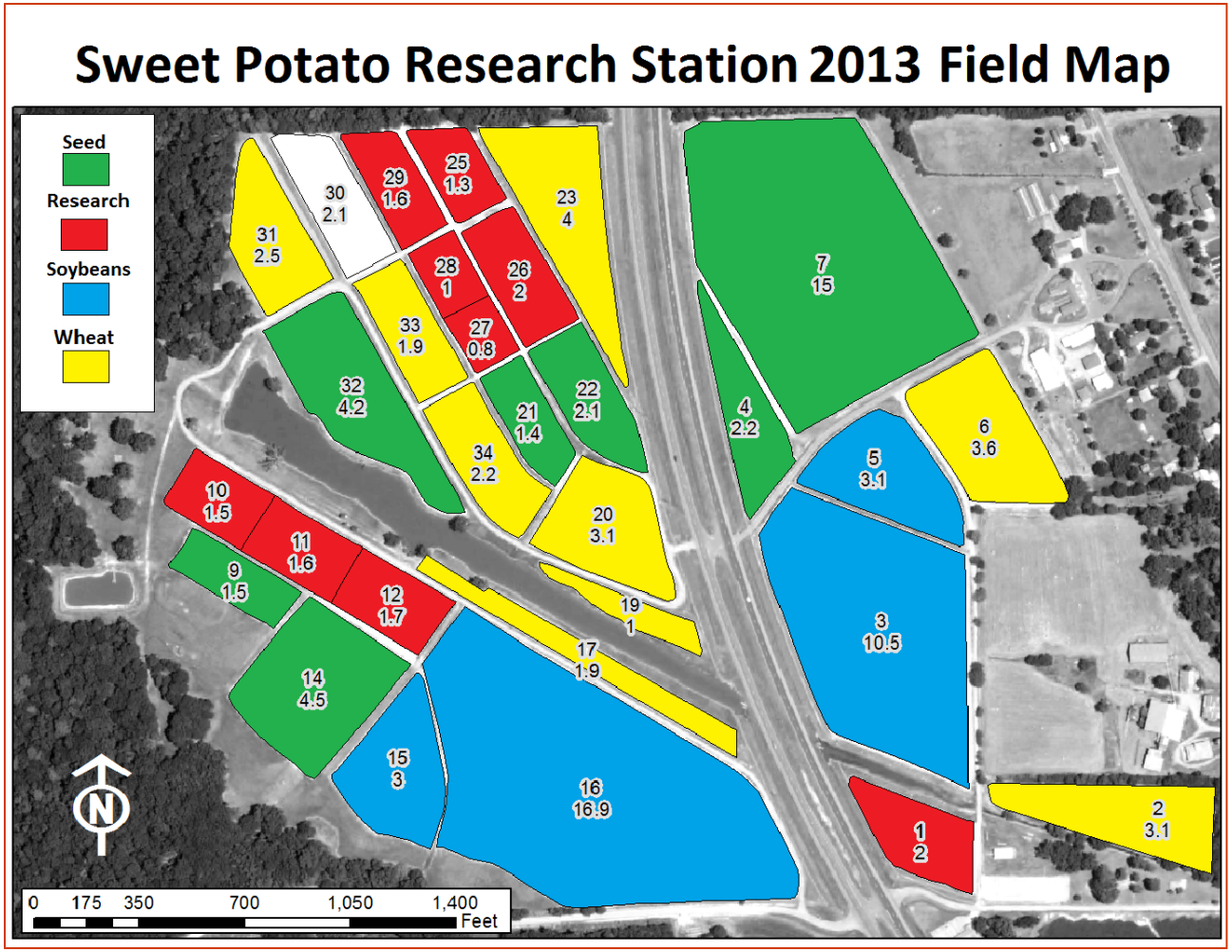




Indexing of 2013 foundation seed

Variety	Field	% Infected
Evangeline	4	0
07-146	7	7
B-14	7	41
B-63	7	42
Orleans	7	14
07-146	9	6
Orleans	14	3
B-14	21	14
B-63	22	0
07-146	32	0
07-146	MR	0
B-14	MR	13
B-63	MR	12
Orleans	MR	13
Beau - + ck	Burden	98

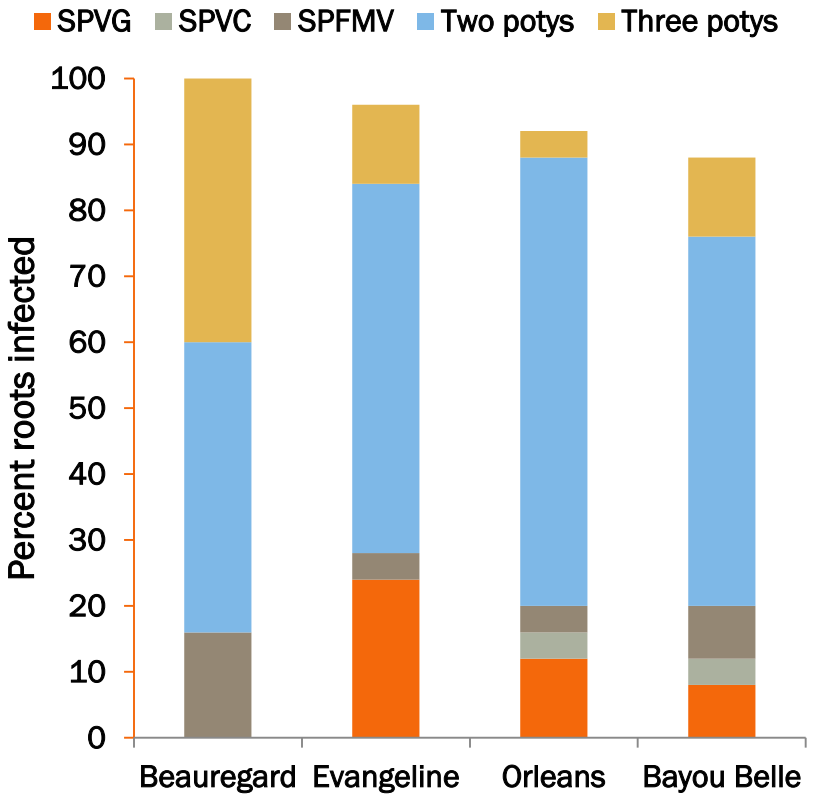
Virus	No. Infected	% of infected with
SPFMV	32	91.4
SPVC	3	8.6
SPVG	1	2.9
SPV2	4	11.4
Total	35	



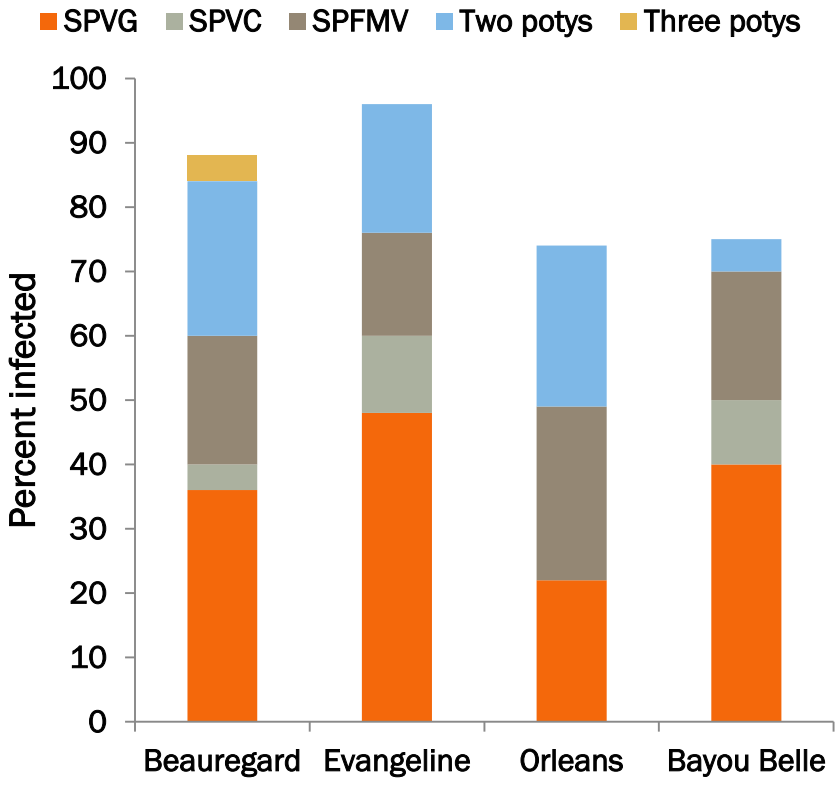
3-year rotation required by state certified seed law & regs.

Viruses transmitted vary by year and location

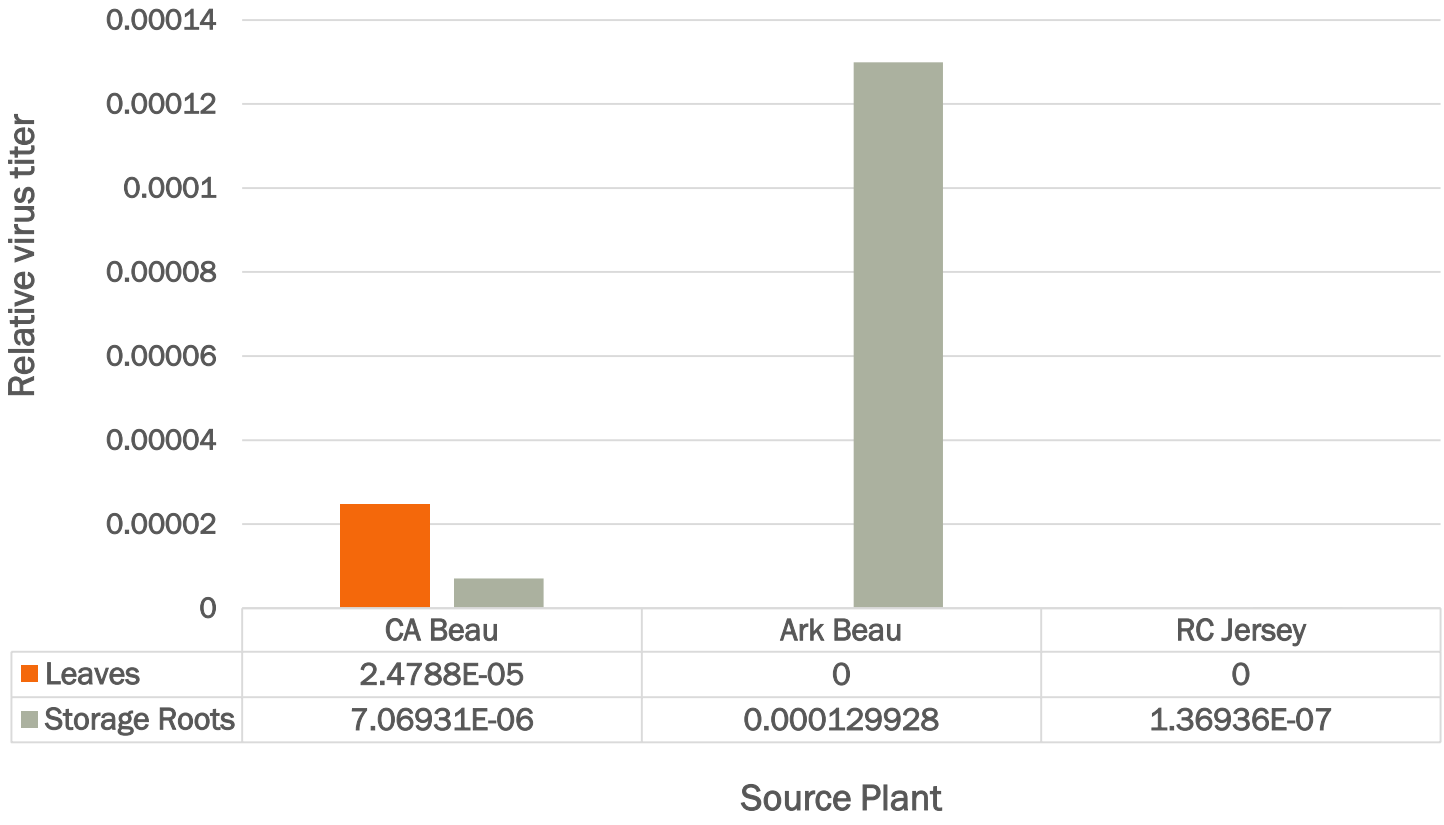
2014



2015



Virus Titers Need to be Studied



	CA Beau	Ark Beau	RC Jersey
Leaves	2.4788E-05	0	0
Storage Roots	7.06931E-06	0.000129928	1.36936E-07