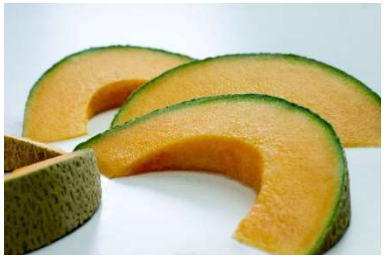

Sakata Seed America
SAKATA
SEED AMERICA, INC.
Quality · Reliability · Service

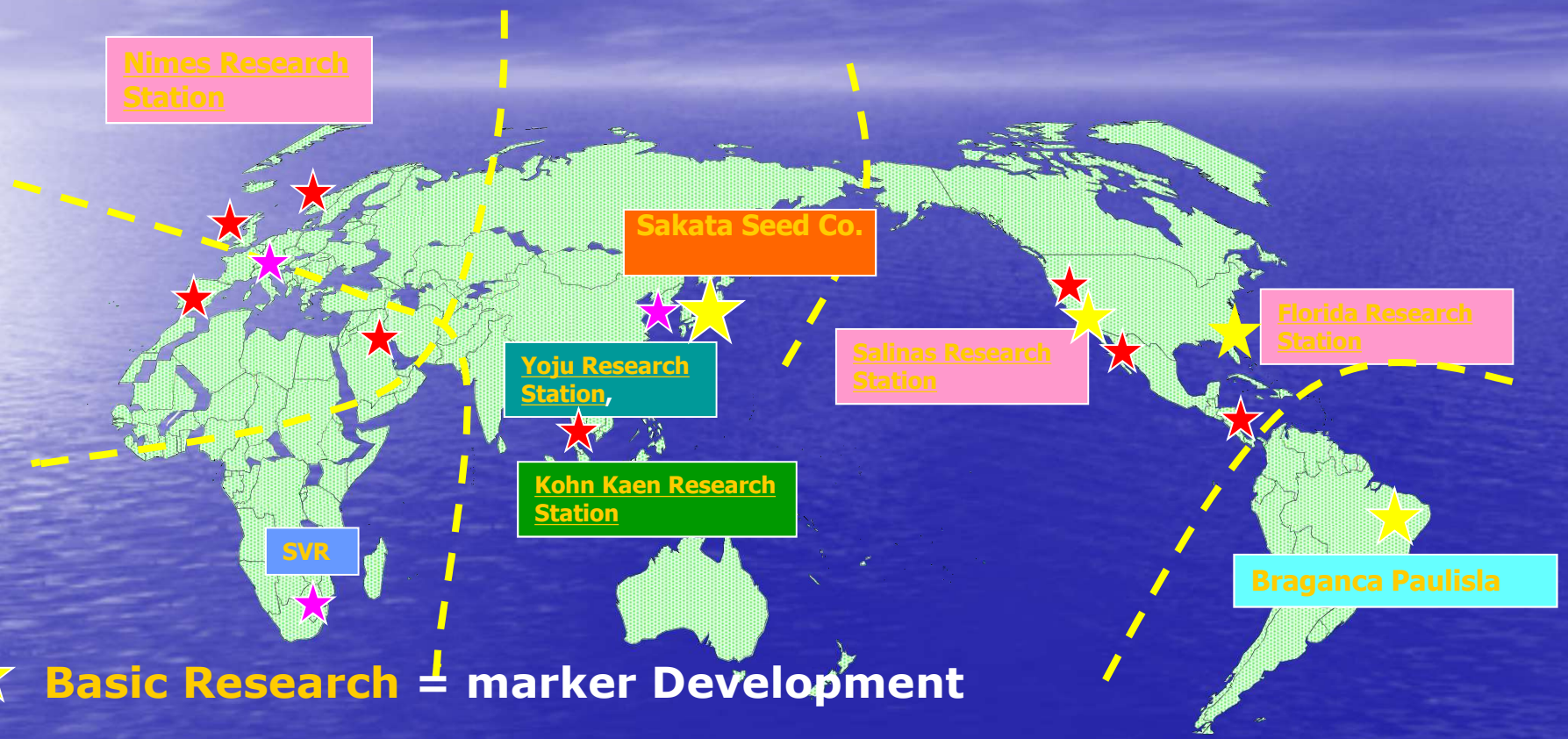
TOP VEGETABLE CROPS



TOP FLOWERS

Breeding Stations

Theoretical Territory



★ **Basic Research = marker Development**

★ **Facility for Practical screening =Hub station**

★ **Breeding station**

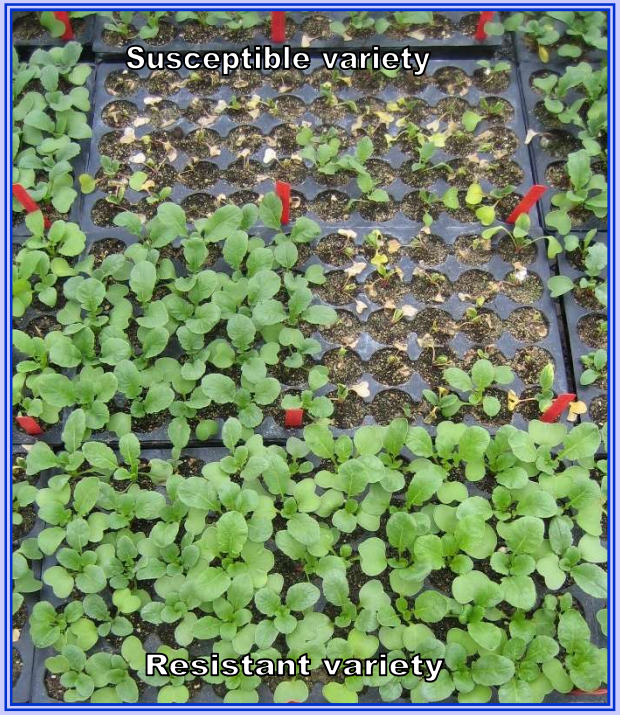

PLANT PATHOLOGY
The science of plant pathology contributes to the success of Sakata Seed by providing support in four major areas:
Breeder Support
Seed Production
Seed Health
Extension
SAKATA SEED AMERICA, INC.
Quality · Reliability · Service

SAKATA® SEED AMERICA, INC.

BREEDER SUPPORT

Inherent resistance of a plant to one or more diseases is often essential for the success of a variety. One key benefit may be a reduction in pesticide use. Breeder support involves the development and application of screening techniques to evaluate and select for resistance to improve varieties.

Differential reaction of tomato lines to the infection of TYLCV-Is in a greenhouse



Reaction of radish selections to the Rhizoctonia scurf pathogen 14 days after inoculation

Verticillium wilt in a Chinese cabbage field trial

SAKATA SEED AMERICA, INC.

SEED PRODUCTION

Field and seed-borne diseases can significantly reduce seed yield and quality. Seed-borne pathogens can also be transmitted to the next generation and cause crop damage. Pathologists work closely with the Production Department and Quality Control in developing strategies to minimize these potential threats.

Inspection of seed production fields

A susceptible broccoli inbred killed by Sclerotinia white mold

Downy mildew lesions on onion scapes

Bacterial blight (*Xanthomonas hortorum* pv. *carotae*) on carrot umbel and leaf

Xanthomonas leaf spot on cauliflower seedlings

Severe black rot infection in a cabbage field. Inset: typical lesion with black veins

SAKATA® SEED AMERICA, INC.

SEED HEALTH

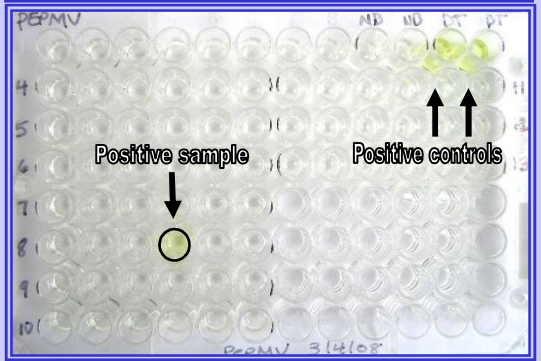
Sakata Seed has made a commitment to producing and marketing healthy seed, and has an extensive seed health testing program as part of its overall commitment to seed quality.

Suspect colonies of *Xanthomonas campestris* pv. *vesicatoria* on isolation medium

Cabbage seedling bioassay to identify virulent black rot bacteria (*Xanthomonas campestris* pv. *campestris*)

Seedling grow-outs for the detection of *Acidovorax avenae* subsp. *citrulli* (Aac) in contaminated seed. Aac lesions on melon (top) and watermelon seedlings

Evaluation of isolation media for the presence of suspect bacterial pathogens



Serological testing by ELISA is used to detect seedborne viruses. The yellow color indicates a positive reaction; colorless wells are negative

SAKATA SEED AMERICA, INC.

EXTENSION

As part of its overall commitment to servicing our customers and growers, Sakata provides technical support, including disease information, prevention, and control. This information may be provided in the form of seminars, technical sheets, or direct advice to the customer.

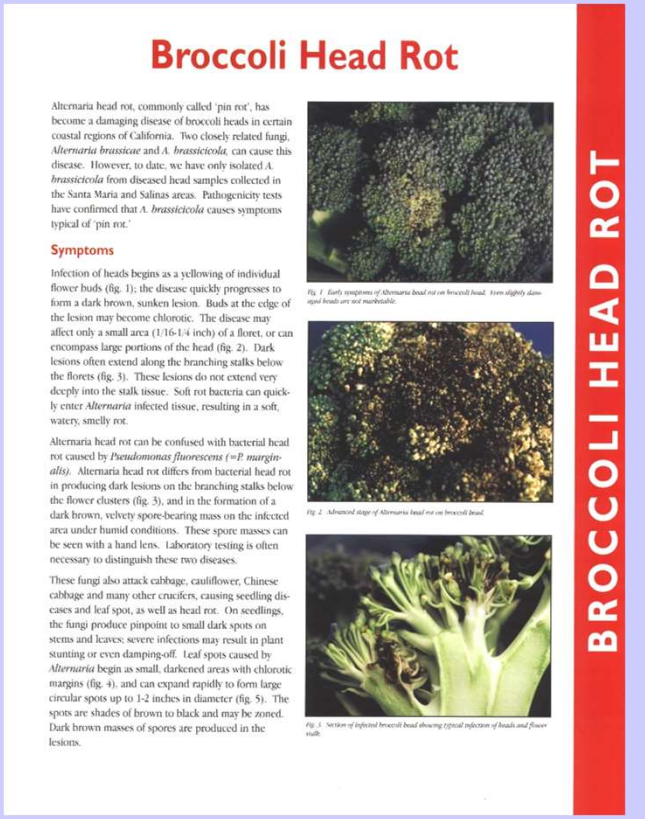
Botrytis blight (*B. cinerea*) on Vinca plants in a greenhouse. Inset: symptoms close-up

Beet field damaged by downy mildew caused by *Peronospora parasitica* f.sp. *beta*

Turnip field damaged by *P. syringae* pv. *maculicola*. Inset: peppery leaf spot lesion

Diagnosis of samples submitted to the clinic

Information on disease etiology, transmission, and control may be provided in the form of a technical sheet



Broccoli Head Rot

Alternaria head rot, commonly called 'pin rot', has become a damaging disease of broccoli heads in certain coastal regions of California. Two closely related fungi, *Alternaria brassicae* and *A. brassicicola*, can cause this disease. However, to date, we have only isolated *A. brassicicola* from diseased head samples collected in the Santa Maria and Salinas areas. Pathogenicity tests have confirmed that *A. brassicicola* causes symptoms typical of 'pin rot.'

Symptoms

Infection of heads begins as a yellowing of individual flower buds (fig. 1); the disease quickly progresses to form a dark brown, sunken lesion. Buds at the edge of the lesion may become chlorotic. The disease may affect only a small area (1/16-1/4 inch) of a floret, or can encompass large portions of the head (fig. 2). Dark lesions often extend along the branching stalks below the florets (fig. 3). These lesions do not extend very deeply into the stalk tissue. Soft rot bacteria can quickly enter *Alternaria* infected tissue, resulting in a soft, watery, smelly rot.

Alternaria head rot can be confused with bacterial head rot caused by *Pseudomonas fluorescens* (=*P. marginalis*). Alternaria head rot differs from bacterial head rot in producing dark lesions on the branching stalks below the flower clusters (fig. 3), and in the formation of a dark brown, velvety spore-bearing mass on the infected area under humid conditions. These spore masses can be seen with a hand lens. Laboratory testing is often necessary to distinguish these two diseases.

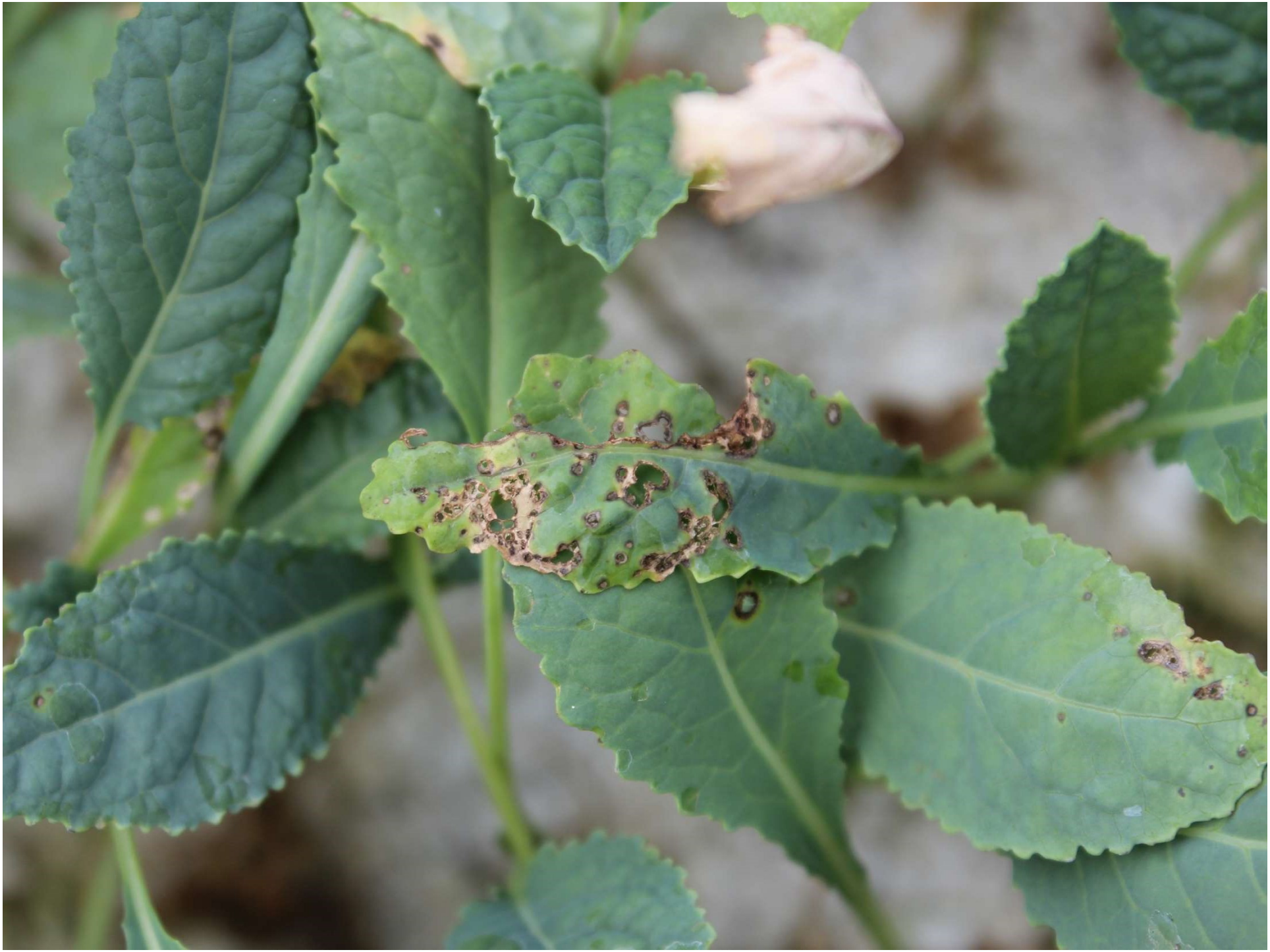
These fungi also attack cabbage, cauliflower, Chinese cabbage and many other crucifers, causing seedling diseases and leaf spot, as well as head rot. On seedlings, the fungi produce pinpoint to small dark spots on stems and leaves; severe infections may result in plant stunting or even damping-off. Leaf spots caused by *Alternaria* begin as small, darkened areas with chlorotic margins (fig. 4), and can expand rapidly to form large circular spots up to 1-2 inches in diameter (fig. 5). The spots are shades of brown to black and may be zoned. Dark brown masses of spores are produced in the lesions.

Fig. 1. Early symptoms of Alternaria head rot on broccoli head. Even slightly damaged heads are not marketable.

Fig. 2. Advanced stage of Alternaria head rot on broccoli head.

Fig. 3. Section of infected broccoli head showing typical infection of heads and flower stalk.

BROCCOLI HEAD ROT




SAKATA
Fort Myers, Florida

Main Sakata FL Campus

SAI Florida Pathology

- Randy Johnson (Branch Manager)
- Marco Bello (Lead Plant Pathologist)
- Traven Bentley (Plant Pathologist)


NOTICE
GAS
SHUT OFF

SAI Florida Pathology

- 70% Breeder support
- 15% Quality Assurance
- 8% Extension
- 7% Administrative

Current Disease Screens

Crop	Disease	Location
BC	Pinrot, downy mildew	CA
BT/SC	Downy mildew, Rhizoctonia, Cercospora, Pythium, yellows	WA
CB	Black rot	FL
CF	Verticillium	CA
CR	Nematodes	CA
	Alternaria	FL
ME	Powdery mildew (1, 2)	FL CA
	Gummy stem blight, downy mildew, Fusarium (1, 2), ZYMV, PRSV, CYSDV	FL
ON	Fusarium basal rot, pink root	NM
PP	Tobamo (*L1, L3, L4*),	CA
	Phytophthora	CA, FL
	Bacterial spot (*Xcvr 1- 6*), *Mi3*, PVY (0.1, 0.1.2), TEV, TSWV	FL
RA	Rhizoctonia, yellows	CA
SP	Downy mildew (4 - 7, 10)	WA
SQ	WMV, PRSV, ZYMV, CMV	FL
TM	ASC, Stemphylium, Vert 1, crown rot, Fus. wilt (I, I2, I3), RKN, corky root, speck, ToMV	FL
	TYLCV, TSWV, Xcv (?)	FL
WM	Anthracnose 1, GSB, Fus. wilt 1, WMV, PRSV, ZYMV	FL

Resistance: SAI definition

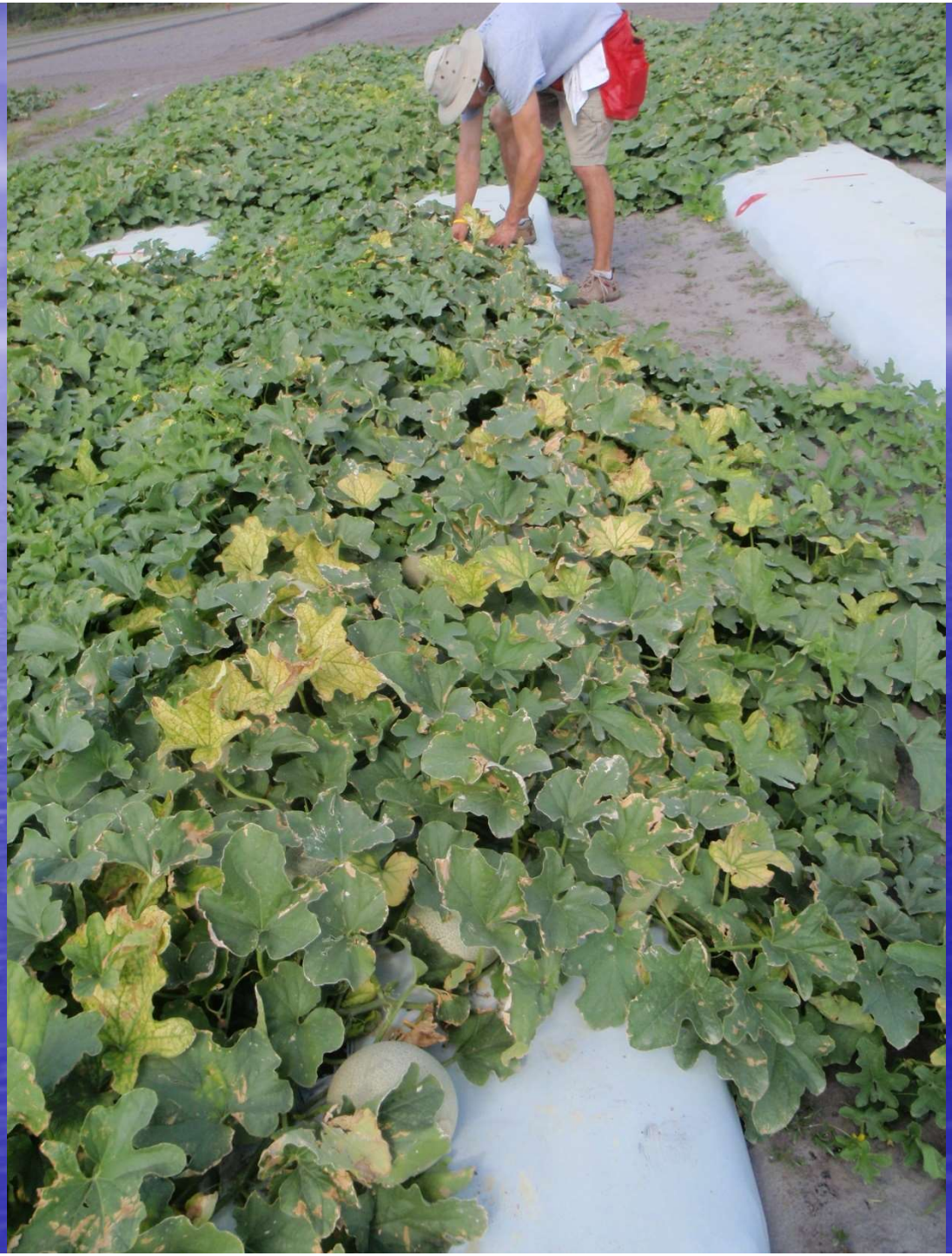
- "The ability of a plant to restrict attack by a specified pathogen or pest when compared to the reaction of susceptible plants grown under similar environmental conditions and pathogen or pest pressure. A resistant plant is expected to suffer very minor damage, but may exhibit some symptoms under heavy pathogen/pest pressure"

Types of resistance

- Immunity
- Classic single dominant genes (vertical)
- Incomplete dominance
- Recessive genes
- Polygenic/quantitative (horizontal)
- Background/vigor
- Systemically acquired resistance
- Cross protection
- GMO's

Resistance Breeding Considerations

- Exotic versus endemic pathogens
- Economic impact of disease
- Production or grower benefits?
- Economic value of Res. or liability of sus.
- Source(s) of resistance available
- Seedling, MAS, Lab, whole plant?
- Selection versus index tests
- Core Disease resistance package
- How/if a claim should be made



Is the resistance relevant?

- Resistance to the pathogen and/or disease
- Years required to achieve commercial resistance or break undesirable linkage
- Grower acceptance
- Screening in the absence of the disease pyramid (vectors-envir.-host-pathogen)
- Disease package – opportunity?

Screening strategy

- World wide ring tests = breadth of resistance
- HR-type resistance = excised leaf
- Poor resolution or difficult tests = MAS?
- Polygenic resistance may need to be assessed under field conditions
- Breed for hort or disease first?
- Population breeding


Jupiter

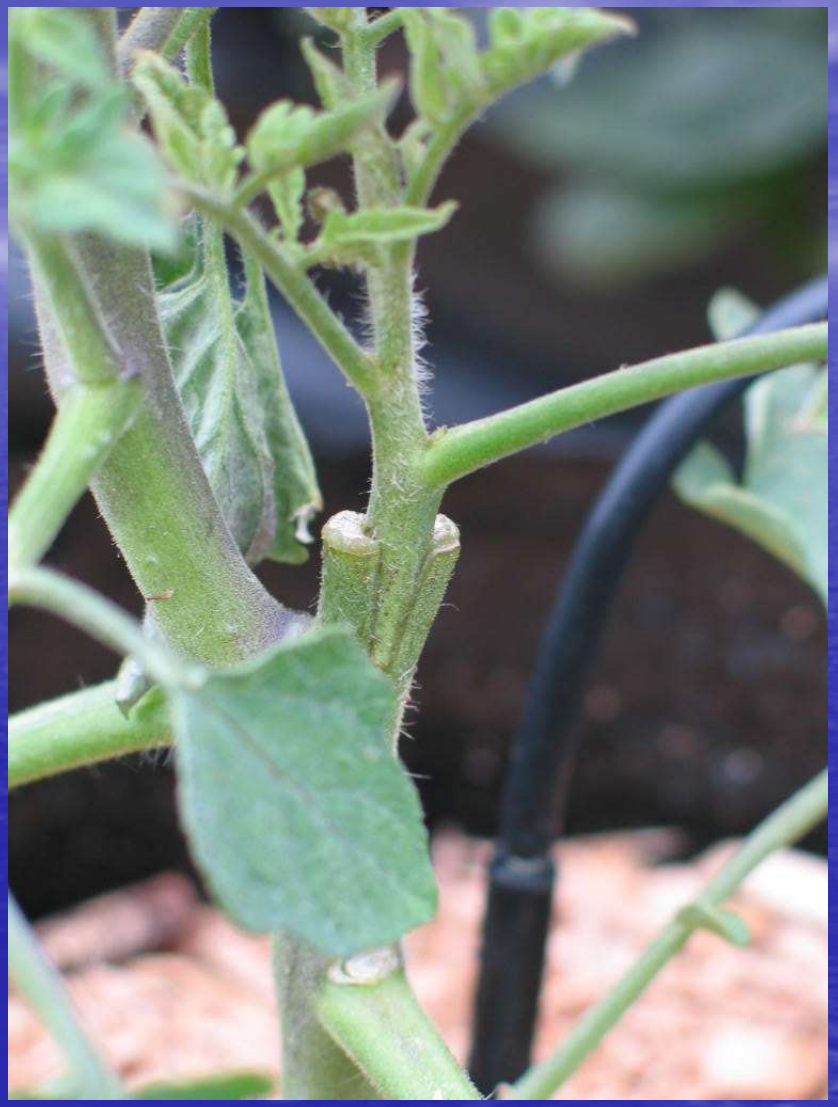

TSWV HR

TYLCV and Whitefly rearing cages


TYLCV Inoculation cages


FE 77 UNINOC


Un-inoculated
Inoculated
UN-INOC



TYLCV – degrees of res./sus.

- Plant/seedling "shut down" (sus)
- Some flower abortions and foliar symptoms and some yield reductions
- Yield OK, but foliar symptoms noted
- No symptoms seen and yield unchanged (Res)
- No symptoms and no virus detected (immune)


SSX 6940
SSX 6940
PRSV


PSDG1
(Zym)

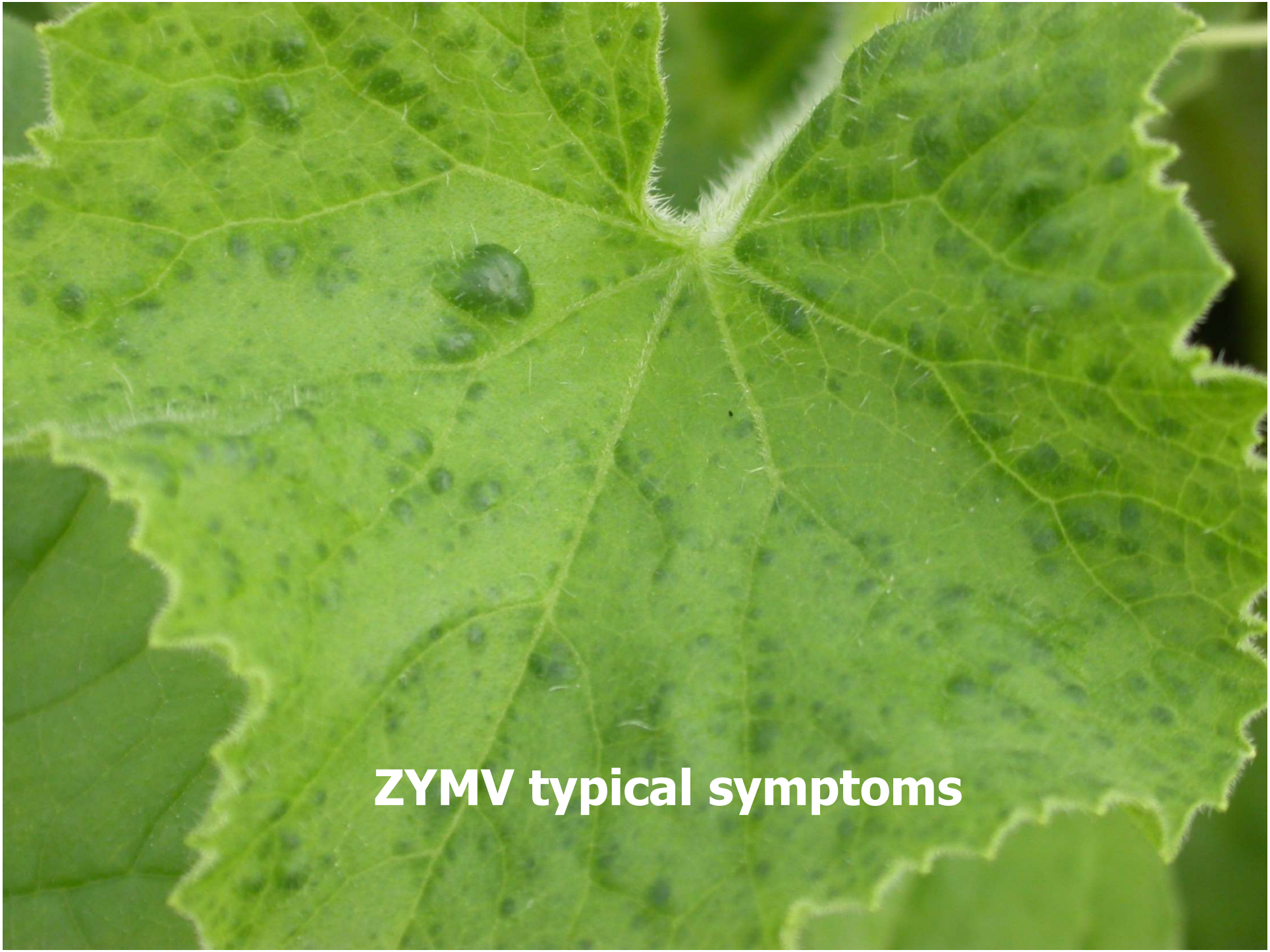
Intermediate Resistance: Sakata definition

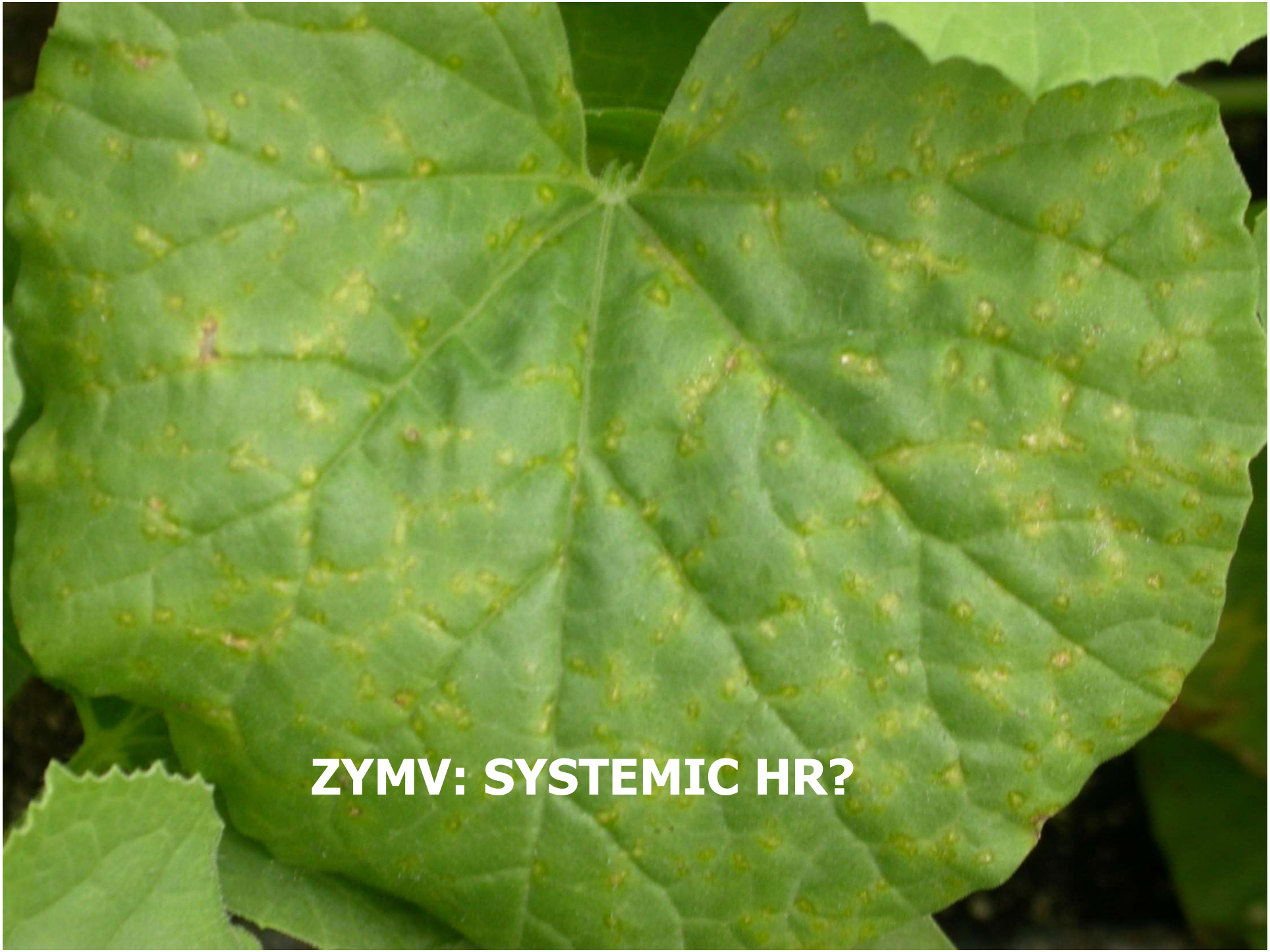
- "The ability of a plant to endure attack by a specified pathogen by performing and producing in spite of the disease. An int. resistant plant my exhibit a wider range of symptoms or damage than a resistant plant, but still should perform better than a susceptible plant grown under the same conditions/disease pressure"


#1
#2
#3
#4

Resistance caveats

- TSWV tomato fruit spotting (*Sw5*)
- TSWV pepper <10% infections (*Tsw*)
- Some foliar diseases where pesticide applications could be reduced
- Cucurbit viruses
- Tobamovirus resistance in GH's


ZYMV typical symptoms


ZYMV: SYSTEMIC HR?

Stages of hybrid development

- G0: Breeder's program (1,000's of lots)
- G1: Breeder's focus
- G2: Experimental hybrids/early production
- G3: "Finished" hybrid (a few dozen) larger production and some sales begin
- G4: named Commercial product

Breeding trait hierarchy in seed industry

- Shelf life
- Yield
- Market niche
- Disease resistance
- Flavor

Traits pursued in the future will be increasingly complex

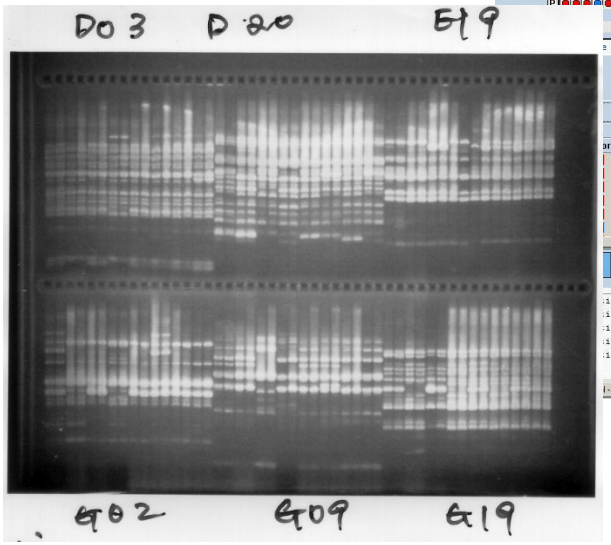
- Gene editing will revolutionize the pursuit of complex traits:
  - Yield
  - Nutrition
  - Flavor
  - Color
  - Shelf Life
  - Drought or salt tolerance
  - Seed performance

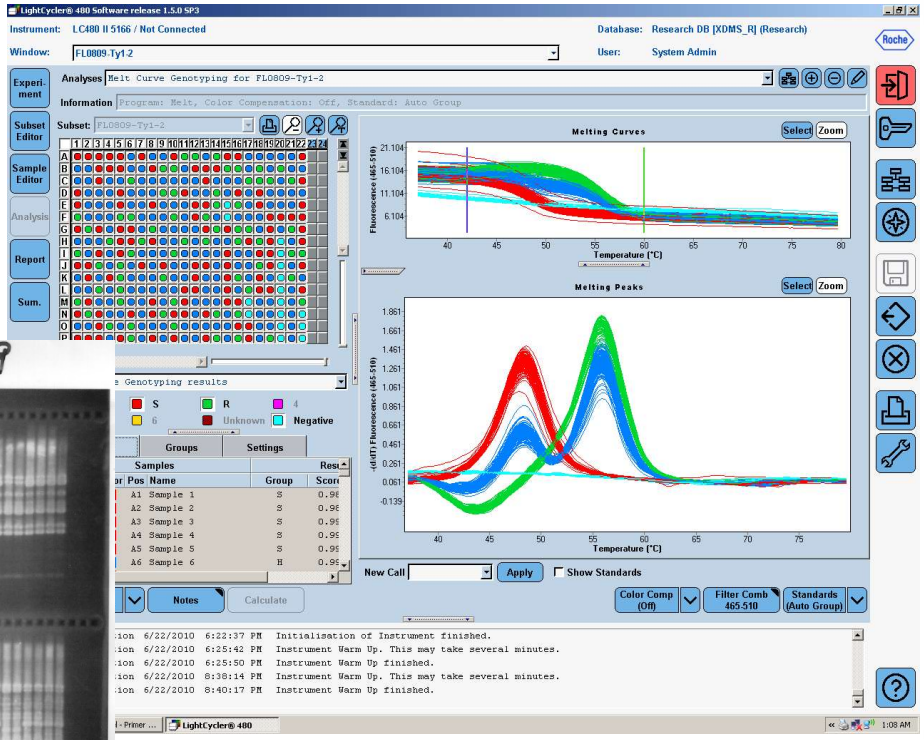
Biotechnology in Use at Sakata

- Molecular markers are an essential tool
  - Reduce costs
  - Allow screening for multiple traits

Reading Molecular Markers Today

- Plant DNA is read for trait of interest





Red dots correspond to plants in a tray that
Are susceptible to a disease. Given this information
The breeder can discard.

Genotyping Each Plant Before Going to the Field – Tray Map

48774 Marker: I3/T 48775

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
R/S	S/R	H/H	H/H	H/R	R/S	R/R	S/S	H/R	R/H	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S
R/H	H/R	S/S	R/R	S/S	R/H	R/S	R/S	S/S	R/R	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S
S/S	H/S	H/H	R/S	R/H	S/S	R/H	H/H	H/H	S/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S
H/H	R/H	H/H	R/S	H/S	S/S	R/H	H/R	R/H	H/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S
H/H	R/H	S/S	H/H	R/H	S/H	S/S	S/S	S/S	R/H	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S
S/S	H/R	H/H	H/H	H/R	R/H	H/H	H/H	S/S	R/R	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S
S/S	H/S	H/H	R/H	S/H	H/S	H/H	R/H	R/H	H/R	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S
S/S	H/R	H/H	H/H	H/R	R/H	H/H	H/H	S/S	R/R	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S
H/H	R/H	H/H	R/S	H/S	S/S	R/H	H/R	R/H	H/H	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S
S/S	H/S	H/H	R/H	S/H	S/S	R/H	H/R	R/H	H/H	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S

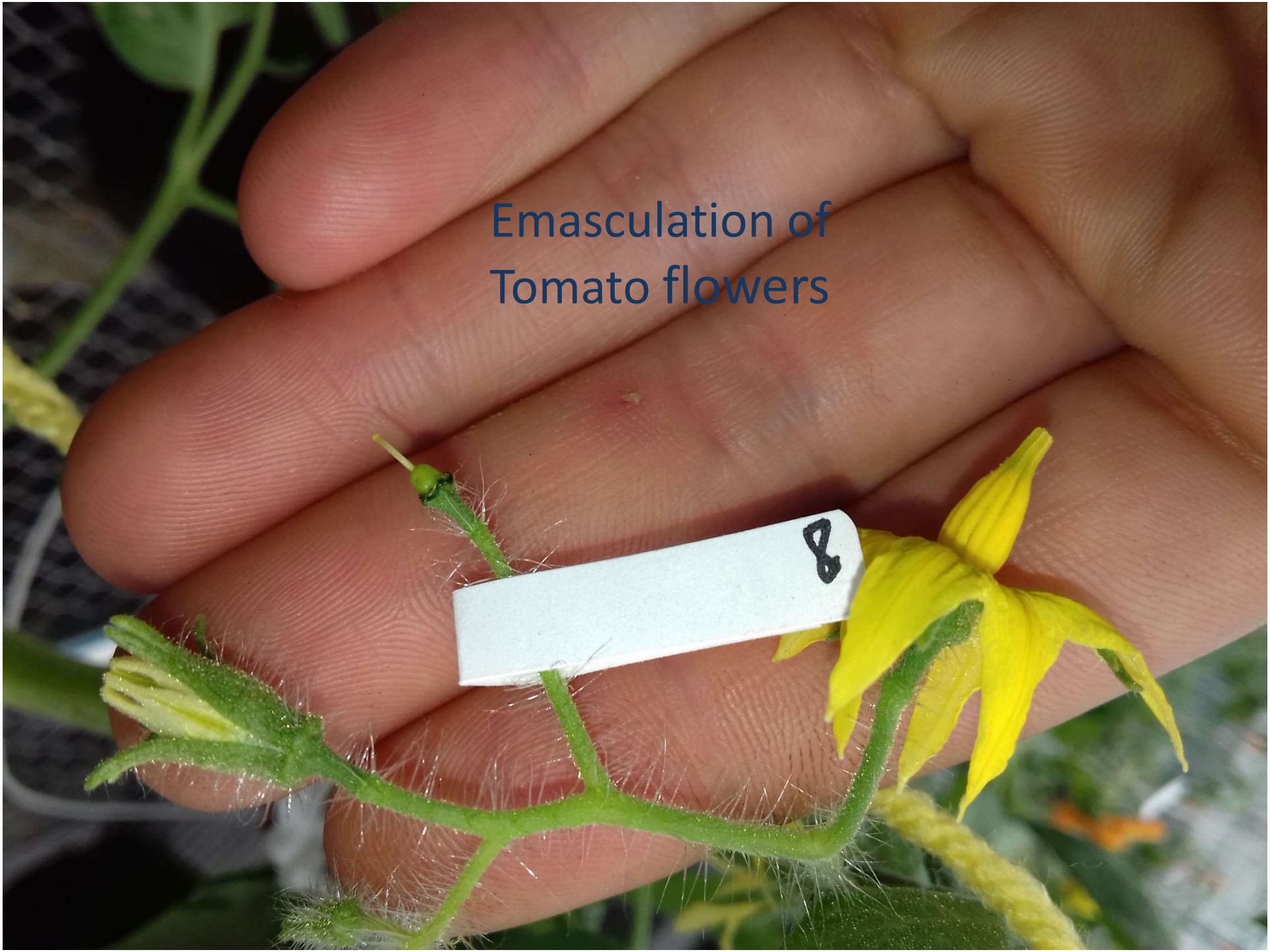
DISCARD 38, SAVE 62

Multiple Markers can be utilized

Marker Assisted Selection (MAS)

- We plant what we have room for.
- MAS enables screening before we use up field space.
- Effectively increases size of program without increasing field use.


Pathology Breeder support


Emasculation of
Tomato flowers
8


2566


SAKATA
SAKATA

Exclusion Protocols

SEED TRANSMITTED DISEASE CONCERNS

CUCURBITS

- ACIDOVORAX AVENAE SUBSP AVENAE (BFB)
- CGMMV (CUCUMBER GREEN MOTTLE MOSAIC VIRUS)
- SQUASH MOSAIC VIRUS
- MNSV
- GUMMY STEM BLIGHT

SOLANACEOUS

- TOBAMOVIRUSES
- CLAVIBACTER MICHIGANENSIS (BACTERIAL CANKER)
- VIROIDS (?)
- PEPINO MOSAIC VIRUS
- XANTHOMONAS SPP (BACTERIAL SPOT)


'99 9 6

Seed Treatments for research seed

Tomato

1.)10% bleach fruit dip

2.)Muriatic Acid (HCl ~7%/20min): Dissolves pulp/some antimicrobial activity

3.)0.5% NaOCl (10% bleach/40 min): Bacteria mainly

4.)10% Tri Sodium Phosphate (TSP):

Tobamoviruses (and PepMV) targeted

5.)Hot water (only if needed)

Seed Treatments for research seed

Cucurbits

1.)10% bleach fruit dip

2.)Fermentation (Pectinase substitute and antimicrobial)

3.)1800ppm Peroxyacetic acid/Hydrogen peroxide (BFB and gummy stem blight)


CGMM
V

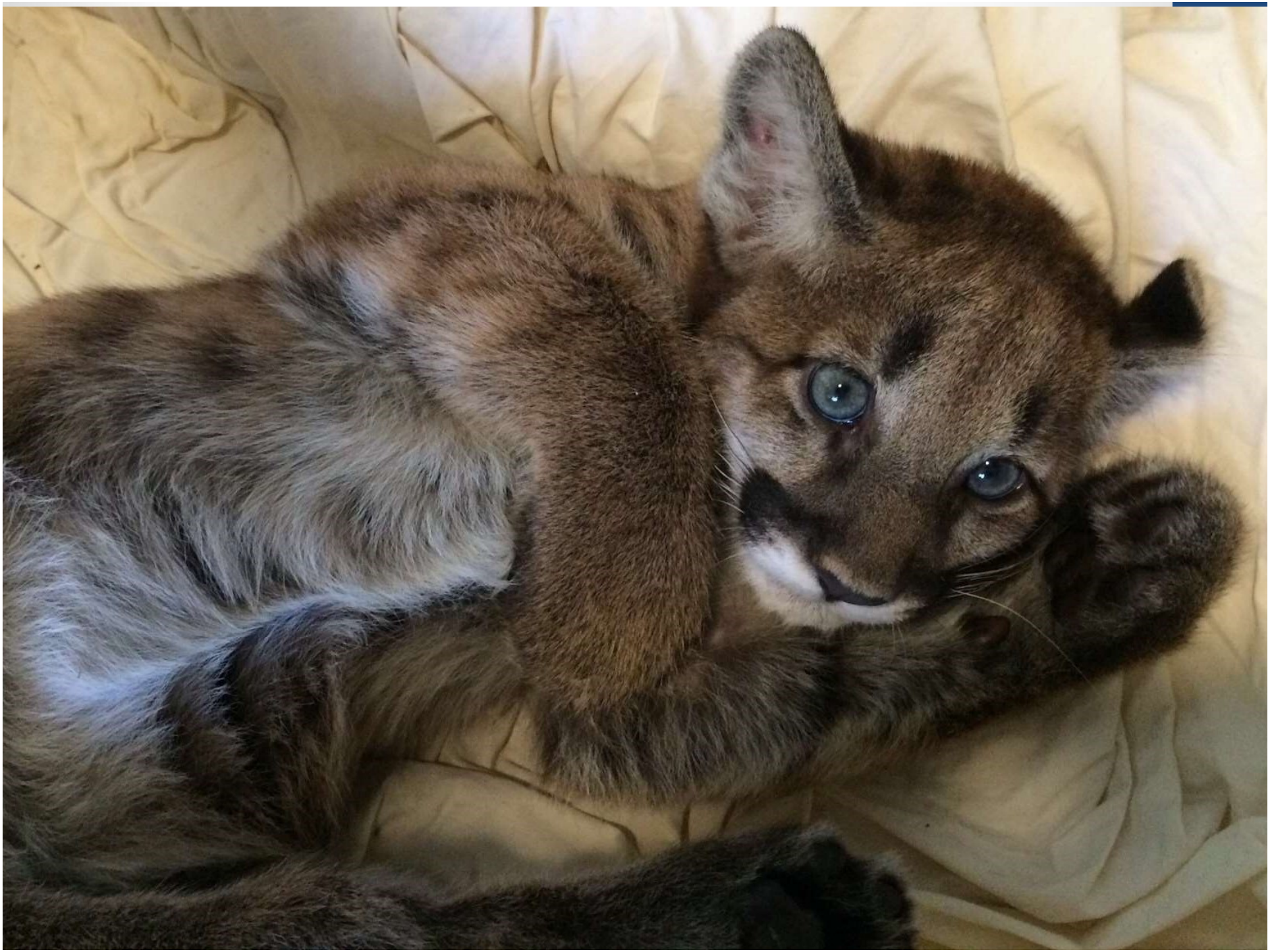
CUCURBIT SEEDBOURNE DISEASES

Disease	transmission	Best symptom expression	Special concerns	Field sprays or cultural practices	Seed treatment
BFB	Seed, mechanical, water, plant debris	Seedling OR harvest(fruit)	Extreme liability due to economic impact	Copper sprays OR peroxide (i.e. Oxidate)	Peroxyacetic acid (Tsunami)
CGMMV	High Seed transmission, mechanical, soil/water, pollen(?)	2 true leaves* to harvest	Symptoms weak early	Sanitation of workers, transplant grower, water source	Heat, Trisodium phosphate.
Squash Mosaic	Seed, mechanical	true leaves and actively growing	Very stable virus, but surprisingly rare	Sanitation of workers, control of cucumber beetle	
Gummy Stem Blight	Wind blown or splashed spores	All stages	Can become a problem in GH	Misc fungicide applications, cull symptomatic or cracked fruit	Peroxyacetic acid and Thiram

SOLANCEOUS SEED BOURNE DISEASES

Disease	transmission	Best symptom expression	Special concerns	Field sprays or cultural practices	Seed treatment
Tobamoviruses	Seed, mechanical	Symptoms are more obvious with mature plants	Extreme liability due to economic impact	Milk solution as hand dip for GH workers when handling	Tri sodium phosphate
Bacterial canker (Cmm)	Seed, mechanical, water	All stages	Infection can be common in GH's with poor sanitation	Bacteriocides, sanitation	Sodium hypochlorite
Viroids (PSTVd)	Seed, mechanical, insect, pollen(?)	Variable and difficult	Can be a hidden problem. Pollen transmission?	Tools/surfaces sanitized with 1% NaOCl; Aphid control	none
Xanthomonas leaf spot.	Seed, wind blown water droplets, mechanical	All stages	Can become a problem in GH and movement of races	Coppers and peroxide based sprays, Actiguard(?)	Sodium hypochlorite


RJohnson@Sakata.com




"Sakata" Now lives at
Homosassa Springs

EXCLUSION PROGRAM PROTOCOLS

- GERMPLASM INTRODUCTION PHYTOSANITARY STATUS CHECKLIST (REVIEWED ANNUALLY)
- SEEDLING PRODUCTION AND TRANSPLANT INTRODUCTION PROTOCOL
- BEST MANAGEMENT AND SANITATION PRACTICES (GSPP)
- SEED PROCESSING AND TREATMENT PROTOCOLS
- DISEASE EPIDEMIC CONTAINMENT PROTOCOL

Seed and Seedling Testing Requirements for Managing Seed-borne Diseases in Melon Breeding Program

Revised: Apr. 21, 2017 (initial draft)

	Pathogens of Concern and Requirements				
Source	SqMV	CGMMV	MNSV	Aac	Db
Florida - old and new	Seedling inspection at 3rd to 4th true leaf followed by ELISA of suspect plants. Note: CGMMV and MNSV symptoms considered hard to determine in seedlings, but risk for these sources considered equally remote.			Visual inspection under normal transplant growing conditions. Immunostrip testing of suspects (minimum entire tray destroy if Aac detected).	For 2017, past increased considered Db negative. Going forward, seed from these sources will be Tsunami treated at the prod location. If not done, presumptively treat seed with Tsunami-100 plus fungicide application to seed.
Guamuchil - Research					
Guamuchil - Production					
CA - Colusa, Woodland					
CA - Colusa, Woodland					
Commercially sold lots from reputable competitors or cooperators with C of A				Such lots considered okay to plant and use seedling inspection as described above.	
External - Univ/USDA	Quarantine SNLS 10% seed test if samples are large enough, otherwise seedling ELISA			Quarantine SNLS Seedling inspection while growing plants in a conducive environment (procedure development needed)	
External - other breeders, competitor seed, cooperators					

GERMPLASM INTRODUCTION CHECKLIST

- LOCATION OF LOT PRODUCTION: SAKATA EXCLUSION SITE OR ELEVATED RISK LOCATION
- SEED CLASSIFICATION (RESEARCH, PRODUCTION, SALES/COMPETITOR VARIETIES
- DISEASE ASSAY STATUS: CERTIFICATE OF ANALYSIS OR INTERNAL TESTING
  - 10% DESTRUCTIVE ASSAY UNDER HIGHLY CONDUCIVE NON-DESTRUCTIVE GROWOUT OR POOLED SAMPLE

SEEDLING PRODUCTION AND TRANSPLANT INTRODUCTION (NORMAL RISK)

- PHYTOSANITARY UNDERSTANDING WITH 3RD PARTY TRANSPLANT GROWER
  - BUFFERED GH SPACE DEDICATED TO SAKATA ONLY
  - DISPOSABLE OR DEDICATED TRAYS FOR SAH USE ONLY
  - PHYTOSANITARY MEASURES OBSERVED AND BEST MANAGEMENT PRACTICES FOLLOWED (BACTERIA)
  - DELIVERY TRAILER SANITIZED PRIOR TO SHIPMENT
  - CLEAN, ORDERLY, NO SURPRISES
- INSPECTIONS AT FULLY EXPANDED COTYLEDONS, 2 TRUE LEAVES, AND 4 TRUE LEAVES (3RD PARTY INSPECTIONS?)
- DOCUMENTATION AND PHOTOS OF ANY SUSPICIOUS SYMPTOMS AND INQUIRY RESULTS
- RESEARCH TRANSPLANT PRODUCTION ON SITE BY 2018

SEEDLING PRODUCTION AND TRANSPLANT INTRODUCTION (ELEVATED RISK)

- SALINAS PATHOLOGISTS OVERSEE DESTRUCTIVE/NON DESTRUCTIVE TESTING
- 10% OF LOT IS GROWN AND POOLED FOR TESTING OR DESTRUCTIVELY ASSAYED UNDER HIGHLY CONDUCIVE CONDITIONS (INSPECTIONS AT FULLY EXPANDED COTYLEDON, 2 TRUE LEAF, AND 4 TRUE LEAF
- OPTION FOR COMPLETING A (SELF) GENERATION IN SNLS
- PRESUMPTIVE "DRY SEED" TREATMENTS (NOT IDEAL) OR HEAT TREATMENTS

BEST MANAGEMENT AND SANITATION PRACTICES

- BEST MANAGEMENT PRACTICES FOR BACTERIA, FUNGI, AND INSECTS
- NO COMINGLING OF RESEARCH AND PRODUCTION PLANT MATERIAL!
- LIMITED ACCESS TO GROWING AREA/MANDATORY SANITATION OF WORKERS AND VISITORS. NO OFF STATION VEHICLES ALLOWED IN GROWING AREAS WITHOUT DIP/SPRAY DOWN. NO FREE MOVEMENT OF WORKERS OR IMPLEMENTS FROM RESEARCH TO PRODUCTION WITHOUT SANITIZING PROTOCOL
- DETERMINE BEST SANITIZING AGENT FOR EACH SITUATION: POTASSIUM PEROXYMONOSULFATE (1% VIRKONS), (1-0.5%) SODIUM HYPOCHLORITE, QUATERNARY AMMONIUM SALTS (MANY FORMULATIONS), ISOPROPYL ALCOHOL
- 3RD PARTY INSPECTION OF CROP VIA USDA OR AFFILIATE OR INTERNAL JUST BEFORE HARVEST FOR PHYTO PURPOSES
- SAH INTERNAL INSPECTION OF FRUIT AT HARVEST (SIGNOFF) AND DURING EXTRACTION

SEED PROCESSING AND TREATMENT PROTOCOLS

- CUCURBITS (FERMENTATION, TSUNAMI)
- TOMATO (ACID, BLEACH, TSP)
- PEPPER (BLEACH, TSP)
- SANITATION OF PROCESSING AREA AND DRYERS

WE HAVE A PROBLEM!
DISEASE CONFIRMATION ONLY BY PATHOLOGY

1. SAH PATHOLOGIST INVESTIGATION INITIATED
2. SOURCE(S) OF INFECTION ANALYZED
3. DOCUMENT SITUATION (PHOTOS/VIDEO)
4. PLAN COURSE OF ACTION WHAT GETS DESTROYED
5. “SCORCHED EARTH” STRATEGY FOR DECONTAMINATION

ANNUAL REVIEW OF EXCLUSION PROGRAM

- ASSESS NEW DISEASE THREATS (RISK DESIGNATION, NEW EMERGING DISEASES)
- EDUCATION AND TRAINING (NEW EMPLOYEES ARE TRAINED IMMEDIATELY)
- AUDITS (ARE WE DOING WHAT WE AGREED TO DO?)
- ALL SEED MUST BE ENTERED INTO THE SYSTEM!!!